Limited neutralization of authentic SARS-CoV-2 variants carrying E484K *in vitro*

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Summary (40 words max):

Authentic SARS-CoV-2 B.1.1.7 (Alpha) is efficiently neutralized by monoclonal antibodies, convalescence and vaccine-elicited sera. However, bamlavimab, casirivimab, convalescence and BNT2b2 or mRNA1273 vaccine-elicited sera are less effective against authentic B.1.351 (Beta) and P.2 (Zeta) both carrying E484K *in vitro*.

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Abstract: Whether monoclonal antibodies are able to neutralise SARS-CoV-2 variants of concern has been investigated using pseudoviruses. In this study we show that bamlanivimab, casirivimab, and imdevimab efficiently neutralise authentic SARS-CoV-2 including variant B.1.1.7 (Alpha) but variants B.1.351 (Beta) and P.2 (Zeta) were resistant against bamlanivimab and partially to casirivimab.

Keywords:

SARS-CoV-2, corona virus, monoclonal antibodies, bamlanivimab, casirivimab, imdevinab

Introduction/Background

As vaccination campaigns against COVID-19 are ongoing, the majority of the world´s population remains unimmunized, and many at risk for severe disease. The availability of both therapeutic and prophylactic agents with proven efficacy are still urgently needed. IgG1 monoclonal antibodies (mAb) prevent viral attachment and entry into human cells by blocking attachment to the ACE2 receptor. However, since several SARS-CoV-2 variants of concern (VoCs), that emerged late 2020, have been associated with increased transmissibility or immune evasion, it is of particular importance to evaluate the effectiveness of mAb against these variants. In particular, substitutions E484K and K417N in S have been associated with immune escape, or increased binding to the ACE-2 receptor (e.g., N501Y) [1].

To evaluate whether variants harboring these substitutions might be also effectively neutralized is critical for effective treatment. So far, studies have been conducted with artificial pseudoviruses indicating that B.1.351 (Beta), P.1 (Gamma) and P.2 (Zeta) variants are expected to be resistant to therapeutic monoclonal antibodies. Furthermore, these variants have been proposed to be partially resistant to neutralization by convalescent sera obtained from COVID-19 patient as well as by vaccine-elicted sera obtained from individuals after immunization with BNT2b2 or mRNA1273. However, since in real world settings additional substitutions that might determine replication efficiency define each VoC, there is still lacking evidence that these artificially performed studies can also be transferred to authentic viruses. Hence, in this study we analysed the ability of bamlanivimab, casirivimab, and imdevimab to neutralize authentic SARS-CoV-2 variants of concern including B.1.1.7 (Alpha), B.1.351 (Beta), and P.2 (Zeta) in infectious cell culture. Additionally, we analyzed vaccine-elicited sera after immunization with BNT162b2 and mRNA1273, and convalescent sera for their ability to neutralize authentic SARS-CoV-2 variants.

Bamlanivimab and REGN-CoV-2 are IgG1 monoclonal antibody (mAb) preparations to treat COVID-19. Bamlanivimab (LY-CoV555) has been demonstrated to accelerate the decline in viral load [2] and has been authorized by the FDA for emergency use in early mild to

moderate COVID-19 disease. REGN-CoV-2 consists of two potent mAb REGN10933 (Casirivimab) and REGN10987 (Imdevimab), both binding non-competitively in different regions of Spike S [3].It is also approved by the FDA for emergency use. Class 1 mAb REGN10933 has an ACE2 blocking property, while class 3 mAB REGN1087 binds outside of the receptor binding domain (RBD).

Emerging SARS-CoV-2 “variants of concern” (VoCs) and “variants of interest” (VoIs) referred to as B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), and P.2 (Zeta), initially observed in the United Kingdom, South Africa, and Brazil, respectively, are in the process of fixation in the population. Mutations in the spike´s receptor binding domain, in particular N501Y, were associated with increased infectivity due to enhanced receptor binding [4]. Further studies have shown that of E484K represents an immunodominant site on the RBD since E484K reduced naturalization capacity of human convalescent sera by >100-fold [1-4]. These amino acid substitutions have also been observed to evade the antibody response elicited by an infection with other SARS-CoV-2 variants, or vaccination.. So far, it is still under debate whether mAb preparations are equally effective against these variants.

