Antibody response of heterologous vs homologous mRNA vaccine boosters against the SARS-CoV-2 Omicron variant: interim results from the PRIBIVAC study, A Randomized Clinical Trial

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Running title COVID-19 vaccine booster immunogenicity

Abstract

Background: Waning antibody levels post-vaccination and the emergence of variants of concern (VOCs) capable of evading protective immunity has raised the need for booster vaccinations. However, which combination of COVID-19 vaccines offers the strongest immune response against Omicron variant is unknown.

Methods: This randomized, subject-blinded, controlled trial assessed the reactogenicity and immunogenicity of different COVID-19 vaccine booster combinations. 100 BNT162b2-vaccinated individuals were enrolled and randomized 1:1 to either homologous (BNT162b2+BNT162b2+BNT162b2; 'BBB') or heterologous mRNA booster vaccine (BNT162b2+BNT162b2+mRNA-1273; 'BBM'). Primary endpoint was the level of neutralizing antibodies against SARS-CoV-2 wild-type and VOCs at Day 28.

Results: 51 participants were allocated to BBB and 49 to BBM; 50 and 48 respectively were analyzed for safety and immunogenicity outcomes. At Day 28 post-boost, mean SARS-CoV-2 spike antibody titers were lower with BBB (22,382 IU/mL 95% CI, 18,210 to 27,517) vs BBM (29,751 IU/mL 95% CI, 25,281 to 35,011, p=0.034) as was the median level of neutralizing antibodies: BBB 99.0% (IQR 97.9 to 99.3%) vs BBM 99.3% (IQR 98.8 to 99.5%, p=0.021). On sub-group analysis, significant differences in mean spike antibody titer and live Omicron neutralization titer was only observed in older adults. Median surrogate neutralizing antibody level against all VOCs was also significantly higher with BBM in older adults, and against Omicron was BBB 72.8% (IQR 54.0 to 84.7%) vs BBM 84.3% (IQR 78.1 to 88.7%, p=0.0073). Both vaccines were well tolerated.

Conclusions: Heterologous mRNA-1273 booster vaccination induced a stronger neutralizing response against the Omicron variant in older individuals compared with homologous BNT123b2.

Keywords: COVID-19 vaccine booster, humoral immunity, omicron, live virus neutralization

Introduction

COVID-19 vaccination programs worldwide have focused on raising population immunity through the primary COVID-19 vaccination series. However, vaccine breakthrough infections have occurred with increasing frequency as a result of waning antibody levels and the emergence of variants of concern (VOCs) such as Omicron which are capable of evading protective immunity.[1,2] All COVID-19 vaccines currently approved by the World Health Organization (WHO) Emergency Use Listing (EUL) were developed with the wild-type SARS-CoV-2 strain that emerged in Wuhan in 2019.[3]

Within a few months from its discovery in November 2021, the Omicron variant supplanted Delta as the dominant strain detected worldwide.[4] Several immunogenicity studies of COVID-19 vaccines have demonstrated that a booster dose is needed to elicit an anti-Omicron neutralizing response.[2,4-6] Vaccine booster combinations tested include homologous mRNA vaccines such as BNT162b2[2,4,6] and mRNA-1273[2], as well as non-replicating viral vector vaccines AD26.COV2.3[2] and AZD1222[6]. However, whether homologous or heterologous mRNA booster vaccination regimens are better at inducing neutralizing antibodies against Omicron, and whether different age groups respond differently to the various vaccine booster combinations is unknown.

In this interim analysis of a phase 4 randomized, subject-blinded clinical trial, we studied the immunogenicity of BNT162b2 versus mRNA-1273 booster vaccinations in individuals who had received the second dose of the BNT162b2 vaccine as a primary series at least six months prior to study enrolment. The study is still ongoing and participants who received mRNA-1273 as their primary series will be included in later phases of the study. Primary endpoint was antibody levels against wild-type SARS-CoV-2 and VOCs as measured by a multiplex surrogate virus neutralization test (sVNT).

Methods

PRIBIVAC is a subject-blinded, randomized-controlled trial to assess the immunogenicity and safety of heterologous booster COVID-19 vaccination compared with a homologous booster regimen. Participants were enrolled at the National Centre for Infectious Diseases (NCID), Singapore. The study protocol is available in Supplement 1.

