COVID-19 vaccines elicit robust cellular immunity and clinical protection in chronic lymphocytic leukemia

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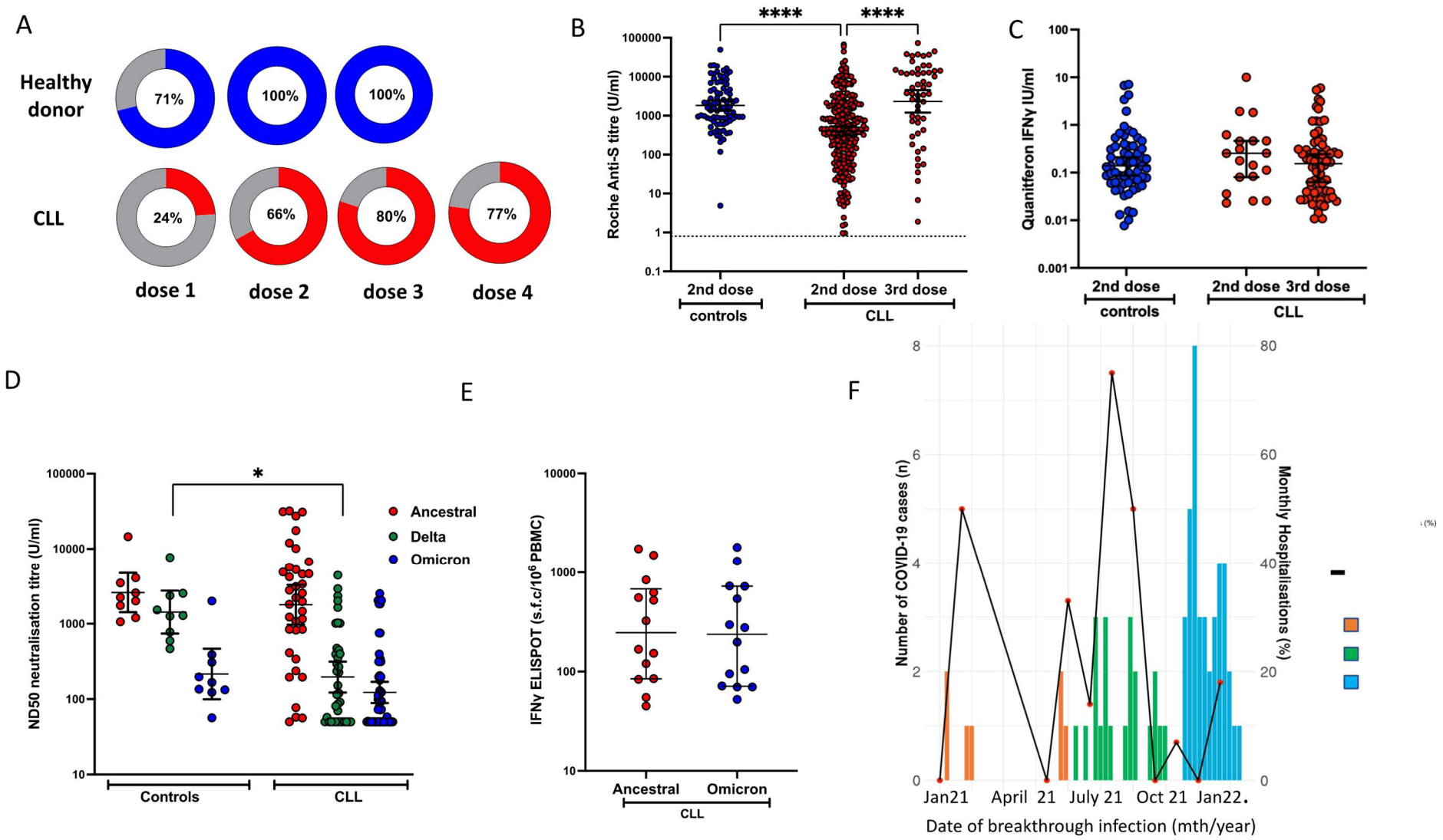
DOI:
10.1016/j.ccell.2022.05.001

