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Cellular Therapies in Acute Lymphoblastic Leukemia

Jae H. Park[*], **Craig Sauter**[*], and **Renier Brentjens**
Department of Medicine Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

Abstract

A majority of adult patients with acute lymphoblastic leukemia (ALL) will die of their disease. While the prognosis for pediatric patients is markedly better, in all cases, the prognosis in patients with relapsed or refractory disease is uniformly poor. Allogeneic hematopoietic stem cell transplantation (HSCT) from a related donor can offer a significant potential therapeutic benefit for some patients. Since most patients lack a suitable related donor, alternative allo-HSCT approaches, including unrelated, umbilical cord blood (UCB), and haploidentical allo-HSCT, have been developed and are increasingly being studied in the clinical setting. Reduced-intensity conditioning further extends access to allo-HSCT for older more comorbid patients. While the use of donor-derived T cell adoptive therapy has a uniformly dismal outcome in patients with relapsed ALL following HSCT, modified adoptive T cell regimens, including the infusion of enriched tumor-targeted donor T cells and genetically targeted T cells, are currently under clinical investigation with promising results. Lastly, natural killer (NK) cells genetically modified to target ALL are also being studied in clinical trials, further expanding therapeutic options for patients with refractory or relapsed ALL. What remains to be seen is whether these novel adoptive cell therapies will ultimately lead to improved clinical outcomes.

Keywords

Acute lymphoblastic leukemia; adoptive cellular therapy; hematopoietic stem cell transplants; chimeric antigen receptor

INTRODUCTION

In general, adult patients diagnosed with acute lymphoblastic leukemia (ALL) have a poor prognosis. Overall, more than 6 out of 10 adult patients diagnosed with ALL will ultimately die of their disease[1]. In the pediatric population, the prognosis is far more favorable with greater than 8 of 10 patients achieving a long-term survival[1, 2]. In most cases, up-front

Correspondence to: Renier Brentjens.
[*]JHP and CS contributed equally to this publication.
Jae H. Park, MD, Assistant Attending, Leukemia Service, 1275 York Avenue, Box 569, New York, NY 10065, parkj6@mskcc.org, 212-639-4048 (phone), 646-422-2288 (fax)
Craig Sauter, MD, Assistant Attending, Bone Marrow Transplant Service, 1275 York Avenue, Box 276, New York, NY 10065, sauterc@mskcc.org, 212-639-3460 (phone), 212-639-3025 (fax)
Renier Brentjens, MD, PhD, Associate Attending, Leukemia Service, 410 E. 68th St., Box 242, New York, NY 10065, brentjer@mskcc.org, 212-639-7053 (phone), 212-772-8441 (fax)

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therapy involves long-term, toxic, and complex chemotherapy regimens. However, for both adult as well as pediatric patients, a failure to respond to up-front chemotherapy or relapse of disease after achieving a remission portends a dismal prognosis[3–5]. These findings suggest that novel approaches to adoptive cell therapies are needed to improve the outcome of patients with ALL. Recent advances in the understanding of tumor biology and immunology, combined with enhanced gene transfer technologies, have made the field of adoptive cell therapy one of great interests to investigators seeking alternative treatment approaches for this disease.

Hematopoietic Stem Cell Transplantation (HSCT)

Allogeneic HSCT (allo-HSCT) is the earliest and most studied form of adoptive cell therapy for leukemia. The original guiding principle of allo-HSCT was that it allows for higher dose chemotherapy with or without additional total body irradiation ideally resulting in consequent ablation of both tumor and normal bone marrow stem cells, the latter of which is subsequently rescued by the infusion of non-malignant HSCs from a healthy allogeneic donor. Clinical studies of allo-HSCT illustrate an additional immunologic benefit of this approach wherein donor T cells may mediate a beneficial graft versus leukemia (GvL) effect through donor T cells recognizing antigens present on residual tumor cells. This GvL effect was first described in patients with acute leukemia, including ALL[6], and is best illustrated by higher relapse rates in patients who have received donor grafts from identical twin siblings and patients treated with T cell depleted grafts designed to minimize graft versus host disease (GvHD)[7]. Consistent with this donor T cell mediated GvL effect is the finding that patients following allo-HSCT who experience acute and/or chronic GvHD are less likely to experience disease relapse when compared to patients who experience little or no GvHD following treatment[7]. Unfortunately as this GvL benefit is met with the untoward consequences of GvHD and associated morbidity and mortality, the benefit of allo-HSCT remains debatable.

HLA matched donor allo-HSCT in ALL as First Remission Therapy—While a large body of clinical data utilizing myeloablative (MA) matched related donors (MRD) allo-HSCT in patients with ALL exists, there remains debate regarding the utilization of MRD allo-HSCT in the setting of adult patients with ALL as a post-remission therapy. Based on the poor overall prognosis of this disease, there is the contention that all patients with suitable MRD should undergo allo-HSCT. However, this contention should take into account a significant treatment related mortality (TRM) of 20–30% associated with allo-HSCT[8], in addition to quality-of-life considerations. Moreover, patients' age and co-morbidities must be taken into careful consideration to determine transplant-eligibility in order to reach the potential benefit of this modality in terms of overall survival (OS).

