


## ORIGINAL PAPER

# Antibody and T-cell responses by ultra-deep T-cell receptor immunosequencing after COVID-19 vaccination in patients with plasma cell dyscrasias

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## Summary

We investigated antibody and coronavirus disease 2019 (COVID-19)-specific T-cell mediated responses via ultra-deep immunosequencing of the T-cell receptor (TCR) repertoire in patients with plasma cell dyscrasias (PCD). We identified 364 patients with PCD who underwent spike antibody testing using commercially available spike-receptor binding domain immunoglobulin G antibodies  $\geq 2$  weeks after completion of the initial two doses of mRNA vaccines or one dose of JNJ-78436735. A total of 56 patients underwent TCR immunosequencing after vaccination. Overall, 86% tested within 6 months of vaccination had detectable spike antibodies. Increasing age, use of anti-CD38 or anti-B-cell maturation antigen therapy, and receipt of BNT162b2 (vs. mRNA-1273) were associated with lower antibody titres. We observed an increased proportion of TCRs associated with surface glycoprotein regions of the COVID-19 genome after vaccination, consistent with spike-specific T-cell responses. The median spike-specific T-cell breadth was  $3.11 \times 10^{-5}$ , comparable to those in healthy populations after vaccination. Although spike-specific T-cell breadth correlated with antibody titres, patients without antibody responses also demonstrated spike-specific T-cell responses. Patients receiving mRNA-1273 had higher median spike-specific T-cell breadth than those receiving BNT162b2 ( $p = 0.01$ ). Although patients with PCD are often immunocompromised due to underlying disease and treatments, COVID-19 vaccination can still elicit humoral and T-cell responses and remain an important intervention in this patient population.

## KEYWORDS

COVID-19, immune, myeloma, T-cell receptor, vaccines, immunization

## INTRODUCTION

Vaccination against coronavirus disease 2019 (COVID-19) has been the main therapeutic strategy for preventing

infection and reducing mortality and morbidity from COVID-19 infection. Previous studies have demonstrated that patients with plasma cell dyscrasias (PCD), including multiple myeloma (MM), have suboptimal responses to

COVID-19 vaccination and increased incidence of severe disease and death upon COVID-19 infection.<sup>1–3</sup> Intrinsic immune dysregulation due to the underlying haematological malignancies, as well as treatment-related factors, are thought to play a role in reducing the effectiveness of vaccination in this patient population. Although antibody responses after vaccination in patients with PCD have been explored, assessment of COVID-19 specific T-cell responses after vaccination in this population has been less well-studied.

We report on our patient cohort with PCD that have undergone COVID-19 vaccination and serological evaluation for COVID-19 antibody responses. Furthermore, we investigate COVID-19 specific T-cell responses after vaccination using T-cell receptor (TCR) $\beta$  immunosequencing, which to our knowledge, has not been previously reported on in this patient population.

## METHODS

Patients treated at University of California San Francisco between January 2021 and December 2021 with a diagnosis of a PCD and who underwent COVID-19 vaccination and subsequent assessment of COVID-19 antibody responses were included in this study. Patient characteristics including age, gender, vaccine type, diagnosis, disease status, and current treatment (within 3 months of completing vaccine series) being received were assessed. Completion of vaccination series was defined as receiving two doses of mRNA vaccines (Moderna mRNA-1273 or Pfizer-BioNTech BNT162b2) or one dose of Johnson & Johnson's/Janssen vaccine (JNJ-78436735). The study population included patients on an Institutional Review Board (IRB)-approved COVID-19 antibody monitoring study, as well as patients identified on a retrospective analysis who had COVID-19 spike antibody testing checked as part of routine care. Patients tested for COVID-19 spike antibodies  $\geq 2$  weeks after vaccination were included. Serological responses were defined as 'positive' or 'negative' based on manufacturer specifications; assays used included DiaSorin LIAISON severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) S1/S2 immunoglobulin G (IgG), Abbott AdviseDx SARS-CoV-2 IgG II, Roche Elecsys Anti-SARS-CoV-2 S, and Siemens Healthcare Diagnostics Atellica IM SARS-CoV-2 IgG. Reference ranges for the assays are outlined in Table S1.

