

Preclinical *in vivo* assessment of the activity of AZD7442 anti-SARS-CoV-2 monoclonal antibodies against Omicron sublineages



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ARTICLE INFO

Keywords:
Preclinical animal model
Monoclonal antibody
SARS-CoV-2
Omicron sublineages
AZD7442
Clinical therapeutics

ABSTRACT

Therapeutic monoclonal antibodies have been successful in protecting vulnerable populations against SARS-CoV-2. However, their effectiveness has been hampered by the emergence of new variants. To adapt the therapeutic landscape, health authorities have based their recommendations mostly on *in vitro* neutralization tests. However, these do not provide a reliable understanding of the changes in the dose-effect relationship and how they may translate into clinical efficacy. Taking the example of Evusheld™ (AZD7442), we aimed to investigate how *in vivo* data can provide critical quantitative results and project clinical effectiveness. We used the Golden Syrian hamster model to estimate 90 % effective concentrations (EC90) of AZD7442 *in vivo* against SARS-CoV-2 Omicron BA.1, BA.2 and BA.5 variants. While our *in vivo* results confirmed the partial loss of AZD7442 activity for BA.1 and BA.2, they showed a much greater loss of efficacy against BA.5 than that obtained *in vitro*. We analyzed *in vivo* EC90s in perspective with antibody levels measured in a cohort of immunocompromised patients who received 300 mg of AZD7442. We found that a substantial proportion of patients had serum levels of anti-SARS-CoV-2 spike protein IgG above the estimated *in vivo* EC90 for BA.1 and BA.2 (21 % and 92 % after 1 month, respectively), but not for BA.5. These findings suggest that AZD7442 is likely to retain clinical efficacy against BA.2 and BA.1, but not against BA.5. Overall, the present study illustrates the importance of complementing *in vitro* investigations by preclinical studies in animal models to help predict the efficacy of monoclonal antibodies in humans.

1. Introduction

Although vaccines have dramatically reduced the risk of severe disease after infection by SARS-CoV-2 virus, complementary tools are still needed, especially to protect vulnerable populations who do not respond adequately to vaccination. In this context, therapeutic monoclonal antibodies (mAbs) such as Ronapreve™ (Casirivimab/imdevimab; marketed by Roche) or Evusheld™ (Cilgavimab/Tixagevimab; marketed by AstraZeneca) (AZD7442) have been used worldwide [1,2]. These antibodies showed high efficacy both therapeutically, reducing the risk of severe infection by 70–90 % when administered during the first week after the onset of symptoms, and prophylactically, where AZD7442 was 76.7 % effective in preventing symptomatic infection with COVID-19 [3–7].

Successive variants of SARS-CoV-2 carrying mutations in particular on the spike protein (the main target of neutralizing antibodies) have emerged over time [8–10]. The Omicron variant (lineage B.1.1.529) is one of the 5 WHO Variants of Concern (VOCs). It includes several sublineages, *i.e.* the original BA.1 sublineage that emerged in South Africa in late 2021 and has rapidly spread worldwide with very high incidence levels [11], the BA.2 sublineage, which became dominant in many countries in March 2022, and the BA.4 and BA.5 sublineages, that emerged in early 2022. A summary of spike mutations associated with the different variants is provided in Fig. 1.

Several *in vitro* studies have shown that therapeutic mAbs became less effective, or even non-effective, against sublineages of the Omicron variant [12–21]. In one of them, conducted with an *in vitro* live-virus assay, the activity against BA.1 sublineage of AZD7442 that targets the SARS-CoV-2 spike receptor binding domain (RBD) [22] was drastically reduced [21]. Several subsequent *in vitro* studies have shown that the activity of Cilgavimab against BA.2, and to a lesser extent BA.5, was partially restored, resulting in a significant improvement of the AZD7442 neutralizing activity [20,23,24].

In vitro studies on the sensitivity of the different variants to therapeutic mAbs can be rapidly implemented and have been pivotal to allow

health authorities to issue recommendations for the management of patients. However, in 2022, a review report concluded that AZD7442 had been used during the Omicron wave without solid clinical data of its efficiency against this variant, and that new randomized controlled trials in immunocompromised vaccine patients were urgently needed [25]. While clinical validation in the target population remains the undisputed gold standard, when variants follow one another rapidly it is possible that results against a given variant are obtained when it has ceased to circulate. In this context, although they cannot completely predict clinical efficacy, preclinical studies in animal models are useful to supplement *in vitro* data in the process of transposing preclinical activity to humans, especially when a partial decrease in mAbs activity is observed. In particular, *in vivo* studies are needed to verify whether efficacy is observed at exposures comparable to those observed in humans. Here, we sought to complement *in vitro* AZD7442 efficacy data with animal studies to shed light on AZD7442's activity against different sublineages *in vivo*. EC90s determined *in vivo* in the hamster model were also compared with anti-SARS-CoV-2 spike protein (S1) IgG levels observed in a prospective cohort of immunocompromised patients treated with AZD7442 (PRECOVIM: Pre-exposure prophylaxis of SARS-CoV-2 infection (COVID-19) by mAbs with early access authorization in immunocompromised patients) [26,27] to help predict efficiency in humans.

