**Supplemental File**

**Humanized CD19-directed CAR-T cell therapy in pediatric relapsed/refractory acute lymphoblastic leukemia with CNSL or neurologic comorbidity**

**Supplemental methods**

**Central nervous system directed bridging chemotherapy**

Patients with high disease burden in central nervous system (CNS) at screening (≥5/μL blasts in cerebrospinal fluid [CSF] or solid mass) were permitted to have CNS-directed bridging chemotherapy before chimeric antigen receptor T (CAR-T) cell infusion. All patients enrolled in our inclusion criteria were over 3 years old. Patients received intrathecal injection (IT) with cytarabine at 35 mg (age ≥ 3), methotrexate at 12.5 mg (age ≥ 3), and dexamethasone at 5 mg every other day. IT was performed every week or until blasts were <5/μL. The aim was to control CSF blasts <5/μL or deplete parenchymal infiltration with no detection of epileptic waves on electroencephalogram.

**Transduction efficiency**

CAR-T cells were centrifugated and suspended to FACS buffer (DPBS containing 1%BSA and 1mM EDTA). The density was adjusted to 1×107/mL. CAR-T cells was stained with B5338-PE (anti-CD19 CAR-T cell–specific detection reagent; Genbase Biotechnology, Shanghai, China) and incubated for 30 mins at 2-8℃. After centrifugation, the cells with B5338-PE were suspended again and determined by BD FACSCanto II flow cytometer. Transduction efficiency was defined as the ratio of CAR-T to CD3+ T cells.

**Immunophenotypes of CAR-T cells**

The phenotype of CAR-T cells was analyzed by flow cytometry (FCM). Cells were stained for surface markers CD3, CD4, CD8, CD45RA, and CCR7 (BD Biosciences). The products included both CD4+ (60.5%±14.9%) and CD8+ T cells (33.2%±16.8%) and did not contain any CD19+ B cells. The corresponding subpopulations of naïve T cells (CD45RA+CCR7+), central memory T cells (CD45RA−CCR7+), effector memory T cells (CD45RA−CCR7−), and effector T cells (CD45RA+CCR7−) were 57.0%±17.0%, 20.5%±7.1%, 10.2%±6.5%, and 12.3%±6.6%, respectively.

**Circulating CAR-T cell detection**

The freshly peripheral blood (usually 500 μL) was hemolysed and resuspended by FACS Buffer. The freshly CSF (usually 1 mL) was centrifuged and resuspended by FACS Buffer. Cells were incubated for 1 hour with B5338 (Genbase Biotechnology, Shanghai, China) as the first antibody, and for 0.5 hours with APC-anti mouse immunoglobulin (Ig) G (BioLegend, San Diego, CA, USA) as the second antibody. The sample was resuspended by 200 μL FACS Buffer and cleaned twice for FCM detection. The proportion of the CD3 and B5338 double-positive population was distinguished as CAR-T cells. CAR-T cells counts were determined using a FACSCalibur flow cytometer (BD Biosciences) and reported as the fraction of live lymphocyte cells. To calculate absolute values of cells, the numbers of lymphocytes on routine clinical blood counts or FCM from the same day were collected. The absolute number of CAR-T cells was defined as the number per microliter. The cut-off frequency for CAR-T cell was 0.1% in our study.

**Anti-epileptic prophylaxis**

Levetiracetam (initial dose at 10 mg/kg, max dose 30 mg/kg, max 1500 mg, twice daily) was given as anti-epileptic prophylaxis to patients with a history of seizure from the day of CAR-T infusion until 1 month after infusion. Seizure prophylaxis with levetiracetam was initiated when patients developed severe cytokine release syndrome (CRS) or new neurologic symptoms. Anti-epileptic prophylaxis is not routinely recommended in patients with CNS disease except patients with a history of seizure. Patients already on anti-epileptics continued their home regimen during CAR-T therapy.

