Head-to-head analysis of immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19-priming and BNT162b2 or mRNA-1273-boosting with homologous COVID-19 vaccine regimens

Martina Sester (martina.sester@uks.eu)
Saarland University https://orcid.org/0000-0001-5482-0002
Verena Klemis
Saarland University
Tina Schmidt
Saarland University https://orcid.org/0000-0001-7929-5283
David Schub
Department of Transplant and Infection Immunology, Saarland University, 66421 Homburg, Germany
https://orcid.org/0000-0002-8995-3080
Janine Mihm
Department of Internal Medicine IV, Saarland University
Stefanie Marx
Saarland University
Amina Abu-Omar
Saarland University
Laura Ziegler
Saarland University
Franziska Hielscher
Saarland University
Candida Guckelmus
Saarland University
Rebecca Urschel
Saarland University
Stefan Wagenpfeil
Saarland University
Sophie Schneitler
Saarland University
Soeren Becker
Center of Infectious disease, Institute of Medical Microbiology and Hygiene, University of Saarland
Barbara Gärtner

Institute of Medical Microbiology and Hygiene, Saarland University

Urban Sester

Saarland University https://orcid.org/0000-0003-4007-5595

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Abstract

Head-to-head analyses of immunogenicity and reactogenicity between the authorized homologous vaccine-regimens and heterologous combinations thereof are currently limited. Using a convenience cohort of 331 healthy individuals, we show that humoral and cellular immunity after vaccination with ChAdOx1-nCoV-19 followed by either BNT162b2 (n=66) or mRNA-1273 (n=101) is equivalent or superior to homologous mRNA-regimens (n=43 BNT162b2, n=59 mRNA-1273), and more pronounced than after homologous ChAdOx1-nCoV-19 vaccination (n=62). Levels of spike-specific CD8 T cells were highest in both heterologous regimens, and significantly higher than all three homologous combinations. Among mRNA-containing combinations, spike-specific CD4 T-cell levels in regimens including mRNA-1273 were higher than respective combinations with BNT162b2. Polyfunctional T-cell levels were highest in regimens based on ChAdOx1-nCoV-19-priming. All five regimens were well tolerated with most pronounced reactogenicity upon ChAdOx1-nCoV-19-priming and mRNA-1273-boosting. In conclusion, immunogenicity after heterologous vector/mRNA-boosting and homologous mRNA-regimens is superior to homologous vector-regimens with notable differences between mRNA vaccines.

Introduction

Three COVID-19 vaccines, the ChAdOx1 nCoV-19 vector-based vaccine (ChAdOx) and the two mRNA vaccines BNT162b2 (BNT) or mRNA-1273, are authorized as homologous dual dose regimens and are widely used in Europe and the USA. In addition, heterologous combinations of vector vaccines followed by boosting with either of the two mRNA vaccines are recommended in some parts of Europe including Germany[1,2].

We and others have recently shown that the heterologous ChAdOx/BNT mRNA vaccine combination elicited similar antibody- and CD4 T-cell levels as the homologous BNT regimen, and both regimens were superior to homologous ChAdOx vaccination[3–7]. Moreover, SARS-CoV-2 specific CD8 T-cell levels after heterologous vaccination were significantly higher than the homologous regimens including either ChAdOx or BNT. Finally, antibodies elicited after heterologous ChAdOx/BNT were shown to have neutralizing activity against the SARS-CoV-2 wild type as well as variants of concern including Delta[8,9].

All homologous vaccine combinations have shown remarkable ability to prevent SARS-CoV-2 infection and COVID-19 disease[10–12], with differences in efficacy and effectiveness between the vaccines, especially between vector-based and mRNA-based compounds. Whether this is associated with differences in immunogenicity is poorly studied due to the lack of head-to-head studies and absence of standardized assays to estimate correlates of protection. Up to now, studies on heterologous vaccine combinations have largely focused on combinations including BNT[3–7]. One study has shown that heterologous boosting with mRNA-1273 after ChAdOx-priming induced higher levels of antibodies than the homologous vector regimen, but cellular immunity induced by this combination was not assessed in parallel[13]. Together this suggests that heterologous mix and match regimens offer similar or superior immunogenicity as homologous mRNA regimens, but head-to-head analyses on all homologous and

heterologous combinations of authorized dual vaccine combinations are currently lacking. We therefore prospectively enrolled a convenience cohort of immunocompetent individuals to study immunogenicity and reactogenicity of the three homologous and two heterologous combinations of authorized vector and mRNA vaccines. This included analyses of SARS-CoV-2 specific antibodies and neutralizing capacity as well as specific CD4 and CD8 T cells and their functional characteristics.

Results

Study population

The study was conducted among 331 healthy individuals mainly including health care personnel at Saarland University Medical Center, who either received homologous regimens with ChAdOx (n=62), BNT (n=43), or mRNA-1273 (n=59), or heterologous vaccinations with ChAdOx-priming followed by a boost with either the BNT (n=66) or the mRNA-1273 vaccine (n=101) (Extended Data Fig. 1 and Table 1). Despite no known history of SARS-CoV-2 infection, one female was positive for nucleocapsid-specific IgG, and was excluded from further analysis. Based on current recommendations, the mean time between the two vaccinations was shorter for the homologous mRNA regimens (5.7±0.7 weeks) as compared to the vector-based regimens (11.9±0.9 weeks). Due to convenience sampling, the group showed some differences in age and gender (Table 1). Blood sampling was carried out at a median of 14 (IQR 2) days after the second vaccination. In differential blood counts, leukocyte and granulocyte numbers differed between the groups with highest numbers found after homologous mRNA-1273 vaccination. The numbers of monocytes, lymphocytes and lymphocyte subpopulations such as B cells, CD4 and CD8 T cells did not differ. Among B cells, plasmablast numbers, which were identified as CD38 positive cells among $IgD^{-}CD27^{+}$ CD19 positive switched-memory B cells were also highest in individuals after homologous mRNA-1273 vaccination (Table 1).