In a subset of patients, T-cell responses were assessed by immunosequencing the complementarity determining region 3 (CDR3) region of TCR $\beta$  using the immunoSEQ<sup>®</sup> Assay (Adaptive Biotechnologies) as previously described.<sup>4,5</sup> Briefly, extracted genomic DNA from peripheral blood samples was amplified by bias-controlled multiplex polymerase chain reaction, followed by high-throughput sequencing. Sequences were collapsed and filtered to identify and quantitate the absolute abundances of each unique TCR $\beta$  CDR3 region for further analysis.<sup>6</sup> The fraction of T-cells was calculated by normalising the TCR $\beta$  template count to the total number of cells sequenced on

the assay using a panel of reference genes found in all nucleated cells. TCR sequences were mapped against a set of TCR sequences known to react to SARS-CoV-2 by matching on V gene, amino acid and J gene. These sequences were previously identified by Multiplex Identification of T-cell Receptor Antigen Specificity (MIRA) experiments as previously described.<sup>7,8</sup> COVID-19-specific TCR sequences identified via MIRA correspond to antigen-specific addresses, which detail major histocompatibility complex class presentation and were used to correlate response with viral open reading frames (ORF) and cellular subsets (e.g., CD8 vs. CD4). Reactive TCRs were further screened for enrichment in a previously COVID-19-infected repertoire compared to COVID-19-negative repertoires collected as part of ImmuneCODE, a publicly available open database, to remove TCRs that may be very frequent or cross-reactive to common antigens. Responses were quantified by the number and/or frequency of COVID-19-specific TCRs after vaccination. Specifically, clonal breadth (defined as the proportion of distinct TCRs that are COVID-19 specific, calculated by the number of unique COVID-19-associated TCRs divided by the number of unique TCRs in the whole repertoire) and clonal depth (defined as the sum frequency of the COVID-19-specific TCRs in the repertoire, calculated by the total number of COVID-19-specific T cells divided by the total number of T cells in the sample) were determined for each sample. Clonal breadth, in particular, provides a measure of diversity and richness of T cells dedicated to COVID-19, and both of these measures have shown good discrimination between COVID-19 infected/vaccinated subjects and unexposed controls in previous studies.<sup>4,5</sup>

Fisher's exact tests and Kruskal–Wallis rank-sum tests were used to compare categorical and continuous variables respectively. Association between spike-specific T-cell breadth and COVID-19 spike antibody titres was evaluated using Spearman's correlation coefficient. Multivariate logistic regressions were used to evaluate the adjusted covariate effects on seroconversion. A two-sided  $p < 0.05$  was considered statistically significant. Statistical analysis was conducted using R (version 4.0.5; [www.r-project.org](http://www.r-project.org)). The study was approved by the University of California San Francisco IRB and was conducted in accordance with principles of the Declaration of Helsinki.

## RESULTS

### Serological testing after vaccination

A total of 364 patients with PCD underwent COVID-19 spike antibody serological testing, including 300 patients with MM (as defined by International Myeloma Working Group criteria). Population characteristics are summarised in Table 1.

Amongst the 341 patients that had COVID-19 spike antibody serological testing done within 6 months of completing the vaccination series, 294 patients (86%) were positive for

**TABLE 1** Baseline characteristics of the patients with plasma cell dyscrasias

	MM (N = 300)	Non-MM (N = 64)	<i>p</i>
Age at time of vaccination, years			0.804 <sup>a</sup>
Mean (SD)	67.0 (9.6)	67.6 (9.6)	
Median	68.3	67.0	
Range	31.7–85.1	33.9–88.3	
Gender, <i>n</i> (%)			1.000 <sup>b</sup>
Female	123 (41)	26 (41)	
Male	177 (59)	38 (59)	
Vaccine received, <i>n</i> (%)			0.704 <sup>b</sup>
JNJ-78436735	7 (2)	2 (3)	
mRNA-1273	153 (51)	30 (47)	
BNT162b2	140 (47)	32 (50)	
Diagnosis, <i>n</i> (%)			<0.001 <sup>b</sup>
MM	294 (98)	0 (0)	
AL	6 (2) <sup>c</sup>	33 (52)	
SMM/MGUS	0 (0)	19 (30)	
WM	0 (0)	5 (8)	
Other <sup>d</sup>	0 (0)	7 (11)	
Number of lines of prior therapies			<0.001 <sup>a</sup>
Mean (SD)	3.0 (2.7)	1.2 (1.2)	
Median	2.0	1.0	
Range	0.0–14.0	0.0–6.0	
COVID-19 infection, <i>n</i> (%)			0.810 <sup>b</sup>
No	274 (91)	58 (91)	
Yes	26 (9)	6 (9)	

Abbreviations: AL, light-chain amyloidosis; COVID-19, coronavirus disease 2019; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; SMM, smouldering multiple myeloma; WM, Waldenström macroglobulinaemia.

<sup>a</sup>Kruskal–Wallis rank-sum test.

<sup>b</sup>Fisher's exact test for count data.

<sup>c</sup>Represents patients with concurrent MM and AL.

<sup>d</sup>Includes POEMS syndrome (Peripheral sensorimotor neuropathy, Organomegaly, Endocrinopathy, Monoclonal Gammopathy and Skin lesions) and monoclonal gammopathy of renal significance.

