An exceptional case of durable remission achieved with reinfusion of CD19-directed CAR-T despite failure to induce B-cell aplasia and review of institutional experience with reinfusion of tisagenlecleucel

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Abstract

CD19-directed chimeric antigen receptor T lymphocytes (CAR-T) have led to durable remissions in children with refractory and/or multiply relapsed B-lymphoblastic leukemia. For those who relapse or lose B-cell aplasia post-CAR-T, the role of CAR-T reinfusion is unclear. We report a case of durable remission with tisagenlecleucel reinfusion despite failure to achieve B-cell aplasia and compare this case to seven additional children who received multiple tisagenlecleucel infusions at our institution. Our experience suggests that reinfusion is safe and may be a definitive therapy for a small subset of patients. Reinfusion can also reintroduce remission and/or B-cell aplasia, allowing for subsequent therapies.

Introduction

Chimeric antigen receptor T lymphocytes (CAR-T) targeting the CD19 surface antigen have led to durable remissions in children and young adults with refractory and/or multiply relapsed B-lymphoblastic leukemia (B-ALL).[1-11] Pediatric trials of CD19-directed CAR-T have shown complete response rates up to 89% with one-year event-free survival around 50%.[1-4,12] Historically, consolidative allogeneic hematopoietic stem cell transplant (HSCT) has been required to cure refractory and relapsed B-ALL, and CAR-T may be used as a bridge to HSCT. However, CAR-T has resulted in long-term remissions for some patients without the need for further therapy, although the ability to predict response is limited.[4,5] CAR-T is an appealing option for heavily pretreated patients unqualified for transplant, or patients at high risk of transplant-related toxicities.[13,14] Most post-CAR-T relapses occur within the first year following infusion, and the role of CAR-T reinfusion for the treatment of post-CAR-T relapse is unclear.[15] Here we report an unusual case of reinfusions achieving durable remission despite failure to re-achieve B-cell aplasia (BCA). For comparison, we briefly update the experience of seven additional children with relapsed or refractory B-ALL, who were reinfused with an additional dose of tisagenlecleucel, manufactured at the same time as the original dose.

Methods

We performed a retrospective analysis of all patients who received multiple infusions of a non-humanized CD19-directed 4-1BB CAR-T, tisagenlecleucel, at Cincinnati Children's Hospital Medical Center between January 2018 and December 2021. This study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board. A comprehensive review of patient, disease, and product characteristics, toxicities, and patient outcomes was performed. Cytokine release syndrome (CRS) and immune effector

cell-associated neurotoxicity syndrome (ICANS) were graded according to the American Society of Transplantation and Cellular Therapy consensus guidelines.[16] Remission status was tested via multiparametric flow cytometry on bone marrow samples collected 28 days following CAR-T infusion, as well as morphological cerebrospinal fluid (CSF) analysis via lumbar puncture. BCA was monitored at least monthly by peripheral blood analysis of lymphocyte subpopulations. Loss of BCA was defined as >1% CD19+ or 100 absolute CD19+ cells on peripheral analysis, confirmed on a subsequent sample.

Case Descriptions

An 8-year-old boy with trisomy 21 and high-risk B-ALL presented with early marrow relapse fourteen months after initial diagnosis. He was diagnosed at age 7 years with an initial WBC of 4,100 cells/μl and was CNS 1. He did not have cytogenetic or molecular genetic alterations besides his known constitutional trisomy 21. He underwent chemotherapy per Children's Oncology Group (COG) AALL0932 with detectable minimal residual disease (MRD) [0.022% by flow cytometry] at the end of induction. He received consolidation chemotherapy per COG AALL1131 but was switched to treatment per COG AALL0232 due to significant methotrexate toxicity during interim maintenance. He received CTL019 (tisagenlecleucel) on clinical trial (NCT02228096) due to the increased risk of treatment-associated toxicity with trisomy 21 and poor prognosis associated with HSCT in the setting of early relapse. He received CTL019 infusion following standard cyclophosphamide and fludarabine lymphodepleting chemotherapy and developed grade 1 CRS without evidence of ICANS. Bone marrow evaluation 28 days following CAR-T infusion was MRD negative by flow cytometry.

Loss of BCA, indicating functional loss of CAR-T cells, occurred three months post-CAR-T. CTL019 was undetectable on study-performed polymerase chain reaction (PCR). Despite this, the patient remained in an MRD-negative complete remission (CR) by flow cytometry. Since loss of BCA prior to six months is highly predictive for subsequent relapse[15], the following options were considered: proceeding immediately with an unrelated donor HSCT while in remission, proceeding with chemotherapy and HSCT if relapse occurred, or administering a second CTL019 infusion through a single-patient Investigational New Drug application to the Food and Drug Administration. The patient received a second CTL019 infusion six months after his original infusion. Parents declined preceding lymphodepleting chemotherapy. Infusion was well tolerated without CRS. Before the second CTL019 infusion bone marrow evaluation showed a tiny population of CD19+ blasts (0.0092%), though the patient remained in a morphologic remission. However, bone marrow evaluation 28 days following the second CAR-T infusion was MRD-positive with 3% blasts. CTL019 was absent by PCR at that time. A third CTL019 infusion was given one month following the second infusion, preceded by lymphodepleting chemotherapy. The patient developed grade 1 CRS but otherwise tolerated treatment well. His bone marrow evaluation 28 days after the third CAR-T infusion demonstrated an MRD-negative remission by flow cytometry. Notably, he failed to achieve BCA; however, he remains in an MRD-negative remission seven years following his third CAR-T infusion.

