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Antibodies: Immunoconjugates and autologous cellular therapy in acute lymphoblastic leukemia

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A B S T R A C T

Using a case study of a 57-year-old man with relapsed/refractory precursor-B (pre-B) acute lymphoblastic leukemia (ALL), this review discusses treatment with immunoconjugates and autologous therapy in acute ALL. Three therapies—blinatumomab, inotuzumab, and CAR T cells—are considered here, each with advantages in specific clinical situations. These therapies represent some of the exciting advances that have been made in the treatment of ALL over the last several years.

Introduction

The incidence of relapse in adults with acute lymphoblastic leukemia (ALL) remains high. At the time of relapse, the prognosis is dismal [1], and novel therapies are clearly needed. In this review, we will focus on three antibody-based therapies currently being evaluated in ALL: blinatumomab, inotuzumab, and chimeric antigen receptor (CAR) T cells.

This review begins with a patient case that will help highlight some of the key aspects of these therapies. Mr W is a 57-year-old gentleman with relapsed/refractory precursor-B (pre-B) ALL who was referred to my clinic for participation on clinical trial S1312: a phase 1 trial of inotuzumab in combination with CVP (cytoxan, vincristine, prednisone). Initially, he had been diagnosed as having an aggressive B-cell lymphoma and received treatment with rituximab-EPOCH (etoposide, prednisone, vincristine, cytoxan, doxorubicin) followed by sacral irradiation. At the time of relapse, it was noted that he had pre-B ALL and he was treated with part B of hyperCVAD (high-dose methotrexate, high-

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dose cytarabine) and then the C10403 induction regimen [2]. Unfortunately, a follow-up bone marrow demonstrated a hypocellular marrow (<10% cellular) with 66% blasts (CD19+, CD22+). His cytogenetics were complex. He was not eligible for the clinical trial because he had Grade 2 peripheral neuropathy. Around the time that I saw him, blinatumomab was FDA approved, and we decided to proceed with this therapy.

Blinatumomab

Blinatumomab is a BiTE (bispecific T-cell engaging) antibody [3]. It has two arms. The one arm (anti-CD3) engages the cytotoxic T cell while the other arm (anti-CD19) engages the lymphoblast (Fig. 1). This in turn leads to activation and proliferation of the cytotoxic T cells and redirected cell lysis and apoptosis of the B-lymphoblasts [4]. Because of its mechanism of action, the drug does lead to significant lymphopenia [4]. However, it does not tend to cause significant myelosuppression. In our patient's case, this was an advantage since he had been heavily pre-treated and had a hypocellular marrow.

A large phase 2 multi-center study of blinatumomab in relapsed/refractory Philadelphia chromosome (Ph chromosome)-negative pre-B ALL was published at the beginning of 2015 [5]. This trial enrolled 189 patients with relapsed/refractory ALL. The median age was 39 years. Approximately one-third of patients had undergone prior allogeneic hematopoietic stem cell transplant (AHSCT) and 39% of the patients were salvage 2 or higher (Table 1). All patients in first relapse had relapsed within 12 months of their initial remission. Sixty-nine percent of patients had a bone marrow blast count ≥50%. The most common adverse events in this trial were fever (60%) and headache (34%) (Table 2). Febrile neutropenia, neutropenia, and anemia were the most common Grade 3–4 toxicities. Two percent of