Enrollment and randomization

During the first phase of the study, from October-November 2021 we enrolled 100 individuals who received BNT162b2 as their primary vaccine series at least six months earlier. Key exclusion criteria included a history of known SARS-CoV-1 or SARS-CoV-2 infection or an immunocompromising medical condition (e.g. active leukemia or lymphoma, generalized malignancy, aplastic anemia, solid organ transplant, bone marrow transplant, current radiation therapy, congenital immunodeficiency, HIV/AIDS with CD4 lymphocyte count < 200 cells/mm^3 and patients on immunosuppressant medications).

Study participants were randomized 1:1 to receive one intramuscular (IM) dose of either BNT162b2 30 mcg (0.3 mL) or mRNA-1273 50 mcg (0.25 mL). Randomization was stratified by age (<60 years, ≥60 years) and time from 2^{nd} vaccine dose administered (6-9 months, >9 months). The study team from Singapore Infectious Disease Clinical Research Network (SCRN) in charge of participant enrolment will perform the randomization using a web-based randomization system hosted by the Singapore Clinical Research Institute (SCRI), in which a randomization list with randomized permuted blocks will be generated by the trial statistician

Blood samples were collected pre-booster (day -28 to day 0), at 7 days (+/-2 days) and 28 days (+/- 7 days) post-booster for assessment of the immune response. Blood samples for immunogenicity assessment will also be collected at 6 months and 12 months. Participants

were given a diary card to record solicited and unsolicited local and general symptoms experienced in the first 7 days after vaccination.

Primary endpoint

The primary objective for this clinical trial is to determine whether a heterologous mRNA-1273 COVID-19 vaccine booster leads to non-inferior humoral immunity against wild-type SARS-CoV-2 and/or VOCs at day 28 compared with homologous BTN162b2. This was assessed by a surrogate virus neutralization test (sVNT) that detects total immuno-dominant neutralizing antibodies targeting the viral spike protein receptor-binding domain in an isotype- and species-independent manner.

Interim analyses and stopping guidelines

Interim analyses were performed for Data Safety Monitoring Board (DSMB) review after 10 participants from each of the intervention arms completed assessments at study day 28. The following criteria were established *a priori* for the DSMB to recommend discontinuation of participant enrolment to either study arm:

- An absolute difference of ≥25% in the proportion of participants with an SAE
- An absolute difference of ≥25% in the proportion of participants with Grade 3 and 4 AEs
- The geometric mean ratio of anti-SARS-CoV-2 antibody between either intervention group falls below 0.60.

Sample size calculation

Based on data from our ongoing COVID-19 vaccine immune-monitoring observational prospective study (SCOPE), the mean level of SARS-CoV-2 anti-spike immunoglobulins by the sVNT was 84% (standard deviation 15%) at 28 days after the second dose.[7] We expect immunogenicity will be boosted back to the same level after the third booster dose in the control

arm. Assuming an immunogenicity level of 84% in the control arm and a non-inferiority margin of -10%, a sample size of 87 subjects per arm is needed to conclude non-inferiority of the intervention arm against the control arm with 80% power. The sample size is calculated at a one-sided 2.5% significance level and accounts for an attrition rate of 15%.

Antibody response assays

Serum samples were tested with a newly developed multiplex-sVNT assay using the Luminex platform.[8] Briefly, AviTag-biotinylated receptor binding domain (RBD) proteins from wild-type SARS-CoV-2 and five VOCs (Alpha, Beta, Gamma, Delta, Omicron) were coated on a MagPlex Avidin microsphere (Luminex) at 5ug/1 million beads. RBD-coated microspheres (600 beads/antigen) were pre-incubated with serum at a final concentration of 1:20 or greater for 15 min at 37°C with 250 rpm agitation. After 15 min incubation, 50uL of phycoerythrin (PE)-conjugated hACE2 (GenScript 2ug/mL) were added to the well and incubated for 15 min at 37°C with agitation, followed by two PBS-1% BSA washes. The final readings were acquired using the MAGPIX system.