Document Version
Peer reviewed version

Citation for published version (Harvard):
Parry, H, Bruton, R, Roberts, T, Mcilroy, G, Damery, S, Sylla, P, Dowell, AC, Tut, G, Lancaster, T, Bone, D, Willett, B, Logan, N, Scott, S, Hulme, S, Jadir, A, Amin, U, Nicol, S, Stephens, C, Faustini, S, Al-taei, S, Richter, A, Blakeway, D, Verma, K, Margielewska-davies, S, Pearce, H, Pratt, G, Zuo, J, Paneesha, S & Moss, P 2022, 'COVID-19 vaccines elicit robust cellular immunity and clinical protection in chronic lymphocytic leukemia', *Cancer Cell*, vol. 40, no. 6, pp. 584-586. https://doi.org/10.1016/j.ccell.2022.05.001


A
Healthy donor
CLL
71%
100%
100%
24%
66%
80%
77%
dose 1
dose 2
dose 3
dose 4
B
****
****
Roche Anti-S titre (U/ml)
100000
10000
1000
100
10
1
0.1
2nd dose
controls
2nd dose 3rd dose
CLL
C
Quanitferon IFNγ IU/ml
100
10
1
0.1
0.01
0.001
2nd dose
controls
2nd dose 3rd dose
CLL
D
*
ND50 neutralisation titre (U/ml)
100000
10000
1000
100
10
Ancestral
Delta
Omicron
Controls
CLL
E
IFNγ ELISPOT (s.f.c/10^6 PBMC)
10000
1000
100
10
Ancestral
Omicron
CLL
F
Number of COVID-19 cases (n)
Monthly Hospitalisations (%)
8
6
4
2
0
80
60
40
20
0
Jan21
April 21
July 21
Oct 21
Jan22.
Date of breakthrough infection (mth/year)

Supplemental Figure S1.
COVID-19 vaccines elicit robust cellular immunity and clinical protection in CLL

A. Proportion of CLL participants who developed a positive antibody response following 1st (n=267), 2nd (n=486), 3rd (n=374) and 4th (n=171) vaccine dose compared to healthy donors following 1st (n=93), 2nd (n=93) and 3rd dose (n=9). Participants with evidence of natural infection were excluded from analysis. B. Spike-specific antibody titer in participants with a positive antibody response following 2nd dose (n=257) compared to controls (n=85) ($p<0.0001$), and in CLL participants following 3rd dose (n=54) ($p<0.0001$) (Post hoc Dunn analysis). Cut-off for positive response is indicated by dotted line (GM and 95% CI shown). C. IFNγ concentration following whole blood overnight stimulation with SARS-CoV-2 peptide pools is shown in CLL participants after 2nd (n=19) and 3rd vaccine doses (n=70) and controls (n=61) following 2nd dose (median and IQR shown). D. ND50 neutralising antibody titers against viral pseudotypes bearing ancestral, Delta and Omicron spike glycoproteins following 3rd vaccine dose from age-matched controls (n=9) and participants with CLL (n=37) (post hoc Dunn for Delta $p= 0.03$) (GM and 95% CI shown). E. IFN-γ ELISpot assay following 3rd vaccine dose in infection-naïve CLL patients following peptide stimulation with either ancestral or Omicron peptide pools ($p=0.33$) (Wilcoxon) (Median and IQR shown) (n=14). F. Bar chart to show the number and timing of breakthrough infections since first vaccine dose (n=66). Colors indicate dominant viral variants over the sample period. The proportion of infected participants who required hospitalisation is shown by the black line.

