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A Phase I study to evaluate the safety, tolerance and pharmacokinetics of anti-Shiga toxin hyperimmune equine F(ab')$_2$ fragments in healthy volunteers

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Abstract

Shiga toxin-producing Escherichia coli-associated hemolytic uremic syndrome (STEC-HUS) is considered a toxemic disorder in which early intervention with neutralizing antibodies may have therapeutic benefits. INM004, composed of F(ab')$_2$ fragments from equine immunoglobulins, neutralizes Stx1/Stx2, potentially preventing the onset of HUS. A single-center, randomized, Phase 1, single-blind, placebo-controlled clinical trial to evaluate INM004 safety, tolerance, and pharmacokinetics (PK) in healthy adult volunteers, was conducted; In Stage I, eight subjects were divided in two cohorts (n=4) to receive a single INM004 dose of 2 or 4 mg.kg-1, or placebo (INM004:placebo rate 3:1). In Stage II six subjects received either three INM004 doses of 4 mg.kg-1 repeated every 24 h, or placebo (INM004:placebo rate of 5:1). Hospital discharged was 24 hours after the last infusion. INM004 was quantified by ELISA in serum samples obtained at predefined times. Safety and tolerability were assessed in both Stages by monitoring adverse events (AEs), laboratory test values, and vital signs. Eight subjects (57.1%) experienced treatment-emergent AEs (TEAEs), that resolved within 24 hours without requiring changes in treatment or additional intervention. No serious AEs were reported. Most TEAEs were of mild or moderate intensity, and four were possibly drug-related. Peak concentrations (C_{max}) of INM004 were 45.1 µg.mL-1 and 77.7 µg.mL-1 for different doses, within two hours after infusion. The serum concentration declined in a biphasic manner ($t_{1/2}$ range 30.7-52.9 hours). Systemic exposures showed accumulation in the repeated dose regimen (C_{max} Day1 85.7 vs.149 µg.mL-1 Day3). These results supporting progression into the phase 2 trial in children with HUS

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Authors

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The authors confirm that the Principal Investigator for this paper is Ventura Simonovich and that he had direct clinical responsibility for patients.

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ABSTRACT

Shiga toxin-producing *Escherichia coli* -associated hemolytic uremic syndrome STEC-HUS is considered a toxemic disorder in which early intervention with neutralizing antibodies may have therapeutic benefits. We developed a novel therapy, INM004, composed of F(ab')$_2$ fragments from equine immunoglobulins that neutralizes Stx1/Stx2, potentially preventing the onset of HUS.

A single-center, randomized, Phase 1, single-blind, placebo-controlled clinical trial to evaluate INM004 safety, tolerance, and pharmacokinetics (PK) in healthy adult volunteers, was conducted in two stages; In Stage I, eight subjects were divided in two cohorts (n=4) to receive a single INM004 dose of 2 or 4 mg.kg-1, or placebo (INM004:placebo rate 3:1). In Stage II six study subjects received either three INM004 doses of 4 mg.kg-1 repeated every 24 h, or placebo (INM004:placebo rate of 5:1). Hospital discharged was 24 hours after the last infusion. INM004 was quantified by ELISA in serum samples obtained at predefined times. Safety and tolerability were assessed in both Stages by monitoring adverse events (AEs), laboratory test values, and vital signs. Eight subjects (57.1%) experienced treatment-emergent AEs (TEAEs), that resolved within 24 hours without requiring changes in treatment or additional intervention. No serious AEs were reported. Most TEAEs were of mild or moderate intensity, and four were possibly drug-related. Peak concentrations of INM004 occurred within 2 hours after infusion, with median Cmax values of 45.1 µg.mL-1 and 77.7 µg.mL-1 for different doses. The serum concentration of INM004 declined in a biphasic manner (t1/2 range 30.7-52.9 hours). Systemic exposures increased with each subsequent dose in a dose-proportional manner, exhibiting accumulation. Geometric median Cmax and AUC values were 149 µg.mL-1 and 10300 µg.h.mL-1, respectively, in the repeated dose regimen. The results obtained in this First in Human Study supporting progression into the phase 2 trial in children with hemolytic uremic syndrome.

