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REVIEW

Post-Hematopoietic Cell Transplantation Relapsed Acute Lymphoblastic Leukemia: Current Challenges and Future Directions

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Abstract: Allogeneic hematopoietic cell transplantation (allo-HCT) represents an important and potentially curative treatment option for adult patients with acute lymphoblastic leukemia. Relapse continues to remain the most important factor influencing overall survival post allo-HCT. We discuss early identification, clinical manifestations, and management of relapsed disease. Routine evaluation of measurable residual disease (MRD) and change in donor chimerism play a crucial role in early detection. Pivotal clinical trials have led to FDA approval of multiple novel agents like blinatumomab and inotuzumab. Combining targeted therapy with cellular immunotherapy serves as the backbone for prolonging overall survival in these patients. Donor lymphocyte infusions have traditionally been used in relapsed disease with suboptimal outcomes. This review provides insight into use of cellular therapy in MRD positivity and decreasing donor chimerism. It also discusses various modalities of combining cellular therapy with novel agents and discussing the impact of chimeric antigen receptor T-cell therapy in the setting of post allo–HCT relapse both as consolidative therapy and as a bridge to second transplant.

Keywords: acute lymphoblastic leukemia, hematopoietic cell transplantation, relapsed disease, measurable residual disease, MRD, ALL, CART, DLI

Introduction

Acute lymphoblastic leukemia (ALL) represents the second most common acute leukemia in adults; it is estimated there are over 6500 new ALL diagnoses per year in the United States.[1] Implementation of intensive, pediatric-inspired regimens for treatment of adult patients and the addition of tyrosine kinase inhibitors (TKIs) for Philadelphia chromosome-positive ALL (Ph+ ALL) have improved the survival rates.[1] Accurate risk stratification and monitoring response to therapy with measurable residual disease (MRD) techniques help determine patients who need a consolidative allogenic hematopoietic cell transplantation (allo-HCT). Despite the relatively high rate of disease response to induction chemotherapy, only 30%–40% of adult patients achieve long-term remission.[1] Elderly patients have even worse outcomes, with 5-year overall survival (OS) of approximately 20%.[2] High-risk mutations and inability to tolerate intensive therapy are the two main factors contributing to their poor prognosis.[2]

Allo-HCT represents a potentially curative treatment option for adult patients with ALL. It offers the ability to administer myeloablative conditioning regimen with the introduction of graft versus leukemia (GVL) effect.[3] Data from the Center of International Blood and Marrow Transplant Research (CIBMTR) showed that 1476 (6%) of all HCTs in 2019 were performed for management of ALL.[4] Patient age, comorbidities, donor availability, degree of social support, disease risk features, including T versus B-ALL, and MRD status post induction are some of the factors that play a role in the choice of therapy.[5] In general, eradication of MRD prior to proceeding to allo-HCT is preferred, given the higher risk of disease relapse in the setting of pre-HCT MRD positivity.[5–7]

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Several risk factors have been identified which impact treatment outcomes and relapse risk for patients with ALL. Specific cytogenetic abnormalities such as t (9:22), t (4:11), −7, or +8 have lower 5-year survival rates than those with normal karyotypes.[5] Inadequate or interrupted therapy has been associated with increased relapse risk.[5] MRD positivity both at the time of allo-HCT and after allo-HCT increases the risk of post-transplant relapse.[7] Retrospective data review has suggested that prognostic factors that are independently associated with poor survival were earlier relapse, increased number of bone marrow and peripheral blasts, and disease status greater than CR1 at the time of transplant.

Primary ALL disease risk, pre-transplant MRD status (defined as absent or <0.1% by flow cytometry), and occurrence of grade 1–3 acute GVHD have been shown to be independent predictors of post-transplant relapse. The phase III Children's Oncology Group / Pediatric Blood and Marrow Consortium trial led by Pulsipher et al was one of the first randomized multi-center studies that showed that MRD positivity (defined as >0.1% blasts) was associated with increased risk of relapse, HR 3.30 (1.32–8.22), and occurrence of grade 1–3 a GVHD predicted lower risk of relapse 0.44 (0.20–0.95), but this effect was not seen in patients with grade 4 GVDH as it was offset by increased treatment-related mortality.[8] Post HCT MRD positivity with levels >0.1% by flow or late (>8 months) have been associated with increase increased risk of relapse.[9,10] In general, eradication of MRD prior to proceeding to allo-HCT is preferred, given the higher risk of disease relapse in the setting of pre-HCT MRD positivity.[5–7]

Relapsed disease is the most important factor affecting OS post allo-HCT, and management of these patients is complex and requires close collaboration between the leukemia and the HCT teams. This review article will discuss the current challenges and future directions of patients with relapsed ALL following allo-HCT.

Clinical and Laboratory Manifestations of Relapsed Disease

Following allo-HCT, ALL relapse remains a major contributor to the suboptimal long-term survival.[6] Furthermore, patients who are MRD-positive have higher risk for post-transplant disease relapse as opposed to patients who are MRD-negative.[7] A number of retrospective studies report higher relapse post allo-HCT when patients are transplanted with MRD positivity. A retrospective analysis of 180 patients who underwent allo-HCT for ALL reported a 5-year OS and progression-free survival (PFS) in the MRD-negative group of 55.1% and 49.6%, respectively. There was a cumulative incidence of relapse of 41% and OS of 33% at 10 years.[6] A retrospective analysis between 2008 and 2018 of patients who were transplanted with active disease showed a 3-yr OS of 34%±3% and 32%±3% for matched sibling and a matched unrelated donor allo-HCT, respectively.[17] Despite relapsed disease being the most common cause impacting OS post allo-HCT, there are no standardized recommendations on management. There is a critical need for clinical trials with novel agents in this patient population.