COVID-19 spike antibodies. The median time from completing vaccination to antibody testing was 2.4 months. The proportion of patients with COVID-19 spike positivity varied according to plasma cell diagnosis: MM 85.9%, light-chain amyloidosis 93.9%, smouldering MM/monoclonal gammopathy of undetermined significance 93.3%, Waldenström macroglobulinaemia 25%, and other PCD 71.4% ( $p = 0.01$ ). After the two-dose series, mRNA-1273 was associated with a higher seroconversion rate when compared to BNT162b2 (90.1% vs. 81.5%,  $p = 0.03$ ), as well as higher antibody titre levels (Abbott AdviseDx SARS-COV-2 IgG II median titres: 1345.7 vs. 309,  $p = 0.02$ ; Roche Elecsys Anti-SARS-CoV-2 S titres: 748 vs. 162,  $p = 0.047$ ) (Figure 1A).

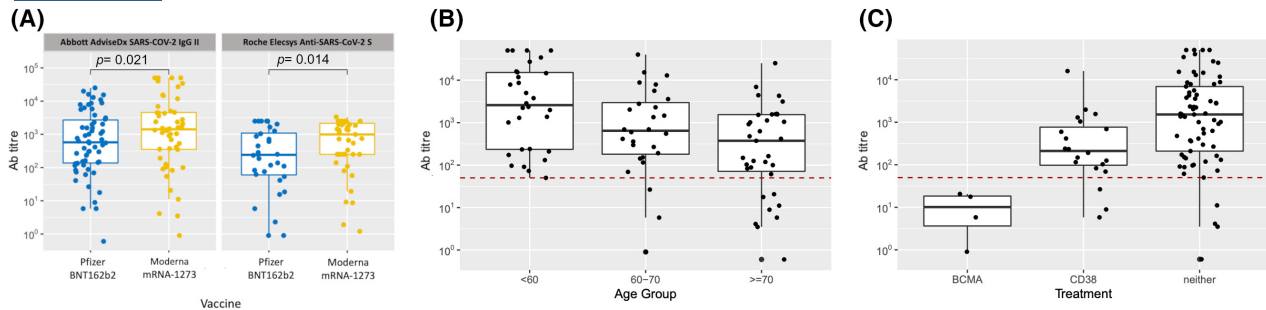
A total of 300 patients with MM were included, with disease and treatment characteristics summarised in Table 2. Among the newly diagnosed and those undergoing first-line therapy, the response rate was 92.8% compared to 79.2% for the relapsed/refractory patients ( $p = 0.001$ ). Furthermore, patients with an ongoing response (complete response [CR]/very good partial response [VGPR]/partial response [PR]) were more likely to have a positive antibody response compared to those with stable disease/progressive disease (89.2% vs. 73.8%,  $p = 0.006$ ). Age strongly influenced antibody response rates: the antibody positivity rates were 100%, 88.6%, and 76.9% for patients who were aged <60, 60–70 and >70 years respectively ( $p < 0.001$ ), with declining antibody titres with increasing age (Figure 1B). Patients receiving intravenous Ig (IVIg) had lower rates of serological response (66.7% vs. 87.6%;  $p = 0.01$ ) than those who did not require IVIg, and those with non-IgG MM who had IgG levels <4 g/l also had lower rates of seroconversion (75% vs. 100%,  $p = < 0.001$ ).

Patients receiving B-cell maturation antigen (BCMA)-directed therapy (belantamab mafadotin or bispecific BCMA T-cell engagers within 3 months or BCMA chimeric antigen receptor T-cell therapy within 6 months of vaccination completion) had significantly reduced rates of COVID-19 seroconversion compared to those not receiving BCMA therapies (33.3% vs. 88.8%,  $p < 0.001$ ). Patients receiving immunomodulatory imide drugs (IMiDs; lenalidomide or pomalidomide), on the other hand, appeared to have favourable rates of antibody responses (91.3% vs. 79.9% for those not receiving IMiDs,  $p = 0.006$ ). There was trend towards lower seroconversion rates for those receiving anti-CD38 treatment compared to those who were not (79.5% vs. 88.1%;  $p = 0.080$ ). There were no significant differences in COVID-19 seropositivity when stratified by steroid exposure or proteasome inhibitor (PI) exposure. With regards to antibody titres, patients receiving anti-CD38 therapy and anti-BCMA therapies had lower titres when compared to those not receiving targeted therapies (Figure 1C).

Multivariate logistic regression analysis suggested increasing age at vaccination, the use of anti-BCMA therapy and receipt of BNT162b2 (as opposed to mRNA-1273) were significantly associated with lower rates of seroconversion (odds ratios 0.91, 0.074 and 0.36 respectively,  $p < 0.05$ ), while the adjusted effect of disease characteristics and exposure to IMiDs, PI, and anti-CD38 therapy did not reach statistical significance (Table S2).

