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Immunotherapy in Pediatric Acute Lymphoblastic Leukemia

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Abstract

Leukemia is the most common childhood malignancy and cause of pediatric cancer death. Significant advances in the cure rates of B-cell acute lymphoblastic leukemia (B-ALL) and T-cell acute lymphoblastic leukemia (T-ALL) have been achieved; however, patients with refractory or relapsed B-ALL or T-ALL continue to have poor outcomes. Immunotherapy is a revolutionary treatment aimed to improve survival and reduce the toxicity of chemotherapy by harnessing the patient's own immune system to target cancer cells. Several immunotherapies have been developed including monoclonal antibodies, antibody drug conjugates, Bispecific T-cell engagers (BiTEs), and chimeric antigen receptor T-cell (CAR-T) therapy. Immunotherapy has been shown to have efficacy in relapsed acute leukemia; however, antigen escape relapse remains a challenge and the duration of effect is unknown. Nevertheless, immunotherapy holds the potential to significantly improve outcomes in relapsed pediatric acute B-ALL and T-ALL and is actively being studied in upfront therapy.

Keywords: B-ALL, T-ALL, Immunotherapy, Monoclonal Antibodies, Antibody-drug Conjugates Bispecific T-Cell Engager (BiTE), Chimeric Antigen Receptor (CAR) T-Cells

Abbreviations: ASMT: American Society for Transplantation and Cellular Therapy; B-ALL: B-cell Acute Lymphoblastic Leukemia; BiTE: Bispecific T-cell Engagers; CAR-T: Chimeric Antigen Receptor T-cell; COG: Children's Oncology Group; CR: Complete Remission; CRh: Complete Remission with partial hematologic recovery; CRS: Cytokine Release Syndrome; FDA: U.S. Food and Drug Administration; HSCT: Hematopoietic Stem Cell Transplant; InO: Inotuzumab Ozogamicin; NK: Natural Killer; MRD: Minimal Residual Disease; OS: Overall Survival; OR: Odds Ratio; PFS: Progression-free Survival; scFv: Single-chain variable fragment; SOC: Standard of Care; SOS: Sinusoidal Obstructive Syndrome; T-All: T-cell acute lymphoblastic leukemia; TCR: T-cell Receptor; VHR: Very High-risk

Introduction

Leukemia is the most common childhood malignancy and is the most common cause of cancer death before the age of 20 [1]. Pediatric leukemia can be subdivided into acute versus chronic and lymphoid versus myeloid leukemia. Acute lymphoid leukemia (ALL) can be further divided into B-cell precursor ALL (B-ALL) and T-cell ALL (T-ALL). The focus of this paper will be pediatric B-ALL and T-ALL.

B-ALL

Approximately 85% of ALL cases are B-ALL [2]. Cure rates for B-ALL significantly rose over the past five decades from 10% to 90% [1-3] due to multi-agent chemotherapy regiments, CNS prophylaxis and better risk stratification [3]. Despite these successes, about 2% of patients are refractory to chemotherapy and another 10% to 15% of patients will relapse [4]. Treatment for these patients remains a therapeutic challenge. Event free survival for

patients with relapsed or refractory ranges from 13% to 40% [4-7]. Attempts to intensify chemotherapy in high risk patients resulted in excessive toxicity [8,9].

T-ALL

T-ALL accounts for approximately 15% of pediatric ALL cases [1,10]; and historically these patients have inferior outcomes to patients with B-ALL with event-free and overall survival around 70% and 80% respectively [11-13]. Survival has improved with intensification of therapy and T-cell focused regimens, such as the addition of nelarabine to treatment paradigms [14]. However, survival after relapse is about 30% due to a lack of effective salvage therapies [15].

Immunotherapy

Immunotherapy is a revolutionary treatment aimed to improve survival and reduce the toxicity of chemotherapy by harnessing the patient's own immune system to target cancer cells. Several different approaches have been developed. Antibody therapy utilizes antigens present on the surface of leukemia cell to aid in the immune system's attack of the cancer cell. Therapies include monoclonal antibodies, antibody drug conjugates and Bispecific T-cell engagers (BiTES). Adaptive therapies manipulate patient's cytolytic immune cells to recognize tumor cells and elicit an anti-tumor response. These therapies include chimeric antigen receptor T-cell (CAR-T) therapy. This review will focus on immunotherapeutic options approved and under investigation for pediatric ALL. Common targets are highlighted in Tables 1 and 2.

Target	Drug
CD10	CD10 CAR-T
CD19	Antibody-drug conjugates • SAR3419 • SGN-CD19A • SGN-CD19B BiTE • Blinatumomab CAR-T • CD19 CAR-T • CARCIK-CD19
CD20	Antibody • Rituximab • Ofatumumab BiTE: Preclinical CAR-T • CD20 CAR-T • CD20/CD19 CAR-T
CD22	Antibody • Epratuzumab • Moxetumomab pasudotox Antibody-drug conjugates • Inotuzumab Ozogamicin CAR-T • CD22 CAR-T • CD19/CD22 CAR-T

Table 1: B-ALL Targets.

Target	Drug
CD1a	CAR-T (Preclinical)
CD3	CD3 NK CART-T (Preclinical)
CD4	CD4 CAR-T
CD5	CAR-T
CD7	CAR-T UCART7 (Preclinical) TruUcar GC027 (Preclinical)
CD38	Daratumab Isatuximab Mogalizumab
CD52	Alemtuzumab
CD194	Mogamulizumab CD194/30 CAR-T
Interleukin-2 receptor alpha	Basiliximab (Preclinical)
Interleukin-7 receptor alpha	Preclinical
TALLA-1	Preclinical
Hedgehog interacting proteins	Preclinical
Human telomerase reverse transcriptase	Preclinical

Table 2: T-ALL targets.

Monoclonal Antibodies/Antibody-drug Conjugates

Antibody therapy is engineered to attack specific antigens on tumor cells. Monoclonal antibodies stimulate antibody-dependent cytotoxicity; whereas, drug-antibody conjugates deliver a cytotoxic drug to the tumor cell when it binds and is phagocytosed by the target cell. In pediatric

leukemia, both monoclonal antibody and antibody-drug conjugates have shown promise.

Inotuzumab ozogamicin (InO)

Mechanism of action: CD22 is expressed on 80% to 90% of B-ALL cells. Inotuzumab ozogamicin (InO) is a humanized anti-CD22 monoclonal antibody conjugated to the cytotoxic drug, calicheamicin [16]. Calichaemicin is cleaved and binds to minor DNA grooves causing double-stranded DNA breaks and apoptosis of the leukemia cell [16]. Clinical trials of InO in B-ALL are highlighted in Table 3.

Adult experience with InO: U.S. Food and Drug Administration (FDA) approval of InO for relapsed/refractory CD22 positive ALL was based on the INO-VATE trial. The study showed a superiority of InO compared to standard of care (SOC) chemotherapy with improved complete remission (CR)/remission with partial hematologic recovery (CRh) rates, 73.8% vs. 35% and progression-free survival (PFS) 5.0 months vs. 1.7 months. More patients proceed to transplant in the InO arm (48% vs. 22%, p<0.0001) [17]. At the two-year follow-up, overall survival (OS) rates were superior with InO 22.8% and 10.0% [17].

