Immunogenicity and safety of a booster dose of a self-amplifying RNA COVID-19 vaccine (ARCT-154) versus BNT162b2 mRNA COVID-19 vaccine: a double-blind, multicentre, randomised, controlled, phase 3, non-inferiority trial

Yoshiaki Oda, Yuji Kumagai, Manabu Kanai, Yasuhiro Iwama, Iori Okura, Takeshi Minamida, Yukihiro Yagi, Toru Kurosawa, Benjamin Greener, Ye Zhang, Judd L Walson

Summary

Background Licensed mRNA COVID-19 vaccines require booster doses to sustain SARS-CoV-2-specific responses, creating the need for novel, broadly immunogenic vaccines. We aimed to compare the immunogenicity, safety, and tolerability of ARCT-154—a self-amplifying mRNA vaccine against SARS-CoV-2 D614G variant—with the BNT162b2 (Comirnaty; Pfizer–BioNTech) mRNA vaccine when administered as a fourth-dose booster.

Methods This double-blind, multicentre, randomised, controlled, phase 3, non-inferiority trial, conducted at 11 outpatient clinical sites in Japan, enrolled healthy adults aged at least 18 years who had previously been immunised with two doses of an mRNA COVID-19 vaccine (BNT162b2 or mRNA-1273 [Spikevax; Moderna]) followed by a third dose of BNT162b2 at least 3 months before enrolment. Participants were randomly assigned, in a 1:1 ratio using an Interactive Response Technology system with a block size of four, and with stratification by age (18–64 years or ≥65 years) and by interval since last COVID-19 vaccination (<5 months or ≥5 months), to receive either ARCT-154 or BNT162b2 as a fourth-dose booster via deltoid intramuscular injection. Participants and investigators assessing outcomes were masked to group assignment. The primary objective, measured in per-protocol set 1 (consisting of participants with no evidence of previous SARS-CoV-2 infection who received their intended injection according to protocol), was to show that the immune response 28 days after the ARCT-154 vaccine was non-inferior to that of the BNT162b2 vaccine, measured in terms of both pseudovirus neutralising antibody geometric mean titre (GMT) ratios and seroresponse rates against the wild-type Wuhan-Hu-1 strain of SARS-CoV-2. Non-inferiority was declared when the lower limit of the 95% CI of the ARCT-154 to BNT162b2 GMT ratio exceeded 0·67, and when the lower limit for the difference in seroresponse rates exceeded –10%. Key secondary endpoints included the immune response against the omicron BA.4/5 subvariant, which was assessed for non-inferiority and superiority in per-protocol set 1. Safety was assessed in the full analysis set. This study was registered on the Japan Registry for Clinical Trials, jRCT 2071220080, and is ongoing.

Findings Between Dec 13, 2022, and Feb 25, 2023, we enrolled and randomly assigned 828 participants to receive ARCT-154 (n=420) or BNT162b2 (n=408) vaccines as a fourth-dose booster. In per-protocol set 1, the GMTs of surrogate neutralising antibodies induced against the Wuhan-Hu-1 SARS-CoV-2 strain in the ARCT-154 group (5641 [95% CI 4321–7363]) were non-inferior to those in the BNT162b2 group (3934 [2993–5169]) when measured at 28 days after boosting, with a GMT ratio of 1·43 (95% CI 1·26–1·63). Seroresponse rates were 65·2% (95% CI 60·2–69·9) in the ARCT-154 group versus 51·6% (46·4–56·8) in the BNT162b2 group, a difference of 13·6% (95% CI 6·8–20·5). GMTs against the omicron BA.4/5 variant on day 29 were 2551 (1687–3859) in the ARCT-154 group and 1958 (1281–2993) in the BNT162b2 group—a GMT ratio of 1·30 (1·07–1·58)—with seroresponse rates of 69·9% (65·0–74·4) and 58·0% (52·8–63·1). Both boosters were equally well tolerated. No treatment-related deaths were reported, nor were there severe or serious adverse events considered to be causally associated related to study vaccination. One serious adverse event, a foot deformity reported in a participant in the BNT162b2 group, was observed but determined not to have a causal relationship to the study vaccination. One severe adverse event, a case of abnormal hepatic function in the ARCT-154 group, was considered to be related to study vaccine. Adverse events of special interest for detection of myocarditis and pericarditis included chest pain (one case in the ARCT-154 group and three cases in the BNT162b2 group) and shortness of breath (two cases in the BNT162b2 group), all of which were considered to have a reasonable possibility of being related to vaccination. Local reactions were reported by 398 (95%) of 420 participants receiving the ARCT-154 vaccine and 395 (97%) of 408 participants receiving the BNT162b2 vaccine, and solicited systemic adverse events by 276 (66%) of those receiving the ARCT-154 vaccine and 255 (63%) of those receiving the BNT162b2 vaccine. Adverse events were mainly mild in severity, occurring and resolving within 3–4 days after vaccination.

Lancet Infect Dis 2023

Published **Online** December 20, 2023 https://doi.org/10.1016/S1473-3099(23)00650-3

See **Online/Comment** https://doi.org/10.1016/S1473-3099(23)00734-X

For the Japanese translation of the abstract see **Online** for appendix 1

Meiji Seika Pharma, Tokyo, Japan (Y Oda PhD, M Kanai MSc, Y Iwama MSc, I Okura MSc, T Minamida BSc, Y Yagi PhD, T Kurosawa PhD)**; Kitasato University Kitasato Institute Hospital, Tokyo, Japan** (Y Kumagai PhD)**; Arcturus Therapeutics, San Diego, CA, USA** (B Greener PharmD, Y Zhang PhD)**; Walson Consulting, Seattle, WA, USA** (Prof J L Walson MD)

Correspondence to: Prof Judd L Walson, Walson Consulting, Seattle, WA 98117, USA **jwalson@walsonconsultingllc.com**

Interpretation In adults who had previously received three doses of an mRNA COVID-19 vaccine, immune responses 28 days after an ARCT-154 booster dose were non-inferior to those observed after a BNT162b2 booster dose for the Wuhan-Hu-1 strain of SARS-CoV-2 and superior for the Omicron BA.4/5 variant. Increased immune responses at 28 days might provide increased likelihood of protection against these strains during this period and could also result in longer duration of protection. Further studies will assess the immunogenicity induced against more recent SARS-CoV-2 variants.

Funding Japanese Ministry of Health, Labour, and Welfare.

