Third-dose BNT162b2 vaccination elicits markedly high-level SARS-CoV-2-neutralizing antibodies in vaccinees who poorly responded to second dose in Japan

Masayuki Amano[1], M.D., Ph.D., Kenji Maeda[2], M.D., Ph.D., Kiyoto Tsuchiya[3], Ph.D., Shinya Shimada[4], M.D., Ph.D., Hiroaki Mitsuya[2*], M.D., Ph.D.

[1]Department of Hematology, Rheumatology, and Infectious Diseases, Kumamoto University Hospital, Kumamoto, 860-8556, Japan; [2]Department of Refractory Viral Infections, National Center for Global Health and Medicine (NCGM) Research Institute, Tokyo, 162-8655, Japan; [3]AIDS Clinical Center, NCGM, Tokyo, 162-8655, Japan; [4]JCHO Kumamoto General Hospital, Kumamoto, 866-8660, Japan.

*Corresponding Author: Hiroaki Mitsuya (hmitsuya@hosp.ncgm.go.jp).
National Center for Global Health and Medicine Research Institute,
1-21-1 Toyama, Shinjuku, Tokyo 162-8655, Japan.
Tel: +81-3-3202-7181

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A running title: 3rd BNT162b2 elicits high neutralization

To the Editor - We read with great interest the article by Saciuk *et al.* demonstrating that, in a retrospective cohort study in Israel, an additional dose of BNT162b2 vaccine 6 months after initial 2-dose vaccination bolsters protection against infection, with a vaccine effectiveness of 89% as assessed during August-October 2021, when the majority of infection was due to the Delta variant [1]. Recent SARS-CoV-2 studies using pseudovirus have shown that 3rd-dose of BNT162b2 well elicits neutralizing antibodies against VOCs (variants of concern) including the Omicron (B.1.1.529, BA.1) [2-4]. However, Cheng *et al*. very recently reported a single source outbreak of Omicron BA.2 sublineage in Hong Kong, which indicates high transmissibility of the Omicron/BA.2 [5], which poses further concerns on the efficacy of anti-SARS-CoV-2 vaccines.

In the present prospective study enrolling 225 health care workers (see demographic characteristics in **Supplementary Table 1**), who received 3-doses of BNT162b2 in Japan, we consecutively determined SARS-CoV-2 neutralizing activity (NT_{50}) of their sera using VeroE6^{TMPRSS2} cells over 300 days following the 1st-dose and its kinetics/profiles, which is the continuation of our previous study [6]. We also determined NT_{50}s of selected sera using VeroE6^{TMPRSS2} and HeLa$^{hACE2-TMPRSS2}$ cells against infectious variants of concern VOCs including Delta and Omicrons (BA.1 and BA.2), whose emergence has been associated with a steep increase in COVID-19 cases and hospitalizations (experimental details are provided in the Methods section of Supplementary materials).

There was significant neutralizing activity on day-28 post-1st dose (NT_{50}=501 at one-week post-2nd dose), while there was a continual decrease until day-280. NT_{50} values further decreased to 51 by day-280 when ~85% of the participants had NT_{50} values of <100 and ~36% had less than 20 NT_{50} or undetectable (**Supplementary Figure 1**). However, 2 weeks after 3rd-dose administration (205 participants [91.1%] had remained in the cohort on day-300), there was a substantial rise in neutralizing activity, achieving an average NT_{50} of 3,531. There was a concern that individuals who poorly responded to 2nd-dose might again fail to produce sufficient neutralizing antibodies. Therefore, we specifically determined neutralization activity in vaccinees, who had achieved the bottom10% levels of neutralization following the 2nd-dose (n=22, average-NT_{50}=110 on day-28; an inset of **Supplementary Figure 1**).

