Phase I Trial of a Multi-Peptide COVID-19 Vaccine for the Induction of SARS-CoV-2 T-Cell Immunity

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Abstract

T-cell immunity is central for the control of viral infections. CoVac-1 is a peptide-based vaccine candidate, composed of SARS-CoV-2 T-cell epitopes derived from various viral proteins, combined with the toll-like receptor 1/2 agonist XS15 emulsified in Montanide™ ISA51 VG, aiming to induce superior SARS-CoV-2 T-cell immunity to combat COVID-19. We conducted a Phase I open-label trial, including 36 participants aged 18 to 80 years, who received one single subcutaneous CoVac-1 vaccination. The primary endpoint was safety analyzed until day 56. Immunogenicity in terms of CoVac-1-induced T-cell response was analyzed as main secondary endpoint until day 28. No serious adverse events and no grade 4 adverse events were observed. Expected local granuloma formation was observed in all study subjects, while systemic reactogenicity was absent or mild. SARS-CoV-2-specific T-cell responses targeting multiple vaccine peptides were induced in all study participants, mediated by multifunctional T-helper 1 CD4⁺ and CD8⁺ T cells. CoVac-1-induced interferon-γ T-cell responses by far surpassed those detected in COVID-19 convalescents and were unaffected by current SARS-CoV-2 variants of concern (VOC). Together, CoVac-1 showed a favorable safety profile and induced broad, potent, and VOC-independent T-cell responses, supporting the presently ongoing evaluation in a Phase II trial for patients with B-cell/antibody deficiency. Funded by the Ministry of Science, Research and the Arts Baden-Württemberg, Germany; ClinicalTrials.gov number, NCT04546841.

Main

The Coronavirus Disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is linked to the death of millions of people.^{3,4} As predominantly individuals with medical comorbidities are affected severely,⁵⁻⁷ vaccines inducing long-lasting immunity, particularly in those high-risk populations, are urgently needed.⁸⁻¹⁰

CoVac-1 is a peptide-based vaccine candidate composed of multiple dominant SARS-CoV-2 T-cell epitopes derived from various viral proteins (*e.g.* spike (spi), nucleocapsid (nuc), membrane (mem), envelope (env)) with proven relevance for T-cell immunity against COVID-19 and the ability to mediate long-term immunity after infection.^{1,2,11} Adjuvanted with the toll-like receptor (TLR) 1/2 agonist XS15¹² emulsified in Montanide™ ISA51 VG,^{13,14} CoVac-1 is intended to induce superior SARS-CoV-2 T-cell immunity, that is not affected by evolving viral variants of concern (VOC) after a single-dose vaccination.

T cells play an important role for COVID-19 outcome and maintenance of long-term SARS-CoV-2 immunity, even in complete absence of humoral immune responses.^{1,15-24} SARS-CoV-2 T-cell immunity thus is a central goal for vaccine development and of particular importance for patients with congenital or acquired B-cell deficiencies. The latter comprise cancer patients after and on B-cell-depleting therapies, as well as leukemia and lymphoma patients with disease-related immunoglobulin deficiency, who develop only limited humoral immunity after infection or vaccination and persist with a high risk for severe course of COVID-19.²⁵⁻²⁸

We here report the results of the primary endpoint (safety up to day 56) and the results of the efficacy (immune response up to day 28) of the open-label first-in-human Phase I trial involving adults aged 18-80 years, to evaluate safety, reactogenicity, and immunogenicity of CoVac-1.

Results

Participants

From November 28th, 2020 to January 15th, 2021, 12 healthy adults were enrolled in Part I (age group 18-55 years) including sentinel dosing in the first participant. From March 24th, 2021 to April 1st, 2021, 24 adults were enrolled in Part II (age group 56-80 years). All participants received one dose of CoVac-1 on day 1 and were available for immunogenicity and safety analyses until day 28 and day 56, respectively (Fig. 1, Extended Data Fig. 1). No major protocol violations occurred. Analyses of follow-up safety and long-term immunogenicity data of participants enrolled in study Part I and II are ongoing. Demographic and clinical characteristics of the participants are provided in Table 1.