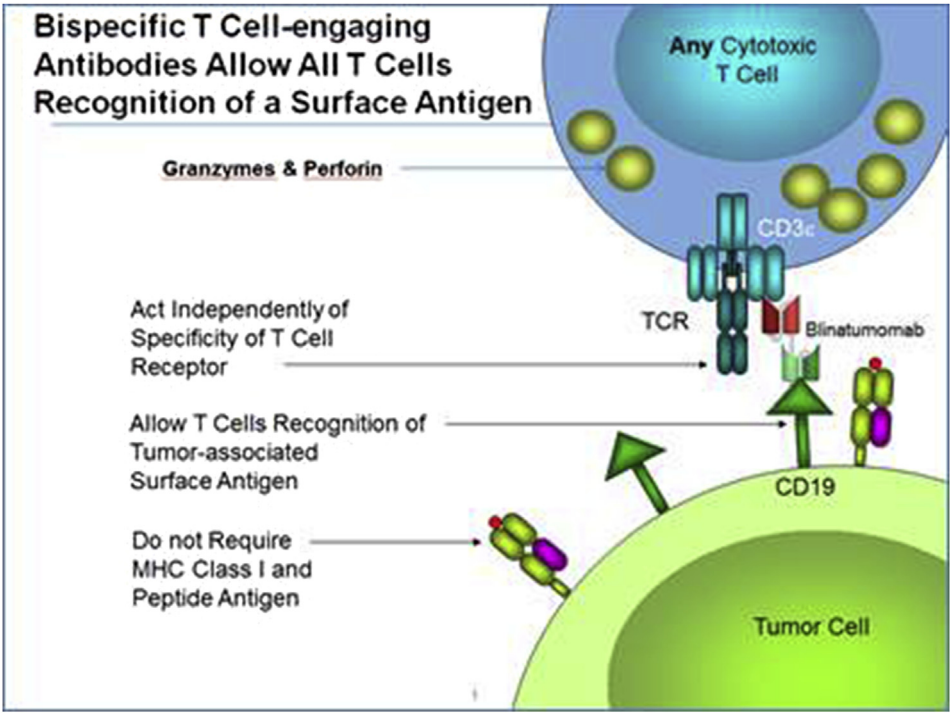

Fig. 1. Mechanism of action of BiTE antibodies. The bispecific T-cell engaging antibody has two arms. One arm (anti-CD3) engages the cytotoxic T cell while the other arm (anti-CD19) engages the lymphoblast. This in turn leads to activation and proliferation of the cytotoxic T cells and redirected cell lysis and apoptosis of the B-lymphoblasts. Figure reproduced with permission of Amgen, Inc.

Table 1
Number of prior lines of salvage therapy in the blinatumomab trial [5].

0	20%
1	41%
2	22%
>2	17%

patients did experience Grade 3 cytokine release syndrome. Fifty-two percent of patients had neurologic events, although 76% of these were Grade 1 or 2 and did not require discontinuation of blinatumomab. Patients with a high tumor burden were pre-treated with dexamethasone, and this likely accounts for the low incidence of the cytokine release syndrome that was observed [5].

The response rate in this trial was 43% [complete remission (CR) and complete remission with incomplete count recovery (CRi)]. Although this number is similar to what has been observed in other relapsed studies, most of these studies have included a combination of good- and poor-risk patients, unlike this trial, which only included poor-risk patients. Eight-two percent of responders had a minimal residual disease (MRD) response and 40% of responders went on to AHSCT. On multivariate analysis, only bone marrow blast <50% was predictive of response [5]. The median relapse-free survival was 5.9 months and overall survival 6.1 months. Based on these encouraging results in a heavily pretreated population, blinatumomab was FDA approved for relapsed/refractory Ph chromosome-negative pre-B ALL in December 2014.

Prior to blinatumomab's FDA approval in the United States, the TOWER study was accruing. This trial randomized patients with Ph chromosome-negative relapsed/refractory pre-B ALL 2:1 to blinatumomab or salvage chemotherapy with one of four standard regimens (FLAG, high-dose cytarabine-based regimen, high-dose methotrexate-based regimen, clofarabine-based regimen). The results of the study are eagerly awaited.

