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Original Article

Clinical trial to compare safety and tolerability between intravenous infusion and bolus intravenous injection of ApTOLL in healthy volunteers

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ApTOLL, a new modulator of Toll-like receptor 4, has demonstrated safety and efficacy in healthy subjects and in stroke patients; however, the route of administration used so far (30 min infusion) can potentially be an issue in the acute stroke units where "time is brain." To safely reduce the time of administration in future clinical trials, a dose-ascending, open-label, phase I clinical trial was conducted in healthy subjects. The objective was to assess the safety and pharmacokinetics of ApTOLL when comparing intravenous infusion (30 min) vs. bolus intravenous injection (1–3 min). The study was divided into three periods: (1) volunteers received 0.1 mg/kg of ApTOLL as a slow intravenous infusion, (2) 0.1 mg/kg of ApTOLL was administered as a single bolus, and (3) subjects received 0.2 mg/kg as a single bolus injection. No adverse events related to ApTOLL administration at any dosing pattern were reported. Maximum concentration was detected at the end of the infusion/injection, and mean half-life was 9.5 h for both routes of administration. These results show that safety and pharmacokinetic profiles were comparable between intravenous infusion and bolus injection of ApTOLL, supporting a change of the route of administration for future clinical practice (ClinicalTrials.gov: NCT05569720).

INTRODUCTION

ApTOLL (the final product of the active-substance aptamer 4FT) is an aptamer selected to antagonize Toll-like receptor 4 and, therefore, to block the inflammatory response developed after different insults, such as acute ischemic stroke (AIS),[1,2] acute myocardial infarction,[3,4] or multiple sclerosis.[5] The main indication for ApTOLL is AIS, where the molecule showed a long-lasting protective effect against brain injury induced by transient and permanent middle cerebral artery occlusion in mice and rats.[1,2] Additionally, ApTOLL has also demonstrated a potent anti-inflammatory effect in rat and pig models of myocardial infarction[3] as well as outstanding cytoprotection in different models of multiple sclerosis,[5] supporting the therapeutic effect in diseases with a substantial inflammatory component.

In clinical trials, ApTOLL has demonstrated an excellent safety profile and a half-life of approximately 9.3 h in plasma from healthy subjects.[6] These results were obtained in the ApTOLL-FIH-01 clinical trial (ClinicalTrials.gov: NCT04742062)[6] conducted in 46 healthy subjects. The results demonstrated the absence of adverse events related to ApTOLL after 30 min intravenous (i.v.) infusion, both with a single-administration pattern and a multiple-dose (every 8 h during 24 h) pattern. Following the mentioned clinical study, the APRIL trial (ClinicalTrials.gov: NCT04734548) in AIS patients was conducted.[7] This was a phase Ib/IIa clinical trial in 151 AIS patients who received ApTOLL (30 min, i.v.) at different doses.[8] The results showed that ApTOLL was associated with a significant reduction of death rates, lower functional impairment, smaller final infarct volume, and lower degrees of disability at 90 days.[7] These results await confirmation in larger clinical trials. However, given the specific characteristics of acute stroke units, where "time is brain," several potential issues related to ApTOLL slow infusion have been identified. Patient management could potentially be affected in larger trials and/or clinical practice by the infusion pump placement and the time-consuming slow infusion of ApTOLL.

With this background, a new phase I clinical trial in healthy subjects, the APTABOLUS trial (ClinicalTrials.gov: NCT05569720), was conducted to compare the safety of ApTOLL when administered at the current 30 min slow i.v. infusion vs. administration as a single i.v. bolus injection. The final objective was to check whether the change of the route of administration is safe and does not affect the pharmacokinetic properties of ApTOLL infusion in order to facilitate clinical practice and the patient's well-being.

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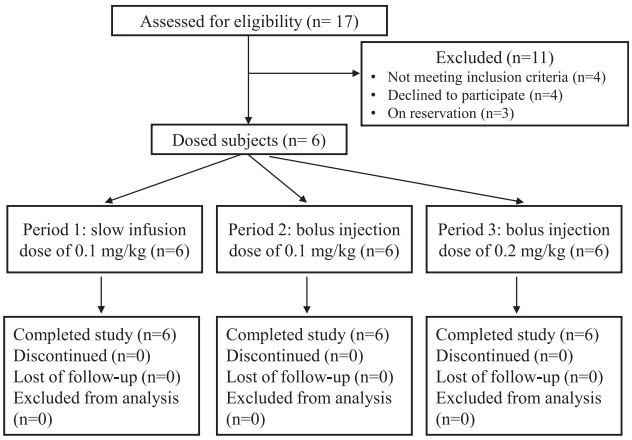

Figure 1. Subject disposition in the APTABOLUS clinical trial
For details, see materials and methods.

RESULTS

Demographics

From the initial 17 subjects screened, 14 signed the informed consent form, and 3 did not. Among them, 4 subjects were excluded for not meeting the eligibility criteria, and 1 decided not to participate. Finally, 6 male healthy volunteers (1 White [16.7%] and 5 Latin American [83.3%]) aged between 26 and 55 years (mean 37.3 ± 11.5 years old), with a body mass index (BMI) of 27.5 ± 1.7 kg/m^2 and a body weight of 83.32 ± 9.2 kg, were enrolled (Figure 1).

All subjects included in the trial met the eligibility criteria and received ApTOLL at the doses and administration routes described in the protocol.