Serological results were obtained using the Elecsys® (Roche, Basel, Switzerland) Anti-SARS-CoV-2 chemiluminescent immunoassays following manufacturer instructions [anti-nucleocapsid (anti-N) and anti-spike protein receptor binding domain (anti-S)]. Antibody titres in U/ml from the Elecsys® anti-S assay are equivalent to the WHO standard Binding Antibody Units (BAU)/ml, with no conversion required.[9]

Live virus inhibition assay

The Omicron variant (B.1.1.529/BA.1) isolate M21021166 was originally isolated by Prof. Gavin Screaton, University of Oxford, UK, and then obtained from Prof. Wendy Barclay, Imperial College London, London UK through the Genotype to Phenotype National Virology Consortium (G2P-UK). Sequencing confirmed it contained the variant defining mutations.[10] Viral stock of

the SARS-CoV-2 Omicron isolate was generated in Vero/hSLAM cells with Dulbecco's minimal essential medium (DMEM) (Sigma) containing 4% fetal bovine serum (FBS) (Sigma), 0.05 mg/ml gentamicin (Merck), and 0.4 mg/ml geneticin (G418; Thermo Fisher) and harvested 72 h post-inoculation. Virus stocks were aliquoted and stored at −80 °C as previously described.[11]

PRNTs were performed using African green monkey kidney C1008 (Vero E6) cells (Public Health England, PHE). Sera were heat-inactivated at 56 °C for 1 h and stored at −20 °C until use. DMEM containing 2% FBS and 0.05 mg/mL gentamicin was used for serial twofold dilutions of patient plasma samples. SARS-CoV-2 at 800 PFU/mL was added to an equal volume of diluted plasma and incubated at 37 °C for 1 h. The virus-plasma dilution was inoculated onto Vero E6 cells in duplicate and incubated at 37 °C for 1 h. They were then overlaid with agarose as in standard plaque assays. Cells were incubated for 72 h at 37 °C and 5% CO2 before being fixed with 10% formalin and stained with crystal violet solution (Sigma-Aldrich). Plaque reduction neutralisation test (PRNT) 90/80/50 was determined by the highest dilution with a 90/80/50% reduction in plaques compared to the control.

Statistical methods

Demographic and baseline characteristics were summarized by vaccine and age groups. For comparison of vaccine reactions, categorical data was compared using Fisher's exact test or Chi-square as appropriate. Anti-spike antibody titers were $\log_{10}$-transformed for all statistical analysis, and compared using student's t-test. A multiple regression model of pre-vaccination antibody titers was constructed, which included age (<60; ≥60 years), sex and time since vaccination (in days) with the $\log_{10}$-transformed antibody titer as the dependent variable. Comparison of sVNT % inhibition level and the neutralization activity of plasma samples against Omicron was conducted by Mann-Whitney U. No adjustments were made for multiple testing.

Statistical significance was defined as $p<0.05$. Analyses were performed using R and figures generated using GraphPad Prism version 9.

Ethics Statement and Data Availability

Written informed consent was obtained from all study participants (Domain Specific Review Board ref no: 2021/00821). The study was registered with ClinicalTrials.gov (NCT05142319). All data sharing requests should be addressed to the corresponding authors.

Results

Participants

Among 100 participants who received two primary doses of BNT162b2, 51 were randomized to receive the homologous mRNA booster BNT162b2 (control group; BBB) and 49 to the heterologous mRNA booster mRNA-1273 (intervention group; BBM) (Figure 1). One participant from each group withdrew from the study, resulting in an analysis sample size of 50 and 48 for BBB and BBM groups, respectively. Baseline demographic characteristics of the participants who received BBB or BBM in the younger (<60 years) or older (≥60 years) age groups are shown in Table 1.

No COVID-19 infections were recorded during the 28-day study period. All participants were negative for anti-N antibody at baseline, Day 7 and Day 28.

Safety

The number of participants with solicited local and systemic adverse reactions (ARs) were similar between the BBB and BBM groups (Supplementary Table 1 and Supplementary Figure 1). The most common local AR was injection site pain, with 89% and 87% of participants who received BBB or BBM respectively experiencing pain at the injection site within 72 hours of a booster dose. The most common systemic AR was fatigue/tiredness (BBB 70% and BBM 67%), followed by muscle pain (BBB 61% and BBM 56%).

Local and systemic ARs between BBB and BBM in each age group were similar, except in the older age group where fever and weakness occurred more frequently in the BBM (35%) than BBB (5%) group.

35 unsolicited adverse events (AEs) were reported by 25 participants, 12 in BBB and 13 in BBM. No serious AEs were reported in the 28 days after vaccination in either age group.

Immunogenicity assessments

Level of SARS-CoV-2 anti-S antibodies and neutralizing antibodies against the wild-type SARS-CoV-2 and VOCs were measured in serum samples collected before the booster dose (day -14 to Day 0) and at Days 7 and 28 after the booster dose. Before the booster dose and across all participants, mean anti-S antibody titer in all participants was 555 IU/mL (95% CI 484 to 635 IU/mL), and median sVNT level 48.0% (inter-quartile range [IQR] 36.5 to 59.3%) and similar between intervention groups. On multiple regression, baseline anti-S titers were significantly lower with older age (p=0.0188) and among men (p=0.0051), but not with time since primary vaccination series.

