Low seropositivity and suboptimal neutralisation rates in patients fully vaccinated against COVID-19 with B-cell malignancies

Patients with haematological malignancies are at increased risk of severe disease and death from coronavirus disease 2019 (COVID-19).[1] Vaccination is essential to increase population immunity and decrease disease burden. The first COVID-19 vaccines were authorised in the UK after phase III trials, which showed that both the BNT162b2 (Pfizer-BioNTech) and ChAdOx1 nCoV-19 (Oxford-AstraZeneca) vaccines were effective at preventing symptomatic disease and hospitalisation.[2,3] Whilst both vaccines have demonstrated robust immune responses in healthy volunteers, patients with haematological malignancies were excluded from clinical trials. Emerging data suggest such patients are less likely to mount a humoral immune response to COVID-19 vaccination, with those who have received Bruton tyrosine kinase inhibitors (BTKi) or cluster of differentiation (CD)20-directed therapies for B-cell malignancies a particularly high-risk group.[4–7]

We report interim results from 55 participants recruited to our ongoing COV-VACC study, exploring the immune response to COVID-19 vaccination in patients with B-cell malignancies (South Central Berkshire B Research Ethics Committee and UK Health Research Authority approval IRAS number: 294547). Patients on treatment or treated within the last 24 months for a B-cell malignancy and receiving either the BNT162b2 (Pfizer-BioNTech; $n = 41$) or ChAdOx1 nCoV-19 (Oxford-AstraZeneca; $n = 14$) vaccines were recruited. The median (range) age of participants was 60 (27–82) years and 50% were receiving systemic anti-cancer therapy (SACT) at the time of vaccination (Fig 1A, B).

Blood samples were taken before vaccination and 1 month after the first and second vaccine doses. At each time-point, a full blood count and enumeration of whole blood lymphocyte subsets (CD3, CD4, CD19, CD56) by flow cytometry (Aquios flow cytometers; Beckman Coulter, Brea, CA, USA) were performed. Serum samples were screened for anti-severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antibodies using quantitative double-antigen sandwich immunoassays for both the nucleocapsid (N) antigen and the spike (S) protein receptor binding domain (RBD) (both Roche, Basel, Switzerland). Samples from participants with detectable anti-S antibodies were then assessed to determine if these antibodies were able to neutralise SARS-CoV-2 infection *in vitro* using a luciferase encoding lentivirus pseudotyped with the SARS-CoV-2 spike as previously described.[8,9] Groups were compared using logistic regression, chi-squared/Fisher's exact tests and Wilcoxon–Mann–Whitney tests.

After a single dose of either BNT162b2 (Pfizer-BioNTech; $n = 41$) or ChAdOx1 nCoV-19 (Oxford-AstraZeneca; $n = 14$) vaccine, 36% overall had detectable anti-S antibodies (15/41 Pfizer-BioNTech and five of 14 Oxford-AstraZeneca) and 42% (23/55) after a second dose (Fig 1C). Three participants had serological evidence of previous infection with SARS-CoV-2.

Where available, sera from seropositive participants after the first or second dose were then used to assess neutralisation activity *in vitro*. Of the seropositive patients after the first dose ($n = 17$), just 41% were able to neutralise SARS-CoV-2 pseudotyped virus with a 50% inhibitory dilution (ID_{50}) of >1:50. After two doses ($n = 23$) 57% of the seropositive patients had detectable neutralisation activity [median (range) ID_{50} of 1:469 (1:70–1:3056)] (Fig 1D).

