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Immunotherapy in Pediatric B-Acute Lymphoblastic Leukemia: Advances and Ongoing Challenges

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Abstract

Leukemia, most commonly B-cell acute lymphoblastic leukemia (B-ALL), accounts for about 30% of childhood cancer diagnoses. While there have been dramatic improvements in childhood ALL outcome, certain subgroups, particularly those who relapse, fare poorly. In addition, cure is associated with significant short- and long-term side effects. Given these challenges, there is great interest in novel, targeted approaches to therapy. A number of new immunotherapeutic agents have proven to be efficacious in relapsed or refractory disease and are now being investigated in frontline treatment regimens. Blinatumomab, a bispecific T cell engager (BiTE) that targets CD19, as well as inotuzumab ozogamicin, a humanized antibody-drug conjugate to CD22, have shown the most promise. Chimeric antigen receptor T (CAR-T) cells, a form of adoptive immunotherapy, rely on the transfer of genetically modified effector T cells that have the potential to persist *in vivo* for years, providing ongoing long-term disease control. In this article, we will discuss the clinical biology and treatment of B-ALL with an emphasis on the role of immunotherapy in overcoming the challenges of conventional cytotoxic therapy. As immunotherapy continues to move into the front-line of pediatric B-ALL therapy, we will also discuss strategies to address unique side effects associated with these agents and efforts to overcome mechanisms of resistance to immunotherapy.

1. Introduction

Leukemia, the most common childhood malignancy, accounts for about 30% of childhood cancer diagnoses [1]. More than three-quarters of cases are acute lymphoblastic leukemia

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(ALL) with 85% of those being B-ALL and the remainder T-ALL and other rare subtypes. There have been dramatic improvements in childhood ALL outcome over the past five decades with 90% of children surviving five years or more from initial diagnosis [2]. Despite these advances, the burden of therapy for the children who are cured is substantial with both short- and long-term toxicities and there are subgroups that do not fare well such as infants, adolescents, young adults, and those with unfavorable genomic alterations [3, 4]. Among the 20% of patients who relapse, no significant improvements in survival have been observed despite intensification of therapy [2, 5]. While allogeneic hematopoietic stem cell transplant (HSCT) can benefit some patients with relapsed ALL, a substantial majority fail retrieval therapy [6]. Therefore, novel approaches are needed to improve outcome for poor risk subsets and to potentially replace components of cytotoxic chemotherapy. Immunotherapy has drawn significant interest particularly for children with B-ALL, the majority of whom have suitable target antigens.

2. Biology and Risk Stratification of Pediatric B-ALL

Childhood ALL is traditionally considered a sporadic disease, with <5% of cases due to an underlying genetic predisposition [7, 8]. However, a number of studies have identified germline polymorphisms associated with an increased risk of ALL [9]. The inciting steps are thought to be due to somatic mutations that may occur *in utero* resulting in preleukemic clones that subsequently acquire additional somatic alterations [10–13]. Breakthroughs in treatment have been orchestrated by collaborative groups worldwide and while approaches may differ somewhat, there is no indication of the superiority of one regimen over another. All prescribe rounds of rotating multiagent chemotherapy, many of which have been augmented in terms of dose and sequence over the past two decades. The recognition of the central nervous system (CNS) as a sanctuary site led to the development of pre-symptomatic therapy, now almost exclusively intrathecal chemotherapy without radiation. Recent experience indicates that further escalation of conventional agents has no impact on disease-free survival (DFS) and causes considerable toxicity [14–17].

Risk stratification is critical for maximizing cure, minimizing side effects and identifying candidates for novel treatment approaches. Clinical features, blast genetics and early treatment response are the fundamental prognostic variables used for risk stratification. A representative algorithm adapted from the Children's Oncology Group (COG) is shown in Table 1 [18–23]. Minimal residual disease (MRD), measured by flow cytometry or molecular detection of antigen receptor rearrangements, at the end of induction is the most significant prognostic factor [24, 25].

In contrast to children with newly diagnosed disease, the prognosis for those who relapse is poor and frequently characterized by the development of resistance to conventional therapeutic agents [26–28]. The identification of relapse specific somatic alterations and backtracking studies support a Darwinian model of clonal evolution with the outgrowth of drug resistant subclones occurring under the selective pressures of chemotherapy [29–41]. Ultimately, the most significant prognostic factors are blast immunophenotype and the timing and site of relapse. The COG approach to risk stratification at relapse is summarized in Table 2 and other cooperative groups follow similar algorithms [42–45].

3. Immunotherapy

Given the challenges associated with conventional treatments, novel targeted approaches are urgently needed. While certain subgroups such as Philadelphia (Ph+) ALL and “Ph-like” ALL benefit from molecularly targeted agents, most patients lack a suitable target. A number of immunotherapeutic agents, however, have ushered in a new dimension in care for relapsed/refractory disease and are now being investigated in frontline treatment regimens. An overview of immunotherapeutic approaches in B-ALL are shown in Figure 1.

3.1. Blinatumomab

CD19 is an early B-lineage restricted antigen expressed in > 90% of leukemic blasts [46, 47]. Blinatumomab (Blinicyto®, Amgen®) is a first-in-class bispecific T cell engager (BiTE) consisting of two single-chain variable antibody fragments (scFv) connected by a flexible linker. One scFv binds to CD19 expressed on B cells, whereas the other binds to the T cell receptor/CD3 complex resulting in the formation of an immunological synapse triggering apoptosis of the CD19 positive cancer cell through production of cytolytic proteins, release of inflammatory cytokines, and promotion of T cell activation and proliferation [46, 48].

