REVIEW

New approaches to the management of adult acute lymphoblastic leukemia

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Acknowledgments

We are grateful to Associazione Italiana per la Ricerca sul Cancro (AIRC), Milan (Italy), Special Program Molecular Clinical Oncology-Extension program, 5 x 1000 (10007), grant to Division of Hematology (recipient: Robin Foà), "Sapienza" University, Rome (Italy); and to Francesca Carobolante (Division of Hematology, Ospedale dell'Angelo, Mestre – Venezia (Italy) for support in editing the manuscript.

Abstract

Traditional treatment regimens for adult acute lymphoblastic leukemia, including allogeneic hematopoietic cell transplantation, result in an overall survival of about 40%, a figure hardly comparable with the extraordinary 80-90% cure rate currently reported in children. When translated to the adult setting, modern pediatric-type regimens improve the survival to about 60% in young adults. The addition of tyrosine kinase inhibitors for patients with Philadelphia chromosome positive disease and the measurement of minimal residual disease to guide risk stratification and post-remission approaches has led to further improvements in outcomes. Relapsed disease and treatment toxicity - sparing no patient but representing a major concern especially in the elderly - are the most critical current issues awaiting further therapeutic advancement. Recently, there has been considerable progress in understanding the disease biology, specifically the Philadelphia-like signature as well as other high-risk subgroups. In addition, there are several new agents that will undoubtedly contribute to further improvement in the current outcomes. The most promising agents are new the monoclonal antibodies, immunomodulators, and chimeric antigen receptor T cells and, to a lesser extent, several new drugs targeting key molecular pathways involved in leukemic cell growth and proliferation. This review examines the evidence supporting the increasing role of the new therapeutic tools and treatment options in different disease subgroups, including frontline and relapsed/refractory disease. It is now possible to define the best individual approach based on to the emerging concepts of precision medicine.

Key words: ALL, adults, immunotherapy, targeted therapy, molecular profiling, drug profiling

Running title: Novel treatments for adult ALL

Introduction

In Western countries, new cases of adult acute lymphoblastic leukemia (ALL) occur at an annual rate of approximately 1/100,000, with a bimodal distribution decreasing at 45-54 years and increasing again above 55 years, totaling in the United States about 2300 new cases/year for patients above 15 years of age (1750 between 15-55 years).[1,2] Over the past decade, we have witnessed an incredible therapeutic improvement. Currently, pediatric patients have an estimated 5-year overall survival (OS) approaching 90%.[3-5] Modern pediatric programs thrive on an intensified use of corticosteroids (mainly dexamethasone), antimetabolites (especially methotrexate and 6-mercaptopurine) and L-asparaginase/pegylated-asparaginase, and rely on minimal residual disease (MRD) analysis for further dose intensification or allogeneic hematopietic cell transplantation (HCT).[6-8]

Recent advances using pediatric regimens in adults

The results in adult ALL have unfortunately not kept pace, with OS rates below 45%[9] despite the addition of central nervous system (CNS) prophylaxis, 'late' intensification with prolonged maintenance chemotherapy and an extensive use of HCT in high risk (HR) subsets. Currently, pediatric inspired regimens are being administered in young adult patients, leading to improvements in EFS (event-free survival) and OS rates as compared to historical controls.[10-13] This approach, initially reserved for adolescents and young adults (AYA, less than 40 year-old),[10,14,15] and later applied to patients up to 50-60 years of age, [11,12,16] has increased 5-year OS rate to ≥ 50%, and up to 70-80% in favorable subsets (AYA, SR [standard risk]), MRD negative) (*supplemental Table 1*),[17] but not in older patients, whose survival decreases progressively to less than 20%.[2-4] Finally, allogeneic HCT is often considered in first complete remission (CR) in adults with HR disease in order to reduce the risk of relapse,[18] but potential benefits may be offset by transplant-related morbidity and mortality, especially in the elderly.[19]

