

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

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9

10 ***Abstract***

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

19 ***Text***

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

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

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

52 **References**

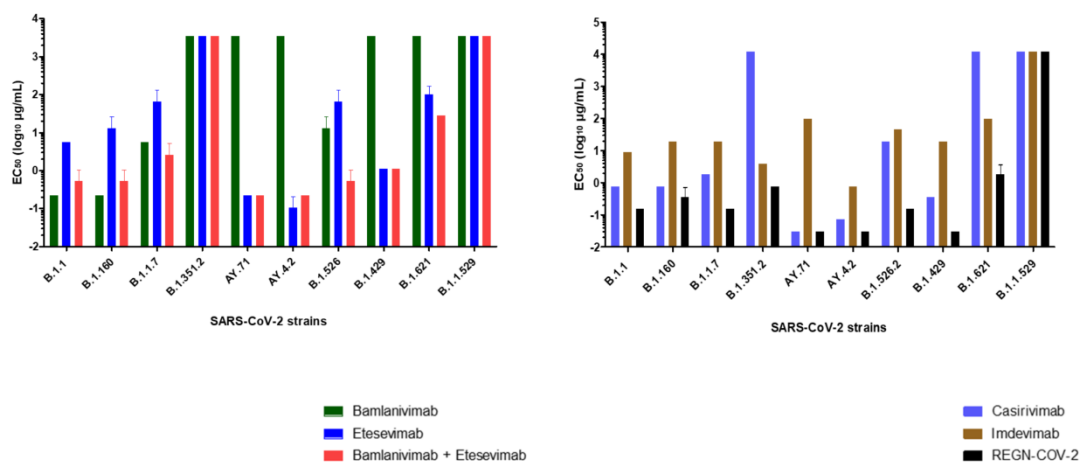
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65 Resistant to Bamlanivimab and Evades Antibodies Induced by Infection and
66 Vaccination. *Cell Rep.* 2021, 36 (3), 109415.
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78 **Figure 1 legend:** Concentrations required obtaining 50% neutralization (EC_{50} \log_{10} $\mu\text{g}/\text{mL}$)
79 for each mAb. (A) bamlanivimab, etesevimab, mixture of bamlanivimab and etesevimab, (B)
80 casirivimab, imdevimab and REGN-CoV-2 on the 10 SARS-CoV-2 strains tested. Each mAb
81 was tested three times (except for Omicron variant 4 times).



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

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

123 Micro-neutralization assay

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

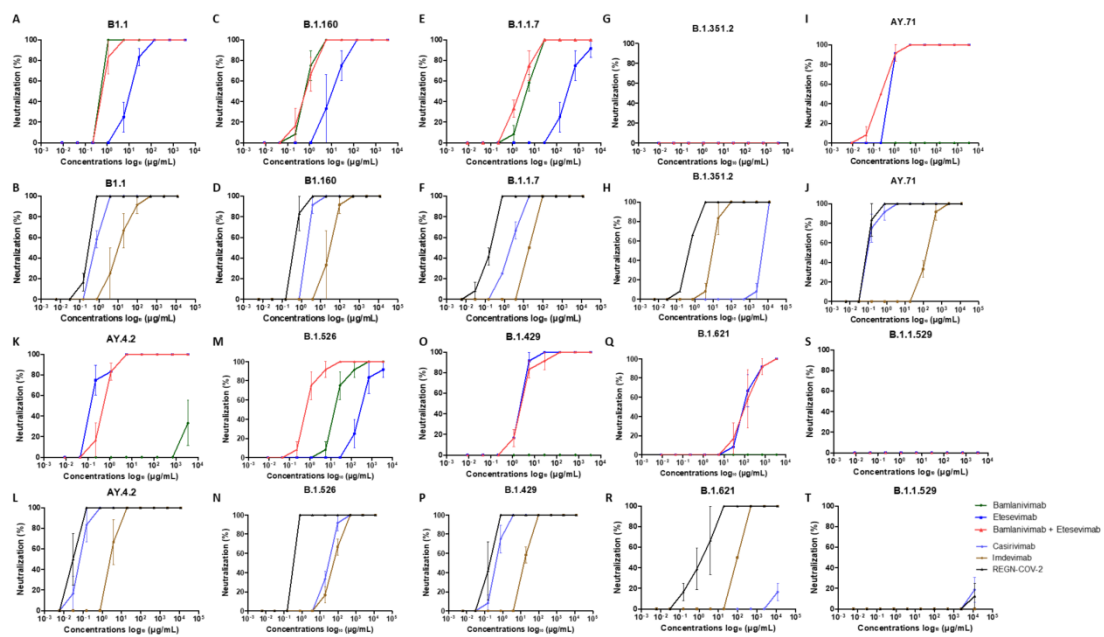
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134 for discharge of SARS-CoV-2 patients from infectious disease wards. *Eur. J. Clin. Microbiol.*
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144 Original Strain and Nine Different Variants of SARS-CoV-2. *Viruses* **13**, 2177 (2021).