Measurable Residual Disease

MRD is a validated tool for prediction of risk of relapse during induction and consolidative therapy for ALL. MRD is widely considered a part of routine follow-up for patients who are post allo-HCT for management of ALL. MRD positivity post allo-HCT is associated with an increased incidence of relapse and frequently predates overt relapsed disease.[7]

MRD in ALL is defined as the presence of leukemic cells below the threshold of detection by conventional morphologic methods. Leukemic cells can be identified by several methods including expression of gene fusions, clonal rearrangement of immunoglobulin, T-cell receptor genes, and immunophenotypic markers.[11] In general, methods of MRD monitoring include multicolor flow cytometry assays, real-time quantitative polymerase (qPCR) chain reaction assays, and next-generation sequencing (NGS)-based assays. MRD positivity is generally defined by the presence of 0.01% or more malignant cells. Next-generation flow cytometry can detect leukemic cells at a sensitivity threshold of $<2.3\times10^{-6}$. PCR and NGS methods can detect leukemic cells at a sensitivity threshold of $<10\times10^{6}$.[12] Genetic markers that can be utilized for MRD analysis in patients who are post allo-HCT include but are not limited to *BCR-ABL*/*ABL* ratio, *NPM1*, *RUNX1*, and *MLL* mutations.[7]

The use and clinical significance of serial MRD assessment post allo-HCT have been predominately studied in the pediatric ALL population. In an analysis of 113 pediatric patients with relapsed ALL post allo-HCT, MRD of $\geq10^{-4}$ leukemic cells was inversely correlated with event-free survival.[13] Data suggest that the relevance of low-level MRD

positivity in the post allo-HCT setting is dependent upon the time elapsed since transplant. A study of pediatric patients revealed higher risk of treatment failure with the presence of low-level MRD as more time elapsed compared to the time of transplant.[14] Relapse risk in pediatric patients with MRD positivity post allo-HCT has also been shown to be modified by the presence of acute GVHD.[15]

Donor Chimerism

Measurement of donor chimerism via peripheral blood and/or bone marrow aspiration samples represents an alternative method to detect residual or impending disease relapse. Although retrospective studies comparing the utility of MRD to donor chimerism assessments have shown MRD to be a more sensitive and specific method of relapse prediction, donor chimerism analysis remains an important tool.[16] Donor chimerism assessment can be particularly valuable in patients for early detection of impending relapse when a reliable MRD marker has been lost and/or cannot be established in the post-transplant setting.[16] Clinical interpretation of chimerism analysis is complex as chimerism kinetics are influenced by several factors including varying transplant practices.[7] It should also be noted that mixed chimerism in T-cells poorly correlate to later disease relapse.[7] Donor chimerism is generally utilized in conjunction with serial MRD testing. Serial laboratory monitoring is also essential to assess for overt manifestations of relapsed disease.

Extramedullary Disease

Although the majority of ALL patients who have undergone allo-HCT develop disease relapse within the medullary compartment, extramedullary relapses do occur.[18] A diminished GVL effect in extramedullary tissues in comparison to bone marrow may contribute to the extramedullary relapse risk in the post-transplant setting.[19] Although the incidence of extramedullary relapse in acute leukemias post-HCT has varied widely in previous studies, the 5-year cumulative incidence of extramedullary relapse is estimated to be around 11.0%, whereas the 5-year cumulative incidence of isolated extramedullary relapse is estimated to be around 5.8%.[20] Isolated extramedullary relapse represents clonal disease in other tissues without concurrent bone marrow involvement. Sites of potential extramedullary tissue involvement are diverse, including the central nervous system (CNS), testes, skin, soft tissues, and lymph nodes.[13] Factors that have been reported to increase the risk of isolated extramedullary relapse include poor cytogenetics and a history of prior extramedullary disease.[20]

The CNS is the most common site of extramedullary relapse post allo-HCT. The inability of the donor T-cells to exert GVL in the CNS, primarily due to the blood–brain barrier, has been postulated to make the CNS a sanctuary site for disease relapse.[21] Given the propensity of leukemic cells of either B- or T-cell origin to infiltrate the CNS, adults routinely receive prophylactic CNS-directed treatment to address occult disease.[22] Retrospective studies have shown an approximately 4% rate of CNS relapse following HCT in patients with ALL.[21]

Approach to Relapsed Disease

FDA-Approved Agents for Relapsed Disease

Historically, cytotoxic chemotherapy regimens have been utilized in patients with relapsed or refractory B-ALL with dismal response rates. In the past decade, several new therapies have been approved that provide more treatment options for relapsed/refractory (R/R) B-ALL patients. With the majority of precursor and mature B-cells expressing CD19 and CD22, blinatumomab and inotuzumab ozogamicin are ideal targeted therapies for patients with R/R B-ALL. Multiple studies are also underway combining these novel agents with each other and with other more conventional cytotoxic chemotherapies. Several cytotoxic chemotherapies can be utilized in the R/R setting, including liposomal vincristine and clofarabine.[23,24] Although response and survival outcomes have improved with the addition of TKIs to the treatment arsenal, relapse in patients with Ph+ disease may be mitigated by TKI resistance and/or kinase-independent pathways, requiring alternative treatment mechanisms. Therapy for post allo-HCT relapse disease is often a combination of chemotherapy and cellular therapy.

Blinatumomab

Blinatumomab is a bispecific T-cell engager (BiTE) antibody against CD19/CD3 which was approved by the US Food and Drug Administration (FDA) in 2014 for patients with R/R ALL, based on a single-arm study.[25] In this phase II, open-label study, treatment with single-agent blinatumomab resulted in complete remission (CR) or CR with partial hematologic recovery (CRh) in 43% of patients. An exploratory analysis of 64 patients who had their disease relapse following allo-HCT before enrollment in the trial was performed. Eighty-six percent of the patients had received salvage therapy after allo-HCT prior to enrollment. The rate of CR/CRh within the first two cycles of treatment was 45%, comparable to 43% of patients who did not receive previous allo-HCT. Median OS was 8.5 months with a median follow-up of 16.6 months. OS rate was 36% (95% CI 24%–48%) at 1 year and 18% (95% CI 9%–29%) at 3 years. The incidence of adverse events was similar to patients who had not received a prior allo-HCT, although rate of grade 4 adverse events was two-fold higher in patients who had previous allo-HCT.[26]

