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Napoli, Italy, October 15-17, 2020

ABSTRACT BOOK

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ABSTRACT BOOK

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MAIN PROGRAM

HYPOTESIS-DRIVEN DISSECTION OF MOLECULAR COMPONENTS OF INNATE IMMUNITY AND INFLAMMATION: FROM CANCER TO COVID-19

A. Gomes,[1] B. Bottazzi,[1] M. Locati,[1,2] C. Garlanda,[1,3] A. Mantovani[1,3,4]

[1]Humanitas Clinical and Research Center – IRCCS, Rozzano (Mi) - Italy; [2]Department of Medical Biotechnologies and Translational Medicine, Università degli Studi di Milano; [3]Humanitas University, Department of Biomedical Sciences, Pieve Emanuele – Milan, Italy; [4]The William Harvey Research Institute, Queen Mary University of London, London EC1M 6BQ, United Kingdom

Inflammation is a manifestation of innate immunity and has emerged as a metanarrative of modern medicine (Mantovani *et al.*, *Immunity*, 2019; Furman et al., *Nature Medicine*, 2019), a component of diverse disease ranging from cancer to COVID-19 (Mantovani *et al., Nature* 2008; Mantovani e Netea, *New England Journal of Medicine*, 2020 in press). Dissection of the diversity and complexity of regulatory pathways of innate immunity has taken advantage of hypothesis driven and non-hypothesis driven approaches.

IL-1 is the prototypic member of a complex family of cytokines and receptors which play a central role in innate immunity and in the activation and regulation of adaptive immune responses (Mantovani et al. *Immunity*, 2019). Based on structure, we originally hypothesized that IL-1R2 should behave as a decoy receptor, a tenet confirmed by extensive experimental data. In the same line, we cloned IL-1R8 and focused on it based on the hypothesis that it would behave as a negative regulator. IL-1R8 was then found to be a negative regulator of signaling downstream of members of the IL-1 and Toll like receptor (TLR) family and a component of the receptor complex recognized by the anti-inflammatory immunosuppression cytokine IL-37.

As a result of a fishing expedition we originally cloned PTX3 as a gene induced by IL-1 (Garlanda *et al*., *Physiol Rev*, 2020). This distant relative of C reactive protein (CRP) was then found to represent an essential component of the humoral of innate immunity playing a role in antimicrobial resistance and in the regulation of inflammation. The latter includes selected solid tumors and hematological malignancies (Garlanda et al., *Physiol Rev*, 2020). We also cloned in silico PTX4, but its function remains to be defined. Interestingly, PTX3 was recently found to be highly expressed in monocytes and lung macrophages at population and single cell level in COVID-19 and to represent a candidate novel biomarker of disease severity (Brunetta et al., medRxiv, 2020). Stemming from our interest in macrophage diversity and polarization (Locati, Curtale, Mantovani, *Ann Rev Pathol* 2020; Mantovani *et al.*, *Nature Rev. Clin. Onc*. 2017; Jaillon et al., *Nature Rev Cancer* 2020). We identified the tetraspan MS4A4A, a gene of unknown function, as a gene associated with M2-like macrophage polarization (Mattiola *et al*., *Nat Immunol*. 2019). We found that MS4A4A partner with Dectin-1 and is essential for full syk signalling downstream of this pattern recognition receptors. The examples discussed above, selected from our previous and ongoing research efforts, highlight the complexity of innate immunity and its regulation. The dissection of the daunting complexity and diversity of cellular and human innate immunity requires the use of unbiased non-hypothesis-driven approaches complemented by hypothesis-driven, biased experimental and clinical testing.

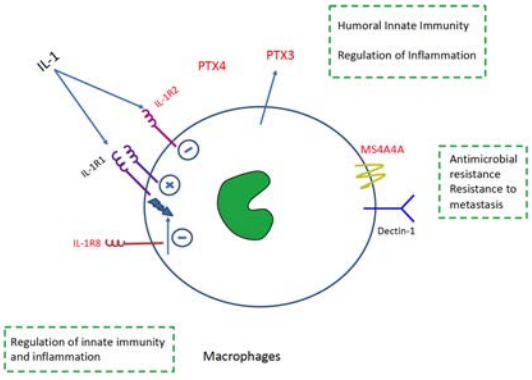

Figure 1. Selected genes (in red) involved in innate immunity inflammation discovered and functionally characterized by the authors. Unbiased fishing was coupled with hypothesis-driven experimental approaches.

References

Mantovani A, Dinarello CA, Molgora M, Garlanda C. Interleukin-1 and Related Cytokines in the Regulation of Inflammation and Immunity. Immunity. 2019;50(4):778-795. doi:10.1016/j.immuni.2019.03.012

Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med. 2019;25(12):1822-1832. doi:10.1038/s41591-019-0675-0

Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454(7203):436-444. doi:10.1038/nature07205

Mantovani A, Netea M
Trained Innate Immunity: epigenetics and Covid-19
New Engl J Med, 2020, in press.

Garlanda C, Bottazzi B, Magrini E, Inforzato A, Mantovani A. PTX3, a Humoral Pattern Recognition Molecule, in Innate Immunity, Tissue Repair, and Cancer. Physiol Rev. 2018;98(2):623-639. doi:10.1152/physrev.00016.2017

Macropahge expression and prognostic significance of the long pentraxin PTX3 in COVID-19
Enrico Brunetta, Marco Folci, Barbara Bottazzi, Maria De Santis, Alessandro Protti, Sarah Mapelli, Roberto Leone, Ilaria My, Monica Bacci, Veronica Zanon, Gianmarco Spata, Andrea Gianatti, Marina Sironi, Claudio Angelini, Cecilia Garlanda, Michele Ciccarelli, Maurizio Cecconi, Alberto Mantovani
medRxiv 2020.06.26.20139923; doi: https://doi.org/10.1101/2020.06.26.20139923

Locati m, Curtale G, Mantovani A. Diversity, Mechanisms and Significance of Macrophage Plasticity. Ann Rev Pathol 2020; 15:123-47

Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol. 2017;14(7):399-416. doi:10.1038/nrclinonc.2016.217

Jaillon S, Ponzetta A, Di Mitri D, Santoni A, Bonecchi R, Mantovani A. Neutrophil diversity and plasticity in tumour progression and therapy. Nat Rev Cancer. 2020;20(9):485-503. doi:10.1038/s41568-020-0281-y

Mattiola I, Tomay F, De Pizzol M, et al. The macrophage tetraspan MS4A4A enhances dectin-1-dependent NK cell-mediated resistance to metastasis. Nat Immunol. 2019;20(8):1012-1022. doi:10.1038/s41590-019-0417-y

"OMICS" APPROACHES AND BIG DATA ANALYSES

G.S. Vassiliou

Cambridge, UK

Throughout human history big advances have followed "disruptive" changes in technology or society. The examples go as far back as the first agricultural revolution of more than 10,000 years ago, which changed human civilisation irrevocably. Biology and Medicine are currently going through their most dramatic period of progress in living memory, driven by advances in omics and high-content data analyses. These approaches are not only useful in improving our understanding and ability to classify, but also represents powerful engines of discovery. Furthermore, these technologies operate on different mathematical scales to romantic human endeavour and promise to effect exponential progress that will transform Medicine as we know it.

Brace yourselves!

THE BIRTH OF ITALIAN HEMATOLOGY AND THE FOUNDATION OF HAEMATOLOGICA

P. Mazzarello

Department of Brain and Behavioral Sciences and University Museum System, University of Pavia, Italy

Since antiquity and in every culture, blood has always been considered emblem of life, a source of nutrition, the seat of the vital spirit and soul. With the development of scientific thought this fluid has progressively been interpreted in a pathophysiological sense, as the basis of the mechanisms underlying the body's metabolism in health and disease. A fundamental turning point came with the publication in 1628 of William Harvey's scientific masterpiece *Exercitatio anatomica de motu cordis et sanguinis in animalibus*. The heterogeneity of body structures found then a common ground for functional unification: the blood.

However the term hematology was used in a scientific and professional medical sense much later. Thomas Schwencke (1694-1767), a professor at the Faculty of Medicine of the University of The Hague, and also a physician to Wolfgang Amadeus Mozart in 1765 during his concert tour in the Netherlands, published in 1743 a volume entitled *Haematologia, sive sanguinis historia,* which is considered the very first textbook of hematology. A book written by Martin Schurig (1656-1733), a doctor from Dresden, was published in 1744 which again bore the term 'hematology' in the title: *Haematologia historico-medica, hoc est sanguinis consideratio physico-medico-curiosa etc.* However, hematology as a separate medical specialty, with its own journals and clinical departments was born much later. The conceptual turning points for the development of the discipline during the nineteenth century were: the formulation of the cell theory, the progress of microscopy with the development of achromatic lenses and the introduction by Paul Ehrlich (1854-1915) of important methods of staining and differentiation of the various cells of the blood. The most important Italian pioneer of modern hematology was undoubtedly the general pathologist Giulio Bizzozero (1846-1901) who was responsible for some fundamental advances: the discovery of the hematopoietic function of the bone marrow, the description of phagocytosis and the observation of platelets.[1] In the first half of the twentieth century, Adolfo Ferrata (1880-1946) played a fundamental role in the application of hematological research to the medical clinic.

Born in Brescia, he studied medicine at the University of Parma and soon showed a great aptitude for laboratory research. During two scientific stays in Germany, he developed a considerable interest in the study of blood under the direction of Julius Morgenroth (1871-1924) and, especially, of Artur Pappenheim (1870-1916). The latter was one of the leaders of the emerging field of hematology and had founded *Folia haematologica*, a prestigious journal dedicated to the study of blood in physiology and pathology. Back in Italy, Ferrata published the monograph *Morfologia del sangue normale e patologico* (1912) that can be considered a cornerstone of Italian hematology. In the following years he quickly became one of the leader in this field of medicine.[2] Ferrata soon felt the need for an Italian scientific journal that would become a means of disseminating research in the hematology field in Europe. The project was developed in Naples in 1920 during a meeting in the "Gambrinus" café, between Ferrata and Carlo Moreschi (1876-1921).[3] The latter had been a pupil of Camillo Golgi (1943-1926) and, subsequently, worked in Paul Ehrlich's laboratory in Frankfurt. In 1907 and in 1908, he developed the antiglobulin test, rediscovered many years later by Robin Coombs (and known nowadays as "Coombs test"). Ferrata and Moreschi therefore launched the *Haematologica* under the best auspices of the Neapolitan sun. Initially published in Naples by the typography "N. Jovene & Co.", in 1924 the editorial office of the journal followed Ferrata who had obtained the chair of medical clinic at the University of Pavia. The first article in the new journal was signed by Camillo Golgi, the most prestigious name of Italian medicine, also known for his hematological studies on malarial infection.

From that moment on, a journal came to life that ferrata has characterized the history of a century of hematology.[4] At the end of its one hundred years of life, *Haematologica* can look forward to the next hundred years with full confidence.

Figure 1. Adolfo Ferrata.

Figure 2. Camillo Golgi.


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HAEMATOLOGICA

ARCHIVIO DI EMATOLOGIA E SIEROLOGIA

Istituto di Patologia Generale dell'Università di Pavia.

Sulla struttura dei globuli rossi dell' uomo e di altri animali. (*)

(con una tavola litografica a colori)

per il prof. Camillo Golgi

Figure 3. The first article published on *Haematologica.*

References

1. Mazzarello P, Calligaro AL, Calligaro A. Giulio Bizzozero: a pioneer of cell biology. Nat Rev Mol Cell Biol. 2001;2(10):776-781.
2. Mazzarello P, Calligaro A, Vannini V. [L'ematologo Adolfo Ferrata: un profilo]. Medicina nei Secoli 2005;17(3):747-768.
3. Garbarino C. [Moreschi, Carlo]. Dizionario Biografico degli Italiani. 2012;76:692-694.
4. Mazzarello P. One hundred years of Haematologica. Haematologica 2020;105(1): 12-21

GUIDELINES FOR MYELOMA COMPLICATIONS

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Multiple myeloma (MM) is a common hematologic malignancy characterized by the accumulation of abnormal plasma cells in the bone marrow. Frequent features of MM include bone destruction, renal impairment (RI), anemia, immunological dysfunction leading to infections, pain, hypercalcemia, thromboembolic events and peripheral neuropathy.

Management of Myeloma-Related Bone Disease. Approximately 70-80% of patients have osteolytic lesions at diagnosis and up to 90% develop lytic lesions during the course of their disease. Myeloma bone disease is due to increased osteoclast activity which is accompanied by suppressed osteoblast function leading to debilitating skeletal complications such as pathologic fractures, severe bone pain and hypercalcemia. Regarding imaging, conventional radiography or recently whole body low dose computed tomography (WBCT) will reveal lytic lesions in 80% of patients at diagnosis. In cases of asymptomatic (smoldering) myeloma whole body MRI (or spine and pelvis MRI) can reveal focal lesions that suggest symptomatic disease with the novel IMWG criteria. Targeting osteoclastic bone resorption represents to-date the most important approach to treat patients with myeloma-related bone disease. Bisphosphonates (BPs) are potent inhibitors of osteoclast activity and function. Zoledronic acid (ZA) is the preferred bone targeted agent for newly-diagnosed MM (NDMM) patients with or without myeloma bone disease. Once patients achieve VGPR or better, the treating physician may consider decreasing frequency or discontinuing ZA, if the patient has received one year of monthly ZA. Denosumab can be also considered for the treatment of myeloma-related bone disease, particularly in patients with RI. Denosumab may prolong PFS among NDMM patients with bone disease, who are eligible for autologous transplantation. Denosumab discontinuation is challenging due to rebound phenomenon. Cement augmentation is effective for painful vertebral compression fractures. Radiotherapy is recommended for uncontrolled pain, impeding or symptomatic spinal cord compression or pathological fractures. Surgery should be used for the prevention and restoration of long-bone pathological fractures, vertebral column instability and SCC with bone fragments within the spinal route. Novel anti-myeloma agents, such as immunomodulatory drugs, proteasome inhibitors and daratumumab alter abnormal bone metabolism in MM. Lenalidomide, thalidomide and bortezomib reduce bone resorption either directly, through the inhibition of osteoclast formation, or indirectly, through the modification of interactions between malignant plasma cells and osteoclasts. However, in terms of the restoration of osteoblast function, bortezomib (and possibly other PIs) and daratumumab are able to directly stimulate osteoblast differentiation and activity and leads to increased bone formation and increased bone mineral density, at least in responders. Novel drugs that may effectively manage myeloma bone disease in the future include anti-sclerostin antibodies (romosozumab).

Management of Renal Impairment. Depending on the definition of RI, this complication is reported in 15-40% of MM patients. RI in MM results primarily from the toxic effects of monoclonal light chains on the kidney, as well as other contributing factors such as dehydration, hypercalcemia, hyperuricemia, the use of nephrotoxic drugs and rarely myeloma cell infiltration or hyperviscosity. Cast nephropathy is the main cause of RI in MM (~90% of cases) and is characterized by tubular atrophy and tubular-interstitial fibrosis. Proximal tubule cells may be also affected. IMWG guidelines support the use of bortezomib plus dexamethasone (or triplets, i.e. VTD, VCD or PAD) in MM patients with RI. Thalidomide maybe used with caution in the absence of results from randomized trials in this setting. Lenalidomide is also a feasible and effective option for patients with mild-to-moderate renal impairment, if it is used at the recommended reduced dose based on renal function. Pomalidomide can be used at the standard dose irrespective of the kidney function, while carfilzomib and ixazomib may be also used in this setting.

Management of Anemia. Treatment with erythropoiesis stimulating agents (ESAs) may be initiated in patients with persistent symptomatic anemia (usually Hb levels <10g/l) in whom other causes of anemia (i.e. iron deficiency) have been excluded. The standard dose of Epo-α is 40,000U/week, of Epo-β 30,000U/week and of darbepoietin 150mg/week or 500mg every three weeks. Hb levels should not increase > 12g/l. ESAs should be stopped after 6–8 weeks without adequate Hb response. True or functional iron deficiency during treatment with an ESA should be treated with intravenous iron.

Management of peripheral neuropathy (PN). In the treatment for chemotherapy-induced PN, prevention is a key strategy for patients' quality of life and ongoing treatment options. All MM patients with potential neurotoxic drugs should be routinely and clinically assessed for signs of PN before undergoing treatment and sequentially graded with validated tools, such as the Total Neuropathy Score. The use for dose modifications for the management of bortezomib- or thalidomide-induced PN remains the 'gold standard' of care. Reduction of PN induced by bortezomib can be achieved by a) prompt dose-modification ($1.3 \rightarrow 1.0 \rightarrow 0.7 mg/m^2$), b) once-per-week instead of twice weekly application and c) s.c. rather than i.v. administration.

Infections Prophylaxis: Vaccination against influenza is appropriate; moreover against Streptococcus pneumonia and Haemophilus influenzae is recommended, but efficacy is not guaranteed, due to suboptimal immune response. Prophylactic aciclovir is recommended for patients receiving bortezomib therapy, ASCT/allogeneic-SCT and possibly lenalidomide.

OPEN QUESTIONS AND SELECTION CRITERIA FOR ADVANCED DIFFUSE LARGE B CELLS LYMPHOMA PATIENTS CANDIDATES TO CAR T-CELLS

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Introduction. The standard treatment of advanced stage Diffuse Large B cell Lymphoma (DLBCL) is the monoclonal antibody anti-CD20 rituximab in combination to cyclophosphamide-doxorubicin-vincristine-prednisone (R-CHOP). Despite the high rate of complete response at the end of the treatment, roughly the 40% of the patients experience relapse or progression[1]. Nowadays, a better characterization of DLBCL in term of cell of origin (COO), immunohistochemical profile and cytogenetic alterations allows to identify patients with a dismal prognosis when treated with R-CHOP. First line more intensive chemotherapy regimens have been tested in these selected subgroups in order to improve prognosis but the best strategy for treatment of patients with high grade or double expressor lymphomas has not yet been stated. Relapsed or refractory patients after R-CHOP chemotherapy have to be evaluated for eligibility to salvage chemotherapy containing platinum-derived drugs (R-DHAP or R-ICE) followed by autologous transplant. In patients eligible to transplant this program is the standard of care and allows to achieve a long disease control in about 50% of patients[2]. Historically, clinical characteristics such as prior rituximab treatment, early relapse (within 12

months) and an high age-adjusted IPI score, were identified as prognostic parameters at relapse correlated to lower 3-years PFS and OS[3]. Overall, patients refractory after a first or a second line therapy and relapsed after autologous transplant have a poor prognosis as showed in the SCHOLAR study, reporting for these patients a median event free survival of 6 months[4]. Among the novel molecules available for treatment of relapsed or refractory DLBCL, three classes of drugs showed an anti-lymphoma activity: BTK inhibitors, lenalidomide and antiapoptotic inhibitors. However, Overall Response Rate (ORR) observed after exposition to ibrutinib in ABC DLBCL was 37%, in DLBCL exposed to lenalidomide was 27% and in patients treated with venetoclax was 18%. Treatment of these patients represents a clinical challenge. Recently, availability of anti-CD19-directed chimeric antigen receptor (CAR) T-cells, both in clinical trials and in commercial use has transformed the treatment approach for these patients.

CAR T-Cells. CAR T-cells are generated using an inactivated viral vectors (lentivirus or retrovirus) to transduce autologous T cells to express an antigen receptor (CD19), a co-stimulatory domain (usually CD28 or 4-1BB) and an intracellular signaling domain (CD3-zeta). Differently from other new compounds, this new treatment is not an "off-the-shelf" drug, indeed the availability of CAR T-cells is not immediate. In fact the T cells are collected from the patients, then the cell product is genetically modified and at last is reinfused to the patients. Before infusion of CAR T-cells a lymphodepleting chemotherapy is given to improve proliferation and expansion and prevent an anti-CAR immune response. Since first use in humans in 2010, many efforts have been made to develop new generations of CAR T-cells and to better understand possible clinical applications and toxicities. Today two different products, axicabtagene ciloleucel (axi-cel) and tisagenlecleucel (t-cel), have been approved from FDA and EMA for treatment of refractory or relapsed DLBCL patients after at least two prior lines of systemic therapy. A third product, the lisocabtagene maraleucel (liso-cel) is pending for approval by FDA. Axicabtagene ciloleucel contains a CD28 costimulatory domain in addition to a CD3 zeta domain. The Phase 2 ZUMA-1 study enrolled 111 patients and 101 of them received axi-cel[5]. Overall Response rate was 82% and 54% of patients achieved a CR, among 77 DLBCL patients CR was achieved in 49%. CR were durable at a median follow-up of 15.4 months in 70% of patients. The updated follow-up at 2 years for these patients showed that the median OS is not reached and 39% of patients have ongoing responses two years after the infusion. Recently published data showed an ORR of 82% and CR of 69% among 298 patients treated outside clinical studies with the axi-cel commercial product in 17 US Institutions[6]. Real life toxicity profile was also similar to the ZUMA-1 study. Tisagenlecleucel contains a 4-1BB costimulatory domain. Following promising results observed in a Phase I study, a large multicenter phase II study was conducted, the JULIET trial[7]. Among 106 infused patients the ORR was 50% and CR rate was 32%, in the subgroup of 16 high grade lymphoma patients the ORR was 50% indicating that the clinical response was independent from the genetic lesions. Liso-cel has a 4-1BB costimulatory domain and is engineered so that the final product has a defined composition of CD4 and CD8 T cells. In the pivotal study TRANSCEND-NHL-001 were enrolled also patients with secondary CNS lymphoma and patients relapsed after prior allogeneic transplant, among 268 infused patients, ORR was 73% and CR rate was 53%[8].

Toxicity. Administration of CAR-T cells products is correlated to a remarkable toxicity. Cytokine Release Syndrome (CRS) and Immune Effector Cell-associated neurotoxicity syndrome (ICANS) are fearsome and potentially life-threatening. CRS is an immune-mediated toxicity caused by an excessive immune reaction. Inflammatory cytokines like Interferon-gamma and TNF-alfa are released from activated T cells and lead to activation of macrophages, dendritic cells and endothelial cells. Early CRS presents with fever and other constitutional symptoms include headache, rash, arthralgia and myalgia. In short time symptoms can progress to hypotension, vascular leakage, DIC and respiratory failure. Clinical conditions can rapidly worsen from flu-like symptoms to multiorgan failure. ICANS is a common toxicity after CAR-T cells infusion, symptoms can range from headache to possible aphasia, seizures and increased intracranial pressure with cerebral edema and coma. Patients develop CRS and ICANS usually during the first 14 days after CAR-T cells infusion, and the onset of ICANS is usually later than CRS. Majority of toxicities resolve in a median of 15 days using immunosuppressive drugs like the anti-IL6 receptor antibody (Tocilizumab) and steroids in case of CRS and corticosteroids alone in case of neurotoxicity. Comparison of toxicity incidence and grading between different products is not easy because of the different toxicity-scale used in the studies. Taken together, all grade CRS incidence is ranging between 37% and 93% with a percentage of Grade≥3 in 1 to 22% of patients. The rate of ICANS of all grades is ranging between 23% and 65% with grade more than 3 in 12 to 31% of patients. Other significant related adverse events are infections, in fact depletion of normal CD19-expressing B cells due to the anti-CD19 activity results in prolonged cytopenias lasting beyond day 90 after infusion. Commonly observed infections are caused by fungal and viral opportunistic pathogens. Today, considering all patients affected by DLBCL treated with CAR T-cells, the rate of mortality related to the treatment is estimated around 4%. Main frequent causes of death are infections and toxicities.

Patients Selection. Considering that, unfortunately, not all patients can be cured by CAR T-cells and that a percentage of them run the risk of early or long term toxicities, a careful evaluation of candidates in the elegibility process is crucial. Appropriate patient selection represents a key point to obtain the maximal benefit in term of disease control and quality of life. The selection process requires evaluation of disease-specific, patient-specific and product-specific factors.

Disease-specific Factors.

Histology. Nowadays commercial CD19-CAR-T cells in Italy are approved for treatment of relapsed/refractory DLBCL (NOS or transformed from follicular lymphoma), High grade B cell Lymphoma (HGBL) and Primary Mediastinal B cell lymphoma (PMBCL) (only axi-cel), after at least two chemotherapic-based regimens. Therefore, the first mandatory step in patient selection is a careful evaluation of histology. Among DLBCL disease different subgroups are represented according to the cell of origin, to the cytogenetic characteristics (such as BCL2, MYC and BCL6 translocation) and to the immunohistochemical profile. Activity of CAR T-cells in different subgroups and optimal timing for treatment are matter of discussion. Based on the available clinical results it seems that CD19-CAR-T is a powerful therapy for all forms of DLBCL, regardless of COO and, more importantly, regardless of DHL/DEL status. Indeed, in the ZUMA-1 study, the ORR in patients with DEL and HGBL was 91%, with a CR rate of 70%, similar to that in the entire cohort[5]. In the JULIET trial, patients with DHL/THL comprised a significant proportion of the cohort (19/70, 27%), and their ORR was the same as patients without DHL/THL[7]. In the recent analysis of 298 patients treated outside clinical trials with axi-cel, subgroups of patients stratified according to COO achieved similar results in term of PFS and OS[6]. Patients with relapsed or refractory PMBCL represent a challenge population with a poor prognosis when treated with conventional chemo and immunotherapy. Results of CAR T-cells treatment in these patients seem promising but detailed analysis for selected cohorts of only PMBCL patients are at the moment not available. CNS involvement is an exclusion criteria for CAR T-cells treatment, however limited cohorts of patients with CNS have been published and no increased cases of neurotoxicity have been reported. These patients should be considered for selected clinical studies.

Previous Therapies. Patients candidates to CAR T-cells treatment are often heavily pretreated, evaluation of number of last therapies and disease response is critical in the selection process. In registration studies no limits of previous regimens were established and results of both ZUMA-1 and JULIET study showed that PFS is not depending on the number of previous chemotherapies; patients pretreated with 3 or more

regimens have similar PFS in comparison to those receiving less than 3 chemo. Same results are reported in commercial use of axi-cel in the study of the US Lymphoma CAR T consortium study and in cases collected from 7 centers in the US[9]. Differently, advantage from early administration of CAR T-cells as second line salvage treatment is matter of debate. Indeed, results of two ongoing clinical trials, ZUMA-7 and BELINDA, could clarify whether patients receiving CAR T early after first line failure have a major benefit from treatment. At the moment no chemotherapy regimens preclude treatment with CAR T-cells, however, administration of highly immunosuppressive regimens such as bendamustine-based or high dose of steroids-containing early before apheresis could have a negative impact on numbers and quality of collected lymphocytes. Patients treated with autologous transplant are eligible, whereas previous allogeneic transplant is not admitted before CAR T-cells commercial use in Italy.

Disease Characteristics. CART cells are an engineered product and availability of final product requires time ranging from 20 to 40 days from apheresis. This is a critical time and the risk of disease progression and clinical deterioration in patients waiting the final product is very high. Therefore, evaluation of the disease in term of progression kinetic, mass expansion, Performance Status and vital organ impairment is another key point in clinical evaluation (Figure 1).

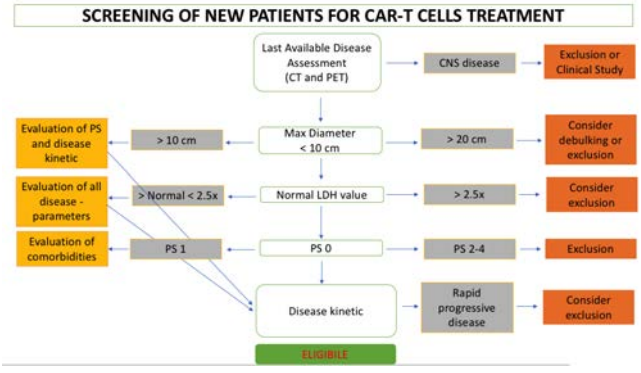

Figure 1. Flow-chart of disease-specific factors evaluation during selection process for CAR T-cells treatment.

Patients with very aggressive disease run the risk not to be alive at infusion day or to receive cells in a critical condition with consequent high risk of life threatening toxicity and insufficient disease control. The tools available to clinicians in order to define disease aggressivity are limited: comparison between last CT scan to evaluate disease progression, LDH value and PS are useful tools. In this complicated evaluation physicians with clinical experience in treating aggressive lymphoma patients and with experience in CAR T-cells therapy have a critical role. In the pivotal studies patients with bulky disease had a reduced probability to achieve a clinical response. In the US Lymphoma CAR T Consortium Study high LDH levels at lymphodepletion correlate with a worse prognosis in term of PFS and OS. Patients with PS > 1 were not eligibile in registration studies, on the contrary in clinical practice patients with PS 2-4 have been treated. Results of two studies on axi-cel outside trial setting showed that patients with PS > 1 experienced more neurotoxicity, less disease responses and lower PFS and OS. Other laboratory parameters like CRP and ferritin have been considered as surrogate of disease aggressivity and CRP at day of infusion seems to be a poor prognostic maker when values are above 30 mg/dl. Sensitivity to last therapy in term of relapsed or refractory disease should reflect disease aggressivity, however available data are not clear in defining refractory disease as less responsive to CAR T-cells: and at the moment it seems that both relapsed and refractory patients have the same probability to respond to CAR T-cells.

Patient-specific Factors. Patients selection requires a careful evaluation of clinical characteristics in terms of age, comorbidities, performance status and risk of infections (Figure 2).

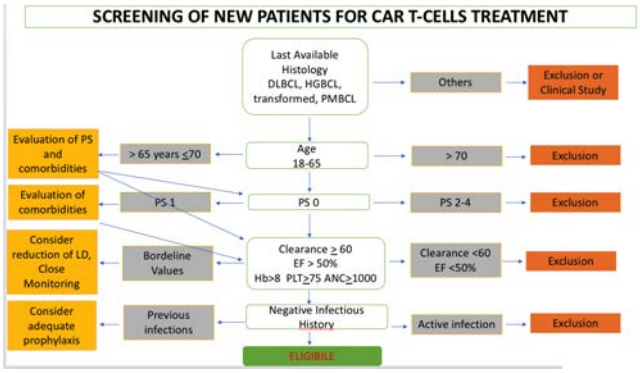

Figure 2. Flow-chart of patients-specific factors evaluation during selection process for CAR T-cells treatment.

In Italy patients older than 70 years are not eligible to CAR T-cells treatment. Patients aged around 70 (i.e between 65 and 70) have to be considered with caution before enrollment, fitness and organ impairment should be evaluated. In a recently published paper, Neelapu et al. observed that these patients are not at higher risk for toxicity and achieve complete remission and durable PFS with the same probability of young patients[10]. However, in our experience development of high grade CRS or neurotoxicity in elderly patients requires at least more assistance, longer hospitalization and more sequelae after discharge than young patients. Evaluation of vital organ function is required for CAR-T eligibility in all patients. Test for kidney, heart and bone marrow function through assessment of creatinine levels and clearance, echocardiogram with ejection fraction measurement, blood test for white blood cells, haemoglobin and platelets counts are part of the screening. Patients with borderline renal function could be treated with CAR-T cells but dose adjustment of lymphodepletive chemotherapy is required to avoid related toxicity. In these patients there is a major risk to develop acute renal failure requiring dialysis in case of high grade CRS, septic shock or complicated infections after CAR-T cells infusion. Similarly, patients with borderline cardiac function or with previous cardiac disease should be closely monitored, in fact, in case of toxicities or infections they are at high risk to require amine support and Intensive Care Unit admission. Infections and progression of disease are the main causes of death in CAR-T cells patients. Bacterial and fungal infections both in the short and in the long term are induced by neutropenia and lymphopenia related to CAR T-cells treatment. Patients with active infections should be excluded from treatment and reconsidered for CAR T-cells program when recovered from infection. Patients with previous severe infection (i.e viral infections, pneumocystis or fungal) should be treated with adequate prophylaxis from lymphodepletion until immune recovery.

Car T-Cells Product Selection. The two CAR T-cells products available for commercial use in Italy, axi-cel and tisa-cel, are different in design and manifacturing. Both are efficacious and safe for eligible patients as showed in pivotal studies and in clinical use. However, direct comparison of the two products in term of activity and risk of toxicity is difficult given that the two registration studies had different study design, eligibility criteria and toxicity grading. The third product lisa-cel, not yet commercialized, seems to have less CRS and neurotoxicity as reported by the TRANSCEND study. The different co-stimulation domain between products, could play a role in different observed toxicities. Axi-cel utilizes the CD28 co-stimulation domain that confer to CAR-T cells rapidity in in-vivo expansion and shorter persistance. On the contrary the 4-1BB co-stimulation domain incorporated in tisa-cel and liso-cel results in more gradual expansion and prolonged persistance of CAR-T cells. Given these differences, frail or old patients with comorbidities

and at high risk for morbidity or mortality in relation to severe toxicities should be considered for a 4-1BB costimulated product that should result in a lower risk of high grade CRS and neurotoxicity. On the contrary, axi-cel, containing the CD28 co-stimulaton domain should be used in young patients requiring a short term disease control due to rapidly progressive disease. Logistic process for CAR-T cells production is different between axi-cel and tisa-cel, manufacturing procedure and turnaround time should be considered in relation to different patients. Clinical status, disease evolution, adequate bone marrow function in relation to last previous therapy, apheresis agenda, lymphocytes pick-up schedule need to be considered separately for every patient. The choice of the product is the result of these careful global evaluation of the patient.

Conclusion. CAR T-cells represent a new, efficacious treatment option for patients affected by relapsed or refractory B cell lymphomas and should be considered in the treatment's landscape for patients with eligible histologies and who failed two or more chemotherapy regimens. A careful evaluation of the patients before starting the CAR T-cells production process is required. Two commercial products with different characteristics are available in Italy at this moment, integration of information about patient, disease characteristics and products availability should allow the correct selection of a specific CAR-T cell product for a given patient. Evaluation of appropriate histology, disease kinetics, performance status, organ function and comorbidities are part of the complex clinical assessment that allows to optimize the real benefit and to reduce toxicities related to CAR T-cells treatment. Results of further studies could help clinicians to better select patients in order to improve outcome.

References

1. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002 Jan 24; 346(4): 235-242.
2. Philip T, Guglielmi C, Hagenbeek A, Somers R, Van der Lelie H, Bron D, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med 1995 Dec 7; 333(23): 1540-1545.
3. Gisselbrecht C, Glass B, Mounier N, Singh Gill D, Linch DC, Trneny M, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol 2010 Sep 20; 28(27): 4184-4190.
4. Crump M, Neelapu SS, Farooq U, Van Den Neste E, Kuruvilla J, Westin J, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. Blood 2017 10; 130(16): 1800-1808.
5. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. N Engl J Med 2017 12; 377(26): 2531-2544.
6. Nastoupil LJ, Jain MD, Feng L, Spiegel JY, Ghobadi A, Lin Y, et al. Standard-of-Care Axicabtagene Ciloleucel for Relapsed or Refractory Large B-Cell Lymphoma: Results From the US Lymphoma CAR T Consortium. J Clin Oncol 2020 May: JCO1902104.
7. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. N Engl J Med 2019 01; 380(1): 45-56.
8. Abramson J, Palomba M, Gordon L, al. e. Pivotal safety and efficacy results from TANSCEND-NHL-001, a multicenter phase 1 study of lisocabtagene maraleucel (liso-cel) in relapsed /refractory (R/R) large B cell lymphomas. Blood 2019; 134: 241.
9. Jacobson CA, Hunter BD, Redd R, Rodig SJ, Chen PH, Wright K, et al. Axicabtagene Ciloleucel in the Non-Trial Setting: Outcomes and Correlates of Response, Resistance, and Toxicity. J Clin Oncol 2020 Jul: JCO1902103.
10. Neelapu SS, Jacobson CA, Oluwole OO, Munoz J, Deol A, Miklos DB, et al. Outcomes of older patients in ZUMA-1, a pivotal study of axicabtagene ciloleucel in refractory large B-cell lymphoma. Blood 2020 Jun; 135(23): 2106-2109.

THE OPTION OF EARLY PALLIATIVE CARE IN HEMATOLOGY

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Early palliative care (ePC) is a novel model of care, which may be delivered also in the ambulatory setting, consisting of delivering dedicated palliative service concurrent with active treatment early in the cancer disease trajectory.[1] Several studies indicate that solid cancer patients with advanced diseases, assigned to receive ePC integrated with standard care, compared with patients assigned to standard oncologic care alone, report improvements in quality of life, mood, distress, satisfaction with care, management of disabling symptoms, mainly pain, healthcare utilization, caregiver outcomes, longer hospice enrollment, lower rates of aggressive treatments near the end of life (EoL), and, in some cases, even survival.[1-3] Moreover, as many patients with cancer hold an inaccurate view of the goals of treatment and their prognosis, an important component of ePC is the cultivation of patients' prognostic awareness as it is associated with earlier enrollment in hospice and lower rates of resuscitation for patients with incurable disease.[1,4] Based on those results, either the American Society of Clinical Oncology or the European Society of Medical Oncology have recognised that patients with advanced solid cancer, should receive dedicated PC services, concurrent with active treatment, as soon as they start active oncologic treatments.[5,6]

Nevertheless, the majority of oncologic patients still do not receive ePC and this is even more apparent in hematologic patients[4]. Although some prospective, either completed or ongoing studies and other observational works have demonstrated the feasibility, acceptability, and efficacy of integrating palliative care in the hematologic setting, especially in allogeneic stem cell transplant and acute myeloid leukemia patients, patients with hematologic malignancies are largely under-utilizers of PC services.[4,7,8] Thus, hematologic patients result to have significantly higher rates of cancer-directed care at the EoL, intensive care unit admissions and in-hospital deaths and are less likely to enrol in hospice or home-care program. The reasons rely mainly on the uncertainty about patients who can benefit of such a model of care, uncertainty about the prognostic definition of hematologic malignancies, misperceptions equating palliative care with EoL care, and scarce availability of specialty palliative care programs (Potenza et al., submitted).[7,8]

Such unmet PC needs may be emphasized by the recent changing landscape of treatment efficacy in the population of patients with hematologic malignancies. The novel therapeutic paradigm represented by anti-CD19 chimeric antigen receptor (CAR) T-cell therapy, by showing an unprecedented effectiveness in patients with relapsed/refractory B-cell non Hodgkin lymphoma and acute lymphoblastic leukemia, may pose to clinical hematologists several challenges to deal with, when discussing with patients and families the opportunity to administer such potentially life-saving immunotherapies.[9] First, although CAR T-cells are less frequently associated with the usual adverse effect of chemotherapy, these treatments have specific toxicities that can, nonetheless, be quite serious. Such toxicities raise significant questions about administering CAR T cells to certain populations, including those with poor performance status. In fact, a significant proportion of potential candidates (37% in a real-life Italian, monocentric, retrospective analysis of relapsed/refractory B-cell non Hodgkin lymphoma) will not be able to undergo the preplanned therapy for clinical reasons (i.e. progressive disease, poor performance status, central nervous system involvement, etc).[9] Second, hematologists may find it challenging to weigh their optimism about CAR T cells against the realities of either response or relapse rates, or toxicity.[9]

EPC may well apply to such an optimistic but demanding scenario. This model may allow hematologic clinicians to help patients and fam-

ilies to be prepared to the possibly different trajectories of treatment, by discussing about the risks and benefits, by articulating and developing a realistic understanding of the likely outcome and also by managing the deep disappointment and loss which would be experienced when CAR T cells cannot be offered, are not effective or result too toxic.

Research studies should be designed and funded, hopefully also by health care systems, to integrate ePC with hematologic patients' care, including the setting of patients who are potential candidates to CAR T-cell therapy products, and compare different models of ePC intervention in different clinical and regional/geographical situations. Much more efforts should also be done to implement education programs for medical and nurse students, as early as possible, which could be continued and refined in the following specialty school courses, including specific medical communication teaching courses[10].

References

1. Temel JS, Greer JA, Muzikansky A, et al. Early palliative care for patients with metastatic non-small-cell lung cancer. N Engl J Med. 2010;363:733-42.
2. Bandieri E, Romero M, Ripamonti CI, et al. Randomized trial of low-dose morphine versus weak opioids in moderate cancer pain. J Clin Oncol. 2016;34:436-42.
3. Bandieri E, Banchelli F, Artioli F, et al. Early versus delayed palliative/supportive care in advanced cancer: an observational study. BMJ Support Palliat Care. Epub ahead of print 14 Jun 2019. DOI: 10.1136/bmjspcare-2019-001794.
4. Gray TF, Temel JS et El-Jawahri A. Illness and prognostic understanding in patients with hematologic malignancies. Blood Rev. Epub ahead of print 6 Apr 2020. DOI: 10.1016/j.blre.2020.100692.
5. Ferrell BR, Temel JS, Temin S, et al. Integration of palliative care into standard oncology care: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2017;35:96-112.
6. Hui D, Bansal S, Strasser F, et al. Indicators of integration of oncology and palliative care programs: an international consensus. Ann Oncol . 2015;26:1953–1959.
7. El-Jawahri A, Nelson AM, Gray TF, et al. Palliative and end-of-life care for patients with hematologic malignancies. J Clin Oncol. 2020;38:944-53.
8. El Jawahri A, LeBlanc TW, Kavanaugh A, et al. Multisite randomized trial of integrated palliative and oncology care for patients with acute myeloid leukemia (AML). J Clin Oncol. Epub ahead of print 25 May 2020. DOI: 10.1200/JCO.2020.38.15_suppl.12000.
9. Potenza L, Luppi M, Efficace F, et al. Early palliative care: a necessary intervention for patients ineligible to approved potentially life-saving CAR T-cell therapy. Clin Lymphoma Myeloma Leuk. 2020;20:201-2.
10. Potenza L, Galli L, Morselli M, et al. Early education for early integrated palliative care. J Pain Symptom Manage 2015 50:e1-2. doi: 10.1016/j.jpainsymman.2015.07.019.

CLINICAL PRATICE GUIDELINES IN HEMATOLOGY

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Clinical practice commonly adheres to some basic normative paradigms: diagnostic criteria, prognostic ranking, treatment recommendations and response criteria. Diagnostic and response criteria are established at consensus conferences celebrated by international institutions, such as WHO or LeukemiaNet, and usually become the unique standard for both clinical trials and clinical practice. Rather, treatment recommendations are pragmatic statements based on the best available evidence and as such elaborated in an evidence-to-recommendation process by national and international scientific societies or networks. Disease-specific practice guidelines usually address or collect all the above normative paradigms and are often intended as "comprehensive baskets/shortcuts of knowledge". However, clinical practice guidelines (CPG) are specific tools translating evidence into appropriate clinical actions and should adhere to the international GRADE (Grading of Recommendations Assessment, Development and Evaluation) standard.[1] As such, CPGs are strategically structured by listing clinical questions, which are subsequently answered by evidence-to-decision flows. Unfortunately, common clinical questions usually show a loose syntax, which especially lacks completeness and transparency. An exemplar: "*Which is the most appropriate therapy for newly diagnosed multiple myeloma (NDMM)?".* In order to build an evidence-to-decision bridge, questions need to be appropriately shaped according to the PICOT (Patient Intervention Comparator Outcome Time) framework which forces all the clinical questions to provide explicit comparative assessments and a formal rank of outcomes. The PICOT format of the above clinical question might therefore be: "Is VRD (Intervention) preferred to the present standard of care (Comparator; stays for VMP or Rd in Italy) for NDMM (Patient) in order to improve the net benefit between progression-free survival (desiderable Outcome) amelioration and possible increase of grade 3-4 adverse events (undesiderable Outcome) in a 5 year time (Time)?". According to the GRADE framework, a systematic review of the PICOT-specific literature is subsequently performed by methodology experts and the certainty of the effects assessed by risk-of-bias tools. A group of clinicians, plus possible lay people such as patient advocates, are reported the evidence review and are in charge of assessing the benefit-to-risk balance and formulating recommendations: the strength of recommendations should be based on the benefit-to-risk ratio.

Over the last 20 years an exponential increase of CPGs devoted to blood cancers has been observed (Table 1). Nevertheless, hematologists still lack a repository of evidence-based practice recommendations developed according to GRADE, fully transparent and regularly updated. The largest set of CPGs were developed by oncology networks/societies but are not really GRADE-based and score poorly at quality appraisal (AGREE-II).[2-4]

Table 1. Hematology CPG (blood cancer management, excluded supportive care and diagnosis/monitoring).

Society/Network/Institution	N° CPG	GRADE	National? Disease-specific?	Dissemination
American Society of Hematology	1 (+2)	Y/N		Web (open), APP Blood Advances Leaflets
National Comprehensive Cancer Network	16	N		Web (open), APP
European Society of Hematology	1^	N		Peer-reviewed papers
European Society of Medical Oncology	20	N		Web (open) Ann Oncol
LeukemiaNet	8	Y/N		Peer-reviewed papers
European Blood and Bone Marrow Transplant	26	N		EBMT manual (online) Peer-reviewed papers
American Society of Clinical Oncology	3	Y/N		Web (open), APP J Clin Oncol
British Committee for Standards in Hematology	31	N	national	Web (open) Br J Hematol
Italian Society of Hematology	23	Y/N	national	Web Peer-reviewed papers
Japanese Society of Hematology	18	N	national	Int J Hematol
Cancer Care Ontario	24	N	national	Web site
German Society for Hematology and Medical Oncology	12	N	national	Web (German)
Swedish Society of Hematology	20	N	national	Web (Swedish)
International Myeloma Working Group	16^^	N	disease-spec	Peer-reviewed papers
European Myeloma Network	8^^	N	disease-spec	Peer-reviewed papers
IWCLL	2	N	disease-spec	Peer-reviewed papers
GELTAMO	7	N	national disease-spec	Web
National Institute of Clinical Excellence	39*	Y/N	national	Web

^ will endorse ESMO guidelines ^^ plus several consensus recommendations on diagnostic criteria, imaging and response criteria *all drug-specific

Since the development of a fully-GRADE guideline is a huge effort and country-specific adaptations are also required, the GRADE-ADAPTE Collaboration proposed a framework for endorsing ("adoption") and/or adapting evidence-based recommendations produced in different cultural and organizational setting.[5] Where high quality guidelines are already available, adaptation may be used as an efficient alternative to *de novo* guideline development for customizing the existing guidelines to the need of local users (The ADAPTE Collaboration 2006-2010). In April 2019 the CNEC (National Center for Clinical Excellence, Quality and Safety of the Therapies) published the 1.3.2 version of the Methodology Manual for CPG development[6] which allowed both de-novo GRADE and GRADE-ADAPTE/ADOLOPT frameworks. In the same year, the Italian Society of Hematology moved from de novo GRADE-based CPGs to GRADE-ADOLOPMENT methodology[7] and

is currently adapting four international high-quality CPGs for inclusion in the CNEC repository.

The formal process of CPG development according to GRADE-ADOLOPT is quite complex and involves many actors (Table 2) and a proper sequence of steps (Table 3), but grants transparency and shared decisions. In particular, for CPGs developed in the patient perspective GRADE suggests to share the rank of relevant outcomes with patient advocates and to discuss with patient representatives country-specific costs and other barriers to adoption of novel technologies as well as equity issues. Moreover, before definite approval, all the CPGs submitted to the CNEC may undergo revision by the registered stakeholders. The CNEC-SNLG repository of high-quality, applicable and regularly updated CPGs is also granted liability value (Italian law n.24, 8th March 2017). Therefore, as soon as the repository is populated with CPGs for the management of blood diseases, Italian hematologists will get appropriate material for regional and local pathways.

Table 2. Actors of the SIE CPG development process.

Actors	Qualify and number	Actions
Strategic Committee	About 3 senior hematologists	To prioritize the CPG To compose the Expert Panel To invite External reviewers
Methodology Committee	Four EBM experts	To select the master CPG (focused, complete, updated, high-quality) To submit the CPG project to the CNEC To update the evidence base for recommendations to be adapted or newly developed. To provide an evidence draft for each PICO To lead the decision process by the Expert Panel To draft the CPG To get feedback from the External Reviewers
Scientific Committee	About 10 clinical specialists from different scientific societies plus a patient advocate	To share the criteria for adaptation (i.e. novel approvals, novel evidence) To select recommendations to be adopted To approve the GRADE evidence-synthesis To adapt recommendations To newly develop recommendations To fill the full GRADE dimensions
Expanded Expert Consultation	Other specialists	Convened for supervision of specific issues
External Committee	About 2 senior hematologists	To revise the scientific and practical aspects
Stakeholders^	Registered user SNLG	Consultation allowed during the assessment phase
CNEC		To check the quality of the proposed master-CPG To approve the CPG project To assess the final CPG
SIE Secretariat		To keep contacts with CNEC To collect COIs To convene Expert panel meetings

^ scientific societies, patient associations, pharma companies, public institutions, universities, research institutes.

Table 3. Steps of CPG ADOLOPMENT.

Steps	Methodology Committee	Scientific Committee
List of clinical questions from master CPG	√	
Check questions for appropriateness	√	
Prioritize questions for relevance		√
List of recommendations from master CPG	√	
Check recommendations for adoption		√
Check recommendations for updating	√	√
Check recommendations for adaptation	√	√
Check missing questions/recommendations	√	√
Build PICOTs for questions to be adapted or newly developed	√	
Select and prioritize the Outcomes		√
Share the definition of Comparator (country-specific SoC)		√
Build evidence-to-decision tables for PICOTs	√	
Review the economic impact of the intervention	√	
Propose new recommendations	√	
Approve wording of new recommendations		√
Vote strength of new recommendations		√
Share other dimensions (equity, applicability, costs..)	√	√

References

1. GRADE working group. Grading quality of evidence and strength of recomendations BMJ 2004; 328: 1490-4. http://www.gradeworkinggroup.org.
2. Brouwers M, Kho ME, Browman GP, Burgers JS, Cluzeau F, Feder G, Fervers B, Graham ID, Grimshaw J, Hanna S, Littlejohns P, Makarski J, Zitzelsberger L for the AGREE Next Steps Consortium. AGREE II: Advancing guideline development, reporting and evaluation in healthcare. Can Med Assoc J. 2010. Available online July 5, 2010. doi:10.1503/cmaj.090449
3. AGREE sito ufficiale e tool interattivo. https://www.agreetrust.org/agree-ii/
4. Versione Italiana di AGREE-II. http://www.gimbe.org/ pubblicazioni /traduzioni/AGREE_IT.pdf
5. Schünemann HJ et al. GRADE Evidence to Decision (EtD) frameworks for adoption, adaptation, and de novo development of trustworthy recommendations: GRADE-ADOLOPMENT. J Clin Epidemiol. 2017 Jan;81:101-110. doi: 10.1016/j.jclinepi.2016.09.009.
6. Sistema Nazionale Lingee-Guida. http://snlg.iss.it
7. CUBE guidelines. Linee-guida SIE. Manuale metodologico. https://www.siematologia.it/files/—CUBE-GUIDELINES-versione-2_5igr-wumc.1-31gen2019—1.pdf

IMMUNOLOGIC NICHE AND HEMATOLOGIC TUMOURS

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Introduction. The bone marrow (BM) is a peculiar primary lymphoid organ. Activated/memory T cells are hosted and maintained in the BM niche, where also mature T cells recirculates and can interact with antigen presenting cells. Therefore, the BM represents a potential source of effector T cells.[1] Notwithstanding BM T cells are maintained in a homeostatic condition, which is required for the proper hematopoietic development. This homeostasis is granted by an elevated frequency of regulatory T cells (a peculiarity of the BM niche, if compared to other lymphoid organs)[2] and immune regulatory activities exerted by the different subsets of BM-mesenchymal cells (BM-MSCs). Chronic signals from inflammatory and autoimmune conditions or tumours may impact on BM immunological homeostasis.[3]. Acute myeloid leukaemia (AML) is a heterogeneous, aggressive malignancy developing within the BM. AML cells exhibit tumour antigens that can trigger anti-leukaemia immune responses. To escape from immune recognition leukemic cells could instruct neighbouring mesenchymal cells to trigger inhibitory T cell pathways (i.e. IDO1). Alternatively, the need for BM-MSCs in creating the BM-tolerogenic environment could be encroached upon AML cells that autonomously gain immunoregulatory properties. ZEB1 has been extensively studied as epithelial-to-mesenchymal transcription factor, but new evidences indicate that ZEB1 may also drive myeloid cell polarization toward an immunosuppressive phenotype.[4] More recently, it has been shown that a particular aggressive AML subset expressed some EMT-related genes including ZEB1,[5] which was correlated with poor overall survival.

Aims. To evaluate whether ZEB1 expression defines a subset of AML with aggressive features and whether the poor outcome of some AML cases is also the consequence of an immunosuppressive BM microenvironment orchestrated by ZEB1+ AML cells.

Methods: To address the involvement of Zeb1 as a driver of BM immunosuppression, we used the ZEB1+ C1498 murine leukaemia cell line in which ZEB1 has been knockdown using lentiviral vectors. The correlation between ZEB1 expression and aggressiveness in AML has been established through a prospective gene expression analysis on newly diagnosed non-preselected AML patients and a retrospective immunohistochemical analysis in a larger AML patient cohort with available clinical annotations. ZEB1 induction of immune suppression has been studied evaluating the interaction between ZEB1-high and ZEB1-low AML cells with the BM immune microenvironment in mouse models.

The expression of immune regulatory markers in bone marrow biopsies from AML patients has been evaluated and correlated with ZEB1 expression.

Results. We analysed the transcriptomic profile of AML samples from The Cancer Genome Atlas and the BEAT-AML dataset and divided cases in two groups, "ZEB1-low" and "ZEB1-high", according to ZEB1 median expression. The two groups showed difference between the karyotypic features, FAB and mutations; in particular, ZEB1-low was enriched for NPM1 mutated cases. Furthermore, we found differences in terms of overall survival among the patients who received conventional chemotherapy. In the BEAT-AML cohort, ZEB1 expression was associated with a trend towards poor outcome, which reached significance in 2 independent cohorts (GSE37642 and GSE6891). Furthermore, GEP analysis of a cohort of patients for which blasts were available at diagnosis and relapse showed that all relapsed occurred with ZEB1-high blasts. IHC analysis performed on archival BM biopsies showed that the frequency of ZEB1+ blasts varies from a 10 to a 90% indicating that ZEB1+ and negative blasts can co-exist in the same patient. AML cases where ZEB1 was significantly expressed by the leukemic clone showed an increased expression of PDL-1 on neoplastic cells as well, at a variance with AML cases with scant ZEB1 expression. Finally, ZEB1+ AML cases showed increased f Foxp3+ cells and of IL17A+ T-cells. Modelling ZEB1+ AML in mice we found that *Zeb1*-gene silencing in C1498 cells promoted the down-regulation of immune-regulatory genes including *Pd-l1*and *Arg1* and of genes involved in Th17 differentiation. Furthermore *Zeb1*-gene silencing down-modulated the expression of the pro-metastatic genes *mmp9*. When injected intra-bone the growth of shZeb-1 cells was significantly lower compared to scramble counterpart. This phenotype was paralleled by a strong modification in the BM immune niche, with a reduction in IL17+Tregs and Th17 cells along with an increase in activated CD8 T cell in the BM of mice injected with shZeb-1 cells. Taken together, our results suggest that ZEB1 expression could recognize a group of particularly aggressive and chemoresistant AML, whose poor outcome results from both intrinsic clone aggressiveness and peculiar immune-niche initiated by this molecule.

References

1. Zhao ED, Xu HB, Wang L, Kryczek I, Wu K, Hu Y, Wang GB, Zou WP. Bone marrow and the control of immunity. Cellular & molecular immunology. 2012 Jan;9(1):11-19. English. doi:10.1038/cmi.2011.47. Cited in: Pubmed; PMID WOS:000299201200006.
2. Hirata Y, Furuhashi K, Ishii H, Li HW, Pinho S, Ding L, Robson SC, Frenette PS, Fujisaki J. CD150(high) Bone Marrow Tregs Maintain Hematopoietic Stem Cell Quiescence and Immune Privilege via Adenosine. Cell Stem Cell. 2018 Mar 1;22(3):445-453 e5. doi:10.1016/j.stem.2018.01.017. Cited in: Pubmed; PMID 29456159.
3. Tripodo C, Burocchi A, Piccaluga PP, Chiodoni C, Portararo P, Cappetti B, Botti L, Gulino A, Isidori A, Liso A, Visani G, Martelli MP, Falini B, Pandolfi PP, Colombo MP, Sangaletti S. Persistent Immune Stimulation Exacerbates Genetically Driven Myeloproliferative Disorders via Stromal Remodeling. Cancer Res. 2017 Jul 1;77(13):3685-3699. doi:10.1158/0008-5472.CAN-17-1098. Cited in: Pubmed; PMID 28536276.
4. Cortes M, Sanchez-Moral L, de Barrios O, Fernandez-Acenero MJ, Martinez-Campanario MC, Esteve-Codina A, Darling DS, Gyorffy B, Lawrence T, Dean DC, Postigo A. Tumor-associated macrophages (TAMs) depend on ZEB1 for their cancer-promoting roles. Embo J. 2017 Nov 15;36(22):3336-3355. English. doi:10.15252/embj.201797345. Cited in: Pubmed; PMID WOS:000415184300007.
5. Stavropoulou V, Kaspar S, Brault L, Sanders MA, Juge S, Morettini S, Tzankov A, Iacovino M, Lau IJ, Milne TA, Royo H, Kyba M, Valk PJM, Peters AHFM, Schwaller J. MLL-AF9 Expression in Hematopoietic Stem Cells Drives a Highly Invasive AML Expressing EMT-Related Genes Linked to Poor Outcome. Cancer Cell. 2016 Jul 11;30(1):43-58. English. doi:10.1016/j.ccell.2016.05.011. Cited in: Pubmed; PMID WOS:000380077100011.

THE ROLE OF STROMAL CELLS IN LEUKEMIAS

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Until recently, leukemia research has been based on the elucidation of hematopoietic stem cell (HSC)-autonomous and disease-specific genetic and molecular events underlying malignant transformation[1]. Despite, major strides have been made, such HSC-centered approach failed to fully disclose the mechanism/s of leukemia initiation and progression. Lately, the contribution of bone marrow (BM) microenvironmental factors has gained increasing interest, challenging the evidence that leukemia derives exclusively from cell-intrinsic defects[2, 3] . Among those factors, mesenchymal stromal cells (MSCs) substantially contribute to the creation of hematopoietic *niche* by regulating HSC fate[4] and have a unique immune-modulating capacity with a pivotal role in the induction of self-tolerance and in the control of inflammatory response[3, 5]. More recently, the other side of the MSC coin has been uncovered. New studies demonstrated that alterations, which first occur in the MSCs, could directly drive the dysfunction of HSCs, favoring leukemia initiation in mice models and in some cases in patients [6, 7]. Moreover, MSCs can supply leukemic stem cells of pro-survival signals contributing to create a protective niche, supporting malignant cells at the expense of normal HSC and potentially affecting the resistance to therapies[8]. All these notions concur to attest a fundamental bi-directional and complex interaction among malignant cells and BM microenvironment, specifically stromal cells, either contributing to leukemia onset and progression. However, the mechanism/s underlying this crosstalk have just been started to get untangled.

Different aspects are emerging as pivotal in MSC-driven pro-leukemia mechanisms:[3, 8]

- The alterations in MSC genome and gene-expression program, affecting MSC functions;
- The MSC aberrant cytokine profile, differently impacting on hematopoietic and/or leukemia cell supporting ability;
- The osteogenic/adipogenic MSC differentiation defective balance, resulting in BM failure and awarding a competitive advantage to leukemia cells;
- MSC immunomodulatory ability, contributing to tumor progression and immune response escape;
- The MSC–dependent mechanism/s of resistance to therapy.

Many gaps remain to be filled in the overall picture, however, is becoming increasingly clear that considering the BM context, besides the malignant cells, is crucial to generate a comprehensive understanding of the hematological diseases. Current leukemia treatments are mainly focused on the eradication of malignant cells, and often neglect BM microenvironment hold. An increasing body of evidence indicates that MSC alterations in the leukemic microenvironment contribute to creating an AML permissive/self-reinforcing niche favorable to escape therapy and immune response. Thus, the design of therapies that, similarly to other malignancies, besides eliminating the root, takes into account the fertile soil where the disease born and grow, could turn out more effective for the control of diseases.

References

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. N Engl J Med 2015 Sep; 373(12): 1136-1152.
2. Méndez-Ferrer S, Bonnet D, Steensma DP, Hasserjian RP, Ghobrial IM, Gribben JG, et al. Bone marrow niches in haematological malignancies. Nature Reviews Cancer 2020 2020/05/01; 20(5): 285-298.
3. Ciciarello M, Corradi G, Loscocco F, Visani G, Monaco F, Cavo M, et al. The Yin and Yang of the Bone Marrow Microenvironment: Pros and Cons of Mesenchymal Stromal Cells in Acute Myeloid Leukemia. Front Oncol 2019; 9: 1135.
4. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature 2014 Jan; 505(7483): 327-334.

5. Meisel R, Zibert A, Laryea M, Gobel U, Daubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 2004 Jun 15; 103(12): 4619-4621.
6. Raaijmakers MH, Mukherjee S, Guo S, Zhang S, Kobayashi T, Schoonmaker JA, et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature 2010 Apr; 464(7290): 852-857.
7. Zambetti NA, Ping Z, Chen S, Kenswil KJG, Mylona MA, Sanders MA, et al. Mesenchymal Inflammation Drives Genotoxic Stress in Hematopoietic Stem Cells and Predicts Disease Evolution in Human Pre-leukemia. Cell Stem Cell 2016 11; 19(5): 613-627.
8. Short NJ, Konopleva M, Kadia TM, Borthakur G, Ravandi F, DiNardo CD, et al. Advances in the Treatment of Acute Myeloid Leukemia: New Drugs and New Challenges. Cancer Discov 2020 Apr; 10(4): 506-525.

MOLECULAR GENETICS FOR MDS BY NGS: WHY, HOW AND WHEN?

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Myelodysplastic syndromes (MDS) are a group of clonal stem cell neoplasms, presenting with cytopenias. MDS diagnosis relies on peripheral blood counts and bone marrow morphology, classifying MDS patients by percentage of blasts and number of cell lineages with dysplasia. Although half of the patients show normal karyotypes, cytogenetic profiles allow risk stratification (IPSS-R). A number of recurrently mutated genes were identified but in the current WHO classification gene mutations are still rarely mentioned or exist only as a footnote, although molecular genetics, nowadays performed mainly by Next generation sequencing (NGS), are part of daily routine diagnostics in clinical and laboratory settings.

NGS is a powerful tool that can investigate mutations as well as copy number changes or structural variants either in targeted or comprehensive manner. Today, this information is mostly delivered by chromosome banding and FISH analyses and is used in various prognostic models that aim to predict the course of the disease for individual patients. Furthermore, targeted treatments are no longer terms, which are irrelevant for MDS diagnostics and resulting treatment. Of course, the question arises whether all-encompassing sequencing also provides a linear increase in information and allows stratification to become more granular and thus better. Initial studies may challenge this, as it is difficult to interpret the obtained data. Nevertheless, in MDS clonal evolution is a feature, so that clonal hematopoiesis of indeterminate potential (CHIP) and aging have found their way into MDS diagnostics, as with this the probability of developing MDS increases. But not only hematology uses this knowledge, also cardiology shows first prognostic connection with MDS typical CHIP mutations. But not only in the early stages of MDS clonal evolution plays an important role, also in the progress towards s-AML since this is accompanied by an increase in number of cytogenetic aberrations and mutations. All these aspects must be taken into account when considering "why, how and when" MDS diagnostics by NGS is needed and provides information that improves the treatment options for our patients.

FUNCTIONAL GENOMICS FOR CLINICAL MANAGEMENT AND THERAPEUTIC TARGETING OF ACUTE MYELOID LEUKEMIA

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Introduction. Acute myeloid leukemia (AML) is a phenotypically and genetically heterogeneous disease. Mutations and cytogenetic abnormalities are progressively acquired over many years in hematopoietic stem cells that gain a selective advantage and outcompete normal hematopoiesis. The occurrence of secondary genetic events promotes fully transformation in leukemic populations. Historically, classification of AML has been relied on morphology, immunophenotype and presence of recurrent genetic abnormalities but only for a small fraction of AML subgroups. Recent genomic sequencing studies have advanced our understanding of the pathogenesis of myeloid malignancies. Unfortunately, these studies have mostly analyzed specific subtypes and/or used targeted DNA-sequencing, thus limiting discovery of novel mutational patterns and gene expression clusters. Here, we performed an integrated genome-wide mutational/transcriptomic analysis of a large cohort of adult AML to accurately define subtypes of diagnostic, prognostic and therapeutic relevance, irrespective of clinicopathological features.

Methods. We performed unbiased whole genome and transcriptome sequencing of 1,304 adult individuals with myeloid malignancies (598 AML and 706 myelodysplastic syndromes, MDS; Fig. 1A), incorporating analysis of somatic and presumed germline sequence mutations, chimeric fusions and structural complex variations. Transcriptomic gene expression data were processed by a rigorous bootstrap procedure to define gene expression subgroups in an unsupervised manner. Associations between genetic variants, gene expression groups and outcome were examined.

Results. Genomic/transcriptome sequencing confirmed diagnosis according to WHO 2016 of AML with recurrent genetic abnormalities in 10.9% of cases. These cases had a distinct gene expression profile (Figure 1A), good prognosis (Figure 1B) and a combination of mutations in the following genes: *KIT*, *ZBTB7A*, *ASXL2*, *RAD21*, *CSF3R* and *DNM2* in RUNX1-RUNXT1 leukemia; *FLT3*, *DDX54*, *WT1* and *CALR* in PML-RARA promyelocytic leukemia; *KIT* and *BCORL1* in CBFB-rearranged leukemia. In addition, 9% of cases showed rearrangements of *KMT2A*, with known (e.g. *MLLT3*) and non-canonical partners (e.g. *ACACA*, and *NCBP1*) and poor outcome. Gene expression analysis identified six groups of AML and/or MDS (over 80% of cases) lacking recurrent cytogenetic abnormalities. The spectrum of the most frequently mutated genes (>10 cases) and associated gene expression subtypes is summarized in Figure 1A. *TET2* (more frequent in MDS than AML, p=0.0011) and *DNMT3A* (more frequent in AML than MDS, p<0.0001) were the most frequently mutated genes. Interestingly, mutations in these genes promoting clonal hematopoiesis were significantly enriched in the subgroup with *NPM1* mutations. Overall, *NPM1* mutations occurred in 27.4% of AML and 1% of MDS and were characterized by four expression signatures with different combination of cooperating mutations in cohesin (*RAD21*), epigenetic modifiers (*DNMT3A*, *IDH1/2*) and signaling genes (*FLT3, NRAS, PTPN11*) and outcome. Co-occurring *NPM1* and *FLT3* mutations conferred poorer outcome compared to only *NPM1*, in contrast co-occurring mutations with cohesin genes had better outcome (p=0.0071). Three gene expression clusters accounted for additional 9% of cases with mutual exclusive mutations in *RUNX1, TP53* and *CEBPA* and co-occurring with a combination of mutations in DNA methylation, splicing and signaling genes. Interestingly, *RUNX1* mutations were significantly associated with *SRSF2* mutations but not with *SF3B1,* showed high expression of *MN1* and poor outcome. *TP53* mutations accounted for 12% of AML and 10% of MDS cases. Alterations included missense, nonsense and frameshift mutations mostly in the DNA binding domain and structural variations leading to *TP53* loss. In half % of cases TP53 mutations were biallelic with two mutations or one mutation of the other allele. Mutations were associated with complex karyotype and older age and conferred poor outcome. In contrast to the distinct, mutation-associated patterns of gene expression in AML samples, the gene expression profile of MDS was less variable despite diversity in patterns of mutation. MDS was enriched in mutations of *SF3B1* (27.2%), mutually exclusive with *SFRS2* (14.4%) and *U2AF1* (5.5%); *TP53* (13.7%) and *RUNX1* (10.5%) and a combination of mutations in epigenetic regulators with outcome dependent on mutational pattern. Moreover, structural variations and/or missense mutations of *MECOM* accounted for 2% of cases.

Conclusions. The integration of mutational and expression data from a large cohort of adult pan myeloid leukemia cases enabled the definition of subtypes and constellations of mutations and have prognostic significance that transcends prior gene panel-based classification schema.

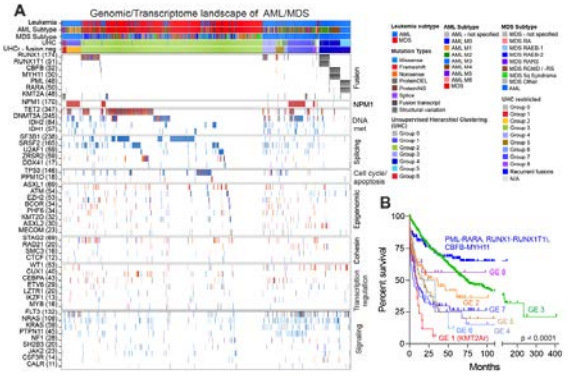

Figure 1. Subtyping and outcome of myeloid malignancies. A) Oncoprint showing the most frequently mutated genes (N > 10 cases; for epigenomic category N > 20 cases), categorized according to their functional annotation. UHC, unsupervised hierarchical clustering; UHCr, UHC restricted to cases negative for PML-RARA, CBFB-MYH11 and RUNX1-RUNX1T1. B) Overall survival (OS) according to UHCr gene expression (GE) groups.

Figure 1.

IMMUNITY IN INVASIVE FUNGAL INFECTIONS: BIOLOGICAL MECHANISMS AND POTENTIAL CLINICAL IMPLICATIONS

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Studies in the mouse have dissected the pathogenesis of Invasive Aspergillosis (IA) and emphasized the role of anti-fungal innate and adaptive immunity. Alveolar macrophages and epithelial cells are the first cells in the lung to engage conidia. Pattern Recognition Receptors (PRRs) on host cells recognize specific fungal motifs, i.e. conserved Pathogen-Associated Molecular Patterns (PAMPs). Engagement of PRRs up-regulates the induction of specific cytokines and chemokines and contribute to the maturation of dendritic cells (DCs). Uptake of fungi by DCs induces DC maturation, which, in turn, promotes the differentiation of naïve T cells into effector T helper (Th) cells and T regulatory (Treg) cells. Th1 cells, producing interferon-gamma are protective and promote fungal clearance. Th17 cells are involved in neutrophil recruitment. Tregs produce interleukin-10, which acts as a homeostatic response to keep inflammation under control but ended by limiting the efficacy of protective immune responses.[1,2]

The importance of innate immunity in vivo, has been confirmed by the discovery of rare inherited immune deficiencies (monogenic disease), associated with severe pyogenic bacterial and/or muco-cutaneous fungal infections. Several molecular studies have identified major single nucleotide polymorphisms (SNPs)/haplotypes associated with susceptibility to fungal infections/diseases and potentially influencing also disease outcome. However, susceptibility to infection in the general population results from polymorphisms of several genes, each having smaller functional contributions (polygenic inheritance) and is influenced by the degree of general immunosuppression. SNPs leading to long pentraxin-3 (PTX-3) deficiency, which hampers the normal alveolar expression of the protein and impairs the antifungal effector mechanisms of neutrophils, have been identified as strong predictor of IA in allogeneic hematopoietic stem cell transplants (HSCT) recipients. This association was the only one to be validated in a large, independent, study and extended across different clinical settings, including acute myeloid leukemia (AML) patients.[2] A prospective, genetically-stratified, randomized, double-blind event-driven multicenter trial is ongoing to assess the efficacy of posaconazole-based anti-fungal prophylaxis allocation strategies for AML patients, under induction chemotherapy, being the allocation strategy based on the risk predicted by genotyping two PTX-3 single nucleotide polymorphisms (NCT03828773).[2] Of note, based on a variety of clearly specific off-target effects on innate immunity, namely on neutrophils, monocytes, pulmonary macrophages, nurse-like cells and platelets, ibrutinib, (but not other more selective Bruton Tyrosine Kinase Inhibitors) has been proposed as a new predisposing factor for invasive fungal infections and incorporated as a novel host factor for the definition of probable invasive pulmonary mold disease by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group.[3,4] The importance of adaptive immunity in vivo has been confirmed by the studies showing that: 1) the HSCT recipients with anti-*Aspergillus Fumigatus* Th1 responses higher than the Th2 responses have a better survival outcome and 2) that the adoptive transfer of interferon gamma producing Th1 cells, stimulated by *Aspergillus* antigens, may cure probable IA in haploidentical transplant patients.[1] Our group was the first to develop and patent enzyme-linked immuno-spot assays to track individual *Aspergillus*-specific, *Mucor* specific and *Fusarium* specific T cells.[5-9] Compared with the results in healthy subjects, patients with IA demonstrated noticeably higher frequencies of *Aspergillus*-specific T cells producing interleukin-10 and interferon-gamma, with wider antigenic specificity. Protective T cells targeted predominantly *Aspergillus* cell wall antigens, tended to increase during the IA course and to be associated with a better clinical outcome.[5,6] In patients with hematologic malignancies at risk for Invasive Mucormycosis (IM), the detection of *Mucorales*-specific T cells polarized to Th2 cytokines production, was associated with proven IM.[7,8] Mold-specific T cells were either CD4+ or CD8+, both central and effector memory cells, and capable to directly induce hyphal damage. Unlike IA and IM, the disseminated fusariosis was associated with the appearance of *Fusarium*-specific T cells, secreting only pro-inflammatory cytokines such as interferon-gamma and interleukin-17A, at disease onset, resembling systemic candidiasis.[9] In the following years, other groups showed that quantification of CD154 positive T cells, after brief stimulation of patient□derived CD4+T cells with fungal lysates, holds the potential to detect the presence of fungal pathogens.[10] Hopefully, immunologic monitoring of fungal infections will be validated for diagnostic purposes, as in the case of interferon-gamma-release-assays for latent tuberculosis or viral infections.

References

1. van de Veerdonk FL, Gresnigt MS, Romani L et al. Aspergillus fumigatus morphology and dynamic host interactions Nat Rev Microbiol 2017; 15: 661-674.
2. Cunha C, Carvalho A. Genetic defects in fungal recognition and susceptibility to invasive pulmonary aspergillosis. Med Mycol 2019; 1; 57: S211-S218.
3. Luppi M, Forghieri F, Potenza L. Ibrutinib is a newly recognized host factor for the definition of probable invasive pulmonary mold disease, based on off-target effects, unrelated to its B cell immunesuppressant activity. Clin Infect Dis 2020 May 15;ciaa323. doi: 10.1093/cid/ciaa323.Online ahead of print.
4. Maffei R, Maccaferri M, Arletti L, et al. Immunomodulatory effect of ibrutinib: reducing the barrier against fungal infections. Blood Rev 2020 Mar;40:100635. doi: 10.1016/j.blre.2019.100635.Epub 2019 Oct 31.
5. Potenza L, Barozzi P, Vallerini D, et al. Diagnosis of invasive aspergillosis by tracking Aspergillus-specific T cells in hematologic patients with pulmonary infiltrates. Leukemia 2007, 21: 578–581.
6. Potenza L, Vallerini D, Barozzi P, et al. Characterization of specific immune responses to different Aspergillus antigens during the course of invasive Aspergillosis in hematologic patients. PLoS One. 2013 Sep 4;8(9):e74326. doi: 10.1371/journal.pone.0074326.
7. Potenza L, Vallerini D, Barozzi P, et al. Mucorales-specific T cells emerge in the course of invasive mucormycosis and may be used as a surrogate diagnostic marker in high-risk patients. Blood. 2011; 118: 5416-5419.
8. Potenza, L, Vallerini D, Barozzi P, et al. Mucorales-Specific T Cells in Patients with Hematologic Malignancies PLoS One 2016 Feb 12;11(2):e0149108. doi: 10.1371/journal.pone.0149108.
9. Vallerini D, Forghieri F, Lagreca I, et al. Detection of Fusarium-specific T cells in hematologic patients with invasive fusariosis. J Infect.2017; 74: 314-318.
10. Bacher P, Steinbach A, Kniemeyer O, et al. Fungus specific CD4(+) T cells for rapid identification of invasive pulmonary mold infection. Am J Respir Crit Care Med. 2015;191:348-352.

TUMOR CELL-ENDOTHELIAL CELL INTERACTIONS IN THROMBOSIS

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Patients with cancer are at increased risk of thromboembolic complications, which involve both venous and arterial districts. Many clinical and biological factors have been identified as accountable for the high thrombotic risk in the malignant disease, including general clinical risk factors (*i.e.* age, previous thrombosis, immobility, etc.), factors typical of cancer (*i.e.* type of cancer, advanced disease stage, and cancer therapies), and biological factors (*i.e.*tumor cell-specific prothrombotic properties and host cell inflammatory response to tumor).[1] The combination in various ways of these factors causes a systemic activation of blood coagulation, favoring the onset of the thrombotic disease on one side, but also the progression and diffusion of the tumor itself on the other side.[2] A common feature of this bidirectional relation between hypercoagulability and tumor progression, is the prothrombotic switch of endothelial cells (EC). Endothelium, the inner cellular monolayer lining the blood vessels significantly regulates blood fluidity and homeostasis.[3] In physiological conditions pro- and anti-coagulant endothelial activities are well balanced. As a barrier, endothelium separates blood clotting factors from exposure to subendothelial prothrombotic extracellular matrix components. The constitutive release by EC of the vasodilator substances nitric oxide and prostacyclin maintains platelets in the resting state, preventing them from aggregating. In case of endothelium damage, as during tissue injury, the subendothelial matrix is exposed and promotes platelet adhesion and blood clotting activation.

In many human diseases associated with a high thrombotic risk, including malignancy, dysfunction of the vascular endothelium, rather than damage, is commonly observed.[3] Endothelial dysfunction is characterized by a shift of the endothelial activities toward a proinflammatory and prothrombotic state. Specific changes consist in: upregulation of cell surface adhesion molecules (*i.e.* ICAM-1, E-selectin, P-selectin, and VCAM-1); loss of surface anticoagulant molecules' expression (*i.e.* thrombomodulin [TM], tissue factor pathway inhibitor [TFPI], and heparan sulphate), and increase in procoagulant (Tissue Factor, TF) and antifibrinolytic proteins (plasminogen activator inhibitor 1, PAI-1). In cancer, several tumor cell-dependent pathogenic mechanisms are involved in EC dysfunction/activation and include: 1. the release of proinflammatory and pro-angiogenic cytokines and microparticles by tumor cells or by host cells, including leukocytes and platelets, 2. the adhesion of tumor cells to EC by means of cell adhesion molecules, and 3. the direct effects of antitumor agents on EC.[4] Tumor cells synthesize and release a variety of proinflammatory cytokines (TNF-α, IL-1β) and proangiogenic factors (VEGF, bFGF), which can act on EC and affect their antithrombotic status, by upregulating TF and PAI-1, and downregulating of TM and TFPI. In addition, these tumor cell-derived cytokines enhance the adhesion potential of the vascular wall, by increasing the expression of surface adhesion molecules of EC, which become more capable to attract tumor cells and support their extravasation. Furthermore, the tumor cells attached to the endothelium can release their cytokine content into a protected milieu that favors their pro-thrombotic and pro-angiogenic activities.

The activated phenotype of EC is reversible and can revert to the quiescent phenotype when stimuli are removed. However, prolonged cellular activation status can induce endothelium dysfunction, causing loss of the of vascular barrier integrity, and apoptosis. Apoptotic cells can bind and activate FXII via phosphatidylserine, representing one of the mechanisms mediating the procoagulant activity of apoptotic cells. This apoptosis-mediated activation procoagulant pathway is also shared by some antitumor drugs that act on EC, including cisplatin and daunorubicin. The progression of endothelial cell death is also associated with an increased release of endothelial microparticles which are highly procoagulant.

Therefore, impairing tumor cell-endothelial cell interaction mechanisms can be relevant for preventing the associated coagulopathy and organ infiltration by tumor cells. Heparins are widely used as anticoagulant drugs for the prevention and treatment of thrombosis in a variety of conditions, including cancer. Interestingly, clinical evidence suggests that heparins, particularly low-molecular-weight heparins (LMWH), may improve survival of cancer patients. Some of the beneficial biological mechanisms of heparins in cancer have been explored by our and other laboratories.[5,6] An important mechanism is related to the heparin effect on vascular endothelium (particularly microvascular) activities involved in inflammation, wound healing, angiogenesis and tumor metastasis. In the last decade, our group demonstrated that heparins (both LMWH and unfractionated heparin, UFH) are able to prevent the thrombogenic switch of EC induced by the pro-inflammatory bacterial endotoxin,[7] by downregulating TF expression and upregulating its main inhibitor, TFPI, and thrombomodulin. Most importantly, we could demonstrate that heparins retained the same anti-inflammatory activity also towards the prothrombotic properties of EC elicited by both leukemic and solid tumor cells.[8] This confers to heparins a specific cellular antithrombotic activity, a mechanism of great importance in cancer, where hypercoagulability is at least in part due to the stimulation of EC activation by tumor cells. Along this line, we could also prove that, in an *in vitro* system of interaction of cancer cells with microvascular EC, LMWH can prevent endothelial cell capillary formation induced by breast cancer and leukemic cells, and by standard proangiogenic factors (i.e. VEGF and FGF-2).[9] We described a similar anti-angiogenic activity also for the "second generation" LMWH bemiparin and for the ultra-LMWH RO-14, on EC stimulated by lung cancer, breast cancer and leukemia cells.[10] Finally, we showed that heparins could impair the adhesion of promyelocytic blasts to the EC monolayer.[11] The relative weight and relevance of these EC-directed activities of heparins on cancer patient's survival remains to be further investigated.

References

1. Falanga A, Schieppati F, Russo L. Pathophysiology 1. Mechanisms of Thrombosis in Cancer Patients. Cancer Treat Res. 2019;179:11-36.
2. Falanga A, Marchetti M, Russo L. The mechanisms of cancer-associated thrombosis. Thrombosis research. 2015 Feb;135 Suppl 1:S8-S11.
3. Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, et al. The vascular endothelium and human diseases. International journal of biological sciences. 2013;9(10):1057-69.
4. Falanga A, Marchetti M. Anticancer treatment and thrombosis. Thrombosis research. 2012 Mar;129(3):353-9.
5. Zacharski LR, Loynes JT. The heparins and cancer. Curr Opin Pulm Med. 2002 Sep;8(5):379-82.
6. Falanga A, Vignoli A, Diani E, Marchetti M. Comparative assessment of low-molecular-weight heparins in cancer from the perspective of patient outcomes and survival. Patient related outcome measures. 2011 Jul;2:175-88.
7. Vignoli A, Marchetti M, Balducci D, Barbui T, Falanga A. Differential effect of the low-molecular-weight heparin, dalteparin, and unfractionated heparin on microvascular endothelial cell hemostatic properties. Haematologica. 2006 Feb;91(2):207-14.
8. Vignoli A, Marchetti M, Falanga A. Heparins inhibit the endothelial prothrombotic features induced by tumor cells. Thrombosis research. 2017 Jul 01;157:55-7.
9. Marchetti M, Vignoli A, Russo L, Balducci D, Pagnoncelli M, Barbui T, et al. Endothelial capillary tube formation and cell proliferation induced by tumor cells are affected by low molecular weight heparins and unfractionated heparin. Thrombosis research. 2008;121(5):637-45.
10. Vignoli A, Marchetti M, Russo L, Cantalino E, Diani E, Bonacina G, et al. LMWH bemiparin and ULMWH RO-14 reduce the endothelial angiogenic features elicited by leukemia, lung cancer, or breast cancer cells. Cancer Invest. 2011 Feb;29(2):153-61.
11. Vignoli A, Marchetti M, Falanga A. Acute promyelocytic leukemia cell adhesion to vascular endothelium is reduced by heparins. Annals of hematology. 2018 Sep;97(9):1555-62.

GAIN OF FUNCTION VARIANTS OF GENES FOR PLATELET GLYCOPROTEINS CAUSING INHERITED BLEEDING DISORDERS: DISCOVERY AND PATHOGENIC MECHANISMS

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Inherited platelet disorders (IPD) are a heterogeneous group of genetic hemorrhagic diseases characterized by mild to severe mucocutaneous bleeding and a wide phenotypic and genotypic heterogeneity.[1,2] IPD include 51 disorders caused by variants in 67 different TIER1 genes,[3] while for several of them the causative molecular defect is unknown. Most IPD are caused by loss-of- function (LoF), this report deals with two specific IPD caused by gain of function (GoF) variants involving platelet surface glycoproteins, i.e. autosomal dominant-GT and PT-VWD.

Autosomal dominant GT (AD-GT). AD-GT is caused by heterozygous GoF variants in the ITGA2B and ITGB3 genes, that encode respectively for the $alpha_{IIb}$ and the $beta_3$ subunits of integrin $alpha_{IIb}beta_3$, the platelet surface receptor for fibrinogen. GoF variants causing AD-GT generate an $alpha_{IIb}beta_3$ complex constitutively activated, locked in a high affinity state. AD-GT is characterized by absence of platelet aggregation and defective clot retraction, in association with macrothrombocytopenia and reduced $alpha_{IIb}beta_3$ expression. Bleeding manifestations are moderate to severe. This disorder is very rare and only 12 causative GoF variants have been reported to date.[4,5,6] Studies in megakaryocytes (MK) from a patient with AD-GT and from transduced murine MK showed that proplatelet formation was severely impaired, with tips decreased in number and larger in size than those of controls. $alpha_{IIb}beta_3$ expression was reduced, the receptor was constitutively activated and activation of proteins of the $alpha_{IIb}beta_3$-mediated outside-in signalling was observed. Therefore, constitutive activation of $alpha_{IIb}beta_3$-mediated outside-in signalling in human MK negatively influences proplatelet formation leading to macrothrombocytopenia.[7,8]

Platelet dysfunction was studied using platelets from two patients carrying the *ITGB3* p.D621_E660del variant and CHO cells expressing 4 different GoF *ITGB3* variants. We demonstrated that reduced surface expression of $alpha_{IIb}beta_3$ is due to receptor internalization and that permanent triggering of $alpha_{IIb}beta_3$-mediated outside-in signaling causes an impairment of cytoskeletal reorganization, arresting actin turnover at the stage of polymerization, leading to impaired platelet function. Therefore, constitutively active $alpha_{IIb}beta_3$ exerts a dominant effect over normal $alpha_{IIb}beta_3$ thus being the main effector of platelet dysfunction in AD-GT.[8] Our group also demonstrated that a GoF variant in $beta_3$ can exert its dominant-negative effect even when associated with a LoF $beta_3$ mutant.[6] In conclusion, reduced platelet number and platelet dysfunction in patients with $alpha_{IIb}beta_3$ GoF variants causing AD-GT are both consequent to the cytoskeletal perturbation induced by the constitutive $alpha_{IIb}beta_3$-mediated outside-in signaling.