After the booster dose, anti-S titer across both intervention groups increased by 35- to 49-fold at Day 7 to a mean of 23,158 IU/mL (95% CI 19,539 to 27,454 IU/mL), with only a modest further increase by Day 28 (25,651 IU/mL (95% CI 22,444 to 29,322 IU/mL). Comparing study groups, antibody titers were higher at both Day 7 (1.4-fold, p=0.0496) and Day 28 (1.3-fold, p=0.0339) in the mRNA-1273 booster group compared with BNT162b2 (Figure 2). This finding was consistent when comparing neutralization levels against wild-type, Omicron and most of the other variants (Figure 3).

At pre-planned sub-group analysis, the anti-S antibody titers between BBB and BBM in the younger age group were not significantly different at Days 7 and 28 post-booster, whereas older participants who received BBM resulted in a significantly higher induction of anti-spike antibody

levels than those who received BBB. Mean anti-S titer was significantly higher with BBM than BBB by 2.1-fold (p=0.0078) at Day 7 and 1.6-fold (p=0.0184) at Day 28.

The same trend was observed in inhibition level measured by sVNT against the wild-type SARS-CoV-2 and VOCs. Older BBM participants had higher levels of neutralizing antibodies against SARS-CoV-2 and all known VOCs, including Omicron (Supplementary Table 2-4). The median wild-type SARS-CoV-2 sVNT inhibition level was modestly different at Day 28 (BBB 98.8% (IQR 95.3 to 99.0%) vs BBM 99.3% (IQR98.7 to 99.5%)) likely due to saturation, although this achieved statistical significance (p=0.003).

The largest absolute difference in inhibition level was observed against the Omicron variant in older participants (BBB 64.6% (IQR 53.7 to 75.2%) vs BBM 89.2% (IQR 75.9 to 91.6%), p=0.0003) at Day 7 post-booster. At Day 28 post-booster, the inhibition % remain significantly higher against the Omicron variant in the BBM group: 84.3% (IQR 78.1 to 88.7%) than BBB 72.8% (IQR 54.0 to 84.7%, p=0.0073).

The neutralizing activity of plasma samples from a subgroup of 40 participants against the Omicron variant isolates was assessed using a live virus neutralization assay. The results corroborated the antibody and sVNT assay data, showing a significant increase in plaque reduction neutralization test 50 (PRNT50) to Omicron at Day 28 after booster vaccination (Figure 4A). In addition, older BBM participants had a higher PRNT50 against Omicron than BBB at Day 28 post-booster [BBB 80 (IQR 40 to 80) vs BBM 160 (IQR 100 to 240), p=0.022] (Figure 4B). Similar results were observed with PRNT80 and 90 (Supplementary Table 5).

Discussion

The Omicron variant of SARS-CoV-2 has opened a new chapter in the COVID-19 pandemic [12] due to its high transmissibility and large number of mutations in the RBD region of the spike protein[13], which may explain its partial or complete resistance to antibody neutralization in fully

vaccinated or previously infected individuals. The increasing frequency of vaccine breakthrough infections and the variable supply for different vaccine products have raised the need and consideration for heterologous booster vaccinations. Recent studies have shown both homologous and heterologous boosting, irrespective of primary vaccine series, to increase neutralizing antibody titers.[14,15] In Singapore, a recent study of data from the Delta variant outbreak found heterologous boosting to be associated with a lower incidence rate of SARS-CoV-2 infection than homologous boosting in adults 60 years and older.[16] However, the comparative effect of different booster vaccine regimens on the serum neutralizing activity against Omicron and other VOCs remains unknown.

This interim analysis describes the safety and immunogenicity of a homologous (BNT162b2, BBB) or heterologous (mRNA-1272, BBM) mRNA booster dose in fully vaccinated adults against clinically important VOCs such as Omicron. The adverse reactions after single booster injections with BNT162b2 or mRNA-1273 were comparable between BBB and BBM groups, and similar to those observed after the BNT162b2 primary series, which commonly include pain at the site of injection, lethargy and muscle pain.