Pediatric experience with InO: Of five pediatric patients with relapsed CD22 positive B-ALL treated with InO as part of an adult phase 2 trial, three had a CR/CRh [18]. A retrospective analysis of compassionate use of InO in fifty-one pediatric patients with relapsed/refractory B-ALL showed a 12-month EFS and OS of 23% and 36% respectively [19]. Twenty-one patients underwent a hematopoietic stem cell transplant (HSCT) after achieving CR [19]. In the Children's Oncology Group (COG) trial AALL1621 (NCT02981628) of InO in heavily pre-treated relapsed/refractory, CD22 positive B-ALL patients, 58.3% had a CR/CRh, with 65.4% of those having a minimal residual disease (MRD) response [20].

Ongoing Trials with InO in pediatrics: There are several ongoing pediatric trials investigating the timing and indications for InO. InO is being studied in upfront therapy, relapsed/refractory MRD positive ALL with chemotherapy, and as consolidation post-transplant (Table 4).

Sinusoidal obstructive syndrome (SOS): Sinusoidal obstructive syndrome (SOS) was seen more commonly in patients who are treated with InO than salvage chemotherapy, a serious concern in patients whom subsequent transplant is a consideration. In INO-VATE trial, rates of SOS were 14.0% (5% fatal) in the InO arm vs. 2.1% in the SOC chemotherapy arm [17]. Risk factors for SOS included conditioning with dual alkylators, hyperbilirubinemia before HSCT, and prior HSCT (OR 6.02; p=0.032) [17]. In the pediatric experience, 4 of the 13

Patients	Phase	N	Dosing	Response	SOS	Group/ study
Adult CD22+ R/R ALL Ph+/-	Phase I/II Multicenter Open Label	N=72 Phase 1 n=-24 Expan-sion n=13 Phase II n=35	1.2mg/m2/cycle (n=3) 1.6mg/m2/cycle (n=12) 1.8mg/m2/cycle (n=9) Days 1, 8, 15 over 28 day cycle Expansion (n=13) Recommended does 1.8mg/m2/cycle	CR/CRh: 49(68%) MRD negative: 41(84%) Median DOR: 4.6 mo (95%CI; 3.8-6.6) Median PFS: 3.9 mo (95%CI; 2.9-5.4) Median OS: 7.4 mo (95%CI; 5.7-9.2)	N=4	[78] NCT01363297

Adult and pediatric CD22+ R/R B-ALL	Phase II Single center	N=49	1.8mg/m2/cycle every 3-4 weeks	CR/CRh: 28 (57%) MRD negative: 19 (39%) Median OS: 5.1 mo (95%CI; 3.8–6.4)	N=22	[79] NCT01134575
Adult R/R CD22+, Ph+/- B-ALL	Phase III Randomized (SOC chemotherapy vs. Inotuzumab ozogamicin)	N=326 Ino n=164 SOC n=162	1.8mg/m2/cycle Days 1, 8, 21	CR/CRh: (73.8% vs. 35%), p<0.001 MRD negative: 78.4% (95% CI; 68.4-86.5] vs. 28.1% (95% CI; 13.7-46.7), p<0.001 DOR: 4.6 mo (95% CI; 3.9-5.4) vs. 3.1 mo (95% CI; 1.4-4.9, p = 0.03 Median PFS: 5.0 mo (95% CI; 3.9-5.8) vs. 1.7 mo (95% CI; 0.34-0.60); HR, 0.45 (97.5% CI; 0.34-0.61), p<0.001) Median OS: 7.7 mo vs. 6.2 mo (95%CI, 4.7-8.3) 2-year OS: 22.8% vs. 10.0% (HR 0.75; 97.5CI, 0.57-0.99), p=0.0105	Patients who proceed to transplant and developed SOS InO-18/79 (22.8%) SOC-3/35 (8.6%)	[17] NCT01564784 INO-VATE

Pediatric R/R CD22+ B-ALL	Phase II	N=5	1.3mg/m2 every 3 weeks (n=3) Increased to 1.8mg/m2 every 3 weeks (n=1 of the 3) Then weekly 0.8mg/m2 on Day 1 followed by 0.5mg/m2 on Days 8 & 15	CR: 1 (20%) CRh: 2 (40%) No response: 2 (40%)		[18]
Pediatric R/R. CD22+ ALL compassionate use	Retrospective analysis	N=51 3 not evaluable for response	Cycle one: three doses: 0.8mg/m2 on week 1 followed by 0.5mg/m2 on weeks 2 and 3 One patient with MRD-only disease received 0.5mg/m2/dose for all three doses. In second and subsequent cycles, assuming CR/CRh during cycle 1, patients received 0.5mg/m2/dose on days 1, 8, and 15.	CR/CRh: 67% CR: 15 (39%) CRh: 13 (25%) MRD negative: 20 (71%) No response: 8 (16%) 12-month EFS 23.4±7.5% 12-month OS 36.3±9.3%		[19]
Pediatric and AYA CD22+ B ALL in 2nd relapse, refractory to two prior regimens, relapse after HSCT, or 1st relapse with DS	Phase II Single arm	N=48		CR/CRh: 28 (58.3%) (95%CI, 43.2-72.4) CR: 19 (40%) CRh: 9 (19%) MRD negative: 17 (65%) Progressive disease: 8 (16.7%)	4 (30.7%)	[20] NCT02981628 AALL1621

Table 3: Inotuzumab ozogamicin studies.

Study	Aim	Design	Age	Status
NCT03959085 AALL1732	InO added to post-induction chemotherapy in HR B-ALL	Phase III Randomized Multicenter	1 y.o. to 24 y.o.	Recruiting
NCT03150693	InO with frontline chemotherapy with young adults with newly diagnosed CD22 + B-ALL	Phase III Randomized Multicenter	18 y.o. to 39 y.o	Recruiting
NCT04307576	Addition of InO in chemotherapy for newly diagnosed B-ALL (IR/HR)	Phase III Randomized	1 y.o. to 45 y.o.	Not yet recruiting
NCT03739814	InO and Blinatumomab in newly diagnosed, or R/R CD22+ B-ALL	Phase II Multicenter	≥ 18 y.o.	Suspended-Request for amendment
NCT03962465	InO with augmented BFM Re-induction for AYA patients with R/R B-ALL	Phase I Single Center-U. Virginia	18 y.o. to 55 y.o.	Recruiting
NCT03991884	InO with chemotherapy in R/R CD22+ B-ALL (Ph+/-)	Phase I Single Center- U. Washington	≥ 18 y.o.	Recruiting
NCT01925131 S1312	InO with chemotherapy in R/R CD22+ B-ALL	Phase I	≥ 18 y.o.	Recruiting
NCT03677596	Study of a lower dose of InO in R/R, transplant eligible B-ALL at risk of liver disease and plan for HSCT	Phase IV Randomized Multicenter	18 y.o. to 75 y.o.	Recruiting
NCT03851081	InO and Vincristine sulfate liposome in R/R CD22+ B-ALL	Phase ib/II Single Center-Roswell Park Cancer Institute	≥ 18 y.o.	Not yet recruiting
NCT02311998	Bosutinib with InO in R/R Ph+ B-ALL	Phase I/II Single Center-MD Anderson	≥ 18 y.o.	
NCT03856216	InO in CD22+ R/R B-ALL in patients who are not eligible for a myeloablative HSCT but eligible for a RIC HCST	Phase II Single Center-MD Anderson	18 y.o. to 70 y.o.	Recruiting
NCT03610438 ALL2418	InO in B-ALL who have MRD+ disease after at least 3 months of any therapy Ph+/-	Phase IIa Exploratory Multicenter	≥ 18 y.o.	Not yet recruiting
NCT03913559	InO for children with MRD+, CD22+ ALL with <5% blasts in BM	Phase II Multicenter	Up to 21 y.o.	Recruiting

