Progress in adult ALL: incorporation of new agents to frontline treatment

Jessica Leonard[1] *and Wendy Stock*[2]

[1]*Division of Hematology and Medical Oncology, Knight Cancer Institute, Oregon Health and Science University, Portland, OR; and* [2]*University of Chicago Medicine, Chicago, IL*

Treatment of acute lymphoblastic leukemia (ALL) in adults remains a challenge, as the delivery of intensive chemotherapeutic regimens in this population is less feasible than it is in the pediatric population. This has led to higher rates of treatment-related toxicity as well as lower overall survival in the adult population. Over the past several years, a host of novel therapies (eg, immunotherapy and targeted therapies) with better tolerability than traditional chemotherapy are now being introduced into the relapsed/refractory population with very encouraging results. Additionally, insights into how to choose effective therapies for patients while minimizing drug toxicity through pharmacogenomics and the use of minimal residual disease (MRD) monitoring to escalate/de-escalate therapy have enhanced our ability to reduce treatment-related toxicity. This has led to the design of a number of clinical trials which incorporate both novel therapeutics as well as MRD-directed treatment pathways into the frontline setting. The use of increasingly personalized treatment strategies for specific disease subsets combined with standardized and rapid molecular diagnostic testing in the initial diagnosis and frontline treatment of ALL will hopefully lead to further improvements in survival for our adult patients.

Learning Objectives

- Review recent progress in treatment of adults with acute lymphoblastic leukemia (ALL)
- Review novel therapies for patients with relapsed disease
- Provide an overview of strategies to introduce new agents to the frontline setting and optimize outcomes

Introduction

Acute lymphoblastic leukemia (ALL) has long been considered a challenging disease to treat in the adult population, with historic cure rates of just 20% to 40%. However, in addition to the significant improvements in survival for adults with Philadelphia chromosome-positive ALL (Ph^+ ALL) (discussed in a separate section of this education session by Dr. Ravandi), the past decade has shown several important advances in the field, which have translated into improved outcomes for adult patients. A host of novel therapies are now being introduced into the relapsed/refractory population with very encouraging results, particularly within the realm of immunotherapy. In addition, new insights into disease pathogenesis, including the recent identification of a new subset of B-precursor ALL, Philadelphia chromosomelike (Ph-like) ALL, is providing new opportunities for personalizing treatment approaches with targeted agents. In this article, we will first briefly review "state-of-the-art ALL therapy," which guides our treatment strategies today. We will then discuss novel therapies being used in the relapsed/refractory setting as well as the evidence behind each of these therapies. Finally, we will discuss current trials as well as other potential avenues through which these new therapies might be introduced into the frontline setting.

State-of-the-art ALL therapy

Learning from the kids. One important change in the management of adult ALL that has occurred in recent years has been a focus on extending the use of intensive pediatric regimens into the adult population. Based on a number of retrospective studies showing a benefit in survival for young adults who were treated with pediatric regimens,[1-3] several prospective phase 2 clinical trials in adults with both B- and T-precursor ALL have now been carried out by large, international cooperative groups. These trials consistently demonstrate significant improvements in event-free and overall survival as compared with historical controls with overall survival rates ranging from 60% to 80%.[4-8] One of the potential benefits to these treatment strategies is the avoidance of allogeneic hematopoietic stem cell transplant (HSCT), because the regimens themselves are delivered with curative intent. The upper age limit for treatment with a pediatric regimen has yet to be defined; several of the studies published[4,5] suggest that these regimens are feasible and effective for adults up to the age of 45 to 50 years. However, due to the age-related increase in toxicities that occur with regimens that intensively employ glucorticoids, vincristine and asparaginase, concern for increasing rates of treatment-related toxicities currently limits the use of pediatric regimens in older adults. Other treatment strategies have yielded high survival rates in younger patients; a single institution phase 2 study has reported similar good treatment outcomes with the use of the hyper-CVAD regimen compared with a pediatric regimen in young

Conflict-of-interest disclosure: J.L. declares no competing financial interests. W.S. is on the Board of Directors or an advisory committee and has consulted for Novartis and Pfizer.

Off-label drug use: None disclosed.

adults.[9] The appropriateness of using hyper-CVAD in the adolescents and young adults (AYA) population is controversial, however, and many would not view this as an appropriate front line therapy. It is important to note that all of these intensive regimens can only result in improved outcomes if the treatment is given according to planned protocol specifications; this requires the support of dedicated, knowledgeable providers to support the patients and successfully deliver these arduous regimens.

Moving toward subset-specific treatment: rituximab and nelarabine. Another advancement in the treatment of ALL is the addition of an anti-CD20 monoclonal antibody, rituximab to standard chemotherapy for the 30% to 50% of adults with CD20-expressing precursor-B ALL. CD20 expression was noted to be an adverse prognostic factor in B-ALL[10] and led to a phase 2 trial that applied rituximab to frontline therapy. The investigators found that addition of rituximab to standard chemotherapy for patients with $CD20^+$ ALL improved treatment outcomes compared with historical controls.[11] A large, multicenter phase 3 trial employing an intensive pediatric-style regimen has now confirmed improved event-free survival in adults up to age 60 years with $CD20^+$ ALL (>20% CD20 expression) treated with rituximab and chemotherapy.[12] Similarly, the addition of rituximab to dose-dense chemotherapy significantly improves event-free survival in adults with Burkitt's lymphoma.[13,14] Although mild hypersensitivity reactions are common, rituximab adds little in the way of toxicity and may now be considered standard of care for patients with $CD20^+$ disease. For patients with precursor T-cell ALL, the addition of nelarabine to an intensive chemotherapy backbone in pediatric patients with newly diagnosed, high risk T-ALL was well-tolerated, with a 5-year EFS significantly higher than historic controls.[15] This strategy is being evaluated in the treatment of adults with T-ALL as well[16]; results from several large phase 3 studies are eagerly awaited to determine the impact of frontline nelarabine that will inform the design of future trials.

