1 **Omicron variant escapes therapeutic mAbs contrary to eight prior main VOC**

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Abstract

Monocolonal antibodies (mAbs) are currently used for active immunization of COVID-19 in immunocompromised patients. We herein show that in spite there are variations in susceptibility to available mAbs that are authorized for clinical use in France tested on the original B.1.1 virus and 9 variants of concern or of interest, the cocktail casirivimab/imdevimab (REGN-CoV-2) showed a major synergistic effect. However, none of the four mAbs either alone or in combination neutralized the new Omicron variant. Our data strongly warrant a reinforcement of protective measures against infection for immunocompromised patients.

Text

Monocolonal antibodies (mAbs) are currently used for active immunization of COVID-19 in immunocompromised patients that do not respond to a complete vaccine schedule. As described previously¹, we tested the neutralizing activity of four mAbs that are authorized for clinical use in France, including bamlanivimab and etesevimab (alone or in combination) and casirivimab and imdevimab (alone or in combination as REGN-CoV-2), against SARS-CoV-2 strains isolated throughout the pandemic. Strains are the French original B.1.1 virus and 9 variants of concern or of interest: B.1.160, Alpha (B.1.1.7), Beta (B.1.351.2), Delta original (AY.71) and of sublineage (AY.4.2), Iota (B.1.526), Epsilon (B.1.429), Mu (B.1.621), and the recent Omicron (B.1.1.529)². Bamlanivimab did not inhibit the Beta and Delta variants as previously reported³, but also of Epsilon and Mu variants (Figure 1 and Supplementary appendix). For etesevimab, 50% of neutralization was below 5 µg/mL for Original/B.1.1 virus, Epsilon variant and both delta variants. For the cocktail of bamlanivimab/etesevimab, efficient neutralization was recovered for Alpha, B.1.160 and Iota variants. Casirivimab efficiently neutralized Original/B.1.1 virus, B.1.160, Alpha, Delta, AY4.2, Epsilon and Iota variants. In contrast, we did not observe any neutralization by

casirivimab of Beta and Mu variants. Imdevimab neutralized all variants except Omicron but concentrations to obtain 50% of neutralization were higher on average than with casirivimab. Unexpectedly, the cocktail casirivimab/imdevimab showed a major synergistic effect, particularly on Delta, AY4.2 and Epsilon variants because 50% of neutralization was observed at 0.03 µg/mL. We observed 50% of neutralization at 0.2 µg/mL for Original/B.1.1 virus, Alpha and Iota variants, at 0.4 µg/mL for B.1.160, 0.7 µg/mL for Beta. For Mu variant, we observed heterogeneity according to the replicates with 50% of neutralization on average at 2 µg/mL. However, none of the four mAbs either alone or in combination neutralized the new Omicron variant.

These results suggest that although the four tested mAbs can have a lowered effect on recently emerging variants, their combination is highly synergistic *in vitro*, a feature clinically reported recently for Delta variant⁴. But we observed also that the 4 mAbs currently used alone or in combination in our country showed a complete loss of their neutralizing activity against Omicron variant, a feature recently reported in comparison to the WA1/2020 D614G parental isolate⁵. Of course, definitive conclusions regarding the inefficiency of mAbs against Omicron await the outcomes of clinical studies but our data strongly warrant a reinforcement of protective measures against infection for immunocompromised patients.

References

- (1) Jaafar, R.; Boschi, C.; Aherfi, S.; Bancod, A.; Le Bideau, M.; Edouard, S.; Colson, P.; Chahinian, H.; Raoult, D.; Yahi, N.; Fantini, J.; La Scola, B. High Individual Heterogeneity of Neutralizing Activities against the Original Strain and Nine Different Variants of SARS-CoV-2. *Viruses* 2021, 13 (11), 2177. <https://doi.org/10.3390/v13112177>.

