Review Article

Novel Therapeutic Strategies in Acute Lymphoblastic Leukemia

Maria K. Angelopoulou, John V. Asimakopoulos, Theodoros P. Vassilakopoulos

Department of Hematology and Bone Marrow Transplantation Unit, National and Kapodistrian University of Athens, School of Medicine, Laikon General Hospital, Athens, Greece

ABSTRACT:

Adult acute lymphoblastic leukemia (ALL) is a rare and difficult to cure disease with a long-term survival of 30-40%. During recent years, a significant progress has been accomplished, especially for young patients. This is mainly due to the adaptation of "pediatric" type inspired protocols. Moreover, minimal residual disease (MRD) monitoring has become the most valuable tool for the discrimination of patients who will benefit from allogeneic hematopoietic stem cell transplantation at first complete remission. The bispecific anti-CD19/CD3 antibody blinatumomab and the anti-CD22 antibody-drug conjugate inotuzumab ozogamicin represent two new effective treatment options for relapsed/refractory disease. The evolving technology of chimeric antigen receptor-T-cells breaks new ground in the treatment of B-ALL. Finally, the introduction of tyrosine-kinase inhibitors has reversed the dismal outcome of Philadelphia-chromosome positive ALL.

KEY WORDS: *ALL, Acute lymphoblastic leukemia, Blinatumomab, Inotuzumab ozogamicin, CAR-T cells, Tyrosine-kinase inhibitors, Philadelphia-chromosome positive ALL*

INTRODUCTION

Acute lymphoblastic leukemia (ALL) in adults is an extremely rare but highly fatal disease with annual incidence 1/100.000.[1] Despite the unfavorable outcome (long-term survival 30-40%), the substantial progress in the treatment of ALL in the last two decades has led to the improvement of overall survival (OS) in all age groups, except from patients >60 years-old; 5-year OS >60% for age group 15-19, 45%, 34% and 24% for age-groups 20-29, 30-44 και 45-59, respectively.[2]

The majority of ALL cases are classified as precursor B-cell type, while T-ALL accounts for 25% of cases.[1] Inferior outcomes in adults can be attributed to the combination of various factors, such as the unfavorable

genetic basis of the adult ALL and its rarity, as well as the presence of co-morbidities, the treatment side-effects of active chemotherapeutic agents which affect the dose administration, and the complexity of these protocols.[1,3]

DEVELOPMENTS IN THERAPEUTIC STRATEGY

Intensification of initial treatment

The backbone of ALL therapeutic strategy during the early 21st century is the intensification of initial chemotherapeutic approaches. Pediatric-inspired therapies of the Berlin-Frankfurt-Munster (BFM) Study Group have revolutionized the management of the disease leading to a significant outcome improvement. Having been adapted from the majority of ALL Study Groups[3,4] (GMALL, GRAALL, GIMEMA, GNILG, PETHEMA, HOVON, UKALL, CALGB) these protocols are characterized by these main features; 1) multiple cycles of relatively low doses of antimetabolites with reduced myelotoxicity and

Corresponding author:
Prof Maria K Angelopoulou
Agiou Thoma 17 str, Athens 11527
Tel: +30 213 2061902, Fax: +30 213 2061498
e-mail: mkangelop@gmail.com

without cross-link resistance, which lead to high cumulative doses, 2) repeated administration of asparaginase, 3) high-dose methotrexate, 4) early and frequent central nervous system (CNS) prophylaxis and 5) long-term consolidation and post-remission therapy. Contrariwise, adult-type approaches have been emerged from Hyper-CVAD protocol philosophy.

With the adoption of pediatric-type combination chemotherapy regimens, the 5-year OS has reached 50-60%. This outcome mainly results from the age group of adolescents and young adults (AYAs), who are in position to manage these intensive regimens. Indeed, the comparison with the historical studies suggests significantly more favorable outcome with the pediatric-inspired therapies than adult approach, at least in young patients. The 2-year event-free survival (EFS) and OS rates of 296 evaluable young adult patients with ALL enrolled in the US Intergroup C10403 trial were as high as 66% and 78%, respectively.[3] The exact cut-off of age for AYAs usually is defined as the 40 years, however it can be expanded up to 45-50 years of age, for patients without co-morbidities.[5]

Despite having been perceived as an extremely toxic drug in adults, asparaginase holds a critical role in modern therapeutic approach of ALL. The increased incidence of certain related side-effects has led to its omission or the use of inadequate doses in many adult-type regimens, a practice that should be avoided in patients <60 years-old, as most of these AEs are manageable. Indeed, hepatotoxicity related to asparaginase is very common as high-grade hyperbilirubinemia and transaminitis occur in a quarter and half of treated patients, respectively. Reversible hyperbilirunemia is mostly observed in the initial infusion and should not prevent further administration of additional doses, as the risk of recurrence is very low in subsequent cycles. However, clinical pancreatitis has a substantial risk of recurrence and it is included in life-threatening complications that preclude the re-administration of asparaginase. Hypersensitivity reactions to asparaginase are related with the generation of antibodies that result in drug inactivation and may not be clinically obvious. Pegylated form (PEG-Asparaginase) is less often involved with these reactions and, thus, it has substituted Escherichia Coli (E.Coli) Asparaginase. A third form of the drug, Erwinia Asparaginase, has been recently approved in the USA for patients who develop hypersensitivity to E Coli Asparaginase. As silent hypersensitivity is not rare and can result in loss of drug activity, measurement of asparaginase activity on day 14 after its administration is strongly recommended in clinical practice, with the target level being >0.1 IU/ml.[3] On the other hand, due to limited tolerance to asparaginase in elderly, the Spanish Group Study has omitted asparaginase from induction leading to decrease in early mortality.[6]

In T-cell ALL subcategory, pediatric-inspired regimen have improved outcomes making them comparable with those of precurcor B-cell ALL. These regimens mainly involve higher doses of methotrexate (5g/m²),[7] as well as intensive use of asparaginase.[8] Nelarabine activity is well-known in relapsed T-ALL, but recently its use has been introduced in upfront or in risk-adapted chemotherapy regimens with promising outcomes. Nelarabine is a purine nucleoside analog that is metabolized to arabinosylguanine nucleotide triphosphate (araGTP), which incorporates into DNA inhibiting its synthesis, and resulting in apoptosis. A phase II French study is evaluating nelarabine incorporated into consolidation and maintenance therapy in high-risk T-cell ALL (NCT02619630), as well as a phase III study will investigate the use of nelarabine as consolidation therapy in T-ALL (NCT02881086).[9] On the contrary, asparaginase-free upfront protocols, such as hyper-CVAD, have demonstrated higher relapse rates in adult T-cell ALL.[10]

At the time of diagnosis, CNS disease is uncommon, but the risk of relapse in this sanctuary site is substantial in the absence of adequate CNS-directed prophylactic therapy. Historically, prophylactic cranial-spinal radiation was an integral part of CNS prophylaxis regimen. However, due to prohitibive late toxicities, this strategy has been abandoned. On the other hand, the mainstay of CNS prophylaxis in modern ALL therapeutic regimens, which has resulted in low risk CNS relapse, consists of early and frequent intrathecal administration of MTX in combination with intravenous chemotherapeutic agents that have sufficient blood-brain barrier penetration. Moreover, the prognosis of CNS involvement at diagnosis remains a matter of debate, while isolated CNS relapse portends systemic relapse and mandates systemic chemotherapy in addition to CNS-directed therapy.[3] Allogeneic stem cell transplantation (allo-SCT) after local clearance of ALL has demonstrated promising outcomes, as the graft-versus-leukemia effect can potentially extend to cerebrospinal fluid. Contrariwise, radiation-based conditioning, administration of cranial radiation prior to allo-SCT or administration of post-allo-SCT prophylactic intrathecal chemotherapy do not seem to offer substantial benefit.[11]

Selection of candidate patients eligible for allogeneic stem-cell transplantation

The second axis of development in therapeutic strategy involves the precise defining of high-risk patients for relapse and their selection for consolidation with allo-SCT. Classic criteria remain the high white blood cell count and certain cytogenetic features, such as t(9;22), t(4;11) and other types of MLL gene rearrangement. However,

recent data suggest a more pronounced benefit with early allo-SCT for standard-risk patients in complete response (CR) 1 compared with the high-risk subset.[12]

In modern era, the classic criteria are tend to be replaced by the detection of the minimal residual disease (MRD) in certain time points; usually between 4th and 16th week from induction.[13] Despite that the molecular-based methods of MRD detection are strenuous, demanding and expensive, the great majority of study-groups are using them, as significant prognostic tools.[14-17] The positive predictive value of MRD negativity, early in the disease course, has been proven in many studies. In example, the German Study Group demonstrated 67% relapse free survival (RFS) in patients with negative MRD compared to 25% in those with positive MRD, for age-group 15-55 years-old.[15] MRD positivity constitutes the main selection criteria for allo-SCT (5-year RFS 44% vs. 11% with or without allo-SCT). In some studies, the risk stratification based on MRD has been expanded not only to standard-risk patients, but also to high-risk ones.[13] Nevertheless, the elimination of MRD through the introduction of targeted therapies may alter the subsequent therapeutic strategy. At the same time, recent scientific data supports the increased relapse risk in MRD negative patients with deletion of IKAROS gene (IKZF1) and the distinct entity of bcr/abl1-like ALL.[18,19] Thus, the incorporation of new molecular entities in ALL classification may further improve therapeutic approach.

