Immunotherapy in Pediatric Hematologic Malignant Neoplasms

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Childhood acute leukemia has achieved tremendous treatment outcome improvement over the past several decades. Given that pediatric leukemia remains the most common type of childhood malignant tumors, there are still unmet needs in relapsed/refractory diseases. Moreover, reducing the toxic adverse effects of chemotherapy is another big challenge. Over the past decades, immunotherapy in pediatric leukemia has achieved significant improvement. This review will focus on the recent development and achievement of bi-specific T-cell engagers, antibody-drug conjugates, and chimeric antigen receptor T cell therapies in pediatric leukemia. Moreover, several prevalent obstacles in administering these treatments will also be discussed. Based on the characteristics of each treatment, a variety of clinical trials are currently underway. As a new treatment modality, immunotherapy should be optimally applied based on disease conditions.

Key Words: Childhood leukemia, Immunotherapy, Bi-specific T-cell engagers, Antibody-drug conjugates, Chimeric antigen receptor T cell

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Introduction

Childhood acute leukemia achieved tremendous treatment outcome improvement over the past several decades, especially in patients with acute lymphoblastic leukemia (ALL). Risk-directed treatment for childhood ALL has achieved a 5-year survival rate of more than 90%, while reducing the relapse incidence to less than 10% [1-3]. However, childhood acute myeloid leukemia (AML) continues to have relatively lower survival rates of around 60% despite intensive chemotherapy and subsequent consolidative hematopoietic stem cell transplantation (HSCT) [4].

Given that pediatric leukemia remains the most common type of childhood malignant tumors, reducing the toxic adverse effects of chemotherapy is another great challenge. Moreover, unmet needs still exist for subsequent treatment in relapsed or refractory ALL. As an immunotherapy for leukemia, HSCT is one of the well-known immunotherapy and relapse risk can be reduced through graft-versus-leukemia effect [5,6]. In recent years, advanced concept of immunotherapy has been widely incorporated into the treatment options for pediatric leukemia. These include bi-specific T-cell engagers (BiTEs), antibody-drug conjugates (ADCs), and chimeric antigen receptor T cell (CAR-T) therapies (Table 1, Fig. 1). This review focuses on the recent development and achievement of these immunotherapies in pediatric leukemia, especially based on papers published within 10 years, and also highlights emerging applications of immunotherapy as upfront treatment.

Table 1. Summary of immunotherapy in pediatric hematologic malignancy

	Bi-specific T-cell engagers	Antibody-drug conjugates	Chimeric antigen receptor T cell
Composition	Recominant antibodies consisting of two different single-chain variable fragments, which brings leukemic blasts in close proximity to a patient's own T cells, inducing T cell activation and expansion	Monoclonal antibodies designed to target tumor specific antigen linked with cytotoxic agent.	Autologous T cells with recombinant receptors comprised of an extracellular single chain variable fragment-based domain that binds a tumor-specific surface antigen, a hinge, a transmembrane domain, and an intracellular signaling domain
Example	Blinatumomab	Inotuzumab ozogamicin	Tisagenlecleucel
Dosing	Continuous infusion 28 days on, 14 days off	Once weekly	One infusion
Complete responses in pediatric relased/ refractory ALL (shown in representative study)	39% [10]	58% [24]	81-90% [34,36]
Major toxicities	CRS, neurotoxicity	hepatotoxicity	CRS, neurotoxicity
Disadvantages	Burdensome infusion regimen Dependent on patient's T cell function	Higher SOS rate after HSCT	Complex process to manufacture High cost
Ongoing clinical trials	AALL1731	AALL1732	AALL1721

ALL, acute lymphoblastic leukemia; CRS, cytokine release syndrome; SOS, sinusoidal obstructive syndrome; HSCT, hematopoietic stem cell transplantation.

