Possible influence of anti-vector immunity and SARS-CoV-2 variants on efficacy of ChAdOx1 nCoV-19 vaccine and the proposal of a new pharmacotherapy

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Abstract

The present work analyses in detail the published data on ChAdOx1 nCoV-19 vaccine and provides arguments for the involvement of anti-vector immunity and of SARS-CoV-2 variants on the efficacy of ChAdOx1 nCoV-19 vaccine. First, it is suggested that anti-vector immunity takes place as the regimen of homologous vaccination with ChAdOx1 nCoV-19 vaccine is applied and interferes with efficacy of the vaccine when the interval between prime and boost doses is less than three months. Second, longitudinal studies suggest that ChAdOx1 nCoV-19 vaccine provides sub-optimal efficacy against UK variant of SARS-CoV-2, which appears to have an increased transmissibility over the ancestral SARS-CoV-2 among vaccinated people. At the moment, ChAdOx1 nCoV-19 vaccine is able to reduce the severity of symptoms and transmissibility; however, if the vaccinated individuals do not maintain everyday preventive actions, they could turn into potential spreaders, thus accelerating the process of generation of new viral variants due to the selective pressure of immune response. Prediction and possible consequences of the SARS-CoV-2 evolution and repeated anti-SARS-CoV-2 vaccinations are discussed. Since the impact of emerging SARS-CoV-2 variants suggests that vaccines are unlikely to be effective in quickly solving the pandemic crisis, it is highlighted the need to keep searching for new and more efficacious pharmacotherapy for COVID-19, such as those targeting ACE2 and ADAM17 zinc-metalloprotease activities.

Possible influence of anti-vector immunity and SARS-CoV-2 variants on efficacy of ChAdOx1 nCoV-19 vaccine and the proposal of a new pharmacotherapy

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Data sharing is not applicable to this article because no new data were created or analysed in this study

Key words: Vaccine; immunity; viral vectors; SARS-CoV-2 variants; zinc-metalloproteases; chelating agents.

Abstract

The present work analyses in detail the published data on ChAdOx1 nCoV-19 vaccine and provides arguments for the involvement of anti-vector immunity and of SARS-CoV-2 variants on the efficacy of ChAdOx1 nCoV-19 vaccine. First, it is suggested that anti-vector immunity takes place as the regimen of homologous

vaccination with ChAdOx1 nCoV-19 vaccine is applied and interferes with efficacy of the vaccine when the interval between prime and boost doses is less than three months. Second, longitudinal studies suggest that ChAdOx1 nCoV-19 vaccine provides sub-optimal efficacy against UK variant of SARS-CoV-2, which appears to have an increased transmissibility over the ancestral SARS-CoV-2 among vaccinated people. At the moment, ChAdOx1 nCoV-19 vaccine is able to reduce the severity of symptoms and transmissibility; however, if the vaccinated individuals do not maintain everyday preventive actions, they could turn into potential spreaders, thus accelerating the process of generation of new viral variants due to the selective pressure of immune response. Prediction and possible consequences of the SARS-CoV-2 evolution and repeated anti-SARS-CoV-2 vaccinations are discussed. Since the impact of emerging SARS-CoV-2 variants suggests that vaccines are unlikely to be effective in quickly solving the pandemic crisis, it is highlighted the need to keep searching for new and more efficacious pharmacotherapy for COVID-19, such as those targeting ACE2 and ADAM17 zinc-metalloprotease activities.