Keywords: HUS treatment, F(ab´)$_2$ fragments, pharmacokinetics, anti-shiga toxins, INM004

What is already known about this subject:

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STEC-HUS is a serious foodborne disease worldwide for which there are currently no specific therapeutic options availables.

STEC-HUS is considered a toxemic disorder more than a bacterial disease, suggesting that treatment with neutralizing antibodies may have a therapeutic benefit.

What this study adds

Equine Anti Shiga Toxin F(ab')$_2$ immunoglobulins fragments (INM004) is a novel therapy for STEC infections which have an excellent profile of safety.

In this phase I study, INM004 showed adequate tolerability and safety in healthy volunteers, providing information on the pharmacokinetics of this novel passive immunotherapy to support further clinical development, including clinical trials in children suffering HUS.

Introduction

Shiga toxin-producing *Escherichia coli* -associated hemolytic uremic syndrome (STEC-HUS) is a disease characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal compromise of varying extent, occurring after an episode of *E. coli* diarrhea with or without blood[1]. STEC-HUS affects predominantly pediatric patients. STEC-HUS poses a high social and economic burden, particularly due to the frequent need for long-term renal replacement therapy and renal transplantation[2]. STEC-HUS was described for the first time in 1955 by Gasser et. al, and 30 years later Karmali et al. demonstrated the association between this syndrome and diarrhea caused by bacteria producing cytotoxins[3]. These cytotoxins, called Shiga-type toxins or shigatoxins (Stx) can cause renal, neurological, intestinal or heart complications leading in some cases to chronic renal impairment, and even death. The development of effective treatments for STEC-HUS remains a priority. Polyclonal antibodies (pAbs) and specifically equine polyclonal antibodies (EpAbs) present an interesting therapeutic option[4]. Their safety and effectiveness have been widely demonstrated in rabies virus or botulinum toxin exposures, and in the treatment of venomous snake and scorpion bites[5–8]. Furthermore, EpAbs present several advantages over mAbs. First, they are composed of F(ab)'$_2$ fragments generated by pepsin digestion that retain the bivalent binding capacity of IgG immunoglobulins but lack the constant region (Fc) potentially responsible for Fc-triggered side effects. Second, EpAbs recognize a vast array of epitopes and tend to develop greater avidity than mAbs for their cognate antigens. Finally, EpAbs are considerably easier than mAbs to produce, allowing manufacturers to rapidly achieve large-scale production.

We developed a novel therapy composed of F(ab')$_2$fragments from equine immunoglobulins that efficiently neutralizes eight variants of Stx1 and Stx2 *in vitro* and *in vivo* in an animal model of STEC-HUS potentially preventing the onset of HUS[9]. No significant toxicity was observed in preclinical studies, both in single-dose and repeated-dose toxicity studies in mice and rabbits[10].

The aim of this phase I study was to evaluate the safety, tolerance, and pharmacokinetics (PK) of INM004 in human volunteers. In addition, the Stx neutralizing capacity of INM004 in the plasma of volunteers was evaluated *in vitro* .

Methods

Study design

We conducted a single-center, randomized, Phase 1, single blind, placebo-controlled clinical trial to evaluate the safety, tolerability, and PK of INM004 in two stages. In stage I, a single dose was given (at 2 dose levels, 2 mg/kg and 4 mg/kg), and in stage II, 3 daily 4 mg/kg doses were given. The study enrolled healthy adult volunteers between December 2017 and September 2018 in a single site in Argentina.

