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REVIEW

SARS-CoV-2 E484K Mutation Narrative Review: Epidemiology, Immune Escape, Clinical Implications, and Future Considerations

Wan-Ting Yang[1,*], Wei-Hsuan Huang[1,*], Tsai-Ling Liao[2], Tzu-Hung Hsiao[2], Han-Ni Chuang[2], Po-Yu Liu[1,3,4]

[1]Division of Infection, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; [2]Department of Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan; [3]Ph.D. Program in Translational Medicine, National Chung Hsing University, Taichung, Taiwan; [4]Rong Hsing Research Center for Translational Medicine, National Chung Hsing University, Taichung, Taiwan

*These authors contributed equally to this work

Correspondence: Po-Yu Liu, Division of Infection, Department of Internal Medicine, Taichung Veterans General Hospital, 1650 Taiwan Boulevard Sect. 4, Taichung, 4070, Taiwan, Tel +886 4-23592525, Fax +886 4-2359-5046, Email pyliu@vghtc.gov.tw

Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread rapidly over the world and claimed million lives. The virus evolves constantly, and a swarm of mutants is a now major concern globally. Distinct variants could have independently converged on same mutation, despite being detected in different geographic regions, which suggested it could confer an evolutionary advantage. E484K has rapidly emerged and has frequently been detected in several SARS-CoV-2 variants of concern. In this study, we review the epidemiology and impact of E484K, its effects on neutralizing effect of several monoclonal antibodies, convalescent plasma, and post-vaccine sera.

Keywords: E484K, SARS-CoV-2 variant, coronavirus spike glycoprotein, SARS-CoV-2 convalescent sera treatment, monoclonal antibody, vaccine

Introduction

The emergence of the SARS-CoV-2 strain of the human coronavirus has led to a new pandemic. According to data from the World Health Organization (WHO), as of 31 July 2021, there have been around 194 million confirmed cases of Covid-19, including more than 4 million deaths globally.[1]

SARS-CoV-2 is an enveloped virus, belonging to the genus *Coronavirus*, with a non-segmented, positive-sense single-strand RNA genome, measuring 29,903 kilobytes.[2] Entry of SARS-CoV-2 into host cells is mediated by the transmembrane spike (S) glycoprotein. The S glycoprotein comprises two functional subunits. S1 subunit amino acids 318–510 (S318–510) contains the receptor-binding domains (RBD), which bind to the host cell receptor, angiotensin-converting enzyme 2, whereas the S2 subunit is responsible for fusing of cellular membranes.[3,4] Mutations on the spike protein are common features of many SARS-CoV-2 variants.

Receptor binding domain (RBD) mutations enhance the structural stability and increase the human ACE2 receptor-binding capacity of the spike protein, which could be an essential characteristic that would explain the high virus spread and the severe infectivity.[5] E484K, a mutation of one of the RBD residues, has a glutamate (E) to lysine (K) substitution at position 484.

The mutation in the spike protein has an impact on the convalescent sera, monoclonal antibody treatment, and vaccine efficacy. E484K has been identified as an escape mutation that emerges during convalescent plasma.[6] E484K reduces the convalescent sera antibody binding affinity by more than order of magnitude. Similar experimental results reported by Andreano et al, Greaney et al, Liu et al and Weisblum et al show that mutations at the site E484 reduce the neutralization potency of some human plasmas by >10-fold.[7–10]

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In the research of Liu et al, 0.03% of sequenced isolates exhibited variation at E484, as of October 2020, and this increased to 0.09% of substitutions at this position by January 2021.[10] The variation at E484 emerged more frequently with viral replication. In an escape mutation study, which using 19 monoclonal antibodies, substitutions were found at E484 with higher incidence than at any other residue, and four variants at this position (E484A, E484D, E484G, and E484K) exhibited resistance to each of the human convalescent sera tested.[10]

There is a gap in our current knowledge, with few available data on E484K mutations. In this review, E484K mutations are discussed particularly in the context of observed frequencies in global sequence datasets. The binding-strengthening mutations of E484K can play a vital role in the development of infectivity, transmissibility, and/or antigenicity, and therefore it is important to gain insights into the underlying mechanisms involved. Herein, we analyze the percentage of E484K present in the variants and the global prevalence based on geographic location in order to surveil the unfolding pandemic, comprehend the roles of epidemiological variables, identify SARS-CoV-2 variants and their genetic sequences, and recommend public health policy strategies.

Significance of E484K in SARS-CoV-2 Variant

The E484K mutation is present in several variants and popped up in rapid succession in different geographical regions of five continents, especially in Southern Africa and South America.

The E484K mutation was first identified in the Beta variant (B1.351) in South Africa and was also detected in the Alpha variant (B.1.1.7) in the United Kingdom, as well as in the Gamma variant (P.1) in Brazil, all of which are classified as SARS-CoV-2 variants of concern (VOC). These variants were found to have higher transmissibility, increased fatality rates, and according to the WHO, the effectiveness of vaccines, therapies, and other health measures used against these variants were significantly lower.[11] According to the data from Outbreak Information, the presence of the E484K mutation in the Gamma variant is 91%, compared with 86% in the Beta variant, and less than 0.5% in the Alpha variant. Table 1 illustrates the prevalence rates of E484K in the other variants.[12]