ChAdOx1 nCoV-19 vaccine clinical trials

A vaccine is a special drug that people do not take every day but only once or a few times. It primes the immune system to fight off an infection; however, like any drug, vaccines can vary in probability of both effectiveness and side effects; benefits and risks that can differ depending on age, comorbidities and other genetic and/or environmental factors. The widespread mortality and morbidity associated with the COVID-19 pandemic has induced the development of several vaccines (**1**), some of which have recently received emergency use authorisation. Among them, ChAdOx1 nCoV-19 vaccine was authorised with a regimen of two standard doses given with an interval of 4–12 weeks on the basis of the interim analysis data (**2**). Following regulatory approval, the optimal dose interval was assessed in a recent report through post-hoc exploratory analyses (**3**). The ChAdOx1 nCoV-19 vaccine consists of a replication-deficient chimpanzee adenoviral vector containing the full-length SARS-CoV-2 spike glycoprotein gene, which was tested across different studies (**2,4,5**). Based on previous experience with ChAdOx1 MERS (a chimpanzee adenovirus-vectored vaccine encoding the spike protein of Middle East respiratory syndrome coronavirus, **7**), the vaccination studies (COV001-UK, COV002-UK, and COV003-Brazil, COV005-South Africa) were initially designed to assess a single-dose (5×10^{10} particles) of ChAdOx1 nCoV-19 (**2,4,5**), although other vaccination protocols consist of first (i.e., prime) and second (i.e., boost) doses. Differently from vectors derived from human viruses for which pre-existing immunity can reduce the vaccine immunogenicity (because of the possibility of anti-vector immunity), a chimpanzee adenovirus-vectored vaccine can bypass this possibility; however, after the prime dose there is the possibility to develop an anti-vector immunity, which could inhibit the potency of the booster effect of a second dose. Preliminary data showed that vaccination of rhesus macaques with a single dose of ChAdOx1 nCoV-19 was able to protect from SARS-CoV-2 infection, indicating the efficacy of the single-dose strategy (**6**). However, once the studies were underway, analysis of immune responses and other factors justified for amendments to the trials including groups receiving different vaccination protocols in the analysis. Induction of both spike-specific neutralising antibody titres and T-cell responses has been shown to provide protection against viral infections in animal models (**6,7**) and the immunogenicity data from phase 1 (COV001-UK, begun on April 23, 2020) showed a substantial increase in SARS-CoV-2 spike neutralising antibodies (but not in interferon-γ ELISpot T cell response to SARS-CoV-2 spike peptides) with a second dose of vaccine given after 28 days (**4**). Based on this observation, the trial protocol was modified to a regime of two doses administered 28 days apart (**4**). A second study (COV002-UK) included participants who received a low dose (LD) of the vaccine ($2.2 \times 10^{1}0$ viral particles) as their first dose and were boosted with a standard dose (SD, 3.5-6.5 $\times 10^{1}0$ virus particles), called LD/SD group, and subsequently participants who were vaccinated with two standard-dose vaccines (SD/SD group). Initial low dosing of viral particles in COV002 was due to an inaccurate quantification of viral particles by spectrophotometric method and further doses were adjusted to the standard dose ($5 \times 10^{1\circ}$ viral particles) using a more accurate qPCR assay (**2**). The trial protocol was amended on June 5, 2020, resulting in enrolment of two distinct groups with different dosing regimens. The LD/SD cohort was enrolled between May 31 and June 10, 2020, while the SD/SD cohort (aged 18–55 years) was enrolled later from June 9 to July 20, 2020 (**2**). Older age cohorts began subsequently in August, all of whom were assigned to two standard dose (SD/SD) cohort.

However, participants who received the prime vaccination of low-dose ChAdOx1 nCoV-19 had similar anti-spike antibody titres by day 28 after both their prime and booster vaccination as those who received a standard dose and were higher than for those who did not receive a booster vaccine, suggesting that immune responses elicited by LD/SD and SD/SD were similar and confirming that prime-boost regimen (28 days apart) could give a higher protection (**5**). Nevertheless, as for previous observations (**4**), IFN-γ ELISpot T cell responses against SARS-CoV-2 spike peptides peaked 14 days after the prime vaccination and did not increase significantly after the booster vaccination (**5**). Based on the above observations, it was offered a second booster dose to the participants who received a low prime dose (originally planned as single-dose cohort) and it produced the LD/SD cohort (protocol modified on July 20, 2020); however, some participants chose not to receive the second dose and constituted a cohort of low single-dose recipients (**2,3**). On the other hand, most participants in the LD/SD group (COV002) received a second dose around 12 weeks after the first, while the interval between doses for the SD/SD group (COV002) was both lower and more heterogeneous (**2**). Boosting began on Aug 3, 2020, resulting in a longer gap between prime and booster vaccines in LD/SD cohort (median 84 days, interquartile range, IQR, 77—91) than for those in SD/SD cohorts (median 69 days, IQR 50–86) (**2**). Moreover, in UK the large number of participants who received the two-dose schedule delayed the administration of the second dose (target 28 days) because of an insufficient production of the vaccine (**2**). Differently, a trial in Brazil (COV003), which began on June 23, 2020, included a two standard dose (SD/SD) group with the majority of participants receiving a second dose within 6 weeks of the first (median 36 days) (**2**). These situations provide the opportunity to analyse the vaccine efficacy of a single dose, and the effect of different dose interval. Unfortunately, there was no overlap in enrolment of participants in these cohorts and participants of LD/SD cohort and single LD cohort were vaccinated (prime dose) before those of SD/SD cohort (**2**).

Possible influence of anti-vector immunity on efficacy of ChAdOx1 nCoV-19 vaccine