New biological insights and targeted treatments will lead to better treatment outcomes. Although each of these approaches represent important progress, survival rates for adults with ALL still lag significantly behind that of the pediatric population. Innovative treatment strategies are now moving into the clinic with the goal of further improving responses and enhancing survival rates. Advancements in our understanding of the differences in disease biology in adult ALL are providing important insight into novel treatment targets. A major advance has been the recent identification of a new biological subset, Ph-like ALL, which was identified from gene-expression array profiling.[17-19] Ph-like ALL is characterized by a large variety of novel fusion genes that result in aberrant kinase signaling and is associated with adverse prognosis in both pediatric and adult ALL. Importantly, Ph-like may comprise nearly one-third of adult precursor B-ALL and may, to a certain extent, explain the adverse treatment outcomes for adults with ALL.[17-19] This is in part because patients with Ph-like ALL are less likely to attain MRD negativity after induction (30% MRD negativity in Ph-like vs 87% for other B-all, with Ph^+ ALL excluded).[20,21] Of note, Ph-like leukemias demonstrate significant in vitro sensitivity to targeted kinase inhibitors,[19] and a number of case studies in both pediatric as well as adult patients have now reported the activity of targeted tyrosine kinase inhibitors (TKIs) used in the treatment of patients identified with this signature who have refractory disease.[19,22,23]

Insights into how to choose effective therapies for patients while minimizing drug toxicity through both individualized drug selection and better supportive care are additional methods by which we can improve our outcomes. Recent advancements in the field of pharmacogenomics[24] allows us to predict, based on specific gene polymorphisms, which patients will develop rapid resistance to medications such as asparaginase[25] or methotrexate,[26,27] whereas other genetic polymorphisms predict specific complications, such as osteonecrosis from steroids[28-30] and neuropathy from vincristine.[31,32] Although not widely employed to guide treatment decisions, the development of commercially available tests to determine drug activity levels is being adopted by many treatment centers to personalize drug delivery. An algorithm for assessing asparaginase activity with associated recommendations for when to switch to *Erwinia* asparaginase in the instance of PEG-asparaginase resistance has been detailed[33] with the goal of detecting patients who have silent inactivation and/or accelerated clearance of asparaginase. Finally, guidelines for the management of asparaginase toxicity[34,35] that is enhanced in the adult population, describe supportive care measures that assist with the safe delivery of this medication. Furthermore, commercially available assays to measure asparaginase levels are now widely available and may facilitate the ability to facilitate individualized drug dosing that may reduce treatment-related toxicity.

These biological insights complement our increasing ability to prospectively use minimal residual disease measurements for individualizing patient prognostication and improving treatment stratification in ALL. Using MRD measurements to assess the impact of novel treatment approaches and to identify when and whom may benefit from allogeneic transplant in first remission is becoming a routine assessment tool[36,37] and will be discussed here by Dr. Bruggeman. We will now turn our discussion to the exciting new therapies that are changing the treatment landscape for adults with relapsed ALL and that are starting to find their way into the frontline with the goal of eradicating MRD and improving survival.

Immunotherapies: new strategies for relapse

Three immunotherapies, blinatumomab, inotuzumab, and CAR-T cells, have demonstrated remarkable activity in patients with relapsed/refractory disease. Each has its unique strength in terms of treatment, as well as unique toxicities. Below, we review the data that led that led to FDA approval for blinatumomab and the currently pending approval for inotuzumab (and fast-track approval for CAR-T cells) for treatment of relapsed/refractory ALL and then discuss how these therapies are being introduced into treatment regimens for patients with newly diagnosed disease.

Blinatumomab

Blinatumomab is a bispecific T-cell engaging (BiTE) antibody composed of the light and heavy chains of an anti-CD19 antibody connected via a non-immunogenic link to an anti-CD3 antibody. When both ends of the antibody are bound to antigen, cytotoxic T cells come in contact with $CD19^+$ cells and lead to perforin-mediated lysis of the target[38] (Figure 1). Because CD19 is almost universally expressed on B-ALL blasts, CD19 is an attractive target for therapy. The target population for the first trial of blinatumomab in ALL focused on treatment of minimal residual disease rather than treatment of patients with overt hematological relapse and included those with MRD relapse after an initial molecular remission after intensive chemotherapy or those who never attained MRD negativity. In this pilot study, patients received 4 cycles of blinatumomab followed by up to 3 additional cycles of blinatumomab if relapse had not occurred. Patients could undergo allogeneic HSCT at any time. Sixteen of 20 patients (80%) attained MRD negativity after the first 4-week cycle of therapy, and with

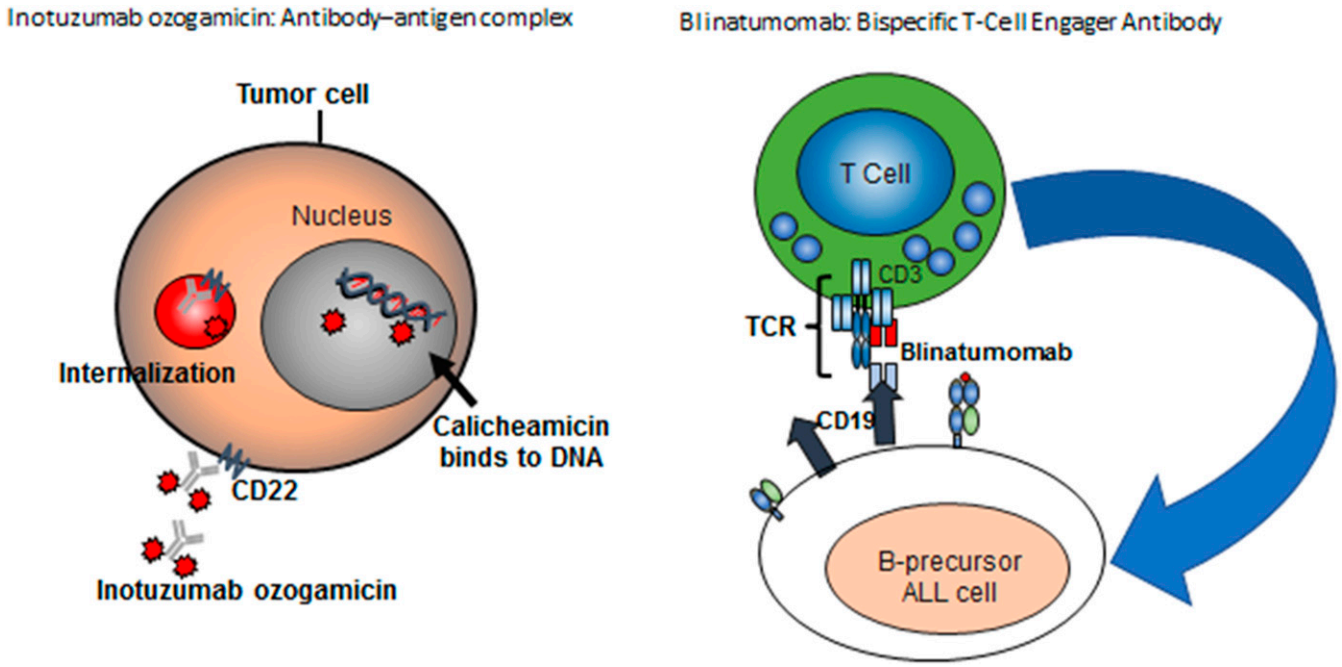

Figure 1. Mechanism of action (inotuzumab, ozogamicin, and blinatumomab).

a median follow up of 405 days, all 16 patients remained in remission.[39] Long-term follow up of this population showed that 60% and 50% of patients remained in remission at 33 months and 5 years, respectively.[40,41] With regards to HSCT, 5 of 9 patients who underwent allo HSCT remain in remission and, intriguingly, 5 of 11 without HSCT remain in long-term remission. Although numbers are small, this early study demonstrated that long term remissions were possible both with and without allogeneic HSCT in patients who initially had suboptimal responses to frontline chemotherapy and suggests a potentially important role for blinatumomab in the MRD setting.