Risk stratification

Current risk stratification criteria reflect the clinical and prognostic heterogeneity of ALL and determine which patients should undergo more intensive treatment including HCT, due to the high risk of relapse. Besides patient-related characteristics, namely advanced age and poor performance status, recognized risk factors include hyperleucocytosis, early T-precursor (ETP) phenotype and adverse cytogenetics/genetics, i.e. t(9;22)/*BCR-ABL1*

rearrangement (Philadelphia chromosome positive [Ph+] ALL), Ph-like ALL, t(4;11)/*KMT2A-AFF1* rearrangement, hipodiploidy, mutated *TP53* and other abnormalities.[20] In all studies, MRD has proven to be a major independent risk factor for relapse.[21] In contrast to MRD-negative patients (typically defined as < 10^{-4}), MRD-positive patients are seldom cured with chemotherapy alone. In prospective trials performed over the past 25 years, enrolling more than 1500 patients,[22-24] OS was between 60-80% with chemotherapy alone in MRD-negative patients, even in HR subsets and Ph+ ALL.[25] Instead MRD-positive patients benefit partially from HCT, however with OS rates of 50% or less in intention-to-treat analyses, due to the cumulative effects of pre-and post-transplantation relapse and tranplant-related deaths.[26-28]

Current therapeutic limitations

The treatment of older patients represents a major obstacle,[29] and, at all ages, relapse affects one third or more of the patients and remains an unsolved issue due to extremely poor results with standard salvage chemotherapy. An international study on 1706 patients with refractory or recurrent (R/R) B-cell precursor (BCP) ALL reported 3-year survival rates of only 10%.[30] Results are worse in Ph+ ALL[31] and T-cell precursor (TCP) ALL, with some mitigation provided by nelarabine.[32] Another concern is high-grade toxicity causing deaths in remission, which increases with age and with transplants (20% or more in most studies).

The challenge of new management options

Despite these constraints, the management of adult ALL can improve further. This new era started with the advent of tyrosine kinase inhibitors (TKI) for Ph+ ALL,[33] flourished with immunotherapy for BCP ALL and is now empowered by novel immunotherapeutics (*Tables 1* and *2*)[13,34-64] and several small molecules targeting critical metabolic pathways (*Figure 1, Table 3*), used alone or in combination in specific ALL subsets (*Figure 2)*. While more robust data on toxicity, dosing and therapeutic implications are required, and will be generated by ongoing trials (Clinical.Trials.gov repository, accessed April 2017; *supplemental Tables 2-7*), some of these agents could improve the cure rate and prompt a shift in the therapeutic regimens for ALL. The most promising agents currently available are those targeting cell membrane antigens (CD19, CD20, CD22) and major molecular pathways controlling cell proliferation and apoptotic response (multiple kinases and members of Bcl-2, TP53, RAS, mTOR/PI3K, pre-B/B-cell receptor and NOTCH networks). Furthermore, new molecular and drug profiling techniques might become essential to

define targets and compounds deserving evaluation in trials or individual patients. At present, this new strategy is still largely speculative, especially in frontline therapy, since both molecular sequencing and new drug sensitivity screening models have not yet been sufficiently tested or validated in early clinical trials.This review will focus on the rationale supporting this change and will illustrate how new treatment approaches and related experimental work are likely to modify and improve the management of adult ALL.

Actionable target and drug screening

Molecular profiling

While targets for immunotherapy can be identified by diagnostic immunophenotype, ALL subtype classification and target identification relies mostly on molecular genetics for the detection of gene rearrangements, translocations and actionable recurrent mutations with genome-wide technologies.[65-69] In the era of precision medicine, molecular profiling has gained in importance for the management of this disease. New concepts for targeted therapies and combinatory approaches with immunotherapy and/or chemotherapy require sophisticated experimental modeling and are now increasingly entering into clinical development (*Figure 2* and *supplemental Tables 2-9*).

Drug profiling platforms

Since the molecular classification of ALL is often insufficient to capture the complex biology of the disease and provide a predictive guide for treatment,[70] functional screening approaches are being explored to generate drug response profiles directly from clinical samples, leading to proof of concept results and raising interest to explore this approach in clinical trials (*Figure 3*). The first screening platform tested a customized library of kinase inhibitors,[71] leading to a prospective trial in relapsed acute myeloid leukemia (AML). The Primary Blood Cancer Encyclopedia project, which integrates short-term drug testing data with transcriptome and DNA methylome analysis, strongly supported the value of phenotypic screening in hemato-oncology.[72] Some platforms are based on large viability assays for high-throughput testing[72-74] with the advantage of simplicity and lower costs, and others more sophisticated are based on automated microscopy which can discriminate leukemia cells with the normal microenvironment at the single cell level.[75,76] Functional screens of ALL samples maintained on mesenchymal stromal cells identified unexpected dependencies in defined HR ALL subtypes[77], captured response

heterogeneity across ALL subtypes, discriminated patients based on drug sensivitiy efficiently[75,78,79] and detected new pathways and vulnerabilities in resistant disease.[75,77-80]

