thereby provoking somatic variants characteristic of classic MDS?

Although these important questions require further investigation, the work by Hirsch *et al.* suggests a clear distinction between MDS and other hematologic diseases with bimodal distributions. For example in aplastic anemia, it has become increasingly clear that younger patients have distinct pathophysiologies and therapeutic vulnerabilities with important clinical implications.[19] However, the analysis by Hirsch *et al.* indicates that the molecular underpinnings of adult MDS, regardless of age, are likely more similar than they are different and that our clinical management, including enrollment in interventional studies, should reflect this.

References

1. Ma X, Does M, Raza A, Mayne ST. Myelodysplastic syndromes: incidence and survival in the United States. Cancer. 2007;109(8):1536-1542.
2. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014;20(12):1472-1478.
3. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488-2498.
4. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371(26):2477-2487.
5. Niemeyer CM, Kratz CP. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia: molecular classification and treatment options. Br J Haematol. 2008;140(6):610-624.
6. Li W, Morrone K, Kambhampati S, Will B, Steidl U, Verma A. Thrombocytopenia in MDS: epidemiology, mechanisms, clinical consequences and novel therapeutic strategies. Leukemia. 2016;30(3):536-544.
7. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011;364(26):2496-2506.
8. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616-3627; quiz 99.
9. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209-21.
10. Hirsch CM, Przychodzen BP, Radivoyevitch T, Patel B, Thota S, Clemente MJ, et al. Molecular features of early onset adult myelodysplastic syndrome. Haematologica. 2017;102(6):1028-1034.
11. McKerrell T, Park N, Moreno T, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. Cell Rep. 2015;10(8):1239-1245.
12. Silva P, Neumann M, Vosberg S, et al. Acute Myeloid leukemia in the elderly is characterized by a distinct genetic landscape. Blood. 2015;126(23):804.
13. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059-2074.
14. Loh ML, Sakai DS, Flotho C, et al. Mutations in CBL occur frequently in juvenile myelomonocytic leukemia. Blood. 2009;114(9):1859-1863.
15. Chan RJ, Cooper T, Kratz CP, Weiss B, Loh ML. Juvenile myelomonocytic leukemia: a report from the 2nd International JMML Symposium. Leuk Res. 2009;33(3):355-362.
16. Emanuel PD. Juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. Leukemia. 2008;22(7):1335-1342.
17. Churpek JE, Pyrtel K, Kanchi KL, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. Blood. 2015;126(22):2484-2490.
18. Montané E, Ibáñez L, Vidal X, et al. Epidemiology of aplastic anemia: a prospective multicenter study. Haematologica. 2008;93(4):518-523.
19. Scheinberg P, Young NS. How I treat acquired aplastic anemia. Blood. 2012;120(6):1185-1196.

Immunoglobulin genes in chronic lymphocytic leukemia: key to understanding the disease and improving risk stratification

Lesley-Ann Sutton,[1,2] Anastasia Hadzidimitriou,[3] Panagiotis Baliakas,[1] Andreas Agathangelidis,[4] Anton W. Langerak,[5] Stephan Stilgenbauer,[6] Sarka Pospisilova,[7] Zadie Davis,[8] Francesco Forconi,[9] Frederic Davi,[10] Paolo Ghia,[4] Richard Rosenquist[1,2] and Kostas Stamatopoulos,[1,3] on behalf of the European Research Initiative on CLL (ERIC)

[1]Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Sweden; [2]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; [3]Institute of Applied Biosciences, Center for Research and Technology Hellas, Thessaloniki, Greece; [4]Division of experimental Oncology, IRCCS Istituto Scientifico San Raffaele e Università Vita-Salute San Raffaele, Milan, Italy; [5]Department of Immunology, Erasmus MC, University Medical Center Rotterdam, The Netherlands; [6]Department of Internal Medicine III, Ulm University, Germany; [7]Central European Institute of Technology, Masaryk University and University Hospital Brno, Czech Republic; [8]Department of Haematology, Royal Bournemouth Hospital, UK; [9]Haematology Department and Cancer Sciences Unit, Cancer Research UK and NIHR Experimental Cancer Medicine Centres, University of Southampton, UK and [10]Hematology Department and University Pierre et Marie Curie, Hopital Pitie-Salpetriere, Paris, France

