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Cellular and humoral responses to SARS-CoV-2 vaccination in immunosuppressed patients

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ABSTRACT

Objective: SARS-CoV-2 vaccinations have demonstrated vaccine-immunogenicity in healthy volunteers, however, efficacy in immunosuppressed patients is less well characterised. There is an urgent need to address the impact of immunosuppression on vaccine immunogenicity.

Methods: Serological, T-cell ELISpot, cytokines and immunophenotyping were used to assess vaccine responses (either BNT162b2 mRNA or ChAdOx1 nCoV-19) in double-vaccinated patients receiving immunosuppression for renal transplants or haematological malignancies (n = 13). Immunological responses in immunosuppressed patients (VACC-IS) were compared to immunocompetent vaccinated (VACC-IC, n = 12), unvaccinated (UNVACC, n = 11) and infection-naïve unvaccinated (HC, n = 3) cohorts.

Results: No significant different differences in T-cell responses were observed between VACC-IS and VACC-IC (92%) to spike-peptide (S) stimulation. UNVACC had the highest T-cell non-responders (n = 3), whereas VACC-IC and VACC-IS both had one T-cell non-responder. No significant differences in humoral responses were observed between VACC-IC and VACC-IS, with 92% (12/13) of VACC-IS patients demonstrating seropositivity. One VACC-IS failed to seroconvert, however had detectable T-cell responses. All VACC-IC participants were seropositive for anti-spike antibodies. VACC-IS and VACC-IC participants elicited strong Th1 cytokine response with immunodominance towards S-peptide. Differences in T-cell immunophenotyping were seen between VACC-IS and VACC-IC, with lower CD8⁺ activation and T-effector memory phenotype observed in VACC-IS.

Conclusion: SARS-CoV-2 vaccines are immunogenic in patients receiving immunosuppressive therapy, with responses comparable to vaccinated immunocompetent participants. Lower humoral responses were seen in patients treated with B-cell depleting therapeutics, but with preserved T-cell responses. We suggest further work to correlate both protective immunity and longevity of these responses in both healthy and immunosuppressed patients.

1. Introduction

In late 2019, identification of a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was described as the causative pathogen of a pneumonia outbreak, known as coronavirus-induced-disease-19 (COVID-19) [1]. What emerged as a local outbreak in Wuhan, China, rapidly progressed into a global pandemic of acute respiratory syndrome evoking mass morbidity, mortality and significant socio-economic turmoil [2]. Currently, mass vaccination programmes, utilising regulatory-approved vaccines, remains the best way to prevent viral transmission [3,4], severe disease, death [5,6] and overwhelming

the already stretched healthcare services.

Currently, four vaccines have been approved by the European Medicines agency [7,8], demonstrating satisfactory safety and immunogenicity. However, these pre-authorisation trials were performed on healthy individuals and excluded immunosuppressed patients as they are poor responders to vaccines [9,10]. Consequently, ambiguity regarding vaccine efficacy in patients on immunosuppression prevails. Furthermore, it has been established immunosuppressed patients, such as kidney transplant recipients, have been considered as clinically vulnerable to SARS-CoV-2 infection [11–13]. This was further demonstrated by a prospective study conducted by Baek *et al* [14], which

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investigated SARS-CoV-2 infection outcomes in 871 immunosuppressed patients. The study defined patients as immunosuppressed, based on a diagnosis of malignancy, organ transplantation recipients or those receiving immunomodulatory treatments. Findings revealed immunosuppressed patients had significantly higher rate of in-hospital mortality, and increased likelihood of developing severe COVID-19 compared to immunocompetent patients.

Moreover, case reports have illustrated the lack of viral clearance in those with immunodeficient states, resulting in elevated serum cytokine profiles, progression into acute respiratory distress syndrome and succumbing to mortality. [15]. Additionally, defective SARS-CoV-2 clearance, due to impaired immune responses, have been associated with persistent SARS-CoV-2 infection and evolution of SARS-CoV-2 variants [16,17]. Choi *et al* [16] described an immunosuppressed patient, who experienced SARS-CoV-2 infection for 152 days. During this time, 31 substitutions and three deletions within the SARS-CoV-2 genome were identified. Importantly, twelve spike mutations were detected, of which seven were linked to immune evasion [16,18,19]. Data from these case reports also cautions against the use of convalescent plasma therapy in immunosuppressed patients [17]. It's been postulated administered antibodies may have inadequate support from cytotoxic *T*-cells, therefore, reducing the capacity of viral clearance, yet driving the evolution of SARS-CoV-2 variants. In view of this, characterising vaccine-induced immune responses, within the immunosuppressed, is crucial for understanding their protective immunity and formulating optimal immunisation regimes.

Both natural infection and SARS-CoV-2 vaccination induce spike protein specific antibodies with neutralising activity [8,20]. Nevertheless, the longevity and duration of such humoral protection is unclear, with several studies demonstrating waning antibody-levels over time [21]. In contrast, several findings have highlighted the role of long-term SARS-CoV-2 *T*-cell responses [22]. Effective cellular immune responses were attributed to mild-COVID-19 [23], alongside development of robust SARS-CoV-2 specific *T*-cells which were detected 6–8 months post-infection [24]. Moreover, both mRNA and adenoviral vaccines stimulated potent *T*-cell mediated responses in study- participants [25,26]. Furthermore, long-term duration of protective *T*-cell responses were identified against SARS-CoV, whereas no antigen-specific B-memory cells or antibodies were detected 6 years post-infection [27]. As such, when assessing vaccine immunogenicity, it is critical to assess both humoral and cellular responses. Such evaluation is of greater importance in immunosuppressed cohorts, as there is an urgent need to understand the impact of immunosuppression on the efficacy of SARS-CoV-2 vaccinations.

To address this knowledge gap, we examined the impact of immunosuppressive therapies on SARS-CoV-2 vaccine responses. SARS-CoV-2 vaccine responses were assessed in adult-vaccinated kidney transplant patients, or those with haematological malignancies. Here we provide a detailed description of the cellular and humoral responses, following two doses of either mRNA or adenoviral-vector SARS-CoV-2 vaccines. Unlike current studies examining findings of early post-vaccine period, we define details of their most current response (median time: 115 days post-second dose). Based on our findings, we were able to conclude that these immunosuppressed patients produced an immunological response, to SARS-CoV-2 vaccines, which were comparable to healthy vaccinated participants. Our findings warrant further investigation to determine correlation between such observed responses with protective immunity and longevity, within this clinically vulnerable cohort. Moreover, such studies are imperative for the understanding of cellular responses towards the continual emergence of SARS-CoV-2 variants of concerns.

2. Methods

2.1. Study design

This study was approved by the institutional research review board

of Portsmouth Hospital University NHS Trust and ethical approval was obtained from a national ethics committee (London-City and East research ethics committee, IRAS: 291009). This study was registered under National Institute of Health Research (NIHR) portfolio (CPMS ID: 48275). The study was conducted in accordance with principles of Good Clinical Practice. All enrolled participants were aged ≥ 18 or over. Participants were assessed for study eligibility by providing a clinical history. Before enrolment, all participants provided written informed consent.

All recruited participants were convalescent donors, except for healthy controls who were COVID-19 infection-naïve and unvaccinated. All convalescent individuals had prior positive real-time polymerase chain reaction (RT-PCR) results before study enrolment. Participants were stratified into the following cohorts: healthy unvaccinated COVID-19-infection naïve (HC, $n = 3$), unvaccinated (UVACC, $n = 11$) and vaccinated immunocompetent healthcare workers (VACC-IC, $n = 12$) and vaccinated immunosuppressed participants (VACC-IS, $n = 13$). VACC-IS participants were put forward to the study by their respective clinicians, whereas the remaining participants were enrolled through hospital communications.

Blood samples were collected upon enrolment, which were taken in heparinized, EDTA and SST-collection tubes. Samples were processed within 8 h of venepuncture. SST-collection tubes were centrifuged at 2000g for 10 min for collection of serum. Collected serum was stored at -20°C until SARS-CoV-2 serological assays were performed. EDTA tubes were used to perform a full-blood count using the DxH Haematology Analysers (Beckman Coulter). Heparinized tubes were processed for peripheral blood mononuclear cells (PBMCs) collection as described below for ELISpot analysis.

2.2. T-cell ELISpot

SARS-CoV-2-specific *T*-cell responses were identified using the *T*-Spot Discovery SARS-CoV-2 (Oxford Immunotec) according to manufacturer's instructions. In brief, leucosep tubes (Oxford Immunotec) were used to isolate PBMCs from lithium-heparinised whole blood. A total of 2.6×10^6 PBMCs were plated into each individual well of *T*-spot plate. Each well is coated with one of the four different SARS-CoV-2 structural peptides; Spike (S1) protein, nucleocapsid (NC), membrane (MN) protein, and homology (segments of similar sequences which were eliminated from NC and MN panel). Negative and positive controls (phytohaemagglutinin) were used to control for cellular contamination and functionality, respectively. PBMCs were incubated overnight (37°C , 5% CO_2) for 20 h and IFN γ -secreting SARS-CoV-2 specific *T*-cells were detected by using an automated plate reader (Autoimmun Diagnostika-ASK JM). IFN γ secreting SARS-CoV-2 *T*-cells were reported as spot forming units (SFU) per well.

2.3. SARS-CoV-2 cellular immunophenotyping

Following SARS-CoV-2 peptide stimulation in ELISpot plate, harvested PBMCs were counted to 2.0×10^6 . Counted PBMCs were resuspended in FACS buffer (phosphate buffered-saline with sodium chloride, Beckman Coulter) and stained with a tetra 1 backbone (CD45-FITC, CD3-PC5, CD4-RD1, CD8-ECD (Beckman Coulter)), added with another two fluorochrome-labelled monoclonal antibodies (mAbs), HLA-DR-PC7 and CD38-Alexa-fluor 750, as markers for *T*-cell activation. PBMCs were incubated at room temperature in dark for 15 min and then resuspended with 250 μl FACS buffer. Up to 2.0×10^6 PBMCs were counted using a 10-laser Navios Flow cytometer (Beckman Coulter).

For *T*-cell subset analysis, harvested PBMCs following S-peptide stimulation were counted as outlined above. PBMCs were then stained with Duraclone IM *T*-cell panel (Beckman Coulter, Miami, FL). Signals from the following different fluorochrome-labelled mAbs were obtained; CD45-Krome Orange, CD3 APC-A750, CD4-APC, CD8-AF700, CD27-PC7, CD57-Pacific Blue, CD279 (PD1)-PC5.5, CD28-ECD, CD197

(CCR7)-PE and HLA-DR-FITC. PBMCs were incubated at room temperature in dark for 15 min and then resuspended with 500 μ l FACS buffer. Up to 2.0×10^6 PBMCs were counted using a 10-laser Navios Flow cytometer (Beckman Coulter).

2.4. Representation of high-dimensional flow cytometry

Flow cytometric *t*-distributed stochastic neighbor embedding (tSNE) and FlowSOM analysis were performed using Cytobank (<https://premium.cytobank.org>). For surface *T*-cell activation marker expression, analysis was performed using the above outlined markers. CD3⁺ gated events from individuals within each cohort were collected and concatenated into a single file. Data from 103,311 CD3⁺ gated events, from all cohorts per peptide, were exported as flow cytometry standard (FCS) files using Kaluza 2.1 software (Beckman Coulter, Miami, FL). Then, data from 4453 CD3⁺ events, with the following settings: 1,000 iterations, perplexity 30, and theta 0.5, subsampling equal each cohort was used to generate tSNE analysis. FlowSOM was performed using above outlined markers, which were performed individually with gated CD4⁺ and CD8⁺ populations from tSNE analysis per cohort. The following parameters were used to conduct FlowSOM analysis: number of clusters: 225; number of metaclusters: 15; iterations: 10; and hierarchical consensus clustering method was used.

For *T*-cell subset, analysis was conducted using above outlined markers. S-peptide stimulated CD4⁺ and CD8⁺ gated events from each individual were concatenated into a single file per cohort; VACC-IC CD4: 83,612 events; VACC-IC CD8: 35,721; VACC-IS CD4: 60,932; VACC-IS CD8: 16,397 events. All CD4 and CD8 events, per cohort, were used to conduct tSNE analysis with aforementioned parameters. For both *T*-cell activation and *T*-subset analysis, heat maps were used to report statistical phenotypic changes in marker expression within CD4 and CD8 populations per cohort.

2.5. Serological testing

Serum was tested for antibodies to Spike (S) protein using the Binding site Anti-spike IgG/A/M ELISA assay according to manufacturer's instructions. Result outcomes are reported as positive or negative with a threshold index-value of ≥ 1.0 . Samples with optical density greater than top-standard of curve were reported as >4.00 index value.

2.6. Cytokine profiling

Cytokine responses (IL-6, TNF α , IL-1 β , IL-10) were measured in supernatant derived from PBMC stimulation with SARS-CoV-2 peptides within ELISpot plate. 20 μ l of supernatant was collected and stored in -80°C until analysis was conducted. IFN- γ was not tested as supernatant was derived from ELISpot plate which captures IFN- γ secretion. Cytokine responses were measured using Multiplex assays as performed by the Clinical Immunology laboratory at Addenbrooke's Hospital, Cambridge.