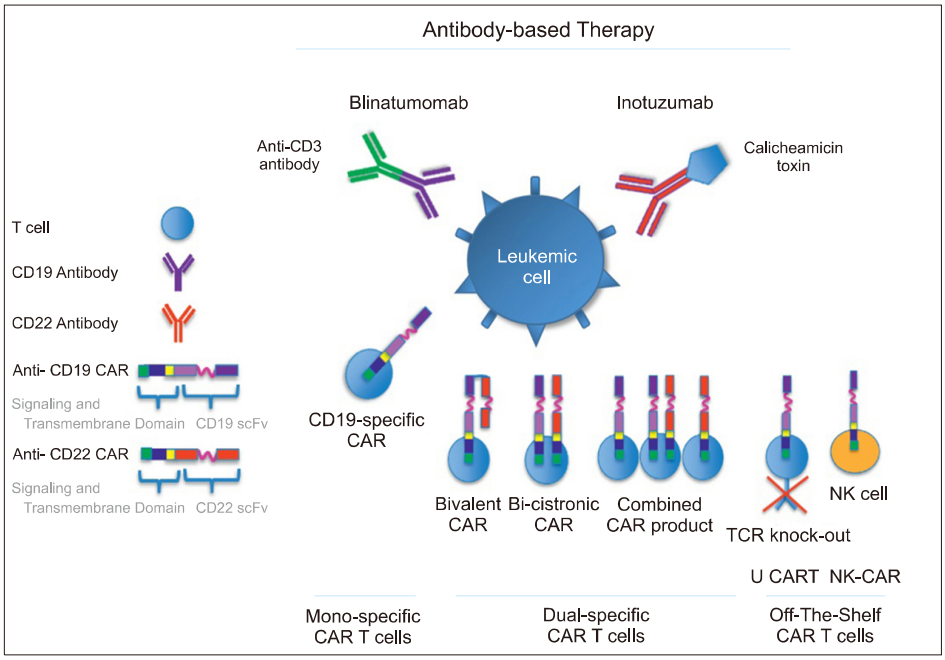

Fig. 1. Schema of immunotherapeutic modalities for hematologic malignancy. Antibody-based and cell-based immunotherapies including blinatumomab, inotuzumab, and chimeric antigen receptor (CAR) T cell, have been clinically translated (Adopted from Schultz L et al. Hematology Am Soc Hematol Educ Program. 2019;2019:226-32.).

Bi-Specific T-cell Engagers (BiTE)

Advances in protein engineering have resulted in the accelerated development of new recombinant antibodies that are capable of binding to tumor-specific antigens, and simultaneously activating effector T cells. BiTEs consist of two different single-chain variable fragments (scFv) joined by a short flexible glycine-serine linker [7]. Blinatumomab is one of the BiTEs which has two specific

binding site, CD19 and CD3. It brings $CD19^+$ leukemic blasts in close proximity to a patient's own $CD3^+$ T cells, inducing T cell activation and expansion, which ultimately leading to leukemic cell death [8,9].

Base on the promising results of blinatumomab in adult, a phase I/phase II dose-escalation/dose-expansion trial of blinatumomab in pediatric patients with relapsed/refractory B cell precursor (BCP) ALL had been conducted [10]. In this study, the recommended blinatumomab dosage for pediatric patients was 5 $\mu g/m^2$/day for the first seven days, followed by 15 $\mu g/m^2$/day thereafter for subsequent 21 days. Complete remission within the first two cycles was achieved in 27 of 70 patients (39%) who were treated at the recommended dose, with 14 of these responders (52%) having negative minimal residual disease (MRD). Median relapse-free survival (RFS) was 4.4 months, median overall survival (OS) was 7.5 months, and 6-month RFS was 42%. Adverse effects observed in this study included cytopenia, transient liver enzyme elevation, neurologic events such as tremor, dizziness in 24% of patients, and cytokine release syndrome (CRS) in 6%. Neurotoxicity and CRS also occurs after CAR-T therapy which is discussed in detail below. Long-term follow-up of previous study has reported that 14 (20%) of the 70 patients who received blinatumomab at the recommend dose were alive at two years, and additional eight patients were still alive at study closure [11]. Moreover, a multicenter expanded access study of 40 patients achieved 63% of complete remission rate after two cycles of blinatumomab therapy [12].

