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ORIGINAL ARTICLE

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# Analysis of BNT162b2- and CVnCoV-elicited sera and of convalescent sera toward SARS-CoV-2 viruses

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## Summary

**Background:** The mRNA vaccine BNT162b2 (Comirnaty, BioNTech/Pfizer) and the vaccine candidate CVnCoV (Curevac) each encode a stabilized spike protein of SARS-CoV2 as antigen but differ with respect to the nature of the mRNA (modified versus unmodified nucleotides) and the mRNA amount (30 μg versus 12 μg RNA). This study characterizes antisera elicited by these two vaccines in comparison to convalescent sera.

**Methods:** Sera from BNT162b2 vaccinated healthcare workers, and sera from participants of a phase I trial vaccinated with 2, 4, 6, 8, or 12 μg CVnCoV and convalescent sera from hospitalized patients were analyzed by ELISA, neutralization tests, surface plasmon resonance (SPR), and peptide arrays.

**Results:** BNT162b2-elicited sera and convalescent sera have a higher titer of spike-RBD-specific antibodies and neutralizing antibodies as compared to the CVnCoV-elicited sera. For all analyzed sera a reduction in binding and neutralizing antibodies was found for the lineage B.1.351 variant of concern. SPR analyses revealed that the CVnCoV-elicited sera have a lower fraction of slow-dissociating antibodies. Accordingly, the CVnCoV sera almost fail to compete with the spike-ACE2 interaction. The significance of common VOC mutations K417N, E484K, or N501Y focused on linear epitopes was analyzed using a peptide array approach. The peptide arrays showed a strong difference between convalescent sera and vaccine-elicited sera. Specifically, the linear epitope at position N501 was affected by the mutation and elucidates the escape of viral variants to antibodies against this linear epitope.

**Conclusion:** These data reveal differences in titer, neutralizing capacity, and affinity of the antibodies between BNT162b2- and CVnCoV-elicited sera, which could contribute to the apparent differences in vaccine efficacy.

**Abbreviations:** Ab, antibody; Alfa, SARS-CoV-2 Alfa variant; Beta, SARS-CoV-2 Beta variant; BNT162b2, mRNA COVID-19 vaccine; COVID-19, coronavirus disease 2019; Ctrl, negative control sera; CVnCoV, mRNA COVID-19 vaccine; mRNA, messenger ribonucleic acid; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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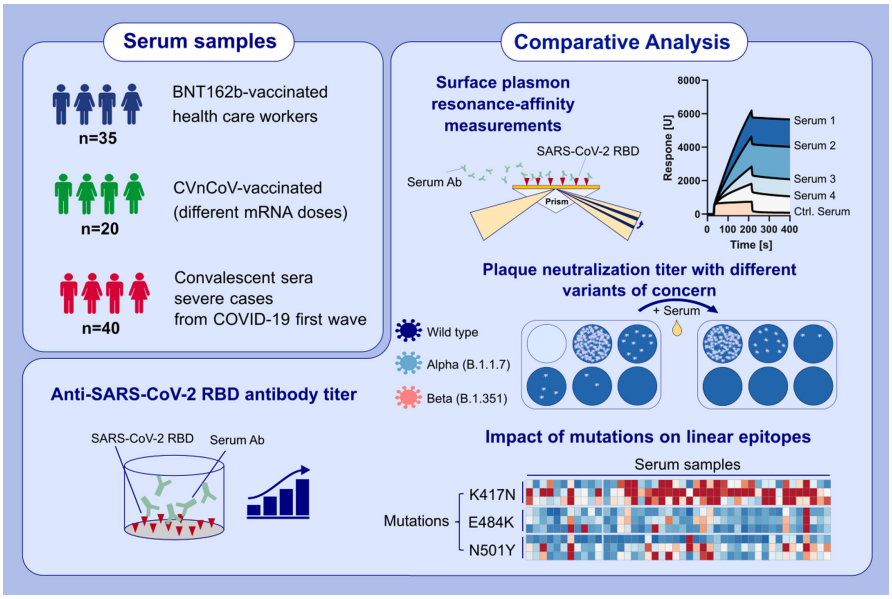
KEYWORDS
antisera, mRNA, SARS-CoV-2, vaccine, variants of concern




## GRAPHICAL ABSTRACT

This study presents comparative characterization of differences between sera derived from BNT162b- or CVnCoV-vaccinated individuals and first-wave convalescent patients. In addition to standard titer measurements, we used novel methods for sera analysis, such as SPR to measure antibody-antigen complexes and SARS-CoV-2 variant-specific peptide arrays to study efficiency against linear epitopes. Our results indicate a strong difference between all three serum groups (also between the two vaccine types), especially with respect to the titer, affinity, and binding pattern to linear epitopes of the elicited antibodies.

Abbreviations: Ab, antibody; Alpha, SARS-CoV-2 Alpha variant; Beta, SARS-CoV-2 Beta variant; BNT162b2, mRNA COVID-19 vaccine; COVID-19, coronavirus disease 2019; Ctrl, negative control sera; CVnCoV, mRNA COVID-19 vaccine; mRNA, messenger ribonucleic acid; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