Eligible participants included fourteen healthy volunteers 18-55 years old, with a body mass index (BMI) of 19-27 kg.m-2, normal laboratory values, chest ray, and electrocardiogram recordings. Exclusion criteria included history of equine serum allergy or any history of allergy, prior administration of equine serum

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for other indications (anti-tetanus serum, anti-ophidic serum, anti-arachnid toxin serum, among others), pregnancy, seropositivity for HIV, hepatitis B or C, mental condition, drug or alcohol abuse, any history of cardiovascular, hepatic, pulmonary, gastrointestinal, hematological, or neurological illness, having taken any prescription drugs within the two weeks before the inclusion date, infectious disease within 30 days before the inclusion, or having a personal bond with any of the study personnel.

All study subjects provided written informed consent after receiving comprehensive information of the objectives, methods, and potential harms related to their participation in the study. The present study was approved by the local ethics committee and the Argentine National Food and Drug Regulatory Agency, Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT). The study was prospectively registered in clinicaltrials.gov (*NCT03388216*).

Blinding and randomization

Study subjects were masked to treatment allocation and were randomized to either treatment or placebo group when admitted to the Hospital at day 0. We established a system of opaque closed envelopes containing the arm designation. The pharmacist of the study randomly selected the envelope for each study subject in the presence of the principal investigator. For safety reasons, the researchers responsible for the clinical trial were aware of treatment allocation. INM004 was provided to the nursing staff in anonymized bags with the preparation already reconstituted and labeled with participant and treatment identification number without the specific content (INM004 or placebo) so that the subject could not infer the assigned treatment. The physician responsible for safety monitoring during the infusion of INM004 or placebo was blinded to treatment allocation.

Procedures

INM004 was formulated by Inmunova and manufactured under GMP standards and supplied in vials containing: Total proteins (equines) 125,0 mg; Sodium chloride 45,0 mg; Hydrochloric Acid/Sodium Hydroxide as much as suffices to keep pH between 6,0 and 7,0; Water for injection as much as suffices to 5 mL.

Safety monitoring and tolerability of INM004 were assessed by monitoring adverse events (AEs), laboratory test values, and vital signs (blood pressure, heart rate and complete physical examination and 12 lead electrocardiogram examinations).

The trial was divided into two stages. In Stage I, 8 study subjects were divided in two cohorts of 4 patients to receive a single dose of INM004 or placebo at an INM004:placebo rate of 3:1 with dose escalation design. Thus, after a screening period of two weeks, study participants in the first and second cohort received 2 mg.kg-1 protein of INM004 or placebo and 4 mg.kg-1 of INM004 or placebo, respectively. We selected an initial dose of 2 mg.kg-1 based on the kinetics and effectiveness determined in preclinical studies. This dose was determined after applying a correction factor of 5 to the maximum dose of 116 mg.kg-1 administered in mice following the allometric approach recommended by the US Food and Drug Agency´s guidelines. We used this correction factor since the NOAEL was not reached with the highest dose tested in mice, and similar equine Fab products already available for human use have demonstrated safety in humans at higher protein doses.

Four participants received sequentially an initial dose of 2 mg.kg-1 intravenously, with ClNa 0.9% in a total volume of 100 ml administered at 2 ml.min-1. Once we established that the drug was well tolerated, four more volunteers were enrolled and received 4 mg.kg-1. Both cohorts of stage I had a INM004:placebo rate of 3:1.

Stage II recruited and randomized sequentially 6 study subjects to receive INM004 or placebo (INM004:placebo rate of 5:1). After a screening period of two weeks, participants received three repeated doses of 4 mg.kg-1 of INM004 or placebo every 24 hours.

Four visits for PK sampling and a final safety follow-up visit at 30 days after hospital discharge were pre-scheduled. In case of occurrence of adverse events (AEs), unscheduled visits or hospitalizations were

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performed until resolution of the event.