### Response rates after booster and/or after third vaccination

Among those with MM, 280 patients underwent third vaccination or booster and 120 had antibody response assessed afterwards. In all, 93% of patients had a positive antibody response. Among the 29 patients who previously had negative antibody responses within 6 months after vaccination completion, 75.9% achieved an antibody response after receiving



**FIGURE 1** COVID-19 antibody titres for mRNA-1273 versus BNT162b2 (A). COVID-19 antibody titres in patients with MM by age group (B) and treatment type (C). Antibody titres were evaluated by Abbott AdviseDx SARS-CoV-2 IgG II assay in (B) and (C). Dashed line represents threshold for positive serological response. Ab, antibody; COVID-19, coronavirus disease 2019; MM, multiple myeloma; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

**TABLE 2** Disease and treatment characteristics of the patients with multiple myeloma (MM)

Characteristic	MM (N = 300)
Disease status, n (%)	
Newly diagnosed	148 (49)
Relapsed/refractory	152 (51)
Number of lines of prior treatment	
Mean (SD)	2.98 (2.718)
Median	2
Range	0–14
Treatment status, n (%)	
Induction	29 (9.7)
Post-autologous transplant or on maintenance	98 (32.7)
On ≥second-line therapy	115 (38.3)
Not receiving therapy	58 (19.3)
Treatments received within 3 months prior to vaccine series completion, n (%)	
Lenalidomide	116 (38.7)
Corticosteroids	96 (32)
Anti-CD38 therapy	76 (25.3)
Carfilzomib	52 (17.3)
Pomalidomide	39 (13)
Bortezomib	27 (9)
Alkylating agent	14 (4.7)
BCMA-directed bispecific/antibody conjugate	13 (4.4)
Ixazomib	6 (2)
Autologous stem cell transplant (within 6 months prior to vaccine series completion), n (%)	25 (8.3)
BCMA-directed CAR-T treatment (within 6 months prior to vaccine series completion), n (%)	4 (1.3)

Abbreviations: BCMA, B-cell maturation antigen; CAR-T, chimeric antigen receptor T-cell therapy.

a third dose/booster dose of vaccination. Among those with antibody titres performed within 6 months of initial vaccination completion and after the third dose/booster using the

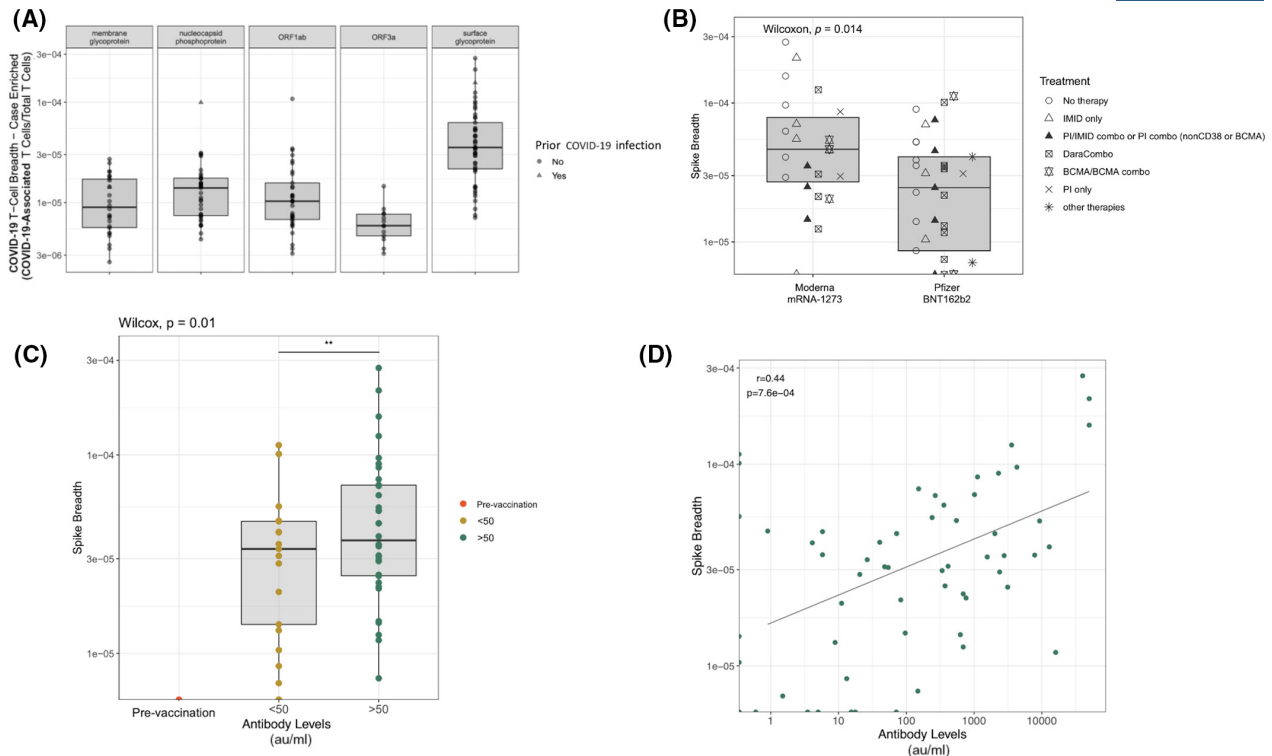
same assay, 40 of 51 patients (78%) had a four-fold increase in titres or reached the assay's limit of quantification.

