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 ($2x10^5$) in 100µL were added to each well of Human ELISpot^{PRO} 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%CO2 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/$2x10^5$ cells (range 0-5). Frequencies of spot forming units (SFU) were reported per $1x10^6$ $CD3^+$ 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 analyse 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™ Instrulent (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®, Rotkreuz, Switzerland. As recommended by the manufacturer, titers ≥0.8 U/mL were considered positive, the highest value being >250.

RESULTS

Table 1: Vaccinated individuals' characteristics

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

*: Humoral Responders
*: Non Humoral Responders
AML: acute myeloid leukemia; MDS: myelodysplastic syndrome ; MUD : matched unrelated donor ; GVHD : graft-versus-host disease; CsA: cyclosporine; MMF: mycophenolate mofetyl; PTCY: 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.

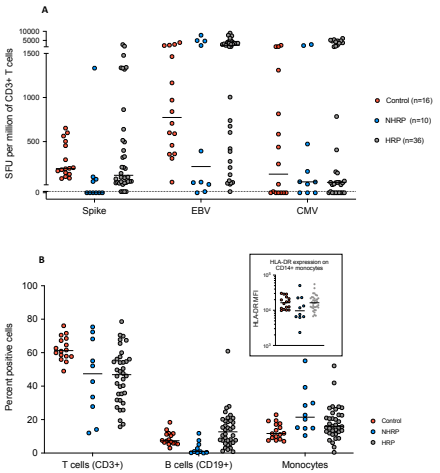

Figure 1: T-cell response (A) and PBMC phenotype (B)

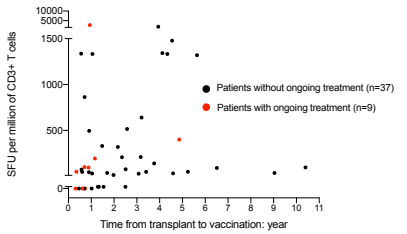

Figure 4 : Relation between time from transplant to vaccination and vaccine response

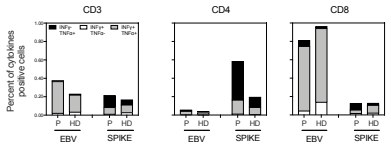

Figure 2 : SARS-CoV-2 Spike-specific INFγ and TNFα production

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.

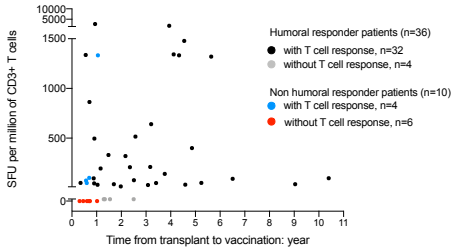

Figure 3: Relation between time from transplant to vaccination and vaccine response

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/10^6 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/10^6 T-cells (range 31-2718). For 8 patients, this T-cell response was higher than that of controls (>800 SFU/10^6 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/10^6 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