Table 1
Demographic and clinical characteristics of the study population.

1° vaccine	**ChAdOx**	**ChAdOx**	**ChAdOx**	**BNT**	**mRNA-1273**	
2° vaccine	**ChAdOx[1]**	**BNT[2]**	**mRNA-1273[3]**	**BNT**	**mRNA-1273**	
	n=62	n=66	n=101	n=43	n=58	p-value
Years of age (mean±SD)	52.5±10.9	45.0±10.9	38.2±13.7	52.9±18.7	41.9±15.2	<0.0001
Female gender, n (%)	41 (66.1)	53 (80.3)	82 (81.2)	26 (60.5)	38 (65.5)	0.020
Weeks between 1° and 2° vaccination, (mean±SD)	11.9±1.1	11.7±1.0	12.0±0.7	5.4±1.0	5.9±0.2	
Analysis time [days after 2° vaccination], median (IQR)	14 (2.25)	14 (1)	14 (1)	14 (2)	15 (2)	
Differential blood cell counts	n=62	n=65	n=101	n=41	n=57	
Leukocytes (cells/µl), median (IQR)	7000 (2400)	6400 (2000)	6800 (1850)	6100 (2300)	7700 (2550)	0.018
Granulocytes (cells/µl), median (IQR)	4014 (1715)	3856 (1391)	4100 (1363)	3611 (2176)	4617 (1981)	0.006
Monocytes (cells/µl), median (IQR)	561 (267)	546 (233)	531 (173)	520 (217)	568 (176)	0.211
Lymphocytes (cells/µl), median (IQR)	2174 (1045)	2103 (899)	2170 (828)	2189 (786)	2241 (1127)	0.720
CD3 T-cells (cells/µl), median (IQR)[#]	1527 (817)	1524 (735)	1498 (696)	1567 (663)	1636 (890)	0.840
CD4 T-cells (cells/µl), median (IQR) [#]	919 (645)	962 (413)	937 (448)	1031 (526)	1066 (627)	0.863

[1]Refers to ChAdOx1 nCoV-19 by AstraZeneca; [2]Refers to BNT162b2 by BioNTech/Pfizer, [3]Refers to mRNA-1273 by Moderna; [#]B and T cell counts were calculated on 61 ChAdOx-ChAdOx, 64 ChAdOx-BNT, 101 ChAdOx-mRNA-1273, 39 BNT-BNT, and 57 mRNA-1273-mRNA-1273 vaccinated individuals, respectively.

1° vaccine	ChAdOx	ChAdOx	ChAdOx	BNT	mRNA-1273	
CD8 T-cells (cells/µl), median (IQR) [#]	383 (236)	385 (271)	396 (241)	340 (259)	388 (266)	0.448
CD19 B cells (cells/µl), median (IQR)[#]	204 (145)	205 (135)	202 (126)	189 (168)	244 (148)	0.529
Plasmablasts (cells/µl), median (IQR)[#]	0.478 (0.696)	0.483 (0.722)	0.528 (0.473)	0.471 (0.476)	0.868 (0.785)	0.001

[1]Refers to ChAdOx1 nCoV-19 by AstraZeneca; [2]Refers to BNT162b2 by BioNTech/Pfizer, [3]Refers to mRNA-1273 by Moderna; [#]B and T cell counts were calculated on 61 ChAdOx-ChAdOx, 64 ChAdOx-BNT, 101 ChAdOx-mRNA-1273, 39 BNT-BNT, and 57 mRNA-1273-mRNA-1273 vaccinated individuals, respectively.

Differential induction of antibodies and T cells after homologous and heterologous vaccination

Spike-specific IgG were detectable in all individuals, but their levels were significantly higher in individuals boosted with mRNA vaccines as compared to individuals after homologous ChAdOx vaccination (Fig. 1a, p<0.0001). When comparing heterologous regimens, boosting with mRNA-1273 led to numerically higher IgG levels (6043 (IQR 4396) BAU/ml) than boosting with BNT (4275 (IQR 4080) BAU/ml). Likewise, among homologous regimens, IgG levels were higher after mRNA-1273 vaccination (5529 (IQR 5755) BAU/ml) than in BNT vaccinated individuals (3438 (IQR 3287) BAU/ml), although the differences did not reach statistical significance. As with IgG levels, neutralizing inhibitory capacity of spike-specific antibodies determined using a surrogate assay was high and reached a maximum of 100% in the majority of mRNA-boosted individuals, which contrasted with significantly lower neutralizing activity after homologous ChAdOx vaccination (median 77.8% (IQR 33.5%), p<0.0001, Fig. 1a).

Vaccine-induced CD4 and CD8 T cells were quantified after stimulation with overlapping peptides encompassing the spike protein. Activation-induced T cells were identified based on CD69 and IFNγ, TNFα and IL-2. A representative example of CD69-positive spike-specific CD4 and CD8 T cells producing IFNγ from a 49-year-old female after homologous mRNA-1273 vaccination is shown in Extended Data Fig. 2, and data from all individuals are summarized in Fig. 1b. Spike-specific CD4 T-cell levels in the homologous ChAdOx vaccine group were significantly lower than in all other groups. Among mRNA-boosted regimens, median levels of spike-specific CD4 T cells were highest after heterologous ChAdOx1/mRNA-1273 vaccination (0.29% (IQR 0.23%)). Not only did a boost with mRNA-1273 outperform heterologous boosting with BNT after ChAdOx-priming (0.18% (IQR 0.17%), p<0.01), but CD4 T-cell levels were also higher after homologous vaccination with mRNA-1273 (0.24% (IQR 0.27%) than with BNT (0.10% (IQR 0.08%), p<0.0001). Interestingly, the two heterologous regimens also led to a strong

