Peer Review File

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Reviewer Comments

The authors report four cases of B-ALL with the E2A-HLF fusions who were refractory or MRD-positive following induction/consolidation chemotherapies, who achieved morphologic and molecular remissions following CAR-T cell therapy. This is a very rare B-ALL subtype associated with dismal outcome. There is currently no defined standard of care treatment for these patients. Therefore, these case reports are of clinical relevance. However, this manuscript could benefit from major revisions:

1. First, it would be important to provide more information regarding the CAR-T cell construct. In addition, it would be important to precise if these 4 patients who went to receive CAR-T cell therapy as part of a clinical trial (same or different) or how was the CAR-T cells obtained. If it was part of a clinical trial, the authors should provide the eligibility criteria (inclusion/exclusion), the CAR-T cell dose, number of infusions and whether HSCT post-CAR-T cell was part of the trial or was left to the treating physician. Clarifications of indications for CAR-T cell therapies for the 4 patients would be important; was it based on the presence of E2A-HLF vs response to initial therapy?

Comment 1: First, it would be important to provide more information regarding the CAR-T cell construct.

Reply 1: Chimeric antigen receptors (CARs) are recombinant receptors consisting of an extracellular antigen-recognition domain-a single-chain variable fragment (scFv) from a CD19 monoclonal antibody-linked to intracellular signaling and costimulatory domains from CD3ζ and CD28/4-1BB, respectively. T cells engineered with CAR was the CAR-T cell construct.

Comment 2: In addition, it would be important to precise if these 4 patients who went to receive CAR-T cell therapy as part of a clinical trial (same or different) or how was the CAR-T cells obtained.

Reply 2: CAR-T cell therapy was a clinical trial in our hospital and the clinical trial was approved by the ethics committee of Children's Hospital of Soochow University

(2016004). The CAR-T cells were obtained referring to the following steps. First, T cells were sorted by flow cytometry from peripheral blood which were collected from patients or donors, and then amplified in vitro. Finally, CAR was constructed into T cells by viral approaches.

Changes in the texts: Page 6, Line124-126.

Comment 3: If it was part of a clinical trial, the authors should provide the eligibility criteria (inclusion/exclusion), the CAR-T cell dose, number of infusions and whether HSCT post-CAR-T cell was part of the trial or was left to the treating physician.

Reply 3: It was a clinical trial, so we provide the criteria in the following.

Inclusion criteria for CAR-T cells therapy: 1. Age 0~18 years old, regardless of gender; 2. Refractory/relapsed B-ALL meets the following criteria: 1) Refractory: there is no remission after chemotherapy, and there is no appropriate targeted drug, or targeted therapy is ineffective; 2) Recurrence: more than 5% blasts appear in the bone marrow, and the peripheral blood smear presents blasts, or there will be leukemic cell infiltration outside the bone marrow after complete remission. 3. CD19 phenotype was positive, and peripheral blood cells could be collected through venous; 4. No obvious damage of important organs: 5. Stable vital signs and more than 3 months life expectancy; 6. No peraspartase was used within 4 weeks before cell collection, cytosine arabinoside, anthracycline and methotrexate were used within 2 weeks, and vincristine, 6-mercaptopurine and asparaginase were not used within 1 week. Blood routine examination was in line with Hb≥70g/L, ANC≥1.5×10^9/L, PLT≥50×10^9/L (blood transfusion or recombinant human granulocyte stimulation factor was acceptable within 1 week before cell collection); 7. No evidence of HIV, hepatitis B, hepatitis C, tuberculosis, syphilis or other infections within 3 months before enrollment; 8. No obvious coagulation dysfunction, and no electrolyte disorder such as hyponatremia and hypokalemia; 9. Adolescent female subjects shall be determined not to be pregnant by pregnancy test if necessary; 10. Sign the informed consent; 11. Subject will be able to comply with the study follow-up schedule and other protocol requirements, including follow-up evaluation, in person or with the assistance of a legal guardian.

Exclusion criteria: 1. Critical condition, requiring mechanical ventilation and other life supports; 2. Active infection, such as virus, bacteria and fungus, which is difficult to control, suspected active or latent tuberculosis infection; 3. Patients with primary

immunodeficiency or bone marrow failure syndrome; 4. Persons with a serious mental illness or autoimmune disease, or who are unable to comply during the trial or follow-up period with the assistance of a legal guardian such as a parent;5. No active CNS leukemia; 6. Received allogeneic lymphocyte infusion (DLI) within 6 weeks before cell collection; 7.After repeated chemoradiotherapy, peripheral blood lymphocytes can hardly be amplified by in vitro stimulation to meet the therapeutic needs of T cells; 8. Allergic to the active ingredients, excipients or products derived from mice or foreign proteins contained in this test.

In addition, the CAR-T cell dose and number of infusions were presented (see Page4, Line67; Page4, Line85; Page5, Line95; Page6, Line110).

HSCT post-CAR-T cell was not part of the trial. Whether or not HSCT was left to the treating physician and the family.

Comment 4: Clarifications of indications for CAR-T cell therapies for the 4 patients would be important; was it based on the presence of E2A-HLF vs response to initial therapy?

Reply 4: According to the indications for CAR-T cell therapies for the 4 cases, the presence of E2A-HLF is the one of the things that we should consider, while we think more about response to initial therapy of them and if the patients were relapsed.

Minor corrections:

1. I would change to the new name of the E2A gene which is TCF3.

Reply 1: Thanks for your suggestion. TCF3, also known as E2A, is a well-studied transcription factor that plays an important role in stem cell maintenance and hematopoietic development. In the text, cited references about this fusion were used the name of E2A. To make it easier for readers, we don't use the new name. Do you think it is appropriate?

2. Line 32, should be high relapse rate or risk.

Reply 2: we have modified our text as advised (see Page 2, line 25)

3. Please check spelling of "authours" on line 40.

Reply 3: we have modified our text as advised (see Page 2, line 33)

4. Line 70, should be infusions and not fusions.

Reply 4: we have corrected our text (see Page 4, line 68)

5. Line 93, correct CAT-T cell.

Reply 5: we have modified our text (see Page 5, line 90).

Review version and the submitted version have some difference, so I modified text in red font. Thanks for your suggestions.