MATERIAL AND METHODS

Peripheral blood sample preparation

Peripheral blood (PB) samples (8 ml of whole blood in EDTA) were collected and immediately used for total mononuclear cell (MC) isolation. Whole blood was diluted with phosphate buffer saline (PBS) 1:1 (v/v), then subjected to a density gradient stratification with Lymphoprep (Euroclone), at 500xg for 30 minutes. The white ring interface containing peripheral blood mononuclear cells (PBMC) was collected, washed in PBS, and then frozen in cryovials in liquid nitrogen containers.

PBMC stimulation

Subsequently, cells were thawed, counted, and resuspended at $2x10^6$/ml in RPMI-1640 medium (Euroclone), supplemented with 10% fetal bovine serum (FBS) (Euroclone), 2 mM l-glutamine (Euroclone), 100 U/ml penicillin and 100 µg/ml streptomycin (Euroclone), 2 hours in incubator at 37°C, 5% CO_2. Next, cells were counted and used for subsequent flow cytometry characterization and IFNγ ELISpot analysis. Cells were stimulated with 6nM of 15-mer peptides overlapping 11 amino acids of full-length Spike protein (Miltenyi Biotec) for 6 hours. The last 4 hours of stimulation, cells received brefeldin (Golgi-Plug, BD Bioscience), according to the manufacturer indications [1].

Flow cytometry analysis

Following cell stimulation, PBMC were stained for 30 minutes, 4°C, at dark with the following anti-human antibodies against the listed surface antigens: CD3-BB700 (clone: OKT3), CD4-APC (clone: SK3), CD8-PECF594 (clone: HIT8a), (all purchased by BD Biosciences). For intracellular cytokine detection, cells were fixed and permeabilized using the Cytofix and Cytoperm fixation and permeabilization kit (BD) for 10 min at 4°C. Cells were then washed in PBS and stained for 30 minutes, 4°C, at dark, with the anti-human IFNγ-BV650 (clone: 4S.B3), antibody (BD Biosciences) [2]. Cells were analyzed using a BD FACS Fortessa x20,

equipped with 5 lasers, for T cell analysis and IFNγ production. Viable cells were identified, based on FSC-A and SSC-A gating. T cell subset populations were set on $CD3^+$ gated cells, then interrogated for $CD4^+$, $CD8^+$, $CD4^+IFN\gamma^+$ and $CD8^+IFN\gamma^+$ cells. Flow data were analyzed using the FlowJo v10 software (TreeStar).

IFNγ ELISpot

IFNγ ELISpot analysis was performed ex vivo using whole PBMC after thawing [3]. Tests were performed in duplicate and with a positive control (anti-CD3 monoclonal antibody 2 µg/well) (Miltenyi Biotec). PVDF filter 96 well plates (R&D Systems) pre-coated with IFNγ-specific antibodies (EL285 ELISpot kit, R&D Systems) were washed with PBS and blocked with complete medium (Euroclone) containing for 1h. PBMC at 5 x 10^5/well were stimulated for 16–20 h with an overlapping peptide pool representing full-length Spike protein. Bound IFNγ was visualized using a secondary anti-IFNγ polyclonal biotinylated antibody for 2h. Then plates were washed 4 times with kit buffer and then incubated with alkaline phosphatase-conjugated streptavidin for other 2h, followed by washing and then incubation with a 5-bromo-4-chloro-3'-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) substrate (EL285 ELISpot kit, R&D Systems). Spot forming cells (SFC) were counted by a stereomicroscope and were summarized as mean values of each duplicate. For the determination of anti-Spike T cell response frequencies negative control values were subtracted from the positive values. Values were defined as positive if there was >2 times the negative value and with at least 5 SFC per well [4,5].

References

1) Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M, *et al*. COVID-19 vaccine BNT162b1 elicits human antibody and T_H1 T cell responses. *Nature* 2020; 586:594-599.
2) Gallazzi M, Baci D, Mortara L, Bosi A, Buono G, Naselli A, *et al*. Prostate Cancer Peripheral Blood NK Cells Show Enhanced CD9, CD49a, CXCR4, CXCL8, MMP-9 Production and Secrete Monocyte-Recruiting and Polarizing Factors. *Front Immunol* 2021; 11:586126.
3) Mortara L, Gras-Masse H, Rommens C, Venet A, Guillet JG, Bourgault-Villada I. Type 1 CD4(+) T-cell help is required for induction of antipeptide multispecific cytotoxic T lymphocytes by a lipopeptidic vaccine in rhesus macaques. *J Virol* 1999; 73:4447-51.
4) Gallais F, Velay A, Nazon C, Wendling MJ, Partisani M, Sibilia J, *et al*. Intrafamilial Exposure to SARS-CoV-2 Associated with Cellular Immune Response without Seroconversion, France. *Emerg Infect Dis* 2021; 27:113–21.
5) Pulendran B, Arunachalam PS. Systems biological assessment of human immunity to BNT162b2 mRNA vaccination. *Res Sq* 2021; e-pub ahead of print 22 April 2021; doi: 10.21203/rs.3.rs-438662/

Table ST1: Main characteristics of the 64 patients with hematological malignancies who underwent autologous stem cell transplantation at the time of anti-SARS-CoV-2 vaccine.

