

First-in-human phase I clinical trial of a TLR4-binding DNA aptamer, ApTOLL: Safety and pharmacokinetics in healthy volunteers

Macarena Hernández-Jiménez,^{1,10} Samuel Martín-Vílchez,^{2,10} Dolores Ochoa,² Gina Mejía-Abril,² Manuel Román,² Paola Camargo-Mamani,² Sergio Luquero-Bueno,² Bernd Jilma,³ María A. Moro,^{4,7,8} Gerónimo Fernández,⁵ David Piñeiro,¹ Marc Ribó,¹ Víctor M. González,^{5,6} Ignacio Lizasoain,^{7,8} and Francisco Abad-Santos^{2,9}

¹AptaTargets S.L., Avda. Cardenal Herrera Oria 298, 28035 Madrid, Spain; ²Clinical Pharmacology Department, Hospital Universitario de La Princesa, Universidad Autónoma de Madrid (UAM), Instituto de Investigación Sanitaria La Princesa (IP), 28006 Madrid, Spain; ³Department of Clinical Pharmacology, Medical University of Vienna, 1090 Vienna, Austria; ⁴Centro Nacional de Investigaciones Cardiovasculares Carlos III (F.S.P.), 28029 Madrid, Spain; ⁵Aptus Biotech S.L., Avda. Cardenal Herrera Oria 298, 28034 Madrid, Spain; ⁶Grupo de Aptámeros, Departamento de Bioquímica-Investigación, IRYCIS-Hospital Ramón y Cajal, 28034 Madrid, Spain; ⁷Unidad de Investigación Neurovascular, Department of Pharmacology and Toxicology, Faculty of Medicine, Universidad Complutense, 28040 Madrid, Spain; ⁸Instituto de Investigación Hospital 12 de Octubre (i+12), 28041 Madrid, Spain; ⁹Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain

ApTOLL is an aptamer that antagonizes Toll-like receptor 4 and improves functional outcomes in models of ischemic stroke and myocardial infarction. The aim of this study was to characterize the safety and pharmacokinetics of ApTOLL in healthy volunteers. A first-in-human dose-ascending, randomized, placebo-controlled phase I clinical trial to assess safety and pharmacokinetics of ApTOLL (30-min infusion intravenously) was performed in 46 healthy adult male volunteers. The study was divided into two parts: part A included seven single ascending dose levels, and part B had one multiple dose cohort. Safety and pharmacokinetic parameters were evaluated. No serious adverse events or biochemistry alterations were detected at any dose nor at any administration pattern studied. Maximum concentration was detected at the end of the infusion and mean half-life was 9.3 h. Interestingly, exposure increased in the first four levels receiving doses from 0.7 mg to 14 mg (AUC of 2,441.26 h*ng/mL to 23,371.11 h*ng/mL) but remained stable thereafter (mean of 23,184.61 h*ng/mL after 70 mg). Consequently, the multiple dose study did not show any accumulation of ApTOLL. These results show an excellent safety and adequate pharmacokinetic profile that, together with the efficacy demonstrated in nonclinical studies, provide the basis to start clinical trials in patients.

INTRODUCTION

ApTOLL is an unmodified single-stranded DNA aptamer (ssDNA) that has been selected to antagonize the Toll-like receptor 4 (TLR4) with high specificity and, therefore, to block the inflammatory response produced after different insults such as acute ischemic stroke (AIS)¹ or acute myocardial infarction (AMI).²

Toll-like receptors (TLRs) are a family of pattern recognition receptors. TLR signaling is initiated by the binding of a TLR with its respec-

tive ligand. TLR4 was the first TLR characterized in mammals,^{3,4} and several authors have demonstrated its implication in the activation of innate immunity and in the inflammatory response elicited during the pathophysiology of different diseases such as ischemic or autoimmune diseases.^{5–9} For this reason, there is a high interest in developing TLR4 inhibitory drugs for the treatment of those diseases where this receptor plays a central role.

Aptamers are single-stranded oligonucleotides selected from combinatorial libraries by systemic evolution of ligands by exponential enrichment (SELEX) technology.^{10,11} Under physiological conditions, and based on their nucleotide sequence, aptamers acquire unique three-dimensional structures that confer the selectivity and affinity for each specific target.^{11,12} Aptamers are obtained by chemical synthesis and possess certain properties that provide advantages over antibody-based approaches.¹³ In particular, they do not require mammalian cells for production and can be synthesized on a large scale. Additionally, they show high stability with long shelf-lives, can be chemically modified to extend half-life or to improve their structures, and their functions can be neutralized by using an antidote sequence.^{14–16} Aptamers also elicit little or no immunogenicity in therapeutic applications.^{17,18} Consequently, aptamers are currently being developed as tools for a wide range of applications, including biosensors, diagnostics, and therapeutics. To determine their applicability in therapeutics, several aptamers are currently in clinical trials,^{19–22} and one aptamer has been commercialized up to date,

Received 9 August 2021; accepted 3 March 2022;
<https://doi.org/10.1016/j.omtn.2022.03.005>.

¹⁰These authors contributed equally

Correspondence: AptaTargets S.L., Avda. Cardenal Herrera Oria 298, 28035 Madrid, Spain.

E-mail: m.hernandez@aptatargets.com

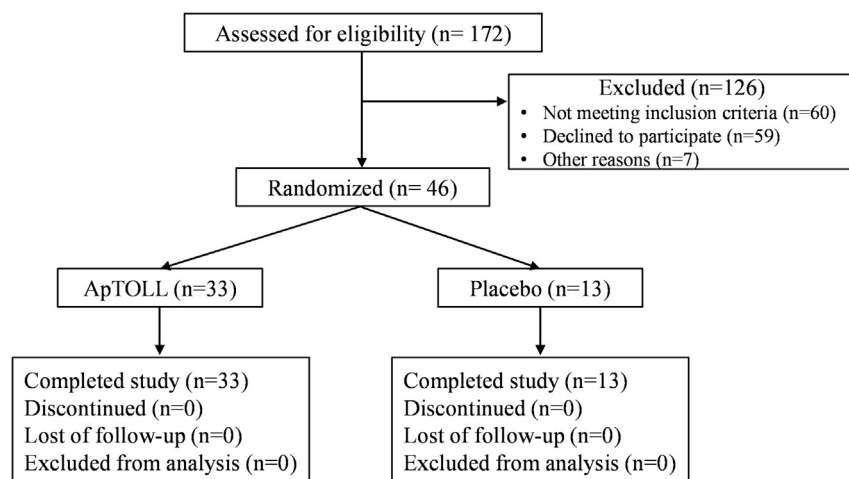


Figure 1. Subject disposition in the ApTOLL-FIH-01 clinical trial (for details see materials and methods section)

No subjects were withdrawn.

Pharmacokinetic analysis

Results from this study show that, in part A, C_{max} was detected 30 min after injection (at the end of the infusion) (Figure 2), and mean half-life of ApTOLL in plasma was 9.3 h. Interestingly, ApTOLL exposure increased in levels 1–4 but remained stable in higher levels (Table 2).

Accordingly, results obtained after multiple dose administration of ApTOLL (part B) showed that administration of several doses did not increase C_{max} . Additionally, there is not any accumulation of ApTOLL levels in plasma from HVs that received multiple doses (Figure 3).

pegaptanib,²³ for the treatment of age-related macular degeneration patients.

ApTOLL specifically binds to human TLR4, showing an antagonistic profile and a great protective potential against ischemic diseases. ApTOLL showed a long-lasting protective effect against experimental cerebral ischemia induced by middle cerebral artery occlusion (MCAO) in rats and mice.¹ Additionally, ApTOLL has also demonstrated a protective effect against cardiac ischemia in pigs,² improving functionality of cardiac tissue and reducing the inflammatory damage. Finally, the characterization of nonclinical pharmacokinetic, safety pharmacology, and toxicology properties of ApTOLL in rats and non-human primates showed that ApTOLL is safe and has a good pharmacokinetic profile to inhibit TLR4 after an ischemic stroke (short half-life with rapid clearance) (data included in the international patent WO2020/230108).