NCT03441061	InO in MRD+ B-ALL	Phase II Single Center- MD Anderson	≥ 18 y.o.	Recruiting
NCT03104491	InO post-transplant in ALL who have a high risk of relapse	Phase I/II Multicenter	16 y.o to 75 y.o.	Recruiting
NCT03564678	Levocarnitine and Vitamin B Complex in treating InO and PEG-Asparaginase hyperbilirubinemia in ALL	Phase II Single Center-MD Anderson	12 y.o and older	Recruiting
*Table is not comprehensive, please see clinicaltrials.gov for additional ongoing trials				

Table 4: Ongoing trials with Inotuzumab ozogamicin in pediatric and AYA patients.

patients (30.7%) who went on to HSCT developed grade 3 SOS in AALL1621 study [20] at 11 of the 21 (52%) patients in compassionate use study [19]. Strategies to prevent SOS include avoiding dual alkylating agents and/or thiotepa and hepatoxic agents, prophylactic ursodiol, proceeding to HCT after two cycles of InO, and close monitoring for SOS [21]. Ongoing trials include Levocarnitine and Vitamin B to reduce hyperbilirubinemia with InO treatment (NCT03564678), lowering the dose of InO pre-transplant (NCT03677596) and reduced intensity transplant (NCT03856216).

Daratumumab

Mechanism of action: CD38 is a type II transmembrane glycoprotein on the surface of thymocytes, activated T-cells and terminally differentiated B cells, with low level expression on other normal lymphoid and myeloid cells [22]. CD38 expression has been seen on T-ALL blasts and remains stable after treatment with chemotherapy [22]. Daratumumab is a human monoclonal antibody directed against CD38 [22]. It is FDA approved for multiple myeloma both as monotherapy and in combination [23,24]. Preclinical data has shown efficacy of Daratumumab in T-ALL models [22,25], and case series have shown efficacy as salvage therapy in relapsed T-ALL [26,27]. There are ongoing clinical trials of Daratumumab in pediatric T-ALL and B-ALL in combination with cytotoxic chemotherapy (NCT03384654). Other monoclonal antibodies being tested in T-ALL are listed in Table 2.

BiTEs

Bispecific T-cell-Engaging (BiTE) antibodies are antibody-based molecules that bind to distinct surface markers on T-cells and tumor cells to form the immunological synapse [28,29]. BiTEs bind the invariant signaling component of the T-cell receptor (TCR), CD3, and a surface target antigen on tumor cells, resulting in T-cell activation, expansion and tumor cell lysis [28,29]. BiTEs are independent of T-cell receptor specificity and do not require MHC presentation of the antigen; thus, bypassing T-cell regulation [29]. Unlike CARTs, BiTEs do not require manufacturing and infusion of T-cells [29].

Blinatumomab

Mechanism of action: CD19 is expressed on approximately 90% of B-ALL cells [30]. Blinatumomab is a BiTE that binds to CD19 on leukemic cells and CD3-subunit of the TCR on T-cells [29]. Clinical trials of Blinatumomab for B-ALL are highlighted in Table 5.

Role in relapsed/refractory B-ALL: In 2014, the FDA granted accelerated approval of blinatumomab for adult Philadelphia chromosome negative (Ph-) relapsed/refractory B-ALL based on a single-arm study of 189 adults that showed efficacy and manageable toxicity [31]. Eighty-one patients (43%) had a CR/CRh within two cycles of blinatumomab [31]. Median overall survival was 6.1 months [31]. This was superior to historical controls who received SOC, salvage chemotherapy [31]. Efficacy was confirmed in the TOWER trial, a multicentered, randomized, phase III trial comparing blinatumomab to chemotherapy in adult relapsed/refractory Ph- ALL [32]. CR was achieved in 91 patients (34%) in the blinatumomab arm compared to 21 patients (16%) in the SOC arm. The median overall survival was significantly longer for the blinatumomab arm (7.7 months versus (4.0 months) in the SOC arm [32].

Role in Philadelphia chromosome-positive (Ph+) ALL: The approval of blinatumomab was extend to Ph+ relapsed/refractory B-ALL based on the ALCANTARA trial showing a 36% CR/CRh, with 88% complete MRD response in patients with relapsed/refractory Ph+ ALL, previously treated with TKI treatment [33]. Blinatumomab as consolidation to treatment with TKI has also been studied in a multicenter phase II trial of Ph+ ALL, patients

were treated with dasatinib, followed by post-induction consolidation with blinatumomab. At the end of two cycles of blinatumomab 19/35 (54%) had a molecular response that further increased after subsequent cycles [34]. Twelve-month OS and DFS are 96.2% and 91.6% respectively [34]. There are several ongoing studies examining the efficacy of TKIs with blinatumomab (Table 6).

Role in MRD positive disease: In 2018 the FDA granted approval for blinatumomab for the treatment of adults and children with B-ALL in a morphological first or second CR with MRD [35]. Eighty-eight of 113 patients (78%) achieved a complete MRD response after one cycle of blinatumomab [35]. Patients who achieved a complete MRD response had a prolonged OS (38.9 vs 12.5 months; p=0.002) and RFS (23.6 vs 5.7 months; p=0.002) [35].

Role in first vs. later relapse: Blinatumomab appears to be a more effective salvage therapy in first versus second or later relapse. In the TOWER study, blinatumomab's effect on overall survival was greater for first salvage therapy (HR 0.59; p=0.016) than second or greater salvage therapy (HR 0.72; p=0.055) [36]. Similarly, in the BLAST MRD trial, patients who had previously relapsed had inferior RFS and OS compared with those treated in first remission (HR 2.02 for CR2 vs CR1 relapse and

Patients	Phase	N	Dosing	Response	Group/study
Adults with Ph- R/R B- ALL	Multicenter, single -arm, open-label, phase II,	189 (185- received blina)	Continuous infusion over 4 wks of a 6 wk cycle Two cycles for induction and 3 for consolidation Cycle 1 initial dose 9µ/day for 7 days then 28µ/day for the remaining 3 wks. Subsequent cycles 28µ/day	CR or CRh 81 (43%) (95%CI; 36-50) CR 63 (33%) CRh 18 (10%) MRD negative: 60 (82%) Median RFS (MRD responders vs. non-responders) 6.9 mo (95% CI, 5.5-10.1) vs 2.3 (95% CI 1.2-NE) Median OS: (MRD responders vs. non-responders 11.5 mo (95% CI, 8.5-NE) vs. 6.7 mo (95% CI 2.0-NE)	[31] MT103-211 (NCT01466179)
Ph- R/R ALL Blinatumomab vs standard of care (SOC) chemotherapy -	Phase III, randomized, open-label, multi-center trial comparing Blinatumomab with conventional chemotherapy (2:1 ratio)	405 pts -271 in the blina arm -134 in the SOC Intent-to treat	Blina 9µ/day days 1-7 and 28 µ/day days 8-28 (induction) Cycles 2-5 days 1-28 in a 42 day cycles (consolidation) Cycles 6-9 in 84 day cycles (maintenance)	CR : 91 (34%) (95%CI, 28-40) vs 21 (16%) (95%CI, 10-23; p<0.001) CRh: 24 (44%) vs. 6 (25%) (p<0.001 MRD negative: 76% vs. 48% (95%CI, 9-47)	[32, 36] TOWER (NCT02013167)