In this study, we examined the ability of bamlanivimab, casirivimab, imdevinab, vaccine-elicited sera after immunization with BNT162b2 and mRNA1273, respectively, and convalescent sera, to neutralize authentic SARS-CoV-2 variants B.1.1.7 (Alpha, mutations include N501Y and Δ69/70), B.1.351 (Beta, mutations include E484K and N501Y), and P.2 (Zeta, mutations include E484K in the absence of a 501 mutation). These isolates have been obtained from travelers from Great Britain, South Africa and Brazil, respectively. Two SARS-CoV-2 isolates collected in early 2020 were also tested (B, FFM1; B.1, FFM7) [5, 6].

Methods:

SARS-CoV-2 IgG levels were quantified using the SARS-CoV-2 IgG II Quant kit (Abbott Diagnostics, Delkenheim, Germany) (**table 1**). The mAb working solutions,convalescence sera, or sera from BNT162b2 and mRNA1273 vaccinated individuals were serially diluted 1:2 and incubated with 4000 $TCID_{50}$ / ml of each SARS-CoV-2 isolate, and subjected to cell based SARS-CoV-2 neutralization assay. The corresponding sample dilution resulting in 50% virus neutralization titer (NT_{50}) was determined. After 3 days of incubation cells were evaluated for the presence of a cytopathic effect (CPE).

All relevant ethical guidelines have been followed and approved by the Ethik-Kommission des Fachbereiches Medizin der Goethe Universitaet Frankfurt (250719). All necessary patient/participant consent has been obtained and the appropriate institutional forms have been archived.

Results:

The variant B.1.1.7 (Alpha), as well as B and B.1 isolates from early 2020 (FFM1 and FFM7), could be efficiently neutralized by bamlanivimab, casirivimab, and imdevinab (titer 1/1280, respectively) (**figure 1a**). However, using bamlanivimab, no neutralization effect could be detected against either B.1.351 (Beta) or P.2 (Zeta), both harboring the E484K substitution. Spike protein alignments confirmed that only E484K substitution occurs exclusively in the two strains that could not be neutralized by bamlanivimab (GeneBank accession numbers: MW822592 (B.1.351; FFM-ZAF1/2021) and MW822593 (P.2; FFM-BRA1/2021)). Imdevimab binding outside the ACE2 receptor binding domain (RBD), was able to neutralize the virus without decline in efficiency. However, for casirivimab a severe drop in neutralization capacity was observed for B.1.351 (Beta) (titer 1/20), and a considerably reduction in the neutralization capacity against P.2 (Zeta) (titer 1/320) (**figure 1a**). These data shows that B.1.351 exerts a 1000-fold reduction of SARS-CoV-2

neutralizing activity of bamlanivimab and casirivimab and confirms the observations made with artificial pseudoviruses published previously [7]. In addition, P.2 (Zeta) virus was resistant to bamlanivimab and partly resistant against casirivimab (>4-fold). However, using the clinically used combination of REGN-CoV-2, full neutralization was observed indicating unrestricted effectiveness of a therapeutic treatment.