The majority of ALL patients (>80%), both adult and pediatric, will achieve disease remission (CR) after one or 2 cycles of induction chemotherapy[1]. Whether patients in first complete remission (CR1) benefit with MRD HSCT versus chemotherapy alone in the adult ALL setting is a critical question with conflicting answers. Adult patients with ALL have traditionally been divided into standard and high-risk groups based on several clinical and genetic criteria. High-risk patients are variably defined as those patients >35 years of age, with an elevated WBC count at diagnosis, a delayed response (>28 days) following initial induction chemotherapy, and with genetically adverse features including the presence of the Philadelphia chromosome (Ph^+), t(1;19) and t(4;11). In high-risk transplant-eligible patients, MA MRD allo-HSCT is currently the consolidation treatment of choice in the setting of CR1[9], given several large clinical trials as well as a meta-analysis conferring benefit when compared to either chemotherapy alone or autologous HSCT[10–13]. However, in contrast to these findings, data from the PETHEMA ALL-93 and MRC UKALL XII/ECOG E2993

trials failed to demonstrate a similar advantage for patients with high risk disease once more placing the role of MRD HSCT for high risk patients into question[14, 15].

An important high-risk category of patients that deserves separate discussion is ALL harboring the Philadelphia chromosome (Ph+) for a multitude of reasons, including: 1) poor prognosis predominately secondary to relative chemo-insensitivity[16] , 2) predilection for older patients which may not be able to tolerate intensive therapy and 3) opportunity for targeted tyrosine-kinase inhibitor therapy (TKI: imatinib, dasatinib and nilotinib). Prior to the incorporation of TKIs into treatment regimens, patients with Ph^+ ALL fared poorly in the setting of MRD HSCT. However, one recently published report of data generated by the MRC UKALL XII/ECOG E2993 trial demonstrated a significantly improved relapse free survival in Ph^+ ALL patients following matched unrelated (MUD) or MRD HSCT when compared to chemotherapy alone, data generated prior to the utilization of TKI[17]. While this is the largest prospective study evaluating chemotherapy versus allo-HSCT as post-remission consolidation for Ph+ ALL, there are important limitations. Given the age restriction for allo-HSCT on the study (95% of patients receiving allo-HSCT were <50 years of age), the patients that received chemotherapy alone were significantly older than those that received allo-HSCT (p=0.004), which may introduce a potentially large confounder effect as age was a significant prognostic factor in the multivariate analysis. Additionally, further analysis by intent-to-treat (ITT) revealed non-significant differences in the two groups, again speaking to the relative chemo-insensitive nature of Ph+ ALL and the intolerance of therapy of advanced-age patients with this disease phenotype. TKIs in combination with chemotherapy has become an accepted standard of care for remission induction in patients with Ph+ ALL with significant improvements in complete remission rates compared to chemotherapy only historical controls[18]. Importantly, the relatively low-toxicity of adjunctive TKI with combination chemotherapy appears to offer access to allo-HSCT for this high-risk and typically advanced age patient cohort. Considering ITT models, the efficacy of TKI therapy for patients with Ph^+ ALL necessitates reevaluation of the role of allo-HSCT in this disease phenotype. Early results of allo-HSCT in patients with high risk Ph^+ disease treated with TKI during induction and consolidation prior to transplantation suggest that the addition of imatinib prior to allo-HSCT results in favorable CR rates and further appears to offer improved disease free survival and overall survival following allo-HSCT transplantation when compared to historical controls[19, 20]. More recent studies, of a larger Ph+ patient cohort, have confirmed favorable outcomes of TKI in combination with chemotherapy followed by transplantation. In the MRC UKALL XII/ECOG E2993 series, patients randomized to allo-HSCT in the post-TKI era had improved 3-year overall survival (OS) compared to pre-TKI patients randomized to allo-HCT based upon same CR criteria (56% vs 40%)[21]. Lastly, a study from Japan demonstrated added efficacy of TKI in first CR patients prior to MA allo-HSCT compared to pre-TKI historic controls (3 year OS 65% and 44% respectively, p=0.005)[22].