Ph- or + R/R **Pediatric** **<18 (2 to 17)** **>25% BM blasts**	Phase I/II open label, multicenter	40 pts- phase I 44 phase II 70 recommended dosage phase I (n = 26) or phase II (n = 44)	Phase I: dosages of 5, 15, and 30 µ/m2/d and a stepwise dosage of 15/30 µg/m2/d (15 µ/m2/d for the first 7 days and 30 µ/m2/d thereafter). Recommended dosage of 5/15 µ/m2/d	Of the 70 pts that received the recommended dosage: CR: 27 (39%) (95% CI, 27-51%) MRD negative: 14/27 (52%) (95% CI, 32-71) Median RFS: 4.4 mo (95% CI, 2.3-7.6) (for patients who achieved CR_ Median OS: 7.5 mo (95%CI, 4.0-11.8) 6 mo estimated EFS (1st relapse): 40.8% (95%CI, 30.1-50.) vs. 25.9% (95%CI; 15.3-37.8) HR 0.7 (95% CI, 0.47-1.03; p=0.11) 6 mo estimated EFS (2nd or later relapse): 24.0% (95% CI, 17.4-31.3%); 1.6% (95% CI, 0.1-7.5%): HR 0.49 (95% CI, 0.29-0.57; p<0.001)	[37] MT103-205 (NCT01471782) AALL1121
R/R Ph+ B ALL, previously treated with at least one 2nd generation TKI or intolerant to 2nd generation TKI and refractory to imatinib **>5% bone marrow blasts**	Phase II, multicenter, single arm trial of blina	45 patients	9 µ/day days 1-7 and 28 µ/day days 8-28 for cycle 1. 28 µ/day days 1-28 subsequent cycles IF CR achieved could receive up to 3 cycles of consolidation unless a HSCT was scheduled	CR/CRh; 16 (36%) (95% CI, 22- 51) CR: 14 (31%) (95% CI, 18-47) CRh: 1 (4%) (95% CI, 1-15) MRD-negative: 14 (88%) (95% CI, 62-98) Median OS: 7.1 mo (95% CI, 5.6-NE) Median RFS: 6.7 mo (95% CI, 4.4-NE)	[33] ALCANTARA (NCT02000427) (Martinelli, JCO, 2017)

Adult R/R B- ALL in first or later hematologic CR but MRD positive	Multicenter, open label, single arm trial	116 113 evaluable patients Excluded no central MRD assay results or a test sensitivity that did not reach 10−4	15 µ/m2/day for 28 days followed by 2-2wk tx free period Up to 4 cycles	CR:48/110 (remained in CR MRD negative: 88/113 (78%) (95% CI, 69-85) Median OS: 36.5mo (95% CI, 19.1-NE) Median RFS (MRD responders vs nonresponsers): (23.6 vs 5.7 months; p=0.002) Median OS (MRD responders vs nonresponsers): (38.9 vs 12.5 months; p=0.002	[35] BLAST MT103-203 NCT01207388
Pediatric and AYA R/R B-ALL after re-induction chemotherapy	Multicenter, randomized phase III trial	208 HR/IR patients were randomized Chemotherapy arm- Blocks 2 and 3 of UKALLR3 Blinatumomab	Two cycles at 15 µ/m2/day for 28 day	2-year DFS: 59.3 ± 5.4% vs. 41.0 ± 6.2% (p=0.05) 2-year OS: 79.4 ± 4.5% vs. 59.2 ± 6.0% (p=0.005)	[38] NCT02101853 AALL1331
Pts with B-ALL 0 to 21 y.o who were transplanted with CR but MRD +	Retrospective analysis, multicenter	N=15 10/15 CR1 with EOC+	Singe 12 day course 15µ/m2/d 2 had course shortened to go to HCT (18 and 20 days of blina) 1 received 2 cycles of blina	MRD negative: 14/15 (93%) 1-yr post HCT relapse incidence 27.8% 1-yr OS: 93.2%	[80]

Table 5: Blinatumomab studies.

HR 3.34 for CR3 vs. CR1 relapse, -p=0.001), suggesting the importance of MRD clearance early in the treatment course [35].

Role in pediatrics: Efficacy and safety of blinatumomab was shown in the pediatric population in the Study MT103-205 a phase I/II study, of the 70 patients. Twenty-seven (39%) achieved a CR with 14 (52%) of the responders having a completed MRD response [37]. Duration of response was 4.4 months [37]. There are several pediatric trials studying the role of blinatumomab in relapsed/refractory B-ALL and as consolidation for transplant (Tables 6 and 7). The COG trial AALL1331 (NCT02101853) is a phase III randomized trial for relapsed B-ALL testing blinatumomab as post-reinduction consolidation, with high risk (HR) and intermediate risk (IR) patients proceeding to HSCT, and low risk (LR) patients receiving maintenance chemotherapy. The HR/IR randomization was terminated early due to evidence of superiority and decrease toxicity of the blinatumomab arm [38]. In these groups blinatumomab arm had an improved 2-year DFS (59.3% vs. 41.0% p=0.05), 2-year OS (79.4% vs. 59.2% p=0.005) and MRD clearance (21% vs. 79% p<0.0001) with fewer and less severe toxicities compared to SOC chemotherapy [38]. Data for the low risk (LR) randomization is pending. There are ongoing studies investigating the role of blinatumomab in upfront therapy including the COG trial AALL1731 (NCT03914625) that is studying the addition of blinatumomab to standard chemotherapy in patients with NCI SR B-ALL at high risk for relapse. Blinatumomab is also being studied in HR/IR newly diagnosed B-ALL in the European Studies AIEOP-BFM ALL 2017 (NCT03643276) and PETHEMA-BLIN-01 (NCT03523429). Lastly, blinatumomab is also being studied as maintenance after allogenic HSCT (NCT02807883 & NCT03114865) (Table 6). Combining blinatumomab with other immunotherapies is also being investigated. There is an ongoing adult trial combining treatment with inotuzumab ozogamicin with mini-HCVD with or without blinatumomab in previously untreated acute lymphoblastic leukemia, (NCT01371630). In AYA patients, blinatumomab and inotuzumab ozogamicin are being studied in newly diagnosed and relapsed/refractory CD22+ B-ALL (NCT03739814). The ability of checkpoint inhibitors to further enhance the efficacy of blinatumomab is also actively being studied (NCT03605589, NCT03512405, NCT03160079, NCT02879695).

