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BNT162b2 COVID-19 and ChAdOx1 nCoV-19 vaccination in patients with myelodysplastic syndromes

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Author contributions
Conceptualization and study design (AK, PP, SI, KD); Investigation (SAJ, RB, TA, RG,TL); Patient recruitment (TA, JV, AK); Clinical database (TA,AK,JV); Formal analysis (SAJ, RB); Data curation (SAJ, RB, RG); Visualization (SAJ, RB, SI, KD); Writing - original draft (SI); Writing - review & editing (RB, AK, PP, KD); Funding acquisition (SI, AK, PP); Supervision (SI, KD).

Disclosure of Conflicts of Interest

The authors declare no conflicts of interests relevant to this study

Data Sharing
Data from this study can be made available to other researchers in the field upon request and approval by the study management committee and subject to appropriate data transfer agreements. Requests should be directed to Dr Irshad**.**

Many patients with haematological cancers are not completely protected after the initial dose or after both primary doses of the vaccines (1, 2). with most failing to seroconvert on completion of the two-dose vaccine schedule (2). These reports only included three patients with myelodysplastic syndrome (MDS). MDS represents a spectrum of clonal bone marrow neoplasms from low-risk disease through to those transforming into acute myeloid leukaemia. Patients with MDS, especially with lower-risk disease, many of whom are minimally treated and who might be expected to have a comparable immune response to healthy volunteers, and as such a better immune response to COVID-19 vaccines than other haematological cancers. Previous studies looking at the immune response to influenza vaccination in those with MDS had shown promising results with immune responses not differing from those of healthy family members (3). However, a recent study which included 6 MDS patients, reported poor seroconversion rates following a single dose of COVID-19 vaccine in a group of 60 myeloid cancer patients, including those who are not on cytoreductive treatments and those in complete haematological remission, suggesting a clear need for more detailed interrogation of COVID-19 vaccination in this group of patients (4). Here, we report the humoral and T-cell responses of 38 patients with MDS two weeks following completion of the second dose vaccine schedules of ChAdOx1 or BNT162b2 nCoV-19 vaccines.

Following approval by the institutional review boards, patients with MDS (n=38) vaccinated with either BNT162b2 mRNA or ChAdOx1 nCoV-19 COVID-19 vaccine provided written informed consent. Eligibility criteria for the study included diagnosis of MDS as per the WHO classification (5) and age ≥18 years. The study also included healthy volunteers (HV) (mainly healthcare workers, n=30) serving as a reference group, included principally to provide an experimental control for study assays and facilitate their comparison with results of other studies of BNT162b2 in healthy populations. Plasma samples were tested for IgG binding the SARS-CoV-2 spike (S) protein and nucleoprotein (N) and neutralisation assays against HIV-1 based virus particles pseudotyped with SARS-CoV-2 Wuhan strain (WT), VOC.B.1.1.7 (alpha) or VOC.B.1.617.2 (delta) Spike as previously described (1, 2, 6). Cellular responses were assessed using IFNγ ELISPOT and flow cytometry (CD25 and CD69 expression) after 24h peptide stimulation. IFNγ ELISpot analysis was performed *ex vivo* for assessment of T cell response following stimulation with SARS-CoV-2 peptide pools, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), and influenza virus positive control (CEF) peptides for 24 hours.

Thirty-eight MDS patients and 30 HV provided a blood sample 2 weeks following a second primary dose of their initial vaccine. Clinical characteristics along with median times to second dose are provided in **Table 1**. We observed significant differences between the ages of the HV and MDS cohorts (Student's t test, equal variance, $p<0.001$). 42% (n=16) of the MDS patients received BNT162b2 and 58% (n=22) received ChAdOx1 nCoV-19 vaccines. All HV received a delayed BNT162b2 second dose. As per UK government guidelines at the time of vaccination, individuals receiving BNT162b2 second doses received these between 8-12 weeks following first dose, representing a delay compared to the licenced administration. Prior SARS-CoV-2 infection can influence the magnitude of the vaccine response (7), and as such we excluded two MDS and four HVs based on being positive for nucleoprotein-specific IgG (IgG(N)) (representing response to prior infection) (**Supplementary Figure 1A**). We observed that the anti-S IgG titres at approximately 2 weeks following the second dose were within the upper quantile in these previously virus-exposed individuals (**Supplementary Figure 1B, red dots)**. These were excluded from the overall immune efficacy analysis.