New disease models

Drug development can be accelerated using humanized mouse models with primary leukemia[81,82] that enable systematic preclinical drug testing.[83,84] Patient-derived xenograft (PDX) biobanks integrate extensive genomic and clinical information,[75,85-88] mirror the clonal architecture of leukemia initiating cells,[89-92] maintain the genetic composition of the xenografted sample,[75,77,89,93] and enable testing of new agents on samples from clinically representative cohorts of patients, providing survival cues and a longer window for combinatorial drug testing. Impressive results have been reported from a first trial assessing drug sensitivity in patients with refractory hematologic malignancies using multi-parametric image-based immunocytometry to distinguish the effect of drugs on malignant and normal blood cells.[76] Out of 48 patients, informative results could be used for 17 patients receiving assay-guided treatment, including 2 BCP ALL patients, resulting in responses in 8 patients (1 with ALL). These results will stimulate the design of larger clinical studies on specific disease entities, in order to capture the full potential of drug response profiling with the aim to avoid unnecessary toxicity of inappropriate salvage regimens and improve responses in selected subgroups.

Functional drug screening for molecularly unclustered ALL

The usefulness of functional drug screening is being explored in patients with ALL not included in specific molecular clusters. For example *BCL2*-dependent ALL was identified by screening PDX models for sensitivity to BH3 mimetics including venetoclax[75,77,85,94,95] and drug combinations established to overcome resistance.[75,96] Similarly, selective sensitivity to alternative RIP-1 dependent cell death pathways (necroptosis by SMAC mimetics) not exploited by current anti-leukemic agents were discovered.[80,97] PDX models have also been used to understand elucidate the critical dependence on altered metabolic function.[98-100] This underscores the importance to cross-reference drug responses over many samples in a structured database to establish the effective and expected dose-response range for relevant outliers, that is a drug sensitivity pattern not predicted by the molecular ALL subset.

New management options with immunotherapeutics

Rituximab

In BCP ALL, the expression of CD20 confers a poor prognosis.[101] Rituximab, a chimeric anti-CD20 antibody, was evaluated in combination with chemotherapy for untreated patients with Ph- CD20+ BCP ALL. At the MD Anderson Cancer Center (MDACC), rituximab was added to the first four courses of the hyper-CVAD regimen.[44] The results demonstrated an improved CR duration, a lower relapse rate and an improved OS, but only in patients younger than 60 years as compared to historical controls (70% vs. 38%, $P < .001$; and 75% vs. 47%, $P = .003$). Comparable data were produced by the German adult ALL Study Group.[45] The French-Belgian-Swiss Group for Research on Adult ALL (GRAALL) evaluated the addition of rituximab in a phase III study using a pediatric inspired regimen:[13] patients 18-59 years old received 16-18 rituximab doses, resulting in improved the 2-year EFS from 52% to 65% ($P = .004$), due to a decreased relapse rate with no increase in toxicity.

Blinatumomab

New antibody constructs have shown promise for R/R ALL.[102] Blinatumomab, a bispecific T-cell engager (BiTE®) construct, received US Food and Drug Administration and European Medicines Agency approval. Blinatumomab simultaneously targets CD19 (present on most BCP ALL cells) and CD3 (present on cytotoxic T-cells) and acts to bring ALL cells into proximity of T-cells, capable of tumor eradication. In a phase II study,[38] 189 adult patients with Ph- R/R BCP ALL received blinatumomab with 43% (81/189) of them achieving CR or CR with defective hematologic recovery (CRh), and 40% of responders able to successful transition to allogeneic HCT Importantly, 60 out of 73 evaluable CR patients (82%) achieved MRD negativity. Results were similar in the phase III trial with a 44% CR/CRh rate in the blinatumomab arm compared to 25% in patients receiving chemotherapy,[39] and 76% compared to 48% turning MRD negative. Although generally well tolerated, grade 3 or higher cytokine release syndrome (CRS) and neurologic toxicity was seen in 4.9% and 9.4% of patients, respectively. Blinatumomab was tested as a single agent in patients with R/R Ph+ ALL, where it induced a CR rate of 36% associated with 88% MRD-negative status,[40] and in Ph- MRD-positive ALL, achieving an excellent response rate of 78%, with prolonged survival, occasionally without HCT.[36,103] Resistance mechanisms include a defective T-memory/regulator cell response, PD1/PD-L1 overexpression[104] and emergence of CD19-negative subclones.[105]