## 1 | INTRODUCTION

Since its emergence in December 2019, infection by SARS-CoV-2 has caused on August 2021 (21.08. 2021) over 211 million cases of coronavirus disease 2019 (COVID-19) and more than 4.9 million deaths worldwide (source: COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at John Hopkins University (JHU)). Exceptional research efforts led to the rapid development of vaccines. Almost all vaccines are based on the viral spike protein (S) [1]. S is a trimeric glycoprotein which is located on the viral surface and mediates the interaction with the receptor angiotensin-converting enzyme 2 (ACE2) [2,3]. Each S-monomer encompasses two domains, S1 and S2. These two domains mediate viral binding to ACE2 and fusion with the host cell membrane, respectively. The receptor-binding domain (RBD) is located within S1 and represents a major target for neutralizing antibodies [4]. In the EU and in the US mRNA-based vaccines were the first approved vaccines. BNT162b2 (Comirnaty/Tozinameran, BioNTech/Pfizer) received a temporary emergency use authorization (EUA) in the UK and, subsequently, a series of approvals or authorizations for emergency use in Bahrain, Canada, Mexico, Saudi Arabia, and the USA. In Switzerland (19 December 2020) and the EU (21 December 2020), BNT162b2 received conditional market authorization for active immunization against COVID-19 for individuals 16 years of age and older [5-7]. The mRNA vaccine produced by Moderna obtained the conditional marked authorization in the EU in January 2021 [8,9]. A further vaccine candidate based on mRNA is developed by Curevac (CVnCoV) [10]. Although these three vaccines are all based on mRNA which encodes SARS-CoV-2 full-length spike protein (S-protein) locked in the prefusion conformation (no further viral proteins are encoded in contrast to a natural infection) and are applied in a two-dose regimen, there are some striking differences between them. CVnCoV is based on unmodified RNA. In contrast to this, the mRNA vaccines produced by BioNTech/Pfizer and Moderna are based on a modified mRNA harboring 1-methylpseudouridine instead of uridine. Moreover, the RNA amount per vaccine dose differs. In case of BNT162b2 30 µg and in case of the Moderna vaccine, 100 µg are used. CVnCoV contains 12 µg per dose [11]. The analysis of the vaccine efficacy showed an efficacy of over 94% for BNT162b2 and Moderna in clinical trials toward symptomatic disease [12].

The final analysis of the pivotal phase 2b/3 HERALD three trial revealed that CVnCoV is 48% effective at staving off disease of any

severity [13-16]. A higher efficacy of about 77% was observed for the group of 18–60-year-old individuals, if prevention of moderate or severe disease was considered endpoint. Although the emergence of novel variants during the trial might have affected the result it seems that there is a lower efficacy as compared to the mRNA vaccines from Moderna and BioNTech/Pfizer [16].

Based on these data, this study aims to perform an analysis of sera derived from BNT162b2- or CVnCoV-vaccinated individuals and of convalescent sera with respect to their neutralizing titers, binding affinities to the spike RBD domain of wild-type SARS-CoV-2 and of variants of concern (VOCs) and to their interference with the ACE2/RBD interaction.

## 2 | MATERIALS AND METHODS

### 2.1 | Cell culture and virus strains

Vero E6 (ATCC® CRL-1586™) cells and HEK293T cells (ATCC CRL-3216™) were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma, Taufkirchen, Germany) with 10% FCS (Sigma, Taufkirchen, Germany), 1% penicillin/streptomycin, and 1% L-glutamine and incubated with 5% $CO_2$ at 37°C. The SARS-CoV-2 virus isolates used belonged to the Pangolin lineages B.1.1.7, B 1.351, and B.1.1.298. As an early isolate, the UVE/SARS-CoV-2/2020/FR/702 (GenBank: MT777677.1; here named as MRS) was used provided by the EVAg.

### 2.2 | RBD mutant generation

For mutagenesis, the *rbd* gene was subcloned from the pCAGGS-sRBD plasmid (Florian Kramer, Icahn School of Medicine at Mount Sinai; [17]) into the expression pcDNA3.1(-)-plasmid (Thermo Fisher Scientific, Waltham, USA) with standard cloning procedures. The mutagenesis was performed by the QuikChange Site-Directed Mutagenesis with the PfuUltra High-Fidelity DNA Polymerase (Agilent, USA, Santa Clara) according to the manufacturer's protocol and the respective primer pair (Table S1). All generated plasmids (Table S2) were validated by sequencing.

### 2.3 | Protein production and purification

HEK293T cells were grown in standard DMEM medium with 10% FCS. For transfection, 4.5x10^6 cells/150mm dish were seeded 6 h before. The transfection mix of 3 ml was prepared with by mixing 15 µg plasmid DNA in 1.5 ml PBD with 1.5 ml of PEI (60 µg/ml). 16h after transfection, the 30 ml fresh DMEM medium was added to the cells. The supernatant was harvest 72 h post transfection. A 5 ml Ni-NTA affinity chromatography column (Cytiva, Uppsala, Sweden) was used and equilibrated with 5 CV of wash puffer with a flow rate of 2 ml/min. After sterilfiltration, the supernatant was mixed 1:1 with wash buffer (40 mM imidazole in PBS, pH 8.0) and was used directly for purification. The column was washed with 15 CV of wash buffer, and the protein was eluted with 250 mM imidazole in PBS.

### 2.4 | Plaque reduction neutralization test (PRNT50)

The plaque reduction neutralization test was performed in duplicate in the BSL-3 facility at PEI. 6x10^5 of Vero E6 cells per well were seeded to six-well plates (Geiner Bio-One, Kremsmünster, Austria) and incubated at 37°C for 24 h. The sera were 2-fold serially diluted (1:20 to 1:640) and incubated with 80 PFU of the early isolate (MRS) and the different SARS-CoV-2 variants in a total volume of 100 µl at 37°C for 1 h as described [18].

### 2.5 | Sera

Sera from 35 health care workers receiving routine COVID-19 vaccination with BNT162b2 and sera from participants of a phase I study who were vaccinated with CVnCoV. A total of 12 negative control sera were collected from healthy PEI workers. 20 CVnCoV sera were preselected by the provider (Curevac AG Tuebingen, Germany (each two high and two low titer sera vaccinated with the following mRNA concentrations: 2, 4, 6, 8, or 12 µg). Convalescent sera were provided by the RWTH centralized Biomaterial Bank (RWTH cBMB) of the Medical Faculty of the RWTH Aachen University.

