Original article

Humoral and cell-mediated response against SARS-CoV-2 variants elicited by mRNA vaccine BNT162b2 in healthcare workers: a longitudinal observational study

Irene Cassaniti [1,2], Federica Bergami [1], Elena Percivalle [1], Elisa Gabanti [1], Josè Camilla Sammartino [1], Alessandro Ferrari [1], Kodjo Messan Guy Adzasehoun [1], Federica Zavaglio [1], Paola Zelini [3], Giuditta Comolli [1,4], Antonella Sarasini [1], Antonio Piralla [1], Alessandra Ricciardi [5], Valentina Zuccaro [5], Fabrizio Maggi [6,7], Federica Novazzi [7], Luca Simonelli [8], Luca Varani [8], Daniele Lilleri [1,*], Fausto Baldanti [1,2]

[1] Molecular Virology Unit, Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[2] Department of Clinical Surgical Diagnostic and Paediatric Sciences, Università degli Studi di Pavia, Italy
[3] Obstetrics and Gynaecology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[4] Laboratory of Biochemistry-Biotechnology and Advanced Diagnostics, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[5] Infectious Diseases I, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[6] Laboratory of Microbiology, ASST Sette Laghi, Varese, Italy
[7] Department of Medicine and Surgery, University of Insubria, Varese, Italy
[8] Institute for Research in Biomedicine, Bellinzona, Switzerland

ARTICLE INFO

ABSTRACT

Objectives: To assess the humoral and cell-mediated response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) elicited by the mRNA BNT162b2 vaccine in SARS-CoV-2-experienced and -naive subjects against a reference strain and SARS-CoV-2 variants.
Methods: The humoral response (including neutralizing antibodies) and T-cell-mediated response elicited by BNT162b2 vaccine in 145 healthcare workers (both naive and positive for previous SARS-CoV-2 infection) were evaluated. In a subset of subjects, the effect of SARS-CoV-2 variants on antibody level and cell-mediated response was also investigated.
Results: Overall, 125/127 naive subjects (98.4%) developed both neutralizing antibodies and specific T cells after the second dose of vaccine. Moreover, the antibody and T-cell responses were effective against viral variants since SARS-CoV-2 NT Abs were still detectable in 55/68 (80.9%) and 25/29 (86.2%) naive subjects when sera were challenged against β and δ variants, respectively. T-cell response was less affected, with no significant difference in the frequency of responders (p 0.369). Of note, two doses of vaccine were able to elicit sustained neutralizing antibody activity against all the SARS-CoV-2 variants tested in SARS-CoV-2-experienced subjects.
Conclusions: BNT162b2 vaccine elicited a sustained humoral and cell-mediated response in immunocompetent subjects after two-dose administration of the vaccine, and the response seemed to be less affected by SARS-CoV-2 variants, the only exceptions being the β and δ variants. Increased immunogenicity, also against SARS-CoV-2 variant strains, was observed in SARS-CoV-2-experienced subjects. These results suggest that triple exposure to SARS-CoV-2 antigens might be proposed as valuable strategy for vaccination campaigns. **Irene Cassaniti, Clin Microbiol Infect 2022;28:301.e1–301.e8**
© 2021 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

---

* Corresponding author. Daniele Lilleri, Molecular Virology Unit, Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.
E-mail address: d.lilleri@smatteo.pv.it (D. Lilleri).

Introduction

The mRNA BNT162b2 vaccine [1], the first authorized for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, showed 95% protection against SARS-CoV-2 infection in a phase II/III trial [2]. Another mRNA-based vaccine, mRNA-1273 [3], showed a similar effect. However, data on the kinetics of the immune response elicited by the vaccines are limited to low numbers of analysed subjects, and limited mainly to antibody responses [1,4–8].

The T-cell response elicited by the vaccine may have a crucial role in the long-term protection against SARS-CoV-2 infection and disease. In convalescent subjects, T- and B-cell memory specific for SARS-CoV-2 was found to persist for at least 6–8 months [9–11].

The emergence of new SARS-CoV-2 variants with mutations in the spike (S) protein has raised significant concerns about vaccine efficacy and reinfection risk in previously infected subjects. The new variant 501Y.V1 lineage B.1.1.7 (UK variant or α) includes multiple mutations in both the receptor binding domain (RBD) and the N-terminal domain of the S protein [12], and the 501Y.V3 lineage P.1 (Brazilian, BZ or γ) [13] and the 501Y.V2 lineage B.1.351 (South African, SAF, or β) variants have mutations in the S protein and, especially, in the RBD [14]. More recently, a lineage B.1.617.2 (δ variant) has emerged.

Preliminary data have suggested that convalescent sera and sera from vaccinated individuals efficiently neutralized the α variant, while a reduction in neutralizing (NT) antibody titres has been observed against the β variant [15,16].

In the present study we evaluated humoral and cell-mediated responses elicited by the BNT162b2 vaccine in subjects previously exposed to SARS-CoV-2 and in naive subjects. Moreover, we aimed to define the level of both antibody and cell-mediated responses against the emerging SARS-CoV-2 variants after vaccination.

Methods

We designed an observational, longitudinal, prospective study to evaluate the immune response elicited by the BNT162b2 vaccine against SARS-CoV-2 in 145 healthcare workers (median age 44 years, range 21–69) who received vaccination between 27th December 2020 and 11th February 2021; of these, 127 were SARS-CoV-2-naive and 18 were SARS-CoV-2-experienced before vaccination. All the subjects were enrolled at Fondazione IRCCS Policlinico San Matteo (Pavia, Italy). The efficacy endpoints were the development of SARS-CoV-2-specific neutralizing antibodies and a T-cell response. Analyses were performed at baseline (before vaccination), at the time of the second vaccine administration (T1), and 21 days after the second vaccine dose (T2).