2.7. Statistical analysis

Statistical analysis was conducted using Prism V9.0 (GraphPad Software, San Diego, California, USA). Unless otherwise stated, all data are reported as median with IQR. Where appropriate, Kruskal-Wallis test with Dunn's post-hoc comparison test was performed to assess differences between >2 groups. Two-sided Mann-Whitney was used to assess perform differences between 2 groups. $P < 0.05$ unless otherwise stated. Other details, if any, for each investigation are provided within relevant figure legends. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

3. Results

3.1. Study participant characteristics

A total of 39 participants were recruited into the study and stratified into appropriate cohorts based on their clinical characteristics. In unvaccinated cohort (UNVACC, supplemental table S1), the median age of participants was 37 years (IQR: 31–47), male-to-female ratio was 3:8, with 81.8% of participants from white-British ethnicity. Co-morbidities of hypothyroidism ($n = 1$), stroke ($n = 1$) and sleep apnoea ($n = 2$) were reported in this cohort. The reported time between positive RT-PCR result and study enrolment was 160 days (IQR: 145–165). Nine UNVACC participants (81.8%) were classified as ambulatory mild disease [28], based on reported signs and symptoms during active SARS-CoV-2 infection. Two participants were hospitalised requiring oxygen therapy (non-invasive ventilation) and treated with dexamethasone, which as shown by the RECOVERY trial (NCT: NCT04381936), lowered mortality in hospitalised adult COVID-19 patients.

Twelve participants were stratified as vaccinated immunocompetent (VACC-IC, supplementary table 2) with a median age of 45 years (IQR: 30–53) and male-to-female ratio of 1:5. Five VACC-IC participants reported co-morbidities of depression ($n = 2$) and mild asthma ($n = 3$); none of these participants, alongside remainder of VACC-IC cohort, were treated with immunosuppressive therapies. Time reported between positive RT-PCR result and study enrolment was 175 days (IQR: 143–431), with all twelve participants classified as having mild COVID-19 disease, and double-vaccinated with BNT162b2 vaccine. Median time between receiving the second vaccine dose and study enrolment was 112.5 days (IQR: 87.2–153).

Thirteen immunosuppressed patients (VACC-IS), with a median age of 61 years (IQR: 55–65), were recruited. Male-to-female ratio was 7:6, with all patients from a British-white ethnic background. Clinical characteristics and immunosuppressive regimes are summarised in Table 1. Seven patients (53.8%) had end-stage renal disease and received renal transplantation with an average of 2043 days (6.5 years) prior to study enrolment. Four patients were diagnosed with haematological malignancies, whilst two patients had autoimmune disorders. Twelve patients had further co-morbidities, of which all were high-risk for severe COVID-19. All VACC-IS were on immunosuppressive treatments, with 61.5% and 46.1% managed on Mycophenolate mofetil and Tacrolimus, respectively, and five patients receiving B-cell depletion therapy (Rituximab, R-CHOP) within last 6 months. $>69\%$ of VACC-IS patients suffered either severe or moderate COVID-19 disease, which required hospitalisation, whereas 25% of patients were managed supportively at home. All 13 patients had prior SARS-CoV-2 infection with median time of 243 days (IQR: 163–292) prior study enrolment. All patients received second-dose SARS-CoV-2 vaccinations with median time of 115 days (IQR 85–143) prior study enrolment; all patients were double-vaccinated with 7:6 ratio to Pfizer/BNT162b2-to-AZ/ChAdOx1 vaccines. Three patients were recruited who were infection-naïve (HC), with median age of 27 years (IQR: 25–38), and of British-white ethnicity. All HC reported no co-morbidities or on active treatments.

3.2. Cellular response

We examined SARS-CoV-2 cellular response to natural infection and vaccination, by stimulating PBMCs with either Spike (S) (Fig. 1a), Nucleocapsid (NC) (Fig. 1b), or Membrane (MN) (Fig. 1c) peptides, and enumerated IFN γ producing SARS-CoV-2 specific *T*-cells by ELISpot. As expected, infection-naïve unvaccinated (HC) individuals produced a very low response against S-peptide stimulation, with 2/3 HC eliciting no S-peptide response (Fig. 1a). One HC participant demonstrated very low spot forming units (SFU) to S-peptide (3 SFU), which could be a result of non-specific activation. There were statistically significant differences in S-peptide responses between vaccinated immunocompetent (VACC-IC) and immunocompromised (VACC-IS) cohorts versus HC

Table 1
Patient characteristics of VACC-IS.

Baseline Characteristics	VACC-IS, n = 13
Age, yr (IQR)	61 (55–65)
Gender	
Male	7
Female	6
Ethnicity	
White (%)	12 (100%)
BMI, kg/m ² (±SEM)	30.6 ± 2.46
Diagnosis, n (%)	
ESRD requiring transplantation	7 (53.8%)
MPO vasculitis	1 (7.7%)
CLL	2 (15.3%)
Diffuse large B-cell lymphoma	2 (15.3%)
MCD	1 (7.7%)
Co-morbidities, n (%)	
Gout	2 (15.3%)
Hypertension	6 (46.1%)
Type 2 Diabetes Mellitus	2 (15.3%)
DVT	1 (7.7%)
Asthma	1 (7.7%)
Time from positive COVID-19 PCR result, days	243 (163–292)
Median (IQR)	
WHO COVID-19 clinical severity scale, n %	
Ambulatory mild disease	4 (30.8%)
Hospitalized: moderate disease	3 (27.7%)
Hospitalized: severe disease	6 (46.1%)
Vaccine received, n %	
AZ/ChAdOx1	6 (46.1%)
Pfizer/BNT162b2	7 (58.3%)
Time from receiving 2nd dose, days	
Median (IQR)	115 (85–143)
Immunosuppressive regimen, n (%)	
Tacrolimus	6 (46.1%)
Ciclosporin	1 (7.7%)
MMF	8 (61.5%)
Prednisolone	7 (53.8%)
Azathioprine	1 (7.7%)
Rituximab	3 (23.0%)
R-CHOP	2 (15.3%)

ESRD, End-stage renal disease; MPO, Myeloperoxidase; CLL, Chronic lymphocytic leukaemia; ITP, Immune thrombocytopenia; MMF, Mycophenolate Mofetil; R-CHOP, rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine, and prednisone.

(VACC-IC, $p = 0.040$ and VACC-IS, $p = 0.025$, respectively). No significant differences in S-peptide responses were observed between unvaccinated (UNVACC) versus HC ($p = 0.471$). Comparison of S-peptide responses between VACC-IC and VACC-IS, demonstrated no significant differences ($p = 0.968$, Fig. 1a), where, VACC-IS mounted a higher T-cell response to spike (median 17.00 SFU, IQR: 8–44) compared to VACC-IC (median 14.50 SFU, IQR: 7.50–26.75). In VACC-IS, there was one non-responder to S-peptide (0 SFU) which was a kidney transplant patient. Whilst every VACC-IC participant produced a SFU to S-peptide, one participant exhibited 1 SFU, which we categorised as non-responder following two doses of SARS-CoV-2 vaccination. UNVACC had the highest non-responders ($n = 3$), whereby, T-cell responses to S-peptide (median: 5.00, IQR: 1–27) were lower by 9.50 and 12.00 SFU compared to VACC-IC and VACC-IS, respectively.

As UNVACC, VACC-IC and VACC-IS cohorts comprised of convalescent individuals, NC and MN responses were detected (Fig. 1b, 1c), albeit without any significant differences between all cohorts. Nevertheless, UNVACC and VACC-IS had equal non-responders to NC ($n = 3$) and MN ($n = 4$), whilst non-responders against NC ($n = 2$) and MN ($n = 1$) were also seen in VACC-IC. Moreover, VACC-IC S-peptide responses were significantly higher compared to NC ($p = 0.005$) and MN ($p = 0.001$) by 11.00 and 12.00 SFU, respectively (Fig. 2a). Similar trend was observed in VACC-IS (Fig. 2b), where S-peptide responses were significantly higher by 14.50 SFU compared to both NC ($p = 0.005$) and MN ($p = 0.003$). Whilst UNVACC S-peptide responses were higher, there were no significant differences between NC and MN (Fig. 2c). Together, this

demonstrated that both vaccination cohorts induced immunodominance towards S-peptide, whereas no significant precedence to either SARS-CoV-2 peptides were seen with natural infection.

3.3. Humoral response

Humoral responses were evaluated using total Ig anti-spike ELISA immunoassay. As expected, convalescent unvaccinated and vaccinated cohorts had significantly higher serological responses compared to HC individuals (Fig. 3a, $p < 0.0001$). UNVACC cohort demonstrated the lowest serological response (median: 2.92 index-value, IQR: 2.28–3.18), with 10/11 participants displaying seropositivity. Seronegative responses were seen in one UNVACC participant, who also displayed absent SARS-CoV-2 T-cell response. All 12 VACC-IC participants were seropositive (median: 4.40 index-value, IQR: 4.15–4.40), with 10/12 participants generating a serological response which was greater than top standard of assay (4.00 index-value). Moreover, whilst humoral responses were higher in both vaccinated cohorts compared to UNVACC; only VACC-IC demonstrated a significantly higher humoral response compared to UNVACC ($p = 0.002$).

In VACC-IS, 12/13 patients were seropositive (median: 4.40 index-value, IQR: 2.49–4.40), with 8/13 patients eliciting responses that were greater than assay top-standard. No significant differences in humoral responses were seen between VACC-IC and VACC-IS. Nevertheless, examining the lower quartile ranges, 25% of VACC-IS participants produced a humoral response (2.49 index-value) which was 1.66 index-value lower than 25% of humoral responses observed in VACC-IC (4.15 index-value). Furthermore, analysing the different immunosuppressive therapies within VACC-IS (Fig. 3b), highlighted patients on tacrolimus, MMF and prednisolone, exhibited higher serological response (median: 4.40 index-value, IQR: 2.90–4.40) than those on B-cell depleting therapy (median 2.62 index-value, IQR: 0.94–4.09). Nevertheless, only 1/4 patients receiving B-cell therapy were seronegative. Overall, the above findings highlight both vaccinated cohorts produce higher anti-S serological responses than unvaccinated cohort. Furthermore, vaccinated immunosuppressed patients elicit humoral responses which were comparable to vaccinated immunocompetent cohort.

3.4. Characterisation of CD4⁺ and CD8⁺ T-cell activation marker expression

To assess phenotypic changes in T-cell activation marker expression post-SARS-CoV-2 peptide stimulation, we performed an unsupervised analysis which evaluates the entire complex scenario depicted by CD4⁺ and CD8⁺ T-cells. Initially, we conducted a dimensionality reduction analysis, flow cytometric and combined *t*-distribution stochastic neighbour embedding (tSNE), to acquire a phenotypic landscape of CD45⁺CD3⁺CD4⁺ and CD8⁺ lymphocytes in all cohorts (Fig S1). We then explored CD4⁺ and CD8⁺ T-cell panel by unsupervised analysis using FlowSOM [29]. Such analysis conducts multivariate clustering of cells based on self-organised map (SOM) algorithm, enabling cells to be stratified into specific meta-clusters based on HLA-DR and CD38 expression [30]. Heat maps were used to statistically report differences in phenotypic expression between cohorts.