Total blood lymphocyte, CD19, CD4, and CD56 counts all showed a significant association with seropositivity (Fig 1E–H). For a 1 log increase in each lymphocyte subset, the odds

Fig 1. (A) Number of patients by diagnostic group recruited to the study to date ($n = 70$); (B) Number of patients (whole cohort) exposed to common therapeutic modalities; (C) Anti-S antibody levels 1 month after second vaccination quantified by Elecsys Roche anti-SARS-CoV-2 S assay (Spike); (D) ID_{50}s of serum (from seropositive patients) able to neutralise SARS-CoV-2 pseudotyped virus after first dose (seven of 17) and second dose (14/21); (E) Peripheral lymphocyte count (excluding patients with CLL) in responders ($n = 22$) and non-responders ($n = 28$) after second vaccination ($P = 0{\cdot}0250$); (F) Peripheral CD19 counts in responders ($n = 23$) and non-responders ($n = 32$) after second vaccination ($P = 0{\cdot}031$); (G) Peripheral blood CD4 count in responders ($n = 23$) and non-responders ($n = 32$) after second vaccination ($P = 0{\cdot}00195$); (H) Peripheral blood CD56 count in responders ($n = 23$) and non-responders ($n = 32$) after second vaccination ($P = 0{\cdot}0034$); (I) Peripheral CD19 count in patients who had received CAR-T ($n = 11$) *versus* those who had received a different SACT ($n = 42$) ($P = 0{\cdot}0074$). 'Responders' = seropositive with anti-S antibody level >0·4 μ/ml. ALL, acute lymphoblastic leukaemia; BTKi, Bruton tyrosine kinase inhibitors; CAR-T, chimeric antigen receptor T-cell; CD, cluster of differentiation; CLL, chronic lymphocytic leukaemia; ID_{50}, 50% inhibitory dilution; S, Spike protein; NHL, non-Hodgkin lymphoma; SACT, systemic anti-cancer therapy; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; WM, Waldenström macroglobulinaemia.