Introduction

Although global rates of SARS-CoV-2 infection have decreased since levels observed in late 2022, many cases continue to occur, primarily driven by the emergence of new variants.[1] Many effective vaccines have been developed to protect against the more severe consequences of SARS-CoV-2 infection; however, these vaccines are less effective against the mild-to-moderate disease caused by the new variants that increasingly predominate.[2] This lower effectiveness is a consequence of the combined effects of waning antibody titres following primary immunisation and the lower sensitivity of the newly emerging SARS-CoV-2 variants and subvariants to antibodies elicited against the earlier viral strains, from which the first vaccines were developed.[3] Lower cross-neutralisation activity is a result of the successive accumulation of mutations in the spike protein, the main antigenic target of COVID-19 vaccines;[4] such mutations alter the epitopes on the glycoprotein to make them less susceptible to vaccine-induced neutralising antibodies. Strategies to overcome these factors and maintain immune protection include the administration of booster doses,[5] the development of new vaccine formulations based on more recent strains

Research in context

Evidence before this study

mRNA vaccines have been successfully developed and used to combat COVID-19. Such vaccines require reasonably high loads of mRNA due to its degradation by host-cell RNAases, which could contribute to the short duration of observed immune responses to such vaccines. Delivering RNA through alternative mechanisms, such as self-amplifying RNA (saRNA), should require lower doses as the mRNA is produced intracellularly, so smaller amounts should lead to higher responses and potentially longer duration of those responses. We searched PubMed from database inception to July 17, 2023, with no language restrictions, using the terms "self-amplifying RNA" and "self-replicating RNA". We found several preclinical studies in mice showing robust immune responses to saRNA vaccines with very low doses of mRNA, and four phase 1 clinical studies of saRNA vaccines other than ARCT-154 in humans. Of these phase 1 studies, one involved an saRNA vaccine against metastatic solid tumours, another was a dose-ranging trial of an experimental saRNA vaccine against COVID-19, and two investigated an saRNA COVID-19 vaccine as a booster dose in older people (≥60 years) who had previously been immunised with an adenovirus-vectored COVID-19 vaccine. All four trials showed the potential of saRNA vaccines, with robust immune responses and generally good safety and tolerability profiles, and the studies of their use as booster doses found broad and durable responses for at least 6 months. ARCT-154, an saRNA vaccine based on the SARS-CoV-2 D614G variant spike protein, has previously been shown to be immunogenic and efficacious when administered as a two-dose primary vaccination regimen in a combined phase 1/2/3a study done in Viet Nam. The present study assessed ARCT-154 as a booster dose in Japanese adults.

Added value of this study

To our knowledge, this is the first use of an saRNA COVID-19 vaccine as a booster dose in adults who have previously received an mRNA vaccine. We show that a 5 μg booster dose of ARCT-154 is safe, is well tolerated, and induces a robust immune response to the Wuhan-Hu-1 strain of SARS-CoV-2 that is non-inferior to the response induced by a homologous 30 μg booster dose of the BNT162b2 mRNA vaccine. Additionally, ARCT-154 elicits a superior immune response against the tested omicron BA.4/5 subvariant to the homologous mRNA vaccine.

Implications of all the available evidence

Preclinical studies in mice found that saRNA vaccines elicit cellular immunity and some clinical data also indicate increased and broader functional T-cell responses than traditional mRNA vaccines. Our study shows that, when administered in adults as a booster dose, the tested saRNA COVID-19 vaccine is safe, is well tolerated, and induces humoral immunity. Compared with an mRNA vaccine, the non-inferior magnitude of the humoral response against the Wuhan-Hu-1 strain of SARS-CoV-2 and the superior humoral response against the omicron BA.4/5 subvariant are positive findings; however, further studies are needed to assess whether this new technology will provide better and longer-lasting protective immunity than existing vaccines against COVID-19, especially in the presence of newly emerging SARS-CoV-2 variants for which new formulations will be required before licensure of such novel vaccines.

of SARS-CoV-2,[6] and the use of heterologous immunisation with different vaccine types to broaden and prolong the immune response.[7]

Some of the most widely used COVID-19 vaccines are based on mRNA that codes for the spike protein. These vaccines are effective, but the immune response wanes in the months immediately following administration.[8,9] Administering a booster dose of the same vaccine type, known as homologous boosting, extends the duration of response but does not broaden it against the antigenic drift that occurs as new variants emerge.[10–12] New booster formulations incorporating an antigen or mRNA that directly target the new variants have been introduced.[6,13] However, novel vaccines might be necessary to extend the protective immunity established by the initial vaccines and to cover new variants that are currently unknown or are yet to emerge. Arcturus Therapeutics (San Diego, CA, USA) has developed an alternative technology for mRNA vaccines using self-amplifying RNA (saRNA). Preclinical studies suggest that saRNA vaccines, which are not restricted to COVID-19, require lower doses to enhance antigen expression than conventional mRNA-based vaccines and could improve the efficacy and duration of protection.[14–16] One such formulation, ARCT-154, encodes the spike protein of the B.1 lineage with the D614G (Asp614Gly) mutation, one of the earliest detected variants of SARS-CoV-2.[17] In collaboration with Meiji Seika Pharma (who are conducting the clinical trial and marketing the vaccine in Japan pursuant to an agreement with Seqirus, the exclusive licensee of ARCT-154), we assessed the safety, tolerability, and immunogenicity of ARCT-154 in comparison with the mRNA vaccine BNT162b2 (Pfizer–BioNTech) when used as a booster dose in healthy adults who had previously been immunised with three doses of approved mRNA COVID-19 vaccines, the third of which was BNT162b2. This study follows the primary phase 1/2/3a/3b study (NCT05012943), which demonstrated the safety of ARCT-154 compared with placebo and from which absolute efficacies were calculated as 56·6% (95% CI 48·7–63·3) against any COVID-19 and 95·3% (80·5–98·9) against severe COVID-19 when used as a primary vaccination regimen in Vietnamese adults.[18]

Methods

Study design

This double-blind, multicentre, randomised, controlled, phase 3, non-inferiority trial was conducted at 11 outpatient clinical sites in Japan (appendix 2 p 2). The study protocol (appendix 2) was approved by the institutional review boards of all participating sites. The study was conducted according to the ethical principles of the Declaration of Helsinki and Good Clinical Practice.

Participants

Participants were recruited through advertising at the study sites. Eligible participants were healthy adults aged at least 18 years who had previously been immunised with three documented doses of one of two authorised mRNA vaccines, either BNT162b2 or mRNA-1273 (Spikevax; Moderna), with the last dose being BNT162b2 and received at least 3 months previously. All enrolled participants had to agree to comply with the study requirements, including all study visits and provision of blood samples, and to use approved methods of contraception from 28 days before study vaccination and then throughout the study duration.

The main exclusion criteria were any indication of a current infection (eg, temperature ≥37·5°C on day 1); any known history of a SARS-CoV-2 infection within the previous 4 months; any chronic infection (eg, HIV, hepatitis B virus, hepatitis C virus, or active tuberculosis); or any history of myocarditis, pericarditis, cardiomyopathy, or a medical or psychological issue that, according to the medical judgement of the principal investigator, put the volunteer at risk or risked preventing completion of the study. Other key exclusion criteria included any known immunosuppressive condition or treatment likely to influence immune responses to vaccination, any known history of adverse reactions to vaccination, any participation in any other drug or vaccine trial, or a positive pregnancy test at the time of screening or intention to become pregnant within 1 year of vaccination. The full list of exclusion criteria is provided in the protocol (appendix 2 p 14). All participants provided written informed consent before enrolment.