In the remaining (HV BNT162b2 n=26, MDS BNT162b2 n=15 and MDS ChAdOx1 n=21) cohort; we assessed the anti-S IgG titres following their second primary dose. Overall serological responses were: HV BNT162b2 100% (26/26); MDS BNT162b2 100% (15/15) and MDS ChAdOx1 76.2% (16/21) (**Figure 1A**); notably, the MDS ChAdOx1 cohort demonstrated significantly decreased serological titres to the MDS BNT162b2 cohort (**Figure 1A**). It is noteworthy that the median titre for the MDS BNT162b2 vaccinated patients is higher ($>10^3$) compared to the median reported in a heterogenous BNT162b2 vaccinated haematological cancer population ($<10^3$) observed in (2). Of the 5 non-responders within the MDS ChAdOx1, 3 patients were on disease-modifying treatments (5-azacytidine, venetoclax and danazol), with the patient on venetoclax/rituximab having a concurrent diagnosis of CLL. None of these patients were noted to be on steroid therapy around the time of vaccination; and no differences in the clinical white blood cells were observed between serological responders or non-responders (**Supplementary Figure 1C**). Similar to our previous reports (1, 2), there was no significant correlation between Spike IgG titres and age or the time between the first and second doses of the vaccine in the two MDS cohorts (**Supplementary Figure 1D**).

Next, we assessed the functional implications of seroconversion by neutralisation assays for SARS-CoV-2 WT and VOC alpha and delta. (**Figure 1B**). All but four MDS patients (**Figure 1B; coloured dots**) could neutralise all variant strains, but MDS cohorts showed significantly reduced median neutralisations for all 3 variant strains compared to HV (**Figure 1B)**; importantly this was the case for both the MDS ChAdOx1 and MDS BNT162b2

cohorts. We acknowledge the younger age of the HV cohort may contribute to this reduction, although age was not a determinant of neutralisation response in cancer patients in our previous reports (1,2). Review of the 4 MDS (2 BNT162b2 mRNA and 2 ChAdOx1 nCoV-19 COVID-19 vaccinated) patients classified as non-responders by neutralisation assay demonstrated that these patients were predominantly low risk MDS on no treatment, except one patient with excess of blasts on 5-azacytidine. These data clearly support the need for a third primary dose for this clinically vulnerable patient group irrespective of the seroconversion rates across cohorts. This is especially the case in those who have seroconverted but have a low anti-S IgG titre after the second dose. Third doses have demonstrated higher anti-S IgG titres in other haematological cohorts (8), and in keeping with our previous reports (1, 2), anti-S IgG titres were highly correlated with neutralisation among all cohorts (**Figure 1C**).