3.1.1. Adult Relapsed/Refractory B-ALL—Blinatumomab was first approved by the U.S. Food and Drug Administration (FDA) based on results from a multicenter, phase 2 study of 189 heavily pretreated adult patients (NCT01209286). Patients received blinatumomab 9 mcg/day via continuous infusion for the first seven days, followed by 28 mcg/day to complete a 28-day cycle [49]. After two cycles of blinatumomab, 81 (43%) patients achieved a complete response (CR) or CR with partial hematologic recovery (CRh), of which 82% also achieved MRD-negativity. Forty percent of those in CR/CRh proceeded to HSCT. The median relapse-free survival (RFS) for those in CR/CRh was 5.9 months and the median overall survival (OS) for all patients was 6.1 months. The most common adverse events (AEs) were pyrexia, headache, febrile neutropenia, edema, nausea, hypokalemia, and anemia. Grade 3 cytokine release syndrome (CRS) and grade 3 neurologic events occurred in 2% and 11% of the population, respectively [47]. CRS is a result of robust immune response due to the release of a large number of cytokines such as IL-6, IL-10, IFN- γ , TNF- α , and GM-CSF. Mild symptoms include fever, mild flu-like symptoms, and hypotension, which in severe cases can progress to shock and multi-organ failure [50]. Comparison of CRS rates in early trials had proven difficult due to the use of individualized grading systems for CRS, which led to the development of a consensus grading system by the American Society of Transplant and Cellular Therapy (ASTCT) and is currently employed today [51].

Subsequently, a multi-national phase 3 trial (TOWER) was conducted to compare blinatumomab (n=271) to standard chemotherapy (n=109) in patients with relapsed/refractory B-ALL (NCT02013167). The median OS was almost doubled in patients receiving blinatumomab (7.7 vs 4 months), accounting for a 29% decreased risk of death and a higher proportion of patients achieving a CR/CRh/CR with incomplete hematologic recovery (CRi) (44% vs 25%) and MRD-negativity (76% vs 48%). Toxicity was acceptable with grade 3 CRS occurring in only 5% of patients [52, 53].

In March 2018, blinatumomab received a second FDA approved indication for B-ALL in first or second CR with MRD $\leq 0.1\%$ based on results from the open-label, multicenter, single-arm BLAST trial (NCT01207388) [54]. Seventy-eight percent of evaluable patients had a complete MRD response after one cycle of blinatumomab and, compared to non-responders, had a longer median RFS (23.6 months versus 5.7 months) and OS (38.9 months versus 12.5 months). Sixty-seven percent proceeded to HSCT and 49% remained in remission at median follow-up of 24 months. CRS and neurologic events occurred less frequently compared to patients with relapsed/refractory disease, likely due to the decreased disease burden.

Lastly, the efficacy and safety of blinatumomab in relapsed/refractory Ph+ ALL in adults who failed tyrosine kinase inhibitors has also been established, resulting in FDA approval to this population in 2017. In a phase 2, single-arm, multicenter study (ALCANTARA), 16/45 (36%) patients achieved a CR/CRh after two cycles of blinatumomab (NCT02000427). The great majority (88%) were MRD-negative and 44% were able to proceed to HSCT [55]. When compared to a historical control, both CR/CRh (OR: 1.54, 95% CI 0.61–3.89) and OS (OR: 0.81, 95% CI, 0.57–1.14) favored blinatumomab [56]. Additional predictors of outcome to blinatumomab are described in Table 3 [47, 54, 57–59].

3.1.2. Pediatric Relapsed/Refractory B-ALL—Initial FDA approval of blinatumomab in children was based on an open-label phase 1/2 study in children with relapsed/refractory B-ALL conducted by the COG and I-BFM (NCT01471782). Forty-nine children were treated in the phase 1 dose escalation study, which established the maximum tolerated dose (MTD) of 15 mcg/m²/day. A recommended phase 2 dose of 5 mcg/m²/day for the first 7 days followed by 15 mcg/m²/day thereafter to complete a 28-day course was established to minimize the risk for CRS in patients with bulk disease at treatment onset [57].

Twenty-seven of seventy (39%) patients achieved a CR after 2 cycles, of which 14 (52%) had a complete MRD response. Among responders, median RFS was 4.4 months. Blinatumomab was associated with a longer OS (HR: 0.65; 95% CI 0.44–0.94) and a trend for a higher CR rates (HR: 1.82; 95% CI, 0.74–4.51) when compared to historical trials [60]. The most common AEs noted were pyrexia (80%), anemia (41%), nausea (33%) and headache (30%), with 8 (11%) patients developing CRS (four of which were grade 3) [57].

A subsequent study conducted by the COG (NCT02101853) evaluated patients aged 1–30 years with B-ALL in first relapse. After one cycle of standard re-induction therapy, patients were randomized to receive two intensive chemotherapy blocks or two 4-week blocks of blinatumomab. Analysis of intermediate-risk and high-risk cohorts revealed a 2-year DFS and OS rates of 59.3% and 79.4%, respectively, with blinatumomab (n=105) versus 41% and 59.2% with standard chemotherapy alone (n=103) [44]. A higher proportion of patients with detectable MRD after re-induction therapy were able to achieve MRD-negativity after one cycle of blinatumomab versus chemotherapy alone (79% versus 21%) and patients receiving blinatumomab were more likely to proceed to HSCT (73% versus 45%). Toxic AEs occurred significantly more frequently in the chemotherapy arm, particularly among adolescents and young adults [61]. CRS and neurotoxicity were frequently observed during cycle 1 of

blinatumomab, but the overall rates of grade 3 or higher events were infrequent. Based on these results, randomization was halted early [44]. Results from the interim analysis of the phase 3 study 20120215 (NCT02393859) had similar findings, with improved event-free survival (EFS) reported in children with high-risk B-ALL in first relapse receiving blinatumomab versus standard chemotherapy alone [62].

3.1.3. Special Pediatric Populations—Blinatumomab has been used successfully in infants and children with Down syndrome with persistent MRD. A multicenter study described 11 infants with *KMT2A*-rearranged ALL in first remission or relapse who received 1–2 cycles of blinatumomab. All patients achieved a CR, with 9 infants achieving MRD-negativity, and were able to proceed to transplant at a median time of 51 days. Blinatumomab was generally well-tolerated with 3 infants experiencing grade 1–2 CRS and 1 with neurotoxicity [63]. A study is ongoing in Europe (EudraCT 2016–004674-17). Furthermore, blinatumomab has been used as a bridge to further antileukemic therapy secondary to overwhelming chemotherapy-associated toxicity [64].

