

Vaccinated and convalescent donor-derived SARS-CoV-2-specific T cells as adoptive immunotherapy for high-risk COVID-19 patients

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Summary

Polyclonal SARS-CoV-2-specific T cell products with a safe and strong cytotoxic profile against SARS-CoV-2-presenting targets, as well as SARS-CoV-2 variants, can be generated from both COVID-19 convalescent or vaccinated donors to be used as adoptive therapy of high-risk patients

Abstract

Background. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic poses an urgent need for the development of effective therapies for Coronavirus Disease 2019 (COVID-19). **Methods.** We first tested SARS-CoV-2-specific T-cell (CoV-2-ST) immunity and expansion in unexposed donors, COVID-19 infected individuals (convalescent), asymptomatic PCR-positive subjects, vaccinated individuals, non-ICU hospitalized patients and ICU patients who either recovered and were discharged (ICU recovered) or had a prolonged stay and/or died (ICU critical). CoV-2-STs were generated from all types of donors and underwent phenotypic and functional assessment. **Results.** We demonstrate causal relationship between the expansion of endogenous CoV-2-STs and the disease outcome; insufficient expansion of circulating CoV-2-STs, identified hospitalized patients at high-risk for an adverse outcome. CoV-2-STs with a similarly functional and non-alloreactive, albeit highly cytotoxic, profile against SARS-CoV-2 could be expanded from both convalescent and vaccinated donors generating clinical-scale, SARS-CoV-2-specific T-cell products with functional activity against both the unmutated virus and its B.1.1.7 variant. In contrast, critical COVID-19 patient-originating CoV-2-STs failed to expand, recapitulating the in vivo failure of CoV-2-specific T-cell immunity to control the infection. CoV-2-STs generated from asymptomatic PCR+ individuals presented only weak responses whereas their counterparts originating from exposed to other

seasonal coronaviruses subjects failed to kill the virus, thus disempowering the hypothesis of protective cross-immunity. **Conclusions.** Overall, we provide evidence on risk stratification of hospitalized COVID-19 patients and the feasibility of generating powerful CoV-2-ST products from both convalescent and vaccinated donors as an “off-the shelf” T-cell immunotherapy for high-risk patients.

Keywords: coronavirus 2, COVID-19, T cell responses, adoptive immunotherapy, virus-specific T cells

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Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to the devastating outbreak of Coronavirus Disease 2019 (COVID-19) pandemic. Despite that several repurposed or novel agents have been evaluated as COVID-19 treatment, no proven therapeutic strategy exists.[1]

Similar to the essential role in viral clearance of the related virus SARS-CoV T-cells shown to persist for >10years after exposure,[2] T-cell responses play a significant role in recovering from SARS-CoV-2.[3,4] The power of T-cells is clearly emphasized in the transplant setting, where the adoptive transfer of graft- or third-party donor-derived virus-specific T-cells (VSTs) into immunocompromised recipients, successfully controls adenovirus, cytomegalovirus, Epstein-Barr virus, BK virus, JC virus, human herpesvirus-6, conferring only minimal risk of GvHD.[5–8] VSTs, ex vivo expanded from seropositive donors, and targeting multiple viral antigens and a plethora of epitopes via stimulation with overlapping peptides, provide the benefits of strong cytotoxic potential and minimization of immune evasion by viral mutants.

We here provide the rationale towards the development of a SARS-CoV-2-specific T-cell (CoV-2-ST) bank from convalescent donors as T-cell immunotherapy against severe COVID-19. Since it is still unclear whether vaccination will provide a similar to natural SARS-CoV-2 infection T-cell “training”, extending also to emerging variants, we compared convalescent donor-derived CoV-2-STs (Conv-CoV-2-STs) with vaccinated donor-derived CoV-2-STs (Vac-CoV-2-STs) as regards phenotype and functionality, against both the unmutated virus and the British B.1.1.7 variant.