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146 **Supplementary figure S1:**

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



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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,
 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 O20-03-26	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 2 2020-08-06	20A	B.1.160	IHUMI-2096	20	15	11	5	C241T, C3037T, C4543T, G5629T, G9526T, C11497T, G13993T, C14408T, G15766T, A16889G, G17019T, C18877T, G22992A, A23403G, G25563T, C25710T, C26735T, T26676C, G28975C, G29399A	23585-23599	N.M234I, N.A376I, ORF1a:M30087I, 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_982232 2 O21-01-08	20I (Alpha, V1)	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:K1333R, ORFb:Q27*, ORFb:R52I, ORFb:Y93C, S.N501Y, S.A570D, S.D614G, S.P681H, S.T716I, S.S982A, S.D1118H	ORF1a:S3675-, ORF1a:G3676-, ORF1a:F3677-, ORF1a:D3926-, ORF1a:I3927-, S.H69-, S.V70-, S.Y144-
IHUMI-3147_EPI_ISL_8033349 2 2021-01-22	20H (Beta, V2)	B.1.351.2	IHUMI-3147	26	18	18	6	G174T, C241T, C1059T, A2692T, C3037T, G5230T, A8052G, A10323G, G10396T, C10632T, C14408T, C21614T, A21801C, A22006G, G22813T, G23012A, A23063T, A23403G, T23560C, C23664T, G25563T, C25504T, C26456T, C28253T, T28729C, C28887T, G210T, C241T, C2509T, C3037T, C5184T, A5584G, C8991T, T11418C, C11514T, C13019T, C14408T, G15451A, C16466T, C16728T, A18931G, C19118T, C21618G, C21987A, G22227T, T22917G, C22995A, A23403G, G24410A, C25317T, C25469T, C26759T, T26767C, T27659C, C27752T, A28461G, G28881T, G29402T, G29742T	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 2 2021-06-07	21I (Delta) AY.71	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, C21987A, G22227T, T22917G, C22995A, A23403G, G24410A, C25317T, C25469T, C26759T, T26767C, T27659C, 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:W82A, ORF3a:T120I, ORF3b:T60A, S.T19R, S.G142D, S.R158G, S.A222V, S.L452R, S.T478K, S.D614G, S.D950N, S.S1252F	ORFb:D119-, ORFb:F120-, S.E156-, S.F157-
IHUMI-5002_EPI_ISL_8033351 2 2021-10-25	21J (Delta) AY.4.2	AY.4.2	IHUMI-5002	40	13	33	4	G210T, C241T, T1391C, C1973T, C3037T, G4181T, G4602T, C7124T, C7851T, C8986T, G9015T, C10029T, A11201G, A11331G, C14408T, G15451A, C16466T, T17040C, C19220T, C21618G, C21846T, G21987A, T21995C, C22227T, T22917G, C22995A, A23403G, C23604G, G24410A, C25469T, C25514T, T26767C, T27659C, C27752T, C27874T, A28461G, G28881T, G28916T, G29402T, G29742T	22029-22034, 28248-28253, 28271	M.I82T, N.D63G, N.R203M, N.G215C, N.D377Y, ORF1a:S376P, ORF1a:A1306S, ORF1a:P2046I, ORF1a:P2287S, ORF1a:A2529V, ORF1a:V2930L, ORF1a:T3255I, ORF1a:T3646A, ORF1b:P314L, ORF1b:G662S, ORF1b:P1000L, ORF1b:A1918V, ORF3a:S26L, ORF3a:W82A, ORF3a:T120I, ORF3b:T40L, ORF3b:T60A, S.T19R, S.T95I, S.G142D, S.Y145H, S.R158G, S.A222V, S.L452R, S.T478K, S.D614G, S.P681R, S.D950N	ORFb:D119-, ORFb:F120-, S.E156-, S.F157-
