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A phase I/II study of adoptive SARS-CoV-2-specific T cells in immunocompromised hosts with or at risk of severe COVID-19 infection

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W.L. and Y.C.L share joint last authorship.

SUMMARY

Background
Post-transplant or haematological cancer patients have a higher risk of mortality after infection with ancestral and early variants of SARS-CoV-2. Adoptive cell therapy (ACT) with virus-specific T cells could augment endogenous T cell immunity to avoid disease deterioration before viral clearance.

Methods
We established a third-party SARS-CoV-2-specific T cell (COVID-T) bank in 2020 (NCT04351659) using convalescent and/or vaccinated donors. In a phase I/II study (NCT04457726), thirteen adult and paediatric patients, acutely positive for SARS-CoV-2 and predicted to have high chance of mortality,
were recruited from September 2021 to February 2022. Twelve patients received a single dose of COVID-T cells, matched on at least 1 HLA.

Results
A dose of either 75,000 or 150,000 IFN-γ+CD3+ cells/m² SARS-COV-2-specific T cells did not cause cytokine release syndrome, acute respiratory distress syndrome, or graft-versus-host disease. In the 8 patients who had detectable donor SARS-COV-2-specific T cells after ACT, none progressed to severe disease or died with COVID-19. In contrast, among the other four patients without evidence of donor micro-chimerism, two died of COVID-19.

Conclusion
Long-acting third-party virus-specific T cells from convalescent or vaccinated donors could be expediently produced and might be clinically useful in future pandemics, particularly before global vaccination is implemented.

Key Words: SARS-CoV2 specific T cells, Adoptive Cell Therapy, Immunocompromised, Haematopoietic Stem Cell Transplant, COVID-19

INTRODUCTION
Early strains of SARS-CoV-2 led to severe respiratory illness resembling severe acute respiratory syndrome (SARS) caused by the SARS-CoV-1 virus, with high mortality rates. Early induction of IFN-γ-secreting SARS-COV-2-specific T cell response and consequently, a coordinated cellular-humoral response, has been associated with effective viral clearance, limited disease severity as well as vaccine efficacy. (1–4) In response to the novel virus pandemic, our group proposed adoptive cell therapy (ACT) using virus-specific T cells (VSTs) from convalescent patients as a potential treatment or preventive measure, especially for high-risk immunocompromised patients.

While vaccines have decreased hospitalizations and deaths, certain patient groups, such as post-transplant and hematologic cancer patients, (5–7) remain vulnerable due to uncertainties about vaccine protection duration and effectiveness. (8–10) These patients often lack the cellular immune
defense, making vaccines and antivirals less effective, (11–13) and face challenges with monoclonal antibodies due to rapid variant resistance. (14–17) In contrast to monoclonal antibodies that target the SARS-CoV-2 spike protein, T cell responses targeting MHC-presented peptides seem consistent across SARS-CoV-2 variants. (1,18). Virus-specific T cells (VSTs) have been effectively used for over 25 years in treating refractory viral infections in immunodeficient patients, especially those post-hematopoietic stem cell transplant, showing increased efficacy with shared HLA alleles. (26–28)

There remains a need to investigate the potential of T cell-based treatments for COVID-19. There are two main approaches, non-specific CD45RA-memory T cells and specific SARS-COV-2-reactive T cells. Many groups, including ours, (19) have shown that clinical-grade SARS-COV-2-specific T cells can be reliably produced either through ex vivo expansion or direct selection of IFN-γ secreting cells. (18,20–28) We report herein the safety and outcomes of 12 immunocompromised patients with or at risk of severe COVID-19 after infusion of adoptive SARS-COV-2-specific T cells that were manufactured using an overnight process. This study contributes to the knowledge of cell therapy for COVID-19, particularly in the setting of patients with haematological malignancies and post-transplant, including children.

Ethics and biosafety
The two-part study was approved by the Institutional Review Board (CIRB 2020/2198 and CIRB 2020/2562) per declarations of Helsinki. The first part (NCT04351659) established a third-party SARS-COV-2-specific T cells cell bank and the second part (NCT04457726) developed a treatment protocol for severe COVID-19. The study was approved by the SingHealth Institutional Biosafety Committee (SHSIBC-2021-050) and authorized by the Health Sciences Authority (PRISM Application ID: 2064173C).