With this background, a first-in-human (FIH) study has been conducted to determine whether ApTOLL is safe in healthy volunteers (HV). This study was a dose-ascending, double-blind, randomized, placebo-controlled clinical trial to assess tolerability and pharmacokinetics of ApTOLL in HVs.

RESULTS

Demographics

From the initial 172 subjects screened, a total number of 46 male HVs aged between 18 and 55 years (mean 30.2 ± 7.5 years), with a body mass index (BMI) of 25.1 ± 2.3 kg/m² and a body weight 76.5 ± 5.6 kg, were enrolled in the clinical trial and divided into the corresponding study groups (Figure 1; Table 1). From the 46 subjects included, 35 were Latin (76%), 10 Caucasian (21.8%), and one Black (2.2%).

Thirty-eight subjects were enrolled in part A and eight subjects in part B. In part A, 27 subjects received ApTOLL and 11 received placebo. In part B (Level 8), six subjects received ApTOLL while two subjects received placebo (for details, see materials and methods section).

Results of the pharmacokinetic main parameters are summarized in Table 2.

Pharmacokinetic parameters from part B subjects were assessed also after every ApTOLL administration (Figure 3; Table 2), and determination of C_{max} showed similar values after each administration ($2,531 \pm 368.8$ ng/mL after the first dose, $1,866.32 \pm 284.82$ ng/mL after the second dose, and $1,626.70 \pm 221.37$ ng/mL after the third dose). Exposure was also evaluated and no statistically significant differences between dose administrations were reported (AUC of $11,453 \pm 2,567$ h*ng/mL after the first dose, $8,800 \pm 2,172$ h*ng/mL after the second dose, and $17,269 \pm 5,513$ h*ng/mL after the third dose). On the other hand, ApTOLL levels in urine samples from part B subjects were also determined, showing quantifiable concentrations from 3 h to 23 h after administration in all subjects from the ApTOLL arm (data not shown). In two subjects, ApTOLL was quantifiable but close to the limit of quantification at 39.22 h (1.3 ng/mL) and 29.88 h (2 ng/mL). Total amount eliminated in urine during 48 h was 0.69 ± 0.35 mg.

No ApTOLL concentrations were found in subjects from the placebo arm, either in plasma nor in urine samples.

Adverse drug reactions

In the context of this clinical study, no adverse events (AEs) or serious adverse events (SAEs) attributable to ApTOLL administration were reported. Additionally, no clinically significant laboratory, vital sign, or electrocardiogram (ECG) findings that were considered possibly related to ApTOLL injection were described.

The safety profile was confirmed both in part A (seven single ascending doses) and in part B (multiple dose cohort).

Table 1. Demographics and Baseline Characteristics of Subjects Enrolled in the ApTOLL-FIH-01 Clinical Trial

	Descriptive Statistics	Age (Years)	Weight (Kg)	Height (m)	BMI ^a
Level 1 (0.7 mg; N = 2)	mean ± SD	37.5 ± 16.3	77.3 ± 8.9	170.0 ± 4.2	26.7 ± 1.7
	minimum value	26	71.0	167.0	25.5
	maximum value	49	83.6	173.0	27.9
Level 2 (2.1 mg; N = 2)	mean ± SD	39.5 ± 0.7	78.4 ± 5.0	177.5 ± 4.9	24.9 ± 2.9
	minimum value	39	74.9	174.0	22.9
	maximum value	40	82.0	181.0	27.1
Level 3 (7 mg; N = 2)	mean ± SD	29.5 ± 3.5	69.0 ± 1.4	167.0 ± 4.2	24.7 ± 0.7
	minimum value	27	68.0	164.0	24.2
	maximum value	32	70.0	170.0	25.2
Level 4 (14 mg; N = 8)	mean ± SD	32.6 ± 9.4	77.6 ± 5.4	179.1 ± 4.8	24.2 ± 1.4
	minimum value	25	69.5	169.0	22.5
	maximum value	54	83.7	184.0	26.7
Level 5 (21 mg; N = 8)	mean ± SD	29.1 ± 9.4	78.0 ± 4.9	171.8 ± 4.2	26.5 ± 1.4
	minimum value	21	67.0	164.0	24.9
	maximum value	48	81.7	178.0	28.3
Level 6 (42 mg; N = 8)	mean ± SD	28.0 ± 4.7	74.6 ± 5.4	173.5 ± 5.9	24.8 ± 1.4
	minimum value	22	65.0	166.0	21.4
	maximum value	36	80.0	181.0	28.6
Level 7 (70 mg; N = 8)	mean ± SD	31.2 ± 5.3	77.7 ± 5.3	176.8 ± 6.3	25.0 ± 3.0
	minimum value	25	70.2	165.0	20.3
	maximum value	43	84.8	186.0	28.9
Level 8 (21 mg × 3; N = 8)	mean ± SD	25.7 ± 3.9	76.0 ± 6.9	175.6 ± 6.9	24.7 ± 2.9
	minimum value	20	66.2	165.0	20.7
	maximum value	30	84.3	188.0	29.8

^aBMI: body mass index = weight (kg)/height² (m²).

Adverse events

During the study, 47 AEs were described. Two of them appeared before the first administered dose and therefore were considered n-TEAE (non-treatment-emerged adverse event), both in the ApTOLL arm, and 45 were TEAEs (treatment-emerged adverse events). From the reported TEAEs, 32 AEs occurred in 20 subjects in the ApTOLL arm, and 13 AEs occurred in seven subjects in the placebo arm. In both arms, some subjects did not show any adverse event, and some subjects showed more than one adverse event. Regarding severity, 64.4% of TEAEs were mild (68.75% ApTOLL and 53.85% placebo), 26.67% were moderate (25.00% ApTOLL and 30.77% placebo), and 8.89% were severe (6.25% ApTOLL and 15.38% placebo). All AEs were considered not related to ApTOLL administration (Table 3). Importantly, no SAEs were reported. Specific AEs by system organ class (SOC) are included in Table 4.