Interestingly, vaccine efficacy against symptomatic or asymptomatic disease in participants (COV002-UK) who received a low dose as their first dose of vaccine (LD/SD) was significantly higher than that of participants who received two standard-dose (SD/SD) vaccines (**2,3**). Indeed, vaccine efficacies in LD/SD group was 90.0% (95% CI 67.4–97.0) and 58.9% (95% CI 1.0–82.9) against symptomatic and asymptomatic (evaluated by mean of weekly self-swab) disease, respectively, whereas they were respectively 60.3% (95% CI 28.0–78.2) and 3.8% (95% CI -72.4–46.3) in SD/SD group (data cutoff on Nov 4, 2020) (**2**), indicating that the two trial protocols produced significantly different protection from SARS-CoV-2 symptomatic and asymptomatic disease and transmission. Moreover, the SD/SD cohort in Brazil displayed a relatively low protection, 64,2% (95% CI 30.7–81.5), which was similar to vaccine efficacy of SD/SD UK cohort (60.3%). These surprising data might suggest that low prime dose would induce a longer and/or a higher SARS-CoV-2 immune protection; however, others factors such as dose interval might be involved in determining the significant differences between LD/SD and SD/SD cohorts. In this regard, both the UK (COV002) and Brazil (COV003) SD/SD cohorts, which displayed relatively low vaccine efficacies against primary symptomatic COVID-19, had shorter dose intervals than LD/SD cohort (data cutoff on Nov 4, 2020) (**2**), suggesting that the longer dose intervals of LD/SD group might give higher protection. Indeed, exploratory subgroup analyses of SD/SD cohorts from both UK and Brazil showed a trend (although not significant) of increase in vaccine efficacy when comparing those with short interval between doses (<6 weeks, 53.4%) and those with longer interval ([?]6 weeks 65.4%) (data cutoff on Nov 4, 2020) (**2**). Notably, a subsequent analysis (data cutoff on Dec 7, 2020) (**3**) revealed that when SD/SD group was restricted to those who received their vaccines more than 84 days (12 weeks) between the two doses (a dose interval similar to LD/SD group), vaccine efficacy of SD/SD cohort (81.3% [95% CI 60.3–91.2]) was similar to that of LD/SD cohort (80.7%, [95% CI 62.1–90.2]) (**3**). Moreover, ChAdOx1 nCoV-19 vaccine had a higher efficacy in those with a longer prime-boost interval (vaccine efficacy 81.3% [95% CI 60.3–91.2] at [?]84 days) than in those with a short interval (vaccine efficacy 55.1% [95% CI 33.0–69.9] at <42 days), further suggesting that long ([?]84 days) dose intervals give higher protection. However, 84 days of dose interval might increase the probability of infection between the two doses. In this regard, although anti-SARS-CoV-2 spike IgG responses after a single standard dose of ChAdOx1 nCoV-19 vaccine showed a decrease from the peak at day 28 (median 5496

AU/ml [IQR 2548-12061] for participants aged 56–69 years and 9807 AU/ml [IQR 5847-17220] for participants aged 18–55 years) of 34% by day 90 (geometric mean ratio [GMR] 0.66 [95% CI 0.59–0.74]), a single standard dose was efficacious (76.0% [95% CI 59.3–85.9]) against primary symptomatic (but not against asymptomatic) SARS-CoV-2 infection in the first 90 days after vaccination, with no significant waning of protection during this period, thus supporting the approach to delay second doses (**3**). As indicated in the report, participants were censored in the analysis of single-dose efficacy at the time of their booster dose (**3**); however, most participants in the single dose analysis received a second dose within 90 days after the first dose. That means that the data analysed for participants from 22 to 90 days since first dose were collected before the data cutoff date indicated in the report (December 7, 2020), possibly between June and October. Instead, the group of participants reaching 91 and 120 days since first dose likely represents people who never received a second dose, for which vaccine efficacy was assessed at the data cutoff date (December 7, 2020). During this last period (the time between the beginning of November and the beginning of December), the vaccine efficacy of single dose appeared to wane, reaching only 31.6% protection (95% CI -141.8–80.7). This is possibly due to a progressive decrease of anti-SARS-CoV-2 spike IgG responses (64% by day 180, GMR 0.36 [0.27–0.47]) from the peak at day 28 and/or other factors (e.g. SARS-CoV-2 variants emerging during the month of November, see later). Altogether the data suggested that a 3-month dose interval provided better protection after a second dose without compromising protection in the period before the booster dose is administered. This conclusion was supported by immunogenicity data that showed that in both LD/SD and SD/SD cohorts, participants who received a second standard vaccine more than 84 days after the first had anti-SARS-CoV-2 spike IgG titres more than two-fold higher than those who received the second dose within 42 days of their initial vaccination. Assuming there is a relationship between the humoral immune response and vaccine efficacy, this evidence suggested that long ([?]84 days) dose intervals were more efficacious than shorter dose intervals and could induce a long protection from SARS-CoV-2 (**3**). These data were recently discussed in a report (**3**); however, a possible hypothesis underlying this observation was not discussed. In this regard, it has already been highlighted that there is the possibility to develop an anti-vector immunity on homologous boosting and this eventuality could be on the origin of the reduced potency of the booster effect when the second dose was administered earlier than 84 days. Indeed, it is likely that immunity against the antigenic proteins of simian adenovirus vector tends to wane during the time (as well as that against spike proteins), providing a rational explanation to the increased anti-SARS-CoV-2 spike IgG responses and vaccine efficacy produced by delayed boosting.