The more contentious debate has been the role of allo-HSCT in standard risk adult patients in CR1. The largest prospective randomized trial to attempt to answer this question was the MRC UKALL XII/ECOG E2993. In this trial, 1646 Philadelphia chromosome (Ph)-negative ALL patients underwent a standardized induction and those that achieved CR were "biologically" randomized to allo-HSCT if an MRD was identified and the patient was of appropriate MA allo-HSCT age either <50 years (ECOG) or <55 years (MRC) or chemotherapy/autologous HSCT if no MRD identified. The standard-risk MRD allo-HSCT arm demonstrated a 5-year OS of 62% which was significantly better than 52% in the no donor arm (p=0.02)[14]. Paradoxically, the high-risk Ph(-) patients did not derive significant advantage from allo-HSCT. In both high and standard risk groups, the relapse risk was significantly abrogated in the MRD allo-HSCT arm, lending credence to a GvL effect. Thus, this study may pose more questions than answered, such as does "intensifying"

consolidation with allo-HSCT truly overcomes the poor-prognosis of traditionally-defined Ph(-) high-risk patients. Additionally, one could argue that, given the relative success of intensified pediatric-inspired chemotherapy programs[23], the control chemotherapy group in this large randomized ITT study may have been suboptimally treated. Lastly, prognostic modeling has improved in the modern era. Thus, strategies to better risk-adapt patients in hope of potentiating benefit from the toxicity of allo-HSCT consolidation based upon clinical response to induction chemotherapy, i.e. time to CR and minimal residual disease following completion of induction, are being largely adapted in clinical trials in Europe [24–26]. In addition to identifying the appropriate standard risk patients with which to escalate therapy with allo-HSCT[27], high-risk patients may be afforded the opportunity to be spared allo-HSCT if prompt minimal residual disease negative status is attained[28]. Thus, despite MRC/ECOG randomized data, the role of allo-HSCT in the contemporary era remains a point of discussion.

Unrelated allo-HSCT and ALL—Overall, it would appear that adult patients with ALL in CR1 benefit from allo-HSCT from an MRD, unfortunately only approximately one-third of these patients have an appropriate MRD. Therefore, a majority of patients rely on identification of either an unrelated donor (URD), an umbilical cord blood (UCB) donor, or a haploidentical donor. In all of these settings, one would expect that the risk of TRM secondary to GvHD would be increased, but with a consequently enhanced GvL effect. However, these presumptions are challenged by published data.

Historically, in the MA setting patients with matched unrelated donors (MUD) have fared poorly compared to patients transplanted from MRD secondary to increased transplant-related mortality (TRM) associated with GVHD[29, 30]. In the contemporary allo-HSCT era of more resolute HLA-matching criteria[31], as well as improved supportive care [32], the relative differences in clinical outcome have become less appreciable between allo-HSCT from MRD and MUD[33–35]. Several studies have addressed this question specifically in the setting of MA allo-HSCT for ALL in CR1 demonstrating similar TRM, relapse rate, and ultimately OS when comparing ALL patients who underwent either a MRD or MUD HSCT [36–38]. A recent study from Japan nicely demonstrated the traditionally increased risk of TRM, but concurrently reduced relapse-rate, in MUD compared to MRD illustrating the enhanced GVL as well as GVHD resulting in comparable OS[39]. These data support the recommendation that eligible patients in CR1, with either a MRD or MUD available, be given equal consideration for an allo-HSCT.

Alternative donor allo-HSCT—Advances in alternative donor transplantation, i.e. umbilical cord blood (UCB) and haploidentical allo-HSCT, offers a transplantation option for those patients lacking a suitably matched related or unrelated donor. With increasing numbers of public cord blood banks, UCB in adults is becoming an increasingly viable option with burgeoning data emerging in only the last 7 years [40]. The early experience demonstrated lower than anticipated degrees of GvHD across greater HLA-barriers compared to traditional volunteer unrelated donor grafts[41–43]. A significant factor in TRM is the total nucleated cell (TNC) dose infused, with patients receiving $<2\times10^7$/kg TNC exhibiting a higher incidence of graft failure and greater TRM compared to those receiving grafts with $>2\times10^7$/kg TNC [44, 45]. To overcome this limitation, many centers have adopted double unit UCB transplants[44, 46]. Interestingly, studies have found that double unit recipients appear to fare better than single unit historical controls despite the vast majority of patients engrafting with only one of the 2 infused UCB units[47]. Theories posited of this benefit include reduced TRM related to larger cell dose and brisk myeloid engraftment[46] as well as enhanced GvL and subsequent protection from progression of primary hematologic malignancy[48].

Since the field of UCB transplantation is relatively new, most reports pool AML and ALL into a single category of patients with acute leukemia, making recommendations regarding this modality in the specific setting of ALL difficult. That having been said, recently reported outcomes of UCB HSCT in a large registry series compared favorably to bone marrow or peripheral blood stem cell allo-HSCT transplants in adult patients with acute leukemia[49]. In this series of over 1500 patients, leukemia-free survival in UCB transplant patients mismatched at 0–2 HLA loci were comparable to matched (8/8 HLA-allele matched) or mismatched (7/8 HLA-allele matched) volunteer unrelated donor transplant patients. TRM was significantly greater for UCB patients compared to 8/8 HLA-matched unrelated donors with both peripheral blood stem cell (HR 1.62, 95% CI 1.18–2.23, p=0.003) or bone marrow (1.69, 95% CI 1.19–2.39, p=0.003).