Inotuzumab ozogamicin (INO)

INO is an anti-CD22 antibody conjugated to calicheamicin in late clinical development. A phase I/II study demonstrated a CR/CRi (incomplete hematologic recovery) rate of 68% with 84% of responding patients achieving MRD-negativity.[53] In a recent phase III trial INO was superior to salvage chemotherapy for R/R ALL. Among the first 218 patients randomized, 81% of those assigned to INO achieved CR compared to 29% who received standard-of-care, with higher percentage of MRD-negative cases (78% versus 28%, $P <$ .0001).[54] Duration of remission and OS favored INO, as confirmed by a long-term update reporting a 2-year rate of 22.8% vs 10% in standard care group (*P* .0001).[106] However, hepatotoxicity was more frequent in the INO group (51% versus 34%), including incidence of sinusoidal obstruction syndrome (SOS) (13% versus < 1%). Although most of the cases occurred after HCT, 5 patients (3%) developed SOS with INO therapy alone.[107]

Given the proven efficacy of this compound on these studies, INO is being combined with chemotherapy in the frontline setting. Using a mini-hyper-CVD regimen (cyclophosphamide, vincristine, dexamethasone) with INO in elderly, 47 out of 48 evaluable patients (98%) achieved a CR/CRi (35 CR), coupled with flow-cytometric MRD-negative status in 76%. Two-year PFS and OS were 52% and 66%, respectively.[56,108]

Chimeric antigen receptor (CAR) T-cells

Cellular immunotherapy with CD19-directed CAR T-cells represents another promising approach for R/R disease. Anti-CD19 CAR T-cells have been the most extensively studied in trials using “second-generation” receptors, which comprise three components: an extracellular antigen-recognition domain derived from the single-chain variable fragment of a monoclonal antibody (scFv), an intracellular signaling domain (the CD3z chain from the T-cell receptor), and a co-stimulatory domain (most commonly, 4-1BB or CD28).[109-111]

Initial phase I/II studies using the CTL019 construct reported a 90% CR rate in 30 patients (25 pediatric, 5 adult).[110] In addition, 88% of the patients who achieved a CR were MRD-negative. Responses were durable with 7 relapses and 19 ongoing remissions (2 to 24 months), with 15 patients receiving no further therapy. High rates of CAR T-cell persistence (68%) and associated B-cell aplasia was reported at 6 months. In collaboration with Novartis, CTL019 was administered to 75 children and young adults, with 81% achieving CR and concurrent MRD-negative status. At a median follow-up of 10.6 months, 29 remained in CR. One-year EFS and OS were 50% and 76%, respectively.[60] This led to the approval of tisagenlecleucel (Kymriah™), the first CAR

product in the US. The outcomes in adult patients treated with CAR T-cells has been less impressive with median EFS and OS of 6.1 months and 12.9 months, respectively.[63] CAR T-cells but not NK cells[112] could also be effective against CNS leukemia.[113] Although anti-CD19 CAR T-cells can generate rapid and impressive responses, therapy is associated with a unique set of severe side effects. The two major toxicities include CRS and neurotoxicity. In the CTL019 study, all patients experienced signs and symptoms of CRS with 8 of 30 patients requiring transfer to intensive care unit.[110] Fortunately, tocilizumab, an anti-IL6 receptor antibody, was found effective and has become the mainstay of management for severe CRS, as it is well-tolerated and rapidly effective in the majority of cases. Current approaches include optimization of the CAR T-cell product in defined proportions of CD4 and CD8 T-cell subsets, development of humanized CARs, CARs with two co-stimulatory domains, allogeneic CARs and CARs against other antigens such as CD22.

