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Personalized medicine in adult acute lymphoblastic leukemia

Dieter Hoelzer

Onkologikum Frankfurt am Museumsufer, Frankfurt, Germany

E-mail: dieter.hoelzer@onkologikum-frankfurt.de / hoelzer@em.uni-frankfurt.de *doi:10.3324/haematol.2015.127837*

Knowledge concerning acute lymphoblastic leukemia (ALL) has increased greatly,[1] and personalized medicine has become a reality. More sophisticated diagnostic procedures, including immunophenotyping, cytogenetics, molecular genetics, and new genomics, have allowed the definition of new ALL sub-entities which, in some cases, has translated into specific therapies. A great achievement is the possibility of evaluating minimal residual disease (MRD), which can now be done in about 95% of ALL patients. MRD is the most important prognostic factor and thus a major component of a personalized treatment algorithm. Progress has also come from targeted therapies, extending the existing backbones of chemotherapy and stem cell transplantation (SCT). Targeted therapy in Philadelphia chromosome-positive ALL (Ph^+ ALL) with tyrosine kinase inhibitors (TKI) and immunotherapy with monoclonal antibodies targeting surface antigens expressed on leukemic blast cells have extended the armamentarium. A new promising approach is the activation of patients' T cells directed against their own leukemic blast cells either through a bispecific antibody, or chimeric antigen receptor modified T cells.

Diagnostics

Immunophenotyping is still the most important diagnostic feature, separating B-lineage ALL (~75%) from T-lineage ALL (~25%), and their subtypes according to the stage of maturation/differentiation (Table 1).

Other diagnostic techniques are standard cytogenetics, fluorescence *in situ* hybridization, and reverse transcriptase polymerase chain reaction. These methods allow the detection of Ph^+ ALL, with the chromosomal translocation t(9;22)(q34;q11), and the detection of the corresponding *BCR-ABL1* gene rearrangement. Further ALL entities that have been identified are t(4;11)(q21;q23)/*MLL-AFA4*, abn11q23/*MLL*, and t(1;19) (q23;p13)/*PBX-E2A*.

Gene expression profiling, single nucleotide polymorphism array analysis, array-comparative genomic hybridization, and next generation sequencing recognize newly defined ALL entities with poor prognosis: Ph-like ALL, and early T precursor ALL.

Ph-like ALL, also called *BCR-ABL1*-like ALL, is characterized by genetic lesions similar to Ph^+ ALL, associated with *IKZF1* deletion, *CLRF2* overexpression and tyrosine kinase activating rearrangements involving *ABL1*, *JAK2*, *PDGFRB* and several other genes.[2,3] The frequency is 10% in children and 25-30% in young adults, but does not increase further with age.[4] Treatment could be directed at the underlying genetic pattern with BCR-ABL inhibitors (e.g. dasatinib) or JAK2 inhibitors (e.g. ruxolitinib).[5]

Early T precursor ALL is characterized by lack of CD1a and CD8, weak CD5 expression, at least one myeloid/stem cell marker, a specific transcriptional profile and the possible involvement of several critical genes.[6] No new treatment approaches are currently available for this subtype, and thus SCT in first complete remission is the preferred option.

Minimal residual disease

MRD is the detection of residual leukemic cells, not detectable by light microscopy.

Methods for determining MRD are based on the detection of leukemia-specific aberrant immunophenotypes by flow cytometry, the evaluation of leukemia-specific rearranged immunoglobulin or T-cell receptor sequences by real-time quantitative polymerase chain reaction, or the detection of fusion genes associated with chromosomal abnormalities (e.g., *BCR-ABL*, *MLL-AF4*). The detection limit with these methods is 10^{-3}-10^{-5} (0.1%– 0.001%). The phenotypic aberrations are unique to each patient with ALL and can be detected in up to 95% of individuals.

Methods for MRD evaluation and standardization of MRD quantification have been extensively described.[7,8]

Minimal residual disease response and terminology

Molecular response can be evaluated only for patients in

Table 1. Diagnostics of major ALL subtypes.