More recent published studies of UCB HSCTs have specifically focused on patients with ALL. In a large retrospective study recently published from Japan, there was no difference in TRM or leukemia-free survival between adult ALL patients that received an UCB graft mismatched at up to two loci and matched or mismatched bone marrow grafts[50]. Kumar et al compared outcomes of ALL patients receiving MRD HSCT, unrelated matched HSCT, mismatched HSCT, and matched or mismatched UCB transplants. The investigators surprisingly found superior 3 year OS rates in the UCB transplant group when compared to all other treatment groups, as well as improved leukemia-free survival, lower relapse rates, and lower TRM [51]. The authors report a statistically significant overall survival in ALL patients treated with UCB transplant when compared to patients treated with URD HSCTs (P=0.01). However, as the authors acknowledge, interpretation of these findings should be tempered by the low numbers of patients analyzed.

A final option for allo-HSCT for patients lacking related, unrelated, or UCB HSCT options, is a haploidentical donor HSCT. In this setting virtually every patient has a suitable related donor (a parent or sibling). Not surprisingly, early studies using haploidentical HSCTs were hampered by significant incidences of GVHD and graft failure[52]. Over time, modifications in preparative conditioning regimens designed to optimize myeloablation and host immunosuppression, combined with enhanced techniques of T cell depletion of the graft, as well as the infusion of markedly high doses of HSCs generated from the donor through mobilization with recombinant human granulocyte colony-stimulating factor, has led to a high rate of engraftment (>95%) with minimal GvHD even in the absence of immune suppression prophylaxis[52–55]. In a recently published report, patients with high risk acute leukemias were evaluated following haploidentical HSCT. Ciceri et al report a leukemia-free 2 year survival of high risk ALL patients treated with haploidentical HSCTs to be 13% for those undergoing transplantation in CR1, 30% for those undergoing HSCT in CR≥ 2, and 7% undergoing HSCT in non-remission[56]. Enhanced survival of ALL patients was recently reported in the setting of unmanipulated, non–T cell depleted haploidentical HSCTs. Huang et al report more favorable leukemia free survival in ALL patients treated with un-manipulated haploidentical grafts in ALL patients with a 3 year leukemia free survival of 60% and 25% in standard risk and high risk disease respectively[57]. However, not surprisingly, these improved survivals were associated with increased incidences of GvHD. Currently, much continued debate surrounds the use of haploidentical graft source as opposed to UCB and vice-versa[58, 59].

Reduced-intensity conditioning (RIC) allo-HSCT for adult ALL—Given the typically higher-risk disease in a growing population of older, more infirm, patients with ALL wherein MA conditioning is prohibitively associated with exceedingly high TRM; the need for extending allo-HSCT options with RIC has never been greater. This modality sacrifices disease control with reduced intensity of conditioning to minimize TRM, thus relying more heavily on GvL. Thus, the gravity of disease control prior to allo-HSCT carries

greater value to outcomes. The feasibility of this approach has been met with somewhat encouraging results given this high-risk patient population[60–63]. In a recent large retrospective series comparing RIC and MA conditioning for Ph(-) ALL, while there was a trend toward more frequent relapse with RIC (35% vs 28% MA, p=0.08) there was no difference in OS in multivariate analysis (p=0.92)[64]. The European Group for Blood and Marrow Transplantation registry data demonstrated decreased non-relapse mortality with RIC compared to MA (21% vs 29%, p=0.03) with associated increased frequency of relapse (47% vs 31%, p<0.001) resulting in a trend toward improved estimated 2-year leukemia-free survival with MA compared to RIC (p=0.07) [65]. These data illustrates the improved safety of RIC at the expense of diminished disease control. Application of RIC with UCB transplantation to the older ALL patient population may be feasible given data from Brunstein et al reporting on predominately double UCB transplants in older patients with hematologic malignancies. Overall this approach was well tolerated with relatively modest TRM at 3 years follow-up (26%) and promising overall and event-free survival (45% and 38% respectively)[66]. A more recent publication from the same institution reported the results of 22 ALL patients with 21/22 in CR1, treated with the same reduced intensity conditioning regimen followed by 4 of 22 patients receiving a MRD HSCT while the remaining 18 patients receiving UCB donor grafts. Collectively, these older (median age 49 years) high risk patients (Ph^+ (n=14), and ≥ CD2 (n=10)) tolerated therapy well with a TRM of 27%, disease relapse of 36%, and an overall survival at 3 years a very promising 50%[67]. Again, interpretation of these studies needs to be tempered by the small number of patients reported in this study and requires further confirmation in larger prospective studies.

