

Research Letter

Title: Inactivated COVID-19 vaccine BBV152/COVAXIN effectively neutralizes recently emerged B 1.1.7 variant of SARS-CoV-2

Running title: Neutralisation of UK variant with vaccinated human serum

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Text

The rapid surge of SARS-CoV-2 cases due to the *Variant of Concern (VOC) 202012/01*, also known as lineage B.1.1.7 or 20B/501Y.V1, in the United Kingdom (UK)¹ in December, 2020 raised concerns in several countries due to its high transmissibility. Many of these countries had direct flights to and from UK. Since identification of the new variants of SARS-CoV-2 in UK and South Africa,² health experts have also expressed their concerns about their potential implications pertaining to vaccine efficacy. The root of such concerns was grounded in the structure of SARS-CoV-2 variant, VOC-202012/01, which came to the centre stage of discussion due to its greater transmissibility in humans compared to the other known SARS-CoV-2 lineages. This variant carries seventeen mutations in the genome, eight of which were found in spike receptor-binding domain (RBD), mediating the attachment of the virus to the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of human cells.² One of these mutations N501Y, at position 501, has asparagine (N) replaced with tyrosine (Y) and has been identified to increase the binding affinity of SARS-CoV-2 to human and murine ACE2.² Therefore, it appeared that the majority of the vaccine candidates, being either recombinant or specifically

targeting the single epitope of original D614G ancestral spike sequence, might not be able to generate an efficient immune response against the new variants.

Here, we successfully isolated and characterized the hCoV-19/India/20203522 SARS-CoV-2 (VOC) 202012/01 from UK-returnees in India with all signature mutations of the UK-variant.³ The VOC 202012/01 hallmarks belonged to the GR clade of the viral isolates recovered from UK returnees coming back to India.³ The method used by us for virus isolation and culture from clinical specimens of COVID-19 are described elsewhere.⁴

The SARS-CoV-2 strain (NIV-2020-770) used in developing the BBV152 vaccine was retrieved from tourists who arrived in New Delhi, India.^{4,5} The virus isolation was performed in the *Vero CCL-81* cells and the genome sequence was deposited in the GISAID (EPI_ISL_420545). The BBV152 vaccine candidate strain is located in the (G clade), containing the Asp614Gly mutation, which is characterized by aspartic acid to glycine shift at the amino acid (AA) position 614 of the spike protein. This was used for PRNT₅₀ assay in the present investigation.⁴

Earlier, we reported development of an inactivated whole-virion SARS-CoV-2 vaccine BBV152, which elicited remarkable neutralizing antibody response in phase I clinical trial against hCoV-19/India/2020770 (homologous), and two heterologous strains from the unclassified cluster namely hCoV-19/India/2020Q111 and hCoV-19/India/2020Q100.⁶ The hCoV-19/India/2020Q111 and hCoV-19/India/2020Q100 virus isolate strains contain the L3606F mutation, where leucine to phenylalanine shifted at AA position 3606 of the ORF1ab⁵ have been observed.

In phase II clinical trial, following two dose immunization schedule at day 0 and 28 with 6 μ g and 3 μ g antigen with imidazoquinoline (TLR7/TLR8 agonist adsorbed on aluminium hydroxide

gel), the vaccine candidate showed noteworthy results in plaque reduction neutralization test (PRNT₅₀) based assay; seroconversion rates of neutralizing antibodies being 98.6%.⁷

Here, we present the NAb titers (PRNT₅₀) of sera collected (four-weeks after the second dose) from 38 vaccine recipients, who received BBV152 vaccine-candidate in phase-II trial⁷ to underline the immunogenicity of BBV152 vaccine candidate against SARS-CoV-2 UK-variant with (VOC) 202012/01 hallmarks belonging to GR clade and strain hCoV-19/India/2020770 belonging to G clade.

A representative set of 20 serum samples of vaccine recipients were also tested against heterologous strains hCoV-19/India/2020Q111 (unclassified cluster) using PRNT₅₀ test assay described earlier.⁸ Briefly, all the sera were heat inactivated and serially diluted 4-fold. Further, these samples were mixed with an equal amount of virus suspension containing 50-60 plaque-forming units (PFU) in 0.1 ml. After incubating the mixtures at 37°C for 1 h, each virus-diluted serum sample (0.1 ml) was inoculated onto a 24-well tissue culture plate containing a confluent monolayer of *Vero CCL-81 cells*. After incubating the plate at 37°C for 60 min, an overlay medium consisting of 2% carboxymethyl cellulose (CMC) with 2% fetal calf serum (FCS) in 2× MEM was added to the cell monolayer, and the plate was further incubated at 37°C in 5% CO₂ for 5 days. Plates were stained with 1% amido black for an hour. Antibody titers were determined as the highest serum dilution that resulted in >50% (PRNT₅₀) reduction in the number of plaques.