Platelet-type von Willebrand disease (PT-VWD). PT-VWD is a very rare autosomal dominant bleeding disorder due to GoF variants in *GP1BA* conferring to platelet GPIbalpha enhanced affinity for VWF and associated with mild thrombocytopenia with enhanced platelet volume and a variable hemorrhagic diathesis. PT-VWD is probably an underdiagnosed bleeding disorder, with only 53 patients reported, 6 GoF variants of *GP1BA* causing PT-VWD have been described so far.[9,10,11]

The cause of bleeding and macrothrombocytopenia remained poorly understood for years, and it was hypothesized to be the result of enhanced platelet clearance from the circulation. Our group explored for the first time the mechanisms of this disorder using MK from a PT-VWD patient carrying the p.M239V variant and from a mouse model of PT-VWD carrying the p.G233V variant.[12] We found that VWF was bound at the surface of PT-VWD MK, and that MK generated proplatelets with a decreased number of enlarged tips. A peculiar finding was that PT-VWD MK formed proplatelets upon contact with type I collagen, a protein present in the osteoblastic niche that typically suppresses proplatelet formation. Concordantly, collagen triggered MK showed defective activation of the RhoA-MLC2 axis, which prevents proplatelet formation, and increased phosphorylation of Lyn, which acts as a negative regulator of GPVI signalling thus preventing ectopic proplatelet-formation on collagen. Consistently, human and murine bone-marrow contained an increased number of extravascular platelets as compared with controls. In addition, platelet survival of mutant mice was shortened.[12]

The possibility that platelet dysfunction may contribute to the bleeding phenotype of PT-VWD has attracted little attention, however, defective platelet fibrinogen binding and P-selectin expression after incubation with thrombin, impaired thrombus formation on a damaged carotid artery, unstable clot formation and delayed aggregation in response to ADP and thrombin in a mouse model of PT-VWD have been reported.[13]

Thrombocytopenia and platelet dysfunction both contribute to PT-VWD bleeding phenotype.

References

1. Gresele P, Falcinelli E, Bury L. Inherited platelet function disorders. Diagnostic approach and management. Hamostaseologie.2016;36:265-78.
2. Gresele P, Falcinelli E, Bury L. Laboratory diagnosis of clinically relevant platelet function disorders. Int J Lab Hematol. 2018;40 Suppl 1:34-45.
3. Megy K, Downes K, Simeoni I, Bury L, Morales J, Mapeta R, Bellissimo DB, Bray PF, Goodeve AC, Gresele P, Lambert M, Reitsma P, Ouwehand WH, Freson K; Subcommittee on Genomics in Thrombosis and Hemostasis. Curated disease-causing genes for bleeding, thrombotic, and platelet disorders: Communication from the SSC of the ISTH. J Thromb Haemost. 2019;17(8):1253-1260.
4. Nurden AT, Pillois X, Fiore M, Heilig R, Nurden P. Glanzmann thrombasthenia-like syndromes associated with Macrothrombocytopenias and mutations in the genes encoding the αIIbβ3 integrin. Semin Thromb Hemost. 2011;37(6):698-706.
5. Gresele P, Falcinelli E, Giannini S, D'Adamo P, D'Eustacchio A, Corazzi T, Mezzasoma AM, Di Bari F, Guglielmini G, Cecchetti L, Noris P, Balduini CL, Savoia A. Dominant inheritance of a novel integrin beta3 mutation associated with a hereditary macrothrombocytopenia and platelet dysfunction in two Italian families. Haematologica. 2009;94(5):663-9.
6. Bury L, Zetterberg E, Leinøe EB, Falcinelli E, Marturano A, Manni G, Nurden AT, Gresele P. A novel variant Glanzmann thrombasthenia due to co-inheritance of a loss- and a gain-of-function mutation of ITGB3: evidence of a dominant effect of gain-of-function mutations. Haematologica. 2018;103(6):e259-e263.
7. Bury L, Malara A, Gresele P, Balduini A. Outside-in signalling generated by a constitutively activated integrin αIIbβ3 impairs proplatelet formation in human MK. PLoS One. 2012;7(4):e34449.
8. Bury L, Falcinelli E, Chiasserini D, Springer TA, Italiano JE Jr, Gresele P. Cytoskeletal perturbation leads to platelet dysfunction and thrombocytopenia in variant forms of Glanzmann thrombasthenia. Haematologica. 2016;101(1):46-56.
9. Othman M, Kaur H, Emsley J. Platelet-type von Willebrand disease: new insights into the molecular pathophysiology of a unique platelet defectSemin Thromb Hemost. 2013;39(6):663-73.
10. Giannini S, Cecchetti L, Mezzasoma AM, Gresele P. Diagnosis of platelet-type von Willebrand disease by flow cytometry. Haematologica. 2010;95(6):1021-4.
11. Othman M, Gresele P. Guidance on the diagnosis and management of platelet-type von Willebrand disease: A communication from the Platelet Physiology Subcommittee of the ISTH. J Thromb Haemost. 2020;18(8):1855-1858.
12. Bury L, Malara A, Momi S, Petito E, Balduini A, Gresele P. Mechanisms of thrombocytopenia in platelet-type von Willebrand disease. Haematologica. 2019;104(7):1473-1481.
13. Kaur H, Corscadden K, Ware J, Othman M. Thrombocytopathy leading to impaired in vivo haemostasis and thrombosis in platelet type von Willebrand disease. Thromb Haemost. 2017;117(3):543-555.

INEFFECTIVE ERYTHROPOIESIS IN MYELODYSPLASTIC SYNDROME

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Background: The process of erythropoiesis is the part of hemopoiesis responsible for the continuous renewal of red blood cells. Every second, the human body generates 2 million red blood cells, through this process. Human erythropoiesis is a incompletely understood, complex, multistep process, from the multipotent hematopoietic stem cell (HSC) to the mature erythrocyte.[1] The erythroid maturation and differentiation are virtually divided into two phases, the first erythropoietin dependent and the second iron dependent. The anatomic-functional unit is called erythron, consisting of commissioned precursor for erythropoiesis (Erythroid Burst Forming Units BFU-E and Erythroid Colony Forming Units CFU-E), erythroid precursors (proerythroblasts and erythroblasts) and mature red blood cells (RBC). Erythron differentiation occurs in anatomic niches known as erythroblastic islands. Erythroblastic islands consist of a central macrophage surrounded by up to 30 erythroid cells at varying degrees of maturation. The central macrophage anchors erythroblasts within the island and provide the cellular interactions necessary to drive erythroid differentiation and proliferation. Particularly donating iron that is fundamental in the late maturation.[2] The first steps of erythroid differentiation involve an engagement phase, in which HSCs differentiate into more committed erythroid progenitors, from a common myeloid progenitor the megakaryocytic-erythroid progenitor and finally the BFU-E. The BFU-Es further differentiate into CFU-E, following which, terminal differentiation occurs. The second level of erythroid maturation implicates the differentiation of the nucleated precursors from proerythroblasts to basophilic, polychromatophilic and orthochromatic erythroblasts. This phase is characterized by the steady increase of hemoglobin, nuclear condensation finally resulting in enucleation. The final phase of erythroid development involves the maturation of the reticulocyte into erythrocytes. So that erythrocyte acquires its biconcave shape through extensive membrane remodeling.[3] Erythroid differentiation occurs in response to specific stimuli from cytokines, transcription factors and transduction signal proteins that cooperate to direct maturation towards the erythroid line, for this reason they are called "restricted lineage". All these molecules have as cell target erythroid progenitors and precursors in different differentiation stages. Unquestionably the key transcription factor of erythropoiesis, known as "master of transcription", is GATA 1 (GATA binding factor 1). It promotes cell survival through the activation of the antiapoptotic genes of the BCL family (Bcl-X) and promotes the expression of the erythropoietin receptor (EPO-R); it also activates the globin synthesis and erythroid-specific heme program. On the contrary, it suppresses the expression of specific non-erythroid transcription factors such as GATA-2, typical instead of megakaryocytopoiesis.[4]

Erythropoietin dependent-phase. Erythropoietin (EPO) is a humoral cytokine synthesized primarily in the kidney and secreted into the blood stream where it targets erythroid progenitor cells in the bone marrow. The production of EPO is overall regulated by the amount of oxygen in the tissues. Tissue hypoxia stimulates the synthesis of HIF-1alpha (hypoxia inducible factor-1-alpha), which induces the production of a transcriptional factor regulating positively the expression of the gene that encodes for the synthesis of EPO. Circulating EPO binds to a specific receptor for growth factors that is present on the surface of the erythroid precursors. One of the main signaling pathways mediated by the EPO/EPO-R interaction is Janus Kinase 2 (JAK2) activation, which subsequently phosphorylates and activates Signal transducer and activator of transcription 5 (STAT5). The JAK2/STAT5 pathway has been shown to activate genes fundamental for erythroid progenitor survival, proliferation and differentiation; in fact STAT5 is capable, if activated, of promoting the expression of various erythroid antiapoptotic genes such as BCL-XL.[5]

Iron dependent phase and late-stage erythropoiesis. Iron is essential in the erythroid maturation and the differentiation of the nucleated precursors from pro-erythroblasts to basophilic, poly-chromatophilic and orthochromatic erythroblasts. This phase is characterized by the steady increase of hemoglobin, nuclear condensation finally resulting in enucleation. Late-stage erythropoiesis is regulated also by transforming growth factor beta ligands (TGF-beta) via the small mother against decapentaplegic 2/3 (Smad2/3) signaling pathway. The TGF- beta receptors are a superfamily of serine/threonine kinase receptors and are grouped into 3 types: type I, type II, and type III. The TGF-beta signaling pathway involves ligand binding to type II receptors, which recruits and phosphorylates type I receptors. The TGF-beta receptor ligands are polypeptide growth factors, (including TGF-beta, activins, bone morphogenetic proteins, and Growth differentiation factor 11 GDF-11). Activin and GDF-11 have inhibitory effects on terminal erythropoiesis, distinct from the EPO early regulatory role.[4] Select TGF-beta superfamily ligands signal via activin receptor type IIB trigger the Smad2/3 signaling pathway. Ligand binding triggers phosphorylation (activation) of Smad2/3, enabling it to form a heteromeric Smad complex. The Smad complex translocates to the nucleus, where it modulates transcription of genes involved in erythroid maturation. In particular they are responsible for aberrant late stage erythroid maturation.[6]

Functional reserve of the erythron and physiological recovery mechanisms. The erythron, in the same way as other organs or cell lines, has an ability to modulate production according to requests. If, under basic conditions, the erythron produces every day 20-30 ml of erythrocytes containing 7 g of hemoglobin, in case of need (hemolysis, hemorrhages, hypoxia) it has the potential ability to increase the production (8 times in the child and 5 times in the adult) of the share of erythrocytes and hemoglobin that it generates in basic conditions.

EPO dependent mechanism. Fas and Fas Ligand (FasL) are members of the tumor necrosis factor (TNF) family and corresponding receptor. Their link determines the activation of the caspase cascade responsible for the initiation of cell apoptosis. Immature erythroblasts express FAS while those in a more advanced maturation state express FAS-L. If the erythroid production is physiologically sufficient, their interaction within the erythroid island of maturation favors the maturation arrest and the triggering of the apoptosis of the immature cells through the activation of the caspases, but, in case of hemorrhages, hemolysis or hypoxia , inside the erythroid island a mechanism that protects immature cells from apoptosis is activated and instead favors their differentiation and maturation. The increase in EPO produced in these circumstances favors the production of heat shock protein 70 (HSP70), which nuclear translocation allows the protection of GATA1 from the physiological cleavage that occurs through the activation of FAS/FAS-L and the cascade of caspases . In the absence of the protective effect of HSP70, GATA1 is degraded by the activation of the caspases and the erythroid progenitor undergoes apoptosis.[4]

Iron dependent mechanism. During stress erythropoiesis, anemia causes increased EPO secretion by the kidneys. Under the stimulus of EPO there is an erythroblastic hyperplasia response that causes an increase in the production of the EPO-STAT5 dependent hormone erythroferron (ERFE). ERFE is secreted into the circulation and in the liver it has the property of suppressing the production of Hepcidin.

Hepcidin is the pivotal regulator of the inflow / outflow of cellular iron. Its suppression results in an increased iron absorption in the intestine, an increased release of iron from hepatic deposits and a greater recycling activity of the splenic macrophages. The result is an increased availability of iron for greater compensatory erythropoiesis.

Ineffective erythropoiesis in myelodysplastic syndrome. Ineffective erythropoiesis is defined as the inability to produce an adequate number of mature erythroid cells. The result is a hyperplasia of immature erythroid cells at the bone marrow level but peripheral anemia. To date, there is no single marker of dyserythropoiesis that can be used from a diagnostic-therapeutic point of view. Indeed, it should quantify in a single value the level of increased proliferation, reduced differentiation and

increased apoptosis, which are the three main characteristics of ineffective erythropoiesis.

In Table 1 are reported the major causes of ineffective erythropoiesis in MDS.

Table 1. Major causes of ineffective erythropoiesis in MDS

Type of dinsfunction	Major exemples
Quantitative or qualitative changes in growth factors for multipotent and early erythopoietic progenitor cells	SFC, G-CSF, Il-3
Mutations of cells that directly or indirectly interfere with erythropoiesis, defects in epigenetic mechanisms, defects in immunological mechanisms and angiogenesis	HSC, microenvironment
Later-acting erythropoietic differentiation factors	EPO, TGF-beta,GDF11, Activin A
Quantitative or qualitative changes in essential transcription factors of erythropoiesis	GATA-1, STAT 5
Intrinsic and extrinsic activation of pro-apoptotic pathways and important survival factors for erythropoietic cells	MCL, BCL-XL, HSP70
Negative growth regulators of erythropoietic progenitor cells ,alteration of the control of Fas-mediated apoptosis	TGF-beta, BID,FAS ligand, FAS, Caspase
Quantitative or qualitative changes in iron and proteins involved in iron distribution and metabolism (es: accumulation of iron in the mitochondrial matrix which generates mutation of mitochondrial DNA)	Ferritin, transferrin, transferrin-R, Ferroportin, Hepcidin, sideroblasts
Mutations in genes involved in hemoglobin production	beta/alpha globin chain

In myelodysplastic syndrome (MDS) the mechanisms underlying dyserythropoiesis are characterized by one or more of the above mechanisms.

Above all, a mechanism that deserves a separate discussion is the pathway of the TGF-beta family which are overexpressed in MDS as in thalassemia.[7] The main components of this family are:

- Activin A (A multi-potent stem cell proapoptotic mechanism mediated by Activin A has been described in MDS, especially in forms with 5q- deletion)
- GDF11 (the increase of this molecule in MDS produces an aberrant terminal erythropoiesis with a mechanism still not fully understood)
- TGF-beta (is defined as a negative regulator of erythroid maturation in its terminal phase. It binds to the membrane receptor ActRIIB, generating phosphorylation and therefore activation of the SMAD 2/3 signal. The activated SMAD complex undergoes translocation into the cell nucleus where it modulates the transcription of genes involved in late erythroid maturation).

Focusing on the MDS with ring sideroblasts, nowadays it offers a target therapy model currently unique among MDS as it is frequently characterized by a mutation of an RNA splicing gene SF3B1. SF3B1 is a fundamental protein for the functioning of the U2 ribonucleoprotein involved in the recognition of the 3 'cut site during the RNA splicing process. In case of mutation of this gene, the correct cleavage site is not recognized and an aberrant mRNA is produced which is degraded as it is not functioning. The result of an SF3B1 mutation may involve one or more of the following mechanisms:

- Altered transcript of the exon containing the ABCB7 gene that codes for an iron transporter which for this reason is down-regulated
- Altered transcript of genes involved in hemoglobin production
- Altered functionality of mitochondrial genes
- Production of alternative transcript encoding erythroferron and consequent constant suppression of hepcidin which in these forms of MDS causes increased iron accumulation
- Hyper expression of TGF-beta

However, the relationship between the mutation of the SF3B1 gene characteristic of MDS with ring sideroblastic and dyserythropoiesis has recently been clarified. In fact, it has been shown on the Zebrafish model how the SF3B1 transcript deficiency creates a hyper-expression of TGF-beta. We have already seen how this factor is a negative regulator of erythroid maturation in particular by creating an arrest of the pro-erythroblast in the G0-G1 phase of the cell cycle.[9]

Ineffective erythropoiesis and link with the iron overload. In anemias with ineffective erythropoiesis, most erythroblasts do not successfully differentiate into mature erythrocytes, leading to anemia and increased EPO production by the kidneys. ERFE secretion is increased both because of EPO but also of the ERFE secreting erythroblasts hyperplasia. Hepcidin is thus suppressed and iron is mobilized but erythrocyte production cannot increase and the additional iron is not utilized, generating non transferrin bound iron (NTBI) and its sub component labile pool iron (LPI) that are free toxic forms of iron. Over the course of time, the combination of tissue hypoxia, increased erythropoietin and ineffective erythropoiesis creates a vicious cycle that may ultimately lead to a massive expansion of erythroblasts and iron overload leading to the organ's damage.[9]

Conclusions. New understanding of the molecular pathways governing effective erythropoiesis and iron homeostasis has led to a greater appreciation of the pathophysiology of ineffective erythropoiesis in MDS and iron related disorders. A pipeline of rationally designed novel therapeutics against the major actors of these process has been developing with the aim to avoid transfusion dependency and iron overload in MDS patients thus prevent organs damages and ameliorate quality of life .

References

1. Zivot A, Lipton JM, Narla A, Blanc L. Erythropoiesis: insights into pathophysiology and treatments in 2017. Mol Med. BioMed Central; 2018; 24(1): 11–5.
2. Leimberg MJ, Prus E, Konijn AM, Fibach E. Macrophages function as a ferritin iron source for cultured human erythroid precursors. J Cell Biochem 2008; 103(4): 1211–8.
3. Gifford SC, Derganc J, Shevkoplyas SS, Yoshida T, Bitensky MW. A detailed study of time- dependent changes in human red blood cells: from reticulocyte maturation to erythrocyte senescence. Br J Haematol 2006; 135(3): 395–404.
4. Valent P, Büsche G, Theurl I, Uras IZ, Germing U, Stauder R, et al. Normal and pathological erythropoiesis in adults: from gene regulation to targeted treatment concepts. Haematologica. Haematologica; 2018; 103(10): 1593–603.
5. Elliott S, Pham E, Macdougall IC. Erythropoietins: a common mechanism of action. Exp Hematol. 2008; 36(12): 1573–84.
6. Suragani RNVS, Cadena SM, Cawley SM, Sako D, Mitchell D, Li R, et al. Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. Nat Med 2014; 20(4): 408–14.
7. Pilo E, Angelucci E. Luspatercept in thalassemia , Drugs of Today 2020, 56(7)
8. De La Garza et all. The splicing factor Sf3b1 regulates erythroid maturation and proliferation via TGFβ .Blood Adv (2019) 3 (14): 2093–2104
9. Ganz Tomas. Erythropoietic regulators of iron metabolism Free radical biology & medicine, 2019; 133(4): 69-74.

NEW THERAPEUTIC TARGETS IN INEFFECTIVE HEMATOPOIESIS IN MYELODYSPLASTIC SYNDROMES

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Introduction. The hallmarks of myelodysplastic syndrome (MDS) are chronic cytopenias, due to ineffective hematopoiesis (IE) associated with morphological dysplasia in one or more myeloid lineages, and a variable risk of progression to acute myeloid leukemia (AML). Morbidity and mortality of most MDS are caused by symptomatic cytopenias, with clinical manifestations including complications of anemia, and less frequently neutropenia and thrombocytopenia. Several mechanisms of IE have been proposed in MDS (Figure 1), related to the MDS clone itself (as cytogenetic abnormalities, somatic mutations, iron overload, epige-

netic changes and other alterations associated with the "aging" process), or to the microenvironment, including functional immune defects, or activation of inflammatory signals. IE prevails in lower-risk (LR) MDS, which are also characterized by increased apoptosis levels.

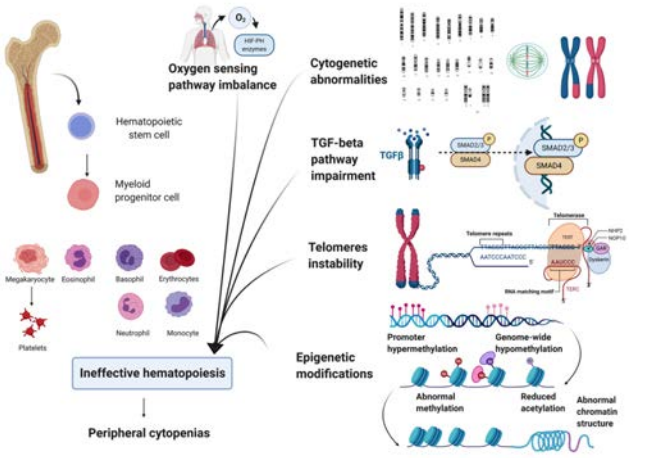

Figure 1. Main "druggable" mechanisms involved in ineffective hematopoiesis in MDS.

Genetic abnormalities: the case of del(5q). Among mechanisms responsible for MDS pathogenesis and with a significant prognostic role are single or multiple recurrent chromosomal abnormalities, included in the revised IPSS (IPSS-R).[1] Of these, the interstitial deletion of the long arm of chromosome 5 (del(5q)) is the most common, present in approximately 10%-15% of primary MDS. MDS with 5q- is paradigmatic for the functional significance of the effects on the hematopoietic process of haploinsufficiency of genes located on the long arm of chromosome 5[2] Lenalidomide(Ln) has been shown to selectively suppress the del(5q) clone, enhancing p53 degradation via protein phosphatase 2a (PP2A) inhibition and consequent MDM2 stabilization. Furthermore, it elicits indirect immunomodulatory and anti-inflammatory effects. Ln is the treatment of choice in del(5q) MDS, where it induces transfusion independence (TI) in 67% of cases, with complete cytogenetic response (CyR) in up to 45% of patients.

Aging and telomeres: Imetelstat. Short telomeres have been shown to characterize both the physiological "aging" process and MDS. Telomerase activity has also been observed in MDS across all disease stages. Imetelstat is a first-in-class inhibitor of the human telomerase enzyme. The ongoing global phase 2/3 randomized (2:1) double-blind, placebo-controlled trial IMerge, is testing the efficacy of imetelstat i.v. in patients with non-del(5q) LR MDS, ESA-resistant and naïve to hypomethylating agents (HMA).[3] The results of the concluded phase 2[4] showed a >8-w TI rate of 42%, with hematological improvement (HI) in 68% of cases. Hematological toxicities are comparable to Ln with 50% of patients experiencing reversible grade 3/4 cytopenias. In particular, this agent seems promising in patients with ring sideroblasts (RS) and SF3B1 mutation, where also CyR and malignant clone reduction have been registered.

TGFbeta pathway: Luspatercept. Increased SMAD2/3 signaling via TGFbeta is another altered pathway responsible for IE in MDS. Luspatercept, a recombinant inhibitor of TGFbeta is able to reduce SMAD2/3 signaling, enabling late-stage erythroid differentiation and improvement of anemia, as shown by the results of the randomized phase 3 MEDALIST trial.[5] In this study performed in LR MDS patients with ringed sideroblasts (RS), ineligible or refractory to ESAs, luspatercept at a dose of 1 to 1.75 mg/kg s.c. every 21 days (d) induced hematologic improvement (HI) in 64% of cases vs 26% with placebo, while the >8w TI rate was 38% vs 13%, respectively.

New formulation of "old" hypomethylatying agents (HMA): oral azacytidine (AZA). Hypermethylation is an important pathogenetic mechanism in MDS and HMA are effective in HR-disease. CC-486, the oral AZA formulation may allow easier and prolonged route of administration, with demethylating effects similar to s.c. AZA. The phase 3, randomized, placebo-controlled study evaluated response to CC-486 in LR MDS patients, treated at a dose of 300 mg for 21d over a 28d course. TI was achieved in 31% vs 11% of patients, and median TI duration was 11 vs 5 months, in the CC-486 vs placebo arm, respectively.[6-7]

Oxygen sensing pathway inhibitors: Roxadustat. Roxadustat is a new inhibitor of hypoxia-inducible factor and prolyl-hydroxylase, able to mimic low oxygen status, thereby enabling erythroid differentiation via increased EPO levels. Along this way, iron metabolism is unblocked and iron absorbtion from gastrointestinal tract is enabled. After the promising results in end-stage chronic kidney disease, roxadustat is now under evaluation for the treatment of anemia of LR MDS.[8-10] Preliminary data on 24 patients showed at least 38% TI rate for >56d over the first 24w.

References

1. Lee JH, List A, Sallman DA. Molecular pathogenesis of myelodysplastic syndromes with deletion 5q. European journal of haematology. 2019;102(3):203-209.
2. Gaballa MR, Besa EC. Myelodysplastic syndromes with 5q deletion: pathophysiology and role of lenalidomide. Annals of hematology. 2014;93(5):723-733.
3. Platzbecker U, Steensma DP, Van Eygen K, et al. Imerge: A Study to Evaluate Imetelstat (GRN163L) in Transfusion-Dependent Subjects with IPSS Low or Intermediate-1 Risk Myelodysplastic Syndromes (MDS) That Is Relapsed/Refractory to Erythropoiesis-Stimulating Agent (ESA) Treatment. Blood. 2019;134(Supplement_1):4248-4248.
4. Platzbecker U, Fenaux, P. , Steensma, D.P., et al. . TREATMENT WITH IMETELSTAT PROVIDES DURABLE TRANSFUSION INDEPENDENCE (TI) IN HEAVILY TRANSFUSED NON-DEL(5Q) LOWER RISK MDS (LR-MDS) RELAPSED/REFRACTORY (R/R) TO ERYTHROPOIESIS STIMULATING AGENTS (ESA). EHA Library. 2020;Abstract S183.
5. Fenaux P, Platzbecker U, Mufti GJ, et al. Luspatercept in Patients with Lower-Risk Myelodysplastic Syndromes. New England Journal of Medicine. 2020;382(2):140-151.
6. Garcia-Manero G, Santini, V., Almeida, A. et al. . A PHASE III PLACEBO-CONTROLLED TRIAL OF CC-486 IN PATIENTS WITH RED BLOOD CELL TRANSFUSION-DEPENDENT (RBC-TD) ANEMIA AND THROMBOCYTOPENIA DUE TO IPSS LOWER-RISK MYELODYSPLASTIC SYNDROMES (LR-MDS). EHA library Abstract S180. 2020.
7. Garcia-Manero G, Gore SD, Kambhampati S, et al. Efficacy and safety of extended dosing schedules of CC-486 (oral azacitidine) in patients with lower-risk myelodysplastic syndromes. Leukemia. 2016; 30:889-96.
8. Chen N , Hao C, Peng X, et al. Roxadustat for Anemia in Patients with Kidney Disease Not Receiving Dialysis. N Engl J Med 2019; 381:1001-1010.
9. Henry DH, Glaspy J, Harrup RA, et al. Roxadustat (FG4592; ASP1517; AZD9941) in the Treatment of Anemia in Patients with Lower Risk Myelodysplastic Syndrome (LR-MDS) and Low Red Blood Cell (RBC) Transfusion Burden (LTB). Blood. 2019; 134 (Supplement_1):843-843.
10. Kubasch AS, Platzbecker U. Setting Fire to ESA and EMA Resistance: New Targeted Treatment Options in Lower Risk Myelodysplastic Syndromes. Int J Mol Sci. 2019; 20(16):3853.

ADOPTIVE IMMUNOTHERAPY OF REFRACTORY CHRONIC GVHD WITH REGULATORY T CELLS: PRELIMINARY RESULTS AND FUTURE PERSPECTIVES

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Chronic GVHD is a major cause of long term morbidity and mortality following allogeneic HSCT. Steroid refractory chronic GVHD has a poor prognosis, with only about 10-20% patients stopping immunosuppressive within 4 years. Although several therapies are employed for steroid refractory chronic GVHD, they are generally poorly effective while causing substantial toxicity. Natural T regulatory cells (Tregs) are a subset of T lymphocytes crucial for the control of the immune response. Their critical importance in the control of the immune system is demonstrated by the observation that their lack is associated with severe autoimmune syndromes both in mice and humans. Recovery of Tregs after allogeneic HSCT is delayed, resulting in reduced Tregs even after >2 years after HSCT. Chronic GVHD has been associated with selectively delayed Tregs recovery, resulting in a lower Treg/Teff ratio in the peripheral blood and target organs. Recently it has been shown that treatment with low dose IL-2 may increase the numbers and function of Tregs and improve chronic GVHD in some patients.[1] However, the effect of IL-2 is rapidly lost and possibly limited by the altered function of Tregs in patients with chronic GVHD. It has been therefore hypothesized that the infusion of healthy Tregs from the donors may overcome this limitation. Infusion of donor T regs at the time of HSCT has been shown to prevent GVHD both in animal models and in patients after UCB and haploidentical transplantation. However, their role in the treatment of established chronic GVHD is still unknown. In animal models, infusion of Tregs ameliorates chronic GVHD. The first attempt to treat chronic GVHD in humans was reported in 2009.[2] More recently, 5 patients were treated with expanded donor Tregs, obtaining 2 PR without significant side effects.[3] The most important trial of donor Tregs infusion in chronic GVHD was presented at ASH in 2017.[4] In this study 25 patients were treated with escalating doses of freshly isolated donor Tregs in combination with low dose IL-2. Although only 4 patients reported a PR, most patients could reduce the dose of prednisone within 6 months. Most importantly, based on NGS analysis of TCR sequences, infused T regs were shown to persist for at least 8 weeks after infusion.

In 2015, the European Union has financed a multicenter project exploring the role of donor Treg treatment of patients with chronic GVHD that is refractory to steroids and at least one line of immune suppressive treatment. The participating centers have each proposed a distinct schedule of Treg infusion, as described in Figure 1.

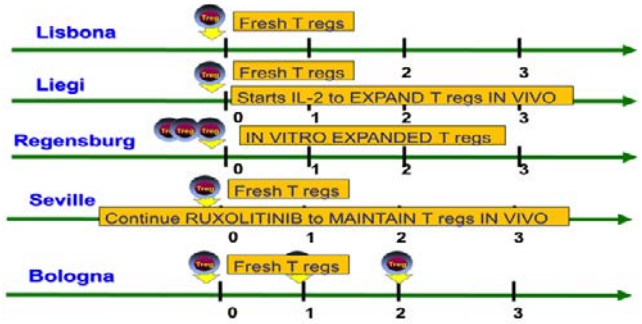

Figure 1. Schedules of Treg infusion across the consortium.

Our center has proposed to treat patients with multiple infusions of cryopreserved isolated Tregs. This design is based on the hypothesis that multiple Treg infusions could induce a more durable immunologic effect. The trial has a dose escalating 3+3 design with safety as the primary end point.

Tregs are isolated in a two step procedure by immunomagnetic depletion of CD8+ and CD19+ cells followed by enrichment of CD25+ cells. To date, we have prepared 10 Treg products, with a median yield of 37-38% and a purity of 67-94%. Contamination with effector T cells was considered acceptable with a median of $2x10^4$ Teff/kg and $4x10^3$ Th17 cells/kg. However, Treg phenotype appears to be altered by the cryopreservation procedure, although the functional consequences are still under investigation.

Nine out of 10 products have been infused, 3 at the first dose level ($0,5x10^5$ Treg/kg total dose), and 6 at the second dose level (10^6 Treg/kg total dose). No infusion related events were observed. One patient developed a DLT (CMV pneumonia) one month after the last infusion. 4 more SAEs were observed during the study, but all were considered unrelated to the infusion. 24 infectious AE were observed but only 4 were grade III. Importantly, no acute GVHD or flares of chronic GVHD were observed. Disease responses observed in the 7 evaluable patients are reported in Figure 2 and are consistent with the results reported by the other participating centers.

	CR	PR	SD
Global cGVHD	0	5 (71%)	2 (29%)

	CR	PR	SD
Skin	0	2 (40%)	3 (60%)
Mouth	0	2 (40%)	3 (60%)
Eyes	0	2 (40%)	3 (60%)
Lung	0	0	5 (100%)

3 MONTHS

Prog	CR	PR	SD	Prog
Global cGVHD	0	2 (29%)	3 (42%)	2 (29%)

	CR	PR	SD	Prog
Skin	0	3 (60%)	2 (40%)	0
Mouth	1 (25%)	2 (50%)	1 (25%)	0
Eyes	1 (15%)	1 (15%)	5 (71%)	0
Lung	0	0	4 (61%)	2 (29%)

12 MONTHS

Figure 2. Global and organ specific responses at 3 and 12 months in the seven evaluable patients who completed follow up. Interestingly, two patients managed to stop Prednisone.

While Treg numbers and percentages did not change significantly during the study, NGS analysis of TCR sequences confirmed the persistence of the infused Treg clones for up to 12 months after treatment.

Taken as a whole, the results of the study show that Treg treatment appears safe but may have limited efficacy. This may be due to several reasons, including low dose, short duration of treatment, low frequency of antigen speficic T reg cells, as well as the contamination by Teff. Methods of ex vivo manipulation may improve the efficacy of Treg treatment in the future. These include ex vivo expansion of Treg cells, ex vivo engineering (e.g. fucosylation) and selection of alloantigen specific Treg cells. A particularly promising approach appears the generation of CAR-Treg cells, as recenty reviewed.[5]

References

1. Koreth J, Matsuoka K, et al. Interleukin-2 and Regulatory T Cells in Graft-versus-Host Disease. N Engl J Med 365:2055-66; 2011.
2. Trzonkowski P et al. First-in-man clinical results of the treatment of patients with graft versus host disease with human ex vivo expanded CD4+CD25+CD127− Tregulatory cells. Clinical Immunology 133, 22–26; 2009.
3. Theil A et al. Adoptive transfer of allogeneic regulatory T cells into patients with chronic graft-versus-host disease. Cytotherapy, 17: 473-486; 2015.
4. Nikiforow S et al. Phase I Trial of Regulatory T-Cell Donor Lymphocyte Infusion Plus Daily Low-Dose Interleukin-2 for Steroid-Refractory Chronic Graft-Versus-Host Disease. Blood 130 (Supplement 1): 511; 2017.
5. Zhang Q et al. Chimeric Antigen Receptor (CAR) Treg: A Promising Approach to Inducing Immunological Tolerance. Front. Immunol. 9:2359; 2018.

OVERCOMING TUMOR RESISTANCE INDUCED BY N-GLYCOSYLATION BOOSTS CAR T CELL EFFICACY AGAINST SOLID MALIGNANCIES

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Chimeric antigen receptors (CARs) are artificial molecules endowing T cells with desired specificities and enhanced antitumor properties. The exciting successes achieved in patients with refractory B-cell leukemia and lymphoma propelled the exploitation of this cutting-edge technology in other tumor settings. However, first-in-man studies against solid tumors revealed unique hurdles contributing to the lack of a sharp demonstration of efficacy. Deciphering the determinants of tumor recognition by CAR T cells should allow leveraging the ensuing knowledge into the design of novel successful strategies. We have recently observed that multiple carcinoma cell lines express branched N-glycans, whose levels negatively correlate with the degree of CAR T cell killing. Accordingly, knocking out the expression of the glycosyltransferase MGAT5 in pancreatic adenocarcinoma (PAC) resulted in the formation of a qualitatively improved immunological synapse with CAR T cells, stronger NFAT/NFkB signals, enhanced cytokine production and cytolytic function. Consistent with previous studies, we hypothesize that the glycans shield might act at multiple levels, either by directly masking antigenic epitopes and hindering close cell-to-cell proximity or by ensuring proper functionality of checkpoint signaling pathways. To overcome the glycosylation barrier and increase the outcome of CAR-T cell therapy against solid tumors, we exploited the glucose/mannose analogue 2-Deoxy-D-glucose (2DG). This compound proved capable of blocking tumor N-glycosylation without interfering with proteins exposure on the cell surface, sensitizing tumor cells to recognition and killing by CAR-T cells. Accordingly, when challenged against high tumor burdens in vivo, CAR-T cells highly benefited from 2DG administration, resulting in more profound antitumor responses and reduced T-cell exhaustion. Thanks to metabolic deregulation (Warburg effect), 2DG is expected to selectively accumulate in cancer cells compared to healthy tissues. Accordingly, the same doses of 2DG able to enhance tumor recognition by CAR-T cells failed to increase the elimination of healthy cells, supporting the safety of the strategy. Remarkably, the combined approach proved successful against multiple carcinomas besides PAC, including those arising from the lung and bladder, and with different clinically relevant CAR specificities, paving the way for the rational design of improved CAR T cell therapies against solid malignancies.

EXOGENOUS AND PHYSIOLOGIC FACTORS AFFECTING TELOMERE LENGTH: RELEVANCE IN THE TRANSPLANT SETTING

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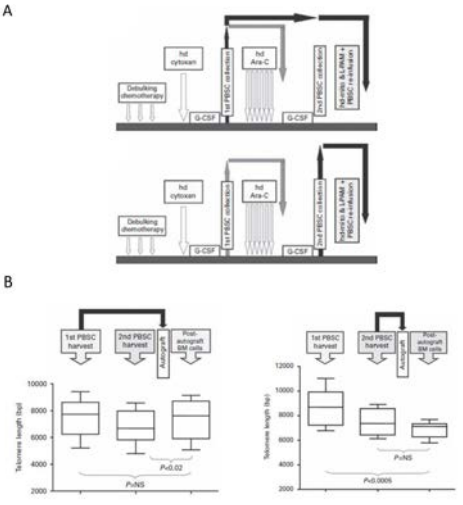
Telomeres are non-coding long tandem repeated sequences at the ends of chromosomes, which prevent chromosomal fusions and accumulation of DNA damage.[1] Telomere maintenance is a major determinant of genomic stability, as telomere shortening leads to accumulation of DNA damage, replicative senescence or cell death.[1,2] Human telomerase (hTERT) is an enzyme able to restore telomere sequences by adding nucleotides pairs de novo thus preventing telomere shortening.[3] Human leucocyte telomere length (TL) correlates with lifespan and has been extensively studied as a biomarker for cancer and age-related diseases.[1,3] hTERT is commonly down-regulated in adults, leading to progressive telomere shortening on each round of cell division.[1,3] In adults telomeres are shorter in males compared to females and accordingly males have shorter life expectancy and higher cancer incidence.[1,4] Exogenous causes of telomere attrition include reactive oxygen species (ROS)-induced DNA damage and inflammation (through ROS generation).[2,4] Chemotherapy exposure, through direct and ROS-mediated DNA damage, is a well established cause of telomere attrition.[5-7] High-dose (HD) sequential chemotherapy and autologous stem cell transplant (ASCT) represents a suitable model to study telomere dynamics. In this setting, previous studies indicate that peripheral blood stem cells (PBSC) harvested after multiple chemotherapy cycles [HD-cyclophosphamide (HD-CY) and HD-ARA-C], showed marked telomere shortening as compared to PBSC harvested after only one HD-CY cycle (Figure 1), leading do to delayed engraftment after ASCT.[6]

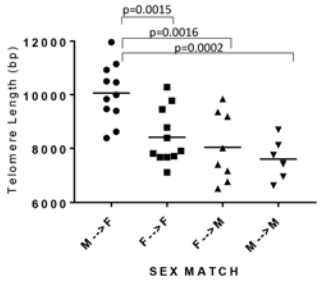


Adapted from Ruella et al. Bone Marrow Transplant. 2010;45(3):505-12

Figure 1.

More important, TL of the grafted cells affected TL measured after ASCT,[7] with a short TL after ASCT being an important recognized predisposing factor for the development of secondary myeloid neoplasms.[7] In general, proliferative stresses, including bone marrow regeneration after autologous and allogeneic hematopoietic stem cell transplantation (HSCT), induce a non-physiological TL shortening, as a consequence of increased number of replicative cycles during engraftment.[8,9] However, besides graft versus host disease (GVHD), which induces telomere attrition as result of systemic inflammation and oxidative stress, endogenous determinants of TL in HSCT and in hematopoietic stem cells are poorly defined. Umbilical cord blood (UCB) transplant (UCB-T) repre-

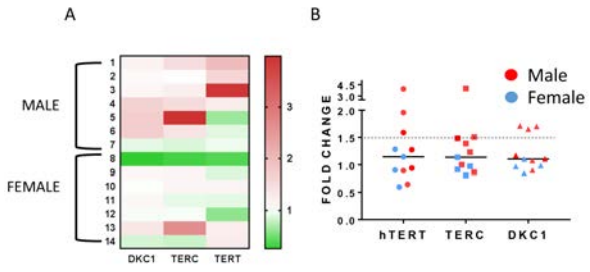
sents an ideal setting to investigate endogenous determinants of TL, being a stem cell source characterized by very long telomeres, not exposed yet to environmental factors. With this goal, we evaluated TL by Southern blot in 36 long-term survivors following UCB-T.[10] We showed that among several clinical factors (n° CD34+ cells reinfused, engraftment parameters, acute and chronic GVHD, recipient age and weight) only recipient and donor gender significantly correlated with TL, with female recipients retaining longer telomeres compared to male recipients. The longest telomeres were observed in females transplanted with male UCBs (9,993 bp, range 8,381-11,960), suggesting that male-derived stem cells may retain long telomeres if placed in a female milieu (Figure 2).



Adapted from Derenzini et al. Biol Blood Marrow Transplant. 2019;25(7):1387-1394

Figure 2.

This difference was particularly evident when considering female recipients of male UCBs with regular menstrual cycles at the time of transplant. *In vitro* experiments confirmed these findings: in fact, *ex vivo* treatment with estradiol resulted in a preferential up-regulation of telomerase subunits (TERT, TERC, DKC1) in male-derived bone marrow mononucleated cells (MNCs) compared to their female counterparts (Figure 3).



Adapted from Derenzini et al. Biol Blood Marrow Transplant. 2019;25(7):1387-1394

Figure 3.

These data indicate that: 1) modifiable factors such as hormonal status and female milieu are major determinants of TL in the transplant setting; 2) estradiol-mediated telomerase up-regulation may prevent telomere erosion in the adult male. To further investigate this finding, we studied telomere dynamics in a series of 34 lymphoma patients undergoing first-line chemoimmunotherapy. TL was measured on PBMCs during chemoimmunotherapy after each cycle and after at least one year from the end of the induction regimen. Telomere shortening was observed as early as after 1 cycle of chemotherapy, being significantly decreased after 3 and 6 cycles. Maximal drop in TL was observed at the end of induction therapy, with no significant recovery after 1 year. In line with our first study, among all patient's subgroups, only premenopausal women did not show a decrease in TL following chemotherapy exposure (*data not shown*).

Taken together, our results suggest a possible role of sex hormones in promoting telomere maintenance. Prospective studies evaluating the role of sex mismatch and age on telomere and stemness maintenance after HSCT are warranted. In conclusion, our observations provide the rationale for investigating hormonal therapy in the allogeneic HSCT setting and beyond, as a strategy to counteract chemotherapy-induced telomere shortening and aging-related diseases.

References

1. Shampay J, Szostak JW, Blackburn EH. DNA sequences of telomeres maintained in yeast. Nature. 1984;310(5973):154-7
2. Blackburn EH, Epel ES, Lin J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. Science. 2015;350(6265):1193-8
3. Blackburn EH, Collins K. Telomerase: an RNP enzyme synthesizes DNA. Cold Spring Harb Perspect Biol 2011, 3: a003558
4. Barrett EL, Richardson DS. Sex differences in telomeres and lifespan. Aging Cell. 2011;10(6):913-21.
5. Buttiglieri S, Ruella M, Risso A, et al. The aging effect of chemotherapy on cultured human mesenchymal stem cells. Exp Hematol. 2011 Dec;39(12):1171-81.
6. Ricca I, Compagno M, Ladetto M, et al. Marked telomere shortening in mobilized peripheral blood progenitor cells (PBPC) following two tightly-spaced high-dose chemotherapy courses with G-CSF. Leukemia 2005; 19: 644–651.
7. Ruella M, Rocci A, Ricca I, et al. Comparative assessment of telomere length before and after hematopoietic SCT: role of grafted cells in determining post-transplant telomere status. Bone Marrow Transplant. 2010;45(3):505-12.
8. Wynn RF, Cross MA, Hatton C, et al. Accelerated telomere shortening in young recipients of allogeneic bone-marrow transplants. Lancet. 1998;351(9097):178-81.
9. Gadalla SM, Aubert G, Wang T, et al. Donor telomere length and causes of death after unrelated hematopoietic cell transplantation in patients with marrow failure. Blood. 2018;131(21):2393-2398.
10. Derenzini E, Risso A, Ruella M, et al. Influence of Donor and Recipient Gender on Telomere Maintenance after Umbilical Cord Blood Cell Transplantation: A Study by the Gruppo Italiano Trapianto Di Midollo Osseo. Biol Blood Marrow Transplant. 2019;25(7):1387-1394. doi:10.1016/j.bbmt.2019.03.026

BEST ABSTRACTS

B001

DUAL TARGETING STRATEGY OF ACUTE MYELOID LEUKEMIA BY ENGINEERED CYTOKINE-INDUCED KILLER CELLS COEXPRESSING AN INTERLEUKIN 3 CHIMERIC ANTIGEN RECEPTOR (CAR) AND AN ANTI-CD33 COSTIMULATORY RECEPTOR

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Acute Myeloid Leukemia (AML) is initially sensitive to chemotherapeutic regimens but only a minority of patients achieve long-term survival. Adoptive cell therapy with Chimeric Antigen Receptor (CAR) engineered T cells could represent a promising approach to prevent relapse by targeting chemoresistant leukemic stem cells (AML-LSCs). CD123 (known as IL-3 receptor alfa) and CD33 satisfy several features of ideal target antigens since they are commonly upregulated on AML-LSCs, especially in NPM1 and FLT-3 mutant AML, and conserved at disease relapse. However, their expression also on normal tissues such as endothelial cells and hematopoietic stem cells, could lead to a potential on-target off-tumor side effect of CAR T-cell therapy. Here, we probe a dual targeting model to improve CAR T-cell selectivity for AML-LSCs while minimizing toxicity against healthy cells, through a first generation anti-CD123 IL-3 zetakine (IL3z.CAR) and an anti-CD33 as costimulatory receptor (CD33.CCR) without activation signalling domains. We evaluated the efficacy and safety profile of dual targeting IL3z.CAR/CD33.CCR CIK cells compared to single targeting first generation IL-3z.CAR CIK cells and third generation anti-CD33.CAR CIK cells. Fresh and frozen peripheral blood mononuclear cells were transduced with retroviral vectors during the CIK cell differentiation process. The functionality of all CAR-CIK cell conditions was assessed by means of short and long term cytotoxicity and cytokine production assays upon challenge with different CD123/CD33 positive AML cell lines or with a CD123 positive human endothelial cell line (TIME). Dual targeting IL-3z.CAR/CD33.CCR CIK cells display a potent and specific *in vitro* anti-leukemic efficacy against all the AML cell lines tested compared to non-transduced CIK cells. The killing efficiency of the dual targeting model is analogous to third generation anti-CD33.CAR.CIK cells, in both short and long term assays. However, since the single targeting IL-3z.CAR CIK cells display also a high anti-leukemic activity, we lowered the anti-CD123 CAR binding affinity to minimize toxicities against CD123+ healthy cells, generating dual targeting low affinity IL-3z.CAR/CD33.CCR CIK cells. These low affinity dual CAR CIK cells show irrelevant cytotoxicity against the TIME endothelial cell line, comparable to non-transduced CIK cells, while preserving high efficacy against THP-1 and KG-1 AML cell lines. Furthermore, in co-culture assays of CD123high THP1 and CD123low TIME cell lines, low affinity dual CAR CIK cells display a preferential killing against the THP-1 cell line with a substantial sparing of endothelial cells. As a confirm of the low reactivity against endothelial cells, low affinity dual CAR CIK cells produce the same levels of IL-2 and INFγ either alone or in the presence of TIME cell line. These preclinical data demonstrate a powerful antitumor efficacy mediated by low affinity dual targeting IL-3z.CAR/CD33.CCR CIK cells against AML targets without any relevant toxicity on endothelial cells, offering a proof-of-concept strategy to increase selectivity for AML-LSCs whilst reducing the risk of "on-target off-tumor toxicity".

B002

REVEALING TRANSCRIPTOME DEREGULATION UPON GENOMIC COMPLEXITY IN MULTIPLE MYELOMA

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Introduction: Multiple Myeloma (MM) is characterized by a huge clonal heterogeneity composed by redundant mutations, copy number aberrations (CNAs), and chromosomal rearrangements (CRs). However, the knowledge about the functional impact implied by its biological complexity at the transcriptomic level is still scanty. Here, we aimed to dissect the transcriptional deregulation promoted by the most recurrent genetic abnormalities and link specific genetic predictors to peculiar gene expression levels improving clustering of cases and identifying patients amenable to personalized therapies.

Patients and methods: We analyzed 517 newly diagnosed patients (NDMM) from the IA12 release of the CoMMpass study, focusing on variants, copy-number segments, seq-FISH, and raw transcript counts. RNAseq data was processed using the VOOM/LIMMA pipeline.

Results: We first analyzed the impact of genetic abnormalities on the number of transcripts deregulated by each. Chr1q amp/gain, followed by HD and IgH translocations showed the highest numbers. Individual mutations had much less impact, apart from NRAS, chr13q genes (DIS3, TGDS, RB1) and MYC transcriptional regulators (MAX, IRF4). Because many genes show a hotspot (HS) mutational pattern, we asked whether this would influence transcription. For KRAS and NRAS only HS mutations caused gene deregulation. In IRF4, non-HS still carried functional relevance, although on different genes. Regarding BRAF, the kinase dead D594 mutation showed huge differences in gene deregulation compared with activating mutations and WT cases. Next, we analyzed the influence on transcriptome of bi-allelic genetic events with known prognostic impact. Known tumor suppressors only had functional relevance upon bi-allelic inactivation. TP53 double-hits were associated with an upregulation of PHF19, a poor prognostic marker in MM. CYLD inactivation correlated with upregulation of BCL2 (no significant correlation with t(11;14)), opening a window for BCL2 targeted therapy in NDMM. Bi-allelic events of genes in del13q showed gene-specific consequences: DIS3 inactivation impacted mostly on lncRNAs, while TDGS influenced cell-cycle machinery genes and cyclin expression. Concerning CNAs, only chr1q-amp (more than 3 copies) were associated with upregulation of the potential therapeutic targets MCL-1 and SLAMF7.

Conclusions: Here, we depicted a link between the genomic architecture and transcriptome in MM. We showed a stronger impact of CNAs and CRs on expression as compared to individual gene mutations. However, the functional relevance of HS mutations needs further testing as they may represent future target of treatment. Moreover, the mutational status is crucially relevant since, while mono-allelic events are of little transcriptional value, compound heterozygosity carries a profound transcriptomic impact. This provides a biological basis for the observed prognostic impact of "double-hit" MM. Finally, we found correlates of BCL2 and MCL-1 expression that could represent future predictive markers for personalized treatment.

B003

IDO1 UPREGULATION AND ATP CATABOLISM INCREASE T REGULATORY CELLS THROUGH DENDRITIC CELLS DURING CHEMOTHERAPY-INDUCED ACUTE MYELOID LEUKEMIA CELL DEATH

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Introduction: Some chemotherapeutic agents as anthracyclines can induce immunogenic cell death (ICD) promoting modifications in leukemia cells which lead to immune system activation. However, chemotherapy is also responsible for inducing immune tolerance. We previously demonstrated that chemotherapy-induced release of ATP from dying AML cells drives dendritic cells (DCs) to upregulate indoleamine 2,3-dyoxigenase 1 (IDO1), which in turn is involved in T regulatory cells (Tregs) induction. In the present work, we investigated the role of P2X7 and P2Y11 ATP receptors for IDO1 regulation in DCs and the influence of ATP catabolism in Tregs induction after chemotherapy treatment in AML.

Methods: For *in vitro* experiments, CD14+-derived DCs were cultured with ATP in presence or absence of P2X7 and P2Y11 inhibitors and then used for flow cytometry staining and IDO1 expression analysis and tested to generate Tregs. IDO1 expression in DCs was correlated with intracellular signaling, *i.e.* non-canonical NF-κB pathway. For *in vivo* experiments, Balbc/J wt mice were injected with WEHI-3B leukemia cells. Daunorubicin (DNR) or Cytarabine (Ara-C) were administered and then, tumor infiltrating lymphocytes were purified and stained by flow cytometry.

Results: Only DNR induced a complete DCs maturation inducing a significant up-regulation of CD83, CD80, CD86 and CCR7. Analysis of IDO1 expression in DCs treated with ATP in presence of P2Rs inhibitors revealed a probable differential regulation of ATP-dependent IDO1 upregulation via P2Y11R (direct positive effect) and P2X7R (indirect negative effect) which was positively correlated with activation of non-canonical NF-κB pathway. As for ATP catabolism mediated by the expression of ATP ectonucleotidases CD39 and CD73 in DCs, DNR and Ara-C induced a significant up-regulation of CD73-the rate limiting step of ATP catabolism. Such increase was paralleled by a significant up-regulation of "fitness markers", in particular OX40/CD39 and OX40/PD-1, on Tregs. Mouse models confirmed the capacity of DNR and Ara-C to induce DCs maturation and activation of ATP catabolism pathways by significantly inducing CD39 and CD73 up-regulation. Moreover, chemotherapy treatment resulted in the stabilization of Tregs suppressive phenotype as demonstrated by the up-regulation of OX40 and ICOS fitness markers in comparison to placebo treatment.

Conclusions: Our data suggest that different mechanisms operate in tumor microenvironment. IDO1 may induce Tregs in an ATP-dependent manner through non-canonical NFkB pathway and seems to be differentially regulated by both P2X7 and P2Y11 ATP receptors, whereas the ATP catabolism may be involved in the stabilization of Tregs suppressive phenotype. In this context, the specific role of the main bioproduct of ATP catabolism, adenosine, is under investigation.

B004

TEMPORAL-WEIGHTED ESTIMATION OF 1384 MULTIPLE MYELOMA COPY NUMBER ALTERATIONS DEFINES AN ANCESTRALITY INDEX IMPACTING PATIENTS SURVIVAL

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Background: MM is a hematological malignancy always evolving from pre-malignant stages, with progressive increase of genomic complexity. MM is characterized by an abundance of copy number alterations (CNA); many of them, regarded as "driver", stack up progressively from early tumor stages, causing biological changes that give rise to tumor hallmarks and malignant phenotypes. The combined application of whole genome analysis and mathematical models allows to deeply describe these alterations and to infer their order of acquisition during oncogenesis from their clonality levels, assuming that clonal ones are more ancestral than subclonal. AIMS: (1) To define the temporal order of acquisition of CNA, leading to the onset of symptomatic MM and (2) to define a scoring model able to stratify patients (pts) according to the ancestrality of the alterations observed in their genomic landscape.

Methods: Genomic data collected from a total of 1384 newly diagnosed MM pts were included in the study: SNPs array data were collected from 514 pts of our Institution (BO dataset); in 870 pts, WES data were downloaded from CoMMpass study. CN calls and clonality levels were harmonized by an analysis pipeline including ASCAT, GISTICv2 and custom R scripts. Timing estimates were obtained with BradleyTerry2 package. Survival analysis were performed on R.

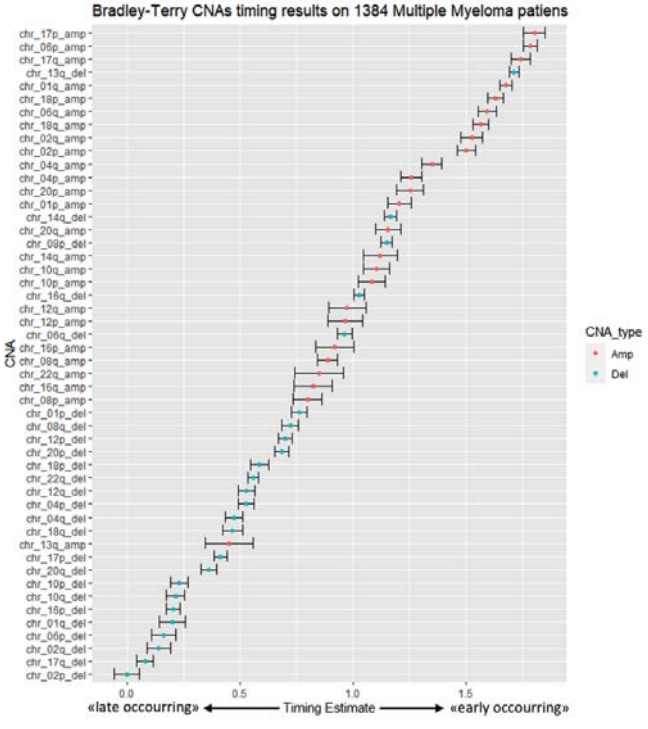

Figure 1.

Results: A full callset of CNAs was obtained by harmonizing BO and CoMMpass datasets. The clonality information was first extrapolated from the whole callset, to define the temporal order of acquisition of non-primary CNAs. CNAs were then accurately ranked, by using the

obtained timing estimates, characterized by a quite narrow confidence interval. Of interest, chr 1q gains and chr 13q losses were frequently clonal and ranked as ancestral events, whereas chr 17p losses were late occurring events. By weighting the CNAs carried by any given pts at diagnosis with their relative timing estimate in a combinatorial process, an Ancestrality Index (AI) was defined for each pts (median AI=3.4, IQR=1.7-6.0). The AI was found to be significantly associated with progression free (PFS) and overall survival (OS) ($p<0.001$). Pts with AI>3.4 (*i.e.* with a more "ancestral" profile) had a worse outcome as compared to the rest of pts (OS 40% *vs.* 58%, PFS 42% *vs.* 56%, at a median follow up of 92m and 34m, $p<0.001$).The risk attributed to this "ancestral" category was independent from other high-risk cytogenetic features (*i.e.* del17p, t(4;14), t(14;20), t(14;20).

Conclusion: By means of whole genome analysis and dataset harmonizing, the temporal order of acquisition of MM CNAs has been confidently described. A score reflecting the disease ancestrality of MM pts at diagnosis was generated and associated to survival outcomes. Overall, these findings support the evidence that MM pts at diagnosis carrying an excess of ancestral alterations, expected to likely be drivers, are prone to have a dismal prognosis.

Acknowledgements: AIRC_IG2014-15839,RF-2016-02362532

B005

ABSTRACT WITHDRAWN

ORAL COMMUNICATIONS

Acute Leukemia 1

C001

THE METABOLOMIC PROFILE DISTINGUISHES TWO SUB-GROUPS OF NPM1-MUTATED ACUTE MYELOID LEUKEMIA WITH DIVERSE GENOMIC, TRANSCRIPTOMIC SIGNATURES AND TARGETED DRUG RESPONSE

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Introduction: Genomic and functional alterations of enzymatic activity drive cancer metabolic reprogramming along with mutations of tumor suppressors and oncogenes. Targeted inhibition of cell metabolism is a promising therapeutic strategy in acute myeloid leukemia (AML). However, metabolic dependencies are largely unexplored. We aimed to classify AML patients based on their metabolic landscape and map connections between their metabolic and genomic profiles.

Methods: Genomic data of AML patients were obtained by whole exome sequencing (n=160) or targeted NGS (n=22). Of them, 119 were evaluated for biofluids (serum and urine) metabolites by nuclear magnetic resonance, along with 145 healthy controls. Intracellular metabolites of bone marrow blasts (n=50) and control cells (n=42) were analyzed by mass spectrometry. RNA-seq data of NPM1-mutated (mut) AML were obtained from TCGA (n=66) and BEAT-AML (n=121) cohorts.

Results: Mutations of metabolic enzymes and regulators were detected in 88% of patients. The presence of mutations in the carbohydrate or lipid pathways had a negative impact on overall survival (HR>1.5, p<0.01), while their co-occurrence had a protective effect. Unsupervised hierarchical clustering of AML based on the intracellular metabolome distinguished three clusters, characterized by enrichment of distinct genomic profiles: (1) NPM1-mut, with high metabolite concentration, (2) chromatin/spliceosome-mut, with low metabolite levels and (3) TP53-mut/aneuploid AML, with a more complex metabolic profile. The clusters were also observed at biofluid level and showed an opposite trend of tyrosine and threonine level between serum and blasts. NPM1-mut AML clustering outside the NPM1-mut metabolic group were enriched for mutations in cohesin and DNA damage-related genes (NPM1-double-mut). They had a higher mutation load (15 vs. 9 on average, p=3.98e-05) and a lower frequency of IDH1-2/TET2 mutations (21.9% *vs.* 46.7%, p=0.014). They were characterized by transcriptomic signatures of reduced inflammatory and more undifferentiated status and showed increased sensitivity to SYK, MET and EGFR inhibitors, while being more resistant to AURKA and FLT3/JAK inhibition. Seven of the differentially-expressed genes between NPM1-double-mut and NPM1-mut AML mapped on the human metabolic network. In silico modelling of the intracellular metabolic perturbations induced by these genes (by flux variability analysis) and network analysis predicted alterations in the purine and nicotinade/nicotinamide metabolic superpathways, that were supported by intracellular metabolic data. Conclusions AML is characterized by genomic-driven and functional metabolic alterations. The integration of genomic and metabolic profiles suggests potential metabolic vulnerabilities to be therapeutically exploited in a subgroup of NPM1-mut AML.

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C002

BCOR AND DNMT3A LOSS COOPERATES IN THE PATHOGENESIS OF ACUTE ERYTHROID LEUKEMIA IN MICE

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Background: In 2011, we identified recurrent loss-of-function mutations of BCL6 co-repressor (BCOR) gene in AML and found that they frequently co-occurred with DNA methyl-transferases 3A (DNMT3A) mutations (40% of cases), were mutually exclusive with FLT3-ITD and NPM1 mutations, and were associated with poor outcome. BCOR deficiency is not itself sufficient to promote leukemia in mice suggesting that other cooperative events are required for AML development. Since BCOR and DNMT3A are known epigenetic modifiers, we hypothesized that their combined disruption may promote AML *in vivo*.

Methods: First, we generated a conditional knock-out (cKO) mouse model, where Bcor deletion mimics truncating BCOR mutations (classically observed in adult AML). Second, we created a Bcor/Dnmt3a double KO mice to investigate combined Bcor and Dnmt3a losses *in vivo*. Third, we characterized both BCOR cKO and BCOR/DNMT3a double KO mice cohorts for resulting phenotypes (histology/morphology; flow-cytometry; RNA-seq of distinct bone marrow populations), response to chemotherapy (cytarabine, ARAC)/hypomethylating agent (decitabine, DEC), and for overall survival.

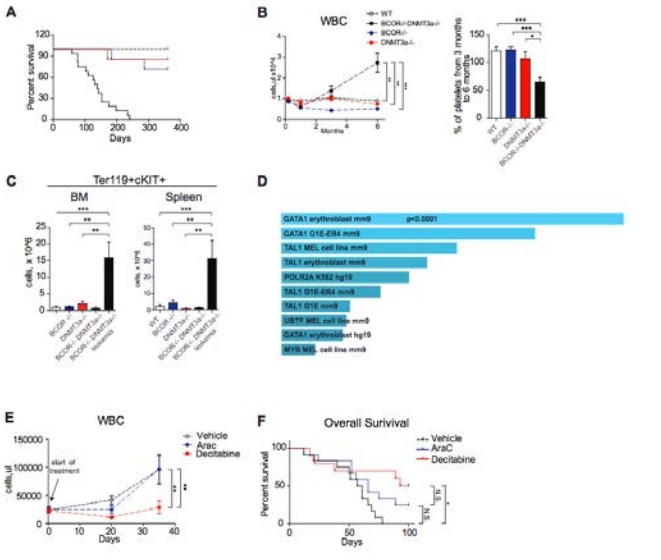

Figure 1.

Results: Mice lacking Bcor only showed an expansion of erythroid-megakaryocytic progenitors, resulting in macrocytic anemia and throm-

bocytosis, with no features of overt acute leukemia. Instead, BCOR/DNMT3a double KO mice developed a fully-penetrant leukemic phenotype, with a median survival of 135 days (range: 59-234 days). Leukemic mice exhibited marked leucocytosis, macrocytic anemia and progressive reduction of platelet counts. Morphological and cytometric analyses showed an expansion of immature erythroid precursors (c-kit+/Ter119+), resembling human acute erythroid leukemia (AEL). In-depth gene-expression profiling of hematopoietic stem/progenitor cells from leukemic mice, indicated that the aberrant erythroid skewing was associated with an altered molecular program, affecting major cell-cycle regulators (Mdm2 and TP53) and erythroid-specific transcriptional factors (GATA1-2). By combining our GEP data with those reported in human AEL, we identified a molecular signature that included several GATA1 interactors. We then tested the efficacy of DEC compared to ARAC in our AEL cohort, thus evincing a significant impact of decitabine on leukemic progression and median overall survival (103 d for DEC *vs.* 66 d for ARAC).

Conclusions: We have demonstrated that BCOR loss perturbs erythro-megakaryopoiesis and cooperates with Dnmt3a loss to drive murine AEL *in vivo*. We identified novel molecular networks acting in AEL development, which affect erythroid-specific transcriptional factors and key cell-cycle regulators. We then showed that decitabine was superior to cytarabine in influencing AEL course and median survival. In summary, our model can be a promising tool to dissect biological underpinnings of AEL formation/maintenance, as serves as a drug-testing platform for rapid translation of therapeutics into human AEL.

C003

GENES BELONGING TO THE SWI/SNF CHROMATIN REMODELING COMPLEXES ARE MUTATED AT HIGH FREQUENCY IN RARE T(11;17)(Q23;Q21)/ZBTB16-RARA ACUTE MYELOID LEUKEMIA PATIENTS

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Introduction: The ZBTB16-RARA fusion gene, resulting from the reciprocal translocation between ZBTB16 on chromosome 11 and the RARA gene on chromosome 17 [t(11;17) (q23;q21)], is rarely observed in AML, and accounts for about 2% of RARA rearrangements. Despite involvement of the RARA gene, carriers of this rare translocation show an unusual bone marrow morphology, with intermediate aspects between APL and AML with maturation. Patients have a high incidence of disseminated intravascular coagulation at diagnosis, are poorly responsive to ATRA and ATO and have an overall poor prognosis. Due to its rarity, the mutational profile of ZBTB16-RARA rearranged AML has not been described so far. The aim of our work was to dissect the molecular landscape of ZBTB16/RARA AML, as compared to classical APL (PML-RARA) and to other AML subtypes, to identify biological differences sustaining the disease phenotype and the resistance to classical APL treatment.

Methods: DNA was extracted from the BM-MNC of 156 AML patients (7 t(11;17) (q23;q21), 46 APL with PML-RARA and 103 non-RARA rearranged AML). Targeted-NGS of 24 genes known to be involved in AML pathogenesis was performed on the entire study cohort. Carriers of the t(11;17) (q23;q21) were then screened for additional mutations using whole exome sequencing (n=3 pts) and the Ion AmpliSeq Comprehensive Cancer Panel (n=4 pts).

Results: Of 7 AML, carriers of the rare ZBTB16/RARA rearrangement, 5 were mutated in at least one gene (71.42%) and the total number of mutated genes was 12 (mean of 1.71 ± SD 1.70 mut/pt). As a control, we analysed the mutational profile of 46 APL with PML-RARA rearrangement and 103 non-RARA rearranged AML. In the 103 non-RARA rearranged AML we found at least one mutation in 97 patients (94.17%), with a mean of 2.86 mut/pt (SD: ±2.03). A significantly lower number of mutations was present in 46 APL (a least one mutation in 32/46 pts, 69.56%, mean 0.89 mut/pt, SD ±0.77, Mann Whitney test, p < 0,0001, Figure 1). Interestingly, ZBTB16/RARA rearranged AML showed an intermediate number of mutations, which affected only 7 genes of the myeloid panel, with frequent involvement of TET2 (4/7 patients, 57%), RUNX1 and CSF3R (2/7 patients each, 29%). The extended mutational profiling of 7 ZBTB16/RARA patients showed a high incidence of ARID1A mutations (5/7 patients, 71%). Of note, we also identified mutations ARID2 and SMARCA4 (1/7, 14%), other tumour suppressor genes belonging to SWI/SNF chromatin remodeling complex.

Conclusions: This is the first report showing the high incidence of ARID1A mutations in the rare cohort of ZBTB16/RARA rearranged AML. ARID1A and other members of the SWI/SNF chromatin remodeling complexes have been previously described as master regulators of hematopoietic differentiation. In this line, we suggest the involvement of the SWI/SNF chromatin remodeling complexes in the clinical presentation, the APL-like morphology and the resistance to ATRA/ATO treatment.

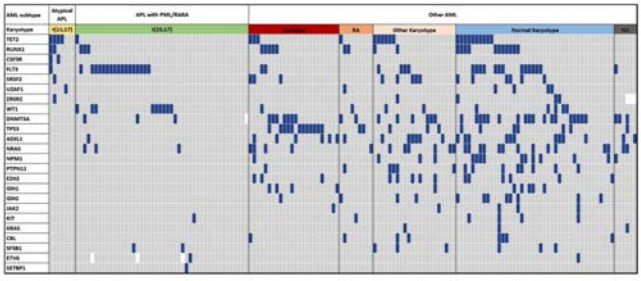

Figure 1.

C004

A THREE-GENE IMMUNE SIGNATURE INCLUDING IDO1, BIN1 AND PLXNC1 PREDICTS SURVIVAL IN ACUTE MYELOID LEUKEMIA

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Introduction: A large body evidence has increasingly demonstrated that the immune tumor microenvironment (TME) critically contributes to acute myeloid leukemia (AML) development. However, current AML prognostic classifications only rely on leukemia cell-intrinsic alterations and do not include immunological markers referring to TME.

Indoleamine 2,3-dioxygenase 1 (IDO1), which is negatively regulated by the BIN1 proto-oncogene, is a central mediator of immune tolerance in the AML TME. This study aimed to identify IDO1-interacting genes in the AML TME and to develop a prognostic immune gene signature.

Methods: Biological and clinical data of 732 patients with *de novo* AML were retrieved from public TCGA and HOVON datasets. Patients >= 65 years were excluded from survival analyses. Co-expression analysis was performed through cBioPortal on TCGA data aiming at discovering new IDO1-interacting genes. Cox regression analysis was used to identified most survival-predicting genes in order to generate a prognostic score. Differential expression (DE) analysis was performed using the nSolver software package (NanoString Technologies, USA).

Results: As expected, BIN1 and IDO1 expression were negatively correlated in HOVON cases (P<0.0001). Further co-expression analyses of RNA-sequencing data from TCGA allowed us to identify PLXNC1, a semaphorin receptor involved in inflammation and immune response, as an IDO1-interacting gene and a strong predictor of survival (P<0.001). PLXNC1 was negatively correlated to IDO1 in the HOVON dataset (P<0.0001). The IDO1-BIN1-PLXNC1 immune gene signature predicted AML survival both in HOVON (P<0.0001) and in TCGA (P=0.001) cases. We, then, sought to identify commonalities between DE genes in $IDO1^{low}$ versus $IDO\text{-}1^{high}$ TCGA cases and in $PLXNC1^{low}$ versus $PLXNC1^{high}$ TCGA cases. Interestingly, CXCR2 was the only shared gene, suggesting the IDO1 and PLXNC1 overexpression reflected non-redundant gene expression programs. Furthermore, we identified IKBKB, FOSL1 and TLR9 as the top DE genes between $IDO1^{low}$ and $IDO\text{-}1^{high}$ cases, whereas GZMH, GNLY, IFIT2 and IFIT3 were the top DE genes between $PLXNC1^{low}$ and $PLXNC1^{high}$ cases. Finally, these newly identified genes, when considered in aggregate, provided a better survival prediction (P<0.001, HR=2.6) compared with the individual signatures deriving from the combination of top DE genes between $IDO1^{low}$ and $IDO\text{-}1^{high}$ (P<0.01, HR=2.2) or between $PLXNC1^{low}$ and $PLXNC1^{high}$ cases (P<0.05, HR=1.8). Conclusions Our data identify PLXNC1 as a novel IDO1-correlated gene. A three-gene immune signature that includes PLXCN1, IDO1 and BIN1 strongly predicted clinical outcome in large AML cohorts. Moreover, IDO1 and PLXNC1 expression-based DE analysis generated an immunological signature highly predictive of prognosis. In light of the emerging role of immunotherapies for AML, our findings support the incorporation of TME-associated immune biomarkers into current AML classification and prognostication algorithms.

C005

CHARACTERIZATION OF T-CELL IMMUNITY AND CD99 IMMUNOTHERAPY IN FLT3-ITD MUTATED AML

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Acute Myeloid Leukemia (AML) with FLT3-ITD mutations remains a therapeutic challenge, with a high relapse rate, due to the persistence of LSCs. T-regulatory cells(Tregs) are known to dampen anti-tumor immunity, by suppressing CD8+ T-cell response mediate programmed death-1(PD-1). We found a strong correlation between the CD34/CD123/CD25/CD99+LSCs phenotype and FLT3-ITDmut positivity, with CD99 expression allowing for separation of LCSs from normal HSCs.The aim of this study was to test the role of an anti-CD99 antibody and of T-cell immunity in FLT3-ITDmutAML. Mutations of the FLT3 gene were investigated in 60AMLs at diagnosis and in 6cases at relapse. Bone marrow(BM) samples were analysed by multiparametric flow cytometry and a sequential gating strategy was carried out to sort the CD34/CD123/CD99/CD25+LSCs from BM samples collected at diagnosis(n=16) and relapse(n=6). The peripheral blood T-cell phenotype was characterized in 10 AML cases and in 18 healthy donors(HDs). Tregs were identified by high CD25 expression on CD4+T-cells and absence of CD127.The expression level of the long and short CD99 isoforms was analysed by a specific RQ-PCR in a cohort of 43 AMLs(FLT3-ITDmut=23, FLT3wt=20) and 7HDs. Sensitivity to the anti-CD99 mAb clone-3.2-3(Diatheva, Cartoceto, PU, Italy) was assessed on leukemia cell lines(MV4-11, MOLM13, THP1, HL60), MNCs(n=13) and LSCs(n=3) from AML patients by cell proliferation, cell death and apoptosis, and transmigration assays.Internalization of the antibody was analyzed by confocal microscopy. Out of 66AMLs, 46 were FLT3mut(n=45 ITDmut, n=1 TKDmut) and 20FLT3wt. Expression of CD123/CD99/CD25+ antigens within the CD34+compartment correlated with FLT3-ITDmut(p=0.001), and CD99-MFI level was significantly higher in FLT3-ITDmutLSCs as compared to leukemic blasts at diagnosis(n=8;p=0.007) and relapse(n=6;p= 0.031) (Figure1A). The CD99-long isoform was prevalent in all subsets of leukemic and control samples, particularly in FLT3-ITDmutAML(p<0.0001)(Figure1B). There were no differences on canonical and memory Treg expression between FLT3-ITDmut and FLT3wtAML (Figure1C-D). Interestingly, CD3+/CD8+cells in FLT3-ITDmut patients expressed very low PD-1 levels, compared to FLT3wtAML(p=0.023) and HDs (p=0.001) (Figure1E). Incubation with the anti-CD99 antibody showed massive internalization in vacuoles accumulating in the cytoplasm of leukemic blasts leading to cell killing through a non-apoptotic pathway, known as methuosis(n=6, Figure1F). The cytotoxic effect was particularly evident on CD34+/CD99+LSCs from 2patients relapsed after Midostaurin treatment(UPN11-UPN15), compared to HDs-CD34+cells(n=2, Figure1G). Furthermore, the anti-CD99 mAb significantly impaired MOLM13-CD34+cell transmigration through a monolayer of endothelial HUVEC cells.Our study shows that PD1+CD8+ T-cells are downregulated in FLT3-ITDmutAML, and provides new insight into the role of CD99 as a promising therapeutic target specifically aimed at eradicating FLT3-ITDmutLSCs.

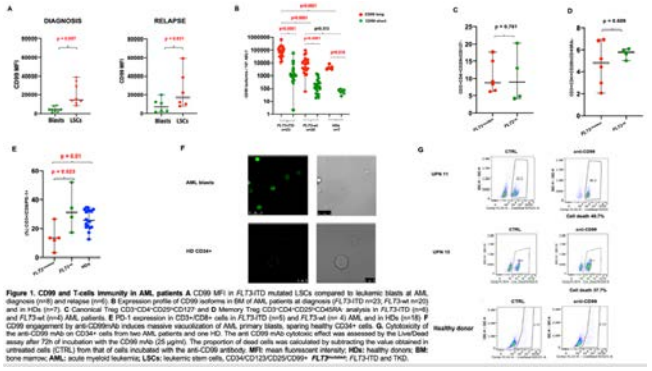

Figure 1.

C006

IDH1-R132H EXPRESSION DRIVES IN HUMAN NORMAL CD34+ HEMATOPOIETIC CELLS A BLOCK OF DIFFERENTIATION RELEASED BY THE SPECIFIC INHIBITOR IVOSIDENIB

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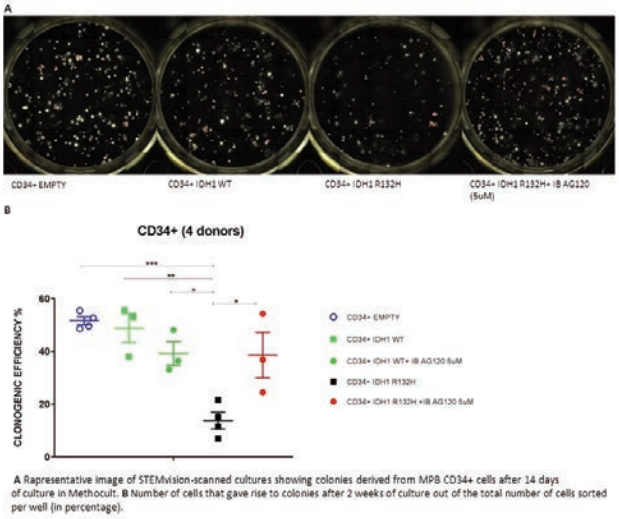
Introduction: Acute myeloid leukemia (AML) arises from multiple and sequential genetic alterations occurring in hematopoietic precursors. Mutation in isocitrate dehydrogenase1 (IDH1) occurs in about 7-10% of all AML and mostly clusters with CN-AML. This gain-of function mutation alters a residue in the active site of IDH1 enzyme reducing its affinity for isocitrate and conferring the ability to convert alpha-ketoglutarate (a-KG) to 2-hydroxyglutarate (2-HG). Studies mostly in murine models showed that supra-physiological concentrations of 2-HG inhibit αKG-

dependent dioxygenases, alter DNA and histone methylation, and inhibit normal differentiation processes likely promoting leukemogenesis. AG-120 (ivosidenib) is a first-in-class, oral, selective, small-molecule inhibitor of IDH1 that has already shown important clinical results in patients carrying this mutation. Notably, in some patients a myeloid cell differentiation with differentiation syndrome has been reported. How human hematopoietic stem cell fate decision is altered by the single different AML-associated genes mutations, including IDH1m, and the specific inhibitors interference are poorly studied.

Methods: Highly purified CD34+ cells derived from mobilized peripheral blood of healthy donors (hCD34+, n=4) were infected by lentivirus containing the gene of interest (either IDH1 wild-type or IDH1-R132H) and ZsGreen gene reporter. After 72h, ZsGreen-positive cells were sorted by flow cytometry and used for colony-forming unit (CFU) methylcellulose differentiation assays, in presence or absence of AG-120 (n=3). Following 14 days, colonies were counted and images acquired using STEMvision (Figure 1A).

Results: Cloning efficiency from hCD34+ expressing empty vector was comparable to hCD34+/IDH1 wt and was not significantly influenced by AG-120 (Figure 1B). In contrast, hCD34+/IDH1-R132H showed an evident block of differentiation compared to counterparts (ANOVA, p=0.0004***, 0.0014**, 0.0165*). In line with being a specific effect of the IDH1-R132H mutation, block of differentiation was released by treatment with the specific inhibitor AG-120 (5uM) (p=0.0193*)(Figure1B). No differences in the proportions of erythroid and myeloid colonies were observed in the different conditions, suggesting that the block of differentiation, and its AG120-induced release, affect both lineages (data not shown). Methylcellulose replating assays and *in vivo* xenograft experiments in mice are ongoing to assess the self-renewal capacity of hCD34+ expressing IDH1-R132H.

Conclusions: We show for the first time in primary human CD34+ cells that expression of mutant IDH1 is sufficient to drive *in vitro* a block of hematopoietic cells differentiation that can be reverted by IDH1i. These preliminary data pave the way to further deeper studies aimed to shed light, in a human cell model, into the specific pathways involved in 0leukemogenesis driven by IDH1 mutant, and other mutations, and in the response to specific inhibitors.



A Rapresentative image of STEMvision-scanned cultures showing colonies derived from MPB CD34+ cells after 14 days of culture in Methocult. B Number of cells that gave rise to colonies after 2 weeks of culture out of the total number of cells sorted per well (in percentage).

Figure 1.

C007

STABLE XPO1 INHIBITION IS REQUIRED TO ACHEIVE MAXIMAL ANTILEUKEMIC EFFECT IN NPM1-MUTATED ACUTE MYELOID LEUKEMIA

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Introduction: NPM1-mutated acute myeloid leukemia (NPM1c AML) is characterized by cytoplasmic delocalization of mutant NPM1 (NPM1c) and sustained HOX expression. We have previously demonstrated that HOX expression is dependent on cytoplasmic localization of NPM1c and that high HOX levels are necessary to maintain the leukemic state of NPM1c AML. Nuclear export of NPM1c is dependent on exportin-1 (XPO1) and pharmacologic XPO1 inhibition by the XPO1 inhibitor selinexor (seli) leads to NPM1c nuclear relocalization, loss of HOX expression and terminal differentiation. Despite promising preclinical results, seli has been tested in clinical trials with no benefits for NPM1c AML patients. This is probably due to its toxicity profile that limits dosing to 2 days/week. The new XPO1 inhibitor eltanexor (elta) is better tolerated and has been administered 5 days/week in Phase I clinical studies. We hypothesized that intermittent XPO1 inhibition (2d/w) does not result in stable NPM1c nuclear relocalization and loss of HOX expression and that constant XPO1 inhibition (5d/w) is necessary to achieve maximal antileukemic effect.

Methods: NPM1c AML cell line OCI-AML3 and CRISPR-edited OCI-AML3 cells with endogenous NPM1c fused to GFP (NPM1c-GFP) were used for *in vitro* experiments. A patient-derived xenograft mouse model of a highly aggressive NPM1c/FLT3-ITD AML engineered to express GFP and Luciferase (PDX) was used for *in vivo* studies.

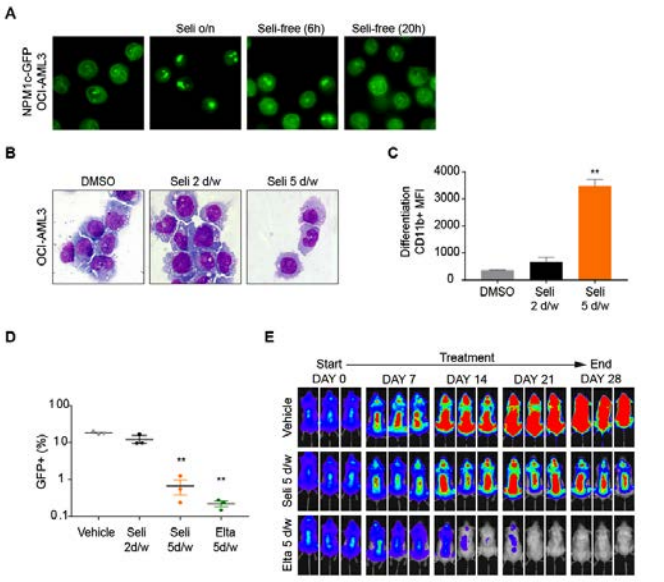

Figure 1.

Results: To establish the effects of intermittent XPO1 inhibition on NPM1c localization and HOX expression, we treated NPM1c-GFP cells with seli for 12 hours, followed by drug washout from culture medium and live cell imaging over 20 hours. As anticipated, intermittent XPO1 inhibition led to only transient NPM1c-GFP nuclear relocalization (A) and did not result in stable HOX downregulation. To compare the outcomes of intermittent and constant XPO1 inhibition on HOX expression and differentiation over time, we treated OCI-AML3 cells with 2d/w and 5d/w seli for a total of 12 days. While 5d/w treatment induced loss of HOX expression and terminal differentiation (B-C), 2d/w treatment led to negligible HOX downregulation and minimal differentiation. To confirm these results *in vivo*, we treated PDX with either seli (2 or 5d/w)

or elta (5d/w) for two weeks. While 2d/w treatment did not show clear efficacy, mice treated with 5d/w seli or elta demonstrated near-complete loss of AML engraftment (D), deep HOX downregulation and differentiation. Finally, we established the effect of constant XPO1 inhibition on PDX survival: mice treated with either 5d/w seli or elta experienced significantly prolonged survival (median 50 and 63 days) compared to vehicle (41.5 days), with elta showing highest antileukemic efficacy (E).

Conclusions: This study demonstrates that stable XPO1 inhibition is necessary to achieve maximal antileukemic effect in NPM1c AML and lays the groundwork for the design of new clinical trials with XPO1 inhibitors in NPM1c AML.