Supplementary Table S1. Patient demographics by vaccine dose

		post 2nd vaccine	Post 3rd vaccine	post 4th vaccine
Number of patients		500	404	186
Age (years)	Median	67	67	68
	IQR	60 to 72	62 to 73	63 to 72
	Range	39 to 89	40 to 89	45 to 84
Sex	Men	267 (53%)	216 (54%)	102 (55%)
	Women	233 (47%)	188 (46%)	84 (45%)
Vaccine received (first two doses)	BNT162b2	204 (41%)	161 (40%)	71 (38%)
	ChAdOx1	296 (59%)	243 (60%)	115 (62%)
Vaccine received (third dose)	BNT162b2		375 (93%)	
	mRNA1273		18 (5%)	
	NVX-Co2373		10 (3%)	
	ChAdOx1		1 (0.2%)	
Vaccine received (fourth dose)	BNT162b2			150 (81%)
	mRNA1273			36 (19%)
Time from vaccine dose to blood test (days)	Median	20	20	21
	IQR	17 to 29	17 to 27	17 to 29
	Range	4 to 133	0 to 163	0 to 125
Time since CLL diagnosis (months)	Median	73	77	75
	IQR	34 to 133	36 to 135	37 to 124
	Range	1 to 408	1 to 408	1 to 373
CLL stage at diagnosis	A	429 (86%)	348 (86%)	158 (85%)
	B	30 (6%)	21 (5%)	10 (5%)
	C	41 (8%)	35 (9%)	18 (10%)
Previous treatment	Watch and Wait	279 (56%)	225 (56%)	102 (55%)
	Treatment planned	13 (3%)	10 (2%)	4 (2%)
	1 line	128 (26%)	104 (26%)	46 (25%)
	2 lines	48 (10%)	40 (10%)	23 (12%)
	3+ lines	32 (6%)	25 (6%)	11 (6%)
On BTKi		99 (20%)	82 (20%)	38 (20%)
On venetoclax		21 (4%)	18 (5%)	11 (6%)
Previous chemotherapy		143 (29%)	117 (29%)	56 (30%)
Previous anti-CD20		153 (31%)	125 (31%)	61 (33%)
History of infection	Frequent infections	145 (29%)	114 (28%)	59 (32%)
	Hospitalisation with infection	95 (19%)	72 (18%)	35 (17%)
Prophylactic antibiotics		37 (7%)	31 (8%)	15 (8%)
IVIG		41 (8%)	33 (8%)	23 (12%)
Immunoglobulin deficiency	Number	471	381	174
	IgG (<6g/L)	236 (50%)	188 (49%)	97 (56%)
	IgA (<0.8g/L)	232 (49%)	189 (50%)	88 (51%)
	IgM (<0.5g/L)	177 (38%)	136 (36%)	65 (37%)
*Patients on a delayed vaccine interval for first and second doses.				

Supplementary methods

Study design and participants

Patients with a diagnosis of CLL or small lymphocytic leukaemia (SLL) were recruited to study with no additional exclusion criteria. Informed consent was obtained by remote consultation and work performed under the CIA UPH IRAS approval (REC 20\NW\0240) from North-West and Preston ethics committee and conducted according to the Declaration of Helsinki. The dates and type of SARS-CoV-2 vaccination were obtained with self-reported information on stage and date of CLL diagnosis, CLL treatment and infection history as previously described. Participant demographics can be found in Table S1.

Samples were obtained 2-3 weeks following the second, third and fourth dose of vaccination. Local participants undertook phlebotomy whilst those more distant donated a dried blood spot sample (DBS). 93 healthy donor controls were recruited from local primary care networks (median age 73; (IQR 68-74.5); 56 were female (60%) and 59 received ChAdOx1 primary course and 34 received BNT162b2) .

Roche Elecsys® electrochemiluminescence immunoassay (ECLIA)

Using ECLIA, qualitative IgG/A/M Anti-nucleocapsid protein (NP) antibodies specific to SARS-CoV-2 were detected (COV2, Product code: 09203079190); cut-off index value ≥1.0 considered positive for anti-nucleocapsid antibodies. Using the quantitative ECLIA assay, anti-spike (S) receptor binding domain antibodies were detected (COV2 S, Product code 09289275190) with values ≥0.8 U/ml considered positive.

Dried blood spot ELISA analysis

Dried blood spot (DBS) analysis was carried out as previously described to ascertain the sero-positive rate amongst donors (Cook et al., 2021). IgG, IgA and IgM antibody isotypes against stabilised trimeric SARS-CoV-2 spike glycoprotein are reported with a positive result classed as a ratio of 1 or more.