Anti-CD20 monoclonal antibodies

CD20 molecule is expressed in 1/3 of B-ALL[20] and is related with increased risk of relapse.[21] Initially, the GMALL study group explored the combination of rituximab and chemotherapy in adult CD20+ precursor B ALL patients (15-55 years of age, n=181) which were compared with patients recruited earlier in the same study without rituximab (n=82), who received the same chemotherapy regimen. Although, there was not improvement in CR rate in the standard risk group, there was a higher rate of molecular CR (57% vs 27% at day 24 and 90% vs 59% at week 16). Moreover, there was a significant improved rate of 3-year continued CR for patients received rituximab (64% vs. 48%) and 5-year OS (80% vs. 47%). Notably, 5-year OS was increased in the high-risk group with addition of rituximab (55% versus 36%).[22] Similar outcomes were presented in another study performed at MD Anderson Cancer Center (MDACC) in precursor B ALL CD20+ patients <60 years of age. However, the backbone chemotherapeutic regimen used was hyper-CVAD and patients >60 years of age were also included. In patients <60 years of age, the addition of rituximab was associated with an improved rate of CR duration and

OS compared with historical controls at 3 years (91% vs. 66% and 89% vs. 53%, respectively). However, this benefit did not extend to patients ≥60 years of age.[23] Recently, a randomized Phase III clinical trial in CD20+ precursor B ALL patients (18-59 years-old) demonstrated that treatment with rituximab is associated with significantly improved event-free survival (EFS) with significantly lower incidence of relapse and higher rates of proceeding allo-SCT during the first remission. Thus, the introduction of Rituximab in 1st line treatment improves the outcome of CD20+ B-ALL.[24] Second generation anti-CD20 molecule ofatumumab in combination with hyper-CVAD led to 98% MRD negativity.[25]

DEVELOPMENTS IN REFRACTORY/ RELAPSE (R/R) DISEASE

Therapeutic Strategy

In R/R disease, therapeutic strategy aims at achieving 2nd CR and proceeding to allo-SCT. In common practice, the outcome of this strategy is limited (20-30% of patients), since the presence of chemoresistance is usual with extremely dismal prognosis (median OS 3-6 months).[26]

The significance of MRD has not been sufficiently clarified in relapsed disease. Recent outcomes demonstrate that MRD negativity in salvage 1 (S1) is related with more favorable outcome, especially for patients who immediately underwent allo-SCT (2-year OS >60%). Contrariwise, patients in >S1 have dismal outcome, regardless of their MRD status.[27]

Newer Chemotherapeutic Agents

Among the chemotherapeutic agents incorporated in the management of R/R disease, mitoxantrone-containing regimens are related with higher response rates compared to that of idarubicin. Newer approaches include clofarabine, cyclophosphamide and etoposide for B-ALL, nelarabine for T-ALL and liposomal vincristine.[28]

Anti-CD22 monoclonal antibodies

Epratuzumab

CD22 antigen is expressed in 60-96% of B-ALL cases.[20] Epratuzumab is naked anti-CD22 humanized monoclonal antibody (mAb). In adults, the addition of epratuzumab to the combination of clofarabine and cytarabine in adults with R/R precursor B ALL led to more favorable CR rate compared to historical studies.[29] However, the combination epratuzumab/hyper-CVAD in CD22+ R/R ALL had disappointing outcomes.[30] After binding, the receptor/antigen complex is internalized, without significant

therapeutic efficacy when used as monotherapy. On the other hand, this molecule is ideal for conjunction with toxins or chemotherapeutic agents.

Inotuzumab ozogamicin (INO)

Inotuzumab ozogamicin (INO) is a mAb against CD22 that is bound to calicheamicin. Initially, the recommended dose was altered from 1 infusion every 3-4 weeks, to 3 weekly infusions every 28 days.[31] The first phase 2 study demonstrated encouraging outcomes with overall response 57%.[32] In the following study, the response rates were comparable, although the AEs were less frequent with the weekly schedule. Allo-SCT was decided for 44% of the patients, whom 17% developed veno-occlusive liver disease, with lower frequency in the arm of weekly administration schedule.[32] Reproducible results have emerged from the following study in 35 B-ALL patients >S1; 67% of patients had CR, while 78% of them were MRD negative. The safety profile of the drug remained stable with main side-effects being haematological, gastroenterological and hepatotoxicity.[33] In a phase III study on 326 patients with R/R B-ALL which compared the effectiveness of INO versus salvage treatment (FLAG, cytarabine and mitoxantrone, high-dose cytarabine), the complete response rates, median disease-free survival (DFS) and OS were better in the arm of INO.[34] MRD response was also higher (78.4% vs 28.1% of those who responded). Moreover, allo-SCT was more likely to be performed in patients who received INO compared to those received systemic chemotherapy (41% vs 11%). From further analysis, the main advantage of INO regarding the achievement of CR has been appeared in all subcategories, except from those with t(4;11). Veno-occlusive liver disease in the INO arm was 11%. Extremely promising results are demonstrated combining INO with unintesified chemotherapy (mini- hyper-CVAD) both in R/R disease and in 1st line treatment in elderly.[35] Main studies of INO are described in Table 1. Based on results of the Phase 3 INO-VATE ALL clinical trial (NCT01564784),[34] which compared the effectiveness, safety and tolerability of INO with those of standard of care chemotherapy, INO received EU approval as monotherapy for the treatment of adults with R/R CD22-positive B cell precursor ALL. Moreover, INO is indicated for patients with Philadelphia positive (Ph+) R/R B cell precursor ALL who have failed treatment with at least one TKI.

Moxetumomab Pasudotox (CAT-8015)

This is another anti-CD22 mAb, which is composed by the variable region against CD22 fused to Pseudomonas Aeruginosa exotoxin A. Its efficacy in R/R disease is currently evaluated.[28]

Combotox

Combotox is a combination immunotoxin that contains a 1:1 mixture of anti-CD19 and anti-CD22 antibodies, both conjugated to the cytotoxin deglycosylated ricin-A chain. A phase 1 trial is currently studying the drug efficacy in combination with cytarabine for adults with R/R disease (NCT01408160).[28]

Anti-CD19 monoclonal antibodies

CD19 is widely expressed in B-ALL (>90%).[20] Like CD22 molecule, CD19 is rapidly internalized upon binding of an antibody making it an ideal candidate for immunoconjugate therapy. Two main immunoconjugates have been developed so far: SAR3419 (Coltuximab Ravtansine), an anti-CD19 humanized mAb conjugated to a semisynthetic maytansinoid combound, an anti-tubulin molecule similar to vincristine, and SGN-CD19 (Denintuzumab Mafodotin), an antibody conjugated to monomethylauristatin. Both antibodies have been studied in phase I trials,[36] whose outcomes were not very promising, while significant AEs from the cornea were described.[28]

Immunotherapies mediated by T-lymphocytes

Blinatumomab

Blinatumomab is the first clinically applied bispecific, single-chain mAb based on BiTE technology (Bi-specific T-cell engagers), a construct specific for CD19 and CD3, which links cytotoxic CD3 positive T cells to malignant cells.[20] It has two single chain variable fragments, each with an antigen-binding domain, linked by a glycine-serine domain. One antigen-binding domain has affinity for CD3ε, which forms part of the T-cell receptor (TCR) complex, while the other engages CD19, a pan-B cell marker expressed in ALL. This technology exploits the anti-neoplasmatic cytotoxic activity of naturally circulating T-lymphocytes, independently of TCR specifity or MHC-mediated target-antigen presentation. The linkage between T-lymphocytes and lymphoblasts provoke the activation of T-lymphocytes leading to cytotoxic effect, through the production of inflammatory molecules and cytokines, such as IL-6, IL-10, IFN-γ, granzymes and perforin. Moreover, each activated T cell is able to engage serially multiple leukemic cells, while there is a polyclonal expansion of activated T cells.