**Management of CRS and neurologic toxicity**

CRS and neurologic toxicity were graded according to the American Society of Transplantation and Cellular Therapy (ASTCT) consensus guidelines (Supplemental Table 1) 1. Management of CRS was performed based on Neelapu et al.,2 with some adaptations. (Supplemental Table 2). Tocilizumab (a monoclonal antibody against IL-6 receptor) was given at 8 mg/kg (weight less than 30kg, 12mg/kg, max 800mg). Tocilizumab was administered no more than four times during one episode of CRS. Siltuximab (monoclonal antibody against IL-6) is not available as a second-line treatment in this study. Other symptomatic measures were conducted meantime, such as non-steroidal anti-inflammatory drugs (NSAIDs) for fever and myalgia, vasopressor for vascular leak syndrome or hypotension, nasal cannula or facemask or CPAP or mechanical ventilation for hypoxia.

Management of immune effector cell-associated neurotoxicity syndrome (ICANS) was performed adapted from Neelapu SS. et al 2 (Supplemental Table 3). Active seizures were managed with benzodiazepines (e.g., diazepam or midazolam) for patients who developed status epilepticus. If seizures persist, phenobarbital treatment was added. Anti-epileptics, such as levetiracetam was given to prevent epilepsy. Mannitol (2.5–5 ml/kg/dose), 3% hypertonic saline (3 ml/kg), and furosemide (1-2 mg/kg/dose) were used to control intracranial hypertension. For patients who developed severe cerebral edema, intravenous injection of corticosteroids was administered together with IT with dexamethasone. Anakinra (anti-IL1R) and anti-IL6 (siltuximab) are not available in this study as a second-line treatment.

**Supplemental Table 1.** ASTCT ICANS Consensus Grading for Children 1

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| Neurotoxicity Domain | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| ICE score for children age ≥12 years  | 7-9 | 3-6 | 0-2 | 0 (patient is unarousable and unable to perform ICE) |
| CAPD score for children age <12 years | 1-8 | 1-8 | ≥9 | Unable to perform CAPD |
| Depressed level of consciousness | Awakens spontaneously  | Awakens to voice | Awakens only to tactile stimulus | Unarousable or requires vigorous or repetitive tactile stimuli to arouse; stupor or coma |
| Seizure (any age) | N/A | N/A | Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention | Life-threatening prolonged seizure (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between |
| Motor weakness (any age) | N/A | N/A | N/A | Deep focal motor weakness, such as hemiparesis or paraparesis |
| Elevated ICP/ cerebral edema (any age) | N/A | N/A | Focal/local edema on neuroimaging | Decerebrate or decorticate posturing, cranial nerve VI palsy, papilledema, Cushing's triad, or signs of diffuse cerebral edema on neuroimaging |

ICE indicates Immune Effector Cell-Associated Encephalopathy; CAPD, Cornell Assessment of Pediatric Delirium; ICP, intracranial pressure; N/A, not applicable.

**Supplemental Table 2.** Management Recommendation of CRS

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| Grade  | Management |
| Grade 1 | Supportive care (NSAIDs for fever and myalgia, IV hydration, and symptomatic management of organ toxicities and constitutional symptoms) Consider empiric broad-spectrum antibiotics and G-CSF if neutropenic. Grade 1＞3 days or refractory fever, consider tocilizumab administration as per grade 2 |
| Grade 2 | Supportive care as per grade 1; Supplemental oxygen as needed;IV fluid bolus of 10–20 ml/kg of normal saline; Can give a second IV fluid bolus if SBP remains less than the median value of SBP of children adjusted by age; Administer tocilizumab 8 mg/kg IV over 1 hour (weight less than 30kg, 12 mg/kg, max 800 mg/dose) with a maximum of four doses total; Start vasopressors if hypotension persists after two fluid boluses and anti-IL-6 therapy.Consider dexamethasone 0.15 mg/kg IV (max 10 mg) every 12 hours for one to two doses, if hypotension persists after two fluid boluses and after one to two doses of tocilizumab.Manage per grade 3 if no improvement within 24 hours of starting tocilizumab |
| Grade 3 | Supportive care as per grade 2; IV fluid and vasopressors as needed;Admit patient to ICU; Conduct echocardiogram and hemodynamic monitoring;Supplemental oxygen including high-flow oxygen delivery and non-invasive positive pressure ventilation;Tocilizumab as per grade 2 if maximum dose is not reached;Dexamethasone 0.15 mg/kg IV (max 10mg) every 6 hours and rapidly taper once symptoms improve; If refractory, manage as per grade 4. |
| Grade 4 | Continue supportive care, IV fluids, and vasopressor as per grade 3; Admit patient to ICU, mechanical ventilation as neededAdminister tocilizumab as per grade 3Initiate high-dose methylprednisolone at a dose of 8 mg/kg (max 1 g/day) every 12 h for 3 days, followed by rapid taper at 4 mg/kg every 12 h for 2 days, 2 mg/kg every 12 h for 2 days, and 1 mg/kg every 12 h for 2 days until CRS improvement to grade 1) |