### 2.6 | Ethics

The study (PEI-SARS-CoV-2) was approved by the local ethics committee (Ethik-Kommission, Landesärztekammer Hessen 60314 Frankfurt am Main) and written informed consent was obtained from all participants (2020-1664_2-evBO). Convalescent sera provided by the RWTH Aachen University Hospital have been collected in a prospective study which was provided by the RWTHcBMB were was approved by the local ethics committee (Ethikvotum 206/09 der Ethikkommission der Medizinischen Fakultät der RWTH Aachen University), and the study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki in its latest revision. The studies performed by Curevac are covered by Trial EudraCT 2020–001286–36; CEC Reference Number 433/2020AMG1, Ethik Kommission Universitätsklinikum Tübingen and CEC Reference Number BC-07807, Commissie voor medische Ethiek University Gent. The study was performed in accordance with the provisions of the Declaration of Helsinki and good clinical practice guidelines.

### 2.7 | ELISA

50 µl purified antigen (4 µg/ml in PBS) was used to coat the 96-well microtiter plates (costar 3590, Corning Incorporated, Kennebunk,

USA) over night at 4°C. The ELISA was performed in duplicate as described [17].

## 2.8 | Peptide Microarray

The wild-type peptides and the mutation carrying peptides were synthesized with the MultiPep2 (Intavis AG, Tuebingen, Germany) according to the principle of Merrifield [19]. Synthesized peptides and DMSO (Blank spots) were spotted onto adhesive foil (Lasertab LAT-34–747–1, Brady, Milwaukee, USA) in a format of 1.5 cm x 1.5 cm. Using a slide-spotting robot (Intavis AG, Tuebingen, Germany) peptides and DMSO (Blank spots) were immobilized in a duplicate on a cellulose membrane (Intavis AG, Tuebingen Germany) attached to a 15 mm ×15 mm slide. Peptide arrays were performed in duplicate according to a modified protocol form [20]. For detection, the arrays were incubated with goat anti-human IgG IRDye680 (LI-COR, Bad Homburg, Germany) diluted 1:10,000. The arrays were scanned with LI-COR Odyssey CLx (LI-COR, Bad Homburg, Germany). Scanned images were analyzed with the software Image Studio Lite (LI-COR, Bad Homburg, Germany) and fluorescence intensity (FI) values extracted. For analysis, the mean of the fluorescence intensity values and the Fold Change (FC = ($FI_{WT}$/$FI_{Mut}$)-1); WT. vs. Mut) were calculated in R (Version 4.0.3, (The R Core Team; 2020)).

## 2.9 | Surface Plasmon Resonance (SPR)

Characterization of the patient sera by surface plasmon resonance was performed using the Biacore T200 system (Cytiva, Uppsala, Sweden). The purified RBD variants were diluted 1:10 to reach a concentration of 20 μM in immobilization buffer (10 mM HEPES +0.05% Tween-20, pH 7.0) for immobilization on two CM5 sensor chips (Cytiva, Uppsala, Sweden). Final response levels of 6622 RU (B.1.1.7), 6227 RU (B.1.1.298), and 6039 RU (MRS) were obtained on chip 1 and 7811 RU (B.1.351), 6656 RU (E484K), and 7031.1 RU (MRS) on chip 2. On each chip, one flow-cell remained blank to allow for background subtraction. Before starting the run, all sera were diluted 1:20 in running buffer (10 mM HEPES pH 7.1, 3 mM EDTA, 150 mM NaCl +0,05% Tween) supplied with 10% NSB reducer (Cytiva, Uppsala, Sweden). After three start-up cycles with negative serum to block unspecific binding, patient sera were applied with a contact time set to 180 s, followed by 180 s dissociation. As described by Ju et al., competition of the patient sera with ACE2-binding was examined by addition of ACE2 (Sino Biological, Jing Dongbei, China) for 60 s at a concentration of 0.1 μM [21]. Subsequently, two regeneration steps were performed using 10 mM Gly-HCl pH 2.5 for 60 s and 30 s, respectively. Anti-His (Santa Cruz Biotechnology, Dallas, USA) as well as anti-RBD antibodies (Sino Biological, Peking, China) served as control samples at the beginning and end of each run as well as five times distributed evenly throughout the run.

To compensate for reduction of binding capacity of the surface over time, patient serum responses were adjusted for the anti-RBD controls using the Biacore T200 evaluation software (Cytiva, Uppsala, Sweden). In addition, results were normalized on the mean anti-His response to compensate different immobilization levels of the RBD variants. Responses of ACE2 binding were adjusted for anti-His controls, as no competition of the anti-His antibody with ACE2 binding was expected.

### 2.9.1 | Statistical Analysis

Statistical analysis was performed with the software Prism 8 (GraphPad) by using the default setting. The analysis of the peptide arrays was performed with R (Version 4.0.3).

# 3 | RESULTS

## 3.1 | Decreased binding of vaccine-elicited and convalescent sera to the B.1.351-derived RBD

In order to characterize the immune response elicited by two different mRNA-based vaccines, we compared sera obtained from individuals vaccinated either with BNT162b2 or with CVnCoV (or fractional doses thereof) and convalescent sera by ELISA for detection of RBD-binding IgGs. For the ELISA, the RBD domains derived from an early isolate (MRS), from the cluster V (lineage B.1.1.298; containing the Y293F mutation in the RBD)-, from the B.1.1.7 (Alpha; containing the N501Y mutation in the RBD)- or from the B.1.351 (Beta; containing the K417N, E484K and N501Y mutations in the RBD)-variant were used as target antigen. Moreover, the RBD domain harboring the exclusively the E484K mutant was instrumental.