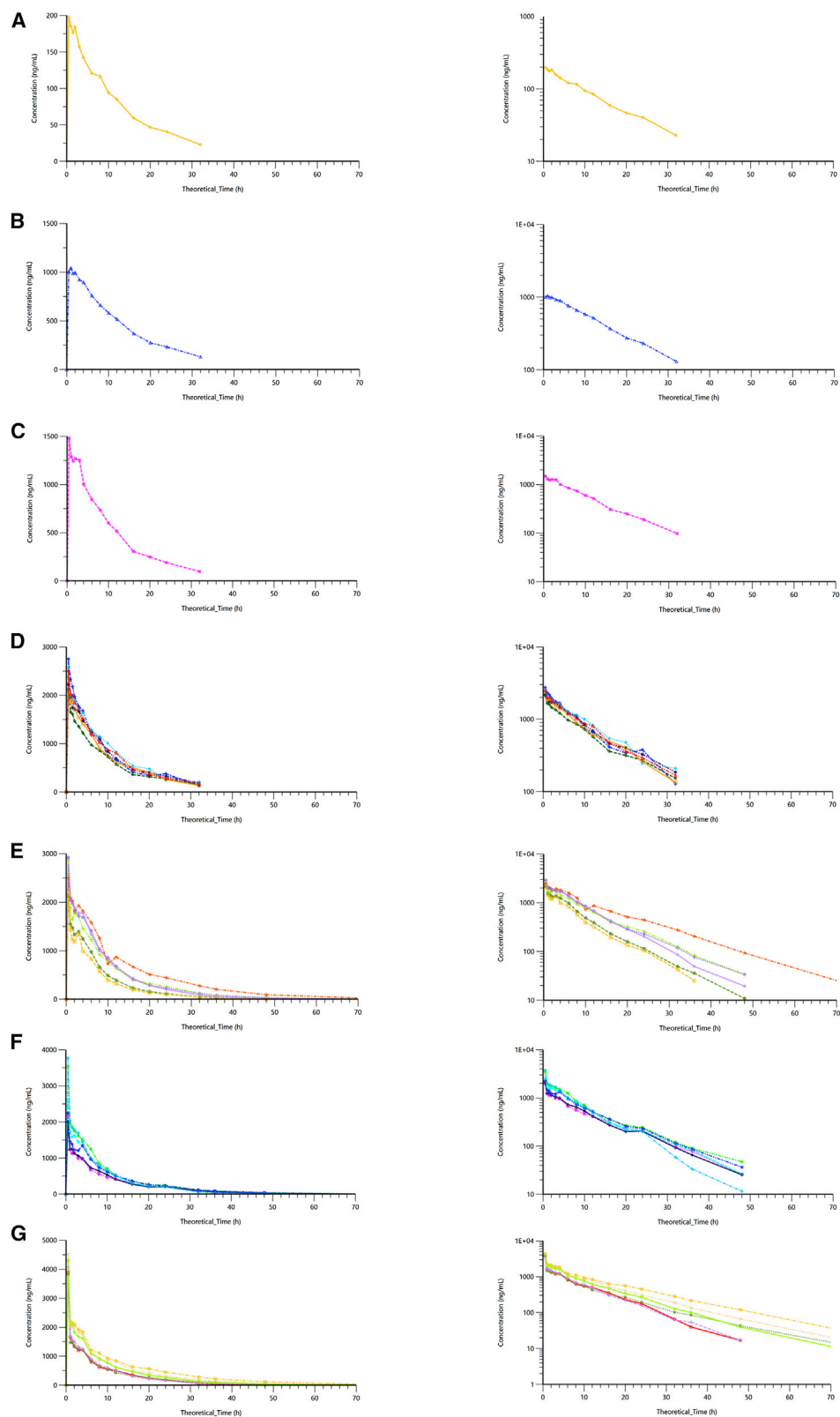
In both arms, headache was the most frequent TEAE (28.13% ApTOLL and 23.08% placebo). The incidence of headache did not increase when ApTOLL dose was increased, confirming that headache is not related to ApTOLL administration. Additionally, as shown in Table 4, the most frequent AEs in the ApTOLL arm were headache,

nasopharyngitis, dizziness, blood creatine phosphokinase increase, and diarrhea, although there were no significant differences compared to the placebo arm.

All events were resolved at the end of the trial. The overall events rates as well as the safety profile, as far as described by the assessment of AEs, were comparable between ApTOLL and placebo.

Clinical laboratory assessment

Clinically significant analytical alterations did not occur in the screening analyses performed. Nevertheless, nine analytical alterations were reported during the clinical trial, mainly at the safety visits on days 8 and 15 (four findings in each treatment arm and one n-TEAE). Volunteers with these alterations (four subjects received ApTOLL and two placebo) remained asymptomatic, but one of them showed myalgia simultaneously with the analytical alteration (creatinine kinase elevation). In addition, volunteers reported an alternative cause that in most cases justified the laboratory finding. Additional safety visits and analyses were performed until the resolution of the analytical alteration. There were no clinically significant alterations in hemogram analysis, except a mild decrease in the mean



(legend on next page)

hemoglobin value (0.65 mg/dL) without clinical relevance that can be explained by multiple blood samplings. There were no major changes that had to be attributed to treatment with ApTOLL or placebo. All serology analysis were negative.

Regarding specific assessment of complement (CH50 and C5b9 factors) and coagulation (PT and aPTT) parameters, no differences were detected after the treatment both in placebo and ApTOLL arms at any time performed.

Vital signs, physical findings, and other observations related to safety

Clinically significant alterations in the physical examinations, vital signs, or ECG were not found during the study. Blood pressure, heart rate, and ECG remained within physiologic limits during the whole study.

DISCUSSION

ApTOLL is a potent Toll-like receptor 4 (TLR4) antagonist, a receptor that is traditionally involved in innate immune response and is directly involved in a large number of diseases such as stroke, myocardial infarction, multiple sclerosis, and asthma,^{6–9,24} among others.

To determine the applicability of aptamers in therapeutics, several clinical studies were conducted. To date, one aptamer has been commercialized, pegaptanib,²³ an RNA aptamer targeting vascular endothelial growth factor in age-related macular degeneration patients. However, other aptamers are still in clinical trials such as Zimura,¹⁹ Fovista,²⁵ NOX-H94,²⁰ or BT200,²¹ among others.

The application of ApTOLL to improve functional outcome after acute ischemic insults, such as stroke or myocardial infarction, has been demonstrated so far in animal trials.^{1,2} Studies in different animal species (mice, rats, and pigs) have been reproduced by different laboratories, supporting the strong effect of ApTOLL in reducing inflammatory damage in different experimental models and under blinded conditions. A battery of studies to characterize distribution, metabolism, elimination, and drug interactions was also included as part of the preclinical characterization of the aptamer (data included in the international patent WO2020/230108). In those studies, ApTOLL levels were detected in different tissues (including kidney, spleen, and liver) early after administration (1 h) but were almost undetectable 24 h after injection. In addition, ApTOLL was present in the ischemic brain tissue but not in brains from naïve animals, indicating that ApTOLL is not able to cross the blood-brain barrier in physiological conditions. On the other hand, studies to determine *in vitro* drug interactions (off-target reactions and absorption, metabolism, and elimination interactions) and toxicity studies in rats and non-human primates (NHP) have demonstrated that ApTOLL is

neither able to produce drug interactions nor to induce any impairment in renal or in hepatic function. The safety and efficacy of ApTOLL together with the proven advantages of aptamers over other therapeutic entities like antibodies¹³ (higher specificity and affinity for the specific target,^{11,12} less toxicity than small molecules due to the endonuclease-mediated metabolism, greater long-term store stability and possibility of customization,^{14–16} reproducibility from batch to batch, lower immunogenic profile,^{17,18} etc.) justify the assessment of ApTOLL safety and pharmacokinetics in HVs in a FIH study.

Therefore, we conducted a phase I clinical trial, FIH dose-ascending, randomized, placebo-controlled clinical trial to assess tolerability and pharmacokinetics of ApTOLL in HVs.

Regarding pharmacokinetics, ApTOLL shows a half-life in plasma of 9.3 h with a C_{max} detected immediately after the end of the infusion (0.5 h) and a rapid clearance during the following hours, being almost undetectable at 32 h after administration and under the limit of quantification at 72 h. Quantifiable levels of ApTOLL were detected in urine samples from part B subjects from 3 h to 23 h after administration, suggesting a rapid clearance by renal filtration. The highest concentrations of ApTOLL in urine were detected approximately 2–3 h after dosing, indicating a rapid renal clearance of the aptamer. However, there is a limitation in the urine pharmacokinetic study since it was not possible to obtain equivalent samples from all subjects and at all time points, so urinary excretion could be underestimated.

The results obtained in this clinical trial fit with the expected pharmacokinetic profile of ApTOLL since it is an unmodified ssDNA aptamer. Pharmacokinetic properties of unmodified aptamers and other unmodified oligonucleotides have been described by several authors.²⁶ It has been established that unmodified aptamers show noticeably short half-lives with detectable levels in urine a few hours after administration. Therefore, pharmacokinetics of unmodified aptamers shows typically poor stability in plasma and a rapid clearance by renal filtration. For this reason, most aptamers are chemically modified to increase the possibilities of therapeutic success. Yet, the half-life was longer than expected for similar sized aptamers (e.g., ARC1779), and C_{max} and ARC showed a ceiling effect. The former may reflect high-affinity binding to the target, which is cell surface expressed. The latter would occur once this target is saturated and free drug would be rapidly filtered renally.