The 2014 FDA-accelerated approval was contingent upon positive results from the follow-up TOWER study. TOWER was a phase III, randomized, open-label multicenter trial which compared blinatumomab with conventional chemotherapy in patients with R/R Ph- B-ALL. Blinatumomab showed superiority over chemotherapy for median OS (7.7 months vs 4.0 months) and CR rate (34% vs 16%). One-third of patients enrolled had previously received allo-HCT; remission rates favored blinatumomab in this patient population [odds ratio 5.56 (95% CI 2.02–15.36)].[27]

Blinatumomab was further investigated for R/R Ph+ B-ALL in the phase II ALCANTRA study. Patients were included who were relapsed or refractory to at least one second-generation or later TKI or were intolerant to second-generation or later TKIs and intolerant or refractory to imatinib. Of 45 patients treated, 44% had a prior allo-HCT. All patients were refractory to, had relapsed on, or exhibited disease progression after prior TKI therapy. Five of the 20 patients with prior allo-HCT had CR/CRh (25%); subgroup analyses of response showed no statistically significant difference based on prior allo-HCT.[28]

Blinatumomab may be a useful agent to decrease risk of relapse in post-HCT settings. A single-center phase II study evaluated blinatumomab in adult patients with B-ALL deemed high risk for relapse after allo-HCT, including patients with MRD positivity, high-risk molecular mutations or karyotype, or disease stage beyond CR1. Of the 21 patients who received at least one cycle of blinatumomab maintenance therapy, 1-year overall survival, progression-free survival, and nonrelapse mortality were 85%, 71%, and 0%, respectively.[29,30] In two pivotal studies assessing blinatumomab for clearance of MRD in ALL, neither trial allowed inclusion of patients who had previously received allo-HCT.[31] However, several ongoing collaborative group studies are evaluating the use of blinatumomab in patients who are MRD-positive post-transplant. The Canadian Transplant and Cellular Therapy Group are evaluating blinatumomab for treatment of MRD in the first year following allo-HCT for B-ALL (ClinicalTrials.gov NCT04044560). Similarly, the FORUM study is evaluating the use of blinatumomab in this setting in children <21 years old (ClinicalTrials.gov NCT04785547). Table 1 lists the clinical trials involving blinatumomab in ALL.

Inotuzumab

Inotuzumab ozogamicin is a CD22-directed humanized monoclonal antibody conjugated to the potent cytotoxin, calicheamicin. Inotuzumab ozogamicin was approved by the FDA in 2017 for patients with R/R B-ALL. Both studies which led to FDA approval included patients who had relapsed after allo-HCT. A phase II, single-arm study to determine the efficacy of inotuzumab ozogamicin enrolled 49 patients; of 7 patients who had previous allo-HCT, 5 (71%) responded to therapy.[32] No other specifics on these 7 patients were included in the publication. In the phase III INO-VATE trial, inotuzumab ozogamicin was compared to standard of care chemotherapy in patients who were in salvage 1 or 2. Twenty percent of patients in standard therapy group and 16% of patients in inotuzumab ozogamicin group had received prior allo-HCT. Patients with and without prior allo-HCT had similar rates of CR/CRh (76.5% and 81.5%). Unfortunately, relapse-free survival and OS in patients with prior allo-HCT were not reported. The rate of veno-occlusive disease (VOD) with previous allo-HCT was 21% compared to 9% in patients without prior transplant; as this toxicity carries high morbidity and mortality, it should be carefully considered along with other patient-specific risk factors when deciding on therapy.[33]

Table 1 Clinical Trials Evaluating Blinatumomab and Inotuzumab Ozogamicin in ALL

Phase	Trial	Rate of Response	AEs	Included Prior Allo-HCT (%)?
II	Safety and activity of blinatumomab for adult patients with R/R B-precursor ALL: a multicenter, single-arm, phase II study[25]	CR: 63/189 (33%) CRh: 18/189 (10%) Median OS: 6.1 months Percentage undergoing allo-HCT after treatment: 40%	Grade 4/5: 45% CRS Neurotoxicity	Y (34%)
III	Blinatumomab versus chemotherapy for advanced ALL[27]	ORR: 44% versus 25% Median OS: 7.7 months vs 4.0 months	Grade 3 or higher: 87% versus 92% Grade 3 neurologic events: 9.4%	Y (34%)
II	Inotuzumab ozogamicin, an anti-CD22–calicheamicin conjugate, for R/R ALL: a phase II study[32]	ORR: 57% Median OS: 5 months Percentage undergoing allo-HCT after treatment: 50%	VOD: 23%	Y (14%)
III	Inotuzumab ozogamicin versus standard therapy for ALL[33]	CR: 36% vs 17% ORR: 81% vs 29% OS 7.7 vs 6.7 months Percentage undergoing allo-HCT after treatment: 48% [41 of 85 patients] vs 32% [10 of 31 patients], *P*=0.12	Rate of VOD in patients with prior allo-HCT: 21%, 9% in those without prior transplant	Y (18%)

Abbreviations: ALL, acute lymphoblastic leukemia; CR, complete remission; CRh, CR with partial hematologic recovery; CRS, cytokine release syndrome; allo-HCT, allogeneic hematopoietic cell transplantation; ORR, overall response rate; OS, overall survival; R/R, relapsed/refractory; VOD, veno-occlusive disease; Y, yes.

In a retrospective subgroup analysis of the phase I/II study 1010 and INO-VATE,[33] outcomes for patients with R/R Ph + B-ALL were assessed, focusing particularly on rates of MRD negativity. Approximately one-third of the patients (n = 16/49) with Ph+ disease in INO-VATE and half of the patients (n = 8/16) in study 1010 had previously undergone allo-HCT. CR/CRi for patients with Ph+ were higher in INO-VATE (73%) versus study 1010 (56%), which the authors attributed to a more heavily pretreated population in study 1010. Outcomes for those with allo-HCT before enrollment were not specified.[33]

A phase I/II clinical trial examined low-dose inotuzumab ozogamicin in patients who have had allo-HCT that are in complete remission but have a high risk for relapse (ClinicalTrials.gov NCT03104491). Inclusion criteria were: MRD positivity before or after allo-HCT, second salvage or beyond, reduced intensity conditioning regimen, lymphoid blast crisis of chronic myeloid leukemia, or Ph-like ALL. Phase I results observed low rates of relapse and a tolerable safety profile. At an interim analysis, 11 out of 12 patients were in CR at 12 months from last dose of inotuzumab ozogamicin.[34] Table 1 lists the clinical trials involving inotuzumab ozogamicin in ALL.