To measure functional SARS-CoV-2 T cell responses to vaccination, PBMCs from our study participants were assessed by ELISpot assays as described. It is noteworthy that no differences in the percentages of T cells amongst the PBMCs plated for ELISpot were observed across healthy and MDS cohorts (**Supplementary Figure 1E**). Using previously published thresholds for response (1, 2), non-T cell responders were seen in all cohorts (**Figure 2A; red dots**). Specifically, SARS-CoV-2-specific IFN-γ T cell responses against the delta variant were: HV BNT162b2 95% (20/21); MDS ChAdOx1 70.6% (12/17) and MDS BNT162b2 71.4% (10/14) (**Figure 2A**); in stark contrast to the comparable control CEF induced effector T cell responses across healthy and MDS samples (**Figure 2A**). Interestingly, significantly reduced T cell responses were seen in MDS BNT162b2 vaccinated patients when challenged with delta compared to *wt* variant strain (**Figure 2B**). Further, 5 MDS ChAdOx1 patients who did not have a serological response, were able to mount T cell responses. Additionally, treatment with either azacytidine or calcineurin inhibitor cyclosporine did not impair appropriate T cell responses. One high risk MDS BNT162b2 patient on 5-azacytidine, who showed no neutralizing activity, showed significantly reduced T cell response to *wt* and alpha, but not to delta variant. During the study period, the delta variant was the predominant variant of concern (VOC) in the UK. We observed non-significant but positive correlations between serological and IFN-γ T cell responses against the delta variant within the MDS vaccinated cohorts (**Figure 2C**). Numbers of individuals who were both serological and T cell responders were as follows: HV 95% (20/21), MDS BNT162b2 71.4% (10/14) and MDS ChAdOx1 52.9% (9/17) (**Figure 2C**). To further investigate the cellular readout of vaccine efficacy, we assessed the activation state of SARS-CoV-2 stimulated CD8 T cells, by measuring activation markers CD25 and CD69 cell surface expression by flow cytometry before and after *in vitro* stimulation. Despite the poorer humoral response observed in MDS-ChAdOx1 vaccinated individuals, we found significantly higher activated $CD25^+$ and $CD69^+$ CD8 T cells across all variants in this group of patients compared to those vaccinated with BNT162b2 vaccine (**Figure 2Di&ii)**. These data are compelling and warrant further investigation with one hypothesis being the ChAdOx1 vector reveals an innate weakness in this patient group inducing a hyper-stimulated but poorly efficacious effector T cell response.

In totality, although ChAdOx1-treated MDS patients do mount both humoral and cellular immune responses, they are weak in comparison to BNT162b2. The overall serological responses in the MDS cohorts were 100% for those who had completed the 2-dose BNT162b2 vaccine schedule compared to 76.2% of patients vaccinated with the ChAdOx1 vaccine. As such, it may be pertinent to advise the clinical community to administer MDS patients with an mRNA-based vaccine to promote enhanced immunity. Finally, we observed that neutralisation in seroconverted patients was significantly weaker for both the ChAdOx-1 and BNT162b2 MDS cohorts compared to HV, highlighting the potential benefit of a third primary dose for this clinically vulnerable patient group, in addition to subsequent booster doses.

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Table 1: Clinical characteristics of patients evaluable for analysis 2 weeks following two primary vaccine doses.

