Cancer Cell, Volume *40*

Supplemental information

Antibody response against SARS-CoV-2 Delta and Omicron variants after third-dose BNT162b2 vaccination in allo-HCT recipients

Lorenzo Canti, Kevin K. Ariën, Isabelle Desombere, Stéphanie Humblet-Baron, Pieter Pannus, Leo Heyndrickx, Aurélie Henry, Sophie Servais, Evelyne Willems, Grégory Ehx, Stanislas Goriely, Laurence Seidel, Johan Michiels, Betty Willems, Maria E. Goossens, Yves Beguin, Arnaud Marchant, and Frédéric Baron

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL METHODS

SARS-CoV-2-specific binding antibodies were quantified using the FDA-approved WANTAI (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China) SARS-CoV-2 Ab ELISA as previously reported (Canti et al., 2021). The limit of quantification (LOQ) of the assay is 5 BAU/mL. Values below LOQ were attributed an arbitrary value of 2.5 BAU/mL in the graphs and statistical analyses (Pannus et al., 2021).

SARS-CoV-2 neutralizing antibodies against wild type (WT), Delta and Omicron variant strains were quantified as previously reported (Mariën et al., 2021) (Ariën et al., 2021). The Reed-Muench method was used to calculate the neutralizing Ab titer that reduced the number of infected wells by 50% (NT50), which was used as a proxy for the neutralizing Ab concentration in the sample. Values below LOQ were arbitrarily attributed a value of 25 in the graphs and statistical analyses while values above 1600 were arbitrarily attributed a value of 1700.

Absolute counts of unswitched memory B cells, class-switched memory B cells, naive B cells, naive T cells, and follicular helper T cells were quantified as previously reported (Canti et al., 2021).

Comparisons of Ab titers between two time points were assessed using Wilcoxon matched-pairs signed rank tests while comparisons of Ab titers between groups were assessed with the Mann-Whitney test. Correlations were calculated using Spearman r correlation tests. Statistical analyses were carried out with Graphpad Prism 9.0 (Graphpad Software, San Diego, CA, USA).

Ab waning after day 49

Of note 3 of the 38 patients were given i.v. Ig (Privigen^{R}) between the second and third dose of the vaccine. Their anti-RBC Ab on days 49 and at booster dose were 179 and 181 BAU/mL, 44 and 11 BAU/ML, and 2652 and 154 BAU/mL, respectively.

Geometric mean of NT50 titers decreased from 70.3 (95% CI: 46.9-105.4) on day 49 to 52.5 (95% CI: 37.3-73.8) on the day of the booster dose (P = 0.0008).

Safety of the booster dose

Four serious adverse events (SAEs) were reported during the 28-day period after the booster dose. Patient #4 experienced catheter infection 28 days after booster dose. Patient #19 was diagnosed with FLT3-ITD AML relapse on day 7 after booster dose. It was her second relapse after transplantation and she has started on gilteritinib. She unfortunately succumbed from AML 116 days after booster dose. Patient #21 was diagnosed with gastric cancer one week before the booster dose and was hospitalized on day 25 after booster vaccination for surgery. Finally, patient #22 was diagnosed with AML relapse on day 28 after booster dose. He was rescued with a second allo-HCT following a sequential conditioning regimen. These four SAEs were considered as not related to the vaccine. In addition to these serious adverse events, one patient (patient #28) reported persistent exertional dyspnea starting after booster vaccine. She had no evidence of myocarditis with normal cardiac echography and normal cardiac enzymes. She also had normal hemoglobin level, normal CT angiography of the chest and normal pulmonary function tests. Finally, one Suspected Unexpected Serious Adverse Reaction (SUSAR) was observed. Patient #7 was diagnosed with transverse myelitis on day 72 after booster dose. She was treated by high-dose steroids with improvement of her neurological condition. This complication was considered possibly related with the vaccine.

Serological response to the booster dose

The only patients who failed to seroconvert with the 3-dose schedule was a patient with extensive chronic GVHD who was on ruxolitinib and photopheresis at the time of the booster dose.

