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Towards robust immune responses after heterologous COVID-19 vaccination and its application perspectives

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The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1], has seriously impacted the global health and economy. Effective vaccination, with homologous or heterologous prime-boost strategies, is the key to controlling the ongoing COVID-19 pandemic [2]. Homologous vaccinations, which are the administration of the same type of COVID-19 vaccine, have been shown to be highly efficient in inducing robust immune responses [3–7]. Due to safety concerns regarding the rare cases of vaccine-induced immune thrombotic thrombocytopenia after the first dose of the ChAdOx1 nCov-19 (AZD1222) vaccine [8], heterologous booster vaccination was assessed and approved in some European countries. There are limited data on the immune responses induced by heterologous vaccination regimens. We summarize the latest data on the immunogenicity, reactogenicity, and neutralizing activity of heterologous COVID-19 vaccinations against variants of concern (VoCs); we further discuss the distinct advantages of their application and critical factors that may influence their efficiency.

Currently, different countries have approved multiple types of COVID-19 vaccines, including mRNA-based vaccines (BNT162b2/BNT and mRNA-1273), adenovirus-vectored vaccines (ChAdOx1 nCov-19/ChAd, Janssen Ad26.COV2.S, and Gam-COVID-Vac), inactivated virus vaccines (Sinovac-CoronaVac and Sinopharm-BBIBP-CorV), and protein subunit vaccines (ZF2001) [2,9,10]. Some of these vaccines have shown high efficacy and safety profiles. The large-scale use of some COVID-19 vaccines has led to the emergence of critical challenges, such as vaccine supply shortage and safety concerns. Therefore, in some countries, the use of homologous vaccinations has been restricted, and heterologous booster vaccinations with vaccines, such as BNT and ZF2001, have been considered. In this alternative vaccination strategy, mRNA or protein subunit vaccines may be used as a booster against COVID-19 to replace the homologous booster immunization with adenovirus-vectored, inactivated virus, or other vaccines.

Humoral immune responses after a heterologous vaccination

Although a heterologous vaccination is a promising alternative strategy, its immunogenicity and re-

actogenicity remain largely unknown. To date, there are limited studies comparing immune responses to homologous and heterologous vaccination schedules in humans (Table 1). In February 2021, Logunov *et al.* [3] reported the interim clinical efficacy results of a heterologous prime-boost vaccination regimen using the rAd26- and rAd5-vectored COVID-19 vaccine Gam-COVID-Vac separately in a randomized, double-blind, placebo-controlled multicentre study. The study found that this rAd26/rAd5 vector-based heterologous vaccination induced robust humoral and cellular immune responses with good safety profiles and had a 91.6% protective efficacy against COVID-19. Homologous immunizations with the ChAd or BNT vaccines have been evaluated in American and European countries. Two observational studies of heterologous prime-boost vaccination regimens, incorporating ChAd and BNT vaccines, have been conducted in healthy adult individuals in Germany [4,5]. In late July 2021, Tenbusch *et al.* [5] reported the vaccine-induced antibody responses in healthcare workers or volunteers who received homologous or heterologous vaccinations using ChAd and BNT as a prime and/or boost vaccine in Germany. Interestingly, the heterologous ChAd/BNT vaccination at a 10-week interval induced a significantly higher surrogate neutralization activity than homologous ChAd/ChAd (at a 10-week interval) or homologous BNT/BNT (at a 3-week interval) vaccination. In September 2021, Schmidt *et al.* [4] reported that a heterologous ChAd/BNT vaccination (10-week interval) induced higher T-cell responses than a homologous BNT/BNT vaccination (3-week interval); nevertheless, the two vaccination strategies yielded similar levels of anti-spike (S) IgG and neutralizing antibodies, consistent with the findings of Tenbusch *et al.* [5]. Moreover, two critical clinical trials, with 1,506 participants from Spain and the UK, were conducted to study the immunogenicity and reactogenicity of heterologous COVID-19 vaccinations [6,7]. Borobia *et al.* [6] conducted the CombiVacS study, a multicentre, open-label, randomized, controlled phase 2 trial in Spain. The study demonstrated significant increases in SARS-CoV-2 anti-S IgG and neutralizing antibody levels and T-cell responses in the ChAd-primed, BNT-boosted participants compared with those in participants not administered a BNT booster, which indicated that humoral immune responses increased following a BNT booster vaccination. However, this study lacked the data regarding the homologous ChAd/ChAd vaccination comparator. Liu *et al.* [7] also observed in their single-blind, randomized, noninferiority trial that heterologous ChAd/BNT and homologous BNT/BNT vaccinations induced higher immunogenicity than a homologous ChAd/ChAd vaccination did. The participants who were vaccinated with ChAd/BNT or BNT/BNT had similar levels of anti-S IgG and neutralizing antibodies, while T-cell responses were higher in the ChAd-primed, BNT-boosted participants. Although both heterologous vaccinations (ChAd/BNT and BNT/ChAd) led to higher levels of anti-S IgG than that induced by the homologous ChAd/ChAd vaccination comparator, the BNT/ChAd regimen did not meet the noninferiority criteria [8]. Collectively, accumulating evidence suggests that heterologous COVID-19 vaccinations with ChAd and BNT boost the vaccine's effectiveness by triggering stronger or broader immune responses.