Randomisation and masking

At enrolment, participants were randomly assigned (1:1) using an Interactive Response Technology system with a block size of four, stratified by age (18–64 years or ≥65 years) and the interval since last COVID-19 vaccination (<5 months or ≥5 months), to receive either the ARCT-154 or the BNT162b2 vaccine. Randomisation lists were generated by external unmasked statisticians who had no further role in endpoint analyses. Vaccines were prepared in opacified syringes and administered by study staff who were unmasked to the randomisation code supplied by the study sponsor and who had no further role in the study. All other study staff and the participants were masked to group assignment.

Procedures

The ARCT-154 study vaccine consists of saRNA encapsulated in lipid nanoparticles. The RNA comprises a replicon based on the Venezuela equine encephalitis virus (VEEV), in which RNA coding for the VEEV structural proteins is replaced with RNA coding for the full-length spike glycoprotein of the SARS-CoV-2 D614G variant, with modifications in the sequence to ensure that the glycoprotein is expressed in the pre-fusion conformation and in the furin cleavage site to stabilise the protein. The vaccine was supplied in a vial containing 100 µg of the active ingredient and was stored at –20°C or

See Online for appendix 2

below before use. After dissolving in 10 mL sterile saline, a 0·5 mL dose, containing 5 µg of the active ingredient, was administered by intramuscular injection into the deltoid.

The control mRNA vaccine, BNT162b2, was supplied as a frozen suspension in vials containing 225 µg of a nucleoside-modified mRNA encoding the viral S glycoprotein of SARS-CoV-2 in 0·45 mL sterile solution, and was stored at –80°C. After further diluting each vial with 1·8 mL sterile saline, the recommended 0·3 mL booster dose of BNT162b2 containing 30 µg was administered by intramuscular injection into the deltoid.

On day 1, enrolled participants had a medical examination and a baseline blood draw before receiving their assigned vaccine. After monitoring for 30 min for any immediate reactions, participants recorded solicited reactogenicity in electronic diaries for 7 days. Solicited local reactions (injection-site pain, tenderness, swelling, erythema, and induration) and systemic adverse events (pyrexia, arthralgia, chills, diarrhoea, dizziness, headache, malaise, nausea, vomiting, and myalgia) were assessed for severity (see appendix 2 pp 3–4 for severity scales). Participants continued to record the occurrence of any unsolicited adverse events and monitor for featured symptoms of interest—chest pain or shortness of breath—given concerns about a potential association between COVID-19 vaccines and myocarditis or pericarditis at the time of the study.[19] Any serious adverse events were reported immediately to the investigator. Electronic diaries were collected at the second study visit on day 29, at which they were reviewed by study staff to ensure that they had been completed correctly and the investigator assessed the causality of any adverse events. Only data up to day 29 are reported here; safety monitoring up to 12 months is ongoing.

For immunogenicity analyses, serum samples were prepared immediately from blood samples drawn on days 1 and 29 and stored at –20°C before shipping to Labcorp Central Laboratory Services (Indianapolis, IN, USA) for the measurement of neutralising antibodies against Wuhan-Hu-1 and omicron BA.4/5 sublineage SARS-CoV-2 pseudoviruses. Antibody levels were expressed as titres (the reciprocal of the serum dilution that achieved 50% killing of the pseudoviruses) and expressed as group GMTs at days 1 and 29. For this calculation, initially seronegative samples were assigned a value of half the lower limit of quantification, which was a dilution of 1:40. Seroresponse rates were calculated for each group at day 29 and were defined as the proportion of participants showing either a four-fold or greater increase in titre from day 1 to day 29 (in participants who were initially seropositive) or a four-fold higher titre than half the lower limit of quantification (for those who were initially seronegative). The presence of antibodies against the SARS-CoV-2 nucleocapsid protein was also qualitatively determined in serum samples using a commercial test kit (Cica Immunotest SARS-CoV-2 IgG EX; Kanto Chemical, Tokyo, Japan); a positive result was considered to be an indicator of a previous SARS-CoV-2 infection.

Outcomes

The primary objective was to show the non-inferiority of the immune response to ARCT-154 compared with the response to BNT162b2, in terms of both the neutralising antibody GMT and the seroresponse rate against the Wuhan-Hu-1 strain of SARS-CoV-2, 28 days after vaccination. A secondary objective was to assess the non-inferiority of these responses against the SARS-CoV-2 omicron BA.4/5 strain pseudovirus on day 29; if non-inferiority was shown, then the superiority of ARCT-154 over BNT162b2 was evaluated.

Solicited adverse events, and any additional symptoms occurring after vaccine administration up to day 7, and unsolicited adverse events, deaths, serious adverse events, medically significant adverse events (appendix 2 p 5), and adverse events of special interest (specifically myocarditis and pericarditis, which have been associated with mRNA vaccines),[19] were also assessed as secondary endpoints.

Statistical analysis

For the immunogenicity evaluations, the ratio of the neutralising antibody GMTs against the Wuhan-Hu-1 SARS-CoV-2 strain measured 28 days after booster vaccination between the two groups was assumed to be 1·0 with a standard deviation of 0·40, and the seroresponse rate of each group was assumed to be 85%. To obtain a statistical power of 90% to detect the non-inferiority of ARCT-154 compared with BNT162b2 in terms of GMTs and seroresponse rates (GMT non-inferiority margin 0·67, seroresponse rate non-inferiority margin –10%, significance level [one-sided] 2·5%), 270 participants per group were required for analysis. Assuming a 10% dropout rate from immunogenicity analyses would require a total of 600 participants, and assuming that a further 20% of randomly assigned participants would be excluded for being seropositive to the SARS-CoV-2 nucleocapsid before administration of the study vaccine, a total of 780 participants were needed.

The full analysis set consisted of all participants for whom immunogenicity data were available. Primary analyses were conducted in a per-protocol subset (per-protocol set 1) and sensitivity analyses were done in a second subset (per-protocol set 2). Both subsets consisted of participants with no protocol violations related to eligibility criteria, dosage and administration, concomitant drugs or therapies, immunogenicity data, and confirmation of appropriate vaccination on the day of study vaccine administration. Per-protocol set 1 consisted of participants who were seronegative for the nucleocapsid of SARS-CoV-2 before administration of

the study vaccine, indicating no recent (within 9–11 months)[20] SARS-CoV-2 infection; per-protocol set 2 includes both participants who were seropositive and those who were seronegative for the SARS-CoV-2 nucleocapsid. Descriptive analyses of safety endpoints were conducted in the safety set, which consisted of all participants who received a study vaccine and who had safety data and no substantial deviations from Good Clinical Practice standards.

For GMTs, ANCOVA was conducted for the log-transformed neutralising antibody titres against the Wuhan-Hu-1 strain of SARS-CoV-2 on day 29 with the vaccination group as a factor, time since the last COVID-19 vaccination (<5 months or ≥5 months) and gender (as participants identified to study staff) as categorical variables, and age as a continuous variable as covariates. The geometric mean ratio (with 95% CI) of neutralising antibody titres against SARS-CoV-2 at day 29 in the ARCT-154 group compared with the BNT162b2 group was calculated as the exponentiation of the difference of the two means of the logarithmically transformed assay results of the two groups and associated two-sided 95% CI using this ANCOVA model; non-inferiority was confirmed when the lower bound of the inverted 95% CI exceeded 0·67.