PK sampling

PK parameters of INM004 levels were determined in all subjects receiving at least one dose of the medication. In stage I the participants were hospitalized for 24 hours after the infusion and PK samples were obtained at the following times: 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, and 480 min, and at 12, 24, 48, and 72 hours after infusion. A baseline and final laboratory tests for immunogenicity were obtained at day 0 and 30, respectively. Ambulatory PK samples, clinical visits, and safety laboratories at days 3, 4, 5, and 30 after infusion were scheduled. During stage II, the participants received three repeated doses of 4 mg.kg-1 every 24 hours during 3 days. The volunteers stayed hospitalized for 24 hours after the last infusion. PK samples were obtained at the following times: 0, 30, 60, 120, 180, 240, 360, and 480 min, and at 12 and 24 hours after first infusion; at 60, 120, 360 min, and 12 and 24 hours after the second infusion; and at 60, 120, 360 min, and 12, 24, 48, 72, 96 and 120 hours, and at day 30 after the third infusion. Ambulatory visits and safety laboratory tests were identical to those scheduled in stage I.

Quantification of the $F(ab´)_2$ equine polyclonal antibody concentrations in plasma was performed by Enzyme-Linked Immunosorbent Assay (ELISA) developed for preclinical studies of INM004 and validated for human plasma samples[11]. Blood samples (5 ml) for PK analyses were collected by venipuncture, and the results analyzed by non-compartmental (NCA) PK methods. The fixed main effects treatment, period and sequence were analyzed for the PK parameters of interest [C_{max}, AUC (0-t) and AUC (0-Inf)]. The AUC was obtained from the graph of the drug plasma levels over time for each subject. The 90% confidence interval limits for PK were calculated. C_{max} and T_{max} were estimated directly from the curve with interpolation. AUC (0-inf) and AUC (0-t) were calculated using the trapezoidal rule based on the individual concentrations observed. The elimination half-time ($t_{\frac{1}{2}}$) was calculated as Ln (2) / λz, where λz was estimated from a log-linear regression of the last 3 to 5 points of the curve, after reaching plateau. Coefficients of variation were calculated for Cmax and AUC(0-inf).

Neutralizing capacity

The neutralizing capacity of INM004 in a subset of study subjects' sera obtained at 1, 24, and 72 hours post-administration was evaluated over the toxic effect of Stxs on Vero cells as previously described[3]. As a positive control we used the pool of baseline samples (before administration) of the three evaluated volunteers, spiked with an estimated average concentration of $F(ab´)_2$. The estimated concentrations of INM004 (determined by ELISA) in the samples at the evaluated times were 40, 15 and 7 μg.mL-1, respectively.

Statistical analyses

Continuous variables were summarized as mean, median, interquartile range (IQR) minimum, maximum and standard deviation (SD) and categorical or ordinal variables as percentages. Analysis was based strictly on observed cases, i.e., no replacement of missing data was planned in general. Statistical significance threshold was set for two-sided probability values at <0.05.

After completing Stage I, an interim analysis of the PK data and safety data was performed to assess if the PK and safety profile were acceptable to initiate Stage II. The blinded analysis was performed by a Medical Monitor not related to the clinical research team or the Sponsor. The dose and interdose interval for Stage II was defined taking into consideration the results obtained in the interim analysis.

All statistical analyses, listing, tabulations, and figures were performed by the Department of Biostatistics of Linical S.L.U., using SAS® Version 9.4 or later. PK analyses were performed using validated Phoenix WinNonlin (v.8.1, Certara USA, Inc., USA).

Results

Twenty-two healthy volunteers were screened, and 14 fulfilled inclusion criteria without reasons for exclusion. These fourteen subjects were randomized to receive INM004 or placebo, without differences in baseline

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characteristics. Table 1 summarizes clinical and demographic data of study participants. Three (21.4%) subjects reported at least one past medical condition.

