Cytomegalovirus (CMV)-specific cytotoxic T lymphocyte therapy resolve CMV diseases and refractory CMV infections in paediatric recipients of allogeneic haematopoietic stem cell transplantation

To the Editor:
The use of CMV-specific cytotoxic T lymphocytes (CMV-CTLs) has shown advantages in controlling refractory and late CMV infection when combined with conventional antiviral treatment in haematopoietic stem cell transplantation (HSCT) recipients [1, 2]. However, the efficacy and safety of using CMV-CTLs in paediatric patients have not been fully evaluated.

A total of 307 paediatric patients received allogeneic HSCT at Shenzhen Children’s Hospital (SZCH), China between May 2018 and September 2020. The rate of CMV-DNAemia for all patients was 40.06% (123 patients). However, only nine patients were diagnosed with CMV end-organ diseases based on published diagnostic criteria [3]. All patients with CMV diseases, together with another patient with refractory CMV infections, were given CMV-CTL therapy in addition to standard antiviral treatment. The analysis of data was conducted with approval from the Institutional Review Board of SZCH. Written consents were obtained from parents or guardians for the analysis and publication of patient data.

PepTivator CMV pp65 peptide stimulated donor-derived CMV-CTLs were generated according to a previously published protocol [4]. The quality of CMV-CTLs was assessed by examining the expressions of IFN-γ and the activation marker NKG2D by CD8⁺ T cells using flow cytometry. The percentage of IFN-γ-producing T cells (CD3⁺IFN-γ⁺ cells) at each dose varied between individuals (ranging from 1.00% to 20.60% of total CD3⁺ T cells). However, it was confirmed that these cells were mainly CD8⁺ T cells (90.60–99.00% of total CD3⁺IFN-γ⁺ cells) and most of them expressed NKG2D (88.90–98.90% of the total CD3⁺IFN-γ⁺ cells).

The clinical characteristics of the patients are summarised in Table 1. Five boys and five girls aged 1–7 years (median 5 years) participated in this study. Primary diseases included thalassaemia and severe aplastic anaemia in 8 and 2 patients, respectively. All except one patient were recipients of haploidentical donors. All patients received myeloablative regimens comprising cyclophosphamide. However, the patient did not receive any steroids during systemic treatment during the active retinitis stage for which might potentially stabilise or restore the best-correlated vision [5]. This patient also received steroids to treat poor graft function due to worsening of refractory CMV diseases. One patient (patient 1) received CMV-CTLs 6 months after the first episode of CMV infection due to worsening of refractory CMV diseases. One patient (patient 2) received a single infusion of CMV-CTLs because of insufficient production of CTLs. However, the patient did not appear to have a significantly delayed resolution of CMV DNAemia or diseases compared to other patients.

In all patients, CMV DNAemia decreased within ~4 weeks (median 23 days, range 15–33 days) after the last CMV-CTL transfusion. In patients with CMV pneumonia, chest computed tomography scans showed that in all patients the lung lesions had resolved within a maximum of 12 weeks. However, one patient (patient 1) experienced blindness caused by CMV retinitis. A plausible cause of the sequelae was that this patient was unable to receive intravitreal ganciclovir therapy in combination with systemic treatment during the active retinitis stage for which might potentially stabilise or restore the best-correlated vision [5]. This patient also received steroids to treat poor graft function 4–5 months after HSCT and may decrease the effectiveness of CMV-CTLs. However, the patient did not receive any steroids during CMV infection. Compared to other patients, patient 1 also had a lower percentage of CD3⁺IFN-γ⁺ effector T cells with each infusion. Nevertheless, whether effector T cells have a dose-dependent effect on the treatment results requires further investigation.