C008

MOLECULAR-CYTOGENETIC DIAGNOSIS TO IMPROVE PROGNOSTIC STRATIFICATION IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Historically considered an aggressive subtype of acute leukemias, pediatric T-ALL have experienced a better outcome since the adoption of MRD to guide standard or intensified treatment. In adults, instead, T-ALL remains a high risk subtype of leukemia with an overall incidence of relapse of about 70%. Neither in children nor in adults, clinical variables or genetic alterations are currently used as predictive/prognostic markers. We sought to assess the potential impact of recurrent genetic markers on probability of relapse in pediatric and adult T-ALL patients who were referred to our laboratory for a comprehensive molecular-cytogenetic study [1].

Methods: We analysed 145 pediatric and 126 adult T-ALL that belong to a previously published cohort [1]. CI-FISH was applied to classify T-ALL into TAL/LMO, HOXA, TLX1, TLX3, NKX2-1/-2, or MEF2C subgroup, and to investigate 26 oncogenes/oncosuppressors recurrently rearranged in T-ALL [1]. NOTCH1 exons 26, 27, 34, FBXW7 exons 8, 9 and PTEN exon 7 mutations were studied by Sanger Sequencing. Cumulative incidences of relapse were estimated by R 3.3.1 program. Gray's test was used to compare subgroups. The analysis was done on the entire cohort and on age stratified subgroups.

Results: Confirming previous reports, CI-FISH allocated about 80% of T-ALL into the main genetic subgroups based on age. As expected, TLX1/NKX2-1 positive cases showed a significantly lower probability of relapse in children (P=0,005) and adults (P=0,028). Interestingly, we observed that the number and the type of additional concurrent aberrations correlated with the probability of relapse. Cases harbouring ≤ 5 abnormalities behaved better than those with >5 (P<0,001). Amongst genomic rearrangements, deletions of TCF7/5q31 (P=0,032), TP53/17p13 (P=0,038), and NF1/17q12 (P=0,008), which were significantly enriched within the HOXA subgroup, resulted to be associated with a higher probability of relapse. Furthermore, a higher incidence of relapse was observed in cases with JAK/STAT involvement (46% *vs.* 31%). Instead, NOTCH1/FBXW7 mutations, PTEN/10q23 inactivation, MYC/8q24 translocations, and deletions of CDKN2AB/9p21, 6q15-21, LEF1/4q25, or WT1/11p13, did not appear to be associated with relapse risk.

Conclusions: Translation of bio-molecular information in the diagnostic work-up of T-ALL is still challenging due to the genetic complexity and heterogeneity of the disease. Although preliminary, our study indicate that genetic diagnosis may serve clinical trials to fine tune MRD-based risk stratification of patients. The TLX1/NKX2-1 was confirmed as the most favourable genetic subgroup, while TCF7, TP53, and/or NF1 deletions, mark a subgroup of chemo-resistant HOXA-positive T-ALL.

References

1. La Starza R, J Mol Diagn 2020 R. La Starza and T. Pierini contributed equally to this work

Chronic Lymphocytic Leukemia 1

C009

COMPLEX KARYOTYPE IS ASSOCIATED WITH A LESS FAVORABLE OUTCOME AFTER IBRUTINIB AND RITUXIMAB AS FRONT-LINE TREATMENT OF UNFIT PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (GIMEMA LLC1114)

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Background: Karyotype (KT) is of clinical relevance in chronic lymphocytic leukemia (CLL), being a prognostic and predictive marker of outcome after chemoimmunotherapy. There are increasing evidences on the suboptimal outcome of CLL patients with complex KT (CKT) after ibrutinib and venetoclax-based regimens, regardless of TP53 gene deletion/mutation. We aim at investigating the impact of CKT on the outcome of unfit CLL patients enrolled in the front-line GIMEMA trial with ibrutinib+rituximab (LLC1114, EudraCT 2014-002714-23).

Methods: Out of 151 patients enrolled, peripheral blood samples from 121 patients were collected and stored in the central laboratory in Rome and sent to Ferrara for the cytogenetic analysis, conducted by stimulation with IL2 and CpG. KT was successfully analyzed in 102/121 (84%) samples. FISH with a 4-probe panel was performed and IGHV mutational status and 4 gene mutations were assessed by Sanger sequencing. In this first subanalysis of the GIMEMA LLC1114 protocol, progression-free survival (PFS) was calculated from the date of treatment initiation to the date of clinical progression, death or last follow-up. Cumulative incidence of progression with deaths as competitive events was also considered. Median follow-up was 40 months.

Results: Of the 102 patients (Table 1), 15 (14.7%) carried a normal KT, 42 (41.2%) one lesion, 17 (16.7%) two lesions, 28 (27.4%) showed ≥3 lesions, *i.e.* CKT. Of the latter, 10 carried ≥5 lesions. As expected, there was a highly significant association between CKT (≥3 lesions, n=28) and del17p (n=8, p=0.0004), TP53 mutations (n=10, p=0.0015) and unmutated IGHV (n=21, p=0.044). Interestingly, patients with ≥5 lesions had a greater enrichment of TP53 deletions and/or mutations (TP53 disruption in 8/10 cases) compared to patients with ≥3 lesions (2/18, p=0.0005). Contrariwise, a CKT was exceptionally found among patients with negative FISH (p=0.0016). No correlation with other FISH lesions, gene mutations or B-cell receptor (BCR) stereotyped subsets was found. In patients with ≥3 lesions, PFS was significantly shorter than in the other (p=0.014), also considering the cumulative incidence of progression (p=0.019). No difference in outcome has so far emerged between patients with ≥3 lesions and those with ≥5 lesions. Patients with a CKT devoid of TP53 deletions and/or mutations (n=18) showed the same poor PFS than those with TP53 disruption (p=0.897). A univariate analysis, including CKT *vs.* non-CKT, TP53 disrupted *vs.* WT and IGHV unmutated *vs.* mutated, showed that CKT was the only parameter with a significant impact on PFS (p=0.019).

Conclusions: Our data add further evidence that KT is a predictive marker of efficacy of ibrutinib used as front-line treatment. Patients with a CKT represent a high-risk subgroup regardless of TP53 deletions/mutations. The analysis of KT should be a tool to further refine CLL patients' stratification and therapeutic decisions in the era of chemo-free combinations.

Table 1. Biologic features of 102 CLL patients.

	All (N=102)	Complex KT (n=28)	Non-complex KT (n=74)	p value
IGHV unmutated (1 NA*)	59	21	38	**0.0437**
FISH lesions (3 NA*):				
Normal	26	1	25	**0.0016**
del13q	33	7	26	0.4733
tris12	15	4	11	1
del11q	15	7	8	0.114
del17p	10	8	2	**0.0004**
TP53 mutated	16	10	6	**0.0015**
NOTCH1 mutated	23	8	15	0.4287
SF3B1 mutated	13	2	11	0.506
BIRC3 mutated	7	2	5	1
WT for 4 genes	55	11	44	0.0787
BCR stereotyped subsets	11	2	9	0.7229

* not available

C010

NOTCH2 CONTRIBUTES TO VENETOCLAX RESISTANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA.

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Background: Chronic lymphocytic leukemia (CLL) has recently experienced an unprecedented revolution thanks to the discovery of crucial pathogenetic mechanisms. Despite considerable therapeutic advancements, the eradication of the disease is not complete and resistance and transformation may occur. Venetoclax is a small-molecule BH3 mimetic that competes for binding in the hydrophobic groove of Bcl-2, a key protein involved in CLL cells survival. Venetoclax monotherapy shows an overall response rate of 79% and complete response rates of 16% and leave some open questions mainly related to intrinsic features of CLL patients that may guide a pattern of resistance.

Aim of the work: We hypothesize that specific intrinsic features of CLL cells may contribute to drive possible mechanisms of resistance to venetoclax (ABT-199) treatment.

Methods: Notch2 expression was monitored by western blot in purified CLL cells. Modulation of Notch2 expression *in vitro* was performed by using siRNA strategy. ABT-199 was used at doses of 0.1 nM or 1 nM. RESULTS: We detected a peculiar high expression of Notch2 in a subgroup of CLL patients carrying trisomy 12. The high expression of Notch2 correlated with high levels of Mcl-1, CD23 and Hes1. Interfering with Notch2 expression in trisomy 12 CLL patients, by siRNA silencing, was able to affect leukemic cells viability, reducing CD23 and Mcl-1 expression. Since Mcl-1 is involved in ABT-199 resistance, we wondered if ABT-199 may have a different effect in CLL cells isolated from patients carrying trisomy 12 comparing to no trisomy 12 cases. After 24h of culture in complete medium, trisomy 12 CLL cells showed a gain in survival rate of 10% during treatment with both 0.1 nM or 1 nM in comparison to no trisomy 12 cases. This advantage in viability reflected the maintenance of Notch2 and Mcl-1 expression in trisomy 12 both at 0.1 nM and 1 nM of ABT-199 compared to control. Conversely, a reduction of Notch2 and consequently Mcl-1 levels were observed in no trisomy 12 cases at both doses of ABT-199. We did not detect any variation in Bcl-2 levels. We wondered if Notch2 expression may reduce the response to ABT-199 in trisomy 12 CLL. To demonstrate this hypothesis, we interfered with Notch2 by silencing strategy. Treatment with Notch2

siRNA decreased the expression of Notch2 and Mcl-1 in combination with ABT-199. We also found that Notch2 down-regulation cooperated with ABT-199 decreasing CLL cells viability.

Conclusions: Our results show a novel mechanism that may compromise the clinical response to venetoclax in CLL patients. The possibility to identify patients with more pronounced expression of Mcl-1 may help to optimize the treatment. The expression of Notch2 identify a subset of CLL patients, mainly harboring trisomy 12 aberration, that through the maintenance of high levels of Mcl-1, may be involved in a reduced response to ABT-199.

C011

DISSECTING DIFFERENT ANATOMICAL COMPARTMENTS OF SMALL LYMPHOCYTIC LYMPHOMA WITH A LIQUID BIOPSY APPROACH

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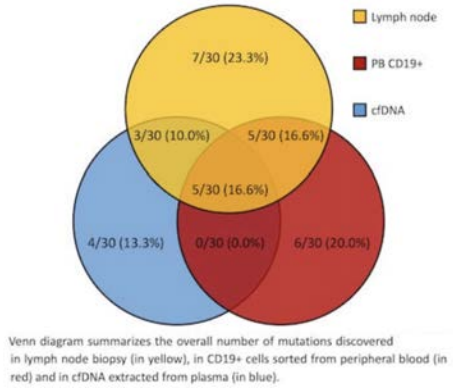
Introduction: According to the WHO classification, small lymphocytic lymphoma (SLL) displays a circulating clonal lymphocyte count < 5 x10^9/L with CLL phenotype coupled to nodal, splenic or other extramedullary involvement. Although SLL may disseminate to different anatomic compartments, tumor burden involves predominantly the lymph nodes and diagnosis is usually achieved on a lymph node biopsy. Molecular analysis of only one anatomic site may miss meaningful biological information that is restricted to other disease sites. Liquid biopsy recapitulates disease genetics in other lymphomas, but its value in SLL has not been explored.

Methods: Patients with SLL (n=7) were referred to our institution and provided with: i) cell free DNA (cfDNA) extracted from plasma; ii) genomic DNA (gDNA) extracted from lymph node biopsies; iii) gDNA extracted from CD19+ cells sorted from the peripheral blood (PB); and iv) gDNA extracted from CD3+ cells for comparative purposes. All biological materials were analysed with a next-generation-sequencing approach in the coding exons plus splice sites of 133 genes relevant to B cell malignancies.



Venn diagram summarizes the overall number of mutations discovered in lymph node biopsy (in yellow), in CD19+ cells sorted from peripheral blood (in red) and in cfDNA extracted from plasma (in blue).

Figure 1.

Results: A total of 30 somatic non synonymous mutations were identified, with at least one mutation identified in every patient. The most frequently mutated gene was ASXL1 in 3/7 (42.8%) patients, followed by NOTCH1, EGR2, SPEN, BRAF and TRAF3 mutated in 2/7 (28.5%) patients each. By comparing mutations identified in different anatomical sites (lymph node, PB, plasma), 5/30 (16.6%) mutations were identified in all three compartments (lymph node, PB and plasma), 7/30 (23.3%) mutations were identified in the lymph node only, 6/30 (20.0%) in the PB CD19+ cell fraction only, and 4/30 (13.3%) in cfDNA only. Three of 30 (10.0%) mutations were shared between cfDNA and lymph node and 5/30 (16.6%) were shared between lymph node and peripheral blood CD19+ cells. Consistently, 26/30 (86.6%) mutations were identified in the lymph node biopsy and in the PB CD19+ cell fraction, and only 4/30 (13.6%) mutations were unique to plasma cfDNA (Figure 1). Three of the 4 mutations identified in cfDNA only (*i.e.* BRAF, TP53 and TRAF3) occurred in a patient with a concomitant diagnosis of multiple myeloma and were restricted to the patient's purified CD138+ plasma cells, confirming that they did not derive from the SLL clone. Thus, only one mutation identified in cfDNA could be ascribed to SLL.

Conclusions: These results suggest that: i) mutational analysis of lymph node biopsies combined with PB CD19+ cell fraction recapitulates the SLL genetic landscape in individual patients; ii) the analysis of the lymph node only or of the PB CD19+ cell fraction only may miss a fraction of mutations of potential relevance; and iii) at variance with aggressive lymphoma, liquid biopsy in SLL may not add significantly to the genetic landscape of the disease. In SLL, PB CD19+ cells conceivably derive from different anatomic sites and might prove to be more useful than cfDNA in SLL genotyping.

C012

COMPLEX KARYOTYPE SUBTYPES AT CHRONIC LYMPHOCYTIC LEUKEMIA DIAGNOSIS ALLOW TO REFINE THE RISK OF DEVELOPING RICHTER SYNDROME

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Introduction: Richter syndrome(RS), the transformation of chronic lymphocytic leukemia(CLL) into an aggressive lymphoma, is a rare but life-threatening complication. Complex karyotype (CK), defined by the presence of >=3 chromosomal lesions, is a heterogeneous cytogenetic category associated to shorter survival in CLL, but its impact on the evolution into RS has not been investigated. Among CK cases, those with >=5 lesions (highCK) and those with major structural abnormalities [type-2 CK (CK2)] forecast a more aggressive disease. The aim of this project was to assess the impact of CK subtypes on the risk of CLL to develop RS.

Methods: We performed a retrospective study in 3 Italian CLL centers. Stimulated cytogenetic was performed in 540 patients within the first year after CLL diagnosis. CK cases with unbalanced translocations, addition, insertion, derivative or marker chromosomes were classified as CK2. An IGHV gene sequence homology >=98% was considered as unmutated(U-IGHV), as opposed to mutated(M-IGHV). TP53 disruptions(TP53dis) include deletions and mutations. Time to Richter syndrome(TTRS) was calculated from CLL diagnosis to histologically confirmed DLBCL transformation or last known follow-up. Survival curves were compared with the log-rank test and $p<0.05$ was considered as significant. Hazard ratio(HR) was calculated by multivariate analysis.

Results: Among the 540 patients 76% were at Binet A stage, 47% were U-IGHV, 11% had TP53dis, 20% harbored a CK including 14% CK2 patients and 9% highCK. Ten% of patients died and 5% developed RS over a median follow-up of 6 years. Overall, the rate of RS after 5 and 10-year after CLL diagnosis was 2.6% and 11.8%. We observed that CK2 and highCK at CLL diagnosis were significantly more common in

patients who subsequently developed a RS compared to no-RS cases (46% *vs.* 12%, 39% *vs.* 8%, both p<0.0001). By univariate and multivariate analysis CK, CK2 and highCK subtypes were all significantly associated with a shorter TTRS, together with U-IGHV, TP53dis, 11q- by FISH and Binet stage B-C. Patients with CK2 (HR=6 p<0.0001) and highCK (HR=7 p<0.0001) harbored the highest risk of developing a RS, being the 10-year TTRS of 37% and 41%, respectively, *vs.* 8% of patients without CK. By integrating statistically significant variables, we developed a hierarchical model based on HR values (Figure 1): 15% patients were classified as highCK or CK2, the 10-year TTRS was 31% and the HR 13; 45% were U-IGHV/TP53dis/11q-/Binet B-C, the 10-year TTRS was 12% and the HR 3; 40% were M-IGHV without CK and TP53 wild-type, the 10-year TTRS was 3%. This model was confirmed in multivariate analysis and internally validated(p<0.0001).

Conclusions: We have herein identified variable associated with a higher risk of developing RS and recapitulated them into a RS prognostic model. Remarkably, patients harboring a CK subtype at the CLL diagnosis have the highest risk of developing RS and should be carefully monitored during the follow-up.

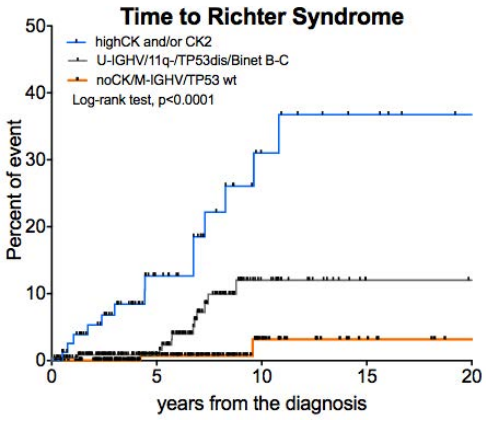

Figure 1.

C013

NOTCH-1 ACTIVATION AND SUBCLONAL MUTATION NEGATIVELY IMPACT ON CHRONIC LYMPHOCYTIC LEUKEMIA OUTCOMES

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Introduction: NOTCH1 mutations and deregulated signal have been commonly found in chronic lymphocytic leukemia (CLL) patients. In particular, NOTCH1 mutation and activation (ICN1+) are detected independently in about 50% of patients. Whereas the impact of NOTCH1 mutations on clinical course of CLL has been widely studied, the prognostic role of NOTCH1 activation in CLL remains to be defined. Here, we analyzed and compared NOTCH1 mutational status and activation in terms of frequency and impact on time to first treatment (TTFT) in a cohort of CLL patients at diagnosis. We also investigated the role of low allelic burden.

Methods: CLL patients were evaluated for NOTCH1 mutation allele burden by ddPCR (N=433) and for NOTCH1 activation by western blot (WB) of ICN1 using the anti-cleaved Val1744 antibody (N=152). Based on ICN1 activation and NOTCH1 mutational status, patients were defined as ICN1-/wild type (WT), ICN1-/mutated, ICN1+/WT or ICN1+/mutated.

Results: NOTCH1 mutation was detected in 178 patients (41.1%). Based on NOTCH1 mutation burden, patients were stratified into three groups: low ratio (LR; allelic frequency from 0.03 to 0.1%); intermediate ratio (IR; allelic frequency from 0.1 to 20%); high ratio (HR; allelic frequency from 20 to 100%). By comparing NOTCH1 WT (N=255; 58.9%) *vs.* LR (N=46; 10.6%), IR (N=104; 24%) and HR (N=28; 6.5%) mutated patients, we found that TTFT was progressively shorter (131 *vs.* 69, 72 and 23 months, respectively; p<0.05; p<0.05; p<0.0001). Overall survival (OS) was significantly lower in the HR, IR and LR groups compared with WT patients (138, 118 and 178 *vs.* 309 months, respectively; p<0.01; p<0.001; p<0.05). According to the IGHV mutational status, NOTCH1 mutation correlated with the unmutated IGHV group independent of allele burden. WB analysis showed that ICN1 was expressed in 112/152 patients (73.7%), 58 of them (51.8%) were NOTCH1 mutated (ICN1+/mutated) and 54 (48.2%) were NOTCH1 WT (ICN1+/WT). We also identified a group of patients (N=9; 22.5%) lacking ICN1 but carrying NOTCH1 mutation at a low allele burden (ICN1-/mutated). By analyzing IGHV mutational status, we found that ICN1+/mutated group correlated with unmutated IGHV status while ICN1+/WT carried mutated IGHV (p<0.01). Analysis of TTFT showed that ICN1+/mutated and ICN1+/WT patients had a similar TTFT (p=0.45) but strikingly, ICN1+/WT group had a significantly reduced TTFT compared to ICN1-/WT patients (67 *vs.* 154 months; p<0.05). OS was similar in ICN1+/WT and ICN1-/WT patients (p=0.15) but was significantly lower in ICN1+/mutated compared to ICN1-WT group (p<0.05).

Conclusion: We provide insight into the prognostic role of NOTCH1 activation in CLL lacking NOTCH1 mutation. Notably, we have defined a group of patients ICN1+/WT with negative outcome, not detectable to date by any molecular marker. Additionally, we have shown that even low levels of NOTCH1 mutation affect the patient outcome.

C014

INHIBITION OF KV1.3 MITOCHONDRIAL POTASSIUM CHANNEL SELECTIVELY TRIGGERS NEOPLASTIC CELL DEATH IN THE E -TCL1 CLL MOUSE MODEL

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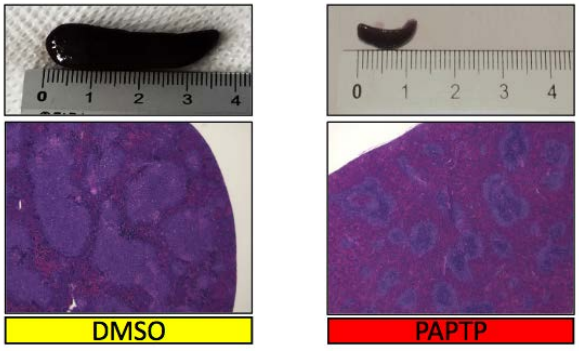
Introduction: Ion channels are emerging and promising oncological targets. The voltage-gated potassium channel Kv1.3 and the calcium-dependent intermediate conductance K+ channel (IKCa) are highly expressed in human Chronic Lymphocytic Leukemia (CLL) B cells' plasma membrane and mitochondria as compared to healthy B lymphocytes. Pharmacological targeting of IKCa using a small molecule inhibitor, TRAM-34 was reported to decrease human CLL proliferation in *ex vivo* setting. On the other hand, direct inhibition of Kv1.3 using a mitochondria-targeted inhibitor, PAPTP that is a derivative of PAP-1, was able to kill 98% of *ex vivo* primary human CLL B cells while sparing healthy B cells. With this as background we evaluated the effect and toxicity of TRAM-34 and PAPTP in the Eμ-TCL1 genetic CLL murine model, that is characterized by a high expression of TCL1 protein in B cells leading to the development of a CLL-like lymphoproliferative disease.

Methods: Since the CLL phenotype is arising late in mouse age, we used only animals characterized by at least 50% of clonal expansion of CD19+/CD5+ in the peripheral blood. Cells were stained with antibodies specific for murine CD45, CD5, CD19, CD3, CD4 and CD8 and evaluated by flow cytometry (FC). Ten mice with overt disease underwent

injection of PAPTP 5 nmol/gbw diluted in DMSO and 10 mice with TRAM34 5 nmol/gbw (5 days/week for 2 weeks). Administration of only DMSO represents the control. At the end of treatment, mice were sacrificed and blood, spleen, bone marrow and intraperitoneal wash were collected, stained and evaluated either for the antigen distribution by FC as well as by histological stain with hematoxylin and eosin. Splenomegaly was also assessed by spleen volume measurements.

Results: After 2 weeks of therapy with PAPTP, we observed a significant decrease in the absolute total lymphocyte number and a more than 50% reduction of pathological B cells in the blood (pre-therapy 56.57%±9.61 *vs.* post-therapy 36.43%±23.04), spleen (DMSO 75.40%±6.14 *vs.* PAPTP 38.44%±8.47; p<0.01), bone marrow (DMSO 33.00%±9.12 *vs.* PAPTP 12.33%±3.73; p<0.05) and intraperitoneal cavity (DMSO 76.00%±11.55 *vs.* PAPTP 33.70%±5.88; p<0.01). Moreover, while untreated mice had enlarged spleen (e.g. 0.70 cm^3) with evidence of CLL infiltration, those treated with PAPTP had smaller spleens (e.g. 0.15 cm^3) with almost no CLL infiltration (Figure 1). In particular, the spleens disclosed well preserved white pulp architecture with clear cut follicles and marginal zones. In contrast to the results obtained with PAPTP, TRAM-34 did not exert any beneficial effect when applied at the highest non-toxic concentration (e.g. Fold induction post/pre therapy white blood cell absolute number: DMSO 7.45±9.39 *vs.* TRAM-34 3.11±1.59; p=ns).

Conclusions: The high selectivity of PAPTP and its capability to selectively induce apoptosis in B CLL cells also *in vivo* in Eμ-TCL1 mouse model suggest the use of this inhibitor for designing new therapeutic strategies.



DMSO: Control mice had enlarged spleen with evidence of CLL infiltration. The spleens showed large nodular lymphoid aggregates, totally effacing the normal architecture of the white pulp.
PAPTP: Treated mice had smaller spleens with minimal (if any) CLL infiltration. In particular, the spleens showed well preserved white pulp architecture with clearcut follicles and marginal zones.

Figure 1.

C015

A DYSREGULATED, DRUGGABLE PP2A/AKT/GSK3β AXIS SUSTAINS NOTCH1 SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Introduction: NOTCH1 is activated independent of NOTCH1 mutations in a large fraction of chronic lymphocytic leukemia (CLL) patients playing an oncogenic role. Progress has been made in identifying the targets of NOTCH1 signaling in CLL but the upstream regulatory mechanisms are unknown. We previously showed that in CLL cells, NOTCH1 activation is sustained by various kinases such as IL-4-induced PI3Kδ/AKT (De Falco *et al.*, Cell Death Dis 2018) and BCR-induced BTK (Del Papa *et al.*, Clin Cancer Res 2019). A target of PI3K/AKT and other dysregulated molecules, such as the protein phosphatase 2A (PP2A), is GSK3β that in turn, controls several oncoproteins. Here, we investigated the role of GSK3β in NOTCH1 regulation and whether targeting GSK3β by modulating its upstream regulators AKT and PP2A has the potential to disrupt NOTCH1 signaling and CLL cell viability.

Methods: In highly purified primary CLL cells, we performed western blot analysis to measure the levels of NOTCH1 intracellular domain (ICD) and GSK3β activity, evaluated as phosphorylation of glycogen synthase and of GSK3β at the inhibitory site S9. GSK3β activity was modulated by pharmacologic and genetic approaches.

Results: Constitutive NOTCH1-ICD levels were reduced by CLL cell transfection with an active GSK3β mutant (p<0.05), whereas they were increased after GSK3β silencing by small interfering RNA (p<0.01) or by the GSK3β inhibitor SB216763 (p<0.05), which enhanced CLL cell viability (p<0.01). SB216763 also increased the NOTCH1-ICD levels induced by stimulation with anti-IgM antibodies. These data indicate that GSK3β downregulates constitutive NOTCH1-ICD levels and also slows down those induced by BCR signaling. In keeping with the negative role of GSK3β in NOTCH1 regulation, we found that CLL cells with high levels of NOTCH1-ICD showed high levels of S9GSK3β phosphorylation indicating a positive correlation between NOTCH1 signaling and GSK3β inactivation. The GSK3β/NOTCH1-ICD interplay was supported by coimmunoprecipitation studies showing a physical interaction between NOTCH1-ICD and GSK3β. We then modulated the activity of a negative (AKT) and a positive (PP2A) regulator of GSK3β and examined the impact on NOTCH1-ICD levels and CLL cell viability. The AKT inhibitor X (AKTiX) reduced NOTCH1-ICD levels (p<0.05) and CLL cell viability (p<0.05). These effects of AKTiX were counteracted by SB216763 suggesting the involvement of GSK3β activity. Even Fingolimod (FTY720), an activator of PP2A and GSK3β, decreased NOTCH1-ICD levels (p<0.05) and was cytotoxic against CLL cells (p<0.05). The anti-NOTCH1 and antileukemic activities of FTY720 were attenuated by either the PP2A inhibitor okadaic acid or SB216763 suggesting that PP2A and GSK3β are involved in FTY720-induced effects.

Conclusions: We identify in a dysregulated PP2A/AKT/GSK3β axis a novel mechanism sustaining NOTCH1-ICD in CLL cells, thus revealing new molecular targets to disrupt NOTCH1 signaling and cell viability in CLL.

C016

FOCAL ADHESION KINASE (FAK) ACTIVATION IS CORRELATED TO HS1 AND CORTACTIN EXPRESSION IN POOR PROGNOSIS CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS

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Introduction: In Chronic Lymphocytic Leukemia (CLL), the complex BCR signaling activates the kinase Lyn that is overexpressed in leukemic B cells. In turn, Lyn phosphorylates the cytoskeletal interactor HS1, as well as its homologous Cortactin that accumulates at sites of actin assembly. Both proteins are overexpressed and involved in survival and migration in CLL B-cells, and have also been shown to correlate with poor prognosis. Focal Adhesion Kinase (FAK) is involved in cellular migration, adhesion and metastasis of many neoplasias. At focal adhesions, FAK recruits adaptors and signaling proteins, such as HS1 and Cortactin, which contribute to the turnover of the actin cytoskeleton, thus leading to cell migration. To date, several studies have focused on FAK expression in solid tumors, but its role in CLL has not yet been established. We then hypothesized that FAK activity, by sustaining cytoskeletal rearrangements pro-migration, may adversely affect the disease outcome in patients with poor prognosis.

Methods: By WB, FAK expression and phosphorylation at Tyr397, regarded as proof of FAK activation, were analyzed in B-cells from 10 healthy subjects and 142 CLL patients, of which 67 IGHV-mutated and 56 unmutated. Expression data were correlated with prognostic parameters. Cortactin and HS1 expression was evaluated in 12 patients by flow cytometry (intracellular staining). Reverse phase protein array (RPPA) analysis was performed on 57 CLL patients and 11 healthy subjects to assess the expression of several proteins, including those related to the cytoskeleton.

Results: By WB analysis we demonstrated a significant down-modulation of FAK expression in CLL patients with poor prognosis, according to the IGHV genes mutation status (IGHV-unmutated 0.39±0.36 *vs.* mutated 0.73±0.72; p<0.001). Moreover, in WB we observed a higher phosphorylation degree of FAK at Tyr397 (pFAK), index of FAK activation, in IGHV-unmutated patients. In terms of RPPA data, patients were divided in pFAK-high *vs.* pFAK-low, according to the obtained median value. A significant positive correlation was proven between HS1 and Cortactin expression levels and FAK activation (values in pFAK-low *vs.* high, respectively: 250±65 *vs.* 298±74; p<0.01 for HS1; 303±122 *vs.* 366±118; p<0.05 for Cortactin). This relationship was also assessed by flow cytometry: Cortactin and HS1 were found to be particularly overexpressed in those CLL patients who showed more activated FAK (697±399 *vs.* 265±137; 1618±1575 *vs.* 232±139, p<0.05, MFI for Cortactin and HS1 respectively).

Conclusions: Our data demonstrate that IGHV-unmutated CLL patients display higher levels of active FAK. Moreover, the malignant phenotype in poor prognosis patients is likely to be related to the overexpression of Cortactin and HS1, two cytoskeletal-connected molecules ultimately accounting for CLL aggressiveness. These results suggest that FAK could be involved in a pathway promoting the malignant phenotype which deserves further investigation.

Multiple Myeloma 1

C017

ABSTRACT WITHDRAWN

C018

NEW INSIGHTS IN PD-L1/PD-1 DISTRIBUTION WITHIN BONE MARROW MICROENVIRONMENT OF PATIENTS WITH MONOCLONAL GAMMOPATHIES

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Introduction: Despite the promising results of immune-checkpoint blockade in the treatment of many tumors, the use anti-PD-1/PD-L1 antibodies in multiple myeloma (MM) still remains debated and under observation for the high toxicity. Literature data on PD-1/PD-L1 expression by CD138+ and bone marrow (BM) cells in MM patients are discordant and none of them compared patients with active and smoldering myeloma (SMM). This suggests the need to better define PD-1/PD-L1 distribution in the BM immune-microenvironment in MM patients also to identify those patients that could benefit from the anti-PD-1/PD-L1 therapy.

Methods: In this study, we isolated mononuclear cells from BM aspirates of 41 patients with SMM and 75 with active MM, including both newly diagnosed and relapsed MM. We compared the expression profile of PD-L1/PD-1 axis on CD138+ cells, CD14+ monocytes and T cells (both CD4+ and CD8+), by flow-cytometry. Results were correlated with clinical parameters, as International Staging system (ISS), cytogenetic risk, bone disease evaluated by whole body low dose TC. BM sera were also collected and used to measure the levels of different soluble factors known to regulate PD-L1 expression or to exert pro/anti-tumor activity in MM (IL-27, IFN-γ, IL-23). Results from ELISA assays were examined in relation with flow-cytometry data.

Results: We found that neither PD-L1 expression on CD138+ cells nor PD-1 on CD4+/CD8+ cells significantly differ between SMM and MM patients; however, we showed for the first time that CD14+PD-L1+% (SMM *vs.* MM: 38.45 *vs.* 56.67, p=0.014) and MFI (15.82vs20.08, p=0.06) increase with disease progression. PD-L1 was also expressed at higher levels in CD14+CD16+ monocytes (17.41vs23.09, p<0.0001), that are known to be increased in MM patients as compared to SMM, and positively correlated with CX3CR1 expression on total monocytes. Among the cytokines tested, we detected a reduction of the anti-tumoral IL-27 BM serum levels in patients with MM as compared to SMM, which inversely correlated with PD-L1 MFI on CD14+ cells (p=0.002) and CD8+PD-1+% (p=0.011), thus supporting a skew toward an immunosuppressive environment. Focusing on patients with active MM, those with ISS=II and III showed increased PD-L1 expression on CD14+ cells (ISS II+III *vs.* I, median MFI 20.73vs16.42, p=0.014) and higher CD8+PD-1+% (II+III *vs.* I, 4.49vs2.58, p=0.008) compared with ISS=I patients. Finally, analysis of PD-L1/PD-1 distribution, in relation with the presence of bone lesions, revealed that not-osteolytic patients have an inverted CD4+/CD8+ ratio and higher CD8+PD-1+% compared with osteolytic ones, even without reaching a statistical significance in our cohort (p=0.07).

Conclusions: All over our data indicate that PD-L1+ monocytes are increased in MM patients as compared to SMM, underling their role in the immune-suppression of MM patients and thereby in tumoral progression.

C019

USEFULNESS OF CIRCULATING CELL-FREE DNA TO DEFINE HOW MULTIPLE MYELOMA SPREAD AND DISSEMINATE THROUGHOUT THE BONE MARROW IN NEWLY DIAGNOSED PATIENTS

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Introduction: Multiple Myeloma (MM) is a plasma cell (PC) disorder characterized by the presence of multiple lytic lesions at the time of diagnosis. Recently, it has been highlighted that cell-free DNA (cfDNA) might resume spatially heterogeneous clones. However, the potential of cfDNA to track the evolutionary dynamics of MM, remains to be confirmed. Aim of this work is to assess the benefit of cfDNA analysis in mimicking the genomic background of plasma cells (PCs) obtained from a single bone marrow (BM) site, by integrating these data with those related to the extent of bone involvement, as evaluated by PET/CT.

Methods: A total of 37 newly diagnosed MM patients (pts) were included in the study and screened at baseline by 18F-FDG PET/CT and NMR. For each pts, Ultra Low Pass-Whole Genome Sequencing (ULP-WGS) was used to characterize both the neoplastic PC clone from BM (BM gDNA) and the cfDNA from peripheral blood. BM CD138+ PCs were isolated with CD138 microbeads. A range of 1 to 10 ng of gDNA and cfDNA was used to obtain libraries, sequenced in a NextSeq 500. Data were analysed by ichorCNA and Clonality R packages.

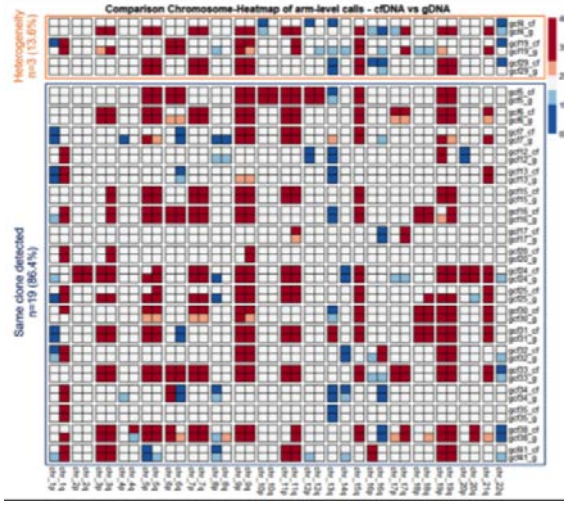

Figure 1.

Results: The ULP-WGS was feasible in 36/37 gDNA (97%) and in 35/37 (95%) cfDNA, with a median coverage of 0,4X (range 0,1-1,4X). By employing the probabilistic ichorCNA algorithm, the tumour fraction (TF) of each sample was determined. As expected, the cfDNA TF was significantly lower than that of gDNA (median, M TF: 4.8% *vs.* 74.4%) but, interestingly, high cfDNA TF (M: 4.8%, range: 0.1-35.2%) correlated with high gDNA TF (M gDNA TF: 84%, range: 5.9-95.2%). Pts with high cfDNA TF were prevalently diagnosed in R-ISS stage III, and showed a higher number of PET lesions and a more active tumour metabolism, as compared to pts with low TF (M n. PET lesions: 4 *vs.* 1; SUVmax: 6.9 *vs.* 3.9). Similarly, bone damage, as detected by NMR, was more evident in pts with high cfDNA TF (M n. focal lesions: 6 *vs.* 1). Finally, evidence of extramedullary disease emerged only in 3 pts, all with high cfDNA TF. Genomic profiles comparison (Figure 1) showed that in almost all patients (19/22; 86.4%) the cfDNA completely resumes the genomic profile of BM clone, except for three patients (3/22; 13.6%): in two of them (gcf19, gcf29) the genomic profiles were almost superimposable, apart from the acquisition or loss of specific copy number alterations (CNAs) in cfDNA (e.g. del1p). In a third pts (gcf4), cfDNA showed a unique CNAs profile, completely different from that of the BM clone. Clonality algorithm further confirmed these discrepancies between gDNA and cfDNA by assigning a probabilistic index of independency of the two lesions (likelihood ratio:$>5x10^3$).

Conclusions cfDNA can resume the genomic complexity of BM clones. Nevertheless, even though just few cases showed genomic evidence of spatial heterogeneity, in pts with high TF in cfDNA, imaging data suggested an overall propensity to a metastatic spread of the disease.

Acknowledgements: AIRC IG2019.

C020

INTERACTION OF JUNCTIONAL ADHESION MOLECULES IN THE BONE MARROW-NICHE: MICE MODEL AND CLINICAL IMPACT FOR MULTIPLE MYELOMA PATIENTS

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Deregulation such as overexpression of adhesion molecules influences cancer progression and survival1–3. Spreading of malignant cells from their primary tumor site to distant organs is the most common reason for cancer-related deaths. Exploring the interaction of multiple myeloma (MM) cells with other cell types within the bone marrow (BM) niche and by different experimental models we found that MM is characterized by dissemination of multiple tumor cells throughout the BM. However, it still remains elusive what mechanisms or interactions within the MM-BM-compartment encourage one or several malignant clones to leave their initial niche and start disseminating. The cell adhesion/migration system in the MM microenvironment has been recognized as a major mechanism of MM cell survival and the development of drug resistance and therefore became a promising target in MM treatment. In particular, we focused Junctional adhesion molecules (JAMs) that are involved in mediating the contact between MM clones and the endothelium/stroma and that deregulation of these molecules leads subsequently to a different signal transduction within heterogeneous subclones. JAM-C, a member of the Ig-like JAM family4, can homodimerize and aid cancer cell migration and dissemination5. To test this hypothesis, we visualized MM cell interactions with the BM endothelium/stroma regarding the involvement of JAM-C in direct and indirect co-culture experiments using fluorescence microscopy, flow cytometry and state-of-the-art light sheet fluorescence microscopy (LSFM) of intact bones as read out systems. Additionally, we determined the mRNA-expression profile of different MM cell populations after having physical contact with BM cells using our syngeneic MM mouse model. Firstly, we investigated the prognostic impact of JAM-C expression on 577 patients enrolled in the COMMPASS trial and we strikingly found that overexpression of JAM-C correlates with worse PFS (P=.023) and OS (P<.0001). Next, we showed that this molecule is dynamically expressed on MM cells in the marrow of mice and patients and co-localizes with blood vessels within the bone marrow of MM and additionally, JAM-C upregulation inversely correlates with the downregulation of the canonical plasma cell marker CD138 (P<.0001), whose expression has recently been found to dynamically regulate a switch between MM growth and MM dissemination. Furthermore, treating MOPC cells with Bortezomib we found that CD138neg cells overexpressing JAM-C were more resistant and could be selected

by the treatment (P<.05). Moreover, targeting JAM-C in a syngeneic *in vivo* MM model ameliorates MM progression and improves outcome. Strikingly, the population of mice not treated with JAM-C specific antibodies developed more frequently extramedullary and rapidly disseminating disease. In particular, an *ex vivo* examination of bones showed that the JAM-C naïve antibody group presented several lesions at the contralateral bone in comparison with the treated group (P<.05). Overall, we showed that JAM-C might serve not only as an additional novel diagnostic biomarker but could also have a pivotal role as a therapeutic target in MM disease in order to avoid clonal selection and dissemination.

C021

CHARACTERIZATION OF CHROMOTRIPSIS IN MULTIPLE MYELOMA: BIOLOGICAL FEATURES AND IMPACT ON PATIENTS' SURVIVAL

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Introduction: Multiple Myeloma (MM) is a genetically complex disease, characterized by the recurrence of several chromosomal aberrations, which impair the disease prognosis. Recently, peculiar massive highly localized chromosomal rearrangements, named "Chromotripsis", have been associated to one-step catastrophic events.

Aims of this study were: (1)to detect and characterize Chromotripsis events (CEs), and (2)to correlate their presence with particular pathways and disease prognosis.

Methods: 488 newly diagnosed(ND) MM patients(pts), whose genomic data obtained from SNP Array experiments were available, have been included in the study. An original and reliable algorithm, able to distinguish and characterize CEs, was set up and tested on genomic data of all pts; most pts (3/4 of the cases) received up-front therapeutic regimens including Proteasome Inhibitor(PI).

Results: Overall, 9.4% of NDMM pts carried CEs; CEs were observed in 46 out of 174 pts with complex chromosomic events. Even if scattered throughout the whole genome, CEs were significantly associated with specific locus: chr1p (p = 8.37E-15), chr2q (p = 4.27E-8), chr11q (p = 6.99E-5) and chr22q (p = 5.15E-7). Pts with CEs were more likely to carry chromosomal aberrations commonly associated to bad prognosis, *i.e.* IgH translocations(p=0.002, HR 3.4) and TP53 deletions(p=1.16E-5, HR=6.26) (Table 1). In particular, CEs were frequently associated with deletions on genes involved in the DNA repair pathways, both NHEJ(non-homologous end-joining) and MMEJ(Microhomology-mediated end joining), (Table 1).

Table 1.

CHROMOTRIPSIs_46pts

gene/event	position	alteration	p-value	ODD RATIO	role
Traslocation			0.002	3.40	
t_4_14			0.003	2.01	
t_14_20			0.010	8.46	
t_14_16			0.040	3.55	
TP53	17p13.1	del	1.157E-05	6.26	Genes of interest in MM
CDKN2C	1p32.3	del	8.890E-05	4.86	Genes of interest in MM
FAF1	1p32.3	del	6.842E-05	5.02	Genes of interest in MM
NRAS	1p13.2	del	1.061E-03	3.05	Genes of interest in MM
XRCC6	22q13.2	del	8.071E-04	3.85	DNA-repair - NHEJ pathway
XBP1	22q12.1	del	1.903E-05	4.68	ER-Stress pathway

The onset of CEs has been shown to impact on pts' progression-free(PFS) and overall survival(OS), with HR of 1.52 (p=0.019) and 1.68 (p=0.019) respectively, and retained an independent prognostic factor when evaluated in multivariate model including also deletion of chr17p and t(4;14) translocation, with HR of 1.42 (p=0.05) in PFS and 1.57(p=0.04) in OS. Since CEs were significantly associated with the deletion of XBP1, a gene involved in the ER stress pathway, whose expression has been linked to the response to PI, we sought to evaluate the correlation between CEs and PIs resistance. Pts were stratified according to up-front treatment (PIs *vs.* not PsI-based regimens). CEs were shown to significantly impact survival only in PIs-treated pts(PFS: 247/438pts, 27 months, p=0.0098; OS: 133/438pts, 57 months, p=0.023). Finally, in 4/55 pts with paired samples (*i.e.* collected at diagnosis and relapse), carrying CEs at diagnosis, the same event was observed at relapse, thus suggesting CEs does not undergo selective pressure during disease progression.

Conclusions: CEs were observed in 9,4% of newly diagnosed MM pts. Since CEs significantly affect MM prognosis, pts carrying these abnormalities should be considered at high risk. The association of CEs and chromosomal aberrations, critical in MM biology, suggests the existence of novel exploitable therapeutic targets in MM.

Acknowledgments: AIRC_IG2014-15839, RF-2016-02362532

C022

THE HEME OXYGENASE-1/CARBON MONOXIDE PATHWAY PROTECTS MULTIPLE MYELOMA CELLS AGAINST BORTEZOMIB-INDUCED APOPTOSIS THROUGH ACTIVATION OF TLR4 SIGNALING

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Heme oxygenase (HO)-1 catalyzes the conversion of heme to biliverdin, iron and carbon monoxide. In myeloma plasma cells, its expression has been demonstrated to increase during bortezomib (BTZ) treatment and localize into the nucleus conferring drug resistance. Recently, our group demonstrated that BTZ also induces up-regulation of Toll like receptor 4 (TLR4) which acts as a stress-responsive mechanism protecting mitochondria during BTZ exposure and sustaining mitochondrial metabolism. Since two studies independently demonstrated that both HO-1 and TLR4 protect myeloma cells from BTZ-induced apoptotic signals, specific functional connections between these two proteins were considered herein. MM cell lines were treated with BTZ alone or in combination with TAK-242, a selective inhibitor of TLR4. We observed a significant increase of apoptosis in TAK-242/BTZ treated cells compared to BTZ alone. Drug combination also led to higher mitochondrial depolarization and decreased mitochondrial mass evaluated using flow cytometry. Accordingly, TAK-242/BTZ treatment activated mitophagy as demonstrated by evaluating co-localization of the autophagosome marker LC3 with mitochondria using confocal microscopy. Since it is known BTZ treated cells increased HO-1 expression as protective mechanism, we next evaluated if BTZ combination with TAK-242 could affect HO-1 expression. Western blot showed a down-regulation of HO-1 after TAK-242/BTZ treatment. Immunofluorescence analysis confirmed that drug combination decreased nuclear HO-1 and increased its cytoplasmic localization compared to BTZ alone. To address this controversy, we administered tin protoporphyrin (SnPP), a well-characterized HO-1 enzymatic inhibitor, alone or in combination with BTZ. Interestingly, SnPP/BTZ treated cells showed lower expression of TLR4 compared to BTZ treated ones. To better investigate if HO-1 enzymatic activity could regulate TLR4 expression, MM cells were exposed to hemin, an inducer of HO-1. We observed a significant up-regulation of TLR4 and NF-kB nuclear localization. Treating cells with rapid or slowly carbon monoxide-releasing molecules (CORM-3 and CORM-A1), an increase of TLR4 expression was observed after 3h with the consequent activation of p-p38, p-ERK and NF-kB nuclear translocation. Moreover, silencing HO-1 confirmed its role in the regulation of TLR4 expression. Moreover, compared to U266 cells, shHO-1/U266 cells howed higher apoptosis after treatment with BTZ, confirming that HO-1/TLR4 signaling protect MM cells from BTZ-induced apoptosis. Our data demonstrate that a functional regulatory link exists between HO1 and TLR4 which in turn impact on drug response. Specifically, inhibition of HO-1/TLR4 axis augmented cytotoxicity of BTZ against MM cells. In conclusion the HO-1/TLR4 axis is involved in BTZ

mediated chemoresistance thus providing an important tool to improve the clinical outcome of MM patients resistant to BTZ.

C023

MODULATION OF THE IMMUNE MICROENVIROMENT IN MULTIPLE MYELOMA PATIENTS TREATED WITH DARATUMUMAB-BASED THERAPY: TUMOR CELL-EXTRINSIC EFFECTS OF DARATUMUMAB TREATMENT

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Introduction: Daratumumab (dara) is a first-in-class anti-CD38 antibody clinically developed for the treatment of multiple myeloma (MM). Anti-CD38 therapy concurrently targets tumor cells and tumor microenvironment because CD38 is expressed not only in MM cells but in immune cells as well, both with an effector function (e.g. NK cells, cytotoxic T cells, Vγ9Vδ2 T cells) and with an inhibitory function (regulatory T cells [Tregs], regulatory B cells [Bregs]). The aims of our study were to investigate tumor cell-extrinsic mechanisms of sensitivity *vs.* resistance in dara-treated patients.

Methods: Relapsed and/or refractory MM patients receiving dara-based treatments included in a prospective observational study (NCT03848676) supported by AIRC were analyzed. Extensive immune cell phenotyping, quantification and functional assays were longitudinally performed on bone marrow (BM) and peripheral blood (PB) mononuclear cells of dara-treated MM patients. In particular, the following populations were examined: T cells, B cells, monocytes, NK cells, Tregs, Bregs. Data were acquired with FACS Calibur cytometers and analyzed using FlowJo software.

Results: As of 01 March 2020, 15 patients were analyzed: 2 received dara monotherapy, 12 dara-Rd, 1 dara-Vd. CD38 expression on immune cells, in terms of percentage and MFI, was highest in NK cells (74%, 51MFI), followed by B cells (50%, 48MFI), monocytes (44%, 58MFI), T cells (24%, 39MFI) and Tregs (17%, 34MFI). After dara treatment, CD38 levels were significantly reduced on all populations but not on monocytes. Paired analysis of immune compartments in PB and BM showed comparable Results: Next, we analyzed the expression of exhaustion markers (PD-1, TIM-3, TIGIT and LAG-3) on the surface of PB T cells. The expression of PD-1 on CD4+ T cells (37% *vs.* 23%, p=0.01) and TIGIT on CD8+ T cells (30% *vs.* 8%, p=0.02) was reduced during dara treatment compared to baseline. Moreover, dara treatment promoted an expansion of CD8 + T cells with an effector memory phenotype and *in vitro* proliferating capacity of CD8+ T cells was augmented as well. Total NK cells (CD56+) were rapidly and persistently reduced after dara treatment (290 cells/ul *vs.* 151 cells/ul). The analyses of NK subsets evidenced an increase of the proliferative subset (CD56+ CD16-) (48% *vs.* 93%, p=0.001), and a reduction of the cytotoxic one (CD56+CD16+) (58% *vs.* 6%, p=0.001). Finally, we investigated the effect of dara on suppressive cells. Interestingly, a specific CD38+ subpopulation of peripheral Tregs (CD4+CD25+CD127dim) showed a severe and persistent shrinkage following dara treatment (4% *vs.* 0.4%, p=0.006). Bregs showed the same marked down-modulation (2.8% *vs.* 0.05%, p=0.005).

Conclusions: Dara-based treatment deeply modifies the immune microenvironment. Integration with MM cell-intrinsic modification and clinical outcome will help identify determinants of resistance to dara therapy and potential strategies to overcome them.

C024

PRECLINICAL EFFICACY FOR A NOVEL MULTI-KINASES INHIBITOR, ARQULE 531 AGAINST MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is an haematological tumor characterized by proliferation of malignant plasma cells (PC) predominantly in the bone marrow, which overproduce monoclonal immunoglobulin proteins. Several studies have demonstrated kinome-related genes significantly linked with MM, thus suggesting their therapeutic interest especially in high-risk patients. We have recently demonstrated that the novel inhibitor ArQule 531 (ARQ 531) has broad anti-tumor activity based on its kinase inhibition profile. As result we pursued its application in pre-clinical models of MM.

Methods: Inhibitory effects of ARQ531 on cell viability were investigated in a panel of MM cell lines and primary tumor cells by CellTiter-Glo luminescent assay over a range of drug concentrations and time. Specific transcriptomic profiling of ARQ531-treated MM cells was performed by using gene expression analysis. Molecular mechanism of ARQ531 on MM cells was further investigated by qPCR and western blot analysis. The therapeutic efficacy of ARQ531 combination with different anti-MM drugs was also explored and CalcuSyn software was employed to confirm synergistic effects.

Results: ARQ531 inhibited cell viability of MM cell lines (n=11) with IC_{50} value of 3±1 μM. Furthermore, inhibitory effects of ARQ531 on cell viability were evaluated in a panel of primary MM cells with different mutational status, with an IC50s value ranging from 0.9 to 4 μM. Consistent with the effect on cell viability, ARQ531 increased the apoptotic rate of MM cells tested. Moreover, anti-MM effects of ARQ531 were not reduced in presence of bone marrow stromal cells (BMSCs), and, more importantly such treatment showed a good therapeutic window. A transcriptome profiling analysis of ARQ 531-treated cells revealed a reversion of the oncogenic MYC-driven transcriptional program as specific event triggered by ARQ531. As result, Myc-targets inhibition was observed in MM cells treated with ARQ531 compared with specific control, in a proteomic analysis. Finally a drug-combination screening revealed IMiDs as most significant sensitizers for ARQ531-based anti-MM effects thus suggesting this approach worth to be investigated in xenograft mice models.

Conclusions: Our data show that the novel multi-kinase inhibitor ARQ531 has a promising therapeutic activity against MM in preclinical models thus supporting the feasibility of targeting oncogenic MYC-driven translation program in MM cells with ARQ531 alone or in combinations with IMiDs.

Benign Hematology

C025

CLINICAL OUTCOMES FOLLOWING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH BETIBEGLOGENE AUTOTEMCEL GENE THERAPY IN THE PHASE 3 NORTHSTAR-2 AND NORTHSTAR-3 STUDIES FOR TRANSFUSION-DEPENDENT β-THALASSEMIA (TDT)

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Introduction: In a phase 1/2 study of betibeglogene autotemcel (beticel; LentiGlobin for β-thalassemia), 8/10 patients with TDT and non-β^0/β^0 genotypes and 3/8 patients with β^0/β^0 genotypes achieved transfusion independence (TI, defined as weighted average Hb of ≥9 g/dL without RBC transfusions for ≥12 months). For patients achieving TI, reductions from baseline to Month 48 were observed in median liver iron concentration (6.3 [n=11] to 2.0 [n=7] mg/g dw), median serum ferritin levels (4829 [n=10] to 937 [n=7] pmol/L) and median transferrin saturation (96.5 [n=10] to 49.0% [n=4]). At up to 5-year follow-up, vector integration was polyclonal. We present interim results of two phase 3 studies, Northstar-2 (NCT02906202; non-β^0/β^0 genotypes) and Northstar-3 (NCT03207009; β^0/β^0, β^0/β^+IVS-I-110 or β^+IVS-I-110/β^+IVS-I-110 genotypes).

Methods: CD34+ hematopoietic stem cells collected via mobilization/apheresis were transduced with BB305 lentiviral vector. Patients were infused with transduced cells following PK-adjusted, single-agent busulfan myeloablation. Statistics presented as median (min/max).

Results: As of 12 Jun and 30 Sept 2019, 34 patients were treated in Northstar-2 and -3 with a follow-up of 11.6 (0.9–26.3) and 8.8 (2.5–20.0) months; 24 patients were aged ≥12 years. Non-hematologic grade ≥3 AEs post-infusion in ≥3 patients in either study were stomatitis (n=17), febrile neutropenia (n=14), pyrexia (n=3), epistaxis (n=3), and liver veno-occlusive disease (VOD; n=3). Drug product-related AEs were abdominal pain (n=3), thrombocytopenia (n=3), leukopenia (n=1), neutropenia (n=1), and pain in extremity (n=1). All patients are alive and a polyclonal vector integration profile was demonstrated. In Northstar-2, 18/20 patients (>5 months follow-up) have not received a transfusion in >3.5 months. The primary endpoint of transfusion independence TI was achieved by 9/10 evaluable patients; duration was 15.2 (12.1–21.3) months. Weighted average Hb during TI was 12.2 (11.4–12.8) g/dL. HbA^T87Q levels were 8.7, 9.3, and 9.4 at Month 6 (n=17), 12 (n=11), 18 (n=8), respectively. Myeloid:erythroid ratios in patients who achieved TI were 0.6–1.9 at Month 12 (n=9) and 0.8–0.9 at Month 24 (n=2) versus 0.1–0.7 at baseline, indicating reduction of ineffective erythropoiesis. In Northstar-3, 9/11 patients followed for >6 months have stopped transfusions for ≥3 months. At Months 6 and 12, total unsupported Hb was 10.2 (8.5–13.2) (n=10) and 13.8 (10.3–14.0) g/dL (n=3), while HbA^T87Q was 8.3 (0–12.0) (n=11) and 11.1 (8.8–12.6) g/dL (n=3), respectively. Two evaluable patients achieved TI.

Conclusions: After beti-cel gene therapy in Northstar-2 and -3, 18/20 patients with TDT and non-β^0/β^0 genotypes and 9/11 patients with β^0/β^0, β^0/β^+IVS-I-110 or β^+IVS-I-110/β^+IVS-I-110 genotypes, with ≥6 months of follow-up, have stopped transfusions. In Northstar-2, 90% of patients achieved TI. The safety profile is consistent with that of single-agent busulfan myeloablation.

C026

CK2 CONTROLS HEMATOPOIESIS DRIVING MATURATION FROM HSC TO MORE DIFFERENTIATED CELLS

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Introduction: Protein kinase CK2 is a pleiotropic serine-threonine kinase composed of two catalytic (α) and two regulatory (β) subunits. CK2 is well known for its role in sustaining hematological malignancies, including acute leukemias, however, the role played by CK2β during normal blood cell development is still poorly investigated. By generating a conditional knockout mouse model for CK2β in the hematopoietic system, we previously demonstrated a negative impact of CK2β lack on erythroid compartment with depletion of more mature cells. Here, we describe further the hematopoietic phenotype of conditional CK2β-deficient mice. We investigated the effects of CK2β KO in other hematopoietic compartments by evaluating: the number and the differentiation ability of HSCs and hematopoietic precursors; the maturation of B lymphocytes, granulocytes and monocytes. This analysis was performed in the fetal livers of control, heterozygous and KO fetuses and in the hematopoietic organs of adult CK2β-deficient heterozygotes mice.

Methods: Knockout mice were generated using the CRE/loxP system, where the CRE recombinase was under the control of the pan-hematopoietic VAV1 promoter. By flow cytometry we detected the amount of HSCs, CLPs, MEPs, CMPs, GMPs. Methocult® of fetal liver cells allowed us to analyse the growth of hematopoietic colonies. To asses the engrafment and self-renewal ability of KO HSCs, we performed transplantation of fetal livers cells at 14.5 days post conception (dpc) (from controls and KO fetuses CD45.2) in recipient adult mice (CD45.1) lethally irradiated. Hematopoietic reconstitution was evaluated by flow cytometry, analysing the frequency of CD45.2 and CD45.1 cells in the different hematopoietic organs. Flow cytometry was also used to characterize pre/pro, pro and pre B cells in fetal livers, more mature B/T cells, granulocyte and monocyte/macrophages, both in fetal and adult context.

Results: We observed a significant increase of HSCs, MEPs, CMPs and GMPs precursors in KO fetal livers, however precursors from KO animals did not efficiently differentiate, colonies were fewer in numbers and finally died after 6 days of culture. Transplantation of fetal liver cells in recipient adult mice showed that the KO cells were unable to engraft and restore hematopoiesis. In the KO samples there was a decrease of more mature B cells (B220high CD19high) and the accumulation of pre/pro and pro B cells. In addition, we observed an expansion of granulocyte and monocyte/macrophages. The CK2β heterozygous animals presented an expansion of HSCs and an intermediate phenotype for B cells and granulocytes/monocytes; no substantial changes were detected for T cells.

Conclusions: Our data supports the hypothesis that CK2β plays a fundamental role in hematopoietic cells survival and maturation starting from HSCs and precursors. This could add some new insights for the involvement of CK2 in pathologic context as in the leukemic stem cell biology.

C027

EFFICACY OF CAPLACIZUMAB IN PATIENTS WITH ATTP IN THE HERCULES STUDY ACCORDING TO INITIAL IMMUNOSUPPRESSION REGIMEN

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This is an Encore abstract Data first presented as Abstract #123126 at the 61st Annual Meeting and Exposition, December 7-10, 2019, Orlando. Introduction: Acquired thrombotic thrombocytopenic purpura (aTTP) is an acute, life-threatening thrombotic microangiopathy that requires urgent and specialized treatment. Prior to the introduction of caplacizumab, the treatment for aTTP was based on daily therapeutic plasma exchange (TPE; to replenish functional ADAMTS13 enzyme) plus immunosuppression (mainly corticosteroids and rituximab; to suppress anti-ADAMTS13 autoantibody production). TPE combined with immunosuppressive therapy improved outcomes in patients; however, episodes of aTTP are still associated with an acute mortality of up to 20% as these therapies do not have an immediate effect on the pathologic microvascular thrombosis. The primary results of the randomized, double-blind, placebo-controlled phase 3 HERCULES study showed that, in combination with TPE and corticosteroids, caplacizumab shortened the time to platelet count response and reduced the incidence of a composite outcome of TTP-related death, exacerbation, or major thromboembolic events, by inhibiting vWF–platelet interaction and, thereby, stopping the formation of microthrombi. As additional immunosuppression per local practice was permitted in HERCULES, the present analysis aimed to determine whether there was any difference in the efficacy of caplacizumab according to the initial immunosuppression regimen. Methods: Data of patients participating in HERCULES were stratified based on the type of first-line immunosuppression regimen (*i.e.* therapy started up to Day 3 of the treatment period) and analyzed descriptively. The main 2 groups analyzed were those receiving corticosteroids only and those receiving a combined regimen of corticosteroids and rituximab. Differences in dose or dosing frequency were not taken into consideration in this descriptive analysis. Results: Of the 145 randomized patients in the HERCULES study, 112 (77.2%) patients received only corticosteroids as first-line immunosuppressive therapy, while 24 (16.6%) patients received corticosteroids and rituximab (initiated within the first 3 days of the study). Three patients (2.1%) received another type of initial immunosuppression (cyclophosphamide + corticosteroids [n=1], hydroxychloroquine [n=1], and mycophenolate mofetil + corticosteroids [n=1]), 1 patient (0.7%) started immunosuppression later in the study (cyclophosphamide + corticosteroids), while 5 patients (3.4%) did not receive any immunosuppressive treatment during the study. Baseline characteristics between the main 2 subgroups were well balanced (Table 1). Immunosuppressive therapy intensification occurred in 38 patients (33.9%) initiated on corticosteroids alone (most often addition of rituximab [n=37], others included splenectomy [n=2], bortezomib [n=1], mycophenolate mofetil [n=1]), and in 3 patients (12.5%) initiated on corticosteroids with rituximab (bortezomib [n=1] and mycophenolate mofetil [n=3]). Caplacizumab treatment improved outcomes in patients with aTTP irrespective of the type of initial immunosuppression. Data on time to platelet count response and clinical outcomes are summarized in Table 2. Caplacizumab reduced the rate of the composite endpoint of TTP-related death, exacerbation, and major thromboembolic events during the double-blind treatment period irrespective of baseline immunosuppression regimen. Notably, in the placebo arm, exacerbations occurred in both subgroups to a similar extent, indicating that corticosteroids, with or without rituximab, are not immediately effective. Overall, recurrences (exacerbations or relapses) during the study were also reduced by caplacizumab in both subgroups (Table 2). Two placebo patients died during the treatment period in the corticosteroid only subgroup versus none in the corticosteroid plus rituximab subgroup (one other placebo patient died during the study drug treatment period while receiving another type of immunosuppression). Conclusion: Immunosuppressive therapy in aTTP aims to control the underlying autoimmune disease but requires time to take effect; this exposes patients to thrombotic complications and death. Caplacizumab treatment prevents disease exacerbations and death, irrespective of the type of initial immunosuppression used, allowing time for immunosuppressive therapy to take effect.

Tables 1 and 2.

Table 1. Baseline characteristics for patients in the HERCULES study (placebo and caplacizumab arms) according to main initial immunosuppression regimen

	Corticosteroids only (n=112)	Corticosteroids + rituximab (n=24)
Previous TTP episodes, n (%)		
Initial	67 (59.8)	13 (54.2)
Recurrent	45 (40.2)	11 (45.8)
ADAMTS13 level, n (%)		
<10%	97 (86.6)	23 (95.8)
≥10%	15 (13.4)	1 (4.2)
Platelet counts (x10^9/L), mean (SD)	35.6 (28.0)	31.6 (28.4)
LDH (U/L), mean (SD)	566 (395)	604 (689)
>ULN, n (%)	92 (89.3)	19 (82.6)
Cardiac Troponin I (µg/L), range (min; max)	0.077 (0.01; 76.0)	0.096 (0.01; 4.44)
>ULN, n (%)	56 (54.4)	13 (56.5)
Serum creatinine (µmol/L), mean (SD)	100 (87)	93.8 (45.3)
>ULN, n (%)	24 (23.3)	5 (21.7)

LDH, lactate dehydrogenase; SD, standard deviation; TTP, thrombotic thrombocytopenic purpura; ULN, upper limit of normal

Table 2. Efficacy outcomes for patients treated with first-line corticosteroids ± rituximab in the HERCULES study

	Corticosteroids only (n=112)		Corticosteroids + rituximab (n=24)	
	Placebo (n=54)	Caplacizumab (n=58)	Placebo (n=16)	Caplacizumab (n=8)
Time to platelet count response Median days (95% CI)	2.88 (2.67; 3.78)	2.69 (1.86; 2.85)	2.76 (2.53; 3.74)	2.67 (1.63; 2.70)
Composite endpoint of ≥1 of TTP-related death, recurrence (exacerbation), or major thromboembolic event during the blinded treatment period, n (%)	26 (48.1)	7 (12.1)	9 (56.3)	1 (12.5)
TTP-related death, n (%)	2 (3.7)	0	0	0
Exacerbation, n (%)	20 (37.0)	3 (5.2)	8 (50.0)	0
Major thromboembolic event, n (%)	5 (9.3)	4 (6.9)	1 (6.3)	1 (12.5)
Overall recurrence during the study (exacerbation + relapse), n (%)	20 (37.0)	7 (12.1)	8 (50.0)	1 (12.5)
Refractory TTP, n (%)	1 (1.9)	0	1 (6.3)	0

CI, confidence interval

C028

INHIBITION OF COMPLEMENT C1S WITH SUTIMLIMAB IN PATIENTS WITH COLD AGGLUTININ DISEASE (CAD): RESULTS FROM THE PHASE 3 CARDINAL STUDY

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Introduction: CAD is a rare autoimmune hemolytic anemia with an estimated prevalence of 16 per 1 million. Hemolysis is driven by activation of the classical complement pathway (CP), resulting in erythrocyte opsonization with predominant extravascular destruction and ensuing anemia. Patients with CAD have an increased early mortality and risk of thromboembolism. There are no approved treatments. Sutimlimab (formerly BIVV009) is a first-in-class humanized monoclonal anti-C1s antibody that selectively inhibits the C1 complex of complement, preventing CP activation, while leaving the alternative and lectin pathways intact. The objective of the Cardinal study (NCT03347396) is to assess efficacy and safety of sutimlimab in adults with CAD who have a recent history of transfusion.

Methods: Cardinal is a pivotal Phase 3, open-label, single-arm, multicenter study of 26 weeks duration (Part A) with an ongoing extension (Part B). Data is available from Part A. Patients with confirmed diagnosis of CAD were enrolled. Eligibility criteria included baseline hemoglobin (Hb) ≤10 g/dL, total bilirubin level above normal, and ≥1 blood transfusion in the prior 6 months. Sutimlimab was administered intravenously on Days 0 and 7, followed by biweekly infusions. Patients weighing <75 kg or ≥75 kg received a 6.5 g or 7.5 g dose, respectively. The primary efficacy endpoint was response rate based on a composite of Hb increase ≥2 g/dL or Hb ≥12 g/dL at treatment assessment (average from Weeks 23, 25, and 26) and transfusion avoidance from Weeks 5 to 26. Secondary efficacy endpoints included change from baseline in hemolytic markers (eg, bilirubin) and quality of life (QOL) measured by the Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F) Scale. The proportion of responders for analysis of the primary endpoint was calculated with a 95% exact Clopper-Pearson confidence interval (CI). All secondary endpoints were analyzed using descriptive statistics, frequency, percentage, or CIs.

Results: Twenty-four patients enrolled and received ≥1 dose of sutimlimab. The mean (standard deviation) age was 71.3 (8.2) years with 62.5% females. Mean (range) baseline Hb was 8.6 (4.9–11.1) g/dL. The median (range) number of transfusions within 6 months prior to enrollment was 2 (1–19) and 62.5% of patients had failed prior therapies. Out of 24 patients, 22 completed Part A; 2 patients were withdrawn early for reasons unrelated to the study drug. The estimated mean (standard error [SE]) Hb increase at treatment assessment time point was 2.6 (0.4) g/dL. Hb improved rapidly after the first dose of sutimlimab with 1.2 g/dL and 2.3 g/dL increases by Weeks 1 and 3, respectively. Mean overall Hb was maintained above 11 g/dL after Week 3 (Figure 1A). Twenty (83.3%) patients had a mean Hb increase ≥1 g/dL. Mean total bilirubin was normalized by Week 3. Seventeen (70.8%) patients remained free of transfusions from Weeks 5 to 26. FACIT-F scores improved within 1 week, peaking by Week 5, and remained stable through Week 26. The estimated mean (SE) FACIT-F score increase at the treatment assessment time point was 10.9 (1.4), consistent with a clinically meaningful response. Hb, bilirubin, and FACIT-F improvements correlated with rapid normalization of complement C4 and near-complete inhibition of CP activity (Figure 1B). The prespecified primary endpoint was met (13 [54.2%] patients). Twenty-two (91.7%) patients experienced ≥1 treatment-emergent adverse event (TEAE), with 7 (29.2%) patients experiencing a serious TEAE (TESAE). There were no TESAEs assessed as related to sutimlimab. There was 1 death in a patient with hepatic cancer that was assessed as unrelated to the study drug. Serious infections were reported, but no meningococcal infections were identified. There were no thromboembolisms and decreases in mean D-dimer and thrombin-antithrombin III complex thrombotic markers were observed. All 22 patients that completed Part A enrolled in Part B.

Conclusions: The Phase 3 Cardinal study shows that sutimlimab, a first-in-class selective inhibitor of the CP, has a rapid and sustained treatment effect in CAD by preventing hemolysis, significantly increasing Hb, and improving QOL (FACIT-F). These results demonstrate that targeting the CP represents a novel, effective therapeutic approach for the management of CAD and indicate that sutimlimab has the potential to change treatment practices for patients with this condition.

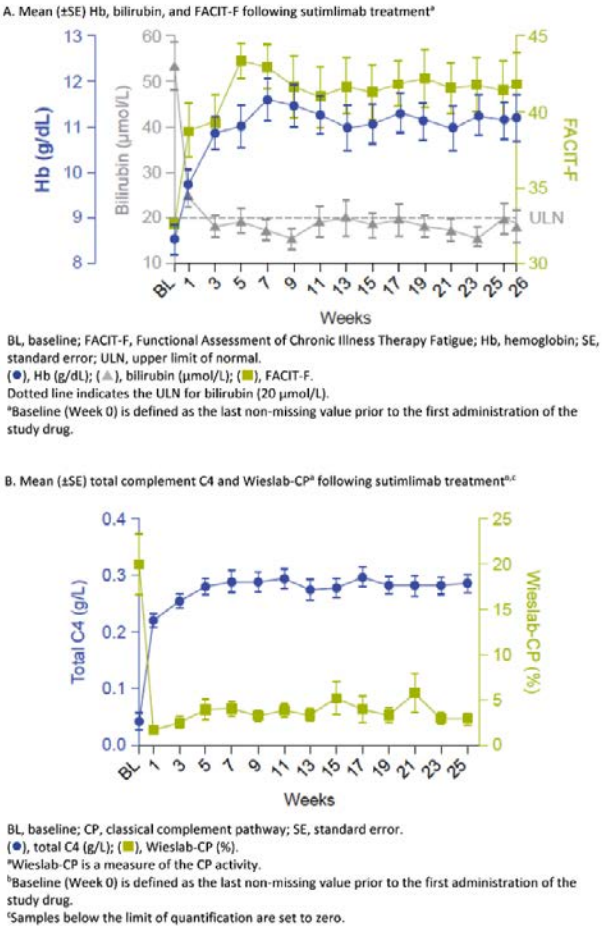

Figure 1.

C029

EFFICACY OF CAPLACIZUMAB IN PATIENTS WITH ATTP IN THE HERCULES STUDY ACCORDING TO BASELINE SEVERITY

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Introduction: Acquired thrombotic thrombocytopenic purpura (aTTP) is a rare, life-threatening autoimmune thrombotic microangiopathy that involves abnormal processing of von-Willebrand factor (vWF) and results in multiple organ dysfunction. Although aTTP remains a very unpredictable disease, risk factors for death include older age, lactate dehydrogenase (LDH) levels >10x the upper limit of normal (ULN), and cerebral involvement (*i.e*., the French severity score) (Benhamou et al. Haematologica 2012;97:1181–1186). In addition, raised cardiac troponin-I (cTnI) levels of >2.5 µg/L have also been linked with a higher risk of mortality or refractoriness (Benhamou *et al*. J Thromb Haemost 2015;13:293–302). In the randomized, double-blind, placebo-controlled phase 3 HERCULES study, patients with aTTP were randomized to placebo or caplacizumab, plus daily therapeutic plasma exchange and immunosuppression. This analysis aimed to determine the efficacy of caplacizumab in patients participating in HERCULES according to baseline disease severity.

Methods: In the HERCULES study, very severe disease was defined as:1. a French severity score ≥3, or 2. severe neurological involvement (*i.e.* coma, seizures, focal deficit), or 3. cardiac involvement (cTnI >2.5xULN). All of these factors have independently been associated with worse outcomes and higher mortality. The French severity score is a discrete score from 0 to 4, involving evaluation of 3 parameters: • Cerebral involvement: yes=1; no=0 • LDH: >10xULN=1; ≤10xULN=0 • Age: >60 years=2; >40 and ≤60 years=1; ≤40 years=0 Scores ≥3 indicate very severe disease. Data from patients participating in HERCULES were extracted and analyzed according to less severe/very severe disease and are presented descriptively.

Results: Overall efficacy outcomes according to baseline disease severity are presented in Table 1. Patients who presented with less severe disease at baseline had a similar risk of mortality compared with patients who presented with very severe disease. Similar trends were observed for other clinically relevant outcomes, such as exacerbations of aTTP and refractoriness. Treatment with caplacizumab improved outcomes in both patient subgroups. Irrespective of disease severity, caplacizumab treatment resulted in faster platelet count normalization and a lower proportion of patients experiencing the composite endpoint of TTP-related death, exacerbation of TTP, or treatment-emergent major thromboembolic event during the double-blind treatment period. No patients who received caplacizumab died, while deaths occurred in 1 (2.1%) and 2 patients (8.0%) in the less severe and very severe subgroups of patients who received placebo, respectively. No patients receiving caplacizumab developed refractory disease, whereas 1 (2.1%) and 2 placebo-treated patients (8.0%) with less severe and very severe disease, respectively, developed refractory disease.

Conclusions: aTTP can be unpredictable, and, although this analysis included a small patient population, our results suggest that patients with less severe disease at baseline are equally at risk of death, refractoriness and exacerbations as patients with very severe disease. A clear treatment benefit was observed in all patients who received caplacizumab irrespective of disease severity at baseline, which highlights the importance of starting therapy early in all patients with aTTP.

Table 1. Overall efficacy outcomes according to baseline disease severity in the HERCULES study, during the double-blind treatment period.

	Less severe		Very severe	
	Caplacizumab (n=42)	Placebo (n=48)	Caplacizumab (n=30)	Placebo (n=25)
Time to platelet count response, HR (95% CI)	1.59 (1.02 to 2.47)		1.69 (0.94 to 3.04)	
≥1 event of the composite of TTP-related death, exacerbation of TTP, or treatment-emergent major thromboembolic event during double-blind treatment, n (%)	2 (4.9)[a]	24 (50.0)	7 (23.3)	12 (48.0)
TTP-related death, n (%)	0	1 (2.1)	0	2 (8.0)
Exacerbation of TTP, n (%)	0	20 (41.7)	3 (10.0)	8 (32.0)
Treatment-emergent major thromboembolic event, n (%)	2 (4.9)	3 (6.3)	4 (13.3)	3 (12.0)
Refractory TTP, n (%)	0	1 (2.1)	0	2 (8.0)

a. 41 patients were assessable for this event
CI, confidence interval; HR, hazard ratio; TTP, thrombotic thrombocytopenic purpura

C030

ELTROMBOPAG TREATMENT FOR APLASTICA ANEMIA REFRACTORY TO IMMUNOSUPPRESSIVE TREATMENT: MULTICENTER RETROSPECTIVE STUDY OF 12 PATIENTS FROM 4 HAEMATOLOGICAL CENTERS

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Introduction: Eltrombopag (ELT) has shown efficacy in acquired severe Aplastic Anemia (SAA) either in the refractory setting or in the first-line therapy, in addition to standard immunosuppressive treatment (IST). However the therapeutic efficacy and safety of ELT for SAA in the real-world setting still need to be explored. Therefore we retrospectively analyzed our multicenter experience on ELT treatment in SAA patients refractory to IST.

Methods: From January 2014, 12 pts (6 males), median age: 69 (27-85) yrs, from 4 Italian Centers, were treated with ELT because of a diagnosis of SAA refractory to previous IST. The following response criteria were used: complete response (CR): Hb >10 g/dL, neutrophil count >1.5 x 10^6/ml and platelet count >100 x 10^6/ml; partial response (PR): transfusion-independence; minimal response (MR): improvement in one or more lineage not fulfilling the criteria of PR.

Results: At the start of ELT, 10 pts showed SAA, and 2 pts a very severe Aplastic Anemia (VSAA); previous IST treatment was: ATG + cyclosporine (CYA) + prednisone (MP) in 6 pts, CYA ± MP in 5 pts, and MP alone in 1 pt. The median time between the start of first-line therapy and the start of ELT was 6.5 (0.5-134) months. ELT was started because

of failure of 1st line therapy (6 pts), failure of 2nd line therapy (2 pts), discontinuation of 1st line therapy (CYA) due to toxicity (2 pts), relapse after 1st line therapy (1 pt), or relapse after 2nd line therapy (1 pt). 6 pts were treated with ELT + CYA, and 6 pts with ELT alone. Maximum daily dose of ELT was 150 mg in 8 pts, 175 mg (1 pt) , 100 mg (1 pt), 75 mg (1 pt) and 50 mg (1 pt). 8/12 pts showed a clinically significant response to ELT (ORR: 66.6%). Best response to ELT was CR in 2 pts (16.6%), PR in 5 pts (41.7%), MR in 1 pt (8.3%), while 4 pts (33.3%) showed no response. Median time to 1st response was 2.5 (1-16) months, and median time to best response was 4.5 (1-28) months. Among the 8 responder pts, 2 pts stopped ELT after 8 and 10 months because of CR or good PR, and are still maintaining response after 25 and 10 months, respectively, under CYA alone. 4 responder pts are still maintaining response under ELT, after 31, 31, 18 and 2 months, respectively. 1 pt discontinued EPAG for personal reasons after 41 months while still on response, and 1 pt stopped ELT after 3 months because of relapse and worsening of clinical condition. With a median follow up of 19 (2-180) months, 4 pts died (after 2, 3, 3 and 9 months, respectively), because of infection (3 pts) and pancreatic cancer (1 pt): median OS not reached. A grade I transient liver toxicity possibly related to ELT was observed in 2 pts. Although some pts received a prolonged ELT treatment, no clonal evolution was observed.

Conclusions: In conclusion, in our experience ELT confirmed to be effective and safe in SAA patients refractory to IST, and some pts (2/12: 16.6%) showed a first response only after > 6 months of treatment.

C031

STUDY OF THE GENETIC HETEROGENEITY IN PATIENTS WITH SICKLE CELL DISEASE: ANALYSIS OF MITOCHONDRIAL DNA

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Introduction: Sickle cell disease (SCD) is an autosomal recessive hemoglobinopathy characterized by the presence of at least one allele that encodes for the HbS variant of hemoglobin (HBB: c20A>T) and a second HBB allele carrying a pathogenetic variant (*i.e.* HbS, HbC, β^° or β^+). The result is an abnormal hemoglobin polymerization and different clinical phenotypes. Interestingly, variants affecting HBA1, HBA2, HBG2, BCL11A and the intergenic region HBS1L-MYB have been related to SCD heterogeneity. Mitochondrial DNA (mtDNA) is widely used to reconstruct populations' dispersal and to infer the maternal origin of individuals. A detailed phylogeny of mtDNA at a global level is widely described, in which the different haplogroups are defined by specific mutational patterns and limited to certain geographical areas and groups of populations. The aim of this work is to study and deepen the genetic heterogeneity of patients suffering from SCD, through the characterization of their mitochondrial haplotypes and phylogeny.

Methods: The study was conducted on 37 SCD patients (25 African, 9 European, 3 American) distributed as follow: 20 (HbS/HbS), 10 (HbS/β^+ or β^0-thalassemia) and 7 (HbS/HbC). The control region mtDNAs were sequenced from nt. 16024 to 210, thus including the hypervariable region (HVR) I and most of the HVRII. The mutational variants relative to the Cambridge Reference Sequence (rCRS) were noted and haplotypes were classified in haplogroups with the software Haplogrep.

Results: Analysis of mtDNA sequence identified 32 different haplotypes: 4 recurrent (3 couples declared to be related by maternal line) while 1 haplotype was identified in 2 African non-relative subjects, 1 from the Ivory Coast and 1 from Benin. The different haplotypes clustered into the two main super-haplogroups R and not-R and have been classified into 30 mitochondrial lines, belonging to 12 different macro-haplogroups: B, D, G, JT, L0, L1, L2, L3, M*, R0, UK and X. Clades identified in HbS/HbS group were: 1 JT, 1 B, 2 G, 6 L2, 4 L1, 1 M*, 1 UK, 2 L3 and 2 L0; in HbS/β-tal were: 3 R0, 1 X, 3 JT, 1 L2, 1 D and 1 L3 and in HbS/HbC group were: 4 L2, 2 L3 and 1 M*.

Conclusions: The analysis of mitochondrial variability and the network topology has allowed characterizing the phylogeographic structure of the 37 individuals affected by SCD. 76% of patient belonged to the super-haplogroup Not-R in agreement with their geographical origin. Integrated genomic and mtDNA sequences analysis will increasingly lead to a precise stratification of patients, which represents a necessary requirement both for diagnostic purposes and for the clinical management of the patient. Since mtDNA is a molecular tool that allows us to reconstruct the movements of human populations over time and space, it offers a strong contribution in understanding the geographical origin of individuals, from the maternal point of view, even when unknown or not declared.

C032

SAFETY OF CAPLACIZUMAB IN PATIENTS WITHOUT DOCUMENTED SEVERE ADAMTS13 DEFICIENCY DURING THE HERCULES STUDY

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Introduction: Acquired thrombotic thrombocytopenic purpura (aTTP) is a rare, life-threatening autoimmune thrombotic microangiopathy caused by a deficiency in the activity of ADAMTS13 leading to the formation of ultra-large multimers of von Willebrand factor (vWF) and abnormal platelet adhesion in the microvasculature. aTTP requires prompt diagnosis and rapid initiation of treatment to limit the risk of negative or fatal outcomes. The clinical diagnosis of aTTP is based on thrombocytopenia and microangiopathic hemolytic anemia and is confirmed by ADAMTS13 <10%. However, the latter confirmation is not always rapidly available, and treatment is typically initiated based on the clinical diagnosis. The HERCULES study, in which patients were enrolled based on the clinical diagnosis of aTTP (ADAMTS13 confirmation was not part of the eligibility criteria) after receiving 1 prior session of therapeutic plasma exchange (TPE), demonstrated the efficacy and safety of caplacizumab in patients experiencing an acute aTTP episode (Scully *et al*. N Engl J Med 2019;380:335–346); caplacizumab targets the A1 domain of vWF, disrupting formation of microthrombi.

The main safety finding in HERCULES was a mild bleeding risk. This analysis aimed to describe the safety of caplacizumab in patients enrolled in HERCULES for whom the diagnosis of aTTP was not confirmed based on documented severe ADAMTS13 deficiency.

Methods: In HERCULES, ADAMTS13 was measured at study baseline (following initial TPE), weekly following cessation of daily TPE during the treatment period, and twice during the follow-up period. Data from patients for whom the diagnosis of aTTP was not confirmed based on documented ADAMTS13 levels <10% were extracted and analyzed descriptively for efficacy and safety outcomes, with a focus on bleeding events.

Table 1. Baseline characteristics and outcomes during the study period for patients with baseline ADAMTS13 ≥10% treated with caplacizumab in the HERCULES study.