Neutralizing Ab response following the booster dose

Seven patients (18%) failed to have NAb against the Delta variant after the booster. This included 4 of 9 patients with moderate/severe chronic GVHD at first dose administration (resolved at the time of the booster dose in one of them), one patient diagnosed with moderate/severe chronic GVHD before the second and the third dose and two patients without moderate/severe chronic GVHD but given rituximab in the year before the first vaccination.

Breakthrough COVID-19 cases

We observed six cases of COVID-19 infection after third vaccination. **Patient #3** was diagnosed with COVID-19 on day 27 (Ay.34 Delta variant) after third dose. She had only mild symptoms and was treated with an injection of casirivimab / imdevimab monoclonal antibodies. She had no detectable anti-RBD Ab at the time of the booster dose administration and 29 IU/ml anti-RBD Ab (and no neutralizing Ab) the day of monoclonal antibody administration. **Patient #4** was diagnosed with COVID-19 on day 160 after third dose. She had mild symptoms and was treated with an injection of sotrovimab monoclonal antibodies. She had high anti-RBD levels (2382 BAU/mL) and NAb against Delta (119) and but not NAb against Omicron variants after the booster dose. **Patient #6** was diagnosed with COVID-19 on day 77 (Ay.43 Delta variant) after third dose and was also treated with casirivimab / imdevimab monoclonal antibodies. She also had mild symptoms. This infection occurred despite high anti-RBD levels (3444 BAU/mL) and detectable NT50 Ab against the Delta variant (133) 28 days after the booster dose. **Patient #12** was diagnosed with COVID-19 on day 146 after third dose. She had mild

symptoms and was treated with an injection of sotrovimab monoclonal antibodies. She had high anti-RBD levels (2468 BAU/mL) and NAb against Delta (269) and Omicron (63) variants after the booster dose. **Patient #20** was diagnosed with COVID-19 on day 154 after third dose. He had mild symptoms and did not receive monoclonal antibodies. He had high anti-RBD levels (10095 BAU/mL) and NAb against Delta (594) and Omicron (126) variants after the booster dose. **Patient #32** was diagnosed with COVID-19 on day 133 after third dose. He had no symptoms and was treated with an injection of sotrovimab monoclonal antibodies. He had high anti-RBD levels (7810 BAU/mL) and NAb against Delta (317) and Omicron (87) variants after the booster dose.

Ethics approval and consent to participate

The study was approved by the Comité d'Ethique Hospitalo-Facultaire Universitaire de Liège (EC2021/73, 11 March 2021) and the Federal Agency for Medicines and Health Products of Belgium (EudractCT # 2021-000673-83). Each patient signed a written informed consent to participate in the study.

SUPPLEMENTAL REFERENCES

Mariën, J., Ceulemans, A., Michiels, J., Heyndrickx, L., Kerkhof, K., Foque, N., Widdowson, M.-A., Mortgat, L., Duysburgh, E., Desombere, I., et al. (2021). Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay. J Virol Methods *288*, 114025.