Six months after the primary vaccine series, mean neutralizing antibody titers against the wild-type SARS-CoV-2 declined to 40-60% in all groups. Additional reduction of neutralizing activity against VOCs compared with the wild-type SARS-CoV-2 is a common trend in all participants that is not influenced by age. Declining neutralization against the wild-type SARS-CoV-2 and low neutralizing activity against Omicron after complete BNT162b2 vaccination calls for an effective booster vaccine regimen to increase immune responses and protection. In this interim analysis, we demonstrate that a booster dose can effectively enhance serum neutralizing activity against the wild-type SARS-CoV-2 and all known VOCs Alpha, Beta, Gamma, Delta and Omicron as early as Day 7 post-booster. More importantly, we evaluated and compared the choice of booster dose for different age groups. For the vulnerable older age group in particular,

a heterologous booster COVID-19 vaccine regimen induces a higher anti-spike antibody titer and a stronger neutralizing antibody response against the highly infectious Omicron variant (~20% higher neutralization) than a homologous booster regimen.

This analysis is limited to healthy individuals receiving the BNT162b2 primary vaccine series, and a recent study has shown that immunogenicity may be affected by the order of vaccine products, though apparently less so than the combination.[17,18] Currently, it is not clear to what extent the higher antibody levels observed in older BBM participants are due to superiority of mRNA-1273 versus BNT126b2 or an effect of heterologous boosting. The PRIBIVAC study is ongoing and in later phases of the study will include individuals who received mRNA-1273 as their primary vaccine series to address this question. In addition, it is not known whether these higher antibody peaks after vaccination will persist for the long term. Study participants will continue to be followed up at 6 months and 12 months after their booster vaccination to measure the rate of waning.

A study of this size is not likely to be able to determine vaccine effectiveness against infection, and the clinical impact of this antibody difference in older adults needs to be determined.[16] Further studies are underway to characterize cell-mediated immunity in this cohort which may indicate effectiveness against severe infection.

This study was initiated initially with only 2 arms (the control arm BBB and intervention arm BBM) as the availability of other vaccine formulations are subjected to rigorous regulatory scrutiny before they can be used in Singapore. We present interim results from this study obtained before reaching our initial planned sample size due to new inclusion of Covaxin as a booster dose to the study platforms adaptive protocol. It is unlikely the study findings will change with a larger sample size given the large difference in the Omicron-specific neutralizing levels among older adults. Singapore has rapidly expanded its COVID-19 booster vaccination campaign, and currently 65% of adults aged ≥ 60 have received a booster dose.

Variant-specific vaccines may be necessary for optimal protection against SARS-SoV-2 variants such as Omicron.[19] Clinical trials are currently ongoing, but even if successful these vaccines are not expected to be available till late in 2022. Thus, there is an urgent need for an effective standard booster vaccination regimen, particularly in vulnerable populations, to reduce the risk of severe disease and the present data provide evidence that a heterologous booster vaccination in older individuals induces a more robust neutralization against the immune-evasive Omicron variant. This information is of paramount importance to inform future COVID-19 booster programs (third dose in other countries or fourth dose in Singapore) for older individuals to better protect them against SARS-CoV-2 infection and severe disease. Future follow-up analyses can provide further insights into the durability of the neutralizing antibody response of the different vaccine booster combinations, as well as the neutralizing ability against new VOCs.

Conclusions

Although the Omicron variant exerts considerable humoral immune escape in BNT162b2 fully vaccinated individuals, a booster dose with BNT162b2 or mRNA-1273 is capable of increasing the serum neutralizing activity against Omicron by more than 50% by Day 7 post-booster. In older individuals who received BNT162b2 as their primary vaccine's series, a heterologous booster regimen with mRNA-1273 induced a higher anti-spike antibody titer and a stronger neutralizing response against the Omicron variant than a homologous booster regimen.

NOTES

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Author contributions: Dr Young had full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

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Conflict of Interest

Young reports personal fees from Astra-Zeneca, Gilead, Roche, Sanofi and Novacyte outside the submitted work and payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Astra-Zeneca, Gilead, Roche, Sanofi, all paid to institution. Tan, Wang and Chia report the following issued patent: United States Patent; Patent No: US 11,054,429 B1, Title of patent: SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-Spike protein binding. All other authors no potential conflicts of interest.