The difference in seroresponse rate (with 95% CI) between the ARCT-154 and BNT162b2 groups against the Wuhan-Hu-1 strain of SARS-CoV-2 on day 29 was calculated by the Miettinen-Nurminen method, with randomisation factors (time since the last COVID-19 vaccination [<5 months, ≥5 months]) and gender and age (<65 years or ≥65 years) as adjusting factors; non-inferiority of ARCT-154 compared with BNT162b2 was concluded if the lower limit of the 95% CI exceeded –10%. The same methodology was applied for the comparison of humoral immune responses against the omicron BA.4/5 strain, in which case superiority was concluded if the lower limit of the 95% CI for the anti-log-transformed GMT ratio was greater than 1 and if the lower bound of the 95% CI for the difference in seroresponse rate exceeded 0%.

We also calculated geometric mean fold rises in neutralising antibody titres against both the Wuhan-Hu-1 and omicron BA.4/5 strains of SARS-CoV-2 on day 29 relative to day 1 before vaccine administration, and summary statistics of the fold increase (with 95% CIs) are presented for each group. In addition, we conducted secondary analyses of neutralising antibody titres against the Wuhan-Hu-1 strain and omicron BA.4/5 subvariant in subgroups striated by age category (<65 years *vs* ≥65 years), gender, type of mRNA vaccine previously received, and time since the last COVID-19 vaccination (<5 months *vs* ≥5 months).

Statistical analyses were done using SAS, version 9.4.

There was no independent data monitoring committee. This study was registered on the Japan Registry for Clinical Trials, jRCT 2071220080.

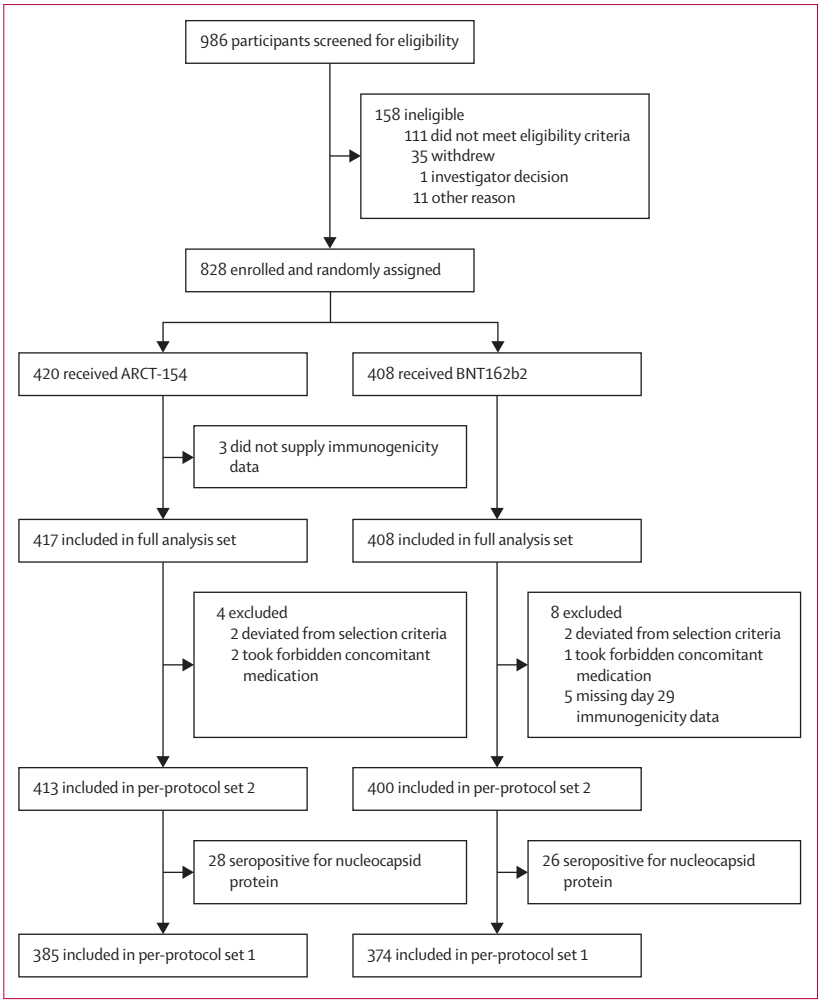

Figure 1: **Trial profile**

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Dec 13, 2022, and Feb 25, 2023, 986 volunteers were screened, of whom 828 were enrolled into the study. 158 were ineligible on screening, mainly because of high blood pressure or a history of COVID-19 with ongoing sequelae within the previous 6 months. The enrolled participants were randomly allocated to the ARCT-154 group (n=420) or the BNT162b2 group (n=408; figure 1). Three participants in the ARCT-154 group did not provide any baseline immunogenicity data, so were excluded from the full analysis set (table 1). The mean age of participants in the full analysis set was 45·7 years (SD 11·8, range 18–77), of whom 485 (59%) were female and 340 (41%) were male. The majority of participants (810 [98%] of 825) had received their last COVID-19

	ARCT-154 (n=417)	BNT162b2 (n=408)	Total (n=825)
Age			
Mean (SD; range), years	45·2 (12·0; 18·0–77·0)	46·2 (11·6; 18·0–76·0)	45·7 (11·8; 18·0–77·0)
<65 years	405 (97%)	400 (98%)	805 (98%)
≥65 years	12 (3%)	8 (2%)	20 (2%)
Gender			
Female	246 (59%)	239 (59%)	485 (59%)
Male	171 (41%)	169 (41%)	340 (41%)
Time since third vaccination			
<5 months	11 (3%)	4 (1%)	15 (2%)
≥5 months	406 (97%)	404 (99%)	810 (98%)
Participants requiring caution in vaccination			
Underlying disease	72 (17%)	62 (15%)	134 (16%)
Previous symptoms indicative of allergic reaction*	90 (22%)	88 (22%)	178 (22%)
History of convulsions	6 (1%)	1 (<1%)	7 (1%)
Neutralising antibodies at baseline			
Wuhan-Hu-1			
Seronegative	5 (1%)	3 (1%)	8 (1%)
Seropositive	412 (99%)	405 (99%)	817 (99%)
Omicron BA.4/5			
Seronegative	84 (20%)	87 (21%)	171 (21%)
Seropositive	333 (80%)	321 (79%)	654 (79%)
SARS-CoV-2 nucleocapsid antibody			
Seronegative	388 (93%)	381 (93%)	769 (93%)
Seropositive	29 (7%)	27 (7%)	56 (7%)
Previous vaccines received			
Three doses of BNT162b2	329 (79%)	329 (81%)	658 (80%)
BNT162b2 + mRNA-1273 + BNT162b2	0	0	0
mRNA-1273 + BNT162b2 + BNT162b2	1 (<1%)	0	1 (<1%)
mRNA-1273 + mRNA-1273 + BNT162b2	87 (21%)	79 (19%)	166 (20%)

Data are n (%) unless otherwise indicated. *Includes previous episodes of pyrexia within 48 h of vaccination.