Blinatumomab is actually the only innovative treatment of B-ALL applied in daily clinical practice. For the time being, its indication is for R/R Ph- ALL. Table 2 represents the characteristics and outcomes of the main clinical studies in detail. Phase II/III studies concluded at significant outcomes in efficacy,[37-39] as they demonstrated CR rates 43-67% and MRD negativity 73-88% of those who responded. Moreo-

TABLE 1. Major clinical studies of inotuzumab ozogamicin in B-cell Acute Lymphoblastic Leukemia

Study Data	Patients' Characteristics	Study Outcomes
B-ALL, infants-adults, R/R, single-arm, single-center **Phase II, NCT01134575 (32)**	N=49 • R1: 27%, R2: 49%, ≥R3: 24% • Ph+: 14% • Previous allo-SCT: 14% • Median age: 36 years-old • Dose: 1.8mg/m2 every 3-4 weeks	• CR/CRh: 57% • CR/CRh in Ph+: 42% • MRD-: 63% of responders • CR/CRh in Ph+: 43% • Median OS: 5.1 m • Median duration of CR/CRh: 6 m

B-ALL infants-adults, R/R, single-arm, single-center **Phase II, NCT01134575 (validation) (31)**	N=90
	• R1: 32%, R2: 38%, ≥R3: 30%
	• Ph+: 17%
	• Previous allo-SCT: 11%
	• Median age: 39.5 years-old
	• Dose:
	  - Single-dose administration 1.3-1.8mg/m2 every 3-4 weeks (N=49)
	  - 3 weekly infusions every 3-4 weeks (day1: 0.8mg/m2, days 8,15: 0.5mg.m2) (N=41)
		• CR/CRh: 58%
		• MRD-: 72% of responders
		• Median OS: 6.2 m (single-dose administration: 5 m, weekly administration: 7.3 m)
		• Median duration of CR/CRh: 7 m

B-ALL, adults R/R, single- arm, multicenter Phase II (33)	N=35 • ≥R2, 17%≥R5 • Ph+: 26% • Previous allo-SCT: 43% • ≥ 18years-old, median age: 34 years-old • Dose: 3 weekly infusions every 4 weeks (day1: 0.8mg/m2, days 8,15: 0.5mg/m2) (N=41)	• CR/CRh: 65,7% • MRD-: 78% of responders • CR/CRh in Ph+: 67% • Median OS: 7.4 m
B-ALL adults, R/R, open-label, 2-arm, randomized (INO vs CHEMO), multicenter, international Phase III (NCT01564784) (34)	N=218, (INO: 109 – CHEMO: 109) • R1-R2 • ≥ 18 years-old, median age: 47 years-old INO CHEMO R1/R2 67%/32% 63%/36% Previous allo-SCT 16% 20% Ph+ 13% 17%	Results INO vs CHEMO • CR/CRh: 80.7% vs 29.4% • MRD-: 78.4% vs 28.1% • Median PFS: 5 m vs 1.8 m. • 41% vs 11% underwent allo-SCT • Median OS: 13.9 m vs 9.9 m (all the differences were statistically significant)
B-ALL, adults R/R, single-arm, single-center, Phase II (NCT01371630) (35)	N=59 • R/R • ≥ 18 years-old, median age: 35 years-old • Combination INO + mini- hyper-CVAD • miniHyperCVAD: without Doxo, CTX και Dexa: decrease 50%, MTX: decrease 75%, ARA-C: 0.5 g/m² × 4 doses), INO: 1.8 -1.3 mg/m², day 3 (cycle 1), 1.3-1.0 mg/m² (next cycles)	• CR: 59% • MRD-: 82% of responders • Post-response allo-SCT: 44% • Median PFS: 8 m • Median OS: 11 m

ALL: Acute lymphoblastic Leukemia, R/R: Relapse/Refractory disease, R: relapse, m: months, N: number of patients, INO: inotuzumab ozogamicin, CHEMO: chemotherapy, CR: complete remission, CRh: Complete remision without normal complete blood count, Ph: Philadelphia chromosome, allo-SCT: allogeneic stem cell transplantation, MRD: minimal residual disease, OS: overall survival, PFS: progression free survival, RFS: relapse free survival

ver, 25-50% of these patients underwent allo-SCT. Median RFS and OS may vary between 5.9-8.8 and 6.1-13 months, respectively. Nevertheless, a group of MRD negative patients seems to have achieved a significant long-term survival. The comparison of blinatumomab with historical studies highlightens the superiority of the first regarding the CR and OS rates.[40] In addition, the use of blinatumomab in older adults seems promising, considering that the therapeutic choices in elderly are extremely limited.[41]

The recently published multinational phase III study (TOWER) compared blinatumomab with standard-of-care S1 chemotherapy.[39] In the blinatumomab arm, OS and RFS were significantly longer (median OS: 7.7 versus 4 months, HR=0.71, p=0.01; median RFS: 7.3 versus 4.6 months). The superiority of blinatumomab on OS extends on all subgroups, except from patiens >S2. It is worth mentioning that OS curves for the blinatumomab and chemotherapy groups separated within 3 months and converged again between 15 and 18 months. This observation indicates that blinatumomab is mostly suitable for the achievement of CR and the immediate proceeding to allo-SCT. Indeed, OS curves were separated showing superiority for blinatumomab, when allo-SCT was taken into account. It should be mentioned that the basic characteristics of TOWER study patients were unfavorable in comparison with phase III study of INO.

Except from its efficacy in R/R B-ALL, blinatumomab could also be applied in the MRD elimination after 1st line treatment: in a limited number of patients with persistent MRD after consolidation treatment, 80% of them achieved MRD negativity.[42] Notably, RFS did not differ between those who respond and underwent allo-SCT from those who achieved MRD negativity and did not received any further treatment. The recent study update revealed that approximately half the patients remain in remission 5 years after treatment with blinatumomab (median follow-up time 50.8 months), regardless of allo-SCT.[43] What remains to be proven is if the elimination of MRD with blinatumomab could substitute the necessity for allo-SCT. Current studies examine the efficacy of blinatumomab in first-line treatment in combination or in comparison with chemotherapy (NCT02877303, NCT02003222, NCT02101853).

Negative predictors of response to blinatumomab include high disease burden (defined as bone marrow blast >50%), the presence of extramedullary disease and the frequency of the circulating T regulatory cells (Tregs).[44] With a cut-off of Tregs <8.525%, Tregs enumeration can identify 100% of all blinatumomab responders and exlude 70% of non-responders.[45] Thus, therapeutic removal of Tregs may convert blinatumomab non-responders to responders. Multiple mechanisms may be involved in resistance to blinatumomab, such as the emergence of CD19-negative clones,[46] the increased frequency of Tregs, the overexpression of programmed cell death-1 receptor (PD-L1) on the leukemic blasts[47] or PD-1 from Tregs.[45] In addition, a group of patients can develop extramedullary disease in relapse following blinatumomab therapy.[44]

Due to its short half-life, blinatumomab should be administered in continuous intravenous infusion, while its final approved dose is 9 μg/day on days 1-7 of the first cycle, followed by 28μg/day on days 8-28. Remarkably, T-cell expansion within 4 weeks of administration is related with increased MRD-negativity rates and more favorable prognosis.[48] Besides the hematologic toxicity, cytokine-release syndrome (CRS) and CNS-related events are the most significant AEs. Thus, pre-emptive administration of dexamethasone is recommended in patients with high leukemic burden (>50% BM blasts) or rapidly progressive disease. CRS management consists of transient discontinuation of treatment, administration of high-dose corticosteroids or the use of tocilizumab, a monoclonal antibody against IL-6 receptor. CNS AEs may be caused by the small size of the antibody, that surpass the blood-brain barrier and results in rapid neurotoxic cytokine release. Grade ≥3 AEs occur in 9-11% of patients. CNS-related events usually present after 1st week of infusion and are reversible with corticosteroids and supportive care.[37-39] Currently, blinatumomab is indicated as monotherapy for the treatment of adults with Ph(-) CD19 positive relapsed or refractory B-precursor ALL, as well as monotherapy for the treatment of paediatric patients aged 1 year or older with Ph(-) CD19 positive B-cell precursor ALL which is refractory or in relapse after receiving at least two prior therapies or in relapse after receiving prior allogeneic hematopoietic stem cell transplantation.