- (2) La Scola, B.; Lavarard, P.; Fournier, P.-E.; Colson, P.; Lacoste, A.; Raoult, D. SARS-CoV-2 Variant from India to Marseille: The Still Active Role of Ports in the Introduction of Epidemics. *Travel Med. Infect. Dis.* 2021, 42, 102085. <https://doi.org/10.1016/j.tmaid.2021.102085>.
- (3) Hoffmann, M.; Hofmann-Winkler, H.; Krüger, N.; Kempf, A.; Nehlmeier, I.; Graichen, L.; Arora, P.; Sidarovich, A.; Moldenhauer, A.-S.; Winkler, M. S.; Schulz, S.; Jäck, H.-M.; Stankov, M. V.; Behrens, G. M. N.; Pöhlmann, S. SARS-CoV-2 Variant B.1.617 Is Resistant to Bamlanivimab and Evades Antibodies Induced by Infection and Vaccination. *Cell Rep.* 2021, 36 (3), 109415. <https://doi.org/10.1016/j.celrep.2021.109415>.
- (4) Kumar V, J.; Banu, S.; Sasikala, M.; Parsa, K. V. L.; Sowpati, D. T.; Yadav, R.; Tallapaka, K. B.; Siva, A. B.; Vishnubhotla, R.; Rao, G. V.; Reddy, D. N. Effectiveness of REGEN-COV Antibody Cocktail against the B.1.617.2 (Delta) Variant of SARS-CoV-2: A Cohort Study. *J. Intern. Med.* 2021. <https://doi.org/10.1111/joim.13408>.
- (5) VanBlargan, L. A.; Errico, J. M.; Halfmann, P. J.; Zost, S. J.; Crowe, J. E.; Purcell, L. A.; Kawaoka, Y.; Corti, D.; Fremont, D. H.; Diamond, M. S. *An Infectious SARS-CoV-2 B.1.1.529 Omicron Virus Escapes Neutralization by Several Therapeutic Monoclonal Antibodies*; 2021; p 2021.12.15.472828. <https://doi.org/10.1101/2021.12.15.472828>.

Figure 1 legend: Concentrations required obtaining 50% neutralization (EC_{50} $\log_{10}$ $\mu\text{g/mL}$) for each mAb. (A) bamlanivimab, etesevimab, mixture of bamlanivimab and etesevimab, (B) casirivimab, imdevimab and REGN-CoV-2 on the 10 SARS-CoV-2 strains tested. Each mAb was tested three times (except for Omicron variant 4 times).

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Author contributions: CB performed microneutralisation tests and co-wrote the first draft of the article, CB performed microneutralisation tests, PC performed genomes analysis co-wrote the first draft of the article, VM co-designed the study, BL conceived and co-designed the

study and co-wrote the first draft of the article. All authors contributed to discussion and interpretation of the results, and to the writing of the manuscript. All authors have read and approved the final manuscript.

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99 *Supplementary data*

100 *Materials and methods*

101 *Cell culture*

102 Vero E6 cells (ATCC-CRL-1586) were cultured without antibiotics in minimal in medium
103 (MEM, Gibco, USA) with 2 mM L-glutamine and 10% foetal bovine serum (FBS) at 37°C in
104 a 5% CO₂ incubator. Vero E6 cells were then prepared at a concentration of 5x10⁵ cells/mL in
105 ninety-six-well plates for the neutralization tests of SARS-CoV-2 in MEM growth medium
106 with glutamine and 4% FBS (M4 media).

107 *SARS-CoV-2 viral strains*

108 The ten SARS-CoV-2 strains used in this study were isolated in cells culture and stored at -
109 80°C from patients's nasopharyngeal swabs tested SARS-CoV-2 positive in our institute IHU-
110 Méditerranée Infection during the pandemic^{1,2}. The supernatant of each strains was then
111 harvested and was genotyped by whole genome next generation (NGS) as previously
112 described³ (Supplementary data). For the neutralization tests, we inoculated the viral strains in
113 96-well Vero E6 cells plate at a concentration of 5x10⁵ cells/mL. Forty eight hours post-viral
114 infection, viral suspension was harvested and quantified by real-time reverse-transcription
115 RT-PCR and TCID50.

116 *Monoclonal antibodies dilutions*

117 Bamlavinimab and etesevimab were diluted each in a 1:5 serial dilutions (from 3500 µg/mL
118 to 0.0089 µg/mL). For the combination of the two mAbs, we tested the mixture in the highest
119 concentration for each mAbs alone with 2 times more etesevimab than bamlavinimab.

120 Casirivimab and imdevimab were diluted each in a 1:5 serial dilutions (from 12 000 µg/mL
121 to 0,00614 µg/mL). For the combination of these two mAbs, we tested the mixture in the
122 highest concentration for each mAbs alone in the same proportion.