Other trials with blinatumomab are currently ongoing. The BLAST trial is enrolling patients with hematologic remission but persistent or relapsed MRD. Preliminary results were presented at this year's American Society of Hematology Meeting by Nicola Goekbuget with encouraging results [6]. Seventy-eight percent of patients achieved a complete MRD response. E1910 is a large US intergroup trial that is trying to address the question of whether blinatumomab improves outcomes of adults with Ph chromosome-negative ALL (30–70 years of age) when added to intensive induction and post-remission therapy. Finally, S1318 recently opened through the Southwest Oncology Group (SWOG) and will soon be open through the other US intergroups. This trial is evaluating blinatumomab as induction and post-remission therapy followed by POMP (prednisone, vincristine, 6-mercaptopurine, prednisone) maintenance in elderly adults (≥65 years of age) not thought to be suitable candidates for intensive therapy. Although blinatumomab is well tolerated, there are still logistical issues with respect to the drug—administration requires continuous infusion, infusion bags must be changed every 48–72 h, approval from insurers is necessary given the high cost of the drug, and patient compliance must be confirmed, since they are receiving continuous infusions.

Going back to our case … Mr W received dexamethasone for a few days prior to admission. He tolerated therapy with blinatumomab well. However, follow-up bone marrow after cycle 1

Table 2
Toxicities in the blinatumomab trial [5].

Adverse events (any grade)	Incidence
Pyrexia	60%
Headache	34%
Febrile neutropenia	28%
Peripheral edema	26%
Nausea	24%
Hypokalemia	24%
Constipation	21%
Anemia	20%

demonstrated 100% blasts. Although patients have received a second cycle of blinatumomab and have responded, Mr W had clearly progressed on therapy with no improvement in his peripheral blood counts. Because of his high blast count prior to treatment (66% blasts in the bone marrow)—he was at higher risk of not responding to therapy (patients with a bone marrow blast count ≥50% have a lower response rate) [5]. A request was then filed for compassionate use inotuzumab.

Inotuzumab

Inotuzumab, unlike blinatumomab, targets CD22 rather than CD19. In addition, it is an antibody-drug conjugate. The anti-CD22 antibody is linked through an acetyl-butyrase linker to the cytotoxic agent, calicheamicin [7]. Although the linkage through CD22 should allow this drug to specifically target CD22-expressing cells, similar to gemtuzumab ozogamicin, off-target effects such as myelosuppression and hepatotoxicity have been noted.

The initial phase 2 trial of inotuzumab was published by Hagop Kantarjian in Lancet Oncology [8]. The drug was given at a dose of 1.8 mg/m^2 on an every 3–4 week basis for a maximum number of 6 cycles. Similar to the blinatumomab study (Table 3), this was a heavily pre-treated group of patients. The majority of patients were salvage 2 or higher. Fourteen percent of patients had undergone prior AHSCT, and 42% of patients had poor-risk cytogenetics. In terms of toxicities, a significant percentage of patients had Grade 3–4 drug-related myelosuppression [8]. Therefore, this was a concern in our patient Mr W, who had a hypocellular bone marrow. Similar to gemtuzumab, increases in transaminase and bilirubin were observed; however, only a small percentage of these were Grade 3–4 (3 out of 49 patients); and the majority were Grade 1–2 (24% hyperbilirubinemia; 55% elevated transaminase).

The response rate in this trial was very encouraging—57% CR/CRis with a high rate of complete molecular remission in responders (63%). However, the overall number of patients treated with inotuzumab (even with pooled data) is much smaller than those treated with blinatumomab and the drug is not yet FDA approved. Median overall survival (5.1 months) and the percentage of patients able to proceed to AHSCT (22 out of 49 patients) were similar to the blinatumomab trial [5,8].

One particular complication that a physician needs to be aware of with inotuzumab is the risk of veno-occlusive disease. In this initial trial, 5 of 22 patients developed veno-occlusive disease [8]. However, on review of the patients, 4 of 5 had received a preparative regimen with clofarabine/thiotepa. The incidence of veno-occlusive disease has been lower in subsequent trials. However, this risk needs to be kept in mind given that in most patients, the goal is to proceed to AHSCT if they achieve a remission. This was the case for Mr W, who had an HLA-matched sibling. Although there is no clear way to predict or reduce the risk of veno-occlusive disease, this risk seems to be related to the number of prior therapies and the preparative regimen. In prior studies with gemtuzumab [9], allowing time between the last dose of gemtuzumab and AHSCT seemed to reduce the risk of relapse. It is also possible that a lower cumulative number of doses of inotuzumab may be important. With the fractionated schedule (as is used on the compassionate use protocol), this is a little easier to do; and this schedule also seems to be slightly better tolerated in terms of other toxicities [10]. I typically try to allow 1 month between the last dose of inotuzumab and AHSCT if the patient's disease will allow.