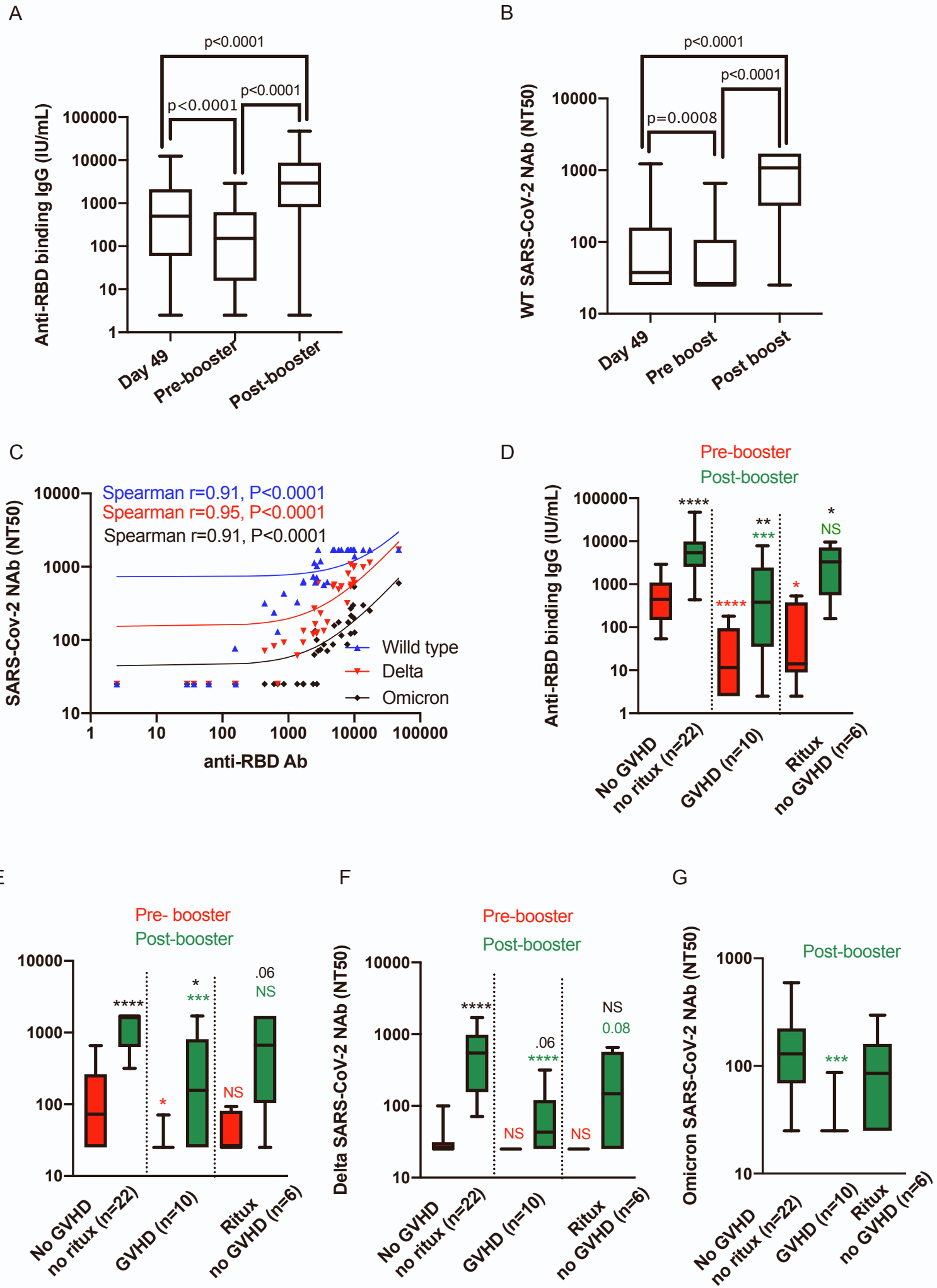
Pannus, P., Neven, K.Y., De Craeye, S., Heyndrickx, L., Vande Kerckhove, S., Georges, D., Michiels, J., Francotte, A., Van Den Bulcke, M., Zrein, M., et al. (2021). Poor antibody response to BioNTech/Pfizer COVID-19 vaccination in SARS-CoV-2 naïve residents of nursing homes. Clin Infect Dis.

SUPPLEMENTAL TABLE S1. CHARACTERISTICS OF THE PATIENTS (n=38).

Age at vaccination (years); median (min, p25, p75, max)	60 (26, 54, 70, 76)
Sex (# males / # females)	19 / 19
Delay between vaccination and transplantation (months); median (min, p25, p75, max)	31 (6, 14, 42, 58)
Donor type (# MSD / MUD/ MMUD / Haplo)	8 / 24 / 1 / 5
Conditioning regimen (# patients)	
Fludarabine + 2 Gy TBI	5
Fludarabine + Melphalan	16
Fludarabine + busulfan	4
Cyclophosphamide + 12 Gy TBI	6
Thiotepa + busulfan + fludarabine	2
Sequential	3
Fludarabine + Cyclophosphamide + 2 or 4 Gy TBI	2
ATG (# yes / no)	27 / 11
PTCY (# yes / no)	6 /32
Chronic GVHD	
Never / only mild	27
Prior moderate/severe solved[(1)]	1
Ongoing moderate / severe[(2)]	10
Rituximab (none or ≥2yrs, ≥ 1 but < 2 yrs, > 6 months but < 1 yr at first vaccine), # of patients	26, 5, 7[(3),(4)]
Systemic immunosuppression at inclusion	
None	24
Tacrolimus	5
Photopheresis	1