Immunophenotype	
B-lineage markers: CD19, CD79a, cCD22 (at least 2); others: TdT, CD10, CD20, CD24, cIgM, sIg (kappa or lambda)	T-lineage markers: cCD3; others: TdT, CD1a, CD2, CD5, CD7 CD4, CD8, TCR α/β or γ/δ
Subtypes:	Subtypes:
Pro-B/B-I (CD19/CD79a/cCD22$^+$)	Pro-T/T-I (cCD3/CD7$^+$)
Common/B-II (CD10$^+$/cIgM-)	Pre-T/T-II (CD2/CD5)
Pre-B/B-III (cIgM$^+$/sIg$^-$)	Cortical-T/T-III (CD1a$^+$)
Mature-B/B-IV (sIg$^+$)	Mature-T/T-IV (CD3$^+$/CD1a$^-$)
Cytogenetics/genetics	
Ph$^+$ ALL t(4;11)$^+$ ALL t(1;19)$^+$ ALL other high-risk cytogenetics	
New genetics/genomics	
Ph-like ALL *IKZF1*, *CLRF2*, *MLL*, *TP53*, *CREBBP*, *RAS* alterations	Early T precursor ALL *NOTCH1/FBW7*-unmutated/*RAS/PTEN*-altered T-ALL

complete cytological remission, with one marker or more for MRD analysis and samples available at diagnosis and followed at specific time points during the course of disease. Results are classified as presented in Table 2.

Molecular response after induction therapy and impact on outcome

Achievement of molecular complete response/molecular remission is the most relevant independent prognostic factor for disease-free survival and overall survival. Patients with molecular complete remission after induction therapy had significantly superior outcome in several studies, with a disease-free survival of 54-74%, compared to 17-40% for MRD-positive patients.[9-12] Patients with molecular failure after induction should proceed to allogeneic hematopoietic SCT.[10]

Prognostic factors, risk stratification, and minimal residual disease

The aim of identification of prognostic parameters at diagnosis, which include age, white blood cell count, specific immunophenotypes, and cytogenetic and genetic aberrations, is to stratify patients into risk groups: standard-risk patients without any risk factor, with a good chance of cure by chemotherapy, and high-risk patients with one or more of those risk factors. High-risk patients are most often candidates for a SCT in first complete remission.

Will minimal risk disease evaluation replace pre-therapeutic risk factors?

The question arises as to whether the evaluation of MRD overcomes all of those pre-therapeutic risk factors, or whether they should be combined.[1,9,13] A practical approach is to enter the conventional prognostic factors and MRD into a decision algorithm. Thereby defined standard-risk patients who will achieve molecular remission (about 90-95%), will remain as standard-risk patients, whereas those who are MRD-positive will be defined accordingly as high-risk patients. Clinically defined high-risk patients are potential candidates for a SCT in first complete remission. However, it is not clear how to proceed if they achieve a complete molecular remission, since some studies suggest a lack of benefit from SCT. If MRD information is not available, the risk stratification should rely on clinical risk factors evaluated at diagnosis.

Unfortunately, 20-30% of adult ALL patients who are MRD-negative after induction will relapse. Potential reasons include loss of sensitivity, evolution of leukemic subclones, and extramedullary origin of disease.

Treatment principles

The goal of induction therapy is achievement of a complete remission, or even better, a molecular complete remission, mostly evaluated within 6-16 weeks of starting chemotherapy. With current regimens the complete remission rate has increased to 80%-90%, being higher for standard-risk patients (90% or more), and lower for high-risk patients (~80%). The outcome of ALL is strictly related to the age of a patient, and treatment protocols considering the age of an individual patient have emerged.

Pediatric-inspired therapies

Pediatric-inspired therapies for adolescents and young adults provide increased drug intensity at several stages of treatment, including larger cumulative doses of drugs such as corticosteroids, vincristine, L-asparaginase, and consequent central nervous system-directed therapy, which should be strictly adhered to, thereby reducing the role of SCT in such cases. In a 2012 meta-analysis of 11 trials including 2489 adolescents and young adults, pediatric-inspired regimens were superior to conventional adult chemotherapy.[14] However none of the trials was a randomized comparison. In recent studies of pediatric-inspired therapies for adolescents and young adults, survival rates at 5 years were 67% to 78% compared to 34% to 41% with the former protocols.

Treatment in adults and elderly patients with acute lymphoblastic leukemia

The treatment results for adult ALL patients have also improved. The overall survival of 38% (54% at 5 years -

Table 2. Response parameters according to MRD.