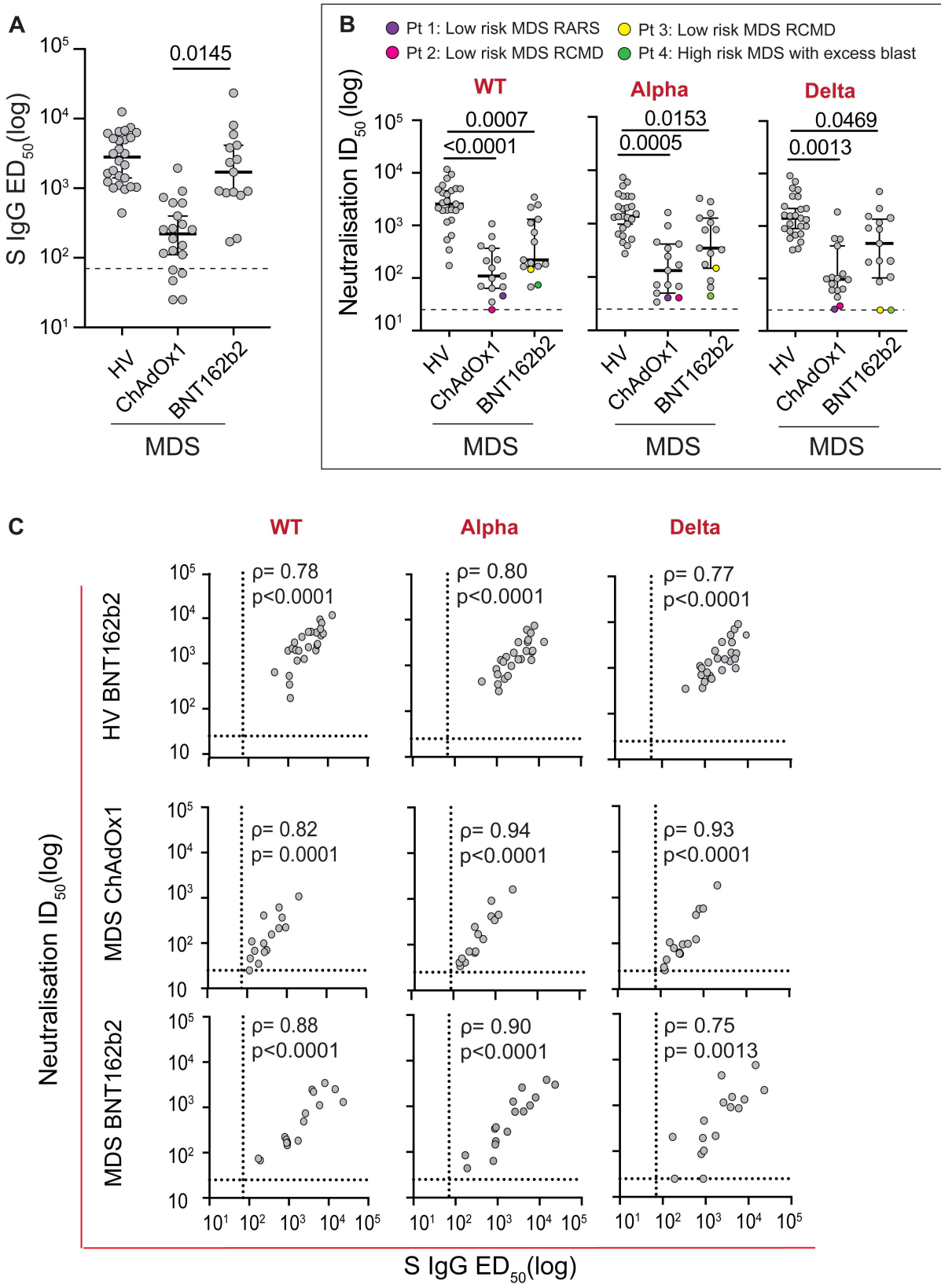
	All MDS patients	BNT162b2 vaccinated MDS patients	ChAdOx1 vaccinated MDS	BNT162b2 vaccinated Healthy volunteers
Total numbers	**38**	**16**	**22**	**30**
Age				
Median (Q1-Q3) years	67.5 (59-73)	69 (60-73)	67 (63-72)	35 (27-49)
Sex				
Male	23/38 (61%)	13	10	19
Female	15/38 (39%)	3	12	11
Race				
Caucasian	36/38 (95%)	16	20	19
BAME	2/38 (5%)	0	2	11
Median time from vaccine 1st dose to second dose				
Median (Q1-Q3) days	75 (68-80)	71 (68-77)	78(70-80)	74 (61-78)
Median time from vaccine second dose to blood sampling				
Median (Q1-Q3) days	19 (16-28)	21(18-30)	18 (15-24)	14 (13-17)
MDS WHO Subtypes				
MDS with single lineage dysplasia	2/38 (5.2%)	0	2	
MDS with ring sideroblasts	5/38 (13.2%)	1	4	
MDS with isolated del5q	1/38 (2.6%)	0	1	
MDS with multilineage dysplasia	20/38 (52.6%)	9	11	
MDS with multilineage dysplasia (hypo)	4/38 (10.5%)	2	2	
MDS with excess blasts	5/38 (13.2%)	3	2	
Chronic myelomonocytic leukemia	1/38 (2.6%)	1	0	
IPSS-R prognostic categories				
Low risk (low/very low/intermediate)	30/38 (78.9%)	11	19	
High risk (high/very high)	8/38 (21.1%)	5	3	
Treatment 15 days pre and post vaccination				
Transfusion support only or watch &wait	22/38 (57.9%)	7/16	15/22	
Growth factors/ TPO mimetics	6/38 (15.8%)	3/16	3/22	
Cyclosporin	3/38 (7.9%)	2/16	1/22	
5-Azacytidine	5/38 (13.2%)	3/16	2/22	
Others*	1/38 (5.2%)	1/16	0/22	

