Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kamar N, Abravanel F, Marion O, et al. Three doses of an mRNA Covid-19 vaccine in solid-organ transplant recipients. N Engl J Med. DOI: 10.1056/NEJMc2108861

Appendix

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Patients:

As we have previously reported [1], when the vaccination campaign started (7 January 2021), all our transplant-patients were invited to be vaccinated. Due to weak immunogenicity of 2-doses of mRNA- based anti-SARS-CoV-2 vaccines, the French National Authority for Health recommended (4 April 2021) the use of a third dose in immunosuppressed patients [2]. This dose had to be given at least one month after the second dose or as soon as possible in patients in whom the second dose was administrated more than one month before this latter recommendation. Hence, all our seropositive or seronegative transplant patients were invited to receive the third dose.

According to the recommendations of the Francophone Society of Transplantation [3], anti–SARS-CoV-2 spike protein antibodies were monitored before and after vaccination. Furthermore, the French authorities recommended that anti–SARS-CoV-2 serologies be performed in immunosuppressed patients [4]. Therefore, all our patients were also invited to perform a serology according to the national recommendations. In the present letter we collected retrospectively the outcome of the first 101 consecutive patients who have been given 3 doses and who have had their anti–SARS-CoV-2 spike protein antibodies assessed. According to French law (*loi Jardé*), anonymous retrospective studies do not require institutional review board approval. However, the study approved by the research department of our institution (Approval provided).

Virological Method

We used the Wantai microplate ELISA that detect total anti-SARS-Cov-2 antibodies (IgG, IgM and IgA) (WANTAI SARS-CoV-2 Ab ELISA). Semi-quantitative results are expressed as signal-to-cut-off ratio (S/C0). Patients were considered as positive if S/Co> 1.1. Samples with a S/C0> 47 were retested after dilution. Using this assay, we have previously reported a 100% specificity and 100% sensitivity in immunocompetent patients tested at 2 to 14 days post symptom-onset and at 15 to 45 days post symptom-onset, suggesting it as the ability to detect low level of antibodies [5].

Results

We compared responders to non-responders after 3-doses vaccine (see supplementary Table 1). Non-responders were older, had a lower total lymphocyte count, a lower CD4-positive T-cell count, a lower CD19-positive count, and a lower estimated glomerular filtration rate before vaccination compared to responders. The proportion of patients given belatacept was higher among non-responders.

None of the patients experienced COVID-19 after the 3 vaccine doses.

No serious adverse events were reported. However, 10 patients who were seropositive before the third dose presented with fatigue and myalgia. Five patients presented transient fever. Ni gastro-intestinal side effects were observed.

Supplementary Table 1: Clinical and biological characteristics of solid organ transplant recipients according to humoral response one month after three doses of mRNA-based vaccine.

	Anti-SARS-CoV2 positive patients (*N*=67)	Anti-SARS-CoV2 negative patients (*N*=32)	*p*-value
Sex ratio (M/F)	2.2 (46/21)	2.6 (23/9)	0.745
Age (years, mean ±SEM)	54 ±2	65 ±3	**<0.001**
Type of organ transplant, n (%)			0.491
- Kidney	51 (76)	25 (78)	
- Liver	9 (13)	3 (9)	
- Thoracic organs	4 (6)	4 (13)	
- Pancreas	3 (4)	-	
History of rejection in the year preceding vaccination, n (%)	2 (3)	1 (3)	1
Time between vaccine and transplantation (months, mean ±SEM)	99 ±10	94 ±16	0.793
No induction therapy, n (%)	26 (39)	14 (44)	0.667
Induction therapy, n (%)	41 (61)	18 (56)	
- Anti-IL2 receptor blockers	26 (63)	9 (50)	
- Polyclonal antibodies	14 (34)	8 (44)	
- Others	1 (2)	1 (6)	
Type of immunosuppressive regimen, n (%)			
- Calcineurin-inhibitors	55 (82)	23 (72)	0.245
○ Tacrolimus	51 (93)	22 (97)	
○ Ciclosporin A	4 (7)	1 (3)	
- Anti-metabolite	41 (61)	24 (75)	0.176
○ Mycophenolic acid	40 (98)	24	
○ Azathioprine	1 (2)	-	
- mTOR inhibitors	22 (33)	7 (22)	0.262
- Steroids	58 (87)	28 (88)	1
- Belatacept	5 (7)	7 (22)	0.052
Neutrophils count before vaccination (/mm^3, mean ±SEM)	5459 ±252	5600 ±664	0.810
Lymphocytes count before vaccination (/mm^3, mean ±SEM)	1561 ±123	1173 ±114	**0.049**
CD4+ T-cells count before vaccination (/mm^3, mean ±SEM)	n=59 529 ±37	n=30 339 ±38	**0.002**
CD8+ T-cells count before vaccination (/mm^3, mean ±SEM)	n=59 440 ±38	n=30 358 ±48	0.201
CD19+ T-cells count before vaccination (/mm^3, mean ±SEM)	n=59 182 ±83	n=30 89 ±33	**0.003**
NK cells count before vaccination (/mm^3, mean ±SEM)	n=59 235 ±18	n=30 216 ±33	0.582
eGFR before vaccination (mL/min/1.73m²)	60 ±3	45 ±4	**0.005**

Abbreviations: IL2, interleukin-2; mTOR, mammalian target of rapamycin; NK, natural killer; eGFR, estimated Glomerular Filtration Rate (Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation).

References:

1. Marion O, Del Bello A, Abravanel F, et al. Safety and Immunogenicity of Anti-SARS-CoV-2 Messenger RNA Vaccines in Recipients of Solid Organ Transplants. Ann Intern Med 2021.

2. https://www.mesvaccins.net/textes/dgs_urgent_n43_vaccination_modalites_d_administration_des_rappels.pdf.

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5. Abravanel F, Miedouge M, Chapuy-Regaud S, Mansuy JM, Izopet J. Clinical performance of a rapid test compared to a microplate test to detect total anti SARS-CoV-2 antibodies directed to the spike protein. J Clin Virol 2020;130:104528.