To determine the neutralisation efficiency of convalescence and vaccine-elicited sera, SARS-CoV-2 specific antibodies were quantified (**table 1**). Antibody titers were detected for the vast majority of the tested samples with an average of 524.0 BAU/ml (± 1256.0) for convalescent sera, 2135.9 BAU/ml (± 1868.4) for BNT162b and 2927.6 BAU/ml (± 1711.4) for mRNA1273 vaccination elicited sera. Comparable to SARS-CoV-2 variants from early 2020 (B and B.1), convalescence (**figure 1b, figure S1a**) and BNT162b2 or mRNA1273 vaccine-elicited sera, respectively, (**figure 1c, figure S1b**) efficiently neutralized SARS-CoV-2 B.1.1.7 (Alpha). A moderate but significant decrease (average of all vaccine-elicited sera: 1.27-fold) in the neutralisation efficiency of sera from vaccinated individuals was observed for SARS-CoV-2 B.1.1.7 (Alpha) compared to SARS-CoV-2 B (p=0.021). Compared to B.1 (FFM7), a slightly higher neutralisation efficiency (0.54-fold) could be observed (p=0.025). All tested sera were significantly less efficient against SARS-CoV-2 B.1.351 (Beta) and P.2 (Zeta) (**figure 1b-c, figure S1**). Testing of convalescent sera revealed a 6.27-6.74-fold lower neutralizing activity against B1.351 (Beta) and a 5.09-fold lower activity against P.2 (Zeta) when compared the variants from early 2020 (**figure 1a, figure S1a**). Serum samples of vaccinated individuals were on average 4.35-fold and 2.67-fold (B and B.1, respectively) less effective against variant B.1.351 (Beta) and 2.84-fold and 2.86-fold (B and B.1, respectively) less effective against variant P.2 (Zeta). Considering the antibody titers the efficacy of both mRNA1273 and BNT162b2 vaccine-elicited sera was comparable in our study. In one serum obtained from an individual who was vaccinated after convalescence (#21) a loss of neutralisation efficiency was observed despite high antibody concentrations (**table1**).

Discussion:

The COVID-19 pandemic continues to set an extraordinary burden on world health. While the proportion of protectively vaccinated individuals is steadily increasing, there are still only limited treatment options available. Furthermore, it is still unclear how long vaccinations will last and whether they are effective against all variants. Since the SARS-CoV-2 variants B.1.1.7 (Alpha) and B.1.351 (Beta) tested in this study are currently displaced by others (such as B.1.617.2; Delta), there will be a constant need to test the available protective and therapeutic options. Previous studies, conducted predominantly with pseudotyped viruses, have shown that the sensitivity of B.1.1.7 (Alpha) to neutralisation by convalescent sera is slightly lower compared to preceding viral isolates, however, the neutralisation sensitivity of variant B.1.351 was shown to be significantly reduced [8]. Our *in vitr*o findings using authentic SARS-CoV-2 confirms that, in contrast to vaccine-elicited sera, bamlanivimab and casirivimab may not provide efficacy against SARS-CoV-2 variants B.1.351 (Beta) and P.2 (Zeta) both harboring the E484K substitution. Since imdevimab was able to efficiently neutralize both variants, therapeutical treatment with the REGN-COV-2 combination is unrestricted effective. In agreement, previous studies using artificial pseudoviruses described that LY-CoV555 and REGN10933 are ineffective against B.1.351 (Beta) but is still effective against B.1.1.7 (Alpha) [7, 9].

This and previous work revealed a lower neutralizing activity against E484K harboring variants B1.351 (Beta) and P.2 (Zeta), which may facilitate re-infection with emerging variants [8, 10, 11]. Amino acid substitutions hinders spike proteins to be bound by antibodies, resulting in reduced protection against SARS-CoV-2 infection [12]. Hence, a higher antibody titer is needed, which might be provided by a second vaccination dose inducing the formation of a critical amount of neutralizing antibodies [13, 14]. Even a single immunization already increased the neutralising titers of convalescents, although with reduced efficiency for B.1.351 (beta) [8]. Of note, immune response after vaccination also includes a broad T cell repertoire, which might be effective despite resistance to antibody

mediated immunity [15]. Hence, viral escape of T cell immunity is unlikely. However, whether this also applicable for tested and future SARS-CoV-2 variants has to be further investigated.

Our study has the following limitations: While we used authentic SARS-CoV-2 variants rather than pseudotyped surrogate models, only a single representative isolate was studied for each variant. Hence, a broader analysis with more isolates should be expanded in future studies. We tested convalescent sera and vaccine-elicited sera (mRNA1273 and BNT162b2) for their neutralisation efficiency, however, the number of sera was relatively small and most of these convalescence sera originate from individuals infected with non-VOCs. In future, it would be also interesting to study sera from individuals infected with VOCs and thus with corresponding antibodies. In addition, sera from individuals vaccinated with vector vaccines and heterologously vaccinated should be included.