All sera had equivalent NAb titers to hCoV-19/India/2020770 homologous strain and two heterologous strains including the characteristic N501Y substitution of the UK-variant; hCoV-19/India/20203522 (UK strain) as well as hCoV-19/India/2020Q111 (Figure 1 A and B). The median ratio of 50% neutralisation of sera was 0.8 compared with hCoV-19/India/2020770

against mutant hCoV-19/India/20203522 (UK-variant), and 0.9 while compared with hCoV-19/India/2020Q111. Non-parametric Kruskal-Wallis test for the comparison of the PRNT₅₀ values from different groups revealed non-significant difference ($p > 0.05$).

Andreano E *et al.* reported an escape of the UK-variant with E484K substitution, which was followed by an 11-amino-acid insertion in the NTD N5 loop (_{248a}KTRNKSTSRRE_{248k}) from high NAb in convalescent plasma, which was a serious concern.⁹ Our study evidently highlighted comparable neutralization activity of vaccinated sera against new UK-variant as well as heterologous SARS-CoV-2 strains. Interestingly, the results showed that the vaccinees' sera could neutralise the new UK-variant strain and heterologous strains with equal efficiency, discounting the uncertainty of possible neutralization escape. The statistically non-significant difference observed (Figure 1) for the UK-variant and other tested strains demonstrated reassuringly similar viral neutralization profile. Similarly, Wu k. et al, assessed the neutralizing capacity of the sera from the humans and nonhuman primates immunized with mRNA-1273 vaccine and demonstrated effective neutralizing response against B.1.1.7 variant.¹⁰

Mutations are expected to occur during viral proliferation in SARS-CoV-2 as witnessed worldwide. Importantly, our study showed that the sera from the vaccine recipients could neutralise the UK-variant strains discounting the uncertainty around potential escape. It was reassuring from the PRNT₅₀ data generated in our laboratory that the indigenous BBV152/COVAXIN, following its roll out in the vaccination program, could be expected to work against the new UK-variant. It is unlikely that the mutation 501Y would dampen the potential benefits of the current vaccination program.

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Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Conflicts of Interest: The authors declared no competing interest.

Author Contributions: P.D.Y and G.N.S contributed to study design, data collection, data analysis, interpretation, writing and critical review. G.R.D and R.E contributed to data analysis and interpretation, writing and critical review. R.R.S, N.G, V.K.M and S.P contributed to data collection, writing and critical review. P.A and B.B contributed to writing and critical review of the manuscript.

References:

1. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations.
<https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563>
2. Starr TN, Greaney AJ, Hilton SK, Et al. Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. Cell. 2020; 182(5):1295-310.

3. Yadav PD, Nyayanit DA, Sahay RR, *et al.* Isolation and characterization of VUI97 202012/01, a SARS-CoV-2 variant in travellers from the United Kingdom to India. *J Travel Medicine*. DOI: 10.1093/jtm/taab009.
4. Sarkale P, Patil S. Yadav PD et al. First isolation of SARS-CoV-2 from clinical samples in India. *Indian J Med Res* 2020; 151(2): 244.
5. Potdar V, Cherian SS, Deshpande GR, et al. Genomic analysis of SARS-CoV-2 strains among Indians returning from Italy, Iran & China, & Italian tourists in India. *Indian J Med Res* 2020;151:255-60
6. Ella R, Vadrevu KM, Jogdand H, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial. *Lancet Infect Dis*. 2021 Jan 21:S1473-3099(20)30942-7. doi: 10.1016/S1473-3099(20)30942-7. Epub ahead of print. PMID: 33485468.
7. Ella R, Reddy S, Jogdand H, et al. Safety and immunogenicity clinical trial of an inactivated SARS-CoV-2 vaccine, BBV152 (a phase 2, double-blind, randomised controlled trial) and the persistence of immune responses from a phase 1 follow-up report medRxiv 2020.12.21.20248643; doi: <https://doi.org/10.1101/2020.12.21.20248643>
8. Deshpande GR, Sapkal GN, Tilekar BN, et al. Neutralizing antibody responses to SARS-CoV-2 in COVID-19 patients. *Indian J Med Res*. 2020; 152 (1): 82-87.
9. Andreano E, Piccini G, Licastro D, et al. SARS-CoV-2 escape in vitro from highly neutralizing COVID-19 convalescent plasma. *bioRxiv*. 2020. doi: 10.1101/2020.12.28.424451

10. Wu k, Werner AP, Moliva JI, *et al.* mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. BioRxiv. 2021.doi: <https://doi.org/10.1101/2021.01.25.427948>