Chimeric antigen receptor (CAR) T cells

Chimeric antigen receptor (CAR) T cells (CAR-Ts) are genetically engineered T cells that express the antigen-binding domain of an immunoglobulin linked via transmembrane domains to the intracellular T-cell receptor signaling moieties. This allows the T cells to recognize unprocessed antigens and to be activated in a major histocomptability complex (MHC)- independent manner. First generation CAR-Ts contain intracellular signaling moieties derived only from the T-cell receptor/ CD3ζ complex. In contrast, second- and third-generation CAR-Ts include co-stimulatory signals in the CAR gene constructs (CD137, CD27, CD28 ή CD134). More recently, fourth-generation CAR-Ts have been engineered to include a cytokine-expressing cassette.[49-55]

The process of CAR-Ts therapy involves collecting T cells, introducing the CAR construct, cell amplification ex-vivo to a goal volume approximately 3×10^6 cells/kg and

TABLE 2. Major clinical studies of blinatumomab in B-cell Acute Lymphoblastic Leukemia

Study Data	Patients' Characteristics	Study Outcomes
B-ALL adults, R/R randomized (blinatumomab vs CHEMO), multicenter international, Phase III, TOWER, NTC02013167 (39)	N=405 Blinatumomab=271, CHEMO=134 • R ≤ 12 m after CR1 or allo-SCT or refractory in 1st line treatment or ≥R2 • Only Ph- patients • Previous allo-SCT: 104/403 (35%) • Median age: 40.9 years-old	Results blinatumomab vs CHEMO: • Median OS 7.7m vs 4.0m after median F/U time 11.7m, $p=0.01$ • CR/CRh in 44% vs 25%, $p<0.001$ • MRD-: 33% vs 12% • Median RFS: 7.3m vs 4.6m • In both groups, 24% underwent allo-SCT
B-ALL adults, R/R, single-arm, multicenter, international, Phase II, NCT01466179, NTC020003612 (38)	N=189 • R ≤ 12 m after CR1 or allo-SCT or refractory in 1st line treatment or ≥R2 • Only Ph- patients • Previous allo-SCT: 64/189 (34%) • Median age: 39 years-old	• CR/CRh: 81/189 (43%) • MRD- after 2 cycles: 60/181 (33%) (1st=59, 2nd=1) • Post response allo-SCT: 32/81 (40%) After median F/U time 8.9m: • In CR/CRh patients: median RFS 5.9m (MRD-: 6.9m vs MRD+:2.3m) • Median OS (189 patients): 6.1m with median F/U time 9.8m
B-ALL adults, R/R, single-arm, multicenter, Germany (GMALL Study Group), Phase I/II NCT01209286 (37)	N=36 • Only Ph- patients • Previous allo-SCT: 15/36 (42%) • Median age: 32 years-old	• CR/CRh: 25/36 (69%) • CR/CRh after allo-SCT: 8/15 (52%) • MRD-: 22/36 (61%) • Post-response allo-SCT: 13/25 (52%) (R=2, TRM=6) • without allo-SCT after CR/CRh: 12/25 (48%) (R=8) After median F/U time 28.9m: • 12 patients with CR (RFS 61%) • Post-response allo-SCT (N=9): CR=6, R=2, TRM=1
B-ALL adults in CR but with persistent/recurrent MRD, single-arm, multicenter, Germany (GMALL Study Group), Phase II, NCT00198991, NCT00198978 (41,43)	N=20/21 • Persistent MRD: 15 • Recurrent MRD: 5 • Ph+ ALL TKI-resistant: 5 • No previous allo-SCT • Median age 47 years-old	• MRD- after 1st cycle: 16/20 (80%) • 3/5 Ph+ ALL achieved response • allo-SCT, N=9 After media F/U time 50.8 m: • CR: 10 (long-term RFS 50%) • Post-response allo-SCT(10): CR=5, R=3, TRM=1 • without allo-SCT(11): CR=5, R=5, TRM=1
B-ALL adults, R/R, single-arm, multicenter, Phase II, NCT02000427 (ALCANTARA) (74)	N=45 • Ph+: all patients • R/R in ≥2nd generation TKI and/or with intolerance to imatinib • ≥18 years-old, median age: 55 years-old • Previous allo-SCT: 44% • Mutation T315I: 27%	• CR/CRh: 36% • MRD-: 88% of CR/CRh • Median RFS: 6.7m • Median OS: 7.1m • 44% underwent allo-SCT

ALL: Acute Lymphoblastic Leukemia, R/R: Relapse/Refractory disease, R: relapse, m: months, N: number of patients, CHEMO: chemotherapy, CR: complete remission, Ph: Philadelphia chromosome, allo-SCT: allogeneic stem cell transplantation, F/U: follow-up, CRh: complete remission without normal complete blood count, MRD: minimal residual disease, RFS: relapse free survival, OS: overall survival, GMALL: German Multistudy Group for Adult Acute Lymphoblastic Leukemia, TRM: treatment related mortality, TKI: tyrosine-kinase inhibitors

then an autologous transplantation of the modified cells into the patient after lymphoablative conditioning regimen. Following infusion, cells can multiple up to 103 fold in vivo within 7-14 days. Degree of expansion in vivo as well as B cell depletion correlates with response to therapy. Options for gene delivery methods include viral vectors and RNA-based methods. The use of viruses induce permanent gene expression, however it involves the risk of oncogene activation through random gene integration.[49-55]

Clinical studies are being elaborated by four major centers: University of Pennsylvania (UPenn), National Cancer Institute (NCI), Fred Hutchinson Cancer Research Center (FHCRC) and Memorial Sloan Kettering Cancer Center (MSKCC)[49-55] (Table 3). The outcomes appear to be outstanding. Near all of these studies have demonstrated CR rates 80-90%, while the vast majority of those who respond achieve MRD negativity.[49-55] Nevertheless, half the patients experience relapse within 1 year during follow-up. Thus, CAR-Ts therapy is proposed as a "bridge" to allo-ASCT.[53] Albeit, scientific data from MSKCC revealed that outcome did not differ regardless performing or not allo-SCT. In addition, the same researchers have highlightened the significant negative predictive value of post-treatment MRD.[54] The persistence of cells in the circulation depends on the manufacturing method of CAR-Ts and the chemotherapeutic regimen.[51] It would be worthy to mention that CAR-Ts therapy is related with serious AEs.[51] Thus, grade III CRS and neurotoxicity are frequently observed (23-100% and 15-50%, respectively). Limitations in efficacy of CAR-Ts include the emergence of CD19-negative clones, the absence of their long-term persistence in patient's circulation after a single administration and the risk of CRS.[49-55] Prognostic factors for the CRS development, as well as the patients' characteristics that will benefit from pre-emptive treatment will be clarified by the running studies. Recently, this technology has also adapted for CD22-negative directed CAR-Ts.[56] In September 2017 tisagenlecleucel (Kymriah, Novartis), a CAR-Ts therapy against CD19, was approved by F.D.A. (U.S. Food and Drug Administration) for R/R B-ALL patients ≤25 years-old.

Escaping m echanisms include loss of target (i.e. truncated protein or splice variants) and checkpoint induced anergy (ie CTLA4, PDL-1). The appearance of CD19-negative clones is involved in up to 30% of relapses after blinatumomab administration, while up to 60% of relapses after treatment with CAR-Ts are characterized by loss of CD19.[37,42] This could be attributed to a disorder of intracellular CD19 movement,[57] or to the proliferation of already existing CD19-negative leukemic stem cells,[58] some of which express IL-3 receptor (CD123). Thus, the simultaneous targeting of both CD19 and CD123 with CAR-Ts technology is under development.[58] Furthermore, the suppression of T-cellular response through PD-1/PD-

L1 axis –escaping mechanism for both blinatumomab and CAR-Ts can be reversed by co-administration of PD-1 inhibitor, pembrolizumab.[59]

Tyrosine-kinase inhibitors and immunomodulatory agents

Proteasome inhibitors

Nuclear factor kappa-B (NF-κB) is a transcriptional activator with anti-apoptotic properties which plays a role in a variety of malignancies. Proteasome inhibitors inactivate NF-κB and impair tumor growth. The induction of apoptosis through the inhibition of proteasome is examined in ALL. Several preclinical studies have suggested a synergestic role of bortezomib, which first approved for the treatment of multiple myeloma, with dexamethasone and additive effects to standard chemotherapy agents in acute leukemias. Yet, a single agent, bortezomib did not produce durable responses in patients with R/R ALL. Current studies examine its efficacy as frontline therapy in combination with chemotherapy in T-ALL, due to its ability to inhibit the NF-κB and NOTCH1 signaling pathways.[28] The phase IIA ALL07P1 COG study investigated the efficacy of bortezomib with re-induction chemotherapy vincristine, prednisone, peg-asparaginase, and doxorubicin for relapsed ALL. The CR rate was 69% in precursor B-ALL (n=61) and 65% in T- ALL (n=17).[9] In adults, recruitment has begun for a phase 2 trial of bortezomib with combination chemotherapy in R/R ALL (NCT01769209), while another phase II trial is investigating the same question in pediatric and young adults patients (NCT02535806). A randomized phase III COG study (AALL1231) is studying combination chemotherapy with or without bortezomib for younger patients with newly diagnosed T-ALL or stage II-IV T-cell lymphoblastic lymphoma (NCT02112916).