E-mail: ghia.paolo@hsr.it

doi:10.3324/haematol.2017.165605

While triggering through the B-cell receptor (BcR) facilitates B-cell development and maintenance, it also carries intertwined risks for the emergence of lymphoid malignancies, since malignant B cells can exploit BcR signaling pathways in order to initiate and fuel clonal expansion. Indeed, substantial research into chronic lymphocytic leukemia (CLL), largely based on immunogenetic data, supports the notion that the clonotypic BcR immunoglobulin (IG) engages in the recognition of and selection by putative (auto)antigen.[1] This highlights the critical role of the BcR IG in the pathophysiology of CLL and implies that disease development is functionally driven and dynamic, rather than being a simple stochastic process. From a clinical perspective, the remarkable therapeutic efficacy of novel drugs such as ibrutinib and idelalisib which target effectors of the BcR signaling pathway (BTK and PI3Kδ, respectively), further vouch for this idea, and herald a major paradigm shift which may ultimately

lead to changes in the natural history of the disease.[2]

The IG molecule is an essential component of the multimeric BcR complex and forms a unique genetic identity that is the perfect contender for a clonal marker since it is present from the birth of every B cell onwards, and thus also includes CLL tumor cells, as they derive from activated B cells. Moreover, in contrast to other markers, most notably genomic aberrations, the clonal BcR IG remains stable and unchanged as the disease evolves.[3] From the inception of immunogenetics analyses in CLL (Figure 1), reports began to emerge indicating pronounced skewing in IG gene usage and differences from the repertoire of normal B cells, alluding to (super)antigen selection.[4] Soon thereafter it was realized that a significant fraction of patients with CLL, approximately 50%, carried somatic hypermutations (SHM) within their BcR IG.[4] Additionally, a varying imprint of SHM was seen in clonal BcR IG utilizing different IG heavy variable (IGHV) genes, pointing to functional selection. A further twist in the CLL immunogenetics story was provided by the discovery that the SHM status of the rearranged IGHV gene segregates CLL cases into two broad categories with markedly different outcomes. Cases with no or a limited SHM burden (germline identity (GI) ≥ 98%) constituted "unmutated CLL" (U-CLL), wherein patients generally follow an aggressive disease course with short time-to-first-treatment (TTFT), poor response to chemoimmunotherapy and inferior overall survival, thus starkly contrasting cases with GI < 98% ("mutated CLL", M-CLL) wherein patients usually have a more indolent form of the disease.[5,6] Within the clinical arena, IGHV mutation burden allowed us to predict the clinical course of the disease based on the number of SHMs within the expressed IG genes. In more recent years, the strongest molecular evidence for antigen selection in CLL emerged from the finding that unrelated patients can carry identical or almost identical BcR IGs, a phenomenon that cannot be attributed to chance alone and is now aptly termed "stereotypy".[4,7]

The aforementioned stratification of patients based on the SHM status of the clonotypic BcR IG has proved to be one of the most robust prognosticators in CLL,[8] superseding the clinical impact of other prognostic markers that may fluctuate or change over time. This division reflects