However, in this context, ApTOLL has been designed for acute indications to reduce the acute inflammatory response after the insult avoiding the interference with the reparative and proliferative phase of the inflammation. As it is well described for acute indications such as AIS and AMI, the inflammation shows a biphasic behavior.^{27–29} First, the acute inflammation starts very early after

Figure 2. ApTOLL plasma concentrations versus time after administration of different dose levels in part A of ApTOLL-FIH-01 clinical trial

(A–G) Plots of same color correspond to one subject enrolled in every single level of part A of the study. (A) Level 1 = 0.7 mg; (B) Level 2 = 2.1 mg; (C) Level 3 = 7 mg; (D) Level 4 = 14 mg; (E) Level 5 = 21 mg; (F) Level 6 = 42 mg; and (G) Level 7 = 70 mg. In every dose level, the concentration is shown in a lineal scale (left graphs) and in semilogarithmic scale (right graphs).

Table 2. Main Pharmacokinetic Results Obtained in ApTOLL-FIH-01 Clinical Trial

Level	Part A					Part B		
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
Dose	0.7 mg	2.1 mg	7 mg	14 mg	21 mg	42 mg	70 mg	3 doses of 21 mg/8 h
AUC _(0-∞) (h*ng/mL) ± SD	2,441.26	14,575.48	15,364.35	23,091.15 ± 2,436.86	21,379.78 ± 6,673.65	18,155.65 ± 2,637.19	23,184.61 ± 6,373.04	36,174.55 ± 10,245.87
AUC _(0-∞) (h*ng/mL) ± SD	2,789.02	16,483.89	16,615.89	25,269.64 ± 2,460.44	21,666.07 ± 6,755.50	18,560.63 ± 2,759.18	23,470.38 ± 6,519.66	37,787.40 ± 10,083.96
C _{max} (ng/mL) ± SD	197.4	1,042.7	1,480.7	2,427.42 ± 232.48	2,576.90 ± 366.20	2,714.60 ± 754.72	4,155.10 ± 265.37	2,531.00 ± 368.80
T _{max} (h) ± SD	0.8	1	0.5	0.50 ± 0.00	0.50 ± 0.00	0.51 ± 0.03	0.50 ± 0.00	0.50 ± 0.00
t _{1/2} (h) ± SD	10.53	10.15	8.86	9.11 ± 1.42	8.03 ± 1.84	9.02 ± 1.94	10.53 ± 2.93	8.49 ± 0.45
Cl (mL/h) ± SD	250.98	127.4	421.28	558.96 ± 61.01	1,057.37 ± 351.67	2,305.47 ± 346.53	3,165.42 ± 801.09	1,783.39 ± 529.88
Vd (mL) ± SD	3,811.42	1,865.9	5,385.61	7,342.05 ± 1,339.48	11,618.07 ± 2,014.84	29,784.02 ± 6,324.11	46,415.69 ± 13,449.9	21,579.23 ± 5,227.68

AUC_(0-∞): area under the plasma concentration curve versus time between 0 and last detected concentration.
 AUC_(0-∞): area under the plasma concentration curve versus time between 0 and infinity.
 C_{max}: maximum concentration.
 T_{max}: time for reaching maximum concentration.
 t_{1/2}: biological half-life.
 Cl: clearance.
 Vd: distribution volume.

onset of ischemic stroke or myocardial infarction, and it is prolonged for a few hours. During this initial phase, necrotic cells release DAMPS (damage associated molecular patterns) activating TLR4 and triggering an intense inflammatory response. Specifically, TLR4 activation promotes the expression of proinflammatory mediators that induce the adhesion, extravasation, and infiltration of immune cells in the damaged tissue. However, after this acute phase of inflammation, a second phase takes place to repair the tissue. The so-called “remodeling phase” starts hours to days after the insult and shares some of the elicitors involved in the acute-harmful phase. Therefore, the short half-life of ApTOLL provides the opportunity of blocking the first acute phase without interfering in the remodeling phase of inflammation.

On the other hand, ApTOLL has demonstrated an exceptional safety profile in this FIH study. No SAEs have been reported even when administering very high doses (up to 70 mg of ApTOLL, corresponding to 10-fold the estimated therapeutic dose in humans), and all AEs reported have been considered not related to the drug. Unfortunately, the MTD (maximum tolerated dose) was not reached due to ethical considerations, since the dose was increased along the part A of the trial up to 10-fold the estimated therapeutic dose (0.1 mg/kg). This dose was calculated based on the conversion by body surface of the efficacious dose in animals (0.45 mg/kg in rats and 0.9 mg/kg in mice) to humans. In this phase I study, doses from 0.01 mg/kg to 1 mg/kg were studied, which is sufficient to obtain safety and pharmacokinetic data that support a clinical trial in patients. Typically, aptamers are considered very safe molecules.^{26,30} To date, no immunological abnormalities have been reported in clinical trials due to aptamers administration. However, some aptamers have shown an increase in plasma coagulation^{31,32} and hematological alterations,³³ although these effects may be partly target related. Additionally, results in nonclinical studies support the possibility of aptamers to display pseudoallergic reactions due to complement activation.³⁴ For this reason, coagulation, hematology, and complement activation were carefully assessed during the ApTOLL-FIH-01 trial, and no abnormalities were reported at any dose level.

The excellent safety profile of ApTOLL offers several advantages compared with the undesirable side effects demonstrated by other TLR4 antagonists such as eritoran, resatorvid, or NI-0101. Clinical trials using eritoran showed phlebitis, increased levels of renal failure, elevated creatinine and transaminases, and an increased rate of atrial fibrillation in patients when compared with placebo arm.^{35,36} Treatment with resatorvid increased the prevalence of anemia, methemoglobinemia, hypokalemia, pyrexia, and urinary tract infections.³⁷ Finally, several studies in HVs and rheumatoid arthritis patients administered with NI-0101 have reported a safety profile similar to ApTOLL. However, NI-0101 half-life was estimated to be approximately 10 days^{38,39} which is a very long half-life for acute indications, as commented before.

In conclusion, this FIH phase I study has demonstrated ApTOLL good safety and tolerability and an appropriate pharmacokinetics

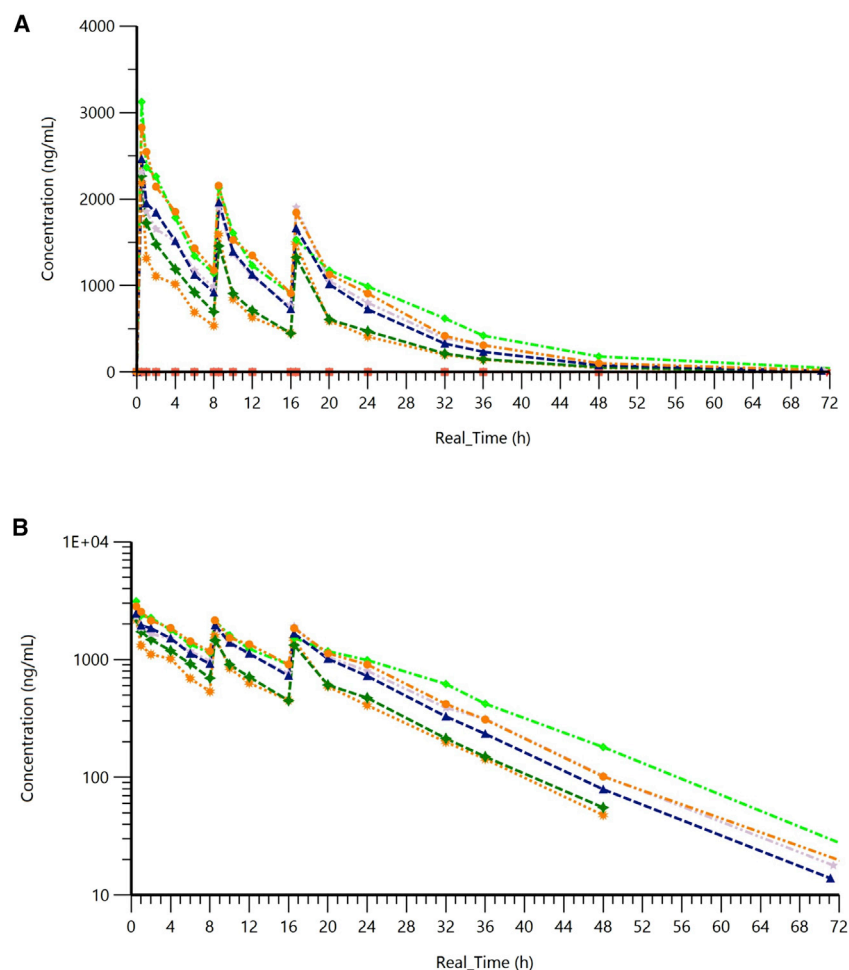


Figure 3. ApTOLL concentration versus time in the part B of ApTOLL-FIH-01 clinical trial