Conclusion:

We conclude that confirmation of the SARS-CoV-2 variant, including screening for E484K, may be needed before initiating mAb treatment with bamlanivimab and to ensure both efficacious and efficient use of the antibody product. Variant-specific mAb agents may be required to treat emerging SARS-CoV-2 variants of concern. To efficiently neutralize VOCs carrying E484K, a high antibody titre is needed to inducing the formation of a critical amount of neutralizing antibodies.

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References:

1. Greaney AJ, Loes AN, Crawford KHD, et al. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell host & microbe **2021**; 29:463-76 e6.

2. Chen P, Nirula A, Heller B, et al. SARS-CoV-2 Neutralizing Antibody LY-CoV555 in Outpatients with Covid-19. New England Journal of Medicine **2020**; 384:229-37.

3. Hansen J, Baum A, Pascal KE, et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. Science **2020**; 369:1010-4.

4. 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**; 182:1295-310 e20.

5. Toptan T, Hoehl S, Westhaus S, et al. Optimized qRT-PCR Approach for the Detection of Intra- and Extra-Cellular SARS-CoV-2 RNAs. International Journal of Molecular Sciences **2020**; 21:4396.

6. Hoehl S, Rabenau H, Berger A, et al. Evidence of SARS-CoV-2 Infection in Returning Travelers from Wuhan, China. The New England journal of medicine **2020**.

7. Hoffmann M, Arora P, Groß R, et al. SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. Cell **2021**.

8. Stamatatos L, Czartoski J, Wan YH, et al. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. Science **2021**.

9. Wang P, Nair MS, Liu L, et al. Antibody Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7. bioRxiv **2021**:2021.01.25.428137.

10. Kustin T, Harel N, Finkel U, et al. Evidence for increased breakthrough rates of SARS-CoV-2 variants of concern in BNT162b2-mRNA-vaccinated individuals. Nature medicine **2021**.

11. Chen RE, Zhang X, Case JB, et al. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. Nature medicine **2021**; 27:717-26.

12. Harvey WT, Carabelli AM, Jackson B, et al. SARS-CoV-2 variants, spike mutations and immune escape. Nature reviews Microbiology **2021**; 19:409-24.

13. Collier DA, De Marco A, Ferreira I, et al. Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. Nature **2021**; 593:136-41.

14. Becker M, Dulovic A, Junker D, et al. Immune response to SARS-CoV-2 variants of concern in vaccinated individuals. Nat Commun **2021**; 12:3109.

15. Tarke A, Sidney J, Kidd CK, et al. Comprehensive analysis of T cell immunodominance and immunoprevalence of SARS-CoV-2 epitopes in COVID-19 cases. Cell Reports Medicine **2021**; 2:100204.

Figure 1: Neutralization titers against SARS-CoV-2 variants.

The monoclonal antibody (mAb) solutions **(A)** and sera from **(B)** convalescence or **(C)** vaccinated individuals was serially diluted (1:2) and incubated with 4000 $TCID_{50}$ / ml of the indicated SARS-CoV-2 variant. Susceptible cells were subsequently inoculated and analyzed for a CPE formation after 72 h incubation. Values indicate reciprocal dilutions of SARS-CoV-2 microneutralization titers (NT_{50}) resulting in 50% virus neutralization. The indicated mAb solutions were used in physiological concentrations according to manufacturer's instructions. Mean values from two replicates, each determined with Caco2 and A549 cells are shown. Colored dots indicate sera from mRNA1273 (red) and BNT162b2 (blue) vaccinated individuals, respectively. Statistical significance compared to SARS-CoV2 B (FFM1) or B.1 (FFM7), respectively, was determined using one-tailed, paired student's t-test. Asterisks indicate p-values as * ($p < 0.05$), ** ($p \leq 0.01$), and *** ($p \leq 0.001$). Sample #21 (convalescence and BNT162b2-vaccinated) was excluded from statistical analysis. Square symbols represent control sera negative tested for SARS-CoV-2 antibodies.