induction of spike-specific CD8 T cells (0.29% (IQR 0.57%) for BNT and 0.40% (IQR 0.60%) for mRNA-1273), with significantly higher levels than all three homologous regimens (Fig. 1b, $p<0.0001$). All vaccine-induced effects on CD4 and CD8 T cells were specific, as no differences in *Staphylococcus aureus* Enterotoxin B (SEB)-reactive CD4 and CD8 T cells were observed between the five groups (Fig. 1c). Finally, in line with a pronounced induction of vaccine-induced T cells, CTLA-4 expression was strongly induced on spike-specific CD4 and CD8 T cells of all individuals after heterologous vaccination and in both homologous mRNA-regimens, whereas CTLA-4 expression on specific T cells after homologous ChAdOx vaccination was significantly lower (Fig. 1d). These differences in CTLA-4 expression were also spike-specific, as CTLA-4 expression on SEB-reactive CD4 and CD8 T cells were similarly low in all five groups (Fig. 1e).

When analyzing correlations between spike-specific IgG levels, neutralizing activity, and spike-specific CD4 and CD8 T cells (Fig. 1f and Supplementary Table 1), neutralizing activity showed a strong correlation with IgG levels in each vaccine subgroup. Likewise, spike-specific CD4 and CD8 T cells showed a significant correlation. It is interesting to note that IgG levels correlated with CD8 T-cell levels in the three homologous vaccine groups only, whereas no such correlation was found for the two heterologous vaccine groups, which may be a result of the exceptionally high CD8 T-cell response in these two groups (see Fig. 1b).

As the five groups differed in age and gender due to convenience sampling and recruitment according to national recommendations (Table 1), a subgroup analysis was performed among 40 individuals per vaccination regimen which were matched for age and gender (Extended Data Fig. 3). As shown in Extended Data Fig. 4, between-group differences in IgG levels, neutralizing activity and spike-specific T cells largely remain the same. In the whole cohort, adjusting for age and gender as confounders in a non-parametric regression analysis showed that both confounders did not have any significant effect on immunological parameters (Extended Data Fig. 5). When testing for interactions of age within each vaccine group with the homologous ChAdOx group as a reference, age had no effect on T-cell levels and neutralizing antibody activity; the only effect of age was found for IgG levels within each of the two homologous regimens ($p=0.003$ for BNT/BNT and $p=0.015$ for mRNA-1273/mRNA-1273, Extended Data Fig. 5).

Functional differences in vaccine-induced T cells after homologous and heterologous vaccination

Apart from IFNγ, we also analyzed spike-specific induction of the cytokines TNFα and IL-2. As with IFNγ, differences between the groups were similar for CD4 T cells producing TNFα or IL-2 (Extended Data Fig. 6a and b), or for cells producing any of the three cytokines alone or in combination (Extended Data Fig. 6c). This also held true for spike specific CD8 T cells, except for IL-2 producing CD8 T cells, where levels were generally lower and only showed subtle differences between the groups (Extended Data Fig. 6b). To assess functionality on a single cell level, cytokine profiles of spike-specific CD4 and CD8 T cells were characterized after Boolean gating (Extended Data Fig. 7). This allowed distinction of seven

subpopulations including polyfunctional cells simultaneously expressing all three cytokines, two cytokines or one cytokine only (Fig. 2). The cytokine expression profiles showed significant differences between the vaccine regimens, and the highest percentage of polyfunctional CD4 T cells was observed for the three vector-primed regimens. These three regimens also showed the highest percentage of CD8 T cells expressing IFNγ and TNFα, which was the dominant fraction among spike-specific CD8 T cells (Fig. 2a). The differences in cytokine expression profiles were spike-specific, as SEB-reactive cytokine expression did not differ among the groups (Fig. 2b).

Differences in reactogenicity after homologous and heterologous vaccination

Local and systemic adverse events within the first week after the first and the second vaccination were self-recorded using a questionnaire (Fig. 3). Irrespective of the vaccine type, local adverse events such as pain at the injection site were reported with similar frequency in individuals after the first vaccination. Swelling at the injection site was overall less frequently observed with the lowest percentage among BNT-primed individuals (Fig. 3b). Systemic adverse events including fever, headache, fatigue, chills, gastrointestinal manifestations, myalgia and arthralgia after priming were most frequent in individuals after ChAdOx vaccination, which also was associated with a more frequent use of antipyretic medication (Fig. 3c). After the second vaccination, local adverse events were least frequent after homologous ChAdOx vaccination, and most frequent in both heterologous and in the homologous mRNA-1273 regimens. The occurrence of systemic adverse events clearly dominated in individuals after heterologous boosting with mRNA-1273, followed by homologous mRNA-1273 vaccination and heterologous BNT-boosting (Fig. 3a and 3c). Individual perception of severity was scored higher after secondary vaccination in both homologous mRNA regimens (Fig. 3d). In contrast, more than 75% of subjects after both the homologous ChAdOx and heterologous BNT vaccination were more affected by the primary vaccination with the vector. Despite the strong reactogenicity after vector-priming, it was interesting to note that a sizable fraction of subjects after heterologous boosting with mRNA-1273 was more severely affected by the secondary vaccination, which contrasts with perception of the heterologous BNT vaccine group. Likewise, among individuals after homologous vaccination, the secondary vaccination with mRNA-1273 was more frequently perceived as more severe, although this vaccine was already strongly reactogenic after the primary vaccination. Overall, it therefore appeared that both the homologous and the heterologous regimens that included BNT were better tolerated than the respective mRNA-1273 regimens.

Discussion

The three vaccines ChAdOx1 nCoV-19, BNT152b2, and mRNA-1273 were developed and authorized to be administered as a homologous prime/boost regimen. Our study now provides the first head-to-head immunogenicity and reactogenicity data comparing all three authorized homologous COVID-19 vaccine regimens with heterologous combinations of ChAdOx-priming followed by either BNT- or mRNA-1273-boosting. We show that all regimens are immunogenic, but show considerable differences in the extent of vaccine-induced antibody and T-cell responses. The most striking finding was that immunogenicity of the

mRNA-1273 containing regimens was more pronounced than the respective BNT vaccine combinations, which held true for both the homologous and the heterologous regimens. Correspondingly, homologous or heterologous boosting with mRNA-1273 was less well tolerated as compared to the other regimens.