MATERIALS AND METHODS
Generation of the third-party donor cell bank
We first described the methods for the rapid 12-hour generation of a third party SARS-COV-2-specific T cell bank in 2020 using the automated CliniMACS Prodigy IFN-γ Cytokine Capture System® (CCS) (Miltenyi Biotec, Germany). (19) Starting material included one unit of whole blood from eight donors
or apheresed lymphocytes from one donor. Leukocytes were stimulated for 4 hours with overlapping peptides of SARS-CoV-2, covering the immunodominant sequence domains of the S protein and the complete sequence of the N and M proteins (GenBank MN908947.3; Proteins QHD43416.1, QHD43423.2 and QHD43419.1). The peptide pools were short 15-mer peptides with 11-amino-acid overlaps, which can bind to MHC class I and class II complexes to stimulate both CD4+ and CD8+ T cells. Functionally reactive cells were enriched using an automated CliniMACS Prodigy device capturing IFN-γ-secreting cells (Miltenyi Biotec, Germany). IFNγ+ T cell frequencies were analyzed using MACSQuant® Analyzer 10 Express Mode.

An initial 6 donors were recruited from COVID19-convalescent individuals between March to June 2020 during the first few months of the pandemic. Eligible donors were aged 21-65, had a history of COVID-19 with a documented positive test for SARS-CoV-2 that later became negative. Once vaccine became available in 2021, a study amendment included healthy vaccinated donors to expand the study aim on production feasibility. They met all blood donation criteria, including a negative blood SARS-CoV-2 PCR, and provided informed consent approved by the hospital Institutional Review Board (clinicaltrials.gov NCT04351659). Those with common HLA-haplotypes, (29) and a moderate to strong positive IFN-γ response to small-scale Spike (S), Membrane (M) and Nucleocapsid protein (N) peptide stimulation were preferentially selected (Supplemental Figure 1).

With the initial 6 convalescent donors, the probability that a recipient would share at least one HLA allele with one of the donors was >88% among Caucasian, >95% among Chinese, >97% among Malay, and >99% among Indian populations. (19) A further 3 vaccinated donors expanded the coverage to >90% for all 4 racial groups.

Inclusion and exclusion criteria for recipients

Patients aged 1 to 90, who tested positive for SARS-CoV-2 by PCR <72 hours prior to enrolment and predicted to have high chance of mortality were eligible. Phase I (Cohort 1) enrolled 6 patients in a 3+3 dose escalation study. Dose level 1 was 75,000 IFN-γ+ SARS COV-2 specific T cells/ m² and dose level 2 was 150,000 IFN-γ+ SARS COV-2 specific T cells/m². Phase II planned to enroll 12 subjects once dose level 2 was established as safe. Patients at both dose levels were categorised into 2
different clinical groups: Group 1 patients were those with NIH severe symptoms requiring high-level care. Group 2 patients had NIH mild to moderate symptoms who had a high risk of progression to severe COVID-19. Patient’s COVID-19 severity and risk profile were determined by an independent Infectious Disease physician before enrolment.

Specific exclusion criteria included rapidly progressive disease; pregnancy or breast feeding, or receiving systemic steroids at a dose greater than 0.5 mg/kg/day of methylprednisolone-equivalent prior to infusion. Concomitant therapy according to prevailing institutional standard of care was allowed for participants at the discretion of their infectious disease physicians. Presence of endogenous SARS-COV-2-specific T cells prior to infusion was not an exclusion criteria as these patients might still benefit from T-cell augmentation therapy.

Choice and administration of the investigational product
We performed high resolution HLA-matching between the donor and recipient, selecting the VST unit with the highest number of HLA-matched loci. A minimum of 1 HLA match (Class I or Class II) between the third-party unit is required for infusion. If > 1 unit was available, the unit with the highest number of HLA matches was chosen. In post-transplant patients, third-party VST had at least 1 HLA allele common to recipient (tissues) and transplant donor (haematopoietic system). Eligible patients received a single infusion of SARS-CoV-2-specific T cells (COVID-T cells), at a cell dose as defined by study phase.

Monitoring plan
During the first month after infusion of SARS-COV-2-specific T cells, patients were evaluated by the recipient’s medical team daily for 7 days and then weekly until discharge. After discharge, follow-up and safety information was collected until at least day 28 and until donor cells were no longer detectable by donor T-cell chimerism.