Possible influence of UK SARS-CoV-2 variant(s) on efficacy of ChAdOx1 nCoV-19 vaccine

Of note, comparison of vaccine efficacy data between the two different cutoff dates for the participants in LD/SD cohort (the only that remained constant in numbers and therefore comparable in longitudinal analyses), the values may suggest a slight decrease of protection (**3**). Indeed, the relative risk of infection in LD/SD group at the first data cutoff date (November 4, 2020) was 0.10 (95% CI 0.03–0.33) and 0.41 (95% CI 0.17–0.99) for symptomatic and asymptomatic disease, respectively (**2**), while they were subsequently estimated to be respectively 0.19 (95% CI 0.10–0.38) and 0.51 (95% CI 0.28–0.93) at the second data cutoff (December 7, 2020) (**3**), possibly suggesting a slight decrease of vaccine efficacy during the last time period of about a month. Indeed, at the first data cutoff date (Nov 4, 2020), within the LD/SD group, the symptomatic infected individuals were 3 in 1367 participants (0.2%) in vaccinated group and 30 in 1374 participants (2.2%) in control group. Instead, at the second data cutoff date (Dec 7, 2020), within the same LD/SD group, the symptomatic infected individuals were 10 in 1396 participants (0.7%) in vaccinated group and 51 in 1402 participants (3.6%) in control group. That means that during the time between the two data cutoff dates (basically the month of November) there were 7 symptomatic infections in 1393 participants (1396 minus 3 already infected) (0.50%) in vaccinated group and 21 in 1372 participants (1402 minus 30 already infected) (1.53%) in control group, which correspond to 0.33 (95% CI 0.14–0.77) of relative risk of symptomatic infection that is about 3 time higher than that of the first time period. Notably, the LD/SD cohort was enrolled between May 31 and June 10, 2020 (**2**) and most of them had a boost dose about 3 months later (median 84 days, interquartile range 77—91) (**3**), that means that booster doses occurred between the end of August and the beginning of September (**2**). Therefore, at the two data cutoff dates

(Nov 4 and Dec 7, 2020), LD/SD cohort was respectively analysed about two and three months after the booster dose. During the longitudinal study, the frequency of infected individuals in control group was 2.2% in the first time period (data cutoff date Nov 4) and 1.53% in the second time period (between the two data cutoff dates). Since in UK the frequency of infected individuals increased during the month of November, it confirms that the second time period was shorter than the first; nevertheless, the frequencies of spontaneous infection were somehow similar (and comparable) between the two groups. In addition, during the time between the two data cutoff dates, a similar trend of the relative risk of infection was observable for the asymptomatic (transmissible) infection, for which the vaccination in LD/SD cohort reached a relative risk of 0.64 ([95% CI 0.28–1.48], it was 0.41 [95% CI 0.17–0.99] on Nov 4, 2020), suggesting that the booster dose provided a protection for two months after which the immune protection seems to start to wane. Instead, no waning of vaccine efficacy was detected between 22 and 90 days after single standard dose. Indeed, the relative risk of symptomatic infection remained stable (median 0.24 [95% CI 0.14–0.41]) until 90 days (3 months) after vaccination, despite a 34% reduction (GMR 0.66 [95% CI 0.59–0.74]) of anti-SARS-CoV-2 spike IgG responses after 90 days from the peak at day 28 (median 5496 AU/ml for participants aged 56–69 years and 9807 AU/ml for participants aged 18–55 years) (**3, 5**). Intriguingly, the relative risk of symptomatic infection after a single standard dose within 90 days (0.24 [95% CI 0.14–0.41]) was similar to that between the two data cutoff dates (about 60 and 90 days after booster dose) in LD/SD group (0.33 [95% CI 0.14–0.77]). In this regard, it is expected that the prime-boost regimen can induce both higher levels of neutralising antibodies and longer time protection (for several months) than a single dose. Indeed, 28 days after the second dose (about one month before the first data cutoff date), the anti-SARS-CoV-2 spike IgG responses in LD/SD group were extremely high (median 39670 AU/ml, [IQR 21068–66338] 9-11 week interval and 49584 AU/ml, [IQR 31122–81163] [?]12 week interval for participants aged 18–55 years) compared with those induced after 28 days by single (standard or low) doses (single low dose, median 6439 AU/ml [IQR 4338-10640] for participants aged 18–55 years) (**3,5**), suggesting that the decrease of anti-SARS-CoV-2 immune protection may not be due to the decline of anti-SARS-CoV-2 antibodies. Since the protection induced by a single dose lasted for at least 3 months (despite a significant reduction of anti-SARS-CoV-2 spike IgG responses) and at 28 days after the second dose (one month earlier than the first data cutoff, November 4, 2020) participants in LD/SD cohort displayed an expression of anti-SARS-CoV-2 spike IgG about five fold higher than single (standard or low) dose (**3,5**), it is unlikely that the decreased vaccine efficacy at the second data cutoff date (Dec 7, 2020) may depend on concentrations of anti-SARS-CoV-2 antibodies. Rather, the relatively low protective efficacy recorded during the month of November after the booster dose of ChAdOx1 nCoV-19 vaccine (relative risk of symptomatic infection 0.33 [95% CI 0.14–0.77]) compared to single dose analyses (relative risk of symptomatic infection 0.24 [95% CI 0.14–0.41]) assessed between 22 and 90 days (for which most data were collected between June and October) suggests that other factors might be at play late after the booster dose. Of note, during the same period of time (basically the month of November), the vaccine efficacy of single standard dose (between 91 and 120 days after administration) similarly wane, reaching a relative risk of symptomatic infection of 0.68 (95% CI 0.19–2.42) (**3**), thus suggesting a common factor that led to a general reduction of ChAdOx1 nCoV-19 vaccine efficacy in that specific temporal period. In this regard, the impairment of vaccine-induced immune protection could be due to reduction not only in concentration, but also in specificity of anti-SARS-CoV-2 neutralizing antibodies. Therefore, it is possible that loss of antibody recognition might be involved in the reduction of immune protection "in vivo". In this regard, evaluation of anti-SARS-CoV-2 spike IgG responses has been assessed using the ancestral spike protein, it is therefore possible that the emergence SARS-CoV-2 variants with spike protein mutations may be at the origin of the discrepancy between anti-SARS-CoV-2 spike IgG responses and vaccine efficacy late during the vaccine trials. In this regard, the B.1.1.7 (UK) variant, which carries several mutations including spike protein, started circulating in England in late September and became the dominant lineage in December (**8**). In the UK, the proportion of the B.1.1.7 variant has increased from 0.1% in early October to 49.7% in late November among sequences available at 19 December 2020 (**8**), suggesting a cause-effect relationship between B.1.1.7 expansion and a possible decrease of efficacy of ChAdOx1 nCoV-19 vaccine against symptomatic infection during the two data cutoff dates of the reports (Nov 4, 2020 and Dec 7, 2020).

