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# Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Leclerc M, Redjoul R, Le Bouter A, et al. Impact of donor vaccination on recipient response to early SARS-CoV-2 mRNA vaccination after allogeneic HSCT. *Lancet Haematol* 2022; published online April 1. https://doi.org/10.1016/S2352-3026(22)00097-7.

**Supplementary appendix**

*Impact of Donor Vaccination on Recipient Response to Early SARS-CoV-2 mRNA Vaccination after Allogeneic Hematopoietic Stem Cell Transplantation* by Leclerc M. et al.

## Methods

*Patients and donors*

All patients and donors signed an informed consent for use of their clinical and biological data for research purposes. The study has been approved by the institutional review board of our institution, which is accredited according to the international JACIE program since 2005. All donors received two vaccine doses before their donation, except one who received only one dose because of a previous symptomatic COVID-19.

*Serological assays for IgG(S-RBD) and anti-N IgG detection*

Serum samples were analyzed for IgG anti-N SARS-CoV-2 detection (ARCHITECT®, Abbott Laboratories) and for IgG(S-RBD) IgG titration with the SARS-CoV-2 IgG Quant II assay (ARCHITECT®, Abbott Laboratories). The assay has been tested and validated against WHO international standards although analytical differences are still detected between commercially available assays.[1] For the anti-N assay, index values (S/CO) reported by the instruments were used in analysis. Interpretation of the results was as follows: S/CO index >1,4 as positive; S/CO index between 0,5 and 1,4 as limit; and S/CO<0,5 as negative. The anti-S assay is an automated immunoassay that quantifies IgG(S-RBD), with 6.8 AU/mL as a limit of detection, 21 AU/mL as a minimum threshold of quantification and 40,000 AU/mL as a maximal threshold of quantification (analytical measuring interval). Samples containing IgG(S-RBD) titers higher than 40.000 AU/mL were further diluted to extend the measuring interval. All assays were performed by trained laboratory technicians according to the manufacturer standard procedures.

*Chimerism analysis*

Chimerism was studied by analyzing various polymorphisms after polymerase chain reaction (PCR) amplification of DNA obtained from whole blood at 1, 2 and 3 months after transplantation. Real-time (RT) quantitative PCR of insertion/deletion polymorphisms was used when the minority chimeric fraction was below 10% and short-tandem-repeat (STR)-PCR was performed when the minority fraction was over 10%. In both cases, analyses were performed according to the kit manufacturer's recommendations (Promega; PowerPlex 16S assay for STR-PCR) and GenDex for Indel RT-PCR (KMRDX Chimerism Assay). When possible, peripheral-blood CD3+ T cells and CD19+ B cells were selected for analysis.

*Statistics*

Categorical variables were compared by Fisher exact tests. Comparisons of continuous variables means were performed using paired or unpaired Student t-tests and one-way ANOVA tests, as appropriate. All tests were two sided and the type 1 error rate was fixed at 0.05.

*Reference*

1 Saker K, Escuret V, Pitiot V, *et al.* Evaluation of commercial anti-SARS-CoV-2 antibody assays and comparison of standardized titers in vaccinated healthcare workers. *J Clin Microbiol* 2021; : JCM0174621.

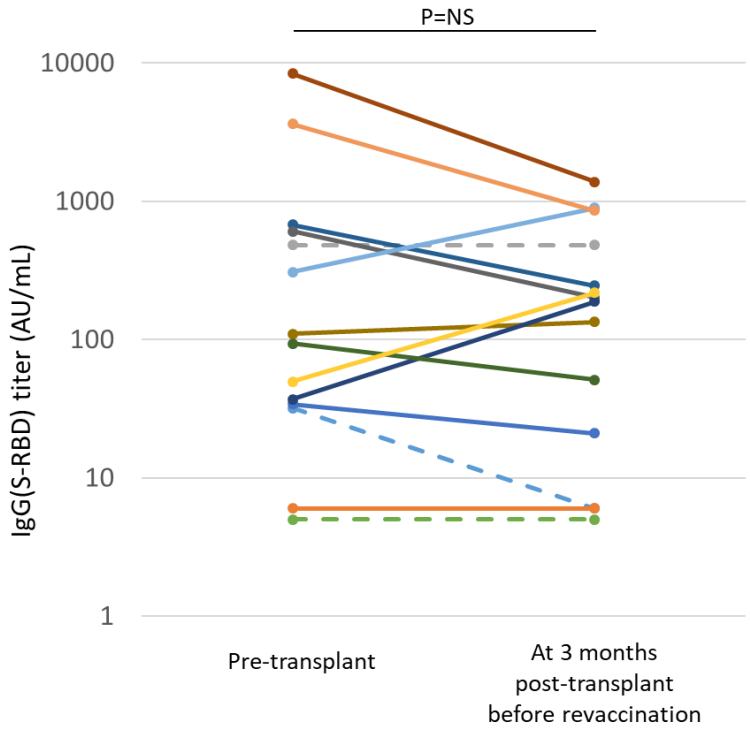


**Figure 1. IgG(S-RBD) titer kinetics in recipients vaccinated before HSCT.**

Solid and dashed lines correspond to recipients further transplanted from vaccinated (n=11) and non-vaccinated (n=3) donors, respectively. All recipients but one received at least two mRNA vaccine doses before HSCT. One patient received only one vaccine dose (one month before HSCT) in consequence of symptomatic COVID-19 occurred 4 months before. Of note, the 3 recipients showing increasing IgG(S-RBD) titers between the two time-points had a vaccinated donor, which could be related to the IgG(S-RBD) passive transfer from donor graft to corresponding recipients.