Antibody response was determined using the chemiluminescent assay Elecsys Anti-SARS-CoV-2 S (Roche Diagnostics Rotkreuz, Switzerland), which provides quantitative measures of antibody (mainly IgG) specific for SARS-CoV-2 RBD. Results are given as units (U)/mL and are considered positive when ≥0.8 U/mL. Moreover, SARS-CoV-2 neutralizing antibodies were quantified using a home-made assay and results higher than 1:10 were considered positive. IgG against RBD of the wild-type (WT) and European (EU, which share the same RBD), α and β strains were determined by ELISA using recombinant proteins.

The SARS-CoV-2-specific T-cell response was quantified by ex-vivo ELISpot assay, and results >10 spot-forming units (SFU)/million peripheral-blood mononuclear cells (PBMCs) were given as positive. Further methodological details are reported in the Supplementary Material.

Results

Antibody response in SARS-CoV-2-naive and -experienced vaccinated subjects

At T1, all the 18 SARS-CoV-2-experienced subjects showed anti-RBD antibody levels above the upper limit of the quantifiable range of the assay, while 122/127 SARS-CoV-2-naive subjects (96.1%) developed anti-RBD antibodies although at significantly lower levels than the experienced subjects did. Moreover, levels of anti-RBD antibodies in naive subjects at T1 were significantly lower (p 0.009) than the baseline levels of experienced subjects.

At T2, all the SARS-CoV-2-naive subjects developed a positive anti-RBD response; however, the median anti-RBD level was still significantly higher in SARS-CoV-2-experienced subjects than in naive ones (p < 0.001) (Fig. 1a).

A similar trend was observed for NT antibodies (Fig. 1b). Serum NT titres were significantly higher in SARS-CoV-2-experienced subjects than in naive ones at any time point.

SARS-CoV-2-specific T-cell response

All but three SARS-CoV-2-experienced subjects (15/18, 83.3%) showed S-specific T cells at baseline, and levels of S-specific T cells increased at T1 (p < 0.001) and had not increased further at T2 (p 0.510).

Among SARS-CoV-2-naïve subjects the percentage of responders was 69.3% at T1 and 98.4% at T2. Levels of S-specific T cells (Fig. 2a) significantly increased from T1 to T2 (p < 0.001); even if the level of S-specific T cells at T2 in experienced subjects was higher than that observed in naive subjects, the difference was not statistically significant (p 0.095). No significant correlation was observed between age and S-specific T-cell response at both T1 (r −0.02, 95%CI −0.21 to 0.17, p 0.8214) and T2 (r −0.17, 95%CI −0.32 to 0.04, p 0.107). Levels of N-specific T cells did not change with time in either experienced or naive subjects (Fig. 2b). Of note, 30% of SARS-CoV-2-naive subjects showed a detectable SARS-CoV-2-specific T-cell response against S and 18.2% against N at baseline. Data on phenotypical characterization of the T-cell response are shown in the Supplementary Material Figs S1 and S2.

At T1, the overall percentage of full responders (i.e. subjects developing both SARS-CoV2 S-specific T cells and NT antibody) was 77.8% (95%CI 70.1–84.0), corresponding to 100% of experienced and 68.8% of naive subjects (95%CI 60.2–76.3). After T2, the overall percentage of full responders was 98.6% (95%CI 95.0–99.8), corresponding to 100% of experienced and 98.4% of naive subjects (95%CI 94.4–99.7).

Antibody and T-cell response against SARS-CoV-2 variants

Sera collected at T2 from 31 SARS-CoV-2-naive subjects were tested against RBD of the D614 WT Chinese-derived strain, and the α variant and β variant strains. Median RBD-specific reciprocal antibody titres were 5838 (IQR 2675–16 351) for WT (and EU) strains, 3220 (801–9263) for α and 60.5 (<50–196) for β strains, respectively (Fig. 3a). A reduction of approximatively 50% and 99% of RBD-specific antibody titres was observed from WT to α and β strains, respectively (Supplementary Material Fig. S3a,b).

