SARS-CoV-2 immunity-escape variants

SUMMARY

1. SARS-CoV-2 variants which show reduced neutralisation by convalescent plasma have been generated experimentally and have been observed in an immunocompromised individual with persistent infection treated with convalescent plasma.

2. Particularly in immune suppressed individuals with prolonged viral replication, viral evasion can occur during antibody-based treatment. However, the overall impact of these escape variants on clinical and virological outcomes are not clear.

3. For both vaccines approved for use in the UK, the virus neutralization titres and efficacy are higher after two doses than one. There is therefore a higher risk of virus replication under partial immunity after one dose than after two doses, so in the short-term, delaying the second dose would be expected to somewhat increase the probability of emergence of vaccine resistance - but probably from a low base.

4. Whilst the neutralization titres seen after one dose of vaccine are lower than the median titre of convalescent plasma, they are within the lower range of responses seen following natural infection.

5. In the current UK circumstances the unquantifiable but likely small probability of the delayed second dose generating a vaccine escape mutant must be weighed against the measurable benefits of doubling the speed with which the most vulnerable can be given vaccine-induced protection.

6. It is a realistic possibility that over time immune escape variants will emerge, most likely driven by increasing population immunity following natural infection.

PURPOSE

1. Concerns have been raised about the possible emergence of SARS-CoV-2 variants that escape immune recognition because of:

   a. The recent identification of two SARS-CoV-2 variants (one in the UK and the other in South Africa) with apparently increased transmission and substitutions in the receptor binding domain (RBD) on the spike protein that theoretically might be associated with immune escape;

   b. High levels of SARS-CoV-2 incidence in the community in the UK associated with a variant B.1.1.7;

   c. The decision in the UK to provide the second dose of SARS-CoV-2 vaccine at 12 weeks rather than 3 weeks after the first dose.

2. This paper explores the possibility that SARS-CoV-2 escape variants that are partially or fully resistant to natural immunity, vaccination or antibody therapies may have arisen or will arise.

BACKGROUND

3. SARS-CoV-2 is prone to making errors in its genetic code during replication, accumulating 1-2 nucleotide changes every month[1] but given that coronaviruses have the capacity for proofreading during replication, mutation rates are lower than in other RNA viruses. However, as SARS-CoV-2 has circulated globally, genetic changes have accumulated and will continue to do so, perhaps at a faster rate as greater immunity develops in the population. [2] [3]

4. Most mutations will be inconsequential, but a few may, by chance, confer a functional advantage over others and, through natural selection may become dominant. A SARS-CoV-2 variant with a D614G substitution that has an apparent fitness advantage has already been seen. This variant emerged in late January or early February 2020 and replaced the initial SARS-CoV-2 strain identified in China and by June 2020 became the dominant form of the virus circulating globally.[4,5]

5. Mutations that allow escape from monoclonal antibodies can be predicted and readily generated in the laboratory, including a single substitution conferring reduced susceptibility to two different monoclonal antibodies (E406W and REGN10933 plus REGN10987). [6–9]

6. Some laboratory generated variants also show at least 2-fold and up to 30 fold reduced neutralisation with polyclonal serum from convalescent subjects. [10,11]

7. There is therefore theoretical and experimental data supporting the possibility that SARS-CoV-2 variants may arise which evade monoclonal antibody therapies, convalescent plasma therapy, vaccine derived immunity, or naturally acquired immunity.

UK VARIANT OF CONCERN B.1.1.7

8. In early December 2020, a new variant (B.1.1.7) with 23 mutations: 14 non-synonymous mutations, 3 deletions and 6 synonymous mutations was identified in the UK. Phylogenetic and transmission modelling analyses suggest this variant has increased transmissibility compared to other variants.[12]

9. B.1.1.7 contains 8 changes in the spike glycoprotein including deletions in the N terminal domain (NTD) and point changes in the receptor binding domain (RBD), notably N501Y.[a]

10. Substitutions at position 501 (although not N501Y specifically) have been associated with reduced viral neutralisation to the monoclonal antibody LY-CoV016. This substitution has not been associated with reduced viral neutralisation to polyclonal sera.[13,14]

SOUTH AFRICA VARIANT OF CONCERN B.1.351

11. At the beginning of October, a different variant was detected in South Africa (B.1.351) which also possessed a large number (22) of mutations, with molecular dating suggesting that it arose at the end of August.[15] This virus is characterised by eight lineage-defining mutations in the spike protein, including three in the receptor binding

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[a] https://virological.org/t/563

domain, and early epidemiological analyses performed by LSHTM suggest a transmission advantage over other co-circulating variants.