(A and B) Plots of same color correspond to one subject enrolled in the part B of the study. 21 mg of ApTOLL was administered intravenously every 8 h during 24 h. The concentration is shown in lineal scale (A) and semi-logarithmic scale (B).

and the Spanish Agency of Medicines and Medical Devices for approval. The study was conducted in accordance with the principles established in the Declaration of Helsinki, the Guidelines of the International Conference on Harmonization (ICH) on Good Clinical Practice (CPMP/ICH/135/95), European Union Directive 95/46/EC, and the current applicable Spanish legislation regarding clinical trials.

All subjects included in this trial provided their written informed consent.

The study was divided in two parts. The first part, part A, was a single ascending dose (SAD) with seven dose levels (0.7 mg–70 mg) (see Table 5). The second, part B, was a multiple dose (MD) where one dose selected in part A (21 mg) was administered to HVs three times, every 8 h, during 24 h.

The main inclusion criteria were as follows: male or female subjects (women without possibility of becoming pregnant because of previous hysterectomy or menopause more than 12 months),

willing and able to give their written consent to participate in the trial; healthy subjects (18–55 years old, body weight between 65 and 85 kg, and BMI between 19.0 and 30.0 kg/m²) with clinical history and physical examination with values within normality (including vital signs and electrocardiogram); and with no clinically significant abnormalities in hematology, biochemistry, serology (HBsAg, HC antibodies, HIV antibodies), and urine tests.

The primary objective of the study was to assess the tolerability and pharmacokinetic characteristics of ApTOLL (both single and multiple doses) in HVs. To determine tolerability, AEs, physical parameters, laboratory data, vital signs, and ECG were collected.

The selected dose levels for single dose escalation (part A) and for the multiple dose cohort (part B) are described in the Table 5.

HVs received during a 30-min intravenous (i.v.) infusion either ApTOLL at the appropriate concentration in saline solution or placebo (saline solution). Therefore, the administration of ApTOLL was

for indications where the reduction of the acute phase of inflammation should be robust but short in time, allowing the second remodeling phase starting hours to days after the insult. These data support the further development of ApTOLL in a wide range of diseases where activation of TLR4 plays a central role, such as AIS or AMI. Additionally, the good safety profile demonstrated by ApTOLL enables the administration of single or multiple doses and opens the door for the applicability of ApTOLL in other sub-acute or chronic conditions.

MATERIALS AND METHODS

Study design

This was a phase I, FIH dose-ascending, randomized, placebo-controlled clinical trial to assess tolerability and pharmacokinetics of ApTOLL in HVs (study code: ApTOLL-FIH-01).

The study was registered on EudraCT (2018-001721-51) and ClinicalTrials.gov: NCT04742062.

Before starting the trial, all the documentation was submitted to the Medical Ethics Committee (La Princesa Hospital, Madrid, Spain)

Table 3. Overview AEs in ApTOLL-FIH-01 trial

Parameter	ApTOLL (N = 33)	Placebo (N = 13)	Overall (N = 46) ^a
AEs reported	34	13	47
n-TEAEs reported	2	0	2
TEAEs reported	32	13	45
Subjects with at least one TEAE ^b	20 (60.61%)	7 (53.85%)	27 (58.70%)
Subjects with at least one drug-related TEAE ^{c,d}	0 (0%)	0 (0%)	0 (0%)
TEAEs relationship ^c			
Related ^d	0 (0%)	0 (0%)	0 (0%)
Not Related	32 (100%)	13 (100%)	45 (100.00%)
TEAEs by severity/intensity ^c			
Mild	22 (68.75%)	7 (53.85%)	29 (64.44%)
Moderate	8 (25.00%)	4 (30.77%)	12 (26.67%)
Severe	2 (6.25%)	2 (15.38%)	4 (8.89%)
SAEs reported ^c	0	0	0

N, number of subjects who received a specific treatment; TEAE, treatment-emergent adverse event.

^aOverall total drug exposures.

^bPercentages are based on the number of subjects in the safety population in each treatment group.

^cPercentages are based on the total number of TEAEs reported in each treatment group.

^dTEAE was reported as reasonable possibility or definite.

performed by dilution of the aptamer in water for injection followed by a dilution in saline buffer for infusion. To guarantee the safety of the subjects, a period of 2 weeks between every dose level was established, and 1 week between sentinels and the rest of the subjects from each level to ensure safety before administration to the whole dose level. A safety monitoring committee evaluated safety parameters before continuing with the study either after sentinel inclusion, or after completion of a dose level.

The allocation was performed using the statistical program Epidat 4.2 (Xunta de Galicia, Spain) randomly assigning each subject to ApTOLL or placebo. All personnel involved in the execution and evaluation of the study (except unblinded nurse and investigator in charge of randomization and preparation of the medication) were blinded.

The start date of the trial was June 18th, 2019, and the last subject follow-up visit was performed on March 20th, 2020. All subjects were followed up to 15 days after administration of ApTOLL or placebo.

Dose selection

The calculation of the starting dose was performed considering the results of animal studies, according to guidelines of the European Medicines Agency (EMA)⁴⁰ and the US Food and Drug Administration.⁴¹ Two approaches, based on the (no observed adverse effects level) NOAEL and based on the (minimum anticipated biological effect level) MABEL from efficacy and toxicity studies, were considered to guarantee the safety of the HVs:

1. NOAEL

- Rats: no adverse effects observed with the highest dose tested, 50 mg/kg/day intravenously for 14 days.

- NHP: no adverse effects observed with the highest dose tested, 13.9 mg/kg/day intravenously for 14 days.

The HED (human equivalent dose) was calculated from the NOAEL considering conversion of animal doses to human equivalent doses based on body surface area.¹⁷ An additional safety factor of 10 was also considered:

- rat: $(50 \text{ mg/kg} \times 0.162)/10 = 0.81 \text{ mg/kg}$
- NHP: $(13.9 \text{ mg/kg} \times 0.32)/10 = 0.45 \text{ mg/kg}$

Therefore, considering the lower calculated dose (0.45 mg/kg), the maximum recommended safe dose (MRSD) for a 70-kg bodyweight person would be 31.5 mg.