Chemotherapy

Several trials have investigated the use of agents approved for R/R B-ALL in combination with conventional cytotoxic chemotherapy; this is an area of ongoing study and exploration to improve rates of response and ability to proceed to allo-HCT.

An ongoing single-center study is evaluating low-intensity chemotherapy plus inotuzumab ozogamicin with or without blinatumomab in R/R Ph- ALL.[35] Low-intensity chemotherapy consists of mini-hyper-CVD, a dose-reduced derivative of hyper-CVAD (cyclophosphamide and dexamethasone at 50% dose reduction, no anthracycline, methotrexate at 75% dose reduction, and cytarabine at 0.5 g/m^2 × 4 doses). In the initial arm, mini-hyper-CVD was combined with inotuzumab ozogamicin for one day each cycle; after higher than anticipated rates of VOD, the trial was amended to

reduce inotuzumab doses. Twenty-five percent of patients had a prior allo-HCT. This subgroup had an ORR in 11 of 15 patients; 1-year event-free survival (EFS) was 40%, and 1-year OS was 47%. In safety analyses of the entire cohort, 9 patients (15%) had VOD; 3 had received allo-HCT prior to enrollment, 5 had received after inotuzumab therapy, and 1 had allo-HCT both before and after inotuzumab therapy. Of patients with prior allo-HCT who experienced VOD, the median time from allo-HCT to start of chemotherapy was 4.9 months (range, 2.5–11.7 months).[36]

The trial was amended to allow sequential addition of blinatumomab after mini-hyper-CVD and inotuzumab ozogamicin, and the dose of inotuzumab ozogamicin was lowered and fractionated. When reviewing patients in first salvage (n = 62), 11% had a previous allo-HCT. Overall, 69% of patients achieved a CR (n = 43/62), and 23% achieved a CRp/Cri (n = 14/62). The overall rate of VOD was 10% (n = 6/62); rate of VOD was 12% in patients who did not receive blinatumomab, and all of these patients had allo-HCT-related VOD. Compared with a similar historical cohort with inotuzumab monotherapy, this regimen resulted in significantly improved survival.[37]

In a subsequent follow-up including 96 patients, the overall response rate was 80% (CR 57%, CRp/CRi 20%), although subgroup analysis was not reported for those with prior allo-HCT. The 3-year OS rates for patients in salvage 1 and salvage 2 were 42% and 13%, respectively. Twenty percent of the cohort had prior allo-HCT (n = 19/96). The incidence of VOD prior to fractionated dose schedule of inotuzumab ozogamicin was 13% (n = 9/67) compared with 3% (n = 1/29) after the amendment, demonstrating that reduced dosing may be essential in future studies combining inotuzumab ozogamicin with chemotherapy in the post allo-HCT relapsed setting.[38]

Role of Cellular Therapy

Cellular therapy forms a cornerstone for therapeutic options for patients with relapse after allogeneic stem cell transplant. Donor lymphocyte infusion is the most traditional approach, where manipulated or unmanipulated donor T-cells are re-introduced into the recipient to elicit graft versus leukemic (GVL) effect. DLI is postulated to induce GVL by reversing T-cell exhaustion especially in the resident T-cells.[39] Administration of DLI is often associated with complications such as GVHD flare and bone marrow hypoplasia.[40] Remission attained from DLI is usually consolidated with a second allogeneic stem cell transplant.

Chimeric antigen receptor therapy or CART cell therapy is a form of cellular therapy that uses genetically modified T-cells that are directly capable of attacking and destroying malignant cells with specific cell surface proteins such as CD19.

Donor Lymphocyte Infusion (DLI) for Decreasing Donor Chimerism

STR-PCR based (short tandem repeat – polymerase chain reaction) serial chimerism has been shown to predict relapse. Patients with rapidly increasing mixed chimerism have the highest rate of relapse.[41,42] Conversion of mixed chimerism to complete chimerism is often achieved by reduction in immunosuppression and repetitive DLIs.[43–45] Bader et al studied 163 children with ALL and attempted to define impending relapse on the basis of increasing mixed chimerism; however, this analysis showed that STR-based chimerism analysis did not always predict an impending relapse, and the time interval between conversion of chimerism and relapse was often very short to allow the implementation of a meaningful intervention to prevent overt relapse.[46]

DLI with MRD Positivity

Prophylactic DLI and MRD-directed therapy has been shown to decrease overt relapses. Theoretically, introduction of the donor leukocytes at a very low disease burden gives time for the GVL effect to occur, enabling effective control of disease; however, this has to be balanced with the morbidity of graft versus host disease (GVHD). Dominetto et al showed that amongst 30 patients who were MRD-positive post-transplant, relapse rate was 87% amongst those who could not receive a DLI, and 7% amongst those who received a DLI.[47]

Prophylactic DLI and MRD-directed DLIs have been shown to improve outcomes in ALL. Yan et al showed a cumulative relapse rate of 32.4% and an OS of 51.4% post-transplant survival in a prospective study that aimed at administering prophylactic DLI to high-risk and multiple DLIs to patients who were MRD-positive after HCT. MRD was tested using 4 color flow and WT-1 PCR. Patients received chemotherapy and DLI for positive MRD. This study also

showed that a positive MRD, limiting to single DLI, and not being able to give all the DLIs were associated with increased rate of relapse.[48]

