In silico evaluation of ten monoclonal antibodies neutralization power of SARS-CoV-2 variants EG.5 and BA.2.86

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Article

In Silico Evaluation of Ten Monoclonal Antibodies Neutralization Power of SARS-CoV-2 Variants EG.5 and BA.2.86

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Abstract: The current globally dominant SARS-CoV-2 variants are showing immune escape and reduced susceptibility to antiviral drugs. Therefore, agencies responsible for drug evaluation and regulation such as the FDA and EMA are revising their emergency authorization use of several COVID-19 neutralizing antibodies. These NAbs proved to be unlikely effective against new variants especially Omicron descendants and several pharmaceutical companies are pursuing the development of more potent neutralizing antibodies. To address the issue of using *in silico* prediction for the rapid assessment of anti-SARS-CoV-2 MAbs neutralization power, we used a computational method we developed previously, to evaluate 10 SARS-CoV-2 antibodies propensity to neutralize the new Omicron's subvariants Eris (EG.5) and Pirola (BA.2.86) based on comparative binding affinity and previous experimental and clinical observations. Nine of these MAbs were once granted emergency use authorization, and one is currently under clinical investigation. The rapid, cost-effective *in silico* evaluation provided reliable predictions consistent with published clinical and experimental data. Furthermore, our data showed new potential therapeutic MAbs combination that could be effective for the treatment countermeasure of the new Omicron's subvariants Eris (EG.5) and Pirola (BA.2.86).

Keywords: *In Silico*; monoclonal; antibodies; COVID-19; EG.5; BA.2.86; neutralization power; Anti-SARS-CoV-2; resistant; variants

1. Introduction

Following the emergence of SARS-CoV-2 virus causing the COVID-19 pandemic in late 2019, several risk reduction strategies have been applied to prevent, overcome and/or migate its health impact. These measures diverse from personal safety measures to avoid contracting the virus to immunity boost via vaccination and treatment using targeted neutralizing antibodies (NAbs). As of early 2021, nearly 18 months into pandemic, the U.S. Food and Drug Administration (FDA) and the European Medicine Agency (EMA) have granted emergency use authorizations (EUA) for several NAbs for the treatment of moderate to severe symptoms and as preventive protection from COVID-19. However, as new variants of SARS-CoV-2 emerge and due to increased frequency of resistant variants especially Omicron and its' subvariants, the EUA has been revoked for many NAbs. Nevertheless, several pharmaceutical companies and organizations continue to invest in developing new Nabs. Several Nabs are now in early clinical development for example, REGN15160 and REGN14256 that are investigated by Regeneron, CPT63, and CTP59 developed by Celltrion and JS026 by Shangai Junshi, which was previously allied with Eli Lilly for the development of Etesevimab [1].

Currently, two Omicron subvariants EG.5 (Eris) and BA.2.86 (Pirola) are monitored closely and are classified by the World Health Organization (WHO) as "variant of interest" (VOI) and "variant under monitoring" (VUM) respectively [2]. EG.5 is a descendant of Omicron XBB.1.9.2, it was first reported on February 2023 and it was related to increased prevalence with no solid evidence of

disease severity [3]. BA.2.86 is a descendant of Omicron BA.2. and it was first reported on July 2023 in Denmark [4]. Up to date little is known about the severity or transmissibility of BA.2.86 variant [5]. According to Stanford University SARS-CoV-2 Variants database [6], EG.5 showed three different additional mutations to its parent subvariant (XBB), G252V, F456L and F486P. One particular mutation appears to be especially important, F456L, it is located at the interface ACE2-RBD within the epitope of several Class I NAbs [6]. Another mutation F490S, is predicted to be resistant to class II antibody (Bamlanivimab). A recent study showed that this mutation is resistant to neutralization and causes antibody evasion [7]. On the other hand, BA.2.86 showed 31 differences in comparison to its parent subvariant (BA.2). It has parent mutations Q493 and R346T besides its own unique mutation L452W and all are linked to antibody evasion. In addition, both variants share four mutations (F486P, E484A/K, K417N, and N460K) that are also predicted to evade neutralization by class I and II NAbs [7,8]. Moreover, studies using BA.2.86 spike-pseudotyped virus model showed that this variant is resistant to several NAbs including Tixagevimab, Cilgavimab (Evusheld), Bebtelovimab (LY-CoV1404) and Sotrovimab (S309) [9–11]. Overall, both EG.5 and BA.2.86 contains three or more of especially important mutations previously reported as NAbs resistant mutations, including residues R346 and P337, G339, S371, N440, F486, V445, and G446 in addition to F456L of EG.3 [9,12–14].