	Patient 1	Patient 2	Patient 3	Patient 4
Baseline ADAMTS13	67%	62%	62%	63%
Range of ADAMTS13 values throughout study period	67–120%	40–86%	62–76%	63–91%
Possible alternative diagnosis	TTP secondary to pancreatitis (pancreatitis reported as concomitant disease)	TTP secondary to pancreatitis (pancreatitis reported as concomitant disease)	Megaloblastic anemia (reported as concomitant disease)	Thrombotic microangiopathy (reported as an AE) General adenopathies (reported as AE)
Discontinued	No	No	Yes (due to non-TTP)	Yes (due to SAE)
Achieved a platelet count response	Yes (within 4 days)	Yes (within 4.7 days)	No	Yes (within 9.8 days)
Duration of daily TPE treatment (on study)	7 days	6 days	2 days	12 days
Duration of caplacizumab treatment	37 days	32 days	2 days	4 days
Platelet counts during daily TPE period, range	13–329 $x10^9$/L	19–234 $x10^9$/L	32–36 $x10^9$/L	16–164 $x10^9$/L[a]
Bleeding-related SAE	No	Gastric ulcer hemorrhage (moderate, recovered without intervention)	No	Epistaxis (moderate, recovered without intervention)
Other SAEs	Bacteremia (moderate, recovered, treated with meropenem, vancomycin, paracetamol)	No	Cardiac tamponade (severe, recovered without intervention)	No
Other bleeding-related AEs	Gingival bleeding, ecchymosis, rectal hemorrage (all three AEs were mild, recovered without intervention)	No	No	No

a. Received caplacizumab for the first 4 days only.
AE, adverse event; TPE, therapeutic plasma exchange; SAE, serious adverse event; TTP, thrombotic thrombocytopenic purpura

Results: Overall, 7 patients in the placebo group (9.6%) and 13 patients in the caplacizumab group (18.1%) had a baseline ADAMTS13 ≥10%; of these, 4 and 9 patients, respectively, had a prior medical history of aTTP and/or ADAMTS13 values <10% at other time points during the study. This left 3 patients in the placebo group and 4 patients in the caplacizumab group for whom the diagnosis of aTTP could not be confirmed based on subsequent ADAMTS13 values or prior medical history, suggesting a diagnosis other than aTTP. The baseline characteristics and outcomes for those patients treated with caplacizumab are summarized in Table 1. Baseline ADAMTS13 levels were >60% for all patients and remained well above 10% throughout the study period. Possible alternative diagnoses included pancreatitis-induced TTP in 2 patients. One patient was reported as having 'thrombotic microangiopathy' and discontinued study drug treatment after 4 days (but continued daily TPE). The fourth patient had a report of 'megaloblastic anemia' and 'general adenopathies' and was withdrawn from the study due to a 'non-TTP diagnosis' after 2 days. The patients who continued daily TPE achieved a platelet count of >150 $x10^9$/L. Two patients experienced a moderate bleeding-related serious adverse event (SAE), 1 case of 'gastric ulcer hemorrhage' (considered unlikely related to study drug and recovered without intervention) and 1 case of epistaxis that led to study drug discontinuation (considered possibly related to study drug and recovered without intervention). Other mild bleeding-related non-serious adverse events (AEs) were reported in 1 patient: gingival bleeding (possibly related), ecchymosis (possibly related), and rectal hemorrhage (not/unlikely related). All events recovered spontaneously without intervention. Two other non-bleeding related SAEs were reported in 2 patients, both considered unrelated to study drug: 1 case of bacteremia and 1 case of cardiac tamponade.

Conclusion: The experience of caplacizumab in patients with a suspected non-aTTP diagnosis to date is limited, and so no definite conclusion can be drawn. Bleeding-related AEs were reported in 3 of the 4 patients; however, the type, nature and manageability of these events appear similar to those reported in the other patients in the study.

Multiple Myeloma 2

C033

AMINO ACIDS DEPLETION TRIGGERED BY L-ASPARAGINASE SENSITIZES MM CELLS TO CARFILZOMIB BY INDUCING ROS-MEDIATED CELL DEATH

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Introduction: Metabolic reprogramming is emerging as cancer vulnerability which could be therapeutically exploitable using different approaches, including amino acid-depletion for those tumors which rely on exogenous ammino acids for their maintenance. L-Asparaginase (ASNase), has contributed to significantly improve outcome in Acute Lymphoblastic Leukemia but toxicity and resistance limit its clinical use in other tumors.

Methods: A targeted-DNA methylation analysis was performed on Carfilzomib-sensitive and resistant MM cells. Next, inhibitory effects on cell viability of ASNase and Carfilzomib co-treatment were investigated in a panel of MM cell lines as well as primary tumor cells (NDMM and RRMM) by CellTiter-Glo luminescent assay. Drug induced molecular effects were investigated by gene expression and extensive western blot analysis. Different biochemical approches were also employed including mithocondrial superoxide measurement and enzymatic cycling assay to analyze Reactive Oxygen Species (ROS) generation and intracellular energetic store levels, respectively. Finally, lentiviral mediated gene transfer strategies were employed to create stable isogenic MM cell lines to get insights into molecular mechanism.

Results: Here, we report that in Multiple Myeloma (MM) cells the DNA methylation status is significantly associated with reduced expression of ASNase related-genes signature, thus suggesting ASNase sensitivity for this tumor. Therefore, we tested therapeutic relevance of ASNase purified from Erwinia Chrysanthemi (Erw-ASNase) combined with the next-generation proteasome inhibitor (PI) Carfilzomib observing an impressive synergistic effects on MM cells, while normal peripheral blood mononuclear cells were not affected. Importantly, this effects was associated with increased ROS generation, compounded mitochondrial damage and Nrf2 deregulation regardless of specific oncogenic program deregulation including cMYC. Furthermore, the co-treatment resulted in genomic instability and DNA repair mechanisms impairment via increased oxidative stress which further enhanced its anti-MM activity.

Conclusions: Overall, we demonstrate that Erw-ASNase treatment, by deregulating several cellular metabolic programs makes MM cells more vulnerable to Carfilzomib and provide proof-of-concept for their clinical use in combination as novel strategy to enhance PIs sensitivity in MM patients.

C034

MYELOMA-INDUCED ALTERATIONS OF GLUTAMINE METABOLISM IMPAIR OSTEOBLAST DIFFERENTIATION OF MESENCHYMAL STROMAL CELLS IN MULTIPLE MYELOMA.

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Introduction: Recent evidence suggests that Multiple Myeloma (MM) metabolism is characterized by high glutamine (Gln) consumption leading to decreased Gln levels in the bone marrow (BM) plasma of MM patients as compared to patients with indolent monoclonal gammopathies. Nevertheless, the potential effect of MM-dependent Gln depletion on bone microenvironment is still unknown and prompted us to investigate the impact of the low-Gln microenvironment imposed by MM cells on osteoblast (OB) differentiation, which is typically impaired as a hallmark of MM-induced osteolysis.

Methods: The effect of extracellular Gln levels on OB differentiation and activity was checked. Several human MM cell lines (HMCLs), human telomerase reverse transcriptase transduced mesenchymal stromal cell line (hTERT-MSCs) and OB cell lines (HOBIT and HOB-01) were used in cell culture experiments. The expression of OB markers (ALP, COL1A1 and RUNX2), as well as ALP activity and expression were assessed to evaluate OB differentiation of hTERT-MSCs. The expression and activity of Gln transporters was monitored during OB differentiation. Lastly, gene expression profiles of hTERT-MSC differentiated in the presence or absence of Gln have been generated by GeneChip ClariomD Arrays technique.

Results: When co-cultured with hTERT-MSCs or OB cell lines, HMCLs accelerated the depletion of extracellular Gln (+25%/day, compared to monocultures of MSC/OBs), promoted the expression of Gln synthetase (GS) by hTERT-MSCs and limited the viability of OBs but not of hMSCs. OB differentiation was suppressed in the absence of Gln. Interestingly, the decrease of extracellular Gln concentration from 0.6 mM (the average physiological BM plasma concentration) to 0.4 mM (the average concentration found in BM plasma of MM patients) was sufficient to negatively affect the expression of OB markers. The expression and activity of the Gln transporter SNAT2 (SLC38A2) were induced during human OB differentiation. SNAT2 expression was also associated with the increased expression of Glutaminase 1, suggesting higher Gln demand and consumption during OB differentiation. Interestingly, in Gln deprivation condition, OB differentiation was restored by supplementation with asparagine (Asn) and characterized by the induction of the expression of Asparagine Synthetase (ASNS), the Gln-dependent enzyme responsible for the synthesis of Asn. Lastly, gene expression profiles analysis indicated that Gln deprivation significantly up-regulated the expression of genes involved in OB inhibition as GREM1 and down-regulated those involved in osteoblastogenesis such as BMP6 and SPARC.

Conclusions: These results suggest that Gln depletion in the bone microenvironment caused by Gln addicted MM cells is critically involved in the MM-induced OB deficiency due to the impairment of Gln-dependent Asn synthesis.

C035

IGH AND IGK CLONAL TRACKING FOR MINIMAL RESIDUAL DISEASE MONITORING IN MULTIPLE MYELOMA BY NEXT GENERATION SEQUENCING: A BOLOGNA EXPERIENCE

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Introduction: The introduction of novel agents has led to improvements in clinical outcomes of patients (pts) with multiple myeloma (MM). Currently, a high proportion of pts achieves minimal residual disease (MRD) negativity: long-lasting maintenance of this status might be considered as a marker of "operational cure". Therefore, molecular tracking of MRD will become critical in the management of MM, and will be included in daily clinical practice. The aim of this study was to evaluate the performances of next generation sequencing (NGS) implemented as best practice in MRD routine evaluation.

Methods: A cohort of 80 newly diagnosed MM pts were screened between 2016 and 2020 to define the ID clonotypes, tracked also during their follow-up (FU). The ID clonotypes screening has been performed by NGS using an assay which fully covers IgH regions (Framework 1, 2, 3) and IgK genes (Invivoscribe®; ION Torrent S5–Thermo Scientific). The MRD clones tracking during FU has been done by both conventional ASO-qPCR and NGS of the whole repertoire of IgH/IgK clones, including a spike-in as internal control. Data were analyzed by Lymphotrack Dx and MRD proprietary softwares (Invivoscribe®).

Results: The screening of ID clonotypes, including both IgH and IgK genes, was successful in 80/80 pts (100%). Despite a small proportion of pts resulted polyclonal (3/80), ID clonotypes were identified in the majority of pts: 62 IgH, 15 IgK and 3 IgH+IgK clones were recognized, mainly restricted to the VH3 family genes. Pts-specific assays have been designed for pts with trackable defined IgH/IgK sequences, according to the EuroMRD guidelines. Assays were tested by ASO-qPCR, including a standard curve and healthy donor cells, to define sensitivity and specificity respectively. Overall, ASO-qPCR was feasible in 15 MM pts, allowing the tracking of a single MRD clone with up to 10^-4 sensitivity, as obtained in 5 cases (more frequently 5x10^-4). In order to achieve a sensitivity of at least 10^-5, 8 FU samples were analyzed in parallel using NGS, reducing time and efforts by simultaneously tracking MRD clones in multiple samples. This method was feasible in all pts, confirming the results obtained by ASO-qPCR, yet with higher sensitivity and confidence >95%. Only in a few cases, NGS allowed to disclose ASO-qPCR detected positive-not-quantifiable results, possibly due to non-specific amplification, confirming MRD negativity with 10^-5 sensitivity. Finally, 8 pts without a designed ASO-qPCR assay, will be monitored by NGS, increasing the percentage of pts achieving an MRD test result.

Conclusions: NGS allowed to detect MRD by efficiently monitor both the dominant and all the possible emerging clones, with higher sensitivity as compared to ASO-qPCR. In order to validate and standardize the method, we aimed at enlarging the cohort of MM pts evaluated by NGS, thus contributing to achieve a novel gold-standard assay for the monitoring of MRD in MM daily clinical practice.

C036

ROLE OF PROTEIN KINASE CK1 IN THE BONE MARROW MICROENVIRONMENT: POTENTIAL THERAPEUTIC TARGET IN THE MULTIPLE MYELOMA ASSOCIATED BONE DISEASE

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Introduction: Multiple myeloma (MM) is a haematological neoplasia characterized by the progressive growth of malignant clonal plasma cells in the bone marrow (BM). Bone destruction is a hallmark of MM: osteolytic lesions result from increased bone resorption due to stimulation of osteoclast formation and activity and inhibition of osteoblasts. The BM microenvironment sustains the multiple myeloma associated bone disease (MMABD) and different pathways, such as Wnt/β-catenin, Hedgehog (Hh) and NF-κB signaling support MM plasma cell clonal expansion and the bone homeostasis imbalance. In particular, Wnt pathway promotes osteogenesis by stimulating the gene expression of RUNX2, the master regulator of bone differentiation. MM cells can hamper osteoblast differentiation also by inhibiting RUNX2 activity in BM mesenchymal stromal cells (BMSC). We have previously demonstrated that the Ser/Thr Protein Kinase CK1α supports MM plasma cells growth and its inactivation causes MM cell apoptosis and cell cycle arrest. Since CK1α plays a pivotal role in the Wnt/β-catenin pathway, as it phosphorylates β-catenin on Ser45 promoting its proteasomal dependent degradation, this kinase could be therapeutically targeted in BMSC to ameliorate MMABD, by recovering the β-catenin and RUNX2 activity, thus reinforcing the osteogenic program.

Methods: We generated isogenic MM INA-6 cell and MSC-HTERT-GFP stromal cell clones bearing an IPTG-inducible CK1α directed shRNA. To recreate the BM microenvironment, we plated INA-6 MM cells on a layer of MSC-HTERT stromal cells (co-colture model) and the CK1α silencing was achieved either in MM cells or in mesenchymal stromal cells (MSC). MM cells and MSC populations were purified through cell sorting. We evaluated the expression of RUNX2 and of osteogenic markers, by qRT-PCR and Western blot analysis. Alizarin Red Staining was performed to identify calcium deposits upon differentiation towards osteoblastic lineage.

Results: CK1α silencing in MSC induced an osteogenic transcriptional program: β-catenin stabilization, increased expression of the osteoblastic markers and calcium deposits. In the MM/MSC co-colture, CK1α silencing in MM cells, was associated to increased RUNX2 expression in the MSC, favoring its osteogenic fate. Remarkably, different from what observed upon CK1α silencing in MSC cells grown alone, CK1α silencing in the MSC grown together with MM cells did not end up in the activation of the osteogenic program.

Conclusions: Our data suggest that protein kinase CK1α is a key regulator of MM pathophysiology not only because it supports the malignant plasma cells survival, but also through its sustenance of the interaction with the BM microenvironment. CK1α could be a potential therapeutic target not only for killing the malignant plasma cellular clone, but also for mitigating the MMABD.

C037

TREATMENT INDUCED POLARIZATION TOWARDS CYTOTOXIC RESPONSE IN MULTIPLE MYELOMA PATIENTS

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The biology of plasma cell discrasias (PCD) involves both genetic and immune-related factors. Considering that genetic lesions are necessary but not sufficient in Multiple Myeloma (MM) evolution, several authors hypothesized that immune dysfunction involving both B and T cell counterparts plays a key role in MM pathogenesis of the disease. The aim of this study is to evaluate the impact of cornerstone treatments for Multiple Myeloma, namely autologous stem cell transplantation (ASCT) and novel agents like bortezomib (Bort) and lenalidomide (Len), into immune system shaping. A total of 585 bone marrow samples out of 375 active MM (aMM) patients were studies at different time points by flow cytometry for CD3, CD4, CD5, CD8, CD16, CD19, CD56, CD57, HLA-DR and Tgd antigens. Even though no differences were found in total T cells (73.7±10.5 *vs.* 72.5±14.6, p=0.2656), NK cells (14.7±8.4 *vs.* 15.1±10.3, p=0.86) and B cells (9.5±6.4 *vs.* 10.5±12.4, p=0.2612), treated MM samples displayed a reduction in CD4+ cells levels (25.2±11.8 *vs.* 38.0±10.0, p<0.0001) and an increase in CD8+ cells (49.9±15.2 *vs.* 39.0±10.5, p<0.0001), CD8+/DR+ cells (14.7±16.3 *vs.* 4.4±4.5, p<0.0001) and CD3+/CD57+ cells (22.4±14.0 *vs.* 15.4±10.9, p<0.0001) levels. No differences were found in Tgd cells (4.0±6.6 *vs.* 3.2±2.5, p=0.1462). Our patients usually received a Bort based induction treatment, with Len generally reserved at patient relapse. Bort treatment reduced CD4+ cells (34.1±11.1 *vs.* 38.0±9.9, p=0.0037) and increased CD8+ cells (39.6±12.6 *vs.* 36.8±9.9, p=0.0426), CD8+/DR+ cells (6.6±9.1 *vs.* 4.4±4.5, p=0.0125) and total NK cells (17.6±11.8 *vs.* 15.0±8.4, p=0.0283). On the opposite, there were no differences in CD3+/CD57+ lymphocytes

(14.0±9.6 *vs.* 15.4±10.9, p=0.3104) and Tgd lymphocytes (3.2±2.9 *vs.* 3.2±2.5, p=0.8037). A more pronounced cytotoxic polarization was evidenced after ASCT and Len treatment. As a matter of fact, samples of patients who received ASCT (n=110) and Len (n=118) were characterized by higher levels of CD8+ towards untreated patients (n=138 and n=130, respectively) (51.0±13.8 *vs.* 43.0±12.9, p<0.0001 and 53.5±12.8 *vs.* 40.3±11.7, p<0.0001, respectively), CD8+/DR+ (12.9±14.9 *vs.* 8.8±10.9, p=0.0252 and 14.4±15.4 *vs.* 7.4±9.6, p=0.0001 respectively) and CD3+/CD57+ cells (22.2±12.5 *vs.* 15.8±10.7, p<0.0001 and 21.6±13.2 *vs.* 16.2±10.1, p=0.0006, respectively) and lower levels of CD4+ lymphocytes (23.0±8.2 *vs.* 32.8±11.5, p<0.0001 and 25.9±10.5 *vs.* 30.9±11.4, p=0.0005, respectively). No significant differences were found in NK cells while Tgd were significantly higher only in Len treated patients (5.6±5.7 *vs.* 3.0±3.3, p<0.0001). We demonstrated that aMM patients are characterized by a profound T cell modulation and that most changes are therapy-related. Current Myeloma treatments, in detail ASCT and Len treatments, polarize immune system toward a dominant cytotoxic response, likely contributing to the anti-Myeloma effect of these regimens.

C038

A NEW NON-INVASIVE METHOD FOR ISOLATION OF CIRCULATING EXTRACELLULAR VESICLES AND EVALUATION OF ITS SUITABILITY FOR HEMATOLOGICAL MALIGNANCY BIOMARKER DISCOVERY

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Introduction: Extracellular vesicles (EVs) are naturally secreted cellular lipid bilayer particles, which carry selected molecular content. As result of their systemic availability in biological fluids, and their biological role in cancers, circulating EVs (cEVs) serve as a source of new biomarkers in diagnostic, prognostication and monitoring of tumors, probably alternative to traditional biopsy. However, a precise approach for isolation and characterization of cEVs as tumor biomarker is not yet well established.

Methods: We developed a novel, home-made procedure performing a bench centrifuge step for isolation of cEVs allowing the characterization of their size, amount and phenotype by nanoparticle tracking analysis, different microscopy and flow cytometry and the EV nucleic acid assessment by digital PCR.

Results: Applied to blood from healthy subjects (HSs) and tumor patients, our approach permitted from small serum volume i) the isolation of a great amount of EVs enriched in small vesicles; ii) a convenient and specific cell origin identification of EVs, and iii) molecular content (DNA and RNA) assessment. In the clonal plasma cell malignancy, such as multiple myeloma (MM), our approach can identify specific MM-EVs and their size, concentration and microRNA content statistically allowing to discriminate between MM and HSs. Finally, EV associated biomarkers statistically correlated with MM clinical parameters.

Conclusions: Overall, our cEV based procedure plays an important role in malignancy biomarker discovery and then in real-time tumor monitoring using a minimal invasive samples. From a practical point of view, it is smart (small sample volume), rapid (two hours), easy (no specific expertise required) and requirements are present in clinical laboratories and, therefore, widely exportable.

C039

FROM HIGH RISK CYTOGENETIC TO HYPERDIPLOIDY: HOW FISH CAN BE USED TO CHARACTERIZE MULTIPLE MYELOMA

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Introduction: Two main types of aberrations such as translocations of the immunoglobulin heavy chain (IGH) locus (tIGH) or trisomies of odd chromosomes define distinct subtypes of multiple myeloma (MM) and affect the prognosis. Based on the Revised-International Scoring System (R-ISS), the assessment of cytogenetic risk by fluorescent in situ hybridization (FISH) is focused on high-risk (HR) abnormalities: t(4;14), t(14;16) and deletion of 17p (del17p), whereas trisomies or standard risk tIGH are not routinely investigated. Despite an increasing interest in the detection of hyperdiploidy due to the predicted high response rates with lenalidomide treatment, this issue remains challenging. The aim of our study was to identify predictive markers of hyperdiploid status through the assessment of HR abnormalities by FISH.

Methods: We retrospectively analysed FISH results obtained with a standard panel of probes on CD138+ plasma cells of 391 MM at diagnosis. All cases were tested for HR abnormalities and for gain of 1q21 (+1q21); other tIGH were also excluded when appropriate. Hyperdiploidy, defined as concurrent presence of two or more trisomies, was assessed with probes for chromosomes 5, 9 and 15. One hundred cells for each marker were analysed considering a cut-off of 10%. A univariate logistic regression model was applied to evaluate the capability of different HR markers to predict the hyperdiploid status.

Results: Among 391 samples, FISH detected a tIGH in 31% (121/391) of cases and HR changes in 21% (83/391), but it was unable to classify the remaining 59% (232/391) of cases in a specific prognostic or pathogenetic category. Among these undefined cases, hyperdiploid status was analysed in 95 samples selected based on gene gains emerged (e.g., 3 copies of FGFR3, MAF) or normal FISH. Hyperdiploid status was confirmed in 84% of samples (80/95), whereas the 16% (15/95) did not show concomitant trisomies. From the clinical point of view, hyperdiploid cases showed low risk disease features, with 68% (42/67) presenting ISS I-II, 80% (49/61) with normal LDH levels and low frequency of renal injury and hypercalcemia (17%, 11/66, and 11%, 7/66, respectively). Considering baseline FISH results and excluding tIGH, the presence of one or more gene gain was significantly predictive of hyperdiploid status (p=0.020) and increased the probability to detect a hyperdiploid MM from 84% to 94% (44/47). None of the other markers investigated, *i.e.* isolated +1q21 (34/94), deletion of 1p32 (8/95) and del17p (10/95), nor normal FISH, resulted predictive of hyperdiploid status. Remarkably, of the 15 negative cases for hyperdiploidy, 33% (5/15) revealed a hypodiploid/hypotetraploid clones in a subsequent karyotype.

Conclusions: We propose to use FISH testing for HR abnormalities to rapidly identify a subgroup of hyperdiploid MM based on the presence of non-specific gene gains. This classification can be helpful to direct treatment in standard risk patients.

C040

PEGFILGRASTIM VERSUS FILGRASTIM IN THE SUPPORTIVE CARE OF HEAVILY PRETREATED MULTIPLE MYELOMA IN TREATMENT WITH POMALIDOMIDE-DEXAMETHASONE

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Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF that extends the half-life and allows for once-per-cycle dosing, requiring less frequent dosing than nonpegylated G-CSF. The objective of this study was to compare the efficacy and safety of pegfilgrastim in patients affected by heavily pretreated MM, treated with pomalidomide-dexamethasone, in order to determine whether a single subcutaneous injection of pegfilgrastim is as effective as daily injections of standard filgrastim, in terms of haematological toxicity, febrile neutropenic episodes, antibiotic usage and hospedalization duration. 57 patients (31 M and 26 F) were enrolled, median age at diagnosis 69 years (r. 52-84), and median age at start of treatment 76 years (r.56-90) treated with several lines of treatments (median 7, r. 2-12), every refractory to all the drugs previously received, received Pomalidomide-Dexamethasone (P 4 mg for 21 days, D 40 mg days 1,8,15,22, pegfilgrastim day +8) every 28 days, until progression. Since first course, received in domestic setting, with a very good compliance, patients performed blood counts once weekly and received, from day +8 to day +19, prophylactic oral chinolonic antibiotics and anti-fungal drugs. During neutropenia after first cycle, Filgrastim (5 µgr/kg/day for 3 days) was given if neutrophils count was $<1500 \times 10^9$ cells/L. Median number of filgrastim administrations was 4.6 (r. 3-6); nadir neutropenia was registered after a median of 10.4 days (r. 7-14); median of nadir neutrophil count was 1.13×10^9 cells/L (r.0.3 – 1.5), with maximum duration of 14 days. From the second course, all patients switched to prophylaxis with pegfilgrastim (6 mg), injected subcutaneously with a single administration on day +3 independently from the neutrophil count at that time. During pegfilgrastim, neutropenia was never longer than 8 days, with a consequent reduction of neutropenia-related infections. Median nadir neutrophil count, evaluated for every patients for at least three courses of therapy (r. 3-6) registered at day +11, was 1.28 (r.0.9-2.2). Only 4 patients needed a supplement of 3 administrations of filgrastim. Pegfilgrastim was well tolerated in all patients: main side effects in our patients were mild fever and bone pain (21.2%). In patients affected by heavily pretreated MM treated with pomalidomide-dexamethasone, pegfilgrastim seems to reduce the incidence of severe neutropenia and infections and may increase the possibility to maintain the scheduled time of treatment.

Acute Leukemia 2

C041

FUSIONS IN "B-OTHER" ACUTE LYMPHOBLASTIC LEUKEMIA: POWERFUL TRANSCRIPTOME FOUR TOOL PIPELINE ANALYSIS REVEALS POTENTIAL DRUGGABLE GENES AND HIGH RATE OF KNOWN AND UNKNOWN REARRANGEMENTS

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Introduction: In B-Other B-ALL [Ph-/-/-; negative for t(9;22); t(1;19); t(4;11); 61% of adult B-ALL (Roberts KG, JCO. 2017)], many chimeric genes have been recently identified (Gu Z, Nat Genet. 2019) leding to a refined classification of B-ALL and to, in some cases, tailored therapies. The largest B-ALL subgroup, the Ph-/-/-, are not routinely screened for fusions and at this point a RNA-seq approach is needed but challenging for many aspects (genetic complexity, low frequency, expensive), among them data analysis. We developed and validated our integrated four fusion tool pipeline in order to assess targetable biomarkers and to better classify Ph-/-/- patients (pts).

Patients and Methods: We performed 1385 RNAseq gene Panel (Illumina) of 63 adult Ph-/-/- B-ALL samples. We developed a combined four tool analysis that is further implemented with a specific filtering strategy with a B-ALL fusion literature filtering (716 curated gene-fusion list) (Figure 1A). We developed a validation strategy using: RT-PCR, FISH SNP Arrays; MLPA and total RNA-seq.

Results: From 797 candidate fusions, we retained 65 of them, not otherwise detected, in 41 pts with a very high fusions rate of 65.1%, denoting that Ph-/-/- are not deeply characterized. We validated 23 fusions that have been already reported in the literature and 13 novel fusion transcripts. We obtained an overall accuracy of validation around 97%. The majority of the samples (25/41) had only one detectable fusion while a smaller group (16/41) was characterized by multiple fusions. In our cohort, the identified fusions were distributed in all chromosomes with the exception of chromosome 6, 15 and 18 (Figure 1B). 43 of these fusions were previously described in B-ALL (e.g. ZNF384-TCF3/EP300/TAF15, MEF2D-BCL9, KMT2A-MLLT1, ABL1/2-RCSD1, IGH-MYC, DUX4-IGH, P2RY8-CRLF2 and PAX5-ETV6) or in other diseases (n 10). The most recurrent fusions detected were ARHGAP26-NR3C1 (9.2%;6/65), ZEB2-CXCR4 (7.7%; 5/65), PAX5-ZCCHC7 (6.1%; 4/65) and BCL7A-NCOR2, EP300-ZNF384 (4.6%; 3/65). 22 fusions out of 65 (33.8%) were never been reported in Ph- ALL cases. In Ph-like pts, we identified and validate five new transcripts: THADA-CDH1, TET3-ETV6, NUMA1-CSF1R, IKZF1-IGKV5-2 and EBF1-LINC02227. Ph-/-/- fusion detection help to sub-classify our fused pts in Ph-/-/- subgroups. We found: a) ZNF384r in 18.5% (8/41); b) Ph-like in 17.1% (7/41); c) MLLr and BCL2/MYC both in 4.9% (2/41); d) MEF2Dr and DUX4r both in 2.4% (1/41) (Figure 1C).

Conclusions: We identified an unexpected high rate of secondary fusions in adult Ph-/-/- B-ALL pts (65.1%) that are not characterized

with conventional diagnostic Methods: One third of detected fusion were never reported in B-ALL. The use, in Ph-/-/- pts, of an NGS approach and a powerful pipeline permit us to detect fusions useful for a better classification (33.3%) and in some cases to find targetable fusions (e.g. ABL1-2/RCSD1 and NUMA1-CSF1R).

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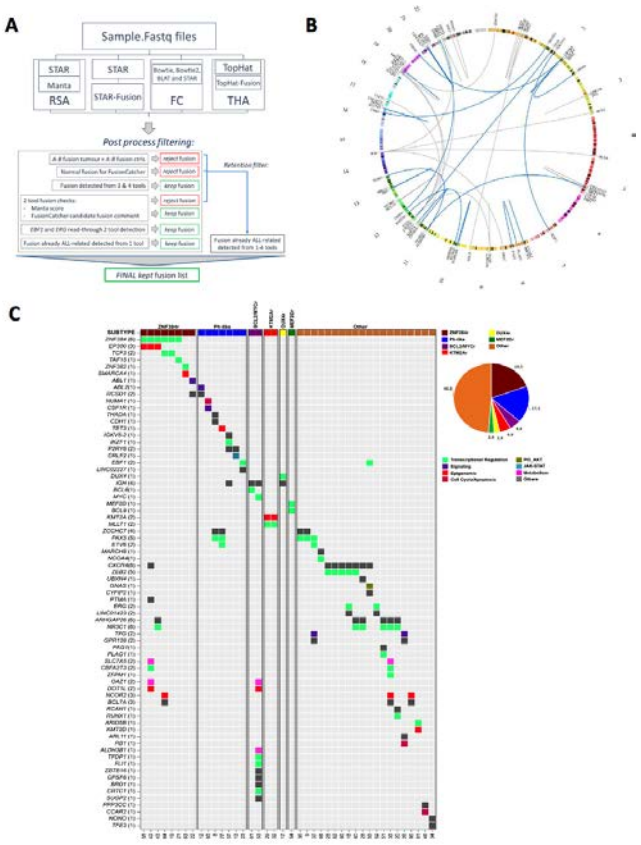

Figure 1.

C042

MLL-AF4 ACUTE LEUKEMIA CIRCRNAOME: HUNTING NEW MECHANISMS OF LEUKEMIC TRANSFORMATION

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Circular RNAs (circRNAs) are abundantly expressed in the haematopoietic compartment with differences across blood cell types (Gaffo *et al.* Sci Rep 2019). In MLL rearranged acute lymphoblastic leukemia (MLLre ALL), fusions of MLL with over 90 partner genes generate leukemogenic fusion proteins whose activity determine deep transcriptome and proteome alterations. Fusion circRNAs expressed from malignant cells with MLL-AF9 translocation can reinforce the oncogenic potential of fusion proteins (Guarnerio *et al.* Cell 2016), and in MLLre ALL we observed circRNA deregulation from genes of the MLL recombinome (Dal Molin *et al*. Front. Oncol. 2019). This study aims to provide a genome-wide picture of circRNA expression specificities of MLL-AF4 leukemia and to discover new molecular mechanisms of the malignant transformation. CirComPara (Gaffo et al. NcRNA J. 2017) software, combining 6 different circRNA detection methods, was used to quantify circRNAs from published and proprietary RNA-seq datasets (ribo-depleted RNA; Illumina® HiSeq2000, paired end 100 nt reads) of blast cells of 6 MLL-AF4 B-ALL patients and patients derived xenografts, the RS4;11 model and human hematopoietic cells (3 CD34+, and 4 mature B-, T-cells and monocytes per cell type). Differential expression (DE) analysis was performed through DESeq2 (p.adj<0.05). Sanger sequencing and targeted quantification by qRT-PCR with divergent primers were used for circRNA validation and screening, respectively. A custom pipeline was used to predict miRNA binding sites and coding potential of circRNAs. Over 74,000 circRNAs were detected by at least two methods of CircComPara in samples of normal and malignant blood cells, of which the 10,000 most expressed were further investigated. CircRNA expression distinguished the different hematopoietic cell types, with MLL-AF4 ALL being closer to CD34+ and to a lesser extent to B-cells (Figure 1A). Comparison of MLL-AF4 ALL with B- and CD34+ cells identified 85 circRNAs significantly DE in both comparisons (Figure 1B), most upregulated in MLL-AF4. These circRNAs derived from leukemia-associated loci, and also from non coding RNAs and newly discovered genes. For all the six circRNAs tested, backplicing sequence, circularity and expression were confirmed. Three isoforms of circAF4 upregulated in MLL-AF4 patients, one previously reportedly (Huang et al. J Hem. Oncol. 2019) and two newly identified were investigated in an extended cohort. In silico functional predictions identified circRNAs with putative binding sites for miRNAs involved in leukemia or with an open reading frame crossing the back-splice junction. For 29 of the circRNAs specific for or deregulated in MLL-AF4 ALL prioritized according to functional predictions, literature data and gene of origin, RNA-seq data confirmed expression in the RS4;11 cell line. In conclusion, here we provide comprehensive data of circRNAs deregulated and potentially involved in leukemogenesis mediated by MLL-AF4 rearrangement. Ongoing functional studies will unveil the impact and the role of specific circRNAs in MLL-AF4 ALL.

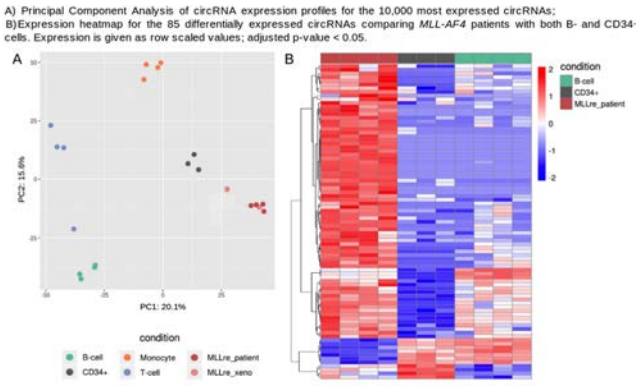

Figure 1.

C043

SELECTIVE BLOCKADE OF ONCOGENIC NOTCH1 WITH THE NEW SERCA INHIBITOR CAD204520

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The discovery of the P-type ATPase Sarco/Endoplasmic Reticulum Ca^{2+} ATPase (SERCA) as a modulator of oncogenic NOTCH1 suggested a new approach for the treatment of T-ALL. In fact, thapsigargin-mediated SERCA inhibition had a stronger effect on the most common type of activating NOTCH1 mutants compared to wild type. However, the binding of thapsigargin to SERCA leads to a rapid increase in cytosolic Ca^{2+} and to a depletion of Ca2+ stored in the endoplasmic reticulum (ER). This shift of Ca^{2+} efflux might cause cardiac toxicity in human, suggesting the need to identify inhibitors with reduced off-target toxicity. Through medicinal chemistry optimization and crystal structure-oriented analysis, we developed the novel oral SERCA inhibitor CAD204520 and we described its anti-leukemic effect *in vitro* and *in vivo* to support a SERCA-based therapeutic modality in T-ALL. From a 191000 small molecules screening targeting P-type ATPase, we identified CAD204520 which showed ~25 and ~79-fold greater selectivity toward human SERCA compared to Na+/K+ and H+ ATPase respectively and promising drug properties. Crystal structure analysis showed that CAD204520 binds to a groove at the membrane interface of SERCA, between the transmembrane helices M1, M2, M3 and M4. This enzymatic pocket is employed for the entry of the Ca^{2+} ions into the pump from the cytosol and compound binding at this groove locks SERCA in a Ca^{2+}-free conformation. This mode of action, that is different from the one of thapsigargin, suggests a lower affinity for Ca^{2+} resulting in a diminished net increase in cytosolic Ca^{2+}. We showed that compared to thapsigargin, CAD204520 minimally alters Ca^{2+} shift and fails to trigger Ca^{2+} dependent programs such as the unfolded protein response mediated by the proteins BIP, EIF and ATF6. We next tested how CAD204520 alters the function of cardiomyocytes and demonstrated that thapsigargin induces a greater negative effect on cardio-mechanics suggesting that the heart will probably tolerate CAD204520 modulation *in vivo*. CAD204520 impairs the proliferation of a panel of T-ALL cell lines carrying activating mutations both in the heterodimerization and in the PEST degradation NOTCH1 domain. Importantly, clinical samples and cell lines carrying NOTCH1 mutations including PEST deletions were more sensitive to CAD204520 compared to normal lymphocytes or wild type NOTCH1 ALL cells. CAD204520 treatment reduces the levels of the activated form of NOTCH1 as consequences of a defect in NOTCH1 trafficking. In anticipation of clinical translation and to explain general mechanisms of acquired resistance to SERCA modulators, we established a thapsigargin- resistant T-ALL cell line. We demonstrated that somatic hotspot mutations in SERCA2 ATPase pocket do not interfere with CAD204520 binding, suggesting that the activity of CAD204520 will be unlikely affected by recurrent resistance genetic variants. Finally, we showed that CAD204520 is well tolerated *in vivo* in CD1 mice without causing loss of weight and cardiac toxicity. In a SKW-3/KE-37 T-ALL xenograft model, CAD204520 reduces circulating and tissue infiltrating human T-ALL cells with no heart related or gastrointestinal toxicities off-target effects. In conclusion, this study presents CAD204520 as a novel orally bioavailable SERCA inhibitor with tolerable off-target toxicity in NOTCH1 dependent tumors. This work provides a foundation for further development of novel drugs targeting Notch-dependent hematopoietic malignancies.

C044

THE MYB ONCOGENE IN PEDIATRIC AND ADULT T-CELL ALL/LBL

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Introduction: MYB is a putative oncogene in different type of human solid and hematological tumors. In T-ALL/LBL, rearrangements of MYB (MYB-R) underlying the up-regulation of the gene are, a t(6;7)(q23;q34), that juxtaposes the TRB@ enhancer to MYB full length, a tandem duplication at 6q23, that involves MYB (MYBtdup) and AHI, and double minutes harbouring extra-copies of the gene. Besides rearrangements, a recurrent somatic mutation of MYB has been recently found in pediatric T-ALL/LBL.(1) This study aimed to investigate incidence and distribution of MYB rearrangements and expression in pediatric and adult T-ALL.

Methods: Our integrated molecular-cytogenetic approach (CI-FISH with two assays to study TRB@-MYB and MYBtdup, and SNPa) (2) was used to investigate 319 cases of T-ALL (193 children, 126 adults). The relative expression of MYB was assessed on 61 patients (6 with MYB-R and 55 without, *i.e.* MYB-wt); in six cases the analysis on paired diagnostic/remission samples could be evaluated. In 56/61, with available data, MYB expression was correlated with overall survival (OS) and probability of relapse (PR) (Cox regression model).

Results: Overall, MYB-R were detected in 17 cases (3 TRB@-MYB and 14 MYB tdup) (5.3%). Notably, SNPa was more sensitive than FISH in detection of MYB tdup (7.7% *vs.* 5%), thus providing the most reliable cytogenetic tool for the diagnosis of this type of CNV. In line with previous report, we identified TRB@-MYB only in pediatric T-ALL (2%), while MYB tdup occurred in both age groups (5% of children and 3% of adults). MYB-R clustered within the HOXA, TLX1/3, or NKX2-1/2-2 subgroups, suggesting that both TRB@-MYB and MYB tdup behave as secondary oncogenic events, which have a strong association with homeobox deregulated subgroups. qRT-PCR showed a wide range of MYB relative expression in our cohort of T-ALL. The level of MYB expression was significantly higher in MYB-R than MYB-wt cases. However, 20 MYB-wt T-ALL expressed levels of MYB as high as MYB-R cases. In keeping with the regulatory positive function of the TAL/LMO complex on MYB transcription, cases with high MYB expression were enriched into the TAL/LMO subgroup. Remarkably, high MYB expression was significantly associated with a lower incidence of relapse (high versus low MYB expression: HR= 0.43; 95% confidence interval: 0.2-0.9, P= 0.03) and showed a trend of better overall survival.

Conclusions: MYB deregulation characterizes leukemic blasts of T-ALL harbouring MYB-R as well as a subgroup of cases with MYB-wt, which mainly belong to the TAL/LMO subgroup. Therefore, high MYB expression should be regarded as a predictive marker of sensitivity for selection of candidates for treatment with MYB inhibitors in clinical trials. Our study also suggests that MYB expression can be used for fine tuning the risk stratification of patients. 1. Liu , Nature Genetics 2017 2. La Starza R, The Journal of Molecular Diagnostics 2020

C045

PAX5 FUSIONS ARE RECURRENT AND ASSOCIATED TO POOR OUTCOME IN MLL-GERMLINE INFANT B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Infant B-Cell Precursor Acute Lymphoblastic Leukemias (BCP-ALL) is a rare and very aggressive leukemia occurring in children younger than 1 year of age and associated to the presence of MLL/KMT2A in 80% of cases. Despite several efforts to better stratify patients and identify efficacious treatments, Infant BCP-ALL is still associated with dismal prognosis, both in MLL-rearranged and -wildtype patients. Recent studies in non-MLL-rearranged Infant patients, reported the NUTM1 gene as frequently involved in fusions. The present study aims at characterizing the presence and prognostic role of other fusion genes in non-MLL-rearranged Infants BCP-ALL patients.

Methods: We applied a custom RNA targeted NGS panel, named Ovation Fusion Custom Panel Target Enrichment System (Nugen/Tecan), with probes capturing 95 leukemia-related genes to detect fusion transcripts, starting from a low amount of total RNA (ranging from 10 to 200ng). The fusion genes were identified by both STAR-Fusion and the bioinformatics pipeline developed in our lab.

Results: Among 38 consecutive non-MLL-rearranged Infant BCPALL cases, 31 had material available and were successfully screened; 30 were enrolled in the Interfant-06 and 1 enrolled in the current AIEOP-BFM ALL2017 protocol. Strikingly, 22/31 (71%) carried a fusion gene. A NUTM1 fusion was identified in 9/31 cases (29%), with Acin1 (n=5), Cux1 (n=2), ZNF618 (n=1) and BRD9 (n=1) as a fusion partner. Remarkably, 6/31 cases (19%) had a PAX5-rearrangement with several partner genes, such as DNAJA1 (n=3), FBRSL1 (n=1), MBNL1 (n=1) and GRHPR (n=1). Moreover, we identified 7 additional fusion genes (grouped as 'others' in following analyses), including TCF3/PBX1 (n=2), TCF3/ZNF384 (n=1), ETV6/ABL1 (n=1), P2RY8/CRLF2 (n=1), and a new KDM2B/GATAD2B fusion in a pair of monozygotic Infant twins. Although aware of the limited cohort, outcome analysis revealed that NUTM1-class patients had a 100% 3-year EFS, with no events (mean age 6.6 months), in agreement with previously published data. Notably, the PAX5-class patients (mean age 11.0 months) had a 3-year EFS of 25.0% (+20.4) whilst the 'others' and negative (negative from panel fusions) cases had a comparable EFS rate of 57.1% (+18.7 and +24.9, respectively).

Conclusions: Overall, we identified an unexpectedly high rate of fusion genes in non-MLL-rearranged BCP-ALL Infant patients. For the first time, we discovered recurrent PAX5 fusions in infants BCP-ALL patients, associated to a worse outcome compared to NUTM1-class. We previously demonstrated in in-vitro and in-vivo experiments in using cells from older children that alternative treatments (*i.e.* Nintedanib) could be applied to PAX5 fusion positive cases.

C046

LARGE-SCALE CIRCULAR RNA DEREGULATION IN T-ALL: UNLOCKING UNIQUE ECTOPIC EXPRESSION OF MOLECULAR SUBTYPES

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Introduction: Circular RNAs (circRNAs) are stable RNA molecules that when deregulated (Gaffo et al. Sci Rep 2019; Dal Molin *et al*. Front Genet 2019) can drive cancer mechanisms, through interactions with microRNAs or proteins, and expressing encoded peptides (Bonizzato *et al.* Blood Cancer J 2016). Previous studies of gene, miRNA and lncRNA expression contributed to determine the molecular networks involved in T-cell Acute Lymphoblastic Leukemia (T-ALL) transformation. Despite recent advances in circRNA research, our understanding of the leukemogenic mechanism involving circRNA remains very limited and translating the current circRNA-related research into clinical practice is a new challenge.

Methods: CirComPara pipeline (Gaffo et al. Noncoding RNA 2017) was used to characterize the expression landscape of circRNAs by analysis of ribosomal RNA-depleted RNA-seq data of 25 T-ALL patients of 5 cytogenetic subgroups, immature, HOXA overexpressing, TLX1, TLX3, TAL1 or LMO2 rearranged, and of 5 sorted populations of developing thymocytes from two healthy donors. CircRNA expression was validated by RT-PCR and Sanger sequencing. CircRNA-miRNA interactions were predicted using miRanda and validated miRNA target genes were retrieved from miRTarBase (Bino, *et al*. PLoS biology 2004). RNA binding proteins recognition motifs and coding potential of circRNAs were predicted by beRBP and ORFfinder, respectively (Hui *et al*. Nucleic acids research 2019, Rombel *et al*. Gene 2002).

Results: Over 68 500 circRNAs were detected and the 3 447 most expressed underwent further study. We revealed deregulation of the circRNAome in T-ALL (Buratin *et al.*, under evaluation): 944 circRNAs were significantly differentially expressed in T-ALL compared with normal thymocytes, mostly upregulated in malignant cells. Next, circRNA signatures of T-ALL subgroups were defined. Comparison with putative-cell of origin of each T-ALL subtype identified 12 group-specific circRNAs for which expression and backsplice sequences were confirmed in T-ALL cell lines. CircRNAs deregulated in T-ALL included circRNAs with well-known oncogenic potential or previously reported associations to specific molecular functions, novel circRNAs from genes linked to leukemogenesis and several not yet characterized circRNAs. For each T-ALL subgroup, a circRNA-miRNA-gene interactions network was obtained, linking group-specific circRNAs to tumour suppressor miRNAs previously associated with T-ALL development whose validated target genes were upregulated in the same T-ALL subgroup. Intriguing new oncogenic axes putatively impacted by ectopically expressed circRNA were identified.

Conclusions: Our findings on circRNAs significantly extend previous data on expression of linear transcripts in human T-ALL, indicating deregulated circRNAs as candidate players of leukemogenesis in T-ALL and exciting further functional investigation.

C047

DEFINITION AND PROGNOSTIC IMPACT OF PH-LIKE AND IKZF1-PLUS FEATURES IN CHILDREN WITH DOWN SYNDROME ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Children with Down Syndrome have an increased risk

for ALL (DS-ALL), which is associated to a high risk of failure due to increased chemotherapy-related toxicity and intrinsic resistance to therapy, thus demanding the development of tailored therapeutic strategies. Cytogenetic abnormalities common in childhood ALL are less frequent in DS-ALL, which is often characterized by alterations in CRLF2 and IKZF1 genes, instead. Although "Philadelphia Chromosome-Like" (Ph-like) and "IKZF1plus" have been described as associated with poor outcome in ALL, no study showed the prognostic relevance of these features in DS-ALL patients. Aim of the study was to evaluate incidence and prognostic value of Ph-like and IKZF1plus features in children with DS-ALL treated in AIEOP-BFM protocols.

Methods: We analyzed the gene expression profile of 70 DS-ALL patients at diagnosis treated in Italian centers from 2000 to 2014. P2RY8-CRLF2 fusion, IKZF1 deletions and IKZF1plus feature were evaluated in a bigger cohort of 134 DS-ALL patients treated in Italian and German centers from 2000 to 2011.

Results: The majority of the AIEOP DS-ALL patients displayed a Ph-like gene expression signature (46/70, 65.7%). Thirty-eight out of 46 (82.6%) Ph-like patients were allocated to non-high risk (non-HR) groups, 33 were positive for CRLF2 alterations, one carried RANBP2-ABL1 fusion and one PAX5-FAM219A. Sixteen showed IKZF1 deletions and 9 of these were IKZF1plus. Conversely, only one out of 23 Ph-like negative patients was positive for CRLF2 alterations, 2 for IKZF1 deletions and one was IKZF1plus, while they more frequently carried ETV6 deletions (45.5% vs. 14.3%). The average number of ALL-specific CNVs per patient was higher in Ph-like group (2.6 *vs.* 1.7; p=0.048). Interestingly, we did not observe any DS-ALL patients positive for ERG deletions. Ph-like DS-ALL patients had an increased cumulative incidence of relapse (CIR) compared to Ph-like negative patients (27.7%±6.8 vs. 13%±7; p=0.04). The joint analysis of the Italian and German cohorts showed that P2RY8-CRLF2 fusion was not associated to a different EFS, despite a tendency for an increased CIR (26.5%±6.6 vs. 18%±4.2; p=0.05). Instead, IKZF1 deletion and IKZF1plus feature were associated with an inferior EFS (42.9%±8.9 vs. 72.7%±4.6; p<0.001 and 37.9%±10.3 vs. 70.8%±4.5; p<0.001, respectively) and with a higher CIR (35.2%±8.6 vs. 17%±3.9; p=0.007 and 36%±10.2 vs. 18.7%±3.9; p=0.004, respectively). Interestingly, 10 out of 11 IKZF1plus relapsed cases were positive for P2RY8-CRLF2 fusion.

Conclusions: The majority of the AIEOP DS-ALL patients displayed a Ph-like ALL gene expression signature, mostly characterized by CRLF2 and IKZF1 alterations. Ph-like signature, IKZF1 deletion and IKZF1plus feature, especially when co-occurring with P2RY8-CRLF2, were associate with poor outcome. These subgroups, that are currently primarily allocated to non-HR protocol arms, need new and tailored therapeutic strategies.

C048

SNP ARRAY IS A VALUABLE TOOL TO DETECT RECURRENT AND NEW GENOMIC REARRANGEMENTS IN T-ALL

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Introduction: T-ALL, an aggressive disease accounting for 15% of pediatric and 20-25% of adult ALL, is extremely heterogeneous at genomic level with co-occurrence of chromosome rearrangements and gene mutations that delineate specific leukemogenic pathways. Genomic CNVs are among the most frequent abnormalities that cause activation of oncogenes or haploinsufficiency/inactivation of suppressors. SNPa is one of the most reliable tool to detect CNVs, cnLOH, and chromothripsis, the highest detectable chromosome instability phenomenon. We here report on SNPa results in a large series of pediatric and adult T-ALL.

Methods: We studied 90 T-ALL cases (children/adults 34/56; male/female 61/29), classified as HOXA (=32), TAL/LMO (=13), TLX1/3 (=10), MEF2C (=1), or unclassified (=34). CNVs and cnLOH were assessed using a High-Density CytoScan HD SNParray platform (Affymetrix/Thermo Fisher Scientific). Filters were set at 200 kb for CNAs and at 10 Mb for cnLOH with at least 50 markers. SNPa findings were validated by CI-FISH.

Results: Overall SNPa detected 656 events in 90 cases (434 losses, 168 gains, 54 cnLOH). The median of events in genetically unclassified and classified cases was similar (4 *vs.* 5 per case). Amongst classified T-ALL no differences emerged between the main genetic subgroups. SNPa properly assigned to the main genetic subgroups 13% of T-ALL, identifying cryptic deletions, *i.e.* SIL-TAL1 (=8) and SET-NUP214 (=4). Moreover, it was helpful to classify 5.5% of cases revealing unbalanced translocations *i.e.* der(5)t(5;14)/BCL11B-TLX3 (=3), der(5)t(5;9)/SQSTM1-NUP214 (=1), and der(5)t(5;11) (q31.1;p11.2)/TCF7-SPI1 (=1). Recurrent T-ALL related secondary events were: loss of CDKN2AB (46%), TCF7 (22%), GRIK2 (15%), CASP8AP2 and NF1/SUZ12 (14%), CDKN1B (13%), ETV6 (12%), PTPRD (11%), TP53 (7%), EZH2 and RB1 (5%), IKZF1 (4%), WT1, ATM and PTEN (3%), and BCL11B (2%); gain of MYB (7%), and amplification of NUP214-ABL1 (3%). New putative oncogenes/oncosuppressors. SNPa detected CNVs of new putative oncogenes/suppressors. Recurrently affected genes encode for: transcription factors (CUX1, TCF4, CAMTA1, NKX2.4, DACH1), tyrosine-kinases (EPHA3, YES1), proteins involved in metabolism (GBE1/LRP1B), or related to the GPCR signaling (ARHGAP15, PRKCB), regulators of cell-cell signaling (CDH4, FAT1, CTNNA3, NRG1) or of cell-cycle/apoptosis (ESCO1, ZWINT, CERKL, TNFF18/4, LYPD6, TAX1BP1), and proteins that play a role in embryonic/postnatal development (SMOC2, LAMA2, SEMA3C, CSMD1). Chromothripsis. Chromothripsis with involvement of one chromosome (5 cases) or 2-4 chromosomes (4 cases) was detected only in ETP ALL.

Conclusions Our study on a large cohort of cases demonstrated that SNPa is a reliable tool to detect known T-ALL associated abnormalities, to enrich our knowledge on abnormal pathways, and to increase our understanding on mechanisms underlying the complex and heterogeneous genomic landscape of T-ALL in both children and adults.

Molecular Hematology

C049

PRECLINICAL EFFICACY OF A NOVEL KINASE INHIBITOR FOR THE TREATMENT OF THE PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA WITH JAK2 GENE REARRANGEMENTS

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Introduction: Although the Event Free Survival for Childhood ALL reaches 85%, the remaining 15% of patients relapse, and 25-40% of them die. Differently from the ABL-class fusions, the JAK/STAT pathway cases, representing 7% of the 'Philadelphia-like' subgroup of B-others, have been less explored. The JAK2 gene encodes for a non-receptor tyrosine kinase fundamental for the hematopoiesis, regulating multiple intracellular signaling pathways. JAK2 mutations have been widely studied in leukemia and lymphoma, while JAK2 fusion genes are still poorly characterized. Our aim has been to identify JAK2 fusion genes among BCP-ALL pediatric patients, to develop a preclinical targeted strategy.

Methods: Using an Illumina targeted TruSight RNA Pan-Cancer NGS strategy, we identified JAK2 fusions among a cohort of PCR MRD high risk BCP-ALL pediatric patients, by an in house bioinformatics pipeline. Patients' primary cells have been *in vivo* expanded in NSG mice. We thus performed ex-vivo drug treatment and apoptosis-viability assays on patients' blasts in co-culture with human bone marrow stroma.

Results: We identified 10 cases carrying a JAK2 fusion gene. Cells were available from 3 cases, carrying PAX5-JAK2, ATF7IP-JAK2 and ZEB2-JAK2, respectively. At basal level, we demonstrated JAK2 signaling pathway activation through phosphorylation on Y1007-1008 JAK2 residues in its catalytic loop of activation, compared to cases wild type for JAK2 and CRLF2 (+70% t test P value 0.024, by phosphoflow) and to cases with P2RY8-CRLF2 rearrangements and JAK2 mutation (+40% t test P value 0.097). JAK2 downstream effectors pS727-STAT3 and pY694-STAT5 were also activated. We thus setup a JAK2 targeted drug treatment using CHZ868, a new class-II tyrosine kinase inhibitor (TKI). We treated the three JAK2 fused cells for 30 minutes and we appreciated a pJAK2 mean inhibition of 42%(range 22-62%) and phosphorylation decrease of 42% of pSTAT3 (range 35-50%) and of 32% of pSTAT5 (range 15-50%). After 48h treatment by CHZ868 or ruxolitinib, we detected apoptosis induction and thus a mean cell viability decrease by CHZ868 of 47% (range 20-75%) using an IC50 100-fold lower than the IC50 determined for ruxolitinib. By CHZ868 in combination with dexamethasone, we assessed a further mean decrease of viability of 53% (range 10-95%), with a biological variability among the three different fusion genes. Only for PAX5-JAK2 fusion, we also treated with the kinase inhibitor BIBF1120/Nintedanib, targeting LCK, activated downstream PAX5 fusions, observing a 20% reduction of cell viability. Combination of BIBF1120 and CHZ868 showed a synergistic effect (-45%, at IC50).

Conclusions: CHZ868 is a promising candidate for the treatment of BCP-ALL carrying JAK2 fusions, with high efficacy and specificity. Combination of TKI treatment with standard chemotherapy agents could be the aim to maintain the efficacy by reducing the intensity and related toxicity of chemotherapy.

C050

MUSASHI-2 SUSTAINS THE GROWTH OF MLL-REARRANGED ALL AND IT IS INVOLVED IN GLUCOCORTICOIDS RESISTANCE

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Introduction: The RNA-binding protein Musashi-2 (MSI2) is a post-transcriptional regulator of protein translation with a role in normal hematopoiesis and in tumors, being involved in proliferation, differentiation and stem cell maintenance. Although several studies have reported the crucial role of MSI2 in myeloid leukemia, the role of MSI2 in acute lymphoblastic leukemia (ALL) is unknown. MSI2 overexpression was reported as a poor prognostic marker in pediatric and adult ALL, however functional data are missing. Here we focused on MLL-rearranged (MLLr) ALL occurring in infant, a rare but very aggressive leukemia with a dismal prognosis, typically associated to therapy resistance and high risk of relapse. The biological mechanisms involved in the pathogenesis of MLLr infant ALL are not completely understood. Therefore, the identification and characterization of novel genes is crucial for future therapeutic approaches.

Methods: CRISPR/CAS9 genome editing was used to generate a MSI2 KO cell line. Cell proliferation and apoptosis were investigated by Flow Cytometry. The leukemogenic potential was assessed in a xenotransplantation mouse model *in vivo*. To identify the mRNA targets of MSI2 an RNA-Immunoprecipitation was performed, followed by microarray analysis of transcripts (RIP-chip).

Results: A human MLL-AF4+ MSI2 KO cell line was successfully generated. Through a long-term competitive assay *in vitro* (in which a mix population was seeded at the initial ratio of 90:10 KO/CNTRL cells), we observed that MSI2 KO cells show a proliferation disadvantage compared to control cells and disappear after 30 days of culture. Notably, MSI2 KO cells show an impaired leukemia-initiating capacity and a lower disease burden *in vivo* (4% *vs.* 59% of hCD19+ cells in BM), and mice have a prolonged survival compared to those transplanted with control cells (mean survival: 59 and 39 days, respectively). Also, we found that abrogation of MSI2 increases by 100-fold the sensitivity of MLLr ALL cells to Glucocorticoids (GCs). Indeed, treatment with low doses of Prednisolone (1ug/mL) or Dexamethasone (0.1ug/mL) induces massive apoptosis in MSI2 KO cells (96.2%±1.1 and 98.2%±0.4 AnnexinV/7AAD+ cells, respectively), but not in control cells (41%±3.4 and 34.9%±7.3 AnnexinV/7AAD+ cells, respectively). By RIP-chip analysis we identified the direct targets of MSI2 and found that they were enriched for genes involved in GC-resistance. Further studies are currently ongoing to elucidate the molecular mechanism of GC sensitization.

Conclusions: In conclusion, we have demonstrated that the lack of MSI2 in MLLr ALL cells: impairs the cell growth *in vitro*, affects the leukemogenic potential and the disease burden *in vivo* and increases the sensitivity to GCs. Given the failure of current therapies, the identification of MSI2 as a crucial gene involved in the maintenance and drug resistance of MLLr leukemia may pave the way for the development of novel therapeutic strategies for infant patients.

C051

COHESINS MUTATIONS AND GENETIC PREDISPOSITION IN CHILDHOOD ALL.

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Introduction: Recent studies indicated genetic predisposition as playing a role in about 5-10% of childhood leukemias, suggesting a possible link between leukemia and genetic disorders. The Cornelia de Lange Syndrome (CdLS) is an example of a genetic disease with potential increased incidence of leukemia. CdLS is caused by germline variants in cohesin genes, which are also involved in a spectrum of developmental disorders, known as "cohesinopathies". Their somatic mutations have been described in myeloid malignancies (10-20% of MRC-AML, 50% of DS- AMKL, 5-15% of MDS and 10% of MPN) and solid tumours (bladder cancer and Ewing's sarcoma), and in a single case of pediatric

Acute Lymphoblastic Leukemia. This study aims to extend the identification of cohesin genes variants in ALL patients to dissect their potential role in leukemogenesis, thus providing new insights in predisposition and pre-leukemic phase. Methods: We setup a targeted Next Generation Sequencing (NGS) Nextera Flex DNA panel of 40 genes (including cohesin genes). According to material availability, we screened consecutive series of diagnosis (n=148) and relapse (n=131) pediatric ALL cases, both at a disease stage and at remission. Bioinformatics analysis has been carried out by Sophia DDM software: we identified variants with VF >5% and coverage 500X.

Results: Overall, among 279 cases we identified 71 variants in cohesin genes, corresponding to 42 unique variants, and distributed as 33 germline (6 at DX/REM/REL samples, 9 at REL/REM samples and 18 at DX/REM), in addition to 9 somatic variants (6 at DX and 3 at REL); the analysis of remission samples is still ongoing. In details, NIPBL is the most frequently affected gene (13 patients with 12 germline and 1 potential somatic variant), followed by SMC1A (11 cases carrying 7 germline, 1 potential somatic and 3 somatic). Moreover, SMC3 germline variants have been detected in 4 cases; 5 germline and 1 somatic in STAG1; 2 germline and 3 somatic variants in STAG2, as well as 2 germline variants in HDAC8 and 1 in RAD21. Of notice, the unique somatic SMC1A variant annotated as pathogenic in ClinVar (rs797045993), was present at relapse, in combination with a rs200093133 germline HDAC8 variant, in a female patient with a modest delay in psychomotor development and IQ at lower limits and malformations, such as minor dimorphisms of the face and partial agenesis of the corpus callosum.

Conclusions: We identified a significant number of ALL samples with multiple variants in cohesin genes, with either a potentially pathogenic or unknown role. Further bioinformatic and functional analysis of cohesin mutations will prove their effective role in leukemia predisposition.

C052

ABSTRACT WITHDRAWN

C053

KINETICS OF MINIMAL RESIDUAL DISEASE (MRD) DURING LENALIDOMIDE MAINTENANCE IN MANTLE CELL LYMPHOMA: RESULTS FROM THE FONDAZIONE ITALIANA LINFOMI (FIL) MCL0208 MULTICENTER, PHASE III, RANDOMIZED TRIAL

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Background: Maintenance treatment with lenalidomide has shown to improve the outcome of mantle cell lymphoma (MCL) patients. However,little is known regarding the impact of these regimens on minimal residual disease (MRD), a recognized predictive tool in MCL. In the Fondazione Italiana Linfomi (FIL) MCL0208 phase III trial (NCT02354313), a 24 months maintenance with LM (15mg days1-21 every 28 days) after ASCT in 300 frontline MCL patients showed substantial clinical activity in terms of PFS *vs.* observation (OBS).

Aims: In the study a systematic MRD detection program at multiple time points (TP) was planned, in order to investigate the MRD kinetics and predictive value in the context of LM *vs.* OBS.

Methods: MRD was assessed with ASO (allele-specific oligonucleotide) primers on either IGH(immunoglobulins heavy chain genes)or BCL-1/IGH rearrangements by Real Time quantitative (RQ)-PCR on bone marrow (BM) samples in a Euro-MRD certified lab at different TPs:here we focus on MRD results obtained after ASCT as well as at 6(M6) and 12 months (M12) after random. Landmark analysis starting at the time of MRD sampling using both Kaplan-Meier and Cox models was performed based on MRD results at each TP, for the whole series and stratified by arm.

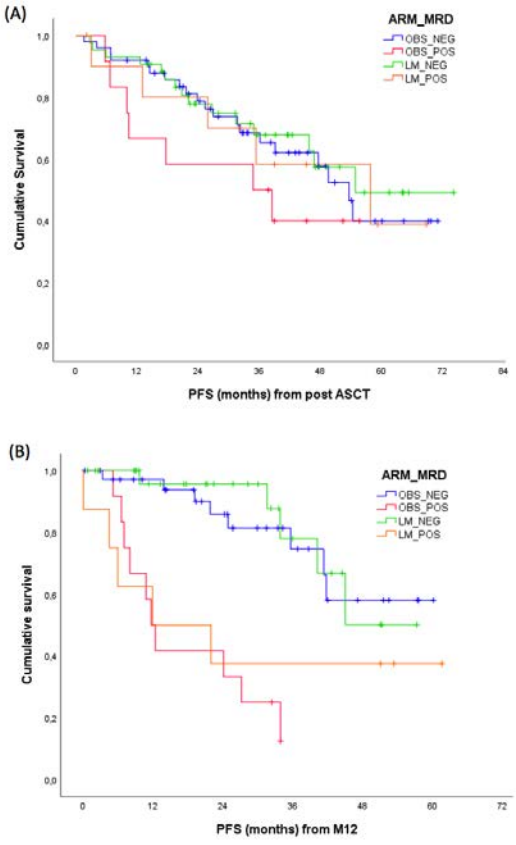

Figure 1. Progression-Free Survival(PFS) plots accordingto MRD status (landmarkanalysis).Randomizedseriesstratifiedby arm: (A) MRD post ASCT, and (B) MRD at M12.

Results: In the randomized population,93 patients scored MRD negative and 23 MRD positive after ASCT. During the first year after ASCT 29/88 patients (33%) showed alternating MRD results: 14 changed their MRD status between ASCT and M6 and 15 between M6 and M12,9 towards MRD negativization (5 in LM) and 20 towards MRD reappearance (8 in LM). In LM arm a lower rate of MRD reappearance than in OBS was registered between ASCT and M6 (8% *vs.* 14%) but not anymore between M6 and M12 (14% *vs.* 14%). This pattern might suggest a biological effect of LM in preventing MRD reappearance. As a conse-

quence,the ability of outcome discrimination of punctual MRD analysis at the post-ASCT TP was suboptimal (3-years PFS of MRD+ *vs.* MRD- patients was 53% *vs.* 68%,HR=1.44,95% CI=0.75-2.78,p=.27, Figure 1A), while a kinetic MRD analysis improved the prognostication:patients persistently MRD- or converting to MRD- had a significantly better 3-years PFS than patients persistently MRD+ or converting to MRD+ (91% *vs.* 42%, respectively, HR=0.23,95%CI=0.11-0.50,p<.001). Therefore, MRD showed a remarkable improvement of its predictive value during the first year post-ASCT, as both M6 and M12 follow-up TPs accounted also for the biological effect of LM: actually,the 3-years PFS of MRD+ *vs.* MRD- patients was 40% *vs.* 77% (HR=2.85,95% CI=1.39-5.87, p<.01) at M6 and 25% *vs.* 75% (HR=4.3,95% CI=2.04-9.05,p<.001) at M12 and was no more influenced by the randomization arm (Figure 1B).

Conclusion: The first MRD data in the context of LM in MCL suggested that:1) the predictive value of MRD analysis during the first year post-ASCT is influenced by the biological effect of LM; 2) the impact of LM is particularly prominent during the first 6 months of delivery,in terms of preservation of MRD negativity,overall contributing to the better performance of LM arm; 3) in contrast,after the first year,the MRD pattern seems not to be affected anymore by LM.

Keywords:Maintenance, MCL, MRD, Prediction.

C054

FOCUS ON PATIENTS WITH TP53 DISRUPTION IN THE FONDAZIONE ITALIANA LINFOMI (FIL) MCL0208 TRIAL: UNIFORM POOR OUTCOME, REGARDLESS OF BASELINE PREDICTORS, MRD STATUS AND LENALIDOMIDE MAINTENANCE

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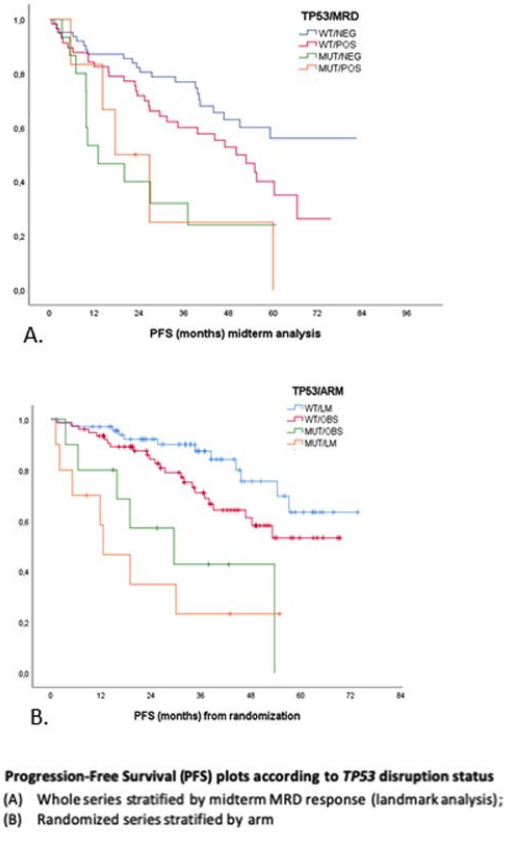
Background: In the FIL MCL0208 phase III trial (NCT02354313), a 24 months maintenance with lenalidomide (LM) after autologous transplantation (ASCT) in 300 younger MCL patients (pts) resulted in better Progression Free Survival (PFS) *vs.* observation (OBS). Moreover, patients achieving minimal residual disease (MRD) negativity in bone marrow (BM) overall showed a better outcome. On the other hand, TP53 disruptions independently predicted dismal outcome. In this abstract we describe the specific clinical features and outcome characteristics of TP53 disrupted pts of our prospective series.

Methods: TP53 disrupted pts were identified by NGS targeting resequencing and copy number variation analysis (on either BM sorted tumoral cells or FFPE lymph node). To identify prognostic factors(PFs) according to PFS, a univariate analysis was applied via Cox modeling via R (3.5.2). Survival analysis was performed using Kaplan-Meier model.



Progression-Free Survival (PFS) plots according to *TP53* disruption status
(A) Whole series stratified by midterm MRD response (landmark analysis);
(B) Randomized series stratified by arm

Figure 1.

Results: Overall, 241 pts had complete mutational data and were thus included in the present analysis: 39 (16%) showed a TP53 disruption. No statistically significant differences were registered at baseline between disrupted and wild type (WT) pts in terms of age, gender, stage, bulky disease or ECOG PS. In contrast, Ki-67 > 30% was more frequent in TP53 disrupted pts (54% *vs.* 24%,p=0.001), as well as blastoid histology (26% *vs.* 5%,p<0.001), abnormal LDH (62% *vs.* 42%,p<0.05) and high risk MIPI (23% *vs.* 11%, p=0.069). Of note, only 24/39 disrupted pts received ASCT *vs.* 177/202 WT (62% *vs.* 88%, p<0.001), as 7 TP53 pts progressed before ASCT, while 3 experienced early death and 5 unacceptable toxicity. No significant differences were recorded in terms of complete response (CR) at midterm (21% *vs.* 25%), however only 17/39 disrupted pts *vs.* 147/202 WT (44% *vs.* 73%, p<0.001) achieved CR before ASCT and 19/39 *vs.* 164/202 (49% *vs.* 81%, p<0.001) after ASCT. No differences were registered in terms of midterm/post-ASCT MRD negativization rates. Finally, 20/39 TP53 disrupted pts were randomized *vs.* 145/202 WT (51% *vs.* 72%,p<0.05). Overall, in the TP53 disrupted series median PFS was 17 months (vs not reached NR, for WT, p<0.001) and median OS 51 months (vs NR for WT, p<0.01). Interestingly, MIPI was no longer able to correctly stratify these high-risk pts (high *vs.* low: HR 1.41,95% CI 0.52-3.84,p=0.50), being the blastoid variant the only PF still significant for shorter PFS (HR 2.35, 95%CI 1.02-5.34,p<0.05). Moreover, neither MRD response at midterm (14/21, 66%) and after ASCT (14/19,74%) nor LM seemed able to overcome the dismal prognostic impact of these aberrations (Figure 1A,B).

Conclusion: The FIL series of 39 TP53 disrupted, younger MCL pts uniformly showed a dismal outcome: nearly 20% of these pts were pri-

mary refractory to treatment and only a half achieved CR after ASCT. Even among CR and MRD negative pts the outcome is still unsatisfactory. LM seems not to add any survival advantage over OBS. Therefore, it might be advisable to implement TP53 disruption testing in clinical routine, in order to develop new upfront and consolidation treatment strategies for this high-risk subgroup.

C055

BROMODOMAIN AND EXTRA-TERMINAL MOTIF PROTEIN INHIBITORS REGULATE LINEAR AND CIRCULAR PVT1 IN ACUTE MYELOID LEUKEMIA CELLS UNDER NORMOXIA AND HYPOXIA

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Introduction: Bromodomain and extra-terminal motif-protein inhibitors (BETi) have shown an antileukemic effect in several subtypes of acute myeloid leukemia (AML), both *in vitro* and *in vivo* (Daniel Gerlach et al. 2018 Oncogene). The effect of BETi is mainly mediated by suppression of bromodomain-containing protein 4 (BRD4) activity, an epigenetic regulator that sustains MYC and c-KIT expression, with consequences on a number of cellular processes, including proliferation and apoptosis (Kazuki Homma *et al.* 2017 Blood). Moreover recent findings suggested that MYC regulates through a feedback loop, a long-non coding RNA, named PVT1, which is located in the same genomic region (8q24) and is often associated with its pro-tumorigenic role (Alberto L'Abbate *et al.* 2018 Leukemia). PVT1 also encodes for a circular isoform (circPVT1), with MYC-dependent and independent functions. The aim of the present study is to investigate the role of BETi in the regulation of the circular and linear forms of PVT1 in AML and the functional consequences of their downregulation.

Methods: OCI-AML3 and KASUMI-1 AML cell lines were treated for 16 hours with BETi under normoxic and hypoxic conditions mimicking the bone marrow microenvironment. circPVT1 and linear PVT1 (PVT1) were quantified by qRT-PCR, using total RNA and di-polisomal fraction. The qRT-PCR results were also confirmed by RNA in situ hybridization (RNA-ISH) in combination with the S6 ribosomal marker. In addition, circPVT1 was silenced by small interfering (siRNA) in order to investigate the effects on apoptosis, proliferation, and its downstream targets in OCI-AML3 cells.

Results: circPVT1 and PVT1 total RNA significantly decreased in both cell lines after treatment with BETi, under normoxia and hypoxia. Notably, KASUMI-1 expressed higher PVT1 levels under hypoxia compared to normoxia. circPVT1 and PVT1 were also detected in the polysomal fraction. Interestingly, PVT1 showed mostly a nuclear localization, while circPVT1 colocalized with ribosomes. Our data on circPVT1 silencing in OCI-AML3 cells showed reduced cell growth, a slight increase in apoptosis and no effects on MYC transcript. Moreover, the interferon (IFN)-inducible serine/threonine protein kinase PKR, which has been previously reported to have a role in the innate immunity and to be inhibited by circRNAs, was induced by circPVT1-silencing in leukemic cells, as observed in T lymphocytes from systemic lupus erythematosus (Chu-Xiao Liu *et al.* 2019 Cell).

Conclusion: Our findings show that circPVT1 and PVT1 are regulated by BETi, along with MYC, in AML cell lines under normoxia and hypoxia and that silencing of circPVT1 dampens cell proliferation. Therefore PVT1 and circPVT1 may contribute to AML pathogenesis and progression, and our results suggest that targeting circPVT1 may have therapeutic potentials in AML.

C056

DIGITAL DROPLET PCR IS A PROMISING TOOL FOR DETECTING ABL1 T315I MUTATION IN CHRONIC MYELOID LEUKEMIA PATIENTS: THE EXPERIENCE OF ITALIAN "CML CAMPUS"

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Background: After introduction of the Tyrosine Kinase Inhibitors (TKIs) into the clinical practice, the outcome of patients affected by Chronic Myeloid Leukemia (CML) is dramatically improved. Nevertheless, about 30% of patients must change TKI for intolerance or resistance. The real mechanisms of resistance are still not fully elucidated, but point mutations in the ABL1 gene are responsible for at least 10% of failures. Among these mutations, the T315I seems to be the worst one, causing resistance to all TKIs except Ponatinib. Thus, the rapid identification of T315I could be a relevant goal.

Aims: To assess feasibility and sensitivity of digital droplet PCR (ddPCR) assays for T315I, both on cDNA and gDNA and to compare these results with those obtained by Sanger and/or NGS. Mutational status was assessed by the FAM/HEX mutation detection ddPCR assay ABL1 p.T315I c.944 C>T (Biorad). Methods: in the context of the "Campus CML" working group, 44 samples from 6 Italian Centers were centralized in the Molecular lab of Hematology of Pisa that performed ddPCR. In order to compare ddPCR results with Sanger and NGS, only cases already detected as T315I-mutated by one of these techniques have been analyzed. In 15 cases ddPCR was compared to both Sanger and NGS; in the remaining samples, only to Sanger or NGS. Two events in the FAM channel were considered as the minimum cut off for defining a sample as mutated.

Results: 1) feasibility: in 42/44 cases the ddPCR was successful: in one case also the BCR-ABL1 QT-PCR failed. 2) sensitivity: the minimum mutational burden detected was 0.02%, so confirming the value of $1x10^{-3}$ stated by producer. 3) ddPCR *vs.* Sanger: 25 samples were concordant; 5 cases resulted mutated by ddPCR but not by Sanger (all mutated by NGS), whereas no samples were wild-type by ddPCR but mutated by Sanger. This comparison showed that ddPCR recovered 16% of mutated cases in respect of Sanger. 4) ddPCR *vs.* NGS: 19 samples were concordant; 2 cases, mutated by NGS, resulted wild-type by ddPCR; on the other hand, other 2 cases wild-type by NGS was mutated by ddPCR. The VAF of these cases was 0.43% and 0.39%, values under the sensitivity limit of NGS. One of the 2 failing cases in ddPCR, one resulted mutated on gDNA but not on cDNA. This comparison showed that ddPCR and NGS are superimposable. 5) cDNA *vs.* gDNA: in 16 samples ddPCR was performed both on cDNA and on gDNA: we observed that 2 cases resulted T315I-mutated only on gDNA. The VAF of these cases was 0.01% and 0.05%, respectively. One of these patients became wild-type on both nucleic acids after Ponatinib. In the other case the test was performed on DNA extracted from liquor during a SNC CML localization; after intratecal chemotherapy, the test became negative.

Conclusions: ddPCR is a promising molecular technique that allows a quantitation of gene expression or detection of mutations without a reference curve, with costs comparable with those of the "classic" QT-PCR. With this work we demonstrated that ddPCR represents a valid tool for assessing in few hours the presence of T315I mutation, either on cDNA or on gDNA. This aspect could be clinically relevant, because many failing patients are not mutated on cDNA. This observation could mirror the persistence of the CML leukemic stem cell in the bone marrow

niche. Our study shows that ddPCR is comparable to NGS and slightly superior to Sanger; nevertheless, differently from NGS, ddPCR is not able to identify "composite" mutations, that sometimes are resistant to Ponatinib alone.

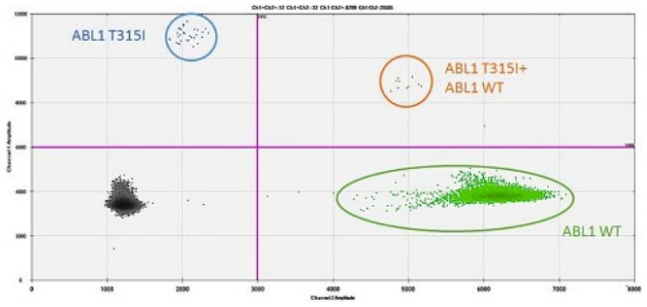

Figure 1.

Lymphomas

C057

ABSTRACT WITHDRAWN

C058

INTERPLAY BETWEEN THE PROTEINS MYC, TCL1A, EZH2 AND RNAS EXPRESSED IN ULTRACONSERVED GENOMIC LOCI IN TYPES OF NON-HODGKIN B-CELL LYMPHOMA

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Introduction: Oncogenes coordinate regulatory circuitries modulating transcription of target genes. MYC controls about 15% of genes encoded in the human genome including many noncoding RNAs. TCL1A activity contributes to the B-cell development and promotes survival of lymphoma cells. The epigenetic modifier EZH2, the catalytic member of the polycomb repressive complex 2 that represses transcription by trimethylating H3-lys27, is mutated or deregulated in subsets of non-Hodgkin B-cell lymphomas (NHBCL). In this study, we investigated the interplay among oncogenes, epigenetic regulation and RNAs in NHBCLs.

Methods: We studied 75 NHBCL tissues including Burkitt lymphoma (BL), diffuse large B cell lymphoma (DLBCL), primary mediastinal B cell lymphoma (PMBL), mantle cell lymphoma (MCL) and follicular lymphoma (FL), and 11 reactive lymph nodes (rLN) as reference. We analysed the immunohistochemical expression of MYC, TCL1A and EZH2 and counted the number of positive cells in NHBCLs and rLNs. We profiled the RNAs expressed in ultraconserved genomic loci including noncoding RNAs, microRNAs and a subgroup of protein coding genes by microarrays. We determined associations and inferred potential regulatory circuitries by correlating probe levels and positive cell counts in each sample and lymphoma type.

Results: The total number of MYC, TCL1A and EZH2 positive cells was in decreasing order of frequency in BL, DLBCL, PMBL, MCL and FL, according to aggressiveness of lymphoma type. EZH2 and MYC positive cell counts correlated positively in MCL cases ($P<0.0001$), as reported in the literature, and also through the five NHBCL subtypes ($P<0.001$). EZH2 and TCL1A positive cell counts correlated inversely through the five NHBCL types ($P<0.0001$). In FL, EZH2 positive cell counts increased in parallel to the lymphoma grade ($P<0.01$). No correlation among MYC, TCL1A and EZH2 positive cells was found in rLNs, indicating the pathological relevance of lymphoma-specific correlations. MYC-, EZH2- and TCL1A-correlated RNA expressions (Pearson's correlation, $R>0.7$) and potential regulatory networks that integrate MYC, TCL1A, EZH2 and coding and noncoding RNAs were identified.

Conclusions: Our findings reveal unique relationships between MYC, TCL1A and EZH2 and the levels of different species of conserved RNAs in five types of NHBCLs. NHBCL type-specific signalling circuitries under control of MYC, TCL1A and EZH2 raise potential therapeutic targets in NHBCLs care.

C059

ABSTRACT WITHDRAWN

C060

PROTEIN KINASES CK1 ALPHA AND CK2 IN MANTLE CELL LYMPHOMA: NOVEL PLAYERS IN BCR INHIBITORS MEDIATED CYTOTOXICITY AND RELATED SIGNALING NETWORK

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Background: Mantle cell Lymphoma (MCL) is a B cell tumor, invariably characterized by relapses. It is distinguished by the chronic activation of multiple signaling pathways, including the BTK, PI3K/AKT and NF-κB. Even if novel drugs targeting the B Cell Receptor (BCR) cascades, such as BTK inhibitors (ibrutinib, acalabrutinib), are promising, new therapeutic strategies are needed in order to uproot and cure this lymphoma. Here, we studied the pathogenetic role in MCL of CK1α and CK2, two Ser-Thr kinases that are regulators of cell survival signaling, such as the NF-κB and AKT, in other blood tumors, as multiple myeloma and acute myeloid leukemia. We described CK1 and CK2 function on BCR signaling and the effects of their inhibition combined with ibrutinib on MCL cell growth.

Methods: CK1α and CK2 expression and BCR signaling components were analyzed in MCL cells from patients, MCL cell lines and controls by western blot (WB). CK1α or CK2 silencing was obtained through the generation of anti-CK1α or CK2 shRNA IPTG-inducible MCL cell clones. CK1 or CK2 chemical inhibition was obtained with D4476 (CK1i) or CX-4945 (CK2i). Cell survival/apoptosis and proliferation were investigated with an array of standard techniques and the therapeutic interaction between drugs was analyzed through the Chou Talalay combination index method. CK2 knock down *in vivo* was obtained in xenograft NOD SCID mouse models.

Results: CK1α and CK2 are overexpressed in MCL cells compared to healthy B lymphocytes. CK1 or CK2 chemical inhibition with D4476 or CX-4945 respectively, caused the deregulation of signaling events downstream of BCR, namely a reduction in the activating phosphorylation of S536 p65/RelA and S473 AKT. CK1 or CK2 inhibition caused MCL cell apoptosis and cell cycle arrest. A novel, unanticipated potential role of these two kinases in regulating BTK was observed: their chemical inhibition or silencing with RNAi, caused a reduction in the activating phosphorylation of BTK on Y223. We confirmed this result also in an *in vivo* xenograft mouse model of CK2 knock down. Consequently, the combination of CK1α or CK2 inactivation with ibrutinib resulted in a synergic increase of cytotoxicity in multiple MCL cell line models, as calculated by the combination index between the drugs.

