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## **SARS-CoV-2 T-cell responses in allogeneic hematopoietic stem cell recipients following two doses of BNT162b2 vaccine.**

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## OBJECTIFS

Virus-specific humoral and cellular immunity act synergistically to protect the host from viral infection. In our recent prospective monocentric study of 117 hematopoietic stem cell adult recipients, we found that 54% and 83 % achieved a humoral response after a first and a second vaccine dose of BNT162b2 anti-SARS-CoV-2 messenger RNA respectively. In this study, we evaluated the T-cell response against the SARS-CoV-2 spike protein after two doses of vaccine in a cohort of patients allografted (N=46) for acute myeloblastic leukemia (AML, N=27) or myelodysplastic syndrome (MDS, N=19) (Table 1) and 16 healthy controls.

For the 18 patients for whom we detected the highest frequencies of anti-spike CD3+ T cells, we evaluated the anti-spike responses of CD4+ and CD8+ T-cell populations as well as their functionality by testing the production of IFN-γ and TNF-α by flow cytometry

## METHODS

### Peripheral blood mononuclear cell (PBMC) isolation

Peripheral blood was collected on Ethylenediaminetetraacetic acid (EDTA). PBMC were isolated using Ficoll density gradient centrifugation and frozen with Fetal Bovine Serum-10% dimethylsulfoxide (DMSO). Immunophenotype was determined by flow cytometry with fluorochrome-conjugated monoclonal antibodies: Fixable Viability Stain-780 , CD45-V500 ; CD3-BUV395, CD14-PE, CD19-BB515 (BD Biosciences) and HLA-DR-APC (Biolegend).

### Peptide pools

The peptide pools consisted of 15mers with an 11 amino acid overlap spanning the whole protein sequence of the SARS-CoV-2 Spike glycoprotein (Prot\_S1; \_S+ and \_S PepTivator peptide pools), 43 peptides from EBV proteins (pepTivator EBV-consensus) and pepTivator CMV pp65 (all from Miltenyi Biotec, Bergisch Gladbach, Germany).

### INF-γ ELISPOT assay

PBMC were thawed and rested overnight in complete culture medium. PBMC (2x10<sup>5</sup>) in 100μL were added to each well of Human ELISpot<sup>PRO</sup> Kit plates (Mabtech 3420-2AST-10, Nacka Strand, Sweden). Cells were incubated with culture medium (negative control); the 3 peptide pools covering the SARS-CoV-2 Spike glycoprotein or EBV or CMVpp65. The plates were incubated at 37°C and 5%CO<sub>2</sub> for 24h, developed according to the manufacturer's instructions and dried before spot-counting on a Bioreader 5000-pro-S (BIOSYS GmbH, Karben, Germany). The median background for the negative control was 0 SFU/2x10<sup>5</sup> cells (range 0-5). Frequencies of spot forming units (SFU) were reported per 1x10<sup>5</sup> CD3+ T-cells, evaluated beforehand in each PBMC suspension.

### Intracellular cytokine staining by flow cytometry

SARS-CoV-2 T cell Analysis Kits (PBMC) from Miltenyi Biotec was used for analysis of CD4+ and CD8+ SARS-CoV-2-reactive T cells. A minimum of 100,000 live CD3+ T-cells were acquired on a BD LSRFortessa™ InSurgent (BD Biosciences, San Jose, CA) and results were analyzed using FlowJo v.10.7.1 software (FlowJo, BD LifeSciences).

Antibody response to the SARS-CoV-2 spike protein receptor-binding domain was tested with anti-SARS-CoV-2 immunoassay, Roche Elecsys<sup>®</sup>, Rotkreuz, Switzerland. As recommended by the manufacturer, titers ≥0.8 U/mL were considered positive, the highest value being >250.

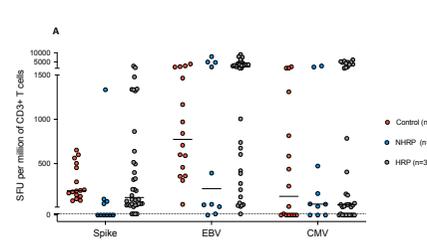
**Table 1: Vaccinated individuals' characteristics**

	Allogeneic hematopoietic stem-cell recipients (N=46)				Healthy controls (N=16)
	Yes (HR) <sup>1</sup> 36 (78%)		No (NHR) <sup>2</sup> 10 (22%)		
Antibody response after two doses of BNT162b2 vaccination	Yes (HR) <sup>1</sup> 36 (78%)		No (NHR) <sup>2</sup> 10 (22%)		Yes 16 (100%)
T-cell response after two doses of BNT162b2 vaccination	Yes 32 (89%)	No 4 (11%)	Yes 4 (40%)	No 6 (60%)	Yes 16 (100%)
Median time from transplant to vaccination (days)	1032 (126-3796)	523 (471-914)	236 (208-384)	237 (112-372)	NA
Range					
Median time from first to second vaccination (days)	21 (19-35)	28 (21-29)	23 (16-29)	21 (21-29)	24 (18-32)
Range					
Median time from second vaccination to T cells response analyses (days)	31 (22-67)	36 (25-69)	62 (56-70)	45 (26-56)	58 (32-70)
Range					
Median time from first vaccination to T cells response analyses (days)	56 (43-95)	64 (52-90)	85 (85-86)	66 (47-85)	81 (62-91)
Range					
Underlying disease	19 AML 13 MDS	4 MDS	3 AML 1 MDS	5 AML 1 MDS	NA
Median age: years (range)	64 (30-75)	58 (49-72)	66 (41-70)	57 (44-66)	52 (37-63)
Gender					
Male	19	2	2	4	3
Female	13	2	1	2	13
Donor type					
Geno-identical	6	1	1	0	NA
MUD	15	2	0	2	NA
Haploidentical	10	1	3	4	NA
9/10 mis-MUD	1	0	0	0	NA
Conditioning					
Myeloablative	1	0	0	0	NA
Reduced-intensity	30	4	4	6	NA
Sequential	1	0	0	0	NA
GVHD prophylaxis					
CSA+MMF+ATG	15	1	0	1	NA
CSA+MMF+ATG	7	3	4	5	NA
PfCY only	10	0	0	0	NA
Previous GVHD					
Yes	19 (59%)	2 (50%)	2 (50%)	3 (50%)	NA
No	13 (41%)	2 (50%)	2 (50%)	3 (50%)	NA
Ongoing treatment *					
No	27 (84%)	4 (100%)	3 (75%)	3 (50%)	NA
Yes	5 (16%)	0	1 (25%)	3 (50%)	NA