In the work we are reporting herein, we applied the computational method we previously described to evaluate SARS-CoV-2 antibodies neutralization power [15], to assess the effectiveness of the available SARS-CoV-2 neutralizing antibodies on the newly emerged variants Eris and Pirola. We modeled nine different neutralizing antibodies previously approved by FDA and EMA for the treatment of mild to moderate COVID-19 symptoms and measured their binding affinities to the new variants in comparison to the original Wuhan strain for which they were granted emergency authorizationuse. These NAbs include Sotrovimab (S309), Bamlanivimab (LY-CoV555), Etesevimab (LY-CoV016, CB6, JS016), Evusheld (Tixagevimab -AZD8895 and Cilgavimab-AZD1061), Regdanvimab (CT-P59), Casirivimab (REGN10933), Imdevimab (REGN10987) and Bebtelovimab (LY-CoV1404). Additionally, one anti-SARS-CoV-2 antibody (JS026) that is currently in early clinical trials and shows excellent results with increased neutralizing efficacy in combination with Etesevimab [1,16] was also evaluated.

2. Materials and Methods

2.1. Selection of the SARS-CoV-2 Neutralizing Antibodies

The selection of the antibodies we studied is based on their granting of the emergency use authorization (EUA). All the antibodies that were granted EUA by the FDA and/or EMA for the first SARS-CoV-2 strain (Wuhan) were retrieved, examined and evaluated in addition to one that is not yet authorized but showed promising clinical results with resistant variants.

2.2. SARS-CoV-2 BA.2.86 (Pirola) and EG.5 (Eris) Variants Sequence Retrieval, Modifications and Modeling

The amino acid sequences of the extracellular domain of SARS-CoV-2 spike proteins were acquired from the National Center for Biological Information (NCBI) protein ID: YP_009724390.1. SARS-CoV-2 variants-specific mutations were introduced to the collected sequence to generate the different variant sequences based on published mutations in the Stanford University SARS-CoV-2 Variants database (https://covdb.stanford.edu/variants/omicron_ba_1_3/) [17]. The spikes 3D monomeric structures were modeled in an open state form as described in our previous report [15]. using the SWISS-MODEL server - User Template Mode [18] (https://swissmodel.expasy.org/interactive#structure) and Omicron's template model.

2.3. NAb/SARS-CoV-2 RBD Reference Models' Selection and Modification

Models representing the interaction of the selected neutralizing antibodies with receptor binding domain of SARS-CoV-2 (Wuhan variant) were extracted from RCSB Protein Data Bank (RCSB PDB)

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(https://www.rcsb.org) (Appendix A: Table A1). Each model was cleaned from any heteroatoms, modified so that only one unit of NAb/RBD is present, and only NAbs' variable domain (Fv) is represented in the model. The modified models were used as reference models to generate complexes with EG.5 and BA.2.86 variants by RBD replacement.

2.4. RBD/S309 Complexes Construction

RBDs of the BA.2.86 and EG.5 SARS-CoV-2 variants were extracted from the generated models and complexes with the ten neutralizing antibodies were constructed by molecular replacement of the RBD domain. All constructed models were energy minimized in one-step energy minimization using Swiss-pdb Viewer 4.1.0 (http://www.expasy.org/spdbv/) [19].