These encouraging results prompted several phase 2 studies of blinatumomab in adult patients with relapsed or refractory ALL. The first trial included patients with relapsed disease, after any number of salvage therapies or after allogeneic HSCT.[42] Overall, the response rate after 2 cycles, including complete response or complete response without hematological recovery (CRh), was 69%. The median overall survival was 9.8 months, and although the numbers were small, the investigators found that patients treated after first salvage had the best response to therapy (11 of 11 attaining complete response [CR] or CRh), followed by those treated after second or greater salvage (6 of 10), or had relapsed after allogeneic HSCT (8 of 15). In the second and larger phase 2 trial, patients were chosen deliberately for their high risk features; patients either had primary refractory disease, had relapsed within 12 months of first remission, had relapsed after allogeneic HSCT, or had not responded to first salvage therapy. A total of 189[43] patients were treated with up to 5 cycles of blinatumomab; in this study 43% attained a CR or CRh after 2 cycles of therapy. The median overall survival in this group was 6.1 months. This is significantly shorter than in the first study, however, patients in this second phase 2 trial had much more advanced and refractory disease.

Finally, a recent phase 3 randomized trial compared blinatumomab to standard chemotherapy for relapsed ALL[44] (see Table 1 for full details). A total of 405 patients with relapsed or refractory ALL, including patients who had relapsed after allogeneic transplant, were randomized in a 2:1 fashion to blinatumomab or to multi-agent chemotherapy. Patients with Philadelphia chromosome-positive ALL were excluded. The primary outcome was overall survival, with rates of CR as a key secondary endpoint. Of the 271 patients that received blinatumomab, 44% attained a CR, CRh, or CR with incomplete platelet recovery (CRi) within the first 12 weeks as compared with 25% in the chemotherapy group. Achievement of MRD-negative status occurred in 76% of the blinatumomab-treated patients who achieved CR as opposed to 48% in the chemotherapy group. Similar to what had been noted in earlier studies, response correlated to percentage of bone marrow blasts present at the initiation of treatment, with 65.5% of patients with $<50\%$ bone marrow blasts responding to treatment vs only 34.4% of those with $>50\%$ blasts responding. The median overall survival for blinatumomab was 7.7 months as compared with 4.0 months with salvage chemotherapy. There was no difference in the percentage of patients who underwent allogeneic HSCT between the groups, with 24% of patients in each group proceeding to transplant. The rate of serious adverse events was similar in each group (87% blinatumomab vs 92% chemotherapy), although blinatumomab did lead to several unique toxicities as described below. The trial was stopped early because of the clear benefit of blinatumomab over standard of care. Blinatumomab was FDA-approved for treatment of relapsed/refractory $CD19^+$ ALL in December 2014. Of note, blinatumomab is contraindicated in patients with active central nervous system disease.

Blinatumomab demonstrates unique toxicities as compared with standard chemotherapy, including cytokine release syndrome (CRS) and neurologic toxicity, seen in 4.9% and 9.4% of patients in the phase 3 trial, respectively. CRS manifests initially with fevers and malaise, however, it can rapidly progress to life-threatening hypotension and hypoxemia requiring intensive care unit level care.[45] The risk of CRS is highest in the first cycle of treatment, and those with higher disease burden are more at risk. Current strategies to mitigate these risks include pre-treatment with steroids and lowering the

Table 1. Comparison of phase 3 studies for blinatumomab vs chemotherapy and inotuzumab vs chemotherapy

	Blinatumomab[44]	Inotuzumab[50]
Study design	Randomized phase 3, open label, 2:1 Prestratification for previous salvage therapy (yes vs no), age <35 vs >35, previous allogeneic HSCT (yes vs no)	Randomized phase 3, open label, 1:1 Pre-stratification for duration of first remission <12 mo vs >12 mo, number of salvage treatments (1st vs 2nd) and age <55 y vs >55 y)
Patient population	Primary refractory, relapse <12 mo after initial treatment, second or greater relapse, relapse after allogeneic HSCT (17%)	Patients after 1-2 salvage treatments or with primary induction failure. Relapse after allogeneic HSCT included.
Investigational treatment (and schedule)	Continuous daily infusion: blinatumomab 9 μg/d during wk 1 of induction, then 28 μg/day; administered via continuous infusion for 28 d with 14 d off in between, cycles 42 d	Weekly infusion: inotuzumab weekly; 0.8 mg IV on d 1, 0.5 mg IV d 8, 15; induction = 21-d cycle, subsequent cycles 28 d
Comparator treatments	FLAG (fludarabine, cytarabine, GCSF) High dose cytarabine High dose methotrexate-based regimen Clofarabine-based regimen	FLAG (fludarabine, cytarabine, GCSF) Cytarabine + mitoxantrone High dose cytarabine
Number of patients	405 (all included in analysis)	326 (281 in ITT analysis)
Inclusion of Ph^+ patients	No	Yes
Response rate (CR + CRh + CRi) vs control	44% (vs 25% in the control) $P = .001$	80.7% (vs 29.4% in control) $P < .004$
% MRD negative (of the responders)	76% (vs 48% in the control) P value not reported	78.4% vs 28.1%, $P < .001$
Responses in subgroups (vs control)	1st salvage 52.6% (vs 35.4%) 2nd salvage 39.6% (vs 16.3%) 3rd or more 34.8% (vs 11.5%) Previous allogeneic HSCT 40.4% (10.9%) No prior transplant 45.8% (vs 31.8%) BM blasts <50% 65.5% (vs 34.2%) BM blasts >50% 34.4% (vs 24.6%) Age <35 43.1% (vs 25.0%) Age >35 44.6% (vs 24.3%)	1st salvage 87.7% (vs 28.8%) 2nd salvage 66.7% (vs 30.6%) Previous allogeneic HSCT 76.5% (vs 27.3%) No prior transplant 81.5% (vs 29.9%) BM blasts <50%-86.7% (vs 41.4%) BM blasts >50%-77.9% (vs 24.4%) Age <55 y 80.3% (vs 31.9%) Age >55 y 81.4% (vs 25.0%) Ph^+ ALL vs normal karyotype 78.6% (vs 44.4%)* t4; 11) vs normal karyotype 33.3% (vs 33.3%)*
Survival, PFS, and OS	PFS: 7.3 mo blinatumomab vs 4.6 mo control OS: 7.7 mo blinatumomab vs 4.0 mo control $P = 0.01$	PFS: 5.0 mo inotuzumab vs 1.8 mo control $P < .001$ OS: 7.7 mo vs 6.7 in control $P = .04$
Unique treatment-related toxicities	Neurologic toxicity 6% blinatumomab vs none in control group CRS in 5% of blinatumomab vs none in control group	Veno-occlusive disease 11% inotuzumab vs 1% control
Subsequent transplant-related outcomes	All patients: 24% in the blinatumomab group vs 24% in the control group OS after transplant: 74% blinatumomab vs 75% control	All patients: 41% inotuzumab vs 11% controls $P < .001$ Duration of remission transplant 5.5 mo. INO vs 5.7 mo control