Table 1: **Baseline demographics of the full analysis set**

vaccination at least 5 months before enrolment; however, 817 (99%) of 825 still had neutralising antibodies against the Wuhan-Hu-1 strain of SARS-CoV-2 before they received the study vaccine, while 654 (79%) had neutralising antibodies against the omicron BA.4/5 subvariant. Relatively few participants—56 (7%) of 825—were seropositive for the SARS-CoV-2 nucleocapsid protein, an indicator of recent infection within the previous 9–11 months. All 825 participants had received BNT162b2 as their third, most recent COVID-19 vaccine. Most participants (658 [80%] of 825) had received the BNT162b2 vaccine exclusively for all three doses; 166 (20%) had received two doses of mRNA-1273 before receiving a third-dose booster of BNT162b2, and one (<1%) received one dose of mRNA-1273 before two doses of BNT162b2.

759 individuals were included in per-protocol set 1 (385 received ARCT-154 and 374 received BNT162b2). Background immunity against the Wuhan-Hu-1 strain of SARS-CoV-2 was evident in both the ARCT-154 and the BNT162b2 groups, with similar baseline GMTs in both groups in per-protocol set 1 (figure 2). At day 29, both groups displayed marked increases in surrogate neutralising antibody GMTs, from 813 (95% CI 716–924) to 5641 (4321–7363) after ARCT-154, a geometric mean fold rise of 6·7 (95% CI 6·0–7·5), and from 866 (755–993) to 3934 (2993–5169) after BNT162b2, a geometric mean fold rise of 4·4 (4·0–4·8). The first primary immunogenicity endpoint, the ratio of surrogate neutralising antibody GMTs at day 29 after ARCT-154 and after BNT162b2, was 1·43 (95% CI 1·26–1·63). The lower limit of the 95% CI exceeded the predefined non-inferiority margin of 0·67, supporting the non-inferiority of the ARCT-154 response (figure 2). Similar analyses of the immune response against the Wuhan-Hu-1 SARS-CoV-2 strain in the full analysis set and in per-protocol set 2 also showed non-inferiority of the ARCT-154 response (appendix 2 p 6).

Seroresponse rates against the Wuhan-Hu-1 strain of SARS-CoV-2 on day 29 were 65·2% (95% CI 60·2–69·9) in the ARCT-154 group and 51·6% (46·4–56·8) in the BNT162b2 group, a difference of 13·6% (95% CI 6·8–20·5). Because the lower limit of the 95% CI exceeded the predefined margin of –10%, the primary objective—the non-inferiority of the immune response to ARCT-154 compared with the response to BNT162b2—was also met, both in terms of GMTs and seroresponse rates.

Regarding secondary endpoints, the GMTs and seroresponse rates against the omicron BA.4/5 SARS-CoV-2 subvariant on day 29 also showed that the response after ARCT-154 was non-inferior to that after BNT162b2 (figure 2). GMTs increased from 275 (95% CI 227–335) on day 1 to 2551 (1687–3859) on day 29 in the ARCT-154 group (geometric mean fold rise of 8·0 [95% CI 7·0–9·1]), and from 292 (236–360) on day 1 to 1958 (1281–2993) on day 29 in the BNT162b2 group (5·7 [5·0–6·4]). The GMT ratio was 1·30 (95% CI 1·07–1·58), in which the lower limit of the 95% CI exceeded the non-inferiority criterion of 0·67, and the difference in seroresponse rate was 11·6% (4·9–18·3), exceeding the –10% non-inferiority criterion. Notably, as the lower limit of the 95% CI for the GMT ratio was greater than 1·0, and the same lower limit for the difference in seroresponse rate was greater than 0%, the criteria for superiority of the ARCT-154 response over the BNT162b2 response were met.

The responses to both the Wuhan-Hu-1 strain and to the omicron BA.4/5 subvariant were not affected by mRNA vaccination history—ie, whether the three previous mRNA vaccinations were all BNT162b2 or some were mRNA-1273 (appendix 2 p 7). In participants who had been previously immunised with three doses of BNT162b2, the GMTs against Wuhan-Hu-1 were 5124 (95% CI 3735–7031; n=306) after ARCT-154 and 3391 (2443–4707; n=298) after a fourth dose of BNT162b2, a GMT ratio of 1·51 (1·31–1·74). In participants who had

previously received two doses of mRNA-1273 and one dose of BNT162b2, the GMT against Wuhan-Hu-1 was 7040 (4270–11608; n=78) after ARCT-154 and 5751 (3534–9357; n=76) after BNT162b2, a GMT ratio of 1·22 (0·94–1·59). Observations for the response against omicron BA.4/5 were similar; after three doses of BNT162b2, the GMTs against omicron BA.4/5 were 2088 (95% CI 1271–3429; n=306) after ARCT-154 and 1479 (884–2473; n=298) after a fourth dose of BNT162b2, a GMT ratio of 1·41 (1·13–1·77). In those previously immunised with two doses of mRNA-1273 and one dose of BNT162b2, GMTs were 4012 (1933–8329; n=78) after ARCT-154 and 3836 (1884–7809; n=298) after BNT162b as fourth doses, a GMT ratio of 1·05 (0·713–1·54).

Non-inferiority of the responses against Wuhan-Hu-1 and omicron BA.4/5 after ARCT-154, compared with the responses after BNT162b2, were also indicated when the groups were further separated according to age category (<65 years *vs* ≥65 years), gender (male *vs* female), or interval since the last COVID-19 booster vaccine before the study vaccine was administered (<5 months *vs* ≥5 months; appendix 2 pp 8–10), although small numbers in some groups (eg, in adults ≥65 years) prevented a statistical demonstration of non-inferiority.

All participants who received a study vaccine according to the randomisation were included in the safety set (table 2). Both the ARCT-154 and the BNT162b2 vaccines were well tolerated as booster doses. Up to day 29, the interim cut-off presented in this analysis, no deaths, adverse events of special interest, or medically significant adverse events were reported (table 2). One serious adverse event, reported in a participant in the BNT162b2 group, was described as a foot deformity, and the investigators did not consider it to have any causal relationship to the study vaccination. No reports of solicited local reactions or of systemic adverse events described as grade 4 or life-threatening were received. Solicited local reactions were reported by 398 (95%) of 420 participants who received the ARCT-154 vaccine and 395 (97%) of 408 participants who received the BNT162b2 vaccine (table 2). Most reported local reactions were transient, starting within 1 day or 2 days of vaccination and resolving by day 4 (appendix 2 p 11), and were described as mild or moderate in severity. Most of these reactions in both groups were mild pain or tenderness at the injection site (figure 3). Seven participants reported severe local reactions: three recipients of the ARCT-154 vaccine reported four severe local reactions (one each of severe pain, tenderness, swelling, and induration at the injection site) and four recipients of the BNT162b2 vaccine reported five severe local reactions (three cases of severe erythema and one each of swelling and tenderness at the injection site). Swelling, erythema, and induration were reported less frequently than pain or tenderness, but all three reactions were more frequent after the BNT162b2 vaccine (17%, 12%, and 12%, respectively) than after the ARCT-154 vaccine (8%, 6%, and 5%, respectively).