2.5. Complex Binding Affinity Analysis

The stability and affinity were assisted based on thermodynamic measure of the formed complex energy, Gibbs free energy, (ΔG). This was performed using an antibody-antigen binding affinity online tool, CSM-AB (https://biosig.lab.uq.edu.au/csm_ab/prediction) [20]. Binding affinity percentage was calculated in reference to NAb/Wuhan complex's binding affinity to which we attributed a 100% value. The interactions of some selected NAbs with the RBD of the new SARS-CoV-2 variants were analyzed based on polar and hydrophobic interaction using the LigPlot+ software [21].

3. Results

3.1. Antibodies Selection

Nine different SARS-CoV-2 neutralizing antibodies were selected. All selected antibodies have been granted authorization by FDA and EMA except for Regdanvimab (CT-P59) that was granted authorization in the European Union only and Bebtelovimab (LY-CoV1404) that was granted authorization by FDA but not EMA. The authorization was for the pre-exposure prophylaxis and/or treatment of COVID-19 symptoms caused by the original strain of SARS-CoV-2 (Wuhan). Currently, and due to the increase frequency of resistant variants especially Omicron and its descendants, FDA revoked the EUA for all the neutralizing antibodies. However, EMA emergency use authorization is yet effective (Table 1). Nevertheless, several clinical trials are conducted to develop new antibodies that are capable of neutralizing the newly emerged subvariants and one of the promising NAbs is JS026 antibody that showed to have increased neutralization effect against wild SARS-CoV-2 and its Alpha, Beta, Gamma, and Delta variants when combined with Etesevimab [16]. Therefore, although it is not an approved NAb yet, we add JS026 to the selected NAbs on this paper to analyze its neutralizing effect with the new variants EG.5 and BA.2.86.

Table 1. Summary of SARS-CoV-2 neutralizing antibodies emergency authorization use by U.S. Food and Drug Administration (FDA) and European Medicine Agency (EMA).

Antibody	FDA [22]	EMA [23]
Sotrovimab (S309)	Authorized May 2021 Revoked April 2022 *	Authorized December 2021 up to date Under the name Xevudy
Bamlanivimab (LY-CoV555)	Authorized September 2021 Administered together as a combination (Bamlanivimab/Etesevimab) Revoked January 2022*	October 2021 Bamlanivimab and Etesevimab EMA ended the rolling review due to withdrawing from the process by the company (Eli Lilly Netherlands BV)
Etesevimab (LY-CoV016, CB6, JS016)		

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Evusheld (Tixagevimab - AZD8895)	Authorized August 2021 Revoked January 2023 Evusheld (tixagevimab co-packaged with cilgavimab) cocktail No longer authorized*	Authorized March 2022 up to date Evusheld (tixagevimab co-packaged with cilgavimab) cocktail
Evusheld (Cilgavimab-AZD1061)		
Regdanvimab (CT-P59)	No authorization	Authorized November 2021 up to date Under the name Regkirona
Casirivimab (REGN10933)	Authorized November 2020 Revoked January 2022 REGEN-COV (Casirivimab / Imdevimab) cocktail No longer authorized*	Authorized November 2021 up to date Under the name Ronapreve (Casirivimab / imdevimab) cocktail
Imdevimab (REGN10987)		
Bebtelovimab (LY-CoV1404)	Authorized February 2022 Revoked November 2022 No longer authorized*	No authorization
JS026	Under clinical trials [1]	

*** Due to increases frequency of resistant variants.**

3.2. Models' Generation

To analyze the binding affinity of the selected NAbs with the new variants EG.5 (Eris) and BA.2.86 (Pirola), 20 models were generated representing the interaction between the NAb's Fv domain and the RBD of SARS-CoV-2 newly emerged variants. In addition to 10 reference models, cleaned and energy minimized, representing the interaction of the selected NAbs with the original Wuhan strain RBD domain (Appendix A: Table A1). Each of the examined NAbs showed to have different binding site on the RBD domain. Moreover, as previously described [15], NAbs are categorized based on binding to a specific RBD epitope or based on whether it is competing with the angiotensin-converting enzyme 2 (ACE2). The selected NAbs were from class I, II and III as shown in Figure 1. Table 2 describes the previously reported neutralizing effect of the selected NAbs on SARS-CoV-2 and its variants.