Recently, the rapid worldwide spread of the B.1.617.2 (Delta) variant has partially nullified the protection effectiveness afforded by COVID-19 vaccines, suggesting that booster vaccination would be beneficial for combating emerging VoCs. Keskin *et al.* designed a small clinical trial to investigate the differences of in antibody titres to S and nucleocapsid (N) proteins in 45 healthcare workers who already received two doses of an inactivated virus vaccine (2IVV) and were administered a third dose of the CoronaVac (3IVV) or BNT (2IVV+BNT) vaccines in Turkey [11]. They found that the levels of anti-S and anti-N IgG antibodies in the 2IVV+BNT group were significantly higher than those in the 3IVV group. Notably, two recent clinical

Table 1 Comparison of humoral immune responses after heterologous COVID-19 vaccinations

Source	Country	Design	Samples (*N*)	Age (years)	Male *N* (%)	Interval of boost	Anti-S protein Ab	Neutralizing Ab	T cell response
Logunov *et al.* [3]	Russia	Randomized, double-blind, placebo-controlled, phase 3 trial	21,977	45.3	12,158 (61.2)	21 days	rAd26/rAd5 > Placebo	rAd26/rAd5 > Placebo	rAd26/rAd5 > Placebo
Schmidt *et al.* [4]	Germany	Observational study	216	40.8	64 (43.0)	10–12 weeks	ChAd/BNT > ChAd/ChAd; ChAd/BNT ≈ BNT/BNT	ChAd/BNT > ChAd/ChAd; ChAd/BNT > BNT/BNT	ChAd/BNT > ChAd/ChAd; ChAd/BNT > BNT/BNT
Tenbusch *et al.* [5]	Germany	Observational study	1085	38–57	354 (32.6)	10–12 weeks	–	ChAd/BNT > ChAd/ChAd; ChAd/BNT > BNT/BNT; BNT/BNT > ChAd/ChAd	–
Borobia *et al.* [6]	Spain	Randomized controlled phase 2 trial	676	43.98	294 (43.0)	8–12 weeks	ChAd/BNT > ChAd	ChAd/BNT > ChAd	ChAd/BNT > ChAd
Liu *et al.* [7]	UK	Randomized, participant-blind trial	830	57.8	450 (54.2)	28 or 84 days	ChAd/BNT ≈ ChAd/ ChAd; ChAd/BNT ≈ BNT/BNT; BNT/ChAd < BNT/ BNT	ChAd/BNT > ChAd/ChAd; ChAd/BNT ≈ BNT/BNT; BNT/ChAd < BNT/ BNT	ChAd/BNT > ChAd/ ChAd ChAd/BNT > BNT/BNT; BNT/ChAd ≈ BNT/ BNT
Cao *et al.* [9]	China	A single-center, open-label, randomized controlled clinical trial	164	~40	47 (28.6)	4 weeks/ 4–8 months	2IVV/ZNF2001 > 3IVV	2IVV+ZNF2001 >3IVV	–
Ai *et al.* [10]	China	A single center prospective, open-label, randomized ontrolled clinical trial	122	24–52	53 (43.4)	4 weeks/ 4–8 months	2IVV/ZNF2001 > 3IVV	2IVV+ZNF2001 >3IVV	2IVV+ZNF2001 >3IVV
Keskin *et al.* [11]	Turkey	Observational study	45	~41	–	6 months	2IVV/BNT > 3IVV	2IVV/BNT > 3IVV	–