Conclusions: Our findings suggest that CK1α and CK2 are kinases key for MCL growth, in part through the regulation of BCR-linked survival signaling cascades. Our data also indicate that they could protect from ibrutinib -induced apoptosis. Therefore, CK1α and CK2 could be promising molecular targets for the treatment of MCL, particularly in aggressive, ibrutinib-resistant cases

C061

18F-FLUORO-DEOXY-GLUCOSE (FDG)-POSITRON EMISSION TOMOGRAPHY (PET)/COMPUTED TOMOGRAPHY (CT) DEAUVILLE SCALE AND CORE-NEEDLE BIOPSY SUCCESSFULLY MANAGE ADVANCED-STAGE HODGKIN LYMPHOMA AFTER SIX DOXORUBICIN, BLEOMYCIN, VINBLASTINE AND DACARBAZINE CYCLES

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FDG-PET is the standard of care for remission assessment in Hodgkin lymphoma (HL) at unfavorable prognosis. Histologic validation data on the diagnostic accuracy of end-of-treatment PET are sparse, also in trials in which imaging results are reported according to the Deauville scale (DS) score. It is very crucial to discriminate the responders from non-responders at the end of frontline doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) program, in order to intensify treatment with salvage regimens and hematopoietic stem cell transplantation, if required. In-vitro studies show that the analog of glucose is trapped in abnormal tissues after phosphorylation by hexokinase and its uptake is not, unfortunately, tumor specific. Inflammatory cells (granulocytes, fibroblasts and macrophages) are FDG-avid and lymph nodes enriched with these elements (probably chemotherapy induced) are a major cause of false-positive Results: To avoid overtreatment, attempts to improve PET oriented therapy are welcome. Imaging technique-assisted core-needle cutting biopsy (CNCB) is a mini-invasive and effective diagnostic strategy that characterizes suspected lesions. In this prospective trial, carried out in the Haematology Unit of the Federico II University of Naples (Italy) from September 2009 to December 2017, patients with stage IIB/IV HL after six ABVD cycles underwent PET (PET6) and CNCB of FDG-avid lymph nodes. If CNCB was positive for HL, patients received high-dose chemotherapy/autologous hematopoietic stem cell rescue (HDCT/AHSCR), alternatively, if PET or CNCB was negative, they received observation or consolidation radio-therapy (cRT) on residual nodal masses, as initially planned. The endpoint was 5-year progression-free survival (PFS). Overall, 43 of the 169 evaluable patients were PET6 positive (DS 4, 32; DS 5, 11). They underwent ultrasonography (n=33) or computed tomography (n=10)-guided CNCB of nodes with the highest SUVmax. Pathologists reported malignancy (HL) in 100% of the CNCB of DS 5 scores and in 12.5% of DS 4 scores. When CNCB results were negative for HL, sarcoid-like granulomatosis, with steato-fibrotic and/or necrotic changes, was the most common histological feature occurring in 53.5% of patients. HDCT/AHSCR was employed in 15 patients with positive CNCB, whereas 28 patients with negative biopsy (DS 4), as well as 126 patients with negative PET6, continued the original plan (cRT, 78 patients; observation, 76 patients). The 5-year PFS in the negative-PET6 group, negative-biopsy group and positive-biopsy group was 95.4%, 100% and 52.5%, respectively. The DS 4 scores with negative histology appeared to benefit from continuing the original non-intensive therapeutic plan as indicated by the similar 5-year PFS to the PET6-negative group, for more than 95% of them. By contrast, the DS 5 score had consistently positive histology promptly requiring treatment intensification or innovative therapeutic approaches.

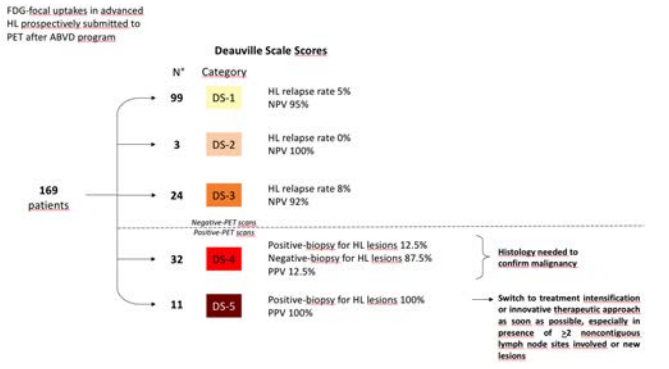

Figure 1.

C062

CASEIN KINASE 2 (CK2) INHIBITION BOOSTS MMAE ACTIVITY IN CLASSICAL HODGKIN LYMPHOMA

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Introduction: Novel treatments including the anti-CD30 monoclonal antibody conjugated with monomethyl auristatin E (MMAE-brentuximab vedotin) and immune check-point inhibitors have become available for the therapy of patients with relapsed classic Hodgkin lymphoma (cHL). However, only one third of patients achieves durable responses with novel targeted drugs. CK2 is a pleiotropic kinase made by 2alpha (catalytic) and 2beta subunits that sustains cancer survival through the activation of NF-kB, PI3K, and JAK/STAT pathways. Even if recent data suggested that CK2 plays a role in the pathogenesis of hematological malignancies, little is known on the role of CK2 in cHL. The aim of this study was to understand CK2 involvement in the signaling pathways of Hodgkin and Reed/Sternberg (HRS) cells and the effects of its inhibition on the efficacy and toxicity of new immunotherapic agents.

Methods: Experiments were performed on 4 cHL cell lines (L-428, L-540, KM-H2, and HDLM-2) cultured from 24 to 72h with silmitasertib (5, 10, and 15 μM), a CK2alpha inhibitor, and 5 nM MMAE. B cells from healthy subjects were used as negative control. By western blot (WB) analysis, CK2alpha, CK2beta, RelA-Serine (S)529, RelA, AKT-S473, AKT-S129, AKT, STAT3-S727, STAT3, PARP cleavage, and actin expression levels were evaluated. By flow cytometry, apoptosis was assessed using Annexin V/Propidium iodide assay. Trypan blue exclusion assay and Chou-Talalay method were used to calculate the combination index (CI) equation for multiple drug effect interactions. A synergistic effect between two drugs is shown by CI < 1.0.

Results: We previously found that cHL is characterized by a skewed expression of CK2 subunits, with CK2alpha overexpressed, and a constitutive serine-phosphorylation of STAT3, NF-kB and AKT at basal conditions. Silmitasertib was able to trigger apoptosis in cHL. After 48h of *in vitro* treatment with 10μM silmitasertib, the rate of alive cells was 58%, 57%, 55%, and 57% for L-428, L-540, HDLM-2 and KM-H2 cell lines (p<0.05 Kruskal-Wallis test), respectively. Our new data highlight that CK2alpha inhibition increases the activity of MMAE: when 5μM silmitasertib was added to 5nM MMAE, the rate of alive cells significantly decreased from 73%, 70% and 68% (MMAE only) to 55%, 59% and 49% (silmitasertib + MMAE) in L-428 (p=0.0038), L-540 (p=0.0237), and HDLM-2 (p=0.0205) cell lines, respectively. The CI analysis of silmitasertib plus MMAE showed synergistic effects in all cHL cell lines. The CIs were 0.91, 0.78, 0.67 for L-428, L-540, and KM-H2, respectively, with the strongest synergic combination for HDLM-2 cells (CI=0.23).

Conclusions: We demonstrated that CK2 inhibition induces time and dose-dependent apoptosis in cHL cell lines. This apoptotic effect was increased in presence of MMAE. Our data suggest that the combination of MMAE with CK2 pharmacological inhibition could lead to an improvement of the brentuximab vedotin efficacy even at very low doses.

C063

BASELINE IGM AMOUNT CAN IDENTIFY PATIENTS WITH POOR OUTCOME: RESULTS FROM A REAL-LIFE SINGLE-CENTERSTUDY IN HODGKIN LYMPHOMA

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Hodgkin Lymphoma (HL) is characterized by an inflammatory background and it has been demonstrated that the reactive myeloid cells may exert an immune suppressive effect that favors progression of disease. Currently, risk models based on clinical variables available at the initial workup and positive early PET scan can predict clinical outcome. Immunoglobulin M (IgM) is the first antibody isotype to be produced during an immune response, thus acting in the front line as a defense against foreign antigenic determinants, but it can also be considered as an immunoregulatory molecule, since it is involved in immunological tolerance and in immune regulation. Since our previous work disclosed that the neutrophils-to-lymphocyte ratio (NLR) can predict outcome also in HL patients treated up-front with a PET-2 risk adapted strategy we investigated if a surrogate of defective B-cell function could have any clinical impact on prognosis. To this end, we reviewed 253 files of HL patients who accessed our Center from January 2002 through December 2016. We identified 212 patients, including 132 advanced stage cases, with a median follow up of 60.3 months (range 60 - 204); 109 (51%) were men, with a median age of 31 years (range 15-77). 64 patients showed bulky disease, while 119 suffered from B symptoms at the onset. The median baseline values for IgM, IgA and IgG were, respectively: 86.0 (range 5.0-336.0 mg/dL), 197.5 (range 5.0- 336.0 mg/dL) and 1110.00 (range 157.0-2763.0 mg/dL). 49/212 (23%) patients had a baseline IgM value lower than 50mg/dL. The clinical variables in univariate analysis that were significantly correlated with shorter PFS included: the presence of bulky disease (p= 0.0209), extranodal sites involvement (p= 0.0317), an increased absolute count of white cells (p=0.0050), a high IPS score (p<0.0001) and PET-2 positivity (p<0.0001). A level of 50 mg/dL of IgM at baseline resulted in 84.1% sensitivity and 45.5% specificity in predicting achievement of complete response in the whole cohort (area under curve, AUC, 0.62, p=0.013). Low levels of IgM (≤ 50 mg/dL) were not associated to age, gender, advanced stage, high WBC, low ALC count, high IPS, positive PET2 or increased LDH, but could predict clinical outcome. PFS at 60 months was 54.1% versus 81.1 % respectively in patients with IgM ≤50 mg/dL or IgM>50 mg/dL (p<0.001). In multivariate analysis, only baseline IgM ≤50 mg/dL and presence of large nodal mass (LNM, >7 cm) were independent baseline variables able to predict clinical outcome, while after two cycles of treatment only IgM ≤50 mg/dL at baseline and PET-2 status were independent predictors of PFS. Thus, we stratified the advanced stage patients in three groups based on clinical variables available at diagnosis: low risk group was defined as absence of LNM and baseline IgM >50 (N=63, 48%); standard-risk was defined by either presence of LNM or baseline IgM ≤50 mg/dL (N=59, 45%); high risk group was defined by both presence of LNM and IgM ≤50 mg/dL at baseline (N=10, 7%). The 60-months PFS estimates were significantly different among the three groups, respectively 83.5, 59.5 and 40.0%, p=<0.0001. After two cycles of treatment, PET-2 negative patients carrying IgM≤50 mg/dL had poor outcome, with PFS at 60 months of 68.0 months versus 84.7%, p=0.0068. Taken together, our findings disclosed that evaluation of IgM amount at diagnosis is a valuable prognostic factor much earlier than PET-2 and can also provide information in PET-2 negative patients. Therefore, it can be added to known predictive factors in order to identify at baseline different HL classes at risk of treatment failure.

C064

THIOREDOXIN SILENCING IN RESISTANT LYMPHOID CELL LINES SENSITIZED CELLS TO THE CITARINOSTAT AND MOMELOTINIB COMBINATION TREATMENT AND REDUCE THE LEVELS OF ANTI-APOPTOTIC PROTEINS BCL-2 AND BCL-XL

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Introduction: Histone deacetylase (HDAC) inhibitors represent an encouraging class of antitumor drugs with minimal toxicity in normal

cells. Citarinostat (Acy-241) is a second generation, orally administered, HDAC6-selective inhibitor. Momelotinib (CYT387) is an orally administered inhibitor of Janus kinase/signal transducer of transcription-3 (JAK/STAT3) signaling. We hypothesize that both HDAC and JAK/STAT pathways are important in lymphoproliferative disorders, and that inhibiting JAK/STAT3 and HDAC simultaneously might enhance their efficacy without increasing toxicity.

Methods: ER stress and apoptosis-related proteins were detected by western blotting. Clonogenic survival was studied with the methylcellulose clonogenic assay. Modulation of Trx expression, *in vitro*, was performed by using siRNA strategy. Transfected cells were incubated for 24 h before the indicated treatment. At 48 h after transfection, cells were collected for a viability assay and for immunoblotting. Transfection efficiency was measured with flow cytometry by detecting cells that expressed GFP.

Results: As previously reported (SIES 2018, abstract n. 0087) 12 lymphoid cell lines were tested with the drug combination showing a good response to the treatment, except for Granta519 (mantle cell lymphoma) and L1236 (Hodgkin's lymphoma), that showed little sensitivity. In these lymphoid cells we observed an antagonistic effect demonstrated by the CI > 1 (combination index). In all sensitive cell lines, the combination treatment induced a large increase in the percentage of apoptosis cells and ROS-positive cells and an upregulation of expression of hallmark ER stress proteins as BIP, CHOP, and PERK. In resistant cells, Granta519 and L1236, the combination drugs caused a not significant increase in ROS production and did not modify ER stress protein expression. We then focus our attention on ROS and ER stress as a possible mechanism of resistance. We observed that Trx, a class of cellular antioxidant proteins expression level was reduced in sensitive lymphoid cells, but was not affected in Granta-519 or L-1236 cells. In order to prove the role of Trx in the cell drug resistance we used siRNAs to knock down Trx in Granta-519 cell and investigated whether these cells were sensitized to the treatment. The knock down was confirmed by the reduction of Trx expression levels that resulted in reduction of the anti-apoptotic protein Bcl-2, Bcl-xL and apoptosis was confirmed by the activation of the caspases. Upon exposure to the combination treatment, Trx-silenced Granta-519 cells showed reduced cell viability and significantly reduced clonogenic survival. We hypothesized that the Trx deficiency in previously-resistant cells contributed to the activation of cytotoxicity, growth arrest, and the loss of clonogenicity.

Conclusion. The resistance to the combination of an HDAC6 inhibitor and a JAK/STAT inhibitor seems to be, at least, partly related to Trx and Trx can represent a potential drug target to overcome drug resistance in lymphoid cells. (In press)

Chronic Lymphocytic Leukemia 2

C065

A HIGH-DEFINITION GENOMIC LANDSCAPE OF CHRONIC LYMPHOPROLIFERATIVE DISORDER OF NK CELLS

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Chronic Lymphoproliferative Disorder of NK cells (CLPD-NK) is included in the 2017 WHO classification of mature T- and NK-cell neoplasms as provisional entity. Similarly to T-cell large granular lymphocyte leukemia (T-LGLL), CLPD-NK is characterized by an indolent clinical course which is clearly distinguished from the clinical features of the aggressive NK cell leukemia (ANKL). The role of the JAK-STAT axis is well established in the development of T-LGLL and ANKL, with a prevalence of STAT3 and JAK2 mutations respectively. Only scattered data are available on the genomic landscape of CLPD-NK and an extensive study is needed to better classify this disorder. An initial cohort of 57 CLPD-NK patients was screened by Sanger sequencing, covering the hotspot regions of STAT3 gene. Thirteen STAT3 wildtype patients with CD16+/CD57±/CD56± immunophenotype were profiled with Whole Exome Sequencing (WES; Illumina sequencing, paired end reads) on both immunomagnetically purified leukemic clone and autologous granulocytes, as control. Sequencing data were analyzed by an in-house bioinformatic pipeline with complementary approaches (MuTect, MuTect2 and Strelka2) to detect somatic variants. Variants were filtered according to population allele frequency (<5%), clinical significance (COSMIC and ClinVar) and predicted functional impact on proteins. Somatic copy number variations (CNV) were analyzed by Varscan2 and copyCaller using the same paired tumor-control WES data. The screening of 57 CLPD-NK patients revealed that the frequency of STAT3 mutations in our cohort is the lowest in literature (9%). WES analysis uncovered a heavy mutational burden comprising mostly subclonal mutations co-occurring with several clonal and deleterious somatic variants. None relevant gene was found recurrently mutated. Nevertheless, genes belonging to Ras-MAPK and PI3K-Akt pathways resulted mutated in at least 70% of the patients, in contrast with JAK-STAT axis which was rarely hit. To note, DDX3X and TET2, recurrently mutated in ANKL, were found also in CLPD-NK. A total amount of 1353 somatic CNV were detected and equally divided between amplification and deletions (Del). CNV recurrence was observed mostly in Del (max 4 patients). Preliminary analysis revealed that several genes affected by CNV are tumor suppressors (e.g. HIC1) or oncogenes (e.g. BIRC6), highlighting their potential pathogenetic role in the disease. A correlation with somatic mutations in the same patient has not yet been ruled out. Despite the indolent nature of the disease, a high genomic instability has been demonstrated in CLPD-NK with somatic SNP and CNV affecting genes involved in cancer proliferation, survival and migration. A high number of genes harboring CNV was observed for the first time, even though WES is not the preferential method for this analysis. By the demonstration of new similarities with ANKL this study contributes to a better definition of the pathogenetic mechanisms accounting for CLPD-NK

C066

ROLE OF NON-LEUKEMIC PERIPHERAL BLOOD CELLS IN SUSTAINING CELL PROLIFERATION OF LEUKEMIC T LARGE GRANULAR LYMPHOCYTES

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T Large granular lymphocytes leukemia (T-LGLL) is a rare lymphoproliferative disorder. According to the immunophenotype, two disease groups (CD8+ and CD4+) have been defined. Sixty % of CD8+ patients are characterized by STAT3 mutations and neutropenia, which represents the most relevant clinical manifestation in T-LGLL. At variance, CD8+ STAT3 wild type and CD4+ cases are usually indolent. Leukemic clone survival is mediated by deregulated pathways that are controlled by somatic mutations and external stimuli. Indeed, many pro-inflammatory cytokines are increased in patients' plasma, including CCL5 and IL-6, this latter being able to sustain LGL survival. Since soluble factors were proven to mediate T-LGLL pathogenesis, we evaluated the contribution of non-leukemic peripheral blood (PB) cells to the disease development. Forty-eight T-LGLL cases and 10 healthy controls were studied. Monocytes (Mo) and LGL were immuno-magnetically purified. IL-6 and CCL5 mRNA expression was measured by RT-qPCR and IL-6 secretion by ELISA. LGL and Mo were stimulated by IL-6 (20 ng/ml) or CCL5 (100 ng/ml) for 12 hours. Apoptosis, Mo classes and T helper 17/T regulatory cells (Th17/Treg) ratio were evaluated by flow cytometry. Protein phosphorylation and expression were analysed by Western Blotting. Plasma cytokinic screening was performed by a commercial array. Mo resulted to be central for leukemic LGL survival, as in-vitro they prevented LGL apoptosis and secreted IL-6 in response to CCL5, which was specifically expressed by the leukemic clone. We identified that non-leukemic cells in PB were biologically different among patients and healthy controls and also between CD8+ and CD4+ cases. CD8+ STAT3 mutated and neutropenic patients harboured increased intermediate and non-classical Mo. Moreover, Mo had a higher STAT3 and p65 expression, not correlated with their activation, indicating a putative role of un-phosphorylated proteins. Th17 cells were also increased, unbalancing the Th17/Treg ratio from the physiological status. The deregulated ratio for CD4+ cases was instead sustained by Treg cells reduction. Mo in this group were not altered in their classes distribution, but Erk activity was increased, suggesting Mo senescence. Moreover, preliminary data indicated that pro-inflammatory cytokines are increased in CD4+ patients' plasma. Our results for the first time demonstrate the crosstalk between leukemic LGL and Mo. Mo, Th17 and Treg cells are altered in some groups of patients, emphasizing that several cells in PB are impacted by the presence of leukemic LGL and, in turn, might be involved in T-LGLL pathogenesis. In particular, CD8+ neutropenic cases are characterized by inflammatory features common to autoimmune diseases. Despite CD4+ forms are seldom symptomatic, also in these patients evidence of inflammation has been provided. We suggest that these differences might be linked to different external stimuli required for CD8+ and CD4+ T-LGLL cells to proliferate.

C067

A NOVEL MECHANISM FOR NOTCH1 ACTIVATIONIN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS: INVOLVEMENT OF NOTCH1 ENDOSOMAL TRAFFICKING AND ALTERATIONS IN RAB PROTEINS

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Introduction: NOTCH1 is activated independent of NOTCH1 mutation in chronic lymphocytic leukemia (CLL) cells raising questions about the involved mechanisms. As NOTCH1 is activated in peripheral blood (PB) cells whose interactions with microenvironment cells bearing NOTCH ligands are unlikely, we suggest the involvement of ligand-independent mechanisms. In other systems, in the absence of ligand interaction, NOTCH1 is endocytosed into vesicles from which it is recycled to the cell surface or sorted to the late endosomes for degradation or activation. A key role in controlling this intracellular trafficking is played by the RAB proteins, small GTPases with distinct subcellular localization. We hypothesized that NOTCH1 endocytosis and alterations in specific RAB proteins contributes to NOTCH1 activation in CLL.

Methods: In purified PB CLL cells (>95%, expression of NOTCH1 intracellular domain (ICD) was analyzed by Western blot (WB) with the anti-cleaved Val1744 antibody (Ab). NOTCH1 intracellular trafficking was examined by Proximity Ligation Assay (PLA) using Abs to NOTCH1 extracellular (ECD) or trans-membrane (TM) domains, RAB5, RAB7 and RAB11 proteins.

Results: To determine the intracellular fate of the membrane-tethered NOTCH1 we analyzed its co-localization with different RAB proteins specific for distinct endosomal vesicles (Figure1): RAB5 (endocytic/early endosomes), RAB11 (recycling endosomes) and RAB7 (late endosomes). For this analysis, we used CLL cells with high activated NOTCH1-ICD evaluated by WB. We showed that both NOTCH1-ECD and -TM localized to RAB5 vesicles with 78.4±21.9% and 35.0±16.3% positive cells, respectively (N=3.NOTCH1-TM was also found in the recycling RAB11 vesicles (N=5, 44.5%±10.7% positive cells. These data indicate NOTCH1 receptor internalization and recycling to plasma membrane in NOTCH1-activated CLL. We then analyzed the localization of NOTCH1-TM in RAB7 vesicles. It has been shown that RAB7 vesicles besides regulating endosome-lysosome fusion for NOTCH1 degradation, also represent a suitable compartment for NOTCH1 cleavage due to their acidic conditions favoring proteolytic activity. We showed that NOTCH1-TM colocalized to RAB7 vesicles with 57.8±15.3% positive cells (N=5). Additionally, treatment of CLL cells with chloroquine, an inhibitor of endosomal acidification and fusion with the lysosome, reduced NOTCH1-ICD levels compared to controls (N=6) suggesting that NOTCH1 cleavage might occur in RAB7 vesicles. Finally, we found that RAB7 protein was overexpressed in CLL with high NOTCH1-ICD levels compared to CLL with low/absent NOTCH1-ICD suggesting an association between NOTCH1 activation and RAB7.

Conclusions: Our results reveal a novel intrinsic mechanism for NOTCH1 deregulation in CLL that is independent of NOTCH1 gene alterations and ligand interactions. They also warrant studies to evaluate if RAB proteins could represent molecular target for NOTCH1 inhibition in CLL with potential treatment implications.

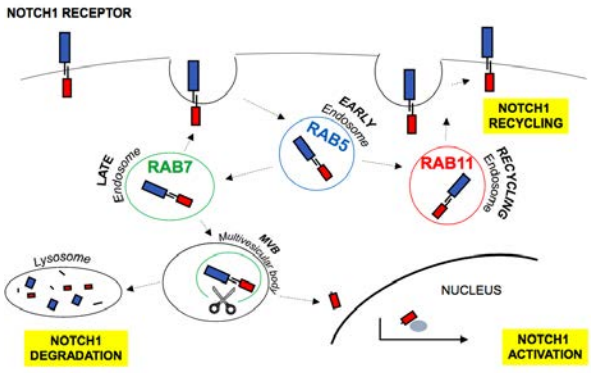

Figure 1.

C068

DROPLET DIGITAL PCR FOR NON-INVASIVE MUTATIONAL SCREENING IN WALDENSTRÖM MACROGLOBULINEMIA AND IGM-MGUS: FIRST RESULTS OF THE FONDAZIONE ITALIANA LINFOMI (FIL) BIO-WM TRIAL

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Introduction: MYD88L265P mutation is gaining relevance in the clinical management of Waldenström's Macroglobulinemia (WM). Tumoral cells sorted from bone marrow (BM) are the standard for mutational screening, however BM aspirate is an invasive procedure and CD19 sorting is not part of routine diagnostics. Liquid biopsy has been tested in WM as a non-invasive alternative to BM aspiration, however no prospective comparison has been performed for MYD88 mutation. These issues have been addressed in the multicenter, observational trial BIO-WM (NCT03521596), sponsored by the Fondazione Italiana Linfomi (FIL) and the IWMFoundation/Leukemia and Lymphoma Society. Primary objective was to demonstrate that MYD88L265P mutation rate in plasma showed a negligible difference compared to BM.

Methods: This trial enrolled both prospective (TR) and retrospective (TE) untreated IgM-MGUS and WM patients. Paired BM, peripheral blood (PB) and plasma for cell-free DNA (cfDNA) were collected: a droplet digital PCR (ddPCR) assay for MYD88L265P was performed. Agreement was estimated by k-statistic and Passing-Bablok regression.

Results: Complete data are available for 173 cases (142 prospective and 31 retrospective, 130 WM and 43 MGUS). Main clinical features were equally distributed between WM and IgM-MGUS patients, except from hemoglobin and albumin (median 12.5 g/dL *vs.* 13.8 and 4 g/dL *vs.* 4.2, respectively) and monoclonal IgM values (median 0.86 *vs.* 0.3 g/dL, respectively). 159 cases had paired tissues for concordance analysis. MYD88L265P rate was 91% in BM *vs.* 78% in PB and 87% in cfDNA in the TR and 92% *vs.* 65% *vs.* 81% in the TE. Similar results were seen separating WM (94% *vs.* 85% *vs.* 92%) from IgM-MGUS, with these showing significantly lower rates than WM in all tissues (77% *vs.* 41% *vs.* 71%). Overall, median MYD88L265P quantification in cfDNA was superimposable to BM, both in TR (both 1.60E-2) and in TE (1.85E-2 *vs.* 4.42E-2), while PB values were always about 1 log lower (2.69E-3 and 2.40E-3, respectively). The Passing-Bablok regression showed a negligible difference in the quantitative dosage of cfDNA compared to BM (intercept p=0.327, slope p=0.189). Therefore, the qualitative concordance was excellent between cfDNA and BM (agreement 96%, K-statistic: 0.796) but only fair between PB and BM (85.2%, 0.464). Moreover, among the 27 patients with CD19-sorted cells, no difference in mutation rate was found between BM and plasma (both 89%): sorted samples showed the highest quantitative mutation levels (median 7.74E-1).

Conclusion: The primary objective of the study was met: MYD88L265P mutation rates detected in plasma by ddPCR were superimposable to BM. PB showed 10% false negative results, as well as lower median quantitative levels. CD19 selection, despite enriching the sample in tumoral content, is dispensable for mutational screening. In conclusion, these data are in favor of implementing MYD88L265P ddPCR assay on plasma as non-invasive, patients friendly, mutational screening in WM and IgM-MGUS.

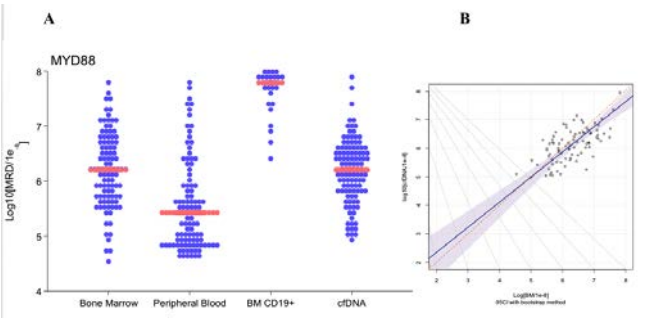

Figure 1. Comparison of MYD88L265P quantitative values among different tissues (TR). (A) Log10 values with medians in red; (B) Passing-Bablok regression: cfDNA (Y-axis) compared to BM (X-axis). TR, training series.

C069

TOWARDS A BETTER DEFINITION OF DISTINCTIVE CLINICAL AND MOLECULAR FEATURES OF CD8+ STAT3 MUTATED T-LGLL

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T-cell Large Granular Lymphocyte Leukemia (LGLL) is a rare and chronic lymphoproliferative disorder characterized by the expansion of CD3+ T-LGLs. Patients affected by T-LGLL can be asymptomatic or develop cytopenia, mostly neutropenia. Recently, somatic STAT3 and STAT5b mutations were discovered in approximately 40% of patients but only limited data on transcriptome are available. By deep RNA analysis, the aim of this study was to identify LGLL patients'subtypes with distinct clinical and biological features. From 1992 to 2019, clinical and biological data of 129 T-LGLL patients were collected. STAT3 exon 21 and STAT5b exon 16-18 mutations analysis was performed by Sanger sequencing. Gene expression profiling was performed by RNA sequencing (RNA-seq) in 20 selected purified samples of T-LGLL patients and in 5 healthy controls. Gene differential expression was assessed using DESeq (adj. p-value<0.01). According to CD4 and CD8 expression, T-LGLL was classified in CD4-/CD8+ LGLL (CD8+ T-LGLL, 65.1%) and CD4+/CD8dim/neg LGLL (CD4+ T-LGLL, 34.9%). DNA samples of 103 and 96 patients were available for STAT3 and STAT5b mutations analysis, respectively. STAT3 and STAT5b mutations were detected in 39 CD8+ patients (37.9%) and in 12 CD4+ patients (12.5%) respectively and were mutually exclusive. From the clinical point of view, STAT5b mutated patients were asymptomatic, with only one patient experiencing

neutropenia. Instead, STAT3 mutated patients were characterized by higher frequency of severe neutropenia (Absolute Neutrophils Count ANC<500/mm3, 43.6% *vs.* 9.4%, p<0.0001), severe anemia (Hb<90g/L, 18% *vs.* 4.7%, p=0.0394), splenomegaly (30.8% *vs.* 9.4%, p=0.0077) and treatment requirement (26.2% *vs.* 0%, p<0.0001). Considering all LGLL patients subgroups, only STAT3 mutated patients displayed significant reduced overall survival (OS, 267 months *vs.* not reached, p=0.0102). To get insight into the molecular features underpinning heterogeneity of the disease, RNA-seq of 20 purified samples of T-LGLL patients, stratified in four groups, according to phenotype (CD8+ and CD4+ LGLL) and STAT mutational status, and in 5 healthy controls was performed. Gene expression profiles clearly separated LGLL patients from controls. As a matter of fact, 2,390 genes resulted differentially expressed comparing LGLL with controls. Of note, among LGLL patients, the STAT3 mutated CD8+ patients presented a distinctive expression profile, whereas the differences between the other groups were more subtle. We identified 1,995 genes with different expression in the group with more symptomatic disease, mainly involved in viral carcinogenesis, autophagy and in Phosphatidylinositol, Sphingolipid, and NF-kappa B signalling pathways. CD8+ STAT3 mutated LGLL represents a distinct clinical and biological entity characterized by symptomatic disease, reduced OS and a specific expression profile towards CD8+ STAT3 wild-type LGLL and CD4+ LGLL.

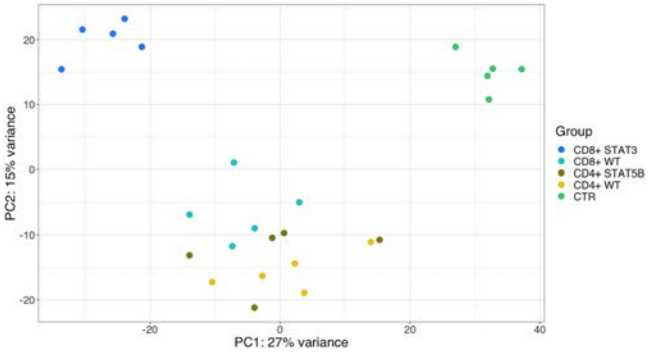

Figure 1.

C070

CHRONIC LYMPHOCYTIC LEUKEMIA CELLS AND TUMOR MICROENVIRONMENT COOPERATE TO MODULATE INDOLEAMINE 2,3-DIOXYGENASE (IDO)/KYNURENINE/ARYL HYDROCARBON RECEPTOR (AHR) AXIS

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Introduction: Chronic lymphocytic leukemia (CLL) is a malignancy of mature B cells in which leukemic cells are "addicted to the host". Extrinsic signals from the microenvironment have a profound influence in establishing progressive immunosuppression for malignant cell growth, survival and drug resistance. Indoleamine 2,3-dioxygenase (IDO) is an enzyme that metabolizes tryptophan (trp) into kynurenine (kyn), which, in turn, inhibits effector T cells and promotes Treg differentiation through activation of aryl hydrocarbon receptor (AhR). IDO protein is expressed in human hematologic malignancies and its level is correlated with a poor prognosis and chemoresistance. The IDO activity, measured as the kyn/trp ratio, was reported to be increased in CLL cases if compared to normal controls.

Methods: Gene transcription and protein expression were evaluated by real time PCR and western blot. Enzymatic activity was assessed through ELISA. Survival was measured with PE/annexin V assay.

Results: We determined the expression of IDO and AhR in our cohort of untreated CLL patients. Our results demonstrated variable expression of IDO and AhR, both at transcriptional and also at protein level. Then we found out that several microenvironmental signaling molecules such as IFNγ, LPS, anti-IgM, CpG, CD40L and TNFα were able to up-regulate IDO mRNA and protein. Interestingly, our set of stimuli increased also Ahr mRNA. To characterize the pathways mediated by IDO induction, we stimulated CLL cells with IFNγ and CD40L respectively. Using ruxolitinib, an inhibitor of JAK-STAT pathway we found that IFNγ induces IDO through STAT1 signaling. Again CD40L overexpressed IDO through the non-canonical NF-kB pathway, as confirmed by the use of NF-κB inducing kinase inhibitor, NIK SMI1. To evaluate if IFNγ-treated CLL cells were able to produce a functional IDO enzyme, we measured kyn production and trp consumption with ELISA assays. IFNγ induced a strong increase in the kyn/trp ratio that was significantly reduced by ruxolitinib treatment. To verify if kyn produced by CLL cells act with an autocrine loop on AhR, leukemic cells were treated with kyn. Activation and translocation of AhR from cytoplasm to nucleus was detected and was confirmed by detecting up-regulation of CYP1A1, a known AhR target gene. Of interest, we detected that kyn treatment improve CLL cells survival. These data were confirmed analyzing the anti-apoptotic protein of the Bcl2 family. Induction of Mcl1 was detected after kyn treatment, while treatment with CH223191, an antagonist of AhR, was able to affect Mcl1 expression.

Conclusion: Our data demonstrate the constitutive expression of IDO and AhR in CLL cells. Furthermore, the tumor microenvironment promotes the induction of IDO and Ahr through a complex signaling crosstalk with leukemic cells. Our data underlined that IDO-kyn-Ahr axis is inducible in CLL cells and that this could be a possible mechanism of survival and immune escape of leukemic cells.

C071

TARGETED ACTIVATION OF THE TYROSINE PHOSPHATASE SHP-1 COUNTERACTS STAT3-MEDIATED SURVIVAL MECHANISM IN T-LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Introduction: T-Large Granular Lymphocytes Leukemia (T-LGLL) is a rare lymphoproliferative disorder characterized by the chronic expansion of clonal cytotoxic T-LGL. STAT3 activation (mediated by Tyr705 phosphorylation) is the molecular hallmark of leukemic T-LGL and it is likely to be central hub of their survival network. In addition, STAT3 activation has a pathogenetic role in neutropenia development, *i.e.* the main clinical manifestation of the disease. Protein phosphorylation is a finely regulated process. However, aberrant phosphorylations can be maintained by the lack of a proper balanced activity between protein kinases and phosphatases. The SH2-domain containing protein Tyr-Phosphatese-1 (SHP-1) acts as a tumor-suppressor, being a negative regulator of cytokine-activated pathways, including the STAT3-axis. Of note, a decreased SHP-1 activity or expression have been reported in several neoplastic conditions (e.g. T/NK-cell lymphomas). The aim of this work was to evaluate whether the targeted activation or induced expression of SHP-1 might affect leukemic T-LGL survival, by reducing STAT3 Tyr705phosphorylation.

Methods: SHP-1 activity/expression and STAT3 phosphorylation were evaluated by immunoprecipitation and western-blot assays. STAT3 transcriptional activity was measured by evaluating the expression of its downstream targets. T-LGL viability was assessed by flow cytometry.

Results: Several compounds, including newly-synthetized molecules,

were evaluated in different concentrations and time points. The most promising data were obtained with SC-78, a regorafenib-like derivate, and Acetyl-11-Keto-β-Boswellic Acid (AKBA), a pentacyclic triterpene, which enhance SHP-1 activity and expression, respectively. The molecular effects of these molecules were investigated in 20 patients, 15/20 (75%) characterized by neutropenia. The basal inactive state of SHP-1, observed in leukemic T-LGL, was reversed by SC-78, as confirmed by the dephosphorylation of [32P]-Band 3, used as an endogenous substrate. Similarly, an increase in the global SHP-1 activity, as a result of its induced overexpression, was observed with AKBA. Both compounds elicited STAT3 Tyr705 dephosphorylation and the reduction of STAT3 transcriptional activity was confirmed by a decreased expression of its downstream targets Mcl-1, survivin and cyclin D1. Consistently, a significant increase in leukemic T-LGL apoptosis was observed even after 24 hours, with a synergistic pro-apoptotic effect combining both treatments. Remarkably, T-LGL treatment with NSC8787, a SHP-1 selective inhibitor, prevented SC-78 and AKBA molecular effects, confirming the selectivity of these molecules towards SHP-1.

Conclusion: Our data confirmed the central role of STAT3 in T-LGLL pathogenesis and showed that targeted activation and overexpression of SHP-1 are novel promising therapeutic strategies to counteract STAT3 hyperactivation and leukemic T-LGL survival, especially for neutropenic patients.

C072

ATOVAQUONE: A NEW STAT3 INHIBITOR ABLE TO CONTRAST THE LEUKEMIC CELL PRO-SURVIVAL IN LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Background: Large granular lymphocyte leukemia (LGLL) is a heterogeneous lymphoproliferative disorder characterized by the chronic proliferation of clonal large granular lymphocytes (LGLs) with cytotoxic activity. The hallmark of leukemic LGLs is a constitutive activation of STAT3 which, in turn, triggers many pro-survival genes. Moreover, 30-40% of patients carry mutations in STAT3 gene likely responsible of the constitutive activation of the malignant clone. LGLL therapy remains an unmet need and is mainly based on immunosuppressive drugs, such as Methotrexate (MTX), Cyclophosphamide (CTX) and Cyclosporin A (CyA). However, the average overall response rate is only about 50%. The crucial role of STAT3 activation in LGLL pathogenesis offers the rationale for using Atovaquone (ATQ), a FDA-approved anti-microbial agent characterized by a low adverse effect profile that has been demonstrated (Xiang *et al*, 2016) to behave as a powerful STAT3 inhibitor with anti-cancer efficacy in both animal models and human cell lines.

Aim: Having in mind to develop a targeted approach to LGLL, likely devoid of adverse effects, we evaluated the *in vitro* effects of ATQ as inhibitor of STAT3 phosphorylation and LGLs survival. METHODS Patients' peripheral blood mononuclear cells (PBMC) were purified to perform cultures to test serial drug concentrations. Using cell culture, the drug's activity has been evaluated by analyzing STAT3 phosphorylation by Western Blot. The cytotoxicity of ATQ, CTX, MTX and CyA was investigated by Annexin V test; by flow cytometry, a staining with anti-CD57, CD56 or CD16 was used to identify leukemic LGLs from PBMCs, and anti-Gp130 to evaluate its expression on LGLs. By RT-PCR, STAT3 activity was analyzed along its transcriptional levels and target genes, as Bcl2 and Mcl1.

Results: Data obtained through cell culture demonstrated that the best concentration of ATQ inhibiting cell viability and STAT3 activation on patients' PBMCs was 25 μM. Results of the apoptosis analysis showed that ATQ has a dose and time dependent cytotoxic effect starting from 48h of treatment and reaching a mortality plateau after 6 days of treatment. The drug was shown to be able to extinguish IL-6-induced STAT3 activation after 1.5h and to significantly decrease STAT3 target genes transcription after 24h. By comparing the *in vitro* cell effect of ATQ with that of the commonly used therapies, ATQ showed a cytotoxic effect superimposable to CTX and CyA, but better than MTX ($p<0.01$). The mechanism of action proposed by Xiang et al, according to which ATQ acts by down-regulating Gp130 from cell membrane, has not been confirmed in leukemic LGLs (MFI treated/untreated=1.01±0.05, $p=0.74$).

Conclusion: These *in vitro* data indicate that ATQ has a promising action on the inhibition of the JAK/STAT pro-survival pathway in leukemic LGLs. Its antagonism to STAT3 activation makes ATQ potentially suitable for other neoplastic diseases sharing the key role of STAT3 activation with LGLL.

Myelodisplastic Syndromes and Acute Leukemia

C073

THE IMMUNOPHENOTYPICAL PROFILE OF CD34 + MYELOID PRECURSORS AND THE NEXT GENERATION SEQUENCING (NGS) MUTATIONAL ANALYSIS IMPROVE DIAGNOSTIC AND PROGNOSTIC EVALUATION OF MYELODYSPLASTIC SYNDROMES (MDS)

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Introduction: Multi parameter flow-cytometry (MFC) and next generation sequencing (NGS) techniques are important tools for diagnosis and prognostic stratification of MDS.

Methods: We evaluated: 1) the diagnostic sensitivity of Ogata score combined with phenotypic aberrancies (PA) on CD34+ cells; 2) the correlation of a scoring system based on Ogata plus PA (O-PA score) with mutational profile; 3) the prognostic significance of a comprehensive score combining O-PA and NGS Results: We investigated in 114 BM samples (87 MDS patients and 27 controls) Ogata score, based on CD34+ myeloblasts (%), CD34+ B-progenitors (%), CD45 expression on myeloblasts, granulocyte to lymphocyte SSC peak channel ratio. Diagnosis of MDS was suggestive for Ogata score ≥ 2. PA were assessed on CD34+ myeloblasts for altered levels of expression of CD13, CD33, CD200, CD25, HLA-DR; asynchronous expression of CD15 and CD64 and lineage infidelity markers including CD2, CD5, CD7 and CD56. Median age was 72 years (range 43-89) and 40 patients (46%) were females. According to WHO criteria 21% had MDS with single lineage dysplasia, 8% MDS with ring sideroblasts, 7% MDS with isolated del (5q), 36% MDS with multilineage dysplasia, 22% MDS with excess blasts, 2% MDS unclassifiable, 4% chronic myelomonocitic leukemia. Revised International Prognostic Scoring System (r-IPSS), was estimated in 82 patients and 17%, 48%, 17%, 12%, 6%, were very low, low, intermediate, high, very high risk, respectively.

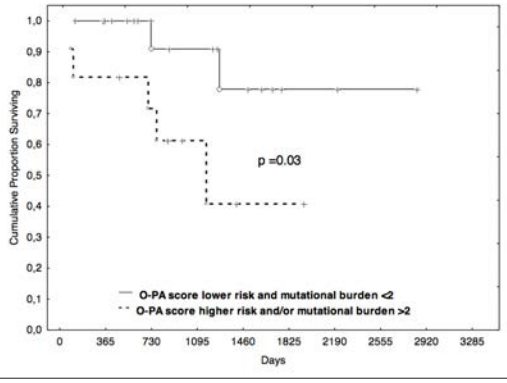

Figure 1.

Results: In control BM samples Ogata score was always < 2 (specificity 100%), whereas in MDS was ≥ 2 in 48 patients (sensitivity 55%). PA were identified in 55 patients (64%). The addition of PA analysis to Ogata score supported the diagnosis of MDS in 17 of 39 patients with an Ogata score <2, increasing the sensitivity to 74%. According to O-PA score patients were divided in "lower risk" (score 0-3) and "higher risk" (score ≥ 4). Lower O-PA risk was significantly associated with r-IPSS very low/low risk group and no progression in AML (p<0.0001 and 0.013, respectively). A subgroup of 43 MDS patients was also studied by NGS. We found a significant direct correlation (p=0.0003) between O-PA score and mutational burden, with SRSF2, TET2 and RUNX1 mutations significantly associated with higher O-PA score (p=0.0004, 0.002 and 0.01, respectively). Finally in a cohort of 31 patients with lower risk r-IPSS, those with favorable risk (O-PA score "lower risk" and mutational burden <2) had significantly longer overall survival than patients with unfavorable risk (p=0.03, Figure 1). These data indicate that in patients without MFC or NGS aberrations the diagnosis of MDS may have to be reconsidered.

Conclusion: 1) Combined assessment of Ogata score and PA on CD34+ myeloid precursors improves diagnostic power of MFC in MDS 2) O-PA score is associated with mutational burden in MDS patients 3) Combined phenotypic and mutational analysis enhances prognostication of lower risk MDS patients

C074

ADOPTIVE IMMUNOTHERAPY WITH REGULATORY AND CONVENTIONAL T CELLS AFFORDS HIGH RATE OF CHRONIC GVHD/RELAPSE-FREE SURVIVAL IN ACUTE MYELOID LEUKEMIA AFTER MYELOABLATIVE HAPLOIDENTICAL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is the most effective treatment for high-risk acute myeloid leukemia (AML). Conventionally best results were obtained using matched siblings or unrelated donors. Over recent years, family HLA haploidentical family donors have become alternative options. In fact, the most recent results show chronic GvHD/relapse-free survival (GRFS) rates in the range of 40-50%, while leukemia relapse and chronic GvHD (cGvHD) still remain major issues. To overcome such limitations, we employed adoptive immunotherapy with regulatory T lymphocytes (Tregs) and conventional T lymphocytes (Tcons) in T-cell depleted HLA haploidentical HSCT. Early protocols resulted in promising clinical outcomes (56% disease-free survival). Here, in a cohort of 50 AML patients we show that an HLA haploidentical HSCT preceded by a myeloablative conditioning regimen that included an age-adapted radiation strategy followed by Treg/Tcon adoptive immunotherapy resulted in a 75% GRFS with 4% of leukemia relapse and 2% of cGvHD rates.

Methods: From 2014 to 2019 50 AML patients received a T cell depleted haploidentical HSCT followed by Treg/Tcon adoptive immunotherapy (clinicaltrials.gov NCT03977103). Median age was 53 years (range 20-65). Twenty patients had adverse genetic risk, 22 intermediate risk, 5 favorable risk and 3 patients could not be classified because of missing karyotype at diagnosis. Favorable risk patients were transplanted because they were in 2nd complete remission (CR) or had minimal residual disease (MRD). Thirty-three (66%) patients had detectable disease at transplant (25 MRD positive, 8 with active disease). The myeloablative conditioning regimen included TBI (single dose 8 Gy or fractionated 13,5 Gy) for fit patients younger than 50 years of age, or total marrow/lymphoid irradiation (13,5 Gy to the marrow, 11,7 Gy to lymph nodes and spleen) for patients who were unfit or older than 50 (up to 65). Irradiation was followed by chemotherapy (thiotepa, fludarabine, cyclophosphamide). Patients received 2x106/Kg freshly isolated donor Tregs on day -4, $1x10^6$/Kg Tcons on day -1 and $10.7x10^6$±3.4/Kg CD34+ hematopoietic progenitor cells on day 0. No pharmacological GvHD prophylaxis was given post-transplant. RESULTS: Patients achieved full-donor type engraftment. Fifteen/50 patients developed grade ≥ II acute (a)GvHD. Twelve/15 patients with aGvHD are alive and off immunosuppressive therapy. The only patient who developed cGvHD died. Non-relapse mortality occurred in 10/50 patients. Only 2 patients relapsed. Consequently, at a median follow up of 29 months, the probability of GRFS is 75%.

Conclusion: A haploidentical transplantation protocol that combined

an age-adapted myeloablative conditioning regimen with Treg/Tcon adoptive immunotherapy provided an unprecedented 75% chronic GvHD/relapse-free survival in 50 AML patients despite 31 were 50 to 65 years old.

C075

RESIDUAL DISEASE MONITORING BY QUANTITATIVE EVALUATION OF WILMS' TUMOR 1 (WT1) GENE EXPRESSION IN ACUTE MYELOID LEUKEMIA TREATED WITH DECITABINE.

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Background: Decitabine (DAC) has recently entered clinical practice for the treatment of acute myeloid leukemia (AML) in patients (pts) unsuitable for intensive chemotherapy allowing a complete cytological remission (cCR) in 25-30% of cases and a stable disease (SD) in 30% of cases with a survival benefit. To date, there are no studies evaluating the effect of DAC therapy on leukemic burden using molecular biology techniques. Aims: In this study we evaluated, in 40 AML pts, the effect of DAC treatment on the bone marrow (BM) leukemic burden by longitudinal evaluation, before each DAC cycle, of quantitative expression of the panleukemic marker Wilms' tumor gene (WT1).

Methods: Leukemic burden was sequentially evaluated in 40 AML cases overexpressing WT1 at baseline before the start of DAC therapy. WT1 quantitative analysis was performed on BM samples using RT Quantitative PCR method by WT1 ProfileQuant kit (Ipsogen), standardized according to the ELN guidelines; the cut-off for BM samples was 250 WT1 copies/104Abl, as reported by ELN. The pre-treatment WT1 levels were 3773 copies/104Abl (range 269-19414); 24 pts were treated with DAC in first-line and 16 pts received DAC as second-line therapy. The mean number of DAC cycles/patient was 7.4±6.6 in the first line and 3.4±1.7 in the second line.

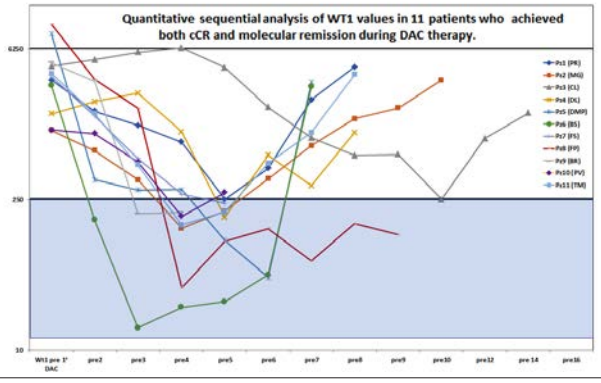

Figure 1.

Results: The cCR rate was 37.5%, the median OS was 40 months for pts who obtained a cCR, 13 months for the remaining pts treated in the first-line and 17.5 months for pts treated in the second-line (of which over 30% had received a SCT). In pts with cCR, the leukemic burden, measured by WT1 expression (obtained on day 1 of each DAC cycle), significantly decreased (P=0.0005; pre-treatment WT1 *vs.* WT1 at the best cytologic response); a molecular remission (WT1 <250 copies/104Abl) was detected in 73% (11/15) of the pts who achieved a cCR. In non responder pts, and/or in those with SD, the leukemic burden (WT1 expression) remained high during entire period of treatment with DAC. The sequential quantitative analysis of WT1 showed, in the 11 pts both in cCR and in molecular remission (WT1 <250 copies/104Abl), a progressive re-increase of WT1 over a few months with a subsequent loss of cCR ending in AML relapse (Figure 1).

Conclusion: These data confirm that DAC therapy improves OS of pts with AML unsuitable for intensive chemotherapy and that it may be a useful bridge to SCT for pts in which age and performance status allow a SCT procedure. DAC is able to achieve, in a proportion of treated cases (27.5%, 11 pts) a molecular remission (WT1 <250 copies/104Abl). However, this molecular remission, when obtained, is short lasting, despite continuing therapy with DAC, resulting in a cytological relapse and death. These biological results on leukemic debulking during DAC therapy support the need for combination therapies (DAC plus venetoclax or FLT3 inhibitors) to obtain a deeper and a long-lasting reduction of leukemic burden improving the patient outcome.

C076

DEFERASIROX TARGETS PHOSPHOLIPASE C BETA1 SIGNALING IN MYELODYSPLASTIC SYNDROMES (MDS)

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Introduction: MDS are characterized by anemia and transfusion requirements. Transfused patients frequently show iron overload that negatively affects hematopoiesis. Iron chelation therapy (ICT) can be effective in these MDS cases, but the molecular consequences of this treatment need to be further investigated. Phospholipase C beta1 (PI-PLCbeta1) regulates hematopoiesis through its downstream targets Cyclin D3 and Protein Kinase C (PKC) alpha. Therefore, here we studied the molecular effect of iron overload and Deferasirox (DFX) therapy on PI-PLCbeta1 inositide signaling.

Methods: We analyzed 24 MDS patients (IPSS Low or Intermediate-1). Before the therapy, all patients were transfusion-dependent and had a serum ferritin level ≥1000 ng/mL. Patients were treated with DFX, with a starting dose that was personalized according to their blood transfusion frequency, with the aim to reduce transfusion-dependent iron overload. Response was assessed according to the IWG response criteria. Flow cytometric analyses to detect cell cycle, apoptosis and oxidative stress, as well as inositide gene and protein expression were carried out in MDS samples and hematopoietic cell lines before and during treatment.

Results: 3/24 patients showed a hematologic response (hematologic improvement) during therapy. At baseline, patients later showing a positive hematologic response displayed higher levels of PI-PLCbeta1/Cyclin D3/PKCalpha expression, as compared with patients not showing any hematologic response and a pool of healthy subjects. In contrast, during treatment, responder patients showed a reduction of PI-PLCbeta1/Cyclin D3/PKCalpha signaling pathway. DFX was also able to specifically decrease the production of Reactive Oxygen Species (ROS). This correlated with a reduction of IL-1A and IL-2, as well as Akt/mTOR phosphorylation. On the contrary, cells from MDS patients who did not show any hematologic response during DFX therapy showed a specific increase of ROS and PI-PLCbeta1/Cyclin D3/PKCalpha expression.

Conclusions: Our data show that PI-PLCbeta1 signaling is a target for iron-induced oxidative stress and suggest that baseline PI-PLCbeta1 quantification could predict hematologic response to ICT in MDS.

C077

FOXM1 DEREGULATION IN MESENCHYMAL STEM CELLS FROM PATIENTS WITH MYELOID NEOPLASMS DE NOVO AND THERAPY-RELATED

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Introduction: Functional and morphological abnormalities of bone marrow (BM) mesenchymal stromal cells (MSCs) have been described in patients with *de novo* (dn) myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). MDS- and AML-MSCs exhibit multiple abnormalities, including impaired proliferative and clonogenic capacity, altered morphology, increased senescence, aneuploidy, impaired immunoregulatory properties and reduced hematopoietic support capacity. The BM-microenvironment may be irreversibly damaged by chemo- and or radiotherapy and play a role in the pathogenesis of therapy-related MN (t-MN). To study the properties of BM-MSCs isolated from patients with a t-MN, we evaluated the morphology and the clonogenic activity in BM-MSCs isolated from a cohort of 45 individuals, including 10 dn-AML, 10 dn-MDS, 15 t-MN and 10 healthy donors (HD). To this end, we evaluated expression of the transcription factor FOXM1 (Forkhead box M1), driving G2/M gene expression, whose repression has been linked to mitotic decline and aneuploidy-driven full senescence in old-aged primary human dermal fibroblasts.

Methods: BM-MSCs at 2nd passage were used for all experiments. mRNA levels of target genes were analyzed using a semi-quantitative real time. The nonparametric Mann-Whitney or Kruskall–Wallis one-way ANOVA tests were used for statistical analysis, considering a p value ≤ 0.05 statistically significant.

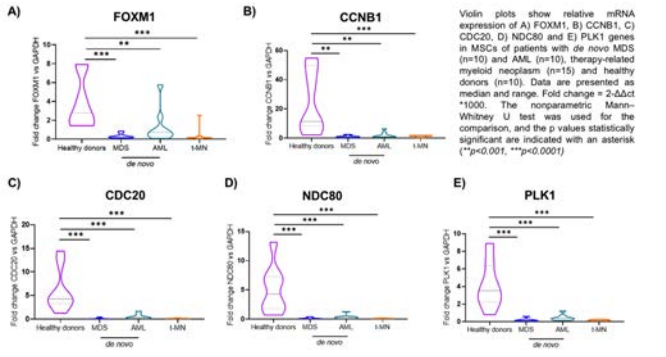

Figure 1.

Results: BM-MSCs cultures have been successfully obtained in all HD, while the expansion was not homogeneous in samples derived from patients with a MN (both de novo and therapy-related). The BM-MSCs expanded from HD showed a fibroblast-like morphology, in contrast to those isolated from patients with a MN, which appeared bigger, flatter and disorganized. Moreover, the clonogenic capacity of BM-MSCs, evaluated after 14 days of culture, was significantly reduced in patients compared to HD (p=0.008). All these functional alterations are suggestive of a senescent phenotype of the BM-MSCs isolated from MN patients, independent from previous cytotoxic therapy and BM-blast counts. A significant decrease of FOXM1 mRNA expression levels was observed in BM-MSCs isolated from patients compared to HD (p=0.0001, Figure 1A). This phenomenon was not evident in paired BM mononuclear cells, indicating that it is a typical alteration of the mesenchymal compartment. In this line, the expression levels of FOXM1 mitotic targets (CCNB1, CDC20, PLK1 and NDC80) were significantly decreased in BM-MSCs isolated from patients compared to HD (Figure 1B-E). Again, all these transcriptional alterations were independent from previous cytotoxic therapy and BM-blast counts.

Conclusions: Our preliminary data show a common senescent phenotype in BM-MSCs isolated from patients with de novo and therapy-related myeloid neoplasms. This may contribute to MSCs dysfunction and may affect their ability to support normal hematopoiesis, and contribute to the onset of MN.

C078

BONE MARROW AND PERIPHERAL BLOOD OF PATIENTS WITH HYPOCELLULAR-MYELODYSPLASTIC SYNDROMES ARE CHARACTERIZED BY PECULIAR CYTOTOXIC T- AND NK-CELL CLONAL EXPANSIONS

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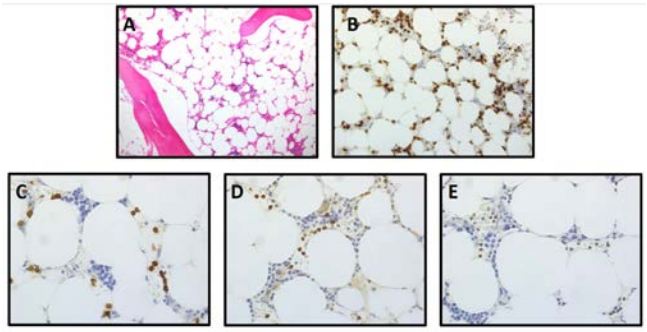
Background: Myelodysplastic Syndromes comprise a heterogeneous group of clonal hematopoietic stem cell (HSC) disorders. A rare subset (10-15%) of MDS, referred as hypocellular MDS (h-MDS), presents with a decreased bone marrow (BM) cellularity (<20-30%) and is characterized by discrete clinical features, including a high response rate to immunosuppressive therapy. This evidence suggests that, beyond HSC genetic/epigenetic alterations, an immune-mediated damage is likely to play a relevant pathogenetic role in this disease subset. However, the immune landscape of h-MDS patients is still poorly characterized. Since MDS have a high risk to evolve into acute myeloid leukemia, the stratification of patients in different risk-categories is central for therapeutic decisions. The aim of this study, through the analysis of T- and NK-cell in newly diagnosed h-MDS, was to identify novel prognostic biomarkers that might help in refining the current prognostic system, thus making appropriate therapeutic choices.

Methods: Thirteen newly diagnosed h-MDS patients were enrolled in a multi-center study promoted by the Italian Federation of MDS (FISM). Immunophenotypic, immunohistochemical and molecular analyses were performed in both BM and peripheral blood (PB) samples. Clinical data allowed to stratify patients according to the Revised-International Prognostic Scoring System (R-IPSS). RESULTS: According to the R-IPSS, we identify 3 very high/high (VH/H), 5 intermediate (INT) and 5 low/very low (L/VL) cases. Immunophenotypic and immunohistochemical analyses revealed an overall immune deregulation, characterized by BM infiltration with cytotoxic T- or NK-cells (Figure 1) and PB lymphocytosis, sustained by clonal T- or NK-cells with an effector memory phenotype. In detail, 3 (3/3, 100%) VH/H and 2 (2/5, 40%) INT risk patients had a clonal CD3+/CD8+/CD57+ T-cell expansion, assessed by TCR-γ gene rearrangement analysis. At variance, in only 1 (1/5, 20%)

L/VL risk patient a T-cell clone was detected. Clonal T-cell of 2 VH/H cases were also characterized by activating mutations in the hot-spot region of STAT3, a feature typically found in leukemic T-Large Granular Lymphocytes which is associated to a reduced OS. A clonal CD3-/CD16bright/CD56dim/neg/NKG2C+ NK-cell expansion, as demonstrated by the expression of a restricted pattern of Killer immunoglobulin-like Receptors, was reported in 1 (1/5, 20%) INT risk case and 3 (3/5, 60%) L/VL risk cases.

Discussion/Conclusions: We found the presence of distinguished innate and adaptive immune alterations in h-MDS, which might represent novel prognostic biomarkers and relevant predictors of response to therapy. Further studies in a large series of patients are warranted to elucidate the actual meaning of these alterations, both in terms of their pathogenetic role (*i.e.* whether they are detrimental, through a reduced immunosurveillance, or protective in controlling clonal HSC outgrowth) and of their clinical implications.



Immunohistochemical analysis of a BM biopsy from a h-MDS patient included in the VH-H R-IPSS risk-category. Staining with hematoxylin-eosin (A) reveals a strongly reduced cellularity (<10%). Staining with CD3 (B), CD57 (C), Perforin (D) and Granzyme B highlight the presence of BM infiltration by CD3+/CD57+ T-lymphocytes with cytotoxic properties.

Figure 1.

C079

POLY(ADP-RIBOSE) POLYMERASE INHIBITORS SYNERGIZE WITH ASCORBATE AND HYPOMETHYLATING AGENTS IN ARSENIC TRIOXIDE-RESISTANT PROMYELOCYTIC LEUKAEMIA CELLS

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Introduction: Poly (ADP-ribose) polymerase inhibitors (PARPi) are a new class of anticancer drugs, approved for ovarian, breast and pancreatic cancers. Recent preclinical studies have shown that PARPi have activity in acute myeloid leukaemia, myelodysplastic syndromes and acute promyelocytic leukaemia (APL). Regarding APL, current treatment with all-trans retinoic acid (ATRA) combined with anthracyclines or arsenic trioxide (ATO) induces long-term remission rates above 80%. However, 5-10% APL patients develop relapsed/refractory disease. Herein, we have investigated PARPi activity against APL cells rendered resistant to ATO, used as single agent or in combination with the pharmacological concentrations of ascorbate (ASC) and the hypomethylating agents azacitidine (AZA) or decitabine (DAC).

Methods: Four different clones (CL1, CL2, CL3, CL4), produced by limiting dilution from the NB4 APL cell line, were exposed to increasing concentrations of ATO (0.1-1 μM) in order to generate ATO-resistant clones (CL1-R, CL2-R, CL3-R, CL4-R). Sensitive and resistant clones were characterized for PML/RARA expression and proliferation rate. APL clones were treated with the PARPi olaparib (OLA, 1.25-10 μM), rucaparib (RUC, 1.25-10 μM), niraparib (NIR, 1.25-10 μM), veliparib (VEL, 5-20 μM) and talazoparib (TAL, 12.5-100 nM), as single agents or in combination with ASC (0.25-1 mM), AZA (1.25-1 μM) or DAC (25-500 nM). Drug concentrations tested included the C(max) reported in clinical trials. Cytotoxicity was evaluated by MTS assay and count of viable cells. Apoptosis was analysed by flow cytometry and detection of cleaved caspases, and DNA damage by γH2AX immunofluorescence and immunoblots.

Results: ATO-resistant cells (ATO IC(50) >1 μM) maintained the APL phenotype with the t(15;17) translocation and PML/RARA expression. These cells lacked the PML A216V/T-mutation that is reported in 40% of ATO-refractory APL cases. Treatment with ATO induced apoptosis in APL ATO-sensitive but not in ATO-resistant cell lines. The PARPi IC(50) values obtained in ATO-sensitive and -resistant clones were far below (OLA and NIR) or within the range (TAL) of the plasma C(max) reached in cancer patients. Conversely, RUC and VEL IC(50)s were above C(max). ASC, AZA or DAC, tested as monotherapy, induced cytotoxicity both in ATO-sensitive and -resistant clones. Furthermore, the combination of ASC, AZA or DAC with PARPi was highly synergic depending on the PARPi used (TAL for ASC, whereas OLA, NIR and TAL for the hypomethylating agents).

Conclusions: Our data on ATO-resistant cells, together with the acceptable toxicity profile of PARPi in cancer patients, suggest the therapeutic potential of the PARPi OLA, NIR and TAL as single agents or in combination with ASC (in the case of TAL) and hypomethylating drugs DAC and AZA (in the case of OLA and NIR) for refractory/recurrent APL. Furthermore, these results support the design of preclinical *in vivo* studies with PARPi in refractory APL.

C080

CD3+CD56+ CELLS (TR3-56) AND IMMUNE TOLERANCE CONTROL IN THE BONE MARROW (BM) OF MYELODYSPLASTYC SYNDROME (MDS) INDIVIDUALS

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Introduction: Immune system has been described as a major player in the pathogenesis of MDS. Indeed, in MDS early stages a deranged pro-inflammatory response has been demonstrated as relevant for damage of hematopoietic precursors in BM; in the advanced stages suppression of immune effectors has been related to the expansion of the dysplastic/leukemic clone/s. The involvement of cytotoxic lymphocytes in both, BM precursor damage as well as immune escape of neoplastic clones has been largely hypothesized. Recently, we described TR3-56 cells as a novel population of regulatory T cell, characterized by the co-expression of CD3 and CD56 molecules. This cell subset has been demonstrated to be specifically involved in the control of cytotoxic effector function (Terrazzano et al Terrazzano G. et al. Nature Metabolism 2, 142–152(2020).

Aim: We previously described that specific alterations of immune profile, as represented by low Treg levels and high expression of CD54 on CD8 effectors in BM, allow the identification of a subgroup of MDS patients in which an immune-mediated pathogenesis of the disease might be inferred. This study aims to analyze the role of TR3-56 cells in the pathogenesis of MDS. The possibility that such cell subset might represent a valuable biomarker of self-tolerance control in the BM of MDS patients has been also evaluated. Materials and methods: We examined BM and peripheral blood (PB) specimens of 65 newly diagnosed MDS patients and 6 healthy BM donors. Patients were classified according to IPSS score in three groups: Low Risk (N=31), Int-1 Risk (N=18), Int-2/High Risk (N=16). TR3-56 regulatory T cells, CD8 lymphocytes, CD4 lymphocytes, Treg and the expression of CD54 on CD8 lymphocytes were analyzed with a multiparametric flow cytometry approach. As in

our previous studies, a multistep cluster analysis algorithm was applied to classify MDS individuals according to BM Treg levels and activation of CD8 BM lymphocytes. Statistical evaluation of data has been performed by Mann-Whitney test.

Results: The analysis of TR3-56 regulatory T cells in the different MDS prognostic groups showed a significant increase ($p<0.05$) of this cell subset in the BM of Int-2/High Risk MDS patients (7,74±1,23) compared to healthy controls (2,85±0.33), and in the BM of Low Risk MDS patients (5,39±0.52) compared to healthy controls (2,85±0.33). Moreover, in Low Risk, significant BM recruitment of TR3-56 cells *vs.* PB in patients with high Treg level (>2% in BM) was observed. When the relationship of TR3-56 cells with activation of CD8 effectors in BM was analyzed, inverse association of BM TR3-56 cells with CD54 expression on CD8 T lymphocytes was found ($p<0.05$).

Conclusion: These data add TR3-56 cells to the complex immune scenario involved in the pathogenesis and progression of MDS. The possibility that such regulatory subset might represent a valuable marker to improve patient classification and clinical management of MDS needs further investigation.

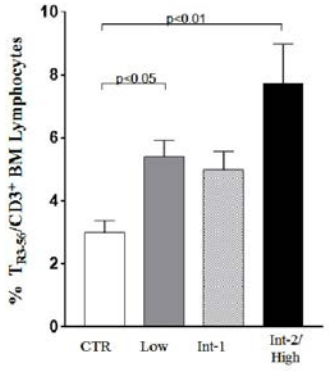

Figure 1.

Myeloproliferative Disorders and Chronic Myeloid Leukemia

C081

STUDY OF MUTATIONAL PROFILE IN MYELOPROLIFERATIVE NEOPLASMS PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS

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Introduction: Myeloproliferative neoplasms (MPN) are the most frequent underlying causes of splanchnic vein thromboses (SVT), including Budd-Chiari syndrome, portal, splenic and mesenteric vein thromboses. Whether there are genetic characteristics that can be correlated with this type of thrombotic events, that are very rare in general population, in the MPN group remains largely to be addressed. Therefore, we aimed to investigate the presence of possible correlation between mutation profile and SVT events in patients with MPN-SVT compared to MPN patients without SVT or with other different thrombotic events.

Methods: A total of 552 MPN cases diagnosed according to WHO 2016 criteria were collected and were stratified in three different group based on thrombotic event: group 1 (G1), consisting of 100 patients with SVT (39 PV, 25 TE, 19 PMF, 17 Pre-PMF), group 2 (G2) of 336 control MPN patient population who did not had an thrombotic events during follow-up (53 PV, 91 TE, 146 PMF, 46 pre-PMF), and group 3 (G3) including 116 patients that had thrombotic events other than SVT (28 PV, 59 TE, 17 PMF, 12 Pre-PMF). The molecular profiling of patients was performed on granulocytes using a NGS 27 genes panel on Ion Torrent S5 platform choosing a threshold of ≥5% VAF. The cumulative probability of Thrombosis-free survival (TFS) and Overall Survival (OS) was estimated using the Kaplan-Meier method; differences were estimated by the log-rank test.

Results: The SVT patients were more frequently mutated in JAK2V617F mutation (87% when compared to the other two groups examined (57.1% in G2 and 74.1% in G3). Moreover, Variant allele fraction (VAF) of JAK2V617F appears to be increased in patients of the first group (average: 48.6%±26.2%) compared to patients in G2 and G3 (average: 43%±21.6% and 38%±22.7% respectively). CALR mutations were found enriched in G2 (26.2%) and inversely correlated with thrombotic risk. ASXL1 mutation was found in 20% of G1, 30.7% of G2 and in 17.2% of G3 resulting to be inversely correlated thrombotic risk ($p<0.005$). SF3B1 was found mutated in none of the cases in G1, 6.9% of G2 and 1.8% in G3 ($p<0.006$). Lastly, somatic variants in DNMT3A, which was found in 8% of the first group cases, in 3.6% of the second and 9.5% of the third ($p<0.02$), were associated with thrombotic events although not specifically with splanchnic thrombosis. MPN-SVT patients displayed a median OS of 23.1 years, longer than other groups (16.3 yrs in G2 and 18.9 yrs in G3). Improved survival trend in the first group may result from the younger age of these patients (47.3 yrs *vs.* 59 yrs in G2 and 59.6 yrs in G3, $p<0.001$) and the earlier diagnosis of MPN.

Conclusions: These results confirm the association between the JAK2V617F mutation and SVT. Conversely, target deep-sequencing, ideally useful to identify patients with a different risk of thrombosis, failed to discriminate SVT patients from the conventional MPNs.

C082

INCIDENCE OF NON-CANONICAL MUTATIONS OF JAK2 AND MPL IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Introduction: The MPNs include Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF). Three usually mutually exclusive driver mutations in MPNs are the JAK2V617F mutation, CALR exon 9 mutations and MPL exon 10 mutation; their presence is indeed one of the major diagnostic criteria of MPN (WHO 2016). Although, around 10% of patients with ET or PMF lacking these driver mutations and they are defined as triple-negative (TN). Recently Whole Exome Sequencing (WES) studies allowed to identify non-canonical, either somatic or germline, mutations of JAK2 and MPL, which contributed to discover the pathogenetic mechanism of the 10% of TN ET or MF. Our study aims firstly to validate novel mutations of JAK2 and MPL genes identified by using target-sequencing (NGS) in patients with MPN diagnosis, secondly to identify their clonal origin, which may be germline or somatic and finally to establish whether these variants, alone or in association with driver mutations, are responsible for the clinical phenotype.

Methods: Our analysis included 14 patients with a suspected diagnosis of MPN. Final diagnostic workup identified the following diagnosis: 6 PMF, 3 MF-PPV, 1 MF-PET, 2 TN thrombocytosis and 2 TN erythrocytosis. Genomic DNA was extracted from granulocytes and only in 5 patients the DNA was extracted also from immunomagnetically purified T-cells (CD3+).

Results: NGS analysis detected 10 novel variant in JAK2 and MPL genes; 7 of them have been confirmed by Sanger sequencing: MPL-R592X (2 PMF), MPL-Y591H (2 MF-PPV), MPL-C322Y (MPN), MPL-G614A (PMF), JAK2-S1115C (1 MF-PET), JAK2-G301R (1 PV, 2 Erythrocytosis), JAK2-G281C (1 PMF). The remained 3 have been identified only in NGS because they had VAF<10% and were localised in MPL: Y591X (1 TN thrombocytosis), W632S (1 PFM) and S42Lfs*5 (1 TN thrombocytosis). All evaluable variants were considered somatic with the exception of JAK2-S1115C that was identified also in CD3+ cells. In 8 patients we observed the co-expression of the novel variants and the driver mutations (n=6 JAK2V617F, n=2 CALR ex9); 6 patients were TN, suggesting their possible pathogenetic role. The MPL-S42Lfs*5 and MPL-C322Y mutations are in extracellular domain (exons 2 and 6) and the rest of mutations in MPL are in intracellular domain (ex12); JAK2-G301R and JAK2-G281C mutations are in FERM domain (ex7) and JAK2-S1115C is in kinase domain (ex25).

Conclusion: The data suggest that novel somatic and germline variants in JAK2 and MPL genes may play a role in the onset of MPNs. It is worth noting that these uncommon variants in phenotypic drivers might lead to constitutive activation of JAK-STAT signalling and is worthy of further functional studies. Moreover, delineation of clonal architecture at the single cell level is key to understanding how the sequential/parallel acquisition of somatic mutations contributes to myeloid transformation.

C083

SOMATIC MUTATIONS ARE SHARED BETWEEN CIRCULATING ENDOTHELIAL CELLS (CEC) AND CD34+ HEMATOPOIETIC STEM CELLS (HSC) IN PATIENTS WITH PRIMARY MYELOFIBROSIS. CAN PMF-INITIATING CELLS DERIVE FROM A COMMON HSC/EC PRECURSOR?

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Introduction: Increased bone marrow vascularity is a hallmark of primary Myelofibrosis (PMF). The JAK2 driver mutation has been detected in micro-laser dissected endothelial cells (EC) (Sozer, 2009), suggesting that PMF clones could derive from a common precursor shared between EC and hematopoietic stem cells (HSC). We investigate this hypothesis, comparing the mutational profiles of paired circulating endothelial cells (CEC) and HSC.

Methods: Since June 2018, 14 PMF patients (pts) untreated with JAK2 inhibitors, along with 4 healthy controls, were enrolled in the MyCEC0617 study. HSC were selected using CD34+ immunomagnetic bead-column separation. CEC were detected using the CellSearch system, which uses immunomagnetic selection and fluorophore-labelled antibodies. CECs were identified as CD146+, CD105 +, DAPI+ and CD45- cells. Putative CEC were then sorted using the DEPArray system, combining di-electrophoresis technology and high-quality image-based cell selection. Sequencing data were then assessed with the MiSeq Illumina NGS platform using a 54-PMF related genes custom panel.

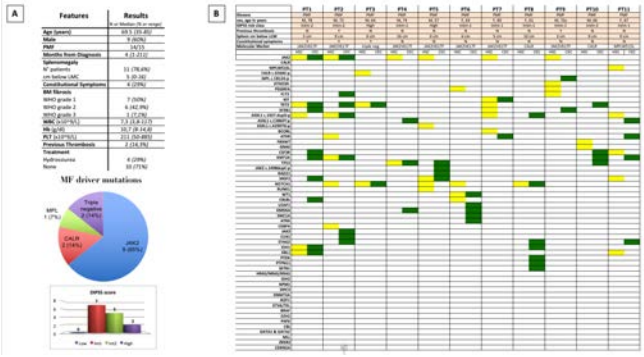

Figure 1. Patients' characteristics (A) and the molecular profile of both CECs and HSCs in pts with PMF (B). The genes mutated in HSCs are in Yellow, while in Green the ones in CECs.

Results: The patients' baseline characteristics are summarize in Figure 1. CECs were successful detected in 15 samples, while for 3 healthy controls the analyze is still ongoing. The PMF pts had a higher number of CEC detected compare to the healthy control [25.5(3.8-362) *vs.* 4.75/ml]. Subsequently, a median of 26 (1-122) CECs in 4 ml of peripheral blood were recovered in 12 of 15 samples. No mutations were found in the CEC or HSC from healthy control. The previously-identified MPN driver mutation was identified in HSC (except for one JAK2-mutated pt and for all CALR and MPL-mutated pts) (Figure 1). Overall, 24 of the 53 genes were mutated in HSC. Surprisingly, CEC presented several somatic mutations (28/53 genes), holding at least 2 mutations per cell (median: 4/CEC [2-9]). Interestingly, PMF pts demonstrated both shared and different mutations when comparing molecular profiles of HSC and CEC. In particular, 8 of 11 pts (73%) shared at least one mutation between ECs and HSCs. 2 pts harbored the JAK2 driver mutation, together with ABL1, IDH1, TET2, and ASXL1, respectively. The presence of JAK2V617F on CEC was not related to a previous history of thrombosis. The most frequently mutated genes shared between both CEC and HSC were JAK2, ASXL1, TET2, NOTCH1, and SRSF2. Considering the clinical characteristics analyzed, no differences were found between pts who shared mutations between HSC and CEC and those who did not.

Conclusion: For the first time, mutations other than JAK2 and MPL have been identified in mature EC in PMF pts, enhancing the role of a neoplastic vascular niche. In most instances, these mutations are shared between CEC and HSC. These preliminary findings support the notion that PMF-initiating cells may be derived from a common HSC/EC precursor. Further data are needed to validate these findings.

C084

PROSPECTIVE EVALUATION OF PERIPHERAL BLOOD CD26+ LEUKEMIA STEM CELLS IN CHRONIC MYELOID LEUKEMIA PATIENTS DURING TKI DISCONTINUATION (FLOWER-TFR STUDY)

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Background and rationale: In order to better identify CML patients (pts) suitable for an efficacious treatment free remission (TFR) are warranted additional biological criteria to molecular response. Leukemia stem cells (LSCs) are supposed to be the reservoir of disease. We first showed in a cross-sectional study that residual circulating CD34+/CD38-/CD26+ CML-specific LSCs are still detectable in the peripheral blood (PB) of the majority CML pts in sustained TFR (66%) despite stable and deep molecular response. Aims In prospective FLOWER-TFR multicenter study we monitored by flow-cytometry the number of circulating CD26+LSCs in CML pts from the time of TKI discontinuation until molecular relapse, if any.

Methods: CML pts meeting the current molecular criteria for TKI withdrawal entered this study. At time of stopping TKI treatment (baseline) and at +1, +2, +3, +6, +12 months (mos) after discontinuation and at any time of molecular relapse, CML pts were evaluated for number of PB CD34+/CD38-/CD26+LSCs by centralized flow-cytometry analysis and for BCR-ABL transcript levels by QRT-PCR assay.

Table 1. Patient's characteristics and Results:

TOTAL PATIENTS		72
Median age at diagnosis		68 (19-71)
Sex	Male	39 (54%)
	Female	33 (46%)
Sokal skore	High	10/72 (14%)
	Intermediate	22/72 (30,5%)
	Low	36/72 (50%)
	n.a.	4/72 (5,5%)
	IMATINIB	44
	NILOTINIB	20
	DASATINIB	8
Median TKI treatment duration before discontinuation (months, range)		103 (38-232)
Median duration of treatment according to TKI (months, range)	IMATINIB	124 (38-232)
	NILOTINIB	92.5 (50-151)
	DASATINIB	65.5 (59-170)
Measurable circulating CD26+ LSCs at time of discontinuation	YES	37/72 (51%)
	NO	35/72 (49%)

	TOTAL (72)	TFR SUSTAINED (52)	TFR LOSS (20)
CD26LSC+ detectable BCR-ABL/ABL ratio detectable	10/72(14%)	6/10 (60%)	4/10 (40%)
CD26LSC+ detectable BCR-ABL/ABL ratio undetectable	27/72 (37,5%)	19/27 (70%)	8/27 (30%)
CD26LSC+ undetectable BCR-ABL/ABL ratio detectable	16/72 (22%)	12/16 (75%)	4/16 (25%)
CD26LSC+ undetectable BCR-ABL/ABL ratio undetectable	19/72 (26,5%)	15/19 (79%)	4/19 (21%)

Results: 72 consecutive CML pts were enrolled. After a median observation time of 11 mos since TKI withdrawal (1-37 mos), 20/72 (28%) pts lost their molecular response and restarted TKI treatment while 52/72 (72%) are still in TFR; of note 12/72 (17%) pts have so far discontinued the treatment for ≤ 6 months. The median time to relapse after discontinuation was 4 mos (range 2-7 mos) (Table 1). At the time of discontinuation, residual CD26+LSCs, were detectable in 37/72 (51%) pts: of those 25/37 (67%) sustained TFR and 12/37 (33%) lost response. The median number of detected CD26+LSCs was 0.0237μ/L (range 0.0077-0.1197) with minimal fluctuation at different time points. On the other hand, 35/72, 49% pts showed no detectable CD26+LSCs at time of discontinuation: 27/35 (77%) pts maintained TFR and 8/35 (23%) pts lost response. No statistical correlation between BCR-ABL/ABLIS ratio and number of residual CD26+LSCs was found. However, we observed that pts in which both LSCs and BCR-ABL copies were detectable had the highest percentage of TFR loss while pts with both undetectable LSCs and BCR-ABL copies had the lowest probability to TFR loss (Table 1).

Conclusions: Our results confirm that CD26+LSCs are detectable at time of TKI discontinuation and during TFR. Moreover, the persistence of "fluctuating" values of CD26+LSCs do not hamper the possibility to maintain a stable TFR. Pts discontinuing TKIs with no detectable CD26+ and no detectable BCR-ABL copies appear to have less probability to undergo TFR loss (21%) compared to pts with both detectable CD26+LSCs and BCR-ABL (TFR loss 40%). However, no correlation between BCR-ABL/ABLIS ratio and number of residual CD26+LSCs was found. Additional studies evaluating CD26+LSCs ability to modulate the immune system through a variable expression of immune response inhibitory molecules are ongoing.

C085

MEK-INHIBITORS/ARSENIC TRIOXIDE COMBINATION THERAPY AS A NOVEL TREATMENT OPTION FOR TYROSINE KINASE INHIBITORS-RESISTANT PH+-LEUKEMIA

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Despite the unprecedented efficacy observed with Bcr-Abl tyrosine kinase inhibitors (TKIs) in the management of chronic myeloid leukemia (CML), resistance and intolerance towards first-, second- and third-generation Bcr-Abl TKIs are frequently reported. This calls upon the need to identify new therapeutic strategies that may improve the therapeutic outcome of TKI-resistant Ph+-leukemia patients. Based upon previous findings, we have hypothesized that combined treatment with MEK inhibitors and arsenic trioxide (ATO) may provide the wanted cytotoxic effects against TKIs-resistant Bcr-Abl leukemic cells. We therefore first analyzed the pharmacologic interactions between the MEK inhibitor PD0325901 (PD) and ATO on Bcr-Abl-positive leukemia cell lines displaying different levels of resistance to TKIs, using a fixed-ratio treatment paradigm. We found that combination treatment with PD and ATO resulted in a synergistic induction of apoptosis in AR230-R, LAMA-R, Ba/F3 p210Y253F, Ba/F3 p210T315I and K562-R TKI-resistant cell lines. We next determined whether PD and ATO also induced apoptosis of primary cells from patients with BCR-ABL-driven CML and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) who had manifested resistance or intolerance to the TKIs Imatinib, Nilotinib and/or Dasatinib, or carried the T315I BCR-ABL mutation. Mononuclear cells derived from blood or bone marrow of patients with CML or Ph+ ALL harboring native BCR-ABL or BCR-ABLT315I were then treated with PD, ATO or both compounds and cell death was monitored by either sub-G1 or Annexin V/PI analysis. Similarly to cell lines, we found that PD significantly ($P<0.02$; n=5) increased ATO-induced cell death also in primary TKI-resistant CML or Ph+ ALL cells. We further observed that the combination PD/ATO promoted the accumulation/activation of the proapoptotic and antiproliferative transcriptionally active (TA)-p73 isoforms and transcription of their proapoptotic target

genes Bax, Bak, Puma and/or P53AIP1. Consistent with these results, we found that PD greatly enhanced the ATO-induced mitochondrial depolarization, caspase-3 activation in all the analyzed BCR-ABL TKI-resistant cell lines, as well as in primary leukemic blast cells derived from patients clinically resistant to TKIs (n=5). Furthermore, in support of the antiapoptotic role of MEK were the findings that experimental overexpression of a constitutively active mutant variant of MEK in TKI-resistant leukemia cells caused enhanced phosphorylation of ERK1/2 and protected against ATO-induced cell death. By contrast, the chemical or genetic loss-of-function of MEK consistently and significantly decreased the basal ERK1/2 activity in these engineered leukemia cells and significantly ($p<0.001$) enhanced their apoptosis in response to ATO. Finally, combination of PD/ATO significantly ($p=0.001$) prolonged survival of mice carrying BCR-ABL-T315I-induced CML, when compared to either treatment alone. The findings highlight the potential of using a therapeutic approaches combining MEK inhibitors and ATO for the treatment of TKI-resistant Ph+-Leukemia.

C086

CIRCULAR RNAS DEREGULATION IN CD34+ CELLS OF MYELOFIBROSIS

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Introduction: Myelofibrosis (MF) is a clonal hematopoietic stem cell disorder belonging to the chronic myeloproliferative neoplasms (MPN). Mutations in JAK2, MPL and CALR genes are considered "phenotypic driver mutations"; however, the mutational landscape of MPN appears to be much more complex, involving genes of epigenetic regulation, of the spliceosome, or other oncogenes. Circular RNAs (circRNAs) is a class of RNAs originated from the backsplicing of 3' and 5' ends covalently linked. CircRNA functions, mostly unknown, can be exerted through interactions with microRNAs and proteins or coding peptides. Recent studies indicate that circRNAs are involved in the pathogenesis of multiple cancers, including leukemias of the myeloid lineage. This study aims to provide an overview of circRNA expression profiles in MF.

Methods: CircRNAs were detected and quantified by CirComPara pipeline (Gaffo et al. NcRNA J. 2017) that implements 6 circRNA detection Methods: CircComPara was applied to high-depth ribodepleted RNA-seq data of 8 MF samples of CD34+ cells purified from peripheral blood and 3 samples sorted from healthy donors (HD). Differential expression (DE) was assessed using DESeq2 (adj. p-value<0.05). Validation of selected circRNAs was performed in CD34+ cells and granulocytes (GN) of MF and HD both by digesting cDNA with RNase R followed by Sanger sequencing and by qRT-PCR.