Dose Limiting Toxicities and Stopping Rules
Protocol-specified dose limiting toxicities were defined as >grade 3 toxicities associated with the therapeutic cells within 7 days, with specific safety assessments for acute graft-versus-host disease (aGVHD), cytokine release syndrome (CRS), and acute deterioration of lung function. CRS was
graded according to the 2018 ASTCT consensus criteria for CRS. (30) Acute GvHD was graded according to the 1994 modified Glucksberg criteria. (31) Serious adverse events were reported in accordance to regulatory stipulated timeframes. Patient enrolment was stopped if there is any death attributable at least in part to the T cell infusion.

**Donor T-cell chimerism assays**

Blood samples were scheduled on days 0, 3, 7, 14, 21 and 28 days post-infusion. T-cells were isolated using the Pan-T cell isolation kit (Miltenyi Biotec, Germany). Genomic DNA was extracted from peripheral blood mononuclear cells (pre-infusion) and from purified T cells (post-infusion). We measured T cell chimerism by standard clinical short tandem repeat PCR assay (limit of detection 1%) and T cell micro-chimerism by real-time quantitative PCR (limit of detection 0.01%). (32) *(Supplemental methodology)*

**Cytokine release assay (CRA) for functional T cell response post-infusion**

CRA of whole peripheral blood stimulated with SARS-CoV-2 spike peptide pools was performed according to the methods of Tan et al. (33) *(Supplemental methodology)*

**Study endpoints and Statistical Analysis Plan**

The primary endpoint of the study was dose-limiting toxicities until day 28 after infusion of SARS-CoV-2-specific T cells. For efficacy, the study aims to estimate the 3-month survival rate post-infusion with exact binomial confidence interval with a sample of 12-18 patients.

With 12 patients, the standard error for survival estimates will be no more than 0.167, ensuring a two-sided 80% confidence interval. *(Exact confidence interval (Clopper-Pearson) formula, PASS software, 2021).* The achieved conditional power with the first 12 patients out of planned 18, is 83.632%, indicating the ability to detect a 0.2 difference in survival rates between proportions of 0.7 and 0.5, using a two-sided one-sample z-test at a significance level of 0.1. The current merged data have a z-value of 2. The futility index stands at 0.16368, with 0.9 indicating statistical futility. *(Conditional Power and Sample Size Reestimation of Tests for One Proportion, PASS 2021).*
The secondary endpoints summarized descriptively were the Daily National or Paediatric Early Warning Score (NEWS or PEWS) until day 28 after infusion of SARS-CoV-2-specific T cells; time to clinical improvement, defined as improvement by one category on a WHO ordinal scale; time to microbiological clearance, defined as SARS-CoV-2 negativity (real-time PCR negativity or Ct value >30) in respiratory samples; duration of persistence of SARS-CoV-2-specific T cells in the recipients’ blood circulation. Donor T cell chimerism in recipients was monitored until negative or 1 month post-infusion, whichever was earlier.

**Analysis**

Our results are summarized by descriptive statistics using GraphPad Prism Version 9.4.1. Where relevant, non-parametric, paired one-way ANOVA was used to compare the means across different timepoints.

**RESULTS**

The CONSORT flow diagram (Figure 1) shows eligible donors and recipients, exclusion, donor groups and recipient allocation to phases and outcomes.

**HLA representation**

Our donor cell bank derived from 9 donors represented the most common HLA alleles (defined as >5% frequency) in Singapore. (Supplemental Table 1)

**Figure 1. Consort Flow Diagram**

- Donors assessed for eligibility (n=12)
  - Excluded (n=3)
    - Non-eligible
    - Low IFNγ+ response
  - Eligible Donors (N=9)
    - Unvaccinated, Convalescent (n=6)
    - Vaccinated, Convalescent (Hybrid) (n=2)
    - Vaccinated, Uninfected (n=1)

- Recipients assessed for eligibility (n=13)
  - Excluded (n=1)
    - Withdrew, condition improved
  - Eligible patients infused (N=12)
    - Phase I (n=6)
      - Group 1, n=0
      - Group 2, n=6
    - Phase II (n=6)
      - Group 1, n=1
      - Group 2, n=5
    - 3 deaths
      - 2 COVID-19 pneumonia
      - 1 Primary Disease
    - 9 Alive
Donor SARS-COV-2-specific T cells