Photopheresis + mPDN < 32 mg/day	1
MMF	1
MMF + mPDN < 32 mg/day	1
Sirolimus	1
Sirolimus + mPDN < 32 mg/day	2
Photopheresis + ruxolitinib	2

(1) > 3 months out of systemic immunosuppression; (2) including one patient diagnosed with moderate/severe chronic GVHD between the second and the third dose (and treated by MMF) and one patient whose moderate/severe chronic GVHD resolved between the second and the third dose; (3) including one patient with moderate/severe chronic GVHD and 6 patients without moderate/severe chronic GVHD; (4) including 2 patients who had received the rituximab < 1 year before the third dose vaccine . MSD, HLA-identical sibling donor; MUD, 10/10 HLA-matched unrelated donor; MMUD, 1/10 HLA-mismatched unrelated donor; Haplo, HLA-haploidentical donor; TBI, total body irradiation; ATG, anti-thymocyte globulin; PTCY, post-transplant cyclophosphamide; MMF, mycophenolate mofetil; mPDN, methyl-prednisolone.


A
p<0.0001
p<0.0001
p<0.0001
Anti-RBD binding IgG (IU/mL)
100000
10000
1000
100
10
1
Day 49
Pre-booster
Post-booster
B
p<0.0001
p<0.0001
p=0.0008
WT SARS-CoV-2 NAb (NT50)
10000
1000
100
10
Day 49
Pre boost
Post boost
C
Spearman r=0.91, P<0.0001
Spearman r=0.95, P<0.0001
Spearman r=0.91, P<0.0001
SARS-Cov-2 NAb (NT50)
10000
1000
100
10
1
10
100
1000
10000
100000
anti-RBD Ab
Willd type
Delta
Omicron
D
Pre-booster
Post-booster
Anti-RBD binding IgG (IU/mL)
100000
10000
1000
100
10
1
****
**
***
*
NS
****
*
No GVHD
no ritux (n=22)
GVHD (n=10)
Ritux
no GVHD (n=6)
E
Pre- booster
Post-booster
WT SARS-CoV-2 NAb (NT50)
10000
1000
100
10
****
*
***
.06
NS
*
NS
No GVHD
no ritux (n=22)
GVHD (n=10)
Ritux
no GVHD (n=6)
F
Pre-booster
Post-booster
Delta SARS-CoV-2 NAb (NT50)
10000
1000
100
10
****
NS
.06
****
NS
0.08
NS
NS
No GVHD
no ritux (n=22)
GVHD (n=10)
Ritux
no GVHD (n=6)
G
Post-booster
Omicron SARS-CoV-2 NAb (NT50)
1000
100
10
***
No GVHD
no ritux (n=22)
GVHD (n=10)
Ritux
no GVHD (n=6)

SUPPLEMENTAL FIGURE S1. (A-B). Ab waning and Ab response to the third vaccine dose. Dynamics of (A) RBD binding Ab (n=38) and (B) NAb (NT50) (n=38) against WT SARS-CoV-2 from day 49 after first dose of mRNA vaccine to pre-booster immunization and 28 days after booster immunization. Ab levels were compared using the Wilcoxon matched-pairs signed rank tests. **(C) Spearman correlations between RBD binding Ab and NAb against WT, Delta and Omicron SARS-CoV-2 variant titers** post-booster immunization. **(D-G): Ab responses to mRNA vaccination according to the presence of moderate/severe chronic GVHD at first and/or third vaccine dose or rituximab administration within the first year before the first vaccine dose**. (D) RBD binding Ab (n=38). (E) NAb (NT50) against WT SARS-CoV-2 (n=38). (F) NAb (NT50) against SARS-CoV-2 Delta variant (n=38). (G) NAb against the Omicron variant (only assessed after booster dose) (n=38) *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$. Red stars refer to Mann-Whitney tests comparing pre-booster titers between subgroups. Green stars refer to Mann-Whitney tests comparing post-booster titers between subgroups. Black stars refer to Wilcoxon matched-pairs signed rank tests comparing pre- and post-boost values within subgroups. In panels A-B and D-G, box extends from the 25^{th} to 75^{th} percentiles, horizontal line shows the median, and whiskers go down to the smallest value and up to the largest.