The Eta variant (B.1.525), Lota variant (B.1.526), Kappa variant (B.1.617.1), and Lambda variant (C.37) are all considered Variants of Interest (VOI). The prevalence of the E484K mutation in the Eta variant is 97%, compared with 43% in Iota, 1% in Lambda, and less than 0.5% in Kappa. According to the WHO, a VOI is a SARS CoV-2 variant with a genetic capability that affects characteristics of the virus, such as disease severity, immune escape, transmissibility, and diagnostic escape. The WHO further confirmed that VOIs are responsible for a consequential volume of community transmission. A global increase in VOI cases poses a public health risk to large portions of populations worldwide.[11,12]

The E484K mutation is present in Zeta variant (P.2) with a prevalence rate of 96%, compared to 85% in P.3. Evidence of the phenotypic or epidemiological impact of the Zeta variant and P.3 variant is currently unclear, and thus enhanced monitoring and repeat assessment is required.[12]

E484K Global Presence Rate

Geographically, E484K mutations are highly prevalent in countries in Southern Africa and neighboring islands. Prevalence of positive E484K mutation over 50% in all confirmed cases with genetic sequence is listed in descending order as follows: Mozambique 66%, Mauritius 65%, Reunion 63%, Malawi 61%, South Africa 54%, and Mayotte 51%. E484K mutation prevalence rates over 50% in Southern America, according to data from Outbreak Information, were reported to be 62% in Suriname, 70% in French Guiana, 72% in Brazil, and 78% in both Trinidad and Tobago.[12] Figure 1 summarizes the E484K global presence rate, and the world map is created by the Microsoft Excel™ on the Bing platform and reprinted under a CC BY license.

E484K Reduced Neutralization by Convalescence Sera

Deep mutational scanning (DMS) is a novel method that analyzes how a mutation changes the structure and function of a protein.[13] DMS creates a library of mutated gene variants, cloned into the appropriate vector, and mostly utilizes yeast-display. Greaney et al recognized that E484 plays a crucial role in neutralization by convalescent sera by mutated spike receptor-binding domain yeast library.[7] Assays such as authentic viruses and pseudoviruses with mutated spike protein were applied to evaluate the impacts of mutations on convalescent sera. Inhibitory concentration 50% (IC50) of mutated

Table 1 E484K Prevalence in SARS-CoV-2 Variant

WHO Label	Country First Detected	First Identification Date	Variant	WHO Classification	Percentage
Beta	South Africa	May-20	B.1.351	VOC	85%
Beta	South Africa	May-20	B.1.351.2	VOC	84%
Beta	South Africa	May-20	B.1.351.3	VOC	97%
Alpha	UK	Sep-20	B.1.1.7	VOC	<0.5%
Delta	India	Oct-20	AY.1	VOC	<0.5%
Delta	India	Oct-20	B.1.617.2	VOC	<0.5%
Gamma	Brazil	Nov-20	P.1	VOC	96%
Gamma	Brazil	Nov-20	P.1.1	VOC	97%
Gamma	Brazil	Nov-20	P.1.2	VOC	100%
Iota	USA	Nov-20	B.1.526	VOI	43%
Eta	UK	Dec-20	B.1.525	VOI	97%
	USA	Mar-20	B.1.427	Monitor	<0.5%
	USA	Mar-20	B.1.429	Monitor	<0.5%
Zeta	Brazil	Apr-20	P.2	Monitor	96%
	Russia	Jan-21	AT.1	Monitor	88%
	Multiple countries	Jan-21	B.1.1.318	Monitor	98%
	Multiple countries	Jan-21	B.1.1.519	Monitor	<0.5%
	Colombia	Jan-21	B.1.621	Monitor	96%
Theta	Philippines	Jan-21	P.3	Monitor	85%
	Multiple countries	Jan-21	R.1	Monitor	100%
	Multiple countries	Jan-21	R.2	Monitor	100%
	Colombia	Jan-21	B.1.621.1	Monitor	81%
	UK	Mar-21	AV.1	Monitor	82%

virus and wild-type virus were compared.[14] Most pseudoviruses assays assessing the neutralization impact on SARS-CoV-2 use lentiviral or vesicular stomatitis virus (VSV) vector-based SARS-CoV-2 pseudoviruses. Convalescence sera obtained from different countries and periods have consistently demonstrated that E484K or triple mutation (K417N/E484K/N501Y) decreased neutralization in pseudo-viral assays.

Jangra et al compared the neutralization of the USA-WA1/2020 virus and a recombinant (r)SARS-CoV-2 virus with the E484K mutation, and found a 2·4-fold to 4·2-fold reduction in neutralization when E484K was present.[6] Greaney et al used lentiviral vector-based SARS-CoV-2 pseudoviruses and reported amino acid changes in E484 to K, P, or Q reduced binding of polyclonal convalescent plasma, and in some individuals, mutations at E484K could decrease neutralization by >100-fold.[7] Gaebler et al and Wang et al obtained convalescent plasma at 1.3 months after infection, and the neutralization against the K417N/E484K/N501Y mutant was reduced 0.5- to 29-fold (P = 0.001).[15,16] Chen et al used Vero-hACE2-TMPRSS2 cells, and found neutralization titer of convalescent plasma decreased fivefold against E484K/N501Y compared with the WA1/2020 D614G virus.[17] Wibmer et al obtained convalescent sera from blood donors in South Africa and discovered 27% of the samples lost neutralization against the pseudovirus containing K417N,