2. Methods

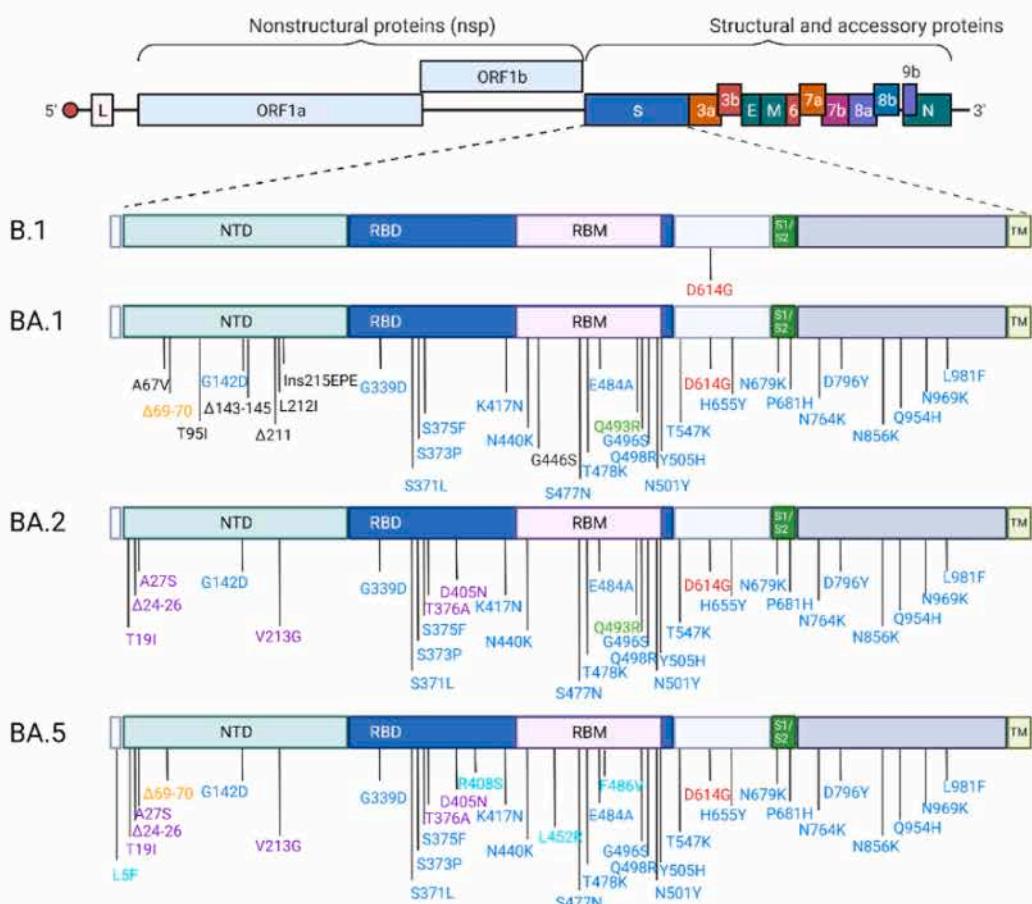
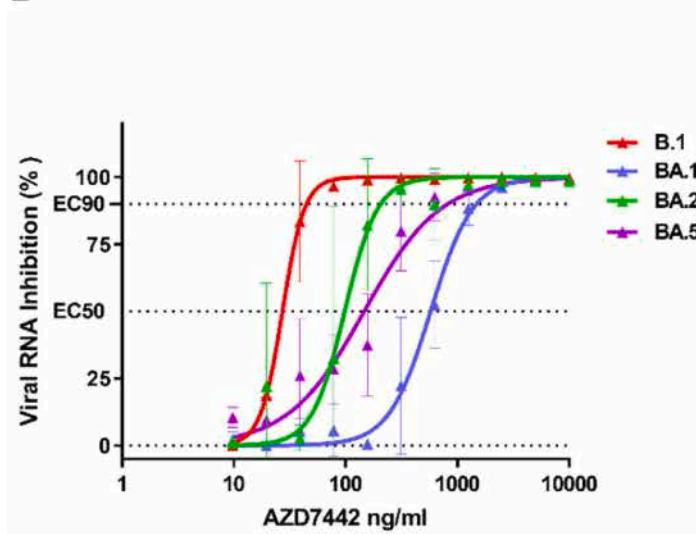
2.1. Cells

VeroE6 cells (ATCC CRL-1586) were cultured at 37 °C with 5 % CO₂ in minimal essential medium (MEM) supplemented with 1 % Penicillin/Streptomycin, 1 % non-essential amino acids and 7 % of heat-inactivated fetal bovine serum (FBS) (all from ThermoFisher Scientific). VeroE6/TMPRSS2 cells (NIBSC 100978) were cultured in the same medium supplemented with 2 % of G-418 (ThermoFisher Scientific).

2.2. Viruses

The B.1 BavPat1 SARS-CoV-2 strain (G614 strain) was obtained from

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Strain	Interpolated value	50	90	Fold Change
B.1	EC (ng.ml ⁻¹)	27.22	44.72	-
	[IC95]	[24.0-31.0]	[35.8-54.2]	-
Omicron BA.1	EC (ng.ml ⁻¹)	575.25	1481.44	33.50
	[IC95]	[498.4-663.6]	[1104.6-1964.6]	-
Omicron BA.2	EC (ng.ml ⁻¹)	98.03	205.64	4.65
	[IC95]	[72.0-125.8]	[125.8-327.6]	-
Omicron BA.5	EC (ng.ml ⁻¹)	146.48	817.25	18.48
	[IC95]	[113.6-188.7]	[484.5-1351.4]	-

Fig. 1. Genomic organization of Omicron sublineages and *in vitro* evaluation of AZD7442. (A) Spike substitutions in SARS-CoV-2 variants Omicron BA.1, BA.2 and BA.5 compared to the ancestral strain B.1. Omicron BA.1, BA.2 and BA.5 sequences used for the graphical representation are those of the viral strains used in this study. Red color indicates the mutation that is present in all strains. The black color indicates mutations specific to BA.1. The blue color indicates the mutations common to BA.1, BA.2 and BA.5. The green color indicates the mutations common to BA.1 and BA.2. The orange color indicates the mutations specific to BA.1 and BA.5. The purple color indicates the mutations specific to BA.2 and BA.5. The cyan color indicates the mutations specific to BA.5. (B) Concentration response curves reporting the susceptibility of the SARS-CoV-2 B.1 ancestral strain and Omicron BA.1, BA.2 and BA.5 sublineages to AZD7742. The data presented are from three technical replicates in VeroE6-TMPRSS2 cells, and error bars show mean \pm SD. (C) Interpolated EC50 values (ng/mL). Interpolated EC90 values (ng/mL) and fold change to B.1. This figure was created with BioRender.com.

Pr. C. Drosten through EVA GLOBAL (<https://www.european-virus-archive.com/>) and contains the D614G mutation. Virus stocks of this strain were produced using VeroE6 cells (passage history: 2 for *in vivo* studies). The clinical strains of the SARS-CoV-2 BA.1, BA.2 and BA.5 Omicron variants used here are respectively named 2021/FR/1514, 2022/FR/TCO and 2022/FR/GPO and are available through EVA GLOBAL (www.european-virus-archive.com, ref: 001 V-04653, GISAID: EPI_ISL_7899754; ref: 001 V-04663, GISAID: EPI_ISL_11926922; ref: 001 V-04746, GISAID: EPI_ISL_13692526; respectively). Virus stocks of these strains were produced using VeroE6/TMPRSS2 cells (passage history: 1 for *in vivo* studies). All virus stocks were characterized by whole-genome sequencing in order to verify the absence of additional mutations, especially in the spike-coding region. All experiments with infectious viruses were performed in a biosafety level 3 laboratory.

