Peer Review File

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Reviewer A

The proposed manuscript provides an extensive overview of the progress that were achieved in the field of CAR-T cells approaches to treat B-ALL. The manuscript is well organized and brings a thorough perspective of where the field stands today, but also where it started from. The context of ALL and the therapeutic options is briefly described then the focus is made on the emergence of CAR-T approaches, and further on the description of the reports on the ELIANA trial, including valuable analysis of long-term follow-up based on the work by Laetsch.

Although the proposed manuscript provides very clear clinical insights it would benefit, in a translational setting, from having consolidated description of the fate of CAR-T cells in patients (PK pattern, including expansion and persistence, but also redistribution) and clinical pharmacology elements on the crucial role of lymphodepletion in the success of the therapeutic approach. Such a paragraph would help putting in perspective statements like the 1000-fold expansion mentioned on lines 94-99 and the exposure to fludarabine on line 225.

Response:

(1) Regarding the first successful use of CAR T-cells in chronic lymphocytic leukemia and acute lymphoblastic leukemia, the dose of CAR T cells and timeline of expansion and persistence were included. Furthermore, in lines 93-94, it states that cells were not only found in peripheral blood and bone marrow, but also in the cerebrospinal fluid. Moreover, supplementary table 1 includes the duration of B-cell aplasia from the clinical trials that it was reported in, which acts as a surrogate for CAR T-cell persistence.

"Infused CAR T-cells, at a dose of 1.46 x 10^5 cells per kilogram of body weight, expanded more than 1000 times by day 21, and the patient had a complete response within 28 days, which had been sustained for 10 months at the time of report. Following this, 19-BBz CAR T-cells were infused in two pediatric patients with relapsed and refractory B-ALL. Again, at a dose of 1.2 x 10^5 and 1.4 x 10^5 cells per kilogram, respectively, these cells expanded more than 1000 times by day 20 in the peripheral blood, and CAR T-cells were observed not only in peripheral blood and bone marrow but also in the cerebrospinal fluid (CSF)."

"In 27 patients with a response, CAR T-cells were detected with the median high peak proportion of 39.8% (range 4.4 to 69.3%) among CD3-positive peripheral blood cells by flow cytometry and remained detectable up to 11 months. With quantitative polymerase-chain-reaction, all patients had peak levels of greater than 5000 copies per microgram of genomic DNA, and 26 had greater than 15000 copies. The probability of persistence at 6 months was 68% and DNA remained detectable in some patients for 2 years."

(2) Line 214 now includes the doses and duration of fludarabine and cyclophosphamide with emphasis on optimal exposure leading to better outcomes.

"When lymphodepletion chemotherapy with fludarabine (30mg/m^2 per day for 4 days) and cyclophosphamide (500mg/m^2 per days for 2 days) were administered prior to CAR T-cell infusion, fludarabine exposure with an area under the curve of ≥13.8 mg x h/L was associated with decreased risk of relapse and loss of B-cell aplasia (20)."

Reviewer B

This is a very well written, well structured, and informative manuscript. Please see some minor suggestions below.

line 1: Chimeric antigen receptor T-cells in B-acute lymphoblastic leukemia "colon": history,
Response: this is fixed in the title.

line 56: "allogeneic" hematopoietic stem cell transplantation
Response: this is added in line 51.

line 108: Consider rewriting this sentence.
Response: this is rewritten.
"Among 30 patients, 19 had received HSCT prior to receiving CAR T-cell therapy, and there was no difference in EFS or OS between patients who had undergone HSCT and those who had not."

line 139: and -space- 50%
Response: space is placed.

line 156: consider removing the word "desperately"
Response: the word is removed.

line 158: that "sometimes requires" an intensivist
Response: the words are changed to "may require".

line 163: "Although tisagenlecleucel showed promising results in the treatment of pediatric and young adults with ..." Consider rewriting this sentence.
Response: this is rewritten.
"Although tisagenlecleucel is a great option for treating children and young adults with relapsed and refractory B-ALL, it is important to confirm the long-term efficacy and safety. In a recently published article, Laetsch et al."

line 192: Consider removing "(after the first 8 weeks of infusion)"
Response: the word is removed.

Reviewer C

Overall, well written succinct broad overview of CART therapy for pediatric ALL.
Manuscript well organized and clear
Please see below minor editorial comments:

1. Line 114: Please define what grade of CRS was classified as severe
Response: the following is added "(grades 3 and 4 in the Penn grading scale)".

2. Throughout the manuscript where MRD is mentioned, please define method used as NGS becoming more widely available
Response: the method is clarified in lines 137 and 168.

3. Line 166: Should read " The median number..."
Response: this is fixed.

4. Please include some additional limitations/challenges with CART therapy such as those associated with:
-Leukapheresis especially in small patients or heavily pretreated patients with poor marrow reserve
-Limitations in manufacturing time in patients with rapidly progressive disease, current trial in adults reducing manufacturing time, see adult study
https://www.novartis.com/news/media-releases/novartis-announces-t-chargetm-next-generation-car-t-platform-first-human-data-ash-2021
-Cost and disparities in equitable access
Response: we appreciate the excellent suggestions. We added the following sentences.

"There are several limitations for CAR T-cell therapy. Cell collection in smaller children can be difficult because of poor venous access, limit inlet rates due to small venous catheters, relatively large extracorporeal volume required by the cell separator device, and metabolic complications such as citrate toxicity (25). Heavily pre-treated patients, especially those who received lymphodepleting therapy such as clofarabine and fludarabine, may have low quantity and quality of T-cells to produce optimal CAR T-cells (26). Furthermore, although there has been continued optimization of the manufacturing process of tisagenlecleucel, especially with commercialization, the median time from leukapheresis to infusion remains at 23 days (range, 21-37 days), which is not feasible for patients with rapidly progressive disease (27). Further refinement of this process is ongoing, including the selection of efficient and non-exhausted T-cell subpopulations, addition of cytokine cocktails, and creation of off-the-shelf allogeneic CAR T-cells (28). Finally, socioeconomic status impacts whether a patient will receive CAR T-cell

therapy. Children with a high disease burden exposed to poverty, as defined by public insurance or those from low opportunity neighborhoods, as defined by the Childhood Opportunity Index, received this therapy less frequently, when compared to those with commercial insurance or from high opportunity neighborhoods, respectively (29). Complete remission rate and overall survival of these patients were comparable between these groups."

5. Expand on future role of CART therapy as possible upfront therapy
Response: we added the following sentences in the conclusion.

"However, further improvements in efficacy, management of toxicity, and risk stratification will be necessary through collaborative research and future clinical trials can assess whether CAR T-cell therapy can be brought to the upfront therapy."