Each donor yielded a median of 6 (range: 4 to 9) bags of cells containing a median dose per bag of 0.13 x 10^6 IFN-γ + SARS CoV2- specific T cells (range: 0.10- 0.3 x 10^6). Immunophenotype and purity of product were characterized by flow cytometry (MACSQuant® Analyzer 10 Flow Cytometer, Miltenyi Biotec, Germany). Product characteristics comparing vaccinated/hybrid donors with milder disease and unvaccinated convalescent donors from earlier phases of the pandemic are summarized in Table 1. Prior to selection, the predominant subset was central memory T cells (TCM), characterized by CD45RO+CD62L+CD3+, and was higher in vaccinated donors. After enrichment, T effector memory cells (CD45RO+CD62L-CD3+) were the most abundant subset in the final product, regardless of vaccination status. We observed an increase in CD4/8 ratio. IFN-γ secreting CD4+ cells were mainly effector memory, while IFN-γ secreting CD8+ cells were either effector memory or terminal effector (expressing CD45RA). Regardless of donor type, an average yield of 54.31% of IFN-γ+ T cells (median 52.71%) was recovered from the starting blood component, with a mean purity of 54% IFN-γ secreting VSTs out of CD3+ (median 61%) in the final product.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Vaccinated or Hybrid Donors (N=3)</th>
<th>Unvaccinated, Convalescent (N=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRE-SELECTION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cells (%Lymph)</td>
<td>70</td>
<td>79</td>
<td>0.03</td>
</tr>
<tr>
<td>TN (% T cells)</td>
<td>6</td>
<td>33</td>
<td>0.0019</td>
</tr>
<tr>
<td>CM (% T cells)</td>
<td>65</td>
<td>40</td>
<td>0.0017</td>
</tr>
<tr>
<td>EM (% T cells)</td>
<td>26</td>
<td>23</td>
<td>0.7408</td>
</tr>
<tr>
<td>TEMRA (% T cells)</td>
<td>4</td>
<td>5</td>
<td>0.8840</td>
</tr>
<tr>
<td>IFN-γ+CD3+ (%CD3)</td>
<td>3</td>
<td>11</td>
<td>0.37</td>
</tr>
<tr>
<td>Total IFN-γ+CD3+ (10^3)</td>
<td>2983</td>
<td>4057</td>
<td>0.0422</td>
</tr>
<tr>
<td>CD4 (%T cells)</td>
<td>53</td>
<td>46</td>
<td>0.37</td>
</tr>
<tr>
<td>CD8 (% T cells)</td>
<td>30</td>
<td>34</td>
<td>0.3165</td>
</tr>
<tr>
<td>NKT (% Lymph)</td>
<td>8</td>
<td>7.5</td>
<td>0.9039</td>
</tr>
<tr>
<td>B cells (%Lymph)</td>
<td>15</td>
<td>12</td>
<td>0.5489</td>
</tr>
<tr>
<td>NK cells (%Lymph)</td>
<td>15</td>
<td>9</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>PRODUCT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viability</td>
<td>93</td>
<td>91</td>
<td>0.5674</td>
</tr>
<tr>
<td>CD3+IFN-γ+ cells (10^6)</td>
<td>1.58</td>
<td>0.87</td>
<td>0.1139</td>
</tr>
<tr>
<td>IFN-γ+ % in CD3</td>
<td>59</td>
<td>52</td>
<td>0.6080</td>
</tr>
<tr>
<td>Total IFN-γ+CD3+ (10^3)</td>
<td>1692</td>
<td>951</td>
<td>0.1811</td>
</tr>
</tbody>
</table>
### Table 1. Mean Donor lymphocyte composition before and after enrichment