CRS, cytokine release syndrome; HSCT, hematopoietic stem cell transplant; ITT, intention to treat; PFS, progression-free survival.
*No significant difference between inotuzumab and control group.

initial infusion rate of blinatumomab for the first 7 days.[43] Neurologic side effects include tremors, somnolence, and seizures and are most common during the first cycle of therapy. Although these side effects can be severe, they are also reversible with administration of steroids and temporary withdrawal of the drug, and they are very manageable when administered by centers accustomed to treating cytokine release syndrome. The administration schedule of blinatumomab is another challenge; not only must it be given as a continuous infusion for 4 weeks, but infusion bags for outpatient use must be changed frequently (every 72 hours to every 7 days) and within a relatively short window. Although patients with access to home health companies experienced with managing blinatumomab may be able to receive this drug at home, others must return to clinic for these bag changes or must be admitted to the inpatient setting for 4 weeks to receive the drug. Increasing familiarity with management of drug toxicities and improved facility of arranging home infusions are essential as this very promising new treatment modality moves into frontline treatment programs.

Inotuzumab ozogamicin

Inotuzumab ozogamicin is a drug antibody conjugate that combines the cytotoxic agent calicheamicin with a humanized anti-CD22 antibody via a non-immunogenic linker (Figure 1). CD22 is rapidly internalized once antigen is bound, and the conjugated calicheamicin is delivered intracellularly to $CD22^+$ cells.[46] Greater than 85% to 90% of patients will express CD22 on their blasts, which will exclude a small percentage of patients from use of this agent. The

initial trial of inotuzumab was carried out in a group of patients with relapsed/refractory B-ALL. Forty-nine patients (including 3 pediatric patients) were enrolled and treated with inotuzumab 1.8 mg/m^2 once every 3 weeks (or longer if delayed recovery) until disease progression or until allogeneic HSCT.[47] The overall response rate (including CR and Cri) was 57% after 2 cycles, and the median overall survival was 5.1 months, with survival rate for the patients who responded of 7.9 months and 2.4 months for the non-responders. Twenty-two patients were able to proceed to allogeneic HSCT, and 5 of these patients developed veno-occlusive disease (VOD). In a follow-up study by the same group, an additional 41 patients with relapsed/refractory B-ALL were treated with inotuzumab but on a weekly schedule rather than every 21 to 28 days.[48] Response rates were similar (59% vs 57% in the prior study), however, adverse events including bilirubin elevation, fever, and hypotension were seen less frequently in the weekly dosing regimen. Additionally, the incidence of VOD after transplant was less frequent with the weekly dosing schedule (7% vs 17%). A subsequent phase 1/2 study confirmed safety and efficacy of the weekly ×3 doses/cycle schedule.[49]

After establishment of safety and efficacy with the weekly ×3 schedule, inotuzumab was tested in a confirmatory randomized phase 3 trial for patients with relapsed/refractory CD22$^+$ ALL[50] (see Table 1 for full details). In this study, 326 patients with Ph$^+$ or Ph$^-$ ALL scheduled to receive their first or second cycle of salvage therapy were randomized to receive inotuzumab as a weekly infusion vs standard salvage chemotherapy. Primary outcomes were rates of CR as well as overall survival. Patients receiving inotuzumab achieved a significantly higher response rate of 81% (CR or CRh) compared with chemotherapy with response rate of only 29% ($P = .001$), Achievement of MRD negative status occurred in 78.4% of the inotuzumab-treated patients achieving CR. Bone marrow blast percentage did not appear to impact overall response rates. The median overall survival in the inotuzumab group was 7.7 months in the inotuzumab group vs 6.7 months in the chemotherapy group. Importantly, a significantly higher percentage of patients who received inotuzumab were able to proceed to allogenic HSCT (41% vs 11%). The rate of serious adverse events was similar between the 2 groups, with 48% of the inotuzumab group and 46% of the standard chemotherapy group experiencing at least 1 serious adverse event. Given the very high response rates with a high percentage of patients achieving MRD negative states, application to the FDA for new drug approval for patients with relapsed/refractory CD22$^+$ ALL is pending; approval is anticipated in 2017.

The unique toxicity seen in the inotuzumab-treated group was liver-associated adverse events, including elevations in bilirubin, aminotransferase level, and VOD. VOD occurred shortly after infusion in 5 patients (2 of whom had undergone prior HSCT), and of the 48 inotuzumab patients who underwent allogeneic HSCT, 10 developed VOD at a median of 16 days, vs 1 of 20 patients who had been treated with standard chemotherapy (11% of patients total). In a multivariate analysis for the risk of developing VOD after transplant, use of a dual-alkylator conditioning regimen vs single-alkylator regimen was the only factor significantly associated with the risk of VOD. The newly FDA-approved drug, defibrotide, is used for the treatment of VOD. Of the 10 patients who developed VOD in the inotuzumab group, 7 received defibrotide, and only 1 patient died although 4 did have ongoing disease. The key to successful management of VOD is early recognition of the syndrome so that supportive care and defibrotide can be administered in a timely manner. Again, increasing familiarity of management of novel drug toxicities will be important as this therapy is introduced into the frontline setting.