Novel approaches to lowering risk of GVHD: T-cell depletion (TCD)—With GVHD as the leading cause of TRM in allo-HSCT, several groups have studied T-cell depletion of a conventional donor graft as a means of lowering the frequency of this often fatal complication. The potential risks of this approach include increased risk of relapse with reduced GVL effect and impaired immune reconstitution[68] leading to increased risk of infectious complications post-allo-HSCT. The group at Memorial Sloan-Kettering Cancer Center (MSKCC) has recently reported result on 35 adult patients receiving unrelated donor allo-HSCT with *ex vivo* TCD for hematologic malignancies, including 13 patients with ALL in remission (3 CR1, 7 CR2 and 3 CR≥3)[69]. Eighteen donors were HLA disparate at 1–3/10 loci. Despite a large proportion of high-risk ALL patients (CR>1), only one patient with ALL relapsed and the relapse incidence of the entire cohort was 6%. The incidence of acute grade II-IV GVHD at 9% and chronic GVHD at 29% were much lower than historical controls with a non-TCD allo-HSCT, especially considering the proportion of mismatched donors in the cohort. There were 5 fatal deaths of the 35 patients[69]. A British group has recently published there experience with the use of the anti-CD52 antibody alemtuzumab as an *in vivo* TCD allo-HSCT in 48 high-risk Ph (-) patients in CR1, with one-third of the patients receiving HLA mismatched grafts[70]. The incidence of acute grade II-IV GVHD and extensive chronic GVHD was 27% and 22% respectively. The OS for the entire group was 61% at 5 years. Both of these key studies demonstrate relatively favorable disease specific outcomes compared with conventional, non-TCD allo-HSCT with a decreased incidence of GVHD. These encouraging results, however, still need to be validated in a randomized prospective fashion.

Allo-HSCT for relapsed and refractory disease—Unfortunately, most patients with relapsed or refractory disease have a less than a 50% chance of responding to salvage chemotherapy[71] and prognosis is extremely poor[3]. In relapsed patients who have not previously received an allo-HSCT, have chemosensitive disease, an appropriate HLA-matched donor, and lack prohibitive comorbidities; an allo-HSCT provides the only chance for long-term disease free survival. For a multitude of reasons, the ability to match these

conditions diminishes steeply in the relapsed and refractory setting. A subset analysis from the MRC UKALL XII/ECOG E2993 study, relapsed patients receiving an allo-HSCT from a RD or an unrelated donor had improved OS at 5 years (23% and 16% respectively) versus those that did not proceed to allo-HSCT (4% OS at 5 years)[3]. Despite the high potential of obvious confounders in this carefully selected subset of patients, it is accepted standard to proceed to allo-HSCT in chemosensitive relapsed and refractory ALL in those who are eligible considering the incurability of chemotherapy alone.

Novel Adoptive Cellular Therapies

Due to conflicting results of these clinical trials, the role of allo-HSCT for patients with ALL remains controversial. Furthermore, novel, less tested approaches to allo-HSCT in ALL, including RIC, UCB and haploidentical HSCTs, lack sufficient numbers or prospective studies to allow for definitive recommendations. While these latter approaches as well as URD HSCT offer viable alternatives for patients requiring allo-HSCT but lacking a MRD, what is equally apparent is the fact that at this time none of these alternative options offers improved outcomes. For this reason, alternative approaches to cell therapies are required for this patient population.

Donor Lymphocyte Infusion (DLI)—DLI has been demonstrated to elicit a good response, mediated through a GvL effect, in patients with chronic myelogenous leukemia (CML) who relapse following allo-HSCT. However, it is rarely successful in relapsed ALL with a long-term disease free survival reported to range between 0% and 13%[72–76].

The reasons for the suboptimal response with DLI in ALL likely stem from several factors: a lack of adequate T-cell mediated GvL, the delayed effects of DLI in patients with aggressive disease, and/or a lack of costimulatory molecule on the tumor [74]. Porter *et al* addressed the latter issue by treating 7 relapsed ALL patients post allo-HSCT with donor lymphocytes that have been activated and co-stimulated *ex vivo* using CD3/CD28 agonist antibodies. In this phase I trial, 4 of 7 patients achieved a CR, but 3 of the 4 patients relapsed with only one patient alive in CR at 11+ months[77], showing the limitation of the conventional DLI in relapsed ALL.

A modified approach to DLI is the enrichment of donor T cells targeted to antigens over expressed on tumor cells. Wilms tumor-1 antigen (WT-1) is one such antigen which is over expressed on both acute myelogenous leukemia (AML) and ALL tumor cells [78, 79]. WT-1 is immunogenic and may represent an attractive target for adoptive T cell therapy, as suggested by a recent study by Rezvani *et al*, who reported WT-1 specific $CD8^+$ T cell responses following allo-HSCT in 5 of 7 patients and a subsequent molecular disease relapse (*i.e.* recurrent WT-1 transcript detection) associated with loss of detectable WT-1 specific $CD8^+$ T cells[80]. Similarly, investigators at MSKCC as well as at the Fred Hutchinson Cancer Research Center (FHCRC) have developed means of enriching for WT-1 specific donor T cell populations through co-culture of donor T cells on antigen presenting cells pulsed with WT-1 peptides. Currently, both MSKCC (NCT00620633) and the FHCRC (NCT00052520) have open phase I clinical trials treating relapsed acute leukemias and myelodysplastic syndrome (MDS) following allo-HSCT with WT-1 specific donor T cells. The completion of these trials will provide more information on the efficacy of tumor-specific, alloreactive T cells in ALL.