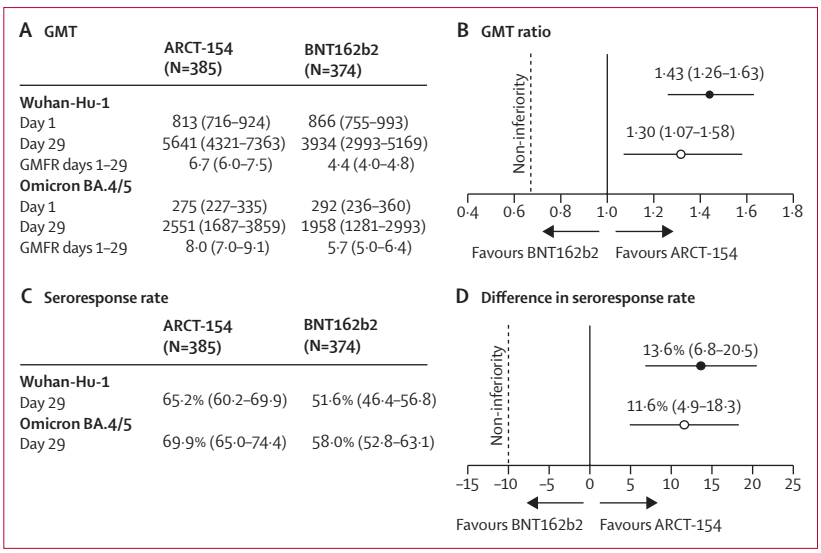

Figure 2: **Surrogate neutralising antibody titres and seroresponse rates**
(A) GMTs of surrogate neutralising antibodies at day 1 (baseline) and day 29, and GMFRs in titres from day 1 to day 29. (B) GMT ratio. (C) Seroresponse rates at day 29. (D) Seroresponse difference. All data are from per-protocol set 1 and are shown with 95% CIs in parentheses. Solid circles represent the Wuhan-Hu-1 variant of SARS-CoV-2, open circles represent the omicron BA.4/5 variant. Vertical dashed lines represent the threshold for non-inferiority. GMT=geometric mean titre. GMFR=geometric mean fold rise.

	ARCT-154 (n=420)	BNT162b2 (n=408)
Any adverse event	402 (96%); 1900	399 (98%); 1910
Solicited local reactions	398 (95%)	395 (97%)
Solicited systemic adverse events	276 (66%)	255 (63%)
Severe adverse events	10 (2%); 18	16 (4%); 30
Unrelated	0	6 (1%); 6
Related	10 (2%); 18	10 (3%); 24
Unsolicited adverse events (day 1–29)	81 (19%)	111 (27%)
Unrelated	26 (6%)	43 (11%)
Related	55 (13%)	68 (17%)
Serious adverse events	0	1 (<1%); 1
Unrelated	0	1 (<1%)
Related	0	0
Medically attended adverse events	0	0
Adverse events of special interest	0	0
Symptoms of interest	2 (<1%)	4 (1%)
Chest pain	2 (<1%)	3 (1%)
Unrelated	1 (<1%)	0
Related	1 (<1%)	3 (1%)
Shortness of breath	0	2 (<1%)
Unrelated	0	0
Related	0	2 (<1%)
Death	0	0

Data are n (%) or n (%); events

Table 2: **Adverse events in the safety population (day 1–29)**

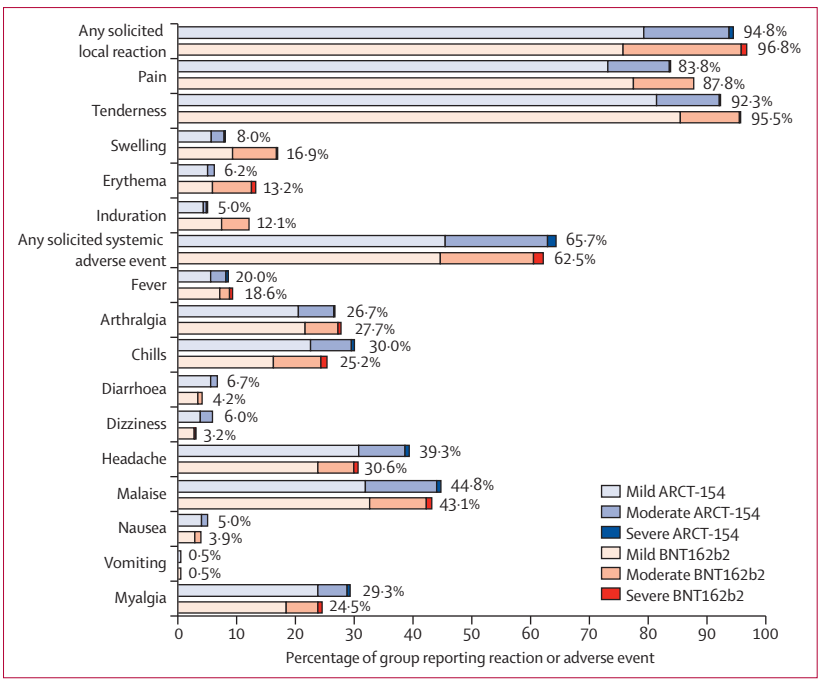

Figure 3: **Rates of solicited local reactions and systemic adverse events and their severity in the ARCT-154 and BNT162b2 groups**

Solicited systemic adverse events were reported by 276 (66%) of 420 participants who received the ARCT-154 vaccine and 255 (63%) of 408 participants who received the BNT162b2 vaccine (table 2). The most frequent was malaise, reported by 188 (45%) participants in the ARCT-154 group and 176 (43%) in the BNT162b2 group, followed by headache (165 [39%] ARCT-154; 125 [31%] BNT162b2), chills (126 [30%] ARCT-154; 103 [25%] BNT162b2), myalgia (123 [29%] ARCT-154; 100 [25%] BNT162b2), and arthralgia (112 [27%] ARCT-154; 113 [28%] BNT162b2). As with the local reactions, solicited systemic adverse events were mainly mild and transient, starting and resolving within 1–3 days after vaccination (figure 3; appendix 2 p 12).

Unsolicited adverse events during the post-vaccination period (up to day 29) were reported by 81 (19%) of 420 participants who received the ARCT-154 vaccine (considered by the investigators to be causally related to vaccination in 55 [13%]) and by 111 (27%) of 408 participants who received the BNT162b2 vaccine (considered causally related in 68 [17%]; table 2). The frequencies of disorders listed according to their classification in the Medical Dictionary for Regulatory Activities are shown in the appendix 2 (p 13). The majority of the reported unsolicited adverse events were mild or moderate, but seven were described as severe (grade 3): one in the ARCT-154 group (a case of abnormal hepatic function, assessed as related to the investigative vaccine by the investigators) and six in the BNT162b2 group (three cases of nasopharyngitis and one case each of pyrexia, ankle fracture, and foot deformity, none of which were considered to be related to the vaccine).