We and others have recently shown that heterologous prime/boost regimens led to a strong induction of antibodies and T cells, although this was so far largely characterized in individuals primed with ChAdOx followed by boosting with the BNT mRNA. We now show that heterologous boosting with mRNA-1273 led to similar antibody and T-cell response patterns with a particular strong induction of CD8 T cells. When comparing the two mRNA vaccines, immunogenicity was generally more pronounced after boosting with mRNA-1273 in both the heterologous and homologous vaccine group. This may be related to a higher dosage of the mRNA (100µg vs. 30µg) and/or different formulations of lipid nanoparticles[11,12]. Our data confirm that a secondary vaccination with the vector is less potent in boosting antibodies and T cells as compared to all mRNA-containing regimens[3–7]. As this may be related to preformed or induced neutralizing immunity towards the vector backbone[14], the boosting effect is superior using either heterologous combinations or homologous regimens with mRNA vaccines that use lipid nanoparticle vaccine carriers. Interestingly, despite poor immunogenicity after homologous ChAdOx-boosting, all three ChAdOx-primed regimens led to the highest percentage of multifunctional T cells upon secondary boosting, which may result from the potent ability of the ChAdOx vector for T-cell priming[15]. In general, spike-specific CD8 T cells known to mediate protection from severe COVID-19[16] were most strongly induced after heterologous boosting. The fact that CD8 T cells did not correlate with antibody levels emphasizes that analysis of antibodies alone may be insufficient to evaluate protection from severe disease. We have previously shown that a pronounced induction of antigen-specific T-cell levels after infection with SARS-CoV-2[17], with varicella zoster virus[18], or after influenza-vaccination[19] is paralleled by an upregulation of CTLA-4 on specific T cells which may serve to counteract excessive T-cell proliferation and/or T-cell mediated immunopathology. Interestingly, we now show that the strongest expression of CTLA-4 on spike-specific CD4 and CD8 T cells was found in the four vaccine groups with the most pronounced induction of CD4 and CD8 T cells after vaccination, whereas CTLA-4 expression in individuals after homologous ChAdOx vaccination was significantly lower, which supports a less potent boost of T-cell immunity. On the B-cell side, a stronger immunogenicity of the heterologous as compared to the homologous BNT regimen was recently found to be associated with a higher percentage of spike-specific activated memory B cells[20]. While this finding may result from a more pronounced T-cell help, this may also explain the higher avidity[4] and the higher neutralizing capacity[20] of antibodies observed after heterologous boosting.

The three homologous regimens have shown remarkable but variable efficacy and effectiveness regarding protection from infection. The differences in immunogenicity between the homologous regimens largely correspond with similar differences in efficacy in the range of 70.4% for ChAdOx[10], 95% for BNT2[12], and 94.1% for mRNA-1273[11]. Similar differences among the three homologous vaccine regimens were also reported for effectiveness in nation-wide observational studies[21–26], whereas real world effectiveness data for heterologous regimens had been limited. In this regard, a recent nationwide

cohort study in ChAdOx-primed individuals from Sweden found an effectiveness of 50% after homologous boosting, 67% after BNT-boosting and the highest effectiveness of 79% after boost with mRNA-1273[27]. This indicates that our observation of a higher immunogenicity of the ChAdOx/mRNA-1273 regimen may also translate into a higher effectiveness. In addition, in line with an equivalent or superior immunogenicity of heterologous mRNA-boosting, a study from France provided evidence for a higher effectiveness of the ChAdOx/BNT regimen as compared to homologous BNT vaccination[20]. Finally, the ChAdOx/BNT regimen in a Danish nationwide study reached a remarkable effectiveness of 88%, although no control groups with other regimens were analyzed in parallel[28].

The strength of our study is the large head-to-head analysis of immunogenicity and reactogenicity of all recommended two-dose homologous and heterologous vaccine combinations, including a large group of individuals vaccinated with the ChAdOx/mRNA-1273 regimen in a real world setting. Our study is limited by convenience sampling in a non-randomized study design, where study participants were enrolled according to national recommendations. Although this led to some differences in age within the five groups, between-group differences of immunological parameters among age-matched subgroups remained largely the same. Within a given regimen, an effect of age was only observed for IgG levels within the homologous mRNA groups. Therefore, at least in our cohort of individuals mainly including health care workers, age is unlikely to have a strong confounding impact on our results. Based on national recommendations, the interval between the first and the second vaccination was longer for the ChAdOx-primed groups (9-12 weeks) as compared to the homologous mRNA-vaccine groups (3-6 weeks). Although this may influence immunogenicity in general, this did not account for the striking differences in immune responses among the three ChAdOx-primed groups, which had the same time interval between priming and boosting. Moreover, we observed notable differences in immunogenicity within the two homologous and the two heterologous regimens containing mRNA vaccines, although the respective intervals between priming and boosting was similar. A further limitation is the fact that we do not have any information on neutralizing activity towards variants of concern. However, the observational study from France showed that the heterologous ChAdOx/BNT regimen had superior activity towards the Delta variant as compared to homologous BNT vaccination[20]. Finally, information on the stability of antibody and T-cell levels over time are not available yet; together with data on effectiveness, collection of these data in follow-up studies is important to guide the potential need and timing for booster vaccinations.