Genetically Modified Tumor-Targeted T Cells—Given the limited GvL effect demonstrated with donor T cells in ALL, several investigators have studied a novel form of adoptive cellular therapy by genetically modifying autologous T cells to target specific tumor antigens. One way of genetically modifying T cells is by gene transfer of the α and β

chain subunits of the T-cell receptors (TCRs) derived from T-cell clones specific to tumor antigens[81–83]. This approach has been shown to be feasible in clinical trials of metastatic melanoma[84, 85], but published data utilizing this approach in hematologic malignancies is limited[86, 87]. Moreover, because the TCR gene transfer approach can only recognize tumor antigens that are processed and presented by human leukocyte antigen (HLA) molecules, specificity of the TCR is restricted to specific patient HLA phenotypes and therefore lacks universal applicability. In addition, many tumor cells downregulate HLA molecules and/or have dysfunctional antigen-presenting machinery so that the targeted tumor-derived peptides are often not adequately presented on the tumor cell surface[88, 89].

One way to circumvent these limitations of TCR gene transfer is the use of chimeric antigen receptors (CARs). CARs are composed of a single-chain variable-fragment (scFv) antibody specific to tumor antigen, fused to a transmembrane domain and a T-cell signaling moiety, most commonly either the CD3-ζ or Fc receptor γ cytoplasmic signaling domains[90]. The resulting receptor, when expressed on the surface of the T cell, mediates binding to the target tumor antigen through the scFv domain which subsequently mediates an activating signal to the T cell inducing target cell lysis.

The use of T cells engineered to express CARs has several advantages over conventional allo-HSCT. First, since this approach utilizes autologous patient derived T cells, there is no risk of GvHD. Second, tumor specific T cells may be relatively rapidly generated *ex vivo* in the laboratory[91]. Third, because CAR recognition of target tumor antigen is HLA independent, CAR-modified T cells can be applied to all HLA types and are less likely to generate resistant tumor cells through down regulation of HLA molecules. And lastly, CARs can be further modified to insert additional genes to express T-cell co-stimulatory molecules or proinflammatory cytokines to enhance anti-tumor efficacy[92–96].

Genetically Modified T Cells in ALL—While there is limited data regarding the use of TCR gene transfer for genetically targeting of T cells in ALL, several groups including our own have investigated the use of CARs as a means of adoptive cellular therapy for ALL.

The first requirement to redirect CAR-modified T cells toward a selected tumor cell is the identification of an appropriate target molecule which is selectively expressed on cancer cells. With regard to ALL of B-cell origin, CD19 is an ideal target for several reasons: 1) in contrast to CD20, which is the target of current antibody-based immunotherapy, CD19 is expressed on the earliest B-precursor lymphocytes; 2) CD19 expression is retained over the process of neoplastic transformation; and 3) CD19 is absent on pluripotent hematopoietic stem cells[94, 97–104]. Furthermore, a recent report demonstrating the efficacy of bispecific single-chain antibody targeting the CD19 antigen (Blinatumomab) suggests that CD19 is an attractive target for cellular immunotherapy in ALL[105].

In fact, human T cells retrovirally modified to express CD19-targeted CAR have been shown to effectively lyse $CD19^+$ tumor cells *in vitro* and eradicate systemic CD19+ tumors in SCID-Beige mice[97]. Further studies in our laboratory have shown *in vivo* efficacy and persistence of these modified T cells is enhanced by co-stimulation[97, 98]. Since most B-cell tumors fail to express co-stimulatory ligands (CD80 and CD86) required to generate optimal activation and proliferation of T cells, we and others have further modified the CAR to include the signaling domain of the T cell co-stimulatory receptors (*e.g.* CD28, 4-1BB, OX40). The resulting "second-generation" CARs exhibit *in vitro* activation and proliferation in the absence of exogenous co-stimulatory ligands, as well as enhanced *in vivo* anti-tumor efficacy in immunodeficient mice bearing systemic human pre-B cell ALL tumors lacking co-stimulatory CD80 and CD86 ligands [94, 98, 102, 103, 106]. More recently, several investigators have constructed and tested "third-generation" CARs containing tandem

cytoplasmic signaling domains from two co-stimulatory receptors (*i.e.,* CD28-4-1BB or CD28-OX40) demonstrating potentially enhanced T cell signaling capacity when compared to second generation CARs[93, 106–108], but these have yet to be studied in the clinical setting.