Safety and reactogenicity

Data regarding both solicited and unsolicited adverse events (AEs) were available for all participants from returned diary cards and safety visits. No participant discontinued the trial because of an AE. No serious AEs occurred and no grade 4 AEs were reported. After vaccination, reactogenicity in terms of solicited AEs occurred in all participants (Fig. 1). Most of the events were mild to moderate (grade 1 to 2) in 81% of participants. All participants showed the expected formation of a granuloma or induration at the injection site, which persisted beyond day 56. Severe AEs (grade 3) comprised local erythema in 19% accompanied by severe swelling in 6% of all participants. 22% of participants reported localized inguinal lymphadenopathy. Local skin ulceration at the vaccination site was reported by 25% of participants, with two participants in Part II showing a grade 2 ulceration. No fever or other inflammatory systemic solicited AEs were reported. Other systemic solicited AEs occurred in 39% of all participants and differed between Part I and II (42% vs. 38%). All reported systemic solicited AEs were mild, with transient fatigue being reported by 31% of participants.

No clinically relevant changes in laboratory values were reported during the conduct of the trial. In 31% of participants, acute phase reaction with elevated C-reactive protein was observed.

In total, 58 unsolicited AEs occurred which were predominantly mild (81%, Extended Data Table 1). Viral re-activations (Varicella-Zoster and Herpes Simplex virus) were reported by two participants ($\leq$ grade 2) in Part II of the trial.

Until day 56, no SARS-CoV-2 infection or immune-mediated medical condition were observed in any participant.

Immunogenicity

Immunogenicity of CoVac-1 was determined in terms of CD4⁺ and CD8⁺ T-cell responses to the six SARS-CoV-2 human leukocyte antigen (HLA)-DR vaccine T-cell epitopes as well as to embedded HLA class I peptides, respectively (Supplementary Table S1). T-cell responses were assessed in all participants (Part I and II) at baseline (day 1), as well as on day 7, day 14, and day 28 after CoVac-1 vaccination. None of the participants showed preexisting SARS-CoV-2 T-cell responses *ex vivo* at baseline. Vaccine-induced interferon (IFN)-g T-cell responses were observed in 100% participants in Part I and Part II on day 28, showing an increase of ≥ 200 -fold and 100-fold (median of calculated spot counts per 500,000 cells) from baseline, respectively (Fig. 2A). Vaccine-induced T-cell responses targeted multiple CoVac-1 peptides with a median of 5/6 peptides recognized by participants' T cells on day 28 (Fig. 2B, Extended Data Fig. 2). The CoVac-1 peptide P6_ORF8 derived from the open-reading frame (ORF) 8 of SARS-CoV-2 showed most frequently induced T-cell responses after vaccination (97%), followed by P5_mem and P4_env (both 94%), P3_spi (89%), P1_nuc (61%), and P2_nuc (53%, Extended Data Fig. 2). Intensity of CoVac-1-induced IFN-g T-cell responses in participants of Part I and Part II (median 488) was up to 38 times higher compared to T-cell responses against CoVac-1 vaccine peptides (median 13) as well as to previously described SARS-CoV-2-specific (median 29) and cross-reactive (median 35) T-cell epitopes^{1,2} in age-matched human COVID-19 convalescents HCs (Fig. 2C). *In vitro* expansion of CoVac-1-specific T cells revealed preexisting low-frequency T-cell responses to single vaccine peptides at baseline in 61% participants of Part I and Part II that could be boosted at least 2-fold by CoVac-1, as observed on day 28 in all but one participant (Extended Data Fig. 3).

CoVac-1-induced CD4⁺ T cells displayed a multifunctional T helper 1 (Th1) phenotype with positivity for IFN-g, tumor necrosis factor (TNF), interleukin-2 (IL-2), and CD107a (Fig. 2D). Magnitude of CoVac-1-induced CD4⁺ T-cell responses did not differ between Part I and Part II participants and was up to 40 times higher compared to SARS-CoV-2-specific CD4⁺ T-cell responses of HCs (Extended Data Fig. 4A). Frequency of functional CD4⁺ T cells could be increased up to 40 times after *in vitro* T-cell expansion, indicating potent expandability of CoVac-1-induced T cells upon SARS-CoV-2 exposure (Extended Data Fig. 4B).