CAR-T cells

One of the most exciting new strategies for the treatment of relapsed/refractory B-ALL is the use of genetically engineered T-cells, the chimeric antigen receptor T-cells (CAR-T cells). In this strategy, autologous T cells from patients are harvested and transduced with an engineered receptor for CD19 that is expressed at high levels on B-lymphoblasts; T cells are then able to exert a cytotoxic effect on tumor cells expressing the target antigen.[51] Receptors also include a co-stimulatory domain derived from 4-1BB or CD28; these "second generation CAR-T cells" show enhanced efficacy.[52] To date, four groups (MSKCC, FHCRC, CHOP, and NCI) have reported results from early trials in adults and children with heavily pretreated disease or relapse after allogeneic HSCT[53-59] (see Table 2). The majority of the data on adult patients with B-ALL comes from MSKCC and FHCRC; each group used a unique CAR-T cell construct, however, both treated a very high risk patient population (see Table 2 for details). The MSKCC group has reported outcomes for 46 patients ages 23 to 74, including 26 with 3 prior lines of treatment and 18 with prior allogeneic HSCT; 37 of 45 attained a CR of which 30 of 36 were MRD negative. The overall 6-month overall survival (OS) was 65%, however, for patients who attained MRD negativity, it was 80%.[55-57] The CAR-T cell product from the FHCRC group uses a unique process by which CD4 cells and CD8 cells are expanded and infused separately in a 1:1 ratio. Of the 26 patients ages 20 to 73 that they have treated thus far, 24 attained a CR, and although MRD negativity was not presented specifically, 22 of 26 were MRD-negative by flow.[58] At this time, data for the use of CAR-T cells in adults is still limited, although the results of two larger studies from Kite and Juno are eagerly awaited.

Challenges that remain for CAR-T cell therapy prior to becoming a more widely adopted therapy include durability of the product and management of adverse events. Although several long-term remissions have been reported, duration of response was an issue with early CAR-T cell studies, with factors such as choice of lymphodepleting conditioning regimen, CAR-T cell construct, host immune response, and resistance all contributing to the lack of persistence of CAR-T cells. The development of third generation CAR-T cells or "armored CAR-T cells" with novel constructs including co-stimulatory domains from cytokine receptors may potentially be a more active and durable product,[60] whereas the addition of fludarabine to conditioning regimens may circumvent immune rejection.[61] CAR-T cells targeting CD22 may be beneficial for patients that relapse with CD19-negative disease.[62] Serious adverse events, including CRS, neurotoxicity, and cerebral edema have been seen with CAR-T cells and have led to several deaths. Although CRS seen in the context of blinatumomab can be managed by withholding the drug, CAR-T cells are a "living drug" and therefore management becomes more challenging, often necessitating intensive care unit level care and administration of siltuximab, an antibody that neutralizes IL-6.[45] Much work remains to be done to enhance durability and better understand and manage the serious toxicities prior to introducing CAR-T cells into frontline treatment. Nevertheless, given the high rates of MRD negativity observed, improvements in management of treatment-specific toxicities, and rapid commercialization of this sophisticated approach, it is reasonable to envision future studies that will incorporate CAR-T therapy into frontline treatment strategies.

Targeted therapies for relapsed disease

As noted above, one of the most interesting findings in the adolescent and young adult age group is the identification of a very high risk group of patients, now defined as having "Ph-like ALL." Patients with this subtype of leukemia have rearrangements in a series of genes that

Table 2. Summary of CAR-T trials in acute lymphoblastic leukemia

Study center	Patients treated (n)	Median age (range)	No. salvage therapies	Lymphodepleting chemotherapy	CAR-T cell dose	Costimulatory domain	Responses: CR, no. of patients attaining MRD negativity
MSKCC55-57	44	45 (22-74)	1-3 (median 1), 4 relapsed after allo	Cy first study, then Flu/Cy	1×10^6/kg vs 3×10^6/kg	CD28 (19-28z)	CR: 37/45 (30/36 MRD-negative) 6 mo OS: 65%
FHCRC58	29	40 (20-73)	1-11 (median 3)	Cy then Flu/Cy	2×10^5/kg vs 2×10^6/kg vs 2×10^7/kg	4-1BB	CR 10/12 in CY group, 14/14 in Flu/Cy group, (23/27 MRD-negative)
CHOP53,59	53	14 (5-60)	1-4, 3 with primary refractory disease	Investigators choice	1.07×10^6 to 17.36×10^6	4-1BB	CR 50/53 (45/50 MRD-negative) 12 mo OS 78%
NCI54	20	14 (5-27)	1-8 (median 2), 7 with primary refractory disease	Flu/Cy	1×10^6/kg vs 3×10^6/kg	CD28	CR 14/20 (12/14 MRD-negative) 7.8 mo OS 52%

Cy, cyclphosphamide; Flu, fludarabine.

give them sensitivity to currently available tyrosine kinase inhibitors; those with ABL1, ABL2, CSF1R, and PDGFR demonstrate in vitro sensitivity to dasatinib and those with rearrangements in CLRF2, EPOR, and JAK2 demonstrate in vitro sensitivity to ruxolitinib.[19] AYA patients tend to have a higher proportion of the ABL-like mutations, whereas in adults >40 years, there is a much higher prevalence of the CRLF2 rearrangements,[20] suggesting that sensitivity kinase inhibitor sensitivity will vary across age groups. Murine xenograft modeling has been done with targeted kinase inhibitors for Ph-like ALL,[63] and there have been a number of anecdotal reports efficacy in the relapsed/refractory setting. As part of the initial cohort of patients that were used for the discovery of Ph-like ALL, 11 patients with poor response to initial therapy received a targeted therapy in addition to their standard therapy (7 dasatinib, 3 imatinib, and 1 ruxolitinib).[19] Although follow-up is not available for all cases, several of these patients have had ongoing durable responses and remain on targeted therapy, including one 82-year-old male who has remained in remission on dasatinib monotherapy for over 8 months. In addition, a number of case reports have now demonstrated the activity of targeted TKIs used in the treatment of patients identified with this gene expression profiling signature who have refractory disease.[22,23] In all instances, the addition of a targeted therapy to chemotherapy has been well tolerated, and patients with initially refractory disease were able to attain MRD-negative remissions. A feasibility trial for adults with relapsed Ph-like ALL adding ruxolitinib or dasatinib to hyper-CVAD therapy is ongoing (NCT02420717).

Given the relatively good safety profile and the wider ability to identify this disease subset using standardized approaches,[64] targeted frontline trials incorporating TKIs with intensive chemotherapy have recently begun. In a trial sponsored by Children's Oncology Group, pediatric and AYA patients up to the age of 30 with Ph-like ALL are first being treated with standard induction therapy. If they harbor a mutation in ABL, CSF1R, or PDGFR, dasatinib is added to standard chemotherapy starting with consolidation (NCT02883049). The primary outcome will be disease-free survival with a secondary outcome measure of change in MRD from the end of induction to the end of consolidation with the addition of dasatinib. In a separate phase 1/2 study for primary children and young adults (up to the age of 21), ruxolitinib will be added to a standard pediatric regimen for patients with CRLF2, EPOR, or JAK mutations (NCT02723994). Outcomes include both safety as well as EFS as compared with historic controls. Because targeted therapies have been shown to be successfully integrated into chemotherapy for patients with Ph^+ ALL, where they also lead to high rates of MRD negativity, the expectation is high that they will also be able to improve the response to induction therapy and lead to deeper remissions for patients with Ph-like ALL.