Results

SARS-CoV-2 boosts long-lasting T-cell immunity

ICU-recovered or critical individuals exhibited profound T-cell lymphopenia over unexposed and convalescent donors and marginally more severe over non-ICU patients, suggesting that T-cell numbers inversely correlate with disease severity (Pearson's $r=-0.6163$; **Figure 1A-B**). Circulating T-lymphocytes of critical ICU-patients were functionally impaired, demonstrating lower activation, higher levels of exhaustion and a varying differentiation status, with decreased memory and naïve subpopulations and elevated percentages of terminally differentiated effector T-cells (TEMRA), than unexposed- or convalescent-donor derived T-cells (**Figure S1**). Strong SARS-CoV-2-specific responses were observed in convalescent donors against both NCAP and spike antigens, suggesting that COVID-19 boosts T-cell immunity. Previous exposure to other seasonal coronaviruses could interpret that 3/16 and 8/15 unexposed donors elicited negligible/low to moderate SARS-CoV-2-specific responses against Spike and NCAP (**Figure 1C-D**), respectively. Irrespective of the varying magnitude of T-cell immunity after natural infection, responses against spike were abundant over NCAP (**Figure S2**). Expectedly, vaccinated donors with BNT162b2 encoding the spike protein, showed almost exclusively, circulating spike-specific CoV-2-STs (**Figure 1E**). CoV-2-STs persisted for at least 8 months post-infection in the majority of convalescents, in whom however, spike-specific IgG showed clear reduction over time (**Figure 1F**).

T-cell responses and clinical outcome

Following the temporal evolution of CoV-2-STs for up to 2 weeks post-admission, we observed that the majority of patients unable to expand their CoV-2-STs in vivo, failed to control the infection and either had a prolonged/complicated ICU stay or succumbed (ICU-critical), whereas patients with CoV-2-ST rebounds, cleared the infection and were discharged (non-ICU and ICU-recovered) (**Figure 2A-C**). The latter presented significant expansion of CoV-2-T-cell immunity over baseline as opposed to ICU-critical patients (**Figure 2D-E**).

The magnitude of CoV-2-ST expansion two weeks post admission (Δ SFCs), rather than baseline CoV-2-STs, was predictive of the patient outcome by receiver operating characteristics (ROC) curve analysis; a threshold of Δ SFC $>$ 35 and Δ SFC $>$ 101 of IFN- γ - and TNF- α -secreting CoV-2-STs, respectively, could predict with high sensitivity and specificity a favorable outcome (**Figure 2F-G;S3;Table S2**), suggesting that the magnitude of CoV-2-ST expansion could serve as a risk stratification tool.

Generation of CoV-2-STs from SARS-CoV-2-convalescent, -vaccinated or -unexposed donors

To generate CoV-2-STs for adoptive immunotherapy (AI), donor PBMCs were stimulated with pepmixes spanning NCAP and spike antigens and cultured as described [5,6,9,10]. Convalescent or vaccinated donor-derived T-cells robustly expanded upon antigen exposure, providing multiple clinical-scale doses per T-cell

product whereas virus-naïve- or asymptomatic donor-derived CoV-2-STs had considerably lower expansion (**Figure 3A-B**).

Characterization of CoV-2-ST products

Convalescent- or vaccinated-donor-derived CoV-2-STs were predominantly CD4+ but also CD8+ T-cells, expressing memory and only at a minimum regulatory T-cell markers (**Figure 3C**) and presenting an activated and non-exhausted profile (**Figure 3D-E**). After re-exposure to initial stimuli, Conv-CoV-2-ST-products showed robust specificity against both targeted antigens, with dominant responses against spike (**Figure 4A-B,S4**). Not unexpectedly, Vac-CoV-2-STs induced strong specificity only against spike, similar to Conv-CoV-2-ST spike specificity, while uninfected or asymptomatic donor-derived CoV-2-ST-products presented significantly milder, albeit specific, responses. Notably, the specificity of same donor Conv-CoV-2-STs, at convalescence (2 months) and post-convalescence (8 months), was almost identical (**Figure S5**), further supporting persistent SARS-CoV-2 T-cell immunity.

To further functionally characterize CoV-2-STs, a cytotoxicity assay against autologous antigen-pulsed PHA blasts was performed in representative products. Convalescent- and vaccinated-donor cell products induced strong, specific and comparable lysis of SARS-CoV-2-pulsed PHA blasts whereas they were non-cytolytic against irrelevant(influenza)-pulsed PHA blasts. Expectedly, NCAP-pulsed lysis was induced only by Conv-CoV-2-STs(**Figure 4C**). Interestingly, unexposed or asymptomatic donor-derived CoV-2-STs, although specific, were non- or barely cytotoxic, respectively. SARS-CoV-2 functional responses of CoV-2-ST products were mapped by HLA-restricted viral epitopes (**Table S3, Figure S7**)

To recapitulate the in vivo performance of CoV-2-STs in severe COVID-19 patients, cell products were also representatively produced from two patients, ICU-1 with a dismal outcome and ICU-2 who recovered. Although either CoV-2-ST-product was specific and cytotoxic against SARS-CoV-2-pulsed autologous PHA-blasts, only ICU-2-COV-2-STs could proliferate upon specific stimulation without expressing exhaustion markers (**Figure S6**), thus confirming the in-vivo inability of critical patients to expand T-cells and control the infection.