2.3. Antibodies

We used the solution for injection Evusheld™ 300 mg (Tixagevimab/Cilgavimab, AZD7442), marketed by AstraZeneca.

2.4. In vitro experiments

In vitro 50 % and 90 % effective concentrations (EC50 and EC90, *i.e.* compound concentration required to inhibit viral RNA replication by 50 % and 90 % respectively) were determined as previously described [21]. Briefly, one day prior to infection, VeroE6/TMPRSS2 cells were seeded in 96 well culture plates. The next day, antibodies were diluted in PBS with 1/2 dilutions from 5000 to 2.4 ng/mL. Twelve twofold serial dilutions of antibodies in triplicate were added to the cells (25 µL/well). Then, 25 µL of a virus mix diluted in medium was added to the wells. The amount of virus working stock used was calibrated prior to the assay so that the viral replication was still in the exponential growth phase for the readout [21,28,29]. Plates were incubated 15 min at room temperature and then 2 days at 37°C prior to quantification of the viral genome by real-time RT-PCR as previously described [21,28–30]. RT-qPCR reactions were performed on QuantStudio 12 K Flex Real-Time PCR System (Applied Biosystems) and analyzed using QuantStudio 12 K Flex Applied Biosystems software v1.2.3. Primers and probe sequences, which target SARS-CoV-2 N gene, are described in Touret et al., 2022 [21]. Viral inhibition was calculated as follow: 100 * (quantity mean “virus control” – sample quantity)/quantity mean “virus control”. The EC50s and EC90s were determined using logarithmic interpolation after performing a nonlinear regression as previously described [21,28,29, 31–33].

2.5. In vivo experiments

All experiments were approved by the local ethical committee (C2EA—14) and the French ‘Ministère de l’Enseignement Supérieur, de la Recherche et de l’Innovation’ (APAFIS#23975). Three-week-old female Syrian hamsters, provided by Janvier Labs (SPF status), were housed in ISOcage P - Bioexclusion System (Techniplast) with unlimited access to food/water and 14 h/10 h light/dark cycle. Wooden gnawing blocks and extra bedding materials were provided as cage enrichment. The animals were monitored and weighed daily to detect the appearance of clinical signs of pain, suffering or distress. Intramuscular administrations and infection were performed under general anesthesia (isoflurane, Isoflurin®, Axience). After 6 days of acclimatization, 0, 2, 4, 8, 16 or 32 mg/kg of the combination Cilgavimab/Tixagevimab were intraperitoneally injected in four-week-old animals. One week later, animals were intranasally infected with 50 µL containing 3.10^4 TCID₅₀ of B.1 or BA.1 strain or 1.10^5 TCID₅₀ of BA.2 or BA.5, diluted in 0.9 % sodium chloride solution.

2.6. Study design

Group size was calculated with an effect size of 2 and a power of 80 %, resulting in 5–9 animals/group. Sample sizes were maximized within the capacity of the BSL3 housing, and compound and virus stock availability. A total of 195 animals were used in this study. Animals were randomly assigned to groups but confounders were not controlled. Since the same experimenters carried out infection/treatment/clinical follow-up, it was impossible to perform a blind trial. Predefined humane endpoints (>20 % weight loss, moribund and a scoring >10 calculated according to a clinical evaluation scale) were set as exclusion criteria. No animals were excluded from the study.

2.7. Organ and blood collection

Nasal washes were performed under general anesthesia (isoflurane, Isoflurin®, Axience), with 150 µL of 0.9 % sodium chloride solution and transferred into 1.5 mL tubes containing 0.5 mL of 0.9 % sodium chloride solution, centrifuged at 16,200 g for 10 minutes and stored at –80°C. Lung and blood samples were collected immediately after euthanasia (cervical dislocation under general anesthesia). Left pulmonary lobes were washed with 10 mL of 0.9 % sodium chloride solution, blotted with filter paper, weighed, transferred into 2 mL tubes containing 1 mL of 0.9 % sodium chloride solution and 3 mm glass beads, crushed using a Tissue Lyser machine (Retsch MM400) for 20 min at 30 cycles/s and centrifuged 10 min at 16,200 g. Supernatant media were transferred into 1.5 mL tubes, centrifuged 10 min at 16,200 g and stored at –80°C. Recovery of plasma: One milliliter of blood was harvested in a 2 mL tube containing 100 µL of 0.5 M EDTA (ThermoFischer Scientific) and centrifuged 10 min at 16,200 g.

2.8. TCID₅₀ assay

Virus titration was performed with 96-well culture plates containing confluent VeroE6/TMPRSS2 cells inoculated with 150 µL per well of four-fold dilutions of crushed lung supernatant samples (dilutions with medium supplemented with 2.5 % FBS). After 6 days of incubation the absence/presence of cytopathic effect in each well was read and infectious titers were estimated using the Reed & Muench calculation method.

2.9. Real time quantitative RT-PCR (RT-qPCR) assays

For viral quantification in lungs clarified homogenates and nasal washes, nucleic acids from each sample were extracted using QIAamp 96 DNA kit and Qiacube HT robot (both from Qiagen). Viral RNA yields were measured using a real time RT-qPCR assay targeting the *rdp* gene as previously described [34].

2.10. Quantification of Cilgavimab/Tixagevimab monoclonal antibodies in blood samples

To estimate the quantity of Cilgavimab/Tixagevimab monoclonal antibodies (AZD7442) in blood samples of animals and humans, we measured the level of human IgG antibodies directed against the S1 domain of the spike protein of the SARS-CoV-2 using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun). Results were expressed in binding antibody units per mL (BAU/mL) following manufacturer instructions and converted to equivalent µg/mL of AZD7442 (eq. µg/mL) using blank plasma from untreated/infected animals spiked with known quantities of AZD7442. This estimate of concentration is based on the assumptions that the measurement concerns AZD7442 antibodies capable of binding to the spike protein used in the ELISA kit, and that the ELISA activity against the spike protein observed in the samples was overwhelmingly linked to the presence of AZD7442, which is a reasonable assumption in the context of the study: (i) in

hamsters, measurement was performed 3 days post infection, before the rise of the natural IgG response against infection; (ii) in humans, blood samples were obtained from immunocompromised patients as a pre-exposure prophylaxis (see below). In the remainder of the article, references to the concentration of AZD7442 must be considered in the context of these assumptions.