Occurrences of the symptoms of interest, chest pain and shortness of breath, between days 1 and 7 were specifically monitored as indicators of potential myocarditis and pericarditis. Two recipients of the ARCT-154 vaccine reported chest pain (table 2): one within 1 day of vaccination, which the respective investigator considered to be related to the vaccine, and the other 6 days after vaccination, which was considered unrelated. Four recipients of the BNT162b2 vaccine reported five symptoms of interest: three cases of chest pain occurring within 1–3 days of vaccination and two cases of shortness of breath occurring within 1 day of vaccination, all of which were considered to be related to vaccination. On clinical follow-up a median of 3·5 days (range 2–16 days) after onset of the symptoms, no indication of myocarditis or pericarditis was detected in participants in either vaccine group.

Discussion

In healthy adults who had previously been immunised with three doses of mRNA COVID-19 vaccines, we observed that a booster dose of an saRNA COVID-19 vaccine, ARCT-154, elicited immune responses against the Wuhan-Hu-1 strain of SARS-CoV-2 that were non-inferior to those elicited by the BNT162b2 mRNA vaccine. Against the omicron BA.4/5 variant, the ARCT-154 response was superior to the BNT162b2 response according to our criteria. Furthermore, both the ARCT-154 and BNT162b2 vaccines were reasonably well tolerated in this population, with no reports of deaths, serious adverse events, or medically significant adverse events that were causally associated with vaccination.

For some COVID-19 vaccines, heterologous boosting has been shown to improve both the magnitude and the breadth of the immune response when compared with homologous boosters;[7,21,22] however, this has not been the case for mRNA vaccines, for which the response to a homologous mRNA booster is usually superior to the response to a heterologous non-mRNA booster.[10–12] The waning of neutralising antibodies induced by mRNA vaccines has been associated with increased susceptibility to infection with the omicron (B.1.1.529) variant of SARS-CoV-2.[23] A systematic meta-analysis found that the effectiveness of mRNA vaccines against the omicron variant wanes to less than 20% within 6 months of primary immunisation and to less than 30% within 9 months of a booster dose.[24] The ongoing evolution of SARS-CoV-2 and the emergence of new variants that might be less susceptible to the immunity induced by current mRNA vaccines will require future immunisation campaigns to consider updated strategies, potentially including annual boosters with different and new vaccines to enhance the breadth of the immune response and minimise vaccine evasion. The saRNA vaccine investigated in this study can self-generate the SARS-CoV-2 spike protein, providing a similar magnitude and

an extended duration of antigen expression at a lower vaccine load than an equivalent mRNA vaccine.

Although antibody titres were high after boosting in this study, the observed seroconversion rates were low: 65% for ARCT-154 and 52% for BNT162b2. These seroconversion rates were probably due to the high level of immunity at baseline following previous immunisations, making a four-fold increase more difficult to achieve. Nevertheless, this saRNA platform appears to induce non-inferior, and for the omicron BA.4/5 subvariant, superior, heterologous responses compared with homologous boosting with an mRNA vaccine—a characteristic that is highly desirable in a period of continued viral evolution. Similar immune responses were observed across subgroups, including in men and women and in older individuals (≥65 years), although the low numbers of participants in some groups (eg, only 19 participants were aged ≥65 years) mean that caution should be exercised in the interpretation of these data (appendix 2 pp 5–7). However, in a small phase 1 trial reported in 2023, another saRNA vaccine elicited neutralising antibodies and T-cell responses against SARS-CoV-2 D614G strain and beta, delta, and omicron variants in older adults (aged ≥60 years) who had previously been immunised with the adenovirus-vectored ChAdOx1 nCoV-19 vaccine.[25] Furthermore, this boost in immunity was shown to persist for at least 6 months.

The immunogenicity of the ARCT-154 vaccine was not accompanied by any observed differences in safety or reactogenicity profile compared with the BNT162b2 mRNA vaccine. This finding is consistent with data from the major phase 1/2/3/3b study of ARCT-154 (NCT05012943), which showed a vaccine safety profile similar to that of the saline placebo.[18] Both ARCT-154 and BNT162b2 were well tolerated with mainly mild-to-moderate solicited adverse events, similar to those reported in previous studies of mRNA vaccine boosters.[21,22] Safety observations are ongoing and are intended to follow up participants for 12 months after vaccination. As mRNA COVID-19 vaccines have been associated with cases of myocarditis in young adults,[19] we monitored for chest pain and shortness of breath in the 7 days after vaccination as indicators of myocarditis and pericarditis. Six participants, two who received the ARCT-154 vaccine and four who received the BNT162b2 vaccine, reported one or both symptoms. Although one case in the ARCT-154 group and all four cases in the BNT162b2 group were considered to be related to the study vaccines, myocarditis and pericarditis were excluded after evaluation by the investigators or a cardiologist.

Our study has some limitations, notably the low numbers of older participants, which limits analysis of the response in older people and a further assessment will be required in this population. Although immunogenicity was assessed by measuring surrogate neutralising antibodies against both the Wuhan-Hu-1 strain of the SARS-CoV-2 virus and the omicron BA.4/5 subvariant, protective responses against the currently globally dominant variants (omicron XBB subvariants EG.5.1 and BA.2.86[26]) and future variants will still need to be assessed, and such studies are currently being planned. This assessment monitored safety and immunogenicity only up to day 29, but the study is ongoing and is continuing to collect safety data; it will also assess the durability of the immune response at 3, 6, and 12 months after vaccination and investigate cellular immunity.

The development of mRNA vaccines against COVID-19 has been a success; however, new technologies—such as saRNA—could help to further reduce the burden of disease. Our data support the further investigation of new saRNA vaccines based on the sequences of more recent SARS-CoV-2 variants to establish whether self-amplification increases the immune response and could potentially enhance the duration of protection.

Contributors

YO, YK, MK, JLW, BG, and YZ conceived and designed the study and contributed to the writing of the protocol. YO, MK, TM, and IO contributed to study oversight. YI designed the statistical aspects of the study and analysed the results. YY was responsible for project administration. TK acquired funding. All authors contributed to the interpretation of the results. JLW led the writing of the initial drafts of the manuscript, on which all authors commented, and all authors had final responsibility for the decision to submit for publication. YO, YY, and JLW verified the data, and all authors had access to the raw data.

Declaration of interests

YO, MK, YI, IO, TM, and YY are full-time employees of, and TK is a board member of, the study sponsor Meiji Seika Pharma. YK received fees from Meiji Seika Pharma for medical consultation during this study. BG and YZ are full-time employees of Arcturus Therapeutics, which developed the vaccine, and JLW is an independent consultant working for Arcturus Therapeutics.

Data sharing

After the final study report is prepared, including the 12-month safety follow-up period, the data generated in this study will be made available to suitably qualified scientific researchers who make a request to the study sponsor with a suitable protocol for a valid research project.