Notably, by day-300, all those low responders achieved markedly greater levels of neutralizing activity with the average-NT_{50} of 2,341 (**Table 1**, range 482-9,113 in VeroE6^{TMPRSS2}). In HeLa$^{hACE2+TMPRSS2}$ cells, sera from those low responders substantially neutralized SARS-CoV-2^{05-2N}, Alpha, Beta, Gamma, and Delta (geometric mean [gMean]-NT_{50}=1,777, 1,350, 480, 1,015, and 959, respectively), but had only marginal activity against Omicron/BA.1 with gMean-NT_{50} being 52 (range ≤ 20-197; **Table 1**). The same sera had similar neutralization profiles in VeroE6^{TMPRSS2} cells. On the other hand, sera from participants who achieved the top10% level neutralization on day-300 (n=22, average-NT_{50}=10,885 in VeroE6^{TMPRSS2}) had high neutralizing activity against SARS-CoV-2^{05-2N}, Alpha, Beta, Gamma, and Delta (gMean-NT_{50}=9,774, 4,906, 2,279, 3,271 and 3,377, respectively) in HeLa$^{hACE2+TMPRSS2}$ cells and good neutralizing activity against Omicron/BA.1 (gMean-NT_{50}=500, range 171-979). Notably, all day-280 sera of the top-10% participants had failed to neutralize both Omicron/BA.1 and BA.2 (**Supplementary Figure 2**, NT_{50} values were ≤20); however, those participants' sera on day-300 also well neutralized Omicron/BA.2 (**Table 1**, gMean-NT_{50}=702, range 262-1,653).

The present data clearly show that 3rd-dose BNT162b2 elicits high-level SARS-CoV-2-neutralizing antibodies even in those who poorly responded to the 2nd-dose although low responders to the vaccines may be vulnerable to infection with Omicron sublineages, BA.1 and BA.2. Of note, however, in terms of the effectiveness of 3rd-dose BNT162b2 against Omicron sublineages, the morbidity and mortality have yet to be determined between individuals who received the 3rd dose but contracted symptomatic Omicron-related COVID-19 and those not receiving the 3rd dose who contracted symptomatic Omicron-related COVID-19.

Notes

Author contributions

MA and HM had access to all data in this study and took and hold all responsibility for the integrity of the data and the accuracy of the data analysis. MA and HM: Concept and design. MA, KM, and KT: Acquisition, analysis, and/or interpretation of data. SS: Administrative and material support. MA and HM: Original draft writing. All authors: Writing and reviewing manuscript.

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Potential conflicts of interest

All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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Corresponding author contact information

Hiroaki Mitsuya (hmitsuya@hosp.ncgm.go.jp).

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Table 1. Neutralization activity of top and bottom 10% responders' sera against SARS-CoV-2s including the Wuhan strain and six VOCs.

	SARS-CoV-$2^{05\text{-}2N}$ (Wuhan)	SARS-CoV-2^{QK002} (Alpha)	SARS-CoV-$2^{TY8\text{-}612}$ (Beta)	SARS-CoV-$2^{TY7\text{-}501}$ (Gamma)	SARS-CoV-2^{1734} (Delta)	SARS-CoV-$2^{NCGM\text{-}929\text{-}1N}$ (Omicron/BA.1)	SARS-CoV-2^{2037} (Omicron/BA.2) In VeroE6^{TMPRSS2}*
gMean NT_{50} of top 10% sera in HeLa$^{hACE2+TMPRSS2}$ cells	9,774 (3,745-27,921)	4,906 (1,926-11,018)	2,279 (844-8,996)	3,271 (1,723-8,849)	3,377 (1,157-8,053)	500 (171-979)	702 (262-1,653)
gMean NT_{50} of bottom 10% sera in VeroE6^{TMPRSS2} cells	1,654 (482-9,113)	1,544 (458-4,335)	483 (114-3,486)	928 (278-2,877)	1,014 (371-4,246)	130 (≤20-487)	115 (≤20-649)
in HeLa$^{hACE2+TMPRSS2}$ cells	1,777 (410-7,608)	1,350 (429-4,337)	480 (96-2,861)	1,015 (528-2,410)	959 (386-5,905)	52 (≤20-197)	n.d.

Geometric mean (gMean) NT_{50} titers of day 300 sera of bottom 10% responders (n=22) and top 10% responders (n=22) against SARS-CoV-$2^{05\text{-}2N}$ and 6 VOCs were determined in cell-based assays using each SARS-CoV-2 strain and VeroE6^{TMPRSS2} cells or HeLa$^{hACE2+TMPRSS2}$ cells. The numbers in parentheses denote ranges of NT_{50} values determined for each strain. *Only VeroE6^{TMPRSS2} cells were used for SARS-CoV-2^{2037} (Omicron/BA.2) since SARS-CoV-2^{2037} did not well propagate in HeLa$^{hACE2+TMPRSS2}$ cells.