Vaccine-induced CD8⁺ T-cell responses, as identified after *in vitro* expansion by tetramer staining and IFN-g enzyme-linked immunospot (ELISPOT) with HLA-matched, CoVac-1-embedded, HLA class I peptides (Supplementary Table S1) were detected in 78% and 80% of participants in Part I and 100% and 95% of participants in Part II with matching HLA allotypes, respectively (Extended Data Fig. 5A, B). CoVac-1-induced CD8⁺ T cells showed a polyfunctional phenotype reflected by IFN-g, TNF, IL-2, and CD107a production/expression upon peptide stimulation (Extended Data Fig. 5C).

Impact of SARS-CoV-2 variants on CoVac-1 vaccine candidate

Analysis of the impact of SARS-CoV-2 VOC declared by the World Health Organization as of 1st July, 2021 (B.1.1.7-Alpha, B.1.351-Beta, P.1-Gamma, B.1.617.2-Delta) on CoVac-1 revealed that 50% vaccine peptides sequences were not affected by any variant-defining or -associated mutations²⁹⁻³³ (Supplementary Table

S2). None of the mutations of the variants P.1-Gamma and B.1.617.2-Delta affect any of the CoVac-1 vaccine peptides. Variant B.1.1.7-Alpha comprises two mutations affecting P2_nuc and P6_ORF8 with a single amino acid change, respectively. Two mutations of variant B.1.351-Beta affect P3_spi with either one or two amino acid changes (Fig. 3A).

IFN- γ T-cell responses to peptide pools comprising the B.1.1.7 and B.1.351 mutated peptides P2_nuc, P3_spi, and P6_ORF8 were detectable in 100% of participants of Part I and Part II with proven CoVac-1-induced T-cell responses to P2_nuc, P3_spi, and P6_ORF8 wild-type (WT) peptides (Fig. 3B). Albeit, intensity of T-cell responses to single peptide variants (P3_spi and P6_ORF8) was reduced compared to WT peptides, intensity of CoVac-1-induced T-cell responses targeting the variant peptide pools was unaffected and at least 10-fold higher than memory T-cell responses to WT and variant peptide pools observed in HCs (Fig. 3C, Extended Data Fig. 6).

Discussion

This analysis of our Phase I trial shows that the CoVac-1 vaccine candidate has a favorable safety profile and induces potent T-cell responses after one single vaccination. As expected and intended, local granuloma formation was observed in all study subjects. These granulomas persisted throughout day 56 and represent a solicited and intended local reaction after Montanide-based vaccination,^{13,14,34} enabling continuous local stimulation of SARS-CoV-2-specific T cells. This is required for induction of long-lasting T-cell responses without systemic inflammation which is frequently reported for messenger-RNA- and vector-based vaccines.⁸⁻¹⁰

The CoVac-1 vaccine candidate is based on T-cell epitopes chosen to induce broad and potent T-cell immunity to SARS-CoV-2 including long-lasting memory. This is supported by data in SARS-CoV-1 convalescents, where T-cell immunity persisted for up to 17 years.²¹ All study subjects displayed CoVac-1-specific T-cell responses and robust release of multiple cytokines, including IFN- γ , IL-2, and TNF. CoVac-1-induced Th1 CD4⁺ T-cell responses are complemented by multifunctional CD8⁺ T cells, counteracting the theoretical risk of vaccine-associated enhanced respiratory disease, which has been associated with a T helper 2 (Th2)-driven cellular immune response.³⁵⁻³⁷ In contrast to other COVID-19 vaccines,³⁸ frequencies of CoVac-1-induced T-cell responses were comparable for younger and older adults and even showed an increased frequency for CD8⁺ T cells in the older participants of Part II. The phenotype of CoVac-1-induced T cells resembles that acquired upon natural infection,^{1,2,15,21} but with a higher magnitude as compared to the SARS-CoV-2 T-cell response in human COVID-19 convalescents. High interindividual heterogeneity, as well as the lack of standards and utilization of different assays complicates comparisons between T-cell responses induced by the different vaccines.³⁹⁻⁴² Nevertheless, the high frequency of T-cell responses as well as the magnitude of functional CD4⁺ T cells with up to 10% SARS-CoV-2-specific T cells detectable *ex vivo* suggest that CoVac-1 induces a superior T-cell immunity. This is further supported by the high diversity of CoVac-1-induced T cells, which target multiple

vaccine peptides derived from different viral proteins, which is of high relevance for anti-viral defense and disease outcome in viral infections including SARS-CoV-2.^{1,11,43-45} Notably, the broad T-cell response induced by CoVac-1 remains unaffected by current SARS-CoV-2 VOC. This is of particular importance since mutations of the VOC are associated with loss of neutralizing antibody capacity in human COVID-19 convalescents and after vaccination.⁴⁶⁻⁴⁸