<table>
<thead>
<tr>
<th></th>
<th>Before Enrichment</th>
<th>After Enrichment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ+ % in CD4</td>
<td>60</td>
<td>62</td>
<td>0.8213</td>
</tr>
<tr>
<td>IFN-γ+ % in CD8</td>
<td>50</td>
<td>55</td>
<td>0.7628</td>
</tr>
<tr>
<td>T cells (%Lymph)</td>
<td>55</td>
<td>56</td>
<td>0.9008</td>
</tr>
<tr>
<td>CD4 (% T cells)</td>
<td>68</td>
<td>68</td>
<td>0.8886</td>
</tr>
<tr>
<td>CD8 (% T cells)</td>
<td>18</td>
<td>29</td>
<td>0.0501</td>
</tr>
<tr>
<td>NKT (% Lymph)</td>
<td>13</td>
<td>21</td>
<td>0.2562</td>
</tr>
<tr>
<td>B cells (%Lymph)</td>
<td>38</td>
<td>41</td>
<td>0.7587</td>
</tr>
<tr>
<td>NK cells (%Lymph)</td>
<td>7</td>
<td>3</td>
<td>0.0091</td>
</tr>
<tr>
<td>CD3+IFN-γ+ T cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%TN</td>
<td>1</td>
<td>2</td>
<td>0.4011</td>
</tr>
<tr>
<td>%CM</td>
<td>9</td>
<td>20</td>
<td>0.1805</td>
</tr>
<tr>
<td>%EM</td>
<td>74</td>
<td>71</td>
<td>0.7750</td>
</tr>
<tr>
<td>%T_{EMRA}</td>
<td>15</td>
<td>8</td>
<td>0.4949</td>
</tr>
<tr>
<td>CD4+ IFN-γ+ T cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%TN</td>
<td>0</td>
<td>2</td>
<td>0.0655</td>
</tr>
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<td>%CM</td>
<td>8</td>
<td>18</td>
<td>0.1507</td>
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<tr>
<td>%EM</td>
<td>90</td>
<td>80</td>
<td>0.1317</td>
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<td>%T_{EMRA}</td>
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<td>1</td>
<td>0.3220</td>
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<tr>
<td>CD8+ IFN-γ+ T cells</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>%TN</td>
<td>2</td>
<td>3</td>
<td>0.8144</td>
</tr>
<tr>
<td>%CM</td>
<td>13</td>
<td>13</td>
<td>0.9734</td>
</tr>
<tr>
<td>%EM</td>
<td>48</td>
<td>54</td>
<td>0.6966</td>
</tr>
<tr>
<td>%T_{EMRA}</td>
<td>37</td>
<td>31</td>
<td>0.7306</td>
</tr>
</tbody>
</table>

TN: Naïve T cells; CM: Central Memory T cells; EM: Effector memory T cells; T_{EMRA}: Effector memory T cells re-expressing CD45RA (Terminal effector T cells); Lymph: Lymphocytes
Recipients

Thirteen patients were recruited to the study in total: 9 adult and 4 paediatric patients. The patients were recruited from September 2021 to February 2022, during the SARS CoV-2 Delta and Omicron BA.1 variant prevalence in Singapore. One child withdrew due to physician’s decision, resulting in 12 evaluable patients. The first 3 patients received dose level 1 75,000/m2 IFN-γ+ SARS-COV-2-specific T cells. The fourth and subsequent patients received the recommended phase 2 dose of 150,000/m2 IFN-γ+ SARS-COV-2-specific T cells. We were able to identify at least one donor for each recipient from the donor cell bank.

Table 2 summarizes the patients’ clinical characteristics, treatment, adverse events and survival outcome. The median time from enrolment to infusion was 2.5 days (0 to 5 d). Nearly all the patients (11/12) received an available product matched at 2 or more HLA loci at the allelic level on HLA-A, B, C and DRB1. One patient received a product from his convalescent haploidential stem cell transplant donor. All patients were high risk for severe COVID-19; one patient had severe COVID-19 with multiorgan complications and viraemia (group 1) and 11 patients had early or persistently symptomatic disease (group 2). Standard-of-care treatment followed prevailing guidelines and managed by independent infectious disease physicians, and included appropriate monoclonal antibodies (Sotrovimab, casirivimab plus imdevimab) and anti-virals (remdesivir) for mild to moderate disease, and dexamethasone for severe disease.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Primary Disease</th>
<th>Immunosuppression</th>
<th>Age / Gender</th>
<th>Comorbidities</th>
<th>Vaccine Doses</th>
<th>Group DL*</th>
<th>AE Donor ID</th>
<th>HLA A Match</th>
<th>Class I Alleles Matched</th>
<th>Class II Alleles Matched</th>
<th>Donor T Cells Detectable after ACT</th>
<th>Outcome 3mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Multiple Myeloma</td>
<td>Post-auto-SCT</td>
<td>65/ M</td>
<td>Splenic rupture</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>Nil</td>
<td>2/8</td>
<td>C<em>01:02; DRB1</em>16:02</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>DLBC L</td>
<td>Post Chemotherapy (#5 R-CHOP)</td>
<td>77/ M</td>
<td>Stage 3 CKD; CA Colon; DM</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>Nil</td>
<td>2/8</td>
<td>A<em>11:01 A</em>11:01</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>AML</td>
<td>Post-haploSC T; Azacytidine</td>
<td>67/F</td>
<td>Parkinson’s Disease</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>Nil</td>
<td>4</td>
<td>1/8</td>
<td>A*02:01 NA</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>R/R Mantle cell</td>
<td>Venetoclax Ibrutinib</td>
<td>69/ M</td>
<td>-</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>Nil</td>
<td>4</td>
<td>2/8</td>
<td>A<em>11:01; DRB1</em>12:02</td>
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<tr>
<td>Lymphoma</td>
<td>Post-Renal transplant</td>
<td>Everolimus/ Prednisone</td>
<td>Diabetes Mellitus</td>
<td>Hypertension</td>
<td>A*24:02;</td>
<td>DRB1*04:05</td>
<td>Yes</td>
<td>Alive</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>Post-Renal transplant</td>
<td>Everolimus/ Prednisone</td>
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<td>Hypertension</td>
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SCT: Stem Cell Transplant; DL: Dose Level; ACT: Adoptive Cell Therapy