Sera collected at T2 from 61 naive and 16 experienced subjects were tested against the WT strain by neutralization assay and challenged against EU strain, α, β, and γ variants (Fig. 3b). Additionally, sera at T2 from 29 naive and 16 SARS-CoV-2-experienced
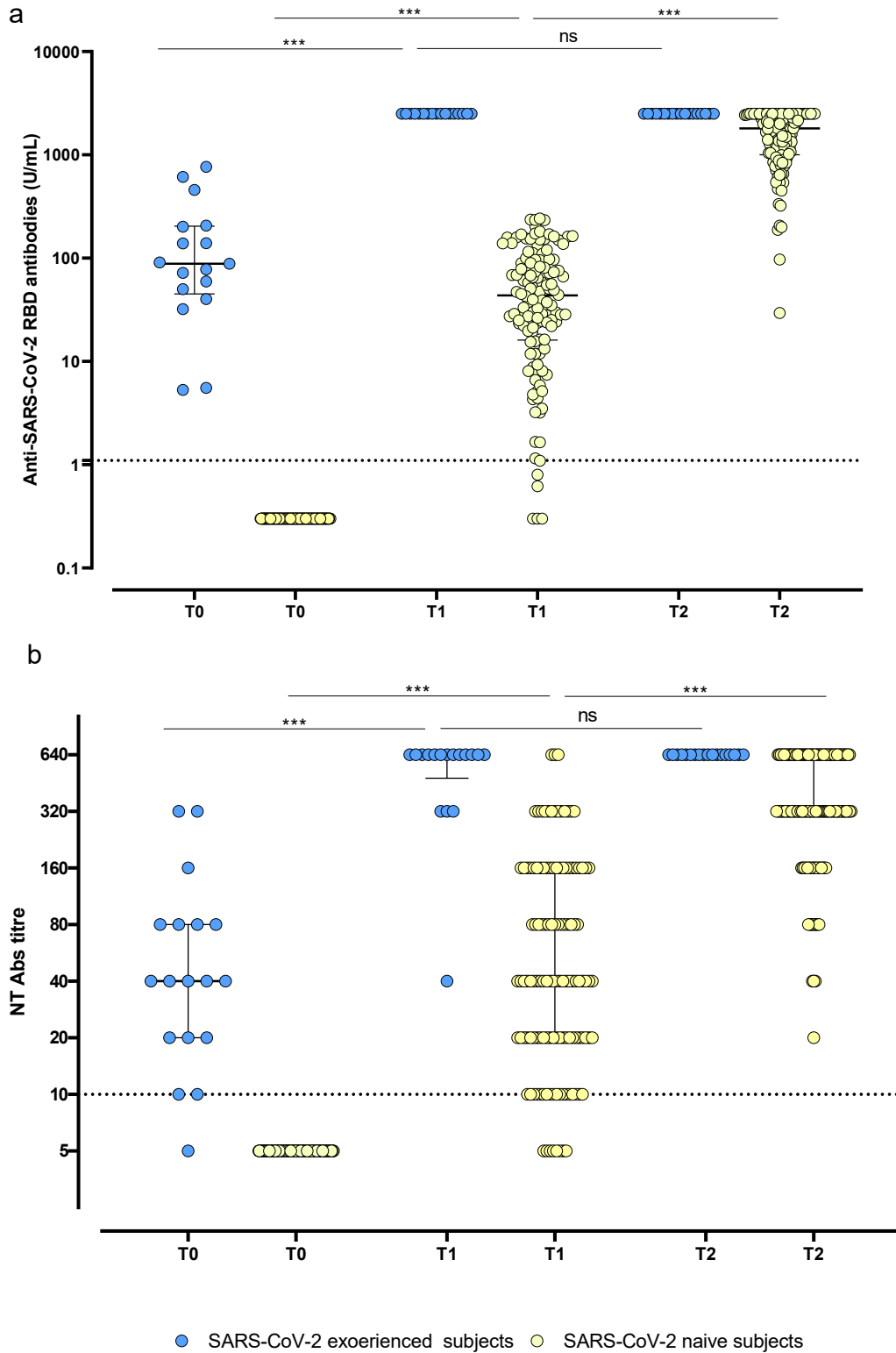
Fig. 1. Anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor binding domain (RBD) response measured by Elecsys Anti-SARS-CoV-2 S assay (A), and anti-SARS-CoV-2 neutralizing (NT) antibodies (B) measured at baseline (day of first dose administration, T0), day 21 (day of second dose administration, T1) and day 42 (21 days after the second dose administration, T2) in 145 healthy healthcare workers. Of these, 127 were naive (seronegative) and 18 were SARS-CoV-2-experienced (seropositive). The dotted horizontal line represents the positive level of responses.