3.1.4. Characteristics of Relapse and Potential Determinants of Response to Blinatumomab—CD19 negativity after treatment with blinatumomab is well-described and is discussed in more detail below [58, 65]. Although pediatric data is minimal, extramedullary (EM) relapses following blinatumomab therapy have been reported in 25–40% of adults [49, 58, 66, 67]. Active CNS disease or CNS pathology were exclusion criteria in most published studies so there are limited data regarding the penetration of blinatumomab into the cerebrospinal fluid (CSF). Alternative treatment options should therefore be considered for patients with active CNS involvement [66]. Additionally, studies suggest low tumor burden, earlier intervention (CR1 vs. others), and its use in patients with Ph-like ALL with high CRLF2 expression might also confer better response to therapy [47, 54, 57–59, 66].

3.2. Inotuzumab Ozogamicin

CD22 is involved in B cell activation and regulation and, like CD19, is present in > 90% of patients with B-ALL [68, 69]. Inotuzumab ozogamicin (InO, Besponsa®, Pfizer) is a humanized antibody-drug conjugate, composed of an IgG4 kappa antibody specific for the cell-surface glycoprotein CD22, and calicheamicin, a cytotoxin derived from *Micromonospora echinospora*. Upon binding to CD22 on leukemic blasts, the complex is rapidly internalized into the cell, where calicheamicin is hydrolyzed and reduced into a reactive intermediate, which induces double strand DNA breaks and results in cell cycle arrest and apoptosis [70–73].

3.2.1. Adult Relapsed/Refractory B-ALL—In 2017, InO received FDA approval for the treatment of relapsed/refractory B-ALL in adults based on data from INO-VATE, an open-label, multicenter, phase 3 trial comparing InO to standard intensive chemotherapy (NCT01564784) [68]. InO was dosed at 0.8 mg/m² on day 1, followed by 0.5 mg/m² on days 8 and 15 for a 21-day cycle followed by subsequent 28-day cycles [74–76]. Compared to chemotherapy, patients who received InO had higher rates of CR and CRi (73.8% versus 35%) and a longer median duration of remission (5.4 months versus 4.2 months), the

majority of which were achieved after the first cycle in both arms [77]. Among patients who received InO and achieved CR/CRi, 62.8% were MRD-negative and more patients receiving InO were able to bridge to HSCT (48% versus 22%) [78]. Importantly, patients who received InO also had a longer progression free survival (5 versus 1.7 months) and OS (7.7 versus 6.2 months) compared to chemotherapy, translating to a 25% lower risk of death [77].

The most common grade 3 or higher AEs in the InO arm were cytopenias and febrile neutropenia. Treatment-associated hepatotoxicity occurred more frequently with InO (50.6% versus 36.4%) and the rate of sinusoidal obstruction syndrome (SOS) at any point during or after InO therapy was 14%, with the highest risk in patients who proceeded to transplant [77].

3.2.2. Pediatric Relapsed/Refractory B-ALL—A retrospective cohort study of 51 pediatric and young adult patients with relapsed/refractory B-ALL who received InO through a compassionate use protocol was also conducted [79]. Of the 42 heavily pretreated patients with either an M2 (5–25% blasts) or M3 (>25% blasts) marrow prior to InO, 28 patients (67%) achieved a CR/CRi, including all 18 patients who had received one or more prior HSCTs. Among the responders, 24 patients (86%) achieved CR/CRi after the first cycle of InO and 20 patients (71%) also achieved MRD-negativity. Twenty-one patients underwent HSCT at a median time from last dose of InO to stem cell infusion of 26 days (range: 13–91 days). The 12-month EFS and OS rates for the entire cohort were $23.4 \pm 7.5\%$ and $36.3 \pm 9.3\%$, respectively. InO was well-tolerated with grade 3 febrile neutropenia and grade 3/4 infections being the most common AEs. Notably, however, 11 of the 21 patients who received HSCT after achieving CR developed SOS, of which two cases were fatal. No cases of SOS occurred during InO therapy [79]. The CR rates were similar to those described in other retrospective studies [80, 81].

Additionally, a phase 1 study of single agent InO in pediatric patients with relapsed/refractory B-ALL (ITCC-059), confirmed that the FDA approved body surface area based dose in adults is appropriate for children [82]. COG is currently conducting a single arm phase 2 trial of InO (NCT02981628). In an interim report of 48 heavily pretreated pediatric and young adult patients with relapsed/refractory CD22-positive B-ALL, 28 patients achieved a CR/CRi (58.3%), 3 patients had a partial response (6.3%), 9 had stable disease (18.8%), and 8 had progressive disease (16.7%) after one cycle of therapy. Two additional patients achieved CR/CRi after cycle 2. Of the 26 patients with available MRD data after cycle 1, 65.4% achieved MRD-negativity. Febrile neutropenia (27%) and infection (18.8%) were the most common AEs with prolonged marrow aplasia being the most common dose-limiting toxicity. SOS only occurred in patients receiving subsequent transplants (4/13, 30.7%), all of which were grade 3 and treated with defibrotide [83]. Results of retrospective studies of the clinical efficacy of InO in pediatric patients are summarized in Table 4 [79–81].

3.2.3. Special Pediatric Populations—Efficacy and safety data for InO in younger children is limited. In a retrospective study, 12 infants and children were administered 1–3 cycles of InO for relapsed/refractory B-ALL. Six patients had a CR (50%), 3 after cycle 1 and 3 after subsequent courses. Five patients (42%) received a HSCT at a median of 34 days

(range 21–70 days). The most common toxicity was hematologic and two deaths were reported, one due to toxic leukoencephalopathy and one due to SOS which occurred during HSCT [82]. InO has been used with success in patients with Down syndrome however, the data remains scarce [79, 83, 84].