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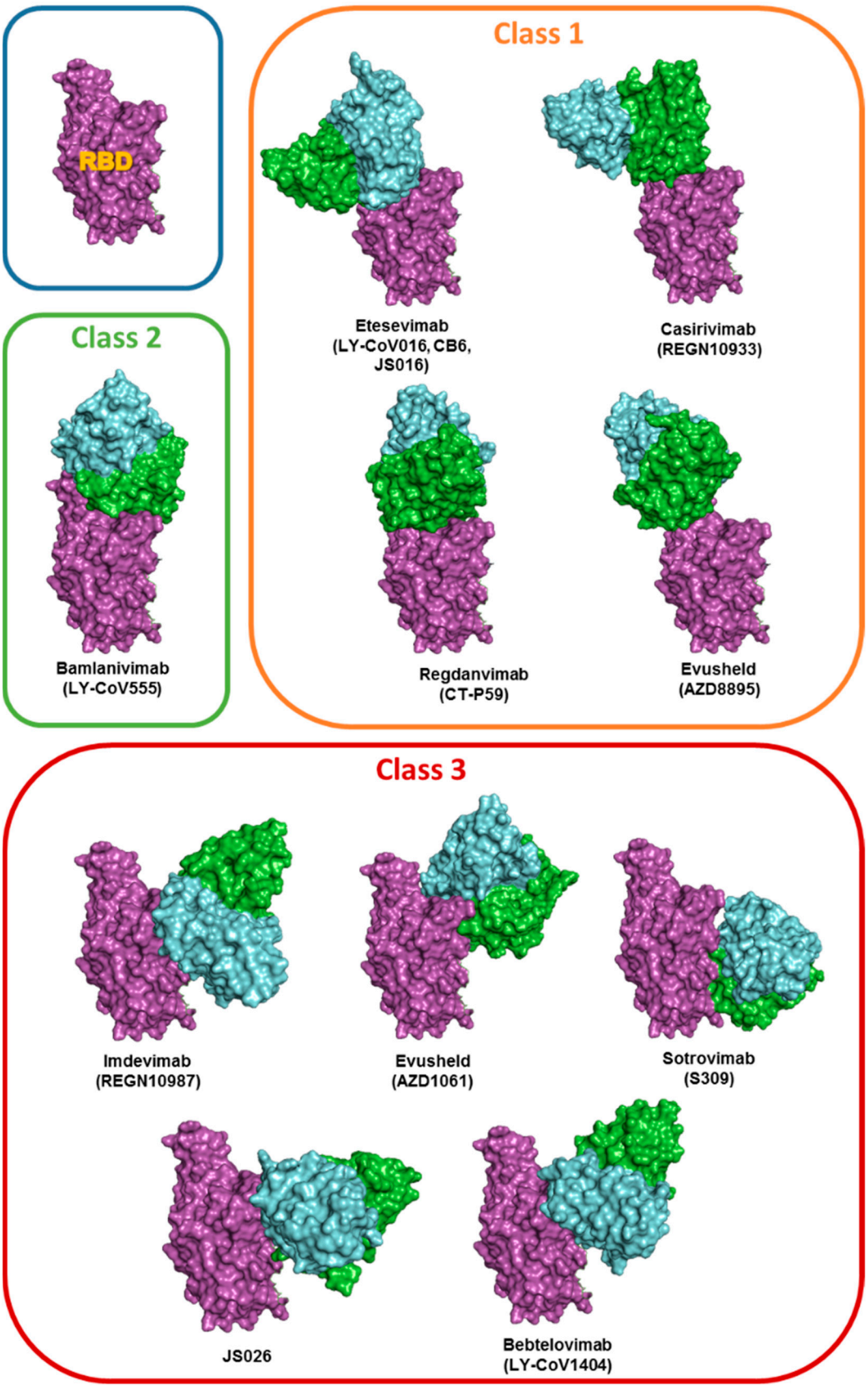