Blinatumomab also showed efficacy and safety in pediatric BCP ALL patients with MRD positive, which further eliminates MRD and serves as a bridging therapy before HSCT [13]. In adult patients where more research has been conducted, blinatumomab yields obvious benefit compared to conventional standard chemotherapy in relapsed or refractory BCP ALL, with manageable toxicities [14]. Additionally, a clinical trial has demonstrated that blinatumomab therapy is effective in adult patients with MRD positive BCP ALL. The 5-year event-free survival (EFS) of these patients was 50%, which is more superior in comparison with generally accepted outcomes of $<25\%$ for adult patients with MRD positive BCP ALL [15]. Moreover, blinatumomab resulted in 36% of complete remission rate in relapsed/refractory Philadelphia-positive (Ph+) adult ALL patients who were refractory to tyrokine kinase inhibitors [16,17].

Based on these results, the US Food and Drug Administration (FDA) granted approval of blinatumomab in Ph^- pediatric patients with relapsed/refractory BCP ALL in September 2016, and Ph+ patients included in July 2017. Furthermore, in March 2018, blinatumomab received accelerated FDA approval for use in both adults and children of first or second remission but with detectable MRD at or above 0.1%. In Korea, blinatumomab received approval in pediatric patients only for Ph^- relapsed/refractory BCP ALL in February 2017 and subsequently, Ph^+ in January 2019.

Blinatumomab is now under evaluation as treatment option for newly diagnosed pediatric BCP ALL. The Children's Oncology Group (COG) is conducting randomized clinical studies of blinatumomab for standard-risk ALL (AALL1731, NCT03914625), and patients were randomly assigned into two groups of blinatumomab versus conventional chemotherapy. This trial aims to find out whether the addition of two cycles of blinatumomab to the standard therapy can improve disease-free survival in standard-risk ALL patients. Another trial, the St. Jude Total 17 protocol (NCT03117751), investigated the effectiveness of blinatumomab in patients with MRD of 0.01 to 1% at the end of treatment induction. Based on these trials, blinatumomab is believed to gradually becoming the standard upfront treatment for pediatric BCP ALL.

Although there are no approved BiTE therapies for pediatric AML, these are actively being investigated, including a few clinical trials targeting CD33 (NCT02520427), CD123 (NCT02152956), and CD371 (NCT03038230) in adult patients. Moreover, the COG Pediatric Early Phase Clinical Trial Network initiated a comprehensive study to evaluate the safety and preliminary antileukemia activity of the CD123-CD3 dual affinity-retargeting antibody flotetuzumab, specifically in children and adolescents with relapsed/refractory AML (NCT04158739).

Antibody-Drug Conjugates (ADC)

Antibody-drug conjugate (ADC) is a monoclonal antibody designed to target tumor specific antigen linked with cytotoxic agent. Unlike BiTEs, this therapy does not require T cells to kill the target cell. Inotuzumab ozogamicin is a humanized CD22 monoclonal antibody conjugated to calicheamicin [18]. CD22 is expressed on more than 90% of BCP ALL cells and mature B lymphocytes, but not on hematopoietic stem cells, or non-hematopoietic lineage cells [19]. After inotuzumab ozogamicin binds to CD22, this molecule can be rapidly internalized and exhibits cytotoxicity via calicheamicin.

Compared with conventional intensive chemotherapy, inotuzumab ozogamicin showed significantly higher complete remission rate (80.7% versus 29.4%), and higher MRD negative rate among patients with remission (78.4% versus 28.1%), compared to conventional intensive chemotherapy in relapsed adult BCP ALL (INOVATE study) [20]. This randomized phase III study in adults with BCP ALL showed significantly longer EFS and OS in inotuzumab treatment arm. Moreover, inotuzumab in combination with low-intensity chemotherapy for older patients BCP ALL with Ph- showed promising results, of which 2-year RFS was 59% [21].