In case of the BNT162b2 vaccine, sera from a cohort of vaccinated health care workers were obtained, in case of the CVnCoV vaccine, a set of sera from participants of a phase I/II trial were provided by Curevac [22]. The participants of the phase I trial were vaccinated with 2, 4, 6, 8, or 12 μg. For each group sera with high and low titer were provided. Convalescent sera were from hospitalized patients who suffered from acute respiratory distress syndrome (ARDS) or had no ARDS (27 patients without ARDS and 13 patients with ARDS). For all tested antigens, the ELISA revealed a significant higher titers in case of sera derived from individuals vaccinated with the standard dose of BNT162b2 (30 μg) as compared to convalescent sera. The titer of sera obtained from CVnCoV-vaccinated individuals were lower even if the full dose of CVnCoV (12 μg) was used for immunization and the "high titer" group was exclusively considered (Figure 1). When RBDs of different VOCs were used as target antigen, it was observed that sera derived from BNT162b2 vaccinated individuals contain a slightly reduced amount of binding antibodies in case of the B.1.1.7 variant and more pronounced if the RBD derived from the B.1.351 isolate was tested (Figure 1A, B). In case of the convalescent sera, binding is strongly reduced when RBDs derived from the B.1.351 variant or of the E484K mutant were used as antigen. The set of sera with

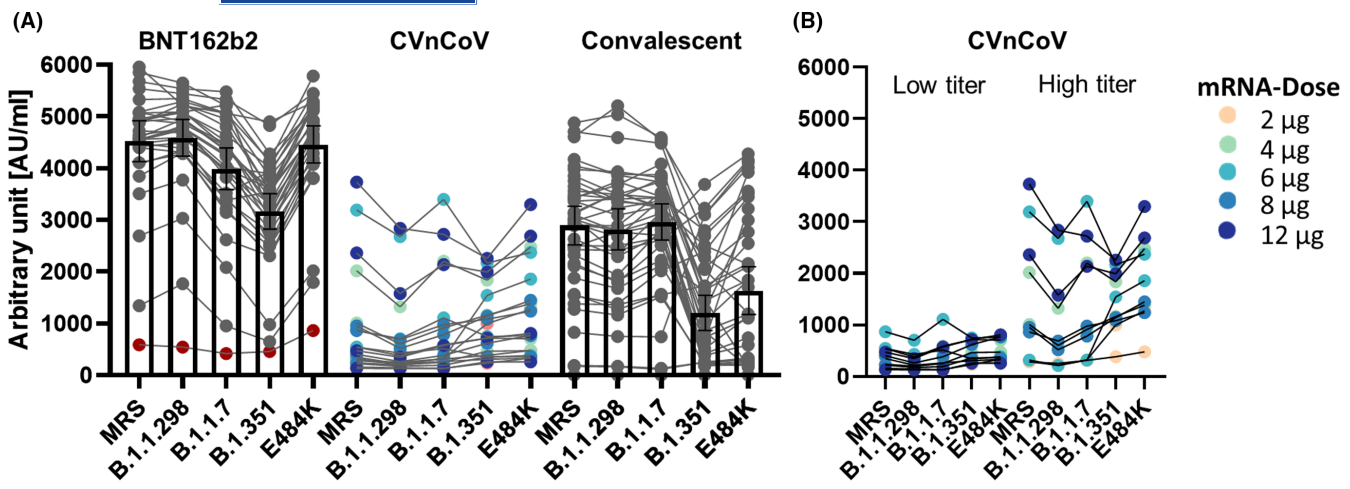



FIGURE 1 Antibody responses against different SARS-CoV-2 RBD proteins. A, Sera from either BNT162b2 (35 sera) or CVnCoV (20 sera) vaccinated individuals or convalescent sera (40 sera) were tested by ELISA using the purified RBD domain from the MRS virus, the B.1.1.7, the B.1.351, the B.1.1.293 variant, or the E484K mutant as immobilized antigen. B, CVnCoV sera from A subdivided into those previously classified as high titer and low titer. The all values measured in duplicate and normalized against the Anti-His coating control. Bars represent the geometric mean including the 95% confidence intervals. The red dot marked patient is immunosuppressed and not included in the statistical analysis. The different CVnCoV dose groups are colored as described in the figure. AU/ml: arbitrary unit per milliliter

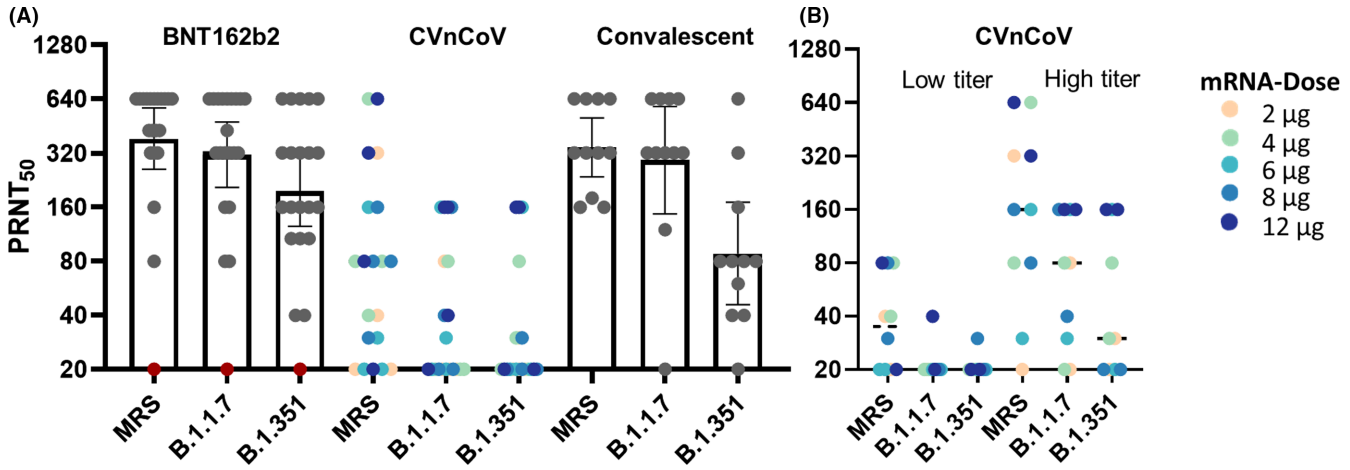



FIGURE 2 Neutralization of SARS-CoV-2 variants. A, Plaque reduction neutralization titer of 21 BNT162b2- or 20 CVnCoV-vaccinated individuals against SARS-CoV-2, the B.1.1.7 variant and the B.1.351 variant. 11 convalescent sera were tested. B, CVnCoV sera from A subdivided into those previously classified as high titer and low titer. Neutralization is represented by the $PRNT_{50}$ (the 50% plaque reduction neutralization titer, the reciprocal of the 50% inhibitory dilution per serum. Bars represent the geometric mean including the 95% confidence intervals. The red dot marked patient is immunosuppressed and not included in the statistical analysis. The different CVnCoV dose groups are colored as described in the figure [Color figure can be viewed at wileyonlinelibrary.com]

a high titer derived from CVnCoV-vaccinated individuals showed a decreased binding to the variants as compared to the BNT162b2-derived sera. For the B.1.1.298 and B.1.351 variants, decreased binding as compared to the other variants was observed.