DLI in Overt Disease Relapse

DLI is a well-established therapeutic intervention for patients with relapsed disease post-HCT. DLI is thought to elicit response through GVL effect; however, it is not successful in ALL. Long-term disease-free survival only ranges between 0% and 13%.[49–51] The European BMT group published on the efficacy of DLI in patients with relapsed disease in acute and chronic leukemia after HCT from May 1992–1994. This study unfortunately showed no remissions among the 12 evaluable patients with ALL.[49] Choi et al conducted a prospective study in 10 patients with relapsed ALL after allo-HCT. Patients received cytoreductive chemotherapy followed by G-CSF-primed DLI. The study achieved 70% CR at a median of 25 days after the DLI. Only 1 of 7 patients remained alive in CR, 907 days after DLI. Two of these patients died of GVHD while in CR, and the remaining 4 relapsed at a median of 153 days after DLI. The study concluded that despite a high induction into CR with chemo DLI the remissions were not durable.[52] The suboptimal response to DLI for patients with relapsed ALL contrasts with its well established GVL effect in chronic myeloid leukemia (CML). An average of 40 days was required to see DLI-related response in patients who had relapsed CML, and responses have occurred as late as 10–12 months post DLI. Increased aggression and rapid turnover in acute leukemia could outpace the ability of the T-cells to expand in control of the disease.[51] The decrease in efficacy is postulated to be secondary due to T-cell anergy by malignant ALL clones and lack of expression of important co-stimulatory and adhesion molecules.[52–55] ALL cell lines have also been reported to be resistant to natural killer cells. Anti-recipient NK cell clones following haplo-identical T-cell-depleted transplant were shown to have a 0% probability of relapse at 5 years for patients with acute myeloid leukemia. However, the probability of relapse at 5 years for 14 patients with ALL who were transplanted with anti-recipient NK cell clones was 85%.[56]

Efficacy, Toxicity of DLI, and Impact on Future Therapeutic Options

Multiple studies have been attempted to improve the efficacy of DLI with manipulations. Activated DLI with donor T-cells (activated by anti-CD3 and anti-CD28 coated beads). Patients were given activated DLI, 12 days after receiving unmanipulated DLI, for 18 patients who had relapsed disease; 38.8% of patients developed aGVHD and 22% cGVHD. CR of 44% was seen.[57] There have been multiple studies that have combined pharmacologic agents and DLI. Most recently, Durer et al published a systematic review combining use of blinatumomab and DLI. A total of 15 patients were reported across 3 studies. Most received 2 to 4 cycles of blinatumomab and 1 to 2 infusions of DLI. Doses of DLI varied from 1×10^7 to 5×10^7. Out of the 15, CR with MRD-negative status was achieved in 3 patients. Three patients developed GVHD within the third dose of blinatumomab, and one patient developed grade 3 GVHD with combined therapy.[58] Inotuzumab has also been very effective in inducing remissions with acceptable toxicity in combination with DLI. Data from an Italian registry showed a median relapse-free survival of 12 months in a total of 8 patients that were treated with this combination.[59] Schroeder et al and Leubert et al have reported interesting results with improved survival in AML and MDS patients, relapsed after transplant. Patients received subcutaneous azacitidine followed by unmanipulated DLI. Time from transplant to relapse and disease burden at the time of relapse influenced outcomes. This study reported aGVHD up to 37% and chronic up to 14%.[60,61] Salvage chemotherapy followed by G-CSF-primed DLI was administered to 57 patients who had relapsed disease, showing a 47% CR; however, this study also reported GVHD 56%, and a treatment-related mortality of 23%.[62]

Lympho-depletion chemotherapy prior to DLI has been postulated to change the immuno-modulatory environment by suppressing the regulatory T-cells. This theory was tested in a few trials. These studies showed higher incidence of severe acute GVHD (cumulative grade 2–4: 60%). Guillaume et al showed a cumulative incidence of grade 2–3 GVHD of 29.4%, OS of 39%.[63]

Factors affecting the incidence of GVHD have not been fully elucidated.[64] Multiple studies have shown that DLI with matched unrelated donor does not have an increased risk of GVHD when compared to matched related donor DLI.[65,66] However, haploidentical DLI has been shown to have higher rate of GVHD unless given at minimal amount such as 1×10^4 CD3+ T-cells/kg. To produce an effective GVL the dose of DLI is often associated with dose-limiting side effects.

Hence to avoid such toxicity haploidentical DLI may often have to be administered with immunosuppression.[67] Higher dose of CD3+ in DLI has also been shown to correlate with GVHD. A study by Bar et al showed that initial DLI doses with CD3+ $>10\times10^7$ resulted in a cumulative incidence of 55% GVHD at 12 months; this contrasted with only 21% GVHD when initial dose contained CD3 of $<1.0\times10^7$. Multivariate analysis of this study showed that DLI doses $>10\times10^7$ correlated with increased risk of GVHD; however, this dose did not decrease the rate of relapse or improve overall survival.[68]

Lack of expected efficacy of DLI has inspired multiple modifications which range from T-cell enrichment to genetic modification of the receptor gene (TCR). Wilms tumor-1 antigen (WT1) is a well-known tumor antigen that is overexpressed in ALL, and WT1-targeted donor cells have shown initial control of relapsed ALL and have been looked into in multiple phase I studies[69] (ClinicalTrials.gov NCT00620633 and NCT00052520).

Hence with the given data, in our institution, DLI is more suitable in situations of MRD positivity and mixed chimerism rather than treatment of an overt relapse especially without chemotherapy.

Second Hematopoietic Cell Transplant

Nagler et al retrospectively studied the outcomes of a second allo-HCT in patients with relapsed ALL using data collected in the European bone marrow transplant network. Two-hundred and forty-five patients received a second salvage allo-HCT between 2000 and 2017. Forty-one percent received transplant from a sibling donor and the rest from matched unrelated donors. The 2-year cumulative incidences of non-relapse mortality, relapse, OS, and GVHD-free relapse-free survival (GRFS) were 24%, 56%, 30%, and 12%, respectively. Five-year OS was only 14%, and GRFS was 7%. Time from first HCT to relapse, performance status at the second allo-HCT, and nature of conditioning that was received for the first transplant seemed to influence outcomes for the second allo-HCT.[70] Older studies which have investigated outcomes of second transplants, following relapse after matched sibling transplant, showed an OS at 5 years of 28%. Younger patients and patients who had relapsed after 6 months from the first transplant had low risk of relapse.[71] Unfortunately, most retrospective studies in ALL patients have reported a poor 2–3-year leukemia-free survival of approximately 9%–27%.[72–74] Retrospective studies have compared DLI versus a second allo-HCT. In a retrospective single-institution study, this study reported that the second allo-HCT had slightly better OS of 65% when compared to 49% for DLI when given in the setting of relapsed disease.[75] However, this has to be confirmed with larger studies from European and CIBMTR database. Ideally a randomized study would be necessary to truly compare DLI versus second transplant; however, such a study may be very difficult to accrue.