Carfilzomib achieves a higher degree of proteasome inhibition compared to bortezomib with fewer off-target effects. The maximum tolerated dose of carfilzomib combined with hyper-CVAD is being studied in a phase I study (NCT02293109). In addition the safety and to-lerability of carfilzomib for relapsed ALL in combination with re-induction therapy are being evaluated as well (NCT02303821). Ixazomib, the newest proteasome inhibitor that is also orally administered, is being studied in a phase I trial, in combination with chemotherapy for ALL (NCT02228772).

JAK inhibitor (ruxolitinib)

Bcr-abl1 -like B-ALL is a recent recognized entity with similar expression profile with bcr/abl+ ALL, which is characterized by IZKF1 deletion, CRLF-rearrangements and coexistence of JAK1/2 mutations, as well as translocations of PDGFRβ, abl1, abl2, JAK2 and EPOR.[19] The JAK/STAT signaling pathway has been identified as a significant

TABLE 3. Major clinical trials of anti-CD19 Chimeric Antigen Receptor T-cells in Acute Lymphoblastic Leukemia

Center/Study Data	Patients' characteristic	Study Outcomes
MSKCC/ B-ALL adults, R/R, single-scale, Phase I (49)	N=16 Median age=50 years-old • ≥R2: 7 (44%) • Ph+ patients: 4 (25%) • CNS disease: 2 (12%) • Previous allo-SCT: 4 (25%) Before CAR-Ts infusion: • Blasts≥50%: 6/15 (40%) • MRD-: 2/15 (13%)	• CR/CRh: 88% (median F/U time 24.5 days) • MRD-: 12/16 (75%) • Post-response allo-SCT: 7/10 (70%)
B-ALL adults, R/R, Phase I (54)	N=44 • Median age=45 years-old • ≥3 treatment lines: 22 (55%) • Ph+ patients: 14/32 (25%) • Previous allo-SCT: 17 (39%) Before CAR-Ts infusion: • Blasts≥5%: 22/43 (51%) • MRD+: 21/43 (49%)	• CR: 36/44 (64%) • MRD-: 29/35 (83%) • 6m-OS: 76% MRD-CR+ vs 14% MRD+CR+ After CAR-Ts administration, post-response allo-SCT (12/35, 33%): • 6m-OS: 70% allo-SCT(+) vs 64% allo-SCT(-)
NCI/ B-ALL infants and young adults, R/R, Phase I (53)	N=51 • primary resistant disease: 11 • Ph+ patients: 5 • CNS disease: 6 Type of lymphoblative treatment LD Flu/Cy: 43 FLAG: 6 Ifo/Eto: 2 HD Flu/Cy: 8	• CR: 31/51 (60.8%) with MRD-: 28/31 (90%) • Median 18m-LFS in MRD-: 49,5% • Median 18m-LFS: 85.7% allo-SCT+ vs 9.5% allo-SCT- • CNS disease CR rate: 100% (6/6) Response rate Flu/Cy(+) 66% vs Flu/Cy(-) 25%, p=0.03: • OS (Flu/Cy+): 34.7% at 38m • Flu/Cy(-): HR=6.35, p=0.0026
MULTICENTER/ B-ALL infants and young adults, R/R, Phase I (ZUMA-3 και -4) (55)	N=11 • age ≥18 years old, 2-21 years-old • LD Flu/Cy: 5/5	• CR: 5/5 (100%)
CHOP/UPenn/ CD19+ ALL infants and young adults, R/R, Phase I (51)	N=53 • CD19+ T-ALL: 1/53 • MRD-: 12/53 (23%)	• CR: 50/53 (94%) (and T-ALL case responded) • MRD-: 42/45 (93%) • After median F/U time 10.6m, CR:29/53 (55%) • 6m-EFS: 70%, 6m-RFS: 72%, 12m-OS: 78%
FHCRC/ B-ALL infants and young adults, R/R after allo-SCT, Phase I (50)	N=43/45	• MRD-: 40/43 (93%) • 12m-EFS: 50.8%, 12m-OS: 69.5%

CAR-Ts: chimeric antigen receptor T-cells, MSKCC: Memorial Sloan Kettering Cancer Center, ALL: acute lymphoblastic leukemia, R/R: relapse/refractory, Ph: Philadelphia chromosome, CNS: central nervous system, allo-SCT: allogeneic stem cell transplantation, MRD: minimal residual disease, CR: complete remission, CRh: complete remission without normal complete blood count, 6m: 6-month, OS: overall survival, LD: low dose, Flu: fludarabine, Cy: cyclophosphamide, FLAG: fludarabine-aracytine, Ifo: ifosamide, Eto: etoposide, HD: high dose, 18m: 18-months, LFS: leukemia free survival, HR: hazard ratio, F/U: follow-up, EFS: event free survival, RFS: relapse free survival

mechanism of leukemogenesis. Preclinical studies have suggested benefit of ruxolitinib for the treatment of Ph-like ALL and CRLF2-rearranged ALL. A phase II trial examine the combination of ruxolitinib with standard multi-agent chemotherapy in newly diagnosed high-risk B-ALL patients with CRLF2 rearrangements.[28]

Hypomethylating agents

DNA methyltransferase (DNMT) inhibition has been investigated as a therapeutic strategy in order to undo or decrease the repression of regulatory genes. Decitabine and azacitidine are cytosine analogs that inhibit DNA methyltransferase by targeting it for degradation, thus causing hypomethylation of key regulatory domains on DNA and leading to differentiation and suppression of tumor growth. In vitro studies of relapsed ALL, cell samples treated with DNMT inhibitors with or without histone deacetylase inhibitors (HDAC) showed restoration of normal gene expression.[9]

A phase I TACL trial studied azacitidine in combination with chemoteherapy for relapsed ALL or AML.[9] Decitabine in combination with hyper-CVAD was shown to have efficacy for R/R ALL in a MD Anderson phase I trial. Moreover, decitabine plus vorinostat (a histone deacetylase inhibitor) -given prior to vinvristine, prednisone, asparaginase and doxorubicin- achieved promising outcomes in a phase II clinical trial for R/R ALL. Decitabine has also been examined in preclinical trials of early T-cell precursor ALL (ETP-ALL), showing synergistic role with conventional chemotherapy.[28]

PI3K/mTOR

The phosphatidylinositol 3-kinase/protein kinase and mammalian target of rapamycin (mTOR) pathways are shown to be constitutively activated in 50-75% of T-ALL. Constitutive activation may also play a role in chemotherapy resistance. Everolimus, temsirolimus and sirolimus inhibit mTOR kinase activity by binding intracellular protein FKBP-1, forming a complex that inhibits mTOR signaling. The significance of PI3K/AKT/mTOR as therapeutic target in T-ALL management has been highlightened in several clinical trials in combination with first line chemotherapy and R/R disease.[28] Ongoing trials include National Cancer Institute (NCI) phase II trial studying temsirolimus in R/R ALL (NCT00084916), phase I COG study ADVL1114 exploring temsirolimus in combination with intensive re-induction therapy for R/R ALL (NCT01403415), phase I trial evaluating sirolimus and corticosteroids in R/R ALL (NCT00874562) and NCI-sponsored multi-center phase II trial with TORC1/2 inhibitor sapanisertib in R/R ALL in adults (NCT02484430). Results from these trials are pending.