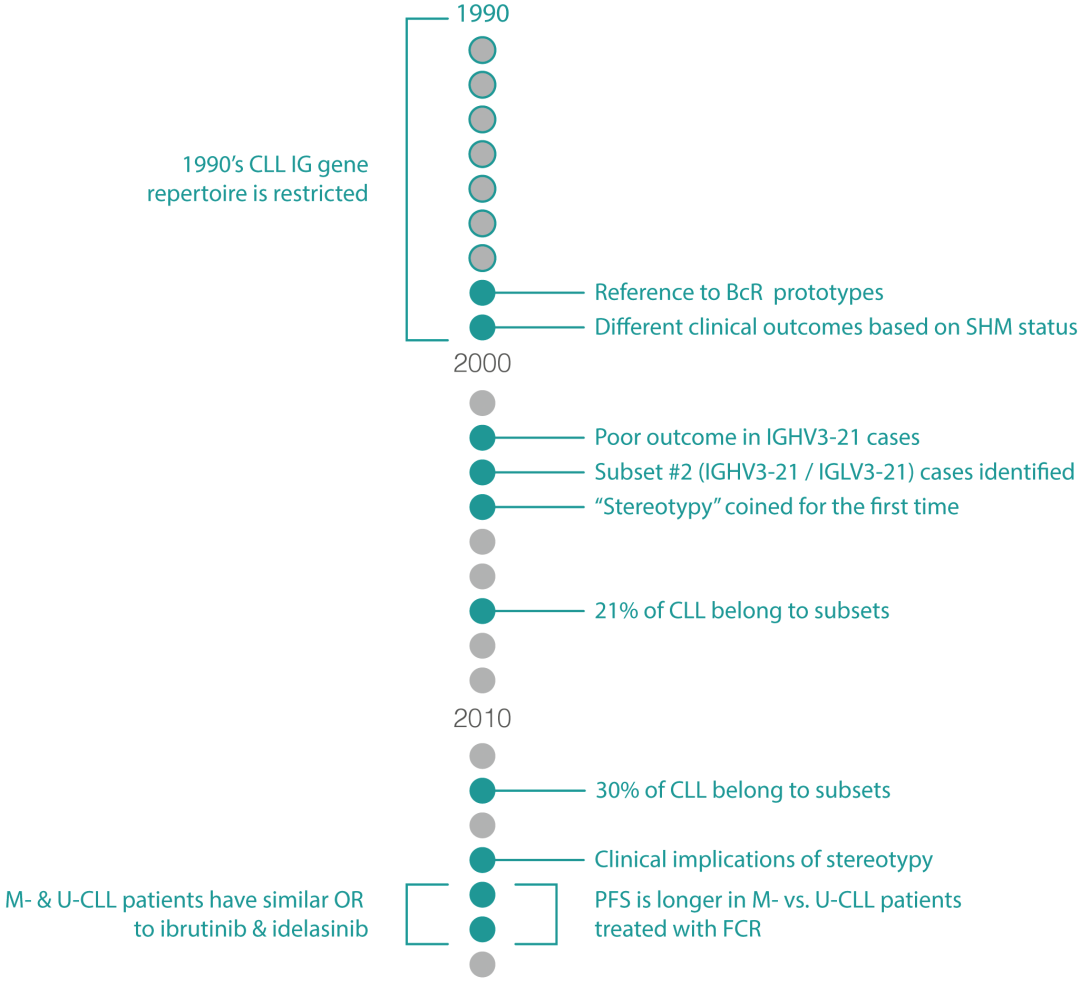

Figure 1. Historical timeline of immunogenetic studies in chronic lymphocytic leukemia (CLL). IG: immunoglobulin; BcR: B cell receptor; SHM: somatic hypermutation; PFS: progression-free survival; M-CLL: mutated CLL; U-CLL: unmutated CLL; FCR: fludarabine, cyclophosphamide and rituximab; OR: overall response.