INM004 infusions were well tolerated in all Stages of the study. Eighteen treatment emergent AEs (TEAEs, i.e. after administration of the study medication) were reported by eight (57.1%) subjects: three in Stage I (one in the placebo group) and five in Stage II (one in the placebo group). All TEAEs lasted for 24 hours or less and resolved completely without need for changes in the treatment schedule or specific treatment. Half of the reported TEAEs were of mild intensity (9/18 TEAEs reported) and the remaining half of moderate intensity, without serious or severe AEs. Of the total reported TEAEs, four were possibly drug related. These related TEAEs included: rhinitis, headache and flushing experienced by three (60.0%) subjects in the INM004 group at Stage II (Supplementary appendix Table s1). For full details on the occurrence of AE, see Tables s2 and s3.

All study subjects remained asymptomatic without the need of any specific intervention. No clinically significant changes in laboratory values were observed from baseline to hospitalization discharge.

PK analyzes

Basic PK parameters of INM004 are summarized in Table 2. Following a single intravenous infusion dose of INM004 to healthy subjects at doses of 2.00 mg.kg-1 and 4.00 mg.kg-1, peak concentrations were observed within 2 hours after the end of the infusion (Figure 2, Panel A and B). Mean C_{max} values were 45.1 µg.mL-1 and 77.7 µg.mL-1 following the 2.00 mg.kg-1 and 4.00 mg.kg-1 doses, respectively. The serum concentration versus time profiles of INM004 declined in a biphasic manner, with $t_{\frac{1}{2}}$ values ranging from 30.7 h to 41.8 h following the 2.00 mg.kg-1 dose and from 33.2 h to 52.9 h following the 4.00 mg.kg-1 dose. The corresponding geometric mean $AUC_{(0\text{-last})}$ values were 1180 µg.h.mL-1 and 2373 µg.h.mL-1, respectively.

In Stage II, following repeated 4.00 mg.kg-1 intravenous infusions of INM004 to healthy subjects, the serum concentrations of INM004 on Day 3 declined in a biphasic manner, with $t_{1/2}$ values ranging from 60.3 h to 95.0 h (Figure 2, Panel C). Systemic INM004 exposures increased with each subsequent dose in an approximately dose-proportional manner, with a 2-fold increase in dose resulting in a 1.7 fold increase in C_{max} and a 2.0-fold increase in AUC(0-inf). Following intravenous administration, the Day 3 geometric mean clearance of INM-004 was 1.63 mL/h/kg and the corresponding Vz value was 172 mL.kg-1.

As expected, accumulation of INM004 was observed on Day 3 when compared to Day 1 in the repeated dose regime, with mean relative accumulation values (RA) of 1.27 and 1.41 for C_{max} and AUC, respectively.

Geometric mean C_{max} value was 149 µg.mL-1 with corresponding geometric mean AUC(0-τ), AUC(0-inf) and AUC(total) (i.e. the total AUC for Days 1 to 3 combined) values of 2460 µg.h.mL-1, 10300 µg.h.mL-1 and 14600 µg.h.mL-1, respectively. The between-subject variability, as assessed from the geometric percent CV, was low (CV% <25%) for both C_{max} and AUC (0-inf) for both Stage I and II measures.

The inter-subject variability, as assessed from the geometric coefficient of variation %, was low (<25%) for both Cmax and AUC(0-inf) in both single and repeated dose regimens.

Neutralizing capacity

Serum samples of 3 study subjects at 1, 24 and 72 hours after INM004 infusion were analyzed to evaluate its Stx neutralizing capacity. All samples evaluated with the in vitro cytotoxic assay in Vero cells showed neutralizing activity (Figure 3), inhibiting the toxicity of the Stx under test conditions in a comparable way to the diluted product in the serum of the same subjects before administration.

Discussion

Our study describes the first in-human use of INM004, an anti-Stx product obtained from serum of equine animals that were previously immunized against epitopes of Stx2B and Stx1B, this is the first study of this kind in Argentina and a very important step regarding potential treatments for neglected diseases such as HUS.