For CD4⁺ characterisation (Fig. 4a-b) against S-peptide stimulation, we clustered all individual cells for each cohort into 15 distinct clusters based on surface HLA-DR and CD38 expression. Subsequently, we reduced complexity by merging similar cluster profiles and conducted further re-clustering. As illustrated in Fig. 4a, 5 distinct clusters in CD4⁺ T-cells were identified in all cohorts. Each metacluster were represented equally within all cohorts, with exception to metacluster 4, which was lower in VACC-IS. Moreover, no significant differences in CD4⁺HLA-DR CD38 phenotypes were seen between cohorts (Fig. 4b). Dual HLA-DR⁺CD38⁺ expression was identified in metacluster 4 and 9, whilst metacluster 7 illustrated CD4⁺ HLA-DR⁺CD38^{wk} expression. Both metacluster 3 and 10 portrayed HLA-DR⁺CD38⁺ and HLA-DR⁺CD38⁺,

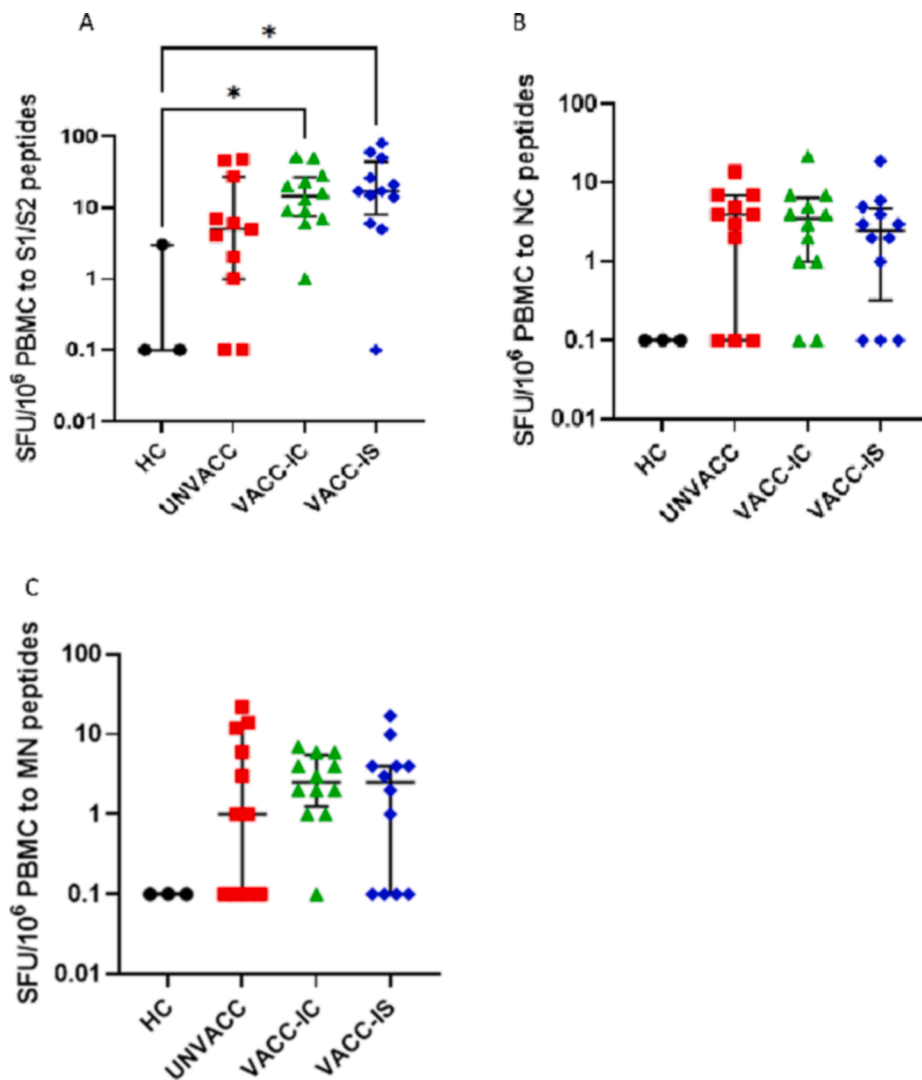


Fig. 1. Cellular responses in unvaccinated infection naïve (HC, $n = 3$), unvaccinated convalescent (UNVACC, $n = 11$), vaccinated immunocompetent (VACC-IC, $n = 12$) and vaccinated immunosuppressed (VACC-IS, $n = 12$) participants to Spike (A), Nucleocapsid (B) and Membrane (C) SARS-CoV-2 peptides. One VACC-IS patient was excluded from analysis due to failed positive control. Data representative of individual values expressed as IFN γ spot forming units (SFUs per 2.5×10^6 PBMCs), median (centre bar) and IQR (upper and lower bars). For visualization of data on log-scale, SFU values = 0 is represented by 0.1. Statistical analysis is performed by Kruskal-Wallis nonparametric test with Dunn's post-hoc test; if not indicated P value is not significant, $* = P < 0.05$. HC, Healthy infection-naïve; UNVACC, unvaccinated convalescent; VACC-IC, vaccinated immunocompetent; VACC-IS, vaccinated immunosuppressed.

respectively. Overall CD4⁺ HLA-DR⁺ expression was in 80% of metaclusters ($n = 4$), whilst CD38⁺, CD38⁻ and CD38^{wk}, were identified in 60% ($n = 3$), 20% ($n = 1$) and 20% ($n = 1$), respectively.

Characterisation of CD8⁺ S-peptide responses demonstrated 5 clusters (Fig. 4c), where metacluster 1 and 8 were under-represented in VACC-IS cohort, whilst remaining metaclusters were similar across cohorts. Unlike CD4⁺, there were differences in HLA-DR and CD38 expression observed between cohorts, as illustrated by metacluster 1 and 2 (Fig. 4d). CD8⁺ HLA-DR⁺CD38^{wk} expression were identified in VACC-IS metacluster 1 (as represented by darker yellow shade for CD38), whereas dual HLA-DR⁺CD38⁺ expression were seen in HC, UNVACC and VACC-IC. Moreover, dual HLA-DR⁺CD38⁺ expression in UNVACC and VACC-IC were identified for metacluster 2, whereas significant different outcomes were observed in HC and VACC-IS; as illustrated by HLA-DR⁺CD38^{wk} expression. Furthermore, all CD8⁺ metaclusters expressed HLA-DR⁺ ($n = 5$), whereas CD38⁺ were reported in 20% and 40% of metaclusters in HC ($n = 1$) and UNVACC, VACC-IC (both $n = 2$), respectively. No CD38⁺ expression were identified in VACC-IS, with 60% of VACC-IS metaclusters ($n = 3$) expressing CD38^{wk}, and 40% ($n = 2$) were CD38⁻. Overall post S-peptide stimulation, both CD4⁺ and CD8⁺ upregulated HLA-DR⁺, with higher CD38⁺ observed in CD4⁺ T-cells.

Evaluation of HLA-DR and CD38 expression was conducted in NC (Fig S2a-d). 7 metaclusters were identified for NC. As with S-peptide, under-representation was observed in VACC-IS (metacluster 6, Fig S2a), which expressed HLA-DR^{wk}CD38⁺ (Fig S2b). Moreover, dual CD4⁺ HLA-

DR⁺ CD38⁺ were seen in metacluster 4 in all cohorts (Fig S2b); albeit expression of HLA-DR and CD38 was lower in both vaccinated cohorts compared to UNVACC and HC. Overall, NC-peptide stimulation preferentially expressed CD38⁺ ($n = 4$) compared to HLA-DR⁺ ($n = 2$) in CD4⁺. For CD8⁺ NC responses, 4 metaclusters were identified (Fig S2c), metacluster 7 and 2 were under-represented in VACC-IS and UNVACC, respectively. Both metacluster 7 and 2 expressed CD8⁺HLA-DR⁺CD38⁺ and CD8⁺HLA-DR⁺CD38⁻, respectively. (Fig S2d). Furthermore, CD8⁺ stimulated with NC-peptide elicited a predominant dual CD8⁺ HLA-DR⁺ CD38⁺ phenotype ($n = 3$) in all cohorts, with only metacluster 2 demonstrating a CD8⁺ HLA-DR⁺ CD38⁻ phenotype. Overall, a greater T-cell activation profile was observed in CD8⁺, compared to CD4⁺, following NC-peptide stimulation.

Stimulation with MN-peptide resulted in characterisation of 7 CD4⁺ metaclusters (Fig S3a). The similar trend of metacluster under-representation was evident in VACC-IS CD4⁺ (metacluster 1), which depicted an HLA-DR^{wk}CD38⁺ profile (Fig S3b). No dual CD4⁺ HLA-DR⁺ CD38⁺ were seen across all cohorts, with HLA-DR^{wk} expression observed across all metaclusters, whilst 57% ($n = 4$) of CD4⁺ metaclusters expressed CD38^{wk}, only 3 metaclusters depicted CD38⁺. However, within CD8⁺ landscape, UNVACC cohort depicted a substantial drop in metacluster 5 and 9 representation (Fig S3c) with HLA-DR⁺ CD38⁻ and HLA-DR⁺ CD38⁺ expression (Fig S3d), respectively. Dual HLA-DR⁺CD38⁺ expression was observed in metacluster 9, with HLA-DR⁺ expressed in all CD8⁺ metaclusters ($n = 5$); albeit at lower levels in

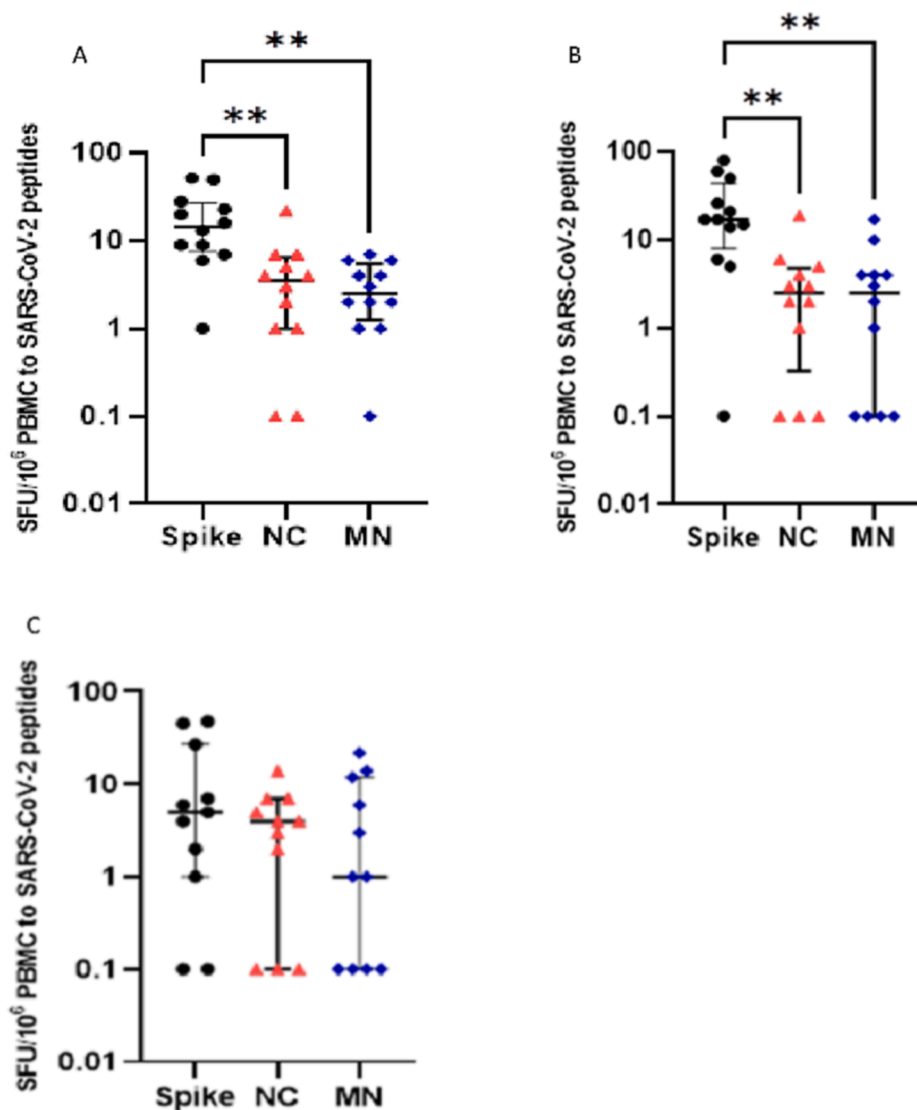


Fig. 2. Comparison of Spike, NC, and MN responses in VACC-IC (A), VACC-IS (B) and UNVACC (C) cohorts. One VACC-IS patient was excluded from analysis due to failed positive control. Data representative of individual values expressed as IFN γ spot forming units (SFUs per 2.5×10^6 PBMCs), median (centre bar) and IQR (upper and lower bars). For visualization of data on log-scale, SFU values = 0 is represented by 0.1. Statistical analysis is performed by Kruskal-Wallis nonparametric test with Dunn's post-hoc test; if not indicated *P* value is not significant, **P* < 0.05, ***P* < 0.01. HC, Healthy infection-naïve; UNVACC, unvaccinated convalescent; VACC-IC, vaccinated immunocompetent; VACC-IS, vaccinated immunosuppressed.

metacluster 14. CD38⁺ expression was generally lower in CD8⁺ metaclusters across all cohorts, with VACC-IS and HC illustrating the lowest CD8⁺CD38⁺ expression. Overall, in S, NC and MN-peptide stimulations, VACC-IS exhibited lower proportions of specific metaclusters. Furthermore, HLA-DR⁺ was preferentially upregulated in CD8⁺ whereas, CD38⁺ expression was skewed towards CD4⁺ T-cells.

3.5. Characterisation of T-cell subsets

As proof-of-principle, we conducted a T-cell subset (TCS) immunophenotyping panel, post-S-peptide stimulation, on VACC-IC (*n* = 3) and VACC-IS (*n* = 2) participants. Initially, we used a manual gating strategy (Fig S4) where we compared both CD4⁺ and CD8⁺ T-cell subsets as illustrated in Fig. 5a-b. Within CD4⁺ and CD8⁺ populations, we examined markers for T-cell differentiation (CD45RA, CD197, CD27 and CD28), senescence and exhaustion (CD57 and CD279 (PD1), respectively). As highlighted in Fig. 5a, there were no significant differences between CD4⁺ T-cell subsets between VACC-IC and VACC-IS. Whilst not significantly different, CD4⁺ naïve (T_n) and effector (T_e) cells were higher, by 10.29% and 6.39%, respectively, in VACC-IC compared to VACC-IS. Whereas CD4⁺ T-effector-memory (T_{em}) was higher by 16.74% in VACC-IS compared to VACC-IC. Both VACC-IC and VACC-IS had very similar T-central memory (T_{cm}) populations. Furthermore, senescent CD4⁺ T-cells (CD4⁺CD57⁺) were also similar between both

cohorts; except for one VACC-IC participant who exhibited higher CD4⁺CD57⁺ cells (19.62%, Fig. 5a).