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Tables

Table 1: Demographics of study participants

	BBB (n = 50)		BBM (n = 48)	
Age group	**< 60**	**≥ 60**	**< 60**	**≥ 60**
N	26	24	25	23
Age, mean (range) year	35 (21-58)	68 (60-78)	37 (23-59)	67 (60-84)
Male sex, No. (%)	9 (35%)	13 (54%)	12 (48%)	9 (39%)
Chinese, No. (%)	20 (77%)	23 (95%)	22 (88%)	23 (100%)
Charlson comorbidity Index, median (IQR)	0 (0-0)	0 (0-0.75)	0 (0-0)	0 (0-0)
Days since 2nd dose, mean (range)	254 (194-297)	219 (190-280)	252 (196-295)	210 (189-257)
Current smoker	1	0	0	1

Figure legends

Figure 1. Consort flow diagram.

Figure 2. Level of SARS-CoV-2 anti-spike receptor binding domain (RBD) antibody in participants (a) below 60 years old and (b) 60 years old and above, and (c) overall. Participants in the older age group (≥60 years old) who received a heterologous COVID-19 vaccine booster (BBM) have significantly higher anti-SARS-CoV-2 IgG antibodies than those who received a homologous mRNA booster (BBB) at day 7 and 28 post-vaccination. Data analyzed using Student's t-test to compare the $\log_{10}$ anti-S titer. Box represents 25^{th} and 75^{th} percentile, line is median, with whiskers denoting extremes. Abbreviations: BBB, BNT162b2-BNT162b2-BNT162b2; BBM, BNT162b2-BNT162b2-mRNA-1273. *, $p < 0.05$; **, $p < 0.01$.

Figure 3. Level of neutralizing antibodies against SARS-CoV-2 and variants of concern in participants (a) below 60 years old and (b) 60 years old and above, and (c) summary data for Omicron. Level of %inhibition was determined using a multiplex surrogate virus neutralization test as previously described.[8] Data was analyzed using Mann-Whitney U test. Red dotted line indicates inhibition of 30% (nominal 'seronegative' threshold). Data presented in box plot and the line in the box indicates median. Abbreviations: BBB, BNT162b2-BNT162b2-BNT162b2; BBM, BNT162b2-BNT162b2-mRNA-1273. **, $p < 0.01$; ***, $p < 0.001$.

Figure 4. The neutralization activity of plasma samples against Omicron variant of SARS-CoV-2. Plasma samples from participants who received a vaccine booster were collected prior to vaccination (day 0) and at day 28 after the booster vaccination were screened for neutralizing activity against Omicron variant of SARS-CoV-2. Plasma neutralizing activity comparison between participants who received the homologous (BBB) and heterologous (BBM) mRNA booster vaccine in the younger (<60 years, n=28) or older (≥60 years, n=12) age groups. Box represents 25th and 75th percentile, line is median, with whiskers denoting extremes. Abbreviations: BBB, BNT162b2- BNT162b2- BNT162b2; BBM, BNT162b2-BNT162b2-mRNA-1273. Data analyzed using Mann-Whitney U test. *, $p < 0.05$.