12. B1.351 in addition to D614G contains 8 lineage defining mutations in the spike glycoprotein including deletions in the NTD and point changes in the RBD, notably E484K, N501Y and K417N. [15]

13. There are published reports indicating that the E484K and K417 substitutions are associated with evasion of monoclonal antibodies.[9,11,13,15,16]

14. The E484K substitution has been reported to confer reduced susceptibility to neutralisation by the Eli Lilly monoclonal antibody product bamlanivimab. Phenotypic data for E484K variants showed a >100-fold reduction in susceptibility to bamlanivimab in a SARS-CoV-2 pseudovirus neutralization assay. The presence of this variant may be associated with virological failure of treatment; however the number of subjects were small, therefore, the overall impact of these emergent resistance-associated variants on clinical and virological outcomes are not clear. [17]

15. In a recent screen of RBD mutations that evaded neutralization by polyclonal sera, there was considerable heterogeneity between sera from 11 different individuals and even between sera taken at different times after infection from the same individual. Nonetheless E484K was the mutation of most concern since it enabled evasion of neutralization by polyclonal sera from several different individuals. E484K, in combination with one deletion and one insertion in the N-terminal domain, was associated with reduced neutralization (>10 fold) by convalescent sera. [13]

ORIGIN OF SARS-COV-2 VARIANTS

16. There is no direct evidence to indicate the exact source of the UK and South African variants. There are several hypothesis why variants may arise, these include zoonotic origin, selective pressure during treatment (including intermittent treatment with antivirals, MAbs or convalescent plasma treatment) or during viral persistence in immunocompromised individuals.

17. Among immunosuppressed individuals, prolonged viral RNA shedding has been observed up to 4 months.[18–20] In one report patients with profound immunosuppression after undergoing hematopoietic stem-cell transplantation or receiving cellular therapies shed viable SARS-CoV-2 for at least 2 months[18]. In two individuals with severe immunosupression and persistent SARS-CoV-2 infection who were treated with monoclonal antibodies (Regeneron) and convalescent plasma, genome sequencing revealed unusually large numbers of nucleotide changes and deletion mutations. [19,20]

18. However, it's important to note that in immunocompetent individuals, the length of RNA shedding and viable virus shedding is much shorter.[21] Therefore, the potential for monoclonal antibody or convalescent plasma therapy to generate escape variants is likely much lower in immunocompetent individuals.

IMPLICATIONS FOR MONOCLONAL ANTIBODY THERAPIES

19. Likelihood that monoclonal antibody therapy in immunocompetent individuals will generate escapte mutants:

    - In a study of 93 patients, the frequency of subjects treated with 700 mg bamlanivimab harbouring a putative resistance-associated variant was similar to that of the placebo arm.[17]

- For emergent variants at an allele fraction ≥50%, there were 6/98 (6.1%) in the 700 mg bamlanivimab group compared with 4/95 (4.1%) in the placebo group.

- These variants occurred disproportionately in high-risk subjects and were associated with higher viral shedding in the high-risk population, but it is not clear whether the variants caused increased viral shedding.[17]

20. Likelihood that escape variants will render monoclonal antibody therapy redundant:

- Of the subjects who met virologic failure, 5.1% harboured a putative resistance-associated variant in the bamlanivimab treatment groups compared with 2.1% in the placebo arm

- A total of 6 (6.1%) of participants in the 700mg bamlanivimab group met the definition of virological failure vs 2 (2.1%) in the placebo group. However, overall clinical and virological outcomes were similar between two groups although the observations are limited.[17]

21. Particularly in immune suppressed individuals where prolonged viral replication is observed, viral evasion can occur during monoclonal antibody treatment. However, according to the available data, the overall impact of these escape variants on clinical and virological outcomes are not clear.

IMPLICATIONS FOR CONVALESCENT PLASMA THERAPY

22. Immune evasion as a result of treatment with polyclonal convalescent plasma is less likely than following treatment with monoclonal antibodies. Convalescent plasma has been used in over 100,000 COVID-19 patients in the USA without any reported cases of immune escape variants. However, the case report by Kemp et al.[20] warrants caution in use of convalescent plasma in patients with severe immune suppression.