A single-center retrospective study that looked at the outcomes of a second allogeneic stem cell transplant in AML patients showed a 2-year OS of 36%, and progression-free survival of 27%. Occurrence of chronic GVHD after the first transplant and a HCT comorbidity index ≥2 was associated with an inferior OS and PFS.[76]

Impact of the donor choice for second transplant remains to be fully explored. Retrospective analysis suggests that there is no difference in leukemia-free survival if second transplant is performed with the same donor as was used for the first transplant or an alternate matched-unrelated donor or a haploidentical donor. However, higher TRM was associated with using a haploidentical donor.[77]

Chimeric Antigen Receptor T-Cell Therapy

Chimeric antigen receptor T-cell (CART) therapy is a novel immunotherapy that improved outcomes for patients with R/R ALL. Tisagenlecleucel (Kymriah) and brexucabtagene autoleucel (Tecartus) are approved by the FDA in pediatric/young adult and adults with R/R ALL which target CD19 antigen present on the leukemic cell surface.[78,79] Maude et al conducted a phase II study with 75 pediatric and young adult patients (<25 years) with R/R B-ALL. Sixty-one percent of these patients had undergone a previous allo-HCT. This study reported an impressive overall remission rate of 81% at 3 months. All patients who had a complete response to treatment had MRD-negative disease by flow cytometry. EFS and OS at 12 months were 50% and 76%, respectively.[80] The long-term results of this study at a 5.9 year follow-up showed a 5-year OS of 55% (95% CI 43%–66%), and the median EFS was 43.8 months. The median time to B-cell recovery was 38.6 months, and probability of B-cell aplasia at 6 months was 83% (95% CI 71%–91%) and 71% (95% CI 57%–82%) at 12 months. Patients with B-cell recovery experienced a 2-year cumulative incidence of relapse of 25.2%. Twenty percent

of patients in remission were noted to have infections even after 1 year following infusions, 14% of patients had long-term persisting cytopenias for more than a year, and 82% of patients received IVIG.[81]

The efficacy and toxicity of KTE-X19 was studied in ZUMA-3. Seventy-one adult patients with relapsed refractory ALL were enrolled, and KTE-X19 was administered to 77% of them. Forty-two percent of patients treated with KTE-X19 had a previous allo-HCT. This study also included patients who had previously received blinatumomab. A median relapse-free survival (RFS) of 11.6 months (2.7–15.5) and median OS of 18.2 months (15.9–not estimable) were reported in this trial. Both studies had notable toxicities. Cytokine release syndrome (CRS) occurred in 77% of patients receiving Kymriah, with 48% needing tocilizumab. Forty percent of patients had neurological events which were managed with steroids and supportive care. KTE-X19 reported two grade 5 events, one patient with brain herniation, and another who succumbed to septic shock. Grade 3 or higher CRS occurred in 24% of patients, and grade 3 and higher neurotoxicity occurred in 25% of patients. The toxicities and efficacy cannot be directly compared as both studies used different systems for grading adverse events. ZUMA-3 reported only 1 episode of grade 2 GVHD amongst the 23 patients that were treated after an allo-HCT.[78,80]

With the FDA approval of 2 anti-CD19 CART-cells for ALL there have been multiple real-world retrospective analyses that have further looked into long-term efficacy. CIBMTR reported real-world experience after approval of tisagenlecleucel. A total of 255 patients were treated with Kymriah for ALL until January 2020. With a median follow-up of 13.4 months, a 85.5% complete remission rate was noted. The study reported an overall survival of 77.2% and event-free survival of 52.4% at 12 months. Approximately 28% of patients had received a prior allogeneic stem cell transplant. A total of 16% of patients had grade ≥3 CRS with 1 death resulting from cytokine release syndrome, and 9% had grade ≥3 ICANS. The overincidence of high-grade CRS is attributed to the fact that 95 of these patients were in a complete remission, and 44 were MRD-negative.[82] A metaanalysis by Anagnostou et al published the outcomes of clinical trials on CART and showed a rate of pooled CR of 80% (95% CI 75.5–84.8), and 1 yr PFS was 37% (95% CI 28.1–47.0). The CR rate did not differ significantly between the constructs; however, autologous T-cell origin CART was associated with higher CR than compared to allogeneic derived T-cell CART.[83]

Impact of Prior Anti-CD19 Therapies

There were concerns that blinatumomab would impair the efficacy of CART therapy, as both target CD19 present on the cell surface of the leukemic stem cells. The published data, however, are conflicting in different studies. CART therapy may not be as efficacious if prior therapy with blinatumomab elicited a CD19 escape. Myers et al performed a multicenter retrospective review amongst 420 children and young adults with relapsed, refractory ALL who received tisagenlecleucel. Seventy-seven of these patients had received prior blinatumomab. This group was also associated with a higher incidence of KMT2A rearrangements, and had undergone HSCT prior to treatment with blinatumomab, when compared to the group that did not receive blinatumomab. Lack of response to blinatumomab had a response rate of 64.5% to CD19 CART cell therapy, when compared to a 92.9% in blinatumomab responders and 72.6% in blinatumomab-naïve patients.[84] Blinatumomab-exposed responders and blinatumomab-naïve patients had comparable outcomes in this study if they had CD19-positive expression prior to receiving CART cell therapy. Downregulation of CD19 after treatment with blinatumomab was associated with high risk of post CART relapse which was CD19-negative. Hence this suggests that although CD19 antigen escape may be contributory to failure of CD19 CART therapy, it is unlikely to be the primary mechanism of resistance. Alteration in T-cell function and resistance from other immunotherapeutic pathways must be further explored to gain insight into the mechanism of relapse post blinatumomab. Similarly, a study from the Children's Hospital of Philadelphia reported that patients treated with prior blinatumomab were seen to have a higher rate of CD19-negative relapse.[85]

In the adult-only trial with axicabtagene ciloleucel, 45% of patients in ZUMA-3 received blinatumomab prior to CD19 CART therapy, and the initial responses were not very different from those who were not exposed to blinatumomab.[78] Hence in the present circumstance the reported data show variations on response to CART cell therapy for patients who are treated with prior blinatumomab. There are multiple confounding factors including different CART cell constructs, prior lines of therapy, and different patient populations. Hence a larger randomized study is warranted to further analyze the impact of CD19-targeting agent prior to the use of CD19-directed CART cell therapy.