IHUMI-3795_EPI_ISL_8033348 1 2021-07-12	21F (Iota)	B.1.526	IHUMI-3795	37	20	18	3	C241T, C1059T, C3037T, G6101A, A7201G, C8909T, T9867C, C14408T, A16500C, A20262G, C21575T, C21846T, A22320T, C22498T, G22992A, A23403G, A24432G, C25517T, G25563T, A25986G, C27739T, C27925T, C28311T, T28879G, G29254T, C29738A, C29753A, 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, ORFb:T11I, ORFb:P105, S.L5F, S.T95I, S.D253V, S.S477N, S.D614G, S.Q957R	ORF1a:S3675-, ORF1a:G3676-, ORF1a:F3677-
IHUMI-4219_EPI_ISL_8033611 1 2021-07-22	21C (Epsilon)	B.1.429	IHUMI-4219	28	3	13	1	C241T, C1059T, C2395T, T2597C, C3037T, C7056A, T7057A, G7059A, G7059A, C8947T, G10282A, G11083T, 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:G2265H, 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 1 2021-07-27	21H (Mu)	B.1.621	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, G22995A, 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, ORFb:T11K, ORFb:P38S, S.T95I, S.Y144S, S.Y145H, S.R346K, S.E484K, S.N501Y, S.D614G, S.P681H, S.D950N	ORF3a:V256-
IHUMI-5227_EPI_ISL_8033859 1 2021-12-01	21K (Omicron)	B.1.1.529	IHUMI-5227	54	36	45	15	C241T, A2892G, C3037T, T5386G, G5924A, G8363A, C10029T, C10449A, A11537S, T131955C, C14408T, C15240T, A18163G, C21967T, C21846T, G22578A, T22673C, C22674T, T22679C, C22686T, G22813T, G22898A, G22992A, C22995A, A23040G, G23048A, A23056G, A23063T, T23075C, C23202A, A23403G, C23525T, T23599G, C23604A, C23664T, C23854A, G23948T, C24130A, A24424T, T24469A, C24503T, C25000T, C25584T, C26270T, A26530G, C26577G, G26709A, A27259C, G28077T, A28271T, C28311T, G28881A, G28882A, G28883C	6513-6515, 11285-11289, 21765-21770, 21987-21995, 28362-28370	E.T9I, M.D3G, M.Q19E, M.A63T, N.P13L, N.R203K, N.G204R, ORF1a:N856R, ORF1a:V180I, ORF1a:L2049I, ORF1a:A2710T, ORF1a:T3255I, ORF1a:P3395H, ORF1a:I3758V, ORF1b:P314L, ORF1b:I1566V, ORFb:P105, S.A67V, S.T95I, S.Y145D, S.G339D, S.S371L, S.S373P, S.S375F, S.K417N, S.G446S, 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:I3674-, ORF1a:S3675-, ORF1a:G3676-, ORFb:R29-, ORFb:R29-, ORFb:A29-, S.H69-, S.V70-, S.G142-, S.V143-, S.Y144-

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