B.1.1.7 variant contains 8 spike mutations in addition to D614G, including one mutation (N501Y) in receptor

binding domain (RBD), two deletions (69-70del and 144del) in the N-terminal domain (NTD) of the spike, and one mutation (P681H) near the furin cleavage site (**9-11**). However, the B.1.1.7 variant seems susceptible to neutralising antibodies elicited by ancestral spike vaccines (**9**), rather it has an enhanced binding to ACE-2, a higher reproduction and an increased transmission that gives it a competitive advantage in humans (**9,10**). Nevertheless, neutralization by serum samples from recipients of vaccines with ancestral spike was moderately reduced and a subset of monoclonal antibodies to the RBD of spike is less effective against the B.1.1.7 variant (**9**), raising the possibility of a moderate increased risk of infection and virus transmission after vaccination with ancestral spike sequences. In line with this possibility, another report found that B.1.1.7 is refractory to neutralization by most monoclonal antibodies to the NTD of the spike and relatively resistant to a few monoclonal to ancestral RBD, which could cause escape from neutralizing antibody control in vivo, thus threatening the protective efficacy of current vaccines (**11**). In this regard, a recent sequencing of the B.1.1.7 variant revealed the presence of the E484K mutation (first identified in South Africa) (**12**) and several studies showed reduced neutralising activity of monoclonal antibodies from convalescent or vaccinated individuals against virus mutants containing the E484K mutation (**13-16**). Moreover, the presence of the N439K mutation, which has emerged independently in multiple variant lineages, has been shown to increase both spike binding affinity for human ACE2 and resistance to several anti-SARS-CoV-2 neutralizing antibodies, which give SARS-CoV-2 variants carrying N439K a selective advantage (**17**). Altogether these observations suggest that neutralising antibodies elicited by ancestral spike vaccines induce cross-protection from B.1.1.7 variant; however, they may not be able to fully protect from the UK variant, and in particular, from its transmission. In this regard, a post-hoc analysis of the efficacy of ChAdOx1 nCoV-19 vaccine against B.1.1.7 variant have shown that clinical efficacy against symptomatic infection was 70.4% (95% CI 43.6–84.5) (while it was 81.5% [95% CI 67.9–89.4] for non-B.1.1.7 lineages) and it was 28.9% (95% CI -77.1–71.4) against asymptomatic infection (**18**); values that are very similar to those calculated in the present report for the LD/SD group between November 4 and December 7, which were respectively 67.2% (95% CI 23.0–86.0) and 35.9% (95% CI -47.6–72.2). In line with these observations, neutralisation activity of vaccine-induced antibodies in a live-virus neutralisation assay has been shown to be about nine times lower against the B.1.1.7 variant than against the ancestral lineage (GMR 8.9 [95% CI 7.2–11.0]) (**18**). Notably, participants were recruited between May 31 and Nov 13, 2020, therefore before expansion and acquired new mutations (evolution) of B.1.1.7 variant. It is therefore likely that the relative increase risk of symptomatic infection in LD/SD cohort between the two data cutoff dates of the reports (Nov 4, 2020 and Dec 7, 2020) may be related to the emerging UK variant(s), which was dominant in that period during the UK pandemic (**8**). Therefore, although actual vaccines are able to protects from severe forms of COVID-19 and its transmission, the present work suggests that ChAdOx1 nCoV-19 vaccine may not be able to block UK variant transmission as for ancestral virus. Whether this possibility may occur only with ChAdOx1 nCoV-19 vaccine or also with other vaccines based on the ancestral spike sequences is still unclear because data for longitudinal studies as those performed for the ChAdOx1 nCoV-19 vaccine are not available. Nevertheless, a recent preprint report (data from 1 December 2020 to 3 April 2021, a period in which B.1.1.7 was dominant) showed no difference of protection between a single dose of ChAdOx1 nCoV-19 and BNT162b2 vaccine (after either one or two vaccine doses) in a representative sample across the UK (**19**).