Strategies for the future

The goal in moving any therapy to the frontline should be to enhance rates of MRD negativity, as the early eradication of disease seems to be one of the most important factors in impacting event-free and overall survival rates. However, no therapy, no matter how effective, should be used in the frontline setting if its toxicity is poorly understood or outweighs its benefits. Below are means through which immunotherapies and targeted therapies are being introduced into upfront treatments for adults with ALL.

Moving immune targeting to the frontline. Both blinatumomab and inotuzumab have proven to be superior to standard salvage

chemotherapy in the relapsed/refractory setting, demonstrating their activity against B-ALL, and it would seem that both of them are promising candidates for use in the frontline setting. However, each has its own unique strengths and toxicities, which have guided how clinical trials incorporating them into the frontline setting have been designed. Blinatumomab seems to provide maximum benefit when used in patients with lower disease burden (<50% blasts or MRD only), and therefore incorporating blinatumomab to modulate MRD in frontline treatment may enhance event-free survival. Inotuzumab, conversely, appears to be highly active irrespective of disease burden, however, the concern of VOD in patients who subsequently undergo allogeneic HSCT suggests that it may best be incorporated into treatments for populations who may not be recommended to receive transplant in CR1.

There are several prospective clinical trials currently evaluating the impact of blinatumomab on eradication of MRD in newly diagnosed ALL. The E1910 trial (NCT02003222) is a North American cooperative group study for adults 30 to 70 years of age. As part of postremission therapy, patients are randomized to receive either receive 2 cycles of blinatumomab prior to proceeding to consolidation, or proceed directly to consolidation. Patients may also proceed to allogeneic HSCT after intensification or after blinatumomab at the investigator's discretion. Additional blinatumomab courses are administered after late intensification. The primary endpoint is overall survival compared between the two groups, and a secondary endpoint will be rates of MRD negativity after treatment with blinatumomab + consolidation therapy vs consolidation therapy alone, prior to the start of maintenance. This study is the first large randomized trial to incorporate the use of MRD as a guide for treatment strategy in the adult population and will facilitate our knowledge of using MRD-directed therapy to improve survival in adult ALL. Additionally, this study is one of the first to evaluate the potential benefit of receiving blinatumomab in the frontline setting. Another smaller phase 2 trial is investigating sequential hyper-CVAD (4 cycles) followed by 4 cycles of blinatumomab and then 12 months of maintenance therapy (NCT02877303). Primary outcome measures are relapse-free survival and overall survival at 3 years. This study will begin to answer the question of whether postremission blinatumomab may replace additional cycles of intensive, myelosuppressive cycles of hyper-CVAD. Because this is a phase 2 trial, outcomes will be compared with historical controls of the hyper-CVAD regimen.

Inotuzumab is highly active against ALL irrespective of disease burden present, and therefore incorporating it into frontline treatment in the AYA population who traditionally have inferior outcomes to pediatric patients may improve outcomes. For young adults ages 18 to 39 years old, a large randomized phase 3 US intergroup study, A041501, is scheduled to begin accrual in the summer of 2017. This will test whether the incorporation of inotuzumab ozogamycin into an intensive pediatric regimen further improves survival for young adults with precursor B-ALL. This study is the successor to the previous US intergroup trial, C10403,[7] which demonstrated the feasibility and efficacy of applying a pediatric regimen in young adults with ALL with EFS and OS at 2 years of 66% and 79%, respectively. Patients with $CD22^+$ ALL will be randomized to receive treatment blocks of weekly inotuzumab ozogamycin during the second and third months of therapy and all patients with $CD20^+$ ALL will receive rituximab during the intensive postremission modules of chemotherapy. One of the important endpoints of this study will be to determine the impact of early eradication of MRD using inotuzumab on event-free and overall survival.

Older adults with Philadelphia chromosome negative ALL remain a tremendous treatment challenge[65,66] with fewer than 10% to 15% of patients achieving long-term survival with standard multiagent chemotherapeutic regimens, and new, less toxic regimens are desired. Therefore, exploration of the immunotherapeutics in this population is warranted. To that end, a trial for older adults is investigating blinatumomab alone for induction (1 or 2 cycles) and consolidation (3 additional cycles). Central nervous system prophylaxis will be given throughout treatment and patients will then receive a traditional POMP (prednisone, vincristine, methotrexate, 6-mercaptopurine) maintenance (NCT02143414). This trial is unique in that it is the first trial to use blinatumomab as a single agent as initial treatment of adult ALL. Given that blinatumomab seems to be more effective when disease burden is lower, it will be very interesting to see how it performs in the frontline setting in patients who do not yet have highly resistant disease.

Already, there are exciting preliminary data demonstrating feasibility and efficacy of combination inotuzumab ozogamycin with low-dose chemotherapy for older adults (>60 years old) with newly diagnosed ALL[67] (NCT01371630). Inotuzumab was added to low-dose chemotherapy (cyclophosphamide, vincristine, and dexamethasone) in sequential cycles of treatment. The overall response rate for 47 patients was 98%; importantly, the regimen was well-tolerated with low treatment-related mortality and with a median follow-up of 24 months, the 3-year survival rate was 54%. Longer follow-up is still required, but this study is an exciting example of the potential for improving outcomes by incorporating targeted immunotherapy into frontline treatment.

These studies represent the first of a new generation of trials in adult ALL that will collectively answer some very important questions regarding the frontline use of immunotherapies; importantly, all will provide useful information about whether strategies that increase the potential to eradicate MRD early in treatment will translate into improved survival for both younger and older adults with ALL.

Conclusion

This is an exciting time for the field! Survival rates are improving, and the new immunotherapeutic approaches, already proven to be highly effective in the relapsed setting, are being introduced into frontline treatment and should contribute significantly to this progress. We are using increasingly " personalized" treatments for patients with ALL through targeted therapies, and as we continue to gain insights into the molecular heterogeneity of this disease, new small molecule inhibitors and other immunotherapies, currently in early-phase development, are likely to be introduced into our treatment regimens for specific disease subsets. Additionally, better pharmacokinetic monitoring and insights from pharmacogenetics will increasingly allow us to personalize drug choice to maximize benefit while avoiding unnecessary toxicities. To achieve these goals, affordable, standardized, and rapid molecular diagnostic and minimal residual disease monitoring tools will be increasingly important to assure optimal treatment of our patients. Robust clinical trial accrual to these innovative trials is essential for future progress and will, hopefully, result in significant further improvements in survival rates for our patients with ALL.

Correspondence

Wendy Stock, Knapp Center For Biological Discovery, 900 E 57th St, Room 8112, Chicago, IL 60637; e-mail: wstock@medicine.bsd.uchicago.edu.