BTK, BCL2 and PD-1 Inhibitors

The BCR signaling pathway plays an essential role in B-cell development and function, which includes the Bruton's tyrosine kinase (BTK). Preclinical studies have investigated the activity of ibrutinib, an oral selective irreversible inhibitor of BTK, in B-ALL, demonstrating suppression of BCR-positive ALL proliferation in vitro and in vivo, These data provide a rationale for clinical testing of B-ALL with active pre-BCR signaling, either as a single agent or in combination with other therapies. A phase II study of ibrutinib and blinatumomab in patients with R/R B-ALL will soon be opened to recruitment (NCT02997761).[9]

The B cell lymphoma-2 (BCL-2) protein tightly regulates cell death. BCL-2 inhibitors block the binding of BCL-2 to anti-apoptotic proteins, triggering cell death. While Navitoclax (ABT-263) has been abandoned due to dose-limiting thrombocytopenia, a second-generation, selective BCL-2 inhibitor Venetoclax (ABT-199) has demonstrated activity against ALL in xenograft models, without yet being tested in ALL.[9]

Nivolumab is a human IgG4 monoclonal antibody against anti-programmed cell death protein-1 (PD-1). Malignant cells evade immunosurveillance as they lack expression of costimulatory molecules and induce T-cell anergy. Nivolumab dampens T-cell anergy and allows immunosurveillance of malignant cells to take place. A phase I study is recruiting patients to investigate nivolumab plus dasatinib in patients with R/R Ph+ ALL (NCT02819804). A phase I study is investigating blinatumomab, nivolumab with or without ipilimumab in patients with poor risk R/R CD19+ precursor B-ALL (NCT02879695).[9]

DEVELOPMENTS IN PHILADELPHIA CHROMOSOME-POSITIVE ALL

Ph+ ALL is the most common B-ALL subtype in adults, comprising 20-30% of all cases, while its incidence increases with age. Traditionally, Ph+ ALL was considered as the subcategory with the worst prognosis having 5-year OS <10%.[60] However, the introduction of TKI inhibitors has improved dramatically the outcome with CR rates and 5-year OS >40%.[61-67] Furthermore, in the majority of studies, the use of TKIs has improved the number of patients who proceed to allo-SCT (in most trials >50%).[61,62,64,65] Thus, the common therapeutic strategy includes the introduction of imatinib or dasatinib in induction, consolidation and maintenance, with continuous administration being more preferable than interrupted.[62]

Issues to be clarified remain: 1. the choice of suitable TKI, 2. the de-escalation of induction chemotherapy regimen, 3. the significance of MRD, 4. the need for allo-SCT and 5.the resistance to TKIs.

Regarding the first issue, only imatinib and dasatanib have gained approval from the FDA. Most scientific studies conducted with imatinib at doses between 600-800mg.[68] Although comparative studies do not exist, the outcomes are similar.[69] However, treatment with dasatinib results in more stable molecular responses, having more toxicities.[60] Moreover, dasatinib has been reported to have a higher CNS penetration.[68] Certainly, the use of second generation TKIs is related with increased incidence of T315I mutations, possibly due to selection of pre-existing mutated clones.[60] Dasatinib was approved for the treatment of patients with Ph+ ALL resistant or intolerant to prior therapy with imatinib following the results of the pivotal START-L study. This study, which included patients with Ph+ ALL resistant or intolerant to imatinib (29% with T315I), revealed complete cytogenetic response in 58%. Major hematologic responses were achieved in 42% of patients, 67% of whom remained progression-free. Meanwhile, dasatinib as first-line treatment leads to 2-year OS>60%.[68] Substantial hematologic and cytogenetic responses have been also achieved with nilotinib and ponatinib.

Especially ponatinib has the potential of minimizing the evolution of TKI-resistant clones with T315I mutation, decreasing the risk of relapse. The T315I mutation is rarely present at the initial diagnosis but more often appears after an initial response to therapy. This mutation can be detected in 70% of patients relapsing after being treated with a second-generation TKI. However, although ponatinib can achieve 41% major haematologic response and 47% major cytogenetic response, many patients relapsed early and the median OS was less than 1 year, according to phase 2 PACE study. The superior outcomes of ponatinib in comparison with dasatinib when they were used in combination with hyper-CVAD, include CR rates 100%, complete molecular response 78% and 3-year overall survival 83%.[70] Yet, the development of novel "compounds" against T315L mutations, which are resistant to ponatinib is a matter of concern.[68]

Regarding the de-escalation of induction treatment, the addition of TKIs in systemic chemotherapy leads to early mortality ~10% in AYAs, while is higher in older patients.[63,65] In contrast, a less toxic therapeutic approach combining steroids or low-dose chemotherapy with a TKI demonstrates CR rates of almost 100%. While this strategy is commonly adopted in older patients,[60] the use of de-escalated chemotherapeutic regimens in AYAs remains strongly doubtful. In a GRAALL group study, 268 patients, 18-59 years old, were randomized between imatinib/vincristine/dexamethasone and imatinib/hyper-CVAD. In the arm of less intensive chemotherapy, increased incidence of CR rates was observed (98% vs. 91%, p=0.006) due to decreased early mortality, but without significant

difference in major molecular response and OS.[71] Similar strategy is followed by other study groups for ALL, such as GIMEMA and PETHEMA.[60]

There is interesting scientific evidence concerning the significance of MRD and the level of the molecular response, during the last 2-3 years.[71-73] The achievement of early complete molecular response is related with lower relapse risk and more favorable outcome. Though, the aim is not clearly defined, in contrast with chronic myeloid leukemia, regarding not only the level of molecular response but also the time it should be studied. Complete molecular response with sensitivity 0.01% within 3 months from treatment initiation using the combination hyper-CVAD/TKI is related with more favorable OS and RFS, while allo-SCT is not performed. (median OS: 127 vs. 38 months and RFS 126 vs. 18 months). The group of patients having WBC<$30X10^9$/L and p190 transcript demonstrate higher possibility of achieving major and complete molecular remission.[71]

Given that the only possibility of cure in Ph+ ALL is hematopoietic stem cell transplantation, even if remission is achieved by chemotherapy combined with a TKI, Ph+ ALL patients in remission should be referred to allo-SCT.[66] However, the long-term results of regimens combining chemotherapy with TKIs suggest the possibility of long-term survival in a proportion of patients who do not undergo a transplant.[71,72] Patients with early complete molecular response are likely to have similar outcome with or without allo-SCT. Currently, the role of autologous transplantation is being also re-examined in these patients.[71]

Concerning the management of TKI-resistance, both blinatumomab and INO are efficient in R/R disease. Despite blinatumomab has not gained formal approval for R/R disease, ALCANTARA study[74] revealed CR rates 36%, while 44% of patients underwent allo-SCT. Similar results have been achieved with INO.[34] An ECOG study group trial compares the efficacy of TKIs/corticosteroid/blinatumomab combination with TKIs/chemotherapy combination, while SWOG study group examines the role of dasatinib/blinatumomab in first-line treatment for older patients (>65 years-old).[60]

CONCLUSION

The adoption of pediatric-type intensive chemotherapeutic protocols has definitely improved the outcome of ALL in younger patients, while the monitoring of MRD is the most important tool for the choice of patients who are eligible for allo-SCT. The development of two novel monoclonal antibodies, blinatumomab and INO constitute important therapeutic option for R/R disease. Yet, the evolving technology of CAR-Ts has already revolutionized the treatment of adult B-ALL.

Conflict of Interest: Advisory Board: Amgen,
Advisory Board-Honoraria: Pfizer, Gilead,
Honoraria: Novartis.

REFERENCES

1. Larson RA, Anastasi J. Acute lymphoblastic leukemia: Clinical presentation, diagnosis and classification. In: Faderl SH, Kantarjian HM, Estey EH, editors. Hematologic malignancies: acute leukemias. Springer-Verlag Berlin Heidelberg; 2008. p. 109-18.

2. Pulte D, Gondos A, Brenner H. Improvement in survival in younger patients with acute lymphoblastic leukemia from the 1980s to the early 21st century. Blood. 2009 Feb;-113(7):1408-11.

3. Aldoss IT, Marcucci G, Pullarkat V. Treatment of acute lymphoblastic leukemia in adults: applying lessons learned in children. Oncology (Williston Park, NY). 2016 Dec;30(12):1080-91.

4. DeAngelo DJ, Stevenson KE, Dahlberg SE, Silverman LB, Couban S, Supko JG, et al. Long-term outcome of a pediatric-inspired regimen used for adults aged 18-50 years with newly diagnosed acute lymphoblastic leukemia. Leukemia. 2015 Mar;29(3):526-34.

5. Huguet F, Leguay T, Raffoux E, Thomas X, Beldjord K, Delabesse E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol. 2009 Feb;27(6):911-8.

6. Sancho JM, Ribera JM, Xicoy B, Morgades M, Oriol A, Tormo M, et al. Results of the PETHEMA ALL-96 trial in elderly patients with Philadelphia chromosome-negative acute lymphoblastic leukemia. Eur J Haematol. 2007 Feb;78(2):102-10.

7. Asselin BL, Devidas M, Wang C, Pullen J, Borowitz MJ, Hutchison R, et al. Effectiveness of high-dose methotrexate in T-cell lymphoblastic leukemia and advanced-stage lymphoblastic lymphoma: a randomized study by the Children's Oncology Group (POG 9404). Blood. 2011 Jul;118(4):874-83.