The possible impact of emerging SARS-CoV-2 variants on vaccine efficacy, SARS-CoV-2 infection age-distribution and severity, and the need to still maintain physical preventive actions

Since no hospital admissions or severe cases were reported in the ChAdOx1 nCoV-19 arm (**2,3**), the data clearly show that ChAdOx1 nCov-19 is still effective against severe and persistent disease during emergence of UK variant. Indeed, a single dose of ChAdOx1 nCoV-19 (or BNT162b2) vaccine significantly reduced rates of both infections and hospitalisations/deaths during a period in which B.1.1.7 was dominant in UK (between 1 December 2020 and 3 April 2021) (**19**). It is clear that the number of both infected individuals and days of infectivity (related to severity) per person substantially influences the probability of both virus transmission and mutation (i.e. generation of variants). Therefore, at the moment, ancestral spike-based vaccines are able to reduce the severity of symptoms and the time of infectivity and transmissibility of UK variant; however, care should be taken because asymptomatic infection in vaccinated individuals may spread

the variant over the non-vaccinated population, albeit at lower efficiency (**19**). Indeed, if the vaccinated individuals do not maintain everyday preventive actions (such as the physical distancing and the use of face masks), they might turn into potential spreaders not only to uninfected and unvaccinated individuals but potentially also to some individuals that were asymptomatic during the first wave but susceptible to new and highly infectious SARS-CoV-2 variants (e.g., B1.1.7).

Under the selective pressure of the immune system in convalescent or vaccinated people, adaptation processes of mutable RNA viruses (such as SARS-CoV-2 and influenza) constantly generates a heterogeneous pool of SARS-CoV-2 variants, which are continuously tested and selected "in vivo" in order to escape immune responses, antibody treatments and herd immunity. For example, SARS-CoV-2 spike variants with increased binding affinity to human ACE2 (such as N439K variants, **17**) can probabilistically lead to a higher number of infected both cells in a patient and individuals in a population. Therefore, they can produce a worse and persistent infection in a broader range of humans, also providing an increased probability of transmission, which is a strong competitive advantage. Moreover, the accidental ability of reinfection or of infection of vaccinated individuals provides a competitive advantage to some SARS-CoV-2 variants, particularly in highly vaccinated countries, in which most people are fully resistant to the ancestral virus. If the vaccinated people become susceptible to a variant infection, this variant will have plenty of people to infect again, potentially leading to a "rebound" effect in highly vaccinated countries (as it may occur for example in Chile, see **20**), which, in this globalisation world, will potentially spread the new variant to less vaccinated countries (potentially turning "vaccinated" countries as well as individuals into potential spreaders that might lead to a sort of an involuntary biological world war). Fortunately, the nature of the new vaccine technology will rapidly allow for new vaccine variants with specific mutations; however, it is not clear how many vaccinations with different vaccine variants will be necessary before ending the pandemic (and recovering the global economy) and what will be the short- and long-term consequences in efficacy and antibody dependent enhancement (ADE) (**21**) of repeated vaccination. In this regard, yearly viral challenge of influenza virus is a good model to try to predict the effect of repeated exposures to mutant viruses and seasonal vaccine variants. Indeed, influenza vaccines successfully control the severe forms of infection; however, it has been observed that some previous infections and/or vaccinations with influenza strains can be sometimes counter-protective (**22**). Interactions between the immune system and mutant pathogens and/or vaccine variants are dynamic processes, which evolve at each exposure on the basis of previous host-pathogen interactions "memorized" by the immune system of each individual and, by extension, of each population/community (**22**). The imprinting event of first influenza infection or of first vaccination generates a pool of long-lasting immunological memory cells which remains throughout life and determinates the response to subsequent infections/vaccinations. It has been hypothesized that an elevated antigenic diversity between previous and subsequent vaccination permits the generation of new immune memory cells that better protect from viral infection. Conversely, repetition of antigenically-related vaccines and previously existing low avidity antibodies derived from memory cells can lead to a deleterious outcome of a subsequent infection by causing ADE (**22**). Therefore, cumulative effects of subsequent influenza virus infections and/or vaccinations can "unpredictably" shape future immune responses that could be either beneficial or deleterious (**22**). Regarding the eventuality of repeated SARS-CoV-2 spike vaccinations, there is a further aspect of unpredictability due to the fact that influenza vaccines include inactivated influenza vaccine, live attenuated influenza vaccine, or recombinant protein influenza vaccine, instead the SARS-CoV-2 vaccines that have recently received emergency use authorisation in Europe include lipid nanoparticle-encapsulated mRNA based vaccines or adenovirus-vectored DNA based vaccines. When compared to traditional vaccines that use dead or weakened forms of the viruses, these new vaccines have an important difference in the envelope that contains the genetic material. The envelope is the vector that determines not only anti-envelope/vector immune responses but also the cells in which the genetic content is inserted and expressed, the vaccine tropism. Differently from traditional vaccine platforms, the novel vaccine strategies induce anti-envelope/vector immune responses which are not functional to generate anti-viral memory cells and insert the spike nucleotide sequence into cells independently on ACE2 expression, possibly driving a non-specific immune response against cells that will never be infected by SARS-CoV-2. Therefore, in order to reduce current pressure on healthcare systems, vaccination should be focused on protecting from severe disease the