Terminology	Definition
Complete (hematologic) remission	Leukemic cells not detectable by light microscopy (<5% blast cells in bone marrow)
Complete molecular remission / *MRD-negativity*	Patient in complete remission, MRD not detectable, ≤0.01% = ≤ 1 leukemia blast cell in 10,000
Molecular failure / *MRD-positivity*	Patient in complete hematologic remission but not in molecular complete remission >0.01%
Molecular relapse / *MRD-positivity*	Patient still in complete remission, had prior molecular complete remission, <5% leukemic blast cells detectable in bone marrow
Hematologic relapse	>5% ALL cells in bone marrow/ blood

27% at 9 years) has improved for standard-risk adult ALL patients to 50-70% with chemotherapy alone, and the outcome for high-risk patients from 20-30% to >50% when they receive an allogeneic SCT in first complete remission.[1] There has also been treatment progress in elderly ALL patients. Since palliative treatments and intensive chemotherapy regimens have failed, with low complete remission rates, high early death rates, and short survival, elderly-specific ALL protocols have been initiated, with less intensive therapy (avoiding anthracyclines and alkylating agents). In nine prospective studies for older ALL patients (55-81 years), with less intensive protocols the complete remission rate was 71% (43-90%), early death decreased to 15% (0-36%) and overall survival was 33 months (range, 16-71).[15]

Targeted therapies with tyrosine kinase inhibitors in Philadelphia-positive acute lymphoblastic leukemia

Patients with Ph$^+$ ALL constitute approximately 25% of adult B-lineage ALL patients, with the incidence increasing to about 50% among elderly patients. In the pre-imatinib era complete remission rates were 60-70%, the survival in patients treated with chemotherapy alone was ~10%, and that of patients undergoing allogeneic SCT was ~30%. The results improved substantially when the first-generation TKI inhibitor imatinib became available, with complete remission rates of 80-90%, but particularly the rate of molecular remissions (*BCR-ABL*-negativity) increased from 5% to ≥50%, and the 5-year survival to 50% or more.[1,16-18]

Treating adult Ph$^+$ ALL with an allogeneic SCT in first complete remission is still the best treatment option. However, in current studies patients not undergoing SCT receiving only chemotherapy plus a TKI also had improved outcome. Thus, a Ph$^+$ group with lower relapse risk, not needing SCT, has to be identified, e.g. by MRD response, absence of additional chromosomal abnormalities, or *IKZF1* gene deletion. Faster and deeper molecular responses can probably be achieved with second-generation TKI (dasatinib, nilotinib).[19] A third-generation TKI is ponatinib, which targets the T315I mutation either present at diagnosis, or developing/remaining after treatment with other TKI.[20] Administration of a TKI after SCT is now standard practice although the optimal duration of this treatment has not yet been established.

Immunologically based treatments with monoclonal antibodies or activated T cells

B-lineage blast cells express a variety of specific antigens, such as CD19, CD20, and CD22. Recently monoclonal antibodies have been developed to target these antigens.[21,22] The anti-CD20 monoclonal antibody rituximab has substantially improved the outcome of patients with Burkitt leukemia/lymphoma. With repeated short cycles of intensive chemotherapy, combined with rituximab the overall survival of such patients increased from 60% to >80%.[23] Monoclonal antibodies directed against CD22, linked to cytotoxic agents, such as calicheamicin (inotuzumab ozogamicin), or to plant or bacterial toxins (epratuzumab) are being explored for use in refractory/relapsed childhood and adult ALL. In a trial of patients with relapsed/refractory ALL treated with inotuzumab ozogamicin, the complete response rate (including responses without blood cell count recovery) was 66%, and of those 78% achieved a molecular complete remission.[24] Targeting CD19 is of great interest, as this antigen is expressed in all B-lineage cells, most likely including early lymphoid precursor cells. A new promising approach is the bi-specific antibody blinatumomab, which combines single chain antibodies to CD19 and CD3, such that T cells lyse the CD19-bearing B cells. This antibody was effective in patients with positive MRD, and 80% converted to MRD-negativity.[25] In a trial of adult patients with refractory/relapsed ALL, the rate of complete remissions/complete remissions with partial recovery of peripheral blood counts with blinatumomab was 43% and the MRD response rate was 82%,[26] leading to a recent approval of this agent by the Food and Drug Administration. Another promising new approach is chimeric antigen receptor modified T cells, targeting CD19$^+$ B-lineage ALL cells.[27] When these genetically engineered T cells were given to children with refractory/relapsed ALL, the complete remission rate was 90%, with an event-free survival of 63% at 6 months, and an overall survival of 78%.[28]

Conclusion and future directions

Progress in the diagnosis of ALL with identification of genomic-defined sub-entities, the evaluation of MRD, and new targeted therapies have led to a substantial realization of personalized medicine in adult ALL. Current options, such as less intensive chemotherapy, reduction of SCT, incorporation of targeted therapies and optimal combinations of treatments require prospective, cooperative research, hereby further refining the individualized approach to each patient.

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