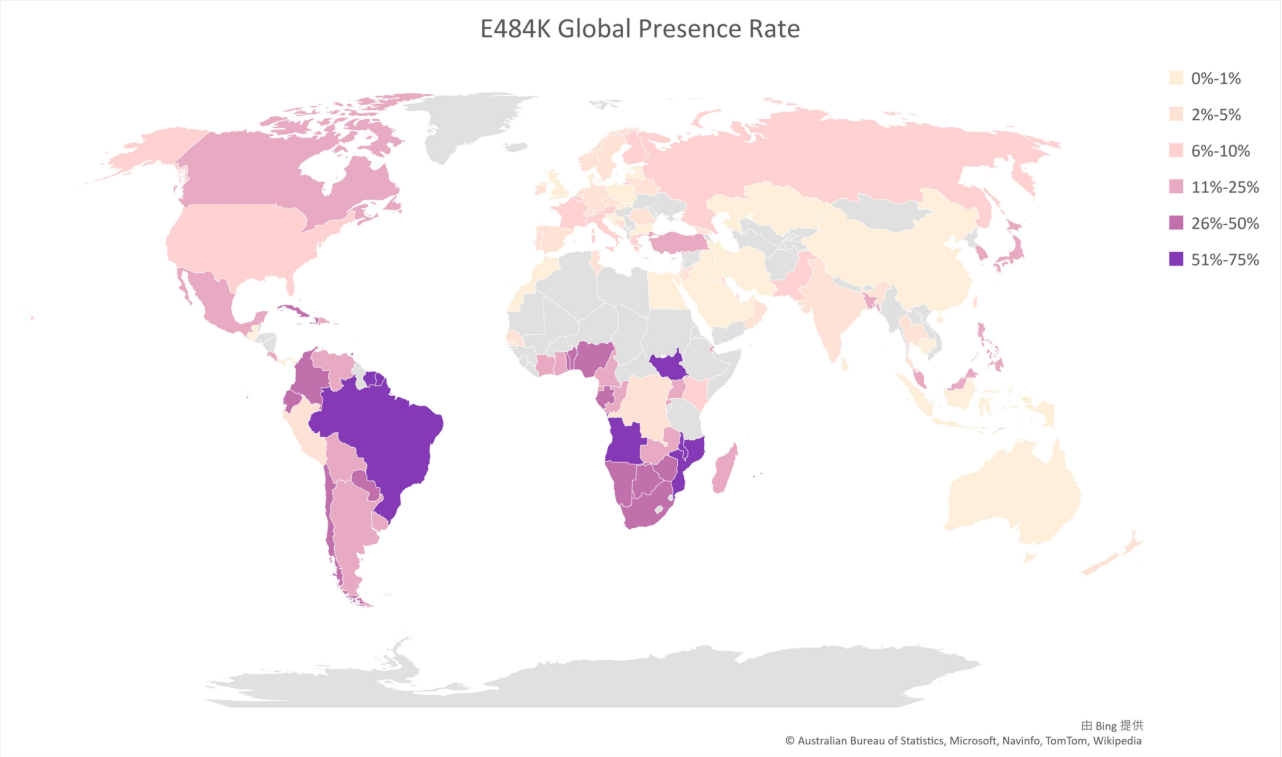

Figure 1 E484K Global Presence Rate.
Notes: No current data for areas in gray color. This map was developed on the Bing platform by Microsoft Excel™, Microsoft product screen shot(s) reprinted with permission from Microsoft Corporation under a CC BY license.

E484K, and N501Y.[18] Collier et al found a similar result showing that the Alpha variant combined with E484K reduced the neutralization 11.4-fold in comparison with the wild-type spike protein.[19] Table 2 summarizes studies on the impact of E484K on the neutralization of convalescent sera.

Monoclonal antibodies derived from convalescent patients were further investigated. Cryo-electron microscopy is an electron microscopy technique capable of capturing images of flash-frozen molecules in liquid nitrogen with a special camera.[20] Cryo-electron microscopy can be used to analyze the structure of RBD-binding neutralizing antibodies and the interaction between RBD mutants and human neutralizing antibodies. Antibodies are categorized into four classes. Class 1 antibodies bind to an epitope only in the RBD "up" conformation, and are the most abundant. Class 2 antibodies bind to the RBD both in "up" and "down" conformations. Class 3 and class 4 antibodies both bind outside the ACE2 binding site. Class 3 antibodies bind the RBD in both the open and closed conformation, while class 4 antibodies bind only in the open conformation. Class 2 antibodies resist E484K mutations.[15,21]

Using Cryo-EM, Barnes et al identified three class 2 antibodies, and all of them commonly interact with E484.[21] They further detected molecular interactions by surface plasmon resonance (SPR). In the SPR assay, class 2 antibodies lose binding affinity with E484K mutations.[21] Other studies using pseudovirus assay similarly proved class 2 antibodies conferred resistance to E484K mutation.[9,10]

E484K Reduced Neutralization by Post-Vaccination Sera

Among the various modalities that have been considered in the fight to bring an end to the COVID-19 pandemic, vaccination offers the most promise. To date, over 100 vaccines have been evaluated in human trials, and over 180 vaccines have been tested in preclinical trials.[22] Several vaccines using different platforms have been approved and are proven to be safe and effective. Platforms of currently available vaccines include inactivated virus vaccines, live attenuated virus vaccines, recombinant protein vaccines, vector vaccines, and RNA and DNA vaccines.[23]