References

1. Stock W, La M, Sanford B, et al; Children's Cancer Group; Cancer and Leukemia Group B studies. What determines the outcomes for adolescents and young adults with acute lymphoblastic leukemia treated on cooperative group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. *Blood*. 2008;112(5): 1646-1654.
2. Barry E, DeAngelo DJ, Neuberg D, et al. Favorable outcome for adolescents with acute lymphoblastic leukemia treated on Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium Protocols. *J Clin Oncol*. 2007;25(7):813-819.
3. Ramanujachar R, Richards S, Hann I, et al. Adolescents with acute lymphoblastic leukaemia: outcome on UK national paediatric (ALL97) and adult (UKALLXII/E2993) trials. *Pediatr Blood Cancer*. 2007;48(3): 254-261.
4. Huguet F, Leguay T, Raffoux E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. *J Clin Oncol*. 2009;27(6):911-918.
5. DeAngelo DJ, Stevenson KE, Dahlberg SE, et al. Long-term outcome of a pediatric-inspired regimen used for adults aged 18-50 years with newly diagnosed acute lymphoblastic leukemia. *Leukemia*. 2014;29(3): 526-534.
6. Gokbuget N. Significant improvement of outcome in adolescents and young adults aged 15 - 35 years with acute lymphoblastic leukemia with a pediatric dervied adult ALL protocol: results of 1529 AYAs in 2 consecutive trials of the German Multicenter Study Group for adult ALL (GMALL) [abstract]. *Blood*. 2013;122(21). Abstract 839.
7. Stock W. Favorable outcomes for older adolescents and young adults (AYA) with acute lymphoblastic leukemia (ALL): early results of U.S. Intergroup Trial C10403 [abstract]. *Blood*. 2014;124(21). Abstract 796.
8. Hocking J, Schwarer AP, Gasiorowski R, et al. Excellent outcomes for adolescents and adults with acute lymphoblastic leukemia and lymphoma without allogeneic stem cell transplant: the FRALLE-93 pediatric protocol. *Leuk Lymphoma*. 2014;55(12):2801-2807.
9. Rytting ME, Jabbour EJ, Jorgensen JL, et al. Final results of a single institution experience with a pediatric-based regimen, the augmented Berlin-Frankfurt-Münster, in adolescents and young adults with acute lymphoblastic leukemia, and comparison to the hyper-CVAD regimen. *Am J Hematol*. 2016;91(8):819-823.
10. Thomas DA, O'Brien S, Kantarjian HM. Monoclonal antibody therapy with rituximab for acute lymphoblastic leukemia. *Hematol Oncol Clin North Am*. 2009;23(5):949-971, v. [v.]
11. Thomas DA, O'Brien S, Faderl S, et al. Chemoimmunotherapy with a modified hyper-CVAD and rituximab regimen improves outcome in de novo Philadelphia chromosome-negative precursor B-lineage acute lymphoblastic leukemia. *J Clin Oncol*. 2010;28(24):3880-3889.
12. Maury S, Chevret S, Thomas X, et al; for GRAALL. Rituximab in B-Lineage Adult Acute Lymphoblastic Leukemia. *N Engl J Med*. 2016; 375(11):1044-1053.
13. Ribrag V, Koscielny S, Bosq J, et al. Rituximab and dose-dense chemotherapy for adults with Burkitt's lymphoma: a randomised, controlled, open-label, phase 3 trial. *Lancet*. 2016;387(10036):2402-2411.
14. Ribera JM, García O, Grande C, et al. Dose-intensive chemotherapy including rituximab in Burkitt's leukemia or lymphoma regardless of human immunodeficiency virus infection status: final results of a phase 2 study (Burkimab). *Cancer*. 2013;119(9):1660-1668.
15. Dunsmore KP, Devidas M, Linda SB, et al. Pilot study of nelarabine in combination with intensive chemotherapy in high-risk T-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. *J Clin Oncol*. 2012;30(22):2753-2759.
16. Jain P, Kantarjian H, Ravandi F, et al. The combination of hyper-CVAD plus nelarabine as frontline therapy in adult T-cell acute lymphoblastic leukemia and T-lymphoblastic lymphoma: MD Anderson Cancer Center experience. *Leukemia*. 2013;28(4):973-975.
17. Den Boer ML, van Slegtenhorst M, De Menezes RX, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. *Lancet Oncol*. 2009;10(2):125-134.
18. Mullighan CG, Downing JR. Genome-wide profiling of genetic alterations in acute lymphoblastic leukemia: recent insights and future directions. *Leukemia*. 2009;23(7):1209-1218.
19. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med*. 2014; 371(11):1005-1015.
20. Jain N, Roberts KG, Jabbour E, et al. Ph-like acute lymphoblastic leukemia: a high-risk subtype in adults. *Blood*. 2016;129(5):572-581.
21. Herold T, Schneider S, Metzeler KH, et al. Adults with Philadelphia chromosome-like acute lymphoblastic leukemia frequently have IGH-CRLF2 and JAK2 mutations, persistence of minimal residual disease and poor prognosis. *Haematologica*. 2016;102(1):130-138.
22. Weston BW, Hayden MA, Roberts KG, et al. Tyrosine kinase inhibitor therapy induces remission in a patient with refractory EBF1-PDGFRB-positive acute lymphoblastic leukemia. *J Clin Oncol*. 2013;31(25): e413-e416.
23. Kobayashi K, Miyagawa N, Mitsui K, et al. TKI dasatinib monotherapy for a patient with Ph-like ALL bearing ATF7IP/PDGFRB translocation. *Pediatr Blood Cancer*. 2014;62(6):1058-1060.
24. Mei L, Ontiveros EP, Griffiths EA, Thompson JE, Wang ES, Wetzler M. Pharmacogenetics predictive of response and toxicity in acute lymphoblastic leukemia therapy. *Blood Rev*. 2015;29(4):243-249.
25. Pastorczak A, Fendler W, Zalewska-Szewczyk B, et al; Polish Pediatric Leukemia/Lymphoma Study Group. Asparagine synthetase (ASNS) gene polymorphism is associated with the outcome of childhood acute lymphoblastic leukemia by affecting early response to treatment. *Leuk Res*. 2014;38(2):180-183.
26. Mishra PJ, Humeniuk R, Mishra PJ, Longo-Sorbello GS, Banerjee D, Bertino JR. A miR-24 microRNA binding-site polymorphism in dihydrofolate reductase gene leads to methotrexate resistance. *Proc Natl Acad Sci USA*. 2007;104(33):13513-13518.
27. Lopez-Lopez E, Ballesteros J, Piñan MA, et al. Polymorphisms in the methotrexate transport pathway: a new tool for MTX plasma level prediction in pediatric acute lymphoblastic leukemia. *Pharmacogenet Genomics*. 2013;23(2):53-61.
28. Relling MV, Yang W, Das S, et al. Pharmacogenetic risk factors for osteonecrosis of the hip among children with leukemia. *J Clin Oncol*. 2004;22(19):3930-3936.
29. French D, Hamilton LH, Mattano LA Jr, et al; Children's Oncology Group. A PAI-1 (SERPINE1) polymorphism predicts osteonecrosis in children with acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Blood*. 2008;111(9):4496-4499.
30. Kawedia JD, Kaste SC, Pei D, et al. Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. *Blood*. 2010;117(8):2340-2347, quiz 2556.
31. Stock W, Diouf B, Crews KR, et al. An inherited genetic variant in CEP72 promoter predisposes to vincristine-induced peripheral neuropathy in adults with acute lymphoblastic leukemia. *Clin Pharmacol Ther*. 2016;101(3): 391-395.
32. Egbelakin A, Ferguson MJ, MacGill EA, et al. Increased risk of vincristine neurotoxicity associated with low CYP3A5 expression genotype in children with acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2010;56(3):361-367.
33. Bleyer A, Asselin BL, Koontz SE, Hunger SP. Clinical application of asparaginase activity levels following treatment with pegaspargase. *Pediatr Blood Cancer*. 2014;62(6):1102-1105.
34. Stock W, Douer D, DeAngelo DJ, et al. Prevention and management of asparaginase/pegasparaginase-associated toxicities in adults and older adolescents: recommendations of an expert panel. *Leuk Lymphoma*. 2011;52(12):2237-2253.
35. Koprivnikar J, McCloskey J, Faderl S. Safety, efficacy, and clinical utility of asparaginase in the treatment of adult patients with acute lymphoblastic leukemia. *Onco Targets Ther*. 2017;10:1413-1422.
36. Dhédin N, Huynh A, Maury S, et al; GRAALL group. Role of allogeneic stem cell transplantation in adult patients with Ph-negative acute lymphoblastic leukemia. *Blood*. 2015;125(16):2486-2496, quiz 2586.