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HUS is an orphan pediatric disease for which no specific therapies have been developed yet. Several overlapping or complementary strategies for preventing and treating STEC-HUS have been proposed previously without success. Specific therapies with monoclonal antibodies (mAbs) that target Stx and prevent its internalization through its specific receptor Gb3 (globotriaosylceramide) were previously evaluated with promising preclinical results, but thus far there is no evidence of conclusive effectiveness from clinical trials[12–14].

In consequence, clinical research and drug development for treatment of STEC-HUS is urgently needed and this study is an important step towards the development of novel targeted therapies for STEC-HUS.

We examined the safety, tolerability, and PK properties of INM004, with encouraging results. The infusion of a single dose and repeated doses was not associated with major side-effects. Laboratory parameters presented transient minor variations without clinical significance, and there was no evidence of immunogenicity against the product in any of the healthy volunteers. In addition, the neutralizing capacity of INM004 was preserved at the concentrations achieved, confirming that INM004 does not lose its neutralizing capacity in the bloodstream after infusion.

INM004 PK were similar to other equine $F(ab´)_2$ products. A study by Lopardo et al. including adult patients with moderate to severe COVID-19 infection treated with RBD-specific polyclonal $F(ab´)_2$ yielded similar PK parameter results among ill patients with COVID-19, with a median half-life of 58.9 h and Cmax1 of 84.6 mg.l-1 and 102.4 at 1 h and 49 h, respectively[15]. With repeated dose regimen, INM004 accumulated and its half-life increased in a dose-proportional manner with low inter-subject variability.

INM004 infusions were not associated with serious or severe AEs and only mild or moderate intensity infusion related TEAEs were reported, mainly headache, flushing and rhinitis, all of which were transient and resolved without sequelae. No hypersensitivity events were reported in our study. In keeping with the aforementioned study by Lopardo et al., rates of adverse events between treatment and placebo groups were similar, including severe and emergent treatment adverse events. Similarly, observational data following the widespread use of these EpAbs for the treatment of COVID-19 in Argentina revealed a low rate of severe AE, with a cumulative incidence of hypersensitivity reactions of 1-2% under real-world conditions among 10,728 patients with COVID-19[16].

This study proves that the production of equine $F(ab')_2$for the treatment of STEC HUS is feasible, since INM004 manufacturing can be easily scaled up. A Phase II open label study, to evaluate the pharmacokinetics, safety, and exploratory efficacy of INM004 in the treatment of pediatric patients with STEC-HUS was started in October 2022 in Argentina (*NCT05569746*), where HUS is an endemic disease with approx. 400 cases annually. This trial is being conducted in 16 clinical sites, and includes children between 1 and 12 years of age, with a clinical diagnosis compatible with STEC-HUS. Should this clinical trial be successful, a Phase III, double blind, clinical trial will be executed to confirm INM004 efficacy in the treatment of STEC-HUS in pediatric patients with the view to become the first line therapy for an orphan disease with global impact.

In conclusion, in this first human study of INM004, the administration was safe and well tolerated, allowing the pharmacokinetic data an adequate allometric conversion for the aforementioned trial in children with HUS whose main objective is to evaluate safety as well as exploratory efficacy of INM004 as a valid new therapeutic approach for STEC-HUS in children.

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* Conflict of interest statement

This study was sponsored by Inmunova. Yanina Hiriart, Linus Spatz, Mariana Colonna and Santiago Sanguineti are employees of Inmunova. Fernando Goldbaum is the scientific director of Inmunova.

*Author contribution statement

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All authors participated in the design, and data analysis of the study. VS, PS, VB and WHB participated in patient enrollment, study procedures and data collection. YH and LM developed and performed the ELISA assay for PK analysis. YH and MC collected and analyzed PK data. YH performed the neutralization assay. All authors participated in writing this manuscript, and read and approved its final version.

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Figure 1. Study flow chart.

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Table 1. Demographic and Baseline Characteristics of participants.