Reconstitution of immune cells provides an important guide for the treatment and prognosis of CMV infection in HSCT recipient [6–8]. We also monitored the reconstitution of immune cells by tracking the numbers and percentages of lymphocyte subsets, including CD4⁺ and CD8⁺ T cells, B cells, and NK cells in the peripheral blood of patients at selected time points (1, 2, 3, 6, and 12 months after HSCT). CD8⁺ T cells have been extensively examined for their vital role in CMV infection [6, 7, 9]. There was a
| Patient No | Sex | Age (years) | Diagnosis | Donor type | CMV serostatus | Time to CMV reactivation after HSCT | CMV infection/disease | Peak CMV DNA titre (IU/mL) | Co-infections | aGVHD | cGVHD | T cell depletion strategy | GvHD prophylaxis | Chimerism | CMV antiviral treatment | Duration from CMV infection to CMV-CTLs therapy | Outcomes |
|------------|-----|-------------|-----------|------------|----------------|-----------------------------------|----------------------|--------------------------|----------------|-------|-------|--------------------------|----------------|-----------|------------------------|---------------------------------|----------|
| 1          | M   | 7           | TM        | Haplo      | D+R−          | 39 days                           | CMV pneumonia         | 1.84 x 10^4 (plasma)    | HBV -          | Liver | ATG   | PT CY + FKS06 + MMF + LDMTX | 40-99% for the first 4 months then >99% after 5 months. | <99% GCV | -                      | 6 months                        | Resolution of CMV DNAemia in 4 weeks. Complete resolution of pneumonia in 2 weeks. Blindness caused by CMV retinitis. |
| 2          | F   | 7           | TM        | Haplo      | D+R−          | 63 days                           | CMV pneumonia         | 6.78 x 10^2 (plasma)    | PVB19, EBV DNAemia | Skin and liver | ATG   | PT CY + FKS06 + MMF + LDMTX | >95%                     | GCV + FOS | 29 days                |                      | Resolution of CMV DNAemia in 4 weeks. Complete resolution of pneumonia in 2 weeks. |
| 3          | M   | 7           | TM        | Haplo      | D+R−          | 79 days                           | CMV pneumonia         | 1.44 x 10^2 (BAL fluid), negative plasma CMV DNA | -              | -     | -     | ATG                       | PT CY + FKS06 + MMF + LDMTX | >99%                     | GCV + FOS | 13 days                |                      | Resolution of CMV DNAemia in 3 weeks. Complete resolution of pneumonia in 2 weeks. |
| 4          | F   | 3           | TM        | MSD        | D+R−          | 28 days                           | CMV pneumonia         | 2.26 x 10^4 (BAL fluid), 4.16 x 10^3 (plasma) | RSV -           | -     | -     | ATG                       | CSA + MMF + LDMTX | >99%                     | GCV                          | 12 days                     | Resolution of CMV DNAemia in 3 weeks. Complete resolution of pneumonia in 12 weeks. |
| 5          | M   | 4           | TM        | Haplo      | D+R−          | 48 days                           | CMV pneumonia         | 7.53 x 10^2 (plasma), 7.70 x 10^1 (BAL fluid) | EBV DNAemia, bacterial enteritis | -     | -     | ATG                       | PT CY + FKS06 + MMF + LDMTX | >99%                     | GCV + FOS | 19 days                |                      | Resolution of CMV DNAemia in 3 weeks. Complete resolution of pneumonia in 4 weeks. |
| 6          | M   | 1           | SAA       | Haplo      | D+R−          | 29 days                           | CMV pneumonia         | 8.48 x 10^2 (plasma), CMV DNA in BAL fluid confirmed by NGS | Fungal meningitis, bacterial enteritis | III   | -     | ATG                       | PT CY + FKS06 + MMF + LDMTX | >99%                     | GCV + FOS | 15 days                |                      | Resolution of CMV DNAemia in 4 weeks. Complete resolution of pneumonia in 6 weeks. |
| 7          | F   | 5           | TM        | Haplo      | D+R−          | 45 days                           | CMV pneumonia         | 4.55 x 10^2 (plasma), 9.35 x 10^1 (BAL fluid) | EBV DNAemia, fungal pneumonia | -     | -     | ATG                       | PT CY + FKS06 + MMF + LDMTX | 60–80% for the first month then >99% after 2 months. | GCV + FOS | 20 days                |                      | Resolution of CMV DNAemia in 3 weeks. Complete resolution of pneumonia in 12 weeks. |
| Patient No | Sex | Age (years) | Diagnosis | Donor type | CMV serostatus | Time to CMV reactivation after HSCT | CMV infection/ disease | Peak CMV DNA titre (IU/mL) | Co-infections | aGVHD | cGVHD | T cell depletion strategy | GVHD prophylaxis | CMV antiviral treatment | Duration from CMV infection to CMV-CTLs therapy | Outcomes |
|------------|-----|-------------|-----------|------------|----------------|-----------------------------------|----------------------|--------------------------|----------------|-------|-------|--------------------------|----------------|--------------------------|-----------------------------|----------|
| 8          | F   | 5           | TM        | Haplo      | D + R+         | 40 days                           | CMV pneumonia         | 7.14 x 10^3 (plasma), 2.48 x 10^2 (BAL fluid) | -             | IV    | -     | ATG                      | PT CY + FKS06 + MMF + LDMTX | >99%         | GCV + OM-IVIG             | 21 days                  | Resolution of CMV DNAemia in 6 weeks. | Completeresolution of pneumonia in 5 weeks. |
| 9          | F   | 4           | TM        | Haplo      | D + R+         | 33 days                           | Refractory CMV DNAemia | 5.78 x 10^3 (plasma)                  | EBV DNAemia II | -     | -     | ATG                      | PT CY + CSA + MMF + LDMTX   | <50% for the first months then >99% after 2 months. | GCV + OM-IVIG             | 19 days                  | Resolution of CMV DNAemia in 2 weeks. |
| 10         | M   | 2           | SAA       | Haplo      | D + R+         | 27 days                           | CMV pneumonia          | 1.33 x 10^3 (plasma), 1.35 x 10^2 (BAL fluid) | Invasive fungal infection, adenoviral enteritis | -     | -     | ATG                      | PT CY + CSA + MMF + LDMTX   | >99%         | GCV + FOS + OM-IVIG       | 23 days                  | Resolution of CMV DNAemia in 4 weeks. | -Complete resolution of pneumonia in 5 weeks. |