Our institution generally tries to avoid use of anti-CD19 agent if a CD19-directed CART therapy is planned for the patient. If the patient has been exposed to prior blinatumomab within 3 months of planned CART cell therapy, a biopsy is performed to assess CD19 expression prior to therapy. Table 2 summarizes the clinical trials involving CART therapies.

Role of Consolidative Hematopoietic Cell Transplant After CART Therapy

CD19 antigen escape and lack of persistence of CART-19 cells are some of the reasons attributed to relapse after therapy with CD19-directed CART therapy.[80,86] Subgroup analysis in the ELIANA study showed that patients with CD19-positive relapses had loss of CART cells persistence compared to those with durable remissions. Patients who had relapsed with persistence of CD19 CART-cells interestingly developed a CD19-negative relapse. CARTs with 4–1BB as compared to those with CD28 as co-stimulatory agents have a longer persistence, leading to longer periods of B-cell aplasia.[87,88] Studies using CD28-CART19 have shown that persistence of the cells may not always correlate with response. This ZUMA-3 trial showed that despite 79% of CART patients not having detectable cells at 6 months, there were ongoing responders even at 1 year.[78] Single-center studies using CD28-CART19 showed a median of CART cells persistence of 14 days, and duration of persistence did not correlate with survival. The long-term outcomes reported by the NCI in their study of the CD28 CD19 CART in 50 children and young adults showed a clear benefit from consolidative allo-HCT. Amongst patients who achieved an MRD-negative CR with CART therapy, 75% proceeded with a HCT and obtained a median OS of 70.2 months. Cumulative incidence of relapse after HCT was 9.5% at 2 years. Patients who did not receive a consolidative HCT relapsed at a median of 152 days (range 94–394).[89] Studies using 41BB-CART19 have also shown a trend towards improved event-free survival when it is used as a bridge to stem cell transplant.[86,90,91]

Hence, with challenges of CART cell persistence and CD19-negative relapse, a preemptive risk versus benefit assessment of a consolidative HCT must be analyzed sooner than later. The choice of consolidative stem cell transplant would primarily depend on patient performance, donor availability, and availability of clinical trials.[92,93] Enrollment into available clinical trials should be considered as the first priority in this high-risk population.

Novel Therapies for ALL

Due to acquired or intrinsic disease resistance, novel, less toxic drugs are needed to improve the response and survival of ALL. Targeted and immune therapies have improved outcomes in patients with other hematologic malignancies. These novel approaches may bring new hope to patients with this challenging disease. Here we discuss the most promising approaches in advanced clinical trials.

Table 2 Clinical Trials Evaluating CART Therapy for ALL

Study Name	CD19 - Co-stimulatory	Age Group	Prior HCT %	Complete Response	OS
Frey NV (2020 Feb)[90]	41BB	Adult	37	90% in HDF	Median OS not reached at 2 yr. 73% in HDF cohort
Hay KA (2019 Apr)[91]	41BB	Adult	43	85%	20 months in MRD-negative group
Park JH (2018 Feb)[92]	CD28	Adult	36	83%	Median 12.9 months (8.7–23.4)
Shah BG (2021)[79]	CD28	Adult	42	71%	18.2 months (15.9–not estimable)
Wang N (2020 Jan)[93]	41BB	Pediatric and adult	23	83%	18.0 months (6.1–NR)
Maude SF (2014)[78]	41BB	Pediatric and adult	60	90%	78% at 1 yr
Maude SL (2018)[80]	41BB	Pediatric and AYA	61	81%	76% at 1 yr

Abbreviations: AYA, adolescents and young adults; HDF, high dose fractionated; MRD, measurable residual disease; NR, not reached; OS, overall survival; yr, year.

Bcl-2 and Mcl-1 Inhibition

Bcl-2 family proteins are a key regulator of apoptosis.[94] Primary ALL cells are dependent on Bcl-2 as the latter is highly expressed in some ALL subtypes.[95] In experiments conducted on adult ALL cell cultures derived from primary cells, BH3 mimetics (venetoclax and navitoclax) had low and narrow IC_{50}, conferring high sensitivity to these drugs.[95] Particularly, venetoclax has shown high activity below 10 nM in B-cell precursor ALL and in some T-cell ALL.[96] As such, BH3 mimetics are being incorporated as chemosensitizers in a multitude of chemotherapy regimens for the treatment of ALL (NCT03319901, NCT03504644, and NCT03808610).

A phase I dose-escalation study including forty-seven pediatric and adult patients with relapsed/refractory acute lymphoblastic leukemia or lymphoblastic lymphoma evaluated the safety and efficacy of venetoclax with low-dose navitoclax and chemotherapy. Complete remission rate was 60%, and 28% of included patients subsequently proceeded to transplant or CART therapy.[97]

However, resistance might emerge to the Bcl-2 inhibition. Mcl-1 overexpression renders ALL cells resistant to venetoclax and navitoclax as Mcl-1 compensate and maintain cell survival whenever Bcl-2 is inhibited.[95] Potentially, if proven safe, the combination of a Bcl-2 inhibitor along with a Mcl-1 inhibitor with chemotherapy could avert the emergence of resistance.