most vulnerable (minority) part of the population for which the risk/benefit balance of vaccination is more favourable. At the same time, this vaccine strategy (successfully applied for highly mutable influenza RNA virus, for which we have never tried and needed to reach a herd immunity) will likely limit vaccine-driven immune selection pressure that, under current conditions of very high levels of virus replication and diffusion, might facilitate viral immune escape mechanisms.

Of particular concern are variants that are able to generate a persistent immune system's fight against viral infection in people with strong immune responses (such as young healthy people). Indeed, the accidental ability of virus variants not only to produce persistent infections in a broader number of individuals including young and healthy people (who are relatively resistant to ancestral infection) but also to "survive" in different environmental conditions (e.g. different seasons) provides a higher probability of transmission and a competitive advantage. Indeed, SARS-CoV-2 variants, which persist during summertime and are more resistant to summer temperatures, humidity and UV rays, are already present in South Africa, Brazil, Chile and India, countries in which the variants emerged during their summer. Moreover, future variants able to produce persistent (asymptomatic and/or symptomatic) infection in a broader spectrum of humans are also expected to be selected. In this regard, during the second wave of SARS-CoV-2 (September 2020 to January 7, 2021), there were more people (and in a shorter time period) in England's hospitals with COVID-19 (weekly incidence per 100000 inhabitants was 19.3 cases, calculated using the 2019 population estimates for the England available from the UK National Statistics) than in the first wave (March to September 2020, weekly incidence per 100000 inhabitants was 6.4 cases), indicating the higher infectivity of UK SARS-CoV-2 variant (see **23**). In particular, there was a relative increase in hospitalization rates in younger age groups (1.72-fold increase for the <17-year age group) compared to the older age groups (1.35-fold increase for the >65-year age group), while relative increase was intermediate (1,46-fold) for the 18-64-year group (see **23**). During the first wave of SARS-CoV-2, the prevalence of hospitalisation for COVID-19 was 1 young (in the <17-year age group of 12023568 individuals based on the 2019 population estimates for the England) every 64 elderly (in the >65-year age group of 10353716 individuals based on the 2019 population estimates for the England), i.e. relative risk ratio 0.016 [99% CI 0.015–0.017], while it significantly increased to 1 every 50 individuals, i.e. relative risk ratio 0.020 [99% CI 0.019–0.021] in the second wave, thus leading to a substantial decrease of the median age of hospitalized patients compared to the first wave. In line with this observation, a recent report observed a shift in the age composition, with significantly more UK variant cases among individuals aged 0-19 and significantly fewer UK variant cases among individuals aged 60-79, as compared to non-UK variant cases (**24**). However, at this time it is not possible to predict whether (spontaneous and vaccine-driven) immune pressure could quickly induce a mild endemic disease or whether this could occur over the course of years, passing through a more aggressive and severe disease, and how mass vaccination may influence its development (this information will rapidly be available in highly vaccinated countries). In this regard, a recent report estimated that infection with a new variant of B.1.1.7 lineage spread in UK during December 2020 has the potential to cause substantial additional mortality compared with previously circulating variants (54906 matched pairs of participants between 1 October 2020 and 29 January 2021), increasing the probability of risk of mortality from 2.5 to 4.1 per 1000 detected cases (**25**). Results that are in agreement with those of another recent report (**26**) and suggest that at the moment the progression of the disease is becoming worse.

For all the above considerations, although vaccine strategies may temporary reduce both disease severity and spread, are unlikely to prevent the appearance of new variants and to be effective in quickly solving the pandemic crisis. It is instead likely that global herd immunity will be slowly achievable by vaccination and neutralizing antibody strategies. Therefore, there is the need to keep searching for new pharmacological therapies and more scientific efforts should be directed towards pharmacological approaches that, working downstream the infection pathways, are independent on virus variant. In this regard, clinical trials employing new safe pharmacological treatments for COVID-19 with a potentially effective mechanism of action that are not tested in clinical trials yet, such as chelating agents, bismuth based or other antiviral drugs, are urgently needed (see **27-30**).