Taken together, these data indicate that the analyzed sera differ with respect to their titer of binding antibodies and the impact of mutations in the RBD on the titer.

## 3.2 | Reduced neutralization of VOCs

As the titer of binding antibodies does not automatically reflect the titer of neutralizing antibodies, plaque reduction tests ($PRNT_{50}$) were performed using the MRS isolate and the B.1.1.7 and B.1.351 variants (Figure 2A, B). The assays revealed for the convalescent sera and for the BNT162b2-elicited sera a high neutralization capacity, which is only slightly reduced in case of the B.1.1.7 isolate. However, a stronger reduction, especially in case of the convalescent sera, is observed in case of the B.1.351 isolate. For the CVnCoV-elicited sera, the neutralizing capacity was lower as compared to the BNT162b2 and to the convalescent sera and does not strictly correlate with the amount of RNA used for vaccination and with the titer of binding antibodies as detected by ELISA. As compared to the MRS isolate, for both analyzed VOCs, a reduction in the neutralization capacity can be observed for the CVnCoV sera for all analyzed dose groups. Moreover, only the 12 µg mRNA dose in the high titer group shows

a neutralization effect against all SARS-CoV2 variants. (Figure 2B). These data indicate that BNT162b2- and CVnCoV-elicited sera differ with respect to their neutralizing capacity. For all analyzed sera groups, a significant impact on the neutralizing capacity against the B.1.351 variant was found.

### 3.3 | BNT162b2-elicited sera have a higher proportion of slow-dissociating antibodies

For a more detailed characterization of the immune response, the stability of the antigen-antibody complexes was analyzed by surface plasmon resonance spectroscopy (Figure 3). The RBD domains (MRS isolate, B.1.1.298, B.1.1.7, B 1.351 variant or the mutant E484K) were immobilized. Based on the dissociation phase, half-lives of the antigen-antibody-complexes were calculated, and two fractions were defined: the fast-dissociating antibodies (low affinity antibodies) characterized by short half-life of the antigen-antibody complex (Figure 3A, B) and the slow-dissociating antibodies (high affinity antibodies) with longer half-lives of the antigen-antibody complexes (Figure 3C, D). In case of the BNT162b2-elicited sera, proportion of the fast-dissociating antibodies was bigger as compared to the sera obtained from the CVnCoV vaccinations and to convalescent sera (Figure 3E, F). Moreover, within the slow fraction of the BNT162b2 sera, there is a major fraction of long-lived complexes while the majority of the complexes in the CVnCoV and convalescent sera have a shorter half-life.

Altogether, the SPR analyses indicate that for the wild-type, the B.1.1.298 and the B.1.1.7 variant the BNT162b2-elicited sera contain a bigger amount of high-affinity antibodies as compared to the CVnCoV-elicited and convalescent sera.

### 3.4 | CVnCoV-induced sera fail to compete with the ACE2/RBD interaction in SPR analyses

To further characterize the relevance of the antigen-antibody complex, the impact of the vaccine-elicited or convalescent sera on the interaction of the RBD domain with ACE2 was studied by SPR as described in the Methods Section. For the interaction of wild-type RBD with insect-cell-derived ACE2, a steady-state $K_D$ value of 189.5 nM was found (Figure 4A). The interaction of ACE2 with the RBD domain of the MRS isolate, the E484K mutant and the B.1.351 variant in the presence of vaccine-elicited or convalescent sera revealed that under these conditions almost all of the CVnCoV-elicited sera have a weak or no impact on the RBD-ACE2 interaction (Figure 4B). In contrast to this, almost all of the BNT162b2 sera and of the convalescent sera were capable of significantly interfere with the ACE2/RBD interaction. Here, the strongest inhibitory effect of ACE2 binding is observed when the MRS-derived RBD is used as ligand, a weaker effect found for the E484K mutant and for the B.1.351 variant. These results fit to the antigen-antibody complex stability data described above, showing that the CVnCoV sera contain a higher fraction of fast-dissociating antibodies and therefore have a lower potential to compete the ACE2/RBD interaction. Taken together, these data indicate in accordance to the affinity data that most of the BNT162b2-elicited sera and of the convalescent impair the binding of ACE2 to RBD, while most of the CvnCoV-elicited sera fail to exert a robust effect on the ACE2-RBD interaction under these assay conditions.

### 3.5 | The pattern of linear epitopes recognized by vaccine-elicited and convalescent sera differs

Finally, the recognition of linear epitopes covering amino acids K417, E484, or N501 by the sera was analyzed by peptide arrays (Figure 5 and Figure S1). These amino acids were chosen as they are frequently mutated in a variety of SARS-CoV-2 variants. Quantification of the antibodies bound to these epitopes and visualization by the heatmap (Figure 5A) revealed that BNT162b2- and CVnCoV-elicited sera strongly recognize linear epitopes harboring K417, while the recognition by convalescent sera is detectable but less pronounced. Linear epitopes covering E484 are moderately recognized by the vaccine-elicited and convalescent sera. Linear epitopes containing N501 are better recognized by the vaccine-elicited sera than by the convalescent sera. Comparison of the binding to the wild-type sequence with the binding to the mutated sequence revealed that the K417N mutation has almost no effect on the binding of the analyzed vaccine-elicited sera and convalescent sera (Figure 5B). The E484K and especially the N501Y mutation affected the binding of convalescent sera to the corresponding linear epitopes but this effect was less pronounced compared to the effect on the CVnCoV- and BNT162b2 elicited sera. Especially in case of the N501Y mutation there was a strong decrease in the binding of BNT162b2- and CVnCoV-elicited sera (Figure 5C). This effect was more distinct for the BNT162b2-sera. (Figure 5B).