Although the effector mechanisms that mediate protection after SARS-CoV-2 vaccination have yet to be fully elucidated, it is undisputed that neutralizing antibodies provide the first line of anti-viral defense. T cell-mediated immunity and in particular CD4⁺ T cells are indispensable for the generation of such protective antibody responses but not limited to it.⁴⁹⁻⁵³ Yet, CD4⁺ T cells are also required to reinforce CD8⁺ T-cell responses⁵⁴⁻⁵⁷ and further act directly as effector cells by degranulation and killing of virus-infected cells.⁵⁸⁻⁶² The relevance of anti-viral T-cell responses during acute infection and for long-term immunity was also proven specifically for SARS-CoV-2^{1,2,17-24}: Early detection of SARS-CoV-2-specific CD4⁺ T-cell responses correlates with mild course of COVID-19^{17,19}, and CD4⁺ T-cell frequencies negatively correlate with viral RNA loads.¹⁷ Moreover, cases of asymptomatic exposure to SARS-CoV-2, as well as reports from patients with congenital B-cell deficiency document occurrence of SARS-CoV-2-specific cellular immune responses without seroconversion, providing evidence for the relevance of T-cell immunity in disease control even in the absence of neutralizing antibodies.^{1,18,63} Accordingly, CoVac-1 may well serve as a (complementary) vaccine candidate to induce T-cell immunity, particularly in elderly and immunocompromised individuals with impaired ability to mount sufficient immune responses after SARS-CoV-2 vaccination with the currently approved vaccines.^{25,26}

Limitations of our trial include the small sample size as well as low ethnic diversity. Further safety and immunogenicity data over a longer observation period are presently being collected during the study's follow-up where participants are monitored for 6 months.

In conclusion, the safety and immunogenicity results of this trial indicate that CoVac-1 is a promising multi-peptide vaccine candidate for induction of superior SARS-CoV-2 T-cell immunity, which builds the basis for a presently ongoing Phase II study evaluating CoVac-1 in patients with congenital or acquired B-cell deficiency, including cancer patients after B-cell-depleting therapy and disease-related immunoglobulin deficiency (NCT04954469).

Methods

Trial design and oversight

The Phase I trial was designed by and conducted at the Clinical Collaboration Unit (CCU) Translational Immunology, University Hospital Tübingen, Germany. Men as well as nonpregnant women aged 18 to 55 years, without any relevant preexisting conditions and adults aged 56 to 80 years with stable medical conditions were included in Part I and II of the study, respectively. A detailed description of the inclusion and exclusion criteria can be found in the Supplement. Health status was based on medical history and

clinical laboratory values, vital signs, and physical examination at screening. Participants with proven history of SARS-CoV-2 infection (real-time polymerase chain reaction (PCR) or antibody test) were excluded. Prior to enrollment, all participants provided their written informed consent. As safety measure, sentinel dosing of the first participant treated in Part I was conducted. The trial was open-label without control arm.

The trial was funded by the Ministry of Science, Research and the Arts Baden-Württemberg, Germany. The trial was approved by the Ethics Committee, University Tübingen (537/2020AMG1) and the Paul Ehrlich Institute and performed in accordance with the International Council for Harmonization Good Clinical Practice guidelines.

Safety assessment to proceed to Part II was performed by an independent data safety monitoring board.