### Table 2. Characteristics of Recipients

The T cell subset analyses of patients [Figure 2] at the time of infusion showed a median CD3 blood count of only 471.5 cells/µl (SD 704.2, range 2-2409 cells/µl), median CD4 125.5 cells/µl (SD 266.6, Range 1-764 cells/µl) and median CD8 225 cells/µl (SD 506.7, Range 1-1728). Fifty percent of the patients were severely T-lymphopenic (< 400 CD3+ cells/ µl), and at a significant risk of COVID-19 mortality (5,34).
Primary safety endpoint

No protocol-defined dose-limiting toxicities until day 28 was observed at both dose levels. The specific safety assessments included incidence of graft-versus-host disease, worsening of cytokine release syndrome, and deterioration of lung function. One patient developed a non-specific mild rash that resolved spontaneously. No deaths were attributable to the T cell infusion.

Secondary endpoints

The National or Paediatric Early Warning Score (NEWS2 or PEWS) measured patient's physiological status for adults and children, respectively. All patients improved by 1 category or remained in the low-risk category in the first 7 days post infusion. Clinical severity was measured using the WHO ordinal score, with the majority of patients having mild disease and no worsening. One patient with mild disease on oxygen improved within 2 days of infusion, while one patient with severe disease worsened within 1 day of infusion.

The median time to SARS-CoV-2 PCR negativity in respiratory secretions after infusion of SARS-CoV-2-specific T cells was 12 days (Range 5-154). High risk group had 75% overall survival at 3 months (95% confidence limits, 50.5% - 99.5%); 3 deaths occurred, one from primary disease relapse (COVID-19 negative) and two from COVID-19 pneumonia. One of the patients who died from progression of COVID-19 pneumonia was infused at an advanced stage of COVID-19 disease (R09) requiring dexamethasone treatment shortly after infusion of T cells.
BIOLOGICAL CORRELATIVES

Donor T cell chimerism

Eleven patients underwent STR and RT-PCR chimerism studies to evaluate adoptive T cell persistence. In one fully-engrafted haplo-identical transplant patient who received haplo-donor SARS-CoV-2-specific T cells, chimerism studies were not applicable. Micro-chimerism assays using RT-qPCR methods detected donor T cells in 8 of the 11 patients at least once after adoptive cell infusion. Three patients showed persistent donor T cell micro-chimerism at 1 month, while one patient missed testing at 1 month, but remained positive at 2 and 3 months (Supplemental Table 2a). Mean T-cell micro-chimerism is summarized graphically in Figure 3. We did not find a correlation between degree of T-cell lymphopenia and level of donor chimerism in this cohort (Supplemental Table 2b). A swimmer’s plot depicts the clinical course of the 12 recipients (Figure 4).

![Donor T cell chimerism graph](image)

Figure 3. Donor T cell Micro-chimerism Post Infusion (% donor T cells among total CD3+ cells)
Remarkably, in the 8 patients who had detectable donor SARS-COV-2-specific T cells after ACT, none progressed to severe disease or died with COVID-19. In contrast, among the other 4 patients without evidence of donor micro-chimerism, 2 died of COVID-19.