Alloreactivity would be an important safety issue in the present context. Coculture of convalescent- or vaccinated-donor-CoV-2-STs with allogeneic PHA-blasts, resulted in very low cell lysis (**Figure 4C**), underlying the specific-only cytotoxic potential of CoV-2-ST-products.

Conv- and vac-CoV-2-STs against B.1.1.7 variant

To address the efficacy of CoV-2-ST-products against the British B.1.1.7-SARS-CoV-2 variant, generated cells were pulsed with mutated virus peptides. Both conv- and vac-CoV-2-STs presented robust IFN- γ and TNF- α responses and strong cytotoxicity against the B.1.1.7-pulsed- and the unmutated-SARS-CoV-2-cell targets(**Figure 4D-E**), implicating effective cytotoxic potential in vivo against the virus and its variant.

Discussion

As the mortality due to COVID-19 continues to rise, developing therapeutic modalities against SARS-CoV-2 remains mandatory. Reasonably, vaccination has generated great optimism,[11–14] however, specific and effective therapeutic approaches are lacking. Even after herd immunity is achieved, vaccine breakthrough cases will exist, emerging mutations may escape antibody binding, vaccine-deniers will be vulnerable and immunocompromised patients always at risk for severe COVID-19.

Based on the safety and the high response rates of post-transplant adoptive immunotherapy (AI) with donor- or third-party-derived VSTs against, most commonly, the human herpes viruses (HHVs) family,[5–8] and by leveraging a previous protocol to generate multi-virus-, [5,6] *Aspergillus fumigatus*-[9] and multi-pathogen-specific T-cells,[10,15] we explored the possibility of producing CoV-2-STs from COVID-19 convalescent donors and, for first time, BNT162b2-vaccinated donors. Furthermore, by monitoring the endogenous CoV-2-ST kinetics, we could identify appropriate candidates for T-cell immunotherapy, i.e. patients at high risk for an adverse outcome.

We here confirmed the observed lymphopenia [16,17] in severely affected patients and the skewing of surviving T cells towards an ineffective differentiation status of exhausted or/and terminally differentiated cells, at the expense of functional memory and naïve subpopulations.

T-cell immunity plays a major role in COVID-19 resolution,[18] but whether protective memory provides long-lasting immunity as with the related SARS-CoV,[2] is still

unclear. By contrast, there is increasing evidence that antibody-based immunity wanes over time.[19,20] We here, further supporting recent findings,[21] demonstrate that the majority of recovered donors maintained SARS-CoV-2 T-cell responses for ≥ 8 months post-infection, suggesting that this branch of immunity is not compromised whereas decreasing antibodies in the same donors, implicated a rather short-lived humoral immunity.[19,22] It remains to be proven however, whether T-cell immunity post natural infection or vaccination could protect from re-infection long-term.

A major finding in our study was the association of immune features with disease outcome. Non-ICU or ICU-recovered patients developed high magnitude SARS-CoV-2 T-cell expansion, in contrast to ICU-critical patients who failed to expand CoV-2-STs and in their majority, succumbed. The kinetics and breadth of SARS-CoV-2 T-cell response over time could predict outcome. Indeed, ROC analysis based on the expansion of endogenous CoV-2-STs -rather than baseline CoV-2-STs- defined threshold levels predicting outcome. Thieme et al.[23] reported that critical COVID-19 patients elicited powerful SARS-CoV-2 T-cell responses not associated with virus clearance. This observation based on the mean frequency of CoV-2-STs in measured samples at different time-points, from our point of view, cannot mirror the magnitude of SARS-CoV-2 T-cell response. In our study, the expansion of endogenous CoV-2-STs was a major indicator of a favorable outcome, discriminating patients with solid immunity from those having low probability to recover. The in vivo performance of endogenous CoV-2-STs was also correlated with their failure or success to expand ex vivo and an exhausted or active phenotype, respectively. T-cell exhaustion implies that checkpoint inhibitors could reverse the CoV-2-ST anergic phenotype to a functional one and be used as a

COVID-19 therapeutic approach. However, several studies on the outcomes of cancer- or non-cancer COVID-19 patients receiving checkpoint inhibitors, remain controversial and inconclusive.[24,25]

Given that no curative therapy exists for COVID-19, adoptive transfer of immunity has emerged as a promising alternative. In this context, convalescent plasma did not reduce mortality over placebo [26] probably reflecting the inherent heterogeneity of plasma therapy providing different immune signatures, and the waning antibody-mediated immunity in convalescent plasma donors.[20]

We here pursued the generation of convalescent and vaccinated donor-derived CoV-2-STs as a feasibility study for future establishment of a CoV-2-ST-cell bank and adoptive transfer of T-cell immunity. Unlike plasma, in which antibodies concentration decreases post-infusion, memory CoV-2-STs expand and proliferate proving a longer-lasting effect.