2. MABEL

Efficacy in experimental models of cerebral ischemia (mice and rats) was found at the following doses¹:

- permanent MCAO mouse model: 0.91 mg/kg intravenously or intraperitoneally
- permanent MCAO rat model: 0.45 mg/kg intravenously
- transient MCAO rat model: 0.45 mg/kg intravenously

The HED was calculated from the nonclinical efficacy dose considering conversion from animal doses to human equivalent dose based on body surface area. An additional safety factor of 10 was also considered to correct differences in target binding and pharmacological activity between species:

- mouse: $(0.91 \text{ mg/kg} \times 0.081)/10 = 0.0073 \text{ mg/kg}$
- rat: $(0.45 \text{ mg/kg} \times 0.162)/10 = 0.0073 \text{ mg/kg}$

Table 4. Summary of Treatment-Emergent Adverse Events by System Organ Class

System Organ Class (SOC)	ApTOLL (AE N = 32) n (%)	Placebo (AE N = 13) n (%)	n-TEAEs (AE N = 2) n (%)
MedDRA Preferred Term (PT)			
Subjects with at least one TEAE	20 (60.61%)	7 (53.85%)	–
Nervous system disorders			
Dizziness	2 (6.25%)	0 (0%)	0 (0%)
Headache	9 (28.13%)	3 (23.08%)	0 (0%)
Somnolence	1 (3.13%)	0 (0%)	0 (0%)
Syncope	1 (3.13%)	0 (0%)	0 (0%)
Investigations			
Blood creatine phosphokinase increased	3 (9.38%)	2 (15.38%)	1 (50.00%)
Blood lactate dehydrogenase increased	0 (0%)	1 (7.69%)	0 (0%)
Gamma-glutamyltransferase increased	1 (3.13%)	0 (0%)	0 (0%)
Hepatic enzyme increased	0 (0%)	1 (7.69%)	0 (0%)
Gastrointestinal disorders			
Abdominal pain	1 (3.13%)	0 (0%)	0 (0%)
Constipation	1 (3.13%)	0 (0%)	0 (0%)
Diarrhea	2 (6.25%)	1 (7.69%)	0 (0%)
Abdominal pain upper	1 (3.13%)	0 (0%)	0 (0%)
Nausea	1 (3.13%)	0 (0%)	0 (0%)
Infections and infestations			
Nasopharyngitis	4 (12.50%)	1 (7.69%)	1 (50.00%)
Pharyngotonsillitis	1 (3.13%)	0 (0%)	0 (0%)
Musculoskeletal and connective tissue disorders			
Back pain	1 (3.13%)	0 (0%)	0 (0%)
Myalgia	1 (3.13%)	0 (0%)	0 (0%)
Neck pain	0 (0%)	1 (7.69%)	0 (0%)
Pain in extremity	1 (3.13%)	0 (0%)	0 (0%)
Injury, poisoning, and procedural complications			
Ligament sprain	0 (0%)	1 (7.69%)	0 (0%)
Limb injury	0 (0%)	1 (7.69%)	0 (0%)
Renal and urinary disorders			
Urine abnormality	1 (3.13%)	0 (0%)	0 (0%)
Respiratory, thoracic, and mediastinal disorders			
Oropharyngeal pain	0 (0%)	1 (7.69%)	0 (0%)

Note: each TEAE was counted only once for each subject within each SOC and MedDRA PT.

Therefore, the MRSD for a 70-kg weight person considering nonclinical efficacy dose would be 0.5 mg.

Taken these data into account, the MRSD finally considered was calculated from MABEL because it was much lower than the MRSD calculated from NOAEL. However, as ApTOLL was prepared in vials of 7 mg, the first dose selected was 0.7 mg to facilitate the dosage.

The maximum theoretical concentration (MTC) in plasma resulting from the selected MRSD was calculated by assuming complete bioavailability, with no plasma-protein binding, no distribution into blood cells or other tissues, and no elimination, using the extracellular fluid (ECF = 18,200 mL for a 70-kg average human bodyweight) as minimum volume of distribution, which resulted in $MTC = 700,000 \text{ ng} / 18,200 \text{ mL} = 38.5 \text{ ng/mL}$. This concentration was lower than the minimum concentration of ApTOLL showing binding

Table 5. Dose Levels Selected for the FIH Study, Part A and Part B

Study Part	Dose Level	Dose of ApTOLL	Number of Subjects Enrolled (Ratio ApTOLL:placebo)
A	1	0.7 mg	2 (1:1)
A	2	2.1 mg	2 (1:1)
A	3	7 mg	2 (1:1)
A	4	14 mg	2 sentinels (1:1) + 6 (5:1)
A	5	21 mg	2 sentinels (1:1) + 6 (5:1)
A	6	42 mg	2 sentinels (1:1) + 6 (5:1)
A	7	70 mg	2 sentinels (1:1) + 6 (5:1)
B	8	21 mg \times 3	2 sentinels (1:1) + 6 (5:1)

affinity to human TLR4 *in vitro* (20 nM = 363.4 ng/mL),¹ which confirmed that the MRSD was below the MABEL.

Physical examination and vital signs

In every HV, physical examination and health assessment, including height and body weight, was performed at screening and at pre-dose.

Immediately after ApTOLL administration and during the in-patient phase, the site of injection was monitored for side effects.

Vital signs (heart rate, blood pressure, and respiratory rate) and 12-lead ECG were measured before the administration and during the in-patient phase. Heart rate, blood pressure, and ECG were registered pre-dose, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 h after ApTOLL administration. Also, vital signs and ECG were recorded at every visit during the out-patient phase.

Subjects left the Clinical Trials Unit 48 h after ApTOLL administration and returned at day 4, day 5, day 6, day 8, day 11, and day 15 after dosing for safety evaluation.

Blood and urine analysis

Blood and urine samples from HVs were obtained at screening and at different time points during the in-patient and out-patient phases.

Hemogram, biochemistry, and urine were analyzed at screening, day 1 (pre-dose), day 2, day 8, and day 15. Serology (HBV, HCV, and HIV) was done at the screening visit. A urine drug abuse test (cannabinoids, cocaine, opiates, and amphetamines) was performed at screening, day 0 (admission night), and day 15. An ethanol breath test was carried out at the screening visit and follow-up phase only if consumption was suspected.

Additionally, for a deeper control of the aptamer's safety, creatine kinase (CK), C-reactive protein (CRP), coagulation (prothrombin activity [PT] and aPTT [activated partial thromboplastin time]) and complement factors (CH50 [50% hemolytic complement] and C5b-9 [terminal complement complex]) were determined at screening, day 1 (pre-dose), day 2, day 8, and day 15.

Pharmacokinetic determinations

For the pharmacokinetic analysis, approximately 9 mL of whole blood was collected in EDTA tubes. Within 30 min of being collected, samples were centrifuged at 1900 g for 10 min at 2–8°C to obtain the plasma. Plasma samples were stored at $-80 \pm 10^\circ\text{C}$ until analysis.

In subjects from levels 1–4, 15 blood samples were taken at the following times: pre-dose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, and 32 h post-dose. In subjects from levels 5–8, 18 blood samples were taken at the following times: pre-dose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 36, 48, and 72 h post-dose, in order to cover the possibility of longer half-lives. Additionally, for part B a sample was taken at 8.5 and 16.5 h (end of second and third doses). A different venous access was used for drug administration and blood sampling.

In subjects from part B of the study (multiple dose study), urine samples from all urination until discharge were also collected for pharmacokinetic analysis. Every urine sample from every volunteer (except sentinels) was collected, measured (the total volume was registered), and snaped frozen immediately after aliquotation (1 mL). Samples were stored at $-80 \pm 10^\circ\text{C}$ until analysis.

ApTOLL plasma and urine concentrations were measured using validated (according to good laboratory practices) dual hybridization assays at Axolabs GmbH (Kulmbach, Germany). Both methods were validated according to criteria from EMA entitled Bioanalytical Method Validation.¹⁹ $\text{AUC}_{(0-t)}$ (area under curve versus time between 0 and last detected concentration), $\text{AUC}_{(0-\infty)}$ (AUC versus time between 0 and infinity), C_{\max} (maximum concentration), T_{\max} (time for reaching the maximum concentration), $T_{1/2}$ (biological half-life), Cl (Clearance), and V_d (Distribution Volume) were calculated in part A, and AUC partial area (AUC during every dose administration), C_{\max} (maximum plasma concentration at each dose administration), T_{\max} (time for reaching maximum concentration at each dose administration), fluctuation, and $T_{1/2}$ were calculated in part B.