3.2.4. Potential Predictors of Response—The rate of EM relapse after receipt of InO has not been clearly elucidated [79]. Similar to blinatumomab, most patients were excluded from prospective studies of InO if they had an isolated EM relapse and/or active CNS leukemia [76, 83]. Because of its large molecular weight (approximately 160 kDa), it is believed that InO and its metabolites do not readily cross the blood brain barrier [70, 85]. In the INO-VATE trial, negative MRD status, baseline hemoglobin level ≥ 10 g/dL, duration of first clinical remission (CR1), CR/CRi, and receipt of HSCT were all associated with improved OS [77]. In the experience described by Bhojwani et al., response was independent of the number of previous relapses or treatment attempts, receipt of CD19- and CD22- directed immunotherapy, and refractoriness to the preceding treatment attempts [79].

4. Chimeric Antigen Receptor T-cell Therapy

Chimeric antigen receptor T cells (CAR-T cells, CARs), a form of adoptive immunotherapy, rely on the transfer of genetically modified effector cells to elicit an anti-leukemic immune response and therefore have the potential to persist *in vivo*, offering long-term disease control. Unlike native T cell receptors which recognize major histocompatibility complex molecules often downregulated by cancer cells, CAR-T cells utilize an HLA-independent approach [86]. While the majority of CARs products employ autologous T-cells, CAR NK cells and universal allogeneic “off the shelf” CARs have also been developed [87, 88]. Tisagenlecleucel (Kymriah®, Novartis), an anti-CD19 CAR-T cell product formerly known as CTL019, became the first FDA approved CAR-T cell therapy for B-ALL in 2017. Unlike most therapies which are first approved in adults, this agent received approval for the treatment of relapsed/refractory B-ALL in children and young adults due to its impressive efficacy in this age cohort [89, 90]. Since its approval, many iterations of CAR-T cells have been developed and deployed in clinical trials (Table 5) [89, 91–94]. While CD19 remains the most commonly targeted antigen, anti-CD22 CAR-T cells have also been developed. These antigens serve as ideal targets as noted above and their effect on normal cells, namely B cell aplasia, can be corrected with intravenous immunoglobulins.

4.1. CAR Biology, Manufacturing and Delivery

After T cells are harvested by leukapheresis, they are reengineered *ex vivo* to contain a monoclonal antibody recognition fragment, specific for a target on the cell of interest, linked to a T cell signaling domain. Upon binding the target cell, the signaling domain activates the T cell’s cytotoxic machinery to ultimately kill the bound antigen-expressing cell [95]. First-generation CAR-T cells joined an extracellular domain, comprised of a scFv antibody fragment, to an intracellular signaling domain, comprised of the CD3 zeta chain of the T cell receptor [96]. After first-generation CARs performed poorly in clinical trials, investigators developed second- and third-generation CARs which were modified to contain additional costimulatory domains (such as CD28, 4-1BB, or OX40). Of the costimulatory domains, 4–

1BB has been shown to ameliorate T cell exhaustion and promote longevity of the CAR *in vivo* [97]. Most current clinical trials employ second- and third-generation CARs due to their superior efficacy and other novel CARs (e.g., fully humanized CARs, “armored” CARs discussed below) [86, 98].

After reengineering and *ex vivo* expansion in culture, the cells are “bedside ready” for reinfusion. Patients undergo lymphodepletion with chemotherapy (most often fludarabine and cyclophosphamide) prior to reinfusion [99]. The CAR-T cell dose is based on the patient’s weight at the time of leukapheresis and the maximum dose that was successfully manufactured within range is infused [100]. Once reinfused, CAR-T cells bind to tumor antigens inciting not only their cytotoxic effects, but also promoting CAR-T cell proliferation *in vivo* allowing for longevity [101]. CARs are widely distributed throughout the body, including the CNS, making them a promising option for EM relapse [90]. For some patients, CAR-T cell therapy has obviated the need for HSCT however it does not prevent recurrence due to antigen escape lack of CAR-T cell persistence or T cell exhaustion. A subset of patients, such as those receiving CD22-directed CARs or those with positive MRD detected by next generation sequencing post-CAR therapy, may still require consolidation with HSCT for definitive treatment [102–104].

4.2. Anti-CD19 CAR-T Cell Clinical Experience

Early clinical trials conducted at the Children’s Hospital of Philadelphia/University of Pennsylvania of CTL019 showed remarkable results. The initial phase I trial which enrolled 30 heavily pretreated relapsed/refractory B-ALL patients (25 pediatric and 5 adult) demonstrated a 90% CR rate at 1 month post-infusion with 67% EFS (95% CI, 51 to 88) and 78% OS (95% CI, 65 to 95) at 6 months [90]. CRS was observed in all patients and was severe in almost one-third of patients, especially those with high tumor burden, but all cases responded to tocilizumab. Tocilizumab, a humanized anti-IL6 receptor antibody, received FDA approval in 2017 for the treatment of CRS related toxicity in children > 2 years of age undergoing CAR-T cell therapy and treatment algorithms have been proposed with tocilizumab indicated for all patients with grade 3 or greater CRS [105, 106]. Thirteen patients developed neurological side effects, all of which were self-limited. Finally, two patients with evidence of CNS leukemia at infusion had no detectable disease in the CSF 6 months post-infusion. The follow-up phase 2 multicenter, global ELIANA trial of tisagenlecleucel expanded the original cohort to 75 evaluable patients less than 21 years of age with relapsed/refractory B-ALL (NCT02435849) and led to FDA approval [89]. At 3 months, 81% of patients achieved remission with 100% being MRD-negative. At 12 months, EFS and OS were 50% (95% CI, 35 to 64) and 76% (95% CI, 63 to 86), respectively. The probability of B cell aplasia at 6 months was 83% (95% CI, 69 to 91), which is significant as early B cell recovery has been linked to a greater risk of relapse [107]. CRS and neurological toxicity were common, occurring in 77% and 40% of patients, respectively. CRS was managed with tocilizumab in 48% of patients, otherwise toxicities were managed with supportive care. Other frequent AEs included infection, febrile neutropenia and cytopenias, most of which occurred within 8 weeks of infusion. Notably, 19 deaths occurred after tisagenlecleucel infusion including one patient who succumbed from cerebral hemorrhage 15 days post-infusion in the setting of CRS-induced coagulopathy. The majority

of deaths, however, occurred more than 8 weeks after infusion and were related to progressive disease or infection.