Conv-CoV-2-STs presented a polyclonal mixture with dominant CD4+ immune signatures and a memory, non-exhaustion phenotype,[27,28] while exerted specific and strong cytolytic activity against SARS-CoV-2 without inducing alloreactivity, thus implicating in-vivo efficacy and safety. Moreover, we report for first time, the feasibility of generating vac-CoV-2-STs sharing similar functional features with conv-CoV-2-STs. Expectedly, due to active immunization with the spike-encoding vaccine, vac-CoV-2-STs presented single-antigen specificity, albeit comparable cytotoxicity to conv-CoV-2-STs. Consequently, vaccinated individuals apart from being potentially protected from future re-infection, may also serve as donors for AI. Given however, that infected subjects are exposed to a plethora of SARS-CoV-2 antigens whereas BNT162b2-vaccinated donors only to spike, conv-CoV-2-STs may, at least

theoretically, have greater potential to conquer immune escape mutations over active immunization or AI with vac-CoV-2-STs. Nevertheless, at least for the British variant, both con- or vac-CoV-2-STs presented strong specificity and cytotoxicity, demonstrating that both natural infection and BNT162b2 vaccination bypass the British mutation. We also investigated whether CoV-2-ST-products could be generated from asymptomatic or unexposed individuals harboring small quantities of circulating CoV-2-STs, presumably as response to other endemic coronaviruses.[4,29,30] Asymptomatic and unexposed donor-derived CoV-2-STs over conv-CoV-2-STs, were expanded but at lower frequencies, secreted moderate to low levels of cytokines upon antigen encounter and importantly, presented moderate or none cytotoxicity, respectively. Despite the limited number of asymptomatic individuals, the observed lower cytotoxic potential of asymptomatic donor-derived CoV-2-STs over conv-CoV-2-STs, suggested a weaker overall immune response in this cohort.[31,32] Moreover, the lack of cytotoxicity of unexposed donor-derived CoV-2-STs, strongly opposes the hypothesis based on studies assessing specificity only, that pre-existing cross-reactive immunity against endemic corona-viruses may provide protection against COVID-19.[27,33]

SARS-CoV-2 is a new virus to humanity which so far, does not seem to evolve to latency; several cases initially thought as reactivations were re-infection from another virus version or fluctuating lab results around the detection threshold. However, in immunocompromised patients, prolonged viral shedding has been described.

The rationale of investigating AI as a COVID-19 therapeutic approach, out of the transplantation context, is challenging, albeit highly justified. Even if SARS-CoV-2 does not perform as a latent virus, the COVID-19-associated lymphopenia will

provide a permissive microenvironment and a “therapeutic window” for the retention and expansion of infused, partially HLA-matched, CoV-2-STs and consequently, virus elimination. Eventually, the patient’s recovered immune system will reject the partially-matched CoV-2-STs at a time however, that their presence may not be necessary. Importantly, hematopoietic cell transplanted patients infused with recovered or vaccinated graft donor-derived CoV-2-STs will maintain long-term protection against SARS-CoV-2 reactivation/re-infection. The different tropism between HHVs and SARS-CoV-2 may also create scepticism for T-cell immunotherapy against COVID-19. SARS-CoV-2 although prevalent in the lung epithelium, usually leads to viremia and systemic invasion as[34] it occurs with HHVs and has been described for other respiratory viruses[35][36]. Despite different kinetics and cellular receptors, both latent HHVs and SARS-CoV-2 show broad and similar organotropism including the lung, intestine, kidneys, liver, heart and immune-privileged territories, like the brain.[37–39] VSTs accumulate at organ sites of virus-induced inflammation controlling disease (colitis, hemorrhagic cystitis/nephritis, lymphoma,pneumonia), even crossing sanctuary sites (progressive multifocal leukoencephalopathy, encephalitis, retinitis).[5,6,40,41] Finally, our findings, including detection of reactive T-cells in the circulation of convalescent COVID-19 donors who successfully cleared SARS-CoV-2 and the relevance of patient endogenous CoV-2-ST expansion in controlling SARS-CoV-2 infection, strongly support the idea of AI with CoV-2-STs for high-risk COVID-19 patients.