Micro-neutralization assay

Each dilution of mAbs was mixed volume by volume with each viral strains with standardized inoculum at 25 Ct as previously described⁴. The mixture of viral suspension and mAbs was incubated 1h at 37 ° C under 5% CO₂. Then, 100μl of medium in the 96-well plates was removed and 100μL of the mixture for each dilution was added in quadruplicate on the Vero E6 cells. Five days post-viral infection, cytopathic effect was determined with the inverted optical microscope to determine the neutralization titer to obtain 50% of neutralization. Each mAbs and combination of mAbs were tested three times against the 10 SARS-CoV-2 strains, except for omicron variant that was tested four times.

References

1. La Scola, B. *et al.* Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *Eur. J. Clin. Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin. Microbiol.* **39**, 1059–1061 (2020).
2. Jaafar, R. *et al.* Correlation Between 3790 Quantitative Polymerase Chain Reaction-Positives Samples and Positive Cell Cultures, Including 1941 Severe Acute Respiratory Syndrome Coronavirus 2 Isolates. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **72**, e921 (2021).
3. Colson, P. *et al.* Spreading of a new SARS-CoV-2 N501Y spike variant in a new lineage. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* **27**, 1352.e1-1352.e5 (2021).
4. Jaafar, R. *et al.* High Individual Heterogeneity of Neutralizing Activities against the Original Strain and Nine Different Variants of SARS-CoV-2. *Viruses* **13**, 2177 (2021).

Supplementary figure S1:

Neutralization curves in Vero E6 cells for each strains tested with each mAb : A, C, E, G, I, K, M, N, O, Q, S : bamlanivimab, etesevimab and mixture of bamlanivimab and etesevimab – B, D, F, H, J, L, N, P, R, T : casirivimab, imdevimab and REGN-CoV-2. Each experiment was done three times, except for Omicron variant four times.

153 **Supplementary Table S2:** Lineage of SARS-CoV-2 isolates and mutations in the spike
154 protein. In this table are indicated for the ten SARS-CoV-2 strains: genome sequence
155 submitted to GISAID database (<https://www.gisaid.org/>), nexstrain clade, Pangolin lineage,
156 IHU name isolate (IHUMI) and the corresponding nucleotide substitutions, nucleotide
157 deletions, amino acid substitutions and amino acid deletion