Table 3
Patient characteristics: Inotuzumab trial [8].

Median age	36 years (Range 16–80)
Salvage status	
Salvage 1	27%
Salvage 2	49%
≥Salvage 3	25%
Prior AHSCT	14%
Poor-risk cytogenetics	
Ph+	14%
MLL+	10%
Complex	18%

The largest study with inotuzumab (B1931022) recently completed accrual. This is an international study randomizing salvage 1 and salvage 2 patients to inotuzumab or standard salvage chemotherapy. The results of this trial are eagerly awaited; and if the results favor inotuzumab, it is hoped they will lead to FDA approval of this drug. Inotuzumab has also demonstrated encouraging results in combination with mini-hyperCVAD in elderly patients with pre-B ALL [11] and this trial is ongoing at MD Anderson Cancer Center. On preliminary review of the data, the 1-year survival was 81%, as compared to 60% with a historical cohort treated with hyperCVAD. Finally, through SWOG, we have S1312 open, as mentioned at the beginning of this review. This trial uses a standard dose of CVP with a dose escalation of inotuzumab given on a fractionated schedule. The advantage of this approach is the potential synergy with cytoxan/prednisone and inotuzumab [12] as well as the possibility that a lower dose of inotuzumab may lead to a lower risk of veno-occlusive disease. Accrual to cohort 2 for this trial is ongoing.

CAR T cells

The last part of this review will focus on CAR T cells. CAR T cells are T cells redirected to specific antigen targets with engineered chimeric antigen receptors (CARs). (Fig. 2). In ALL, most of the CARs to date have focused on CD19, although the NIH is developing a CD22 CAR. CARs typically engage the target (B lymphoblast) via a single chain variable fragment [13]. The generation of CARs refers to the