Table 2 Studies Demonstrating E484K Reduced Neutralization of Convalescent Sera

Author	Plasma Collected Period	Country	Neutralization Method	Mutation/Variant Evaluated	Result (Decreased Neutralization Relative to Wild-Type Virus)
Jangra et al[6]	No data	USA	Recombinant SARS-CoV-2s	E484K	Decrease neutralization 2.4-fold to 4.2-fold
Greaney et al[7]	February–July 2020	USA	Lentiviral particles pseudotyped	E484K	Decrease neutralization by >100-fold
Liu et al[10]	Before May 2020	USA	VSV particles pseudotyped	E484 (including E484A, E484D, E484G and E484K)	Decrease neutralization
Gaebler et al[16] Wang et al[15]	August 31–October 16, 2020	USA	HIV-1 particles pseudotyped	K417N/E484K/N501Y	Decrease neutralization by 0.5- to 29-fold
Chen et al[17]	No data	USA	Vero-hACE2-TMPRSS2 cells	Alpha variant	No difference
				E484K/N501Y	Decrease neutralization by 5-fold
Wibmer et al[18]	May–September 2020	South Africa	Lentiviral particles pseudotyped	K417N/E484K/N501Y	Loss of neutralization in 27% of the samples
Collier et al[19]	No data	UK	Lentiviral particles pseudotyped	Alpha variant combined with E484K	Decrease neutralization by 11.4-fold
Shen et al[28]	No data	USA	Lentiviral particles pseudotyped	Beta variant	Decrease neutralization by 13.1-fold (relative to D614G)

Several methods assess the impacts of mutations on vaccine efficacy both in vitro and in vivo. With respect to in vitro studies, as previously mentioned, authentic viruses and pseudoviruses holding spike mutations were utilized and the neutralization activity has been assessed. In vivo clinical trials of vaccine efficacy have also been conducted.

Studies have evaluated the post-vaccine sera from individuals who received mRNA vaccine, adenovirus vaccine, and protein subunit vaccine, including studies using authentic viruses and pseudovirus assays. Not only human post-vaccine sera were evaluated but also post-vaccine sera from macaque monkeys.[24]

In one study, sera from individuals who received BNT162b2 vaccine were obtained, and the analysis found a 3.4-fold reduction in serum neutralization efficiency when E484K was present.[6] Pseudotyped viruses carrying the E484K mutation reduced the effectiveness by at least tenfold against the 17 most potent antibodies selected from eight vaccinated individuals who received the mRNA-1273 or BNT162b2 vaccine.[15] Another study obtained mRNA-1273 post-vaccine sera and compared the neutralizing activity against VSV-pseudovirus of the Alpha variant and the Alpha+E484K variant, and a significant reduction in neutralizing titers was evident when the E484K mutation was present.[24] BNT162b2 post-vaccine sera also showed a similar result. The Alpha variant alone reduced neutralization 1.9-fold; when adding E484K to the Alpha variant, the neutralization was reduced 6.7-fold relative to the wild-type virus.[19] In addition, a similar trend was found in mRNA-1273 post-vaccine sera from macaque monkeys when assessed in VSV and lentiviral pseudovirus neutralization assays.[24] The above studies verified that E484K decreased the neutralization in pseudovirus assays of post-mRNA-vaccine sera from human and macaque monkeys.

E484K together with other mutations, also decreased the neutralization effect of post-vaccine sera. Chen et al evaluated post-BNT162b vaccine sera using Vero-hACE2-TMPRSS2 cells and found E484K/N501Y mutation decreased neutralization fourfold compared to the WA1/2020 D614G variant.[17] Post-vaccine sera of mRNA-1273 against recombinant SARS-CoV-2 pseudovirus containing RBD mutations (K417N-E484K-N501Y- D614G) in the Beta variant decreased neutralization 2.7-fold.[24] Sera obtained from macaque monkeys showed a similar trend.[24] Wang et al obtained mRNA-1273 or BNT162b2 post-vaccine sera from 20 volunteers.[15] The neutralization decreased one- to three-fold when human immunodeficiency virus-1 (HIV-1) pseudoviruses possessed the E484K-, N501Y-, or K417N/E484K/N501-mutant. Adenoviral vector vaccine demonstrated similar results. Madhi et al investigated antibodies induced by

ChAdOx1 nCoV-19 vaccine in a pseudovirus assay, and found that the geometric mean titers of the RBD triple mutant (K417N, E484K, and N501Y) decreased 3.49-fold relative to the original virus, and the live-virus assay showed lower neutralization than the pseudo-viral assay.[25]