These data indicate that sera elicited by the RNA vaccines BNT162b2 and CVnCoV strongly recognize linear epitopes encompassing K417 and less distinct N501. However, the binding to the linear epitope around aa417 is not significantly affected by the K417N mutation, while the E484K and the N501Y mutation caused a stronger decrease of the binding to these epitopes, especially in case of the BNT162b2-elicited sera.

## 4 | DISCUSSION

The study characterizes the SARS-CoV-2 spike-RBD-specific humoral immune response elicited by two different mRNA vaccines (BNT162b2 and CVnCoV) and by convalescent sera. The analysis revealed that there is a variety of differences between the investigated sera. It has to be mentioned that most of the CVnCoV-elicited sera were derived from individuals vaccinated with doses lower than the finally selected 12 μg RNA, which was used in the phase III trials. However, published data from the phase I trial and our data indicate that there is only a minor impact of the dose on the titer of

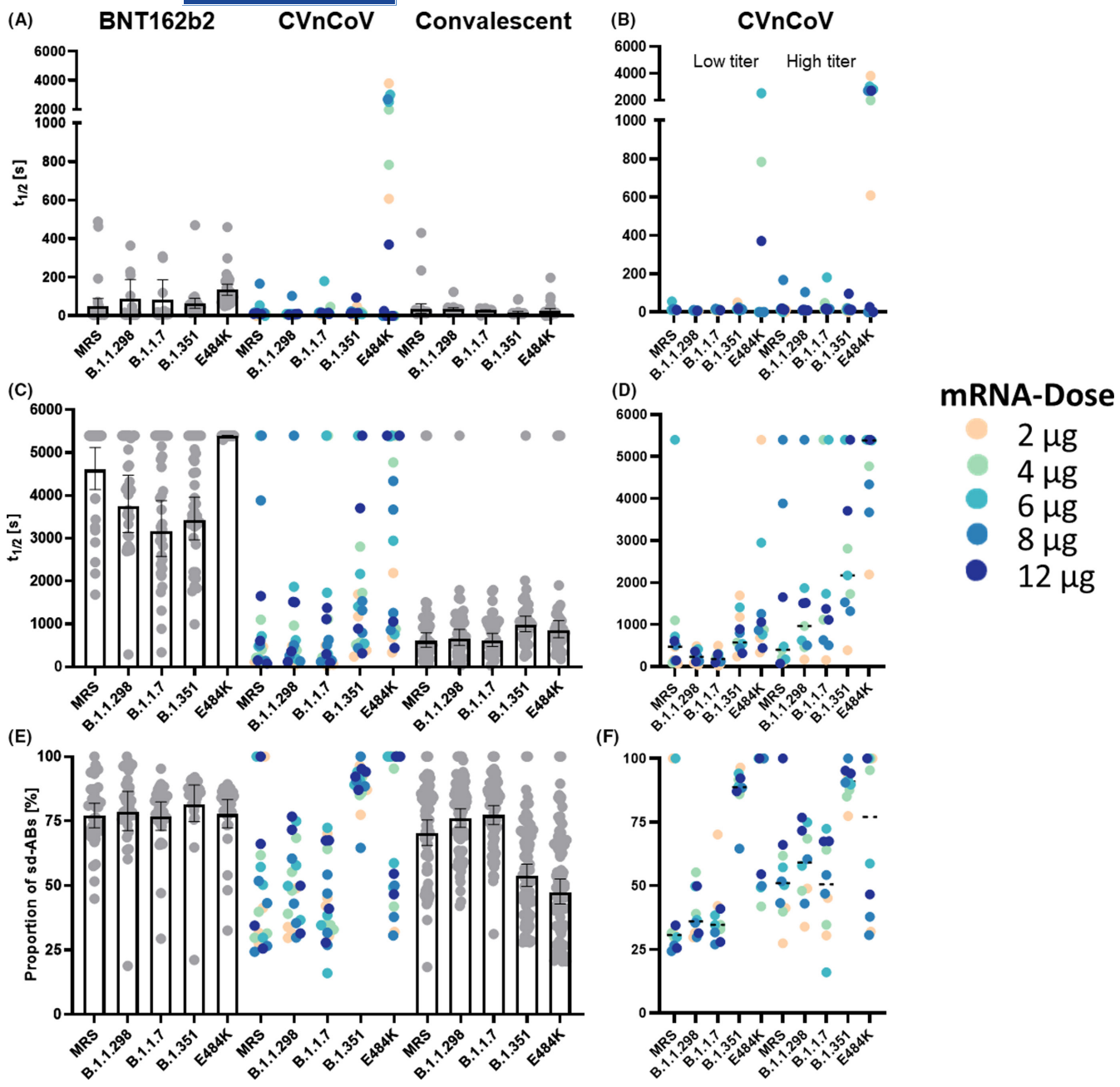



FIGURE 3 Stability of the RBD-antibody complexes. Purified RBD proteins corresponding to the wild-type, B.1.1.298, the B.1.1.7, and the B.1.351 isolate, and E484K variants were immobilized on CM5 sensor chips. The SPR measurement was performed using a Biacore T200 system (see experimental section for details). Sera from 35 BNT162b2 and from 20 CVnCoV-vaccinated individuals and 40 convalescent sera were analyzed. A, Half-lives of the fast dissociating antibodies anti-RBD antibodies. C, Half-lives of the slow-dissociating antibodies anti-RBD antibodies. E, Calculated percentage of slow-dissociating antibodies. B, D and F CVnCoV sera from A, C and E subdivided into those previously classified as high titer and low titer. The different CVnCoV dose groups are colored as described in the figure. To determine the stability of the antigen-antibody complex, sera were applied with a contact time of 180 s followed by a dissociation time of 180 s. The calculation was performed in the Biacore T200 evaluation software using the equation describing a simultaneous dissociation of two independent populations. sd-AB: slow-dissociating antibodies anti-RBD antibodies [Color figure can be viewed at wileyonlinelibrary.com]

spike-specific IgG or on the neutralizing capacity after the second vaccination [22].