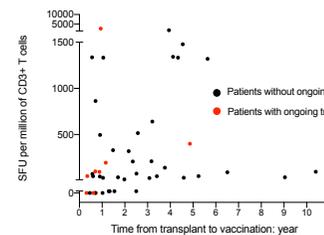
<sup>1</sup>: Humoral Responders  
<sup>2</sup>: Non Humoral Responders  
AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; MUD: matched unrelated donor; GVHD: graft-versus-host disease; CSA: cyclosporine; MMF: mycophenolate mofetil; PfCY: post-transplant Cyclophosphamide; NA: not applicable.  
\*: immunosuppressive drugs or chemotherapy.

- For the 46 patients , the median time between Allo-HSCT and first vaccination was 590 days (range: 112-3796)
- 80% of patients were free of immunosuppressive or chemotherapy treatment (n=37)
- Nine patients were on on-going treatment including 5 for active chronic GVHD (cyclosporine n=2, cyclosporine + corticosteroid n=3), while one patient was under corticosteroid for a chronic rheumatic disease and another one received 5' azacytidine for relapse prevention. Also, two early patients were on their way to stop cyclosporine. No patients had acute graft-versus-host disease (GVHD) at time of analysis.

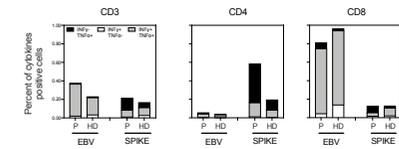
## RESULTS



**Figure 1: T-cell response (A) and PBMC phenotype (B)**

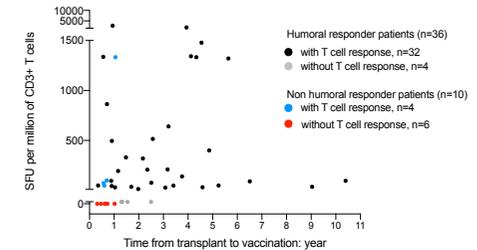


**Figure 4 : Relation between time from transplant to vaccination and vaccine response**



**Figure 2 : SARS-CoV-2 Spike-specific INFγ and TNFα production**

Bar graphs showing the expression of INFγ and TNFα among SARS-CoV-2 Spike- and EBV-specific CD3+, CD4+ and CD8+ T cells in Allo-HSCT recipients (n=18 (P) and 12 healthy donors (HD). Data are shown as means of the percentage of T-cell responders.



**Figure 3: Relation between time from transplant to vaccination and vaccine response**

### After the second dose of vaccine :

- 100 % of healthy donors became seropositive and developed a specific SARS-CoV-2 T-cell response (median: 195 SFU/10<sup>6</sup> T-cells, range 81-653) (Figure 1)
- 78% of patients (n=36/46) had achieved a humoral response and 69% achieved a T-cell response (median of 119 SFU/10<sup>6</sup> T-cells (range 31-2718). For 8 patients, this T-cell response was higher than that of controls (>800 SFU/10<sup>6</sup> T-cells)
- Among the 36 humoral responders (HR), 89% also had a positive anti-Spike CD3+ T-cell response.
- Among the 10 patients who were non humoral responders (NHR), 4 (40%) had developed cellular immunity, including one with a very high CD3+ T-cell response (1333 SFU/10<sup>6</sup> T-cells) (Figure 1)
- In NHR patients : higher frequency of CD14+ monocytes with low/neg HLA-DR expression, potentially corresponding to myeloid-derived suppressor cells (MDSCs).
- Predominance of anti-Spike CD4+ T-cell responses characterized by a high proportion of cells with an IFN-γ/TNF-α cytokine pattern (Figure 2)
- As expected, patients who do not develop a humoral response (n=10) are within one year of transplantation (Figure 3).
- 6 of the 9 patients on treatment developed a T cell response (Figure 4)

## CONCLUSION

### After the second dose of vaccine,

- 78% of patients (n=36/46) had achieved a humoral response and 69% achieved a T-cell response with a predominance of anti-Spike CD4+ T-cell responses characterized by a high proportion of cells with an IFN-γ/TNF-α cytokine pattern
- As expected, patients who do not develop a humoral response (n=10) are within one year of transplantation
- Among the 10 patients who were non humoral responders (NHR), 4 (40%) had developed cellular immunity
- Impact of the CD14+ monocytes with low/neg HLA-DR expression (myeloid-derived suppressor cells) on the vaccine immune response deserves further evaluation