Clinical Trials with CD19-Targeted Modified T Cells in ALL—These promising preclinical data have led to a robust translation of CD19-targeted CAR^+ T cells to the clinical setting for various B cell hematologic malignancies. Currently, there are 11 active and 3 soon-to-open phase I clinical trials targeting CD19 (Table 1). While many of these trials have recently opened, several published preliminary results of these trials suggest that adoptive cellular therapy using autologous CD19-targeted CAR^+ T cells is a promising treatment approach for B cell malignancies. For example, investigators at the National Cancer Institute (NCI) reported a dramatic regression of lymphadenopathy lasting 32 weeks in a patient with advanced follicular lymphoma who was treated with a preparative chemotherapy (60mg/kg cyclophosphamide for 2 days and 25mg/m^2 fludarabine for 5 days) followed by autologous CD19-targeted CAR^+ T cells and Interleukin-2[109]. We, too, have observed a dramatic reduction of lymphadenopathy that lasted for 9 months in one patient and stable disease in two patients with chemotherapy-refractory relapsed chronic lymphocytic leukemia (CLL) who were treated with 1.5g/m^2 cyclophosphamide conditioning one day prior to infusion of autologous CD19-targeted CAR^+ T cells[110]. Investigators from the University of Pennsylvania have recently reported complete responses in 3 patients with advanced stages of indolent B cell lymphomas and CLL, all treated with autologous CD19-targeted CAR^+ T cells[111, 112].

While the majority of the clinical experience using CD19-targeted CARs has been in the setting of indolent or chronic B-cell malignancies, 8 of the 14 clinical trials involve patients with ALL: three using autologous T cells, four using donor-derived T cells, and one using UCB-modified T cells. At our center, we are currently conducting a phase I clinical trial in ALL wherein patients with relapsed disease or with minimal residual disease following the initial induction chemotherapy will be treated with cyclophosphamide conditioning followed by autologous CD19-targeted CAR^+ T cells (NCT01044069). To date, we have enrolled 3 patients to the trial, and two patients have been treated. Notably, a persistent B cell aplasia was observed in the first treated patient despite prompt recovery of other blood cell counts, which lasted for 8 weeks prior to undergoing allo-HSCT from a related sibling[110]. The second patient has just received the modified T cells, and the results of the peripheral blood analysis are not yet available. Similarly, investigators at the University of Pennsylvania are conducting a phase I clinical trial with autologous CD19-targeted T cells in patients with B cell malignancies including ALL (NCT01029366).

Other investigators have examined the use of a CD19-targeted CAR to modify donor-derived T cells for patients with relapsed ALL following allo-HSCT. In order to reduce the risk of GvHD associated with infusion of donor-derived T cells and to offer protection against common post-transplant virus infections, the approach using virus-specific T cells has been commonly utilized in this setting. Specifically, investigators at Baylor College of Medicine are conducting a phase I/II dose-escalation trial in ALL wherein patients, following allo-HSCT, will be infused with allogeneic multi-virus targeted T cells, specific to cytomegalovirus (CMV), Epstein-Barr virus (EBV) and adenovirus, further modified to express a CD19 targeted CAR[113] (NCT00840853). MSKCC has an open phase I dose-escalation clinical trial wherein patients with relapsed or minimal residual disease-positive ALL will be treated with donor-derived EBV-specific CAR-modified T cells (NCT01430390). Finally, investigators in Europe (NCT01195480) will soon open a phase I trial to evaluate the safety of donor-derived EBV-specific CAR-modified T cells in patients with high-risk or relapsed B-cell ALL after allo-HSCT.

Similarly, UCB cells can be modified to express a CD19-targeted CAR, and preclinical data exploring the CAR-modified UCB cells have been published[114, 115]. Based on these preclinical data, investigators at MD Anderson Cancer Center are planning a phase I clinical trial wherein patients with B cell malignancies will be infused with CD19-targeted CAR^+ T cells derived from UCB at 49 days (+/− 7 days) following UCB transplantation (NCT01362452).

Genetically Modified CD19-Targeted Natural Killer (NK) Cells—NK cells are lymphoid cells of the innate immune system which express CD56 and CD16 but fail to express a TCR ($CD3^-$). NK cells express killer immunoglobulin-like receptors (KIRs) which predominantly serve as inhibitory receptors that bind specific matched HLA class I molecules (KIR ligands) on target cells. Expression by target cells of cognate KIR ligands induces a KIR-mediated inhibitory signal to the autologous NK cell, sparing the target cell from NK cell mediated lysis. In the allogeneic setting, however, NK cells encounter target cells with mismatched KIR ligands and as a result trigger NK cell alloreactivity[116].