Figure 1. Neutralizing antibodies' binding position on SARS-CoV-2 RBD domain (magenta). Antibody (Fv domain) heavy chain (green) and light chain (cyan).

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Table 2. ACE2 competition and neutalization effect of the emergency authorized SARS-CoV-2 nutralization antibodies.

Antibody	NAb's Class	RBD Access	ACE2 Competing	Viruses Neutralized	Reference
Sotrovimab (S309)	Class 3	Up/Down	No	SARS-CoV-2; Alpha, Beta, Gamma, Delta, BA.1, BA.2, BA.3, BA.4/5, and BA.2.75	[24]
Bamlanivimab (LY-CoV555)	Class 2	Up/Down	Yes	SARS-CoV-2; Alpha	[24–26]
Etesevimab (LY-CoV016, CB6, JS016	Class 1	UP	Yes	SARS-CoV-2; Alpha and Delta	
Evusheld (Tixagevimab - AZD8895)	Class 1	Up	Yes	SARS-CoV-2; Alpha, Beta, Gamma, Delta	[25,27,28]
Evusheld (Cilgavimab-AZD1061)	Class 3	Up/Down	No	SARS-CoV-2; Alpha, Beta, Gamma, Delta, BA.2, BA.2.75, and BA.5	
Regdanvimab (CT-P59)	Class1	Up	Yes	SARS-CoV-2; Alpha, Beta, Gamma and Delta	[24]
Casirivimab (REGN10933)	Class 1	UP	Yes	SARS-CoV-2; Alpha and Delta, BA.2.75	[24]
Imdevimab (REGN10987)	Class 3	Up/Down	No	SARS-CoV-2; Alpha, Beta, Gamma, Delta, BA.1, BA.2, and BA.4/5	
Bebtelovimab (LY-CoV1404)	Class 3	Up/Down	No	SARS-CoV-2; Alpha, Beta, Gamma, Delta, BA.1, BA.2, BA.3, BA.4/5, and BA.2.75	[24]
JS026	Class 3	Up/Down	No	In combination with Etesevimab. SARS-CoV-2; Alpha, Beta, Gamma and Delta	[16]

3.3. Binding Affinity Analysis

The generated (RBD/NAb-Fv) interaction models were energy minimized and the binding energy of the 3D models were calculated by computational prediction of Gibbs free energy (ΔG). The percentage of the binding energy for each antibody was calculated in comparison to that of the same NAb with Wuhan strain (Figure 2). The results showed that the binding energy is decreases with the newly emerged variants indicating that the new SARS-CoV-2 variant escapes neutralization of most of the available therapeutic NAbs. However, two antibody class I Evusheld (Cilgavimab-AZD1061) and class III Bebtelovimab (LY-CoV1404) retain more or less the same neutralization effect of Wuhan strain with minor increase toward BA.286 variant with 1.1- and 1.2-fold increase respectively (Figure 2).

Interestingly, one NAb JS026 that is currently under clinical trials, showed a significant increase of about 3 and 2.9 folds in the binding affinity with the new descendant EG.5 and BA.2.86 respectively. Additionally, NAbs Imdevimab (REGN10987) showed 2.8-fold increase of affinity

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binding with BA.2.86 only. While JS026 is to date under clinical evaluation, Imdevimab is still under emergency use authorization by EMA under the name Ronapreve and as a cocktail with Casirivimab for the treatment of COVID-19 symptoms in adults while its EUA was revoked by FDA on 2022 (Table 1).