subjects were challenged against the δ variant. Among the 61 SARS-CoV-2-naive subjects, an increase in four-fold dilution for SARS-CoV-2 NT antibodies was observed against the EU strain with respect to the WT strain (p < 0.001) (Supplementary Material Fig. S3d), while the level of SARS-CoV-2 NT antibodies was not affected when sera were tested against α and γ strains
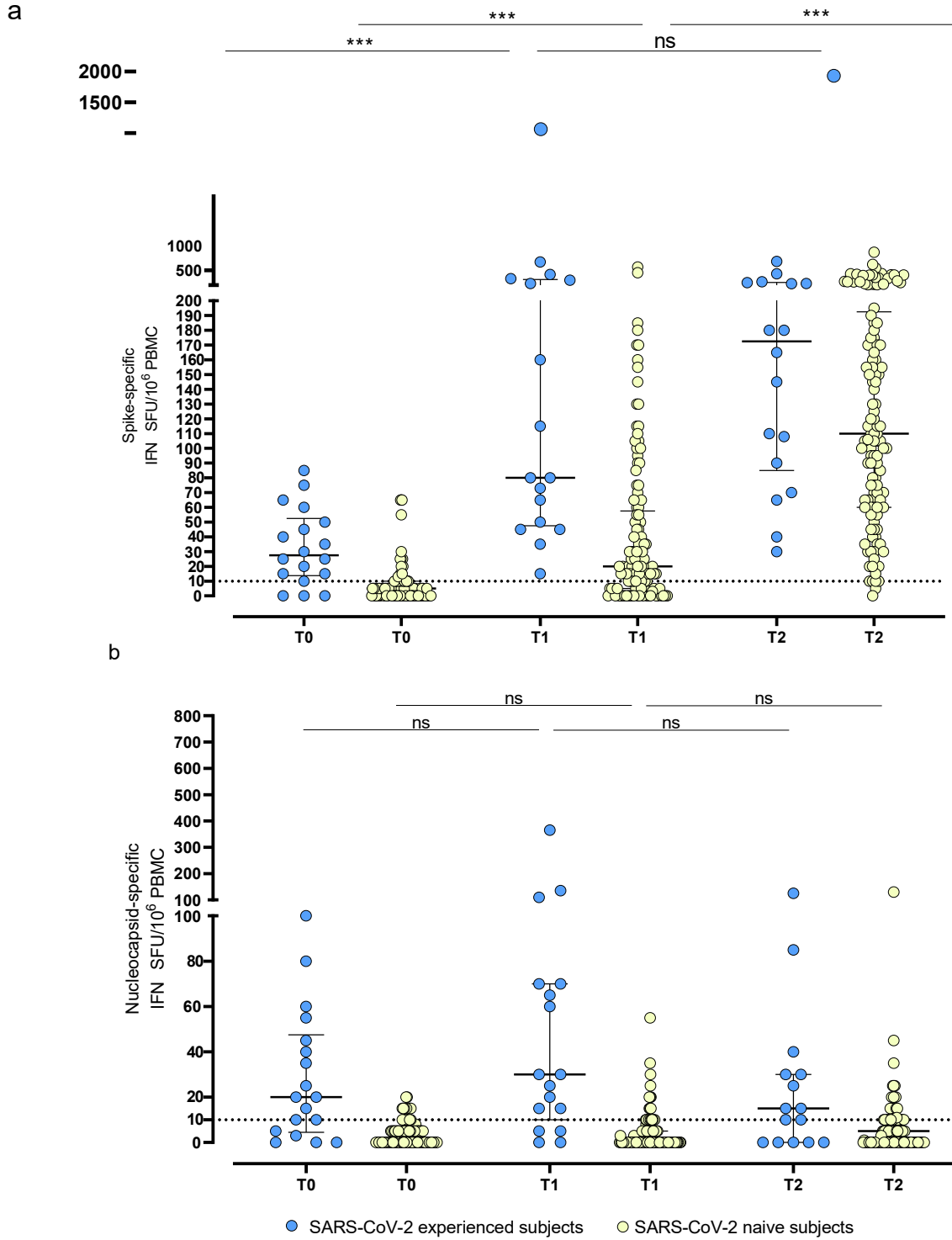
Fig. 2. Cell-mediated immune response against spike (S) and nucleocapsid (N) peptide pools in 145 vaccinated subjects—127 naive (seronegative) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and 18 who had experienced SARS-CoV-2 infection (seropositive)—at baseline (day of first dose administration, T0), at day 21 (day of second dose administration, T1) and at day 42 (21 days after the second dose administration, T2). The dotted horizontal line represents a positive cut-off for SARS-CoV-2 T-cell response.