Early phase trials of other CAR-T constructs have also been completed. A phase 1 trial of a CD19 CAR product with CD3- ξ / CD28 / 4-1BB costimulatory domains in 45 children and young adults with relapsed/refractory B-ALL was conducted at Seattle Children's Research Institute ([NCT02028455](#)) [92]. An intent-to-treat analysis revealed MRD-negative remission rates in 93% of patients with an estimated 12-month EFS of 50.8% (95% CI, 37 to 70) and OS of 69.5% (95% CI, 56 to 87). The MTD was 1×10^6 CAR-T cells/kg. Seven patients had received treatment with anti-CD19 therapy previously, of which 6 received blinatumomab and only 4 achieved MRD-negative status. Of the 40 patients who achieved MRD-negativity, there were 18 relapses, 17 of which were isolated to the bone marrow and one was isolated to the CNS. Seven relapses were characterized by loss of CD19 surface expression and one underwent lineage switch to acute myeloid leukemia. Loss of functional CAR-T cells, as measured by loss of B cell aplasia, greatly increased the risk of CD19+ relapse, compared to the risk of CD19- relapse, in the remaining patients. The overall incidence of CRS and neurotoxicity were 93% (of which 40% required tocilizumab) and 49%, respectively. No deaths were attributed to these toxicities.

While the products discussed above utilize a 4-1BB costimulatory domain, CAR-T cells utilizing a CD28 costimulatory domain have been investigated at Memorial Sloan Kettering Cancer Center (MSKCC) ([NCT01860937](#)) and The National Institute of Health (NIH) ([NCT01593696](#)) [91, 93]. MSKCC reported the result of 25 pediatric/young adult patients with relapsed/refractory B-ALL treated with a second generation CD19 CAR termed 19-28z. The overall response rate was 75%, 89% of which achieved MRD-negativity. Any grade CRS occurred in 80% of patients, however, only 4 required treatment with tocilizumab. The authors attribute the low incidence of severe CRS to the low tumor burden in the majority of patients at enrollment. Neurological toxicity occurred in 72% of patients and was self-limited in all cases. The trial also investigated low- versus high-dose cytarabine monotherapy for lymphodepletion in patients with evidence of morphological disease at the time of infusion and found that patients who received high-dose cytarabine had improved response rates without increased toxicity. Similarly, the NIH phase 1 dose escalation trial of their CD19-CAR, harboring TCR zeta and CD28 costimulatory domains, in 20 children and young adults had similar results. The MTD was determined to be 1×10^6 cells/kg and all toxicities were reversible. The authors reported a 70% CR rate with an OS of 51.6% at a median follow up of 10 months. Updated results showed a CR rate of 61% with the best long-term survival rates seen in patients who subsequently received a HSCT [108].

4.3. Anti-CD22 CAR-T Cell Clinical Experience

While there has been unprecedented success with CD19-directed immunotherapy, antigen escape leading to CD19-negative relapse remains a frequent cause of relapse. As a result, the NIH developed a novel CD22-CAR with a 4-1BB costimulatory domain and results from their initial phase 1 trial of 21 pediatric and adult patients with relapsed/refractory B-ALL post-HSCT have been reported [109]. Among the 21 patients, 17 had failed CD-19 directed immunotherapy. Grade 1-2 CRS occurred in 16/21 (76%) patients and the MTD was

determined to be 1×10^6 CAR-T cells/kg. CR was achieved in 57% of patients. Among the CD19-negative/dim patients, there was a 100% response rate, including one who failed both blinatumomab and CD19 CAR-T cell therapy, indicating prior treatment with anti-CD19 therapy did not impact response to anti-CD22 CARs. Relapse occurred in 8 patients, 7 of which were associated with diminished CD22 density on relapsed blasts. An updated analysis of this trial reported a CR rate of 72.7% [94]. Of the patients who achieved a CR, 63.6% were MRD-negative. Notably, a history of prior CD22-directed therapy correlated with lower rates of MRD-negativity and worse outcomes. The median OS and RFS were 13.4 months (95% CI, 7.7 to 20.3 months) and 6 months (95% CI, 4.1 to 6.5 months), respectively. Toxicity rates were comparable except for one patient with grade 5 CRS.

4.4. CAR-T Cell Therapy Adverse Effects

Although usually self-limited, severe and life-threatening toxicities have been reported with CAR-T cell therapy. The most common and potentially severe toxicity is CRS. The risk of CRS has been shown to be greatest in patients with high tumor burden, however, its development has also been linked to lymphodepletion with cyclophosphamide and fludarabine, CAR-T cell dose and CD28-based CAR constructs [110, 111]. The development of CRS has also been associated with disease response, as patients who do not develop this complication are less likely to respond to therapy [112].

Neurotoxicity, termed immune effector cell associated neurotoxicity syndrome (ICANS), follows CRS as the second most common toxicity associated with CAR-T cell therapy [89, 90, 113]. It is most often characterized by global encephalopathy, but can also manifest as aphasia, confusion, focal neurological deficits and less often, seizures, the latter attributed to an increase in endogenous excitatory neurotransmitters in the CSF [114]. Pediatric ICANS grading has been standardized by the ASTCT and is based on the Cornell Assessment of Pediatric Delirium (CAPD) screening tool [51, 115]. Steroids and supportive care are the mainstay of treatment and most patients recover without any long-term neurologic sequelae. Lastly, chronic B cell aplasia remains a universal side effect for patients undergoing CAR-T cell therapy for B-ALL [90]. Persistent B cell aplasia beyond 6-months after infusion has been associated with superior outcomes.