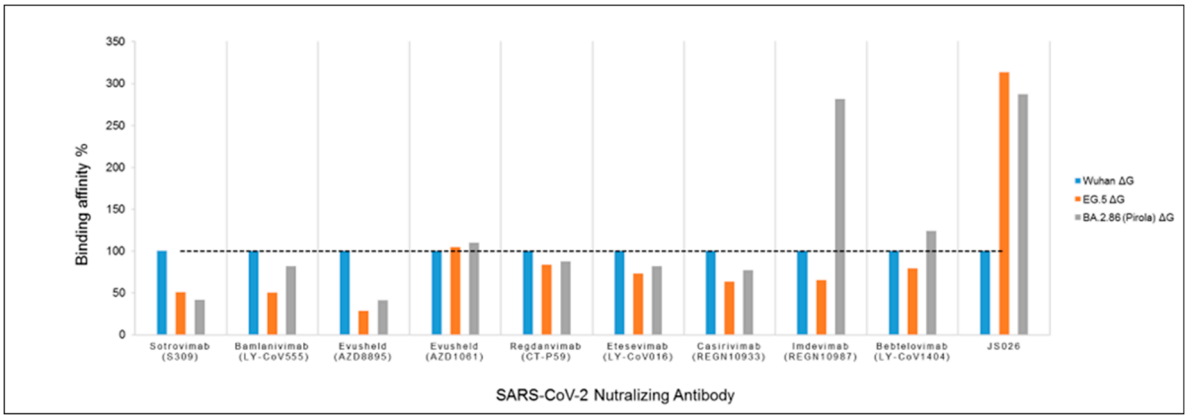

Figure 2. Neutralization efficiency of nine different antibodies against the new SARS-CoV-2 variants EG.5 and BA2.86. based on increased/decreased binding affinity: The (ΔG) percentages are compared to the 100% affinity value attributed to the reference Wuhan strain.

3.4. Analysis of Imdevimab and JS026 Molecular Interactions with the RBD Domain of the New Variants EG.5 and BA.2.86

Imdevimab and JS026 showed the highest binding affinity with the new SARS-CoV-2 variants EG.5 and BA.2.86. Both NAbs are class III antibodies and share similar binding epitope. Imdevimab showed a decrease in the binding affinity with EG.5 variant that is an XBB descendant but on the other hand, it showed a 2.8-fold increase with BA.2 dscendant BA.2.86. Analyses of the interaction pattern of Imdevimad with BA.2.86 using LigPlot+ software, revealed one polar and 33 hydrophobic interaction in addition to one salt bridge. Interaction of Imdevimab with BA.2.86 involves the same residues of Wuhan in addition to three new interacting residues, Asp450, Gln498, and Pro499. However, interaction with EG.5 includes 5 out of 6 residues with an additional four new ones. conversely, JS026 binding affinity increases with both variant EG.5 and BA.2.86. Interaction analysis revealed that interacting residues for both antibodies are the same with fourteen residues as that of Wuhan except for Asn343. Table 3 lists the interaction residues, number and type of interaction and mutated residues for each variant.

Table 3. The interaction of Imedivima and JS026 with epitope residues of the spike RBD of the new variants in comparison to Wuhan strain. The Number and position of polar interactions are indicated by an asterisk (*). Mutated residues are in italic. Wuhan reside and numbering are in-between brackets.