(Supplementary Material Fig. S3e,f). A five-fold and 20-fold reduction in median titre of SARS-CoV-2 NT antibodies was observed in response to the β strain in comparison to the WT virus and EU strain, respectively (p < 0.001) (Supplementary Material Fig. S3g). However, although at lower levels, NT antibodies against the β variant were still detectable in 55/68 subjects (80.9%). Similarly, a decrease of five-fold and 20-fold of median titre of SARS-CoV-2 NT antibodies was observed in response to the δ strain
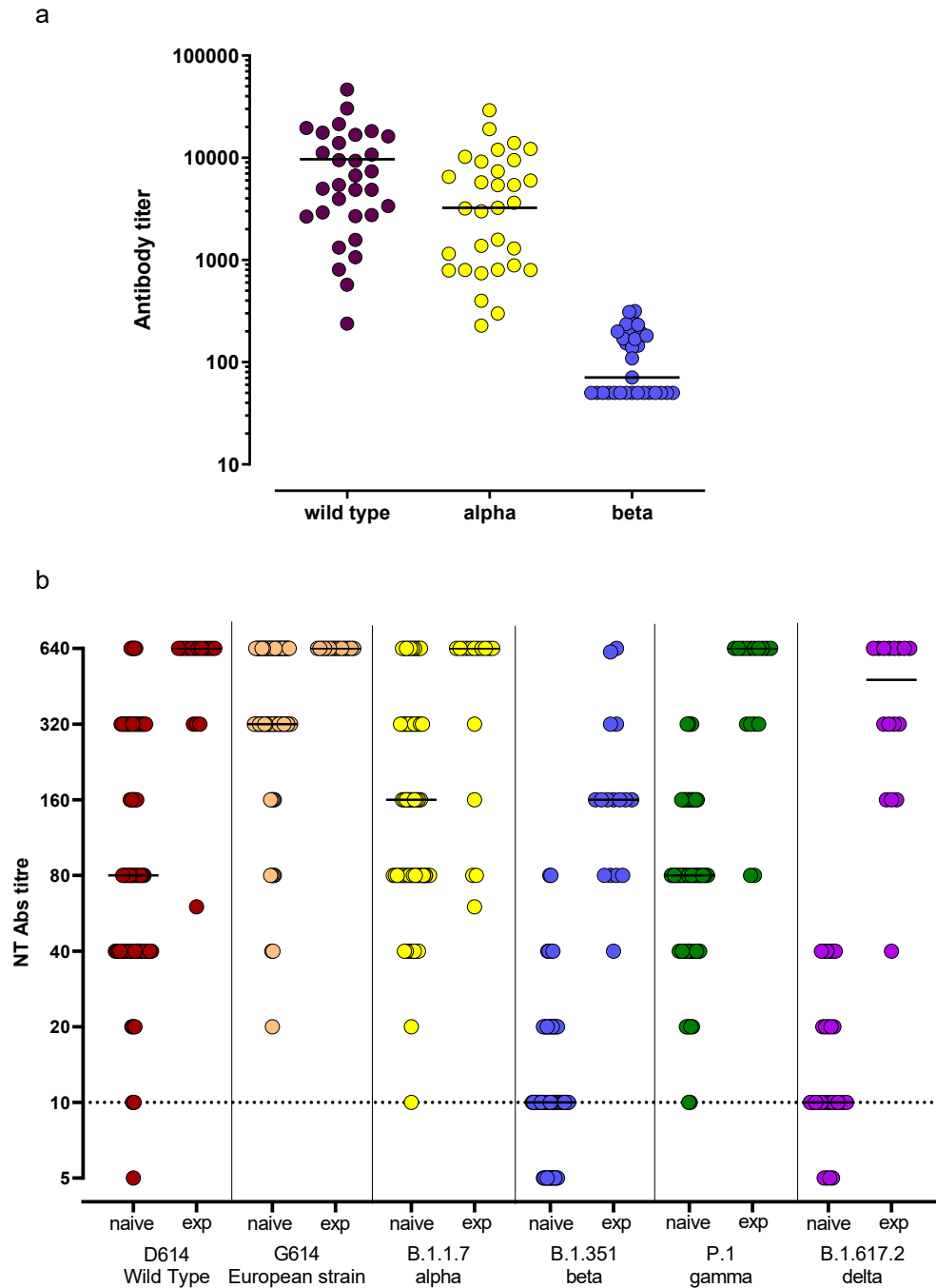
Fig. 3. (a) Median receptor binding domain (RBD)-specific antibody levels against wild type, α and β strains. (b) Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing (NT) antibodies against all the variants measured in SARS-CoV-2-naive and SARS-CoV-2-experienced (exp) SARS-CoV-2 infection.