A similar trend was seen in CD8⁺ subsets, where T_n and T_e were higher in VACC-IC, whilst T_{em} populations higher in VACC-IS (Fig. 5b). Moreover, both VACC-IC and VACC-IS demonstrated a 15.19% and 10.14% increase in CD8⁺ T_e, respectively, compared to CD4⁺ T_e. These observations were supported with elevated senescent-terminally differentiated CD8⁺ (CD8⁺CD57⁺) levels compared to CD4⁺. Overall, with exception of T_n, CD4⁺ and CD8⁺ T_{em}, displayed highest percentage values across both cohorts. Subsequently, T_{em} was identified as the predominant memory T-cell subset. Furthermore, no exhausted CD4⁺ and 8⁺ T-cells (CD4⁺CD57⁺PD1⁺) were seen in both cohorts using manual gating strategy.

Subsequently, we used the same unsupervised analysis, as used for activation-markers, where tSNE analysis identified CD4⁺ and CD8⁺ TCS, whose percentages are represented alongside the heatmap; which portrays TCS marker expression in both cohorts (Fig. 5c-d). Immediately, it can be recognised VACC-IS had absent CD4⁺ T_e subset (Fig. 5d), whereas a low percentage (1.55%) were identified for VACC-IC (Fig. 5c). Unlike manual gating strategy, tSNE analysis demonstrated CD4⁺ T_{cm} were dominant for VACC-IC (19.29%) and VACC-IS (18.39%); which were similar between both cohorts. Whereas, VACC-IS evoked a 7.91% increase in CD4⁺ T_{em} compared to VACC-IC. Furthermore, as highlighted in Fig. 5d, VACC-IS CD4⁺ T_n exhibited considerably weaker

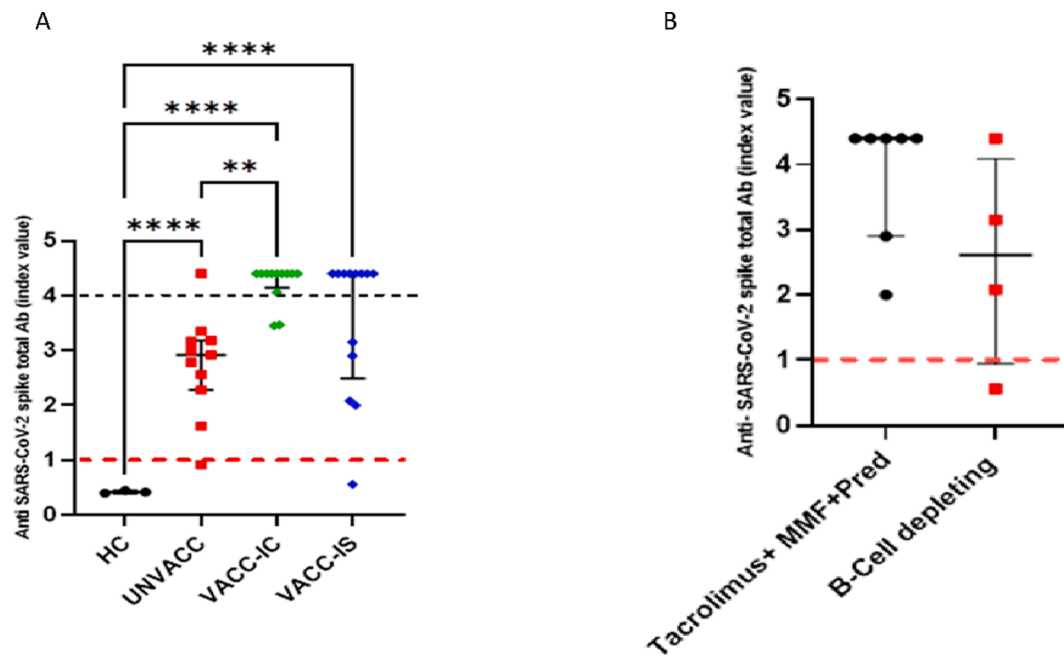


Fig. 3. Characterisation of humoral responses in all four cohorts. A.) Anti-SARS-CoV-2 humoral response was evaluated in infection naïve unvaccinated (HC, $n = 3$), convalescent unvaccinated (UNVACC, $n = 11$), convalescent vaccinated immunocompetent (VACC-IC, $n = 12$), and convalescent vaccinated immunocompromised (VACC-IS, $n = 13$) cohorts. Data representative of individual anti-spike SARS CoV-2 total Ab values (index-value), median (centre bar) and IQR (upper and lower bars). B.) Humoral responses in VACC-IS cohort based on immunosuppressive regimes. Responses were categorised into either combinative therapy of Tacrolimus, MMF and Prednisolone versus B-cell depleting therapy (Rituximab and R-CHOP). Median time between patients receiving previous B-cell depleting infusion and serological analysis was 105 days (IQR: 37.50–240.0). Data representative of individual anti-spike SARS CoV-2 total Ab values (index-value), median (centre bar) and IQR (upper and lower bars) for each therapeutic regime. Black dotted lines indicative of upper limit of assay (4.00 index value). Red dotted lines indicative of assay cut-off threshold for positivity (1.00 index-value). Statistical analysis is performed by Kruskal-Wallis nonparametric test with Dunn's post-hoc test; if not indicated p value is not significant, ** $p < 0.01$, **** $p < 0.0001$. MMF, Mycophenolate mofetil; Pred, Prednisolone; R-CHOP, Rituximab, Cyclophosphamide, Doxorubicin Hydrochloride, vincristine, and prednisolone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CD27 and CD28 expression, compared to VACC-IC. Furthermore, VACC-IS $CD4^+ T_n$, along with T_{cm} , T_{em} and $CD8^+ T_{em}$ exhibited weak expression of exhausted T -cells (CD57 PD1, dark green on heatmap); whereas such CD57 PD1 phenotypes were absent in these subsets in VACC-IC. These subtle variations were not detected from use of manual gating strategy.

Both VACC-IS $CD8^+ T_{cm}$ and T_{em} were greater by 7.12% and 14.94% (Fig. 5d), respectively, compared to VACC-IC (Fig. 5c). Whereas VACC-IC depicted a 6.50% increase in $CD8^+ T_e$ compared to VACC-IS. Overall, tSNE analysis demonstrated $CD4^+ T_{cm}$ as the dominant memory T -cell subset in both VACC-IC and VACC-IS. Whereas $CD8^+ T_e$ and T_{em} were the dominant subset in VACC-IC and VACC-IS, respectively, post S-peptide stimulation.

3.6. Ex vivo production of pro-inflammatory cytokines

Multiplex cytokine analysis (IL-6, $TNF\alpha$, IL-1 β and IL-10) was performed on study cohorts after antigen-specific stimulation in PBMCs with SARS-CoV-2 S, NC, and MN peptides (Fig. 6a-c). We recognise IFN γ secretion as a key cytokine signature in viral infections [31], however as PBMCs were harvested within an IFN γ capture ELISpot plate this cytokine was excluded.

Of the four cytokines analysed, three (IL-6, $TNF\alpha$ and IL-10) showed no significant differences in secretion between cohorts following S-peptide stimulation (Fig. 6a). IL-1 β levels were significantly elevated in both VACC-IC (median 55.85 pg/ml IQR: 30.58–241.4) and VACC-IS (median 77.25 pg/ml, IQR: 30.58–241.4) compared to UNVACC (Fig. 6d, $p = 0.023$ and $p = 0.008$, respectively). IL-6 secretion demonstrated the highest magnitude of cytokine secretion in all cohorts after S-peptide stimulation. Interestingly, HC participants secreted the

highest IL-6 levels (median 2711 pg/ml, IQR: 33.61–3779), whereas within convalescent cohorts, VACC-IC produced the highest IL-6 levels (median 482.4 pg/ml, IQR: 61.16–2894), which was 300.7 and 372.7 pg/ml greater than UNVACC and VACC-IS, respectively. Similarly, VACC-IC secreted the highest $TNF\alpha$ levels (median 126.7 pg/ml, IQR: 55.97–362.5) which were 50.6, 80.47, and 31.29 pg/ml higher than HC, UNVACC and VACC-IS.

Following NC-peptide stimulation, no significant differences in IL-6, $TNF\alpha$ and IL-10, were observed between cohorts (Fig. 6b). Similar to S-peptide, significantly elevated IL-1 β levels were detected in VACC-IC and VACC-IS compared to UNVACC in both NC ($p = 0.023$ and $p = 0.004$, respectively) and MN-peptide stimulation ($p = 0.005$ and $p = 0.025$, respectively, Fig. 6c). Furthermore, following MN-peptide stimulation (Fig. 6c), IL-10 levels were modestly elevated in VACC-IC compared to VACC-IS ($p = 0.033$). Overall, the magnitude of cytokine secretion observed across convalescent cohorts (UNVACC, VACC-IC and VACC-IS) were all significantly greater for IL-6, $TNF\alpha$ and IL-1 β than IL-10 (Fig S5). These findings are indicative of a strong bias towards Th1 cytokine secretion following S, NC and MN-peptide stimulation.

4. Discussion

Immunisation represents the most effective intervention against infectious diseases, such as SARS-CoV-2; as evident by success of mass global vaccination programmes reducing viral spread and preventing severe disease [32]. Nevertheless, there are very few studies exploring the immunogenicity of COVID-19 vaccines in immunocompromised patients, such as solid-organ transplant recipients (SOTs) and haematological malignancies. Moreover, reduced vaccine-induced immune responses have been associated in SOTs, or in general, in patients on

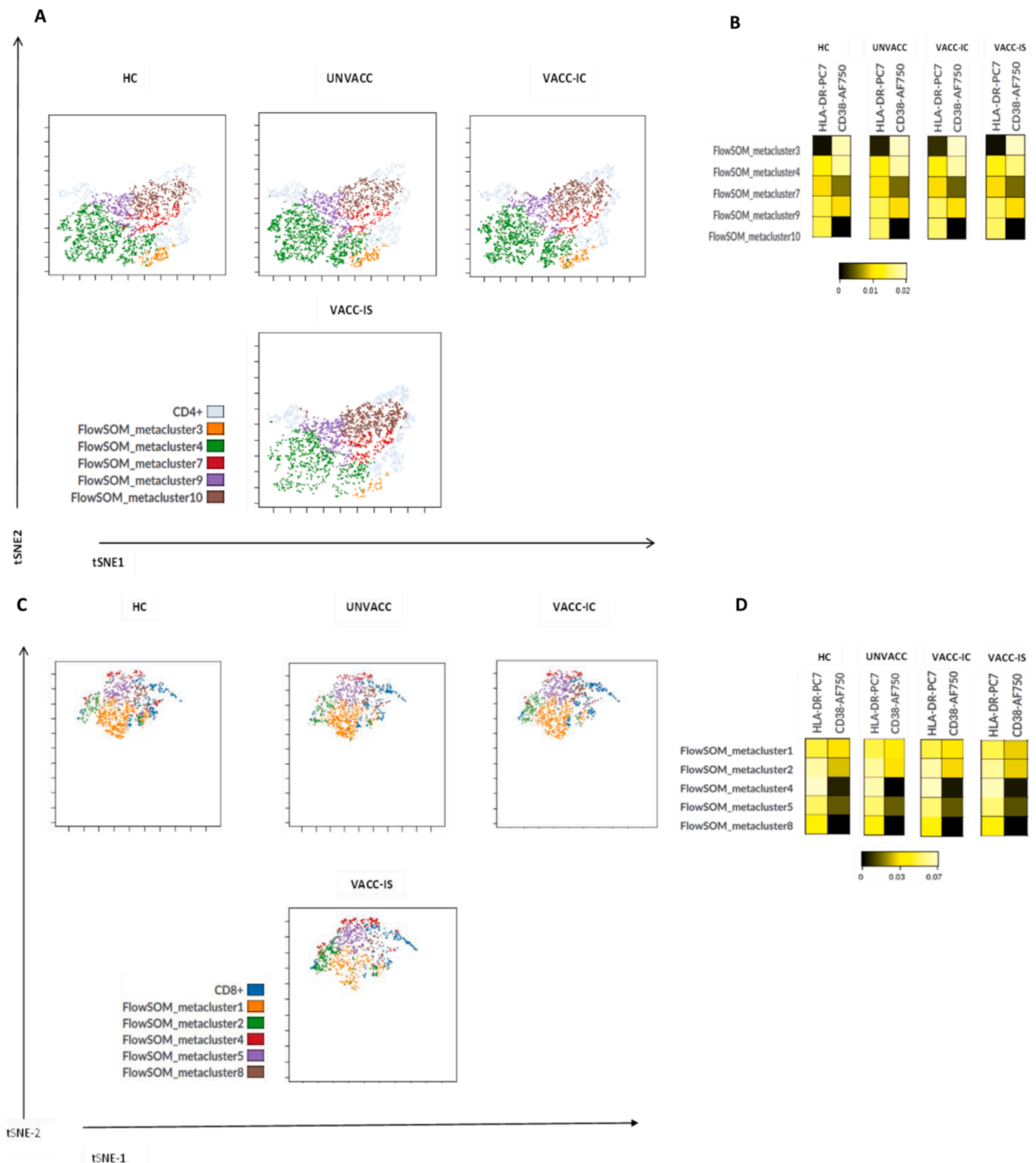


Fig. 4. Unsupervised analysis of CD4⁺ and CD8⁺ T-cells post S-peptide stimulation. **A** Representation of CD4⁺ phenotypic landscape, by coupling tSNE dimensional-reduction analysis with FlowSOM which was used to identify specific CD4⁺ T-cell metaclusters based on HLA-DR and CD38 expression for each cohort. **B** Heat map representing the different CD4⁺ metaclusters identified by FlowSOM for each cohort, where the colours in the heatmap represent the median arcsinh ratio for HLA-DR and CD38 expression of each metacluster. Heatmap colours vary from black for lower expression, to yellow for higher expression of each surface marker (HLA-DR, CD38). **C** The same unsupervised analysis was used to define the CD8⁺ phenotypic landscape, coupled with FlowSOM, for identification of CD8⁺ metaclusters between cohorts. **D** Heat map representing the median arcsinh ratio of HLA-DR and CD38 observed for each cohort. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