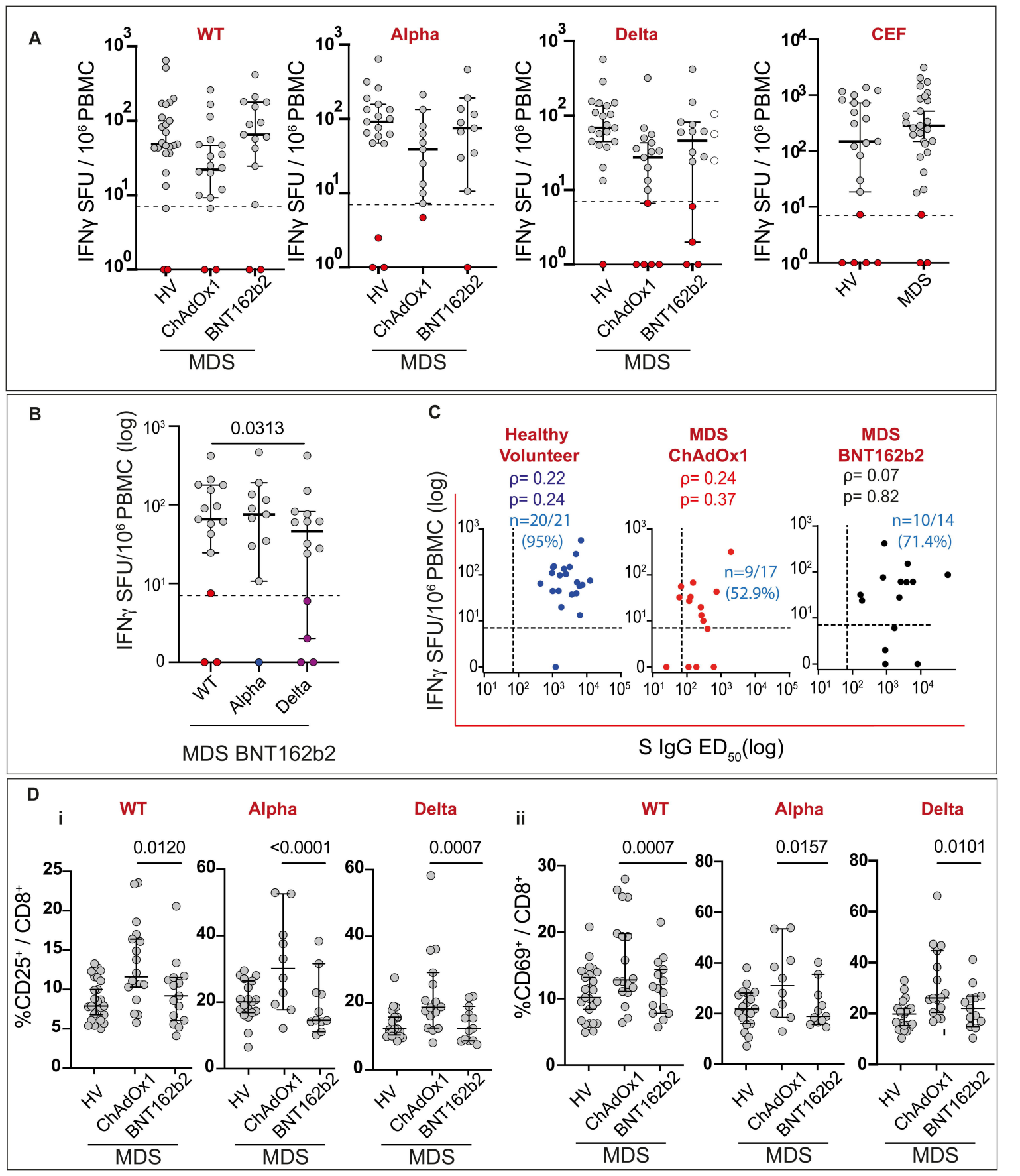
*This patient had concurrent CLL which was the indication for therapy with Venetoclax and Rituximab.
TPO- thrombopoietin. IPSS-R- Revised International Prognostic Scoring System