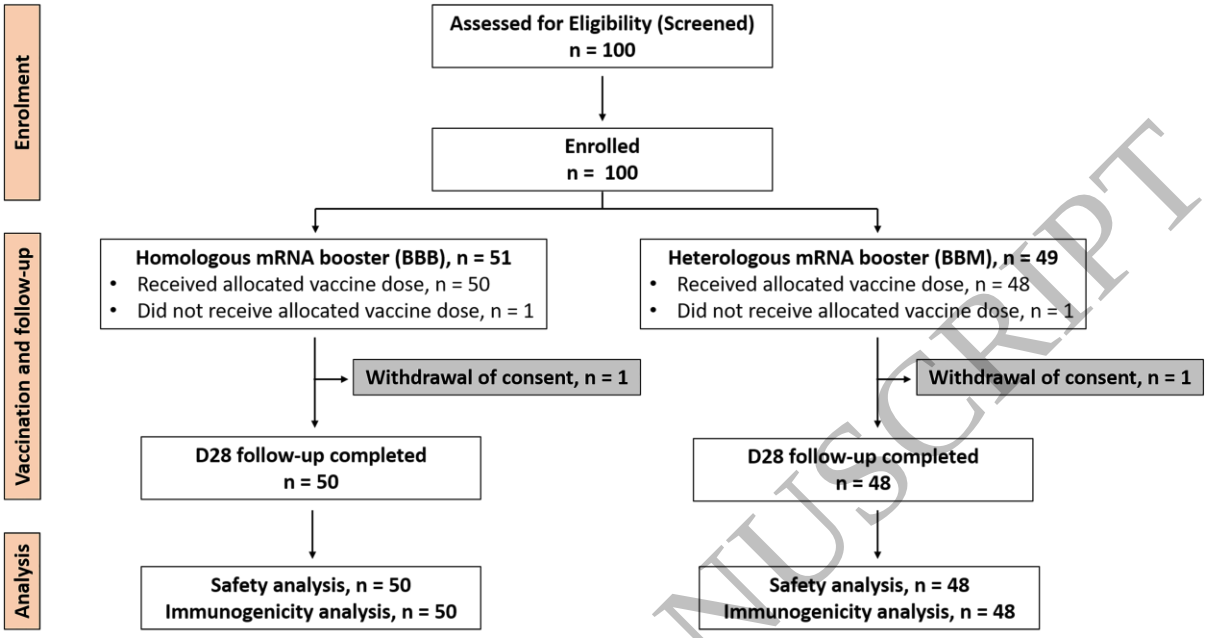
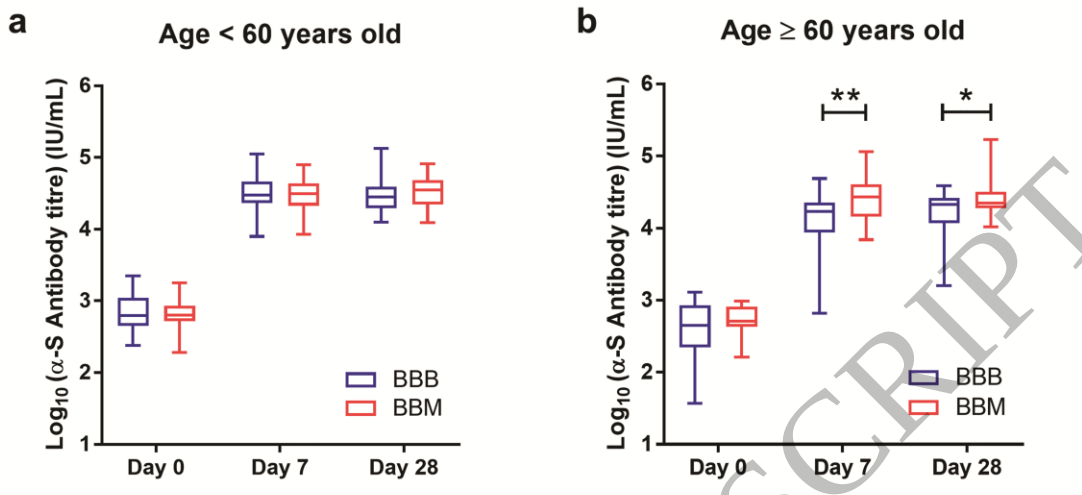


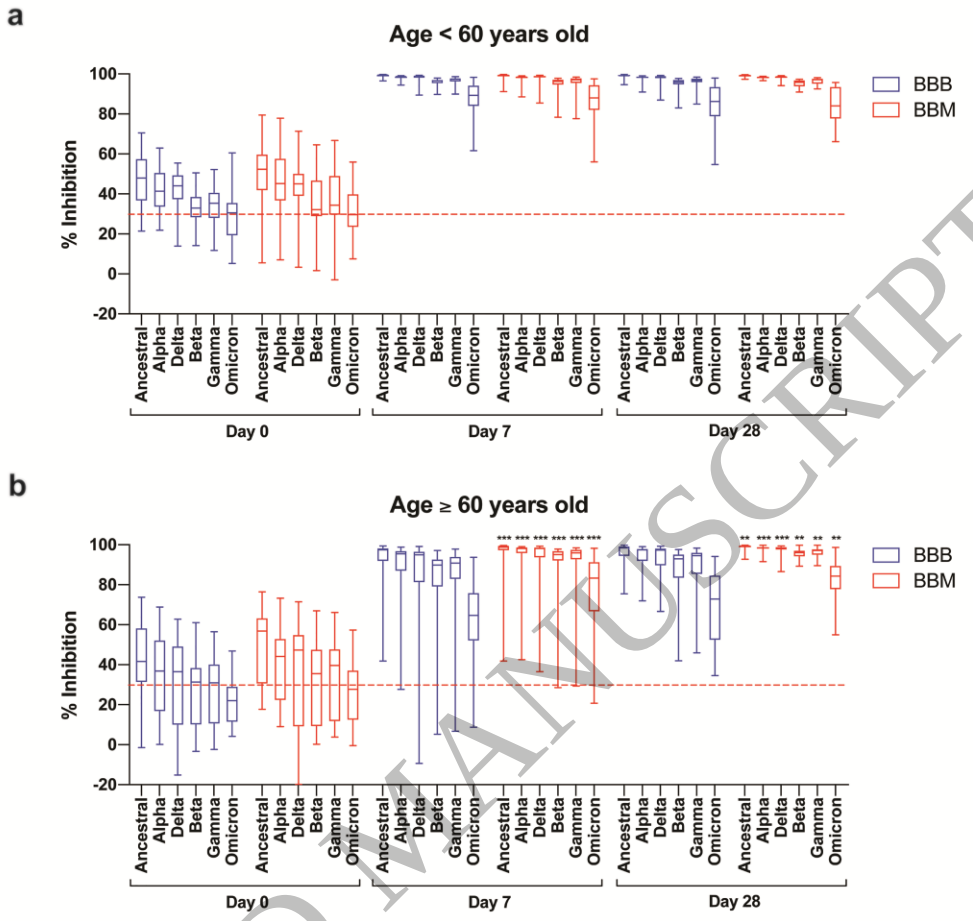
Figure 1
159x84 mm (.23 x DPI)