## T-cell Immunosequencing

In all, 56 patients were enrolled on our prospective study assessing T-cell response to COVID-19 vaccination. Patients with known history of COVID-19 spike antibody negativity after vaccination were preferentially enrolled, and samples from COVID-19 spike antibody positive patients, including those matched for age and time since vaccination were selected as controls. The median time from last vaccination to testing was 121 days. All patients had concurrent COVID-19 spike antibody titre assessed via Abbott AdviseDx SARS-CoV-2 IgG II assay on the same blood draw. Additionally, there were three patients with serial samples pre/post vaccination/booster. Among this cohort, one patient had a prior COVID-19 infection. In all, 25 samples were ultimately negative for COVID-19 antibodies and 34 samples were positive for COVID-19 spike antibodies by Abbott AdviseDx SARS-CoV-2 IgG II assay.

To better characterise spike-specific responses after vaccination, TCR sequences were assessed by immunoSEQ® T-MAP™ COVID and evaluated based on the specific ORF of the COVID-19 genome. There was greater spike-specific T-cell breadth (number of unique COVID-19-associated TCR rearrangements) (Figure 2A) and depth (total frequency of COVID-19-associated TCRs) (Figure S1) for ORFs associated with the surface glycoprotein compared to other regions, consistent with a spike-specific T-cell response after vaccination. The median spike-specific T-cell breadth was  $3.11 \times 10^{-5}$ , similar to those observed in healthy populations after vaccination and after COVID-19 infection.<sup>4,5</sup> There was greater spike-specific T-cell breadth in the surface glycoproteins in both the CD4 and CD8 subsets, with a more significant increase in the CD4 TCR subset (Figure S2). A higher spike breadth after vaccination with mRNA-1273 was observed compared to BNT162b2 (median  $4.63 \times 10^{-5}$  vs.  $2.46 \times 10^{-5}$ ,  $p = 0.01$ ) (Figure 2B).

With regards to spike specific T-cell breadth in relation to serological COVID-19 antibody status, median spike specific



**FIGURE 2** COVID-19 associated TCR breadth observed in patients with PCD classified by ORF region. (A) and stratified by vaccination type (B) and antibody titres (C). Scatterplot of TCR breadth by antibody titres (D). Antibody titres were evaluated by Abbott AdviseDx SARS-CoV-2 IgG II assay.  $\geq 50$  au/ml corresponds to positive serological response. au, arbitrary units; BCMA, B-cell maturation antigen; COVID-19, coronavirus disease 2019; IMiD, immunomodulatory imide drug; ORF, open reading frames; PCD, plasma cell dyscrasias; PI, proteasome inhibitor; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; TCR, T-cell receptor. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

T-cell breadth was higher for patients with positive antibody status compared to those that were negative ( $3.54 \times 10^{-5}$  vs.  $1.40 \times 10^{-5}$ ;  $p < 0.01$ ) (Figure 2C). A moderate correlation was observed between spike-specific T-cell breadth and COVID-19 spike antibody titres by Spearman's correlation coefficient ( $r = 0.44$ ,  $p < 0.001$ ) (Figure 2D). Three patients had serial samples. Of these, two patients had samples obtained before vaccination and subsequently after vaccination and displayed an increase in T-cell breadth from zero to greater than  $3 \times 10^{-5}$ , consistent with a spike-specific T-cell response. For the third patient, serial samples were available after completion of two and three doses of BNT162b2; his antibody levels seroconverted from negative to positive and he had a twofold increase in spike breadth. Of the 24 patients with COVID-19 antibody negative status after vaccination, 15 patients achieved spike-specific T-cell breadth of  $>1 \times 10^{-5}$  (62.5%). For those with COVID-19 antibody positive status after vaccination, all but one of the 32 patients had spike breadth of  $>1 \times 10^{-5}$ , and none of the patients had undetectable spike-specific T-cell breadth.