Knowledge on the differences in immunogenicity and reactogenicity of homologous and heterologous vaccine combinations is of increasing importance for clinical practice. First, mixing different vaccine principles in heterologous vaccination regimens is already implemented for regular COVID-19 vaccination procedures in many countries due to the frequent occurrence of rare, but serious adverse events after ChAdOx-priming[29, 30]. Moreover, vaccine shortage in many countries may necessitate the use of heterologous combinations to ensure broad vaccine coverage. Finally, serial use of heterologous combinations of different vaccines is of increasing importance to optimize immunogenicity of single dose or poorly immunogenic homologous regimens. As illustrated by the favorable immunogenicity of heterologous regimens in solid organ transplant recipients[15], this is of particular relevance for

immunocompromised patients who exhibit a severely impaired immunogenicity after regular homologous vaccination; as reactogenicity is less of a concern in immunocompromised patients[15], mix-and-match regimens may offer the most favorable risk-benefit ratio for this population. Finally, as with other widely used vaccines, deviation from homologous series may become common practice for booster vaccinations after waning of vaccine-induced protection.

Methods

Study design and subjects

Study participants with no known history of SARS-CoV-2 infection were enrolled in this observational study prior to their secondary vaccination as described before[3]. We enrolled participants on all five possible authorized dual dose vaccine combinations as per recommendations in Germany including homologous regimens with ChAdOx, BNT or mRNA-1273, or heterologous regimens with ChAdOx-priming and boosting with either BNT or mRNA-1273 (Extended Data Fig. 1)[1]. The choice of regimen, including the time interval between the first and the second vaccination (3-6 weeks for homologous mRNA regimens, and 9-12 weeks for all regimens with ChAdOx-priming) was based on recommendations[31] and not determined by the study. Study participants were enrolled prior to the second and in part prior to the first vaccination, and received a questionnaire for self-reporting of local and systemic adverse events within the first week after the first and second vaccination. Blood samples were collected during an interval of 13-18 days after secondary vaccination to determine lymphocyte subpopulations and SARS-CoV-2 specific humoral and cellular immunity. Primary vaccinations were performed between 18th of January and 10th of June 2021. Thirty-six individuals (12 ChAdOx/ChAdOx, 22 ChAdOx/BNT, 1 ChAdOx/mRNA-1273, 1 BNT/BNT) were enrolled in a separate observational study (SaarTxVac study). Their results on induction of humoral and cellular immunity were part of a subgroup of 70 immunocompetent individuals to comparatively study vaccine-responses in transplant recipients[15]. The study was approved by the ethics committee of the Ärztekammer des Saarlandes (reference 76/20), and all individuals gave written informed consent.

Quantification of lymphocyte populations and plasmablasts

T cells, B cells and plasmablasts were quantified from 100 µl heparinized whole blood exactly as described before using monoclonal antibodies towards CD3 (clone SK7, final dilution 1:25), CD19 (clone HIB19, 1:40), CD27 (clone L128, 1:200), CD38 (clone HB7, 1:20) and IgD (clone IA6-2, 1:33.3). T and B cells were identified among total lymphocytes by expression of CD3 and CD19, respectively. CD4 and CD8 T cells were quantified after staining of CD4 (clone SK3, 1:100) and CD8 (clone RPA-T8). Plasmablasts were defined by expression of CD38 among IgD-CD27+ CD19 positive switched-memory B cells. Antibodies are listed in Supplementary Table 2. Analysis was performed on a BD FACSLyric flow-cytometer and BD FACSuite software v1.4.0.7047 followed by data analysis using FlowJo software 10.6.2. Analyses of T cells, B cells and plasmablasts were performed using a gating strategy as described before[3]. Absolute lymphocyte numbers were calculated based on differential blood counts.

Quantification of vaccine-induced SARS-CoV-2 specific T cells

SARS-CoV-2 specific T cells were determined from heparinized whole blood after a 6h-stimulation with overlapping peptides spanning the SARS-CoV-2 spike protein (N-terminal receptor binding domain and C-terminal portion including the transmembrane domain, each peptide 2µg/ml; JPT, Berlin, Germany) exactly as described previously[3, 17]. Stimulations with 0.64% DMSO and with 2.5µg/ml of *Staphylococcus aureus* enterotoxin B (SEB; Sigma) served as negative and positive controls, respectively. All stimulations were carried out in presence of co-stimulatory antibodies against CD28 and CD49d (clone L293 and clone 9F10, 1µg/ml each). Immunostaining was performed using anti-CD4 (clone SK3, 1:33.3), anti-CD8 (clone SK1, 1:12.5), anti-CD69 (clone L78, 1:33.3), anti-IFNγ (clone 4S.B3, 1:100), anti-IL-2 (clone MQ1-17H12, 1:12.5), anti-TNFα (clone MAb11, 1:20), and anti-CTLA-4 (clone BNI3) and analyzed using flow-cytometry (BD FACS Canto II including BD FACSDiva software 6.1.3). Antibodies are listed in Supplementary Table 2. SARS-CoV-2-reactive CD4 or CD8 T cells were identified as activated CD69-positive T cells producing IFNγ. Moreover, co-expression of IL-2 and TNFα was analyzed to characterize cytokine-expression profiles using a gating strategy as described in Extended Data Fig. 7). Reactive CD4 and CD8 T-cell levels after control stimulations were subtracted from levels obtained after SARS-CoV-2 specific stimulation, and 0.03% of reactive T cells was set as detection limit as described before[3].

Determination of SARS-CoV-2 specific antibodies and neutralization capacity

All antibody tests were performed according to the manufacturer´s instructions (Euroimmun, Lübeck, Germany) as described before[3]. SARS-CoV-2 specific IgG antibodies towards the receptor binding domain of SARS-CoV-2 spike protein were quantified using an enzyme-linked immunosorbent assay (ELISA, SARS-CoV-2-QuantiVac). Antibody binding units (BAU/ml) <25.6 were scored negative, ≥25.6 and <35.2 were scored intermediate, and ≥35.2 were scored positive. SARS-CoV-2 specific IgG towards the nucleocapsid (N) protein were quantified using the anti-SARS-CoV-2-NCP-ELISA. A neutralization assay based on antibody-mediated inhibition of soluble ACE2 binding to the plate-bound S1 receptor binding domain (SARS-CoV-2-NeutraLISA) was used at a single serum dilution. Surrogate neutralizing capacity was calculated as percentage of inhibition (IH) by 1 minus the ratio of the extinction of the respective sample and the extinction of the blank value. IH<20% was scored negative, IH≥20 and <35 intermediate, and IH≥35% positive.