Immune Checkpoint Inhibitors

Immune checkpoint inhibition with drugs targeting program cell death protein 1 (PD1) and its ligand (PDL1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) are now approved for a multitude of solid tumors and some hematologic malignancies. Compared to healthy controls or patients in remission, bone marrow samples from ALL patients showed increased T-cells PD1 expression.[98,99] In patient-derived xenograft cells from patients with B-ALL, pembrolizumab in combination with blinatumomab improved clearance of B-ALL.[100] In a phase I study, combination therapy with blinatumomab and nivolumab in R/R ALL was feasible with acceptable toxicity. The combination induced a high MRD-negative CR rate (80%) in this heavily pre-treated patient population.[101] Multiple studies are currently evaluating this approach with eagerly anticipated results (NCT02879695, NCT04546399).

The potential increased risk of GVHD in patients receiving immune checkpoint inhibitors after allo-HCT is a risk/benefit consideration. A literature review included 150 patients that received immune checkpoint inhibitors after allo-HCT for management of various hematologic malignancies. Literature review revealed that 14% of patients developed acute GVHD and 9% developed chronic GVHD who received immune checkpoint inhibitors post allo-HCT.[102]

Furthermore, PD-1/PD-L1 expression on ALL cells can mediate T-cell inhibition. Therefore, augmenting anti-CD19 and anti-CD22 CART cell therapy using PD-1 checkpoint inhibitions has shown effective in vitro and in vivo.[103] Moreover, CART cells targeting the PD-1 axis through PD-1 knockout showed enhanced functionality.[104–106]

Menin Inhibition

The *KMT2A* (MLL) rearrangement on chromosome 11q23 is associated with a poor prognosis in ALL. MLL fusion proteins maintain leukemogenic gene expression through interaction with chromatin-associated protein complexes, among them menin.[107] Preclinical studies have shown clinical activity of selective, orally bioavailable menin inhibitors in cell lines and murine models.[107,108] Early studies of menin inhibitors in ALL are underway (NCT04811560, NCT05153330, and NCT04988555).

Monoclonal Antibodies Targeting CD38

CD38 is a type II transmembrane glycoprotein expressed on many cells, including immature T and B lymphocytes.[109] CD38 expression has been also demonstrated in leukemic blasts of T-ALL.[110,111] Daratumumab, an IgG1K monoclonal antibody directed against CD38 and approved for myeloma therapy, has shown efficacy in human xenograft models and has been used for eradication of measurable residual disease in a preclinical model of pediatric T-ALL.[112,113] Furthermore, daratumumab, provided on a compassionate basis, eradicated residual disease in three patients with ALL relapsed post-allogeneic hematopoietic cell transplantation.[114] CD38 is now being evaluated in a multitude of clinical

trials, whether using monoclonal antibodies or CART cell immunotherapy (NCT03754764, NCT05038644, and NCT03860844).

Recent Trials in CART Cell Therapy

Cytosine-based quadruple-edited allogeneic off-the-shelf CART cells targeting CD7 have shown great promise in preclinical models for treating relapse/refractory T–ALL. Cytosine-based editors are effective in silencing gene expression without increased incidence of double-stranded DNA breaks, and hence this new technology could definitely create a new pathway towards off-the-shelf CART cell therapy. 7CAR8 has been shown to have increased efficacy in multiple cell lines. Phase I and II clinical trials, NCT04984356 and NCT05377827, are currently recruiting to further determine tolerability and efficacy.[115]

Institutional Approach

We make every possible attempt to convert patients to an MRD-negative state either with use of blinatumomab or CD19-directed CART cell therapy. MRD testing is routinely measured at day 90 in the marrow along with donor chimerism. Immunosuppression is rapidly tapered with 25% reduction in dose every week with absence of GVHD, while the patient is started on salvage therapy such as inotuzumab or blinatumomab. The donor is also contacted and mobilized for a DLI when chemotherapy is started. Since it can take up to 45 days for DLIs to function, we prefer to start with salvage therapy for quick response and consolidate the response with DLI. CART cell therapy is considered in patients who do not achieve remission after salvage therapy. Autologous T-cells are harvested between cycles of chemotherapy, to avoid interruption of ongoing treatment. Finally, once remission is achieved, a second allogeneic transplant with an alternative donor is considered in patients with an acceptable performance status.

Conclusion

Relapse post allo-HCT for adult patients with ALL has a poor prognosis. However, in the current era there has been improvement in the outcomes and management of this patient population. Advent of immunotherapy, deep insights into molecular mechanisms leading to resistance, and discovery of novel targeted therapy have led to numerous clinical trials and reinstating hope for this patient population.

Summary Points

1. Relapsed disease remains the most important cause for decreased OS and RFS for patient post allo-HCT for ALL.
2. Monitoring donor chimerism and MRD at regular interval enables early detection of relapse and early interventions.
3. The CNS is the most common site of extramedullary relapse post allo-HCT. Inability for donor T-cell to exert GVL in the CNS, due to the blood–brain barrier, has been postulated to make the CNS a sanctuary for relapse.
4. Relapsed disease is managed by combining chemo and targeted therapy with cellular therapeutic intervention.
5. Blinatumomab and inotuzumab are some of the agents that should be considered when aiming to achieve CR.
6. DLI is likely more efficacious in the setting of MRD-positive disease or prophylaxis rather than in situations of overt relapse.
7. CART therapy is a novel therapy that has shown very impressive responses. However, the remissions are short-lived, necessitating further consolidative HCT.
8. The risk-versus-benefit assessment of a consolidative HCT, or a second allo-HCT, must be analyzed sooner rather than later for every patient with relapsed disease. The choice of consolidative HCT would primarily depend on patient performance, donor availability, and availability of clinical trials.
9. Multiple novel anti-leukemic agents targeting menin inhibition, CD38, and Bcl-2 and McL-1 provide new avenues for improving outcomes.
10. There is a crucial need for designing clinical trials with novel agents to this specific population to improve outcomes. Utmost effort must be made to ensure that patients relapsing post HCT are enrolled in clinical trials and are referred to high-volume centers for further management.

Disclosure

The authors report no conflicts of interest in this work.

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