Patients receiving anti-CD38 antibodies and corticosteroids appeared to have a lower COVID-19 spike-specific T-cell breadth compared to those not on these therapies, but this was not statistically significant. Despite reduction in COVID-19 spike antibodies in patients receiving anti-BCMA therapy, there was no significant difference in spike-specific T-cell breadth for those receiving anti-BCMA

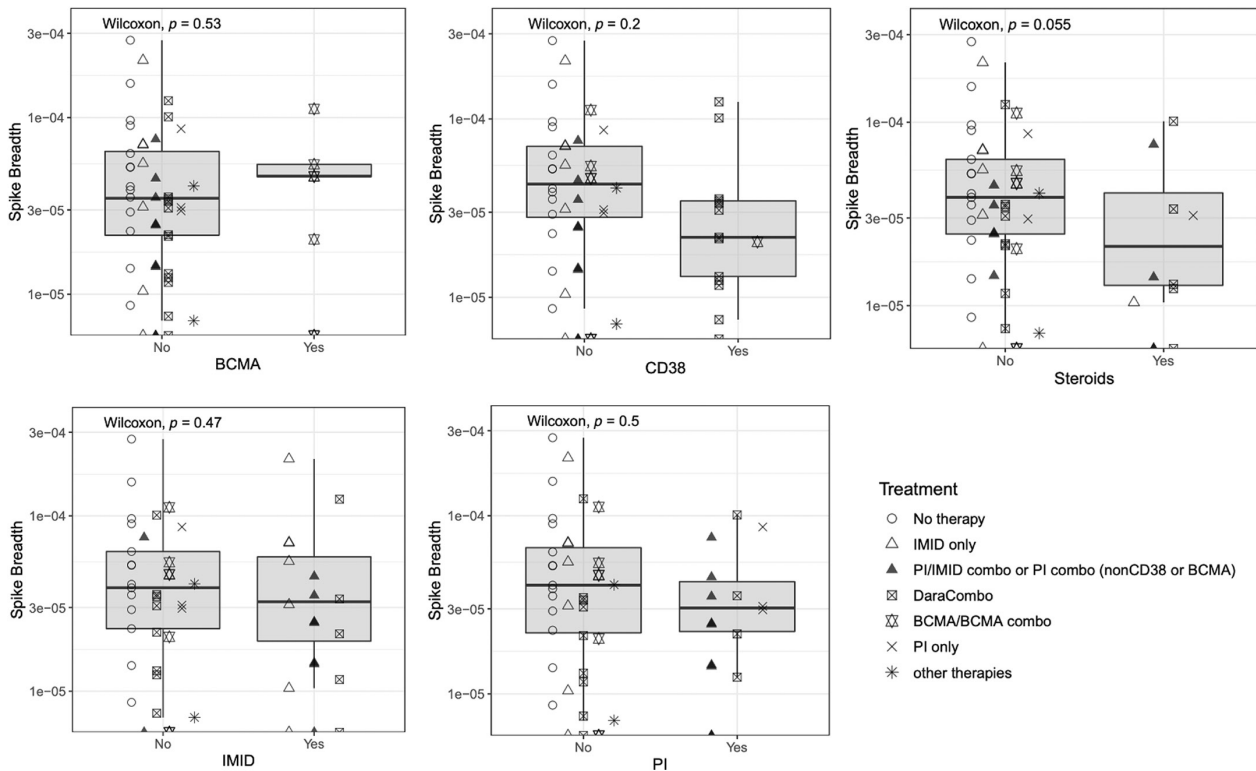
therapy ( $p = 0.53$ ; Figure 3). Spike-specific T-cell breadth was also similar when stratified by IMiD and PI exposure. There was no clear association observed between spike-specific T-cell breadth and age.

## COVID-19 infection

In this cohort, 32 patients (8.8%) had documented COVID-19 infections, with two patients being infected twice during the follow-up period (data cut-off: end of February 2022). Nine patients were infected prior to vaccination and 23 patients had infection after vaccination. With regards to time period of infection, nine infections occurred from January 2020 to May 2021, eight infections occurred from June 2021 to November 2021, and 17 infections occurred from December 2021 to February 2022.

Only five patients required hospitalisation, including three cases post-COVID-19 vaccination; no patients required intensive level care or died due to COVID-19. In all, 12 patients received monoclonal COVID-19 antibodies, four patients received molnupiravir and three patients received remdesivir.

Among the 23 patients that developed COVID-19 infection after vaccination, the median age was 67 years, with a median (range) time from last vaccination to infection of 122 (2–256) days. At the time of infection,



**FIGURE 3** COVID-19 spike-specific TCR breadth stratified by treatment exposures. COVID-19, coronavirus disease 2019; BCMA, B-cell maturation antigen; IMiD, immunomodulatory imide drug; PI, proteasome inhibitor; TCR, T-cell receptor.

anti-CD38-containing regimens were the most common plasma cell-directed therapy being received (nine patients), followed by carfilzomib-containing regimens (six patients); only one patient had COVID-19 infection post-BCMA therapy at the time of analysis. The median antibody titre after vaccination series completion and prior to COVID-19 infection was 55.6 arbitrary units (au)/ml by the Abbott AdviseDx SAR-COV-2 IgG II assay, which was notably lower than for the whole study cohort post-vaccination (554.3 au/ml). Only three patients had TCR immunosequencing prior to COVID-19 infection: two patients had a T-cell breadth  $>6 \times 10^{-5}$  and both had mild disease, not requiring hospitalisation or COVID-19-directed treatments; one patient had a T-cell breadth of  $1.3 \times 10^{-5}$  and required non-intensive care unit hospitalisation, as well as monoclonal antibody treatment and remdesivir.

