

## 1 **Materials and methods**

### 2 Cell culture

3 Vero E6 cells (ATCC-CRL-1586) were cultured without antibiotics in minimal in medium  
4 (MEM, Gibco, USA) with 2 mM L-glutamine and 10% foetal bovine serum (FBS) at 37°C in  
5 a 5% CO<sub>2</sub> incubator. Vero E6 cells were then prepared at a concentration of 5x10<sup>5</sup> cells/mL in  
6 ninety-six-well plates for the neutralization tests of SARS-CoV-2 in MEM growth medium  
7 with glutamine and 4% FBS (M4 media).

### 9 SARS-CoV-2 viral strains

10 The ten SARS-CoV-2 strains used in this study were isolated in cells culture and stored at -  
11 80°C from patients's nasopharyngeal swabs tested SARS-CoV-2 positive in our institute IHU-  
12 Méditerranée Infection during the pandemic [1,2]. The supernatant of each strains was then  
13 harvested and was genotyped by whole genome next generation (NGS) as previously  
14 described[3] (Supplementary table S2). For the neutralization tests, we inoculated the viral  
15 strains in 96-well Vero E6 cells plate at a concentration of 5x10<sup>5</sup> cells/mL. Forty-eight hours  
16 post-viral infection, viral suspension was harvested and quantified by real-time reverse-  
17 transcription RT-PCR and TCID<sub>50</sub>.

### 19 Monoclonal antibodies dilutions

20 Bamlavinimab and etesevimab were first diluted at 1:10 then each mAbs was diluted in a 1:5  
21 serial dilutions (from 3500 µg/mL to 0.0089 µg/mL). For the combination of the two mAbs,  
22 we tested the mixture in the highest concentration for each mAbs alone with 2 times more  
23 etesevimab than bamlavinimab.

24 Casirivimab and imdevimab were first diluted at 1:10 then each mAbs was diluted each in a  
25 1:5 serial dilutions (from 12 000 µg/mL to 0,00614 µg/mL). For the combination of these two

26 mAbs, we tested the mixture in the highest concentration for each mAbs alone in the same  
27 proportion.

28 Tixagevimab and cilgavimab were first diluted at 1:10 then each mAbs was diluted each in a  
29 1:5 serial dilutions (from 10 000 µg/mL to 0,0512 µg/mL). For the combination of these two  
30 mAbs, we tested the mixture in the highest concentration for each mAbs alone in the same  
31 proportion

### 32 Micro-neutralization assay

33 Each dilution of mAbs was mixed volume by volume with each viral strains with standardized  
34 inoculum at 25 Ct as previously described[4]. The mixture of viral suspension and mAbs was  
35 incubated 1h at 37 ° C under 5% CO<sub>2</sub>. Then, 100µl of medium in the 96-well plates was  
36 removed and 100µL of the mixture for each dilution was added in quadruplicate on the Vero E6  
37 cells. Five days post-viral infection, cytopathic effect was determined with the inverted  
38 optical microscope to determine the neutralization titer to obtain 50% of neutralization. Each  
39 mAbs and combination of mAbs were tested three times against the 9 SARS-CoV-2 strains,  
40 except for omicron variant that was tested four times.

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### 42 References

43 [1] La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, et al. Viral  
44 RNA load as determined by cell culture as a management tool for discharge of SARS-  
45 CoV-2 patients from infectious disease wards. *Eur J Clin Microbiol Infect Dis Off Publ*  
46 *Eur Soc Clin Microbiol* 2020;39:1059–61. <https://doi.org/10.1007/s10096-020-03913-9>.

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48 [2] Jaafar R, Aherfi S, Wurtz N, Grimaldier C, Van Hoang T, Colson P, et al. Correlation  
49 Between 3790 Quantitative Polymerase Chain Reaction-Positives Samples and Positive  
50 Cell Cultures, Including 1941 Severe Acute Respiratory Syndrome Coronavirus 2  
51 Isolates. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2021;72:e921.  
52 <https://doi.org/10.1093/cid/ciaa1491>.

53

54 [3] Colson P, Levasseur A, Delerce J, Pinault L, Dudouet P, Devaux C, et al. Spreading of a  
55 new SARS-CoV-2 N501Y spike variant in a new lineage. *Clin Microbiol Infect Off Publ*

56 Eur Soc Clin Microbiol Infect Dis 2021;27:1352.e1-1352.e5.  
57 <https://doi.org/10.1016/j.cmi.2021.05.006>.

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59 [4] Jaafar R, Boschi C, Aherfi S, Bancod A, Le Bideau M, Edouard S, et al. High Individual  
60 Heterogeneity of Neutralizing Activities against the Original Strain and Nine Different  
61 Variants of SARS-CoV-2. *Viruses* 2021;13:2177. <https://doi.org/10.3390/v13112177>.

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63 **Supplementary data S1: Neutralization curves in Vero E6 cells for each strains tested**

64 **with each mAb** : A, C, E, G, I, K, M, N, O, Q, S : bamlanivimab, etesevimab and mixture of

65 bamlanivimab and etesevimab – B, D, F, H, J, L, N, P, R, T : casirivimab, imdevimab and

66 REGN-CoV-2. Each experiment was done three times, except for Omicron variant four times.

67 **Supplementary data S2 : Neutralization curves in Vero E6 cells for the three strains**

68 **tested with new mAb** : A : B.1.1 – B : AY.71 – C : B.1.1.529 with tixagevimab, cilgavimab

69 and combination of both. Each experiment was done four times.

70 **Supplementary table S3:** Lineage of SARS-CoV-2 isolates and mutations in the spike

71 protein. In this table are indicated for the ten SARS-CoV-2 strains: genome sequence

72 submitted to GISAID databe (<https://www.gisaid.org/>), nexstrain clade, Pangolin lineage,

73 IHU name isolate (IHUMI) and the corresponding nucleotide substitutions, nucleotide

74 deletions, amino acid substitutions and amino acid deletion

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