Statistical analysis

Kruskal-Wallis test, followed by Dunn's multiple comparisons test, was performed to compare unpaired non-parametric data between groups (lymphocyte subpopulations, T-cell and antibody levels, CTLA-4 expression). Data with normal distribution were analyzed using ordinary one-way ANOVA (cytokine-expression profiles, age). Categorial analyses on gender and adverse events were performed using X^2 test. Correlations between levels of T cells, antibodies, and neutralizing activities were analyzed

according to Spearman. A p-value <0.05 was considered statistically significant. Analysis was carried out using GraphPad Prism 9.0 software (GraphPad, San Diego, CA, USA) using two-tailed tests. SPSS V27 including an R 3.6 plug-in for non-parametric regression analysis was used to determine the effect of confounders on SARS-CoV-2 specific immunity as described before[32].

Declarations

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Author Contributions

V.K., T.S., D.S., U.S., B.C.G., S.S., and M.S. designed the study; V.K., T.S., D.S., U.S. and M.S. designed the experiments, V.K., S.M., F.H., A.A.-O., L.Z., C.G., R.U., and T.S. performed experiments; S.S., B.C.G., J.M., L.Z., S.L.B. and U.S. contributed to study design, patient recruitment, and clinical data acquisition. T.S., M.S., U.S. and S.W. performed statistical analysis. V.K., T.S., D.S., U.S., J.M. and M.S. supervised all parts of the study, performed analyses and wrote the manuscript. All authors approved the final version of the manuscript.

Competing interest statement

M.S. has received grant support from Astellas and Biotest to the organization Saarland University outside the submitted work, and honoraria for lectures from Biotest and Novartis. All other authors of this manuscript have no conflicts of interest to disclose.

Data availability

Figures 1, 2, 3, Table 1 and Extended Data figures 3, 4, 5, and 6 have associated raw data. The data that support the findings of this study are available from the corresponding author upon request and data will be made available in a public repository upon acceptance of the manuscript.