5. Immunotherapy in Upcoming Pediatric Trials

Due to efficacy and favorable toxicity profiles in the relapsed/refractory setting, current clinical trials are investigating promising immunotherapeutics in the frontline setting [116]. COG AALL1731 ([NCT03914625](#)) is evaluating whether the addition of blinatumomab to standard chemotherapy can improve outcomes in a subset of newly diagnosed National Cancer Institute (NCI) standard-risk B-ALL patients. Because of excessive treatment related mortality, blinatumomab replaces components of cytotoxic chemotherapy in children with Down syndrome on this trial [117–119]. The AIEOP-BFM group is investigating whether the addition of one cycle of post-reintensification blinatumomab for intermediate-risk patients and substitution of two highly intensive chemotherapy courses with two cycles of blinatumomab for high-risk patients can improve outcomes ([NCT03643276](#)) [116, 120]. COG AALL1732 ([NCT03959085](#)) is investigating whether the addition of two cycles of InO

can improve DFS in patients with newly diagnosed NCI high-risk B-ALL. In parallel, InO is being investigated in combination with rituximab in adolescent/young adult patients with newly diagnosed B-ALL on the Alliance A041501 trial ([NCT03150693](#)). Lastly, COG AALL1721 is currently investigating tisagenlecleucel in CR1 in NCI high-risk B-ALL patients with end consolidation MRD positivity ([NCT03876769](#)).

6. Overcoming Mechanisms of Resistance to Immunotherapy

The success of immunotherapy in treating B-ALL is unprecedented. However, while the majority of patients respond, over 50% will recur. The major reasons for therapy failure are antigen down-modulation and, in the case of CAR-T cells, loss of T cell function. Target antigen modulation could have been predicted based on experience with conventional chemotherapy, where tumor heterogeneity leads to the outgrowth of tumor subclones with a competitive advantage under the selective pressures of therapy. In the case of blinatumomab, antigen loss is seen in about 20% of patients who relapse, while incidence after CD19-directed CAR-T cell therapy is not clearly defined, with pediatric and adult clinical reports ranging from 7% to 63%. Data variability could be explained by length of follow-up, prior CD19-directed therapy and patterns of subsequent HSCT [89, 121–124]. Antigen loss most often occurs due to CD19 splice variants and mutations that lead to misfolded proteins that become trapped in the endoplasmic reticulum [125, 126]. There is some suggestion that such variants exist before treatment, but most studies have not identified these clones pre-treatment [127]. Decreased antigen density, as opposed to complete loss, is associated with relapse in the case of CD22-directed CAR-T therapy [109]. This down modulation of CD22 is not associated with genetic alterations of the locus suggesting post-translational mechanisms of modulating CD22 expression. Down-modulation may be less of a problem following InO possibly because of the associated toxin moiety.

The selection of antigen negative/low subclones after therapy with blinatumomab and InO raises an important clinical dilemma: does treatment with either agent impact success for those patients who proceed to CAR-T therapy? While not completely settled, it does appear that prior treatment with blinatumomab and InO decreases the effectiveness of CD19- and CD22-directed therapies respectively [94, 128]. The influence of pretreatment on CAR-T efficacy will require close monitoring given the fact that both blinatumomab and InO are now being evaluated in the frontline setting.

Antigen escape through lineage switch to a myeloid phenotype at relapse has also been seen, specifically, in patients with *KMT2A*-rearrangements, *BCR/ABL1* and other stem cell like subtypes [129, 130]. Such patients might be optimally treated with eventual HSCT following immunotherapy. Another potential mechanism of antigen modulation seen in preclinical models is trogocytosis, whereby the leukemia cells transfer the target antigen to T cells which in turn leads to killing of the CARs by themselves, a process called fratricide. Finally, a particularly unusual case of CD19 negative relapse was observed due to integration of the CAR construct into a single B-ALL cell during the manufacturing process. The aberrant surface expression of the anti-CD19 CAR led to binding of CD19 resident episodes thereby masking it from recognition [131].

To overcome the challenge of antigen modulation, many investigators have resorted to pre-emptive therapy targeting multiple antigens. Sequential InO/blinatumomab (+/- rituximab) with a reduced intensity chemotherapy backbone has been used successfully in the therapy of older adults with ALL as well as patients at relapse [132, 133]. Likewise, combination CD19 CAR-T and CD22 CAR-T therapy has been explored. This approach can be implemented through either an infusion of a “cocktail” of CD19 and CD22 CAR-T cells sequentially administered or bivalent CAR-T cells [134]. A recent report of sequential CD19 and CD22 CAR T-cells demonstrated impressive activity in 20 children with relapsed/refractory ALL. The leukemia free survival of 79.5% at 18 months was far better than prior reports with single antigen specific CAR-T cells and none of these patients proceeded to HSCT, indicating durability of the response [135]. The development of dual antigen CD19/CD22 targeting CAR-T cells, although challenging, has been accomplished and clinical trials are underway ([NCT03448393](#)) [136]. Preliminary results from a phase 1 trial of a bivalent CD19/CD22 CAR-T in heavily pretreated, relapsed adults and children showed that 11 of 12 patients achieved a CR at day 28 [137].

Another mechanism of relapse following CAR-T cell therapy is lack of persistence of the infused T cells, which can be heralded by loss of B cell aplasia. These are antigen positive relapses and usually occur early. Many factors mediate CAR-T cell persistence, including the co-stimulatory domain in the CAR construct with 4-1BB CAR-T cells persisting longer than those containing CD28 [138]. The site of genomic integration of the CAR gene also plays a role, leading to the potential of CRISPR-Cas9 gene editing technology to select for favorable insertion sites [139, 140]. Integration in genetic loci directing cell signaling and chromatin modification correlate with tumor response [141]. More recently, investigators have studied the starting material and manufactured product to determine if unique subsets of T cells lead to improved persistence, cytolytic activity and ultimately outcome. For example, CAR-T cells showing expression of T cell memory related genes including IL-6/STAT3 signatures led to a better response in CD19 CAR-T cell treated patients with chronic lymphocytic leukemia, whereas Finney et al. reported that therapeutic products with increased frequency of TNF- α -secreting CAR CD8+ cells were associated with more favorable responses in pediatric and young adults with B-ALL [139, 142]. Changes to the manufacturing process can have unanticipated consequences. Follow up analysis of the phase 1 anti-CD22 CAR-T cell trial revealed that CD4/8 T cell selection of the apheresis product led to improved CAR expansion, but also an increased incidence of hemophagocytic lymphohistiocytosis, necessitating a dose reduction [94]. Finally, the CAR-T cell product's phenotypical and functional outputs as well as the patient's CD19+ tumor burden at infusion have been correlated with durable persistence of CD19 CAR-T cells [142]. To address this, trials are underway to assess whether co-administration of antigen presenting cells, either in the form of patient derived T cells engineered to express CD19 ([NCT03186118](#)) or off the shelf antigen presenting cells, would improve CAR-T persistence.