New management options in molecularly-defined ALL subsets

Ph+ ALL

Outcome of Ph+ ALL was dramatically improved by TKIs.[114-118] Single-agent imatinib or dasatinib plus corticosteroids therapy, pioneered by the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA)[114,119] induced CR virtually in all patients without risk of induction death. With TKI-chemotherapy combinations, CR rate exceeded 95% but death occurred in 2-7% of the cases. In a randomized trial from GRAALL,[116] a combination of de-escalated chemotherapy plus TKI resulted in less induction toxicity and non-inferior CR and survival results compared to standard chemotherapy plus TKI. In a MDACC study, ponatinib combined with Hyper-CVAD led to an excellent 83% 2-year OS, even without HCT[115]. In elderly and/or frail patients (median age 68 years, range 27-85 years), ponatinib monotherapy (GIMEMA) resulted in 87.5% 1-year OS, associated with a 45% molecular response rate.[120] Postremission consolidation is still based on intensive chemotherapy (plus TKI) and HCT, when feasible. This "global" strategy led to survival rates approaching 50%, thus meaning we still need to improve. Chemotherapy-free trials with TKI-immunotherapy combinations (e.g. TKI-blinatumomab) are ongoing (NCT02744768) and will clarify the place of this antibody construct especially in eradicating MRD. As for other ALL subsets, MRD persistence is associated with recurrence while its negativity may identify patients with favorable prognosis in whom the indication for HCT could be

reconsidered to spare morbidity and mortality.[25]

With these premises, relapse remains relatively frequent ~~event~~ and is often sustained by mutations, the most deleterious being T315I. New potentially active agents include axitinib,[73] a vascular endothelial growth factor receptor inhibitor active in T315I mutant, a new TKI, danusertib,[121] and ABL001 (asciminib),[122] a novel allosteric TKI that binds to the myristoyl pocket of ABL1, causing an inactive kinase conformation (NCT02081378 phase I trial for patients intolerant/refractory to standard TKI). Notably, a drug sensitivity testing platform[123] allowed the identification of axitinib as a selective inhibitor of the T315I mutant.[73] As for combinatory studies, of interest is the simultaneous administration of dasatinib, ruxolitinib and dexamethasone, which *in vitro* was shown to restore cytokine dependency, inhibit STAT3 and STAT5 activation and prevent leukemia initiating cell growth and acquisition of mutations (NCT02494882),[124] and the combination of ruxolitinib with nilotinib (NCT01914484). In cases with IKZF1 impairment, retinoids can induce *IKZF1* re-expression, stimulate cell maturation and restore *in vitro* TKI sensitivity.[125] Moreover, promoters of myelomonocytic differentiation can successfully induce Ph+ ALL cells into non-leukemic monocytes/macrophages.[126]

Ph-like ALL

The Ph-like subgroup, initially identified by means of gene expression profiling, accounts for about 20% of adult BCP ALL cases,with a prevalence in AYA. These cases are characterized by a transcriptional profile similar to that of Ph+ ALL but lacking the t(9;22)/*BCR-ABL1* rearrangement.[127-130] Instead, the underlying genomic lesions are heterogeneous making its recognition difficult and uneven among trials. *CRLF2* rearrangements are detected in about 50%, lesions affecting ABL class genes (i.e. *ABL1*, *ABL2*, *CSF1R*, *PDGFRA*, *PDGFRB*) in roughly 10%, JAK/STAT genes (i.e. *JAK1-3*, *IL7R,* and *CRLF2* mutations) in <10%. Rearrangements in other TKs and *EPOR* gene are extremely rare. *IKZF1* deletions occur in up to 80% of the cases. Patients with Ph-like ALL have a poorer outcome when compared to other BCP ALL subsets and is not yet clear whether they should receive a HCT upfront based on MRD persistence only.[128,131] Given the activated kinome profile several groups are currently testing the combination of TKIs with chemotherapy. Children's Oncology Group (COG) is testing ruxolitinib in *CRLF2*-rearranged and/or JAK-STAT deregulated patients (NCT02723994) or dasatinib in untreated patients (NCT02883049), while MDACC is testing these drugs in pre-treated patients (NCT02420717), with disappointing results.[132] Other experimental approaches

employ a variety of inhibitors based on the individual molecular profile. The pan-TKI ponatinib could be effective regardless of the underlying genetic lesion.[133]