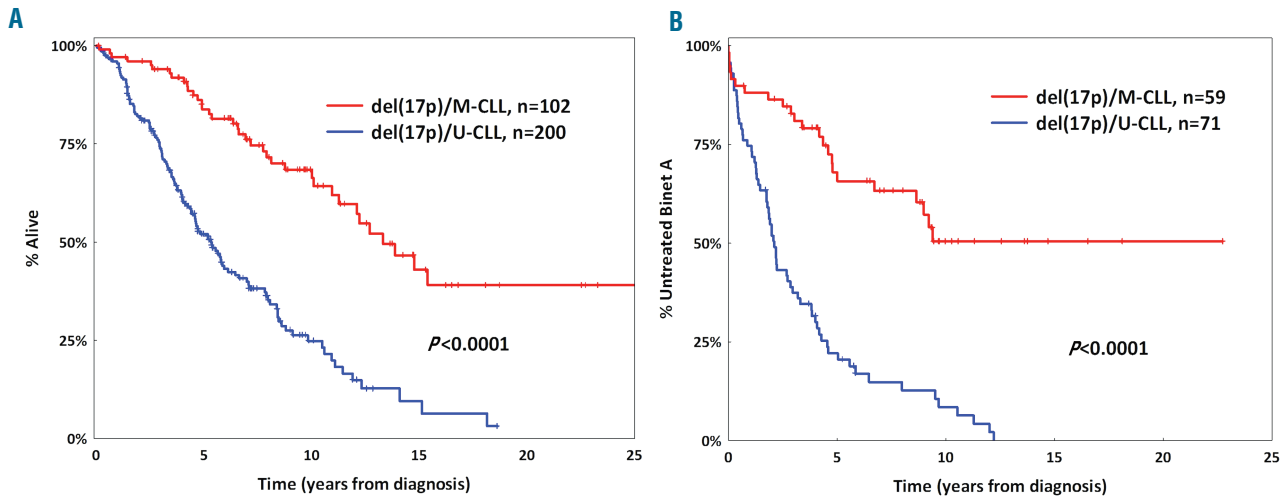

Figure 2. Kaplan-Meier curves for overall survival (OS) and time-to-first-treatment (TTFT) in CLL patients carrying del(17p). M-CLL harboring del(17p) exhibit significantly longer OS (A) and TTFT (B) compared to U-CLL carrying the same genetic defect. Cases included in this analysis are part of a multi-institutional cohort from our collaborative consortium comprising 8563 CLL patients. TTFT analysis was performed in early stage (Binet A) patients. M-CLL: mutated CLL; U-CLL: unmutated CLL.

fundamental biologic differences alluding to a different ontogeny for the two mutational groups and, as a consequence, the BcR IG holds much promise and may be central for developing a biologically-oriented prognostication scheme for CLL.[8-10] However, it should be kept in mind that within both M-CLL and U-CLL, a sizeable proportion of cases exhibit clinicobiological behavior that deviates from that associated with its mutational categorization. This highlights the fact that the renowned heterogeneity of CLL persists even after categorization based on the level of SHMs within the IG molecule.[9-11] A classical example is offered by stereotyped CLL subset #2, defined by the expression of a distinctive BcR IG utilizing IGHV3-21/IGLV3-21, which has emerged as a prototype of aggressive disease independently of the SHM load.[9,10,12] Indeed, evidence suggests that the immunogenetic subclassification of CLL based on BcR IG stereotypy has clinical potential beyond subset #2, with individual subsets differing significantly in terms of demographics, clinical presentation and the presence or absence of prognostically relevant mutations or cytogenetic aberrations.[9]

Furthermore, it appears that the particular clinical and cellular background of a genetic lesion, shaped by distinctive signaling through a particular clonotypic BcR IG, may mediate the prognostic or predictive value of recurrent genetic lesions. This idea is exemplified by the finding that within M-CLL, patients harboring trisomy 12 have a TTFT similar to that of patients carrying *TP53* aberrations, whereas, in contrast, trisomy 12 is associated with a favorable outcome within U-CLL.[13] These findings may explain why trisomy 12 emerges as an intermediate-risk aberration in prognostic indices when the SHM status of the CLL IG is not taken into consideration.[14] A similar finding is evidenced when analyzing patients carrying del(17p), with M-CLL cases exhibiting a significantly longer overall survival and TTFT compared to U-CLL patients carrying the same genetic defect (Figure 2). Also worth mentioning is the finding of an asymmetric distribution of certain gene mutations amongst patients bearing distinct immunogenetic features e.g., *MYD88* mutations are exclusively found within M-CLL while the vast majority of *NOTCH1* mutations are detected within U-CLL.[15,16] Taken collectively, and bearing in mind that the clinical impact of several biological features is strongly influenced by the SHM status, it is increasingly apparent that definitive conclusions about the clinical implications of any given biomarker should be drawn only after also taking into consideration the SHM status of the rearranged BcR IG. This holds even for well-established prognosticators such as *TP53* aberrations.