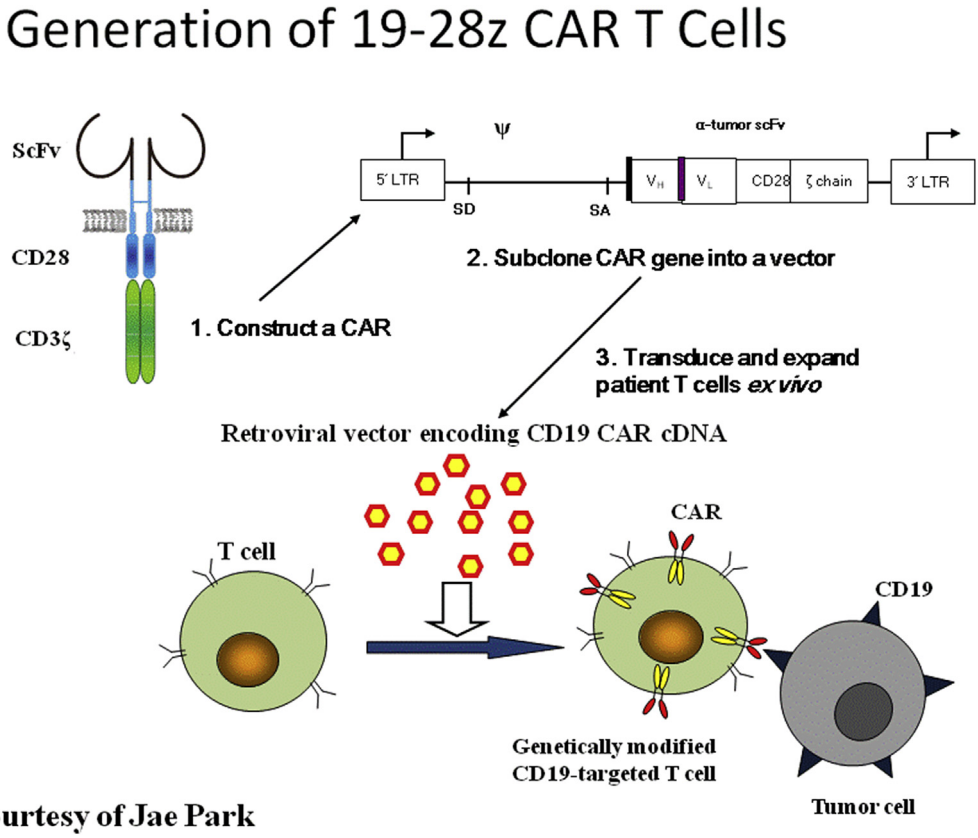

Fig. 2. Generation of 19-28z CAR T cells. The second generation of chimeric antigen receptor (CAR), illustrated here, includes a single co-stimulatory domain derived from CD28 or 4-1BB. Most investigators use retroviruses to transduce T cells with the CAR construct, which then engage the target (B lymphoblast). Illustration courtesy of Jae Park.

intracellular signaling domain. First generation CARs only included CD3. Second generation CARs include a single co-stimulatory domain derived from CD28 or 4-1BB. Most investigators have used retroviruses to transduce T cells with the CAR construct [13]. In clinical trials, high levels of in vivo proliferation of CARs correlate with high response rates [14]. In addition, longer-term persistence may allow for longer-term disease control, although the length of persistence needed for long-term disease control is unknown [14].

Fig. 3 shows the study design for the ALL CAR T-cell trial at Memorial Sloan Kettering Cancer Center led by Jae Park [15]. This schema is similar to other CAR T-cell trials. Patients undergo leukapheresis and their T cells are then transduced with the CAR construct (in the case of 19-28z, CD28 is the co-stimulatory domain) [15]. It is important to note that patients typically receive salvage chemotherapy after the leukapheresis since the T-cell production can take 7–10 days. Currently, the waiting list for these trials is also long since limited centers have these trials open. In the case of our patient Mr W, he had been placed on a waiting list at the time he was referred to see me. After a patient receives salvage chemotherapy, the patient is given time to recover and a repeat bone marrow biopsy is performed to evaluate the response. Prior to CAR T-cell infusion (Day 1), the patient receives cyclophosphamide conditioning on Day −2. Disease is then assessed on Day 28 or so.

The following discussion is based on the results from the Memorial Sloan Kettering trial that were presented at the American Society of Hematology 2014 [15]. The patient characteristics are shown in Table 4. Twenty-eight patients were treated. Notably, 32% of patients were ≥60 years of age and 54% of patients had morphologic disease prior to T-cell infusion. Baseline disease characteristics are shown in Table 5. Thirty-six percent of patients had received three or more lines of therapy and 71% had a prior remission duration of <12 months. Twenty-nine percent of patients had undergone prior AHSCT and 32% of patients were Ph positive [15]. Of the 27 evaluable patients, 89% achieved a CR. Eighty-eight