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Figures

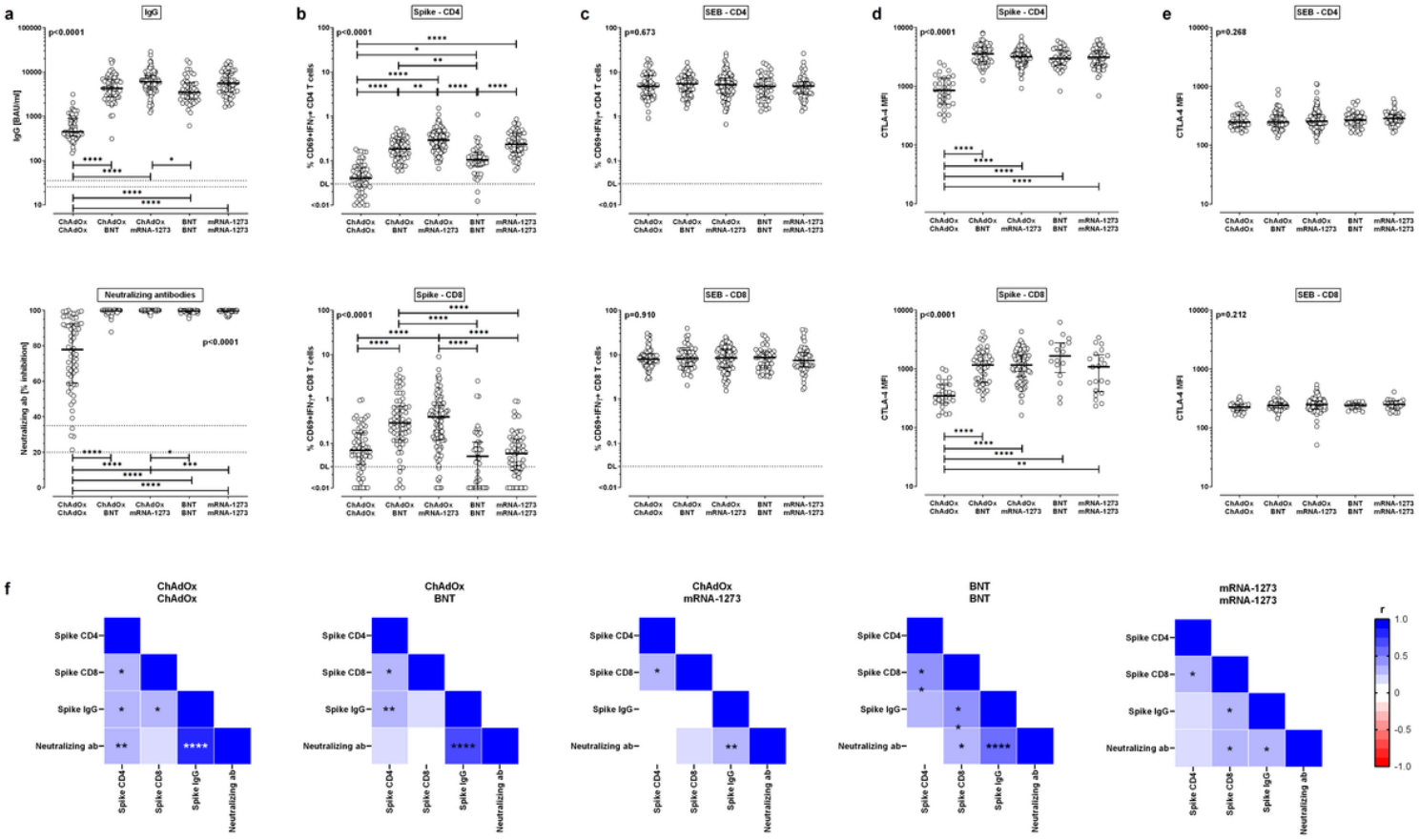

Figure 1

Antibody and T-cell responses against the SARS-CoV-2 spike protein after homologous COVID-19 vaccine regimens or heterologous ChAdOx-priming and BNT- or mRNA-1273-boosting. Cellular and humoral immune parameters were analyzed 13-18 days post vaccination and compared between individuals with different homologous or heterologous COVID-19 vaccine regimens: homologous ChAdOx-vaccination (n=62), heterologous ChAdOx/BNT-vaccination (n=66), heterologous ChAdOx/mRNA-1273-vaccination (n=101), homologous BNT-vaccination (n=43) or homologous mRNA-1273-vaccination (n=58). (a) ELISA and surrogate neutralization assays were performed to quantify levels of spike-specific IgG and neutralizing antibodies. Intracellular cytokine staining after antigen-specific stimulation of whole blood samples allowed for flow-cytometrical determination of SARS-CoV-2 spike-specific (b) and SEB-reactive (c) CD4 and CD8 T-cell levels. Reactive cells were identified by co-expression of CD69 and IFNγ among CD4 or CD8 T cells and subtraction of reactivity of respective negative control stimulations. CTLA-4 expression was determined on (d) spike-specific and (e) SEB-reactive CD4 and CD8 T cells in all samples with at least 20 cytokine-positive CD4 and CD8 T cells. (f) Correlation matrix of spike-specific T-cell and antibody responses among each group. Bars in (a)-(e) represent medians with interquartile ranges.

Differences between the groups were calculated using two-sided Kruskal-Wallis test with Dunn´s multiple comparisons post-test. Correlations in (f) were analyzed according to two-tailed Spearman (see also Supplementary Table 1). Dotted lines indicate detection limits for antibodies in (a), indicating negative, intermediate and positive levels or levels of inhibition, respectively as per manufacturer's instructions, and detection limits for SARS-CoV-2-specific CD4 T cells in (b) and (c). IFN, Interferon; MFI, median fluorescence intensitiy; SEB, Staphylococcus aureus enterotoxin B.