Data for the other seven patients are summarized in Table 1. Patients received initial tisagenlecleucel infusion for either refractory or multiply relapsed B-ALL. Two patients had extramedullary isolated CNS relapses. Two patients experienced grade 1 CRS, and no patients experienced ICANS with initial infusion. No patients required corticosteroid or anti-IL-6 treatment. All seven patients achieved an MRD-negative CR and negative CNS status at evaluation 28 days post-CAR-T.

Four of seven patients received reinfusion in disease remission for early B cell recovery, as B cell recovery less than six months from infusion is known to portend a higher risk of relapse.[15] Three patients received reinfusion for frank disease relapse. Only one relapsed patient experienced CRS with reinfusion, for which he required tocilizumab, and this same patient was the only one to experience ICANS. Five of seven patients achieved an MRD-negative CR following reinfusion, and one patient had 1.2% residual disease. The remaining patient achieved a marrow MRD-negative CR and had biopsy-proven cutaneous B-ALL at assessment 28 days following CAR-T reinfusion. Two patients who were MRD-negative failed to re-establish BCA and required subsequent therapies. For one patient CAR-T reinfusion was used as a direct bridge to HSCT, so the presence of BCA was not monitored. CAR-T reinfusion remained the definitive therapy for one patient,

who experienced BCA for only two additional months and is alive with no evidence of disease.

Discussion

We present an exceptional case of durable remission despite lacking on-target loss of B cells following reinfusion, as well as a case series of seven other pediatric patients who received tisagenlecleucel reinfusion for treatment of post-CAR-T relapse or early loss of BCA. Approximately 40-50% of pediatric patients with B-ALL relapse post-CAR-T, and consolidative HSCT is currently the standard of care. The feasibility of reinfusion with the same product depends on the availability of additional CAR-T cells, as well the presence of CD19+ blasts if in relapse.[17,18] Our institutional experience has suggested that tisagenlecleucel reinfusion has a limited, albeit non-zero, likelihood of being a definitive therapy. There is a subset of patients for whom it is successful, though the identification of these patients remains unclear. For some, reinfusion can provide temporary benefit with remission and/or BCA reintroduction.

Our institutional experience has shown mixed success. CAR-T reinfusion remained the definitive therapy for 25% of patients, who are alive without evidence of disease. Prior studies have reported similar experiences with CAR-T reinfusion for either loss of BCA or disease relapse, where reinfusion was the definitive therapy for 20-25% of patients.[6,19-22] Five of the remaining six patients reinfused are alive, following additional therapy after tisagenlecleucel reinfusion including HSCT, humanized CAR-T, and/or immunotherapy. In our cohort CAR-T reinfusion was safe and generally well tolerated, with only one patient requiring tocilizumab for CRS.

For our patient who remains in remission seven years following third CAR-T infusion, his second CAR-T infusion, which was not preceded by lymphodepleting chemotherapy, failed with disease progression 28 days post-infusion. Gardner et al. reported a similar experience: of eight patients reinfused for loss of CAR-T cells, only the two that received lymphodepleting chemotherapy had CAR-T re-expansion.[2]For our patient we hypothesized that the third infusion irradicated the remaining leukemic clone, as the lack of BCA argues against the presence of persistent, active CAR-T cells for surveillance against recurrence. While monitoring BCA allowed for the early detection of MRD by flow cytometry prior to a morphologic relapse following his initial CAR-T infusion, our current institutional practice includes measuring MRD by next generation sequencing. This allows for significantly higher sensitivity and earlier relapse detection but was not commercially available when our patient was treated.[15]

Our experience suggests that tisagenlecleucel reinfusion is safe and can induce remissions in patients with CD19+ relapse, possibly as an alternative to HSCT or other therapies for post-CAR-T relapse. However, the subset of patients for whom this is definitive therapy is small. Our study is limited by sample size, and pooling experiences among pediatric institutions is needed to make conclusions about tisagenlecleucel reinfusion. Clinical trials of humanized CAR-T products may also offer a pathway to durable remission.[9] Other potential strategies to deepen remission include increasing lymphodepleting chemotherapy dosing, using PD-1 inhibitors to augment T cell response, and empirically reinfusing as a "boost" while in remission with ongoing BCA.[23,24] CAR-T cell dose has been associated with improved event-free and overall survival, and multiple CAR-T infusions improved survival in preclinical mouse studies.[25,26] Such studies suggest that reinfusion may reduce the rate of B cell recovery, allowing for prolonged CAR-T cell persistence, and a pediatric phase II study of scheduled reinfusion is ongoing (NCT05460533).[27]

Conflicts of Interest

S.M.D. has received research support from Alexion Pharmaceuticals and has had consultancies with Novartis, Rocket Pharma, CIRM, Allovir, and Neurogene. C.L.P. has served on an advisory committee for Novartis. The remaining authors declare no competing financial interests.

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