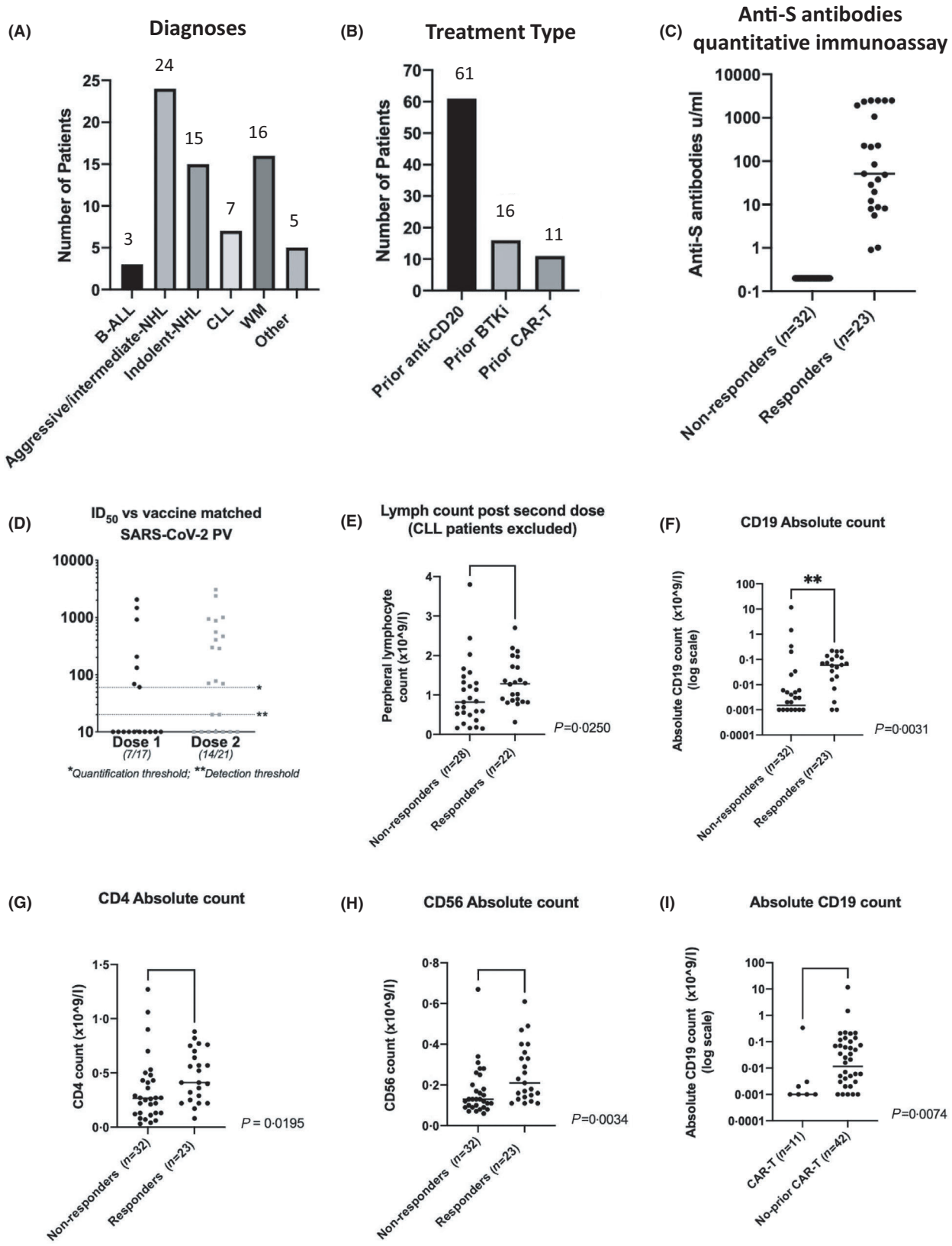

Table I. Logistic regression analysis.

	All patients: anti-S Ab positivity			Seropositive patients only: neutralising activity		
	Seropositive, *n/N*	OR (95% CI)	*P*	Neutralising, *n/N*	OR (95% CI)	*P*
Lymphocyte subsets						
CD19 (1 log increase)	23/54	1·32 (1·06–1·66)	0·013	12/17	2·04 (0·99–4·22)	0·054
CD4 (1 log increase)	23/53	2·50 (1·12–5·55)	0·025	12/19	1·23 (0·28–5·39)	0·78
CD56 (1 log increase)	23/53	4·47 (1·46–13·06)	0·008	12/19	6·79 (0·62–73·92)	0·12
Treatments						
Rituximab						
No	1/4	1·00	0·49	0/1	—	0·37*
Yes	22/51	2·28 (0·22–23·39)		12/18	—	
BTKi						
No	17/42	1·00	0·72	11/16	1·00	0·54
Yes	6/13	1·26 (0·36–4·41)		2/4	0·57 (0·05–4·67)	
CAR-T						
No	221/46	1·00	0·21	13/18	—	0·37*
Yes	2/9	0·34 (0·06–1·82)		0/1	—	
Vaccine time-point						
On treatment	9/25	1·00	0·026	3/7	—	0·034*
Within 6 months of treatment	5/18	0·68 (0·18–2·55)		2/5	—	
>6 months from treatment	9/12	5·33 (1·14–24·90)		7/7	—	

Ab, antibody; BTKi, Bruton tyrosine kinase inhibitors; CAR-T, chimeric antigen receptor T-cell; CD, cluster of differentiation; CI, confidence interval; OR, odds ratio; S, Spike protein.

*OR not estimable, Fisher's exact test used ($P = 0·017$) for on treatment/within 6 months *versus* >6 months.

of developing antibodies in response to vaccination were 1·32 [95% confidence interval (CI) 1·05–1·66, $P = 0·013$], 2·5 (95% CI 1·12–5·55, $P = 0·025$) and 4·47 (95% CI 1·46–13·06, $P = 0·0008$) times higher respectively for CD19, CD4 and CD56 counts (Table I). Timing of vaccination in relation to SACT was important ($P = 0·0126$), with participants vaccinated >6 months after completing therapy more likely to develop antibodies; odds ratio (OR) 5·33 (95% CI1·14–24·90). Patients on or within 6 months of treatment had significantly lower CD56 and CD19 counts ($P = 0·003$ and $P = 0·014$) and a trend towards lower CD4 ($P = 0·11$). Chimeric antigen receptor (CAR) T-cell recipients had very low rates of seropositivity (two of nine, 22·2%; Table I). No difference was seen for patients treated with CD20 antibody therapies or BTKis (Supplementary Table S1).