8. Hoelzer D, Thiel E, Arnold R, Beck J, Beelen DW, Bornhäuser M, et al. Successful subtype oriented treatment strategies in adult T-All; Results of 744 patients treated in three consecutive GMALL studies. Blood. 2009;114(22):324.

9. Man LM, Morris AL, Keng M. New therapeutic strategies in acute lymphocytic leukemia. Curr Hematol Malig Rep. 2017 Jun;12(3):197-206.

10. Kozlowski P, Åström M, Ahlberg L, Bernell P, Hulegårdh E, Hägglund H, et al. High relapse rate of T cell acute lymphoblastic leukemia in adults treated with Hyper-CVAD chemotherapy in Sweden. Eur J Haematol. 2014;92(5):377-81.

11. Aldoss I, Al Malki MM, Stiller T, Cao T, Sanchez JF, Palmer J, et al. Implications and management of central nervous system involvement before allogeneic hematopoietic cell transplantation in acute lymphoblastic leukemia. Biol Blood Marrow Transplant. 2016 Mar;22(3):575-8.

12. Goldstone AH, Richards SM, Lazarus HM, Tallman MS, Buck G, Fielding AK, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance

13. chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). Blood. 2008 Feb;111(4):1827-33.

13. Bassan R, Intermesoli T, Scattolin A, Viero P, Maino E, Sancetta R, et al. Minimal residual disease assessment and risk-based therapy in acute lymphoblastic leukemia. Clin Lymphoma Myeloma Leuk. 2017 Jul;17S:S2-S9.

14. Bruggemann M, Raff T, Flohr T, Gokbuget N, Nakao M, Droese J, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood. 2006 Feb;107(3):1116-23.

15. Gokbuget N, Kneba M, Raff T, Trautmann H, Bartram CR, Arnold R, et al. Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. Blood. 2012 Aug;120(9):1868-76.

16. Bassan R, Spinelli O, Oldani E, Intermesoli T, Tosi M, Peruta B, et al. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). Blood. 2009 Apr;113(18):4153-62.

17. Bassan R, Spinelli O, Oldani E, Intermesoli T, Tosi M, Peruta B, et al. Different molecular levels of post-induction minimal residual disease may predict hematopoietic stem cell transplantation outcome in adult Philadelphia-negative acute lymphoblastic leukemia. Blood Cancer J. 2014 Jul;4:e225.

18. Beldjord K, Chevret S, Asnafi V, Huguet F, Boulland ML, Leguay T, et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. Blood. 2014 Jun;123(24):3739-49.

19. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009 Jan;360(5):470-80.

20. Hoelzer D. Novel antibody-based therapies for acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2011;2011:243-9.

21. Maury S, Huguet F, Leguay T, Lacombe F, Maynadie M, Girard S, et al. Adverse prognostic significance of CD20 expression in adults with Philadelphia chromosome-negative B-cell precursor acute lymphoblastic leukemia. Haematologica. 2010 Feb;95(2):324-8.

22. Hoelzer D, Huettmann A, Kaul F, Irmer S, Jaekel N, Mohren M, et al. Immunochemotherapy with rituximab improves molecular CR rate and outcome in CD20+ B-Lineage standard and high risk patients; results of 263 CD20+ patients studied prospectively in GMALL study 07/2003. Blood. 2010;116(21):170.

23. Thomas DA, Faderl S, O'Brien S, Bueso-Ramos C, Cortes J, Garcia-Manero G, et al. Chemoimmunotherapy with hyper-CVAD plus rituximab for the treatment of adult Burkitt and Burkitt-type lymphoma or acute lymphoblastic leukemia. Cancer. 2006 Apr;106(7):1569-80.

24. Maury S, Chevret S, Thomas X, Heim D, Leguay T, Huguet F, et al. Rituximab in B-Lineage adult acute lymphoblastic leukemia. N Engl J Med. 2016 Sep;375(11):1044-53.

25. Sasaki K, Kantarjian HM, Ravandi F, Daver N, Kadia TM, Khouri RB, et al. Frontline ofatumumab in combination with Hyper-CVAD for adult patients with CD-20 positive acute lymphoblastic leukemia (ALL): interim result of a phase ii clinical trial. Blood. 2016;128(22):2783.

26. Kantarjian HM, Thomas D, Ravandi F, Faderl S, Jabbour E, Garcia-Manero G, et al. Defining the course and prognosis of adults with acute lymphocytic leukemia in first salvage after induction failure or short first remission duration. Cancer. 2010 Dec;116(24):5568-74.

27. Jabbour E, Short NJ, Jorgensen JL, Yilmaz M, Ravandi F, Wang SA, et al. Differential impact of minimal residual disease negativity according to the salvage status in patients with relapsed/refractory B-cell acute lymphoblastic leukemia. Cancer. 2017 Jan;123(2):294-302.

28. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J. 2017 Jun;7(6):e577.

29. Advani AS, McDonough S, Coutre S, Wood B, Radich J, Mims M, et al. SWOG S0910: a phase 2 trial of clofarabine/cytarabine/epratuzumab for relapsed/refractory acute lymphocytic leukaemia. Br J Haematol. 2014 May;165(4):504-9.

30. Chevallier P, Chantepie S, Huguet F, Raffoux E, Thomas X, Leguay T, et al. Hyper-CVAD plus epratuzumab as salvage regimen for younger relapsed/refractory CD22+ B acute lymphoblastic leukemia (ALL) patients: results of the phase 2 prospective cheprall study. Blood. 2016;128(22):4018.

31. Kantarjian H, Thomas D, Jorgensen J, Kebriaei P, Jabbour E, Rytting M, et al. Results of inotuzumab ozogamicin, a CD22 monoclonal antibody, in refractory and relapsed acute lymphocytic leukemia. Cancer. 2013 Aug;119(15):2728-36.

32. Kantarjian H, Thomas D, Jorgensen J, Jabbour E, Kebriaei P, Rytting M, et al. Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. Lancet Oncol. 2012 Apr;13(4):403-11.

33. Advani AS, Stein AS, Kantarjian HM, Shustov AR, DeAngelo DJ, Ananthakrishnan R, et al. A phase II study of weekly inotuzumab ozogamicin (InO) in adult patients with CD22-positive acute lymphoblastic leukemia (ALL) in second or later salvage. Blood. 2014;124(21):2255.

34. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med. 2016 Aug;375(8):740-53.

35. Jabbour E, Ravandi F, Kebriaei P, Huang X, Short NJ, Thomas D, et al. Salvage chemoimmunotherapy with inotuzumab ozogamicin combined with mini-hyper-CVD for patients with relapsed or refractory Philadelphia chromosome-negative acute lymphoblastic leukemia: a phase 2 clinical trial. JAMA Oncol. 2018 Feb 1;4(2):230-4.

36. Kantarjian HM, Lioure B, Kim SK, Atallah E, Leguay T, Kelly K, et al. A phase II study of coltuximab ravtansine (SAR3419) monotherapy in patients with relapsed or refractory acute lymphoblastic leukemia. Clin Lymphoma Myeloma Leuk. 2016 Mar;16(3):139-45.

37. Topp MS, Gokbuget N, Zugmaier G, Klappers P, Stelljes M, Neumann S, et al. Phase II trial of the anti-CD19 bispecific T cell-engager blinatumomab shows hematologic and molecular remissions in patients with relapsed or refractory B-precursor acute lymphoblastic leukemia. J Clin Oncol. 2014 Dec;32(36):4134-40.

38. Topp MS, Gokbuget N, Stein AS, Zugmaier G, O'Brien S, Bargou RC, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. Lancet Oncol. 2015 Jan;16(1):57-66.

39. Kantarjian H, Stein A, Gokbuget N, Fielding AK, Schuh AC, Ribera JM, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. N Engl J Med. 2017 Mar;376(9):836-47.

40. Gokbuget N, Kelsh M, Chia V, Advani A, Bassan R, Dombret H, et al. Blinatumomab vs historical standard therapy of adult relapsed/refractory acute lymphoblastic leukemia. Blood Cancer J. 2016 Sep;6(9):e473.

41. Kantarjian HM, Stein AS, Bargou RC, Grande Garcia C, Larson RA, Stelljes M, et al. Blinatumomab treatment of older adults with relapsed/refractory B-precursor acute lymphoblastic leukemia: Results from 2 phase 2 studies. Cancer. 2016 Jul;122(14):2178-85.

42. Topp MS, Gokbuget N, Zugmaier G, Degenhard E, Goebeler ME, Klinger M, et al. Long-term follow-up of hematologic relapse-free survival in a phase 2 study of blinatumomab in patients with MRD in B-lineage ALL. Blood. 2012 Dec;120(26):5185-7.