Supplementary data S2

Sequence name (including GISAID)	Nexstrain clade	Pangolin lineage	IHU name	Nucleotide substitutions	Nucleotide deletions	Amino acid substitutions	Amino acid deletions	Nucleotide substitutions	Nucleotide deletions	Amino acid substitutions	Amino acid deletions
IHUMI- 717_EPI_ISL_8033347 2 2020-09-26	20B	B.1.1	IHUMI-717	12	0	7	0	C241T, C313T, C3037T, A7903G, C14408T, G19518T, A23403G, G26143T, G28845T, G28881A, G28882A, G28883C		N:R191L, N:R203K, N:G204R, ORF1b:P314L, ORF1b:L2017F, ORF3a:G251C, S:D614G	
IHUMI- 2096_EPI_ISL_8033348 20A 2020-08-06	20A	B.1.160	IHUMI-2096	20	15	11	5	C241T, C3037T, C4543T, G5629T, G9526T, C11497T, G13993T, C14408T, G15766T, G16889G, G17019T, C18877T, G22992A, A23403G, G25563T, C25710T, C26735T, T26876C, G28975C, G29399A	23585-23599	N:M234I, N:A376T, ORF1a:M3087I, ORF1b:A176S, ORF1b:P314L, ORF1b:V767I, ORF1b:K1141R, ORF1b:E1184D, ORF3a:Q57H, S:5477N, S:D614G	S:Q675-, S:T676-, S:Q677-, S:T678-, S:N679-
IHUMI- 3076_EPI_ISL_8033350 20I (Alpha, V1) 2021-01-08	20I	B.1.1.7	IHUMI-3076	31	25	20	8	C241T, C913T, A1399G, C3037T, C3267T, C5388A, C5986T, T6954C, C14408T, C14676T, C15279T, T16176C, A17615G, A23063T, C23271A, A23403G, C23604A, C23709T, T24506G, G24914C, G26730C, C27972T, G28048T, A28111G, G28280C, A28281T, T28282A, G28881A, G28882A, G28883C, C28977T	11288-11296, 12041- 12046, 21765-21770, 21992-21994, 28271	M:V70L, N:D3L, N:R203K, N:G204R, N:S235F, ORF1a:T1001I, ORF1a:A1708D, ORF1a:I2230T, ORF1b:P314L, ORF1b:K1833R, ORF3a:Q27*, ORF3a:R52I, ORF3a:V73C, S:N501Y, S:A570D, S:D614G, S:P681H, S:T716I, S:5982A, S:D1118H	ORF1a:S3675-, ORF1a:G3676-, ORF1a:F3677- , ORF1a:D3926-, ORF1a:I3927-, S:H69-, S:V70-, S:Y144-
IHUMI- 3147_EPI_ISL_8033349 20H (Beta V2) 2021-01-22	20H	B.1.351.2	IHUMI-3147	26	18	18	6	G174T, C241T, C1059T, A2692T, C3037T, G5230T, A8052G, A10323G, G10396T, C10632T, C14408T, C21614T, A21801C, A22066G, G22813T, G23012A, A23063T, A23403G, T23560G, C23664T, G25563T, C25904T, C26456T, C28253T, T27829C, C28887T	11288-11296, 22283- 22291	E:P71L, N:T205I, ORF1a:T265I, ORF1a:K1655N, ORF1a:N2596S, ORF1a:K3353R, ORF1a:A3456V, ORF1b:P314L, ORF3a:Q57H, ORF3a:S171L, S:L18F, S:D80A, S:D219G, S:K417N, S:E484K, S:N501Y, S:D614G, S:A701V	ORF1a:S3675-, ORF1a:G3676-, ORF1a:F3677- , S:L241-, S:L242-, S:A243-
IHUMI- 3630_EPI_ISL_8033350 21I (Delta) 2021-06-07	21I	AY.71	IHUMI-3630	33	13	27	4	G210T, C241T, C2509T, C3037T, C5184T, A5584G, C8991T, T11418C, C11514T, C13019T, C14408T, G15451A, C16466T, C16728T, A18931G, C19118T, C21618G, G21987A, G22227T, T22917G, C22995A, A23403G, G24410A, C25317T, C25469T, C26753T, T26767C, T27698C, C27752T, A28461G, G28881T, G29402T, G29742T	22029-22034, 28248- 28253, 28271	M:I82T, N:D63G, N:R203M, N:D377Y, ORF1a:P1640L, ORF1a:A3209V, ORF1a:V3718A, ORF1a:T3750L, ORF1b:P314L, ORF1b:G662S, ORF1b:P1000L, ORF1b:H1087Y, ORF1b:I1822V, ORF1b:A1884V, ORF3a:S26L, ORF3a:V82A, ORF3a:T120I, ORF3b:T60A, S:T19R, S:G142D, S:R158G, S:A222V, S:L452R, S:T478K, S:D614G, S:D950N, S:S1252F	ORF8:D119-, ORF8:F120-, S:E156-, S:F157-
IHUMI- 5002_EPI_ISL_8033351 21J (Delta) 2021-10-25	21J	AY.4.2	IHUMI-5002	40	13	33	4	G210T, C241T, T1391C, C1973T, C3037T, G4181T, G4402T, C7124T, C7851T, C8986T, G9053T, C10029T, A11201G, A11331G, C14408T, G15451A, C16466T, T17040C, C19220T, C21618G, C21846T, G21987A, T21995C, C22227T, T21917G, C22995A, A23403G, C23604G, G24410A, C25469T, C25561T, T26767C, T27698C, C27752T, C27874T, A28461G, G28881T, G28916T, G29402T, G29742T	22029-22034, 28248-28253, 28271	M:I82T, N:D63G, N:R203M, N:D377Y, ORF1a:P1640L, ORF1a:A3209V, ORF1a:V3718A, ORF1a:T3750L, ORF1b:P314L, ORF1b:G662S, ORF1b:P1000L, ORF1b:H1087Y, ORF1b:I1822V, ORF1b:A1884V, ORF3a:S26L, ORF3a:V82A, ORF3a:T120I, ORF3b:T60A, S:T19R, S:G142D, S:R158G, S:A222V, S:L452R, S:T478K, S:D614G, S:D950N	ORF8:D119-, ORF8:F120-, S:E156-, S:F157-