**Trajectory of T cell reconstitution after adoptive T-cells.**

There was a trend towards increased CD3 and CD4/CD8 counts at day 7 and day 14 post infusion, although this did not meet statistical significance (Figure 5), in part due to missing data after rapid COVID-19 recovery and discharge to step-down facilities as early as day 7.
To further evaluate the effects of adoptive T cell therapy, we looked at bulk T cell responses in IFN-γ secretion to Spike, Membrane and Nucleocapsid antigen before and after infusion at days 0, 3 and 7 in 5 patients. In 4 patients, pre-existing reactive T cells were observed before ACT (Supplemental Figure 2). In the remaining patient (R09), reactive T cells were found only after ACT. In 2 of the 5 patients (R09 and R10), a transient increase in IFN-γ secretion on day 3 in response to Spike protein with concomitant increments in CD3/4/8 between day 3 to 7 was observed. Patient R10 also showed a corresponding increase in donor T cell chimerism (Supplemental Table 2); whereas T cell chimerism testing was not performed in patient R09 who was a haploidentical stem cell transplant recipient with full donor chimerism receiving donor SARS-CoV2 specific T cells.

Discussion

Our study showed the feasibility and safety of rapid manufacturing of SARS-COV-2-specific T cells for clinical use. We infused a comparatively lower dose of VST at 150,000 IFN-γ+CD3+ cells/m² for in vivo expansion, in contrast to ex vivo expansion protocols. (28,35) The T-cell infusions did not cause cytokine release syndrome, acute respiratory distress syndrome, or graft-versus-host disease, all of which were primary concerns that resulted in a cautious initial acceptance of the protocol. None of the 8 patients who had detectable donor SARS-COV-2-specific T cells after ACT had disease progression or died with COVID-19, whereas 2 of 4 patients who did not have evidence of donor T cell microchimerism succumbed to COVID-19 pneumonia as a cause of death.
In our high-risk patients who had received SARS-CoV-2-specific T cells, the time to clearance of SARS-CoV-2 virus, defined as PCR CT value >30 in respiratory tract samples, was approximately 12 days. This compared favorably to healthy comparator cohorts studied during the SARS-CoV-2 Delta variant wave in a multicenter Singapore cohort (9 days in a healthy vaccinated break-through infected cohort and 14 days in a healthy unvaccinated cohort). (36) The time to viral clearance after ACT infusion was also shorter than in a comparator cohort of paediatric oncology patients treated with remdesivir or had no treatment during the same study period in our hospital (median 12 days vs 31 days respectively). (37)

The ideal dose of VST remains uncertain and should be tailored based on product purity, staying below the GvHD threshold of approximately 2.5 x 10^4 cells/kg. (38) In a VIRCTLC trial, multiple infusions were administered with target doses of 0.5 x 10^4 CD3+/kg for HLA mismatched related donors and 2.5 x 10^4 CD3+/kg for matched related donors. (39) Direct selection of IFN-γ+ VST reduces culture time compared to traditional expansion methods, which may promote T-cell proliferation in vivo and reduce exhaustion. (40–42) Despite a lower yield and requiring a minimum number of responding T cells, lower infusion doses, as much as 100-fold, have shown efficacy which rely on robust in vivo expansion for effectiveness. (39,43)

Prospective trials conducted during the early pandemic stages support the role of adoptive T cell therapy. Studies using directly selected allogeneic VSTs are limited to case series. (44) A phase 1 study (n=9) demonstrated the feasibility and safety of using third-party adoptive central memory (CD45RA-depleted) T cells containing SARS-CoV-2-specific T cells for moderate to severe COVID-19. Subsequently, a phase 2 study (n=84) revealed that patients receiving 1x10^6/kg of CD45RA-memory T cells from unvaccinated donors with SARS-CoV-2-specific IFNg response showed accelerated lymphocyte recovery and met primary recovery outcomes. (25,45)

A phase 1/2 study showed favorable outcomes in patients receiving ex-vivo expanded SARS-CoV-2 specific T cells with standard care compared to standard care only (recovery rate 65% versus 38%, median recovery time 11 days versus not reached, respectively). In contrast to our study, this study
used higher doses of ex-vivo expanded VST and excluded immunocompromised patients and children on active T-depleting chemotherapies. (35)

In a descriptive series, four high-risk patients safely received partially HLA-matched third-party cryopreserved ex-vivo expanded SARS-CoV-2-specific T cells (ALVR109) along with other antiviral agents, resulting in detectable donor T cells, increased VSTs post-infusion, and recovery in three out of four patients. The BATIT study terminated early due to poor accrual. (28)

Clearance of virus is important from an individual and community perspective. Inability to clear virus could lead to lower respiratory tract infections and high mortality in the most severely immunocompromised hosts (such as R09). Prolonged COVID-19 infection may significantly delay intensive chemotherapy and increase the risk of a relapsing symptomatic infection (such as R04). In addition, chronic COVID-19 infection raises community concerns as prolonged viral replication in the context of an inadequate immune response facilitates the emergence of immune-pressure escape mutations. (46–49)