43. Gokbuget N, Zugmaier G, Klinger M, Kufer P, Stelljes M, Viardot A, et al. Long-term relapse-free survival in a phase 2 study of blinatumomab for the treatment of patients with minimal residual disease in B-lineage acute lymphoblastic leukemia. Haematologica. 2017 Apr;102(4):e132-e5.

44. Aldoss I, Song J, Stiller T, Nguyen T, Palmer J, O'Donnell M, et al. Correlates of resistance and relapse during blinatumomab therapy for relapsed/refractory acute lymphoblastic leukemia. Am J Hematol. 2017 Sep;92(9):858-65.

45. Duell J, Dittrich M, Bedke T, Mueller T, Eisele F, Rosenwald A, et al. Frequency of regulatory T cells determines the outcome of the T-cell-engaging antibody blinatumomab in patients with B-precursor ALL. Leukemia. 2017 Oct;31(10):2181-90.

46. Kantarjian H, Jabbour E, Topp MS. Blinatumomab for Acute Lymphoblastic Leukemia. N Engl J Med. 2017 Jun;376(23):e49.

47. Kohnke T, Krupka C, Tischer J, Knosel T, Subklewe M. Increase of PD-L1 expressing B-precursor ALL cells in a patient resistant to the CD19/CD3-bispecific T cell engager antibody blinatumomab. J Hematol Oncol. 2015 Oct;8:111.

48. Zugmaier G, Gokbuget N, Klinger M, Viardot A, Stelljes M, Neumann S, et al. Long-term survival and T-cell kinetics in relapsed/refractory ALL patients who achieved MRD response after blinatumomab treatment. Blood. 2015 Dec;126(24):2578-84.

49. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med. 2014 Feb;6(224):224ra25.

50. Gardner R, Finney O, Smithers H, Leger KJ, Annesley CE, Summers C, et al. CD19CAR T cell products of defined CD4:CD8 composition and transgene expression show prolonged persistence and durable MRD-negative remission in pediatric and young adult B-Cell ALL. Blood. 2016;128(22):219.

51. Grupp SA, Maude SL, Shaw PA, Aplenc R, Barrett DM, Callahan C, et al. Durable Remissions in Children with Relapsed/Refractory ALL Treated with T Cells Engineered with a CD19-Targeted Chimeric Antigen Receptor (CTL019). Blood. 2015;126(23):681.

52. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet (London, England)2015 Feb;385(9967):517-28.

53. Lee DW, Stetler-Stevenson M, Yuan CM, Shah NN, Delbrook C, Yates B, et al. Long-term outcomes following CD19 CAR T cell therapy for B-ALL Are superior in patients receiving a fludarabine/cyclophosphamide preparative regimen and Post-CAR hematopoietic stem cell transplantation. Blood. 2016;128(22):218.

54. Park JH, Riviere I, Wang X, Bernal Y, Purdon T, Halton E, et al. Implications of minimal residual disease negative complete remission (MRD-CR) and allogeneic stem cell transplant on safety and clinical outcome of CD19-Targeted 19-28z CAR modified t cells in adult patients with relapsed, refractory B-Cell ALL. Blood. 2015;126(23):682.

55. Shah B, Huynh V, Sender LS, Lee DW, Castro JE, Wierda WG, et al. High Rates of Minimal Residual Disease-Negative (MRD−) Complete Responses (CR) in Adult and Pediatric and Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia (R/R ALL) Treated With KTE-C19 (Anti-CD19 Chimeric Antigen Receptor [CAR] T Cells): Preliminary Results of the ZUMA-3 and ZUMA-4 Trials. Blood. 2016;128(22):2803.

56. Shah NN, Stetler-Stevenson M, Yuan CM, Shalabi H, Yates B, Delbrook C, et al. Minimal Residual Disease Negative Complete Remissions Following Anti-CD22 Chimeric Antigen Receptor (CAR) in Children and Young Adults with Relapsed/Refractory Acute Lymphoblastic Leukemia (ALL). Blood. 2016;128(22):650.

57. Braig F, Brandt A, Goebeler M, Tony HP, Kurze AK, Nollau P, et al. Resistance to anti-CD19/CD3 BiTE in acute lymphoblastic leukemia may be mediated by disrupted CD19 membrane trafficking. Blood. 2017 Jan;129(1):100-4.

58. Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J, et al. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J Clin Invest. 2016 Oct;126(10):3814-26.

59. Feucht J, Kayser S, Gorodezki D, Hamieh M, Doring M, Blaeschke F, et al. T-cell responses against CD19+ pediatric acute lymphoblastic leukemia mediated by bispecific T-cell engager (BiTE) are regulated contrarily by PD-L1 and CD80/CD86 on leukemic blasts. Oncotarget. 2016 Nov;7(47):76902-19.

60. Chiaretti S, Foa R. Management of adult Ph-positive acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2015;2015:406-13.

61. Ribera JM, Garcia O, Montesinos P, Brunet S, Abella E, Barrios M, et al. Treatment of young patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia using increased dose of imatinib and deintensified chemotherapy before allogeneic stem cell transplantation. Br J Haematol. 2012 Oct;159(1):78-81.

62. Wassmann B, Pfeifer H, Goekbuget N, Beelen DW, Beck J, Stelljes M, et al. Alternating versus concurrent schedules of imatinib and chemotherapy as front-line therapy for Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). Blood. 2006 Sep;108(5):1469-77.

63. de Labarthe A, Rousselot P, Huguet-Rigal F, Delabesse E, Witz F, Maury S, et al. Imatinib combined with induction or consolidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the GRAAPH-2003 study. Blood. 2007 Feb;109(4):1408-13.

64. Ribera JM, Oriol A, Gonzalez M, Vidriales B, Brunet S, Esteve J, et al. Concurrent intensive chemotherapy and imatinib before and after stem cell transplantation in newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Final results of the CSTIBES02 trial. Haematologica. 2010 Jan;95(1):87-95.

65. Bassan R, Rossi G, Pogliani EM, Di Bona E, Angelucci E, Cavattoni I, et al. Chemotherapy-phased imatinib pulses improve long-term outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Northern Italy Leukemia Group protocol 09/00. J Clin Oncol. 2010 Aug;28(22):3644-52.

66. Fielding AK, Rowe JM, Buck G, Foroni L, Gerrard G, Litzow MR, et al. UKALLXII/ECOG2993: addition of imatinib to a standard treatment regimen enhances long-term outcomes in Philadelphia positive acute lymphoblastic leukemia. Blood. 2014 Feb;123(6):843-50.

67. Daver N, Thomas D, Ravandi F, Cortes J, Garris R, Jabbour E, et al. Final report of a phase II study of imatinib mesylate with hyper-CVAD for the front-line treatment of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Haematologica. 2015 May;100(5):653-61.

68. Ronson A, Tvito A, Rowe JM. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia. Curr Treat Options Oncol. 2017 Mar;18(3):20.

69. Ravandi F, Jorgensen JL, Thomas DA, O'Brien S, Garris R, Faderl S, et al. Detection of MRD may predict the outcome of patients with Philadelphia chromosome-positive ALL treated with tyrosine kinase inhibitors plus chemotherapy. Blood. 2013 Aug;122(7):1214-21.

70. Jabbour E, Kantarjian H, Ravandi F, Thomas D, Huang X, Faderl S, et al. Combination of hyper-CVAD with ponatinib as first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia: a single-centre, phase 2 study. Lancet Oncol. 2015 Nov;16(15):1547-55.

71. Chalandon Y, Thomas X, Hayette S, Cayuela JM, Abbal C, Huguet F, et al. Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia. Blood. 2015 Jun;125(24):3711-9.

72. Lussana F, Intermesoli T, Gianni F, Boschini C, Masciulli A, Spinelli O, et al. Achieving molecular remission before allogeneic stem cell transplantation in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: impact on relapse and long-term outcome. Biol Blood Marrow Transplant. 2016 Nov;22(11):1983-7.

73. Short NJ, Jabbour E, Sasaki K, Patel K, O'Brien SM, Cortes JE, et al. Impact of complete molecular response on survival in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood. 2016 Jul;128(4):504-7.

74. Martinelli G, Boissel N, Chevallier P, Ottmann O, Gokbuget N, Topp MS, et al. Complete Hematologic and Molecular Response in Adult Patients With Relapsed/Refractory Philadelphia Chromosome-Positive B-Precursor Acute Lymphoblastic Leukemia Following Treatment With Blinatumomab: Results From a Phase II, Single-Arm, Multicenter Study. J Clin Oncol. 2017 Jun;35(16):1795-802.