in comparison to the WT and EU strains (p < 0.001), overall, the response was positive for the δ variant in 25/29 naive subjects (86.2%).

Among the 16 SARS-CoV-2-experienced subjects, a sustained NT level against the all tested variants was observed. The median reduction in SARS-CoV-2 NT against the β variant was four-fold with respect to the WT (p 0.008), and no reduction was observed when sera were challenged against the other variants.

In addition, sera from six vaccinated subjects, five of them naive for SARS-CoV-2 infection, were used to assess the NT level also against the C.36 and B.1.525 variants. No significant difference was

observed between SARS-CoV-2 NT titre against WT, α, β and Thai variants (p 0.999). Similar to what was observed for the β variant, a six-fold decrease in NT level was documented against the B.1.525 strain (p 0.081, Supplementary Material Fig. S4). Of note, no correlation was observed between RBD level and NT antibody titre for any variant (Supplementary Material Fig. S5).

In 36 vaccinated subjects (53%), residual PBMCs isolated at T2 were challenged against inactivated virus preparations from the supernatant of Vero E6 cells infected with five SARS-CoV-2 strains (WT, EU, α, β and γ) and the cell-mediated response was analysed by ELISpot assay. The ELISpot assay was less sensitive using WT
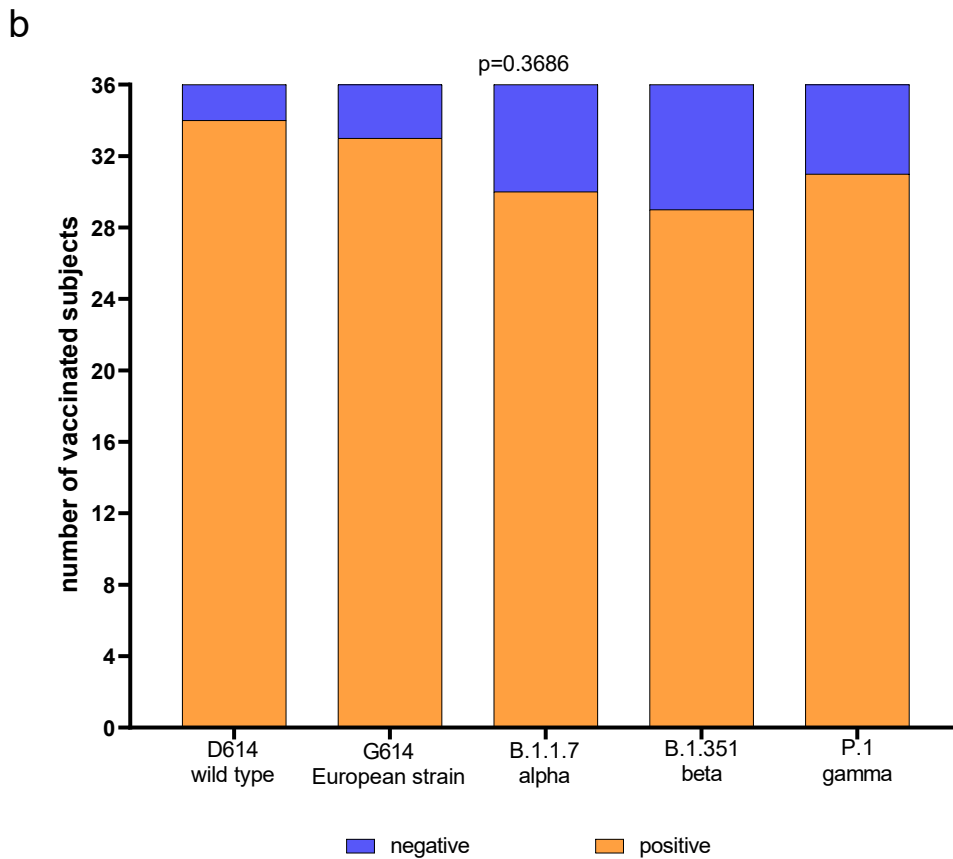
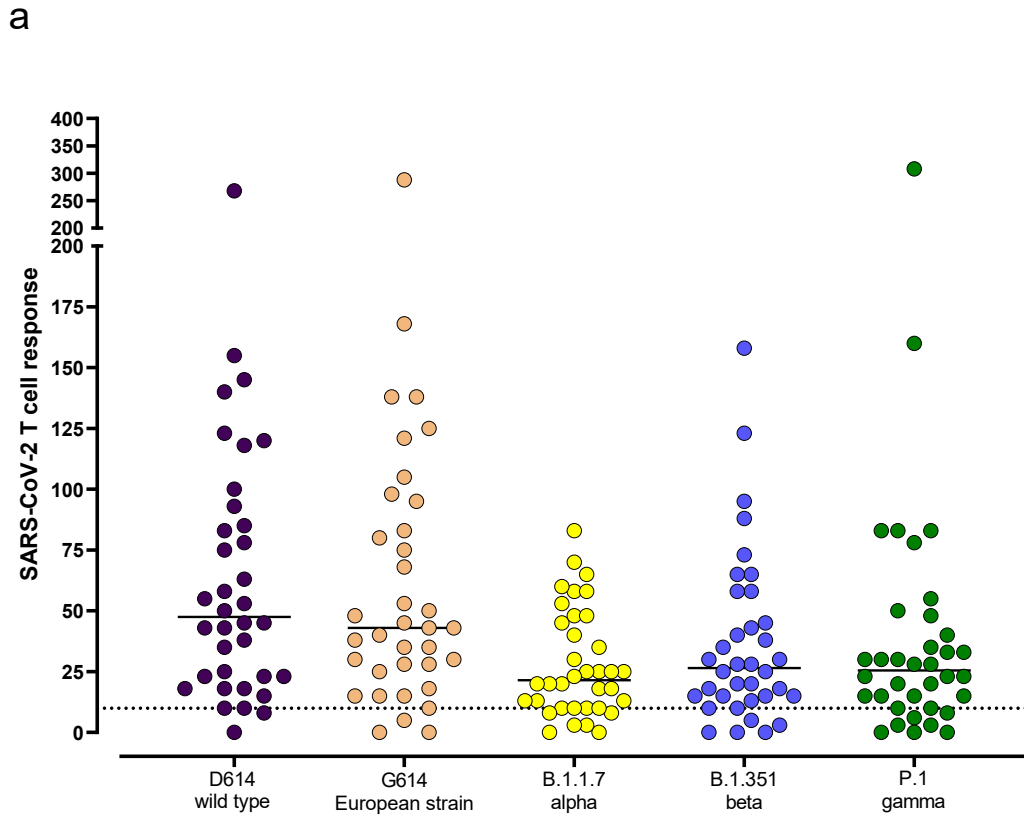a

b

Fig. 4. (a) Median T-cell response against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants. (b) Numbers of subjects with a positive SARS-CoV-2 T-cell response and a negative SARS-CoV-2 T-cell response.

virus than peptide pools from the homologous S protein. However, even if a slightly lower level of cell-mediated T-cell response was observed against α, β and γ variants (Fig. 4a), SARS-CoV-2-specific T cells were detected in the large majority of the subjects, with no significant difference in the frequency of responders. In detail, 34/36 (94.4%) were positive for WT SARS-CoV-2 T-cell response and 33/36 (91.7%) were positive for EU SARS-CoV-2 T-cell response; α, β and γ variant-specific T-cell response was detectable in 30/36 (83.3%), 29/36 (80.5%) and 31/36 (86.1%) vaccinated subjects (Fig. 4b), indicating that the BNT162b2 vaccine was able to elicit T cells able to recognize conserved epitopes from any virus variant tested.

Discussion

In this study, we reported that almost all vaccinated healthy subjects developed a sustained cellular and humoral response after complete vaccination. Moreover, the response level was higher in those subjects with previous exposure to SARS-CoV-2.

SARS-CoV-2-naive individuals developed a humoral response higher than that observed after natural infection after two vaccine doses, but not after one vaccine dose, when about 6% of individuals were still negative for SARS-CoV-2 NT antibodies. On the other hand, the cell-mediated response was detectable in 98.4% naive individuals after two doses of vaccine, whereas one vaccine dose was not sufficient to elicit a detectable SARS-CoV-2 T-cell response in 32.1% of subjects.

Conversely, both humoral and cellular responses were strongly induced after the first dose of the BNT162b2 vaccine in SARS-CoV-2-experienced subjects; therefore, it appears that the prime vaccine dose acts as a boost in these subjects. Moreover, both antibody and T-cell responses did not appear to increase after the second dose in these individuals. According to previous observations [17,18], this evidence strengthens the concept that just one vaccine dose may be sufficient in SARS-CoV-2-experienced subjects [19]. The peptides chosen for T-cell stimulation activated both CD4$^+$ and CD8$^+$ T-cell responses, since they were 15 amino acids long with 10–11 overlap. The peptide pools spanned the entire protein code of the wild-type strain.