NK cell alloreactivity does not appear to be of benefit in the setting of ALL[117]. However, genetic modification of NK cells may be a means of overcoming ALL tumor cell resistance to NK cell mediated lysis. To this end, several investigators have successfully expanded large numbers of NK cells through co-culture of peripheral blood mononuclear cells on an irradiated K562 leukemia cell line genetically modified to express the NK cell stimulatory 4-1BB ligand as well as membrane bound IL-15 (K562-mb15-41BBL) [118], and generated ALL targeted NK cells either by retroviral transduction[119] or by electroporation[120] of a CD19-targeted CAR. These CD19-targeted NK cells effectively lysed NK-cell resistant ALL tumor cells, and further modification of the CAR by incorporation of the 4-1BB receptor cytoplasmic signaling domain enhanced NK-cell killing of $CD19^+$ ALL tumor cell lines and patient derived ALL cells[119]. Significantly, these investigators at St. Jude Children's Research Hospital have successfully modified the NK cell transduction and expansion protocol to large scale cGMP conditions, and have opened a phase I dose-escalating clinical trial wherein children with refractory or relapsed ALL are treated with CD19-targeted NK cells from a haploidentical donor. To date, they have enrolled 2 patients at the lowest dose cohort (D. Campana, personal communication).

CONCLUSIONS

ALL remains a difficult disease to treat. In the adult setting, most patients will ultimately die of their disease, while in the pediatric setting, relapsed and refractory disease remains a therapeutic challenge. Cellular therapy through allo-HSCT remains an option for these patients and recent advances in alternative forms of allo-HSCT including URD transplants, UCB transplants, and haploidentical transplants have expanded the numbers of patients eligible for allo-HSCT, but to date have not improved outcomes when compared to HLA-matched related allo-HSCTs. In light of this persistent failure, several novel adoptive cellular approaches are currently being investigated to treat patients with ALL. The use of enriched WT-1 specific donor T cells to treat patients with ALL is currently under investigation in phase I trials at several centers. Treatment of ALL with genetically modified T cells targeted to the CD19 antigen through the expression of a CD19-specific CAR have entered phase I clinical trials at several centers as well. Similarly, a clinical trial treating ALL patients with genetically modified NK cells targeted to the CD19 antigen has recently opened for accrual. Collectively, these ongoing and anticipated trials provide promising role for adoptive cellular therapies in the treatment of ALL. What remains to be seen is whether this promise will translate into either improved outcomes for these patients or provide significant insights upon which to design "second generation" adoptive cell therapeutic clinical trials for ALL in the future.

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Table 1

Summary of Open and Planned Clinical Trials with CD19-Targeted Chimeric T Cell

Cell Source	Patient Population	Trial Design	Accompanying Lymphodepleting Cytotoxic Therapy	Trial Status	Trial Site
Autologous T cells	Relapsed or refractory CLL	Phase I dose-escalation trial	Cy	Open	US, New York
Autologous T cells	Relapsed or refractory, or MRD+ ALL	Phase I dose-escalation trial	Cy	Open	US, New York
Autologous T cells	MRD+ or residual disease following frontline CLL therapy	Phase I dose escalation trial; consolidation therapy following upfront chemotherapy	Cy	Open	US, New York
Autologous T cells	Relapsed or refractory low-intermediate grade NHL & CLL	Phase I dose escalation trial	None	Open	US, Texas
Autologous T cells	Relapsed or refractory B-cell leukemia/lymphoma (ALL, CLL, FL, MCL, DLBCL)	Phase I dose escalation trial	None	Open	US, Pennsylvania
Autologous T cells	Relapsed or refractory FL	Phase I	Fludarabine + rituximab	Open	US, California
Autologous T cells	CD19-expressing B cell malignancy of any type	Phase I	Fludarabine + Cy	Open	US, NIH
Autologous T cells	Relapsed or refractory low-intermediate grade NHL & CLL	Phase I; modified T cell infusion following autologous-HSCT	Autologous HSCT regimen (carmustine, etoposide, cytarabine, melphalan, rituximab)	Open	US, Texas
Donor-derived EBV-specific T cells	MRD+ or relapsed ALL following allo-HSCT	Phase I dose escalation trial	Cy	Open	US, New York
Donor-derived multivirus-specific T cells	Relapsed B-ALL following allo-HSCT	Phase I dose-escalation trial	None	Open	US, Texas
Donor-derived T cells	Relapsed B-cell leukemia/lymphoma following allo-HSCT	Phase I dose-escalation trial	N/A	Open	US, NIH
UCB-derived T cells	Relapsed or refractory ALL, NHL, SLL, CLL	N/A	N/A	Pending	US, Texas
Autologous T cells	High-risk, intermediate-grade NHL	Phase I/II trial; modified T cell infusion following auto-HSCT	N/A	Pending	US, California

Cell Source	Patient Population	Trial Design	Accompanying Lymphodepleting Cytotoxic Therapy	Trial Status	Trial Site
Donor-derived EBV-specific T cells	High-risk or relapsed B-ALL after allo-HSCT	Phase I	N/A	Pending	Europe (France, Germany, Italy, UK)

CLL: chronic lymphocytic leukemia; Cy: cyclophosphamide; MRD: minimal residual disease; ALL: acute lymphocytic leukemia; NHL: non-Hodgkin's lymphoma; FL: follicular lymphoma; MCL: mantle cell lymphoma; DLBCL: diffuse large B cell lymphoma; HSCT: hematopoietic stem cell transplant; SLL: small lymphocytic lymphoma