Trial vaccine and adjuvant

CoVac-1, developed and produced by the Good Manufacturing Practices (GMP) Peptide Laboratory of the Department of Immunology, University Tübingen, is a peptide-based vaccine comprising six promiscuous HLA-DR-restricted SARS-CoV-2 peptides derived from various SARS-CoV-2 proteins and the adjuvant lipopeptide synthetic TLR1/2 ligand XS15¹² (manufactured by Bachem AG, Bubendorf, Switzerland) emulsified in Montanide^{TM} ISA51 VG (manufactured by Seppic, Paris, France).¹³ CoVac-1 peptides represent dominant SARS-CoV-2 T-cell epitopes validated in human convalescents after SARS-CoV-2 infection to mediate long-term immunity.^{1,2} CoVac-1 HLA-DR T-cell epitopes contain embedded HLA class I sequences for induction of both, CD4⁺ and CD8⁺ T-cell responses (Supplementary Table S1). The linear 15-amino acid peptides are characterized by a free N-terminal amino group and a free C-terminal carboxy group. All amino acid residues are in the L-configuration and not chemically modified at any position. Synthetic peptides were manufactured by established solid phase peptide synthesis procedures using Fmoc chemistry.^{64,65} The adjuvant XS15 hydrochloride is a synthetic linear, 9-amino acid peptide with a palmitoylated N-terminus (Pam₃Cys-GDPKHPKSF). CoVac-1 peptides (250 µg/peptide) and XS15 (50 µg) are prepared as a water-oil emulsion 1:1 with Montanide^{TM} ISA51 VG to yield an injectable volume of 500 µL. Each participant received one subcutaneous injection of the vaccine at the lower abdomen on day 1.

Safety assessment

Primary safety outcomes reflect the nature, frequency, and severity of solicited AEs until day 56 after vaccination. The documentation was facilitated by use of a volunteer diary and graded by the investigators according to a modified Common Terminology Criteria for Adverse Events (CTCAE) V5.0 grading scale (Supplementary Table S3). In addition, the number and percentage of participants with unsolicited events until day 56 were reported (documented according to CTCAE V5.0). Safety assessment included clinically significant changes in laboratory values (hematology and blood chemistry), serious adverse events (SAE), and adverse events of special interest (AESI), which included desired

induration/granuloma formation, SARS-CoV-2 infection, COVID-19 manifestations, and immune-mediated medical conditions (Supplementary Tables S4 and S5).

Immunogenicity assessment

Secondary efficacy outcome was the induction of CoVac-1-specific T-cell responses to at least one of the CoVac-1 vaccine peptides evaluated on day 7, day 14, and day 28 by IFN-g ELISPOT assay *ex vivo* and after *in vitro* T-cell expansion (baseline day 1, prior to vaccination). T-cell responses were considered positive if the mean spot count was ≥ 3 -fold higher than the mean spot count of the negative control and defined as CoVac-1-induced if the mean spot count post vaccination was ≥ 2 -fold higher than the respective spot count on day 1. CoVac-1-induced T-cell responses were further characterized using cell surface marker, intracellular cytokine, and tetramer staining. The gating strategy applied for the analyses of flow cytometry-acquired data is provided in Supplementary Fig. S1. Immunogenicity results were compared with HCs with PCR-confirmed SARS-CoV-2 infection (Supplementary Table S6). All assays were conducted in a blinded fashion and are described in detail in the Supplement.

Statistical analysis

The sample size calculation (36 participants) of the trial was based on the assumption that incidence of SAE associated with administration of CoVac-1 does not exceed a predetermined rate of 5%. Safety data are displayed by counting the respective AE that has occurred at least once in a patient. The highest grading of this AE is indicated. Details regarding the statistical analysis plan and sample size calculation are provided in the Supplement and the protocol.

Declarations

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Author Contributions

J.S.H., H.-G.R., H.R.S., and J.S.W. were involved in the design of the overall study and strategy. C.M., H.-G.R., I.F., and M.W.L. provided feedback on the study design. T.B., C.T., A.N., Y.M., M.Ro., J.B., J.Ri., and M.W. performed the immunogenicity analyses. J.S.H., M.M., J.Re., S.J., L.A., L.M.W, S.H., A.P., R.K., and J.S.W. conducted patient data and sample collection as well as medical evaluation and analysis. J.S.H., M.M., J.Re., S.J., H.R.S., and J.S.W. collected data as study investigators. C.M. and I.F. developed the statistical design and oversaw the data analysis. M.D. and M.Ri. conducted GMP production of CoVac-1. J.S.H., T.B., C.T., M.W.L., H.R.S., and J.S.W. drafted the manuscript. All authors supported the review of the manuscript.