## DISCUSSION

Patients with MM and other PCD are known to be at increased risk of severe illness and death from COVID-19.<sup>2,9,10</sup> Vaccination remains the most important strategy in protecting patients with MM and other plasma cell diseases against COVID-19. This study highlights that despite an impaired immune system, a large proportion of patients with PCD were able to mount a humoral and cellular response to COVID-19 vaccination. Together with the high vaccination rates and relatively low case rates in the San Francisco

Bay area, this translated to a relatively low incidence of COVID-19 infections during the study time frame and reassuringly low level of hospitalisations and severe illness for our highly vaccinated cohort.

Using commercially available anti-spike antibody testing, we observed that ~85% of our patients with PCD mounted a positive serological response within 6 months of completing the initial vaccination series, comparable to other previous studies.<sup>11–18</sup> In studies assessing neutralising antibodies, the serological response has been lower, ranging from 41% to 74%.<sup>12,19–22</sup> Although the presence and titre of neutralising antibodies may more directly reflect viral protection, these assays are not widely available in clinical practice. In most studies, good correlation has been observed between spike receptor-binding domain (RBD) IgG antibody titres and inhibition capacity of neutralising antibodies, including in studies of MM<sup>12,23,24</sup> and for different anti-spike RBD IgG assays utilised in this study.<sup>25–29</sup> As such, we postulate that the presence and level of total anti-spike binding IgG antibodies may be a surrogate marker for humoral immunity to COVID-19 infection, acknowledging, however, that a subset of patients with positive RBD antibodies may have impaired neutralisation ability as highlighted in other studies.<sup>12,21</sup>

We observed several factors associated with lower seroconversion rates and titres. Among those with MM, factors associated with lower seroconversion included: advanced age, lack of treatment response to current myeloma therapy, IgG levels  $<4$  g/l, and recent use of anti-BCMA-directed therapies.

We observed lower responses and titres after vaccination with BNT162b2 compared to mRNA-1273 and this has been reported by others, both in a non-immunocompromised cohort,<sup>30,31</sup> as well as those with haematological malignancies, including MM.<sup>12,13,32</sup> Spike antibody titres were significantly lower in those treated with anti-CD38 antibodies and especially low for those treated with anti-BCMA-directed therapies, consistent with findings in other studies.<sup>3,21</sup>

Whereas most studies have used spike protein peptide-stimulated interferon-gamma (IFN $\gamma$ ) release assays to determine T-cell responses to vaccination, our study is unique in that it utilises ultra-deep next-generation sequencing (NGS) to identify T-cell clones specific to COVID-19, and to our knowledge, is the first study to report on these results in patients with PCD. Assessing T-cell breadth provides a quantitative measurement of T-cell diversity and robustness of T-cell immune response after vaccination. Previous studies using this technique have demonstrated higher breadth of spike-specific TCRs in healthy subjects undergoing vaccination compared to unvaccinated subjects receiving placebo, without a difference in non-spike TCR breadth.<sup>4,5</sup> Unlike with IFN $\gamma$  release assays, NGS allows for the direct identification of COVID-19-specific T-cell clones from preserved cells, which can potentially be followed over time. In our study, sequencing of TCR repertoire confirmed an increase in T-cell breadth and depth for TCRs specific for spike surface glycoprotein after vaccination, and in our immunocompromised patients, we observed a similar median spike specific T-cell breadth as those previously reported for healthy populations after vaccination.<sup>4,5</sup> We did observe a lower spike-specific T-cell breadth for those with negative spike antibody titres; however, >60% of these patients mounted a spike-specific T-cell breadth of  $>1 \times 10^{-5}$ , suggesting a T-cell response despite the absence of a detectable antibody response, although the spike-specific T-cell breadth threshold necessary for clinical protection remains to be determined. The presence of a T-cell response in the absence of antibody response has been observed in other studies for patients with myeloma and other lymphoid malignancies.<sup>28,33,34</sup> Of note, among the seronegative patients, we observed a higher proportion of patients having a detectable T-cell response when compared to prior studies utilising IFN $\gamma$  and other cytokine release assays.<sup>35</sup> One possible explanation is that although TCR immunosequencing captures the presence and diversity of COVID-19-specific T-cell clones in response to vaccination, it is unable to assess the fitness and functionality of these T cells, which may be negatively impacted by the underlying PCD and their treatments resulting in lower response rates when assessed by *in vitro* functional assays. Whether these assays can be correlated with clinical outcomes remain an area of ongoing investigation.