Both BiTE and CAR-T cells depend on a robust T cell response against targeted leukemia cells, however, tumor cells can up-regulate ligands such as PDL1 which bind to inhibitory receptors on T cells and blunt the response to these agents. Multiple studies are now underway combining blinatumomab with checkpoint inhibitors to augment effectiveness, including the upcoming COG AALL1821 trial. Likewise, the effectiveness of CAR-T cells

can be augmented by inhibiting tumor associated inhibitory signals as well as the immunosuppressive effects of the microenvironment. Such signals can also lead to T cell exhaustion and lack of long-term persistence. Trials combining CAR-T cells with checkpoint inhibitors are also underway, but such combinations might trigger excessive immune stimulation. One way to circumvent this potential toxicity is through the creation of “armored” CARs secreting PD-1 blocking scFvs, cytokines (e.g. IL-12, IL-18 and others) or co-expression of dominant-negative TGF- β receptor type II that converts the local microenvironment to a pro-inflammatory state [143].

The resistance mechanisms discussed above can be described as acquired resistance, however a subset of patients are refractory to treatment. Impaired death receptor signaling in the leukemia cells is associated with CAR-T failure through antigen-driven T cell exhaustion without evidence of typical exhaustion epigenetic hallmarks [144]. Likewise, multimodal genetic analysis of CAR-T cell refractory leukemia samples identified the presence of *CREBBP*-fusions, methylation-based upregulation of *JUND/JUN* and a high ratio of open chromatin genome regions in non-responders compared to responders, all suggesting that further analysis of epigenetic modifications in the leukemic cells could explain primary resistance [145]. Finally, high throughput small molecule and genome-scale CRISPR-Cas9 loss of function screens have identified the importance of death receptor signaling (via FADD and TNFRSF10B) and potential sensitization of cytotoxicity with SMAC mimetics [146].

7. Conclusion

Immunotherapy has led to dramatic improvements in outcome for relapsed/refractory B-ALL and a number of agents are now being integrated into frontline therapy. Based on initial experience, a variety of treatment modifications such as dual antigen targeting, integration of therapies designed to overcome the immunosuppressive microenvironment, and optimized design of third- and fourth-generation CAR-T cells are expected to augment efficacy. It is anticipated that immunotherapy will not only improve conventional treatment, but will replace components of cytotoxic therapy with the goal of improving outcomes and decreasing long-term effects.

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Compliance with Ethical Standards

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Key Points

- Although the overall prognosis for childhood acute lymphoblastic leukemia (ALL) is excellent, it remains a leading cause of morbidity and mortality in pediatric cancer, especially in certain subgroups of patients such as infants, adolescents and young adults and those with unfavorable genomic alterations.
- The use of targeted immunotherapeutics has led to significant advances in the treatment of relapsed/refractory B-ALL. Moving these agents to frontline therapy has become the focus of many clinical trials.
- Immunotherapeutic agents are associated with a unique spectrum of side effects mandating anticipation and proactive approaches, to minimize their impact, by clinicians incorporating these agents into multiagent chemotherapy regimens and potential stem cell transplantation.
- Despite the promise of immunotherapy, resistance can develop and novel approaches are needed to mitigate these mechanisms of tumor escape.

Figure 1: Immunotherapeutic approaches to treatment of acute lymphoblastic leukemia (ALL). A. Conventional approaches using blinatumomab, inotuzumab ozogamicin (InO), rituximab and anti-CD19 and anti-CD22 chimeric antigen receptor T cells (CAR-T cells). B. Overcoming mechanisms of resistance utilizing sequential CAR-T cells, dual anti-CD19 and anti-CD22 CAR-T cells, armored CAR-T cells such as those expressing an anti-PDL1 single-chain variable fragment (scFV) and the use of checkpoint inhibitors.

Table 1:**B-ALL Risk Classification at Initial Diagnosis**

Risk Group	Low	Standard		High					Very High
5-year EFS	>95%	90–95%		65–90%					<50%
NCI Risk Group	SR	SR	SR	SR	SR	SR	SR	HR	HR
CNS [^]	1/2	1/2	1	2	1/2	3	1/2	Any	Any
Genetics	Fav	Fav	Neut	Neut	Any	Any	Unfav	Any	Any
MRD Day 8 (PB)	<1%	1% (Any for DT)	Any	Any	Any	Any	Any	NA	NA
MRD Day 29 (BM)	<0.01 %	<0.01% (<0.1% for DT) [*]	<0.01 %	Any	0.01% (0.1% for DT) [*]	Any	Any	Any	0.01%
MRD EOC (BM)								<0.01% ^{&}	0.01%

Abbreviations and definitions:

EFS: event-free survival; SR: standard risk (age < 10 years and initial WBC <50,000/μL); HR: high risk (age ≥ 10 years and/or initial WBC ≥ 50,000/μL); CNS: central nervous system: CNS 1: absence of blasts; CNS 2: WBC < 5/μL with blasts or traumatic LP; CNS3: WBC ≥ 5/μL and blasts; Fav: favorable (double trisomies of chromosomes 4 and 10 [DT] or *ETV6-RUNX1* translocation; Neut: neutral (absence of favorable or unfavorable genetics); Unfav: unfavorable (iAMP21, *KMT2A-R* [formerly *MLL-R*], hypodiploidy [modal chromosome number less than 44, DNA index <0.81, or other clear evidence of a hypodiploid clone], Philadelphia chromosome positive OR t(17;19); NA: not assessed; PB: peripheral blood; BM: bone marrow; Day 8: day 8 of induction; Day 29: end of induction; EOC: end of consolidation.