In the current imperfect landscape of COVID-19 therapeutics, our findings suggest that suboptimal or failed expansion of endogenous CoV-2-STs, identifies patients at high-risk for an adverse outcome for whom, an off-the-shelf, conv- or vac- CoV-2-ST cell product targeting SARS-CoV-2 via a shared HLA, may represent an effective

treatment. Whether this intervention will fulfill expectations, remains to be answered in clinical trials, some of which have already started (NCT04401410, NCT04457726, NCT04351659) or are close to initiation, including one in our center (EudraCT # 2021-001022-22).

Methods

Study approval

The protocol and informed consent forms were approved by the Institutional Review Board.

Participants

The study subjects were unexposed donors with no COVID-19 history or contact with affected individuals, vaccinated subjects, asymptomatic PCR-COV-2-positive subjects and SARS-CoV-2 infected individuals; with ≥ 1 month recovery before sampling (convalescent), non-ICU hospitalized patients, ICU-recovered or ICU critical patients having a prolonged/complicated stay (>25 days) and/or who died **(Table S1)**.

Enzyme-Linked Immunospot (ELISpot) assay

Circulating CoV-2-STs were measured post hospitalization or ICU admission weekly. Peripheral blood monocytes (PBMCs) or T-cell products were pulsed with spike, B.1.1.7 or NCAP protein and the secretion of interferon-gamma (IFN- γ) or tumor-necrosis factor- α (TNF- α) was measured by Elispot. Spot-forming cells (SFCs) were counted on Eli.Scan Elispot scanner (A.EL.VIS; Eli.Analyse software V6.2.SFC). Cell response was considered positive if the total SFCs against antigens tested, were ≥ 30 per 5×10^5 PBMCs or 2×10^5 CoV-2-STs.

CoV-2-ST generation

PBMCs pulsed with $0.5 \mu\text{g/ml}$ of spike and NCAP pepmixes were cultured as described,[5,6,9] in G-Rex10, in media supplemented with 10ng/ml interleukin-7 and 400U/ml interleukin-4 until day 9-11.

Statistical analysis

Results are expressed as mean \pm standard error of the mean (SEM). Differences between data sets were analyzed using nonparametric Kruskal-Wallis test for multiple comparisons or Mann-Whitney test or 2-tailed Student's t-test for two group comparisons. T-cell numbers and disease severity were correlated using a linear regression model and Pearson's correlation. The outcome in association with CoV-2-ST absolute number or expansion was assessed by ROC-curve analysis. Statistical analysis was performed using GraphPad Prism. P-values ≤ 0.05 were considered significant.

NOTES

Author contributions: Conceptualization, AP and EY; Methodology, PGP, DC, KK, AG, AI, EG, CG, MB, E-GE, PT, D-CL, AF, IK, MT, SDB, GK, AKB, ES and AP; Formal analysis, EG, AP and EY; Investigation, PGP, DC, KK, AG, AI, EG, CG, MB, E-GE, PT, D-CL, AF, IK, MT, SDB, GK, AKB, ES and AP; Resources, AA and EY; Writing – original draft preparation, AP and EY; Writing – review and editing, PGP, AP and EY; Visualization, AP and EY; Supervision, AA, AP and EY; Project administration, AA, AP and EY; Funding acquisition, AA and EY. PGP, AP and EY have verified the underlying data. All authors had full access to all the data in the study and accept responsibility to submit for publication. All data associated with this study are available in the main text or the supplementary materials.

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Figure legends

Figure 1. SARS-CoV-2 T-cell immunity in healthy individuals, vaccinated subjects and patients with SARS-CoV-2 infection.

A) Absolute lymphocyte counts in unexposed donors (black dots; n=14), convalescent donors (grey dots; n=10) and COVID-19 patients stratified by disease severity to ICU critical (pink dots; n=16) ICU recovered (orange dots; n=8) and non ICU (blue dots; n=12). Differences between data sets were analyzed using Kruskal-Wallis test. * $p \leq 0.036$; ** $p \leq 0.0054$; *** $p \leq 0.0002$. **B)** Pearson's correlation analysis of the absolute T-cell number correlation with disease severity; (n=58) $p < 0.0001$. **C-D)** IFN- γ (C) and TNF- α (D) secretion of peripheral blood mononuclear cells of unexposed donors (black dots, n=15-16), convalescent donors (grey dots; n=17 and n=7, respectively) and COVID-19 patients stratified by disease severity to ICU critical (pink dots; n=16) ICU recovered (orange dots; n=7) and non ICU (blue dots; n=12 and n=9, respectively). Each dot represents an individual donor. Differences between data sets were analyzed using Kruskal-Wallis test. * $p \leq 0.04$; ** $p \leq 0.0085$; *** $p \leq 0.0006$; **** $p < 0.0001$. **E)** IFN- γ secretion of peripheral blood mononuclear cells of convalescent donors (grey dots; n=17) and vaccinated donors (purple dots, n=11). Each dot represents an individual donor. Differences between data sets were analyzed using Mann-Whitney test. **** $p < 0.0001$. ICU: Intensive care unit.