Figure Legends

Figure 1. *Humoral responses to BNT162b2 COVID-19 and ChAdOx1 nCoV-19 in patients with myelodysplastic syndromes.* **A.** Serum concentrations of IgG antibodies reactive to the spike protein of SARS-CoV-2 (S IgG) with cases positive for N IgG removed. Healthy volunteer (HV; n=26), MDS patients vaccinated with ChAdOx1 (MDS ChAdOx1; n=20), MDS patients vaccinated with BNT162b2 (MDS BNT162b2; n=15). Mean (95% CI): HV 3611 (2455-4768), MDS ChAdOx1 360.9 (149.9-572.2) and MDS BNT162b2 3781 (523.9-7037). Dashed line represents seroconversion threshold. Tukey's multiple comparison's test. **B.** Neutralisation of variants (as indicated in red) by plasma antibodies. Dashed line represents neutralisation threshold. Individual cases on the threshold line are coloured as indicated, as are their matched responses to other variants. HV (n=26); MDS ChAdOx1 (n=15); MDS BNT162b2 (n=15). Tukey's multiple comparison's test. **C.** Correlation matrices showing serum S IgG ED_{50} (log) against neutralisation for each indicated variant in the MDS ChAdOx1 (n=20) and MDS BNT162b2 (n=15) cohorts. Correlation coefficients (rho;r) and p values are given. Dashed lines represent threshold as previously described. Pearson's correlation test.

Figure 2: *Cellular responses to BNT162b2 COVID-19 and ChAdOx1 nCoV-19 in patients with myelodysplastic syndromes.*
A. IFNγ SFU formed after stimulation of PBMC from indicated cohorts in response to indicated variants. Samples were classed as responders if >7 cytokine secreting cells/10^6 PBMCs after correcting for background; as indicated by dashed line. Non-responders are coloured as indicated. Wt; (HV [n=26]; MDS ChAdOx1 [n=20]; MDS BNT162b2 [n=15]); B.1.1.7; (HV [n=11]; MDS ChAdOx1 [n=11]; MDS BNT162b2 [n=15]); B.1.617.2; (HV [n=21]; MDS ChAdOx1 [n=17]; MDS BNT162b2 [n=14]). Tukey's multiple comparison's test. CEF = CMV, EBV and influenza virus positive control peptides **B.** IFNg SFU formed after stimulation of PBMC from MDS BNT162b2 cases to indicated variants. Wt (n=15); B.1.1.7 (n=11); B.1.617.2 (n=14). Tukey's multiple comparison's test. **C.** Correlation matrices showing IFNg SFU formed after PBMCs were stimulated with the B.1.617.2 variant and paired S IgG ED_{50} values for indicated cohorts. Correlation coefficients (rho;r), p values, n numbers and % double positivity are given. Dashed lines represent thresholds as previously described. Pearson's correlation test. **E (i&ii).** $CD8^+CD25^+$ cells (**i**) and $CD8^+CD69^+$ cells (**ii**) within the live $CD3^+$ population after stimulation of PBMC from indicated cohorts in response to indicated variants. HV (n=26); MDS ChAdOx1 (n=20); MDS BNT162b2 (n=15). Tukey's multiple comparison's test.


