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

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Patients with haematological malignancies are at increased risk of severe disease and death from COVID-19.[1] Vaccination is essential to increase population immunity and decrease disease burden. The first COVID-19 vaccines were authorised in the United Kingdom (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 suggests such patients are less likely to mount a humoral immune response to COVID-19 vaccination, with those who have received Bruton's Tyrosine Kinase inhibitors (BTKi) or CD20-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 age of participants was 60 years (range: 27-82) and 50% were receiving systemic anti-cancer therapy (SACT) at the time of vaccination (Figures 1A, B).

Blood samples were taken prior to vaccination and 1 month after 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 (Beckman Coulter (CA, USA) Aquios flow cytometers) were performed. Serum samples were screened for anti-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). 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 5/14 AZ), and 42% (23/55) after a second dose (Figure 1C). Three participants had serological evidence of previous infection with SARS-CoV-2.

Where available, sera from seropositive participants after first or second dose were then used to assess neutralisation activity in vitro. Of the seropositive patients after first dose (n=17), just 41% were able to neutralise SARS-CoV-2 pseudotyped virus with a 50% inhibitory dilution factor (ID50) of >1:50. After two doses (n=23) 57% of the seropositive patients had detectable neutralisation activity (median ID50 of 1:469, range 1:70 – 1:3056) (Figure 1D).

Total blood lymphocyte, CD19, CD4, and CD56 counts all showed a significant association with seropositivity (Figures 1E, F, G, H). For a 1 log increase in each lymphocyte subset, the odds of developing antibodies in response to vaccination were 1.32 (95% 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 1). Timing of vaccination in relation to SACT was important (p=0.0126), with participants vaccinated more than 6 months after completing therapy more likely to develop antibodies; OR: 5.33 (1.14 – 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). CAR-T cell therapy recipients had very low rates of seropositivity (2/9, 22.2%) (Table 1).

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 (0.62 – 73.9), p = 0.12 and 2.04 (0.99 – 4.22), p = 0.054. All seropositive patients (7/7) who were >6 months from treatment had neutralising antibodies compared to 5/12 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 following COVID-19 vaccination.[4-7] In our cohort 42% had detectable anti-S antibodies following 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.

This data provides 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 revaccination 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 at least 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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References

1. Vijenthira A, Gong IY, Fox TA, Booth S, Cook G, Fattizzo B, et al. Outcomes of patients with hematologic malignancies and COVID-19: A systematic review and meta-analysis of 3377 patients. Blood. 2020.
2. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383(27):2603-15.
3. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2020.
4. Herishanu Y, Avivi I, Aharon A, Shefer G, Levi S, Bronstein Y, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. Blood. 2021;137(23):3165-73.
5. Tzarfati KH, Gutwein O, Apel A, Rahimi-Levene N, Sadovnik M, Harel L, et al. BNT162b2 COVID-19 Vaccine is significantly less effective in patients with hematologic malignancies. Am J Hematol. 2021.
6. Lim SH, Campbell N, Johnson M, Joseph-Pietras D, Collins GP, O'Callaghan A, et al. Antibody responses after SARS-CoV-2 vaccination in patients with lymphoma. Lancet Haematol. 2021.
7. Vijenthira A, Gong I, Betschel SD, Cheung M, Hicks LK. Vaccine response following anti-CD20 therapy: a systematic review and meta-analysis of 905 patients. Blood Adv. 2021;5(12):2624-43.
8. Brouwer PJM, Caniels TG, van der Straten K, Snitselaar JL, Aldon Y, Bangaru S, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science. 2020;369(6504):643-50.
9. Seow J, Graham C, Merrick B, Acors S, Pickering S, Steel KJA, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nat Microbiol. 2020;5(12):1598-607.
10. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet. 2020;396(10249):467-78.
11. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med. 2021;27(7):1205-11.
12. Dougan M, Nirula A, Azizad M, Mocherla B, Gottlieb RL, Chen P, et al. Bamlanivimab plus Etesevimab in Mild or Moderate Covid-19. N Engl J Med. 2021.
13. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. N Engl J Med. 2021;384(3):238-51.

	All patients: Anti-S Ab positivity			Seropositive patients only: neutralising activity		
	Seropositive /N	**OR (95% CI)**	**p-value**	**Neutralising /N**	**OR (95% CI)**	**p-value**
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	-	

*OR not estimable, Fisher's exact test used (p = 0.017) for on treatment/within 6 months vs >6 months)

Table 1. Logistic regression analysis

Legend to Figure

Figure 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 post 2nd vaccination quantified by Elecsys Roche Anti-SARS-CoV-2 S assay (Spike); (D) ID50s of serum (from seropositive patients) able to neutralise SARS-CoV-2 pseudotyped virus after first dose (7/17) and second dose (14/21); (E) Peripheral lymphocyte count (excluding CLL patients) in responders (n=22) and non-responders (n=28) post 2nd vaccination (p=0.0250); (F) Peripheral CD19 counts in responders (n=23) and non-responders (n=32) post 2nd vaccination (p=0.031); (G) Peripheral blood CD4 count in responders (n=23) and non-responders (n=32) post 2nd vaccination (p=0.00195); (H) Peripheral blood CD56 count in responders (n=23) and non-responders (n=32) post 2nd vaccination (p=0.0034); (I) Peripheral CD19 count in patients who had received CAR-T therapy (n=11) versus those who had received a different SACT (n=42) (p=0.0074). 'Responders' = seropositive with anti-S antibody level >0.4U/ml.