A retrospective analysis of pediatric compassionate use program of inotuzumab provided important information [22]. Among 51 pediatric patients with relapsed/refractory BCP ALL, 67% of patients showed complete morphologic responses, with negative MRD in 71% of these responders. Sinusoidal obstructive syndrome is a well-known adverse event of inotuzumab, which occurred in 22% of total patients and 52% of HSCT recipients. In this study, CD22 downregulation was suggested as a mechanism of resistance, which has also been reported in other pediatric cases [23]. Furthermore, a phase II COG trial of inotuzumab in children and young adults with relapsed/refractory CD22 positive BCP ALL (AALL1621) recently reported the results of 48 patients. Complete remission rate was 58% of all patients, of whom 65% achieved MRD negative; and sinusoidal obstructive syndrome occurred in 8% of all patients and in 31% of HSCT recipients [24]. This agent is now being investigated as upfront treatment for high risk BCP ALL to evaluate efficacy and safety of adding two blocks of inotuzumab to chemotherapy backbone (AALL1732). Based on this trial, inotuzumab could be used an effective salvage therapy in refractory/relapsed $CD22^{+}$ BCP ALL, as well as an upfront chemotherapy in the near future.

In pediatric AML, a CD33-targeting ADC called gemtuzumab ozogamicin, has shown promising results. The COG first evaluated gemtuzumab in pediatric relapsed AML to find out the maximum tolerated dose and safety (AAML03P1) [25]. A subsequent COG randomized trial (AAML0531) compared the outcome of children with *de novo* AML who received either standard chemotherapy or standard chemotherapy with gemtuzumab during the induction I and intensification II phases of therapy, which resulted in a significant improvement of EFS in the gemtuzumab arm [26]. Response to gemtuzumab was related to the elevated CD33 expression [27] and a CD33 splicing polymorphism in the coding region of exon 2, which is the antibody-binding site for gemtuzumab [28]. Based on these data, gemtuzumab ozogamicin will be incorporated into the frontline therapy for children, adolescents and young adults with CD33+ AML in the phase III AAML1831 trial.

Chimeric Antigen Receptor T cell (CAR-T)

Chimeric antigen receptors (CARs) are recombinant receptors comprised of an extracellular single chain variable fragment (scFv)-based domain that binds a tumor-specific surface antigen, a hinge, a transmembrane domain, and an intracellular signaling domain CD3 z chain; and, in the case of second-generation CAR-T, it includes one or more additional costimulatory domain. These recombinant products are usually manufactured from autologous T cells obtained from patients via apheresis. These T cells are modified ex vivo by introducing a gene that codes for CARs. CAR-T interaction with target cells occurs in a human leukocyte antigen–independent fashion which is effective in patients with

cancers that express the target antigens [29].

The second-generation CAR-T that incorporated a second intracellular T-cell signaling domain (e.g., CD28, 4-1BB [CD137]) had resulted in greater proliferative capacity and better antitumor efficacy with persistence [30-32]. This therapy targeting CD19 and/or CD22 has shown tremendous efficacy in pediatric relapsed/refractory BCP ALL patients. The first success in using CAR-T cells to treat two BCP ALL patients was reported in 2013 [33]. Thereafter, University of Pennsylvania research group reported cases of 30 children and adults with BCP ALL treated with 19-BBz CAR-T cells [34]. Complete remission was achieved in 27 patients who were able to receive CAR-T cells successfully (90%), including 2 patients with blinatumomab-refractory condition, and 15 who had undergone allogeneic HSCT. Six-month EFS and OS rates were 67% and 78%, respectively. This was a remarkable outcome for these patients who were previously thought to be incurable. Another initial trials using 19-28z CAR-T cells have shown similar complete remission rates which were observed in 67% of 21 pediatric and young adult patients [35]. However, more patients treated with 19-28z CAR-T cells required a subsequent HSCT for long-term survival compared to those treated with 19-BBz CAR-T cells.

A phase II global collaborative study (ELIANA) of tisagenlecleucel (19-BBz CAR-T) of 75 pediatric and young adult patients confirmed previous reported efficacy [36]. At three months, the complete remission rate was 81%, with all patients being MRD negative as detected by flow cytometry. Twelve-month EFS and OS were 50% and 76%, respectively. Remarkably, persistence of CAR-T cells was observed for up to 20 months, which led to long-term survival without subsequent HSCT. To decrease T cell differentiation and maximize CAR expression, predefined T cell product with 1:1 ratio of $CD8^+$:$CD4^+$ T cells was used at Seattle Children's Hospital, which yielded a 93% complete remission rate, a 1-year EFS of 50%, and OS of 66% [37]. Furthermore, to enhance CAR-T cell expansion and prolonged persistence, the use of a low-affinity CD19 scFv CAR-T cells was reported [38]. More results of pediatric and young adults CAR-T studies are shown in Table 2 [39-41].