Biomarkers to predict response: Predictive biomarkers of response to blinatumomab are emerging. Patients who have a lower baseline disease burden [31] and day 15 MRD have a better response [39]. In addition, superior response was correlated with greater T-cell expansion of effector memory T-cells [40] and a higher percentage of regulatory T-cells [41]. Identifying additional biomarkers to determine response is actively being studied.

Study	Aim	Design	Age	Status
NCT02101853 **AALL1331**	Blinatumomab compared to SOC chemotherapy in pediatric and AYA patients as a bridge to transplant in relapsed ALL after re-induction with block 1 of UKALLR3/mitoxantrone arm	Phase III trial Multicenter Randomized	1 y.o. to 30 y.o.	Active, not recruiting HR/IR arm closed early due to trend to superiority
NCT02393859	Blinatumomab vs. SOC in pediatric patient with Ph-, HR, first relapsed B-ALL	Phase III Multicenter Randomized	>28 days to <18 y.o.	Active, not recruiting
NCT03914625 **AALL1731**	Blinatumomab in combination with chemotherapy in newly diagnosed SR B-ALL Role of immunotherapy in patients with Down Syndrome and B-ALL	Phase III Randomized Multicenter	1 y.o. to 21 y.o.	Recruiting

NCT03117751 **St. Jude Total Therapy XVII**	Blinatumomab for Newly diagnosed SR ALL Blinatumomab for patients with HR ALL (and MRD of 0.01 to 1% at the end of induction)	Phase II/III Randomized Multicenter	1 y.o. to 18 y.o.	Recruiting
NCT03643276 **AIEOP-BFM ALL 2017**	Incorporating Blinatumomab with standard chemotherapy regimens in newly diagnosed HR/IR B-ALL	Phase III Randomized Multicenter[ed]	<18 y.o.	Recruiting
NCT02877303	Blinatumomab and chemotherapy (Hyper-CVAD) in newly diagnosed B-ALL	Phase II Single center-MD Anderson	14 y.o and older	Recruiting
NCT03367299	Chemotherapy and blinatumomab in newly diagnosed Ph- ALL	Phase II Multicenter	18 y.o. to 65 y.o	Recruiting
NCT03541083 **HOVON146ALL**	Blinatumomab in the prephase and consolidation in newly diagnoses B-ALL	Phase II Multicenter	18 y.o. to 70 y.o.	Recruiting
NCT02807883	Blinatumomab maintenance following allo HSCT in Ph+ BALL	Phase II Single center-MD Anderson	1-70 y.o.	Recruiting
NCT03114865	Blinatumomab in B-ALL post allo HSCT as remission maintenance	Phase I Single Center-SKCC	≥18 y.o.	Recruiting
NCT04044560 **(OZM-097)**	Blinatumomab for MRD in Pre-B ALL following stem cell transplant	Phase II Single arm Open label Multicenter	1 y.o and older	Active, not yet recruiting
NCT03982992 **DLI-TARGET**	Allogeneic donor lymphocyte infusions combined with Blinatumomab in B-ALL who have mixed chimerism (MC) or are MRD after allo HSCT and are refractory to at least one MRD-or MC targeted therapy (i.e blinatumomab, DLI, TKI, etc)	Phase II Single center-Klinikum der Universität München	≥18 y.o.	Recruiting
NCT02790515 **NCT03849651**	Blinatumomab in naïve T-cell depleted haploidentical donor HCT for R/R ALL	Phase II Single center-St. Jude	Up to 21 y.o.	Recruiting
NCT02879695	Blinatumomab and Nivolumab with or without ipilimumab in patients with poor risk relapsed or refractory CD19+ precursor B-ALL	Phase I Multicenter	16 y.o. and older	Recruiting

NCT03605589	Pembrolizumab Blinatumomab in pediatric and AYA R/R ALL	Phase I Pilot Single Center-Cincinnati Children's Hospital	1 y.o. to 40 y.o.	Recruiting
NCT03512405	Pembrolizumab and blinatumomab in R/R ALL	Phase I/II Single center-City of Hope Medical Center	≥18 y.o.	Recruiting
NCT03160079	Pembrolizumab and Blinatumomab in R/R B-ALL with high marrow lymphoblasts (>50% blasts)	Phase i/II Multicenter	≥18 y.o.	Recruiting
NCT02744768	Dasatinib and Blinatumomab in newly diagnosed Ph+ ALL	Phase II Multicenter	≥18 y.o.	Recruiting
NCT03318770 **GIMEMA 2116**	Dasatinib and blinatumomab following chemotherapy in Ph+ ALL	Observational Case-Control Prospective	≥18 y.o.	Not yet recruiting
NCT04329325	Blinatumomab and TKI (Dasatinib) in patients with Ph+ ALL	Phase II Single Group-MSKCC	≥18 y.o.	Recruiting
NCT02997761	Ibrutinib and Blinatumomab in R/R B-ALL	Phase II Single group-University of California Davis	≥18 y.o.	Recruiting
NCT03263572	Blinatumomab, Methotrexate, cytarabine and ponatinib in Ph+ R/R ALL	Phase II Single center-MD Anderson	≥18 y.o.	Recruiting
NCT03147612	Low-intensity chemotherapy, ponatinib and blinatumomab in newly diagnosed and R/R Ph+ ALL	Phase II Single center- MD Anderson	≥18 y.o.	Recruiting
NCT03628053 **OBERON**	Tisagenlecleucel vs Blinatumomab or Inotuzumab for patients with R/R B-ALL	Phase III Randomized Multicenter	≥18 y.o.	Not yet recruiting
NCT03739814	Inotuzumab ozogamicin and blinatumomab in patients with newly diagnosed or R/R CD22+ B-ALL	Phase II Multicenter	≥18 y.o.	Suspended-Request for amendment
*Table is not comprehensive, please see clinicaltrials.gov for additional ongoing trials				

Table 6: Ongoing trials with Blinatumomab in pediatric and AYA patients.

Patients	Phase	N	Response	Group/study
Pediatric and adult R/R ALL **(CTL119) (4-1BBz CAR)**	Phase I/IIa	N=30 25 pediatric 5 adult	CR: 27 (90%) MRD negative: 22/27 (81%) 6-mo EFS: 67% (95% CI, 51-88) 6-mo OS: 78% (95% CI, 65-95)	[60] NCT01626495 NCT01029366
Pediatric and AYA R/R B-ALL **(CTL119) (4-BBz CAR)**	Phase I/II	N=45	ITT MRD: (40/45) 89% 12-mo EFS: 50.8% (95% CI, 36.9-69.9) 12-mo OS: 69.5% (95% CI, 55.8-86.5)	[46] NCT02028455 PLAT-02
Children and AYA CD19+ ALL **(CTL119) (4-1BBz)**	Pilot protocol	N=53	CR: 50 (94%) MRD negative: 45 (90%) 6-mo EFS: 70% (95% CI, 58-85) 6-mo RFS: is 72% (95% CI, 59-87%) 12-mo EFS: 45% (95% CI, 31-66) 12-mo RFS: 44% (95% CI, 30-65) 12-mo OS: 78% (95% CI, 67-91)	[81]
Pediatric and AYA R/R B-ALL **KTE-C19 (CD28 CAR)**	Phase 1	N=5 4 treated	MRD negative CR: 4	[82] (NCT02625480) ZUMA-4
Pediatric and AYA with R/R B-ALL or NHL **(TCR zeta and CD28 signaling domain)**	Phase 1	N=21	CR: 14/21 (66.7%) (95% CI, 43.0–85.4) MRD negative: 12/20 (60%) (95% CI, 36.1–80.9) OS: 51.6%	[45] NCT01593696
Pediatric and AYA R/R B-ALL CD19+ Tisagenlecleucel (CD3-zeta: 4-1BB) **5% blasts in BM**	Phase II Multicenter	N=113 screened N=97 enrolled N=75 infused	RR: 65 (81%) (95% CI, 71-89) CR: 45 (60%) CRh: 16 (12%) MRD negative: 64 (98%) 6-mo RFS: 80% (95% CI, 65-89) 12-mo RFS: 59% (95% CI, 41-73) 18 mo RFS: 66% (95% CI, 52-77) 6-mo EFS: 73% (95% CI; 60-82) 6-mo OS: 90% (95% CI 63-86) 12-mo EFS: 50% (95%CI, 35-64) 12-mo OS: 76% (95% CI, 63-86) 18 mo OS: 70% (95% CI, 58-79)	[43, 47] NCT02435849 ELIANA