C

		Overall			<60			≥60		
		BBB	**BBM**	**p-value**	**BBB**	**BBM**	**p-value**	**BBB**	**BBM**	**p-value**
Day 0	**N**	50	48		26	25		24	23	
	Mean [95% CI] (IU/mL)	527 [418 to 665]	585 [507 to 675]	0.45	704 [566 to 876]	649 [526 to 800]	0.58	385 [257 to 576]	523 [429 to 639]	0.17
Day 7	**N**	50	47		26	24		24	23	
	Mean [95% CI] (IU/mL)	19,683 [14,870 to 26,050]	27,542 [22,925 to 33,083]	**0.049**	31,326 [25,015 to 39,327]	29,943 [23,632 to 37,940]	0.78	11,893 [7,451 to 18,989]	25,235 [18,750 to 33,970]	**0.0078**
Day 28	**N**	50	46		26	25		24	21	
	Mean [95% CI] (IU/mL)	22,382 [18,210 to 27,517]	29,751 [25,281 to 35,011]	**0.034**	30,116 [123,736 to 38,203]	32,576 [26,693 to 39.747]	0.61	16,229 [11,901 to 22,131]	26,712 [20,216 to 35,286]	**0.018**

Figure 2
159x166 mm (.23 x DPI)



c

		Overall			<60			≥60		
		BBB	BBM	p-value	BBB	BBM	p-value	BBB	BBM	p-value
Day 0	N	50	48		26	25		24	23	
	Median [IQR] (%)	26.2 [14.7 to 33.0]	28.6 [17.9 to 37.9]	0.16	30.6 (19.8 to 35.6)	29.6 (24.0 to 39.8)	0.59	17.8 (11.0 to 28.0)	27.8 (12.9 to 35.6)	0.16
Day 7	N	50	47		26	24		24	23	
	Median [IQR] (%)	79.4 [63.4 to 90.6]	88.0 [79.6 to 92.5]	**0.029**	89.3 (86.1 to 93.2)	88.0 (82.3 to 94.6)	0.63	64.6 (53.7 to 75.2)	89.2 (75.9 to 91.6)	**0.0003**
Day 28	N	50	46		26	25		24	21	
	Median [IQR] (%)	82.5 [68.5 to 90.3]	84.2 [78.2 to 91.0]	0.11	86.2 (78.7 to 93.7)	84.0 (78.8 to 93.6)	0.74	72.8 (54.0 to 84.7)	84.3 (78.1 to 88.7)	**0.0073**

Figure 3
159x234 mm (.23 x DPI)

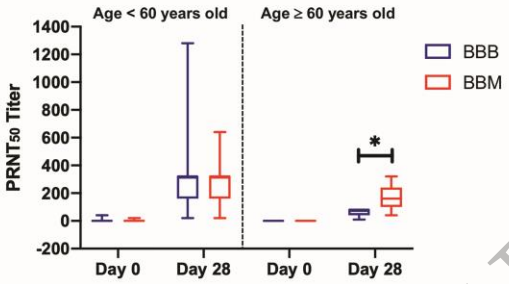


Figure 4
159x110 mm (.23 x DPI)