Although CAR-T cell therapy marks a new era of immunotherapy for hematologic malignant neoplasms, several severe complications should be considered. First, CRS occurs when activated T cells produce abundant inflammatory cytokines including interleukin-6 (IL-6) or interferon-γ, has to be closely monitored after CAR-T infusion [42]. To ameliorate this severe immune hyperactivation, an anti-IL-6 receptor antagonist, tocilizumab, has been approved by the FDA for treating CAR-T cell-induced CRS. A recent study demonstrated that early intervention for CRS with tocilizumab and/or corticosteroids not only reduced the incidence of transition from mild to severe CRS, but also had no detrimental effect on the MRD negative complete remission rates or functional CAR-T cell persistence [43]. Neurotoxicity including encephalopathy, tremor, delirium, and seizures have been reported, which are related to high disease burden, CRS, high CAR-T expansion, and pre-existing neurologic problems [44]. An updated guideline of CRS and neurotoxicity induced by CAR-T cell therapy have been recently published by the American Society for Transplantation and Cellular Therapy [45].

Several obstacles of CAR-T cell therapy have been reported. One mechanism of resistance to CAR-T cell treatment is the loss of CAR-T cell persistence. Early exhaustion of robust CAR-T cells can limit their persistent anti-leukemia activity. Costimulation using 4-1BB has been shown to ameliorate T cell exhaustion more effectively, compared to CD28 which appears to augment the exhaustion induced by persistent CAR signaling [46]. This could be the reason for longer median duration of 19-BBz CAR-T cells (168 days) than that of 19-28z CAR-T cells (30 days), and why 19-BBz CAR-T cells were associated with longer remission without HSCT [35,36]. Moreover, recent finding of prolonged persistence of low-affinity CD19 scFv CAR-T cells could help overcome this obstacle [38]. In addition, target antigen loss is another resistance mechanism. The loss of CD19 antigen can result from mutations, alternate splice variants, and lineage switch [47]. To overcome CD19 antigen loss, CD22 can be a better target, which is expressed on most

Table 2. Pediatric and young adults chimeric antigen recetor T cell studies

Institutution (reference)	Phase	Number of patients	CAR construct and vector	Response+consolidation	Survival	Adverse effects
Children's Hospital of Philadelphia (Maude SL et al. *N Engl J Med* 2014) [34]	I/II	30 (18 post-HSCT)	19-BBz, Lentivirus	27 CR (90%), (MRD-[a], 8%) 3→HSCT	6-month OS 78%	CRS (severe) 27%, Neurologic (any) 43%
Global Multicenter (Maude SL et al. *N Engl J Med* 2018) [36]	II	75 (44 post-HSCT)	19-BBz, Lentivirus	61 CR (81%) (MRD-[a], 100%) 8→HSCT	6-month OS 90%	CRS (≥grade 3) 46%, Neurologic (grade 3) 13%
National Cancer Institute (Lee DW et al. *Lancet* 2015) [35]	I	21 (7 post-HSCT)	19-28z, Retrovirus	14 CR (67%) (MRD-[a], 86%) 10→HSCT	10-month OS 51.6%	CRS (≥grade 3) 29%
Seattle Children's Research Institute (Gardner R et al. *Blood* 2017) [37]	I	43 (27 post-HSCT)	19-BBz, Lentivirus	40 CR (93%) (MRD-[a], 100%) 11→HSCT	12-month OS 69.5%	CRS (severe) 23%, Neurologic (≥grade 3) 21%
United Kingdom (Ghorashian S et al. *Nat Med* 2019) [38]	I	14 (10 post-HSCT)	19 [low affinity]-BBz, Lentivirus	12 CR (86%) (MRD-[a], 100%) HSCT (−)	12-month OS 63%	CRS (≥grade 3) 0%, no severe neurotoxicity
Memorial Sloan Kettering Cancer Center & Dana-Farber Cancer Institute (Curran KJ et al. *Blood* 2019) [40]	I	25[b] (5 post-HSCT)	19-28z, Retrovirus	18 CR (75%) (MRD-[a], 89%) 15→HSCT	12-month OS 48%	CRS (≥grade 3) 16%, Neurologic (grade 3) 28%
National Institutes of Health, (Fry TJ et al. *Nat Med* 2018) [39]	I/II	21 (21 post-HSCT, 15 post CD19 CAR-T)	22-BBz, Lentivirus	11 CR (73%[c]) (MRD-[a], 75%) HSCT (−)	Median remission duration, 6 months	CRS (≥grade 3) 0%, no severe neurotoxicity
China (Pan J et al. *Leukemia* 2019) [41]	I	34 (13 post-HSCT, 31 post CD19 CAR-T)	22-BBz, Lentivirus	24 CR (71%) MRD-[a], 88%) 11→HSCT	12-month LFS 58% (among 24 CR patients)	CRS (≥grade 3) 6%, no severe neurotoxicity