2.11. In vivo EC90 determination

We used the following non-linear function to determine the concentration/effect relationship:

$$VL = VL_0 \times \left(1 - \frac{C_{AZD7442}^\gamma}{EC_{50}^\gamma + C_{AZD7442}^\gamma} \right)$$

Where VL_0 is the lung viral load in untreated animals, $C_{AZD7442}^\gamma$ is the estimation of the animal plasma level of anti-SARS-CoV-2 spike protein (S1) IgG (calculated as explained above), EC_{50} is the drug concentration required to decrease viral load by 50 % compared to untreated animals, and γ is a sigmoidicity parameter, chosen as the one maximizing likelihood of the model. We extrapolated the EC_{90} using $EC_{90} = \sqrt[9]{9 \times EC_{50}^\gamma}$. Parameters were estimated separately according to strain, using non-linear regression. Values under the limit of detection (LoD) for lung infectious titers or antibody levels were imputed to this LoD, respectively 2.43 TCID₅₀/g and 0.54 µg/mL.

2.12. PRECOVIM study

The prospective cohort PRECOVIM (Pre-exposure prophylaxis of SARS-CoV-2 infection (COVID-19) by monoclonal antibodies with early access authorization in immunocompromised patients) is a cohort of immunocompromised patients that received pre-exposure prophylaxis by AZD7442 (ClinicalTrials.gov Identifier: NCT05216588). It received the ethical approval of the « Comité de Protection des Personnes Sud-Ouest et Outre-Mer II ». This study, sponsored by ANRS-MIE, included from January to February 2022 100 patients who remain seronegative after a complete Covid-19 vaccination schedule and who received as PrEP 300 mg of AZD7442 (150 mg of each mAb). Study was designed to evaluate neutralizing antibody activity at different time points (Month 1, 2, 3, 4, 5, 6, 9 and 12 post-administration). To put into perspective the results found in our animal model to this cohort [26,27], we explored the proportion of patients at M1, M2 and M3 with levels of anti-SARS-CoV-2 spike protein (S1) IgG in serum (expressed as equivalent µg/mL of AZD7442) above EC90s determined *in vivo* with the hamster model.

2.13. Graphical representations and statistical analysis

Experimental timelines were generated on biorender.com. Graphical representations and statistical analyses were realized using Graphpad Prism 7 software, except nonlinear regressions and their corresponding graphical representations that were performed using the package *nlsreg*, implemented in R statistical software (<http://www.R-project.org>). Two-sided statistical analysis were performed using Shapiro-Wilk normality test, Fisher's exact test, Student t-test, Mann-Whitney test and Welch's test. P-values lower than 0.05 were considered statistically significant. Statistical details for each experiment are described in the figure legends and in the *Supplementary Data* file.

2.14. Role of funders

The funders of the research had no role in the study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit for publication. The corresponding authors had full access to all data and had taken on full responsibility for the decision to

submit the research for publication.

3. Results

3.1. AZD7442 *in vitro* evaluation against Omicron sublineages

First, we evaluated, *in vitro*, the neutralization ability of AZD7442 against Omicron sublineages BA.1, BA.2 and BA.5 using the B.1 ancestral strain as reference. The sensitivity of the variants to AZD7442 was assessed with a standardized concentration-response experiment developed for the evaluation of antivirals against SARS-CoV-2 [32,35] and now applied to therapeutic antibodies [21] (Fig. 1B). BA.1 exhibited reduced EC50 and EC90 (575.25 and 1481.44 ng/mL respectively) compared to the ancestral strain with a fold change of 33.50 for the EC90. Regarding BA.2, we confirmed the restoration of AZD7442 neutralizing ability with EC50 and EC90 of 98.03 and 205.64 ng/mL respectively resulting in a smaller fold change (4.65) (Fig. 1C). Finally, BA.5 showed an intermediate profile, with an EC90 fold change of 18.48. These results are in accordance with other published studies [20, 36–38].

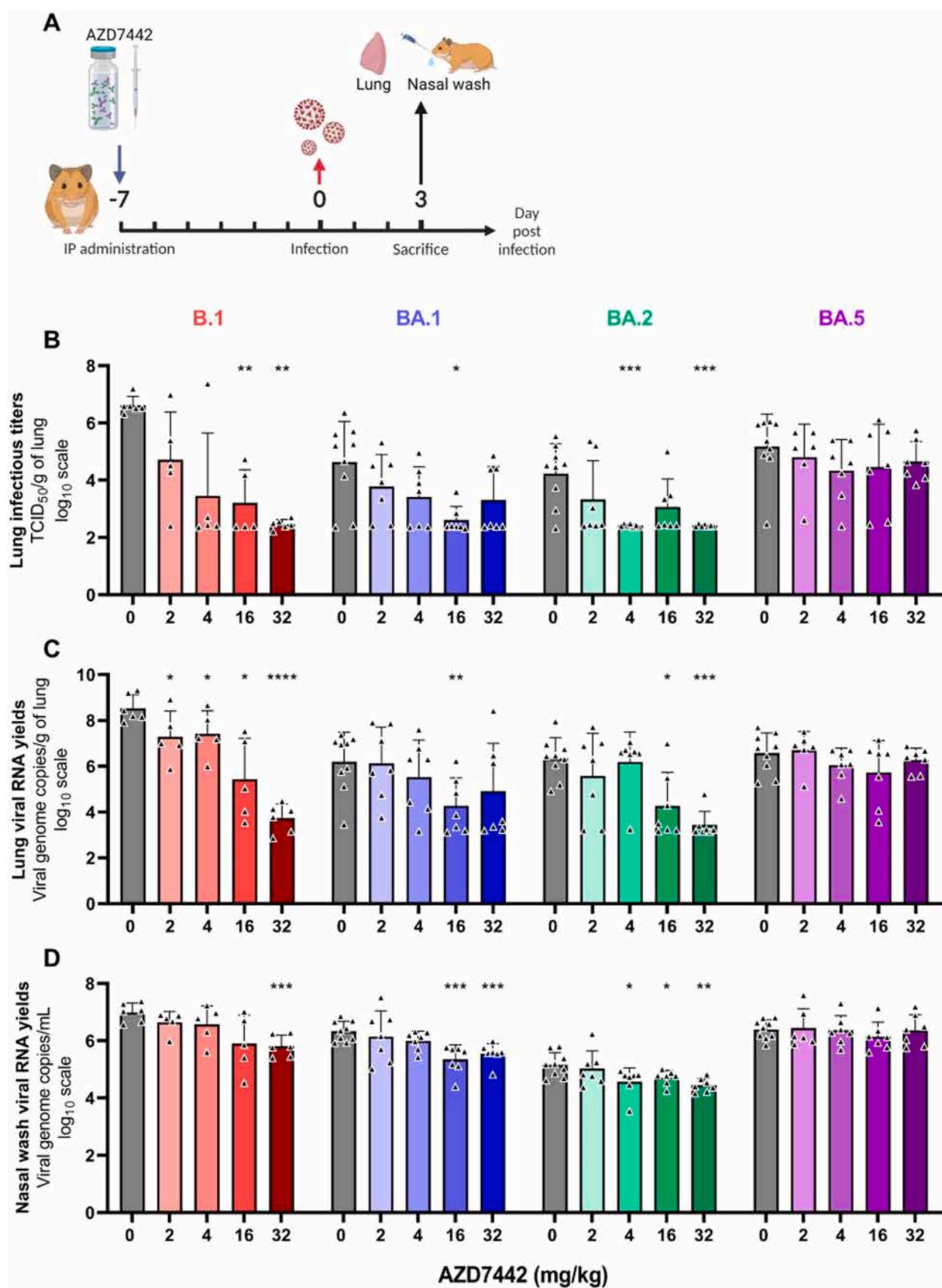
3.2. AZD7442 evaluation in the hamster model