active immunosuppressive therapies [33]. To address this, we explored the immunogenicity of two SARS-CoV-2-19 licenced vaccines (either BNT162b2 mRNA or ChAdOx1 nCoV-19 adenoviral-vector) in double-vaccinated adult renal transplant recipients and those diagnosed with

haematological malignancies. Unlike previous studies where vaccine immunogenicity was limited to early post-vaccine period [34], we enrolled immunosuppressed patients with a median time of 115 days post second-dose, thus, providing an up-to-date snapshot of their

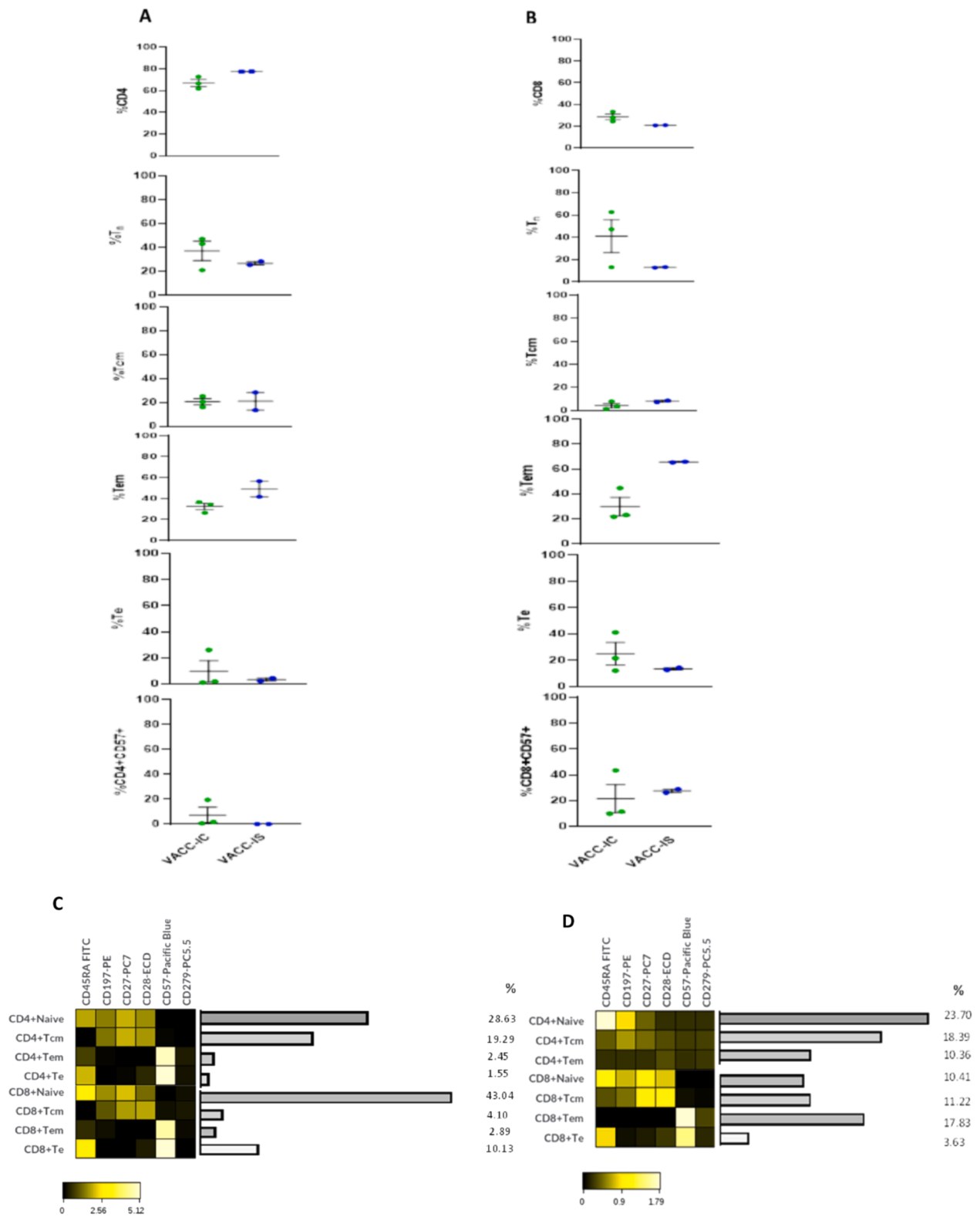


Fig. 5. Characterisation of T-cell subsets. Percentages of different CD4⁺ T-cell (A) and CD8⁺ T-cell subpopulations (B) are shown for VACC-IC (n = 3) and VACC-IS (n = 2), as obtained by manual gating strategy. Data representative of individual values, mean (centre bar) ± SEM (upper and lower bars). Statistical analysis conducted using two-sided Mann-Whitney test; if not indicated, p-value not significant. C. Heat map representing different CD4⁺ and CD8⁺ T-cell subpopulations for VACC-IC (C) and VACC-IS (D) cohorts, as identified by tSNE. The tSNE plot was designed by concatenation of samples per cohort where equal sampling of 60,845 and 16,388 for CD4⁺ and CD8⁺, respectively, were used for VACC-IC. For VACC-IS, 60,932 CD4⁺ and 16,397 CD8⁺ events were used. The colours in the heat map represents the median arcsinh ratio for each surface marker expression. Heatmap colours vary from black for lower expression, to yellow for higher expression. Tn is identified as CD45RA⁺CD197⁺CD27⁺CD28⁺; Tcm are CD45RA⁺CD197⁺CD28⁺CD27^{+/+}; Tem are CD45RA⁺CD197⁺CD28⁺CD27^{+/+}; Te are CD45RA⁺CD197⁺CD28⁺CD27⁺CD57⁺; Exhausted cells express CD57⁺CD279⁺ (PD1). Tn, Naïve T-cells; Tcm, T-central memory; Tem, T-effector memory; Te, T-effector. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

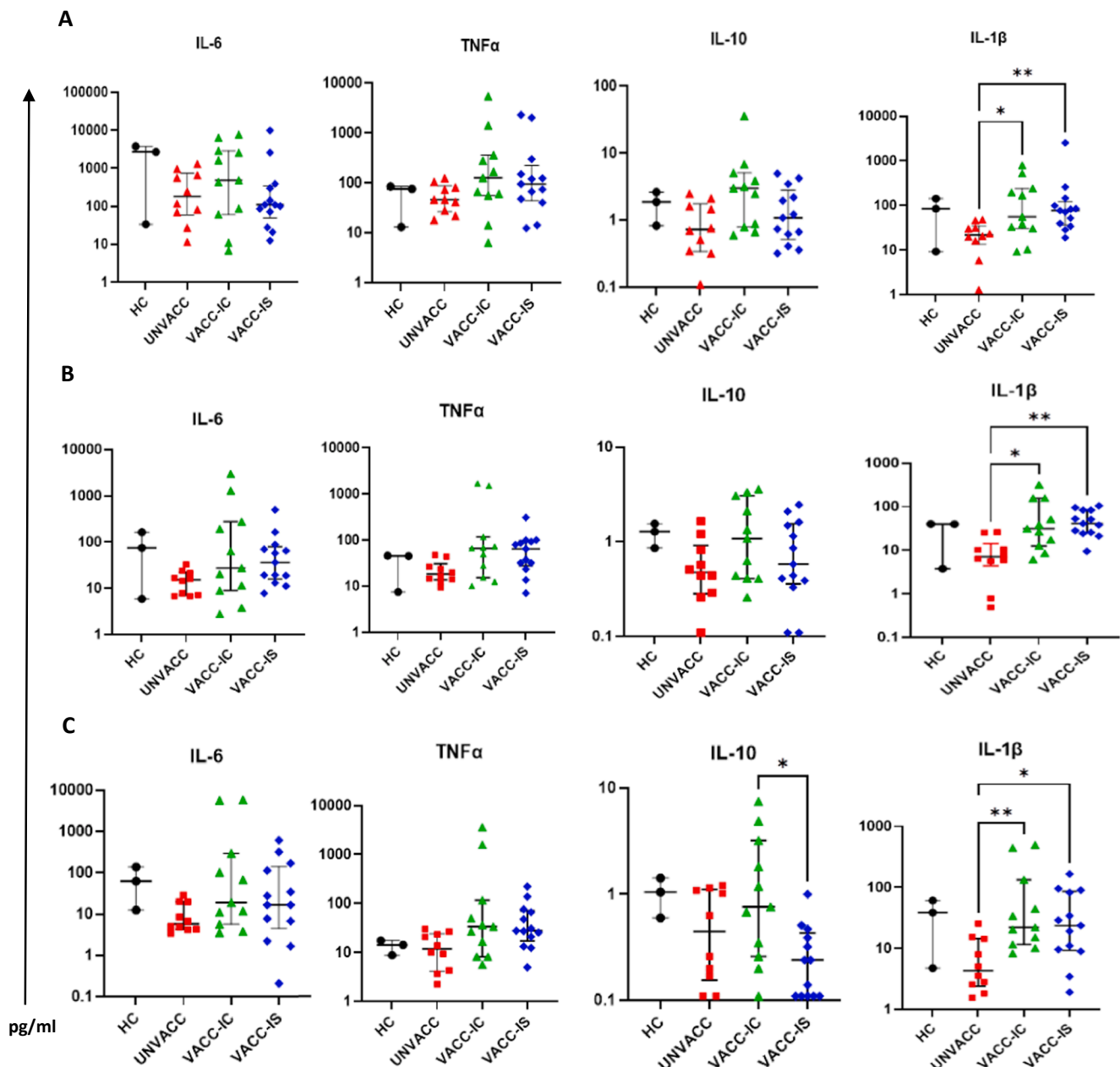


Fig. 6. Cytokine secretion following SARS-CoV-2 peptide stimulation. Multiplex cytokine analysis was conducted using supernatant after antigenic-specific stimulation with Spike (A), Nucleocapsid (NC), and Membrane (MN) peptides in HC (n = 3), UNVACC (n = 10), VACC-IC (n = 11) and VACC-IS (n = 13) participants. Two samples from UNVACC (n = 1) and VACC-IC (n = 1) were excluded due to laboratory technical error. Individual data points are illustrated as individual scatter plots for each cytokine, expressed as pg/ml, with median (centre bar) and IQR (upper and lower bars). Statistical analyses were determined using nonparametric Kruskal-Wallis test with Dunn's post-hoc test for multiple comparisons. * $P < 0.05$, ** $P < 0.01$. HC, Healthy infection-naïve; UNVACC, unvaccinated convalescent; VACC-IC, vaccinated immunocompetent; VACC-IS, vaccinated immunosuppressed.

immune response to SARS-CoV-2 vaccination. We compared the humoral and cellular responses of this immunocompromised group (VACC-IS) to healthy vaccinated (VACC-IC), unvaccinated (UNVACC) and infection-naïve (HC) cohorts. Our data demonstrates that VACC-IS patients responded to the vaccine by producing comparable cellular and humoral responses to VACC-IC. However, findings from large prospective studies [35] are required to correlate such vaccine-induced response with protective immunity.

Recent reports have highlighted diminished T-cell responses against COVID-19 vaccines in renal transplants patients receiving T-cell directed therapies [34] and in haematological cancer patients [36]. In response to these studies, we examined vaccine-induced SARS-CoV-2 specific T-cell responses in these patients through using an IFN γ release assay.