The proposal of a new pharmacotherapy: the targeting of ACE2 and ADAM17 zinc-

metalloproteases

In previous works, we have highlighted that the binding of the SARS-CoV spike proteins to the zinc-metalloprotease ACE2 has been shown to induce ACE2 shedding by activating the zinc-metalloprotease ADAM17, which ultimately leads to systemic upregulation of ACE2 activity (**27,28, 31**). Indeed, the interaction of ACE2 with spike protein of SARS-CoV is able to induce a cellular "protective" ACE2 shedding feedback response that initially limits viral entry (**31**). However, an excessive systemic ACE2 activity produced by ACE2 shedding would be at the origin of COVID-19, which would be induced by positive feedback-loops initially triggered by SARS-CoV-2 and subsequently sustained independently on viral trigger (see **27,28**). Indeed, a few experimental models have shown that an upregulation of pathways downstream ACE2 activity (i.e. Mas receptor and angiotensin receptor 2) can leads to "clinical" manifestations resembling COVID-19 (see**28**). Moreover, strong upregulation of circulating ACE2 activity was recently reported in COVID-19 patients (**32**) and different comorbidities associated with patients' critical status are characterised by pre-existing increased ACE2 activity (see**27,28**), thus supporting the hypothesis that COVID-19 may derive from excessive upregulation of ACE2 activity. Notably, in contrast to SARS-CoVs, HNL63-CoV, which similarly binds to ACE2 through its spike protein, infects ACE2-bearing cells that leads to common cold without inducing both ACE2 shedding and SARS (**31**). That means that the recruitment of ADAM17 in close proximity to ACE2 is a crucial event in order to induce both ACE2 shedding and consequent deleterious effects of SARS-CoVs. It is well known that spike protein has a receptor binding domain (RBD) that is decisively involved in the ACE2-mediated viral binding/entry. Within the RBD, there is an immunodominant SARS-CoV-2 receptor binding motif (RBM, spanning residues 438 to 506), which is the primary target of the neutralizing antibody response induced by SARS-CoV-2 vaccination or infection. The selective pressure of the immune system in vaccinated or convalescent people is known to induce an adaptation process of SARS-CoV-2 that constantly generates a heterogeneous pool of SARS-CoV-2 variants. As expected, the RBM is one the highest variable regions of the S protein (**17**) and SARS-CoV-2 spike variants that increase the binding affinity to human ACE2 and/or confer resistance to neutralising antibodies (such as N439K, Y453F, E484K and N501Y mutations, **17**) are often located in the RBM. Notably, the highest variable region of the entire S protein is surprisingly the N-terminal domain (**17**) and recurrent deletions are preferentially detected in the NTD of the spike glycoprotein (**33**), suggesting that this region may have yet unknown functions involved in SARS-CoV-2 infection. Interestingly, a two-amino-acid deletion at position 69-70 in the NTD often co-occurs with one of the mutations in RBM (N501Y, N439K, or Y453F) (**9, 17, 24**), suggesting that RBM and NTD could participate together in the binding process and in the infectious development of SARS-CoV-2 variants. If this would be the case, these mutations could possibly affect both the binding affinity of SARS-CoV-2 virions to ACE2-ADAM17 expressing cells and the shedding of ACE2 (mediated by ADAM17 recruitment) that leads downstream to induction of pathological events.

Based on this, a reasonable hypothesis of using inhibitors that curb the upregulation of both ACE2 and ADAM17 zinc-metalloprotease activities can be proposed as therapy for COVID-19. In particular, zinc-chelating agents such as citrate and ethylenediaminetetraacetic acid (EDTA) alone or in combination are expected to act in protecting from COVID-19 at different levels thanks to their both anticoagulant properties and inhibitory activity on zinc-metalloproteases (see **27,28**). Several zinc-chelating agents as well as specific RAS pathway inhibitors (e.g., phytates, nicotianamine, zeolites, MLN-4760, Dx600, A779, aliskiren) and specific ADAM17 inhibitors (**34**) have been already proposed as anti-SARS-CoV-2 agents whose administration routes and safety concerns have been widely discussed in previous works (see**27,28**). Among them, chelating agent supplementation during the SARS-CoV-2 pandemic as an adjunct treatment to reduce the risk of infection and severe disease progression is a feasible treatment because oral supplementation of chelating agents is well-tolerated, safe, promptly available, easily deliverable and storable, inexpensive and practically usable also in developing countries. Unfortunately, clinical trials employing chelating agents in COVID-19 are currently absent and urgently needed in order to shed more light on the efficacy of zinc chelators against SARS-CoV-2 infection in vivo.

Competing interests

The authors declare no competing interests.

Author Contributions

L.Z. conceived of the article and wrote it. M.B.L.R. elaborated the statistical analyses and corrected the manuscript. L.Z. and M.B.L.R. read and approved the final manuscript.

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