We evaluated the efficacy of AZD7442 on clinical strains of the Omicron sublineages BA.1, BA.2 and BA.5 using the hamster model and an ancestral B.1 strain as reference. Animals received pre-exposure prophylaxis by intraperitoneal injection of 2, 4, 16 or 32 mg/kg of AZD7442. Seven days later, animals were infected intranasally with the B.1 strain (3×10^4 TCID₅₀), the BA.1 variant (3×10^4 TCID₅₀), the BA.2 variant (1×10^5 TCID₅₀) or the BA.5 variant (1×10^5 TCID₅₀). Animals were sacrificed at 3 days post-infection (dpi) (Fig. 2A).

Overall, the intraperitoneal administration of AZD7442 dose-dependently reduced the viral replication in lungs and upper airways of the B.1 strain, the Omicron BA.1 and BA.2 variants. In contrast, no effect was observed on the viral replication of the Omicron BA.5 variant (Fig. 2B).

When the analysis of virus replication in clarified lung homogenates was based on infectious titers, the administration of AZD7442 resulted in a titer reduction for the B.1 strain, the Omicron BA.1 and BA.2 variants (Fig. 2B). When compared with corresponding untreated animals, this decrease was significant with the dose of 16 and 32 mg/kg for the B.1 strain ($p=0.0043$ and $p=0.0022$ respectively), with the dose of 16 mg/kg for the Omicron BA.1 variant ($p=0.0115$) and with the doses of 4 and 32 mg/kg for the Omicron BA.2 variant ($p=0.0008$ and $p=0.0008$ respectively). When the analysis of virus replication in clarified lung homogenates was assessed on viral RNA yields, the administration of AZD7442 resulted in a reduction of this endpoint for the B.1 strain, the Omicron BA.1 and BA.2 variants (Fig. 2C). When compared with corresponding untreated animals, this decrease was significant with the doses of 2, 4, 16 and 32 mg/kg for the B.1 strain ($p=0.0425$, $p=0.0468$, $p=0.0156$ and $p<0.0001$ respectively), with the dose of 16 mg/kg for the Omicron BA.1 variant ($p=0.0088$) and with the doses of 16 and 32 mg/kg for the Omicron BA.2 variant ($p=0.0229$ and $p=0.0002$ respectively). Finally, when the analysis of virus replication in nasal washes was assessed on viral RNA yields, the administration of AZD7442 resulted in a reduction of this parameter for the B.1 strain, the Omicron BA.1 and BA.2 variants (Fig. 2D). When compared with corresponding untreated animals, this decrease was significant with the dose of 32 mg/kg for the B.1 strain ($p=0.0002$), with the doses of 16 and 32 mg/kg for the Omicron BA.1 variant ($p=0.0004$ and $p=0.0006$ respectively) and with the doses of 4, 16 and 32 mg/kg for the Omicron BA.2 variant ($p=0.0311$, $p=0.0263$ and $p=0.0015$ respectively).

In animals infected with the Omicron BA.5 variant, the preventive administration of AZD7442 did not reduce viral replication in the upper and lower airways despite an *in vitro* efficacy (Fig. 1B-C) higher than that of the Omicron BA.1 variant. To confirm this unexpected result, we



(caption on next page)

Fig. 2. In vivo efficacy of pre-exposure prophylaxis of AZD7442 on B.1 strain and Omicron BA.1, BA.2 and BA.5 variants. (A) Experimental timeline (conceived on biorender.com). Groups of hamsters were intraperitoneally treated with 0 (n=6 for the B.1 strain and n=9 for the Omicron variants; untreated groups), 2, 4, 16 or 32 mg/kg of AZD7442 (n=5 per group for the B.1 strain and n=7 per group for the Omicron variants). Seven days later, animals were intranasally infected with the corresponding virus and sacrificed at 3 dpi. (B) Lung infectious titers measured using a TCID₅₀ assay. (C) Viral RNA yields in lungs measured using a RT-qPCR assay. (D) Viral RNA yields in nasal washes measured using a RT-qPCR assay. (B-D) Data represent mean \pm SD of individual data of hamsters. ****, ** and * symbols indicate that the average value for the group is significantly lower than that of the untreated group with a p-value < 0.0001 , ranging between 0.0001–0.001, 0.001–0.01, and 0.01–0.05, respectively (Two-sided statistical analysis were performed using Shapiro-Wilk normality test, Student t-test, Mann-Whitney test, and Welch's test) (details in [supplementary data 1 & 2](#)). Clinical follow-up of this experiment is presented in [Supplemental Figure 2](#).