Acknowledgments

We thank all participants for volunteering and all study staff and the local investigators (Takashi Eto [Hakata CL, Fukuoka, Japan], Kenji Takazawa [Shinanozaka CL, Shinjuku, Japan], Hiroaki Kondo [Higashi-Shinanozaka CL, Shinjuku, Japan], Kenichi Furihata [P-One CL, Hachioji, Japan], Hidetoshi Furuie [Osaka Pharmacology Clinical Research Hospital, Osaka, Japan], Osamu Matsuoka [ToCROM, Shinjuku, Japan], Shinya Mitsui [Shin-Sapporo HP, Sapporo, Japan], Yuki Sekiguchi [LUNA, Yokohama, Japan], Shokei Kim-Mitsuyama [Medimesse Sakurajyuji, Kumamoto, Japan], Masao Kobayakawa [Fukishima Medical HP, Fukushima, Japan], and Toshio Naito [Juntendo, Bunkyo, Japan]) for their contributions to the study. We are grateful to Keith Veitch (keithveitch communications, Amsterdam, the Netherlands) for his editorial assistance in the preparation of the manuscript, and to Jonathan Edelman (CSL Seqirus, Summit, NJ, USA) and Igor Smolenov (Arcturus Therapeutics, San Diego, CA, USA) for their critical review of the manuscript.

References

1 Araf Y, Akter F, Tang YD, et al. Omicron variant of SARS-CoV-2: genomics, transmissibility, and responses to current COVID-19 vaccines. *J Med Virol* 2022; **94:** 1825–32.

2 Khoury J, Najjar-Debbi R, Hanna A, et al. COVID-19 vaccine—long term immune decline and breakthrough infections. *Vaccine* 2021; **39:** 6984–89.

3 Ai J, Zhang H, Zhang Y, et al. Omicron variant showed lower neutralizing sensitivity than other SARS-CoV-2 variants to immune sera elicited by vaccines after boost. *Emerg Microbes Infect* 2022; **11:** 337–43.

4 Jung C, Kmiec D, Koepke L, et al. Omicron: what makes the latest SARS-CoV-2 variant of concern so concerning? *J Virol* 2022; **96:** e0207721.

5 Adams K, Rhoads JP, Surie D, et al. Vaccine effectiveness of primary series and booster doses against COVID-19 associated hospital admissions in the United States: living test negative design study. *BMJ* 2022; **379:** e072065.

6 Rubin R. Updated COVID-19 vaccine now available in US, recommended for everyone older than 6 months. *JAMA* 2023; **330:** 1420.

7 Au WY, Cheung PP-H. Effectiveness of heterologous and homologous COVID-19 vaccine regimens: living systematic review with network meta-analysis. *BMJ* 2022; **377:** e069989.

8 Andrews N, Stowe J, Kirsebom F, et al. Covid-19 vaccine effectiveness against the omicron (B.1.1.529) variant. *N Engl J Med* 2022; **386:** 1532–46.

9 Andrejko KL, Pry JM, Myers JF, et al. Waning of 2-dose BNT162b2 and mRNA-1273 vaccine effectiveness against symptomatic SARS-CoV-2 infection accounting for depletion-of-susceptibles bias. *Am J Epidemiol* 2023; **192:** 895–907.

10 Atmar RL, Lyke KE, Deming ME, et al. Homologous and heterologous COVID-19 booster vaccinations. *N Engl J Med* 2022; **386:** 1046–57.

11 Zhang B, Huo J, Huang Y, et al. mRNA booster vaccination enhances antibody responses against SARS-CoV2 omicron variant in individuals primed with mRNA or inactivated virus vaccines. *Vaccines (Basel)* 2022; **10:** 1057.

12 Poh XY, Tan CW, Lee IR, et al. Antibody response of heterologous vs homologous messenger RNA vaccine boosters against the severe acute respiratory syndrome coronavirus 2 omicron variant: interim results from the PRIBIVAC study, a randomized clinical trial. *Clin Infect Dis* 2022; **75:** 2088–96.

13 Davis-Gardner ME, Lai L, Wali B, et al. Neutralization against BA.2.75.2, BQ.1.1, and XBB from mRNA bivalent booster. *N Engl J Med* 2023; **388:** 183–85.

14 Lundstrom K. Self-replicating RNA viruses for vaccine development against infectious diseases and cancer. *Vaccines (Basel)* 2021; **9:** 1187.

15 Vogel AB, Lambert L, Kinnear E, et al. Self-amplifying RNA vaccines give equivalent protection against influenza to mRNA vaccines but at much lower doses. *Mol Ther* 2018; **26:** 446–55.

16 Bloom K, van den Berg F, Arbuthnot P. Self-amplifying RNA vaccines for infectious diseases. *Gene Ther* 2021; **28:** 117–29.

17 Korber B, Fischer WM, Gnanakaran S, et al. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. *Cell* 2020; **182:** 812–827.e19.

18 Nguyen X-H, Hô N, Hughes S, et al. Safety and immunogenicity and efficacy of the self-amplifying mRNA ARCT-154 COVID-19 vaccine. *Res Sq* 2023; published online Sept 20. https://doi.org/10.21203/rs.3.rs-3329097/v1 (preprint).

19 Alami A, Krewski D, Farhat N, et al. Risk of myocarditis and pericarditis in mRNA COVID-19-vaccinated and unvaccinated populations: a systematic review and meta-analysis. *BMJ Open* 2023; **13**: e0656.

20 Van Elslande J, Oyaert M, Lorent N, et al. Lower persistence of anti-nucleocapsid compared to anti-spike antibodies up to one year after SARS-CoV-2 infection. *Diagn Microbiol Infect Dis* 2022; **103:** 115659.

21 Munro APS, Feng S, Janani L, et al. Safety, immunogenicity, and reactogenicity of BNT162b2 and mRNA-1273 COVID-19 vaccines given as fourth-dose boosters following two doses of ChAdOx1 nCoV-19 or BNT162b2 and a third dose of BNT162b2 (COV-BOOST): a multicentre, blinded, phase 2, randomised trial. *Lancet Infect Dis* 2022; **22:** 1131–41.

22 Naito T, Tsuchida N, Kusunoki S, et al. Reactogenicity and immunogenicity of BNT162b2 or mRNA-1273 COVID-19 booster vaccinations after two doses of BNT162b2 among healthcare workers in Japan: a prospective observational study. *Expert Rev Vaccines* 2022; **21:** 1319–29.

23 Gilboa M, Regev-Yochay G, Mandelboim M, et al. Durability of immune response after COVID-19 booster vaccination and association with COVID-19 omicron infection. *JAMA Netw Open* 2022; **5:** e2231778.

24 Menegale F, Manica M, Zardini A, et al. Evaluation of waning SARS-CoV-2 vaccine-induced immunity: a systematic review and meta-analysis. *JAMA Netw Open* 2023; **6:** e2310650.

25 Palmer CD, Scallan CD, Kraemer Tardif LD, et al. GRT-R910: a self-amplifying mRNA SARS-CoV-2 vaccine boosts immunity for ≥6 months in previously-vaccinated older adults. *Nat Commun* 2023; **14:** 3274.

26 European Centre for Disease Prevention and Control. ECDC classifies XBB.1.5-like lineages with the amino acid change F456L as variants of interest following an increase in SARS-CoV-2 transmission in EU/EEA countries and abroad. Aug 17, 2023. https://www.ecdc.europa.eu/en/news-events/ecdc-classifies-xbb15-lineages-amino-acid-change-f456l-variants-interest-following (accessed Sept 23, 2023).