A
S IgG ED50(log)
0.0145
HV
ChAdOx1
BNT162b2
MDS
B
Pt 1: Low risk MDS RARS
Pt 2: Low risk MDS RCMD
Pt 3: Low risk MDS RCMD
Pt 4: High risk MDS with excess blast
Neutralisation ID50 (log)
WT
Alpha
Delta
0.0007
<0.0001
0.0153
0.0005
0.0469
0.0013
C
WT
Alpha
Delta
HV BNT162b2
MDS ChAdOx1
MDS BNT162b2
ρ= 0.78
p<0.0001
ρ= 0.80
p<0.0001
ρ= 0.77
p<0.0001
ρ= 0.82
p= 0.0001
ρ= 0.94
p<0.0001
ρ= 0.93
p<0.0001
ρ= 0.88
p<0.0001
ρ= 0.90
p<0.0001
ρ= 0.75
p= 0.0013
Neutralisation ID50(log)
S IgG ED50(log)


A
WT
Alpha
Delta
CEF
IFNγ SFU / 10⁶ PBMC
HV
ChAdOx1
BNT162b2
MDS
B
0.0313
IFNγ SFU/10⁶ PBMC (log)
WT
Alpha
Delta
MDS BNT162b2
C
Healthy Volunteer
ρ= 0.22
p= 0.24
n=20/21 (95%)
MDS ChAdOx1
ρ= 0.24
p= 0.37
n=9/17 (52.9%)
MDS BNT162b2
ρ= 0.07
p= 0.82
n=10/14 (71.4%)
IFNγ SFU/10⁶ PBMC (log)
S IgG ED50(log)
D
i
ii
%CD25⁺ / CD8⁺
%CD69⁺ / CD8⁺
0.0120
<0.0001
0.0007
0.0007
0.0157
0.0101