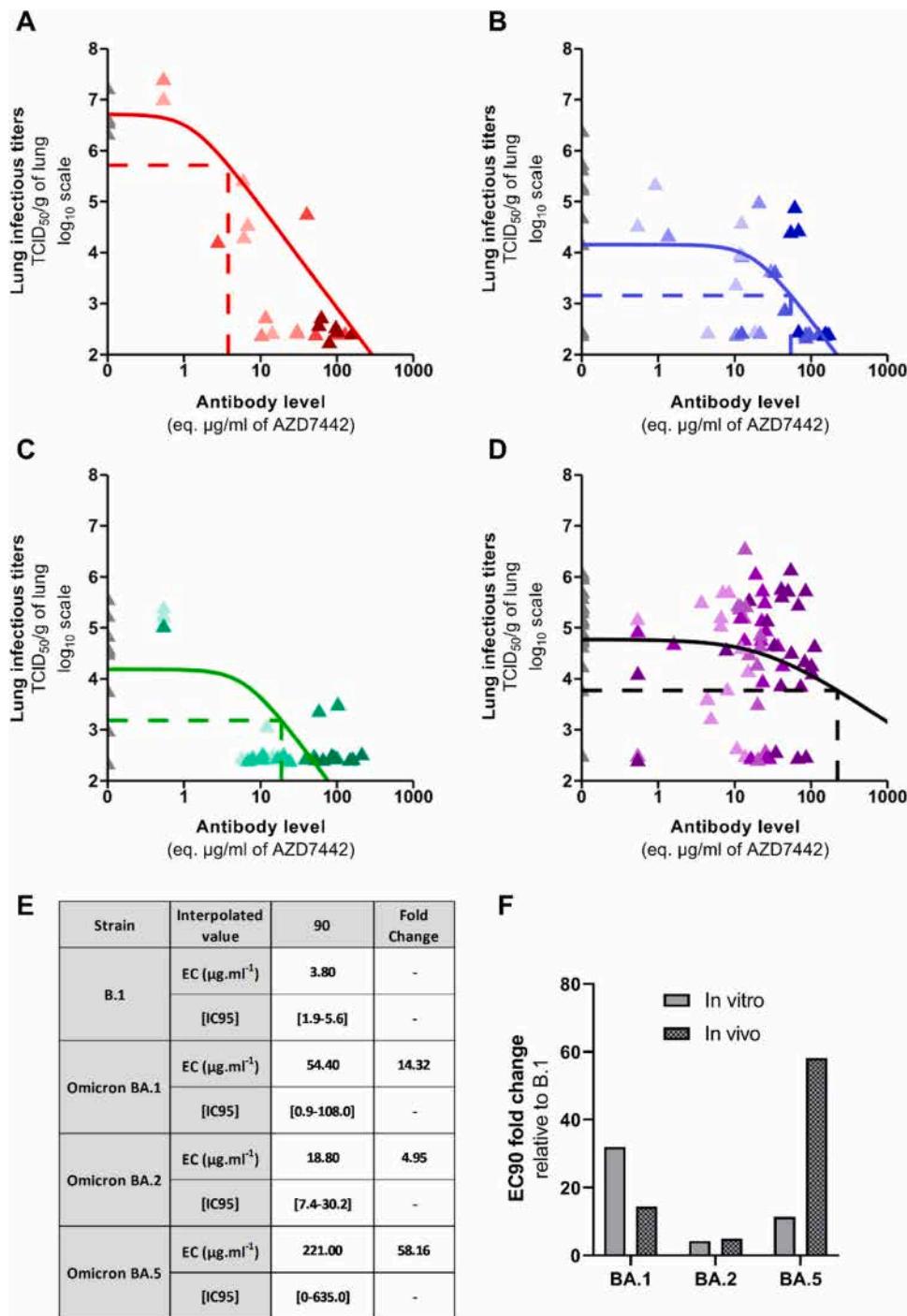


Fig. 3. Plasma antibody concentration-response curves based on lung infectious titers. Anti-SARS-CoV-2 spike protein (S1) IgG concentration-response curves for B.1 strain (A), Omicron BA.1 variant (B), Omicron BA.2 variant (C) and Omicron BA.5 variant (D). In order to construct the concentration-response curves for Omicron BA.5 variant (D), the data concerning the infectious titers of the viruses found in the lungs of the animals of the two duplicates were pooled, after verifying that there was no significant difference between the two untreated groups (Mann-Whitney test; $p=0.2244$). (E) These curves were established to determine 90 % effective concentrations (EC₉₀; dotted lines) in plasma of animals. (F) *In vitro* and *in vivo* EC₉₀ fold changes relative to B.1.

repeated *in vivo* experiments with BA.5. Animals received, by intraperitoneal injection, 2, 4, 8 or 16 mg/kg of AZD7442, seven days before being infected by the Omicron BA.5 variant (2×10^6 TCID₅₀). They were then sacrificed at 3 dpi (Fig. 2A). Despite a significant reduction observed only with lung infectious titers after the intake of 2 mg/kg of AZD7442, this second experiment does not conclusively establish the *in vivo* efficacy of AZD7442 on the Omicron BA.5 variant, since for all other doses used, infectious titers and viral RNA yields observed in lungs and nasal washes were unaffected.

Assuming that the measurement of a biological activity (antibody binding to antigen) correlates with antibody concentrations, plasma antibody levels in animals sacrificed at 3 dpi were estimated by ELISA and expressed as $\mu\text{g/mL}$ AZD7442 equivalents (see method section for details). Variable levels were observed for identical doses, which may be related to the intraperitoneal route of administration. This variability allowed investigating the relationship between animal antibody levels and infectious viral loads in the lungs (Fig. 2B). We conducted a nonlinear regression of infectious viral titers against plasma concentration of anti-SARS-CoV-2 spike protein (S1) IgG. It allowed estimating antibody 90 % effective concentrations (EC90) in plasma to reduce lung infectious titers with each viral strain. In accordance with data presented in Fig. 2B these EC90 ranged from 3.8 $\mu\text{g/mL}$ for the strain B.1 to 221.0 $\mu\text{g/mL}$ for Omicron BA.5 variant, and with values of 54.4 and 18.8 for Omicron BA.1 and BA.2 variants respectively (Fig. 3A-E). *In vivo* EC90 fold changes relative to B.1 were 14.32, 4.95 and 58.16 respectively for BA.1, BA.2 and BA.5. These are presented along with the *in vitro* EC90 fold changes in Fig. 3F.