Characteristics	Number, unless otherwise specified
Number of patients	64
Median age, years (range)	62 (29-75)
Male (%)	37 (58)
ASCT as last therapy (%)	32 (50)
HM types (%)	
Multiple myeloma	45 (70)
Plasma cell leukemia	1 (2)
Follicular lymphoma	6 (9)
Hodgkin lymphoma	5 (8)
Mantle cell lymphoma	2 (3)
Mediastinal B-cell lymphoma	2 (3)
Diffuse large B-cell lymphoma	1 (2)
Angioimmunoblastic T-cell lymphoma	1 (2)
NK/T-cell lymphoma	1 (2)
Stable/progressive disease at vaccination (%)	9 (14)
Previous Covid-19 (%)	2 (3)
Median time from ASCT to vaccination, months (range)	25.6 (1.2-58.1)
Median time from vaccination to test, days (range)	28 (25-48)

ASCT: autologous stem cell transplantation; HM: hematological malignancy.

Table ST2: Seroconversion rates according to last therapy.

LAST THERAPY	N° of PATIENTS (%)	SEROLOGY RESULTS	
		POSITIVE (%)	NEGATIVE (%)
ASCT	32 (50)	31 (48)	1 (2)
IMIDs	15 (23)	14 (21)	1 (2)
ongoing	*13 (20)*	*12 (18)*	*1 (2)*
not ongoing	*2 (3)*	*2 (3)*	*0*
Dara-RD	9 (14)	5 (8)	4 (6)
ongoing	*8 (12)*	*4 (6)*	*4 (6)*
not ongoing	*1 (2)**	*1 (2)**	*0*
Rituximab	1 (2)	0	1 (2)
ongoing	*1 (2)*	*0*	*1 (2)*
BsAb	1 (2)	0	1 (2)
ongoing	*1 (2)*	*0*	*1 (2)*
PIs	2 (3)	2 (3)	0
ongoing	*2 (3)*	*2 (3)*	*0*
CHT/RT	2 (3)	2 (3)	0
ongoing	*1 (2)*	*1 (2)*	*0*
not ongoing	*1 (2)*	*1 (2)*	*0*
Allo-SCT	1 (2)	1 (2)	0
Supportive	1 (2)	1 (2)	0
ALL	**64 (100)**	**56 (87)**	**8 (13)**

*: Dara-RD therapy was discontinued 2.7 months before vaccination.

ASCT: autologous stem cell transplantation; IMIDs: immunomodulatory drugs; Dara-RD: daratumumab/lenalidomide/dexamethasone; BsAb: bispecific antibody; PIs: proteasome inhibitors; CHT/RT: chemo/radiotherapy; Allo-SCT: allogeneic stem cell transplantation.

Table ST3: Detailed immune data per individual patient.

Patient ID	Disease	Last treatment	Serology (POS/NEG)	Ab titer (BAU/ml)	ELISpot (SFC/10^6PBMC)
1	Plasma Cell Myeloma	IMIDs	POS	1767	42
2	Classic Hodgkin Lymphoma	Palliative	POS	1112	0
5	Plasma Cell Myeloma	IMIDs	POS	1092	102
6	Follicular Lymphoma	ASCT	NEG	-	0
7	Follicular Lymphoma	ASCT	POS	1254	158
944-2	Plasma Cell Myeloma	Dara-RD	NEG	-	130
944-5	Plasma Cell Myeloma	ASCT	POS	109	180
944-7	Plasma Cell Myeloma	Dara-RD	POS	112	0
944-24	Follicular Lymphoma	BsAb	NEG	-	72
944-30	Plasma Cell Myeloma	Dara-RD	POS	101	30
944-47	Plasma Cell Myeloma	Dara-RD	NEG	-	0
944-50	Angioimmunoblastic T-cell Lymphoma	IMIDs	NEG	-	57
944-54	Plasma Cell Myeloma	ASCT	POS	166	9
944-57	Plasma Cell Myeloma	Dara-RD	NEG	-	0
944-62	Follicular Lymphoma	Rituximab	NEG	-	92
944-80	Plasma Cell Myeloma	Dara-RD	NEG	-	0

SFC: spot forming cells; PBMC: peripheral blood mononuclear cells; ASCT: autologous stem cell transplantation; IMIDs: immunomodulatory drugs; Dara-RD: daratumumab/lenalidomide/dexamethasone; BsAb: bispecific antibody.