ELISAs using the wild-type RBD or RBD variants (B.1.1.7, B.1.351 B.1.1.298 or the point mutation E484K) revealed for the CVnCoV-elicited sera, even if 12 µg were used for immunization, a lower titer of binding IgGs as compared to the BNT162b2-elicited sera or to the majority of convalescent sera. This correlates with the data from the neutralization experiments that demonstrate a lower neutralizing capacity of the CVnCoV-elicited sera as compared to convalescent or BNT162b2-elicited sera.

Apart from the lower titer, the weaker binding of CVnCoV-elicited antibodies to the RBD as determined by SPR could be a relevant

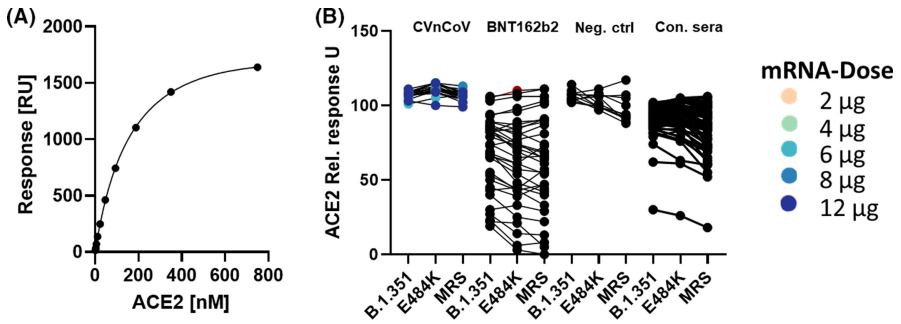



FIGURE 4 BNT162b2 and convalescent sera contain higher amounts of antibodies against the ACE2 interface of RBD. Purified RBD proteins corresponding to the wild-type, the B.1.351 isolate and E484K variant were immobilized on a CM5 sensor. The analysis was performed using a BiacoreT200 system. A, Kinetic properties of insect cell-derived ACE2 against the wild-type RBD. Serial 2-fold dilutions of ACE2 (starting with 750 nM) were injected. After an association of 200 s, the respective RUs were visualized and used for the calculation of steady-state affinity. ACE2-RBD $K_D$ of 189.5 nM was calculated. Sera from 35 BNT162b2 and from 20 CVnCoV-vaccinated individuals and 40 convalescent sera were analyzed. B, SPR measurements were performed as shown in Figure 2. After the dissociation step of 180 s, ACE2 was added for 60 s followed by a second dissociation time of 180 s. The ACE2 binding stability was calculated after 60 s and normalized against the ACE2 binding stability from the anti-His-Tag antibody (immobilization control; see experimental section for details). The red dot marked patient is immunosuppressed. The different CVnCoV dose groups are colored as described in the figure. Neg. ctrl: negative control sera; Con. Sera: convalescent sera [Color figure can be viewed at wileyonlinelibrary.com]