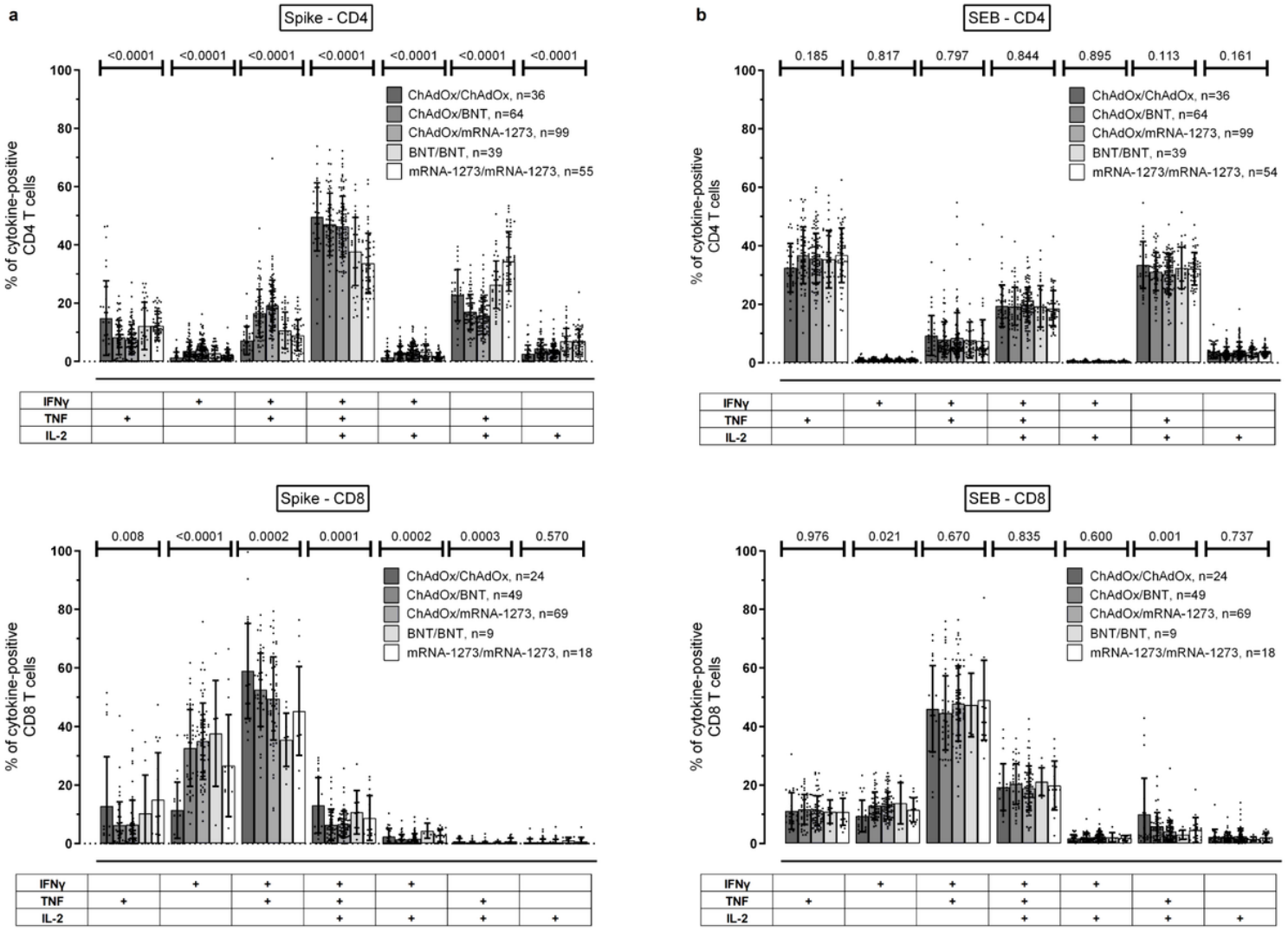

Figure 2

Antigen-specific cytokine-expression profiles of T cells in individuals with different homologous and heterologous COVID-19 vaccination regimens. After antigen-specific stimulation (a) or polyclonal stimulation with Staphylococcus aureus enterotoxin B (SEB, b) of whole blood samples from individuals with different homologous or heterologous vaccination regimens, cytokine expressing CD4 and CD8 T cells were subclassified into 7 subpopulations according to single or combined expression of IFNγ, IL-2 and TNFα. Blood samples from all individuals were analyzed. To ensure robust statistics, only samples with at least 30 cytokine-expressing CD4 or CD8 T cells after normalization to the negative control stimulation were considered (with the number of samples in each vaccine group indicated in the figures). IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

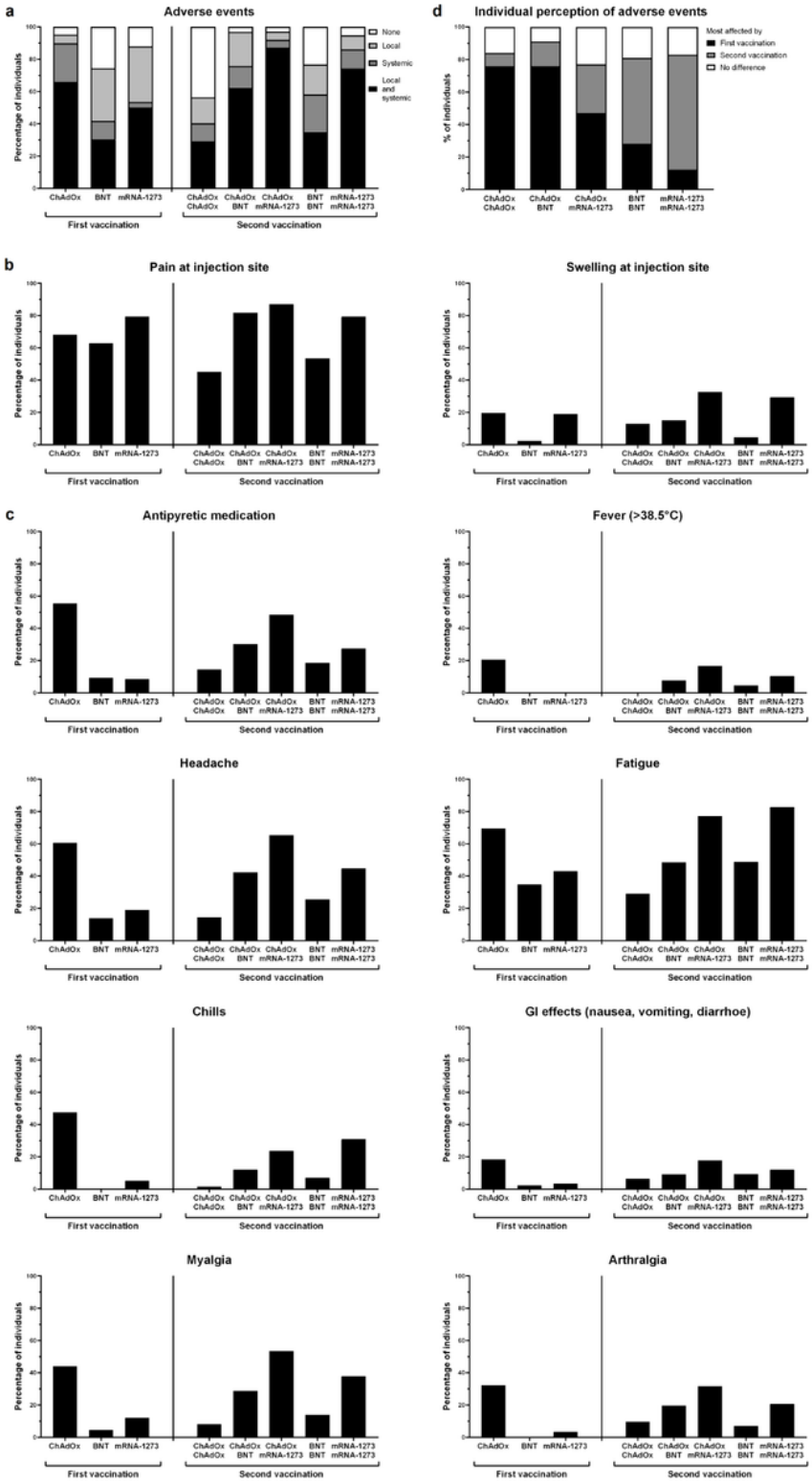

Figure 3

Reactogenicity after primary and secondary vaccination with homologous and heterologous COVID-19 vaccine regimens. According to their COVID-19 vaccine regimens, individuals were classified into three groups after dose 1 (ChAdOx vector (n=229), BNT (n=43) or mRNA-1273 vaccine (n=58)) and five groups after dose 2 (homologous: ChAdOx/ChAdOx, n=62; BNT/BNT, n=43; mRNA-1273/mRNA-1273, n=58; heterologous: ChAdOx/BNT, n=66; ChAdOx/mRNA-1273, n=101). Self-reported reactogenicity within the

first week after each vaccine dose was assessed using a standardized questionnaire. Presence of local or systemic adverse events in general (a), substantial local (b) or systemic adverse events (c) and individual perception of which of the two vaccinations affected more (d) are shown. Comparisons between the groups were performed using X^2 test.

Supplementary Files

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