Variants containing the E484K mutation, such as the Beta and Gamma variants, reduced neutralization of post-vaccine sera, whereas there was a minimal effect on the Alpha variant. Garcia-Beltran et al utilized a lentiviral vector-based SARS-CoV-2 pseudovirus neutralization assay and compared the neutralizing capacity of BNT162b2 vaccine (n = 30) and mRNA-1273 vaccine (n = 35) post-vaccination sera against eight SARS-CoV-2 variants of interest or concern.[26] Pseudoviruses with Alpha variant mutants revealed non-statistically significant reductions in neutralization compared to wild-type viruses. The Beta variants showed significant decreases in neutralization activity; for BNT162b2 post-vaccination sera, the neutralizing responses decreased 34-fold to 42-fold; and for mRNA-1273 post-vaccination sera, the titer decreased 19.2-fold to 27.7-fold. The Gamma variant showed significant decreases in neutralizing antibody responses, with a 6.7-fold reduction for BNT162b2 and a 4.5-fold reduction for mRNA-1273. Both the Beta and Gamma variants contain E484K, and Alpha variants do not. Therefore, these data collectively indicate that the primary determinant responsible for decreased neutralization is related to E484K.[26] Wu et al demonstrated neutralization of mRNA-1273 postvaccine sera against pseudoviruses containing the complete set of S mutations in the Beta variant decreased 6.4-fold; conversely, there was a minimal effect on neutralization of the Alpha variant.[24] Liu et al obtained BNT162b2 post-vaccine sera from 15 participants.[27] They utilized recombinant isogenic SARS-CoV-2s possessing spike mutations, and implemented a conventional 50% plaque reduction neutralization test (PRNT50) to measure virus suppression. Geometric mean neutralizing titers against wild type, Alpha-variant-spike, Gamma-variant-spike, Beta-variant-spike were 532, 663, 437, and 194, respectively. Thus, they concluded that the neutralizing of Alpha-spike and Gamma-spike viruses were not significantly different from the wild type, and the neutralizing of Beta-spike virus decreased significantly.[27] Studies on the neutralization of post-mRNA vaccine sera consistently demonstrated that variants containing E484K, such as the Beta and Gamma variants, decreased the neutralizing effects. Although neutralization levels were lower against the Beta variant, the post-vaccine sera with mRNA-1273 retained neutralizing activity against the Beta variant.[24,28] Antibodies induced by adenovirus vector vaccine, protein subunit vaccines, and inactivated vaccine also showed the neutralization effect was decreased against the Beta variant. For antibodies induced by the Ad26.COV2.S vaccine, the neutralization against the Beta variant decreased 5-fold when compared with the original virus in a pseudovirus neutralizing assay.[29] Antibodies induced by NVX-CoV2373, a recombinant protein nanoparticle vaccine, reduced neutralization 14.5-fold against the Beta variant relative to D614G in a pseudovirus assay.[28] Studies on BBIBP-CorV, an inactivated SARS-CoV-2 vaccine, also showed a similar trend. Huang et al demonstrated that the neutralizing effect of BBIBP-CorV against the Beta variant was 1.6-fold lower than against the original SARS-CoV-2.[30] However, Zhang et al measured the neutralizing activity of post-vaccine sera of individuals who received the BBIBP-CorV vaccine. Neutralizing activity against the Alpha lineage declined 2.2-fold compared to the wild-type strains, the Gamma variant declined 1.9-fold, the Lota variant declined 3.8-fold, and the Beta variant declined 4.6-fold.[31]

In conclusion, studies on post-vaccine sera showed that E484K alone, or combined with other mutations or variants containing E484K, reduced the neutralization titer, regardless of the vaccine platform used. Studies evaluating the impact of E484K, as well as the mutations and variants of concern on post-vaccine sera are summarized in Table 3.

The Impact of E484K on Vaccine Efficacy and Effectiveness

Changes in the in vitro neutralization of strains by vaccine-induced antibodies do not necessarily indicate reduced effectiveness; nevertheless, striking differences may correlate with clinical effectiveness.[32] We assessed the impact of E484K based on vaccine efficacy and effectiveness against variants containing the E484K mutation using real-world data.

A nationwide study evaluated the effectiveness of BNT162b2 in Qatar and the results demonstrated that the vaccine effectiveness was 90% (95% CI 86–92) for Alpha infection, compared with 75% (95% CI 71–79) for Beta infection.[33] Vaccine efficacy of adenovirus-based vaccines was also evaluated in South Africa where the Beta variant predominated. In a randomized, double-blind controlled trial of the ChAdOx1 nCoV-19 vaccine in South Africa, the vaccine efficacy for preventing mild-to-moderate Covid-19 was 21.9% (95% confidence interval [CI], −49.9 to 59.8), and against B.1.351 variant was 10.4%. Nevertheless, the trial was small, the confidence interval of vaccine efficacy was wide, and the trial concluded that

Table 3 Summary of Studies Evaluating the Impact of E484K, Mutations and Variants on Post-Vaccine Sera

Author	Vaccine	Neutralization Method	Mutation/Variant Evaluated	Result (Decreased Neutralization Relative to Wild-Type Virus)
Jangra et al[6]	BNT162b (n = 5)	Recombinant SARS-CoV-2s	E484K	Decreased neutralization 3.4-fold
Collier et al[19]	BNT162b2 (n=21)	Lentiviral particles pseudotyped	Alpha variant	Decreased neutralization 1.9-fold
			E484K-containing Alpha variant	Decreased neutralization 6.7-fold
Wu et al[24]	mRNA-1273 (n=8)	VSV particles pseudotyped	K417N-E484K-N501Y-D614G	Decreased neutralization 2.7-fold
			Alpha variant	No change
			Beta variant	Decreased neutralization 6.4-fold
	mRNA-1273, macaque monkeys	Lentiviral particles pseudotyped	E484K	Decreased neutralization 5.2-fold
		VSV particles pseudotyped	K417N-E484K-N501Y	Decreased neutralization 9.6-fold
Wang et al[15]	mRNA-1273 (n = 14) BNT162b (n=6)	Human immunodeficiency virus-1 (HIV-1) pseudotyped	E484K-, N501Y- or K417N/E484K/N501	Decreased neutralization 1 to 3-fold
Chen et al[17]	BNT162b (n=24)	Vero-hACE2-TMPRSS2 cells	E484K/N501Y	Decreased neutralization 4-fold
			Alpha variant	Decreased neutralization 2-fold
			Beta variant	Decreased neutralization 10-fold
Madhi et al[25]	ChAdOx1 nCoV-19 (n=13)	Lentiviral particles pseudotyped	K417N, E484K, and N501Y	Decreased neutralization 3.5-fold
Garcia-Beltran et al[26]	BNT162b (n = 30)	Lentiviral particles pseudotyped	Alpha variant	No change
			Beta variant	Decreased neutralization 34-fold to 42-fold
			Gamma variant	Decreased neutralization 6.7-fold
	mRNA-1273 (n = 35)	Lentiviral particles pseudotyped	Alpha variant	No change
			Beta variant	Decreased neutralization 19.2- to 27.7-fold
			Gamma variant	Decreased neutralization 4.5-fold
Shen et al[28]	mRNA-1273 (n=26)	Lentiviral particles pseudotyped	Beta variant	Decreased neutralization 9.7-fold (relative to D614G)
	Novavax (n=23)	Lentiviral particles pseudotyped	Beta variant	Decreased neutralization 14.5-fold (relative to D614G)
Liu et al[27]	BNT162b (n = 15)	Recombinant isogenic SARS-CoV-2s	Alpha variant	No change
			Beta variant	Decreased neutralization 2.7-fold
			Gamma variant	No change
Alter et al[29]	Ad26.COV2.S (n=20)	Lentiviral particles pseudotyped	Beta variant	Decreased neutralization 5.0-fold
			Gamma variant	Decreased neutralization 3.3-fold
Huang et al[30]	BBIBP-CorV (n=12)	Authentic virus	Beta variant	Decreased neutralization 1.6-fold