[a]Percentage of MRD negative patients among those with CR. [b]As a conditioning regimen, 17 patients received high-dose cyclophosphamide (3 g/m^2) and 8 received low-dose cyclophosphamide (≤1.5 g/m^2). [c]Of 21 patients, 15 patients received ≥1x10^6/kg CD22-CAR T cells. CR rate was calculated from these 15 patients.
CAR, chimeric antigen receptor; HSCT, hematopoietic stem cell transplantation; CR, complete remission; MRD, minimal residual disease; OS, overall survival; CRS, cytokine release syndrome.

B ALL cells. A phase I study of an anti-CD22 CAR-T cell was conducted in 21 children and adults with refractory/relapse BCP ALL, of which a complete remission was reported for 17 (73%) of whom had received CD19-targeted immunotherapy [39], including five patients in whose ALL cells CD19 expression had been completely lost or reduced. Manufacturing of CAR-T cells is another issue. In ELIANA trial, seven out of 92 enrolled patients had product-related issues [36]. It was observed that heavily pretreated patients could not have enough T cells, therefore manufacturing failure should be considered for CAR-T cell therapy. In such cases, 'universal' CAR-T cells using HLA-mismatched cells from a healthy donor could be a viable option in the future, which was first reported in a United Kingdom study group [48]. Furthermore, the CAR gene can be inserted into B-ALL cells during CAR-T manufacture, which bind in cis to CD19$^+$ ALL cells, to mask the target molecule and inhibit recognition by CAR-T cells [49].

Given this outstanding result of CAR-T cell therapy, many CAR-T trials are ongoing globally using various viral vectors to target a variety of antigens. The COG has entered a phase II open-label study (AALL1721) for patients ages 1 to 25 years with initial diagnosis of CD19$^+$

expressing B-ALL, have *de novo* National Cancer Institute high-risk features, and with MRD ≥0.01% at the end of consolidation chemotherapy. Furthermore, many dual antigen-targeting CAR-T cells are being developed and used in clinical trials. However, development of CAR-T cells for patients with AML has been challenging due to the lack of universal myeloid antigens for therapeutic targeting and the considerable risk of "on-target/off-tumor" hematologic, and nonhematologic toxicity. Several phase I clinical trials of CD33 or CD123 CAR-T are underway in adults with AML (NCT03126864, NCT03904069, NCT02159495, NCT03766126).

Conclusion

The immunotherapy of childhood leukemia has entered a new treatment paradigm. New immunotherapeutic agents are being rapidly developed and numerous clinical trials are under way, globally. However, therapeutic challenges remain to maximize treatment efficacy with minimized toxicities, which include CRS, neurotoxicity, and on-target/off-tumor effect. Future immunotherapy trials should aim to optimize treatment with or without conventional chemotherapy, and to individualize treatment plan more specifically.

Conflict of Interest Statement

The author has no conflict of interest to declare.

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