Seropositive patients could be divided into those whose sera did or did not demonstrate neutralising activity. Neutralising activity was associated with higher median anti-S antibody levels ($P = 0·0005$). Further, both higher CD56 and CD19 counts showed trends towards increased odds of developing neutralising antibodies; OR 6·79 (95% CI 0·62–73·9), $P = 0·12$ and 2·04 (95% CI 0·99–4·22), $P = 0·054$. All seropositive patients (seven of seven) who were >6 months from treatment had neutralising antibodies compared to five of 12 on or within 6 months of treatment (Fisher's exact, $P = 0·017$).

This interim analysis adds to increasing evidence that immunocompromised patients are less likely to produce robust immune responses after COVID-19 vaccination.[4–7] In our cohort, 42% had detectable anti-S antibodies after two doses of an approved vaccine compared to 91–100% in healthy individuals in phase I/II trials.[2,10] Even when seroconversion occurs, the protective humoral response may be limited. Just 23% of the cohort ($n = 56$; 57% of seropositive participants) neutralised virus *in vitro*. Others have shown neutralising antibody levels to be highly predictive of immune protection from symptomatic infection.[11] Our data identifies several factors associated with vaccine response such as peripheral blood lymphocyte, CD19, CD4 and CD56 counts, which if validated in larger cohorts may enable the identification of patients unlikely to respond to vaccination.

These data provide further evidence that patients on SACT are less likely to produce antibodies following COVID-19 vaccination.[6] Anti-S seropositivity does not necessarily correlate with serum neutralisation and is unlikely predictive of an effective antibody response based on current estimates of correlates of protection. Urgent validation in larger cohorts is required, as many patients with B-cell malignancies may remain at high risk of infection regardless of anti-S antibody status. Clinically vulnerable patients, regardless of vaccination status, should be considered for neutralising monoclonal antibody therapies if they develop COVID-19.[12,13]

Urgent consideration needs to be given to provision of booster doses or full re-vaccination to this group of patients, particularly if they have been vaccinated within 6 months of active therapy. The correlation between peripheral blood lymphocyte, CD19, CD4 and CD56 counts suggest that booster doses or vaccination may be most effective if given when an individual has recovered lymphocytes and are ≥6 months following SACT.

This interim analysis is limited by cohort size and heterogeneity. However, we demonstrate a disconnect between seropositivity and virus neutralisation *in vitro*, following vaccination against COVID-19.

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Thomas A. Fox[1,2]
Amy A. Kirkwood[3]
Louise Enfield[2]
Maeve O'Reilly[2]
Suzanne Arulogun[2]
Shirley D'Sa[2]
Jenny O'Nions[2]
Janki Kavi[4]
Evan Vitsaras[5]
William Townsend[2]
Siobhan O. Burns[1,4]
Satyen H. Gohil[2]
Kate Cwynarski[2]
Kirsty J. Thomson[2]
Mahdad Noursadeghi[6,7]
Robert S. Heyderman[6,7]
Tommy Rampling[7]
Kirit M. Ardeshna[2]
Laura E. McCoy[1,6,*]
Emma C. Morris[1,2,*]

[1]UCL Institute of Immunity and Transplantation, University College London, [2]Department of Clinical Haematology, University College London Hospitals NHS Foundation Trust, [3]CR UK and UCL Cancer Trials Centre, UCL Cancer Institute, UCL, [4]Immunology, Royal Free London Hospitals NHS Foundation Trust, [5]Health Services Laboratories, [6]Research Department of Infection, University College London and [7]Department of Infectious Disease, University College London Hospitals NHS Foundation Trust, London, UK.
E-mail: e.morris@ucl.ac.uk
*Joint senior authors.

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Supporting Information

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

Table SI. Anti-S antibody results and corresponding *in vitro* neutralisation data for all 55 patients who had samples available for analysis after two doses of vaccine.

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