SARS-CoV-2 Variant	Wuhan Interaction Epitope	EG.5 Interaction Epitope	BA.2.86 Interaction Epitope
Imdevimab	Arg346		Arg339 (Arg346)
	Asn440*	*Lys436 (Asn440)*	*Lys433 (Asn440)*
	Leu441	Leu437 (Leu441)	Leu434 (Leu441)
	Lys444	Lys440 (Lys444)	Lys437 (Lys444)
	Val445	*Pro441* (Val445)*	*His438* (Val445)*
	Gly446	*Ser442 (Gly446)*	*Ser439 (Gly446)*
		Gly443 (Gly447)	

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		Asn444 (Asn448)	
			Asp443 (Asp450)
		Arg494 (Gln498)	Arg490 (Gln498)
		Pro495 (Pro499)	Pro491 (Pro499)
Bonds	1 polar 14 hydrophobic	1 polar 28 hydrophobic 1 salt bridge	1 polar 33 hydrophobic 1 salt bridge
JS026	Asn343*		
	Thr345*	Thr341 (Thr345)	Thr338 (Thr345)
	Arg346	*Thr342 (Arg346)*	Arg339 (Arg346)
	Asn439	Asn435 (Asn439)	Asn432 (Asn439)
	Asn440**	*Lys436* (Asn440)*	*Lys433* (Asn440)*
	Leu441*	Leu437 (Leu441)	Leu434 (Leu441)
	Asp442*	Asp438* (Asp442)	Asp435* (Asp442)
	Ser443*	Ser439* (Ser443)	Ser436* (Ser443)
	Lys444	Lys440* (Lys444)	Lys437* (Lys444)
	Val445	*Pro441 (Val455)*	*His438 (Val445)*
	Asn448	Asn444* (Asn448)	Asn441** (Asn448)
	Tyr451	Tyr447 (Tyr451)	Tyr444 (Tyr451)
	Pro499	Pro495 (Pro499)	Pro491 (Pro499)
	Thr500	Thr496 (Thr500)	Thr492 (Thr500)
	Arg509	Arg505 (Arg509)	Arg501 (Arg509)
Bonds	7 polar 54 hydrophobic	5 polar 66 hydrophobic	6 polar 72 hydrophobic

4. Discussion

Four years into pandemic and SARS-CoV-2 virus is still evolving. New variants are rising every day and so far, all lineages currently circulating are classified as Omicron variant sub lineages. As of October 2023, WHO listed three variants as variants of interest (VOIs); XBB.1.5, XBB.1.16, and EG.5 and six variants as variants under monitoring (VUMs); DV.7, XBB, XBB.1.9.1, XBB.1.9.2, Xbb.2.3 and BA.2.86 [29]. The most recent two variants are VOI EG.5 (Eris) and VUM BA.2.86 (Pirola). EG.5 is denoted as the most prevalent VOI as it has been reported by 37 countries and represents 33.6% of shared sequences according to GISAD. The other variant under monitoring Pirola, was reported in 21 countries with limited information about its public health potential impact [2,30].

Several recent studies [31–34] experimentally evaluated the immune evasion of neutralizing antibodies by the new evolving SARS-CoV-2 variants showing that Omicron's new subvariants have high capacity of immune evasion and accordingly emergency use authorization revoked for almost all the currently available NAbs. Here we evaluated the neutralization effect on the new SARS-CoV-2 variants (EG.5, BA.2.86) of ten of different classes of neutralizing antibodies, nine of them are granted or previously granted emergency use authorization and one (JS026) is under clinical trials. The neutralization effect was calculated in the form of ΔG percentage in comparison to Wuhan original strain. Our *in silico* method showed similar results for the neutralization resistance of the new

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variants as seen in Figure 2. It is clearly showing that almost all of the available neutralizing antibodies are ineffective against the new SARS-CoV-2 variants EG.5 and Ba.2.86. Moreover, our data came in consistency with recent published clinical data where pseudo-virus neutralizing antibody assays showed that NAb responses to BA.2.86 were lower than that of BA.2 but were similar or slightly higher than EG.5. These studies demonstrated that XBB descendant particularly EG.5 and EG.5.1 evades neutralizing antibodies with increased efficiency than BA.2 descendant BA.2.86 [7,35]. This enhanced neutralization of SARS-CoV-2 variant EG.5 over BA.2.86 could be the result of its unique spike protein mutations of confirmed reduced sensitivity to neutralizing antibody that are not acquired by BA.2.86 including F456L and F490S mutations [14,30,36]