Additional support for the pivotal role of immunogenetic analysis in CLL was recently provided by studies demonstrating that IGHV gene SHM status is a strong marker for predicting the response to chemoimmunotherapy, in particular the fludarabine, cyclophosphamide and rituximab (FCR) regimen which is the gold standard treatment for medically fit CLL patients lacking *TP53* defects.[17] More specifically, M-CLL cases treated with FCR in the context of clinical trials or general practice were independently reported to achieve prolonged responses, often with no detectable minimal residual disease, thus differing significantly from U-CLL cases.[18-20] Interestingly, upon treatment with newer therapeutic agents such as ibrutinib and idelalisib, CLL patients appear to benefit equally and experience similar overall responses irrespective of the IGHV mutational status;[21-23] however, the follow-up time is still limited thus precluding definitive conclusions. That said, differences have been noted between the two mutational groups regarding certain clinical parameters following the administration of novel drugs, for example, the initial ibrutinib-induced rise in lymphocyte count and also the duration of lymphocytosis is reported to be greater in M-CLL than that found in U-CLL patients.[21] While further investigations into the patterns of lymphocytosis and their association with a clinical response are warranted, these observations may hold value for follow-up assessment.

Altogether, the aforementioned examples strongly indicate that determining the SHM status of the IG molecule

is imperative not only for general assessment of the disease course in CLL, but also for guiding treatment decisions; put simply, IG gene analysis should no longer be viewed only as a prognostic test but also as a predictive test for the use of certain therapies. This idea of following an IG-centric model in order to better stratify CLL patients will likely continue to gain value in the near future due to the emergence of novel treatments and the growing concept of precision therapy, and will have a direct impact on the clinical management of patients with CLL.

When broaching the topic of immunogenetic analysis in CLL, what is irrefutable is that the accurate reporting of results obtained from such analyses is paramount, and rigorous standards and meticulous attention to detail are critically important. ERIC, the European Research Initiative on CLL, has been at the forefront of setting standards for immunogenetic research in CLL through pioneering the adoption of good practices by: (i) arranging dedicated educational workshops for the international community; (ii) formulating recommendations for determining the SHM status of IG genes in CLL aimed at harmonizing IG gene sequence analysis in CLL in order to ensure that results are reliable and comparable among different laboratories;[24,25] (iii) establishing the IG Network, which promotes and advances immunogenetic analysis across the medical community; and (iv) launching a certification system with external quality control, an asset for accreditation of laboratories performing IG analysis.

In conclusion, immunogenetic analysis has proved essential for understanding CLL pathophysiology. We argue that it is equally essential for predicting responses to therapies in this most unpredictable and clinically heterogeneous disease. Indeed, IG-centric risk stratification appears more appealing and relevant today now that signaling inhibition has emerged as a powerful, non-chemotherapeutic approach towards eventually curing CLL, a still incurable disease.

Acknowledgements

This work was supported in part by the Swedish Cancer Society, the Swedish Research Council, the Lion's Cancer Research Foundation, the Marcus Borgström Foundation and the Selander's Foundation, Uppsala; H2020 "AEGLE, An analytics framework for integrated and personalized healthcare services in Europe" by the EU; "MEDGENET, Medical Genomics and Epigenomics Network" (H2020, No.692298) by the EU; research grant AZV-MZ-CR 15-30015A-4/2015, Bloodwise, UK (grants 16003 and 14037), GCH-CLL: funded by the General Secretariat for Research and Technology (GSRT) of Greece and the Italian Ministry of Health (MoH) and IMI2 "HARMONY" funded by the EU. AWL receives unrestricted research grants from Roche-Genentech.