3.2.1. Extrapolation to clinical data

Next, we put into perspective the results found *in vivo* to a cohort of 100 immunosuppressed patients, namely the prospective cohort PRE-COVIM (average age 57, 36 % female, 64 % male). Among these

patients, 22 were suffering from haemopathy (chronic lymphocytic leukaemia, non-Hodgkin lymphoma), 19 received hematopoietic stem cell transplantation, 7 were treated with immunomodulators/immunosuppressants (anti-CD20/BTK inhibitor/azathioprine, cyclophosphamide and mycophenolate) and 60 received solid organ transplantation [26,27]. We used plasma/serum anti-SARS-CoV-2 spike protein (S1) IgG levels (expressed as equivalent $\mu\text{g/mL}$ of AZD7442; see method section) as a point of comparison. We hypothesized that human serum antibody levels higher than the EC90 estimated *in vivo* in hamsters should be favorably associated with clinical efficacy.

For each virus studied, we determined the proportion of patients at M1, M2 and M3 with anti-SARS-CoV-2 spike protein (S1) IgG levels in serum above EC90s determined *in vivo* with the hamster model (Fig. 3). Depending on the sampling month, these proportions are very high for the B.1 strain (>97 %), intermediate for the BA.2 variant (between 61 % and 92 %) and low for the BA.1 variant (between 5 % and 21 %). No patient had a serum antibody level higher than the EC90 calculated against the BA.5 variant.

At the end of the year 2022, treatment guidelines for the use of AZD7442 as pre-exposure prophylaxis recommended increasing the dose to 600 mg (300 mg of each mAb). Therefore, we also predicted the serum anti-SARS-CoV-2 spike protein (S1) IgG levels that would have been found in these patients if they had taken a dose of 600 mg. These predictions were obtained by assuming a doubling of the observed serum levels, consistent with the fact that the pharmacokinetics of cilgavimab and tixagevimab are linear and dose-proportional [39]. Thus, this extrapolation increases the proportion of patients whose serum antibody level is higher than the EC90s (Fig. 3C-D). Whatever the month, all the patients have a serum antibody level higher than the EC90 calculated against the B.1 strain. Depending on the month, these proportions are very high for the BA.2 variant (between 91 % and 99 %) and intermediate for the BA.1 variant (between 35 % and 62 %). Finally,

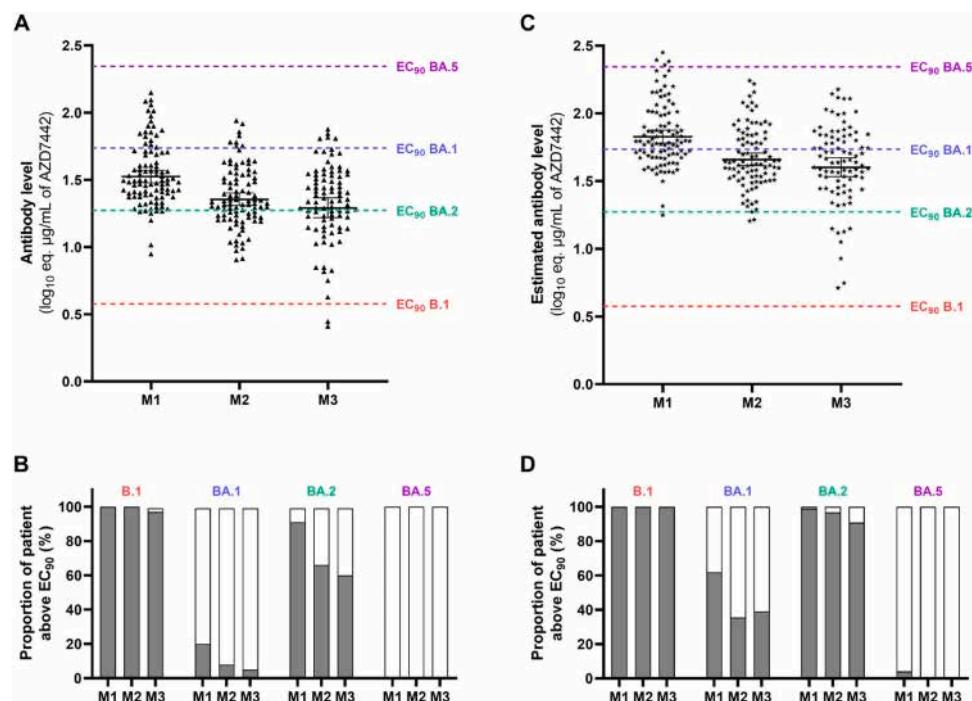


Fig. 4. Levels of anti-SARS-CoV-2 spike protein (S1) IgG in serum of patients at M1, M2 and M3. (A) Observed antibody levels in serum of patients treated with 300 mg of AZD7442 expressed as equivalent $\mu\text{g/mL}$ of AZD7442 (eq. $\mu\text{g/mL}$) ($n=97$, $n=93$ and $n=87$ at M1, M2 and M3 respectively). Dots indicate individual samples and data represent geometric means \pm IC95 %. (B) Observed proportion of patients (%) with an observed antibody level above EC90 against each virus at M1, M2 and M3. (C) Predicted antibody levels in serum of patients assuming a treatment dose of 600 mg expressed as equivalent $\mu\text{g/mL}$ of AZD7442 (eq. $\mu\text{g/mL}$). These predictions were obtained by doubling the antibody serum levels obtained in (A), consistent with the linear drug pharmacokinetics of AZD7442. Dots indicate individual samples and data represent geometric means \pm IC95 %. (D) Predicted proportion of patients (%) with a predicted antibody level above EC90 against each virus at M1, M2 and M3 after a dose of 600 mg.

after one month, only 4 % of the patients exhibited a serum antibody level higher than the EC90 calculated against the BA.5 variant.

4. Discussion

Here, we conducted a preclinical study using data from *in vitro* and *in vivo* models to quantify the efficacy of AZD7442 against several sub-lineages of the SARS-CoV-2 Omicron variant. Using the data generated with the hamster model, we compared our results with serum levels observed in a cohort of immunocompromised patients treated with this mAb cocktail.

This study was performed in the specific context of the emergence of variants with increasingly reduced sensitivity to AZD7442. We observed a striking and unexpected discrepancy between the *in vitro* and *in vivo* results for BA.5. In brief, the *in vitro* and *in vivo* results obtained for BA.1 and BA.2 were consistent, whereas BA.5 *in vitro* results suggested a significant remaining activity and *in vivo* results showed a complete loss of activity in a hamster model. This contradictory finding is highlighted by the comparison of the EC90 fold change relative to B.1 observed *in vitro* and *in vivo* (11.41 and 58.16 respectively).