F) IFN- γ - and TNF- α - producing circulating CoV-2-STs and IgG levels in individual donors during convalescence phase (black boxes; n=4) and beyond convalescence phase (blue boxes; n=4). Differences between data sets were analyzed using a 2-tailed Student's t-test. * $p \leq 0.04$.

Figure 2. Kinetics of endogenous circulating CoV-2-STs in COVID-19 patients.

A) The expansion of IFN- γ - and TNF- α -secreting endogenous CoV-2-STs in response to CoV-2 antigens post admission to the clinic or intensive care unit is associated with a favorable disease outcome (IFN- γ Non ICU & ICU recovered; n=14; IFN- γ ICU critical; n=14; TNF- α Non ICU- ICU recovered; n=14; TNF- α ICU critical; n=14). Differences between data sets were analyzed using Mann-Whitney test. **p=0.0093; ***p=0.0002; ****p<0.0001. **B-C)** The expansion of IFN- γ - (**B**) and TNF- α - (**C**) secreting endogenous CoV-2-STs in response to NCAP or spike antigens post admission to the clinic or intensive care unit is associated with a favorable disease outcome (IFN- γ Non ICU & ICU recovered; n=14; IFN- γ ICU critical; n=14; TNF- α Non ICU-ICU recovered; n=14; TNF- α ICU critical; n=14). Differences between data sets were analyzed using Mann-Whitney test. **p=0.0032; ***p=0.0006; ****p<0.0001. **D-E)** Δ SFC (**D**) or fold change (**E**) of IFN- γ - and TNF- α - secreting circulating CoV-2-STs post admission to the clinic or intensive care unit (Non ICU and ICU recovered; n=15; ICU critical; n=14). Differences between data sets were analyzed using Mann-Whitney test. *p \leq 0.0184; **p \leq 0.0061. **F-G)** Receiver operating characteristic (ROC) curves of the Δ SFC of circulating IFN- γ - (n=29) (**F**) and TNF- α - (n=24) (**G**) secreting CoV-2-STs showing the predictive power of COVID-19 favorable outcome (high probability to self-control the infection).

Figure 3. Generation and phenotypic characterization of CoV-2-STs from convalescent, unexposed, vaccinated and asymptomatic donors. A-B) Absolute cell numbers **(A)** and fold expansion **(B)** of T-cell products generated after a 10-day culture from convalescent (blue dots; n=14), unexposed (black dots; n=16), vaccinated (purple dots; n=11) and asymptomatic (red dots; n=4) individuals. **C-E)** Immunophenotype of T-cell products generated after a 10-day culture from convalescent (blue dots; n=14), unexposed (black dots; n=16), vaccinated (purple dots; n=11) and asymptomatic (red dots; n=4) individuals. Each dot represents a single T-cell product. Differences between data sets were analyzed using Kruskal-Wallis test. *** $p \leq 0.001$; **** $p < 0.0001$. CM: central memory; EM: effector memory; TEMRA: terminally differentiated effector memory expressing CD45RA.

Figure 4. Functional characterization of CoV-2-STs from convalescent, unexposed, vaccinated and asymptomatic donors. A-B) IFN- γ and TNF- α secretion of the CoV-2-STs generated from convalescent (blue dots; n=14), unexposed (black dots; n=16), vaccinated (purple dots; n=11) and asymptomatic (red dots; n=4) individuals upon stimulation with their initial stimuli. Each dot represents a single T-cell product. Differences between data sets were analyzed using Kruskal-Wallis test. * $p \leq 0.04$; ** $p \leq 0.0043$, *** $p \leq 0.0007$; **** $p < 0.0001$. **C)** Percent of killing of autologous, peptide-pulsed or allogeneic unpulsed PHA blasts by CoV-2-STs. Differences between data sets were analyzed using Kruskal-Wallis test versus the respective unexposed donor condition (black asterisks) or allogeneic unpulsed PHA blasts (light blue and pink asterisks,) or irrelevant-peptide condition (blue and purple asterisks). * $p \leq 0.03$, ** $p = 0.0064$, *** $p \leq 0.0005$; **** $p < 0.0001$ Conv:

convalescent donor derived T cell product; Vac: vaccinated donor-derived T cell product; Unexp: unexposed individual derived T cell product. **D)** IFN- γ and TNF- α secretion of the CoV-2-STs generated from convalescent (blue boxes; n=8) and vaccinated (purple boxes; n=11) individuals upon stimulation with spike antigen of SARS-CoV-2 or its B.1.1.7 variant. **E)** Percent of killing of autologous, unmutated spike- or B.1.1.7 spike-pulsed PHA blasts by CoV-2-STs. Conv: convalescent donor derived T cell product; Vac: vaccinated donor-derived T cell product