VACCINATION AND ESCAPE-VARIANTS

23. The UK Government has decided to delay the second dose of current SARS-CoV-2 vaccines to 12 weeks rather than 3 weeks after the first dose to allow greater coverage of high-risk groups with a first dose. This has raised concerns about increased risk of SARS-CoV-2 virus immune-escape variants emerging in a period of partial immunity that may exist between first and second doses.

24. The risks of developing vaccine escape variants are different from the risk associated with therapeutics. Vaccines overall are less vulnerable to pathogen evolution because of differences in the way drugs and vaccines work.

a. Vaccines are used to prevent infection whilst (most) drugs are used to treat established infections. This means that the opportunities for resistant variants to emerge under within-host selection from vaccines are many orders of magnitude smaller than for drugs.

b. Secondly, in immunocompetent hosts the immune response continues to evolve during a single infection.

c. Thirdly vaccines produce responses from several arms of the immune response against many targets on a pathogen, whilst drugs target comparatively fewer sites.

25. These reasons have been proposed as explanations for the observation that, for many infectious diseases, it has been possible to drive them to the verge of extinction, and hold them there for decades using vaccines, without vaccine escape mutations emerging.[22] Nevertheless, viral variation requiring changes in vaccines does occur – most notably requiring the annual adjustment of influenza vaccines. Although it should be noted that the antigenic variation of influenza is not driven by vaccine use but by immunity following natural infection.

26. The question is whether the nine-week delay to a second dose of SARS-CoV-2 vaccine will materially increase the probability of emergence of an escape mutant. This has two important sub-questions: 1- is the probability increased, and if so, 2- is that increase material.

27. For both vaccines approved for use in the UK, the virus neutralization titres and efficacy are higher after two doses than one. There is therefore an increased risk of virus replication under partial immunity after one dose than after two doses, so in the short-term, delaying the second dose would be expected to somewhat increase the probability of emergence of vaccine resistance - but probably from a low base.

28. Is such an increase material? It is not currently possible to quantify the probability of emergence of vaccine resistance as a result of the delayed second dose, but it is likely to be small. The UK currently has more than 1,000 COVID-19 related deaths each day and has limited supplies of vaccine. In the current UK circumstances the unquantifiable but likely small probability of the delayed second dose generating a vaccine escape mutant must be weighed against the measurable benefits of doubling the speed with which the most vulnerable can be given vaccine-induced protection.

29. In the medium term, the risk of emergence may be reduced since delaying the second ChAdOx vaccination until >12 weeks after the first dose gave rise to antibody titres that were 3 fold higher than those achieved if the boosting dose were given from 3 to 8 weeks after the prime.

30. Whilst the neutralization titres seen after one dose of vaccine are lower than the median titre of convalescent plasma, they are within the lower range of responses seen following natural infection. So a single dose of vaccine does not generate a new/novel risk.

31. Given what we have observed recently with the variants B.1.1.7 and B1.351, it is a realistic possibility that over time immune escape variants will emerge, most likely driven by increasing population immunity following natural infection.

MONITORING IMMUNE-ESCAPE

- Vaccine breakthrough should be closely monitored.

- Vaccinated cases who develop COVID-19 should undergo virus sequencing and genotype to phenotype characterization as quickly as possible to understand whether viral variation may explain the breakthrough.

- Sequencing pillar 1 and pillar 2 samples will be used to understand whether new clusters with hallmark antigenic changes accumulate in the next few months of vaccine roll out.

- More intense study of vaccine recipients in immunosuppressed groups are planned that will reveal whether mutants may arise in this cohort.

6

- It might prove useful to create a cohort of immunosuppressed, vaccinated individuals for active surveillance and sequencing to serve as a sentinel population in whom we watch for vaccine escape with particular care.

- Vaccine efficacy after one dose should be carefully monitored to inform future vaccine policy.

REFERENCES

1. Duchene, S. *et al.* Temporal signal and the phylodynamic threshold of SARS-CoV-2. *Virus Evol.* **6**, veaa061 (2020).

2. Cevik, M., Kuppalli, K., Kindrachuk, J. & Peiris, M. Virology, transmission, and pathogenesis of SARS-CoV-2. *BMJ* m3862 (2020) doi:10.1136/bmj.m3862.