Table 1: Sample characteristics SARS-CoV-2 antibody concentrations were tested with SARS-CoV-2 IgG II Quant (Abbott Diagnostics, Delkenheim, Germany) using the automated Alinity i device. The quantitative assay is for the detection of - among others - neutralizing antibodies against the receptor binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2. Results are given as binding antibody units per milliliter (BAU/mL, analytical measurement range: 2.98 – 5,680). Values labelled with B, B.1, B1.1.7, B.1351, and P.2 represent microneutralization titers (NT_{50}) resulting in 50% neutralization of the indicated SARS-CoV-2 isolate. GeneBank Accession numbers of the strains: MT358638 (B; FFM1/2020), MT358643 (B1; FFM7/2020), MW822592 (B.1.351; FFM-ZAF1/2021), MW822593 (P.2; FFM-BRA1/2021), MW822594* (B.1.1.7; FFM-UK4604/2020), and MZ427280 (B.1.1.7; FFM-UK7931/2021). The values indicate mean values from two replicates. n= no neutralization. NC = negative control sera. n.t.: not tested.

Sample ID	BAU/ml	Sample description	B	B.1	B.1.1.7 (Alpha)	B.1.351 (Beta)	P.2 (Zeta)
A	>5680	LY-CoV555 (Bamlanivimab)	1280	1280	1280*	n	n
B	>5680	REGN10933 (Casirivimab)	1280	1280	1280*	20	320
C	>5680	REGN10987 (Imdevimab)	1280	1280	1280*	1280	1280
D	>5680	REGN-COV-2 combination	1280	1280	1280*	1280	1280
#1	<3.0	negative control serum	n	n	n	n	n
#2	<3.0	negative control serum	n	n	n	n	n
#3	1927.1	mRNA-1273	40	40	80	20	20
#4	1035.3	mRNA-1273	20	40	20	10	n
#5	>5680	mRNA-1273	640	320	320	20	40
#6	709.0	mRNA-1273	40	20	10	n	n
#7	4008.3	mRNA-1273	80	80	80	20	20
#8	4620.0	mRNA-1273	320	80	160	10	20
#9	1940.0	mRNA-1273	80	40	20	n	10
#10	4167.0	mRNA-1273	160	40	80	40	10
#11	2357.0	mRNA-1273	80	40	80	20	20
#12	5584.4	mRNA-1273	80	40	80	10	20
#13	310.7	BNT162b2	20	n	10	n	n
#14	151.6	BNT162b2	n	n	n	n	n
#15	1040.1	BNT162b2	20	10	10	n	n

#16	4465.1	BNT162b2	160	80	80	20	20
#17	>5680	BNT162b2	320	40	40	20	20
#18	2441.9	BNT162b2	40	40	80	20	20
#19	1019.3	BNT162b2	40	80	n.t.	10	10
#20	1811.8	BNT162b2	320	160	n.t.	40	20
#21	2302.8	convalescence & BNT162b2	1280	1280	n.t.	160	80
#22	23.7	convalescence	160	160	n.t.	n	n
#23	132.9	convalescence	40	20	n.t.	n	10
#24	127.8	convalescence	40	40	n.t.	n	n
#25	62.5	convalescence	320	160	n.t.	n	n
#26	412.2	convalescence	80	160	n.t.	10	10
#27	302.2	convalescence	160	80	n.t.	10	10
#28	46.3	convalescence	40	10	n	n	n
#29	<3.0	convalescence	80	40	n	20	10
#30	159.5	convalescence	20	20	n	n	n
#31	134.3	convalescence	160	160	n	10	20
#32	240.0	convalescence	40	40	40	10	10
#33	119.0	convalescence	10	10	10	n	n
#34	119.0	convalescence	10	10	10	n	n
#35	62.5	convalescence	160	80	160	n	n
#36	314.5	convalescence	80	80	160	40	20
#37	>5680	convalescence	1280	1280	1280	640	640
#38	1286.8	convalescence	40	80	160	10	40
#39	987.6	convalescence	40	40	40	20	20
#40	134.3	convalescence	20	20	20	n	10
#41	132.9	convalescence	10	10	10	n	n

Figure 1