NSAIDs indicates non-steroidal anti-inflammatory drugs; G-CSF, granulocyte-colony stimulating factor; ICU, intensive care unit; IV, intravenous; systolic blood pressure, SBP; Methylprednisolone dose of 1 mg/kg is equivalent to dexamethasone of 0.15 mg/kg.

**Supplemental Table 3.** Neurotoxicity Management Recommendation

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| Grade | Management |
| Grade 1  | Supportive care (IV hydration, aspiration precautions, convert oral medications and nutrition to IV if swallowing is impaired; continuous cardiac telemetry and pulse oximetry); Monitor neurologic status (imaging examination of brain or spine, EEG, diagnostic lumbar puncture); Consider prophylactic antiepileptic (levetiracetam, 10mg/kg, twice).With concurrent CRS: administer tocilizumab 8 mg/kg IV over 1 hour (weight less than 30kg, 12 mg/kg, max 800 mg/dose). |
| Grade 2 | Offer supportive care and neurological monitor as per Grade 1;Administer antiepileptics for patients with seizures;With concurrent CRS, administer tocilizumab as per Grade 1; Administer dexamethasone 0.15 mg/kg IV (max 10mg) every 6-12 hours or methylprednisolone equivalent (1 mg/kg IV every 12 hours) if refractory to anti-IL-6 therapy, or for ICANS without CRS; Continue corticosteroids until improvement to grade 1, and then rapidly taper as clinically appropriate; |
| Grade 3 | Offer supportive care and neurological monitor as per Grade 2;Transfer patient to ICU; Administer antiepileptics for patients with seizures;Tocilizumab as Grade 2 and if not administered previously;Administer dexamethasone 0.15 mg/kg IV every 6 hours; Taper corticosteroids if patient improves;Lumbar puncture with dexamethasone 10 mg injection every day or twice a day. |
| Grade 4 | Transfer patient to ICU; Mechanical ventilation may be required;Supportive care and neurological monitor as per Grade 3;Administer antiepileptics for convulsive and non- convulsive status epilepticus;Tocilizumab as Grade 3;Lumbar puncture with dexamethasone 10 mg injection every day to twice a day.Administer high-dose corticosteroids (eg, methylprednisone 16 mg/kg/d × 3 days, max 1g/d, followed by rapid taper at 4 mg/kg every 12 h for 2 days, 2 mg/kg every 12 h for 2 days, and 1 mg/kg every 12 h for 2 days). |

ICU indicates intensive care unit; IV, intravenous; EEG, electroencephalogram; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome.

**References**

1. Lee DW, Santomasso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant*. 2019;25:625-638.

2. Neelapu SS, Tummala S, Kebriaei P, et al. [Chimeric antigen receptor T-cell therapy - assessment and management of toxicities.](https://pubmed.ncbi.nlm.nih.gov/28925994/) *Nat Rev Clin Oncol.* 2018;15:47-62.

**Supplemental Figures Legends**

Supplemental Figure 1: Fluorescence activated cell sorting plots of peak serum CAR-T cells among lymphocytes in 12 patients after CD19 CAR-T therapy by flow cytometry. GB5005 indicates CAR‑T cells expressing humanized anti‑CD19 single‑chain Fvs.

Supplemental Figure 2: Cytokine release in CSF after CAR-T therapy. “Acute” indicates peak level obtained at CRS and/or neurotoxicity (days 1–13); d14, day 14 after treatment; d28, day 28 after treatment; pre, sample obtained before CAR-T infusion (days -7–0).

Supplemental Figure 3: Cytokine release in serum after CAR-T therapy. “Acute” indicates peak level obtained at CRS and/or neurotoxicity (days 1–13); d14, day 14 after treatment; d28, day 28 after treatment; pre, sample obtained before CAR-T infusion (days -7–0).