Statistical methods

Appropriate rounding was performed for the summary statistics: geometric mean, median, arithmetic mean, standard deviation, coefficient of variation, minimum, and maximum were presented with two decimals. Percentages were presented with two decimals.

All subjects who received placebo (from all cohorts) were pooled into one large placebo group, independently from the cohort into which they were enrolled. The baseline was defined as the value at Study Day-1 pre-dose assessment. If a Study Day-1 pre-dose value was missing, the baseline was assumed equal to the value at screening.

If data were missing due to “not done,” then no replacement rules were applied, and data remained missing for tabulation purposes. In case of pharmacokinetic data, concentrations that were “below lower limit of quantification” were considered as 0. Subjects who

discontinued before dosing were replaced. However, all available data of these “dropouts” were included in the database.

The statistical analysis was done using Microsoft Excel and WinNonlin Professional Edition current version (Pharsight Corporation, Cary, USA). Output was saved and imported into Microsoft Word. A non-compartment analysis was used for both single dose (part A) and multiple dose (part B) studies.

Calculation of non-compartmental pharmacokinetic parameters was also done using WinNonlin Professional Edition current version (Pharsight Corporation, Cary, USA). The total $AUC_{0-\infty}$ was calculated by adding together two partial AUCs: (1) AUC_{0-t} between the previous time and the first with detectable concentrations and the last with detectable concentrations, calculated using the trapezoidal rule and (2) $AUC_{t-\infty}$, calculated as the C/K_e ratio, where C was the last detectable concentration and K_e was the slope of the line obtained by linear regression from the points corresponding to the drug's elimination phase. To determine the number of points used to calculate K_e , WinNonlin started the regression from the last three detectable points, calculating R^2 adjusted to the number of points, adding a fourth point, a fifth point, and so on at each step. The gradient of the elimination line with the points providing the highest adjusted R^2 value was then estimated by a linear regression of the natural logarithm of the concentrations. A non-compartmental model was used to calculate the volume of distribution (V_d) adjusted to the bioavailability, the elimination constant (K_e), the half-life ($T_{1/2}$), the drug's clearance (CL), and mean residence time (MRT). Peak concentration (C_{max}) and time to peak concentration (T_{max}) were obtained directly from the plasma concentration information.

Finally, the Spanish Pharmacovigilance System algorithm was used to evaluate the relationship between the AEs and the treatment (causal-ity determination).⁴²

ACKNOWLEDGMENTS

This study was sponsored by aptaTargets S.L. (Madrid, Spain) and was conducted at the Clinical Trials Unit (La Princesa Hospital, Madrid, Spain). The study was supported by a grant from the Spanish Ministry of Science, Innovation and Universities (RTC-2017-6651-1). Authors acknowledge David Segarra and M. Eugenia Zarabozo (aptaTargets S.L.) for their contribution in the management and funding of the trial, and Alba Singla (Anagram; Barcelona, Spain) for her contribution in the monitoring of the trial.

AUTHOR CONTRIBUTIONS

M.H.-J. and S.M.-V. equally contributed to the study. Conceptualization, M.H.-J., F.A.S., B.J., and D.O.; Methodology, S.M.-V., F.A.S., D.O., M.R., and M.H.-J.; Investigation S.M.-V., G.M., D.O., M.R., S.L.-B., and M.H.-J.; Formal analysis, G.M. and P.C.-M.; Writing - Original Draft, M.H.-J.; Writing - Review and Editing, M.H.-J., F.A.S., I.L., B.J., and M.A.M.; Resources, F.A.S., D.O., G.F., and D.P.R.; Supervision D.O., F.A.S., B.J., M.A.M., M.Ri, V.M.G., and I.L. M.R. is Manuel Román and M.Ri. is Marc Ribó.

DECLARATION OF INTERESTS

M.H.-J. and D.P.R. are employees of aptaTargets S.L. V.M.G. is researcher from FIBio-HRC. G.F. is employee of Aptus Biotech S.L. M.Ri. receives payment from Philips as Co-principal investigator of the WE TRUST study and he has a consulting agreement with Medtronic, Stryker, Cerenovus, CVAid, Methinks, Anaconda Biomed, and aptaTargets S.L. B.J. served as consultant to aptaTargets S.L. F.A.S. and D.O. have been consultants or investigators in clinical trials sponsored by the following pharmaceutical companies: Abbott, Alter, aptaTargets, Chemo, Cinfa, FAES, Farmalider, Ferrer, GlaxoSmithKline, Galenicum, Gilead, Italfarmaco, Janssen-Cilag, Kern, Normon, Novartis, Servier, Silverpharma, Teva, and Zambon. The information disclosed in this article is protected by the international patent application WO2015197706 and its extensions to different countries; and by the international patent WO2020/230108.

REFERENCES

- Fernández, G., Moraga, A., Cuartero, M.I., García-Culebras, A., Peña-Martínez, C., Pradillo, J.M., Hernández-Jiménez, M., Sacristán, S., Ayuso, M.I., Gonzalo-Gobernado, R., et al. (2018). TLR4-Binding DNA aptamers show a protective effect against acute stroke in animal models. *Mol. Ther. J. Am. Soc. Gene Ther.* 26, 2047–2059.
- Ramírez-Carracedo, R., Tesoro, L., Hernandez, I., Díez-Mata, J., Piñeiro, D., Hernandez-Jimenez, M., Zamorano, J.L., and Zaragoza, C. (2020). Targeting TLR4 with ApTOLL improves heart function in response to coronary ischemia reperfusion in pigs undergoing acute myocardial infarction. *Biomolecules* 10, 1167.
- Medzhitov, R., Preston-Hurlburt, P., and Janeway, C.A., Jr. (1997). A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388, 394–397.
- Medzhitov, R. (2001). Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* 1, 135–145.
- Sabroe, I., Read, R.C., Whyte, M.K., Dockrell, D.H., Vogel, S.N., and Dower, S.K. (2003). Toll-like receptors in health and disease: complex questions remain. *J. Immunol.* 171, 1630–1635.
- Oyama, J., Blais, C., Jr., Liu, X., Pu, M., Kobzik, L., Kelly, R.A., and Bourcier, T. (2004). Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4-deficient mice. *Circulation* 109, 784–789.
- Caso, J.R., Pradillo, J.M., Hurtado, O., Lorenzo, P., Moro, M.A., and Lizasoain, I. (2007). Toll-like receptor 4 is involved in brain damage and inflammation after experimental stroke. *Circulation* 115, 1599–1608.
- Kerfoot, S.M., Long, E.M., Hickey, M.J., Andonegui, G., Lapointe, B.M., Zanardo, R.C., Bonder, C., James, W.G., Robbins, S.M., and Kubes, P. (2004). TLR4 contributes to disease-inducing mechanisms resulting in central nervous system autoimmune disease. *J. Immunol.* 173, 7070–7077.
- Schwartz, D.A. (2002). TLR4 and LPS hyporesponsiveness in humans. *Int. J. Hyg. Environ. Health* 205, 221–227.
- Tuerk, C., and Gold, L. (1990). Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 249, 505–510.
- Ellington, A.D., and Szostak, J.W. (1990). In vitro selection of RNA molecules that bind specific ligands. *Nature* 346, 818–822.
- White, R.R., Sullenger, B.A., and Rusconi, C.P. (2000). Developing aptamers into therapeutics. *J. Clin. Invest.* 106, 929–934.
- Jayasena, S.D. (1999). Aptamers: an emerging class of molecules that rival antibodies in diagnostics. *Clin. Chem.* 45, 1628–1650.
- Rusconi, C.P., Roberts, J.D., Pitoc, G.A., Nimjee, S.M., White, R.R., Quick, G., Jr., Scardino, E., Fay, W.P., and Sullenger, B.A. (2004). Antidote-mediated control of an anticoagulant aptamer in vivo. *Nat. Biotechnol.* 22, 1423–1428.