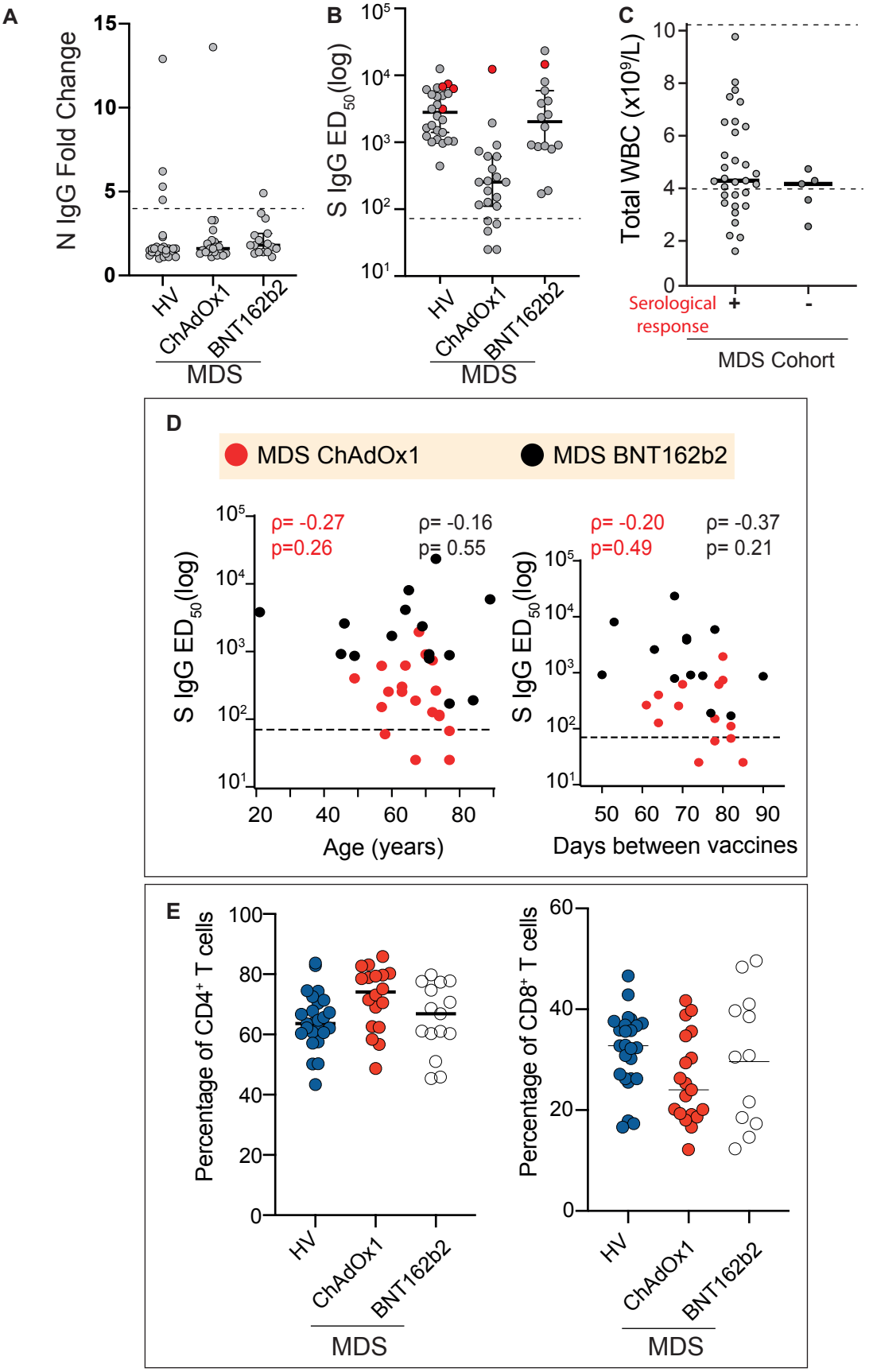

A
N IgG Fold Change
15
10
5
0
HV
ChAdOx1
BNT162b2
MDS
B
S IgG ED50 (log)
10^5
10^4
10^3
10^2
10^1
HV
ChAdOx1
BNT162b2
MDS
C
Total WBC (x10^9/L)
10
8
6
4
2
0
Serological response
+
-
MDS Cohort
D
MDS ChAdOx1
MDS BNT162b2
S IgG ED50 (log)
ρ= -0.27
p=0.26
ρ= -0.16
p= 0.55
20
40
60
80
Age (years)
ρ= -0.20
p=0.49
ρ= -0.37
p= 0.21
50
60
70
80
90
Days between vaccines
E
Percentage of CD4+ T cells
100
80
60
40
20
0
HV
ChAdOx1
BNT162b2
MDS
Percentage of CD8+ T cells
60
40
20
0
HV
ChAdOx1
BNT162b2
MDS

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Figure Legends

Supplementary Figure 1. A. Plasma concentrations of IgG antibodies reactive to the N-protein of SARS-CoV-2 (N IgG). Healthy volunteer (HV; n=30), MDS patients vaccinated with ChAdOx1 (MDS ChAdOx1; n=21), MDS patients vaccinated with BNT162b2 (MDS BNT162b2; n=16). **B.** Plasma concentrations of IgG antibodies reactive to the spike protein of SARS-CoV-2 (S IgG). Red indicates individuals also positive for N IgG. N numbers as in A. **C.** Quantification of clinical white blood cells on routine blood tests around the time of vaccination amongst MDS patients comparing serological responders with serological non-responder. Dashed lines represent healthy ranges. **D:** Correlation matrices showing plasma S IgG dilution at 50% maximum binding (ED_{50}) (log) against age and days between vaccination in the MDS ChAdOx1 (n=20) and MDS BNT162b2 (n=15) cohorts. Correlation coefficients (rho;r) and p values are given. Dashed lines represent threshold as previously described. Pearson's correlation test. **E:** Percentage of $CD4^+$ and $CD8^+$ T cells amongst the PBMCs plated for ELISpot for each of the cohorts.