ATG anti-thymoey globulin, BAL fluid bronchoalveolar lavage fluid, CMV cytomegalovirus, CSA cyclosporine, CMV-CTLs cytomegalovirus specific cytotoxic T lymphocytes, D donor, F female, FKS06 tacrolimus, FOS foscarnet, GCV ganciclovir, Haplo haploidentical, HBV hepatitis B virus, IVG intravenous immunoglobulin, LDMTX low dose methotrexate, M male, MMF mycophenolate mofetile, MSD matched sibling donor, NGS next-generation sequencing, PT CY post-transplant cyclophosphamide, PVB19 parvovirus B19, R recipient, RSV respiratory syncytial virus, SAA severe aplastic anemia, TM thalassemia major.
significant increase in CD8+ T cells in most patients after CTL transfusion except in patient 1. This can help to clear an active viral infection faster and shorten the duration of treatment, as observed in other studies. CD4+ T cells are also important in the adaptive immune response against CMV. CD4+ T cells is required to facilitate and maintain the classic cytotoxic response mediated by the CD8+ T cell response after adoptive transfer of CMV CTLs in HSCT patients [10]. In children, impaired CD4+ T cell immunity has been shown to be associated with delayed regression of CMV-related diseases. However, no correlation was found between CMV infection and the severity of CMV diseases [11]. In this study, each CTL product contained 1–9.1% of CD3+ CD4+ T cells. However, significant increases in CD4+ T cells were only observed in two patients (patients 8 and 9) after CTL transfusion. Instead, a steady increase in CD4+ T cells was observed over time in most patients. CD4+ T cells have been suggested to be important for the detection of CMV during latency and can therefore offer protection against late CMV infection [11]. Another interesting finding from this study is that adoptive transfer of CMV-CTLs also facilitates the recovery of non-T cell populations, including B cells and NK cells, in some patients, suggesting that CTLs may offer universal benefits for immune reconstitution after HSCT. However, the exact mechanism requires further investigation.

A major concern with the use of CMV-CTLs is the potential to develop or extravee in cases of all-leucocytes caused by CTLs. In the current study, aGVHD was observed in four patients at the time of CMV infection, of which only two patients had grade III–IV aGVHD. To prevent the development or aggravate of aGVHD, patients received a lower dose of CMV-CTLs on the first infusion and a higher dose on the second infusion. In accordance with other published studies, we did not observe any increase in the severity of aGVHD after CMV-CTL transfusion [12]. However, an optimisation of the dose of CMV-CTLs for paediatric patients is still required in adequately large, randomised and prospective studies.

In conclusion, our results demonstrated that adoptive transfusion of donor-derived CMV-specific CTLs is effective and safe for the treatment of CMV diseases and refractory CMV infection in paediatric HSCT recipients. Early intervention with CMV-CTLs combined with standard antiviral treatment can facilitate immune recovery, resolve CMV diseases, and prevent latent CMV infection.

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AUTHOR CONTRIBUTIONS
XW, UY and CY drafted and revised the paper with equal contributions. CW, XZ and SL contributed equally. These authors contributed equally: Xiaodong Wang, Uet Yu, Chunlan Yang. email: tiger647@126.com

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COMPETING INTERESTS
The authors declare no competing interests.

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