**Table 1.** Patient characteristics in various D/R pairs according to D/R pre-transplant mRNA vaccination against SARS-CoV-2

| | **D-/R-** **(n=14)** | **D-/R+** **(n=3)** | **D+/R±** **(n=13)** |
|---|---|---|---|
| **Male sex, n (%)** | 9 (64) | 2 (67) | 9 (69) |
| **Recipient age at HSCT (years), mean±SD** | 51±14 | 43±12 | 53±17 |
| **Donor age at HSCT (years), mean±SD** | 34±14 | 32±12 | 33±9 |
| **Disease type, n (%)** | | | |
| myeloid malignancy | 10 (72) | 0 | 9 (69) |
| lymphoid malignancy | 3 (21) | 3 (100) | 4 (31) |
| non-malignant | 1 (7) | 0 | 0 |
| **Donor Type, n (%)** | | | |
| HLA-identical sibling | 5 (36) | 0 | 1 (8) |
| matched unrelated | 5 (36) | 3 (100) | 8 (61) |
| haplo-identical | 4 (28) | 0 | 4 (31) |
| **Anti-thymocyte globulins given within conditioning regimen*** | 5 (36) | 1 (33) | 5 (38) |
| **History of GVHD requiring systemic treatment, n (%)** | 3 (21) | 1 (33) | 3 (23) |
| **Disease relapse after HSCT, n (%)** | 3 (21) | 0 | 1 (8) |
| **Systemic immunosuppression at time of vaccination**, n (%)** | 14 (100) | 3 (100) | 13 (100) |
| **Rituximab given within the 6 months before initiation of vaccination** | 3 (21) | 0 | 1 (8) |
| **Time between HSCT and initiation of post-HSCT vaccination (months), mean±SD** | 3.7±0.8 | 4.0±0.2 | 4.0±0.2 |
| **Time between first vaccine dose and IgG(S-RBD) quantitation (days), mean±SD** | 52±7 | 49±16 | 46±12 |
| **Immune cell counts over study period in PB (cells/µL), mean±SD** | | | |
| *Lymphocytes* | 567±405 | 500±88 | 795±388 |
| *T cells* | 851±749 | 444±107 | 719±364 |
| *B cells* | 97±115 | 56±19 | 119±127 |
| **Prior COVID-19 in recipient (prior positive PCR and/or positive anti-N IgG detection***), n (%)** | 1 (7) | 1 (33) | 1 (8) |
| **Positive anti-N IgG detection*** at time of analysis of response to post-transplant vaccination, n (%)** | 1 (7) | 1 (33) | 0 |

*One additional patient in the D-/R- group received campath as part of the conditioning regimen. ** Drugs received within the 3 months preceding vaccination consisted of cyclosporine alone (n=22), cyclosporine + steroids (n=7) or sirolimus (n=1). All patients transplanted with a haplo-identical donor (and only them) received post-transplant cyclophosphamide. *** anti-N IgG detection corresponded to S/CO index >1.4

**Table 2.** Post-transplant chimerism sequential monitoring in the 13 D+/R± pairs.

| | % Residual Recipient Cells | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Patient*** | **Month 1 post-HSCT** | | | **Month 2 post-HSCT** | | | **Month 3 post-HSCT** | | |
| | **PBMC** | **CD3+** | **CD19+** | **PBMC** | **CD3+** | **CD19+** | **PBMC** | **CD3+** | **CD19+** |
| 1 | <0.1% | ND | ND | <0.1% | 0.9% | 1.3% | <0.1% | 0.2% | 1.7% |
| 2 | 0.1% | 4.3% | ND | <0.1% | 0.1% | 0.5% | <0.1% | 0.2% | 1.6% |
| 3 | <0.1% | ND | ND | <0.1% | <0.1% | 2.8% | <0.1% | <0.1% | 2.7% |
| 4 | 3.0% | 40.0% | 10.0% | 0.4% | 12.0% | 5.0% | <0.1% | 7.1% | 0.2% |
| 5 | <0.1% | ND | ND | <0.1% | <0.1% | 3.1% | <0.1% | <0.1% | ND |
| 6 | <0.1% | <0.1% | 3.3% | <0.1% | <0.1% | 1.6% | <0.1% | <0.1% | ND |
| 7 | <0.1% | 0.2% | 0.2% | <0.1% | <0.1% | 0.7% | 0.1% | <0.1% | 0.3% |
| 8 | <0.1% | ND | ND | 0.2% | 0.3% | 0.8% | <0.1% | 1.5% | 6.6% |
| 9 | 2.6% | ND | ND | <0.1% | ND | ND | <0.1% | 0.2% | 2.3% |
| 10 | 0.3% | 3.9% | ND | <0.1% | 0.2% | 0.4% | <0.1% | <0.1% | <0.1% |
| 11 | 0.3% | 1.3% | 3.4% | 0.4% | 0.9% | 1.8% | 0.3% | <0.1% | 0.5% |
| 12 | <0.1% | ND | ND | <0.1% | ND | ND | <0.1% | 0.3% | 5.4% |
| 13 | <0.1% | ND | ND | 0.5% | ND | ND | 1.2% | ND | ND |

* ND=not done. Patients 1 to 11 were D+/R+ pairs. Patients 12 and 13 were D+/R-.