	Stage I				Stage II	
	Cohort I		Cohort II			
	Placebo (N=1)	INM 004 (N=3)	Placebo (N=1)	INM 004 (N=3)	Placebo (N=1)	INM 004 (N=5)
Age (years)						
Mean (SD)	38 (N/A)	30.33 (7.51)	33 (N/A)	27 (9.85)	21 (N/A)	29.4 (12.46)
Range	N/A	23 - 38	N/A	19 - 38	N/A	19 - 50
Sex [n (%)]						
Male	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)	2 (66.7%)	0.0 (0.0%)	1 (20.0%)
Female	1 (100.0%)	3 (100.0%)	1 (100.0%)	1 (33.3%)	1 (100.0%)	4 (80.0%)
Self-perceived ethnicity [n (%)]						
Hispanic or Latino	0.0 (0.0%)	1 (33.3%)	0.0 (0.0%)	1 (33.3%)	1 (100.0%)	5 (100.0%)
Not Hispanic or Latino	1 (100.0%)	2 (66.7%)	1 (100.0%)	2 (66.7%)	0.0 (0.0%)	0.0 (0.0%)
BMI						
Mean (SD)	24.47 (N/A)	22.9 (2.44)	23.6 (N/A)	25.06 (2.74)	23.57 (N/A)	24.23 (1.78)
Range	N/A	20.41 - 25.29	N/A	21.9 - 26.69	N/A	21.65 - 25.89

BMI: body mass index; SD: standard deviation.

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Figure 2. Combined individual PK serum concentrations (µg.mL-1) of INM 004 vs time (h) of study subjects. Panel A Stage I, Cohort I (2.00 mg.kg-1 single dose regime). Panel B Stage I, Cohort II (4.00 mg.kg-1 single dose). Panel C, Stage II (4.00 mg.kg-1 repeated dose regime).

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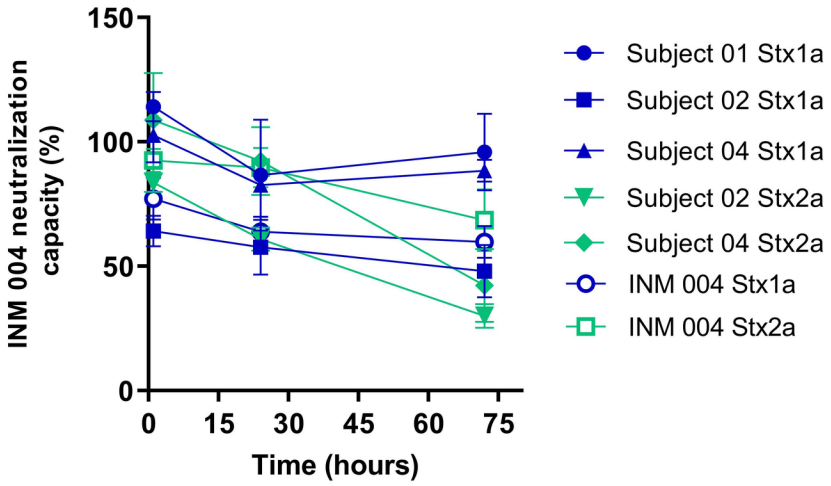

Figure 3. Neutralizing capacity of INM 004 in 3 study subjects.

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Table 2. PK characteristics of INM 004 at single dose and repeated dose regime.

Dose	Median Cmax (µg/mL)	Median tmax (hours)	AUC_{0-last} (µg.h/mL)	t1/2 λz (h)
Single 2.00 mg.kg-1	45.1	1.5	1180	37.7
Single 4.00 mg.kg-1	77.7	0.75	2373	44.3
Repeated 4.00 mg.kg-1, Day 1	85.7	0.5	1810	N/A
Repeated 4.00 mg.kg-1, Day 2	113.1	1	2570	N/A
Repeated 4.00 mg.kg-1, Day 3	149	2	7040	74.4