(*Continued*)

Table 3 (Continued).

Author	Vaccine	Neutralization Method	Mutation/Variant Evaluated	Result (Decreased Neutralization Relative to Wild-Type Virus)
Zhang et al[31]	BBIBP-CorV (n=470)	250 $TCID_{50}$ SARS-CoV-2 pseudoviruses	Alpha variant	Decreased neutralization 2.2-fold
			Beta variant	Decreased neutralization 4.6-fold
			Gamma variant	Decreased neutralization 1.9-fold
			Iota variant	Decreased neutralization 3.8-fold

two doses of the ChAdOx1 nCoV-19 vaccine were ineffective in preventing mild-to-moderate symptoms of the Beta variant.[25] In the international Phase 3 ENSEMBLE trial of Ad26.COV2.S vaccine, the overall vaccine efficacy varied by country: 74% in the United States, 66% in Brazil, and 52% in South Africa. In this trial, 95% of the Covid-19 cases in South Africa were caused by the Beta variant, whereas 69% of the cases in Brazil were caused by the Zeta variant (P.2 lineage), and both carry the E484K mutation.[34] NVX-CoV2373, in a Phase 2 trial in South Africa, where the Beta variant caused most COVID-19 cases, the vaccine efficacy was estimated at 49.4% (95% CI 6.1–72.8), which was lower than the vaccine efficacy 89.7% (95% CI 802–94.6) in the UK.[35,36] Although the above trials showed most vaccines retained efficacy/effectiveness against the Beta and Zeta variants, a trend toward a lower level of vaccine efficacy was observed.

The available evidence suggests the vaccine efficacy of Ad26.COV2.S, NVX-CoV2373, and ChAdOx1 nCoV-19/AZD1222 were lowered but retained in Southern Africa, where the Beta variant is dominant.[25,34–36] The effectiveness of BNT162b2 was also slightly lower with the Beta variant than with the Alpha variant in the observational study.[33] Table 4 summarizes studies showing the impact of variants containing E484K on vaccine efficacy and effectiveness.

E484K and Vaccine Breakthrough Infection

Several factors contribute to breakthrough infection, such as inadequate post-vaccination immune response, the immune response fading over time, and the constant change of the virus over time.[37] Several studies have analyzed how SARS-CoV-2 variants and mutations impact vaccine breakthrough infection.

Table 4 Vaccine Efficacy/Effectiveness Against Variants Possessing E484K

Vaccine	Number of Participants	Country	Period	Vaccine Efficacy/ Effectiveness	Ref.
BNT162b2	265,410	Qatar	December 21, 2020–March 31, 2021	Vaccine efficacy against any documented infection -Alpha variant: 89.5% (95% CI, 85.9 to 92.3) -Beta variant: 75.0% (95% CI, 70.5 to 78.9)	[33]
ChAdOx1 nCoV-19/AZD1222	2026	South Africa	June 24–November 9, 2020	-Vaccine efficacy for preventing mild-to-moderate infection: 21.9% (95% CI, −49.9 to 59.8) -Vaccine efficacy for beta variant infection: 10.4%	[25]
Ad26.COV2.S	43,783	USA and South Africa	September 21, 2020-January 22, 2021	Overall vaccine efficacy: -74% in the United States -52% in South Africa (Beta variant accounts for 95% of the Covid-19 infection in South Africa)	[34]
NVX-CoV2373	29,960	USA and Mexico	January 25- April 30, 2021	-Mostly Alpha variant -Vaccine efficacy: 90.4% (95% CI 82.9–94.6) for preventing mild, moderate, or severe infection	[59]
	4387	South Africa	August 17, 2020-November 25, 2020	-Mostly Beta variant -Vaccine efficacy: 49.4% (95% CI 6.1–72.8) for preventing mild-to-moderate Covid-19	[36]