[^] Patients with testicular leukemia are classified as high risk

^{*} NCI SR patients with CNS 1 or 2, and double trisomies of chromosomes 4 and 10 are classified as SR if Day 29 MRD <0.1%, High Risk if Day 29 MRD ≥ 0.1%

[&] If MRD ≥ 0.01% on Day 29

Table 2:**Risk Stratification of Relapsed Pediatric B-ALL**

Risk Group	Low		Intermediate		High		
5-year EFS	70–75%		40–50%		25–40%		
Site	Marrow	IEM	Marrow	IEM	Marrow	IEM	Any
Immunophenotype	B-cell	B-cell	B-cell	B-cell	B-cell	B-cell	T-cell
Timing	Late	Late	Late	Late	Early	Early	Any
MRD Reinduction I	<0.1%	<0.1%	0.1%	0.1%	Any	Any	Any

Abbreviations and definitions:

Early isolated extramedullary (IEM) relapse (< 18 months from diagnosis), late IEM (≥ 18 months from diagnosis); early marrow relapse (< 36 months from diagnosis), late marrow relapse (≥ 36 months from diagnosis); reinduction I (end of the first cycle of reinduction therapy)

Table 3:**Potential Determinants of Response to Blinatumomab**

High leukemia burden	Patients with bone marrow blasts < 50% had higher CR/CRh rates in both adult (73% vs 29%) and pediatric studies (56% vs 33%) [47, 57, 58]. This is also reflected in the higher overall CR rates observed in patients with MRD positive disease, suggesting that a lower leukemia burden is predictive of response to therapy [54].
Extramedullary (EM) disease	In a multivariable analysis of 65 patients treated with blinatumomab, both a history of EM disease and active EM disease at time of blinatumomab initiation were predictive of poor response. Upon multivariable analysis, the presence of EM-ALL at treatment start was the only independent predictor of poor response (OR= 13.16; p=0.03) [58]. Of note, there is little data on the penetration of blinatumomab to the CNS, as it was often an exclusion criteria in clinical trials.
Treatment after first clinical remission (CR1)	The BLAST study (NCT01207388) showed that patients treated after first CR had longer RFS (24.6 vs 11 months), OS (36.5 vs 19.1 months), and duration of hematologic remission (not reached versus 19.1 months) compared to patients treated after second/third CR, suggesting that earlier intervention with blinatumomab may be beneficial [54].
Ph-like ALL with <i>CRLF2</i> expression	In a study looking at genomic determinants of response to blinatumomab (n=29), patients with Ph-like ALL with <i>CRLF2</i> expression had higher response rates (83.3% or 10/12 patients) compared to Ph-like non- <i>CRLF2</i> patients (60% or 3/5 cases) and other genomic subtypes (33% or 4/12 cases). GSEA showed activation of IFN-gamma, JAK-STAT, and chemokine/cytokine signaling were enhanced in responders [59].

Abbreviations and definitions: CR: complete response, CRh: complete hematologic response, MRD: minimal residual disease, CNS: central nervous system, RFS: relapse-free survival, OS: overall survival, GSEA: Gene Set Enrichment Analysis

Table 4:**Clinical Experience of Inotuzumab in Pediatric B-ALL**

	Multinational [79]	French [80]	Spanish Society of Pediatric Hematology and Oncology (SEHOP) [81]
Population	N=51 (range 2.2–21.3 years) -First relapse: n=10 -Second or greater relapse: n=40 -Primary refractory: n=1	N=11 (range 3–18 years old) -First relapse: n=6 -Second or greater relapse: n=5	N=16 (range 0.5–18 years old) -First relapse: n=6 -Second or greater relapse: n=7 -No prior relapses: n=3
Prior transplant	22/51 (43%) patients	4/11 (36.4%) patients	7/16 (43.7%) patients
Bone marrow status	M1: n=8 M2: n=4 M3: n=38 Unknown: n=1	M1: n=1 M2: n=2 M3: n=8	M1: n=5 M2 or M3: n=11
Complete remission in patients with overt disease (M2-M3)	28/42 (67%)	7/10 (70%)	6/11 (54.5%)
MRD negativity	20/28 (71%) of complete responders with overt disease 4/8 (50%) of patients with M1 disease	2/8 (25%) of complete responders with M1-M3 disease	6/6 (100%) of complete responders with over disease 5/5 (100%) of patients with M1 disease
Grade 3/4 hematologic toxicities	Not reported	10/11 (90.9%)	11/16 (68.8%)
Grade 3/4 febrile neutropenia	6/51 (11.8%)	5/11 (45.5%)	Not reported
Incidence of sinusoidal obstruction syndrome (SOS)	Eleven of 21 patients who subsequently underwent HSCT	One patient who had previously received a HSCT Two patients who subsequently underwent HSCT	One patient who had previously received a HSCT One patient who subsequently underwent HSCT

Abbreviations and definitions: HSCT: hematopoietic stem cell transplant, CR1: first clinical remission, CR2: second clinical remission, M1: <5% blasts in bone marrow, M2: 5–25% blasts in bone marrow, M3: >25% blasts in bone marrow, MRD: minimal residual disease, SOS: sinusoidal obstruction syndrome

Table 5:

Clinical experience of CAR-T cell therapy

Agent (Target)	CAR Construct	Age (years)	N	CR	MRD(-)	Survival (EFS/OS)
CTL019 (CD19) [89] (NCT02435849)	CD3-ξ / 4-1BB	3–23	75	81%	100%	50%/76% (1 year)
MSKCC (CD19) [91] (NCT01860937)	CD3-ξ / CD28	1–22.5	25	75%	89%	Not reported
Seattle (CD19) [92] (NCT02028455)	CD3-ξ / CD28 / 4-1BB 1:1CD4+/CD8+	1–26	45	89%	93%	51%/66% (1 year)
NCI (CD19) [93] (NCT01593696)	CD3-ξ / CD28	5–27	21	67%	60%	79% (4.8 months) / 52% (9.7 months)
NCI (CD22) [94] (NCT02315612)	CD3-ξ / 4-1BB (fully humanized)	4–30	55	73%	64%	25%/38% (median 10 months)

Abbreviations and definitions:

CAR: Chimeric Antigen Receptor, N: number, CR: complete response, MRD (-): minimal residual disease negative, EFS: event-free survival, OS: overall survival