Results: Of the 48,775 circRNAs detected by at least two methods, 94% derived from exonic regions, with a mean of 5.8 circRNAs per gene. Focusing on the 3,455 circRNAs most expressed, comparison of CD34+ cells of MF patients and HD, revealed a dramatic deregulation of the circRNAome, with 1,166 significantly DE circRNAs, mostly (96%) up-regulated in MF. We selected 4 circRNAs highly expressed in MF and absent in controls according to RNA-seq data, including one circRNA previously associated to myeloid leukemias (Hirsch S, Haematologica 2017): circPLOD2, circL3MBTL4, circNRIP1 and circNPM1. For all of them, upregulation in CD34+ cells of MF compared with HD was confirmed by qRT-PCR analysis. We then asked whether abnormal expression of the four circRNAs could also be demonstrated in GN, which would represent a more convenient source for analysis than CD34+ cells. Preliminary results showed that CD34+ cells displayed a more pronounced expression of circRNAs than in GN, suggesting their possible contribution to MF abnormal myeloproliferation. Moreover, we did not get any difference in circRNAs expression according to driver mutation status.

Conclusions: In the present study, the expression profiles of circRNAs in myelofibrosis were investigated. We obtained robust data on the upregulation in MF of four circRNAs, opening the way to the ongoing functional investigation by in silico functional predictions and, *in vitro*, by circRNA silencing.

C087

RESPONSE TO AVAPRITINIB IN PATIENTS (PTS) WITH ADVANCED SYSTEMIC MASTOCYTOSIS (ADVSM) WITH AND WITHOUT PRIOR MIDOSTAURIN THERAPY

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Introduction: Systemic mastocytosis (SM), a rare clonal mast cell (MC) neoplasm is KIT D816V mutation-driven in ≈95% of pts. AdvSM treatments are few, with limited efficacy. EXPLORER phase 1 study in AdvSM investigated the recommended phase 2 dose (RP2D), safety and preliminary efficacy of avapritinib, a novel selective and potent KIT D816V inhibitor.

Methods: Primary objectives: maximum tolerated dose (MTD), RP2D and safety. Secondary objective: centrally reviewed overall response rate (ORR) by modified International Working Group-Myeloproliferative Neoplasms Research and Treatment and European Competence Network on Mastocytosis (mIWG-MRT-ECNM) criteria (complete remission [CR]+CR with partial peripheral blood counts recovery [CRh]+partial remission [PR]+clinical improvement [CI] lasting ≥12 weeks). Pt-reported outcomes (dose expansion phase): use of AdvSM-Symptom Assessment Form (AdvSM-SAF).

Results: As of Aug 30, 2019, 80 pts were enrolled: 7 aggressive SM (ASM); 44 SM with associated hematologic neoplasm (SM-AHN); 11 MC leukemia (MCL); 16 indolent SM/smoldering SM; 1 non-SM diagnosis; 1 diagnosis pending central adjudication. Dose escalation: 32 pts received avapritinib 30–400 mg orally once daily. MTD was not reached. Dose expansion: 48 pts received 200 or 300 mg. Of 62 AdvSM pts, 48 were ORR-evaluable; 14 were not evaluable. Pts receiving 200 or 300 mg had similar efficacy and time to response. Most frequent adverse events (AEs; all grades/grade ≥3) were periorbital edema (71%/4%), anemia (55%/29%), diarrhea (41%/1%), fatigue (40%/9%), peripheral edema (40%/0%), nausea (39%/4%), thrombocytopenia (39%/26%), vomiting (34%/4%), and cognitive effects (34%/4%). 44% of pts (4/9) with baseline grade 3 thrombocytopenia (platelets <50,000/μL) had an intracranial bleeding (ICB) event. Of 71 pts with baseline platelets ≥50,000/uL, 2 (3%) had ICB events and grade 3 thrombocytopenia developed after treatment start. 12 (15%) pts discontinued treatment due to clinical progression and 6 (8%) due to treatment-related AEs. All pts exhibited ≥50% serum tryptase reduction, marrow MC aggregates were eliminated in 85% of pts, and KIT D816V allele fraction decreased by ≥50% and to <1% in 92% and 68% of pts, respectively. ORR and best responses (all evaluable pts) by AdvSM subtype, and prior midostaurin exposure are listed in the Table 1. Median duration of response and overall survival were not reached for the whole cohort or each AdvSM sub-

type. AdvSM-SAF scores were significantly improved by cycle 3 (P=0.0037) and sustained at cycle 11 (P=0.015).

Conclusions: Optimal avapritinib starting dose was 200 mg once daily. ICB emerged as a treatment-related AE in pts with severe thrombocytopenia at baseline. Regardless of prior midostaurin exposure or AdvSM subtype, avapritinib induced rapid, deep and durable reductions in measures of MC burden and disease-related symptoms. Safety and efficacy of avapritinib in AdvSM are being further studied in the PATHFINDER phase 2 study.

Table 1.

Outcome	All (n=48)	ASM (n=3)	SM-AHN (n=35)	MCL (n=10)	All (n=48)	
					Prior Mido (n=15)	No prior Mido (n=33)
ORR, %	77 (63–88[a])	100	77	70	60	85
CR, n (%)	4 (8)	0	2 (6)	2 (20)	0	4 (12)
CRh, n (%)	9 (19)	2 (67)	7 (20)	0	0	9 (27)
PR, n (%)	20 (42)	1 (33)	16 (46)	3 (30)	8 (53)	12 (36)
CI, n (%)	4 (8)	0	2 (6)	2 (20)	1 (7)	3 (9)

[a]95% confidence interval. Mido, midostaurin.

C088

IDENTIFICATION OF POLYCYTHEMIA VERA-SPECIFIC BIOMARKERS OF OUTCOME BY NOVEL MULTIDIMENSIONAL ANALYSIS APPROACHES

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Polycythemia Vera (PV) is a myeloproliferative neoplasm with increased risk of thrombosis and progression to myelofibrosis. PV-specific biomarkers of outcome are an unmet need. Extracellular vesicles (EVs) are increased in thrombosis and cancer. Most circulating EVs are of megakaryocyte (MK-EVs) and platelet (PLT-EVs) origin. Phosphatidylethanolamine (PE)/tissue factor (TF)-EVs play a role in malignant thrombosis. A dysbiotic gut microbiota (GM) can contribute to cardiovascular diseases; moreover, recent evidence points to circulating microbial components or even microbes, as potential players in blood homeostasis. Here we aimed to identify PV-specific biomarkers of outcome by evaluating the phenotype and microbial DNA cargo of circulating EVs and the GM. Peripheral blood and feces were collected from PV patients (pts; n=38) and healthy donors (HD; n=30). Pts (high (n=27)/low (n=11) risk) were under phlebotomy (PLE; n=9) or hydroxyurea (HY; n=15) only, PLE+HY (n=12) and cardioaspirin (n=26). Thrombosis history was recorded in 14 pts (before/at/after diagnosis). Circulating MK-(CD61+/CD62P-), PLT-(CD61+/CD62P+), PE-, and TF-EVs were analyzed by flow cytometry in PLT poor plasma (PPP). EVs were isolated from PPP by ultracentrifugation and lipopolysaccharide (LPS) associated-EVs were evaluated by flow cytometry. After microbial DNA extraction from feces/EVs, the 16S rDNA V3-V4 region was sequenced on Illumina MiSeq. Raw sequences were processed using QIIME 2. Statistical analysis: R and Graphpad 8. MK-/PLT-EVs were decreased/increased in PV pts ($p<0.001$) (Figure 1a, b). Notably, pts with thrombosis history showed increased/decreased proportion of MK-/PLT-EVs compared to pts without thrombosis ($p<0.05$; Figure 1c, d). PE-/TF-EVs were also increased in PV pts ($p<0.001$; Figure 1e, f). Of note, PE-EVs were increased in pts with bone marrow (BM) fibrosis ($p<0.05$; Figure 1g). Interestingly, also LPS-associated EVs were increased in PV pts (Figure 1h; $p<0.05$). The analysis of the microbial DNA cargo of isolated EVs revealed a peculiar profile in PV, with higher diversity (inverse Simpson, $p<0.001$) and a microbial composition distinct from HD ($p<0.001$; Fig 1l, m). In particular, we found a depletion of Bradyrhizobium in PV pts ($p<0.001$; Figure 1n). Of note, EVs from pts with thrombosis history were depleted in Staphylococcus compared to pts without thrombosis history ($p<0.01$; Figure 1o). It is worth noting that pts with thrombosis history also showed lower levels of LPS-associated EVs ($p<0.05$; Figure 1i). Conversely, EVs of pts with BM fibrosis were enriched in Coriobacteriaceae ($p<0.05$; Figure 1p). The GM analysis failed to identify distinct layouts between PV and HD. However, a significant depletion in Veillonellaceae was observed in pts with BM fibrosis compared to pts without BM fibrosis ($p<0.01$; Figure 1q). Here we identified a disease-specific and EVs-based signature associated with thrombosis and BM fibrosis in PV. These data may contribute to refine PV prognosis and therapy.

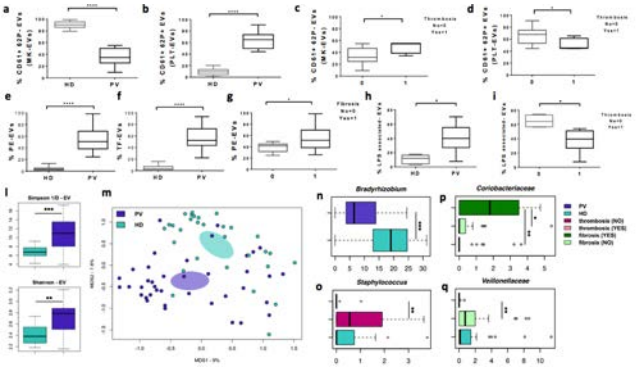

Figure 1.

Stem Cell Transplantation

C089

CORRELATION BETWEEN PLASMATIC EXTRACELLULAR VESICLES AND ACUTE GRAFT VERSUS HOST DISEASE AFTER POST-TRANSPLANT CYCLOPHOSHAMIDE HAPLOIDENTICAL STEM CELL TRANSPLANTATION

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Introduction: Acute Graft-versus-Host-Disease (aGVHD) is a frequent complication after allogeneic stem cell transplantation (SCT) despite significant advances in transplant procedures. We recently explored the diagnostic value of extracellular vesicles (EVs) as non-invasive biomarkers of aGVHD (Lia G *et al*. Leukemia 2018). In this new study we further investigated the correlation of plasma EVs surface proteins and their content in miRNAs with the risk of developing aGVHD in the setting of post-transplant cyclophosphamide haploidentical-SCT.

Methods: Plasma samples were collected at given time-points from 32 patients (pre-transplant, on day 0, 3, 7, 14, 21, 28, 35, 45, 60, 75 and 90 after transplant). EVs were extracted by a precipitative method and characterized by Nano-tracking Particle and by flow-cytometry with a panel of 14 antibodies (Lia G *et al*. Leukemia 2018). Total RNA content was extracted from EVs and three miRNAs (miR100, miR194, miR155) were quantified by qRT-PCR. Concomitant plasma concentrations of human Tumor Necrosis Factor Receptor I (TNFR1), Regenerating Family Member 3 Alpha (REG3a) and Suppression of Tumorigenicity 2 (ST2) were also quantified. Associations between plasma EVs and their miRNAs content and soluble biomarkers was evaluated by logistic regression models for each marker.

Results: Cumulative incidence of aGVHD at day 100 was 21.9% (95% confidence interval: 9.6–37.2%) with an onset median time of 41 days. Logistic regression model showed that CD146 fluorescence and CD30 EVs concentration were associated with a significantly increased risk of acute GVHD (OR 2.93 p<0.001 and OR 1.40, p=0.051, respectively); whereas, a significant decrease risk was associated with concentration of Total Evs (OR 0.53, p=0.01), CD120a (OR 0.58, p=0.018), CD140a (OR 0.55, p=0.013), CD26 (OR 0.59, p=0.017), CD31 (OR 0.62, p=0.047) and CD144 (OR 0.70, p=0.034). MiR100, miR155 and miR194 were all correlated with aGVHD (OR 3.90 p<0.001; OR 1.84 p=0.008; OR 2.68 p<0.001, respectively). Plasmatic hTNFR1 and ST2 were also correlated with aGVHD (OR 1.47 p=0.04; OR 1.55 p=0.05, respectively). In addition, we found that two combinations of CD146-miR100-miR194-TNFR1 increase their discrimination ability to predict aGVHD (multivariable AUROC > 0.975).

Conclusion: Most of the biomarkers correlated with aGVHD are highly expressed on endothelial cells suggesting an important role of endothelium damage in the pathogenesis of aGVHD. The association of miRNA100, miRNA155 and miRNA194 and aGVHD was also significant. Interestingly, MiRNA100 was reported to regulate inflammatory neovascularization during GvHD while miR-155 plays a role in donor T cell expansion. Moreover, using three markers combination (CD146-miR100-TNFR1 or CD146-miR194-TNFR1) could greatly improve aGVHD predictivity. A larger prospective study on patients is in progress to confirm our preliminary findings.

C090

IMMUNOTHERAPY IN ACUTE LEUKEMIA: A GITMO (GRUPPO ITALIANO TRAPIANTO MIDOLLO OSSEO) SURVEY ON EFFICACY AND TOXICITY OF DONOR LYMPHOCYTE INFUSIONS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background and aims: Donor lymphocyte infusions (DLIs) have provided a way to enhance the graft-versus-leukemia (GVL) effect after allogeneic stem cell transplantation (HSCT) for decades, but their role in the treatment strategy of acute leukemias (AL) is still poorly defined. We conducted a retrospective multicentre study including all consecutive pediatric and adult patients with AL who received DLIs after HSCT between January 1, 2010 to December 31, 2015, in order to determine the efficacy and the toxicity of the treatment.

Methods: 252 patients, median age 45.1 years (1.6-73.4) were enrolled from 35 Italian Transplant Centres. Median follow-up was 878 days (55-6754) after HSCT. The main characteristics of patients, donors and transplants were provided by the Italian GITMO Registry; other DLI-specific information were requested to the centre in a study-specific database. OS was calculated from HSCT and first DLI, and the impact of prognostic factors was evaluated.

Results: The underlying disease was AML in 180 cases (71%), ALL in 68 patients (27%), biphenotipic AL in 4 patients (2%). Donors were HLA identical sibling (39%), unrelated (40%) or haploidentical (21%). Conditioning regimens were myeloablative in 71% of patients. The first DLI was administered at a median of 258 days (55-6754) after HSCT. The main indication was leukemia relapse or persistence (73%), followed by mixed chimerism (17%) and preemptive/prophylactic use (10%). 40% of patients received no treatment before the first DLI, while radiotherapy, conventional chemotherapy or targeted treatment were administered in 3%, 39% and 18% of patients, respectively. 156 (62%), 91 (36%), 49 patients (19%) received 2, 3 or ≥ 4 infusions, respectively, with a median of 31 days between 2 subsequent DLI. An escalating schedule was mainly chosen, ranging from a median dose of 1 to 10 $x10^6$/kg CD3+ lymphocytes. Severe adverse events were a few, including 3% grade III-IV GVHD, 11% grade III-IV haematological toxicity and 3 DLI-related deaths. 46 patients (18%) performed a second HSCT after a median of 232 days (32-1390) from the first DLI. Median survival was 915 days (55-6754) from the first HSCT and 466 days (2-3255) from the first DLI, respectively. In multivariate analysis older recipient age and haploidentical donors significantly reduced OS (HR 1.02; 95% CI 1.01-1.03; p=0.001 and HR 3.1; 95% CI 1.92-5.01; p=0.000, respectively), whereas DLI administration caused by mixed chimerism or preemptive/prophylactic treatment in comparison with AL relapse and an escalating schedule in comparison with a single dose significantly prolonged OS (HR 0.44; 95% CI 0.26-0.75; p=0.002; HR 0.25; 95% CI 0.13-0.50; p=0.000; HR 0.87; 95% CI 0.76-0.99; p=0.033).

Conclusion: This GITMO survey confirms that earlier DLI administration and escalating schedule were associated with a favourable outcome in AL patients. DLI from haploidentical donors had a poor outcome and may represent an area of further investigation.

C091

REGULATORY T CELL ADOPTIVE IMMUNOTHERAPY PROMOTES B CELL IMMUNITY AFTER HAPLOIDENTICAL TRANSPLANTATION

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Introduction: Mouse bone marrow (BM) CD4+CD25+FOXP3+ regulatory T cells (Tregs) localize in the hematopoietic stem cell (HSC) niche, where they contribute to HSCs maintenance and promote donor engraftment and B cell lymphopoiesis. Adoptive transfer of donor Tregs is employed to prevent Graft versus Host Disease (GvHD) mediated by conventional T cell (Tcons) in haploidentical hematopoietic cell transplantation (haplo-HCT). We are investigating if human Tregs promote B cell reconstitution and immunity in preclinical models and in patients undergoing haplo-HCT with Treg/Tcon immunotherapy.

Methods: Human Tregs and purified CD34+ HSCs from healthy donors were co-infused in sublethally irradiated (2 Gy) immune-deficient NSG mice. Donor engraftment and B cell reconstitution were analysed by flow cytometry and histology. B cell reconstitution in BM and peripheral blood (PB) were assessed monthly by flow cytometry in 66 patients with hematologic malignancies who underwent either Treg/Tcon haplo-HCT, or T-cell depleted haplo-HCT or haplo-HCT with post-transplant cyclophosphamide. PB total immunoglobulin (Ig), anti-Cytomegalovirus (CMV) IgM and CMV viremia were monitored.

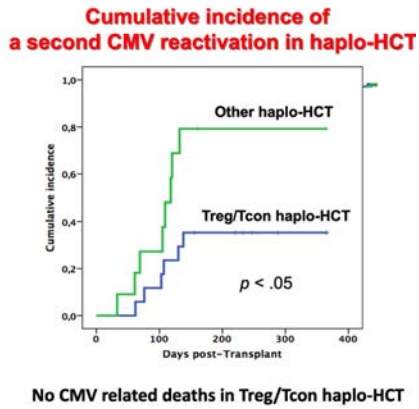

Figure 1.

Results: In preclinical xenogeneic models, treatment with Treg adoptive immunotherapy increased BM and PB human chimerism (p=0.009). One month after the infusion, *in vivo* expanded human CXCR4+ Tregs were detected in histological sections of the femurs of mice co-infused with Tregs. Moreover, these mice had a lower number of HSCs, which localized preferentially in the epiphyseal areas of the femurs, where donor HSCs started to engraft. These results suggest human Tregs facilitate donor HSC engraftment and differentiation. HSC-derived mature B cells were more abundant in PB of Treg-treated mice starting from 30 days after infusion. B cell reconstitution was faster after Treg/Tcon haplo-HCT compared to other protocols. We could detect early frequencies of common lymphoid progenitors and B cell precursors in the BM of these patients, that resulted in an increased production of immature, transitional and mature B cells. B cell counts in PB were also higher and were comparable to those of healthy subjects by 4 months after transplant. Hypogammaglobulinemia was rapidly corrected in Treg/Tcon haplo-HCT patients. Total IgM were higher and reached normal levels by 3 months after transplant (p=0.008). CMV reactivation rate was lower (41% *vs.* 79%). Anti-CMV specific IgM were documented in 41% of CMV seropositive patients by 4 months after Treg/Tcon haplo-HCT, while they were almost undetectable until 6 months after other haplo-HCT transplants. Such rapid primary B cell responses contributed to reduce second CMV reactivations (p<0.05, Figure 1) and no patients died because of CMV disease.

Conclusions: Adoptive transfer of human Tregs boosts donor HSC engraftment and facilitates the reconstitution of functional donor B cells. Treg/Tcon immunotherapy promotes B cell immunity and control of infections in patients undergoi

C092

DETECTION AND CHARACTERIZATION OF MMAA-SPECIFIC T CELL RESPONSES IN PATIENTS WITH MGUS, SMM AND MM

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Introduction: This project aims at screening the presence of antineoplastic T-cell responses in high-risk MGUS/SMM patients, selecting the most immunogenic multiple myeloma associated antigens (MMAAs) able to induce robust T cell immunity, possibly in association with better disease control, and testing the feasibility of expanding effective autologous cytotoxic T lymphocytes (CTLs), in order to develop immunotherapy protocols to prevent progression from high-risk MGUS/SMM to MM.

Methods: Quantitation and functional profiling of MMAA-specific T-cell responses were performed by IFNγ-Elispot and Cytokine Secretion Assay (CSA). As myeloma-specific stimulation we used pools of peptides derived from different MMAAs, namely WT1, NY-ESO-1, Melan-A/MART-1, MAGE-A1, SOX-2, JAM-1, SLAMF-7, DKK-1 and BCMA. Short-term expansion of MMAA-specific T lymphocytes was performed pulsing PBMCs with all the antigens aforementioned. At day +15 responder cells were tested for anti-myeloma activity by 51Cr-release cytotoxicity assay against autologous PHA blasts pulsed with each antigen separately.

Results: We enrolled 19 case patients (9 high risk MGUS and 10 SMM patients) and 14 control patients (9 low risk MGUS and 5 MM patients). PB samples were collected at the enrolment and at follow-up visits and used for immunologic analysis. The IFNγ-Elispot assay documented the presence of antineoplastic T-cell responses against at least one antigen in 26 out of 33 (78%) patients. Although MMAA-specific T cells were detected both in patients at pre-neoplastic stages of disease and in patients with MM, patients with high risk MGUS and SMM (n=19) showed the wider and more intense responses, mainly directed against SLAMF-7 (63%), Ny-ESO and h-TERT (58%, both). Both Effector Memory (CD62L-/CCR7-;CD45RA+/-) and Central Memory (CD62L+/CCR7+/ CD45RA-) T-cell phenotypes, mainly either CD8+ or CD4+ respectively, were observed. Interestingly, the presence of MMAA-specific T cells seems to correlate with a better disease control. In all 5 case patients tested, the short-term expanded CTLs were able to induce cytotoxic responses, with a percentage of specific lysis at least >10%, mainly against JAM-1, DKK-1, SLAMF-7, hTERT and SOX-2. Specific cytotoxicity was mediated by both CD8+ and CD4+ T cells, as lysis was observed both with the 5-hr and 12-hr assay.

Conclusions: These results show the presence of robust and wide antineoplastic T cells in patients with high risk MGUS and SMM, able to exert anti-myeloma cytolytic activity against different MMAAs. Moreover, such MMAA-specific T-cell immunity seems to correlate with patients' clinical outcome and may represent a novel prognostic factor

in MGUS patients. Further experiments will aim at identifying anti-myeloma T-cell responses in association with better disease control and/or useful as prognostic factor and selecting the most promising MMAAs to be exploited for CTL expansion.

C093

CLINICAL-GRADE EXPANDED TREGS ARE ENRICHED ON HIGHLY SUPPRESSIVE CELLS PRODUCING IL10, GRANZYME B AND IL35

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Introduction: In T cell depleted full-haplotype mismatched transplantation for high-risk acute leukaemia adoptive immunotherapy with Tregs and Tcons prevented GvHD, improved post-transplant immunological reconstitution and is associated with a powerful GvL effect. To improve purity and numbers of tinfused Tregs, GMP-compatible expansion protocols are needed. Here we expanded Tregs by using an automated clinical grade protocol and cells were extensively characterized *in vitro* and their efficiency was tested *in vivo* in a mouse model.

Methods: Tregs and Tcons were collected from 3 healthy donors. Tregs were selected by CliniMacs and expanded for 14 days with CliniMACS Prodigy System. Expanded cells were sorted with anti-CD39, anti-CD62L and anti-TIM3 moAbs and mRNAs for IFNg, IL10, TGFb, CD39, CTLA4, Granzyme B (GrB), Perforin and IL35 were analysed. NSG mice were irradiated with 2.5 Gy and infused with $3x10^6$/mouse expanded Tregs and Tcons.

Results: The leukapheresis contained $11x10^9$ (7-14) cells (CD4+CD25+4%±1.7). After immune separation, $237x10^6$ (126-250) cells were recovered (CD4+CD25+ 98.5%±1.3; CD4+CD25+CD127-FoxP3+ 67%±13 and CD4+CD25+CD127+ 20%±3). An aliquot of 100×10^6 cells were expanded for 14 days, obtaining a mean of 684×10^6±279 cells (fold increase 12, range 5-13). CD4+CD25+ cells were 99.6%±0.2, FOXP3 expression raised from 67%±13 (day 0) to 82±8 (day 14). CD127+ and CD45RA+ cells disappeared while the percentage of CD45RO+ cells increased to 97% ± 8 ($p<0.01$). The expression of CD39 and CTLA4 both increased from day 0 to day +14 (from 22.4%±7.4 to 58.1%±7.6, $p<0.05$ and from 20.4%±3.9 to 85.4%±5.7 $p<0.01$). TIM3 levels significantly increased from 0.4% ± 0.03 (day 0) to 29% ±9 (day +14) ($p<0.05$). The Tregs suppressive capacity improved after expansion (from 60%±7 to 75%±12). Central Memory-Tregs (CD45RO+CD62L+CD95+) and Effector Memory-Tregs (CD45RO+CD95+) were the most prevalent population (56.7%±7 and 23.6%±11 respectively). mRNA analysis showed expanded Tregs displayed a significant increase in IL10, GrB, CD39 and IL35 ($p<0.05$ *vs.* day 0). Conversely, IFNg significantly decreased on day +14. Expanded Tregs were sorted according to the CD39, CD62L and TIM3 expressions (purity >95%). When sorted populations were analysed, TIM3+ cells showed the greatest levels of IL-10 and GrB. CD39, IL35, TGFb and CTLA4 were equally represented in all subpopulations. Sorted cells strongly inhibited Tcons (CD39+ 55%±24, CD62L+ 60%±28, TIM3+ 53%±7). When expanded Tregs were infused in NGS mice, Tcon-treated mice died of GVHD; mice infused with $3x10^6$ expanded Tregs or coinfused with $3x10^6$ Tcons and $3x10^6$ Tregs survived without GVHD.

Conclusions: Expanded cells for clinical purposes can be obtained in a completely automated system and display phenotypic and functional Tregs features. Treg suppression is mediated by multiple overlapping mechanisms (CTLA4; CD39; IL10; IL35; TGFb; GrB). TIM3+ cells emerge as a potentially highly suppressive population.

C094

HLA-HAPLOIDENTICAL TRANSPLANTATION WITH REGULATORY AND CONVENTIONAL T-CELL ADOPTIVE IMMUNOTHERAPY IN PEDIATRIC PATIENTS WITH HIGH-RISK ACUTE LEUKEMIA

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Introduction: Post-transplant relapse is still a major cause of treatment failure in high-risk acute leukemia (AL) patients. Allogeneic hematopoietic stem cell transplantation from an HLA-haploidentical familial donor is a suitable option for pediatric patients with high-risk acute leukemia lacking a suitable HLA-match donor. As reported, HLA-haploidentical HSCT (haplo-HSCT) is based on *ex vivo* α/β T and B-cells depletion or on *in vivo* T cell depletion with post-transplant high-dose cyclophosphamide. The Perugia center reported results from adult high-risk AL patients who received an haplo-HSCT with donor regulatory T (Treg) and conventional T (Tcon) cells (without post-transplant pharmacologic immunosuppressive GvHD prophylaxis). Data demonstrated this transplant protocol prevented post-transplant leukemia relapse and protected from GvHD. Since September 2016 even the pediatric onco-hematology Unit of Perugia adhered to haplo-HSCT with adoptive Treg/Tcon immunotherapy program and treated sixteen pediatric patients with very high risk AL.

Methods: Sixteen pediatric patients, median age of nine years (range,4-19) with high-risk AL underwent Treg/Tcon haplo-HSCT between September 2016 and March 2020. Eleven patients had acute lymphoblastic leukemia (ALL, three Ph+), five were acute myeloid leukemia (AML). Nine patients were transplanted in CR1 (3 Ph+ ALL were in CR after second-line induction,1 ALL with extramedullary leukemia,1 ALL with t(19;11), 1 secondary AML after medulloblastoma, 2 AML with primary induction failure), four patients were in CR2, three in CR3. Median time from diagnosis to transplantation was 18.5 months (range, 5-48), median time from relapse to transplantation for CR2 and CR3 patients was 3 months (range, 3-15). The conditioning regimen was based on fractionated total body irradiation, thiotepa, fludarabine, cyclophosphamide. Grafts included CD34+ cells (mean $12.5x10^6$/Kg,7-24.5) at day 0, $2x10^6$/Kg Tregs at day -4 and $0.5\text{-}1x10^6$/Kg Tcons at day -1. Five patients were transplanted from NK alloreactive donors.

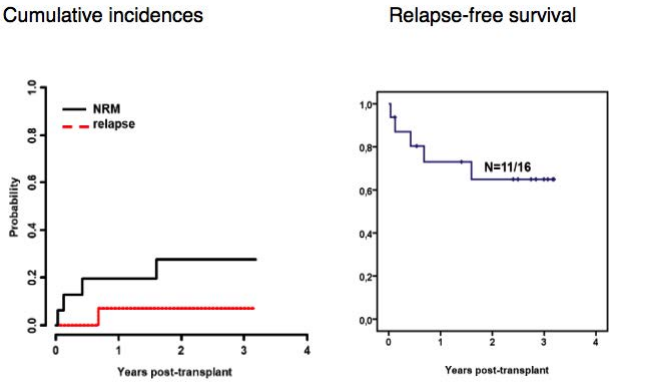

Figure 1.

Results: All patients achieved full-donor engraftment (median neutrophils engraftment 14 days, median platelets 15 days). Four patients

(25%) developed ≥ grade 2 aGvHD, one developed liver cGvHD. The immune recovery was good in all patients despite immune suppressive therapy in patients with aGvHD. Median values of T-cells at 100 days were: CD3+ 927/µL, CD4+ 244/µL, 1CD8+ 51/µL. Only one patient relapsed, the NRM was 25% (4/16). Causes of NRM were:1 aGvHD,1 invasive aspergillosis,1 thrombotic microangiopathy, 1 acute respiratory distress post engraftment. Eleven patients are alive at a median follow-up of 33 months (2-38 months), cGvHD/leukemia-free survival is 63%.

Conclusions These preliminary data showed that Treg/Tcon haplo-HSCT immunotherapy greatly reduced the incidence of leukemia relapse (1/16, 6%) also in pediatric patients with high risk AL and was associated with an encouraging DFS (69%). Even if the incidence of aGVHD is 25%, most of the patients are alive and only one affected by cGVHD. The immunosuppressive therapy is not associated with increased risk of relapse.

C095

HAPLOIDENTICAL TRANSPLANTATION WITH TREG-TCON ADOPTIVE IMMUNOTHERAPY FOR THE CURE OF ACUTE MYELOID LEUKEMIA WITH HIGH-RISK CYTOGENETICS

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Introduction: Acute myeloid leukemia (AML) with high risk cytogenetics (AML-HRC) is a biologically heterogeneous entity. It includes chromosome 3, 5, 7 aberrations, complex karyotypes (CK) (>= 3 abnormalities) and monosomal karyotypes (MK) (1 monosomy and a structural abnormality or >= 2 monosomies). AML-HRC is generally featured by chemoresistance and poor outcomes. Hematopoietic stem cell transplantation (HSCT) is the only potentially curative option, although limited by high rates of disease relapse (often > 50%) and dismal survival (10-20%, Ciurea, Cancer 2018). Thus, new HSCT strategies are needed to improve outcomes of AML-HRC patients. HLA-haploidentical T cell depleted HSCT with adoptive immunotherapy with regulatory T cells and conventional T cells (Treg-Tcon HSCT) resulted in low relapse rate in AML patients transplanted in complete remission (Martelli, Blood 2014). We explored the efficacy of such approach in AML-HRC.

Methods: We retrospectively analyzed outcomes of patients with AML-HRC that received Treg-Tcon HSCT. All patients received a myeloablative conditioning followed by an infusion of $2x10^6$/Kg freshly isolated donor Tregs on day -4, followed by $1x10^6$/Kg Tcons on day -1 and a "megadose" of purified CD34+ hematopoietic progenitor cells on day 0. No pharmacological GvHD prophylaxis was given post-transplant. We also performed a molecular-barcoded deep sequencing that targeted 39 genes that are recurrently mutated in myeloid neoplasms on available samples, to detect mutational burden at the time of transplant.

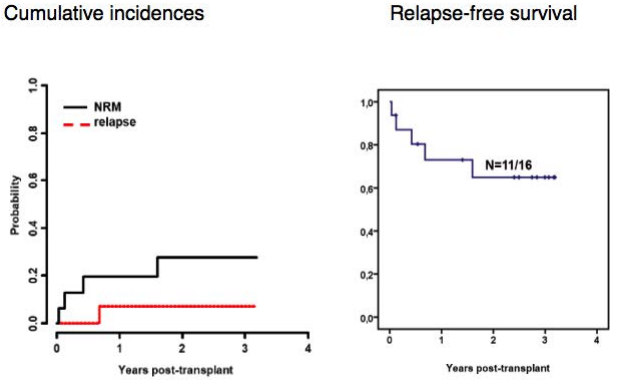

Figure 1.

Results: From January 1st 2007 to April 30th 2020, 26 AML-HRC patients received Treg-Tcon HSCT. Median age was 49 years. Six/26 had chromosome 7 abnormalities, 1 isolated del(5q), 1 inv(3), 18 CK and 4 MK. Five patients had secondary AML and 6 AML with myelodysplasia-related changes (2016 WHO). Twenty-two/26 patients were transplanted in hematologic complete remission, 4 with active disease. Fifteen/26 patients were transplanted with detectable disease by standard methods and 7/14 (50%) were transplanted with molecularly active disease. DNMT3A and TP53 were the most recurrently mutated genes. TP53 mutations were present in 3/14 (21%) patients. Twenty-four/26 patients achieved full-donor type engraftment, 1 patient rejected the graft and 1 patient died during the aplastic phase because of intracranial hemorrhage. Grade ≥ II acute GvHD occurred in 6/24 (25%) patients, 4 of them are alive and off immunosuppressive therapy. Only one patient developed cGvHD and experienced leukemia-relapse late after transplant (46 months). Non-relapse mortality occurred in 4/26 patients. Despite the high-risk disease, only 2 patients relapsed. The probability of GRFS was 72% at a median follow-up of 42 months (Figure 1).

Conclusions: The present study demonstrates that Treg-Tcon HSCT is an effective approach to eradicate AML-HRC and that it should be tested in a prospective trial with a larger cohort of patients.

C096

HEMATOPOIETIC CHIMERISM AND CD3+ RECONSTITUTION MONITORING AS PROGNOSTIC MARKERS IN ACUTE MIELOID LEUKEMIA PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction: Hematopoietic chimerism (HC) assessment after allogeneic stem cell transplantation (alloHSCT) gives engraftment informations and could reflect a reapparence of leukemic cells. The CD3+ donor chimerism impact also on acute graft versus host disease (aGvHD) process. However the use of HC assessment remains controversial as MRD marker and early GvHD predictor factor after transplantation. Our study was designed to relate prognostic significance of HC monitoring and CD3+ reconstitution in AML trasplanted patients with relapse and GvHD occurence.

Methods: We have retrospectively considered 84 consecutive AML patients transplanted by the Hematological Division of Udine from January 2014 to June 2018 in order to have almost three follow-up timepoints after alloHSCT. Through an STR approach we have analysed HC in bone marrow (BM), whole peripheral blood (WPB) and CD3+ cells at 30, 60, 90, 180 an 360 days after HSCT. We defined Mixed chimerism (MC) as a the donor signal level was below 95%.

Results: 40.4% of patients showed a MC value during the first year. 23 patients (27.3%) relapsed at a mean time of 176±110 days after transplant. Of this 74% relapsed by the sixth month. MC of the BM samples correlated with relapse at the third (p=0.018) and sixth month (p= 0.0001), while the WPB samples only correlated at the sixth month (p=0.02). CD3+ chimerism showed no significant correlation with relapse at any time points. MC of the BM samples at the third month determined a worse disease free survival (DFS) (p=0.0003) and Overall Survival (OS) (p=0.002). Complete remission (CR) status before alloHSCT improved DFS (p=0.05) and OS (p= 0.03), but CR status and MRD negativity before alloHSCT did not have correlations with MC status at the third month (p=0.09 and p=0.5, respectively). Acute GvHD occurred in 62% of the patients at a median time of 24 days (range 12-96). While MC detection in every fraction did not show correlation with aGvHD development, aGvHD positive patients presented higher CD3+ levels at the first month than negative ones (314 *vs.* 84/µl, p=0.014, Figure 1). Using a ROC analysis with an AUC= 0,68 (p=0.015), a circulating CD3+ cell levels greater than 263,6/µl defined a subgroup of patients

that could develop aGvHD with a specificity of 96% and a sensitivity of 30%. The absolute count of CD3+ cells at the first month was correlated with ATG administration (p=0.02) but not with myeloablative conditioning regimen (p=0.85), disease status (p=0.55) or infused CD3+/kg of recipient body weight (p=0.09).

Conclusions: In our experience, the HC in AML transplanted patients shows a dynamic profile with transient fluctuations. However, evaluation of chimerism on BM samples at the third month seems to be a fundamental time point to predict relapse. Finally, absolute CD3+ levels higher than 263,6/µl at the first month can help in identifying a patients with high risk of aGvHD development, that need more caution with immunosoppressive therapies.

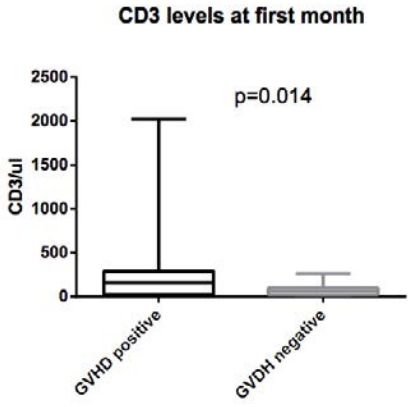

Figure 1.

POSTERS

Stem Cells and Growth Factors

P001

ROLE OF ERYTHROPOIESIS STIMULATING AGENTS (ESAS) IN SUPPORTIVE CARE OF LOW-RISK MYELODYSPLASTIC SYNDROMES

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Erythropoiesis stimulating agents (ESAs) are the frontline treatment in low-risk anemic MDS patients and an employment of this therapy in the earlier stage of the disease can delay the need for RBC transfusion, hypothetically by slowing the disease course. It's matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation of residual normal erythropoiesis by ESAs. Macrocytosis is one of the cytological hallmarks of dyserithropoiesis in MDS: an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non trasfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDS, subgroup of Italian register, was performed. 183 patients, treated with standard-dose ESAs, have been retrospectively analyzed. Data analysis was performed, according to IWG 2006 criteria, at the baseline, after 3 and 6 months of continuous treatment, with a subanalysis of the patients according to WHO and R-IPSS risk stratification. ESAs were started at mean Hb concentration of 9.31 g/dl, mean serum EPO concentration: 51 mU/L, after a mean time from diagnosis of 6 months (r.1-118). ORR was 83.6% (153/183), no difference among WHO and IPSS subgroups was found: 132/183 (72.1%) achieved response after 3 months of treatment, while other 21/183 (11.2%) after 6 months. 19 patients with stable disease (non-responders, according to IWG criteria), in which treatment was continued, achieved response after 9 months. In the macrocytic-responders group 83.2% exhibits again macrocytosis after 3 months, while 16.8% become normocytic. In the normocytic-responders group 89.8% exhibits again normocytosis, while 10.2% become macrocytic: in these patients, after 3 months, there was a contemporary worsening in neutropenia and thrombocytopenia, with transfusion-dependence, regarded as first signs of progression of disease. Non-responders were 30/183 (16.3%): in the macrocytic non-responders group 89% exhibit again macrocytosis after 3 months, while 11% become normocytic; in the normocytic group 76% exhibits again macrocytosis, while 24% become normocytic. These preliminary data can suggest that, in the majority of MDS patients responsive to ESAs, the increase of Hb concentration occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESAs last even when the expression of dysplasia progresses.

Table 1.

MDS PATIENTS	183
M	89 (49%)
F	94 (51%)
ERYTHROPOIESIS	
BASELINE HB (mean, g/dL)	9.31 g/dL (r. 7.1-11.3)
BASELINE SERUM EPO (mean, mU/mL)	51 mU/mL (r.3-84)
OVERALL RESPONSE RATIO	
RESPONDERS	153/183 (83.6%)
RESPONDERS AT 3 MONTHS	132/183 (72.1%)
RESPONDERS AT 6 MONTHS	21/183 (11.4%)
RESPONDERS AT 9 MONTHS (NON RESPONDERS IN IWG 2006)	19/183 (10.3%)
NON RESPONDERS	30/183 (16.3%)

Molecular Hematology

P002

DIGITAT PCR IS A PRECISE, ACCURATE AND SENSITIVE METHOD FOR THE DETECTION OF THE KIT D816V MUTATION IN PATIENTS WITH SUSPECTED SYSTEMIC MASTOCYTOSIS

M. Martelli[1], L. Bavaro[1], G. De Matteis[3], M. Rondoni[2], R. Zanotti[3], F. Grifoni[4], M. Bonifacio[3], C. Papayannidis[1], C. Sartor[1], A. Curti[1], C. Monaldi[1], S. De Santis[1], M. Mancini[1], M. Cavo[1], S. Soverini[1]

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Introduction: Detection of the D816V KIT mutation in the bone marrow (BM) is one of the minor criteria for the diagnosis of systemic mastocytosis (SM). The European Competence Network on Mastocytosis recommends routine use of assays like ASO-qPCR, conjugating high sensitivity with the possibility to quantitate the allele burden (which may provide diagnostic and prognostic information). Recent studies have also demonstrated that ASO-qPCR has the potential to detect the D816V KIT mutation even in a noninvasive manner in peripheral blood (PB) in the majority of patients with positive BM. Droplet digital PCR (ddPCR) is a promising alternative to ASO-qPCR. We evaluated a commercial ddPCR assay for the detection of KIT D816V in SM.

Methods: PB samples from 13 healthy donors (HDs) were used as negative controls, to calculate the limit of blank; the D816V-positive HMC-1.2 cell line was used as positive control. 35 BM samples from pts with suspected SM, either with neoplastic MC infiltration <0.01% by flow cytometry and low level positivity by ASO-qPCR or negative both by flow cytometry and ASO-qPCR were used for ddPCR validation. 151 pts with suspected SM were prospectively analyzed: 87 BM samples, 39 PBL samples and 25 matched BM and PBL samples. ddPCR was performed on a BioRad QX200 instrument using the ddPCR™ Mutation Assay: KIT p.D816V, Human (Bio-Rad). The allele burden (VAF) of KIT D816V was calculated by dividing the number of mutated copies by the total number of KIT copies.

Results: The threshold between the FAM and HEX droplets was set for both fluorescence channels based on control samples. In HMC-1.2 cells, dPCR measured a VAF of 50%, consistent with the heterozygous mutation status. No KIT D816V-positive events were detected in any of the HD samples. Comparison between ddPCR and ASO-qPCR results revealed high concordance in mutation detection and quantitation, even at very low VAF levels. ddPCR identified the D816V mutation in 70/151 pts prospectively evaluated for diagnosis of SM. According to WHO criteria, 49 were later diagnosed with ISM, 13 with aggressive SM, 4 with smoldering SM, 2 with SM with an associated clonal hematopoietic non MC disease and 2 with MC leukemia. The median allele burden in all positive SM patients was 0.34%. Pts with advanced SM showed a significantly higher VAF (median, 15.23%) compared with patients with ISM and smoldering SM (median, 0.27%). All pts positive for the D816V in BM also tested positive in the PB by ddPCR.

Conclusions: ddPCR for the detection of the D816V KIT mutation proved precise, accurate and sensitive. When compared to the gold standard, ASO-qPCR, ddPCR yielded highly concordant results, also in terms of VAF measurement, with no need of standard curves. ddPCR was always capable to detect the D816V mutation in PB when the BM was positive. We thus propose ddPCR as an attractive alternative to ASO-qPCR for routine D816V KIT testing.

P003

IDENTIFICATION OF IDH2 MUTATION IN ACUTE MYELOID LEUKEMIA IS TODAY A CLINICAL NEED: HOW DROP OFF DIGITAL DROPLET PCR CAN HELP PHYSICIANS

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Background: IDH2 mutations characterize 20% of *de novo* acute myeloid leukemias (AML), especially those with normal karyotype. Mutations are single-nucleotide variants involving the exon 4 at the arginine hotspots (R140 or R172). Today, the screening of IDH2 mutations plays an important role firstly for better categorizing AML and then for leading the therapy, by adding to the induction treatment the IDH2 inhibitor Enadisenib. Generally, IDH2 mutations are detected by Sanger or, more recently, by NGS. Nevertheless, NGS is not available everywhere and it could be an expensive and laborious technique. In this study, we investigated a new easier, cheaper and effective molecular tool that could be employed for detecting and quantifying IDH2 mutations. Aims: To assess feasibility and sensitivity of drop off digital droplet PCR (ddPCR) assays for IDH2 mutations and the possibility of adopting it as tool for MRD monitoring. The evaluation of prognostic value of IDH2 mutation in a series of 60 AML patients.

Methods: A drop-off ddPCR FAM/HEX Assay (Biorad®) method was used as basis for setting our new technique for IDH2 mutations detection. The FAM-labeled probe binds a reference sequence distant from the target but still within the same amplicon, while the HEX probe binds only the wild-type sequence in the target site. We included in the study 60 AML cases at diagnosis observed at the Division of Haematology, University of Pisa, Italy.

Results: 1) feasibility: we identified 8/60 (13.5%) IDH2-mutated patients by Sanger; by ddPCR, as by ARMS PCR, we identified as mutated 13/60 (21.6%). In discrepant cases, the VAF was 0.4-12%, so lower than the detection power of the Sanger. 2) sensitivity: using GeneArt Strings DNA Fragments (ThermoFisher, Waltham, Massachusetts, USA) the sensitivity of the method resulted 1x10-3. 3) clinical impact: when we tested if IDH2 mutations might condition the quality of response after induction/consolidation treatment, we found that there was not statistically significant differences, either in terms of ORR (69% in IDH2-WT *vs.* 84% in the IDH2-mutated subgroup), CR rate (53% in IDH2-WT *vs.* 68% in the IDH2-mutated subgroup), 3-year OS (34% for IDH2-unmutated vs. 30% for the IDH2-mutated subjects), and 3-year PFS (23% for IDH2-unmutated versus 19% for the IDH2-mutated subjects). 4) MRD: Finally, we used IDH2 mutations as marker for assessing MRD in 37 samples from 9 patients, In 6 cases IDH2 was a good marker of disease status; indeed, in 4 of them IDH2 mutational load well correlated with CR while in other 2 the increased levels of IDH2 mutations predicted disease progression. On the contrary, in other 3 other cases the behavior of IDH2 mutations did not correlate with clinical outcome.

Conclusions: ddPCR is a promising molecular technique that allows a quantitation of gene expression or detection of mutations without a reference curve. With this work we demonstrated that the new "drop off" ddPCR represents a valid tool for assessing in few hours the presence of IDH2 mutations. In particular, the high sensitivity of this method could allow to employ it as tool for MRD monitoring, but the role of IDH2 mutations as marker of MRD is still a matter of debate. Indeed, ELN guidelines state that this is not a good marker of MRD, but 2 groups recently reported for IDH2 to be a valid MRD marker. Larger studies are needed to resolve this issue.

P004

CELL-FREE DNA FOR MINIMAL RESIDUAL DISEASE ANALYSIS IN HEMATOLOGIC MALIGNANCIES

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Background: The analysis of circulating cell-free DNA (cfDNA) in solid tumors has led to explore its use in hematologic malignancies. Before its wide clinical implementation, different pre-analytical aspects need to be defined. We studied cfDNA in different hematologic diseases to: a) validate an extraction system which allows an adequate yield of material with optimal purity; b) determine if the amount of cfDNA released by nodal diseases is different from leukemic conditions; c) evaluate if the quantity of cfDNA allows MRD monitoring with IG/TR and BCL2/IGH markers; d) set up a system to increase the yield of cfDNA.

Methods: Fourty-four diagnostic plasma samples (6 ALL, 5 CLL, 8 CML, 10 early stage FL, 3 FL in stage III/IV, 12 DLBCL) and 10 from healthy donors were collected. Three ml of plasma per patient were pooled and extracted by both the Maxwell RSC48 instrument (Promega Corp. Madison, WI) and QIAVAC 24 Plus Vacuum system (Qiagen, Hilden, GE). cfDNA quality was tested by the Bioanalyzer High Sensitivity instrument and 3 housekeeping genes were quantified by digital-droplet-PCR (ddPCR). Finally, in 13 diagnostic samples (4 ALL, 2 I/II stage FL, 2 III/IV stage FL and 5 CLL) a SsoAdvancedTM PreAmp Supermix was used to increase the amount of cfDNA. All post-amplification products were sequenced, compared with genomic DNA (gDNA) and analyzed by ddPCR for quantification.

Results: a) Promega and Qiagen methods provided similar yields, but Promega had an inferior genomic contamination than Qiagen (92% of purity *vs.* 80%, respectively). b) cfDNA concentration varied among patients and differed among diseases: ALL and CML released a higher amount of cfDNA than CLL, with a mean yield of 148 ng (range: 57.6-300) and 258 ng (range: 288-609) *vs.* 26 ng (range:19.35-38.85), respectively. Among lymphomas, DLBCLs released a higher quantity of cfDNA than FL I/II and III/IV stage: mean 147 ng (range 5.3-137.2) *vs.* 10 (range 4.7-10) and 40 ng (range 12-74) ng, respectively. In healthy donors, cfDNA mean value was 11 ng (range 7.5-23.4). c) Variable amounts of cfDNA in different hematologic neoplasms, as well as the inter-patient variability, restrict a widely applicable MRD monitoring. d) In ALL and CLL patients, the circulating tumor DNA quantification at diagnosis by IG/TR gene rearrangements was greater after preAmp amplification, and equal between gDNA and cfDNA, with an improvement of 2-3 logarithms; similar results were observed in III/IV stage FL, but not in early stage FL. Importantly, the sequence analysis showed a complete correspondence between gDNA and cfDNA targets.

Conclusion: Pre-analytical factors have different effects on cfDNA yield, quality and applications. The preAmp system increases the amount of cfDNA. However, it is necessary to test post-treatment samples with different MRD levels in larger series of patients to assess the feasibility of the system, especially among lymphomas, where MRD is hampered by the lack of circulating cells.

Myeloproliferative Disorders and Chronic Myeloid Leukemia

P005

BCR-ABL1 LEVELS INCREASE AT FIRST MONTH AFTER TKI DISCONTINUATION PREDICT LOSS OF MAJOR MOLECULAR RESPONSE

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Introduction: The possibility to discontinue TKI therapy and maintaining molecular response ("treatment-free remission" – TFR) has become a common clinical practice in chronic myeloid leukemia (CML). It is now accepted to attempt TFR in patients with sustained deep molecular response (DMR) and resume therapy if major molecular response (MMR) is lost. The reported TFR rates range between 30% and 70%, but, at present, it remains difficult to identify factors that may predict TFR and, after TKI stop, to early detect patients who will eventually relapse.

Aims: The present study was designed to evaluate BCR-ABL1 expression in patients attempting TFR, to investigate if baseline values and/or trends after TKI suspension could predict CML recurrence. Methods. We analyzed the BCR-ABL1 RNA expression at therapy discontinuation (baseline) and monthly during the first year of monitoring in 38 CML patients receiving imatinib (n=24) or 2nd generation TKI (n=14). Patients were divided between those who maintained MMR (group 1, n=32) and those who needed to restart therapy for MMR loss (group 2, n=6). Molecular response was classified according to the standardized International Scale.

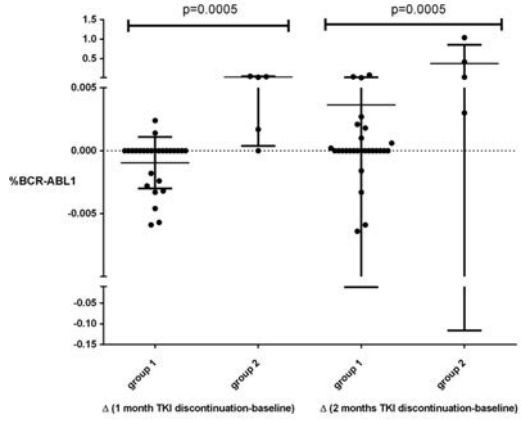

Figure 1.

Results: Median time from TKI stop and restart in group 2 was 3.5 months (range: 3-10). Mean BCR-ABL1 RNA expression at baseline for groups 1 and 2 was 0.0017±0.0024 and 0.0031±0.0028, respectively (p=0.2). BCR-ABL1 RNA levels increased during the first month of therapy discontinuation only in group 2 (0.0212±0.0183), compared to a slight reduction in group 1 (0.0008±0.0018; p=0.0013) and increased during the second month of discontinuation more in group 2 (0.3345±0.4271) then in group 1 (0.0056±0.0183; p=0.0006). This finding was confirmed comparing the differences between the BCR-ABL1 expression at baseline and at one (-0.0010±0.0020 for group 1 *vs.* 0.0188±0.0184 for group 2; p=0.0005) or two months (0.0036±0.0152 for group 1 *vs.* 0.3688±0.4848 for group 2; p=0.0005) after TKI discontinuation (Figure 1). Moreover, even the slopes obtained with the values at baseline and one month (-0.0009±0.0021 in group 1 *vs.* 0.0131±0.0180 in group 2, p=0.0129) and between one and two months (0.0013±0.0071 in group 1 *vs.* 0.1409±0.2010 in group 2; p<0.0001) demonstrate a significant difference between patients with or without sustained molecular response. For the determination of a threshold value of BCR-ABL1 RNA at one month after discontinuation, the ROC analysis was performed, defining an AUC=0.86 (p=0.006): the cut-off value for BCR-ABL1 was defined as 0.0047%. The chosen range (>0.0047% BCR-ABL1) has 96.15% specificity, 66.7% sensitivity and a likelihood ratio of 17.33.

Conclusions: With the limits of a relatively small number of patients, our data suggest that the chance of a successful TFR could be foreseen already at one month after TKI discontinuation: the 0.0047% BCR-ABL1 cut-off value identify over 95% of patients who will lose MMR and thus need to restart TKI therapy.

P006

IN SYSTEMIC MASOCYTOSIS, MIDOSTAURIN TARGETS BOTH KIT AND AURORA KINASE A REVERTING H3K36ME3 DEFICIENCY AND SYNERGIZES WITH NILOTINIB AND DASATINIB

M. Mancini[1], C. Monaldi[1], S. De Santis[1], C. Papayannidis[1], M. Rondoni[2], C. Sartor[1], L. Bavaro[1], M. Martelli[1], S. Bruno[1], A. Curti[1], M.C. Fontana[3], R. Zanotti[4], M. Bonifacio[4], L. Scaffidi[5], L. Pagano[5], M. Criscuolo[5], F. Albano[6], F. Ciceri[7], C. Elena[8], P. Tosi[9], P. Valent[10], M. Cavo[1], S. Soverini[1]

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Introduction: Trimethylation of lysine 36 on histone 3 (H3K36me3) is a highly conserved chromatin modification that plays a pivotal role in the control of transcription, splicing fidelity and DNA damage repair. The predominant writer of the tri-methyl mark on H3K36 is SETD2. SETD2 non genomic loss of function due to proteasome mediated protein degradation has recently been reported in advanced SM (advSM).

Methods: Western blotting and co-immunoprecipitation assays were performed to evaluate protein expression, interactions and activity. Apoptotic cell death was evaluated by flow cytometry after Annexin V/PI staining, reduction of clonogenic capacity was tested by clonogenic assays.

Results: After proteasome inhibition, we found that SETD2 binds Aurora Kinase A (AKA), that was overexpressed and hyper-activated in a series of patients (pts) with advSM compared to ISM pts and to a pool of healthy donors. AKA phosphorylated SETD2, raising the hypothesis that phosphorylation by AKA might be implicated in proteasome-mediated degradation of SETD2. The new standard of therapy in advSM is midostaurin, that inhibits KIT as well as various other kinases including AKA. Therefore we investigated if midostaurin treatment may result in AKA inhibition and consequent SETD2/H3K36me3 rescue. HMC-1 cells were treated with 5μM midostaurin for 24 h and phospho-AKA (T288), SETD2 and H3K36me3 expression were evaluated. AKA phosphorylation was reduced by about 60% and SETD2 expression and activity were partially restored, but only cytostatic effects were observed. In neoplastic mast cells from 7 pts with advSM, a rescue of SEDT2 expression and activity associated with partial de-phosphorylation of AKA was observed after 3 months of midostaurin treatment. Our subsequent experimental step was to test the efficacy of combined treatment with midostaurin and second generation TKIs (nilotinib and dasatinib) in inducing cytotoxic effects. Cytofluorimetric analysis of apoptosis in HMC-1 cells showed an important advantage in using the midostaurin + TKI combination compared to each single agent. We observed in our *in vitro* models that midostaurin combined with nilotinib completely dephosphorylated AKA and restored SETD2 expression and activity. Final-

ly, we found that KDM4A, an histone demethylase involved in H3K36me3 de-methylation, is overexpressed in HMC-1.2 cells (D816V+) compared to HMC-1.1 and in 4 advSM who had normal SETD2 levels, yet reduced H3K36me3 (in the absence of inactivating mutations). This suggests that KDM4A overexpression may result from KIT D816V oncogenic signaling and may be involved in H3K36me3 loss.

Conclusions: Our results indicate that inhibition of c-KIT and AKA by midostaurin in combination with second generation TKIs is a promising therapeutic strategy in patients with SETD2/H3K36Me3 deficiency and that KDM4A may be a new druggable oncogene, overexpressed in a proportion of advSM pts. Supported by AIRC (project 23001) and AIL.

P007

MODELLING CHRONIC MYELOID LEUKEMIA IN ZEBRAFISH

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Zebrafish has proven to be a versatile experimental model to study human hematopoiesis and it is a reliable *in vivo* tool for modeling hematological malignancies. The powerful genome editing, genome-wide forward genetic screens and chemical screening tools generated models that recapitulate human malignant hematopoietic pathologies and unraveled cellular mechanisms involved in these diseases. Several Authors described zebrafish models of acute myeloid and lymphoid leukemia, myeloproliferative diseases and myelodysplastic syndromes. To model Philadelphia positive (Ph+) chronic myeloid leukemia, we generated a transgenic fish expressing the human cDNA (BCR-ABL1) encoding for the human fusion protein P210 by using the Gal4/UAS system. We then crossed this line with the HSP70-Gal4 transgenic line and we obtained a new transgenic line named bcr/abl-pUAS-CFPY//HSP70-Gal4. Characterization of the transgenic fish was carried out using whole mount in situ hybridization and Real Time PCR for genes involved in hematopoiesis (gata1, scl, runx1, mpx, I-plastin). In both cases, at 24 and 48 hours of the embryonic development we observed an increase of ematopoietic markers in transgenic fish compare to controls. BrdU was used to label proliferating hematopoietic cells in the caudal hematopoietic tissue (CHT) at 30 hours. All CHT cells were labeled in transgenic bcr/abl-pUAS-CFPY/HSP70-Gal4 larvae following exposure to BrdU, whereas fewer CHT cells were BrdU labeled in control larva (Figure 1A).

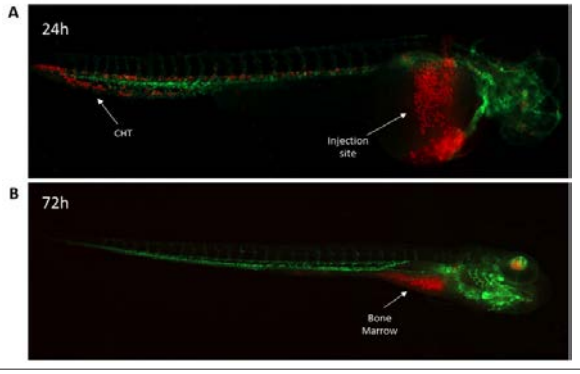

Figure 1.

Hematologic malignancies are frequently characterized by dissemination and homing of leukemic cells to the bone marrow. Because of his optical clarity, zebrafish is the perfect *in vivo* model to study and monitor cancer cell dissemination and homing processes. At 48 hours of the embryonic development, we performed intracardiac injection of a leukemia cell line and monitored the migration to the caudal hematopoietic tissue, the region where hematopoiesis occurs in the zebrafish embryo. We investigated the behavior of the injected leukemia cells and observed that during the embryo development they move across various anatomical districts following the subsequent activation of the different hematopoietic tissues (Figure 1B). The presented transgenic and leukemia xenograft model could help to elucidate the mechanisms of Ph+ chronic myeloid leukemia progression and will probably allow high-throughput drug screening of putative targeted molecules with therapeutic effect by monitoring the down-regulation or the deactivation of BCR-ABL1 p210 protein and the behavior modifications of the leukemic injected cells.

P008

TYROSINE KINASE INHIBITORS SUSTAIN IMMUNITY AND ANTI-VIRAL RESPONSE OF PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA

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Background: In addition to be effective in terms of hematological, cytogenetic and molecular responses, tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML) have been reported to play also a positive effect on the immune system by reducing myeloid-derived suppressor cells, re-activating T and NK lymphocytes, and deactivating the PD1-PDL1 axis. Torque Teno virus (TTV) is a DNA-virus detectable in more than 60% of the asymptomatic healthy subjects that mimics the immunocompetence status because its load significantly increases after immunosuppressive therapy or transplant.

Aim of the Study: to assess the immunomodulating effect of TKIs in CML 1) by measuring TTV infection and replication rate in a cohort of CML patients receiving Nilotinib; 2) by analysing the immune profile of 5 CML patients at diagnosis and after 6 months of Imatinib by the Nanostring technology.

Results: 1) TTV load was measured by quantitative real-time PCR in 60 peripheral blood samples from 10 CML patients receiving Nilotinib. At diagnosis, only 2 patients showed detectable TTV genome. When we analyzed the TTV load at different time points, TTV did not replicate in 68% of cases and in positive samples the viral load was low (2.8 log/mL *vs.* 3.9-8.5 log/mL of the transplanted patients and of 2.3 log/mL of healthy subjects). When we analyzed TTV load in respect of molecular response assessed by the BCR-ABL1/ABL1 ratio, a statistically significant correlation between genome TTV detection and absence of optimal response was found. 2) in the second part of our study we employed the Nanostring technology for analyzing the expression of 770 inflammation- and immunity-related genes in 5 CML patients before and after 6 months of treatment with Imatinib. Overall, 58 genes were deregulated, with 18 genes resulting up-regulated and 40 down-regulated. Interestingly, 20 of de-regulated genes were strictly correlated with immune or anti-viral response. On the contrary, 6 of the 9 immune response-related genes that were down-regulated by Imatinib were already known as inhibitors of T and NK lymphocytes activity. Consequently, their reduced expression during Imatinib treatment might play a positive effect on the patients innate immunity. Among the down-regulated genes we found: ARG1, C3AR1, CEACAM1, GSN, NECTIN1 and FUT4. ARG-1 (ARGINASE-1) in CML it has been reported to be highly expressed at diagnosis, when myeloid-derived suppressor cells belonging to the neoplastic clone are very active in inhibiting T cells activity. Also the reduced expression of FUT4 might be positive for the host's immune system, because this gene is correlated with PD1. Among pro-immune genes up-regulated by Imatinib, we observed CD28, IFN gamma, CCL5, and CCR5. Particularly interesting are CD28, located immediately down-

stream of PD1, and CCL5 that make mice resistant to the viral infection.

Conclusion: about the low TTV infection rate, we might hypothesize that TTV receptors are lower/absent in CML cells or that BCR-ABL1 fusion gene might be able to change the conformational status of the host's cell, probably modifying the cytoscheleton function; it has been reported that an actin adaptor gene, FAK, necessary to viral entrance into the human cell, is expressed at a very low level in CML. About the gene expression profiling data, they clearly confirmed that Imatinib restores and perhaps supports CML patients' immune competence.

P009

JAK2V617F MUTATION AND HIGH SCORE CHART FOR CARDIOVASCULAR RISK CAN PREDICT THROMBOTIC RISK IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background: The introduction of anti-thrombotic and/or cytoreductive treatment for patients with Ph negative chronic myeloproliferative neoplasms (MPNs) can modify the impact of pro-thrombotic clinical and biological features at time of diagnosis.

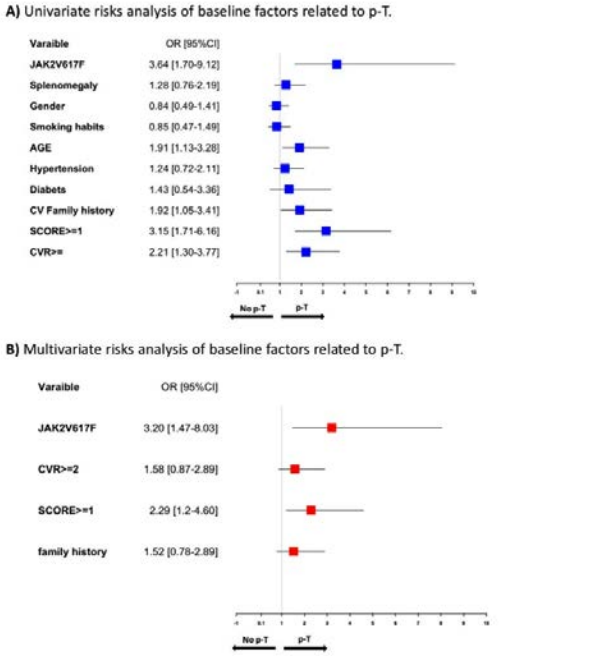

Figure 1.

Methods: In order to avoid the potential confounding effect of therapy, we investigated the relationship between previous thrombosis (p-T) occurred before MPN diagnosis and the characteristics at diagnosis in 372 patients with MPN. The main patients' characteristics at diagnosis, analysed to explore their relationship with p-T, were classified as host-related (gender, age, SCORE risk, cardiovascular risk factors (CVR)), and disease related (platelet (PLT) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (HCT), splenomegaly, JAK2V617F mutation). SCORE chart was evaluated at the time of MPN diagnosis, it represents the 10-year risk of fatal cardiovascular disease (CVD) in countries at low CVD risk (Italy included) based on the following risk factors: age, sex, smoking, systolic blood pressure and total cholesterol, as above described. CVR factors included were hypertension, diabetes, dyslipidemia and smoking habits. Categorical and continuous were respectively analysed using the chi-square and Student's t-test, according to the presence/absence of p-T. Logistic regression models (univariate and multivariate) were estimated to investigate the relationships between p-T and patient features at diagnosis. The results are reported as odds ratios (OR) and related 95%CI. All statistical analyses were performed using R version 3.6.0.

Results: p-T occurred in 70/372 (18.8%) patients, with similar rates in the different MPNs (6/109, 5.5% for PV, 19/215, 8.8% for ET, 0/18, 0% for PMF and 3/32, 9.3% for early-MF). In univariate analysis, p-T rate was significantly related to JAK2V617F mutation (p=0.0015), SCORE 1 (p=0.0003), CVR2 (p=0.004), family history positive for CV events (p=0.038), hypertension (p=0.02) and age (p=0.0014) (figure 1A). Others disease related features (such as PLT, WBC, Hb, HCT, splenomegaly) and gender were not associated to p-T. For multivariate analysis, we exclude hypertension, being included in CVR evaluation and age because it was included in SCORE assessment. Thus, p-T rate was significantly related to JAK2V617F mutation (3.2 [1.47-8.03], p=0.006) and SCORE1 (2.29 [1.2-4.59], p=0.014), whereas CVR2 and family history positive for CV events lost their significance (figure 1B).

Conclusion: This analysis in MPN patients disclosed the unbiased characteristics at diagnosis with a pro-thrombotic effect. Moreover, it suggests that SCORE risk calculation could be introduced in clinical practice. Maybe, the optimal strategy could be to start cytoreductive therapy in patients with JAK2V617F mutation and SCORE risk 1 or to control modifiable risk factors included in the SCORE chart to prevent thrombosis during the follow-up. This data need to be confirmed in a larger MPN patient cohort.

P010

SECOND-LINE BOSUTINIB IN ELDERLY CML PATIENTS: FINAL RESULTS OF BEST STUDY

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Introduction: The median age of CML patients failing a first-line TKI because of resistance or intolerance is higher than 60 years. Bosutinib (BOS), dasatinib (DAS) and nilotinib (NIL) have similar second-line efficacy, but in elderly patients DAS and NIL toxicity is more frequent and more clinically relevant. BOS safety profile may be an added value in this setting, but the approved initial dose of 500 mg OAD may be higher than necessary.

Aims: All TKIs have been tested in CML patients at a fixed initial dose, with dose reductions in case of toxicity. On the contrary, the aim of our study was to evaluate the efficacy and the tolerability of low-dose second-line BOS in elderly CML patients, using the molecular response at given timepoints to increase the dose only in selected patients, thus finding the minimum effective dose.

Methods: A prospective phase 2 single-arm multicenter study has been designed by the GIMEMA CML Working Party (NCT02810990). Study design: all patients started BOS 200 mg OAD for 2 weeks ("run-in" period), then the dose was increased to 300 mg OAD; after 3 months, patients with BCR-ABLIS transcript ≤ 1% continued 300 mg OAD, while in patients with transcript > 1% the dose is furtherly increased to 400 mg OAD, in absence of relevant toxicity. The primary endpoint was the rate of MR3 at 12 months. Key inclusion criteria: > 60 yrs old, chronic phase CML, intolerance or failure of any first-line TKI (2013 ELN criteria), absence of T315I or V299L mutation.

Results: Sixty-three patients have been enrolled. Median age: 73 yrs (range 60-90). Reasons for switching to BOS: intolerance 63%, resistance 37%. First-line TKI: imatinib 83%, DAS 11%, NIL 6%. All patients reached at least 1-year observation. Due to the emergency situation caused by SARS CoV2 spread in Italy, few data are still missing, but final results will be presented onsite. Maximum BOS dose: 400 mg OAD, 19%; 300 mg OAD, 76%; 200 mg OAD, 5%. At baseline, 17% of patients were already in MR3; MR3 rates at 3, 6 and 12 months were 44%, 54% and 59%, respectively. The cumulative rate of patients achieving or maintaining a MR3 by 12 months was 67%; patients achieving MR4 or MR4.5 by 12 months were 44% and 24%, respectively. Overall, 30%, 29% and 8% of patients had 1 log, 2 logs or > 3 logs reduction from baseline BCR-ABLIS transcript level (67% of patients had a molecular improvement from baseline). Selected adverse events: acute coronary syndromes, 4 patients; pericarditis, 2 patients; peripheral arterial thrombosis, 1 patient; no pleural effusions were observed. Events leading to permanent treatment discontinuation: 2 unrelated deaths, 7 adverse events, 4 unsatisfactory responses (without progressions), 1 second neoplasia. Fourty-nine patients are still on BOS at the last contact: 10% of them on 400 mg OAD, 61% on 300 mg OAD, 29% on 200 mg OAD.

Conclusions: These results trial showed that in elderly patients intolerant to or failing a first-line TKI BOS may be highly effective and better tolerated at a dose lower than 500 mg OAD, namely at 300 mg OAD.

P011

MARROW BCR-ABL+ ENDOTHELIAL CELLS SHARE MYELOID-LINEAGE ANTIGENS FORMING 2D AUTOCRINE BRANCHING PATTERNS *IN VITRO* THAT SUPPORT TKI-RESISTANT CML STEM/PROGENITOR CELLS

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BCR-ABL tyrosine kinase inhibitors (TKIs) approved for the treatment of chronic myeloid leukemia (CML) have adverse effects mostly including hemodynamic instability and pulmonary edema. Whilst imatinib prevents vascular leakage and edema formation, other TKIs such as dasatinib, nilotinib or ponatinib are much stronger associated with endothelial barrier dysfunction in endothelial cells (ECs) isolated from multiple origins. At present, however, the intrinsic TKI-responsiveness of CML-patient derived ECs and their antigen identity (including that related to a myeloid-lineage origin) remain yet unexploited. This study reports that > 80% of marrow BCR-ABL+ ECs (BECs) isolated from newly-diagnosed CML patients are phenotypically and functionally distinguishable from their normal counterpart or tumor-activated ECs of multiple myeloma (MM). Besides the expression of vascular-associated antigens (e.g. CD31, Tie-2/Tek and VEGF-R2) and the uptake of acetyl-low-density lipoprotein, BECs harbor themselves the myelomonocytic markers CD14 and CD68, both suggestive of an ongoing neovascularization via mimicry and/or cooption of leukemic lineage progenitor cells. Moreover, BECs seeded on 2D serum/matrix-free culture dishes rapidly form capillary lumen-like structures and highly adhesive and anastomosed architectures while remaining less proliferative and angiogenic on matrigel® than MM-activated ECs. Such 2D autocrine branching patterns of BECs are markedly delayed by dasatinib and ponatinib and at a lesser extent by nilotinib and imatinib but without induction of apoptosis, mainly depending on the presence of their own CD14+ subpopulation and N-cadherin mediated cell-to-cell interactions that support quiescence and TKI-resistance of CML stem/progenitor cells. To sum up, these data start to uncover unique functional features of CML marrow endotheliopoiesis that may be associated to TKI-resistance suggesting a refinement of current treatment concepts.

P012

AUTOIMMUNE DISEASES AND MYELOID HEMATOLOGICAL DISORDERS: A POSSIBLE PATHOGENETIC RELATIONSHIP.

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Background: The association between autoimmune diseases (ADs) and lymphomas is well established; nonetheless, few studies have investigated the relationship between myeloid malignancies and ADs. In a series of more than 11,000 patients with myeloproliferative neoplasms (MPN), a Swedish group reported that a prior history of AD was significantly associated with a higher risk of MPN. More recently, our group showed that in chronic myeloid leukemia (CML) some genes correlated with AD (GLYPR1, PCARD, S100) were highly expressed at diagnosis and that the treatment with Imatinib impacted on the "inflammatory" profile of CML patients. Aim of the study: to investigate the frequency of myeloid malignancies, such as myelodysplastic syndromes (MDS) and chronic, either Philadelphia-positive (CML) or Philadelphia-negative (MPN), myeloproliferative disorders in patients with ADs, and to identify several distinctive clinical and biological features.

Methods: A retrospective systematic search through the electronic health records of the patients admitted at our Rheumatology of Pisa from 2009 to 2019 was performed to select those presenting with ADs and MDS or MPNs. Categorical variables were compared using chi square test and Fisher's test; continuous variables were compared using Student's t-test. A 2-tailed value of $p < 0.05$ was taken to indicate statistical significance.

Results: Out of the medical records of 5040 patients, we identified 112 patients (67 F: 45 M, mean age: 63 years) with ADs and hematological malignancies (2.2%): 41% with AD and MPN, 28% with AD and MDS, and 20% with AD and CML. No demographic differences were observed in the two subgroups. Regarding MDS, AR was the most common hematologic presenting finding, with diagnosis of refractory anemia with excess of blasts (RAEB I/II) done in 16% of cases. In the MPNs subgroup, 31% had a diagnosis of CML, 31% had a myelofibrosis (MF), 15% had an essential thrombocythemia (ET) and 13% a polycythemia vera (PV). The JAK2 V617F mutation was detected in 80%, 92%, and 61% of MF, PV, and ET patients respectively, and CALR was mutated in 15% of ET and in 10% of MF. Regarding the temporal appearance of ADs in respect of myeloid disorders, ADs preceded hematological diseases in 53% of all cases, especially in MPNs. Both kind of disorders were synchronously diagnosed in 35% of MDS and 32% of MPNs, while

in 45% of CML the hematological diagnosis anticipated that of AD. In MDS, the most commonly diagnosed ADs were seronegative arthritis (25%) and large and small vessel vasculitis (20%). In patients with MPNs, the most frequent diagnoses were connective tissue disorders (30%) and rheumatoid arthritis (26%); arteritis was more frequent in CML. The anti-Ro52 (TRIM21)-positive systemic connective tissue disorders were more frequently detected in MPN (55% *vs.* 22% of CML and MDS). Cardiovascular events were observed in 27% of patients: 23% in MDS, 25% in CML and 32% in MPNs, with a significant correlation with JAK2V617F mutation.

Conclusion: Our study is limited by its retrospective design. However, it showed that the frequency of MDS and MPNs in ADs is not negligible. It has been already reported that, under viral infection, TRIM21 is upregulated by activation of the IFN/JAK/STAT pathway; interestingly, anti-Ro52 (TRIM21) were over-represented in our MPN cases, where the JAK/STAT signal is hyper activated. This might be a factor explaining the frequent association between ADs and MPN, and support the use of anti-JAK2 compounds as anti-inflammatory drugs.

P013

ABSTRACT WITHDRAWN

P014

SONIC HEDGEHOG PATHWAY AS POTENTIAL NOVEL MOLECULAR TARGET FOR PRIMARY MYELOFIBROSIS

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Myelofibrosis (MF), including primary MF (PMF), post-essential thrombocythemia MF and post-polycythemia MF, are characterized by ineffective clonal hematopoiesis, splenomegaly, bone marrow fibrosis and the propensity for transformation to acute myeloid leukemia. The identification of driver mutations in JAK2, CALR, and MPL has contributed to a better understanding of disease pathogenesis leading to the development of the only targeted therapy for MF, the JAK2 inhibitor ruxolitinib. Although this drug has contributed to relief from inflammatory symptoms and splenomegaly, it is not sufficient in producing long-term disease remissions and reversal of BM fibrosis. A defective balance between the vascular niche and the endosteal niche is a cause of MF. Studies have demonstrated that an increased production of osteoprotegerin by stromal and endothelial cells contributes to the unbalanced osteoblast production leading to the osteosclerosis, frequently associated with MF and to vascular complications. In this work we focused on the role of sonic hedgehog pathways (SHH) in the microenvironment alterations in PMF. SHH signaling is based on SHH receptor Patched1 (PTCH1) that represses protein Smoothened (SMO); following binding to its ligand SHH, PTCH1 is internalized and the SMO repression is alleviated, thus, inducing a complex and potent activation of the GLI-Kruppel family members (GLI1, GLI2, and GLI3) acting as transcription activators/repressors. We observed that purmorphamine (PMO, an activator of SHH pathway) was able to induce MSC differentiation towards cancer associated fibroblast (CAF) phenotypes. In particular, PMO induced up-regulation of CAF-associated markers such as TGFB, aSMA and FAP1. In accordance with these observations, we also observed that activation of SHH pathway by PMO led to activation of Toll-like receptor (TLR3), which drives MSC polarization toward a pro-tumoral phenotype. Moreover, multiparametric assay highlighted that activation of SHH pathway led to higher secretion of BMP2, osteoprotegerin and osteonectin confirming that this pathway is involved in pre-osteoblat differentiation. Next, we evaluated if SHH pathway could have a role in the inflammation status observed in PMF microenvironment. Analyzing expression of inflammation and oxidative stress-related genes, we found that PMO significantly increased the expression of TGFB, IL6, IL1 beta, IL8, but also of the HMOX1 and SOD, highlighting that SHH pathway could play an important role in the pathogenic mechanism of inflammation observed in PMF patients. Finally, transcriptome analysis of CD34+ cells from PMF patients showed an alteration of SHH related genes expression. In conclusion, our data demonstrated an involvement of SHH pathway in the crosstalk between MSC and CD34+ cells in PMF. The inhibition of this pathway may constitute a promising strategy to counteract inflammation, osteosclerosis and fibrosis of the PMF patients.

P015

THE ROLE OF IGFB6 IN THE PATHOGENESIS OF BONE MARROW (BM) FIBROSIS IN MYELOPROLIFERATIVE DISEASE

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Primary Myelofibrosis (PMF) is a Philadelphia-negative chronic myeloproliferative neoplasm (MPN) characterized by the uncontrolled proliferation of bone marrow stem cells sometimes with an increase in bone marrow fibrosis and consequent extramedullary hematopoiesis. Oncogenic driver mutations in PMF include Janus kinase 2 (JAK2), calreticulin (CALR), and MPL. There are evidences that myelofibrosis mainly results from a stromal reaction to the clonal hematopoiesis as a consequence of the release of profibrotic cytokines. Megakaryocytes (MKs) are the key cells involved in the myelofibrosis because they can release, in the bone marrow, large amounts of growth factors such as profibrotic (transforming growth factor β1 (TGF-β1), basic fibroblast growth factor, and platelet-derived growth factor), angiogenic and pro-inflammatory cytokines. In recent years, an increasing number of studies pointed towards an important involvement of the mesenchymal stem cells (MSCs) niche in the pathophysiology of PMF. The insulin-like growth factor-binding proteins (IGFBPs) are a family of six high-affinity key regulators of IGFs action. The insulin-like growth factor binding protein 6 (IGFBP6) is expressed in a variety of tissues and its expression is developmentally regulated; it is an inhibitor of IGF-II actions and several studies have shown that it plays an important role in inhibiting survival and migration of tumor cells. In this work we study the role of IGFBP6 in BM fibrosis which characterize PMF patients. In particular, we observed that IGFBP6 was highly expressed in CD34+ cells from PMF patients, suggesting that IGFBP6 release by tumor cells could shape the human mesenchymal stem cells phenotype thus contributing to BM fibrosis. In this work we also treated healthy mesenchymal stem cells with recombinant IGFBP6 *in vitro*. We demonstrate that IGFBP6 induceed a significant increase of -SMA, FAP1 and TGF-β protein expression, suggesting that IGFBP6 activated Cancer-associated fibroblasts (CAFs). Quantitative multiplex assay showed that IGFPB6 induced a significant increase of OPG and osteopontin both at 24h and 48h after the treatment, while increased BMP2, MMP-9, MCP-1/CCL2 and RANTES/CCL5 levels only after 48h. We also observed that TIMP-1 levels were increased after 48h of IGFBP6 treatment. We confirmed by RT-PCR assay, a significant increase in mRNA expression of BMP2, RUNX2 suggesting that IGFBP6 was able to stimulate MSC differentiation in pre-osteoblast cells. Finally, immunocytochemistry analysis showed that IGFB6 induced TLR3 activation pathways suggesting that this molecule was able to stimulate a pro-tumoral phenotype of MSCs. We demonstrated that IGFBP6 participate to the BM microenvironment transformation stimulating osteosclerosis, extracellular matrix deposition and favoring a pro tumoral phenotype of mesenchymal stem cells. In conclusion, IGFBP6 could be an interesting novel key player and a therapeutic target to explore for the treatment of BM fibrosis in PMF patients.

P016

ABSTRACT WITHDRAWN

Myelodysplastic Syndromes

P017

HEMATOPOIETIC STEM CELLS (HSC) AND GRANULOCYTE MACROPHAGES PROGENITORS (GMP): THE TARGETED VICTIMS OF OXIDATIVE STRESS IN MDS

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Introduction: Reactive oxygen species (ROS) play a role in the pathogenesis and clinical evolution of MDS (Novotna B, Leuk Res, 2009; Chung, Y. J., Leuk Res, 2014), contributing to hematopoietic stem and progenitor cells (HSPC) genetic instability. Less is known about ROS levels in the sub-populations of MDS HSPC. We aim to analyze ROS in MDS hematopoietic stem cells (HSC), common myeloid progenitors (CMP), granulocyte macrophages progenitors (GMP) and megakaryocyte-erythrocyte progenitors (MEP); also, we want to analyze the relationship between ROS and clinical data.

Methods: thirty-eight MDS and 27 normal bone marrow (NBM) samples were collected; via multicolor flow cytometry, we analyzed ROS levels (as mean fluorescence intensity) in MDS and NBM hematopoietic progenitors cells (HPC) and HSC.

Results: In both NBM and MDS, HSC showed much higher ROS levels than HPC (3 to 4 folds, $p < 0.00001$); HSC ROS were significantly more elevated in MDS-no excess blasts versus MDS with excess blasts (16.7 ± 7.2 *vs.* 9.2 ± 4.9, p: 0.01) and NBM. GMP from MDS-no excess blasts showed higher ROS compared to NBM GMP (1.8 ± 0.7 *vs.* 2.8 ± 0.9, $p < 0.0001$). The 3 MDS with ringed sideroblasts (RS) showed higher ROS in HSC and GMP compared to the not RS low/int-1 MDS (HSC: 29.9, 26.6, 15.2 in MDS-RS *vs.* median 14,7 in MDS not RS; GMP: 4.5, 3.8, 2.9 in MDS-RA *vs.* median 2.6 in MDS not RS). The 2 monosomy 7 patients displayed higher ROS levels in each subpopulation compared to the normal karyotype MDS (HSC: 20, 15 *vs.* median 14.8; CMP: 3.4, 3 *vs.* median 1.8; GMP: 4.8, 2.9 *vs.* median 2.5; MEP: 2.8, 3.2 *vs.* median 1.8). The only del(5q) patient did not show relevant differences in ROS levels compared to the median of the normal karyotype MDS ROS. The one complex karyotype patient displayed low ROS values in all subpopulations, except for GMP, where ROS levels were similar to the median of normal karyotype MDS (HSC: 3.9 *vs.* median 14.8. CMP: 1 *vs.* median 1.8, GMP 2.5 *vs.* median 2.5, MEP 1 *vs.* median 1.8). The 9 high transfusion burden patients showed higher ROS in HSC and GMP compared to NBM HSC and GMP. These data were not seen in low transfusion burden (n:2) and non-transfused patients (n:26). In low/int-1 MDS, a direct correlation between ferritin values and ROS levels in HPC, but not in HSC, was spotted (CMP p: 0.02; GMP p: 0.004; MEP p: 0.02). GMP and HSC from low/int-1 risk patients that lost response to EPO tended to show higher ROS levels: GMP pre EPO 2.8 ± 0.8; GMP on EPO 2.7 ± 0.8; GMP post EPO 3.8 ± 0.7; HSC pre EPO 15.5 ± 6.5, HSC on EPO 14.81 ± 9.6, HSC stop EPO 21.33 ± 8.1. Looking at OS, patients with HSC ROS higher than 14.5 had a probability of OS of 240 months, while patients with HSC ROS less than 14.5, 94 months (p: 0.033).

Conclusions: Our data show that in low/int-1 risk MDS, ROS are particularly high in HSC and GMP, possibly contributing to genetic instability and AML evolution (Goardon N, Cancer Cell, 2011). Use of antioxidant agents combined with standard therapies should be further investigated in order to ameliorate MDS clinical outcomes.