Competing Interests

A.N., T.B., H.-G.R., and J.S.W. are listed as inventors on a patent related to the SARS-CoV-2 T cell epitopes included in CoVac-1. H.-G.R. is listed as inventor on a patent related to the adjuvant XS15 included in CoVac-1. The other authors declare no competing interests.

Data Availability Statement

Further supporting data related to the findings of this study are available upon reasonable request to the corresponding author J.S.W.

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Tables

Table 1: Participants' characteristics

Characteristics	All	Part I	Part II
Participants – n	36	12	24
Age – years			
Median	59.5	38	62.0
Range	23 - 70	23 - 50	56 - 70
Sex – n (%)			
Female	16 (44)	4 (33)	12 (50)
Male	20 (56)	8 (67)	12 (50)
Race – n (%)			
White	36 (100)	12 (100)	24 (100)
Other	0 (0)	0 (0)	0 (0)
Body-mass index (BMI)*			
Median	24.4	24.9	24.4
Range	18.5 - 30.1	20.1 - 30.1	18.5 - 29.3
Relevant preexisting disease [‡] – n (%)			
Hypertension	6 (17)	0 (0)	6 (25)
Previous malignant disease	1 (3)	0 (0)	1 (4)
Mild psoriasis	1 (3)	0 (0)	1 (4)

Abbreviations: n, number. * the body-mass index (BMI) is calculated by the weight in kilograms divided by the square of the height in meters. Assessment was done at the time of screening; [‡] Relevant preexisting disease includes conditions with increased risk of severe COVID-19 and with higher risk for side effects due to CoVac-1.

Figures

Figure 1

Local and systemic solicited adverse events. Related (A) local and (B) systemic solicited AEs occurring within 56 days after vaccination. Severity was graded as mild (grade 1), moderate (grade 2), or severe (grade 3) based on the definition provided in the methods section. Healthy adults of 18-55 years were included in Part I, and participants of 56-80 years were included in Part II.

Figure 2

CoVac-1-induced T-cell responses. CoVac-1-induced T-cell responses were assessed ex vivo by IFN- γ ELISPOT assays using PBMCs from study participants of Part I and II collected before vaccination (day 1) and at different time points after vaccination (day 7, day 14, day 28). (A) The intensity of T-cell responses is depicted as calculated spot counts (mean spot count of replicates normalized to 500,000 cells minus the normalized mean spot count of the respective negative control). (B) Number of CoVac-1 T-

cell epitopes (n = 6) per participant that elicited a vaccine-induced T-cell response. (C) Intensities of CoVac-1-induced IFN- γ T-cell responses assessed ex vivo in study participants of Part I and Part II (pCoVs, n = 24, day 28, left y-axis) were compared to T-cell responses detected in human convalescents (HCs, right y-axis) after SARS-CoV-2 infection against CoVac-1 vaccine peptides and previously published^{1,2} SARS-CoV-2-specific (spec) and cross-reactive (cross) T-cell epitope compositions (ECs, CoVac-1 n = 24, cross EC n = 27, spec EC n = 26). (D) Frequencies of functional CoVac-1-specific CD4⁺ T cells in study participants prior to vaccination (day 1) were compared to day 28 following vaccination. Functionality of CD4⁺ T cells was assessed for upregulation of the degranulation marker CD107a and for production of the Th1 cytokines IFN- γ , TNF, and IL 2. The right graph displays the proportion of samples revealing difunctional (2), trifunctional (3), or tetrafunctional (4) T cells. Box plots or combined box-line plots show median with 25th or 75th percentiles, and min/max whiskers.

Figure 3

Role of SARS-CoV-2 variants of concern (VOC) on CoVac-1 peptides and immunogenicity. (A) Color-coded mutations described for VOC are shown together with corresponding affected CoVac-1 peptides. (B,C) Intensities of T cell responses (calculated spot counts) to CoVac 1 peptides as well as to the corresponding peptide pools comprising the CoVac-1-affecting mutations of B.1.1.7 and B.1.351 were assessed ex vivo by IFN- γ ELISPOT assays using PBMCs from (B) study participants of Part I and Part II collected on day 28 after vaccination (pCoVs) or from (C) human COVID-19 convalescents (HCs).

Supplementary Files

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