Adult R/R ALL **KTE-C19 (CD28 CAR)**	Phase 1	N=53	CR: 44/53 (83%) (95% CI, 70-92) MRD: 32 (67%; 95% CI, 52-80) Median EFS: 6.1 months (95% CI, 5.0-11.5) Median OS: 12.9 months (95% CI, 8.7-23.4)	[44] NCT01044069
R/R B-ALL treated with CD22 BBz CAR, **(4-1BB domain)**	Phase I	21 Children and adults 17-previously treated with CD19	CR: 12 (57%) CR MRD negative 9 (75%)	[48] NCT02315612
R/R B-Cell malignances, sequential infusion of CD19 and CD22 3rd generation CAR-T	Pilot	N=89 N=51- ALL	MRD Negative: 96% (95% CI, 86.3-99.5) Median PFS: 13.6 mo (95% CI, 6.5 to NE) Median OS: 31.0 mo (95% CI, 10.6-NE)	[83] ChiCTR-OPN-16008526
Pediatric R/R B-ALL **AUTO3-Bicistronic CD19 and CD22 CAR** **Ox40 co-stim for CD19 CAR** **41BB co-stim for CD22**	Phase I	N=10 7-Treated	CR and MRD: 7/10 1 year follow up: -3 relapses -4 patients in ongoing CR/CRh with B-Cell	[84] Amelia Study
CD19/CD22 bispecific CAR-T in children and AYA patients with B-ALL **Lentiviral transduction bivalent CAR** **fmc63 CD19** **m971 CD22** **41BB costimulatory endo-domain**	Phase I	N=4	CR: 4/4 (100%) MRD negative: 3/4 (75%)	[85]
CD19 and CD22 CART cocktail for R/RB-ALL	Phase I	N=16	CR/CRh: 15/15 (100%) MRD negative: 14/15 (93.3%)	[86]

Table 7: B-cell CART studies.

CAR-T Therapy

Chimeric antigen receptors (CARs) are T-cells that are engineered to recognize tumor associated antigens. CARs are composed of T-cell signaling moiety and a tumor specific antigen binding domain, commonly a single-chain variable-fragment monoclonal antibody that is fused to a transmembrane domain [42]. Various generations of CARs have been developed to heighten function based on the knowledge that T-cells require two signals to be activated, T-cell receptor (TCR) engagement and co-stimulation. First generation CARs consisted of T-cell receptor complex domain and antigen recognition domains, only providing signal 1; whereas, second generation CARs were constructed to contain co-stimulatory signaling domains including CD28, 4-1BB (CD137), and OX40 (CD134) [42]. Third generation CARs further enhanced T-cell signaling by containing tandem cytoplasmic signaling from two co-stimulator receptors (CD28-4-1BB or CD28-OX40) [42]. Fourth generation CARs have pro-proliferative T-cell costimulatory ligands (4-1BBL) or proinflammatory cytokines (IL-12) [42]. Advantages of CAR-T therapy include HLA-independent recognition of tumor antigen; allowing T-cells to recognize the antigen as foreign and activity is unaffected by HLA down regulation in tumor cells. In addition, both CD4+ and CD8+ T-cell subsets are transduced, allowing for both helper and cytotoxic activity.

CD19 CAR-T

Commercial approval: There are two FDA approved CD19 directed CAR-T products, tisagenlecleucel (CTL019) and axicabtagene ciloleucel. Both are second generation CAR-T-cells. Tisagenlecleucel uses a 41BB costimulatory domain and is transduced by lentivirus, whereas axicabtagene ciloleucel uses the CD28 costimulatory domain and is produced by retroviral transduction. Tisagenlecleucel is FDA approved for relapsed/refractory B-ALL in pediatric and young adult patients. Axicabtagene ciloleucel is approved for relapsed/refractory B-cell lymphoma in adults and is being studied for the treatment of pediatric B-ALL.

The FDA approval of tisagenlecleucel was based on a pivotal, global multicenter trial of tisagenlecleucel in pediatric relapsed/refractory, CD19+, B-ALL that showed an overall remission rate of 81%, all with MRD response [43]. Six- and 12-month relapse-free survival rates were 80% and 59% respectively [43].

Clinical trials: Trials have shown second generation CD19-CAR-T therapy induced remission in heavily pre-treated patients with multiple relapsed/refractory B-ALL [43-46]. Axicabtagene ciloleucel is currently being studied in pediatric patients with relapsed/refractory B-ALL previously treated with salvage therapy or HSCT (NCT02625480). In adult relapsed/refractory B-ALL, 44 of the 53 patients (83%) had a CR and 32 patients (67%) had a MRD response [44]. In a Phase 1 trial of 21 pediatric patients with relapsed/refractory B-ALL or Non-Hodgkin's Lymphoma, CR was seen in 66.7% (14 of 21) of patients with a MRD response occurring in 60% of patients [45]. CD19-CAR-T therapy is being studied in newly diagnosed very high-risk (VHR) B-ALL patients in the COG trial AALL1721 (NCT03876769) and St Jude Total Therapy XVII (NCT03117751). In addition, CD19-CAR-T is being studied in combination with checkpoint inhibitors to enhance efficacy for the CAR-Ts and decrease T-cell exhaustion (Table 8).

Unanswered questions: Several unanswered questions remain including the role of CD19-CAR-T in upfront therapy and if it should be used as monotherapy versus a bridge to HSCT. Historically, patients at high risk of relapse, myeloablative transplant is recommended. In the Park et al. study, subsequent transplant did not influence EFS or OS for the patients who had a MRD response after CD19-CAR-T [44]. In the Eliana study, the 18-month EFS was 66% with a median persistence of CAR-T of 168 days [43,47]. Eight patients underwent allogeneic hematopoietic stem-cell transplantation while in remission [43,47]. Conversely, 29 (45%) patients had an ongoing response without additional treatment, and 19 patients (29%) relapsed without receiving additional therapy [43,47]. An association has been seen between early loss of B-cell aplasia with relapse. In patients with early loss of B-cell aplasia, if there is an available donor and the patient is in good functional status, early transplant is recommended. Long term follow-up is needed to better assess which patient's CAR-T can be used as monotherapy.