Hacisuleyman et al investigated fully vaccinated patients who received mRNA-1273 and developed a breakthrough infection. Whole viral genome sequencing found E484K along with other mutations.[38] An institute in Washington state reported 20 vaccine breakthrough infections. All of the breakthrough infections were caused by variants of concern. Sixty percent of vaccine breakthrough cases were caused by variants reported to reduce the neutralization of post-vaccination sera, such as the Gamma, Beta, and Epsilon variants; while these variants only accounted for 36.7% of non-vaccinated cases, and a 1.63-fold change (p=0.037) was found.[39] Kustin et al conducted a matched cohort study in Israel to evaluate the distribution of variants of concern in individuals who had a BNT162b2 mRNA vaccine breakthrough infection. An increased proportion of the Beta variant was found in vaccinated cases with breakthrough infection when compared with unvaccinated cases.[40] Other reports also demonstrated that some variants of concern were more prevalent in breakthrough infections.[38,40,41]

While some studies reported a significantly higher proportion of variants of concern among vaccine breakthrough infection cases relative to the general population, other reports have suggested the rate of variants of concern was no different from that of the general population. A report on fully vaccinated health care workers in Israel found 85% were caused by the Alpha variant, which was similar to the prevalence in non-vaccinated people.[42] The United States Centers for Disease Control and Prevention (CDC) reported that among 101 million fully vaccinated individuals, 10,262 breakthrough infections occurred, with a breakthrough rate of 0.01%, from January 1 to April 30, 2021. Sixty-four percent of the viral sequences obtained were variants of concern, including the Alpha variant (56%), the Epsilon variant (33%), the Gamma variant (8%), and the Beta variant (4%). Nevertheless, the proportions caused by variants of concern were no different from the circulating rates of those variants, suggesting that the proportions of SARS-CoV-2 variants were not significantly greater in breakthrough cases.[14] Other studies also demonstrated that the proportions of variants of concern in vaccinated and non-vaccinated individuals were similar.[43–45] Table 5 lists reports on vaccine breakthrough infection and proportions of variants in vaccinated and non-vaccinated individuals.

Despite inconsistent data, some studies demonstrated that in comparison to non-vaccinated individuals, a higher proportion of variants of concern was observed in vaccinated individuals who had a breakthrough infection. A few studies revealed an increased proportion of variants containing E484K (such as Beta and Gamma variants) in cases of

Table 5 Reports of Vaccine Breakthrough Infection and Proportions of Variants in Vaccinated and Non-Vaccinated Individuals

Author	Country	Period	Vaccine	Number of Sequenced Data	Result
Hacisuleyman et al[38]	New York City, USA	March 2021	mRNA-1273	N=1	Whole viral genome sequencing revealed E484K
McEwen et al[39]	Washington state	February 23 and April 27, 2021	BNT162b2 (n=14), mRNA-1273 (n=19)	N=20	The proportion of variants of concern: -60% in vaccinated cases -36.7% in non-vaccinated cases -1.63-fold change (p=0.037)
Kustin et al[40]	Israel	January 23, 2021 to March 7, 2021	BNT162b2	N=813	Increased proportion of the Beta variant compared with non-vaccinated cases
The Centers for Disease Control and Prevention[14]	USA	January 1– April 30, 2021	No data	N=555	No different from the circulating rates of the variants in vaccinated and non-vaccinated individuals
Bergwerk et al[42]	Israel	January 20, 2021 to April 28	BNT162b2	N=33	Ratio of variants in vaccinated people similar to the prevalence in non-vaccinated people
Jacobson et al[44]	USA	December 2020 to March 2021	BNT162b2 and mRNA-1273	N= 115	Vaccinated people do not have statistically significantly elevated risk ratios for infection with variants

breakthrough. Whether variants possessing E484K play a role in breakthrough infection is not clear currently. The correlation between mutation and vaccine breakthrough infection warrants further investigation.

The Effects of E484K on Therapeutic Monoclonal Antibodies

Many therapeutic monoclonal antibodies are currently under development. Monoclonal antibodies for Covid-19 have been granted emergency use authorization (EUA) from the US Food and Drug Administration (FDA) to treat out-patients with mild-to-moderate disease, and include casirivimab–imdevimab, sotrovimab, bamlanivimab–etesevimab.[46,47]

Bamlanivimab (also known as LY-CoV555 and LY3819253) and Etesevimab (LY-CoV016 and LY3832479) are neutralizing monoclonal antibodies that bind to the RBD of the SARS-CoV-2 S protein. In phase 3 of BLAZE-1, treatment with bamlanivimab and etesevimab reduced Covid-19-related hospitalizations and deaths by 70% relative to placebo treatment.[48] Based on these data, EUAs for bamlanivimab and etesevimab were issued, but later revoked due to the growing number of SARS-CoV-2 variants resistant to bamlanivimab.[49] In a pseudovirus harboring the E484K substitution, bamlanivimab and etesevimab reduced susceptibility by 17-fold.[49] Moreover, it was reported that in patients with a high risk of severe Covid-19 treated with bamlanivimab, viral rebound was found in five of six severely immunodeficient patients, and E484K was found in the five patients. The study revealed that the E484K escape mutation can develop during treatment with monoclonal antibodies.[50]

E484K reduced susceptibility to casirivimab (25-fold). In contrast, imdevimab and casirivimab combined with imdevimab retained their activity against VSV pseudovirus with E484K.[49,51,52] REGN-COV2 is an antibody cocktail composed of Casirivimab and imdevimab. In the preliminary results of a phase 3 randomized controlled trial, casirivimab-imdevimab reduced hospitalization and death compared with placebo.[49] It has been granted an EUA for treatment of mild-to-moderate Covid-19.