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Figure 1

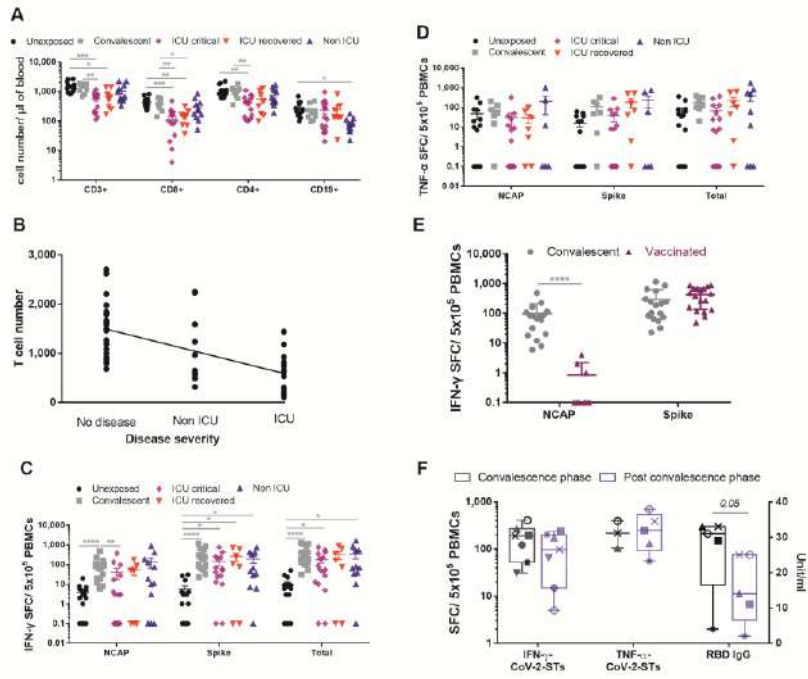
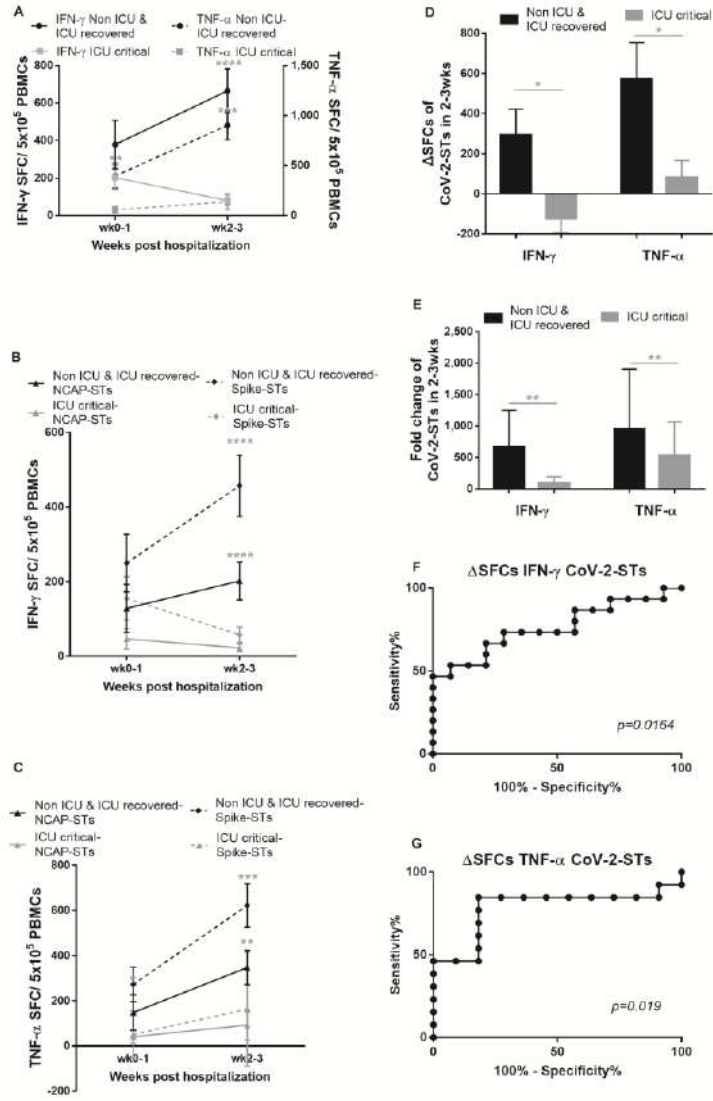