We compared the EC90 estimated *in vivo* in hamsters with anti-SARS-CoV-2 spike protein (S1) IgG blood levels in a cohort of immunocompromised patients receiving 300 mg of this treatment. Observed antibody levels predicted significant activity against BA.2 and to a lesser extent BA.1, but no patient had serum antibody levels above the EC90 against BA.5. Furthermore, we calculated that an increase in the dose (*i.e.*, 600 mg) would bring only minimal improvement (*e.g.* for BA.5: 4 % of patients one month after injection). This is fully in line with the results of a recent study on patients with haematological malignancies treated with 300 mg AZD7442 during the successive onset of BA.1, BA.2 and BA.5 sublines in the USA (January to August 2022) [40]: the incidence of new SARS-CoV-2 infections fell sharply during the BA.1 and BA.2 waves of Omicron (January to May), then gradually rose to almost that of the untreated control group at week 34, when the BA.5 sub-lineage became predominant [41].

Overall, our study was part of the monitoring of mAbs efficacy during the successive emergences of SARS-CoV-2 variants. For obvious reasons, clinical studies remain the gold standard for assessing the efficacy of antiviral treatments. However, the time required to allow a robust clinical evaluation of mAb activity is commonly much longer than that needed for the emergence of a new variant of SARS-CoV-2. Thus, *in vitro* studies have been at the forefront of enabling health authorities and clinicians to rapidly adapt the therapeutic landscape of clinical management, based on the rapid determination of EC50 and EC90 values against emerging variants. When the first SARS-CoV-2 variants emerged (B.1.1.7, B.1.351, P.1, B.1.617.2), the *in vitro* efficacy of AZD7442 was initially only slightly affected, with EC50 and EC90 values remaining within the range of those determined with the original B.1 strain [42]. In this context, *in vitro* and *in vivo* data remained very coherent. However, when the Omicron sublineages emerged, the *in vitro* activity of AZD7442 decreased (EC90 fold change compared to B.1 of 31.98, 4.25 and 11.41 for BA.1, BA.2 and BA.5, respectively). It is in this specific context, when the fold change compared to B.1 was greater than 10, that we observed a loss of coherence between *in vitro* and *in vivo* results. This could be the rough outline of a "warning zone" from which data become less clear and *in vivo* experiments become crucial to anticipate clinical efficacy of mAbs.

This phenomenon could be more general in scope, if we refer to another study we carried out recently, which also showed a discrepancy in results between *in vitro* and *in vivo* studies. The human neutralizing monoclonal antibody Sotrovimab (S309) showed substantial reduction in *in vitro* activity with the emergence of Omicron variants, notably BQ.1.1 and XBB (EC50 fold change compared to B.1 of 27 and 17.5 respectively) [43]. Nevertheless, experiments using hamster models revealed that S309 retained significant activity in reducing the infectious titers of viruses present in the lungs and the quantities of viral RNA

present in the nasal washes [44]. These results were corroborated by experiments on non-human primates [45].

Taken together, these observations suggest that, when the *in vitro* sensitivity of newly-emerged variants is very high (*i.e.*, in our case, in the range of that of the original strains), the efficacy can probably be safely extrapolated to *in vivo* experimentation and, ultimately, to the clinic. However, beyond a certain threshold, *in vitro* and *in vivo* results may diverge, hence the need to pay particular attention to *in vivo* efficacy data. We have provided two examples which illustrate that *in vitro* activity can be both over- and underestimated. Additional evaluation also seems necessary when *in vitro* evaluation of two mAbs used in combination shows that one of the two has lost its efficacy (*e.g.* Tixagevimab against Omicron sublineages).

Our study has several limitations due to the use of ELISA that measure the level of human IgG antibodies directed against the S1 domain of the SARS-CoV-2 spike protein to estimate AZD7442 concentrations. Firstly, this assay relies on the measurement of a biological activity (antibody binding to antigen). Furthermore, although the prospective PRECOVIM cohort is a cohort of immunocompromised patients who remained seronegative after a complete Covid-19 vaccination schedule, it is not possible to deduce completely that anti-SARS-CoV-2 spike protein (S1) IgG detected by ELISA correspond only to Cilgavimab and Tixagevimab, due to the infectious history and complexity/variability of immune responses in these patients. In hamsters, a much better-controlled *in vivo* model (genomic homogeneity, naïve to infection), the correlation between anti-SARS-CoV-2 spike protein (S1) human IgG levels and plasma AZD7442 concentrations is much more reliable, even if not quite perfect. In both cases, AZD7442 concentrations could have been quantified by high-resolution liquid chromatography/mass spectrometry (LC/HRMS/MS), as recently described [46].

In conclusion, our study supports the importance of developing and using relevant *in vivo* models for monitoring the antiviral activity of monoclonal antibodies whose efficacy against new variants has been reduced beyond a certain threshold, in order to better anticipate their clinical efficacy.

CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Pr C Drosten for providing the SARS-CoV-2 B.1 strain through EVA GLOBAL. This work was conducted in the framework of the Preclinical Study Group of the French agency for emerging infectious diseases (ANRS-MIE). The study was supported by the BIOVAR and EMERGEN-PRI projects (#22275) of ANRS I MIE (INSERM), by the European Commission (European Virus Archive Global project (EVA GLOBAL, grant agreement #871029) of the Horizon 2020 research and innovation programme) and by the European virus archive-Marseille (EVAM) under the label technological platforms of Aix-Marseille. The ANRS0166s PRECOVIM cohort is conducted with the support of ANRS|MIE and funded by French ministries: « Ministère des Solidarités et de la Santé » and « Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation ». We thank the pharmacy of the University hospital of La Timone (Marseille, France) for providing the combination Cilgavimab/Tixagevimab (AZD7442, Evusheld™, AstraZeneca). We thank Pr Yazdan Yazdanpanah and all the ANRS-MIE team for their invaluable support and help. We also thank Pr. Azeddine Driouch (GLYCOMEV UR 4358 - University of Rouen Normandie) for his careful reading of the manuscript and English language editing.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.biopha.2024.116988](https://doi.org/10.1016/j.biopha.2024.116988).

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