Sotrovimab, a recombinant human IgG1 monoclonal antibody, interacts with a highly conserved epitope on the RBD.[53] The epitope is different from the sites of mutations among SARS-CoV-2 variants of concern and interest. In an unpublished randomized trial for patients with one or more risk factors for severe disease, who have mild-to-moderate Covid-19, sotrovimab reduced the combined rates of hospitalization and death. In both pseudovirus and authentic virus assays, sotrovimab retains activity against the Alpha, Beta, Gamma, Epsilon and Iota variants.[53]

AZD7442 is a combination of two human monoclonal antibodies, AZD8895 and AZD1061.[54] In vitro, AZD8895, AZD1061, and AZD8895+ AZD1061 reduced the inhibitory capacity slightly (2- to fivefold increases in IC50 values) when tested in recombinant isogenic SARS-CoV-2s possessing the E484K mutation.[55] Other studies also proved that AZD7442 is effective against the Beta variant.[15,55,56]

Regdanvimab, a monoclonal antibody, is also known as CT-P59. A pseudovirus with the E484K mutation resulted in a 2-fold reduction individually. The neutralization against the Beta variant strain was reduced 19-fold compared to the wild type.[57]

In vitro assays have demonstrated that sotrovimab, AZD7442, regdanvimab, and REGN-CoV2 retained their activity against SARS-CoV-2 variants containing E484K, while bamlanivimab and etesevimab reduced neutralization. Table 6 summarizes monoclonal antibodies under Phase III clinical trials and the impact of E484K on neutralization of these monoclonal antibodies.

Conclusion

E484K has emerged frequently in several SARS-CoV-2 variants of concern/interest. E484K escapes the neutralizing effect of several monoclonal antibodies, convalescent plasma, and post-vaccine sera.

Several studies have revealed that the E484K mutation reduces the neutralization of convalescent sera, as proved by a pseudoviral assay. Monoclonal antibodies derived from convalescent patients were further studied, and class 2 antibodies, which bind to RBD both in "up" and "down" conformations of the spike, were proved to be resistant to the E484K mutation.

E484K mutation also reduced neutralization of post-vaccination sera. An in vitro analysis of post-vaccination sera from different vaccines simultaneously showed significantly decreased activity against the variants of concern

Table 6 The Impact of E484K on Neutralization of Monoclonal Antibodies Under Phase III Clinical Trial

Drug	Developer	Status	Trial ID	The Impact of E484K on Neutralization
Bamlanivimab and etesevimab	Eli Lilly and Company	EUA	NCT04427501 (Phase 2/3)	Reduced by 17-fold[49]
REGN-COV2 (casirivimab and imdevimab)	Regeneron Pharmaceuticals	EUA	NCT04452318	No change[15,49,51]
Sotrovimab (VIR-7831/ GSK4182136)	Vir Biotechnology Inc and GlaxoSmithKline	PhaseII/III clinical trials	NCT04545060	No change[53]
AZD7442 (AZD8895 and AZD1061)	AstraZeneca	Phase I; phase III pending	NCT04507256 (phase 1) NCT04625725 (phase 3) NCT04625972 (phase 3)	Minor reduction[15,55,60]
TY027	Tychan Pte Ltd	Phase I; phase III pending	NCT04429529 NCT04649515	No data
Regdanvimab (CT-P59)	Celltrion	Phase 3 clinical trials	NCT04525079	2-fold against E484K[57]

containing E484K. Clinical trials of Ad26.COV2.S, NVX-CoV2373, and ChAdOx1 nCoV-19 showed reduced efficacy in Southern Africa, where the Beta variant is dominant.[25,34–36] Despite the inconsistency of reported data, some studies have revealed an increased proportion of variants of concern, especially the Beta variant, in vaccine breakthrough infections.[39,40]

Therapeutic monoclonal antibodies are being developed rapidly. The impact of E484K on the susceptibility to monoclonal antibodies were assessed. E484K reduced the susceptibility to casirivimab (25-fold), but retained susceptibility when casirivimab was combined with imdevimab. Other monoclonal antibodies, such as sotrovimab, AZD7442, regdanvimab, and REGN-COV2, also retained their activity against E484K in vitro. However, bamlanivimab and etesevimab exhibited significantly reduced neutralization. Due to the reduced susceptibility of variants to bamlanivimab and etesevimab, the FDA has revoked the EUA for this combination therapy.[49,51,53,56,57]

E484K can be selected when a culture recombinant virus expressing SARS-CoV-2 S is treated with antibody cocktails, and new variants with novel mutations are on the rise.[8–10,15] Studies are being conducted with the aim of developing a new vaccine booster or a modified vaccine to provide increased protection against variants. In January 2021, Moderna developed a modified vaccine, a boosting dose of mRNA-1273.351, targeting the beta variant and three mutations (E484K, N501Y, and K417N). mRNA-1273.351 resulted in increased neutralizing antibody levels against the Beta and Gamma (P1) variants compared with levels elicited following the initial vaccine series.[58] As the virus is rapidly evolving and new mutations are emerging, it is necessary to continue to develop new vaccines and antibody therapies for Covid-19 over time.

Abbreviations

WHO, World Health Organization; RBD, receptor binding domain; E, glutamate; K, lysine; VOC, variants of concern; VOI, variants of interest; DMS, deep mutational scanning; IC50, Inhibitory concentration 50%; VSV, vesicular stomatitis virus; SPR, surface plasmon resonance; HIV-1, human immunodeficiency virus-1; PRNT50, conventional 50% plaque reduction neutralization testing; CDC, Centers for Disease Control and Prevention; EUA, emergency use authorization; FDA, Food and Drug Administration.

Disclosure

The authors report no conflicts of interest in this work.

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