Levels of antibody and, especially, cell-mediated response after one vaccine dose was weak or undetectable in SARS-CoV-2-naïve subjects. This contrasts with what was observed in a previous report analysing the antibody response in a small number of subjects [17], and suggests that only one dose was not sufficient to elicit complete protection in naive subjects. Furthermore, in these subjects the emergence of viral resistance or escape might be increased if a suboptimal immune response is elicited. Thus far, a single vaccine dose can be unfavourable in SARS-CoV-2-naive subjects.

Only β and δ variants significantly impacted neutralizing antibody levels in vaccinated subjects, while SARS-CoV-2 T-cell response was not significantly affected. NT antibody titres are extremely variable after SARS-CoV-2 infection [20]. Those with low titres could be reinfected, and due to the presence of incomplete protective neutralizing antibodies might develop escape variants [21,22]. The accumulation of mutations in RBD and the N-terminal domain of spike protein might be associated with increased escape from neutralization. Mutation at the 484 site has been related to a reduction in neutralization sensitivity [23,24]. Spike mutations in the β variant are linked to immune escape from several classes of anti-SARS-CoV-2 monoclonal antibodies, and a significant decrease in neutralization titre against the β variant was observed in plasma from convalescent subjects [25]. Usually, after natural infection or vaccination, polyclonal immune responses arise against multiple antigenic epitopes. Consequently, small numbers of variations in

antigen sequence should have little impact on recognition by the immune system, including both NT antibodies and T cells.

Our results show that vaccinated individuals developed an equally effective NT antibody response against the WT, α, and γ variants, while the NT response against the EU variant was increased. On the other hand, sera from vaccinated subjects showed a significant reduction in SARS-CoV-2 NT antibodies when challenged against the β and δ variants. Nevertheless, serum from the majority of vaccinated subjects (almost 80%) maintained a neutralizing activity against these variants that could be effective in preventing the development of severe disease. These results are in contrast to those of another report showing a higher reduction in neutralization titres against pseudoviruses with S protein from α, β and γ variants, with respect to WT, and no increase in NT against the European variant [26]. Although the binding titre to β RBD was highly reduced with respect to WT (about 100-fold), the NT titre was less affected, showing a five-fold reduction. In addition, we observed that anti-RBD antibody levels, measured in vaccinated subjects with either commercial or in-house assays, have a poor correlation with NT titres. This may suggest that anti-S antibodies binding to sites outside the RBD, which are less involved by the mutations reported, could contribute to virus neutralization. Importantly, our studies rely on the use of wild-type viruses rather than pseudoviruses, which likely could give discrepant results with respect to natural strains [27] because the artificial lentiviral particles cannot resemble the complete biology of actual clinical isolates.

The level of NT antibodies against SARS-CoV-2 variants in SARS-CoV-experienced vaccinated subjects was robust. This preliminary observation might be helpful in the design of vaccination strategies, suggesting that three exposures to SARS-CoV-2 Spike antigen could increase the level of neutralizing antibodies against SARS-CoV-2 variants.

However, and most considerably, our studies on cell-mediated response revealed that SARS-CoV-2 T-cell response is minimally affected by the mutations occurring in SARS-CoV-2 variants, as reported [28], and most subjects tested showed a detectable T-cell response against each virus strain. Unlike Tarke and colleagues, we used inactivated preparations of different SARS-CoV-2 variant isolates in order to recapitulate realistically antigen presenting mechanisms and natural epitope recognition from T cells. The levels of T-cell response and NT antibodies elicited by the vaccine were not correlated; therefore, a low NT titre does not exclude the presence of a protective T-cell response able to control SARS-CoV-2 infection and avoid the development of severe disease.

Potentially cross-reactive T cells originally elicited by the common cold coronaviruses may be boosted by the vaccine and contribute to protection. In our study, about 20–30% of SARS-CoV-2-naive subjects showed a T-cell response specific for S or N proteins. This might be related to the presence of cross-reactive epitopes from common cold coronaviruses that circulate seasonally [11,29–32].

Our study has some limitations. First, the number of SARS-CoV-2-experienced subjects was much lower than that of SARS-CoV-2-naive subjects, and elderly people were not represented. Second, neutralizing and T-cell responses against variants were tested only in a subset of subjects.

In conclusion, the BNT162b2 vaccine appears able to induce roust antibody and T-cell responses in all immunocompetent individuals and paves the way for the clinical evaluation of other mRNA vaccines currently in development [33]. Although the effectiveness of the antibody response may be partially reduced by some mutations naturally arising in virus strains, the T-cell response appears to be marginally affected. A sustained immune response against SARS-CoV-2 variants was observed in vaccinated

SARS-CoV-2-experienced subjects, thus suggesting that a triple exposure to SARS-CoV-2 antigen might represent a valuable strategy for vaccination campaigns.

Transparency declaration

Author contributions

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.09.016.

References

[1] Walsh EE, Frenk RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and immunogenicity of two RNA-based COVID-19 vaccine candidates. N Engl J Med 2020;383:2439—50.

[2] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med 2020;383:2603—15.

[3] Baden LR, Rubin LJ. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med 2021;382:1851—2.

[4] Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehret M, et al. COVID-19 vaccine BNT162b1 elicits human antibody and T(H)1 T cell responses. Nature 2020;585:107—12.