Reassuringly, 92% of VACC-IS patients (n = 12) elicited a detectable T-cell response following spike (S) peptide stimulation. An identical T-cell response rate were observed in VACC-IC participants, demonstrating no differences in vaccine-induced T-cell responses between VACC-IC and VACC-IS. Interestingly, a 17.24% increase in IFN γ -secreting SARS-CoV-2 specific T-cells were identified in VACC-IS compared to VACC-IC. Our findings are consistent with the preliminary OCTAVE trial data (ISRCTN: 12821688), where T-cell responses were similar across immunosuppressed and immunocompetent cohorts [35]. Similarly, both VACC-IC and VACC-IS PBMCs stimulated with SARS-CoV-2 peptides induced a predominantly Th1 response, with significantly elevated TNF α and IL-1 β compared to IL-10. Moreover, cytokine and T-cell responses in vaccinated cohorts demonstrated immunodominance towards S-peptide

compared to NC an MN peptides; findings that are consistent with both vaccine clinical trials [25,26].

Both VACC-IC and VACC-IS had one T-cell non-responder each; however, both these participants demonstrated positive serology for anti-spike SARS-CoV-2 antibodies. Moreover, 3 VACC-IS patients had received B-cell depleting therapy (Rituximab) 2 months following their second SARS-CoV-2 vaccine dose. Whilst 2/3 of these patients, were serologically positive, their anti-spike antibody levels were lower in comparison to those receiving T-cell targeted therapies. Such correlation between diminished vaccine specific humoral responses and B-cell depleting therapies have been reported in prior studies [37]. Nevertheless, all 3 patients receiving B-cell therapy, including the patient who failed to seroconvert, elicited T-cell responses to S-peptide stimulation. These results highlight that B-cell negative patients, due to primary or therapy-induced aetiologies, can still reap benefit from T-cell compartment of vaccination.

A plausible explanation for comparable T-cell responses observed in our data, which were not seen in a study conducted by Predecki *et al* [34], could be that our immunosuppressed patients had prior natural infection, subsequently it could represent an augmented response to second-dose vaccine ("third" challenge in these convalescent patients). In fact, the same study reported a 54% increase in T-cell response in their immunosuppressed patients following second-dose vaccination [34]. Such findings support additional vaccine doses could provide an immunogenic "top-up" in immunosuppressed patients. Going forward, we propose a comparative evaluation of assessing vaccine immunogenicity between convalescent and infection-naïve vaccinated immunosuppressed patients. Findings from these studies could provide evidence-based data for optimal vaccine type and dosing schedule in these patients.

All study participants, except HC-infection naïve, had prior natural infection, where unvaccinated (UNVACC) had the highest T-cell non-responders ($n = 3$) to S-peptide stimulation. Interestingly, one UNVACC T-cell non-responder was also seronegative for anti-spike antibodies. This participant represented a house-hold case of COVID-19; with positive real-time polymerase chain-reaction nasopharyngeal result, and no significant medical history. We speculate one of two reasonings; firstly, this could represent a case of natural waning immunity, or, secondly, a false-positive result. We believe the latter is unlikely, as the house-hold contact was tested in our study and had detectable serology and T-cell response. Furthermore, all but one HC participant had no detectable T-cell responses. One HC participant had a weak response of 3 SFU to S-peptide. We favour two hypothetical models which could explain this. Firstly, this result could represent a cross-reaction with other six human pathogenic coronaviruses [38]. Secondly, as these were healthcare workers, both occupational and household exposure could evoke very low concentration of SARS-CoV-2, which may be insufficient to elicit a B-cell response but may induce a T-cell response.

Investigating the CD4⁺ and CD8⁺ vaccine-induced landscape highlighted key differences between VACC-IC and VACC-IS. Firstly, whilst no significant differences in CD4⁺ surface activation markers (CD38 and HLA-DR) were observed between VACC-IC and VACC-IS, the abundance of the dominant metacluster population were reduced in VACC-IS. Similarly, reduction of metacluster abundance were identified in VACC-IS CD8⁺, however, with notable differences in T-cell activation marker expression. Over 40% of VACC-IC CD8⁺ metaclusters depicted dual HLA-DR⁺CD38⁺ expression with elevated levels of CD8⁺ T_e (CD45RA⁺CD197⁺CD27⁺CD28⁺) cells post-S-peptide stimulation. Such finding is consistent with prior studies which have highlighted terminal effector T-cells overexpress the activation markers CD38 and HLA-DR [39]. However, the same metaclusters were identified as HLA-DR⁺CD38^{wk} in VACC-IS. Moreover, VACC-IS demonstrated a greater increase in CD8⁺ T_{em} (CD45RA⁺CD197⁺CD27^{+/−}CD28[−]CD57⁺) subsets compared to VACC-IC. Such findings may explain the increased levels of IFN γ secreting SARS-CoV-2 specific T-cells observed in our ELISpot data;

as CD8 + T_{em} have shown to secrete the greatest IFN γ levels compared to other T-cell memory subsets [40].

Similar findings have been reported in a recent study investigating vaccine-induced response in multiple sclerosis patients on anti-CD20 therapy [41]. No differences in T-cell activation were seen in CD4⁺ compartments post-vaccination in both healthy and MS-patient cohorts. However, CD8⁺ HLA-DR⁺CD38^{wk} metaclusters were seen in MS patients, which were predominantly of the T_{em} subset in line with our findings in VACC-IS cohort. The authors concluded such findings in MS-patients are indicative of a robust CD8⁺ T-cell response compared to healthy controls. However, we hypothesise the lack of CD4⁺ T-follicular helper cells and vaccine-induced antibodies could have preferentially driven and augmented CD8⁺ T-cell responses. Whilst these findings are encouraging, we believe extensive deep-immune profiling comprising a broader range of immunosuppressed patients are required to achieve a definitive illustration of vaccine-induced T-cell responses. We propose undertaking activation-induced marker (AIM) assays on CD4⁺ and CD8⁺ antigenic specific cells. Such experimental design may provide in-depth information surrounding CD4⁺ T-cell priming by examining co-expression of CD200 and CD40L. Functional CD8⁺ T-cell responses can be investigated through examining IFN γ , TNF α , IL-2 and granzyme B expression. Such functionality could be correlated with polyfunctional status of SARS-CoV-2 specific T-cells as it remains unclear whether mono- or polyfunctional T-cells are of greater protective value [25].

Our study has some limitations. We are aware of the small sample size in this study, which makes it challenging to draw firm conclusions. Moreover, demographical risk factors for COVID-19, such as age, BMI and ethnicity [42] were not controlled for. This was mainly due to patient enrolment was restricted only within Portsmouth NHS trust; therefore, limited patients were available for enrolment who satisfied the requirements of being both convalescence and vaccinated. We suggest a multi-site recruitment from a diverse set of clinical specialities, which would enhance sample power and lead to fewer demographic differences between cohorts. Regardless the small sample size, we have demonstrated vaccine responses and immunophenotypic landscape can be successfully derived using the methodologies within this study and provides a blueprint for larger-scale multi-site studies. Secondly, we were only able to re-bleed a small proportion of our VACC-IC and VACC-IS study participants for T-cell subset analysis. Going forward, we propose to extend the T-cell subset panel along with drop-in markers of activation and proliferation (such as Ki-67) to validate these findings. This would provide a more detailed phenotypic landscape of T-cell memory subsets found in vaccinated healthy and immunosuppressed cohorts.

Overall, our data confirms an immunological response to SARS-CoV-2 vaccines in immunosuppressed patients, when assessed by combination of cellular and serological assays. The observed vaccine-induced responses within this immunosuppressed cohort were comparable to healthy vaccinated participants. Furthermore, our data highlights the robust and broad capacities of SARS-CoV-2 specific T-cells. Further work is required to decipher these responses with the continual emergence of global SARS-CoV-2 variants of concerns. Our findings warrant further work correlating the observed immunological responses with protective immunity and evaluate if longevity of these responses is comparable to healthy individuals. Such information may aid development of a standardised immunisation schedule required to optimise the vaccine-induced responses observed in this clinically vulnerable patient group.

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Institutional Review Board Statement

Study proposal was evaluated and approved by Portsmouth NHS Trust Research Ethics Committee (Study reference: 21/BPR10029) and national Health Research Authority ethics committee (London-City and East research ethics committee, IRAS: 291009).

Data Availability Statement

Data available upon request.

CRedit authorship contribution statement

Dinesh Mohanraj: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Samuel Baldwin:** Investigation, Data curation. **Satbeer Singh:** Validation, Resources. **Alun Gordon:** Validation. **Alison Whitelegg:** Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cellimm.2022.104501>.