Despite mutations in the Spike protein affecting antibody neutralization efficacy, (16,17) most T cell epitopes remain fully conserved. (1) Spike-directed vaccination induces robust T cell responses against both wild-type and variant strains, making vaccinated individuals a convenient source of adoptive T cell donors. (50) SARS-CoV-2-specific T cells from vaccinated or hybrid immunity donors maintain functionality and specificity against ancestral SARS-CoV-2 antigens, yielding higher doses of IFN-γ+ CD3+ cells compared to convalescent donors.

In our study, vaccinated and hybrid donors, who received mRNA Spike-directed vaccines, displayed a higher CD4/CD8 proportion, potentially due to the vaccine’s specific immunogenicity. About 50% of the CD4+ T cell response to SARS-CoV-2 targets the spike protein, with the remainder directed towards other viral proteins. CD8+ T cells in convalescent patients show reactivity against a wider range of antigens, including the spike protein, N protein, and others. (51)

The IFN-γ+ cell product includes around 40% B cells, which differs from expanded VST cultures. (52) We postulate that IFN-γ-secreting B cells may be captured via CD45-IFN-γ Cytokine Capture System
during the manufacturing process, suggesting a need for further study. B-cell-driven autocrine feedback loops and innate responses could be advantageous in viral immune responses. (53–55)

Clinical trials using VST have used various methods of generating antigen-specific T cells, most of which yield a mixed population of CD4+ and CD8+ T cells, unless they are generated against a single CD8+-restricted epitope. The presence of both CD4 and CD8 T cells support persistence of VSTs in vivo (43), however, it is not clear whether differing proportions of CD4+/CD8+ T cells are associated with increased or decreased clinical efficacy.

In our third-party bank, we prioritize products with a shared HLA allele to ensure shared antiviral activity. This matching can involve either class I or class II, depending on the virus. For instance, adenovirus response relies on HLA class II matching, while CMV typically requires class I matching. (56) In COVID-19, both HLA I and II mediate the endogenous T cell response and correlate with immunodominant peptides. (44) Third-party VSTs generally match the recipient at less than 4 out of 10 HLA alleles; (57,58) therefore, only a fraction of the infused cells are likely able to kill recipient infected cells. Furthermore, it has been shown that patients who responded were more likely to be matched at the restricting alleles and that this is the critical determinant of response more than the overall degree of match per se. (59) Depending on the donor/recipient match at restricting alleles, the response of VST may differ considerably.

There are several limitations of the study conducted during the pandemic. The study did not include a randomized control group; however, healthy adult patients and paediatric oncology cohort receiving remdesivir alone during the same study period served as reference cohorts. (36,37) Comprehensive correlative studies were limited by stringent biosafety restrictions. Missing data from those who had been discharged to community step-down wards could potentially bias immune reconstitution data towards sicker inpatients beyond day 7. For ethical reasons, the study allowed adjuvant drugs and steroids in critically sick patients; consequently, the efficacy of adoptive T cell therapy could not be evaluated independently of other treatments, and was negatively impacted by the addition of steroids.
in the most severe cases. As the SARS-CoV2 variant evolved rapidly from the Delta to the Omicron strain, phase 2 recruitment stopped due to the milder course of Omicron. The small number of patients in the study did not allow conclusive recommendations for adoptive cell therapy.

**Future directions**

We postulate that ACT may be particularly useful in haematological or post-transplant patients who are profoundly T-cell depleted, especially if this was given as early as possible prior to the need for steroids. Larger studies incorporating a control group will be required to verify the clinical efficacy of COVID-T cells and define these conditions.

(3739 words)

**Data sharing statement**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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**Authorship Contributions**

Conceptualization W.L.; Methodology W.L, K.P.N., M.S.S; Formal Analysis W.L., K.P.N., M.S.S, L.Y.C; Investigation All Authors; Resources W.L., S.T.G, M.C, T.L.K.; Data Curation L.Y.C, K.P.N,
M.S.S, M.C; Writing M.S.S (first draft), W.L; Visualization M.S.S, K.P.N; Supervision W.L, L.Y.C;
Project Administration M.S.S; Funding Acquisition W.L, M.S.S
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Conflict of Interest Disclosures
W.L. is a part-time employee of Miltenyi Biotec. All authors declare no competing financial interests.

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