[5] Wang Z, Schmidt F, Weisblum Y, Muecksch F, Barnes CO, Finkin S, et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature 2021;592:616—22.

[6] Abu Jabal K, Ben-Amran H, Beiruti K, Batheesh Y, Sussan C, Zarka S, et al. Impact of age, ethnicity, sex and prior infection status on immunogenicity following a single dose of the BNT162b2 mRNA COVID-19 vaccine: real-world evidence from healthcare workers, Israel, December 2020 to January 2021. Euro Surveill 2021;26:2100096.

[7] Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. mRNA-1273 study group. An mRNA vaccine against SARS-CoV-2—preliminary report. N Engl J Med 2020;383:1920—31.

[8] Anderson EJ, Rouphael N, Lackson LA, Anderson EJ, Roberts PC, Makhene M, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med 2020;384:80—2.

[9] Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021;371:eabf4063.

[10] Sherina N, Piralla A, Du L, Wan H, Kumagai-Braesch M, Andréll J, et al. Persistence of SARS-CoV-2-specific B and T cell responses in convalescent COVID-19 patients 6—8 months after the infection. Med (NY) 2021;2:281—95. e4.

[11] Cassaniti I, Percivalle E, Bergami F, Comolli G, Piralla A, Bruno R, et al. SARS-CoV-2 specific T cell immunity in COVID-19 convalescent patients and un-exposed controls measured by ex-vivo ELISpot assay. Clin Microb Infect 2021;27:1029—34.

[12] Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Mundayet JD, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 2021;372. eabg3055.

[13] Voloch CM, da Silva FR, de Almeida LGP, Cardoso CC, Brustolini OJ, Gerberet AL, et al. Genomic characterization of a novel SARS-CoV-2 lineage from Rio de Janeiro, Brazil. J Virol 2021. JVI.00119-21.

[14] Tegally H, Wilkinson E, Lessells RJ, Giandhari J, Pillay S, Msomi N, et al. Sixteen novel lineages of SARS-CoV-2 in South Africa. Nat Med 2021;27:440—6.

[15] Shi PY, Xie X, Zou J, Fontes-Garfias C, Xia H, Swanson K, et al. Neutralization of N501Y mutant SARS-CoV-2 by BNT162b2 vaccine-elicited sera. Res Sq 2021. rs.3.rs-143532.

[16] Rees-Spear C, Muir R, Griffith SR, Heaney J, Aldon Y, Snitselaar JL, et al. The effect of spike mutations on SARS-CoV-2 neutralization. Cell Rep 2021;34: 108890.

[17] Manisty C, Otter AD, Treibel TA, McKnight A, Altmann DM, Brooks T, et al. Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals. Lancet 2021;397:1057—8.

[18] Prendecki M, Clarke C, Brown J, Cox A, Gleeson S, Guckian M, et al. Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine. Lancet 2021;397:1178—81.

[19] Bradley T, Grundberg E, Selvarangan R. Antibody responses boosted in sero-positive healthcare workers after single dose of SARS-CoV-2 mRNA vaccine. medRxiv 2021. 2021.02.03.21251078.

[20] Robbiani DF, Gaebler C, Muecksh F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature 2020;584:437—42.

[21] Van Elslande J, Vermeersch P, Vandervoort C, Wawina-Bokalanga T, Vanmechelen B, Wollants E, et al. Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain. Clin Infect Dis 2020. ciaa1330.

[22] Larson D, Brodniak SL, Voegtly LJ, Cer RZ, Glang LA, Malagon FJ, et al. A case of early re-infection with SARS-CoV-2. Clin Infect Dis 2020:ciaa1436.

[23] Greaney AJ, Starr TN, Barnes CO, Weisblum Y, Schmidt F, Caskey M, et al. Mutational escape from the polyclonal antibody response to SARS-CoV-2 infection is largely shaped by a single class of antibodies. bioRxiv 2021. 2021.03.17.435863.

[24] Weisblum Y, Schmidt F, Zhang F, DaSilva J, Poston D, Lorenzi JCC, et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. ELife 2020;9:e613122020.

[25] Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysenet B, et al. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. bioRxiv 2021. 2021.01.18.427166.

[26] Garcia-Beltran WF, Lam EC, Astudillo MG, Yang D, Miller TE, Feldmanet J, et al. COVID-19-neutralizing antibodies predict disease severity and survival. Cell 2021;184:476—88. e11.

[27] Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 2021;593:130—5.

[28] Tarke A, Sidney J, Methot N, Zhang Y, Dan JM, Goodwin B, et al. Negligible impact of SARS-CoV-2 variants on CD4+ and CD8+ T cell reactivity in COVID-19 exposed donors and vaccines. BioRxiv 2021. 2021.02.27.433180.

[29] Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Rydyznski Moderbacheret C, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell 2020;181: 1489—501. e15.

[30] Sette A, Crotty S. Pre-existing immunity to SARS-CoV-2: the knowns and unknowns. Nat Rev Immunol 2020;20:457—8.

[31] Le Bert N, Tan AT, Kunasegaran K, Tham CYL, Hafezi M, Chia A, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature 2020;584:457—62.

[32] Meckiff BJ, Suastegui CR, Fajardo V, Chee SJ, Kusnadi A, Simon H, et al. Single-cell transcriptomic analysis of SARS-CoV-2 reactive CD4+ T cells. Cell 2020;183:1340—53. e16.

[33] Liu MA. A comparison of plasmid DNA and mRNA as vaccine technologies. Vaccines 2019;7:37.