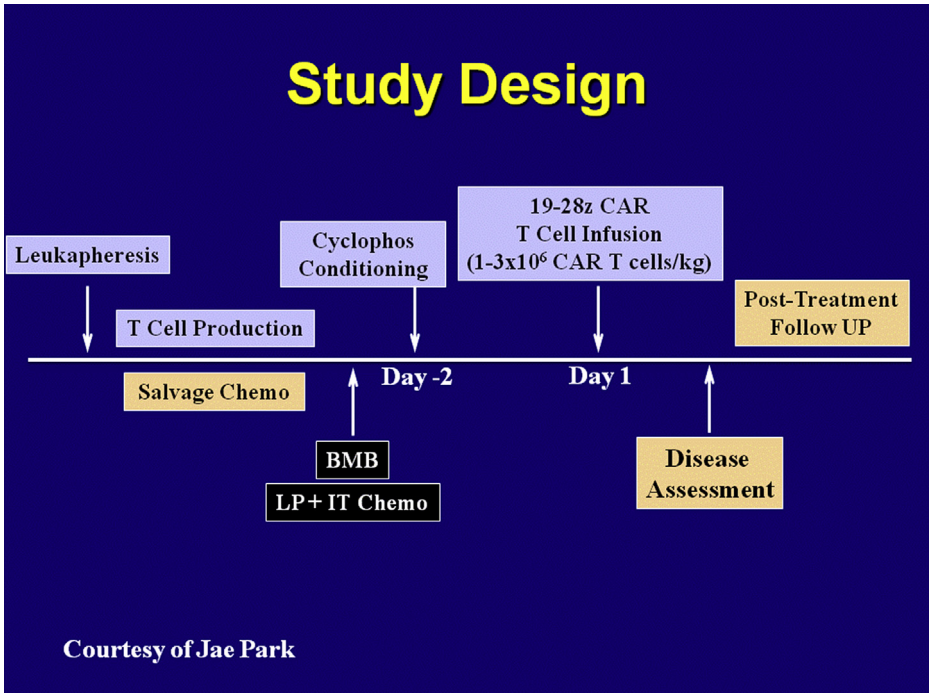

Fig. 3. Study design of CAR T-cell trial conducted at MSKCC. Patient T cells are collected by leukapheresis. While their chimeric antigen T cells are being transduced, the patients receive salvage chemotherapy, a second bone marrow biopsy, and cyclophosphamide conditioning on Day −2. The CARs are infused on Day 1. This schema is similar to other CAR T-cell trials. Figure courtesy of Jae Park.

Table 4
Baseline patient characteristics: CAR T-cell trial [15].

Characteristics	Number of patients (N = 28) (%)
Age at infusion (years)	
18–29	7 (25)
30–59	12 (43)
≥60	9 (32)
Disease burden immediately prior to T cells	
Morphologic disease (5–100%, median 63%)	15 (54)
Minimal disease (<5%)	13 (46)

percent of patients achieving a CR also achieved a complete molecular remission. The median survival of patients was 8.5 months. Although this is a small trial and patients had to be well enough to proceed with therapy, these results are very exciting given the poor-risk population. Of particular interest, when analyzing patients proceeding to AHSCT versus not proceeding to AHSCT after receiving CAR T cells, there was no statistically significant difference in median survival although the numbers of patients are small and follow-up short [15].

If these results bear out, this may make CAR T cells a particularly attractive therapy for those patients relapsing after AHSCT. However, this therapy is associated with toxicity and requires a center with excellent intensive care unit support. In Park's trial, 18% of patients experienced severe cytokine release syndrome, defined as requiring vasopressors and/or mechanical ventilation [15]. Twenty-five percent of patients experienced Grade 3/4 neurotoxicity and 7% of patients died. There was a significant correlation of morphologic disease with the incidence of severe cytokine release syndrome and Grade 3/4 neurotoxicity. No graft-versus-host disease was observed in patients with prior AHSCT. Cytokine release syndrome was managed with an IL-6 receptor inhibitor in 3 patients, steroids in 2 patients, and IL-6 receptor inhibitor plus steroids in 6 patients. Neurologic symptoms were reversible in patients and could occur independently of cytokine release syndrome.

Conclusions

Exciting advances in the treatment of ALL have been made over the last several years. There is no good way to compare the three therapies discussed, but each has its advantages in specific clinical situations. CARs are a particularly exciting new therapy and may be particularly attractive as a therapy post-transplant; however, widespread application of this therapy requires further study.

Table 5
Baseline disease characteristics: CAR T-cell trial [15].

Characteristics	Number of patients N = 28 (%)
Prior lines of therapy	
2	18 (64)
3	5 (18)
≥4	5 (18)
Prior remission duration	
Primary refractory	2 (7)
<12 months	20 (71)
12–24 months	0 (0)
≥24 months	6 (22)
Prior AHSCT	
Yes	8 (29)
No	20 (71)
Philadelphia chromosome (Ph)+	9 (32)
T315I mutation	3 (11)

Conflict of interest

Consulting Fees: EUSA; Sigma Tau; Pfizer.

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