3. Dearlove, B. *et al.* A SARS-CoV-2 vaccine candidate would likely match all currently circulating variants. *Proc. Natl. Acad. Sci.* **117**, 23652–23662 (2020).

4. Volz, E. *et al.* Evaluating the Effects of SARS-CoV-2 Spike Mutation D614G on Transmissibility and Pathogenicity. *Cell* S0092867420315373 (2020) doi:10.1016/j.cell.2020.11.020.

5. Plante, J. A. *et al.* Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* (2020) doi:10.1038/s41586-020-2895-3.

6. Starr, T. N. *et al. Prospective mapping of viral mutations that escape antibodies used to treat COVID-19.* http://biorxiv.org/lookup/doi/10.1101/2020.11.30.405472 (2020) doi:10.1101/2020.11.30.405472.

7. Baum, A. *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* **369**, 1014–1018 (2020).

8. Weiss, S. H. & Wormser, G. P. COVID-19: Understanding the science of antibody testing and lessons from the HIV epidemic. *Diagn. Microbiol. Infect. Dis.* **98**, 115078 (2020).

9. Greaney, A. J. *et al.* Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host Microbe* S1931312820306247 (2020) doi:10.1016/j.chom.2020.11.007.

10. Andreano, E. *et al. SARS-CoV-2 escape* in vitro *from a highly neutralizing COVID-19 convalescent plasma*. http://biorxiv.org/lookup/doi/10.1101/2020.12.28.424451 (2020) doi:10.1101/2020.12.28.424451.

11. Liu, Z. *et al. Landscape analysis of escape variants identifies SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization*. http://biorxiv.org/lookup/doi/10.1101/2020.11.06.372037 (2020) doi:10.1101/2020.11.06.372037.

12. NERVTAG. NERVTAG meeting on SARS-CoV-2 variant under investigation VUI-202012/01.

13. Greaney, A. J. *et al. Comprehensive mapping of mutations to the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human serum antibodies*. http://biorxiv.org/lookup/doi/10.1101/2020.12.31.425021 (2021) doi:10.1101/2020.12.31.425021.

14. The Menachery Lab. Viral neutralisation with N501Y. https://twitter.com/TheMenacheryLab/status/1341460341293723650?s=20.

15. Tegally, H. *et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa*. http://medrxiv.org/lookup/doi/10.1101/2020.12.21.20248640 (2020) doi:10.1101/2020.12.21.20248640.

16. Weisblum, Y. *et al.* Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *eLife* **9**, e61312 (2020).

17. CDER Review. Emergency Use Authorization (EUA) for bamlanivimab 700mg IV. Center for Drug Evaluation and Research (CDER) Review, 2020.

18. Aydillo, T. *et al.* Shedding of Viable SARS-CoV-2 after Immunosuppressive Therapy for Cancer. *N. Engl. J. Med.* **383**, 2586–2588 (2020).

19. Choi, B. *et al.* Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host. *N. Engl. J. Med.* **383**, 2291–2293 (2020).

20. Kemp, S. *et al.* *Neutralising antibodies in Spike mediated SARS-CoV-2 adaptation*. http://medrxiv.org/lookup/doi/10.1101/2020.12.05.20241927 (2020) doi:10.1101/2020.12.05.20241927.

21. Cevik, M. *et al.* SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *Lancet Microbe* S2666524720301725 (2020) doi:10.1016/S2666-5247(20)30172-5.

22. Kennedy, D. A. & Read, A. F. Why does drug resistance readily evolve but vaccine resistance does not? *Proc. R. Soc. B Biol. Sci.* **284**, 20162562 (2017).

23. MHRA. REG 174 INFORMATION FOR UK HEALTHCARE PROFESSIONALS.

24. Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* **383**, 2603–2615 (2020).

25. Mulligan, M. J. *et al.* Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* **586**, 589–593 (2020).

26. Sahin, U. *et al.* COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature* **586**, 594–599 (2020).

27. Voysey, M. *et al.* Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *The Lancet* S0140673620326611 (2020) doi:10.1016/S0140-6736(20)32661-1.

28. Folegatti, P. M. *et al.* Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *The Lancet* **396**, 467–478 (2020).

29. Ramasamy, M. N. *et al.* Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. *The Lancet* **396**, 1979–1993 (2020).