References

- [1] P. Zhou, X.-L. Yang, X.-G. Wang, B. Hu, L. Zhang, W. Zhang, H.-R. Si, Y. Zhu, B. Li, C.-L. Huang, H.-D. Chen, J. Chen, Y. Luo, H. Guo, R.-D. Jiang, M.-Q. Liu, Y. Chen, X.-R. Shen, X.-i. Wang, X.-S. Zheng, K. Zhao, Q.-J. Chen, F. Deng, L.-L. Liu, B. Yan, F.-X. Zhan, Y.-Y. Wang, G.-F. Xiao, Z.-L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, *Nature* 579 (7798) (2020) 270–273, <https://doi.org/10.1038/s41586-020-2012-7>.
- [2] Z. Wu, J.M. McGoogan, Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention, *JAMA* 323 (13) (2020) 1239–1242, <https://doi.org/10.1001/jama.2020.2648>.
- [3] J. Keehner, L.E. Horton, M.A. Pfeffer, C.A. Longhurst, R.T. Schooley, J.S. Currier, S. R. Abeles, F.J. Torriani, SARS-CoV-2 Infection after Vaccination in Health Care Workers in California, *The New England journal of medicine* 384 (18) (2021) 1774–1775, <https://doi.org/10.1056/NEJMc2101927>.
- [4] M. Levine-Tiefenbrun, I. Yelin, R. Katz, E. Herzal, Z. Golan, L. Schreiber, T. Wolf, V. Nadler, A. Ben-Tov, J. Kuint, S. Gazit, T. Patalon, G. Chodick, R. Kishony, Initial report of decreased SARS-CoV-2 viral load after inoculation with the BNT162b2 vaccine, *Nature medicine* 27 (5) (2021) 790–792, <https://doi.org/10.1038/s41591-021-01316-7>.
- [5] F.P. Polack, S.J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J. L. Perez, G. Pérez Marc, E.D. Moreira, C. Zerbini, R. Bailey, K.A. Swanson, S. Roychoudhury, K. Koury, P. Li, W.V. Kalina, D. Cooper, R.W. Frenck, L. L. Hammitt, Ö. Türeci, H. Nell, A. Schaefer, S. Ünal, D.B. Tresnan, S. Mather, P. R. Dormitzer, U. Şahin, K.U. Jansen, W.C. Gruber, Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine, *The New England journal of medicine* 383 (27) (2020) 2603–2615, <https://doi.org/10.1056/NEJMoa2034577>.
- [6] L.R. Baden, H.M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, D. Diemert, S. A. Spector, N. Roupachel, C.B. Creech, J. McGettigan, S. Khetan, N. Segall, J. Solis, A. Brosz, C. Fierro, H. Schwartz, K. Neuzil, L. Corey, P. Gilbert, H. Janes, D. Follmann, M. Marovich, J. Mascola, L. Polakowski, J. Ledgerwood, B.S. Graham, H. Bennett, R. Pajon, C. Knightly, B. Leav, W. Deng, H. Zhou, S. Han, M. Ivarsson, J. Miller, T. Zaks, Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine, *The New England journal of medicine* 384 (5) (2021) 403–416, <https://doi.org/10.1056/NEJMoa2035389>.
- [7] D.L. Longo, M.C. Castells, E.J. Phillips, Maintaining Safety with SARS-CoV-2 Vaccines, *The New England journal of medicine* 384 (7) (2021) 643–649, <https://doi.org/10.1056/NEJMra2035343>.
- [8] E.E. Walsh, R.W. Frenck, A.R. Falsey, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, K. Neuzil, M.J. Mulligan, R. Bailey, K.A. Swanson, P. Li, K. Koury, W. Kalina, D. Cooper, C. Fontes-Garfias, P.-Y. Shi, Ö. Türeci, K.R. Tompkins, K. E. Lyke, V. Raabe, P.R. Dormitzer, K.U. Jansen, U. Şahin, W.C. Gruber, Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates, *The New England journal of medicine* 383 (25) (2020) 2439–2450, <https://doi.org/10.1056/NEJMoa2027906>.
- [9] K.M. Krueger, M.G. Ison, C. Ghossein, Practical Guide to Vaccination in All Stages of CKD, Including Patients Treated by Dialysis or Kidney Transplantation, *American journal of kidney diseases : the official journal of the National Kidney Foundation* 75 (3) (2020) 417–425, <https://doi.org/10.1053/j.ajkd.2019.06.014>.
- [10] C. Hua, T. Barnetche, B. Combe, J. Morel, Effect of methotrexate, anti-tumor necrosis factor α , and rituximab on the immune response to influenza and pneumococcal vaccines in patients with rheumatoid arthritis: a systematic review and meta-analysis, *Arthritis care & research* 67 (7) (2014) 1016–1026, <https://doi.org/10.1002/acr.22246>.
- [11] E.J. Williamson, A.J. Walker, K. Bhaskaran, S. Bacon, C.E. Morton, H. J. Curtis, A. Mehrkar, D. Evans, P. Inglesby, J. Cockburn, H.I. McDonald, B. MacKenna, L. Tomlinson, I.J. Douglas, C.T. Rentsch, R. Mathur, A.Y.S. Wong, R. Grieve, D. Harrison, H. Forbes, A. Schultze, R. Croker, J. Parry, F. Hester, S. Harper, R. Perera, S.J.W. Evans, L. Smeeth, B. Goldacre, Factors associated with COVID-19-related death using OpenSAFELY, *Nature* 584 (7821) (2020) 430–436, <https://doi.org/10.1038/s41586-020-2521-4>.
- [12] O. Thanaat, C. Legeai, D. Anglicheau, L. Couzi, G. Blanche, M. Hazzan, M. Pastural, E. Savoye, F. Bayer, E. Morelon, Y. Le Meur, O. Bastien, S. Caillard, O. Thanaat, E. Morelon, C. Levi, F. Buron, A. Koenig, T. Barba, S. Caillard, B. Moulin, S. Fafi-Kremer, M. Hazzan, A. Dany, A. Hertig, J. Tourret, B. Barrou, L. Couzi, P. Merville, A. Kaminski, V. Moal, T. Legris, P.-F. Westeel, M. Jauregui, L. Frimat, D. Ducloux, J. Bamouid, D. Bertrand, M. Tsimaratos, F. Garaix-Gilardo, J. Dumortier, S. Mussot, A. Roux, L. Sebbag, Y. Le Meur, G. Blanche, C. Masset, N. Kamar, H. Francois, E. Rondeau, N. Bouvier, C. Mousson, M. Buchler, P. Gatault, J.-F. Augusto, A. Duveau, C. Vigneau, M.-C. Morin, J. Chemouny, L. Golbin, P. Frimbert, M. Matignon, A. Durrbach, C. Greze, R. Snanoudj, C. Colosio, B. Schvartz, P. Malvezzi, C. Mariat, A. Thierry, M. Le Quintrec, A. Sicard, J. P. Rerolle, A.-É. Heng, C. Garrouste, H.V. Coponat, É. Epailly, O. Brugiere, S. Dharancy, É. Salame, F. Saliba, IMPact of the COVID-19 epidemic on the mortality of kidney transplant recipients and candidates in a French Nationwide registry sTudy (IMPORTANT), *Kidney international* 98 (6) (2020) 1568–1577, <https://doi.org/10.1016/j.kint.2020.10.008>.
- [13] K.J. Jager, A. Kramer, N.C. Chesnaye, C. Couchoud, J.E. Sánchez-Álvarez, L. Garneata, F. Collart, M.H. Hemmeler, P. Ambühl, J. Kerschbaum, C. Legeai, Y. Del Pino, M.D. Pino, G. Mircescu, L. Mazzoleni, T. Hoekstra, R. Winzeler, G. Mayer, V.S. Stel, C. Wanner, C. Zoccali, Z.A. Massy, Results from the ERA-EDTA Registry indicate a high mortality due to COVID-19 in dialysis patients and kidney transplant recipients across Europe, *Kidney international* 98 (6) (2020) 1540–1548, <https://doi.org/10.1016/j.kint.2020.09.006>.
- [14] M.S. Baek, M.-T. Lee, W.-Y. Kim, J.C. Choi, S.-Y. Jung, S. Stepkowski, COVID-19-related outcomes in immunocompromised patients: A nationwide study in Korea, *PLoS one* 16 (10) (2021) e0257641, <https://doi.org/10.1371/journal.pone.0257641>.
- [15] J. Wei, J. Zhao, M. Han, F. Meng, J. Zhou, SARS-CoV-2 infection in immunocompromised patients: humoral versus cell-mediated immunity, *Journal for immunotherapy of cancer* 8 (2) (2020) e000862, <https://doi.org/10.1136/jitc-2020-000862>.
- [16] B. Choi, M.C. Choudhary, J. Regan, J.A. Sparks, R.F. Padera, X. Qiu, I.H. Solomon, H.-H. Kuo, J. Boucau, K. Bowman, U.D. Adhikari, M.L. Winkler, A.A. Mueller, T.-T. Hsu, M. Desjardins, L.R. Baden, B.T. Chan, B.D. Walker, M. Lichterfeld, M. Brigg, D.S. Kwon, S. Kanjilal, E.T. Richardson, A.H. Jonsson, G. Alter, A.K. Barczak, W. P. Hanage, X.G. Yu, G.D. Gaiha, M.S. Seaman, M. Cernadas, J.Z. Li, Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host, *The New England journal of medicine* 383 (23) (2020) 2291–2293, <https://doi.org/10.1056/NEJMc2031364>.
- [17] S.A. Kemp, D.A. Collier, R.P. Datt, I.A.T.M. Ferreira, S. Gayed, A. Jahun, M. Hosmillo, C. Rees-Spear, P. Mlcochova, I.U. Lumb, D.J. Roberts, A. Chandra, N. Temperton, K. Sharrocks, E. Blane, Y. Modis, K.E. Leigh, J.A.G. Briggs, M.J. van Gils, K.G.C. Smith, J.R. Bradley, C. Smith, R. Doffinger, L. Ceron-Gutierrez, G. Barcenas-Morales, D.D. Pollock, R.A. Goldstein, A. Smielewska, J.P. Skittrall, T. Gouliouris, I.G. Goodfellow, E. Gkrania-Klotsas, C.J.R. Illingworth, L.E. McCoy, R.K. Gupta, SARS-CoV-2 evolution during treatment of chronic infection, *Nature* 592 (7853) (2021) 277–282, <https://doi.org/10.1038/s41586-021-03291-y>.
- [18] A. Addetia, K. Crawford, A. Dingens, H. Zhu, P. Roychoudhury, M.L. Huang, K. R. Jerome, J.D. Bloom, A.L. Greninger, Neutralizing Antibodies Correlate with Protection from SARS-CoV-2 in Humans during a Fishery Vessel Outbreak with a High Attack Rate, *Journal of clinical microbiology* 58 (11) (2020) e02107–e2120, <https://doi.org/10.1128/JCM.02107-20>.
- [19] S.F. Lumley, D. O'Donnell, N.E. Stoesser, P.C. Matthews, A. Howarth, S.B. Hatch, B. D. Marsden, S. Cox, T. James, F. Warren, L.J. Peck, T.G. Ritter, Z. de Toledo, L. Warren, D. Axten, R.J. Cornall, E.Y. Jones, D.I. Stuart, G. Sreaton, D. Ebner, S. Hoosdally, M. Chand, D.W. Crook, A.-M. O'Donnell, C.P. Conlon, K.B. Pouwels, A.S. Walker, T.E.A. Peto, S. Hopkins, T.M. Walker, K. Jeffery, D.W. Eyre, Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers, *The New England journal of medicine* 384 (6) (2021) 533–540, <https://doi.org/10.1056/NEJMoa2034545>.
- [20] N. Okba, M.A. Müller, W. Li, C. Wang, C.H. GeurtsvanKessel, V.M. Corman, M. M. Lamers, R.S. Sikkema, E. de Bruin, F.D. Chandler, Y. Yazdanpanah, Q. Le Hingrat, D. Descamps, N. Houhou-Fidouh, C. Reusken, B.J. Bosch, C. Drosten, M. Koopmans, B.L. Haagmans, Severe Acute Respiratory Syndrome Coronavirus 2-