P018

CLONAL EVOLUTION OF MYELODYSPLASTIC SYNDROME IN CML. CASE REPORT OF A PATIENT WITH RETROPERITONEAL FIBROSIS

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Introduction: Retroperitoneal fibrosis is a rare idiophatic auto fibroinflammatory condition of the retroperitoneum , called secondary (about one third of cases). Few cases are reported in literature of a secondary malignancy as CML after MDS and none with retroperitoneal fibrosis and complex clonal evolution.

Methods: We report a case of a 76 year old male who was referred to our institution in July 2018 for anemia,joint pains,fatigue,weight loss. CT(computed tomography) imaging studies showed a fibrotic process in the retroperitoneum between second and forth lumbar vertebrae and compression of left ureter, abdominalis aorta,with consequent periaortitis,aneurisma abdominalis aorta and bilateral hydroureteronephrosis .Positron emission tomography (PET) revealed the active inflammation in retroperitoneal fibrotic mass.Laboratory findings were anemia normocytic, serum monoclonal gammopathy (IgG lambda), presence of antinuclear antibodies with elevation in ferritin,urea,creatinine, erythrocyte sedimentation rate and C reactive rate.

Resuts: The diagnosis of retroperitoneal fibrosis was made by interprofessional collegial team. Morphologic evaluation of the bone marrow revealed a Myelodysplastic syndrome (MDS) and trisomy of chromosome 21 in 3/20 metaphase cells (G-banded chromosome).For IPSS-R int1 MDS he was treated with epoetin alfa , while for retroperitoneal fibrosis he required indwelling left ureteral stent and steroid therapy with prednisone 1mg/kg for day for 4 weeks and after tapered during which for worsening of chronic failure(in october 2018)was administrated Rituximab (antiCD20 :375mg/m^2) for only one somministration and not reapeated for recurrent urinary infections and requirement right iliac artery embolization surgery and replacement stent. In January 2020, he presented, suddenly,a worsening of anemia, leukocytosis,and thrombocytopenia and MDS transformed to accelerated phase Ph+ Chronic Myelogenous Leukemia (CML) with RT-PCR positive for BCR/ABL1 transcript p210 and cytogenetic analysis of the bone marrow (BM) raised a complex karyotype including 5,7 and 21 chromosome besides BCR/ABL translocation . The patient was treated with tyrosine kinase inhibitor imatinib 600mg each day and after a month he did not reached a haematologic response, so mutational analysis with NGS was made and showed VAF(variant Allele Frequency)c.1423_142ins35fsX with clinical and therapeutic significance unclear and started multi-target TKI active also against mutated forms of BCR-ABL1 ponatinib (15mg/day dosage adjusted for vasculitic patient history)and it is ongoing from 20 days .

Conclusions:The transformation from MDS to CML has rarely been reported and the translocation with additional chromosome abnormalities could be arise during the evolution of the haematological disease .To our knowledge, transformation of mds disease associated with retroperitoneal fibrosis in CML Ph+ with this complex karyotype has never been described.

Acute Leukemia

P019

METABOLIC CHARACTERIZATION OF ACUTE PROMYELOCYTIC LEUKEMIA AND ARSENIC TRIOXIDE RESISTANCE

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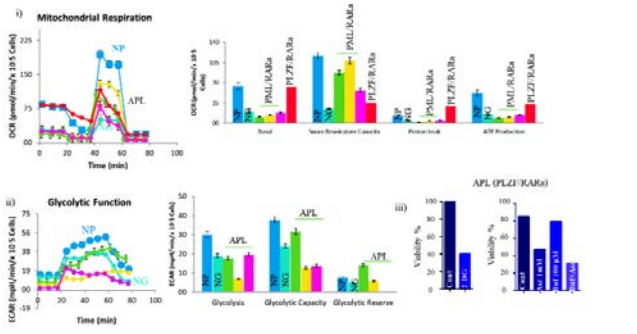
Introduction: Central in the pathogenesis of APL, PML/RARa rewires metabolic pathways to feed anabolic cell growth. ATRA and ATO-based therapies render APL the most curable subtype of AML, yet approximately 5% of cases are resistant and 5 to 10% relapse.

Aim: To characterize the metabolic peculiarity, to ascertain fuel requirement of PML-RARa expressing cancer cells, to identify new therapeutic targets and strategies.

Methods: We analyzed primary samples from 3 APL with PMLRARa, 1 from a PMLZF/RARa+ AML, and normal CD34+ cells differentiated to promyelocytes and granulocyte, to test the ability to switch oxidative pathways in meeting basal energetic requirements. The U937-PR9 cell line (PML-RARa inducible system); NB4 (APL cell line) and two ATO-resistant clones were studied. Metabolic profile was analyzed by the Seahorse XFe96; protein and mRNA expression by western blot and q-RTPCR; cellular viability by ATP Glow Assay and ROS by mytosox assay.



i) Mytocondrial respiration measured by Seahorse assay in normal promyelocyte (NP), normal granulocyte (NG) and APL primary blasts ii) Glycolytic respiration measured by Seahorse assay in normal promyelocyte (NP), normal granulocyte (NG) and APL primary blasts iii) Viability of APL primary blasts after treatment with 2-Deoxiglucose (2-DG), Ascorbate 1mM (A), Buf 100 µM and Buformin + Ascorbate.

Figure 1.

Results: We observed reduced respiratory and glycolytic capacity in APL samples, which renders these cells sensitive to metabolic treatments using 2-DG o Buformin + Ascorbate (Figure1). Expression of PML-RARa in U937-PR9 cell lines increases mitochondrial respiration rate (OXPHOS) and inhibits glycolysis. Differentiation of NB4 cells using ATRA and of normal CD34+ cells is associated with reduced OXPHOS and reduced glycolysis. Normal promyelocyte depends of FA and glucose for mitochondrial respiration, conversely APL blasts show enhanced FAO, increase in the carnitine transporter CT2, and lose pyruvate dependence, featuring flexibility for glucose utilization, and high lactate production when mitochondrial respiration is inhibited. The enhanced FAO and CT2 over-expression was confirmed in the PR9 system after PML/RARa induction. ATO resistance involves a switch towards glycolysis, reduced viability at low glucose cc (p=0.0008) and 1.5 times increase of the glucose transporter Glut1. Resistance induces further enhancement of FAO. ATO resistant clones shows an increase in glycolytic and in mitochondrial ATP production; an increase in FAO confirmed by a higher sensitivity to Perexilin, inhibitor of CPTA1 (p=0.003), and a 1.7 fold increase in the expression of CT2. One clone shows also high level of ROS (p=0.0001), the cellular integrity is protected by increased levels of Periredoxin in both, and a 1.5 fold increase in pAMPK that induces FAO and glycolysis. The apoptosis escape in ATO resistant cells has been related to a concomitant increase (1.7 folds) in the expression of Mcl1.

Conclusions: PML/RARa induces FAO. Metabolic shift from respiration to glycolysis and FAO in ATO resistant cells is mediated by pAMPK, the increase of glycolytic flux contributes to NADPH production and cell survival under oxidative stress. The increase in Mcl1 contributes to apoptotic resistance. ATO resistant patients could benefit from treatment with ATO in combination with glycolysis' and FAO inhibitors.

P020

MUTATION PROFILE AND FLT3-ITD CLONAL DYNAMICS IN AML PATIENTS RELAPSED AFTER TYROSINE KINASE INHIBITORS TREATMENT

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Acute Myeloid Leukemia (AML) is characterized by heterogeneous genetic abnormalities, which significantly influence clinical outcome. FLT3-ITD mutations (FLT3-ITDmut) characterize approximately 25% of AML patients and represent an independent predictor of poor prognosis, being associated with an increased risk of relapse. Recently, addition of FLT3 inhibitors to chemotherapy has significantly improved patients' survival, and midostaurin was the first FLT3 inhibitor approved for treatment of FLT3mut patients. However, AMLs with FLT3-ITDmut remain a therapeutic challenge, with a still high relapse rate, despite tyrosine-kinase inhibitors (TKIs) treatment. Here, we report FLT3-ITDmut clonal selection, and the profiles of additional mutations during disease progression after TKIs. A total of 58 consecutive patients aged 19-81 years with newly diagnosed AML, were observed and treated at the Hematology Unit of the Department of Biomedicine and Prevention of the University of Tor Vergata, during the period 2018-2020. Patients were treated according to fitness and following the ELN 2017 recommendations for management of AML patients. Molecular status of FLT3 was investigated at the DNA level on MNCs isolated from bone marrow at AML diagnosis using standard protocols. FLT3-ITDmut burden was reported as allelic ratio (AR) of mutant/wild-type alleles obtained by a semi-quantitative assay (Genescan analysis). For selected cases, FLT3-ITDmut clones were sequenced. Out of 58 tested patients, 15 (26%) and 3 (5%) were FLT3-ITDmut or FLT3-TKDmut, respectively and in particular, the median FLT3-ITDmut AR was 0.69 (range 0.05-0.86). Five FLT3-ITDmut patients relapsed after 3+7/midostaurin or quizartinib combination at a median of 10.5 months (range 6-14) from TKI treatment (Figure 1). In patients relapsed after midostaurin, two were characterized by 2 different FLT3-ITDmut clones at diagnosis (UPN1/UPN2) and only one ITD+ clone was retained at relapse, while one patient became FLT3-ITD negative (UPN3). On the contrary, the same FLT3-ITDmut pattern was present both at diagnosis and at relapse in 2 patients treated with quizartinib (UPN4/UPN5). No associations with specific FLT3-ITDmut nucleotide sequences were found in patients who lost the FLT3-ITDmut clone at relapse, but clonal selection of a minor FLT3-ITDmut clone present at diagnosis was observed in 2 pts after midostaurin. Using NGS, we then studied the pattern of clonal evolution during disease progression in these patients (Figure 1). In cases treated with midostaurin, RUNX1,

ASLX1, and WT1 mutated clones expanded at relapse, while in one patient the second RUNX1-mutated clone was lost. For patients who received quizartinib, clonal selection of a N-RAS mutation occurred at disease relapse. The load of NPM1 mutations was almost stable at disease onset and at relapse, and allelic burden of DNMT3AR882H and IDH2R140Q mutations remained constant, compatible with their role as driver (NPM1) and background mutations in AML. Our results demonstrate that TKIs treatment may impact on clonal evolution of FLT3-ITD-mut AML, downregulating specific clones. These data, underscore the importance of repeated mutation testing for FLT3-ITDmut to distinguish patients where TKI may induce long-lasting remission, from those where relapse may originate from subclones, which may be FLT3wt.

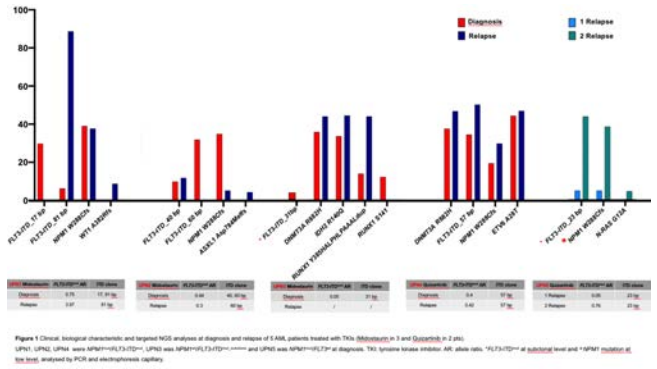

Figure 1.

P021

RNA-SEQUENCING REVEALS ZNF384 REARRANGEMENTS AS THE MOST FREQUENT LESION IN ADULT PHILADELPHIA NEGATIVE, NON PH-LIKE, PATIENTS ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): BIOLOGICAL AND CLINICAL FINDINGS

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Introduction: Genome-wide techniques identified new recurrent fusions in Philadelphia negative B-lineage ALL (B-ALL), which allow to define new subtypes of ALL. ZNF384 encodes a zinc finger protein that regulates transcription of the extracellular matrix genes. Fusion genes involving ZNF384 have been recently reported in B-ALL, with 9 different partner genes: EWSR1, TAF15, TCF3, EP300, SYNRG, CREBBP, BMP2K, SMARCA2 and ARID1B. The presence of ZNF384 rearrangements (ZNF384-r) was reported in 6% of children and 7% of adults.

Methods: 164 newly diagnosed adult B-ALL patients enrolled in the GIMEMA LAL1913 and LAL2317 front-line clinical trials were first analyzed by our in-house BCR/ABL1-like predictor and, subsequently, by TruSight RNA Pan-Cancer Panel kit (Illumina, San Diego, CA). To confirm ZNF384-r, RT-PCR and Sanger sequencing were performed. Copy number aberration (CNA) analysis was performed by MLPA using the SALSA MLPA kit P335 ALL-IKZF1 (MRC Holland, Amsterdam, The Netherlands). MRD status was monitored at various time points, as per protocol.

Results: By RNA sequencing, we identified 15 cases with ZNF384-r (12.8%): the most frequent fusion was ZNF384-EP300 (n=7), followed by ZNF384-TAF15 (n=4), ZNF384-TCF3 (n=2) and ZNF384-ARID1B (n=1). We also identified a novel ZNF384-r - *i.e.* ZNF384-SPI1 -in a patient; results were confirmed by RT-PCR. All cases were non-Ph-like ALLs. The median age of ZNF384-r patients was 39 years (19-64), median WBC was 4.x109/l. Patients with ZNF384-r displayed a non-typical immunophenotype with a heterogeneous or negative expression of CD10 (3 pro-B, 11 Common, 1 pre-B), ranging from 86% to 0% (mean 43.3 ± 29.2%); 7 and 14 patients of the 15 ZNF384-r patients (46.6% and 93.3%, respectively) expressed > 20% CD13 (mean 36.8 ± 35.2%) and CD33 (mean 59.2 ± 28.4%). CNA analysis, performed in 10/15 ZNF384-r cases revealed IKZF1, BTG1, CDKN2A/2B, PAX5 and EBF1 deletions in 3 (30%), 1 (10%), 4 (40%), 1 (10%) and 0 cases, respectively. Only 2 patients, both with ZNF384-TAF15, had IKZF1 plus deletions. MRD evaluation - feasible in 11 patients at TP1 and TP2, 9 at TP3 and 5 at TP4 - showed that 36.4%, 72.7%, 77.8% and 100% of ZNF384-r cases were negative or positive non-quantifiable at TP1, TP2, TP3 and TP4 respectively. No relapses have so far been reported (median follow-up: 18 months, range 4-62). Finally, this subtype expressed high levels of FLT3, the median FLT3 expression being 891.5 in ZNF384-r *vs.* 460.3 in non-ZNF384-r cases (p=0.002).

Conclusions: The frequency of ZNF384-r in in our cohort of adult non-Ph+ and non-Ph-like ALL is 12.8%, higher than previously reported. EP300-ZNF384 was the most recurrent fusion, with a frequency of 6%. These cases have some peculiar features, represented by a low expression of CD10, frequent co-expression of myeloid antigens, and overexpression of FLT3; from a clinical standpoint they are frequently MRD - from TP2 onwards and, in line with this, no relapses have so far been recorded.

P022

METABOLIC ORIENTED TREATMENT: EFFICACY OF BUFORMIN PLUS ASCORBATE IN ACUTE MYELOID LEUKEMIA

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Introduction: Metabolic targeted therapies may represent an innovative strategy in elderly AML patients unfit for intensive chemotherapy. Metabolic peculiarities of leukemia stem cells are indeed involved in tumor growth, survival and resistance to therapy. Aim: Since AML cells mainly depend on mitochondrial respiration for ATP production, we challenged AML cells *in vitro* with Buformin® (Buf 0,1 and 0,5 mM), that inhibits complex I and shuts down mitochondrial contribution in ATP production, and Ascorbate (Asc 1mM) that blocks hexokinase.

Methods: We characterized the metabolic profile of different cell lines: OCI-AML2 (DNMT3A+); OCI-AML3 (NPMc+,DNMT3A+); U937AETO (RUNX1/RUNX1T1 inducible); MV411 (MLL/AF4, FLT3+), and primary blast from AML patients. The metabolic profile was studied using the Seahorse XF Analyzer. Viability was evaluated with MTS and ATP Glow Assays. Western blot analysis was performed for apoptotic signature. Colony formation after treatment was evaluated in MethoCultTM and apoptosis was assessed by flow cytometry.

Results: Buf plus Asc shoot down respiratory capacity and mainly mitochondrial ATP production in primary AML. In all cells lines we observed an arrest of mitochondrial respiration and ATP production after combined treatment, but only in Oci-AML3 and in U937-AETO cells the glycolytic capacity decreased 4.7 times and 10.5 times respectively, coincident with a decrease of GAPDH protein expression (p=0,001) and underline the higher sensitivity of this cells lines. To evaluated possible mechanism of death we treated cells with specific inhibitors: N-Acetyl-Cysteine (NAC) 10 mM (Ros production), Zvad 20 µM (caspases), MG132 0,3 µM (proteasome) and Ammonium Chloride 10 mM (autophagy). NAC restored 100% viability, thus Ros production was mainly responsible for Buf plus Asc induced cell death. Primary blasts from seventeen AML patients, assayed for annexin and live/dead exclusion by flow cytometry, showed an increase in the apoptotic effect using the combination, as compared with Ascorbate alone (Figure 1A).

The combination completely inhibited colony formation in MethoCult™, colonies number by OCI AML3 (Control 110; Asc 50; Buf + Asc: 14) and MV4;11 (Control 121; Asc 50; Buf + Asc: 20) cell lines, but not by normal bone marrow CD34+ cells (Control 111; Asc 149; Buf + Asc: 109) (Figure 1B). The combination is not toxic for normal cells and can be useful to target leukemic stem cells. Western blot analysis for BCL2 expression, using nuclear extracts of the OCI AML2 ($p<0,05$), OCI AML3 ($p<0,0001$) and MV4;11 ($p<0,0001$) cell lines showed an evident decrease after treatment in a time dependent manner (Figure 1C).

Conclusions: Our data show that Buf combined with Asc decreases ATP production and downregulates glycolysis, adding to the apoptotic effect of Ascorbate in primary AML blasts, sparing normal CD34+ bone marrow cells. The Buf-Asc combination could be an innovative therapeutic option for AML.

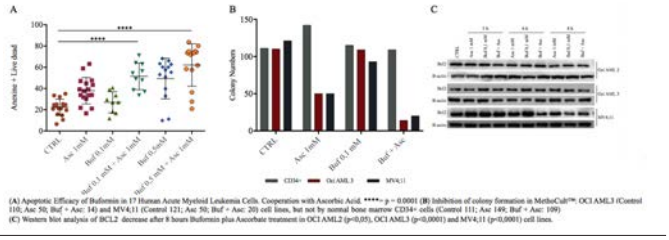

Figure 1.

P023

THE STIMULATION OF BITTER TASTE RECEPTORS HAS A SYNERGISTIC EFFECT WITH CHEMOTHERAPY IN ACUTE MYELOID LEUKEMIA CELLS

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**These authors contributed equally to this work as the last author*

Background: Bitter taste receptors (T2Rs) are G protein-coupled receptors known for their primary role as a warning signal to induce aversion towards noxious/harmful substances. Nevertheless, the increasing amount of evidence about their extra-oral localization has suggested alternative functions and there is growing interest in their potential role in cancer biology. Interestingly, it has been demonstrated that T2R agonists displayed anti-cancer effects against various cancer cell types and improved the efficacy of chemotherapy. Recently, we reported that AML cells express functional T2Rs and their activation with the agonist denatonium benzoate (DEN) modified the AML cell functions, reducing their proliferation or inducing apoptosis. We hypothesized that T2R activation may potentiate the cytotoxic effect of antineoplastic drugs on AML cells.

Methods: Primary AML samples, THP-1 and OCI-AML3 leukemia cell lines, and cord blood-derived CD34+ cells were used. T2R expression was analyzed by RT-PCR. Functional analyses were assessed by MTT assay. Expression assay were evaluated by FACS analysis.

Results: We investigated whether the combination of DEN with cytarabine (ARA-C) interacts in a synergistic way to enhance their antineoplastic activity. Our data show that DEN potentiates the cytotoxic effect of ARA-C, significantly reducing AML cell proliferation and allowing to reach a high toxicity using lower doses of the chemotherapic agent. Multidrug resistance is a frequently encountered phenomenon contributing to treatment failure in AML and several members of ATP-binding cassette (ABC) transports are involved in it. It has been shown that T2R triggering modulates their expression in cancer settings. Interestingly, we observed a reduced expression of the ABC transporter associated to ARA-C, ABCC4, after AML cell exposure to DEN, suggesting the ABC transporter inhibition as a possible mechanism responsible for enhanced chemotherapy response after T2R activation. Preliminary data showed that other T2R agonists, Chloroquine and Quinine, also synergistically increase the cytotoxic effect of ARA-C. Afterward, we analyzed the T2Rs expression and function on normal hematopoietic stem cells (HSCs). Interestingly, even if HSCs expressed several T2R subtypes, T2Rs activation by DEN did not affect their viability and apoptosis, conversely to AML cells.

Conclusions: Overall, our results show that T2R system could be used as target for the development of new therapeutic strategy aiming to potentiate the cytotoxic effect of chemotherapic drugs reducing their impact on normal hemopoiesis.

P024

PAN-AURORA KINASE INHIBITOR TOZASERTIB INDUCES THE ACTIVATION OF DNA DAMAGE REPAIR NETWORK IN ACUTE MYELOID LEUKEMIA

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Introduction: Aurora kinases A and B play a pivotal role in cell cycle regulation, and increased expression has been described in acute myeloid leukemia (AML) making them a promising therapeutic target. Phase I/II studies of Aurora kinase inhibitors combined with induction chemotherapy have demonstrated a good tolerability and efficacy in patients with high-risk AML (Fathi et al, Haematologica 2017; Brunner, Blood 2018). However, the molecular mechanisms underlying their anti-tumor effects still remain to be elucidated.

Methods: AML cell lines (NOMO-1: t(9;11); MOLM-13: FLT3-ITD; KASUMI-1 t(8;21), c-KIT and p53 mutated; OCI-AML3: NPM1 and DNMT3A mutated) were treated for 24 h with the pan Aurora inhibitor Tozasertib (50 and 100 nM). Apoptosis and cell cycle evaluations were performed by Annexin V and PI staining, respectively. Protein level alterations were analyzed by western blot (WB).

Results: Tozasertib strongly inhibited growth and proliferation in AML models. In particular, all tested cell lines showed a significant accumulation of cells in G2/M phase after 24h of treatment, in a dose-independent way. Increased apoptotic cell death was induced in a dose-dependent manner after 24h of treatment in NOMO-1 (38,2% at 100 nM and 46,6% at 250 nM) MOLM-13 (41,5% at 100 nM and 56,4% at 250 nM) and KASUMI-1 (55,5% at 100 nM and 61,7% at 250 nM) models, while OCI-AML3 showed no significant apoptotic increase. WB analysis demonstrated that inhibition of Aurora kinases was associated with activation of the DNA damage repair (DDR) network, with the expression of p-p53(ser15) and γH2AX in all tested cell lines. Interestingly, the induction of apoptosis observed in NOMO-1, MOLM-13 and KASUMI-1 models correlated with a strong increase of trimethylation levels at lysine 36 of histone H3 (H3K36me3) that is involved in regulation of DDR network.

Conclusions: Aurora kinase inhibition induced an evident cell-cycle arrest in G2/M phase through induction of the DDR network in AML models. Increased apoptotic cell death was correlated with up-regulation of H3K36me3, suggesting its involvement in AML cell persistence and survival. Notably, Aurora kinase inhibition induced cytostatic and cytotoxic effects in MLL-AF9 translocated, FLT3-ITD, and both wt and mutant TP53 AML models, which represents an important goal in AML therapy. In conclusion, our results highlight the new pivotal role of Aurora kinase pathway in regulation of DDR response as an overall pro-survival mechanism involved in AML pathogenesis.

P025

UNCOUPLING HOX GENES OVEREXPRESSION AND TRILINEAGE CLONAL TERMINAL DIFFERENTIATION BY FLT3-INHIBITORS TREATMENT IN NPM1MUT/FLT3-ITD AML

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Introduction: Homeobox (HOX) and cofactors MEIS1 and PBX3 genes, expressed in hematopoietic stem cells (HSC) are involved in self-renewal and are typically silenced in mature blood cells. Their dysregulation in HSC favours leukemogenesis. We previously reported that HOX and cofactors up-regulation is associated with NPM1 mutation, independently from co-occurring mutations, and maintains the undifferentiated state of leukemic cells. Indeed, silencing NPM1mut in AML leads to their downregulation, followed by cell differentiation (Brunetti *et al.*, Cancer Cell 2018). We recently observed that FLT3-inhibitors (FLT3i) may induce terminal clonal trilinear differentiation of leukemic cells in NPM1mut/FLT3-ITD AML not followed by eradication of the leukemic clone (Martelli *et al.*, SIES 2018, CO03), leading ultimately to disease progression. Here, we investigated how HOX and their cofactor genes expression changed with this phenomenon.

Methods: qRT-PCR was used to quantify expression of HOXA9, HOXA10, MEIS1 and PBX3 known to be involved in leukemogenesis and upregulated in NPM1-mut AML. qRT-PCR products were normalized to GADPH before estimating Fold-change values. GraphPad Prism was used for statistical analysis (CI 95%; p-value <0.05). Analysis was performed on cells harvested from bone marrow (BM, n=3), either at baseline or upon FLT3i (Gilteritinib:2/Sorafenib:1). Pre- *vs.* post-FLT3i PB samples were studied in 2 patients (Gilteritinib:2). The same analysis was performed also on samples (BM/PB) from 3 healthy donors, as comparison.

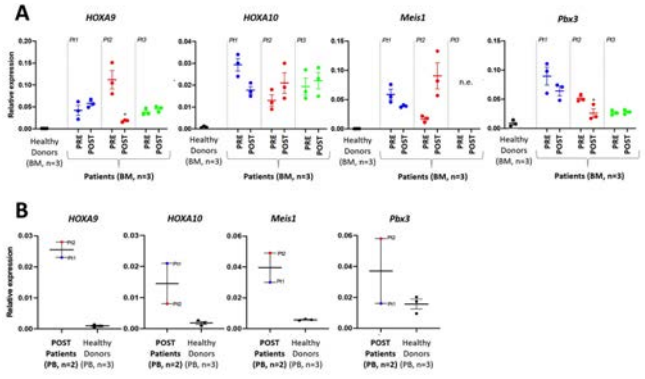

Figure 1.

Results: As predicted, compared to healthy donors, HOXA9/10, MEIS1 and PBX3 were overexpressed in pre-FLT3i, either BM (n=3) (Figure 1A) or PB (n=2) (not shown), patients samples (blasts>90%). Strikingly, although BM cells of patients (n=3) treated with FLT3i appeared terminally differentiated, mimicking a morphological CR (blasts<5%), no statistically significant downregulation of HOX and their cofactors occurred, with the exception of HOXA9 and PBX3 (2.6 and 1.1 fold reduction) in pt.2 (Fig.1A). As compared to baseline, high levels were confirmed also in post-FLT3i PB patients samples (n=2), enriched in granulocytes of leukemic origin. Consistently, comparing these terminally differentiated leukemic cells from BM (n=3) and PB (n=2) patients upon FLT3i, respectively, with BM and PB cells from healthy donors (n=3), we further confirmed overexpression of HOX and their cofactors genes in patients' samples (Figure 1A and B).

Conclusions: We show that high levels of HOX and their cofactor genes are maintained in mature cells with NPM1 and FLT3-ITD mutations derived from patients treated with FLT3i, suggesting for the first time that the morphological state of the terminally differentiated hematopoietic leukemic cells can be uncoupled from the HOX-related 'self-renewal signature' when NPM1 mutation is sustained. Functional studies to assess leukemogenic and self-renewal potential of these terminally differentiated leukemic cells are warranted.

P026

MOLECULAR MINIMAL RESIDUAL DISEASE MONITORING IN NPM1-MUTATED ACUTE MYELOID LEUKEMIA: A SINGLE INSTITUTION EXPERIENCE

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Introduction: The NPM1mut identification by RQ-PCR at diagnosis is important for AML risk stratification and represents a reliable marker to track minimal residual disease (MRD) and to early detect relapse. We retrospectively analyzed clinical and biological features of NPM1mut AML pts consecutively treated with intensive therapy in our Institution to evaluate the role of MRD monitoring in relation to overall survival (OS) and disease free survival (DFS).

Methods: We analyzed 104 NPM1mut AML pts eligible for intensive therapy, diagnosed between 2008 and 2018. Median age was 52 years (y) (range, 8-75). NPM1mut was detected in the bone marrow (BM) by RQ-PCR at diagnosis and at different time points. MRD levels were expressed as a percentage (ratio of the NPM1 copies-cp to the housekeeping gene ABL cp × 100); MRD positivity was defined as any level above 0.01%. FLT3mut was detected in 50 pts (48.1%) (7 FLT3-TKD; 43 FLT3-ITD). All pts received intensive treatment according to local institutional standard. After induction and consolidation, pts received either high-dose (HD) chemotherapy followed by autologous stem cell transplantation (ASCT) or 2/3 further cycles of HDAra-C if not eligible for ASCT.

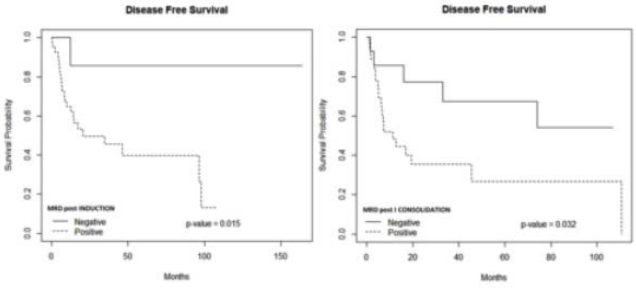

Figure 1.

Results: Eighty-nine pts achieved a complete remission (CR) (85.6%), 7 proved refractory (6.7%), while 8 died during induction (7.7%). After induction, MRD was available for 50 pts (61.7%): 8 (16%) were MRD negative (-) (7 FLT3wt; 1 FLT3mut) and 42 (84%) were MRD positive (+) (18 FLT3wt; 24 FLT3mut); MRD evaluation after I consolidation was performed in 52 pts (64.2%): 16 (30.8%) were MRD- (9 FLT3wt; 7 FLT3mut), 36 (69.2%) were MRD+ (16 FLT3wt; 20 FLT3mut). For the whole cohort, the 5y-OS was 40.8% (95% CI, 31.5-52.9%) and 5y-DFS was 38.2% (95% CI, 28.8-50.7%). Achieving an MRD- after induction or I consolidation, identified pts with better 5y-DFS than pts with persisting MRD+ (85.6% and 65.5% *vs.* 40% and 26%; p= 0.015, p= 0.032). This also translated into significant differences in 5y-OS (100% and 75.8% *vs.*

44% and 30.5%; p= 0.009, p= 0.045); 5y-cumulative incidence of relapse (CIR) was 43.7% (95% CI: 30.2-54.6%). No statistically significant differences were observed in OS (p= 0.535) and DFS (p= 0.224) according to FLT3 status. However, the CIR was higher in FLT3mut pts (p= 0.063). In the whole cohort, ASCT were 24, allogenic (allo)SCT were 26. Reasons for alloSCT in CR1 were: 1) FLT3-ITDmut; 2) secondary-AML; 3) raising MRD levels, 4) slow clearance of MRD reduction; 5) primary refractory pts. MRD- pts before alloSCT showed a trend towards better OS (p= 0.074) and DFS (p= 0.065) than MRD+ pts.

Conclusions: Our study underlines the clinical relevance of achieving an early molecular response in NPM1mut AML. MRD monitoring is a valuable tool for the early identification of pts who might benefit from alloSCT/new drugs and pts with molecular relapse. The most relevant time points for collecting samples and the prognostic MRD thresholds remain to be defined.

P027

EXTENSION OF MRD ANALYSIS PH- ALL PATIENTS FOLLOWING THE CLOSURE OF THE GIMEMA LAL 1913 TRIAL

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Background: Minimal residual disease (MRD) monitoring is the major prognostic indicator in adult acute lymphoblastic leukemia (ALL). MRD analysis must be performed in highly skilled and certified laboratories and this approach has been carried out in the GIMEMA LAL 1913 trial for Ph- ALL. Following the closure to enrolment of the trial, MRD could not be performed despite the many requests, reducing the quality of treatment provided to the newly diagnosed cases. We obtained an unrestricted support from Shire in order to continue to provide an optimal characterization and monitoring of Ph- ALL patients.

Methods: Between January 2018 and February 2020, 156 newly diagnosed ALL samples and 259 follow-up (FU) samples from 42 Italian centers were sent to the Hematology Center of the 'Sapienza' University. Multi-parametric flow cytometry (MFC) analysis was carried out at diagnosis. Molecular analyses - gene fusion and case-specific gene rearrangements - were performed.

Table 1.

71 B-lineage ALL cases analyzed for gene fusions

76 T-lineage ALL cases analyzed for gene fusions

N. of cases	Translocation	Fusion gene	Positive	Negative
71/71	t(9;22)(q34;q11)	BCR/ABL1		x
5/71	t(4;11)(q21;q23)	ALL1/AF4	x	
3/71	t(1;19)(q23;p13)	E2A/PBX1	x	
1/76	t(11;19)(q23;p13)	MLL/ENL	x	
1/76	t(1;14)(p32;q11)	SIL/TAL1	x	
4/76	del9(9q34.11; 9q34.13)	NUP214/TAF1	x	
1/76	9q34 episome amplification	NUP214/ABL1	x	
1/76	Mutations of Fms-related tyrosine kinase 3 (FLT3) internal tandem duplication (FLT3-ITD)	FLT3-ITD	x	

Results: MFC analyses were carried out on the 156 diagnostic patients and showed different immunophenotypes: 71/156 were B-lineage ALLs, 76/156 were T-lineage ALLs, 6/156 were mixed-phenotype acute leukemias (MPAL), 1/156 was not evaluable due to the hypocellularity of the sample, 1/156 was an acute undifferentiated leukemia (AUL), 1/156 was a second tumor derived from a myelodysplastic syndrome. Molecular analyses of gene fusions were performed in all cases. Informative results were reported in 147/156 cases and are summarized in Table 1. Overall, a total of 131 cases proved eligible for IG/TR gene rearrangement analysis: 76/131 (58%) underwent a IG/TR screening, while 55/131 (42%) could not be analyzed because of lack of material at the time points (TP) following diagnosis. Of the 76 cases analyzed, 5 (6.6%) resulted with no marker and 11 (14.5%) were marker-positive but without a sensitive probe: 60/76 (78.9%) cases were suitable for MRD quantification. The cases without a sensitive probe have been monitored by qualitative PCR. The MRD analysis was performed by the two molecular methods at four TPs and at several post-induction FU: 259 FU samples from 87 patients - 71 IG/TR-positive and 16 gene fusion-positive - were analyzed.

Conclusions: At diagnosis, all adult Ph- ALL cases were studied by MFC and molecular approaches in order to apply a methodologic algorithm to classify biologically the disease and to identify leukemia-associated targets for MRD monitoring. Since, the IG/TR screening is laborious, expensive and time-consuming, we decided not to study patients who did not have at least one monitoring TP: in 42% of cases, the referring centers did not send samples to monitor MRD. This resulted in an inferior number of cases analyzed compared to the total of cases potentially eligible to IG/TR screening. Overall, MRD monitoring was possible in 87 cases that were marker positive, but in a relevant proportion of patients monitoring could not be carried out because of lack of compliance to the expected TPs.

P028

ABSTRACT WITHDRAWN

P029

KEVETRIN TARGETS TP53 WILD-TYPE AND MUTANT ACUTE MYELOID LEUKEMIA CELLS

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Introduction: Thioureidobutyronitrile (kevetrin) is a small compound that showed activity against Tumor protein p53 (TP53) wt and mutant solid tumors (lung, breast, colon and ovarian) both in cell lines and xenograft models (Kumar, Cancer Res, 2011, 2012, 2017). TP53 is mutated in 8-14% of acute myeloid leukemia (AML) cases (Haferlach, Leukemia, 2008), associating with typical complex karyotype (Leung, Am J Hematol, 2019) and chromothripsis (Fontana, Leukemia, 2018; Rücker, Haematologica, 2018) and conferring a very poor prognosis (Haferlach, Leukemia, 2008). The aim of this study was to evaluate the consequences of kevetrin exposure in TP53 mutant and wt AML cell lines and primary cultures.

Methods: TP53-wt MOLM-13 and OCI-AML3 and TP53-mut KASUMI-1 and NOMO-1 were treated with kevetrin [85-340 μM]. After 24 and 48 h MTS, Annexin-V and cell cycle alterations were evaluated. Western blot and immunofluorescence analysis were performed after 48 h exposure to increasing kevetrin doses. In primary samples cell viability

was evaluated by trypan blue exclusion assay and Annexin V staining was combined with surface markers (CD45, CD33, CD14, CD3 and CD19). Patients' mutational profile was determined using SOPHiA Myeloid SolutionTM.

Results: MOLM-13 and OCI-AML3 TP53-wt models, showed a significant decrease of cell viability and apoptosis induction only after 48 h at the highest concentration. A dose- and time-dependent inhibition of cellular viability, was detected in KASUMI-1 and NOMO-1 cell lines with a significant increase in Annexin V+ cells after 48 h in NOMO-1 (60.93% vs. 22.90% in the control), and a dose-dependent response in KASUMI-1 cells, with 79.70% of apoptotic cells after 48 h at 340 μM (compared to 13.18% of the control). NOMO-1 and OCI-AML3 also showed a significant increase of cells in the G0/G1 phase and a reduction of S phase cells after treatment. Immunofluorescence analysis showed an increased p53 expression at the highest kevetrin dose in all cell lines, and in particular in TP53-mutated ones. In primary AML blast cells we observed a dose-dependent decrease of cell viability and a significant increase of apoptotic cells (170 μM: 31.8 ± 13.3% vs. CTRL: 11.4 ± 6.5, 340 μM: 54.3 ± 13.9% vs. CTRL) with a preferential activity on AML blasts, while monocytes and lymphocytes were marginally affected. One of the cases carried a TP53 mutation (NM_000546, c.764T>A, p.(Ile255Asn), VAF% 92.3%), showing a higher effect compared to TP53-wt ones.

Conclusion: Our results showed an activity of kevetrin on both TP53 wt and mutated AML cells, the latter being more sensitive. Moreover, they provide the rationale for an experimental trial in AML patient, especially those carrying TP53 mutation, who currently have very few therapeutic options.

P030

GERMLINE TP53 MUTATIONS IN A COHORT OF PAEDIATRIC HYPODIPLOID ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Hypodiploid acute lymphoblastic leukaemia is a rare subtype of childhood ALL and it is known to be associated with a poor prognosis. Currently, haematopoietic stem cell transplantation is recommended as the main therapeutic strategy. Previous studies showed the hallmark of low-hypodiploid ALL are variants in TP53, the majority of which are germline. This finding suggests that hypodiploid ALL may be one possible manifestation of Li-Fraumeni syndrome. This study aims to identify TP53 variants in hypodiploid paediatric ALL patients focusing on frequency and features of germline variants.

Methods. We performed a targeted Next Generation Sequencing (NGS) Nextera Flex DNA panel of 40 genes, including TP53, of hypodiploid paediatric ALL Italian patients enrolled in four nationwide frontline protocols. Ploidy was defined based on DNAindex and/or cytogenetics/FISH. BM at diagnosis and at MRD-controlled remission was analyzed. Only P53 variants with VF >5% and coverage 500X were considered. Bioinformatics analysis has been carried out by Sophia DDM software. Cases have been analysed both in disease-hematopoietic and germline tissues (remission or buccal brush samples.

Results. TP53 variants were observed in 21/41 (51%) hypodiploid ALL patients. Interestingly, 20/21 of TP53 mutated and 12/20 (60%) of TP53 germline were low-hypodiploid ALL (DNAi between 0,6 and 0,8). 12 patients out of 18 (67%) were found to carry a germline variant, while 6 patients (33%) presented a somatic variant. In 3 cases a remission sample was not available, but we can hypothesize that 2 of them carry a somatic variant (VF < 20%) and 1 carries a germline variant (VF 50%). Among the germline variants, 1 was found to be a germline mosaicism, presenting a nonsense pathogenic TP53 variant both in remission (VF 10%) and buccal brush sample (VF 8,5%). Overall, we filtered 20 different TP53 variants (two patients shared the same variant); 13/20 were known to be pathogenic and 7/20 were classified as VUS. Considering the VUS variants, 3/7 were missense, 2/20 frameshift and 2/20 in-frame. 16/20 variants (including 6/7 VUS) reside in the p53 core DNA binding domain, known to be a fundamental site that mediates the transcription of p53 regulated genes, where most of the pathogenic mutations in cancerous cells occur.

Conclusion. These results confirm the high frequency of deleterious TP53 germline mutations in low-hypodiploid ALL and suggest the importance of mutational testing of TP53 in these patients, to ensure a proper genetic counselling to patients and families, a tailored therapy (*i.e.* condition regimen for hematopoietic stem cell transplantation) and a specific clinical surveillance.

P031

TERMINAL DEOXYNUCLEOTIDYL TRANFERASE (TDT) EXPRESSION IS ASSOCIATED WITH FLT3-ITD MUTATIONS IN ACUTE MYELOID LEUKEMIA (AML)

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Introduction: The TdT enzyme is a DNA polymerase expressed in immature lymphoid cells and ALL. It is also expressed by one third of AML cases. Recent studies showed that TdT may be involved in the generation of NPM1 and FLT3-ITD mutations by adding nucleotide stretches at N-regions of the duplication junctions of these genes. We analyzed the correlation between TdT expression, prevalence of FLT3-ITD and other genetic features, and their influence on survival in a cohort of AML patients.

Methods: Between 2011 and 2018, 143 AML patients (median age: 62.5 years, range 21–86 years) diagnosed and treated at the Hematology Unit of Policlinico Tor Vergata in Rome, were studied for TdT expression, measured by flow cytometry as percentage of positive blasts and mean fluorescence intensity (MFI). Since the mutational status of TP53, ASXL1 and RUNX1 genes was not available for all patients, we stratified our population according to 2010 ELN genetic risk assessment. Treatment schedules were defined by age and performance status, according to the international standards.

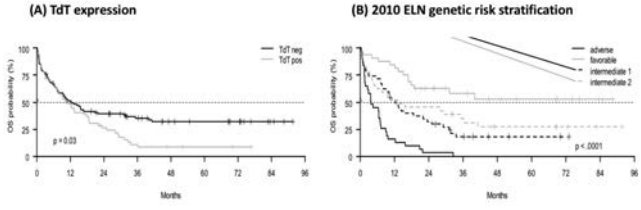

Figure 1. Overall survival

Results: TdT was positive in 49 cases (34.2%), with a median of 48% (range 7-98%) TdT+ blasts and a median MFI of 2.70 (range 1.23-30.54). Clinical characteristics were similar in TdT+ and TdT- patients. FLT3-ITD mutations were present in 24 patients (16.7%): 12 NPM1mut/FLT3-ITD+ (5 high-, 7 low-AR) and 12 NPM1wt/FLT3-ITD+ (6 high-, 6 low-AR). Twenty-two of 34 NPM1 mutated patients were FLT3-ITD-. The proportion of TdT+ blasts was significantly higher in FLT3-ITD+ patients (median 8%, range 0-98%), as compared with FLT3-ITD- cases (median 0%, range 0-98%, p=0.035). The predictivity index of TdT+ for FLT3-ITD mutations was 0.69. FLT3-ITD AR, karyotype and NPM1 mutational status did not correlate with percentage of TdT+ blasts or MFI. Analysing the N-regions of the duplication junctions in 12 FLT3-ITD+ patients (7 TdT+ and 5 TdT-) the additional fillers were identified

in 2 out of 7 TdT+ patients (28.6%). Despite no impact on CR achievement, TdT+ patients had poorer survival as compared to TdT- (5-year OS 10% *vs.*35%, p=0.03) (Figure 1A). Stratifying population by 2010 ELN genetic risk, 5-year OS was 55% for favourable, 25% for intermediate I, 18% for intermediate II groups, while none of the patients in the adverse group survived at 5 years (p<.0001) (Figure 1B). In the multivariable analysis, ELN risk stratification was an independent prognostic factor for OS (fav. *vs.* adv. p<.0001; int-I *vs.* adv. p=0.004; int-II *vs.* adv. p<.0001), together with age (p=0.006) and type of treatment (intensive chemotherapy *vs.* supportive care, p<.0001), while TdT positivity lost its prognostic value (p=0.196).

Conclusions: In summary, our results are in line with the previous data about the possible implication of TdT in the generation of FLT3-ITD mutations in AML. This translates into a significant association between TdT expression and FLT3-ITD positivity. Moreover, TdT expression seems to be associated with reduced survival.

P032

OCCULT OR MANIFEST INVOLVEMENT OF CENTRAL NERVOUS SYSTEM (CNS) IMPACTS ON OUTCOME IN ADULT ACUTE MYELOID LEUKEMIA (AML)

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Background: Several studies demonstrated that flow cytometry (FCM) is superior to conventional cytology (CC) for detection of Central Nervous System (CNS) involvement in lymphoproliferative disorders. At the opposite, the role of FCM to investigate cerebrospinal fluid (CSF) in acute myeloid leukemia (AML) is still unknown.

Design and Methods. The aims of our study were 1) to determine the incidence of occult/manifest CNS disease in a homogenous series of AML patients 2) to correlate CNS disease with clinical/biological parameters 3) to examine the impact of CNS involvement on outcome. We collected CSF samples from 126 newly diagnosed AML patients, 81 males and 47 females, median age 55 years (range 18-80). Of these, 98 patients received standard (SDARAC) and 23 high-dose-ARA-C-based (HDARAC) regimens, 5 supportive care. All CSF samples were examined by CC whereas FCM was performed in 118/126 (91%) samples. A cluster of at least 10 phenotypically abnormal events was regarded as a proof of FCM positivity and then of CNS infiltration by leukemia.

Results: Eighty-five patients were CNS negative (CNS-), while forty (31,7%) patients were CNS positive (CNS+). Of these, 12 (9,5%) presented positivity both CC than FCM (manifest CNS+) and 23 (18,3%) were only FCM positivity (occult CNS+). The median age and the median LDH were similar in both groups. Instead, higher levels of WBCs at diagnosis were observed in CNS+ than CNS- patients (p=. 02, median WBC 9680/mmc *vs.* 37000/mmc). No significant difference in cytogenetic/genetic features between CNS+ and CNS- patients were observed. The complete remission (CR) rate showed no significant difference between CNS+ and CNS- patients (69% *vs.* 81% p= NS). The 5-years DFS and OS were significantly shorter in occult or manifest CNS+ patients than CNS-(13,6% *vs.* 32,5% p= .006 and 19% *vs.* 46,5%, p=.008, respectively, Figure 1a). We observed that DFS and OS were also shorter in CNS+ pts who received SDARAC (0% *vs.* 29,7% p= .000 and 7,9% *vs.* 45,5%, p=.000, respectively, Figure 1b).

Conclusion: Our data suggest that incidence of CNS involvement in newly diagnosed AML pts is higher than currently expected. Manifest and also occult CNS positivity should always be investigated at diagnosis, regardless of neurologic symptoms, since it may occur in asymptomatic pts and significantly affect outcome. Particularly, pts candidate to receive SDAC could benefit the FCM evaluation to detect occult CNS disease. Further prospective studies on larger series are warranted to confirm this data.

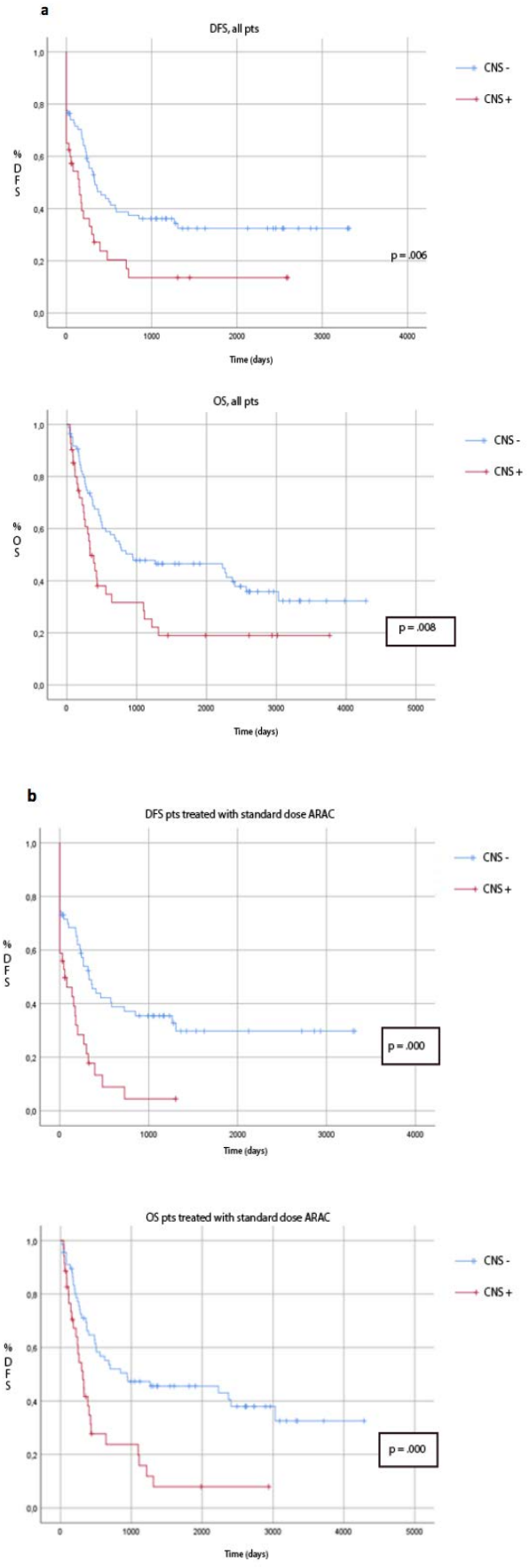

Figure 1.

P033

INOTUZUMAB OZOGAMICIN AND DONOR LYMPHOCYTE INFUSION ARE A SAFE AND PROMISING COMBINATION IN RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA AFTER ALLOGENEIC TRANSPLANT

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Introduction: Post allogeneic hematopoietic stem cell transplant (HSCT) relapse of B-cell ALL is associated with a dismal outcome. Calicheamicin-immunoconjugate anti-CD22 Inotuzumab Ozagamicin (IO) allows, in the setting of R/R B-ALL patients, high response rates, even molecular, but with limited duration. We report the outcome of 8 B-ALL adult patients, relapsed after allogeneic HSCT, and treated at three Italian Institutions with IO in association with donor lymphocyte infusion (DLI), either in a sequential or alternate schedule.

Methods: Complete remission (CR) was defined as bone marrow (BM) blasts < 5% and no evidence of extramedullary (EM) disease (PET-documented in PET+ patients pre-IO). Minimal residual disease (MRD) was evaluated with BCR-ABL fusion transcript quantification or V(D)J IgH/TCR disease-specific rearrangement on BM aspirate in Ph-positive and Ph-negative patients, respectively. Disease Free Survival (DFS) and Overall Survival (OS) were calculated from start of IO therapy. Toxicity was graded according to CTCAE version. 4.03.

Table 1.

Patient ID	1st DLI	2nd DLI	3rd DLI	4th DLI
1	5 x 10^6/kg CD3+	1 x 10^7/kg CD3+	5 x 10^7/kg CD3+	
2	1 x 10^4/ kg CD3+	1 x 10^5/kg CD3+		
3	5 x 10^6/kg CD3+	1 x 10^7/kg CD3+	5 x 10^7/kg CD3+	
4	5 x 10^6/kg CD3+	1 x 10^7/kg CD3+	5 x 10^7/kg CD3+	
5	1 x 10^4/ kg CD3+	1 x 10^5/kg CD3+	1 x 10^5/kg CD3+	5 x 10^5/kg CD3+
6	5 x 10^6/kg CD3+	1 x 10^7/kg CD3+	4 x 10^7/kg CD3+	
7	1 x 10^4/kg CD3+	1 x 10^5/kg CD3+		
8	3.8 x 10^6/kg CD3+	5.81 x 10^6/kg CD3+	11.8 x 10^6/kg CD3+	

Results: Five Ph-negative and 3 Ph-positive B-ALL patients received IO. All patients had BM disease (6 morphological relapse and 2 MRD-positivity); 2 patients had additionally active EM disease. Three patients were in salvage1, 4 patients in salvage2, 1 patient in salvage3; 50% of the patients (4/8) had previously received Blinatumomab. Median time from transplant to relapse was 11 (range 2-21) months; median time from relapse to IO treatment was 2.5 (range 0-65) months. Patients received a median of 3 (range 2-6) courses of IO, at the standard dose. Four patients received IO and DLI in an alternate schedule and 4 patients sequentially after IO treatment; patients received a median of 3 (range 2-4) DLI infusions at escalating dose according to center policy (Table 1). All patients evaluable for morphological CR achieved CR after 1st IO cycle. Six of 8 patients evaluable for MRD obtained MRD negativity after 2nd IO cycle (4 of 6 patients after 1st cycle). Both PET+ patients achieved PET-negativity. With a median follow-up of 23.5 (range 3-58) months, 6 of 8 (75%) patients are alive. Four of 8 (50%) patients have relapsed, of which 2 with CNS localization. Median DFS is 12 months (range 3-58) and median OS is 23.5 (3-58) months. Of the 2 patients with CNS relapse, 1 had not received CNS prophylaxis. Concerning ≥ G3 toxicities, 4 of 8 patients experienced G4 thrombocytopenia, 3 patients ≥ G3 neutropenia, 1 patient had a G3 fungal infection, 1 patient a G3 CMV-enteritis and 1 patient G3 constipation; no > G2 hypertransaminasemia or hyperbiliruinemia was observed. Importantly, considering the setting, 2 patients experienced grade 1 GvHD and no veno-occlusive (VOD) diseases were reported.

Conclusions: IO and DLI are a safe combination in the post-HSCT setting that may ameliorate the dismal prognosis of this patient subset; CNS prophylaxis is highly recommended

P034

PREVALENCE AND PROGNOSTIC ROLE OF IDH MUTATIONS IN ACUTE MYELOID LEUKEMIA: FIRST RESULTS OF THE AML1516 GIMEMA PROTOCOL

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Introduction: Somatic mutations have been shown to play a diagnostic and prognostic role in AML, and some of them are "targetable" by specific inhibitors. Among these, are mutations of the isocytrate dehydrogenase IDH1 and IDH2 enzymes.

Methods: The GIMEMA AML1516 protocol was designed to study the prevalence of IDH1 and IDH2 mutations in patients with AML, at the time of initial diagnosis and/or at relapse. Sanger sequencing or NGS technologies were used, and molecular testing was performed at local labs or through the LabNET AML platform. Associations between IDH mutations and patient or disease characteristics were also evaluated, together with the impact on treatment response and survival.

Results: Between 5/2017 and 1/2020, 381 patients were diagnosed with AML at 17 Italian Hematology Centers, members of the GIMEMA working group, and enrolled in the AML1516 study. The present analysis is based on 324 pts with available IDH mutation status (310 at diagnosis, 14 at relapse). At diagnosis, 51% of patients were males, of a median age of 64.9 yrs (range 18.8-85.6). IDH1 and IDH2 mutations were present in 13.5% and 18.7% of cases, respectively. None of the patients had concurrent IDH1/IDH2 mutations. At relapse, 3 and 1 out of 14 pts were mutated for IDH1 or IDH2, respectively. IDH1/IDH2 mutations were significantly associated with lower white blood cell counts WHO performance status ≥ 2, and non-complex karyotype. Patients with IDH1

mutations had higher platelet counts and were more frequently NPM1-mutated (Table 1). Of 256 pts evaluable for outcome, 71.5% received conventional chemotherapy (CHT), while 26.2% received hypomethylating treatment (HMT), and 2.3% other regimens. The logistic regression model showed that complete remission (CR) was significantly associated with younger age, performance status, type of AML (*de novo vs.* secondary), NPM1 mutations and CHT, but not with IDH mutation status. Similarly, the univariate analysis for survival showed that overall and event-free survival were significantly longer in patients treated with CHT *vs.* HMT (p<0.0001 and p=0.0018, respectively). Also, age, WBC, WHO PS, type of AML, complex karyotype, FLT3 mutations and treatment were significant prognostic factors for OS and EFS. Age and complex karyotype were independent prognostic factors for OS and EFS in the multivariable model. IDH1 or IDH2 mutation had no impact on outcome, also when subgrouping according to IDH mutation type (IDH1mut *vs.* IDH2R140 and R172 *vs.* IDH-wildtype).

Conclusions: The preliminary results of the AML1516 study confirm that IDH1 and IDH2 mutations are frequent in AML, accounting for 34% of patients at the time of initial diagnosis. IDH mutations did not impact on achievement of CR or on survival. However, since IDH mutations are stable during disease course, IDH-mutation testing may track a substantial patient subgroup who may benefit from treatment with the specific inhibitors ivosidenib or enasidenib.

Table 1.

	IDH1/IDH2 WT	IDH1^{mut}	IDH2^{mut}	*p*
Patients (n)	210	42	58	
Sex (F, %)	111 (53.1)	17 (40.5)	23 (39.7)	0.097
Age* (years)	64 [18.8-85.4]	66.4 [22-85.6]	64.9 [32.1-84.7]	0.571
WBC* (10^9/L)	8.99 [0.47-347.00]	5.33 [1.15-720.00]	3.30 [0.36-800.00]	**0.025**
Hb* (g/dl)	8.90 [4.50-28.70]	8.80 [7.20-13.40]	9.30 [2.50-14.90]	0.490
PLTS* (10^9/L)	56.00 [4-664]	114.00 [6.00-2400]	53.00 [10-789]	**0.049**
BM-blasts* (%)	50.00 [0.00-99.00]	67.50 [3.00-96.00]	68.00 [0.00-98.00]	0.070
WHO PS 1 2 3	89 (47.8) 19 (10.2) 2 (1.1)	10 (29.4) 7 (20.6) 3 (8.8)	10 (20.4) 12 (24.5) 3 (6.1)	**0.001**
AML type (%) **Secondary** **Therapy-related**	 28 (13.5) 16 (7.7)	 8 (19.0) 2 (4.8)	 5 (8.6) 1 (1.7)	 0.233
***FLT3*-mut (%)** **ITD** **TKD**	 32 (15.5) 10 (4.9)	 11 (29.7) 2 (5.4)	 9 (18.4) 0 (0.0)	 0.289
***NPM1*^{mut} (%)**	49 (24.1)	17 (47.2)	10 (22.2)	**0.012**
Karyotype (%) Normal CBF (RUNX1T1-RUNX1, n=4,	 99 (47.1)	 22 (52.4)	 20 (34.5)	 0.144
CBF-MYH11, n=9) del5q/-5 or del7q/-7 +8 Complex Other alterations Not evaluable/not done	12 (5.7) 34 (16.2) 16 (7.6) 31 (14.8) 52 (24.8) 13 (6.2)	0 (0.0) 1 (2.4) 3 (7.1) 1 (2.4) 11 (26.2) 6 (14.2)	1 (1.7) 8 (13.8) 8 (13.8) 4 (6.9) 15 (25.9) 6 (10.3)	0.140 0.061 0312 **0.033** 0.97 0.166

* median, range

P035

IMPLICATION OF THROMBOTIC EVENTS ON SURVIVAL IN NON-M3 ACUTE MYELOID LEUKEMIA PATIENTS TREATED WITH INTENSIVE CHEMOTHERAPY

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Background: Thrombosis is one of the most frequent and serious complications in cancer patients. Several thrombosis risk-scoring systems, as Khorana Risk Score (KRS) and the International Society of Thrombosis and Haemostasis (ISTH) Disseminated Intravascular Coagulation (DIC) score, are currently used to evaluate the risk of thrombosis in patients with solid tumors. Thrombotic events (TE) in patients with hematologic malignancies (HM) are considered less frequent than in solid tumors, and much of the attention is directed towards bleeding and infectious complications due to the condition of thrombocytopenia and neutropenia frequently affecting hematologic patients. Among HM, the incidence of TE is better known in myeloma, non-Hodgkin lymphoma, and acute lymphoblastic leukemia, while the information in non-M3 acute myeloid leukemia (AML) is sparse.

Methods: We retrospectively analyzed 166 consecutive patients with newly diagnosed non-M3 AML, diagnosed and treated at the Hematology Unit of Policlinico Tor Vergata in Rome, between January 2010 and December 2019. Treatment schedules were defined by age and performance status, according to international standards. TE were confirmed by Doppler ultrasonography, computed tomography, or magnetic resonance imaging. The thrombosis scoring was assessed according to KRS and ISTH-DIC score. Patients were stratified by European Leukemia Net (ELN) 2017 genetic/cytogenetic risk assessment.

Results: A total of 166 AML patients submitted to intensive chemotherapy were included in the analysis. The median age at AML diagnosis was 58 years (range, 21-78), and the majority were male (54,2%, n =90). Based on ELN2017, patients were stratified in favorable-risk (21,7%, n=36), intermediate-risk (46,4%, n=77) and adverse-risk (29,5%, n=59), whereas a minority were not classifiable (2,4%, n=4). The median follow-up was 76 months (range, 4 – 121). A total of 32 TE (18.8%) were observed: 12/32 (37,5%) before starting any treatment and 20/32 (62,5%) during the subsequent course of the disease. In detail, 22 deep venous thromboses of various districts, 3 pulmonary embolisms, 3 myocardial infarctions, 3 thrombosis of others arterial district, and 1 thrombosis of the middle cerebral artery were observed. No statistical differences in the risk of thrombosis between patients with KRS<3 and KRS ≥3, as well as ISTH-DIC score <5 and ISTH-DIC score≥5 were observed. In the multivariate analysis, we did not find a correlation between hemocromocitometric parameters and TE. Patients with TE experienced a significant reduction of DFS compared to patients with no TE (2-years DFS 12,9% *vs.* 30,1%, p= 0.036, Figure 1) while no significant statistical differences were observed in terms OS between the two groups (5-years OS 21,7% *vs.* 29,9%, p=0.80).

Conclusions: In patients with non-M3 AML, the risk of thrombosis is not negligible. Thrombosis may present at diagnosis or at any time during the subsequent course of the disease. As the thrombotic risk-scoring system used for non-hematological malignancies do not apply to AML patients, the development of an adequate thrombotic risk-scoring system for these patients is warranted. To note, our observation of worse DFS in patients who experienced thrombosis may reflect different biological characteristics of the disease. Further investigation on a larger cohort of patients is necessary to better understand the pathogenesis that leads to TE in AML, and the correlations with prognosis.

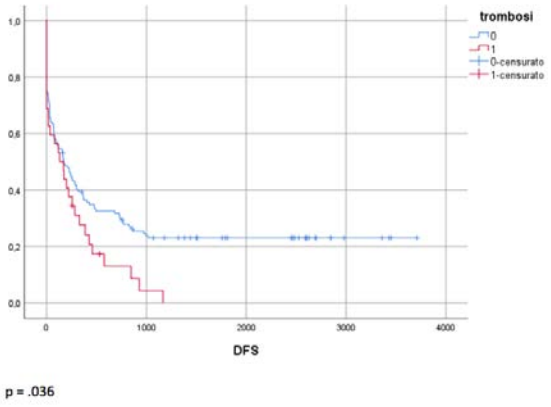

Figure 1.

P036

PROGNOSTIC IMPACT OF MINIMAL RESIDUAL DISEASE AND COMPLETE MOLECULAR EVALUATION IN NPM1 MUTATED ACUTE MYELOID LEUKEMIA: A SINGLE CENTER STUDY

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Despite the recent unravelling of the mutational landscape of NPM1-mutated acute myeloid leukemia (AML), treatment decisions remain based on a limited number of factors, and mainly on FLT3-ITD co-occurrence. The independent prognostic impact of NPM1 measurable residual disease (MRD) monitoring is now well described. However, few studies investigated the prognostic impact of NPM1 MRD combined with complete initial molecular evaluation. We aimed to evaluate prognostic factors, including MRD, in a "real life" cohort of patients with mutated NPM1 AML. We retrospectively collected data on all adult patients diagnosed with NPM1 mutated AML between 2008 and 2018 for which MRD data and complete molecular evaluation at diagnosis were available. Analysis of co-mutations at diagnosis was performed by NGS targeted resequencing of 41 genes. Molecular MRD was assessed on bone marrow by specific PCR after one and/or two courses of chemotherapy. The median follow-up period was 27.4 (range, 2.6-137.5) months. A total of 85 consecutive adult patients were included. The median age was 60 (range 20-85) years and 13 patients had secondary AML. The most frequent mutations involved epigenetic modifiers like DNMT3A, TET2, IDH1 or IDH2 or proliferation pathways like FLT3 and NRAS. Treatment was mainly based on 7+3 or similar. 81 patients (95%) achieved complete remission (CR), including 66 (78%) after a single course of induction therapy. 23 (27%) patients underwent allogeneic hematopoietic stem cell transplantation (allo HSCT) in first CR. Overall survival (OS) was 80 ± 5% at 2 years. Leukemia free survival (LFS) was 57 ± 6%. Relapse occurred in 33 (39%) patients in a median time from CR of 14,5 (range 0,53 – 119,5) months. Among them, 28 were classified into favorable risk category according to the ELN 2017, including 11 patients DNMT3A mutated. Post-induction NPM1 MRD was available for 82 patients, with 16 (19%) negative and 66 (80%) having detectable MRD, including 32 (39%) with NPM > 0.1%.

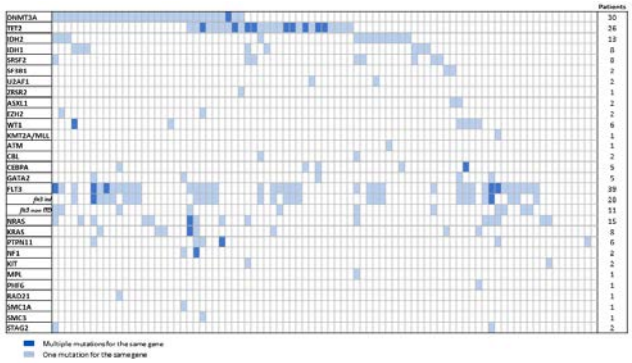

Figure 1.

For NPM1 MRD assessed after two cycles of chemotherapy, available in 70 patients, 31 (44%) had a detectable and 39 (56%) a negative MRD. In multivariate analysis, predictive factors for LFS were: age (p=0.02), NPM 1 > 0.1% after induction (p<0.01), preleukemic hematological disease (p= 0.01) and a trend for DNMT3A mutation (p= 0.06). Independent predictive factors for OS were: NPM > 0.1% after induction (p=0.04), presence of FLT3 TKD mutations or other non-ITD mutations (p=0.02), FLT3-ITD mutation with allelic ratio > 0.5 (p<0.01) and age (p<0.01). In this cohort of NPM1 AML patients, we confirm that NPM1 MRD monitoring after induction is an important prognostic factor. Mutational status of FLT3 (ITD with high allelic ratio and other mutations) and DNMT3A have also an independent prognostic value and should be combined with an accurate evaluation of MRD after induction and consolidation for a better selection of patients who can benefit from allo-HSCT in first line treatment.

P037

INVOLVEMENT OF CENTRAL NERVOUS SYSTEM IN BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM: A SINGLE CENTER EXPERIENCE

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Introduction: Blastic plasmacytoid dendritic cell neoplasm (BPDC) is a rare and highly aggressive hematopoietic malignancy associated with a very poor prognosis, and a median overall survival (OS) of 12-14 months, in responding patients (pts). In BPDC, extramedullary localization such as skin, lymphnodes and central nervous system (CNS) can be seen. The reported rate of CNS localization is 10% but this figure may be underestimated since screening for CNS involvement is not routinely performed at diagnosis.

Methods: We studied 9 pts with BPDC (median age: 67 yrs, range 60-80 yrs, 8 males, 1 female) admitted to the Hematology Unit of Policlinico Tor Vergata in Rome, between 2008-2020. Treatment schedules were defined by age and performance status according to international standards. CNS involvement, as routinely assessed at baseline for all BPDC pts at our center, was evaluated by conventional cytology (CC) and flow cytometry immunophenotype (FC). FC positivity was defined as the presence of a cluster of at least 10 events with the typical BPDC phenotype.

Table 1.

Pt id	Age	Sex	Therapy	RC	CNS assessment at diagnosis	CC (cell count)	FC (events)	relapse	CNS assessment at relapse	CC (cell count)	FC(events)
_01	60	M	IDA-HDARAC-ETOPOSIDE	yes	positive	621	4500	yes	positive	7216	9500
_02	63	M	MICE	yes	positive	35	50	yes	positive	35	50
_03	72	M	FLA IDA	yes	positive	12	15	no			
_04	70	M	MICE	yes	not evaluated			yes	positive only by FC	1	15
_05	80	M	supportive care		positive	49	40				
_06	68	M	supportive care		not evaluated						
_07	60	M	FLA IDA	yes	not evaluated			yes	positive only by FC	1	40
_08	62	F	FLA IDA	yes	not evaluated			no			
_09	67	M	FLA IDA	died during aplasia	positive	540	7650				

Results: Seven pts received induction therapy: 4 received FLA-IDA (fludarabine, cytarabine, idarubicin), 1 MICE (mitoxantrone, cytarabine, etoposide) 1 was treated with high-dose cytarabine, daunorubicin and etoposide. Two pts received supportive care only. Among intensively treated pts, CNS involvement was assessed in 5 out of 7 at diagnosis, while the remaining 2 were evaluated only upon disease recurrence. Overall, all patient showed CNS involvement, demonstrated by CC and FC while neurological symptoms at diagnosis were present in only 2 pts. Upon demonstration of CNS involvement, the pts received additional intrathecal therapy (IT) until 2 consecutive CC-negative samples were

obtained. Two pts who received IT during the induction since CNS positive at the initial diagnosis was observed, showed also FC positivity at CNS evaluation upon relapse (Table 1). Among the 7 pts who received induction therapy, 1 of them died during aplasia, while 6 pts obtained a CR (85.7%). Four pts underwent allogeneic stem cell transplantation: 3 of them relapsed and died because of disease progression, while 1 patient remained alive and in remission with a follow-up of 47 months. Median OS was 24,9 months, median disease-free survival (DFS) was 24 months. The 5 pts with an initial CNS involvement had a median OS and DFS of 3 months.

Conclusions: BPDCN is characterized by aggressive behavior with rapid systemic dissemination. Despite an initial response to systemic chemotherapy, the disease regularly relapses. Our experience suggests that the rate of CNS involvement could be even higher than reported in the literature because of a high frequency of occult CNS disease, also in the absence of neurological symptoms. CNS examination should be carried out in all pts affected by BPDC, and early intrathecal prophylaxis should be given.

P038

ABSTRACT WITHDRAWN

P039

VENETOCLAX PLUS HYPOMETHYLATING AGENTS (HMAS) FOR RELAPSED/REFRACTORY (R/R) ACUTE MYELOID LEUKEMIA (AML) IS AN EFFECTIVE AND MANAGEABLE REGIMEN IN THE OUTPATIENT SETTING: A REAL-LIFE EXPERIENCE

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Introduction: In the setting of clinical trials, Venetoclax combined with HMAs has shown fair activity in R/R AML and impressive results in treatment-naïve elderly AML patients. However, no clear guidelines are available on real-life management, especially in the outpatient setting.

Methods: This is a single-center retrospective study involving R/R AML patients treated with Venetoclax combined with HMAs. Data were collected in accordance with GCP and Helsinky declaration. Adverse events (AEs) were graded according to CTCAE v4.03. Survival is estimated with Kaplan-Meyer method.

Results: Thirty-one R/R AML patients have been treated with Venetoclax plus HMAs from March 2018 to March 2020 and completed at least 1 therapy course (range 1-9, median 2, IQR 1.0-5.0), being evaluable for this analysis. Patients' characteristics are summarized in Table 1. The median age was 67 years (range 22-84, IQR 60.0–75.0) and ECOG performance status was greater than or equal to 2 in 6/31 (19,4%) patients. Seventeen out of 31 (54,8%) had received intensive chemotherapy as induction therapy. Twenty-two patients (71 %) had already received HMAs, of which 14/31 (45,2%) as first and only previous line of therapy. Venetoclax was combined with 5-Azacitidine in 13/31 (41,9%), and with Decitabine in 18/31 patients (58.1%). For clinical reasons, 9/31 (29,1 %) patients received the first cycle as in-patients, whereas the majority (22/31, 70,9%) in the outpatient setting, main focus of our analysis, where Venetoclax dose escalation was managed with weekly laboratory and clinical monitoring. No cases of tumor lysis syndrome (TLS) or AEs during ramp up phase were detected. Nine AEs were documented during the 1st cycle of therapy with a median time of occurrence at day 20 (range 8–24); of which 2 required subsequent hospitalization. The early 30-days and 60-days mortalities were 4,5% (1/22) and 13,6% (3/22), respectively, comparable to percentages observed in the 9 patients who underwent the first cycle hospitalized (0% and 11,1%). Overall, with a median follow-up of 138 days (IQR 69 – 285), we reported 48 AEs, of which 27 were grade III-IV and the most common were hematological (12/27, 44,4 %) or infective (14/27, 51,8 %). Eleven AEs required hospitalization, whereas 24 were followed by a Venetoclax temporary withdrawn (median duration 14 days, range 5–120) and 5 by a discontinuation. As for the rate of response, a CR rate of 16,1% and an Overall Response Rate (CR + CRi + HI) of 38,7% were observed. Four out of 31 (12,9%) patients received subsequent HSCT. In this setting, with a median follow up of 138 days, median OS was 285 days (95% C.I. 179 - 381).

Conclusions: With the limitations of a single-center retrospective study, our real-life data indicate that Venetoclax plus HMAs is feasible in an outpatient management, without TLS or other limiting toxicities. Further studies evaluating the clinical, social and economic impact of outpatient Venetoclax-based treatment are highly warranted.

Table 1.

STUDY POPULATION CHARACTERISTICS (n = 31)		
MEDIAN AGE	67.0 years (IQR 60.0 - 75.0)	
ECOG at Venetoclax therapy	0: 10/31 (32,3%) 1: 15/31 (48,4 %) 2: 6/31 (19,4%)	
WBC/mmc at Venetoclax therapy	Median 2.410 (min 500 – max 60.000)	
Status at Venetoclax therapy	Primary induction failure: 17/31 (54,8 %) 1st Relapse: 8/31 (25,8 %) 2nd or later Relapse: 6/31 (19,4 %)	
ELN risk classification	Favorable risk: 3/31 (9,6 %) Intermediate risk: 16/31 (51,6 %) Adverse risk: 9/31 (29%)	
CLASSIFICATION	De novo AML AML secondary to MDS AML secondary to MPN Therapy-related AML	19/31 (61,3%) 7/31 (22,6%) 4/31 (12,9%) 1/31 (3,2 %)
CYTOGENENTICS	Normal karyotype 46 XY, 17p- inv(3) inv(16) 47 XX, 5q-, +8 Complex karyotype Other alterations Not available	11/31 (35,5 %) 1/31 (3,3 %) 1/31 (3,3 %) 2/31 (3,3 %) 1/31 (3,3 %) 6/31 (19,3 %) 4/31 (12,9 %) 5/31 (16,1 %)
MOLECULAR BIOLOGY	FLT3-ITD NPM1 mutation tp53 mutation IDH1 mutation IDH2 mutation	3/31 (9,7 %) 1/31 (3,3 %) 5/31 (16,1 %) 1/31 (3,3 %) 1/31 (3,3 %)

P040

IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA, STEROID INDUCED HYPOFIBRINOGENEMIA IS ASSOCIATED WITH BCR/ABL REARRANGEMENT

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Introduction: Hypofibrinogenemia in adult acute lymphoblastic leukemia (ALL) is typically associated with asparaginase (ASP) delivery. Since we have noticed significant reduction of fibrinogen (FBG) plasma levels even before the first ASP dose, we aimed at assessing the levels

of FBG at diagnosis and during induction treatment and exploring if the FBG fall correlate with biologic B-ALL features.

Methods. We retrospectively analyzed FBG levels in 110 B-ALL patients (pts): Fifty-two (47%) were females and 58(53%) were males, median age was 54,5 years (range 18-89), median bone marrow (BM) blast cell infiltration was 90% (range 20-100), median white blood cell count (WBCc) was 11.9 x10^9/L (range 0.840-407). Fifty (45%) pts had a BCR/ABL + ALL and were given tyrosine kinase inhibitors (TKI) plus steroids, with or without chemotherapy, while 60 (55%) had a BCR/ABL- ALL and were treated with intensive chemotherapy, mostly according to current GIMEMA protocols. FBG changes were graded according to the Common Toxicity Criteria for Adverse Events version 4.

Results. Overall, 6/110 pts (5% - 2 BCR/ABL+ and 4 BCR/ABL-) showed a modest pre-treatment hypofibrinogenemia of grade (G)=1. More evident falls in FBG plasma levels were observed during several phases of treatment, mainly in BCR/ABL+ pts. In this subset, hypofibrinogenemia was observed in 38/50 (76%) pts, during the steroid pre-phase. Of these 38, 16 (42%) scored G=1-2 and 22 (58%) G=3-4. Ten additional pts developed a G=3-4 hypofibrinogenemia while receiving TKI, for a total of 48/50 (96%) pts experiencing a fall of FBG. In BCR/ABL- subset, 34/60 (56%) pts had an FBG decrease; 29 (48%) during the steroid pre-phase [10 (34%) pts G=1-2 and 19 (66%) G=3-4]. The remaining 5 (8%) pts, had hypofibrinogenemia during the induction phase before the administration of ASP. In univariate analysis, FBG decrease occurring in the steroid pre-phase had a significant correlation with the BCR/ABL positivity (p=.005) and, in this cohort, also with age =/>60 years (p=.019). Overall, no correlation with gender, WBCc, extramedullary disease or BM blast count was detected. In the BCR/ABL+ subset, 29/38 pts (76%) with hypofibrinogenemia carried a p190 or p190/p210 transcript. In multivariable analysis, BCR/ABL positivity was independently associated with FBG fall (p=.037). Whatever the genetic pattern (BCR/ABL+ or -), none of those experiencing hypofibrinogenemia had significant modifications of ISTH DIC score and liver function during the steroid pre-phase.

Conclusions: The ability of steroids to induce hypofibrinogenemia has already been described. Our retrospective study shows that in B-ALL, FBG fall is associated with BCR/ABL positivity and takes place mainly during the steroid pre-phase. Since this phenomenon occurs without increment of the ISTH DIC score, it is likely due to primary fibrinolysis activation. Additional studies are needed to clarify further the mechanisms of hypofibrinogenemia in this subset of pts.

P041

INOTUZUMAB OZAGAMICIN IS SAFE AND EFFECTIVE IN RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE ITALIAN CENTER EXPERIENCE

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Background: Despite novel agents, B-ALL is still associated with dismal prognosis in the R/R patient. Anti-CD22 calicheamicin-immunoconjugate Inotuzumab Ozagamicin (IO) has demonstrated efficacy in the R/R, extramedullary (EM) and minimal residual disease (MRD)-positive setting.

Methods: Data of 33 consecutive patients treated with IO as standard of care for R/R B-ALL were retrospectively collected. Response assessment was evaluated after each IO course. ORR was defined as no evidence of EM disease (PET-documented in PET-positive patients pre-IO) and bone marrow (BM) blasts < 5% after 1 or 2 IO cycles. Molecular ORR (mORR) was defined as BCR-ABL, V(D)J IgH/TCR disease-specific rearrangement negativity on BM blood and no evidence of EM disease after 1 or 2 IO cycles. Adverse events were graded according to CTCAE v4.03.

Results: Thirty-three patients (M/F = 17/16); median age 45, (range 20-78) with B-ALL were treated with IO from February 2013 to January 2020. Twenty-five of 33 (75,7%) patients received IO for R/R BM disease, 11/33 (33,3%) had EM disease and 4/33 (12,1%) for BM MRD-positivity. Twelve of 33 (36,4%) patients were Ph-positive, 10/33 (30,3%) had a normal karyotype, 4/33 (12,1%) a complex karyotype, 1/33 (3%) had t(4;11), 1/33 (3%) t(1;19) and 1/33 (3%) t(2;16); 4/34 (12,1%) were not evaluable. Patients had received a median of 2 (range 1-7) previous therapy lines. Seventeen of 33 (51,5%) patients had previously received Blinatumomab and 17/33 (51,5%) had undergone allogeneic stem cell transplant (ASCT). Patients received a median of 2 IO (range 1-6) courses. Twenty-four of 33 (72,7%) patients were hospitalized for the beginning of the therapy, while 9/33 (27,3%) began in outpatient setting. The following courses were also administered as outpatient. Thirty of 33 (90,1%) patients were evaluable for response; ORR was 70% (21/30), 3/30 (10%) patients achieved a PR and 6/30 (20%) were refractory. Of the 21 responsive patients MRD negativity was achieved in 12 of 20 (60%) patients (1 patient not evaluable for MRD status). Eight of 30 (26,6%) patients proceeded to ASCT. Median OS in our cohort was 6,9 months (95% CI 2,7-11,0), OS at 1 year was 40,0% and at 2 years 24.9%. Median DFS was of 8,7 months (95% CI 3,1-14,3); DFS at 1 year was 36,0% and at 2 years 24,7%. CD22-expression, Ph-chromosome status, duration of 1st remission or previous Blinatumomab therapy did not influence IO response. The most frequent adverse event (AE) involving 13/30 (43,3%) patients was ≥ G3 thrombocytopenia; 7/30 (23,3%) patients had G3 febrile neutropenia, 2/30 patients (6,6%) G3 and G4 invasive fungal infection. Other ≥ G3 AEs include 1 G3 hepatic failure with mild reversible encephalopathy, 1 G3 constipation, 1 G4 sepsis from P. aeruginosa and 1 pneumonia. One patient died of VOD after ASCT.

Conclusions: Our results confirm IO's efficacy and high response rate in a heavily pre-treated patient population. IO can be safely administered in out-patients setting.

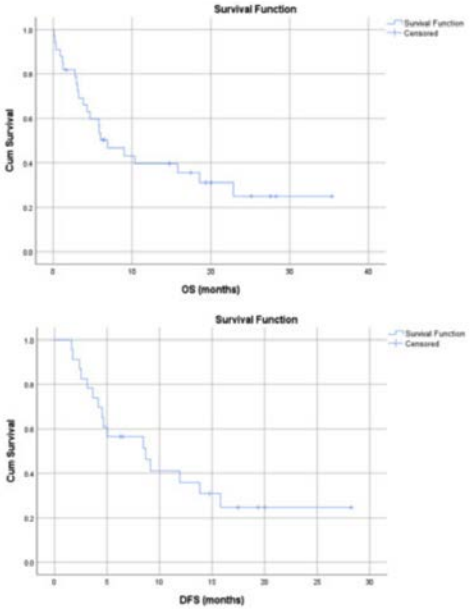

Figure 1.

P042

REAL-WORLD TREATMENT PATTERNS AND CLINICAL OUTCOMES IN UNFIT PATIENTS WITH AML RECEIVING FIRST LINE SYSTEMIC TREATMENT OR BEST SUPPORTIVE CARE (CURRENT): AN INTERIM ANALYSIS OF THE ITALIAN STUDY POPULATION

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Introduction: As the population continues to age, the number of new AML cases increases 2.2% each year. With the increasing incidence and rising cost of treatment, there is a need to understand current AML treatment pathways and their associated treatment outcomes. The aim of the CURRENT study is to evaluate the overall survival (OS) and other clinical outcomes (e.g. CR+CRi), clinico-pathologic characteristics of AML patients (pts) unfit for intensive chemotherapy and who received systemic treatment or Best Supportive Care (BSC) in the real world setting.

Methods: This is an ongoing multicountry non-interventional, retrospective chart review of AML pts who have initiated first line treatment (or Best Supportive care) between Jan15 and Dec18 and were ineligible for intensive induction chemotherapy. Patients are followed up until the last recorded visit or death. We report here the interim results of the Italian population. The primary endpoint is OS from diagnosis of AML. Secondary endpoints include time to treatment failure (TTF) and CR+CRi. Analyses are descriptive, with the Kaplan-Meier method used to estimate time-to-event outcome measures.

Table 1. Patient characteristics and treatment patterns.

	Fist-Line Systemic Therapy (n=48)	BSC only (n=10)
Male	25/48 (52.1%)	9/10 (90%)
Median age at diagnosis, years (range)	76.0 (58-88)	80.5 (52-89)
Secondary AML	14/45 (31.1%)	3/10 (30%)
ECOG performance status		
• 0-1	15/27 (31.3%)	3/6 (30%)
• ≥2	12/27 (25%)	3/6 (30%)
• unknown	21 (43.8%)	4 (40%)
Molecular profile		
Any mutation	7 (14.6%)	1 (10%)
• *RUNX1* mutation	1 (14.3%)	0
• *ASXL1* mutation	1 (14.3%)	0
• *FLT3* mutation	0	1 (10%)
• *FLT3*^{ITD} mutation	3 (42.9%)	0
• *CEBPA* mutation	1 (14.3%)	0
• *NPM1* mutation	4 (57.1%)	0
No mutation	33 (68.8%)	8 (80%)
Unknown molecular profile	8 (16.7%)	1 (10%)
Cytogenetic risk		
• Favourable	5 (10.4%)	0
• Intermediate	24 (50%)	5 (50%)
• Poor	15 (31.3%)	3 (30%)
• Unknown	4	2
First-line treatment received*:		
Systemic therapy	48 (100%)	-
• HMA (azacitidine)	29 (60.4%)	
• HMA (decitabine)	18 (37.5%)	-
• LDAC	1 (2.1%)	-
• Venetoclax	1 (2.1%)	-
• Other	1 (2.1%)	-
		-
BSC only	-	10 (100%)
Treatment combinations:		-
• Azacitidine + Decitabine	1 (1.7)	-
• Azacitidine + Venetoclax	1 (1.7)	

*Patients could be treated also with combination of therapies

Results: At the Feb2020 interim analysis, 58 consecutive pts were enrolled, 48 with a first line systemic therapy and 10 with BSC (Table 1). Mean age (SD) at diagnosis was 75.7(6.1) and 77.6(10.2) years, respectively. AML subtype was unknow for 12 (25%) first-line and for 3 (30%) BSC pts; 6 first-line pts (12.5%) had AML with recurrent genetic abnormalities and 5 (10.4%) had therapy-related myeloid neoplasms; 9 first-line (18.8%) and 2 BSC (20%) pts had AML with myelodysplasia-related changes; 16 first-line, (30.3%) and 5 BSC pts, (50%) had AML not otherwise specified. First line systemic treatments were mainly azacitidine (29, 60.4%) and decitabine (18, 37.5%), and these patients underwent a median of 10 cycles. Of the 48 pts who received a first line treatment, 25 (52%) achieved a response (CR+CRi+PR), with a median duration of 198 days; no CR/CRi/PR was reached in the BSC group. Sixteen first line pts (33.1%) achieved a CR+CRi, and 9 (18.8%) achieved a PR. Median OS was 14.9 months in the HMA first-line pts (44/48), and 2.7 months in BSC pts. Median time to best response was 119 days in first-line pts. Median TTF was 212 and 88 days for first line and BSC pts, respectively. During treatment, 64.6% and 60% of first line and BSC pts were hospitalized. Main reason for hospitalization of first-line patients was infections (45.6%); therapy administration was reported for 17.5% of pts. The majority of patients underwent blood transfusions (91.7% of first line and 100% of BSC pts) during treatment.