Legends to figure:

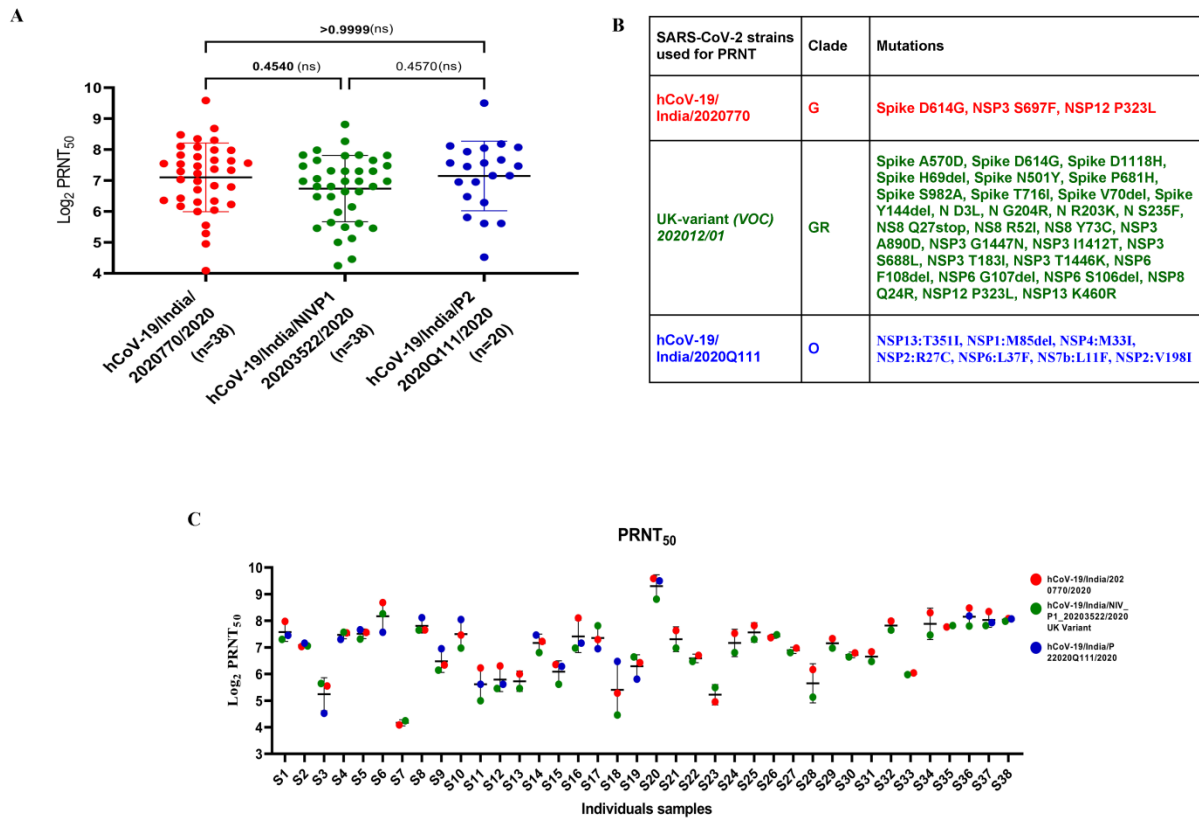


Figure 1: (A) Neutralising antibody response of BBV152/COVAXIN vaccinated sera against SARS CoV-2 strains: Neutralizing antibody titres (PRNT_{50} value) of vaccinees' sera against hCoV-19/India/2020770 (homologous Asp614Gly mutation G clade), hCoV-19/India/20203522 (heterologous UK-variant (VOC) 202012/01 hallmarks belonged to GR clade) and hCoV-19/India/2020Q111 (heterologous unclassified cluster with L3606F mutation). The bar represents the geometric mean and standard deviation of the respective group titers.

Non-parametric Kruskal-Wallis test was used for the comparison of the PRNT₅₀ values from different groups. The p-values above 0.05 were considered non-significant and are marked on the figure (ns-non-significant). **(B) Details of SARS-CoV-2 strains used for plaque reduction neutralization test with respective mutations (C) Comparison of PRNT₅₀ value of each vaccine recipient's sera with three strains of SARS CoV-2:** Neutralizing antibody titres, (PRNT₅₀ value) of each vaccinees' sera against hCoV-19/India/2020770 (homologous Asp614Gly mutation G clade), hCoV-19/India/20203522 (heterologous UK-variant (*VOC*) 202012/01 hallmarks belonged to GR clade) and hCoV-19/India/2020Q111 (heterologous unclassified cluster with L3606F mutation).The bar represents the mean and standard deviation of the respective sera.

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