References

1. Vardi A, Agathangelidis A, Sutton LA, Ghia P, Rosenquist R, Stamatopoulos K. Immunogenetic studies of chronic lymphocytic leukemia: revelations and speculations about ontogeny and clinical evolution. Cancer Res. 2014;74(16):4211-4216.
2. Wiestner A. BCR pathway inhibition as therapy for chronic lymphocytic leukemia and lymphoplasmacytic lymphoma. Hematology Am Soc Hematol Educ Program. 2014;2014(1):125-134.
3. Sutton LA, Rosenquist R. The complex interplay between cell-intrinsic and cell-extrinsic factors driving the evolution of chronic lymphocytic leukemia. Semin Cancer Biol. 2015;34:22-35.
4. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest. 1998;102(8):1515-1525.
5. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94(6):1840-1847.
6. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94(6):1848-1854.
7. Agathangelidis A, Darzentas N, Hadzidimitriou A, et al. Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. Blood. 2012;119(19):4467-4475.
8. International CLLIPIwg. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol. 2016;17(6):779-790.
9. Baliakas P, Hadzidimitriou A, Sutton L, et al. Clinical effect of stereotyped B-cell receptor immunoglobulins in chronic lymphocytic leukaemia: a retrospective multicentre study. Lancet Haematol. 2014;1(2):74-84.
10. Baliakas P, Agathangelidis A, Hadzidimitriou A, et al. Not all IGHV3-21 chronic lymphocytic leukemias are equal: prognostic considerations. Blood. 2015;125(5):856-859.
11. Jeromin S, Haferlach C, Dicker F, Alpermann T, Haferlach T, Kern W. Differences in prognosis of stereotyped IGHV3-21 chronic lymphocytic leukaemia according to additional molecular and cytogenetic aberrations. Leukemia. 2016;30(11):2251-2253.
12. Tobin G, Thunberg U, Johnson A, et al. Chronic lymphocytic leukemias utilizing the VH3-21 gene display highly restricted Vlambda2-14 gene use and homologous CDR3s: implicating recognition of a common antigen epitope. Blood. 2003;101(12):4952-4957.
13. Baliakas P, Moysiadis T, Hadzidimitriou A, et al. Tailored approaches for refined prognostication in chronic lymphocytic leukemia patients with mutated versus unmutated immunoglobulin receptors. Blood. 2016;128(22):3199.
14. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343(26):1910-1916.
15. Baliakas P, Hadzidimitriou A, Sutton LA, et al. Recurrent mutations refine prognosis in chronic lymphocytic leukemia. Leukemia. 2015;29(2):329-336.
16. Sutton LA, Young E, Baliakas P, et al. Different spectra of recurrent gene mutations in subsets of chronic lymphocytic leukemia harboring stereotyped B-cell receptors. Haematologica. 2016;101(8):959-967.
17. Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet. 2010;376(9747):1164-1174.
18. Rossi D, Terzi-di-Bergamo L, De Paoli L, et al. Molecular prediction of durable remission after first-line fludarabine-cyclophosphamide-rituximab in chronic lymphocytic leukemia. Blood. 2015;126(16):1921-1924.
19. Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. Blood. 2016;127(2):208-215.
20. Thompson PA, Tam CS, O'Brien SM, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia. Blood. 2016;127(3):303-309.
21. Farooqui MZ, Valdez J, Martyr S, et al. Ibrutinib for previously untreated and relapsed or refractory chronic lymphocytic leukaemia with TP53 aberrations: a phase 2, single-arm trial. Lancet Oncol. 2015;16(2):169-176.
22. Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med. 2014;370(11):997-1007.
23. Burger JA, Tedeschi A, Barr PM, et al. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. N Engl J Med. 2015;373(25):2425-2437.
24. Ghia P, Stamatopoulos K, Belessi C, et al. ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. Leukemia. 2007;21(1):1-3.
25. Langerak AW, Davi F, Ghia P, et al. Immunoglobulin sequence analysis and prognostication in CLL: guidelines from the ERIC review board for reliable interpretation of problematic cases. Leukemia. 2011;25(6):979-984.