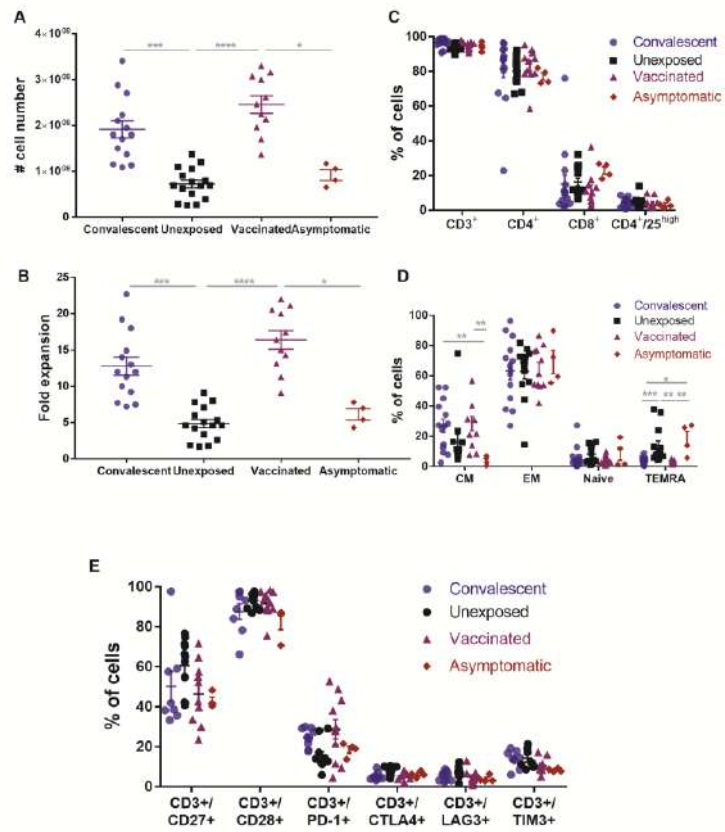
Figure 2



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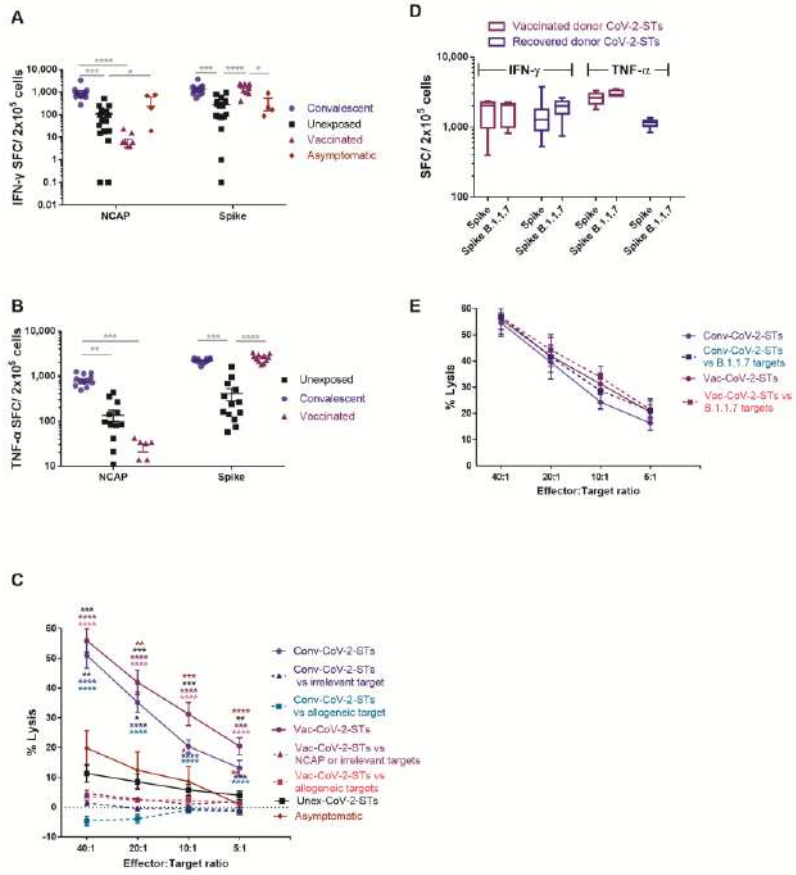
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Figure 3



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Figure 4



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