IHUMI- 3795_EPI_ISL_8033348 21F (Iota) 2021-07-12	21F		IHUMI-3795	37	20	18	3	C241T, C1059T, C3037T, G6101A, A7201G, C8809T, T9867C, C14408T, A16500C, A20262G, C21575T, C21846T, A22320T, C22498T, G22992A, A23403G, A24432G, C25517T, G25563T, A25986G, C27739T, C27925T, C28311T, T28879G, G29254T, C29738A, T29753A, G29755A, G29757T, T29758G, G29759C, G29762G, A29763G, T29765A, A29768G, G29779A, T29785C	11288-11296, 28271, 29740- 29745, 29749- 29750, 29770- 29771	N:P13L, N:S202R, ORF1a:T265I, ORF1a:G1946S, ORF1a:L3201P, ORF1b:P314L, ORF1b:Q1011H, ORF3a:P42L, ORF3a:Q57H, ORF3a:L116F, ORF8:T11I, ORF9b:P10S, S:L5F, S:T95I, S:D253V, S:5477N, S:D614G, S:Q957R	ORF1a:S3675-, ORF1a:G3676-, ORF1a:F3677-
IHUMI- 4219_EPI_ISL_8033611 21C (Epsilon) 2021-07-22	21C		IHUMI-4219	28	3	13	1	C241T, C1059T, C2395T, T2597C, C3037T, C7056A, T7057A, C7058A, G7059A, G8047T, G10282A, C11083T, C12100T, A12878G, C14408T, G17014T, G21600T, G22018T, T22917G, A23403G, T24349C, G25563T, C26681T, G27890T, G28001T, A28272T, C28887T, C29362T	518-520	N:T205I, ORF1a:T265I, ORF1a:T2264K, ORF1a:G2265N, ORF1a:L3606F, ORF1a:I4205V, ORF1b:P314L, ORF1b:D1183Y, ORF3a:Q57H, S:S13I, S:W152C, S:L452R, S:D614G	ORF1a:M85-
IHUMI- 3964_EPI_ISL_8033762 21H (Mu) 2021-07-27	21H		IHUMI-3964	32	4	21	1	C241T, C3037T, A3428G, C4878T, C5192T, G6037T, C10029T, C11344T, A11451G, A13057T, C14408T, C17491T, C17707T, C18877T, T19035C, C20148T, C21846T, A21993C, T21995A, G22599A, G23012A, A23063T, A23403G, C23604A, G24410A, G25563T, A26492T, G27008C, C27925A, C28005T, A28272T, C28887T	26158-26161	M:K162N, N:T205I, ORF1a:T1055A, ORF1a:T1538I, ORF1a:T3255I, ORF1a:Q3729R, ORF1b:P314L, ORF1b:P1342S, ORF1b:P1414S, ORF3a:Q57H, ORF8:T11K, ORF8:P38S, S:T95I, S:Y144S, S:Y145N, S:R346K, S:E484K, S:N501Y, S:D614G, S:P681H, S:D950N	ORF3a:V256-
IHUMI- 5227_EPI_ISL_8033859 21K (Omicron) 2021-12-01	21K		IHUMI-5227	54	36	45	15	C241T, A2892G, C3037T, T5386G, G5924A, G8383A, C10029T, C10448A, A11537S, T19195C, C14408T, C15240T, A18163G, C21762T, C21846T, G22578A, T22673C, C22674T, T22679C, C22686T, G22813T, G22898A, G22992A, C22995A, A23040G, G23048A, A23055G, A23063T, T23075C, C23202A, A23403G, C23525T, T23599G, C23604A, C23664T, C23854A, G23948T, C24130A, A24424T, T24469A, C24503T, C25000T, C25584T, C26270T, A26530G, C26577G, G26709A, A27259C, C27807T, A28271T, C28311T, G28881A, G28882A, G28883C	6513-6515, 11285-11293, 21765-21770, 21987-21995, 28362-28370	E:T9I, M:Q3G, M:Q19E, M:A63T, N:P13L, N:R203K, N:G204R, ORF1a:N856R, ORF1a:V1870I, ORF1a:L3084I, ORF1a:A2710T, ORF1a:T3255I, ORF1a:P3395H, ORF1a:I3758V, ORF1b:P314L, ORF1b:I1566V, ORF9b:P10S, S:A67V, S:T95I, S:Y1450, S:G539D, S:S371L, S:S373P, S:S375F, S:K417N, S:G446G, S:S477N, S:T478K, S:Q493R, S:G496S, S:Q498R, S:N501Y, S:Y505H, S:T547K, S:D614G, S:H655Y, S:N679K, S:P681H, S:A701V, S:N764K, S:D796V, S:N856K, S:Q954H, S:N969K, S:L981F	N:E31-, N:R32-, N:S33-, ORF1a:S2083-, ORF1a:L3674-, ORF1a:S3675-, ORF1a:G3676-, ORF9b:E27-, ORF9b:N29-, ORF9b:A29-, S:H69-, S:V70-, S:G142-, S:Y143-, S:Y144-

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