Serum Immunoglobulin concentration

Quantification of IgG, IgA and IgM was evaluated using the COBAS 6000 (Roche) at the University of Birmingham Clinical Immunology Service as previously described (Parry *et al.*, 2021).

Neutralization assays

HEK293, HEK293T and 293-ACE2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, 200mM L-glutamine, 100µg/ml streptomycin and 100 IU/ml penicillin. HEK293T cells were transfected with the appropriate SARS-CoV-2 spike gene expression vector in conjunction with lentiviral vectors p8.91 and pCSFLW using polyethylenimine (PEI, Polysciences, Warrington, USA). HIV (SARS-CoV-2) pseudotype-containing supernatants were harvested 48 hours post-transfection, aliquoted and frozen at -80^{o}C prior to use. The SARS-CoV-2 spike glycoprotein expression constructs for ancestral Hu-1, B.1.617.2 and Omicron have been described previously (Willett et al., 2022). The delta construct bore the following mutations relative to the ancestral Hu-1 sequence (GenBank: MN908947): T19R, G142D, E156del, F157del, R158G, L452R, T478K, D614G, P681R, D950N. 293-ACE2 target cells were maintained in complete DMEM supplemented with 2µg/ml puromycin.

Neutralising activity in each sample was measured by a serial dilution approach. Each sample was serially diluted in triplicate from 1:50 to 1:36,450 in complete DMEM prior to incubation with approximately 1x10^{6} CPS per well of HIV (SARS-CoV-2) pseudotypes, incubated for 1 hour, and plated onto 239-ACE2 target cells. Luciferase activity was quantified after 48-72 hours by the addition of Steadylite Plus chemiluminescence substrate and analysis on a Perkin Elmer EnSight multimode plate reader (Perkin Elmer, Beaconsfield, UK). Antibody titer was then estimated by interpolating the point at which infectivity had been reduced to 50% of the value for the 'no serum' control samples.

QuantiFERON assay

T cell responses were measured by QuantiFERON assay, using the QuantiFERON SARS-CoV-2 assay (Catalogue 626715, QuantiFERON SARS-CoV-2 RUO, Qiagen). 1ml of whole blood was added to the test tubes, including QuantiFERON Nil (negative control), QuantiFERON Mitogen BCTs (positive control) and a QFN SARS CoV-2 Ag2 tube containing epitopes from whole spike that simulate both CD4+ and CD8+ T cells. After 18hr incubation, plasma was retrieved from each tube and an ELISA for IFN-γ release performed (Catalogue number 626410 QuanitFERON ELISA, Qiagen). The concentration of IFN-γ in IU/ml was confirmed after deduction of the QFN-SARS-CoV-2 Nil concentration.

ELISpot assay

250,000 PBMC were incubated overnight with peptide pools containing 15-mer peptides overlapping by 10aa from SARS-CoV-2 spike S1 or S2 domains for either the ancestral

strain or the Omicron variant (PepMix™ SARS-CoV-2 (Spike B.1.1.529 / Omicron) Product Code: PM-SARS2-SMUT08-1
PepMix™ SARS-CoV-2 (Spike Glycoprotein) Product Code: PM-WCPV-S-2
JPT Peptide Technologies, Germany). T cell responses were determined using a Human IFNγ ELISpot PRO kit (3420-3PT Mabtech, Sweden) and plates read using the Bioreader5000 (Bio-Sys, Germany)

Statistical analysis

For comparative analysis, Mann-Whitney U-tests or Spearman rank correlation were performed and antibody data presented either as median or geometric means + 95% confidence intervals. Kruskal-Wallis was performed with post-hoc Dunn's analysis for comparative groups and Wilcoxon's matched-pairs signed rank test for paired responses. Logistic regression of clinical variables was tested for associations with positive antibody response after each vaccine dose. Chi-square analysis was used to compare proportions of responders and Kaplan-Meier for time to first treatment with Gehan-Breslow-Wilcoxon test reported. Analysis was performed using Graphpad prism v9.1.0 for Mac (San Diego, California USA) and SPSS Statistics v27.0 for Windows (Armonk, NY: IBM Corp.)

Supplementary references

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