MLL-rearranged ALL

The prognosis of t(4;11)/*KMT2A-AFF1*+ and other *MLL*-rearranged ALLs is poor and could be improved by new targeted approaches. *MLL* (i.e. *KMT2A*) rearrangements are associated with high levels of H3K79 methylation catalyzed by the DOTL1 enzyme. Therefore DOT1L inhibitors, particularly EPZ-5676 (pinometostat) have been tested in R/R cases (NCT02141828, NCT01684150) in both pediatric and adult cohorts.[134] Furthermore, *MLL*-rearranged cases express high levels of Bcl-2, BAX, and BIM but relatively low levels of BCL-XL and MCL-1, a mechanism ~~is~~ directly sustained by *KMT2A* rearrangement on *BCL2* expression and ~~is~~ partly mediated by interaction with H3K79me2/3. As a consequence, *in vitro* and xenograft model studies showed that the Bcl-2 inhibitor venetoclax induces cell killing in synergy with chemotherapy.[85,135,136] Additionally, histone deacetylase inhibitors (HDACi) can exert synergistic activity with cytarabine by repressing cytidine deaminase.[137]

TCF3-rearranged ALL

TCF3-PBX1+ ALL associated with t(1;19) represents about half the cases of the newly recognized pre-BCR (B-cell receptor)+ subset and is characterized by a favorable outcome with intensive treatment. These cases could be targeted by dasatinib since they overexpress a large number of TKs[138] including the BCR-dependent TK ROR1[139] and Mer TK which correlates with risk of CNS progression[140], by idealisib due to the high levels of *PIK3CD* [141] and ibrutinib via downmodulation of the pre-BCR signaling on *BCL6*.[98,142-143] Instead, *TCF3-HLF*+ ALL is a very high risk subset associated with t(17;19), often with high levels of *BCL2* expression recalling venetoclax as a potential therapeutic compound.[77] Drug response profiling predicted robustly resistance to conventional drugs and confirmed a unique sensitivity to venetoclax. Combination therapy with dexamethasone, vincristine and venetoclax in PDX from two patients maintained CR for up to one year.[77]

Hypodiploid BCP ALL

Hypodiploid ALL is a rare poor prognostic subtype including near haploid (24–31 chromosomes), low hypodiploid (32–39 chromosomes) and high hypodiploid (40–43 chromosomes) ALL.[144] RAS and PI3K pathways are frequently altered in near haploid

ALL, while TP53 and IKZF members are often mutated in low hypodiploid ALL, pinpointing to functional targeting using PI3K and PI3K/mTOR inhibitors.[144,145] Germline mutational screening of *TP53* should always be performed in these cases.

Other BCP ALL subsets

Many other actionable deletions or mutations are emerging in BCP ALL (and sometimes TCP ALL).[127,144,146-148] These involve pathways affecting lymphoid development, cell cycle, regulation of transcription, lymphoid and RAS signaling, epigenetic modifications, cytokine receptors, TK expression and the JAK/STAT phosphorylation system (*Table 3*). Focus is now on downstream members of the RAS pathway, namely with the MEK and PI3K inhibitor BEZ235 (NCT01756118), the allosteric MEK1/2 inhibitor selutetinib, trametinib and steroids, and FLT3 inhibitors, i.e. lestaurtinib, midostaurin and quizartinib – all being evaluated in phase I-II and III trials, respectively (NCT 00866281, NCT00557193 and NCT01411267). Among epigenetic regulators, HDACi vorinostat and panobinostat are being investigated in phase I-II trials for R/R disease (NCT01483690, NCT01321346 and NCT01321346), however with reports of toxicity. JAK2 inhibitors (ruxotilinb) and BCL2 inhibitors might be used in cases harboring target mutations. SMAC mimetics, directly acting on apoptosis/necroptosis pathways, proteasome inhibitors and checkpoint inhibitors, have shown *in vitro* activity and are being studied (*Supplemental files*). The role of inhibitors of molecules involved in interaction with the marrow niche (NOTCH3 and NOTCH4) is still largely undetermined,[149] while targeting SCD and SPP1 gene/proteins[150] and vascular endothelial growth factor A (with bevacizumab) could be useful against CNS leukemia.[151]

B-ALL (mature B/Burkitt leukemia)

MYC rearrangements are the hallmark of B-ALL, leading to escape from cell cycle control and high proliferative rate. Thus inhibition of *MYC*-related pathways is an attractive option for refractory disease. MYC inhibitors JQ1 and THZ1 target MYC/MAX heterodimerization and CDK7 (THZ1), while dependency of MYC activation on multiple enhancers and 'super-enhancers', such as a BET proteins and PI3K are targeted by mTOR or HDACi, Aurora kinase A and B and other BET inhibitors (I-BET 151, GSK525762, CPI-0610).[152] New phase I trials are underway.