Although, most of the NAbs responses (ΔG percentage) were higher for BA.2.86 than that for EG.5, they all fell below the neutralization of the Wuhan strain except for Evusheld (AZD1061), Imdevimab (REGN10987), Bebtelovimab (LY-CoV1404) and the new JS026 antibody. Both Evusheld (AZD1061) and Bebtelovimab (LY-CoV1404) showed a very slight increase above the Wuhan threshold that is almost negligible with 1.1- and 1.2-fold increase respectively. Nevertheless, Imdevimab (REGN10987) which is one of the REGEN-COV (Ronapreve) cocktail showed 2.8-fold increase in the neutralization effect of BA.2.86. Additionally the new potential therapeutic antibody JS026 [1] which is currently under clinical trials showed a promising results with a neutralization effect displayed 3 and 2.9 folds increase for both variant, EG.5 and BA.2.86 respectively. Clinically the potential effect of JS026 antibody was discussed in newly published research investigating the use of new therapeutic cocktails to countermeasure residual changes on the spike protein of new SARS-CoV-2 variants. It showed that using combinations of NAbs from different classes, in this case JS026 (class III) with Etesevimab (class I) can increase neutralizing efficacy [16]. Class I and class III were shown to be the best choice for NAbs cocktails as they have distinct epitopes on the RBD domain unlike class II that may overlap with the ACE2-binding site of class I causing steric clashes.

In conclusion, our previously described *In silico* method [15] to evaluate available SARS-CoV-2 antibodies neutralizing power with the new emerging variants EG.5 and BA.2.86 showed to be reliable and effective for preliminary evaluation of new neutralizing antibodies and their cocktails. This paper supports our described method, and it is the first paper to discuss the neutralizing efficiency of all available emergency authorized neutralizing antibodies with the new SARS-CoV-variants. Moreover, we believe that the available NAb cocktail Ronapreve also known as REGEN-COV (Casirivimab / imdevimab) can be still effective against BA.2.86 variant but not EG.5. Our data support and add to what has been published before on the potential therapeutic cocktail of Etesevimab in combination with JS026 against new variants [16]. Furthermore, we propose to look into different NAbs cocktails' mix that increases neutralizing efficacy against the two new variants such as Regdanvimab (class I) with either of JS026 or imdevimab (class III) for strain BA.2.86 and Regdanvimab with JS026 for EG.5.

Author Contributions: DA: *In silico* analysis, methodology, Illustrations and figures, data curation, writing, and editing. MDF: Project conception, data analysis, writing, editing, and supervision.

Data Availability Statement: All data generated or analysis during this study are included in this published article.

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Conflicts of Interest: The authors declare no conflict of interest.

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Appendix A

Table A1. list of template models.

#	Model	Antibody	Heavy Chain (Green)	Light Chain (Cyan)	RBD	Reference
1	7R6W	Sotrovimab (S309)	A	B	R	[37]
2	7KMG	Bamlanivimab (LY-CoV555)	A	B	C	[38]
3	7L7E	Evusheld (AZD8895)	A	B	G	[39]
4	7L7E	Evusheld (AZD1061)	E	F	G	[39]
5	7CM4	Regdanvimab (CT-P59)	H	L	A	[40]
6	6XDG	Casirivimab (REGN10933)	B	D	E	[41]
7	6XDG	Imdevimab (REGN10987)	C	A	E	[41]
8	7MMO	Bebtelovimab (LY-CoV1404)	A	B	C	[42]
9	7C01	Etesevimab (LY-CoV016) CB6, JS016, LY3832479	H	L	A	[43] [26]
10	7F7E	JS026	C	L	E	[16]

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