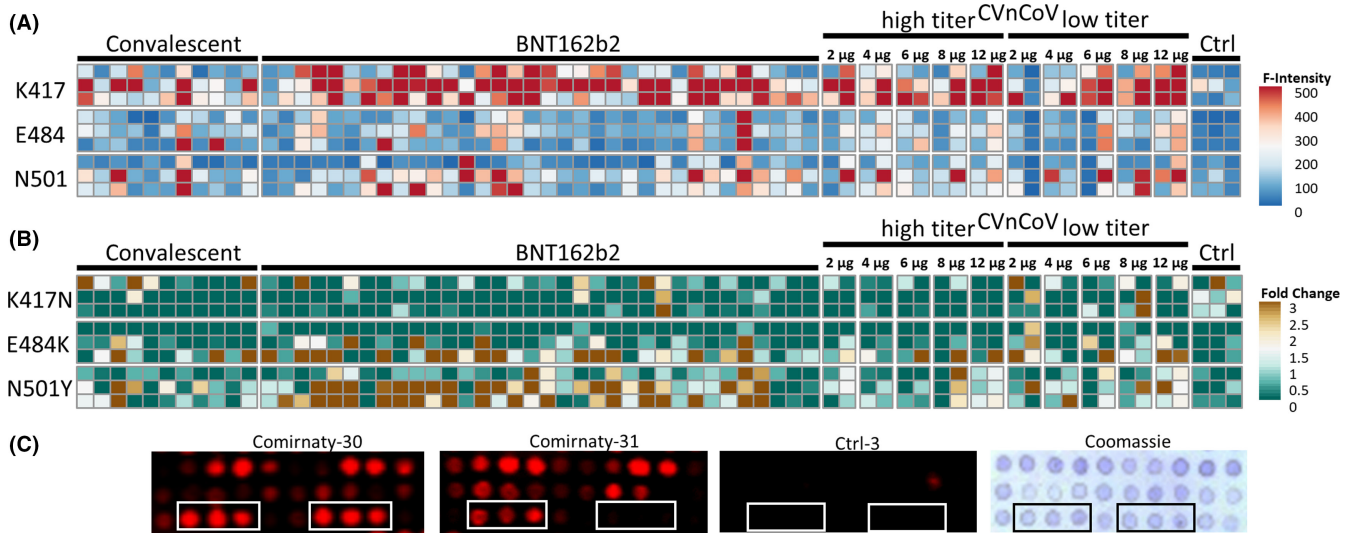



FIGURE 5 Linear epitopes are discriminated by mutation E484K and N501Y. A, Fluorescence intensities of recognized wild-type peptides. Sera from 34 BNT162b2 and from 20 CVnCoV-vaccinated individuals, 40 convalescent sera and three negative control sera were analyzed. B, Fold-change values of the fluorescence intensity of wild-type peptides compared with the respective mutated peptide (WT vs. Mut). As a threshold for possible epitope discrimination by the mutation, an FC greater than 2 was set in at least two adjacent peptides. C, Peptide arrays of selected sera. Shown are the peptide arrays of three different sera and a Coomassie-stained array. In the sera BNT162b2-30 and BNT162b2-31 the influence of the mutation in the peptide array can be seen directly (white boxes). The right white boxes show the wild-type N501 peptides, the left boxes the mutation-bearing N501Y peptides. The negative serum shows no significant signal for any peptide. To rule out the possibility that the difference in intensity was due to a difference in the amount on peptide of the spots, 5 arrays were stained with Coomassie after each synthesis. No difference is detectable the Coomassie-stained array for the N501 and N501Y peptides. The peptide sequences are shown in Figure S1. Ctrl: negative control sera; F-Intensity: fluorescence intensity [Color figure can be viewed at wileyonlinelibrary.com]

factor for the decreased neutralizing activity. In case of BNT162b2-elicited sera and convalescent sera, a higher fraction of highly stable antibody-RBD complexes was found. In contrast to SARS-CoV-2, the interaction of SARS-CoV-2 with ACE2 is characterized by high affinity [23]. In light of this, the weaker binding of RBD antibodies in case of the CVnCoV-elicited sera fail to inhibit the RBD-ACE2 interaction. This might be a relevant factor that contributes to the reduced neutralization capacity in vitro and the lower vaccine efficacy of CVnCoV in clinical trials as compared to BNT162b2 [13,14]. In the meantime further variants of interest or variants of concern (B.1.617.2 (Delta), B.1.525 (Eta), Iota (B.1.526), B.1.617.1 (kappa), C.37 (Lambda)) emerged. As these variants were not circulating in the period when the efficacy data were generated they were not analyzed in this study. The data from the peptide arrays reveal that there are qualitative

differences between the vaccine-elicited sera on the one hand and the convalescent sera on the other hand. While the vaccines studied here are based on a stabilized spike, the spike in the wild-type virus exists in a non-stabilized form and in a context with further structural proteins. This might affect the antigenicity and thereby the pattern of recognized linear epitopes by the analyzed sera.

CVnCoV-immunized hamsters evolve a protective immune response [10]. However, as compared to the 12 μg RNA used for the vaccination of humans, the 2 or 10 μg used for the hamsters represent an artificially high concentration. An interim analysis of the efficacy analysis revealed for the CVnCoV vaccine a significant lower vaccine effectiveness in comparison to BNT162b2 [13,24]. For CVnCoV, the vaccine efficacy against symptomatic COVID-19 was approximately 48% in the overall study population and 53% in the 18–60 years of age subgroup. A higher efficacy of about 77% was observed in the prevention of moderate to severe disease for the group of 18–60-year-old individuals.

There might be a variety of factors contributing to this result. The study was performed under conditions in which especially the B.1.1.7 lineage and other variants were dominating among the circulating viruses. For the B.1.1.7 neutralization analyses and especially "real world" vaccine effectiveness, data revealed for BNT162b2 a slightly reduced but still high vaccine effectiveness of 93,4% [25]. For the B.1.351 variant neutralization assays revealed a reduced but still robust neutralization capacity of BNT162b2-elicited sera [26]. As the structure of the antigen used for both mRNA vaccines is comparable (all are based on a stabilized spike protein in the prefusion conformation), other factors could contribute to the lower titer and vaccine efficacy. As compared to BNT162b2 or mRNA-1273, which are based on modified RNA and are applied in amounts of 30 μg and 100 μg respectively, CVnCoV is based on unmodified RNA. The reactogenicity of unmodified RNA limits the amount of mRNA, which can be used for vaccination. The amount of RNA and their translation efficacy affect the amount of produced antigen. It can be speculated that modified RNA and unmodified RNA differ with respect to their half-life. Differences in the stability and in the amount of delivered mRNA might affect the persistence of spike protein production and the amount of spike protein. Apart from the effect on the titer, both parameters might have an impact on the immune response ie, by affecting the affinity maturation and the T helper cell [27]. In light of this, attempts to increase the amount of antigen produced were started by development of a second generation of the vaccine (CV2CoV). Here, a higher amount of produced antigen is found that is expected to contribute to a higher vaccine effectiveness [10,28,29].

Taken together, analyses of antisera elicited by regular vaccination with BNT162b2 or by vaccination with different amounts of CVnCoV (2–12 μg) and of convalescent sera revealed that the two RNA vaccines that encode the same antigen (spike in the prefusion conformation) significantly differ with respect to the elicited humoral immune response. Even for the 12 μg CVnCoV-vaccinated group, differences in the titer, neutralization capacity and stability of the antigen-antibody complex were observed as compared to BNT162b2. Although effectiveness of a RNA vaccine depends on more parameters than serological parameters, the lower titer and neutralizing activity, the high fraction of low affinity antibodies that fail to compete the neutralization of ACE2 binding could contribute to a decreased vaccine effectiveness of CVnCoV.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTION

SH, MLH, IM, DO, and EH designed the research. SH, MLH, IM, DB, VH, NB, JE JS, and CM performed experiments. CH, MD, and JCS collected the clinical material, JCS organized provision of the clinical samples. JS, CM, MB, and TW provided virus isolates. SH, MLH, IM, DB, and EH analyzed the data, SH, MLH, IM, and EH wrote the manuscript. All authors read the manuscript.

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## SUPPORTING INFORMATION

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