TCP ALL

TCP ALL accounts for about 25% of ALL cases and is further classified according to

maturation stage (early-, cortical- and mature-T). With modern pediatric-based regimens adopting MRD/risk-oriented intensification, outcome of TCP ALL may be excellent and superior to that of BCP ALL. Among actionable molecular lesions,[153] the most frequent is *NOTCH1* mutation. NOTCH1 and the strictly associated gamma-secretase inhibitors (GSI) were tested in late stage disease, with some responses of short duration and considerable gut toxicity.[154] The best study reported one CR and an overall 32% response rate in 25 relapsed patients.[155] Theoretically, targeting NOTCH1-related overexpression of chemokine receptor CCR7 and its ligand CCL19 could reduce the risk of CNS disease.[156] Many other targeting agents are being investigated, often in combination, like GSI and AKT inhibitors to revert glucocorticoid resistance[157-159] (*Figure 2*, *Table 3*). Moreover, induction of T-cell receptor signaling led to apoptosis mimicking thymic negative selection,[160] while targeting contact structures with the marrow microenvironment (CXCR4, CXCL12) reduces proliferation and the propagation potential of leukemic stem cells.[161,162] Notably PDX and drug screening models identified a subset of refractory T-ALL responsive to dasatinib in a nanomolar range, correlating with strong responses *in vivo* after resistance to multiple other treatments.[75]

Early thymic precursor (ETP) ALL

This peculiar diagnostic subset (weak/absent CD5 expression and early T/myeloid phenotype/genotype) is associated with poor outcome unless treated with very intensive MRD-based chemotherapy or HCT in first CR.[163] ETP ALL is characterized by abnormalities typically observed in myeloid disorders including mutations in *RUNX1*, *ETV6, GATA3, IDH1*, *IDH2*, *DNMT3A*[164,165] and the JAK/STAT pathway. In an experimental PDX model ETP ALL was exquisitely sensitive to ruxolitinib, which abrogated IL7-induced STAT5 phosphorylation.[166] Furthermore, FLT3 inhibitors might be considered since mutations are detected in about 35% of cases.[167]

Future directions

We are entering an intensive phase of clinical investigations with new agents. To take advantage of these new treatment options we will have to gradually shift from R/R ALL to the frontline setting, where treatment resistance is less likely to occur.[168] We will certainly need to develop solutions to integrate functional and genomic data for reference bioinformatics tools supporting clinical decisions, in accordance with studies in cancer patients including AML and childhood ALL[169-171] For the exploration of individualized or

subset-specific treatment forms it will be crucial to design prospective clinical studies with modular elements to evaluate optimal strategies for chemotherapy,[172] immunotherapy and combinations of molecularly targeted drugs and synergistic drug pairs,[74,173] and detect activity in the early clinical trials more rapidly to pilot subsequent therapeutic developments.

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Legends to the figures

Figure 1. Actionable targets and drugs for innovative therapeutics in adult ALL.

New therapeutic targets are membrane markers associated with B- or T-cell functions (type A), intracellular molecules involved in the regulation of key cell proliferation and differentiation pathways (type B) and receptors involved in the interaction with the supportive marrow niche (type C). Examples are shown for each category. Multi-targeted therapy is possible and synergy with chemotherapy is reported. Molecular profiling and ew drug profiling techniques can help identify suitable targets and the more active compounds and drug combinations, to be exploited in clinical trials of subset- and patient-specific therapy.

Figure 2. Subset-specific approaches with new therapeutics in adult ALL.

Clinical and pre-clinical experimental approaches with new management options for adult ALL and subsets (detailed sourcing and referencing in text and supplemental tables). Clinical trial evidence extracted from ClinicalTrials.gov repository, accessed April, 2017.

Figure 3. Drug Response Profiling (DRP) of primary patient samples.

(A) Workflow for phenotypical screens of co-cultures of primary ALL cells on human mesenchymal stromal cells using large scale automated microscopy. Generation of patient-derived xenografts (PDX) provide a renewable source of representative ALL cells for mechanistic research but may also be invaluable for deeper co-clinical validation experiments depending on the clinical situation. (B) Example of DRP output. IC50 values that were obtained based on dose-response curves with 8 datapoints after 72 h exposure of ALL cells to a selection of drugs are shown as a heatmap (red responses in the nM range, deep blue; resistance in the 10 µM range). Two examples of individual strong activity to the SMAC mimetic birinapant and to dasatinib are provided, with validation in an extended set of ALL PDX. MRD: minimal residual disease.