Conclusions: These real-world efficacy data for HMA and BSC showed consistent results with clinical trials. HMA are the most common first-line treatment choice in Italy, when patients are not eligible to intensive chemotherapy. Outcome for AML patients remains poor and novel agents/combinations are needed.

P043

PROMISING MANAGEMENT OF EARLY MOLECULAR RELAPSE WITH VENETOCLAX AND HYPOMETILATING AGENTS IN NPM1-POSITIVE ACUTE MYELOID LEUKEMIA: A SINGLE-CENTER CASE SERIES

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Background: Venetoclax (VEN) and Hypomethylating agents (HMA) have demonstrated remarkable activity in elderly front-line and R/R AML patients. NPM1-mutated (NPM1+) AML seems to benefit most from such therapy. In this patient subset exploring new applications of VEN-HMAs could be an interesting and promising strategy.

Methods: We report 3 patients with NPM+ AML treated with VEN-HMAs in early molecular relapse (EMR) after first line chemotherapy. EMR was evaluated as 1 log increase of NPM1 qRT-PCR transcript, minimal residual disease (MRD) negativity was defined as ratio NPM1+/ABL x 100 transcript < 0.01.

Results: From December 2019 to April 2020 we treated three fit AML NPM1+ patients in EMR with Azacitidine (AZA) 75 mg/m2 from days 1-5 in association with VEN at standard dosage of 400 mg. Patient's characteristics and previous therapy lines are summarized in Table 1. All patients had received an intensive induction chemotherapy-program and, considering the favorable disease risk, had not received a front-line allogeneic stem cell transplant (ASCT). Patient 1 experienced EMR after 1 year from the end of chemotherapy program. Patient 2 and patient 3 developed a log increase of NPM1 transcript during consolidation chemotherapy. In all cases an ASCT could not be performed quickly as first choice due to logistic and concomitant SARS-CoV-2 management complications. In patient 2 and 3 a further chemotherapy approach was contraindicated for recent severe infections occurred during the previous hospitalization. All patients were treated in out-patient setting and VEN full 400 mg dose was reached after a quick 6-days ramp-up. Blood count and chemistry were performed weekly during 1st cycle. Patient 1 received 3 cycles of VEN-AZA and achieved CR MRD- after the 1st

cycle, confirmed after 3rd cycle. No AEs were reported and patient 1 is now proceeding to ASCT in optimal disease conditions. Patient 2 received 2 cycles of VEN-AZA, 1 cycle of AZA single-agent and achieved CR MRD- after 3rd. Patient 2 suspended VEN after 2nd cycle for G3 neutropenia and G2 thrombocytopenia; WBC recovered after 2 weeks of suspension. Patient 2 is now proceeding to consolidation with natural killer (NK) adoptive immunotherapy. Patient 3 received 3 cycles of VEN-AZA and achieved CR MRD- at 3rd cycle evaluation. AEs reported for patient 3 were G4 neutropenia during cycle 2 for which VEN was suspended from C2 day 21 to day 28. Patient 3 is also now proceeding to ASCT in optimal disease conditions.

Conclusions: VEN-HMAs represent a promising approach for the treatment of the unexplored EMR setting in NPM1+ AML patients as bridge to intensified consolidation strategies. This approach allowed to avoid disease expansion, recover from infective complications and even more to achieve MRD-.

Table 1.

Table 1. Baseline characteristics. FLAI: Fludarabine-ARA-C-Idarubicin chemotherapy regimen, IDARAC: Intermediate-dose ARA-C, HDARAC: High Dose ARA-C, *KPC: Klebsiella pneumonia carbapenemase.*

Characteristic	Patient 1	Patient 2	Patient 3
Sex	Male	Female	Female
Age @ diagnosis	41	67	58
WHO 2016 AML classification	AML with mutated *NPM1*	AML with mutated *NPM1*	AML with mutated *NPM1*
ELN 2017 AML disease risk	Favorable	Favorable	Favorable
Leukocytes @ diagnosis /μL	59.650	43.940	103.000
Karyotype	46XY	46XX	46XX
NPM1 status	mutated	mutated	mutated
FLT3 status	wild type	wild type	wild type
IDH1/2 status	wild type	*IDH1* mutated	*IDH1* mutated
Induction chemotherapy	FLAI-5	FLAI-3	3+7 and Midostaurin
Consolidation therapy	FLAI-5, IDARAC x 2	IDARAC, HDARAC	HDARAC + Midostaurin x 2
Total n° of chemotherapy cycles received	4	3	3
G ≥ 3 infectious complications during chemotherapy	G4 *KPC* sepsis, and *KPC* colonization	*P. aeruginosa* G4 pneumonia	G3 pneumonia

Chronic Lymphocytic Leukemia and Chronic Lymphoproliferative Disorders

P044

PRECLINICAL STUDIES ON THE ROLE OF HUMAN ENDOGENOUS RETROVIRUSES AS POTENTIAL MARKERS OF DISEASE AND PROGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: The transcriptional activity, proteins, and viral particles production of human endogenous retrovirus K (HERV-K) have been demonstrated in tissue, patient serum, and cell lines isolated from different types of tumors, such as ovarian, breast, prostate, teratocarcinoma, lymphomas, leukemias, sarcomas, and melanoma. The mechanisms underlying HERV-K oncogenic activity could depend on: the expression of oncogenic viral proteins, the induction of immune escape mechanisms, the regulation of gene expression mediated by the long terminal repeat sequences, the ability of retro-transposition to determine genomic instability and alteration of the expression of neighbouring genes. In the onco-hematology field, few studies have identified alterations of HERVs messengers and protein expression in human lymphoid leukemic cells and the presence of circulating antibodies to HERV-K. On these bases, the objective of the study was to evaluate the potential use of distinct HERVs families as biomarkers of disease and prognosis of chronic lymphocytic leukemia (CLL).

Materials and methods: Fifty patients with CLL diagnosis [median age 69.11 (50-84), gender M/F 30/20 (ratio 1.5)] and 26 healthy donors [median age 68.87 (60-80), gender M/F 17/9 (ratio 1.8)] were recruited. CLL patients have been divided into three groups: 18 naïve/untreated, 13 undergoing chemotherapy, and 19 treated with biological drugs. Data on diagnosis, therapy, outcome, biochemical parameters, and mutational status were collected. The transcriptional activity of HERV-K (HML-2), HERV-W, and HERV-H, as well as of the embryonic genes OCT4 and KLF4 was analyzed by RT-Real Time PCR. The non-parametric Mann-Whitney test and the calculation of the Rho coefficient of Spearman were used for statistical analysis.

Results: The molecular analysis showed a significant higher expression of HERV-K, HERV-W, HERV-H, OCT4, and KLF4 in the patients compared to healthy donors. Moreover, a positive significant correlation among HERVs expression and embryonic genes in patients was demonstrated. Of note, we are able to discriminate the two distinct populations of healthy subjects and patients based on the HERVs expression. We found significant differences in HERV-K, HERV-W, OCT4, and KLF4 expression between untreated patients and those treated with chemotherapeutic or biological drugs patients.

Discussion and conclusion: The results suggest HERVs expression as distinctive marker of CLL and their involvement in the etiopathogenesis of the disease. The ongoing study could provide an indication of the role of HERVs as markers associated with genetic instability and as prognostic factors, in order to identify subgroups of CLL patients who could benefit from targeted therapeutic approaches.

P045

STRATEGIES TO FIGHT IBRUTINIB-RESISTANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: The Bruton's tyrosine kinase inhibitor Ibrutinib has significantly changed the management of patients with Chronic Lymphocytic Leukemia (CLL) achieving high efficacy even in poor-risk and chemo-refractory patients. Despite this, relapses may occur and outcomes after Ibrutinib failure are dismal due to a lack of effective drugs. Besides BTK and PLCγ mutations, mechanisms of Ibrutinib-resistance remain to be clarified. We previously demonstrated that the Heat Shock Protein of 70kDa (HSP70) and its transcription factor Heat Shock Factor 1 (HSF1) play a role in mediating survival and progression of CLL. Since both proteins have been proven to be involved in drug-resistance in several cancers, we are facing the implication of the HSP70/HSF1 axis in Ibrutinib-resistance in CLL. In this regard, we focused on a main topic: the *in vitro* use of HSP70/HSF1 inhibitors in patients who failed Ibrutinib treatment.

Methods: Leukemic B cells from 8 CLL patients who failed Ibrutinib treatment were cultured *in vitro* with molecules targeting the HSP70/HSF1 axis, as follows: 15μM Fisetin; 10μM Cantharidin; 10μM Resveratrol and related molecules, *i.e.* Pterostilbene, Triacetyl Resveratrol and Honokiol. Apoptosis was evaluated by Annexin V/Propidium iodide flow cytometry test and by the presence of cleaved PARP in Western blotting (WB). HSP70 and HSF1 expression was evaluated by WB in CLL B cells after *in vitro* treatment.

Results: We demonstrated that HSP70/HSF1 inhibitors were able to induce *in vitro* apoptosis in cells from 8 patients who failed Ibrutinib treatment. Particularly, a significantly relevant apoptosis was induced by Fisetin (+70.28% of apoptotic cells *vs.* untreated condition, $p<0.01$), Cantharidin (+55,60%, $p<0.05$), Resveratrol (+23.67, $p<0.05$), and Pterostilbene (+45,41%, $p<0.01$). HSP70 and HSF1 levels decreased following *in vitro* treatment, according to the amount of induced apoptosis.

Conclusions: The inhibition provided by HSP70/HSF1 on *in vitro* apoptosis in cells from Ibrutinib refractory patients suggests an involvement of HSP70/HSF1 molecules in controlling the pharmacological resistance to Ibrutinib in CLL cells. Our results offer the rationale for a novel treatment of patients who relapsed after Ibrutinib based on targeting the HSP70/HSF1 axis.

P046

CURCUMIN EXHIBITS *IN VITRO* AND *IN VIVO* ANTI-LEUKEMIC ACTIVITY INTERFERING WITH THE NOTCH1 PATHWAY AND INDUCING ENDOPLASMIC RETICULUM STRESS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: Chronic lymphocytic leukemia (CLL) shows high genomic heterogeneity that explains its variable clinical course. NOTCH1 (NT1) mutations are one of the most common genetic lesions in CLL with poor prognosis.Several studies showed that the natural product curcumin has anti-cancerous properties including anti-CLL potential,and modulates NOTCH signaling in preclinical models of solid tumors but still,its mode of action in CLL is not entirely known.We investigated the anti-CLL effect of curcumin and its ability to interfere with the NT1 pathway and the functions of endoplasmic reticulum(ER).

Methods: Highly purified CD5+/CD19+ cells were obtained from CLL patients.We used ddPCR for NT1 mutation assessment (cut-off=12%); flow cytometry for Annexin-V/PI assay;Western blot for NT1-intracellular domain (ICD),MCL-1,AKT,PARP-1,BAP31,GRP78/BiP and caspase-4 analysis;RT-PCR for HES1 and c-MYC mRNA quantitation.Eμ-TCL1 mice were treated for 8 weeks with curcumin (50mg/kg/day) by intraperitoneal injection.

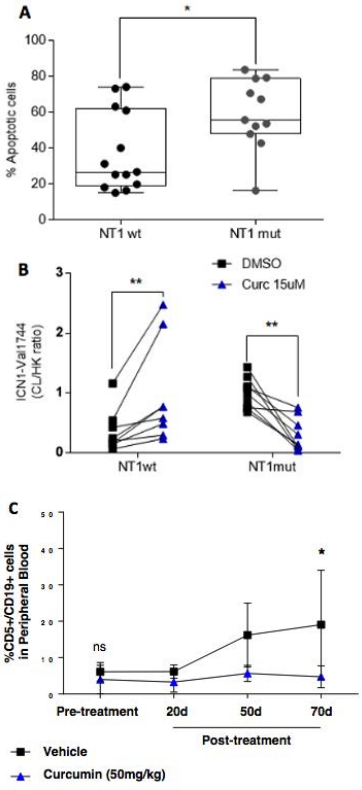

Figure 1.

Results: In NT1-mutated CLL,curcumin *in vitro* treatment (15uM for 24h) induced significant higher levels of apoptosis ($N=11;p<0.05$; Figure 1 panelA) and PARP-1 cleavage ($N=6;p<0.01$) than in NT1-wild type (WT) cells ($N=13$; Figure 1panelA). Notably, these effects were associated with a significant decrease in the activated NT1-ICD protein ($N=9;p<0.01$;fig.panelB) and a tendency toward HES1 mRNA reduction in NT1-mutated CLL. Conversely, NT1-WT cells showed increased NT1-ICD levels ($n=8;p<0.01$; Figure 1 panelB) upon curcumin exposure. The anti-NT1 activity of curcumin was accompanied by a reduction of different NT1 targets including c-MYC ($N=6;p<0.05$),AKT ($N=4$; $p<0.05$) and the anti-apoptotic MCL-1 ($N=9$; $p<0.05$),in NT1-mutated compared to WT cells.To get further insight into the biological effects of curcumin in NT1-mutated CLL,we analyzed ER-associated markers. Curcumin increased intracellular Ca^{2+} influx,caspase-4 cleavage ($N=4;p<0.05$), BAP31 degradation and GRP78/BiP expression ($N=6;p<0.05$) suggesting the induction of an apoptotic ER stress-associated response. Finally,we showed that curcumin treatment of Eμ-TCL1 mice delayed the growth of peripheral blood CD5+/CD19+ cells ($N=4;p<0.05$; Figure 1 panelC) overtime.At sacrifice,curcumin caused a significant reduction of CD5+/CD19+% cells ($p<0.05$) in bone marrow and liver ($p<0.05$) compared to controls.Analysis of BM sorted CD5+/CD19+ cells showed a reduction of NT1-ICD ($p<0.05$) and MCL-1 ($p<0.05$) expression in curcumin-treated mice compared to controls.

Conclusions: Curcumin is cytotoxic against CLL cells *in vitro* and *in vivo* showing a selective efficacy in NT1-mutated cells.This effect is associated with NT1 inhibition and ER stress induction suggesting that a crosstalk between the NT1 pathway and ER machinery could be a plausible explanation for the better efficacy of curcumin in these CLL.Defining the mechanisms of the differential effects of curcumin in NT1-mutated and WT CLL might enhance its usefulness as a potential anti-CLL drug.

P047

DEVELOPMENT OF A SINGLE HIGH-THROUGHPUT NGS-BASED METHODOLOGY FOR IGH/TP53 MUTATIONAL STATUS AND CHROMOSOMAL COPY NUMBER ABERRATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: In Chronic lymphocytic leukemia (CLL) the worst prognosis is associated with TP53 defects (mutations an deletion) and IGHV gene somatic hyper-mutation status (SHM) with the affected patients being potentially directed to alternative treatment. For this reason , to date, these biomarkers represent the key decision-making in CLL. Inactivation of the TP53 locus due to del(17p) is frequently associated with mutation(s) on the second TP53 allele, however, TP53 mutations also occur in the absence of del(17p) and are associated with a poor outcome, similar to the disease course observed in del(17p) CLL patients. Moreover, the presence of complex karyotype (CK) abnormalities is an adverse prognostic factor and associated with inferior outcomes in patients with CLL after treatment with chemotherapies and targeted therapies.

Methods: We development a NGS approach to detect simultaneously the analysis of IGHV gene SHM status, TP53 mutations of the entire coding region and genome-wide copy number aberrations (CNA). For all biomarkers a total of 120 ng of genomic DNA from peripheral blood samples was employed for an amplicon-based library preparation and analysis was performed by Ion Torrent Chef-S5 platform. IGHV gene library was performed according EuroClonality-NGS protocol. At least 10.000 reads was evaluated for IGHV gene SHM determination and at least 1 million reads for chromosomal CNA. For Tp53 gene analysis the cut off of variant reads for reliable variant calling was at least 10 and at least a 100x coverage of the entire gene as suggested by ERIC recommendation.

Results: We studied 150 consecutive CLL patients referred to our institution for genetic assays for IGHV/TP53. In total 49 TP53 pathogenic mutations in 44 (29%) patients were found; among them, 41 missense substitutions predominated (84 % of detected mutations) and the other 8 mutations were one splice site, two nonsense and five indel. A single mutation was detected in 90 % of mutated cases with 10 % of mutated patients presenting 2 or more mutations. Moreover, minor TP53-mutated subclones were disclosed in 11/44 TP53 mutated patients (VAF <15%, range 5-12%), the mutated subclone is the only detected in 9 of them. All patients were studied for del(17p) by FISH and we performed NGS-CNA in a subgroup of 50 samples. In all 17p deleted cases NGS-CNA was able to detect the deletion, moreover we detect CK in several patients associated with TP53 mutations.

Conclusions: We have developed a NGS-based methodolog inclusive all routine diagnostic biomarkers that improve patient stratification, detecting both TP53-mutate subclones and CK, and optimize therapeutic decisions. The NGS approach shows good concordance with Sanger sequencing and FISH, excellent intra-laboratory reproducibility and reduction of time consuming. The laboratory is certified by ERIC consortium. This grant was supported by Assessorato alla Salute Regione Sicilia, PSN2016.

P048

ABSTRACT WITHDRAWN

P049

DEFECTIVE PLATELET AGGREGATION IN CHRONIC LYNPHOCYTIC LEUKEMIA PATIENTS: MONOCENTRIC EXPERIENCE

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Introduction: Chronic Lymphocytic Leukemia (CLL) is associated with a major risk of minor bleedings in absence of any treatment and during the therapy with Ibrutinib, a Bruton's tyrosin kinase inhibitor. CLL is featured by an immunosuppressed microenvironment where ATP, ADP, AMP are degraded to adenosine by different ectonucleotidases, namely CD39 an CD73. The increased adenosine levels promote the development and progression of cancer and inhibit platelet (PLT) aggregation by engaging the adenosine A2A and A2B receptors. Ibrutinib causes defects in collagen-dependent platelet responses caused by the central role of BTK (its target) in glycoprotein VI signaling.

Methods: 20 CLL patients followed at Hematology of University Hospital of Pisa underwent to aggregation test in the context of clinical routine assessment. For aggregation test, we used the Born method on platelet-rich plasma (PRP) from these patients in two different times of the pathology course: 1) in absence of any treatment and 2) during treatment with Ibrutinib. Platelet (PLT) aggregation test measures the ability of various agonist (ADP, Collagen, Ristocetin) to induce PLTs aggregation.

Results: In absence of any treatment, we observed 7 patients with an ADP 1 uM-induced maximum aggregation <10% and with lower aggregation values at all further ADP concentrations also (group 1). The remaining 13 patients (with values of maximum aggregation at ADP 1uM >10%) presented a different behavior when we used ADP intermediate concentrations: in fact, in 7 patients we observed an initial aggregation wave followed by a secondary wave of irreversible aggregation (group 2), while in other 6 subjects we did not observe the "physiological" biphasic waveform (group 3). When we used collagen, all patients presented a normal maximum aggregation, even if 17/20 cases showed a prolonged latency time. Then, we assessed 5 patients during Ibrutinib therapy: in all patients a reduced PLT aggregation and a longer prolonged latency time to collagen were observed. Concerning ADP, we observed a different non-uniform behavior: 2 patients have mantained lower aggregation values, other 2 patients presented increased values and the remaining patient presented aggregation reduction compared to basal conditions. Aggregation to ristocetin resulted normal, before and after therapy in all patients.

Conclusions: This study supports the old idea that CLL patients present an impaired PLT aggregation. The absence of the normal biphasic waveform at intermediate ADP concentrations and the observed longer latency to collagen might be useful for immediately recognizing cases with a "basal" impaired PLT aggregation. This could be useful in the clinical practice, when physician has to choice among different therapies (BTK *vs.* PI3K *vs.* BCL2 inhibitors). Our experience, also if small, confirms what already reported in literature, that Ibrutinib impairs the PLT aggregation to collagen. This phenomenon might be the consequence of inhibition of other members of the TEC family, to which BTK belongs, that control different PLT signaling routes.

P050

GENE MUTATIONS ANALYSIS AND NEW INSIGHTS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): PRELIMINARY RESULTS FROM A CLINICAL RESEARCH PROJECT BASED ON NEXT GENERATION SEQUENCING (NGS)

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Introduction: Genetic complexity of CLL has been revealed by NGS analysis, obtaining a comprehensive view of structural variants, somatic mutations and different layers of epigenetic changes. Using a research-based NGS gene panel which targets 12 genes that have been suggested, predicted or clinically proven to be associated with risk of CLL, this project explored clinical impact of recurrent gene mutations in a real life CLL pilot sample and explored any additional insights toward the genetic risk of CLL.

Methods: TruSeq Custom Amplicon Kit (Illumina) was used to produce libraries of exonic regions from 12 genes (BIRC3, DDX3X, KLHL6, MED12, MYD88, NOTCH1, POT1, PTPN6, SAMHD1, SF3B1, TP53, XPO1) starting from genomic DNA from PB samples. Sequencing was performed on Illumina HiSeq3000 (average target coverage>3000X) and variants were identified using Mutect2 according to GATK Best Practices. Variants (VAF 0.1) were annotated with dbSNP, COSMIC, ExAC and gnomAD databases and in silico tools were used for predictions of pathogenicity. Gene mutations were then classified and reported according to ACMG guidelines.

Results: One-hundred genomic DNA samples were sequenced as part of a preliminary analysis of a clinical research regional project (L. 7/2007, Sardinia), still in progress (254 pts). Blood samples were collected at different stage of disease, while 58% of patients (pts) have already been treated with at least one line of therapy at time of sampling. Gene mutation data and concurrent biological and clinical characteristics (age, Binet stage, Dohner-prognosticator FISH, IGHV) were analyzed. A total of 154 sequence variants were detected in 81 pts, 45 of whom underwent at least one line of therapy. Recurrent mutations were classified as pathogenetic (n°42), likely pathogenetic (n°12) and variant of uncertain significance (n°100,VUS). The most frequently mutated genes were TP53(12%), NOTCH1(11%), SF3B1(5%), DDX3X(5%), MYD88(3%), XPO1 (3%), in accordance with data reported in literature. No mutation was detected in BIRC3, PTPN6, KLHL6 and SAMHD1 genes. Median follow up from diagnosis for the entire cohort was 9.63 yrs (2.78-30.6) while median OS was not reached for both mutated (at least 1 mut) and wild type pts (p=ns). When number of mutations per patient were analyzed 27% had 1 mut (mainly TP53), 29% with 2, 19% with 3, 11% with >3. Pts had a mean of 1.9 mut. Pts with ≥3 mut were mutated in TP53 which co-occurred with DDX3X, SF3B1 and NOTCH1 genes mutation. No statistical difference in terms of TTFT has been found, due to limited number of pts examined so far. We stratified pts in three categories based on n° of mutations (0-1 *vs.* 2 *vs.* ≥3), with a trend for worse OS in ≥3 mut group (n°11 pts)(p=ns).

Conclusion: Our proof-of-concept demonstration of targeted gene sequencing confirmed that NGS reveals the feasibility of this approach but identifies specific challenges to be dealt with in future projects. Prospective trials are needed to stratify CLL pts in order to confirm mutations clinical impact and to address the possible future use as biomarkers.

Lymphomas

P051

NEW PROGNOSTIC FACTORS IN MANTLE CELL LYMPHOMA: A MONOCENTRIC RETROSPECTIVE STUDY

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Background: The clinical course of Mantle Cell Lymphoma (MCL) is variable and involves both indolent and highly aggressive forms. Mutations in TP53, biologic MIPI high risk and blastoid or pleomorphic cytomorphology are associated with aggressive disease course and inferior outcome. Advances in molecular techniques have led to identification of novel genomic alterations in MCL with different prognosis.

Aims: Using a next-generation sequencing (NGS) platform we analyzed in FFPE tissues samples collected at the diagnosis a panel of 11 genes frequently mutated in MCL (ATM, TP53, BIRC3, KMT2D, NOTCH1, NOTCH2, UBR5, WHSC1, CCND1, MEF2B, TLR2), trying to assess whether point mutation and copy number variation had a role on progression-free survival (PFS) and overall survival (OS). Immunohistochemistry (IHC) analysis were also perfmormed to study SOX11, P53 and Ki67 status.

Results: In this study we enrolled 68 patients MCL referred to the Institute of Hematology AOU Careggi from 1999 to 2017; 79% were male (54) 21% female (14), with a median age of 69. Only 5 patients (7.35%) were IHC positive for P53, 29 (42.65%) were weakly positive and 24 835.29%) were negative. 42 (61.76%) were positive for SOX11, 8 (11.76%) were weakly positive, and 10 (14.71%) were negative. 50 (79%) were positive for Ki67. Overall, we identified 6770 point mutations and 19 Copy number variation, conventionally we considered only alterations occurred in at least 5 samples (605 SNV). Among them, seven mutations resulted to impair PFS: NOTCH2 D1306N (median PFS 3.458 years for the reference allele, versus 0.227 for the alterate allele), S1407L (3.458 *vs.* 0.928 years), NOTCH1 E2543K (3.504 *vs.* 0.886 years), KMT2D P523 (3.458 *vs.* 0.838 years), T4193M (3.46 *vs.* 1.03 years), R2282W (3.458 *vs.* 0.227 years) and TP53 R183Q (3.46 *vs.* 0.78 years). Four mutations were significant for OS: NOTCH2 D1306N (median PFS 8.08 years versus 0.611), NOTCH1 V1379M (1.29 years), KMT2D R2282W (0.611 years), TP53 R183Q (0.887 years). In addition, we found that age, elevated LDH blood levels and elevated ki67 proliferation index percentage had a severe impact on OS (p=0.007; p=0.027; p=0.002 respectively). Moreover, elevated LDH blood levels and ki67 proliferation index percentage impacted on PFS as well (p=0.029 and p<0.001 respectively). Interestingly, IHC analysis did not show any impact on OS and PFS.

Conclusion: Our data seem to suggest that mutational status of MCL patients at the diagnosis may allow to identify cases with particular poor prognosis, independently from other conventional risk factors. They might be used to select high risk patients for novel therapy. Further studies possibly in a prospective setting are warranted.

P052

SERUM MIR-22 AS NOVEL NON-INVASIVE PREDICTOR OF POOR CLINICAL OUTCOME IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: PRELIMINARY RESULTS OF AN ONGOING PROSPECTIVE STUDY

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Introduction: DLBCL are a heterogeneous group of tumors with aggressive clinical course. To date, their diagnosis requires tissue biopsy and the available prognostic tools are not able to identify all the patients refractory to therapy with R-CHOP. Liquid biopsies introduced with the benefit of being non-invasive, facilitate serial sampling and dynamic monitoring. MiRNAs, frequently deregulated in cancer, are present in body fluids in a stable form, making them interesting candidates as biomarkers. We have, previously performed a pilot study on serum miRNAs profile in DLBCL patients, and found that serum miR-22-3p was significantly correlated with PFS. Evaluating the relationship between circulating miRNAs and their tumor origin may support their use as reliable biomarkers. In addition, it is widely recognized that a single biomarker approach may not be robust enough to have prognostic and predictive utility. Thus, the evaluation of a multiple miRNA signature could be a more valuable and reliable approach. Our aims are a) to validate serum miR-22 as novel and reliable prognostic biomarker in DLBCL; b) to compare miR-22 expression in serum and matched tumor samples and c) to perform a global serum miRNA profiling in order to identify circulating miRNAs predictive of treatment response.

Methods: Multicentric prospective study on *de novo* DLBCL patients uniformly treated with R-CHOP. MiR-22 expression profile was evaluated by qRT-PCR in serum samples and in matched formalin fixed, paraffin embedded-FFPE sections from biopsies or surgical specimes. Survival analysis was performed by Kaplan–Meier method. Global miRNAs expression profiling in serum samples of a cohort of responsive to R-CHOP versus primary refractory patients was performed by small-RNA Seq. Predictive accuracy was evaluated by ROC curve analysis.

Results: (a) Serum miR-22 profiling in a validation cohort of 78 DLBCL patients shows a significant correlation of miR-22 levels at diagnosis with patients 2-year PFS (p=0,003). (b) The comparison results indicate a positive correlation (Spearman's Rho = 0.42; p = 0.05) between serum miR-22 and its expression in tumor tissue. (c) Our ongoing experiments in serum samples from a cohort of responsive to R-CHOP versus primary refractory patients show 9 serum miRNAs (miR-143-3p, -185-3p, -200c-3p, -27a-3p, -324-5p, -421, -4462, -4729, -6731-3p) differentially expressed (p< 0,05) depending on to treatment response with an area under ROC curve > 0,7 demonstrating a predictive accuracy of this signature.

Conclusions: Our preliminary results suggest that serum miR-22, alone or in combination with a signature of circulating miRNAs, are of potential interest as novel and reliable prognostic and predictive biomarkers in DLBCL to early identify the chemo-resistant patients. The potential transfer of the study results to clinical settings could significantly contribute to improve DLBCL management.

P053

PROLONGED IMPAIRMENT OF NAÏVE T CELLS AFTER BENDAMUSTINE TREATMENT IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Introduction: Treatment of follicular lymphoma (FL) with immunochemotherapy can induce severe and prolonged lymphocytopenia, more common for patients (pts) treated with rituximab-bendamustine (RB) compared to RCHOP. Limited data exist on the composition of the peripheral T cell pool after RB therapy.

Methods: A single-platform method using the Lyotube Immunomonitoring was used to determine the percentages and absolute count of lymphocyte subsets in peripheral blood. T cell maturation was studied by staining with the following monoclonal antibodies: CD45RA-FITC, CCR7-PE, CD95-PerCP-Cy5.5, CD4-PE-Cy7, CD27-APC, CD8-APC-H7, CD3-BV450, CD31-BV500. Data were acquired on FACSCantoII and analyzed with Diva Software (BD Biosciences). A total of $0.5X10^6$ events for the first tube and 30.000 CD3+ events for the second one was collected. CD4 and CD8 maturational subsets were defined as Naïve (CD45RA+CCR7+), Central Memory (CD45RA-CCR7+), and Effector Memory (CD45RA-CCR7-). Wilcoxon-Mann-Whitney test was used for statistical analysis.

Results: We prospectively studied 15 pts with FL in complete remission between 18 and 24 months from the end of first-line treatment during rituximab maintenance. Induction therapy was RB in 9 pts (M 1/F 8, median age 60, range 49-71) and RCHOP in 6 pts (M 1/F 5, median age 57, range 51-72). No significant cluster of CD19+ events was found. We observed significant lower absolute CD4+ counts in RB-treated pts compared to RCHOP (median value $225x10^6$/L *vs.* $605x10^6$/L, p=0.02). The absolute count of CD8+ T cells did not differ between the two groups (median $453x10^6$/L *vs.* $532x10^6$/L). Therefore, the CD4/CD8 ratio was significant inferior in RB pts (median 0.5 *vs.* 1.45, p=0.02). We next analyzed the maturation status of T cell population. The proportion of naive CD4+ cells was significantly lower in RB group compared to RCHOP (median 13% *vs.* 42%, p=0.039), while CD4+ total memory cells was higher in RB-treated pts (median 85% *vs.* 55%, p=0.034). These findings were also observed in CD8+ cells, with a lower proportion of naïve (median 14% *vs.* 31.5%, p=0.025) and a higher percentage of total memory cells (median 47% *vs.* 38%, p=0.029) in RB pts. In the group of 9 RB-treated pts, 4 pts developed infections during maintenance phase: pneumonia in 2 pts (Haemophilus Influenzae, Klebsiella), recurrent cystitis with ESBL in 1 pt, recurrent bronchitis and Pseudomonas cellulitis in 1 pt. Median CD4+ count and naïve CD4+ T cell percentage in these pts was $229x10^6$/L and 20%, respectively. In the RCHOP group only one herpes labialis infection was registered.

Conclusions: The CD4+ count is significantly reduced for a prolonged period in FL pts during maintenance after RB. Our most striking finding is the marked long-term reduction of naïve T cells in both CD4+ and CD8+ cells following RB compared to RCHOP that appears to associate with the risk of infections. Further studies are warranted to corroborate the clinical significance of our finding.

P054

BRENTUXIMAB VEDOTIN FOLLOWED BY BENDAMUSTINE SUPERCHARGE FOR REFRACTORY OR RELAPSED HODGKIN LYMPHOMA: MATURE RESULTS OF A MONOCENTRIC PROSPECTIVE TRIAL

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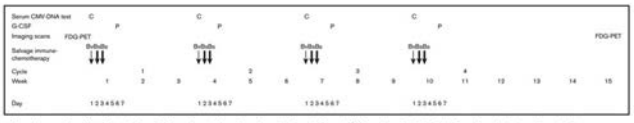
Introduction: Brentuximab vedotin (Bv) and bendamustine show encouraging results in the most challenging subset of patients with classic Hodgkin lymphoma (cHL). Emerging *in vitro* data allowed the speculation that high-dose bendamustine, administered right after Bv, facilitated intracellular trafficking, internalization, and metabolism of anti-CD30–auristatin conjugates and thus targeted delivery of anticancer therapeutics. We evaluated the impact of a deep metabolic response at ^{18}F-fluoro-deoxy-glucose (FDG) positron emission tomography (PET) on progression-free survival (PFS) in patients with refractory or relapsed (R/R) cHL following a new salvage regimen of brentuximab vedotin+bendamustine supercharge, every 21 days (Bv+Bs-21). Methods In this prospective trial, from 2013 to 2020, 26 consecutive patients (median age 43 years; range 23-59) with R/R cHL after failure of ≥1 salvage treatments received Bv+Bs regimen consisting of 3-days outpatient i.v. infusions of 1.8 mg/kg of Bv on day 1 in sequence with bendamustine on days 2 and 3 of the 3-weeks cycle at a fixed dose of 120 mg/m^2 per day (4 courses total). A robust primary prophylaxis including premedication, antimicrobials, colony-stimulating factors, and cytomegalovirus monitoring, was performed. Results All 26 patients underwent the scheduled 4 courses of Bv+Bs (median dose intensity of 100%) and the 38% of them experienced grade ≥3 treatment-related adverse events (CMV reactivation in 7 cases and neutropenia in 3 cases), without requiring hospitalization. All patients achieved deep metabolic responses with FDG/PET Deauville 5-point scale scores ≤3. Thereafter, three patients (12%) received two additional courses of Bv+Bs, four patients (15%) received allogeneic hematopoietic stem cell transplantation (HSCT) and the remaining 19 patients (73%) received autologous HSCT. In this last sub-group, for 12 patients peripheral blood stem cells (PBSC) were previously harvested after two courses of Ifosfamide, Gemcitabine, Vinorelbine and Prednisolone; in the remaining seven cases PBSC were successfully collected after Bv+Bs, with mobilization with G-CSF, vinorelbine-cyclophosphamide and/or plerixafor regimen. The median peak value of CD34+ cells was on day 12 after mobilization treatment (median number of harvest CD34+ cells: 3.1 x 10^6 per kilogram of body weight; range 1.6-4.2 x 10^6). After HSCT, median day of engraftment of neutrophils and platelets was recorded on day 11 (range 9-21) and day 12 (range 9-25), respectively. At a median follow-up of 33 months (range 1-79) from Bv+Bs regimen termination, the estimated 3-year PFS of the entire population was 94.4% (95% confidence interval, 84.4%-100%). Conclusions Bv+Bs-21 is an effective salvage regimen able to induce lasting complete remission in a high proportion of patients aged <60 years with R/R cHL. A deep metabolic response with a PET-negative status achieved with salvage therapy is the most important determinant of favorable outcome after HSCT.



Bv+Bs regimen schedule. Bv, at 1.8 mg/kg IV on day 1 of the 3-week cycle; Bs, at 120 mg/m^2 IV on days 2 and 3 of the 3-week cycle; P, peg-filgrastim 6 mg subcutaneous on day 6 of the 3-week cycle; FDG-PET, at baseline and 5 weeks after salvage treatment completion; C, serum CMV-DNA monitoring on day 1 of the 3-week cycle. Moreover, as support prophylactic treatment: methylprednisolone at 200 mg IV on days 1 to 3 of the 3-week cycle, diphenhydramine at 50 mg IV on days 1 to 3 of the 3-week cycle, febuxostat at 80 mg orally on days 1 to 5 of the 3-week cycle (plus hyperhydration); trimethropin-sulfamethoxazole at 960 (160 + 800) mg orally every 12 hours 2 times a week and acyclovir at 800 mg orally daily from the start of Bv+Bs regimen until 1 month after the last cycle. Clinical visits were performed on days 1 to 3 of the 3-week cycle. Routine laboratory tests were performed every 2 weeks.

Figure 1.

P055

RITUXIMAB-BASED RISK-ADAPTED TREATMENT STRATEGY IN NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA: 7-YEARS FOLLOW-UP

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Background: Nodular lymphocyte-predominant Hodgkin lymphoma (NLP-HL) is a rare variant of HL (5% of all HL). CD20 on neoplastic lymphocytes is a suitable target for novel treatments based on Rituximab (R). In vitro studies suggest that the expanded meshwork of follicular dendritic cells and germinal center T-lymphocytes (CD4+/CD57+) can mediate R-induced antibody dependent cellular cytotoxicity. Furthermore, R-induced signaling can enhance antineoplastic effect with anthracycline-based cytotoxic therapy, by the inhibition of p38-MAPK, NF-κB and ERK pathways deregulated in NLP-HL. Due to its rarity, consolidated and widely accepted treatment guidelines still lack for this disease.

Methods: Between 1 December 2007 and 28 February 2018 in the Hematology Unit of the Federico II University of Naples (Italy), sixteen consecutive newly diagnosed adult patients with NLP-HL received R alone or combined with ABVD (Doxorubicin, Bleomycin, Vinblastine, Dacarbazine) according to the baseline risk (GHSG prognostic score system). Among them, six patients with early favorable disease received R as a single agent, once per week for four weeks, followed by R maintenance (once every three months for 2 years); three patients with early unfavorable disease received R once per month plus 4 ABVD cycles; the remaining seven patients with advanced-stage disease received R twice a month plus 6 ABVD cycles. The treatment efficacy (according to the 2007 Revised Response Criteria for Malignant Lymphoma) and safety (according to the NCTCAE, v4.03) were compared to those of a historical cohort of 12 patients with NLP-HL who received 4 ABVD courses followed by involved-field radiotherapy if at stage I-II (n= 9), or 6 ABVD courses for III-IV stages (n= 3). The primary outcome was PFS, and secondary outcomes were OS and side-effects.

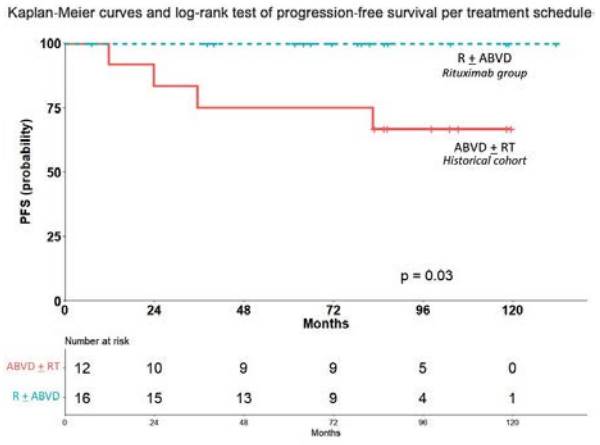

Figure 1.

Results: After a 7-year follow-up (range, 1-11 years), the K-M estimated PFS were 100% for the patients treated with R-containing regimen versus 66% (95% CI, 44.7-100) for the historical cohort (P=0.036). Four patients in the latter group, showed insufficient response (one relapse, in the early-stage sub-set; one refractory and two relapses, in the advanced-stage sub-set). The OS was similar for the two treatment groups. Short and long-term side-effects were more frequently observed in the historical cohort. Grade 3-4 neutropenia was more frequent in the historical cohort compared with R group (58.3% vs. 18.7%, P=0.03). Long-term non-hematological toxicities were observed only in the historical cohort. Among the 12 cases, six suffered from thyroid disease, two from lung fibrosis, two from avascular necrosis of the femoral head, and one from valvular heart disease, most likely related to irradiation.

Conclusion: Our results confirm the value of R in NLP-HL and show that R induction and maintenance in a limited-stage, or R with ABVD only in presence of risk factors, give excellent results compared to conventional chemo-radiotherapy, while sparing cytotoxic agent and/or irradiation-related damage.

P056

ONE DAY ANTIEMETIC PROPHYLAXIS WITH NEPA (NETUPITANT/PALONOSETRON) AND DEXAMETHASONE TO PREVENT CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING (CINV) IN HODGKIN'S LYMPHOMA NAÏVE PATIENTS RECEIVING ABVD REGIMEN: A MULTICENTER PHASE IIA STUDY

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Introduction: Cancer chemotherapy may be associated with a high incidence of nausea and vomiting, particularly when highly emetogenic antineoplastic drugs are used. Uncontrolled emesis can profoundly impact on the patient's quality of life and ability to survive, by causing dehydration, electrolyte imbalance, malnutrition and treatment discontinuation. The ABVD regimen (Adriamycin, Bleomycin, Vinblastine and Dacarbazine) is considered the standard of care for first-line treatment of Hodgkin's Lymphoma. Among these drugs, dacarbazine and adriamycin are the most emetogenic, being classified as highly and moderately emetogenic chemotherapy, respectively. NEPA is the first fixed antiemetic combination composed by the pharmacologically and clinically distinct 5HT3 receptor antagonist (5HT3-RA) palonosetron and the highly selective Neurokinin1/Substance P receptor antagonist (NK1-RA) netupitant available as oral formulation. A single dose of NEPA per chemotherapy cycle acts on the principal pathways involved in the mechanisms controlling nausea and vomiting in a synergistic way with an appropriate half-life to cover both the acute (0-24 hours from chemotherapy) and delayed (25-120 hours) phase.

Methods. In this open label, multicenter study chemo-naïve Hodgkin's Lymphoma patients who were addressed to receive their first cycle of ABVD regimen (2 doses in 28 days, on days 1 and 15) were given a single administration of NEPA plus 4 mg dexamethasone. The primary endpoint was complete response (CR), defined as no emesis and no rescue medication during the overall phase (0-120 hours) on the first dose of the first ABVD cycle.

Results. A total of 77 patients were evaluated. According to the adopted Fleming one-stage design, the primary endpoint of this study was achieved. Indeed, the number of the complete responders for the overall phase among the first 70 consecutive patients was 66, which is greater than the pre-determined cut-off of 46, representing the minimum frequency of responders for which the treatment is considered effective. In addition to the primary efficacy endpoint, several additional endpoints were evaluated. The CR values were 93.5% for the acute phase, 81.8% for the delayed phase and 80.5% for the overall phase, while levels of no significant nausea were 94.6% (acute phase), 83.1% (delayed phase), 81.8% (overall phase) and 90.9% (inter-dose phase, days 6 to 14).

Conclusions: This study demonstrated the efficacy of NEPA plus dexamethasone in preventing the nausea and vomiting induced by the highly emetogenic ABVD regimen.

P057

INCIDENTAL DIAGNOSIS OF BURKITT LYMPHOMA ON CECAL APPENDIX: CASE REPORT AND LITERATURE REVIEW

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Primary lymphoid organs are bone marrow and thymus. The secondary lymphoid organs are spleen, lymphonodes and lymphoid tissue associated to mucous membranes.Cecal appendix, therefore, can be site of NHL such as Burkitt lymphoma. BL is an aggressive NHL characterized by translocation and deregulation of the MYC gene on chromosome 8 and the heavy chain gene on chromosome 14. The t(8;14) is the most common translocation in BL occurring in 70-80% of cases. BL is derived from germinal center B cells and hystologicaly is characterized by a monomorphic population of matur lymphocytes with basophilic cytoplasm and prominent vacuoles. The result is a "starry sky" apparence due to macrophages containing cellular debris. The malignant cells express B-cell markers including CD19-CD20-CD79A-PAX5 and for germinal center markers CD10 and bcl6 but are negative for bcl2. There are only 30 cases of appendiceal Burkitt lymphoma in literature. In this cohort, the patients are predominantly male with an average age of 20 years. The majority of cases clinically presented as classical acute appendicitis, the remainder of cases presented atypical clinic but radiological signs of appendicitis. In february 2020, a 44-years old man underwent appendicectomy for the diagnosis of acute appendicitis. Intraoperatively, a significant inflamed, edematous appendix without perforation was identified. Biopsy histology demonstrated marked expansion by lymphoid infiltrate composed by monomorphic intermediate-sized and the characteristic starry sky appaerance. In immunochemistry, the cells are CD20+ CD10+ BCL6+ BCL2- cMyc + with Ki67 proliferative index equal to 90%. The translocation t(8;14) in FISH confirms the diagnosis of Burkitt lymphoma. The patient had no B syntoms and was subjected to bone marrow biopsy without marrow infiltration by Burkitt Lymphoma. Total body CT and whole body PET did not demonstrate evidence of residual disease at any nodal or extranodal side. CSF was negative for neoplastic cells. LDH was normal. The Ann Arbor staging classification corresponde to stage IE, low risk. The patient was started on a multiagent steroid and chemotherapy regimen(R-CODOX-M-IVAC)and medicated lumbar punctures. Our therapeutic program is to subject the patient to other cycles of therapy and to peripheral stem cells collection according to NCCN guidelines. Approximately 1% of appendicetomies have an incidental finding of an appendiceal neoplasm. A primary appendiceal lymphoma is extremely rare. The predominant PAL histological subtype was DLBCL followed by Burkitt lymphoma. Treatment for Burkitt Lymphoma is stratified on patient age and stage. In adult, current recomandations include multiagent regimens with intensive chemotherapy and immunotherapy with anti CD20. Newer anti-CD20, anti-CD19, CD22 monoclonal antibody are under investigation. Follow up is carried out in the basis of the disease responce according to the Lugano criteria. Ours is one of the rare cases of appendiceal BL. This case allows us to underline the importance of hystology after appendicectomy to discover incidental diagnosis of NHL and other pathology with poor prognosis if not treated.

Monoclonal Gammopathies and Multiple Myeloma

P058

BENDAMUSTINE-BORTEZOMIB-DEXAMETHASONE (BVD) IN HEAVILY PRETREATED MULTIPLE MYELOMA: OLD/NEW IN NOVEL AGENTS' ERA

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Bendamustine is an old bi-functional alkylating agent which has proved to be effective in relapsed, refractory and in new diagnosed Multiple Myeloma (MM). Thus, aiming to provide further insights in this field, also in novel agents'era, we present here a retrospective, real-life analysis of patients with relapsed/refractory MM (rrMM), who had received salvage therapy with bendamustine in combination with bortezomib and dexamethasone (BVD) 81 patients (44 M/37 F), with rrMM, median age at diagnosis 59.4 years (r. 36-82), median age at start of treatment 63.6 years (r.37-86) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (B 90 mg/sqm days 1,2; V 1.3 mg/sqm days 1,4,8,11, D 20 mg days 1,2,4,5,8,9,11,12, Pegfilgrastim day +4) every 28 days, until progression. All patients had previously received bortezomib-based and IMIDs-based treatments, and 32% (26/81) had also received radiotherapy. 69% (56/81) had undergone single or double autologous and three (2%) allogeneic stem cell transplant. All patients were relapsed and refractory to last therapies received before BVD. Bendamustine was well tolerated, with grade 3-4 transfusion-dependent anemia in 56% (46/81) of patients, and 43% (35/81) grade 3-4 neutropenia (no ospedalization was required, no septic shocks were observed). No severe extrahematologic toxicity was observed, only grade 1 gastrointestinal side effect (nausea), treated by common antiemetic drugs. According to IMWG, ORR was 63% (51/81: 7 CR, 18 VGPR, 15 PR, 11 MR) with 11 PD and 19 patients in SD, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 11 patients, BVD was, after having achieved at least a PR, a bridge to second auSCT, and for two patients a bridge to alloSCT. Eight patients have surprisingly achieved a notable PR after failure of novel agents (*i.e.* Carfilzomib, Daratumumab and Pomalidomide). Median time to response was 1.3 months (r.1-3), median OS from diagnosis was 67.3 months (r.6-151), median OS from start of Bendamustine was 9.6 months (r.2-36). The triplet Bendamustine-Bortezomib-Dexamethasone has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT, also after failure of novel agents.

P059

CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF LENALIDOMIDE-REFRACTORY MULTIPLE MYELOMA

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Carfilzomib is an epoxyketone proteasome inhibitor of second generation, proved to be effective and safe in relapsed and refractory Multiple Myeloma (rrMM), in combination with dexamethasone or lenalidomide and dexamethasone. In this retrospective observational trial, it has been evaluated efficacy and safety of carfilzomib, in combination with lenalidomide-dexamethasone (KRD) as salvage regimen in patients with rrMM, refractory to lenalidomide, where lenalidomide-based regimens have no proven efficacy. 41 patients (23 M/18 F), with rrMM, median age at diagnosis 63.7 years (r. 43-82), median age at start of treatment 67 years (r. 48-84) previously treated with several lines of treatments (median 3, r. 2-11), underwent to KRD regimen (ASPIRE trial schedule) for a median treatment cycles of 8 (r 2-18). ISS was equally distributed, and all patients had previously been treated with bortezomib and IMIDs, and were refractory to this agents. 61% (19/31) of them had undergone at least to a single ASCT. According to IMWG criteria, after a median follow-up of 9 months (r. 2-18), ORR was 68,2% (28/41: 9 CR, 12 VGPR, 7 PR) with 5 progressive diseases (PD) and 8 patients in stable disease (SD): this can be considered as an impressive result in this subset of rrMM patients, refractory to lenalidomide. In particular, for 11 patients, KRD was, after having achieved at least a PR, a bridge to second/third autologous SCT. Median time to response was 1.3 months (r.1-4), median OS from diagnosis was 62 months (r. 9-170), median OS from start of Carfilzomib was 11 months (r. 2-18). Carfilzomib was well tolerated, with grade 2 anemia in 39%(16/41) of patients, successfully managed by ESAs, without necessity of blood transfusions; 29% (12/41) grade 3-4 neutropenia (pegfilgrastim in primary prophylaxis was given, no ospedalization was required, no septic shocks were observed); 34% (14/41) grade 2, 21% (9/41) grade 3 and 12% (5/41) grade 4 thrombocytopenia, without hemorrhagic events and transfusion-dependency. Moreover, it was observed pneumonia in 39% (16/41) of patients, treated by common antibiotic drugs and always solved. A cardiac monitoring was performed for all patients: hypertension (grade 2-3) in 34% (14/41) of patients; fatigue in 39% (16/31) of patients. Carfilzomib-Lenalidomide-Dexamethasone has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, also lenalidomide, and it could be considered as a bridge to a second autologous or allogenic SCT.

P060

CAN THE BONE MARROW MULTIPARAMETER FLOW CYTOMETRY (MFC) HAVE IMPACT ON THE PROGNOSIS OF NEW DIAGNOSED SYSTEMIC LIGHT CHAINS AMYLOIDOSIS?

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Introduction: Light chains amyloidosis (AL) is characterized by the deposition of free light chains in different organs with progressive damage; a bone marrow (BM) plasmacells (PCs) clone is the responsible. The MFC can assess PCs number, clonality and expressed antigens We evaluated the prognostic role of MFC in AL at diagnosis exploring a possible relation between the MFC PCs number and the expression of normal(CD38,CD138) and aberrant(CD56) antigens with disease localization and overall survival(OS).

Methods: We retrospectively analyzed data from 57 patients We first evaluated the relationship between PCs number and antigen expression with heart involvement. Secondly, we estimated the impact of MFC features on the OS The diagnosis and the organ damage were assessed according to the Italian Society of Amyloidosis(SIA)Guidelines. The patients underwent to first line therapy according to the best therapeutic choice MFC was practiced for all the patients and the following characteristics were analyzed: PCs number, clonality and CD38, CD138, CD45, CD56 expression MFC allowed the quantification and differentiation

between normal and clonal PCs with a sensitivity of 10−4 Comparisons between groups were based on Chi square tests or MannWhitney. The value of PCs number in predicting cardiac involvement was evaluated by Receiver Operating Characteristic (ROC). Statistical analyses and modelling were performed by R software. P-values <0.05 were considered statistically significant. OS was estimated by the Kaplan-Meier method, log-rank test compared 2 or more groups of stratified patients. Multivariate analyses were performed by the Cox proportional hazard regression model.

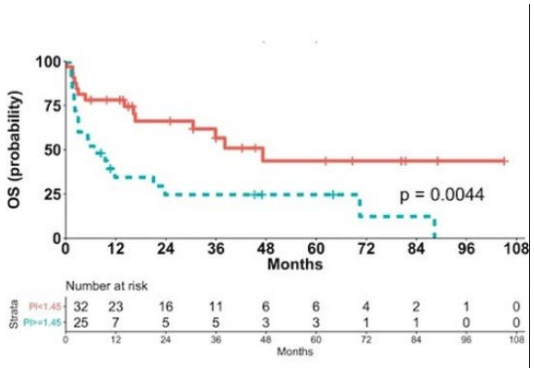

Figure 1.

Results: We detected BM monoclonal PCs by MFC in 51(89%) patients; the median of PCs was 1.4% PCs number showed a significant association with AL localization. In particular, when treated as continuous variable, higher PCs number was associated with heart involvement (p=0.001). ROC curve analysis supported the predictive role of PC number in the heart localization and the "best" cut-off point was 1.45% Median OS of the population was 11.8 months (0.3-105). Cardiac involvement was detected in 39 (68%) cases. The 6 patients, who were negative for PCs in BM, didn't show cardiac involvement. When we compared the OS of patients with PC number <1.45% *vs.* >1.45%, in the second case a worse outcome was showed (p=0.004). In multivariate analysis, this data lost its significance due to the strong correlation with cardiac localization The expression of PCs antigens was: CD38 in 51 (100%), CD138 in 50 (98%), CD45 in 15 (29%), CD56 in 39 (76%). No studied antigen is related to cardiac involvement or had an impact on OS.

Conclusions: MFC role in the prognostic stratification of amyloidosis is still unclear. We identified a PCs cut off point of 1.45% above which the risk of cardiac localization increases and OS is shorter. Notably, no one of the patients with no detectable BM PCs showed heart involvement.

P061

POMALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF HEAVILY PRETREATED MULTIPLE MYELOMA

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Pomalidomide is a new generation IMID, with a very good compliance, thanks to oral administration, which can be used also in heavily pretreated patients, in a domestic setting. In this retrospective observational trial, It has been evaluated efficacy and tolerance of pomalidomide plus dexamethasone (PD) as salvage regimen in heavily pretreated patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe. 57 patients (31 M/26 F), with rrMM, median age at diagnosis 69 years (r. 52-86), and median age at start of treatment 76 years (r.56-90) treated with several lines of treatments (median 7, r. 2-11), every refractory to all the drugs previously received (also Bortezomib, Thalidomide and Lenalidomide), received Pomalidomide-Dexamethasone (Pomalidomide 4 mg for 21 days, Dexamethasone 40 mg days 1,8,15,22, pegfilgrastim day +8) every 28 days, until progression. ISS was equally distributed, and cytogenetic at relapse was evaluable in 14 patients. All the patients had previously been treated with schedule containing bortezomib and IMIDs. 63% (36/57) had undergone at least to a single ASCT. All patients were relapsed and refractory to last therapies received before PD. Pomalidomide was well tolerated, with grade 3-4 transfusion-dependent anemia in 58% (33/57) of patients, 44% (23/57) grade 3-4 neutropenia (pegfilgrastim in primary prophylaxis was given, no hospitalization was required, no septic shocks were observed), 40% (23/57) grade 3-4 thrombocytopenia without hemorrhagic events and transfusion-dependence. No severe extra-hematologic toxicity was observed. According to IMWG, ORR1 (≥PR) was 47.3% (27/57: 5 CR, 11 VGPR, 7 PR, 4 MR), but, considering that we are evaluating a cohort of heavily pretreated patients, with poor prognosis, another parameter should be considered, ORR2 (≥SD), considering stable disease as a successful result in progressive MM. ORR2 was 77.1% (17 SD). These can be considered as impressive result in this subset of patients. Oral treatment gives a really good compliance, in frail and unfit patients, and response, when present, is always really fast (median time to response: 2 months (r.1-6)), median OS from diagnosis was 94 months (range 21-234), median OS from start of pomalidomide was 9 months (range 1-25). Nine patients have surprisingly achieved a notable response (3 VGPR, 4 PR, 2 MR) after failure of novel agents (*i.e.* Carfilzomib, Daratumumab and Pomalidomide). Pomalidomide-dexamethasone has shown significant efficacy and a very good compliance, thanks to oral administration, in a particularly severe setting of heavily pretreated patients, relapsed and refractory to all available therapeutic resources, also after failure of novel agents.

P062

BENDAMUSTINE-POMALIDOMIDE-DEXAMETHASONE (BPD) IN PATIENTS EXPOSED TO MANY CHEMOTHERAPEUTICAL REGIMENS WITH HIGHLY REFRACTORY MULTIPLE MYELOMA. A SINGLE CENTER EXPERIENCE

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Introduction: Despite in the last years many new therapeutic strategies have emerged for the treatment of patients with RRMM but the prognosis of patients who relapsed after proteasome inhibitor (PI) IMIDs or monoclonal antibody (MoAB) is very poor. Thus, we assessed a strategy that could determine a benefit without frequent access to the hospital. Our choice fell on the pomalidomide, whose use is associated to a good ORR and PFS in RRMM, associated with bendamustine and dexamethasone. Therefore, we decided to treat patients with RRMM refractory to PI, IMIDs and/or MoAB and in an acceptable clinical condition (ECOG≤2). Our aim was to describe efficacy of BPD in terms of OS, PFS and safety.

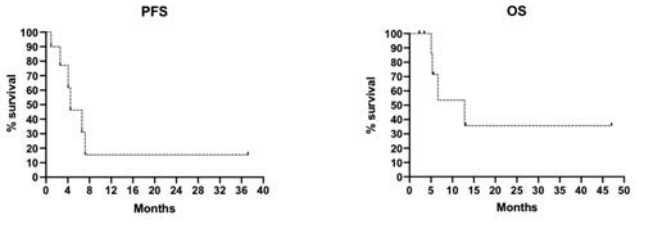

Figure 1.

Patients and methods: From 2016 to 2020, we treated 10 patients with a median age of 70 years and a confirmed diagnosis of RRMM. All patients were refractory to PI and IMIDs with a median number of pre-

viously therapies of 5,5 (range 4-8). Eight patients (80%) had undergo to an ASCT, 8 patients (80%) were refractory to carfilzomib, 5 patients (50%) to daratumumab and 3 patients (30%) even to pomalidomide and they were treated with the addition of bendamustine. Before treatment all patients underwent to laboratory assessment and, in case of non-secretory MM, to a BM aspiration and a PET-TC. Laboratory assessment was repeated after the first cycle and every 3 months or in case of PD. Bendamustine was administered I.V at dose of 70 mg/m^2 on D1-2, pomalidomide 4mg (D1 to D14) and dexamethasone 20mg (D1-2, 8 - 15 – 22) every 28 days. BPD was performed till disease progression or unacceptable toxicity. No primary prophylaxis with G-CSF was performed but G-CSF was used in case of febrile neutropenia (FN) as secondary prophylaxis. In case of FN in any previous cycle, the patient started G-CSF as primary prophylaxis with blood count and biochemistry control at home every 5 days till resolution of fever and ANC recovery.

Results: Patients received a median of 4 cycles (range 1-12) with 3 patients which are still on treatment. Patients without a response ≥ to SD after first cycle were considered refractory. The ORR was 50% with 1 patient (10%) achieving a CR and 4 patients (40%) achieving a PR. Three patients achieved a SD and underwent to progression after a median of 4,1 months while 2 patients underwent to PD after 1 and 3 months respectively. After a median FU of 5,5 months the median PFS was 4,5 months and the median OS was 12,8 months. The 5 patients exposed to daratumumab achieved an ORR of 60% with 3 patients achieving a PR while 1 patient achieved a SD and 1 patient a PD. The most common grade≥3 AEs were anemia (50%), neutropenia (50%), thrombocytopenia (30%). No patients need hospitalization and no extra-hematological toxicities of grade≥3 were observed.

Discussion: BPD represent an effective and safety chemotherapy regime and our data compares favorably with the ORR of carfilzomib or daratumumab-based regimens in RRMM patients. BPD represent a feasible choice provided that an appropriate domiciliary laboratory assessment is ensured with a frequent contact, even by phone or email, with the hospital of reference.

P063

CAN A PERSISTENTLY POSITIVE SERUM OR URINARY IMMUNOFIXATION (SIFE/UIFE), WITH NORMALIZATION OF SERUM FREE LIGHT CHAINS (FLC) ASSAY AND KAPPA/LAMBDA RATIO AFTER TREATMENT, HAVE A PROGNOSTIC IMPACT IN SYSTEMIC LIGHT CHAIN AMYLOIDOSIS (AL)?

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Background: AL is characterized by an excess of FLC and insoluble amyloid deposits in different tissues. Hematological complete response (HCR) requires normal FLC, normal kappa/lambda ratio and negative sIFE and uIFE, according to ICC guidelines; after treatment a normalization of FLC and kappa/lambda ratio with still positive (POS) sIFE or uIFE can occur.

Methods: We retrospectively selected 29 patients with systemic AL in our unit with the following features: age ≥18years, normalization of FLC assay and kappa/lambda ratio after bortezomib-based (B) regimen in first line or Lenalidomide-Dexamethasone (RD) in refractory/relapsed disease. Autologous stem cell transplant (ASCT) was performed as consolidation when possible. Hematological Response assessment was performed with FLC assay, kappa/lambda ratio and sIFE/uIFE. It was evaluated every 2 cycles during the therapy and every 4 months during the follow up. The number of cycles and therapy lines employed were established according to the clinician. Our aim was to evaluate if the persistently POS sIFE/ uIFE with normal FLC and kappa/lambda ratio, after treatment, has an impact on progression free survival (PFS) and overall survival (OS) rates.

Results: Median age was 61years (48-83). All patients had measurable disease at diagnosis with altered FLC; 24(83%) and 14 (48%) had, respectively, POS sIFE and POS uIFE. The median PFS and OS of the entire population were respectively 48(39-72) and 68 months (42-94). All Patients received B-based regimen as first line and 2 of them could consolidate with ASCT; 6 patients relapsed and received RD as second line; Ten (34%) patients showed normal FLC and kappa/lambda ratio,9 after the first line and 1 after the second and sIFE or uIFE were always negative. Nineteen (66%) patients achieved normal FLC and kappa/lambda ratio, 15 after the first line and 5 after the second. In this case sIFE or uIFE were persistently positive after treatment. Median PFS for POS sIFE /uIFE group was 67months *vs.* 40 for patients with negative findings. (P=0.089). Median OS was 72 *vs.* 42 months(P=0.04) Notably, PFS and OS for POS sIFE group at diagnosis were longer compared with patients with negative sIFE (50 *vs.* 15 months, P=0.014 and 72 *vs.* 19 months, P=0.0046)

Conclusion: The prognostic role of POS sIFE/uIFE with normal FLC and kappa/lambda ratio after therapy it's still unknown. A worse PFS and OS was found when sIFE was negative at diagnosis. This condition can be the consequence of a delayed AL diagnosis with advanced disease and shorter PFS and OS. However, we showed no adverse impact for persistence of POS IFE after the treatment. We assumed that, in a small percentage of patients, the residual monoclonal component detected by sIFE/uIFE might be not amyloidogenic and therefore unable in forming amyloid fibrils, leading to the possibility of considering this condition clinically similar to a CR. Our results need to be validated in a wider study.

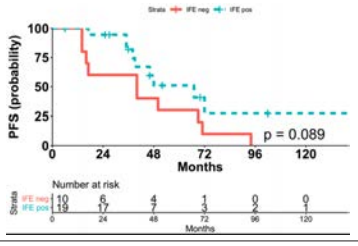

Figure 1.

Stem Cell Transplantation

P064

IMMUNE RECONSTITUTION AND CLINICAL OUTCOMES IN THE SETTING OF HLA-IDENTICAL ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION

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Introduction: Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) can lead to prolonged immunodeficiency due to conditioning regimens and immunosuppressors, albeit its curative role in diverse settings. Deficits both in innate and adaptative immunity are major contributing factors to treatment-related mortality (TRM). We investigate thymus-dependent and independent role in immune reconstitution (IR) kinetics and long-term clinical outcomes.

Methods: Sixty-four patients (median age 56) undergoing HLA-identical sibling or unrelated donor allo-HSCT after a reduced intensity conditioning (RIC) regimen were enrolled. Peripheral blood samples were collected before conditioning and at 1, 3, 6, 12, 18, 24 months after allo-HSCT from patients and at the time of donation from healthy donors as controls. Evaluation of IR was conducted by flow-cytometry analyses of CD4+ and CD8+ T-cell subsets [naïve, central memory (CM), effector memory (EM), CD45RA-expressing terminal effector memory (EMRA) and revertant] and Real-Time PCR quantification of signal joint T-cell receptor excision DNA circles (sjTRECs), specific markers of naïve T cells thymopoiesis. sjTRECs real-time PCR was performed on genomic DNA extracted from sorted CD4+ and CD8+ T cells. Median, maximum and minimum values were summarized using descriptive statistics. Associations between sjTRECs and T-cell subsets and trends over time were evaluated by Generalized Linear Models.

P065

THE ENDOTHELIAL DAMAGE POST ALLOTRANSPLANTATION: BIOMARKERS AND CLINICAL CORRELATION

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Introduction: Among endothelial damage manifestations after HSCT, TTP, Diffuse Alveolar Hemorrhage, Engraftment syndrome, Capillary leak syndrome, PRES and VOD, have been extensively described, but other clinical manifestations and laboratory abnormalities are often misdiagnosed. Several markers of endothelial damage have been identified, some of them shared within steroid-refractory aGVHD (TM and ST2).

Methods: We prospectively correlate clinical manifestations with biomarkers of endothelial damage (by ELISA assays: IL6, IL8, TNF, Thrombomoduline, ST2, IFNγ and PAI1) in 18 patients underwent HSCT, at specific timepoints (before HSCT, at the day of infusion, at phase pre-engraftment (7d), periengraftment (14d), early engraftment (30d), intermediate (60d) and late engraftment (120 d). Characteristic of patients (pts): Median age 55 y (27-67); 61% MAC, 39% RIC; 22% sibling, 33% MUD, 39% haploidentical, 6% MMUD; 61% ATG, 39% PTCy. Median follow up was 340 days (153-974).

Results: We observed 7 TTP, 1 VOD, 1 ES, 14 aGVHD. In one case, TTP was associated with steroid-refractory aGVHD. Three patients died for TRM (aGVHD grade 3) and 3 for relapse. Among the 13 pts receiving ATG, 5 developed endothelial syndromes (TTP, ES, VOD). Among the 7 pts receiving PTCy, 2 developed TTP and 6 aGVHD (with one overlap). A minor clinical-laboratory signs, suggestive of endothelial damage has been observed: hypertension, nephrotic syndrome (direct); increase of: creatinine, LDH, bilirubin, BNP; albuminuria ≥ 30 mg/dl; reduction of haptoglobin, refractoriness or 20% reduction in PLT, any changes in kPa on elastosonography (indirect). 17 pts showed direct and indirect signs not attributable to anything else. In 10 cases endothelial damage anticipated the aGVHD. We observed a typical pattern of biomarkers dynamic: first peak of IL6 and IL 8 at 7d; a second peak of TNF at 14d; a third peak of TM at 30d; surprisingly ST2 and PAI1 were normal, but with higher values at 14d. In TTP we always observed high levels of IL 6 (49 *vs.* 47 pg/ml p=0,022), TNFα (341 *vs.* 274 pg/ml; p=0,008) and TM (22,9 *vs.* 14,9 ng/ml; p=0,007), compared to non-TTP cases, with normal ST2 values (4616 pg/ml in TTP *vs.* 5825 pg/ml non-TTP; p=0,03). In pts developing GVHD, higher TM (20 *vs.* 14 pg/ml in non-GVHD; p= 0,002) and higher ST2 have been observed. Interestingly in pts with both direct and indirect signs of endothelial damage, high levels of IL6, IL8 (at 7d), TNFα(at 14d) and TM (from 30d) have been observed, even if the manifestations occurred later.

Conclusions: In this preliminary series IL6, TNFα, TM and ST2 correlated with endothelial damage: higher levels of TM and TNF, and lower levels of ST2 correlate with a TTP while elevated levels of TM correlate with aGVHD. We recommend to evaluate both direct and indirect changes that can anticipate the clinical syndromes; finally patients experiencing a reaction to ATG had higher incidence of endothelial damage associated with high IL6 and ST2 levels.

P066

EVALUATION OF RISK FACTORS AND OUTCOME OF TOXIGENIC CLOSTRIDIUM DIFFICILE INFECTION IN PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: C. difficile infection (CDI), the most common cause of hospital-acquired diarrhea, can occur after allogeneic hematopoietic stem cell transplantation (HSCT) and it is reported to increase the risk of graft versus host disease (GVHD).

Methods: We evaluated incidence, risk factors and outcome of toxigenic CDI in 202 patients who received HSCT from January 2016 to September 2019. All the patients performed rectal swab before conditioning and received Levofloxacin routine prophylaxis. At the first episode of diarrhea occurring between day - 10 and day + 100 after HSCT, they underwent stool specimen collection: CDI diagnosis was carried out by Enzyme Immune Essay (EIA) until March 2019 and then by microarray-based Comparative Genome Hybridisation (CGH), identifying toxin producing strains. CDI infections were treated with oral Vancomicin in all patients. Fidaxomicin was given in case of lack of stool negativization after a 7 day-course of Vancomicin treatment.

Results: 12 out of 202 patients (6%) developed toxigenic CDI at a median of 25 days (range -5; 100) after HSCT. Comparing patients with and without toxigen CDI infection, we observed that history of Multidrug Resistant Gram negative bacterial infection in the previous 2 years before HSCT (p 0.05), previous abdominal surgery (p 0.00001), HBV/HCV infection or sieropositivity (p 0.00001), previous hospitalization one month before HSCT (0.000098) and previous CDI infection or colonization (0.01) are significantly associated with toxigen CDI development during HSCT. Disease status at HSCT, conditioning regimen intensity,

mucosyte severity, total parenteral nutrition use and development of Bacteria infections during aplasia period were equally distributed between the 2 groups. Moreover, we did not identify any significant differences in the rate of patients developing acute GVHD (p=0.2), while use of more of two antibacterial therapies (p=0.006) and CMV reactivation (p=0.02) are significantly more frequent in patients with CDI infection. Median time to CDI negativization was 16 days (range 5-30) after Vancomicin (10 patients) or Fidaxomicin (2 cases). At a median follow-up of 1 year after HSCT, 1-year OS was 58,3% for CDI group and 56,3% for control group.

Conclusions: We conclude that in our experience risk of CDI infection after HSCT was increased by pre-trasplant factors such as previous Multidrug resistant Gram negative bacterial infections and prior abdominal surgery, and by post-transplant factors such as use of more than 2 antibacterial therapies. Stool negativity was obtained in all patients with appropriate treatment and there was no increased risk of GVHD or OS impairment, suggesting that search for CDI at the first episode of diarrhea after HSCT and prompt treatment can definitively solve CDI infections without sequelae in allogeneic HSCT recipients.

P067

HEMATOPOIETIC STEM CELL (HSC) COLLECTION, PRODUCT'S QUALITY AND ENGRAFTMENT: THE IMPACT OF PLERIXAFOR "ON DEMAND"

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Background: The standard therapy to treat several hematological malignant diseases is autologous hematopoietic stem cell transplantation ASCT. For poor mobilizer patients (5-40%), a CXCR4 inhibitor Plerixafor can induce mobilization of PBSC but the best timing to use Plerixafor is matter of debate. In order to avoid failure's mobilization, Plerixafor has shown its efficacy when given "on demand". Further, it's known that the quality of the PBSC collection impacts on the transplant outcome and the high PMN count negatively affects the platelet recovery but data about the effects of Plerixafor on graft composition and engraftment lack. AIM of our study was to evaluate the impact of Plerixafor use "on demand" on the quality of the PBSC collection and engraftment, related to the collection timing and WBC count.

Methods: We retrospectively examined 404 collection procedures from 264 hematological neoplastic adult patients, 31 of whom received Plerixafor 'on demand' at the recommended dose of 0.24 mg/kg/day, while 233 received standard mobilization regimen. Overall, 168 ASCT (17 Plerixafor *vs.* 151 no Plerixafor) were performed and data outcome were analyzed. Moreover, 12/17 patients, who underwent ASCT, achieved a collection ratio CD34+/WBC>1 upon Plerixafor and were considered as "early Plerixafor use group".

Results: Patients treated with Plerixafor "on demand" achieved a median value of pre collection CD34+ and WBC count, significantly lower (p=0.0012) and higher (p=0.000000021) than no Plerixafor group (CD34+: 43 *vs.* 94/µl and WBC: 27 *vs.* 14 x103/µl), respectively. Collection efficiency was significantly higher (p=0.04) in the Plerixafor group. Moreover, the collection yield of CD34+x106/Kg was significantly higher (p=0.00012) in the no Plerixafor group while the PMN count of the collected product was significantly higher (p=0.00000003) in the Plerixafor group. No differences between collection length (p=0.36), processed volume (p=0.4) and platelets count of collected product (p=0.12) were observed between the 2 groups. Concerning the transplant outcome, median time of PMN and PLTS engraftment was 12 *vs.* 11 (p=0.72) and 20 *vs.* 13 days (p=0.51) in the Plerixafor and no Plerixafor group, respectively. The same differences occurred in the "early Plerixafor use group", except for collected platelets, that were significantly lower (p=0.005) compared to no Plerixafor group. CONCLUSIONS Our data confirm that the products collected with Plerixafor "on demand" have significantly higher PMN count than those collected with standard mobilization whereas the products collected by the "early Plerixafor use group" have a significant lower content of PLTS. However, no clinical significant impact on PMN and PLTS engraftment was observed in the 2 subsets. Further studies are needed in order to understand if CD34+/WBC ratio >1, associated or regardless to the WBC count, may be identified as the best timing for Plerixafor introduction in the clinical practice.

P068

TREATMENT WITH RUXOLITINIB IN STEROID-REFRACTORY GVHD

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Background: More than 50% of patients who develop acute and chronic graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) will not respond adequately and permanently to first-line treatment with steroids. Although these patients have poor prognosis and high treatment-mortality, a standard salvage treatment has not been established yet. Preclinical evidences and initial clinical studies support the efficacy of the selective JAK1/2 inhibitor ruxolitinib (RUXO) in this setting.

Methods: We conducted a retrospective observational study recruiting 9 patients who underwent HSCT between February 2012 to April 2020, developed steroid-refractory (SR) aGvHD or cGvHD and received "off-label" RUXO. AGVHD was evaluated according to the IBMDR criteria and cGVHD according to NIH scale. Treatment was considered effective if complete or partial responses were reached on day + 30 after RUXO initiation.

Results: Median age of patients was 50 years (range, 27-69). The most frequent underlying diseases were acute myeloid leukemia (4) and myelodisplastic syndrome (2). Three patients had a related HLA identical donor, 4 a matched unrelated donor (MUD) and 1 an haploidentical related donor. Six out 9 patients received a reduced-intensity conditioning while the remaining 3 patients underwent myeloablative regimens. As GvHD prophylaxis methotrexate was used in all patients in combination with cyclosporine (5) or tacrolimus (4). In MUD transplants anti-thymocyte globulin (ATG) was added. Post-transplant cyclophosphamide was administered in the haploidentical transplant. Nine patients received RUXO for SR grade 3-4 aGvHD; 5/8 were previously treated with ≥ 2 immunosuppressive treatments. Five patients (62%) were responsive (3 CR and 2 PR) after a median of 3 weeks of treatment. The median dose of RUXO was 20 mg/day divided into 2 doses and it was continued for a median of 12 months (range, 1-42). One patient with refractory severe cGvHD previously treated with 3 lines obtained CR and maintained it on RUXO treatment for 2 years. Overall, only 1 patient definitively interrupted RUXO, because of severe infection. Grade III-IV cytopenias were also observed in all patients and were managed with dose reduction. At a median follow-up of 21 months (1-48), 7 out of 9 patients died because of progressive aGVHD (3 RUXO refractory patients and 2 RUXO responsive patients who developed subsequent severe cGVHD), systemic mycosis caused by Fusarium Solani (1), methastatic squamocellular cancer (1). Relapse of the underlying malignancy occurred in 1 RUXO unresponsive patient. In our experience the use of ruxolitinib in the setting of refractory severe acute or chronic GVHD was safe and well tolerated. Although globally 6/9 patients (66%) showed complete or partial response, the long-term outcome was hampered by progression to severe chronic GVHD and late complications, suggesting the opportunity of an earlier RUXO initiation.

P069

SUCCESSFUL USE OF LETERMOVIR IN SECONDARY CYTOMEGALOVIRUS PROPHYLAXIS IN A HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENT AFFECTED BY GANCICLOVIR RESISTANT CYTOMEGALOVIRUS REACTIVATION.

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Background: Letermovir has been approved in 2017 for primary prophylaxis of Cytomegalovirus (CMV) infection and disease in adult CMV seropositive recipients [R+] of an allogenic hematopoietic stem cell transplant (HSCT). It has a good safety profile and can be given orally, properties that make it very manageable. Compared to other antiviral agents with activity against CMV, it has a novel mechanism of action as it targets the CMV terminase complex. Here we report a case where Letermovir was used for secondary CMV prophylaxis in a HSCT recipient affected by recurrent Ganciclovir resistant CMV infection.

Case report: A 55-year-old female patient affected by angioimmunoblastic T-cell lymphoma experienced CMV reactivation on the 26th day from HSCT. At first, she was treated successfully with Foscarnet followed by Valganciclovir (ValGCV), which was later continued as maintenance therapy. Due to still uncomplete hematologic recovery, ValGCV dose was reduced because of fear of myelotoxicity. After 20 days from negativization, CMV again became detectable and Foscarnet was restarted. Due to rapid increase of viral loads the combined antiviral therapy of Foscarnet and ValGCV was potentiated with important toxicities such electrolytic imbalance and myelotoxicity. After detection of ValGCV/GCV resistance, ValGCV was dismissed and Letermovir was started on day +146 as a combined antiviral therapy with Foscarnet. On day +152 CMV DNA finally became undetectable, Foscarnet was then interrupted and Letermovir was continued successfully as secondary prophylaxis.

Conclusions: Letermovir is approved for primary prophylaxis use. However, it can be an important aid in patients with CMV infections who have failed prior antiviral therapies or cannot tolerate them. Moreover, particularly when there is a high risk of CMV recurrence, its use as secondary prophylaxis should be favored.

Immunotherapy and Cell Therapy

P070

THE IMPACT OF CRYOPRESERVATION ON PURIFIED DONOR TREG CELLS FOR MULTIPLE INFUSIONS IN PATIENTS WITH REFRACTORY CHRONIC GVHD

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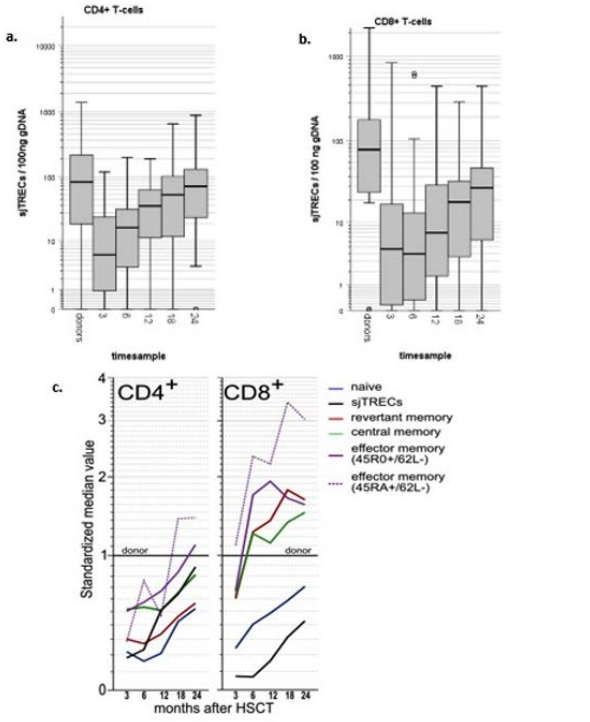
We are evaluating the role of multiple infusions of purified cryopreserved donor T regulatory cells (Tregs) in patients with refractory chronic Graft Versus Host Disease (cGVHD). The infusion program includes three escalating total doses of cryopreserved Tregs for three patient cohorts :1st cohort $0,5x10^6$ cells/Kg; 2nd cohort $1x10^6$ cells/Kg; 3rd cohort $2x10^6$ cells/Kg. The patients of the first cohort have completed the treatment, while the patients of the second cohort have started the infusion program. One of the project aim is to study the effects of cryopreservation on Tregs population that is still not defined. Ten purified Tregs products were prepared from leukapheresis of the original stem cells donor. The purification of Tregs were performed in GMP conditions by Cell Factory "Calori" using immunomagnetic selection, in particular by depletion of CD8 and CD19 positive cells and enrichment of CD25high positive cells. After purification, Tregs products were divided in three fractions, then cryopreserved and shipped to Bologna. The cells were stored in liquid nitrogen until to infusion and thawed in 37°C water bath. All analyses have been performed by Flow Cytometry. We have analysed donor Tregs viability, purity, phenotype and inhibitory function before and after thawing. The recovery of Tregs was 38,8% (CD45+7AAD-CD4+CD25+ median) from donor leukapheresis, the contamination of CD8+, CD19+ e CD56+ cells was not significant in the final Tregs products. We observed a mild, but significant decrease of the viability of donor cryopreserved Tregs (shown as % of 7-AAD cells): thawed Tregs median +/- IQ 75 (71-84) % *vs.* fresh Tregs median +/- IQ 89,5 (87-92) %($p \leq 0.05$). The purity (CD4+/CD25+/CD127 low) was stable after thawing (thawed Tregs median +/- IQ:87,2 (81-88) % *vs.* fresh Tregs median +/- IQ: 90,3 (90-94) %). We studied the expression of specific Tregs markers such as FoxP3, CD15s and CD62L. The expression of Foxp3 decreases in thawed T reg cells. The CD15s expression is variable following thawing, while CD62L expression is reduced in thawed donor Tregs (median +/- IQ:33 [11-56] %) *vs.* fresh donor Tregs (median +/- IQ: 77 [74-84] %) ($p \leq 0.05$). The inhibitory capacity of thawed Tregs seems to be reduced compared to fresh Tregs, however we are still studying Tregs function. The percentage of thawed Treg inhibition at the ratio 1:1 (Treg: Teff) was respectively 40%, 28% and 15% for the three patients of first cohort. Cryopreservation of T reg cells for immunotherapy appears feasible. However, the expression of functionally relevant molecules, such as FoxP3, CD15s and CD62L may be altered possibly resulting in reduced Tregs function.

Results: A constant gradual increase in absolute numbers of T-cell subsets and sjTRECs from the first month up to two years post-transplant was observed. Overall, at two years, median CD4+ and CD8+ T-cell and sjTRECs levels were lower than those observed in healthy donors. Molecular analysis of the sjTRECs kinetics was associated with CD4+ naïve T cells increase (global $p < 0,001$). This correlation clearly suggests that most of CD4+ naïve T cells derived from thymic re-education of donor precursor stem cells, whereas CD8+ naive T cells underwent peripheral expansion. By contrast, CM and EM T cells showed a faster thymic-independent expansion. By multivariate analysis, moderate-severe chronic GVHD (p 0.004 and p 0.032 in CD4+ and CD8+, respectively) and age older than 60 years old ($p < 0.001$ and p 0.015 in CD4+ and CD8+, respectively) were significantly associated with low thymic output 1 year after allo-HSCT. We also observed a significant effect of 3-month post allo-HSCT CD4+ sjTRECs levels on the risk of CMV reac-

tivation: cumulative incidence within 2 years post-alloHSCT was 69.6% in patients with CD4+ sjTRECs levels below the median *vs.* 40.2% in those with levels above the median (p 0.008).

Conclusion: Active thymic function despite age-dependent involution substantially contributes to T-cell reconstitution after allo-HSCT. Chronic GVHD and older age are significantly correlated with thymic activity. Correlation between IR and clinical outcomes need further investigations and prospective analyses to be confirmed.



a) sjTRECs copy number variation in CD4+ T cells. (dashed line=median healthy donor levels). b) sjTRECs copy number variation in CD8+ T cells. (dashed line=median healthy donor levels). c) standardized values of CD4+ and CD8+ T-cell subsets and sjTRECs reconstitution at different time-points after allo-HSCT. (1=donor median values).

Figure 1.

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ABSTRACT WITHDRAWN