CD22 CAR-T

Studies of CD22 showed a similar anti-leukemic effect and safety profile to CD19-CARs. In a phase I trial of CD22 BB.z CART in heavily pretreated relapsed/refractory patients, 12/21 (57%) of patients had a CR, with nine patients having a MRD response (NCT02315612) [48]. To further potentate the efficacy of CD22, CAR-T Bryostatin 1 has been seen to upregulate CD22 on leukemia cell lines and improve CART function and persistence [49].

Dual targeting CAR-T

Antigen-escape relapse after CD19 directed therapies is a major challenge, thus dual targeting of CD19 and CD22 is being developed. Bi-cistronic CAR-T that express CD19 and CD22 scFv simultaneously on every cell and mono-CARs that express CD19 and CD22 scFv separately have been developed as dual-target CAR-T cells. Phase 1 trials of different dual targeting CAR-T therapies are highlighted in Table 8.

Study	Aim	Design	Age	Status
NCT03117751 **St. Jude Total Therapy XVII**	19-BBzCART for MRD positive B-ALL or isolated CNS relapse	Randomized Single Center	1 y.o. to 18 y.o.	Recruiting
NCT03876769 **CASSIOPEIA**	Tisagenlecleucel in newly diagnosed HR B-ALL with EOC MRD positive disease (CTL019)	Phase II Multicenter	1 y.o. to 25 y.o.	Recruiting
NCT02625480 **ZUMA-4**	KTE-C19 in Pediatric and adolescents with R/R B-ALL	Phase I/II Multicenter	Up to 21	Recruiting
NCT02808442	UCART19 in R/R pediatric B-ALL	Phase I Multicenter	Upt 17 y.o.	Recruiting
NCT04154709	CTA101 UCAR-T for R/R CD19+ B-ALL	Phase I	3 y.o. to 70 y.o.	Recruiting
NCT03876769	Tisagenlecleucel in HR B-ALL with EOC + disease	Phase II Multicenter	1 y.o. to 25 y.o.	Recruiting
NCT02435849	CTL019 in R/R or relapse <6 mot after allo-HSCT	Phase II Multicenter	Up to 25 y.o.	Active not Recruiting
NCT03263208	CD19 CAR-T for R/R CD19 B-ALL	Phase I/II	2 y.o. to 70 y.o.	Unknown
NCT02924753	CD19 CAR-T in B-ALL	Phase I Single center-China	4 y.o. to 70 y.o.	Unknown
NCT04276870	CD19 autologous CAR-T Cohort A- hypodiloid Cohort B- t(17; 19) Cohort C- infants with very high risk KMT2A B-ALL Cohort D- CNS relapse who did not receive XRT or BMT CART19 cells transduced with a lentiviral vector to express anti-CD19 scFv:41-BB:TCRζ	Phase II Single Center-UPenn	Up to 29 y.o.	Recruiting
NCT03768310 **CARMA**	CD19 multivirus-specific CAR-for CD19+ B-ALL of NHL undergoing related allo HSCT	Phase I Single Center-Baylor	1 y.o. to 75 y.o.	Not yet recruiting
NCT04214886	CD19 CAR-T for R/R B-cell malignancies (CD19-CD34 CAR transduced T cells)	Phase I Single Center-Loyola University	18 y.o. and older	Recruiting

NCT04404660 AUTO1	CD19 CAR-T in R/R B-ALL	Phase I/II Multicenter	18 y.o. and older	Recruiting
NCT04225676	Efficacy and safety of reinfusion of Tisagenlecleucel in pediatric and AYA patients with B-ALL	Phase II	Up to 25 y.o.	Not yet recruiting
NCT04094311	Study of out of specification for release as commercial product for Tisagenlecleucel in pediatric and AYA R/R B-ALL and B-NHL CTL019	Phase III	Child, AYA, adult	Recruiting
NCT03743246 (JCAR017)	JCAR017 in R/R B-ALL or B-NHL	Phase I/II Multicenter	Up to 25 y.o.	Recruiting
NCT03103971	huJCAR014 for R/R B-Cell ALL and NHL (CD19CAR-4-1BB-CD3zeta-EGFRt-expressing CD4+/CD8+ T-lymphocytes)	Phase I Single Center-University of Washington	18 y.o. and older	Recruiting
NCT04264039	Anti-CD19 U-CAR-T for B cell hematologic malignancies	Early Phase I Single Center-China	2 to 70 y.o.	Not yet recruiting
NCT03389035 CARCIK	CARCIK-CD19 in R/R ALL post HSCT	Phase I/II Multicenter-Italy	1 y.o. to 75 y.o.	Recruiting
NCT03666000	PBCAR0191 for patients with R/R NHL and R/R B-ALL	Phase I/IIa Multicenter	18 y.o. and older	Recruiting
NCT04088890	Autologous CD22 CAR-T in R/R B-ALL	Phase I Single Center-Stanford University	18 y.o. and older	Recruiting
NCT03241940	CD19/CD22 CAR-T for R/R B-cell malignancies	Phase I Single center-Lucile Packard Children's Hospital, Stanford	1 y.o. to 30 y.o.	Recruiting
NCT03289455 AMELIA	CD19/22 CART (AUTO3) for R/ALL	Phase I/II Multicenter-UK	1 y.o. to 24 y.o.	Active, not recruiting

NCT03448393	CD19/CD22 CAR-T in R/R B-ALL	Phase I Single center-NCI	3 y.o. to 30 y.o.	Recruiting
NCT03233854	CD19/CD22 CAR-T in R/R B-cell malignancies	Phase I Single center-Stanford University	18 y.o. and older	Recruiting
NCT03330691	CD19/CD22 CAR for R/R B-ALL	Phase I Single center-Seattle	Up to 30 y.o.	Recruiting
NCT04049383	CD20/19 CAR-T for R/R B-ALL	Phase I Single center-Medical College Wisconsin	1 y.o. to 39 y.o.	Not yet recruiting
NCT02906371	Optimization of Tocilizumab timing for CD19 CAR-T associated CRS (CTL019)	Pilot study Single Center-CHOP	1 y.o. to 24 y.o.	Active, not recruiting
NCT02445222	Long term follow-up of CAR-T. Patients are followed for 15 years following their last CAR-T infusion	Not applicable Multicenter	All ages	Recruiting
*Table is not comprehensive, please see clinicaltrials.gov for additional ongoing trials				

Table 8: Ongoing CAR-T trials for B-ALL.

CAR-T for T-ALL

Several challenges have been encountered in the development of CAR-T including disease heterogeneity, T-cell aplasia, fratricide, and increased side effects in T-ALL. There is a large amount of disease heterogeneity in T-ALL due to distinct stages when T-cell differentiation arrest occurs, making identifying a target difficult [50]. Furthermore, targets on T-lymphoblasts are likely to be on normal T-cells leading to a severe immunocompromised state and fratricide of CAR-T. Fratricide of the CAR-T product or the destruction of the CAR-T due to the target being on both the malignant T-cells and on the CAR-T, leads to decreased CAR-T expansion and persistence. CD3 and CD7 CAR-Ts are more prone to fratricide compared to other targets such as CD1a and CD5 [51,52]. Gene editing to decrease expression of the target antigen on the CAR-T is being studied [53,54] in addition to "off-the-shelf" CAR-T without the target antigen [55]. Preclinical efficacy has been shown in NK-CARs [56,57].