We also observed a statistically higher induced spike-specific T-cell breadth for patients receiving mRNA-1273 compared to BNT162b2. This, together with higher serological response rates and anti-spike antibody titres observed in our study, suggest that mRNA-1273 may be the preferred vaccine for patients with PCD. These findings were further supported by a recent study by Nooka et al.,<sup>12</sup> which also found

higher neutralising antibodies for mRNA-1273-vaccinated patients with MM compared to those receiving BNT162b2. Although there are not yet data for whether these differences affect clinical outcomes in patients with PCD, a recent large study of the Veterans Affairs registry of >200 000 vaccinated veterans found that recipients of BNT162b2 had higher risks of COVID-19 infection (5.75 vs. 4.52 events/1000 persons) and higher risk of hospitalisation compared to those that received mRNA-1273, providing clinical evidence that mRNA-1273 may translate to better protection.<sup>36</sup>

Our study has several limitations. Due to its predominantly retrospective nature, timing of antibody testing was not predefined. As the study was initiated after most patients had already received vaccination, we do not have testing pre-vaccination; however, given the low rates of COVID-19 infection prior to vaccination in our cohort, the results are primarily driven by COVID-19 vaccination alone. With regards to serological testing, we did not have access to neutralising antibody testing, and most of our data were obtained retrospectively. Our study utilises ultra-deep immunosequencing to characterise T-cell responses on a genomic level, but one limitation is the inability to assess the functional responses of these T-cell clones. However, this study is novel in that it utilises another tool to study T-cell responses in the immunocompromised population and has the advantage of only requiring whole blood samples without the need for peripheral blood mononuclear cells isolation, which may facilitate use in clinical practice. Nevertheless, further studies on T-cell responses are needed, and integrating deep sequencing approaches with functional T-cell testing may provide a more comprehensive assessment of the T-cell response to COVID-19 vaccination. Lastly, our patient population was highly vaccinated, and the community rates of COVID-19 infection were among the lowest in the country at time of the study, reducing the generalisability of our observed infection rates. Future studies will need to assess the impact of antibody development and T-cell responses with clinical protection, including to new variants and the role of waxing immunity over time.

In conclusion, our study demonstrates that a large proportion of patients with PCD develop a serological spike-specific antibody response. COVID-19-specific T-cell responses after vaccination can be observed regardless of antibody levels. Despite the immunosuppressive nature of PCD and their treatment, aggressive COVID-19 vaccination does appear to bolster immune protection against COVID-19 and in our study, translated to low rates of severe disease.

## AUTHOR CONTRIBUTIONS

Alfred Chung: data collection, data validation, data analysis, figure development, manuscript writing, manuscript revision and editing; Barbara Banbury: data analysis, figure development, manuscript revision and editing; Marissa Vignali: data analysis, figure development, manuscript revision and editing; Chiung-Yu Huang: data analysis, figure development, manuscript revision and editing; Sireesha Asoori: data collection, manuscript revision and editing;

Rachel Johnson: data collection, manuscript revision and editing; Theodore Kurtz: data collection, manuscript revision and editing. Shagun Arora: conceptualisation, manuscript revision and editing. Sandy W. Wong: conceptualisation, manuscript revision and editing. Nina Shah: conceptualisation, manuscript revision and editing. Thomas G Martin: conceptualisation, manuscript revision and editing. Jeffrey L Wolf: conceptualisation, manuscript revision and editing.

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## CONFLICT OF INTEREST

Alfred Chung reports the following disclosures: Caelum (research), Merck (research), Sanofi (honoraria). Barbara Banbury and Marissa Vignali report the following disclosures: Adaptive Biotechnologies (employment). Sandy W. Wong reports the following disclosures: Amgen (consulting), BMS (research), Caelum (research), Catalent Biologics (consulting), Dren Biosciences (consulting), Genentech (research), Fortis (research), GSK (research), Janssen (research), and Sanofi (consulting). Nina Shah reports the following disclosures: Amgen (consulting), Bluebird Bio (research), BMS/Celgene (consulting/research), CareDx (consulting), GSK (consulting), Indapta Therapeutics (consulting), Janssen (research), Karyopharm (consulting), Kite (consulting), Nektar (research), Poseida (research), Sanofi (consulting), Sutro Biopharma (research), and Teneobio (research). Jeffrey L Wolf reports the following disclosures: Amgen (consulting), Celgene (consulting), Janssen (consulting), Novartis (consulting), and Takeda (consulting). Thomas G Martin reports the following disclosures: Amgen (research), GSK (consulting), Janssen (research), Juno (consulting), Roche (consulting), Sanofi (research), and Seattle Genetics (research). The remaining authors report no relevant disclosures.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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