15. Ulrich, H., Trujillo, C.A., Nery, A.A., Alves, J.M., Majumder, P., Resende, R.R., and Martins, A.H. (2006). DNA and RNA aptamers: from tools for basic research towards therapeutic applications. *Comb. Chem. High Throughput Screen.* 9, 619–632.
16. Elskens, J.P., Elskens, J.M., and Madder, A. (2020). Chemical modification of aptamers for increased binding affinity in diagnostic applications: current status and future prospects. *Int. J. Mol. Sci.* 21, 4522.
17. Lakhin, A.V., Tarantul, V.Z., and Gening, L.V. (2013). Aptamers: problems, solutions and prospects. *Acta Naturae* 5, 34–43.
18. Zhou, J., and Rossi, J. (2017). Aptamers as targeted therapeutics: current potential and challenges. *Nat. Rev. Drug Discov.* 16, 181–202.
19. Jaffe, G.J., Westby, K., Csaky, K.G., Monés, J., Pearlman, J.A., Patel, S.S., Joondeph, B.C., Randolph, J., Masonson, H., and Rezaei, K.A. (2020). C5 inhibitor avacincaptad pegol for geographic atrophy due to age-related macular degeneration: a randomized pivotal phase 2/3 trial. *Ophthalmology* 128, 576–586.
20. Boyce, M., Warrington, S., Cortez, B., Zöllner, S., Vauléon, S., Swinkels, D.W., Summo, L., Schwoebel, F., and Riecke, K. (2016). Safety, pharmacokinetics and pharmacodynamics of the anti-hepcidin Spiegelmer lexapted pegol in healthy subjects. *Br. J. Pharmacol.* 173, 1580–1588.
21. Zhu, S., Gilbert, J.C., Hatala, P., Harvey, W., Liang, Z., Gao, S., Kang, D., and Jilma, B. (2020). The development and characterization of a long acting anti-thrombotic von Willebrand factor (VWF) aptamer. *J. Thromb. Haemost.* 18, 1113–1123.
22. Steurer, M., Montillo, M., Scarfò, L., Mauro, F.R., Andel, J., Wildner, S., Trentin, L., Janssens, A., Burgstaller, S., Frömming, A., et al. (2019). Olaptased pegol (NOX-A12) with bendamustine and rituximab: a phase IIa study in patients with relapsed/refractory chronic lymphocytic leukemia. *Haematologica* 104, 2053–2060.
23. Ruckman, J., Green, L.S., Beeson, J., Waugh, S., Gillette, W.L., Henninger, D.D., Claesson-Welsh, L., and Janjić, N. (1998). 2'-Fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). Inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain. *J. Biol. Chem.* 273, 20556–20567.
24. Poltorak, A., He, X., Smirnova, I., Liu, M.Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., et al. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282, 2085–2088.
25. Dunn, E.N., Hariprasad, S.M., and Sheth, V.S. (2017). An overview of the Fovista and rinucumab trials and the fate of anti-PDGF medications. *Ophthalmic Surg. Laser. Imag.* 48, 100–104.
26. Kovacevic, K.D., Gilbert, J.C., and Jilma, B. (2018). Pharmacokinetics, pharmacodynamics and safety of aptamers. *Adv. Drug Deliv. Rev.* 134, 36–50.
27. Dirnagl, U., Iadecola, C., and Moskowitz, M.A. (1999). Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci.* 22, 391–397.
28. Prabhu, S.D., and Frangogiannis, N.G. (2016). The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. *Circ. Res.* 119, 91–112.
29. Maki, T., Hayakawa, K., Pham, L.D., Xing, C., Lo, E.H., and Arai, K. (2013). Biphasic mechanisms of neurovascular unit injury and protection in CNS diseases. *CNS Neurol. Disord. Drug Targets* 12, 302–315.
30. Dobrovolskaia, M.A., and McNeil, S.E. (2015). Immunological and hematological toxicities challenging clinical translation of nucleic acid-based therapeutics. *Expert Opin. Biol. Ther.* 15, 1023–1048.
31. Gorczyca, M.E., Nair, S.C., Jilma, B., Priya, S., Male, C., Reitter, S., Knoebl, P., Gilbert, J.C., Schaub, R.G., Dockal, M., et al. (2012). Inhibition of tissue factor pathway inhibitor by the aptamer BAX499 improves clotting of hemophilic blood and plasma. *J. Thromb. Haemost.* 10, 1581–1590.
32. Dyke, C.K., Steinhubl, S.R., Kleiman, N.S., Cannon, R.O., Aberle, L.G., Lin, M., Myles, S.K., Melloni, C., Harrington, R.A., Alexander, J.H., et al. (2006). First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology: a phase Ia pharmacodynamic evaluation of a drug-antidote pair for the controlled regulation of factor IXa activity. *Circulation* 114, 2490–2497.
33. Rosenberg, J.E., Bambury, R.M., Van Allen, E.M., Drabkin, H.A., Lara, P.N., Jr., Harzstark, A.L., Wagle, N., Figlin, R.A., Smith, G.W., Garraway, L.A., et al. (2014). A phase II trial of AS1411 (a novel nucleolin-targeted DNA aptamer) in metastatic renal cell carcinoma. *Invest. New Drugs* 32, 178–187.
34. Szebeni, J. (2014). Complement activation-related pseudoallergy: a stress reaction in blood triggered by nanomedicines and biologicals. *Mol. Immunol.* 61, 163–173.
35. Rossignol, D.P., Wasan, K.M., Choo, E., Yau, E., Wong, N., Rose, J., Moran, J., and Lynn, M. (2004). Safety, pharmacokinetics, pharmacodynamics, and plasma lipoprotein distribution of eritoran (E5564) during continuous intravenous infusion into healthy volunteers. *Antimicrob. Agents Chemother.* 48, 3233–3240.
36. Tidswell, M., Tillis, W., Larosa, S.P., Lynn, M., Wittek, A.E., Kao, R., Wheeler, J., Gogate, J., and Opal, S.M. (2010). Phase 2 trial of eritoran tetrasodium (E5564), a toll-like receptor 4 antagonist, in patients with severe sepsis. *Crit. Care Med.* 38, 72–83.
37. Rice, T.W., Wheeler, A.P., Bernard, G.R., Vincent, J.L., Angus, D.C., Aikawa, N., Demeyer, I., Sainati, S., Amlot, N., Cao, C., et al. (2010). A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. *Crit. Care Med.* 38, 1685–1694.
38. Monnet, E., Lapeyre, G., Poelgeest, E.V., Jacqmin, P., Graaf, K., Reijers, J., Moerland, M., Burggraaf, J., and Min, C. (2017). Evidence of NI-0101 pharmacological activity, an anti-TLR4 antibody, in a randomized phase I dose escalation study in healthy volunteers receiving LPS. *Clin. Pharmacol. Ther.* 101, 200–208.
39. Monnet, E., Choy, E.H., McInnes, I., Kobakhidze, T., de Graaf, K., Jacqmin, P., Lapeyre, G., and de Min, C. (2020). Efficacy and safety of NI-0101, an anti-toll-like receptor 4 monoclonal antibody, in patients with rheumatoid arthritis after inadequate response to methotrexate: a phase II study. *Ann. Rheum. Dis.* 79, 316–323.
40. van Gerven, J., and Bonelli, M. (2018). Commentary on the EMA Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. *Br. J. Clin. Pharmacol.* 84, 1401–1409.
41. Food; Evaluation, D. A. J. C. f. D (2005). Research. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers (Food and Drug Administration), p. 7.
42. Aguirre, C., and García, M. (2016). [Causality assessment in reports on adverse drug reactions. Algorithm of Spanish pharmacovigilance system]. *Med. Clin.* 147, 461–464.