37. Leonard JT, Stock W. The persistence of minimal residual disease in Philadelphia chromosome-positive acute lymphoblastic leukemia: we know it's bad, now what? *Biol Blood Marrow Transplant*. 2016;22(11):1913-1914.
38. Hoffmann P, Hofmeister R, Brischwein K, et al. Serial killing of tumor cells by cytotoxic T cells redirected with a CD19-/CD3-bispecific single-chain antibody construct. *Int J Cancer*. 2005;115(1):98-104.
39. Topp MS, Kufer P, Gökbuget N, et al. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. *J Clin Oncol*. 2011;29(18):2493-2498.
40. Topp MS, Gökbuget N, Zugmaier G, 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;120(26):5185-5187.
41. Gökbuget N, Zugmaier G, Klinger M, 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;102(4):e132-e135.
42. Topp MS, Gökbuget N, Zugmaier G, 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;32(36):4134-4140.
43. Topp MS, Gökbuget N, Stein AS, 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;16(1):57-66.
44. Kantarjian H, Stein A, Gökbuget N, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. *N Engl J Med*. 2017;376(9):836-847.
45. Frey NV, Porter DL. Cytokine release syndrome with novel therapeutics for acute lymphoblastic leukemia. *Hematology (Am Soc Hematol Educ Program)*. 2016;2016(1):567-572.
46. de Vries JF, Zwaan CM, De Bie M, et al. The novel calicheamicin-conjugated CD22 antibody inotuzumab ozogamicin (CMC-544) effectively kills primary pediatric acute lymphoblastic leukemia cells. *Leukemia*. 2011;26(2):255-264.
47. Kantarjian H, Thomas D, Jorgensen J, et al. Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. *Lancet Oncol*. 2012;13(4):403-411.
48. Kantarjian H, Thomas D, Jorgensen J, et al. Results of inotuzumab ozogamicin, a CD22 monoclonal antibody, in refractory and relapsed acute lymphocytic leukemia. *Cancer*. 2013;119(15):2728-2736.
49. DeAngelo D, Stock W, Stein A, et al. Inotuzumab ozogamycin in adults with relapsed or refractory CD22 positive ALL: a phase 1/2 study. *Blood Adv*. 2017;1:1167-1180.
50. Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *N Engl J Med*. 2016;375(8):740-753.
51. Maude SL. Future directions in chimeric antigen receptor T cell therapy. *Curr Opin Pediatr*. 2017;29(1):27-33.
52. van der Stegen SJ, Hamieh M, Sadelain M. The pharmacology of second-generation chimeric antigen receptors. *Nat Rev Drug Discov*. 2015;14(7):499-509.
53. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;371(16):1507-1517.
54. Lee DW, Kochenderfer JN, Stetler-Stevenson M, 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*. 2015;385(9967):517-528.
55. Brentjens RJ, Davila ML, Riviere I, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci Transl Med*. 2013;5(177):177ra38.
56. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*. 2014;6(224):224ra25.
57. Park J. 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 [abstract]. *Blood*. 2015;126:682. Abstract 682.
58. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *J Clin Invest*. 2016;126(6):2123-2138.
59. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;371:1507-1517.
60. Yeku OO, Brentjens RJ. Armored CAR T-cells: utilizing cytokines and pro-inflammatory ligands to enhance CAR T-cell anti-tumour efficacy. *Biochem Soc Trans*. 2016;44(2):412-418.
61. Turtle CJ. Addition of fludarabine to cyclophosphamide lymphodepletion improves in vivo expansion of CD19 chimeric antigen receptor-modified T cells and clinical outcome in adults with B cell acute lymphoblastic leukemia [abstract]. *Blood*. 2015;126. Abstract 614.
62. Shah NN. 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) [abstract]. *Blood*. 2016;128. Abstract 650.
63. Maude SL, Tasian SK, Vincent T, et al. Targeting JAK1/2 and mTOR in murine xenograft models of Ph-like acute lymphoblastic leukemia. *Blood*. 2012;120(17):3510-3518.
64. Reshmi SC, Harvey RC, Roberts KG, et al. Targetable kinase gene fusions in high-risk B-ALL: a study from the Children's Oncology Group. *Blood*. 2017;129(25):3352-3361.
65. Gökbuget N. How I treat older patients with ALL. *Blood*. 2013;122(8):1366-1375.
66. Marks DI. The challenges of managing older patients with acute lymphoblastic leukemia. *Am Soc Clin Oncol Educ Book*. 2015;e343-e351.
67. Sasaki K. Inotuzumab ozogamicin in combination with low-intensity chemotherapy (mini-hyper-CVD) as frontline therapy for older patients with acute lymphoblastic leukemia (ALL): interim result of a phase II clinical trial [abstract]. *Blood*. 2016;126. Abstract 588.