Challenges in CAR-T manufacturing

One of the challenges of CAR-T is manufacturing the product. For adequate collection of T-cells, it requires an absolute lymphocyte count ≥ 500 cells/μL or an absolute CD3 count ≥ 150 cells/μL. This is particularly challenging in heavily pre-treated patients due to poor bone marrow in patients with a higher cumulative dose of chemotherapy [58] and in younger patients due to their size. In the phase II study of tisagenlecleucel, eight patients did not receive the CAR-T infusion due to manufacturing related issues and another seven died before infusion [43,47]. Early collection is suggested for high risk patients; and gene-edited, universal CAR-T-cells are in development. Allogeneic CD19-CAR-T-cells successfully treated two

infants with B-ALL using non–HLA-matched, universal, CAR19 (UCART19) T-cells manufactured from a healthy female donor [59].

Persistence of CAR

There have been several mechanisms proposed explaining why certain patients do not respond or have a durable remission following treatment with CD19-CAR-T-cells. One mechanism of relapse is poor persistence of the CAR-T-cell [46,60]; however, the length of CAR persistence required to induce a durable response or cure is unknown. There is no commercially available test to detect CAR-T persistence. B-cell aplasia has been used as a marker with early emergence of CD19-positive B-cells, within six months of CAR-T infusion, being associated with early relapse [46,60]. The presence of hematogones in the bone marrow has also been suggested as an earlier marker of loss of persistence and can occur while B-cell aplasia is still present [61]. Persistence may also be influenced by the CAR-T construct. The 19-BBz CAR are more persistent (168 days) than 19-28zCAR (~28 days), and 19-BBz CAR-T are associated with longer remission without HSCT [43,45]. Another contributor to decreased persistence is the development of T-cell mediated anti-CAR immune response related to the murine CD19 scFV [45]. Re-infusion with humanized anti-CD19 CAR T-cells has induced remissions in children and young adults with relapsed/refractory B-ALL previously treated with murine-CD19-CAR-T [62]. Lastly, expansion and persistence are improved with CAR-T generated from early linage T-cells (naïve T-cells and stem central memory T-cells) versus more differentiated T-cells (effector memory and terminal effector cells) [63,64]. Similar to studies in blinatumomab, T-cell exhaustion and high levels of T- regulatory cells have also been thought to contribute to treatment failure due to poor CAR-T persistence [65,66]. Most CAR-T protocols include lymphodepletion prior to CAR-T infusion, which leads to depletion of regulatory T-cells and greater engraftment. In addition, it is felt checkpoint inhibition may mitigate T-cell exhaustion. Re-expansion of CAR-T-cells has been seen in patients who are treated PD-1 inhibitors after early loss of CAR-T-cells or relapse [67].

Antigen escape

In antigen directed therapy, escape, or loss of the therapy directed antigen on tumor cells is commonly seen in relapse [37,43]. Mechanisms of CD19 escape seen with blinatumomab and CD19-CAR-T include: selecting for pre-existing antigen negative leukemia, trogocytosis, the development of mutations or alternate splice variants of CD19, or lineage switching [68-74]. There are ongoing trials using two immunotherapies targeting different antigens and bispecific CAR-T-cells (Table 8) to mitigate this effect.

Side effects of immunotherapy

Cytokine release syndrome (CRS): Cytokine release syndrome (CRS) is a systemic inflammatory response due to a rise in cytokine levels during T-cell activation and expansion. Symptoms range from mild and self-limiting to severe and life- threatening and consists of fever, myalgia, capillary leak, hemodynamic instability, coagulopathy and multi-organ failure [75]. Higher disease burden has been associated with higher grade CRS [60]. Varying grading symptoms have been developed, and ASBMT consensus grading system was developed last year [76]. Tocilizumab, an IL-6 receptor antagonist, has been shown to be effective in treating CRS [76]. Other medications that have been considered include infliximab, etanercept, and anakinra [75]. There are ongoing studies regarding the optimal timing of Tocilizumab administration where patients with a higher disease burden will receive early Tocilizumab (NCT0290637).

Neurotoxicity: Neurotoxicity has also been seen with immunotherapy. Symptoms include delirium, encephalopathy, aphasia, lethargy, seizures and cerebral edema [76]. Symptoms typically occur either during or more commonly after CRS. The ASBMT similarly recently created an Immune effector-cell associated encephalopathy (ICE) score [76]. Corticosteroids are recommended for severe neurotoxicity.

B-cell aplasia: B-cell aplasia is an on-target, off tumor adverse effect of immunotherapy directed to antigens on normal B-cells including CD19, CD20 and CD22. B-cell aplasia occurred in all patients who responded to Tisagenlecleucel, and 83% experienced B-cell aplasia for at least 6 months [43,47]. Immunoglobulin replacement is recommended following treatment while there are signs of B-cell aplasia.

Future Directions

The development of immunotherapy is a major advancement in treating pediatric ALL. Particularly in relapsed and refractory B-ALL, immunotherapy has been able to induce remission in chemotherapy refractory patients who had limited treatment options. The timing of immunotherapy in treatment paradigms is being investigated including the role in upfront, salvage, consolidation, and maintenance therapy. Furthermore, checkpoint inhibitors are being studied to further enhance the efficacy of many immunotherapies (Table 9). The role of immunotherapy in T-ALL has remained a challenge, and further research into optimal targets to limit effects on normal T-cells and maximize the efficacy of the therapy is ongoing. Preclinical and clinical research has shown significant promise in improving survival for these patients.

Study	Aim	Design	Age	Status
NCT02767934	Pembrolizumab in ALL with MRD			
NCT03605589	Pembrolizumab Blinatumomab in pediatric and AYA R/R ALL	Phase I Pilot Single Center-Cincinnati Children's Hospital	1 y.o. to 40 y.o.	Recruiting
NCT03512405	Pembrolizumab and blinatumomab in R/R ALL	Phase I/II Single center-City of Hope Medical Center	≥18 y.o.	Recruiting
NCT03160079	Pembrolizumab and Blinatumomab in R/R B-ALL with high marrow lymphoblasts (>50% blasts)	Phase I/II Multicenter	≥18 y.o.	Recruiting
NCT02767934	Pembrolizumab in MRD+ ALL	Phase II Single Center- University of Washington	≥18 y.o.	Terminated due to lack of efficacy
NCT03286114	Augmentation of GVL effect with Pembrolizumab	Phase 1b Single center-University of Michigan	≥18 y.o.	Recruiting
NCT02879695	Blinatumomab and Nivolumab with or without Ipilimumab in patients with poor risk relapsed or refractory CD19+ precursor B-ALL	Phase I Multicenter	16 y.o. and older	Recruiting
NCT01822509	Ipilimumab or Nivolumab in patients with relapsed ALL	Phase I	≥18 y.o.	Active, not recruiting

Table 9: Checkpoint inhibitors.

Conflicts of Interest

Brown: Scientific Advisory Boards – Novartis, Servier, Jazz, Janssen.

Author Contribution Statement

All authors wrote manuscript.

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