- Specific Antibody Responses in Coronavirus Disease Patients, *Emerging infectious diseases* 26 (7) (2020) 1478–1488, <https://doi.org/10.3201/eid2607.200841>.
- [21] N. Post, D. Eddy, C. Huntley, M.C.I. van Schalkwyk, M. Shrotri, D. Leeman, S. Piva, S.V. Williams, W.H. Bermingham, P. Kellam, J. Maher, A.M. Shields, G. Amirhalingam, S.J. Peacock, S.A. Ismail, N.J. Mantis, Antibody response to SARS-CoV-2 infection in humans: A systematic review, *PLoS one* 15 (12) (2020) e0244126, <https://doi.org/10.1371/journal.pone.0244126>.
- [22] A. Grifoni, D. Weiskopf, S.I. Ramirez, J. Mateus, J.M. Dan, C.R. Moderbacher, S. A. Rawlings, A. Sutherland, L. Premkumar, R.S. Jodi, D. Marrama, A.M. de Silva, A. Frazier, A.F. Carlin, J.A. Greenbaum, B. Peters, F. Krammer, D.M. Smith, S. Crotty, A. Sette, Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals, *Cell* 181 (7) (2020) 1489–1501.e15, <https://doi.org/10.1016/j.cell.2020.05.015>.
- [23] M. Liao, Y. Liu, J. Yuan, Y. Wen, G. Xu, J. Zhao, L. Cheng, J. Li, X. Wang, F. Wang, L. Liu, I. Amit, S. Zhang, Z. Zhang, Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19, *Nature medicine* 26 (6) (2020) 842–844, <https://doi.org/10.1038/s41591-020-0901-9>.
- [24] J.M. Dan, J. Mateus, Y. Kato, K.M. Hastie, E.D. Yu, C.E. Faliti, A. Grifoni, S. I. Ramirez, S. Haupt, A. Frazier, C. Nakao, V. Rayaprolu, S.A. Rawlings, B. Peters, F. Krammer, V. Simon, E.O. Saphire, D.M. Smith, D. Weiskopf, A. Sette, S. Crotty, Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection, *Science (New York, N.Y.)* 371 (6529) (2021) eabf4063, <https://doi.org/10.1126/science.abf4063>.
- [25] K.J. Ewer, J.R. Barrett, S. Belij-Rammerstorfer, H. Sharpe, R. Makinson, R. Mörter, A. Flaxman, D. Wright, D. Bellamy, M. Bittaye, C. Dold, N.M. Provine, J. Aboagye, J. Fowler, S.E. Silk, J. Alderson, P.K. Aley, B. Angus, E. Berrie, S. Bibi, P. Cicconi, E. A. Clutterbuck, I. Chelysheva, P.M. Folegatti, M. Fuskova, C.M. Green, D. Jenkin, S. Kerridge, A. Lawrie, A.M. Minassian, M. Moore, Y. Mujajidi, E. Plested, I. Poulton, M.N. Ramasamy, H. Robinson, R. Song, M.D. Snape, R. Tarrant, M. Voysey, M.E.E. Watson, A.D. Douglas, A.V.S. Hill, S.C. Gilbert, A.J. Pollard, T. Lambe, A. Ali, E. Allen, M. Baker, E. Barnes, N. Borthwick, A. Boyd, C. Brown-O'Sullivan, J. Burgoyne, N. Byard, I.C. Puig, F. Cappuccini, J.-S. Cho, P. Cicconi, E. Clark, W.E.M. Crocker, M.S. Dattoo, H. Davies, F.R. Donnellan, S.J. Dunachie, N. J. Edwards, S.C. Elias, J. Furze, C. Gilbride, G. Gorini, G. Gupta, S.A. Harris, S.H. C. Hodgson, M.M. Hou, S. Jackson, K. Jones, R. Kailath, L. King, C.W. Larkworthy, Y. Li, A.M. Lias, A. Linder, S. Lipworth, R.L. Ramon, M. Madhavan, E. Marlow, J. L. Marshall, A.J. Mentzer, H. Morrison, N. Moya, E. Mukhopadhyay, A. Noé, F. L. Nugent, D. Pipini, D. Pulido-Gomez, F.R. Lopez, A.J. Ritchie, I. Rudiansyah, S. Salvador, H. Sanders, I. Satti, A. Shea, S. Silk, A.J. Spencer, R. Tanner, I. J. Taylor, Y. Themistocleous, M. Thomas, N. Tran, A. Truby, C. Turner, N. Turner, M. Ulaszewska, A.T. Worth, L. Kingham-Page, M.P.P. Alvarez, R. Anslow, L. Bates, K. Beadon, R. Beckley, A. Beveridge, E.M. Bijker, L. Blackwell, J. Burbage, S. Camara, M. Carr, R. Colin-Jones, R. Cooper, C.J. Cunningham, T. Demissie, C. D. Maso, N. Douglas, R. Drake-Brockman, R.E. Drury, K.R.W. Emary, S. Felle, S. Feng, C.F.D. Silva, K.J. Ford, E. Francis, L. Gracie, J. Hamlyn, B. Hanumanthadu, D. Harrison, T.C. Hart, S. Hawkins, J. Hill, E. Howe, N. Howell, E. Jones, J. Keen, S. Kelly, D. Kerr, L. Khan, J. Kinch, S. Koleva, E.A. Lees, A. Lelliott, X. Liu, N. G. Marchevsky, S. Marinou, J. McEwan, E. Morey, G. Morshead, J. Muller, C. Munro, S. Murphy, P. Mweu, E. Nuthall, K. O'Brien, D. O'Connor, P.J. O'Reilly, B. Oguti, P. Osborne, N. Owino, K. Parker, K. Pfafferott, D. Phillips, S. Provtsgaard-Morys, H. Ratcliffe, T. Rawlinson, S. Rhead, H. Roberts, K. Sanders, L. Silva-Reyes, C.S. Rollier, C.C. Smith, D.J. Smith, L. Stockdale, A. Szigeti, T.M. Thomas, A. Thompson, A. Tomic, S. Tonks, R. Varughese, M.K. Verheul, I. Vichos, L. Walker, C. White, R. White, X.L. Yao, C.P. Conlon, J. Frater, L. Cifuentes, I. Baleanu, E. Bolam, E. Boland, T. Brenner, B.E. Damratowski, C. Datta, O.E. Muhanna, R. Fisher, P. Galian-Rubio, G. Hodges, F. Jackson, S. Liu, L. Loew, R. Morgans, S. J. Morris, V. Olchawski, C. Oliveria, H. Parracho, E.R. Pabon, A. Tahiri-Alaoui, K. Taylor, P. Williams, D. Zizi, E.H. Arbe-Barnes, P. Baker, A. Batten, C. Downing, J. Drake, M.R. English, J.A. Henry, P. Iveson, A. Killen, T.B. King, J.P.J. Larwood, G. Mallett, K. Mansatta, N. Mirtorabi, M. Patrick-Smith, J. Perring, K. Radia, S. Roche, E. Schofield, R.t.W. Naude, J. Towner, N. Baker, K.R. Bewley, E. Brunt, K. R. Buttigieg, M.W. Carroll, S. Charlton, N.S. Coombes, M.J. Elmore, K. Godwin, B. Hallis, D. Knott, L. McInroy, I. Shaik, K. Thomas, J.A. Tree, C.L. Blundell, M. Cao, D. Kelly, A. Schmid, D.T. Skelly, A. Themistocleous, T. Dong, S. Field, E. Hamilton, E. Kelly, P. Klenerman, J.C. Knight, Y. Lie, C. Petropoulos, C. Sedik, T. Wrin, G. Meddaugh, Y. Peng, G. Sreaton, E. Stafford, T cell and antibody responses induced by a single dose of ChAdOx1 nCoV-19 (AZD1222) vaccine in a phase 1/2 clinical trial, *Nature medicine* 27 (2) (2021) 270–278, <https://doi.org/10.1038/s41591-020-01194-5>.
- [26] U. Sahin, A. Muik, E. Derhovanessian, I. Vogler, L.M. Kranz, M. Vormehr, A. Baum, K. Pascal, J. Quandt, D. Maurus, S. Brachtendorf, V. Lörks, J. Sikorski, R. Hilker, D. Becker, A.-K. Eller, J. Grützner, C. Boesler, C. Rosenbaum, M.-C. Kühnle, U. Luxemburger, A. Kemmer-Brück, D. Langer, M. Bexon, S. Bolte, K. Karikó, T. Palanche, B. Fischer, A. Schultz, P.-Y. Shi, C. Fontes-Garfias, J.L. Perez, K. A. Swanson, J. Loschko, L.L. Scully, M. Cutler, W. Kalina, C.A. Kyratoussou, D. Cooper, P.R. Dormitzer, K.U. Jansen, Ö. Türeci, COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses, *Nature* 586 (7830) (2020) 594–599, <https://doi.org/10.1038/s41586-020-2814-7>.
- [27] F. Tang, Y. Quan, Z.-T. Xin, J. Wrarmert, M.-J. Ma, H. Lv, T.-B. Wang, H. Yang, J. H. Richardus, W. Liu, W.-C. Cao, Lack of Peripheral Memory B Cell Responses in Recovered Patients with Severe Acute Respiratory Syndrome: A Six-Year Follow-Up Study, *J. I.* 186 (12) (2011) 7264–7268.
- [28] J.C. Marshall, S. Murthy, J. Diaz, N.K. Adhikari, D.C. Angus, Y.M. Arabi, K. Baillie, M. Bauer, S. Berry, B. Blackwood, M. Bonten, F. Bozza, F. Brunckhorst, A. Cheng, M. Clarke, V.Q. Dat, M. de Jong, J. Denholm, L. Derde, J. Dunning, X. Feng, T. Fletcher, N. Foster, R. Fowler, N. Gobat, C. Gomersall, A. Gordon, T. Glueck, M. Harhay, C. Hodgson, P. Horby, YaeJean Kim, R. Kojan, B. Kumar, J. Laffey, D. Malvey, I. Martin-Loeches, C. McArthur, D. McAuley, S. McBride, S. McGuinness, L. Merson, S. Morpeth, D. Needham, M. Netea, M.-D. Oh, S. Phyu, S. Piva, R. Qiu, H. Salisu-Kabara, L. Shi, N. Shimizu, J. Sinclair, S. Tong, A. Turgeon, T. Uyeki, F. van de Veerdonk, S. Webb, P. Williamson, T. Wolf, J. Zhang, A minimal common outcome measure set for COVID-19 clinical research, *The Lancet. Infectious diseases* 20 (8) (2020) e192–e197, [https://doi.org/10.1016/S1473-3099\(20\)30483-7](https://doi.org/10.1016/S1473-3099(20)30483-7).
- [29] Van Gassen, S., Callebaut, B., Van Helden, M. J., Lambrecht, B. N., Demeester, P., Dhaene, T., & Saey, Y. (2015). FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data. *Cytometry, Part A: the journal of the International Society for Analytical Cytology*, 87(7), 636–645. <https://doi.org/10.1002/cyto.a.22625>.
- [30] S. De Biasi, M. Meschiar, L. Gibellini, C. Bellinazzi, R. Borella, L. Fidanza, L. Gozzi, A. Iannone, D. Lo Tartaro, M. Mattioli, A. Paolini, M. Menozzi, J. Milic, G. Franceschi, R. Fantini, R. Tonelli, M. Sita, M. Sarti, T. Trenti, L. Brugioni, L. Cicchetti, F. Facchinetti, A. Pietrangelo, E. Clini, M. Girardis, G. Guaraldi, C. Mussini, A. Cossarizza, Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia, *Nature communications* 11 (1) (2020), <https://doi.org/10.1038/s41467-020-17292-4>.
- [31] J. Zuo, A.C. Dowell, H. Pearce, K. Verma, H.M. Long, J. Begum, F. Aiano, Z. Amin-Chowdhury, K. Hoschler, T. Brooks, S. Taylor, J. Hewson, B. Hallis, L. Stapley, R. Borrow, E. Linley, S. Ahmad, B. Parker, A. Horsley, G. Amirhalingam, K. Brown, M.E. Ramsay, S. Ladhani, P. Moss, Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection, *Nature immunology* 22 (5) (2021) 620–626, <https://doi.org/10.1038/s41590-021-00902-8>.
- [32] N. Dagan, N. Barda, E. Kepten, O. Miron, S. Perchik, M.A. Katz, M.A. Hernán, M. Lipsitch, B. Reis, R.D. Balicer, BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting, *The New England journal of medicine* 384 (15) (2021) 1412–1423, <https://doi.org/10.1056/NEJMoa2101765>.
- [33] K.A. Birdwell, M.R. Kizler, E.C. Sannella, L. Wang, D.W. Byrne, T.A. Izkizler, P. F. Wright, Decreased antibody response to influenza vaccination in kidney transplant recipients: a prospective cohort study, *American journal of kidney diseases: the official journal of the National Kidney Foundation* 54 (1) (2009) 112–121, <https://doi.org/10.1053/j.ajkd.2008.09.023>.
- [34] M. Prendecki, C. Clarke, H. Edwards, S. McIntyre, P. Mortimer, S. Gleeson, P. Martin, T. Thomson, P. Randell, A. Shah, A. Singanayagam, L. Lightstone, A. Cox, P. Kelleher, M. Willicombe, S.P. McAdoo, Humoral and T-cell responses to SARS-CoV-2 vaccination in patients receiving immunosuppression, *Annals of the rheumatic diseases* 80 (10) (2021) 1322–1329, <https://doi.org/10.1136/annrheumdis-2021-220626>.
- [35] Kearns, Pamela., Siebert, Stefan., Willicombe, michelle., Gaskell, Charlotte., Kirkham, Amanda., Pirrie, Sarah., Bowden, Sarah., Magwaro, Sophia., Hughes, Ana., Lim, Zixiang., Dimitriadis, Stavros., Murray, Sam M., Marjot, Thomas., Win, Zay., Irwin, Sophie L., Meacham, Georgina., Richter, Alex G., Kelleher, Peter., Satsangi, Jack., Miller, Paul., Rea Daniel., Cook, Gordon., Turtle, Lance., Klenerman, Paul., Dunachie, Susanna., Basu, Neil., de Silva, Thushan L., Thomas, David., Barnes, Eleanor., Goodyear, Carl S., McInnes, Iain. (2021). *Examining the Immunological Effects of COVID-19 Vaccination in Patients with Conditions Potentially Leading to Diminished Immune Response Capacity – The OCTAVE Trial*. Available at SSRN: <https://ssrn.com/abstract=3910058> or <https://doi.org/10.2139/ssrn.3910058>.
- [36] L. Monin, A.G. Laing, M. Muñoz-Ruiz, D.R. McKenzie, I. del Molino del Barrio, T. Alaguthurai, C. Domingo-Vila, T.S. Hayday, C. Graham, J. Seow, S. Abdul-Jawad, S. Kamdar, E. Harvey-Jones, R. Graham, J. Cooper, M. Khan, J. Vidler, H. Kakkassery, S. Sinha, R. Davis, L. Dupont, I. Francos Quijorna, C. O'Brien-Gore, P.L. Lee, J. Eum, M. Conde Poole, M. Joseph, D. Davies, Y. Wu, A. Swampillai, B. V. North, A. Montes, M. Harries, A. Rigg, J. Spicer, M.H. Malim, P. Fields, P. Patten, F. Di Rosa, S. Papa, T. Tree, K.J. Doores, A.C. Hayday, S. Irshad, Safety and immunogenicity of one versus two doses of the COVID-19 vaccine BNT162b2 for patients with cancer: interim analysis of a prospective observational study, *The Lancet. Oncology* 22 (6) (2021) 765–778, [https://doi.org/10.1016/S1470-2045\(21\)00213-8](https://doi.org/10.1016/S1470-2045(21)00213-8).
- [37] M.M. Bonelli, D. Mrak, T. Perkmann, H. Haslacher, D. Aletaha, SARS-CoV-2 vaccination in rituximab-treated patients: evidence for impaired humoral but inducible cellular immune response, *Annals of the rheumatic diseases* 80 (10) (2021) 1355–1356, <https://doi.org/10.1136/annrheumdis-2021-220408>.
- [38] D.S. Kim, S. Rowland-Jones, E. Gea-Mallorquí, Will SARS-CoV-2 Infection Elicit Long-Lasting Protective or Sterilising Immunity? Implications for Vaccine Strategies (2020), *Frontiers in immunology* 11 (2020), 571481, <https://doi.org/10.3389/fimmu.2020.571481>.
- [39] O.A. Odumade, J.A. Knight, D.O. Schmeling, D. Masopust, H.H. Balfour Jr, K. A. Hogquist, Primary Epstein-Barr virus infection does not erode preexisting CD8* T cell memory in humans, *The Journal of experimental medicine* 209 (3) (2012) 471–478, <https://doi.org/10.1084/jem.20112401>.
- [40] L. Gattinoni, E. Lugli, Y. Ji, Z. Pos, C.M. Paulos, M.F. Quigley, J.R. Almeida, E. Gostick, Z. Yu, C. Carpenito, E. Wang, D.C. Douek, D.A. Price, C.H. June, F. M. Marincola, M. Roederer, N.P. Restifo, A human memory T cell subset with stem

- cell-like properties, *Nature medicine* 17 (10) (2011) 1290–1297, <https://doi.org/10.1038/nm.2446>.
- [41] S.A. Apostolidis, M. Kakara, M.M. Painter, R.R. Goel, D. Mathew, K. Lenzi, A. Rezk, K.R. Patterson, D.A. Espinoza, J.C. Kadri, D.M. Markowitz, C. E. Markowitz, I. Mexhitaj, D. Jacobs, A. Babb, M.R. Betts, E.T.L. Prak, D. Weiskopf, A. Grifoni, K. A. Lundgreen, S. Gouma, A. Sette, P. Bates, S.E. Hensley, A.R. Greenplate, E. J. Wherry, R. Li, A. Bar-Or, Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy, *Nature medicine* 27 (11) (2021) 1990–2001, <https://doi.org/10.1038/s41591-021-01507-2>.
- [42] D. Wolff, S. Nee, N.S. Hickey, M. Marscholke, Risk factors for Covid-19 severity and fatality: a structured literature review, *Infection* 49 (1) (2021) 15–28, <https://doi.org/10.1007/s15010-020-01509->.