Abbreviations: Ab, antibody; BNT, BNT162b2 vaccine; BNT/BNT: homologous vaccination with BNT as both prime and booster; BNT/ChAd: prime with BNT followed by a ChAdOx1 booster; ChAd, ChAdOx1 COVID-19 vaccine; ChAd/BNT: prime with ChAd followed by a BNT booster; ChAd/ChAd: homologous vaccination with ChAd as both prime and booster; IVV, inactivated whole virus vaccine; PBMCs, peripheral blood mononuclear cells; S, spike protein; 2IVV, two-dose inactivated whole virus vaccine; 2IVV+BNT, two-dose inactivated whole virus vaccine followed by a third BNT booster; 3IVV, three times inactivated whole virus vaccine.

studies separately, conducted by Cao *et al.* [9] and Ai *et al.* [10], evaluated the safety and immunogenicity of a heterologous third booster dose with the recombinant protein subunit vaccine ZF2001 primed with two doses of IVVs (CoronaVac or BBIBP-CorV) in China. Both studies showed that the third booster dose of ZF2001 was well tolerated and could induce a significantly high humoral immunogenicity in adult recipients. Therefore, these studies indicate that a heterologous third booster dose induces a much stronger B-cell recall response than its homologous counterpart.

To date, there are limited data regarding the neutralization capacities of heterologous vaccination regimens against different emerging VoCs [12], including B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma, formerly B.1.1.28.1), and B.1.617.2 (Delta) variants. Barros-Martins *et al.* [13] reported that the heterologous ChAd/BNT vaccination elicited significantly higher neutralizing antibody titres against the B.1.1.7, B.1.351, and P.1 variants than homologous ChAd/ChAd vaccination. Interestingly, their subsequent study further found that the homologous BNT/BNT vaccination more efficiently induced neutralizing antibodies against the B.1.617.2 variant than the heterologous ChAd/BNT vaccination. However, the heterologous ChAd/BNT vaccination also supported strong inhibition of the B.1.617.2 variant by inducing robust neutralizing antibodies. Recently, two independent clinical studies reported that a heterologous third booster dose with ZF2001 was more efficient than a homologous third booster dose with IVVs in terms of inducing humoral immunity against different SARS-CoV-2 variants, including the Delta strain [9,10]. Heterologous prime-boost COVID-19 vaccination as well as additional heterologous vaccine doses might be a suitable strategy to combat emerging VoCs. Therefore, there is an urgent need for more detailed studies to evaluate and compare the immunogenicity of heterologous and homologous COVID-19 vaccinations against emerging VoCs.

Distinct advantages of heterologous vaccinations

Given the ongoing COVID-19 pandemic, heterologous COVID-19 vaccination may have distinct advantages. First, mRNA vaccines may serve as an alternative heterologous booster for the ChAd vaccine to overcome their safety concerns. Second, heterologous vaccinations may boost more durable and robust humoral and cellular immune responses than those induced by already authorized homologous vaccinations and greatly contribute to effective control of emerging or future VoCs [4,6,10]. Finally, as a feasible and reasonable vaccination strategy, heterologous vaccinations also have the potential to compensate for vaccine shortfalls and accelerate the worldwide vaccine rollout, especially in low- and middle-income countries.

Factors influencing the efficiency of heterologous vaccinations

Some of the critical factors that may influence the high efficiency of heterologous COVID-19 vaccinations are as follows: 1) the vaccine types used in the heterologous prime-boost schedules [9–11]; 2) the order of candidate vaccines in the prime-boost process of a heterologous vaccination regimen [7]; and 3) the differences in time interval between doses of the vaccines in heterologous vaccinations [14].

Overall, accumulating evidence supports the fact that certain heterologous COVID-19 vaccination regimens have good safety profiles and represent a feasible and promising vaccination strategy to boost durable

and robust humoral and cellular immune responses and effectively control the emerging VoCs [4,6,10]. Notably, a feasible heterologous vaccination is a powerful weapon against the ongoing COVID-19 pandemic. Further studies are needed to confirm the clinical effectiveness of heterologous vaccinations against emerging VoCs and optimize the combinations, orders, doses, and intervals of heterologous prime-boost vaccination schedules.

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Author contributions

AH conceived this article; YL wrote the original draft and prepared the table; YL and AH edited and reviewed the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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