Adverse drug reactions

In the context of this clinical study, no adverse events (AEs) or serious AEs (SAEs) attributable to ApTOLL administration were reported. Additionally, no clinically significant laboratory, vital sign, or electrocardiogram (ECG) findings that were considered related to ApTOLL with any route of administration or dose were described. The safety profile was confirmed in the three periods of the study.

During the study, 7 AEs were reported (Table 1). The ApTOLL 0.2 mg/kg dose showed a higher number of AEs than the 0.1 mg/kg dose, which only reported one AE for each type of administration. Some subjects did not show any AEs, and some subjects showed more than one AE. Regarding severity, 85.71% of AEs were mild, 14.29% were moderate, and no AEs were severe. Specific AEs by system organ class (SOC) are included in Table 2. The most frequent AE was headache (66.66%). Additionally, other AEs reported were back pain, alterations in blood creatin kinase (CK), and epistaxis (16.66% each). Alterations in CK were reported in only one subject; the values increased within the context of strenuous physical exercise and decreased to normal values in the next follow-up analysis.

All events were resolved at the end of the trial. The overall event rates as well as the safety profile, as far as described by the assessment of AEs, demonstrated that both routes of administration have a similar safety profile. These results suggest that ApTOLL administered either as a slow infusion or as a bolus injection was equally safe.

Table 1. Overview of AEs in the APTABOLUS trial

Parameter	ApTOLL 0.1 mg/kg slow infusion (N = 6)	ApTOLL 0.1 mg/kg bolus (N = 6)	ApTOLL 0.2 mg/kg bolus (N = 6)
AEs reported, *n*	1	1	5
Subjects with at least one AE, n (%)[a]	1 (16.67%)	1 (16.67%)	4 (66.67%)
AE relationship[b]			
Related [n(%)]	0 (0%)	0 (0%)	0 (0%)
Not Related [n (%)]	1 (100%)	1 (100%)	5 (100%)
AEs by severity/intensity[b]			
Mild, *n* (%)	1 (100%)	1 (100%)	4 (80.00%)
Moderate, *n* (%)	0 (0%)	0 (0%)	1 (20.00%)
Severe, n (%)	0 (0%)	0 (0%)	0 (0%)
SAEs reported[b]			
Subjects with at least one SAE, *n* (%)[a]	0 (0%)	0 (0%)	0 (0%)

N, number of subjects who received a specific treatment.
[a]Percentages are based on the number of subjects in the safety population in each treatment group.
[b]Percentages are based on the total number of TEAEs reported in each treatment group.

Clinical laboratory assessment

Clinically significant analytical alterations did not occur during the screening or at follow-up analyses. The biochemistry and hematology parameters did not show any remarkable difference between the screening and follow-up analysis for the rest of the subjects and periods. All serology analyses were negative.

Regarding specific assessment of complement (terminal complement complex [C3 and C4] and CH50 [50% hemolytic complement]) and coagulation (prothrombin activity [PT] and activated partial thromboplastin time [aPTT]) parameters, no effect was detected after the treatment at any study period.

Pharmacokinetic analysis

Results from this study show that the means of the primary endpoints of ApTOLL for bioequivalence study were similar after ApTOLL 0.1 mg/kg bolus injection and after ApTOLL 0.1 mg/kg as a slow infusion: differences were −3.63% in $AUC_{0\text{-}t}$ and −5.53% in C_{max} of bolus injection respect to slow infusion. In addition, when comparing ApTOLL 0.2 mg/kg bolus injection and ApTOLL 0.1 mg/kg slow infusion, differences increase to 15.55% and 41.24% for $AUC_{0\text{-}t}$ and C_{max}.

T_{max} (time to reach C_{max}) for ApTOLL 0.1 mg/kg single bolus injection and ApTOLL 0.2 mg/kg single bolus injection was 0.17 (0.17–0.50) and 0.10 (0.03–0.17), respectively.

Table 2. Summary of AEs by SOC

System organ class (SOC) MedDRA preferred term (PT)	ApTOLL 0.1 mg/kg slow infusion ($N = 6$) n (%)	ApTOLL 0.1 mg/kg bolus ($N = 6$) n (%)	ApTOLL 0.2 mg/kg bolus ($N = 6$) n (%)
Headache	0 (0%)	1 (100%)	3 (60%)
Blood creatine phosphokinase increased	(0%)	0 (0%)	1 (20.00%)
Back pain	1 (100%)	0 (0%)	0 (0%)
Epistaxis	0 (0%)	0 (0%)	1 (20%)
TOTAL GENERAL	1	1	5

Each treatment-emergent AE was counted only once for each subject within each SOC and MedDRA PT.

Table 3. Main pharmacokinetic parameters obtained in the APTABOLUS clinical trial

Period	1 (0.1 mg/kg, slow infusion) ($n = 6$)	2 (0.1 mg/kg, bolus injection) ($n = 6$)	3 (0.2 mg/kg, bolus injection) ($n = 6$)
$AUC_{(0\text{-}t)}$ (h*ng/mL) ± SD	27,673.13 ± 3,105.44	26,669.43 ± 4,249.40	31,976.75 ± 4,121.29
$AUC_{(0\text{-}\infty)}$ (h*ng/mL) ± SD	28,609.12 ± 3,198.70	27,560.18 ± 4,653.64	32,939.91 ± 3,920.66
C_{max} (ng/mL) ± SD	2,062.82 ± 261.89	1,948.58 ± 183.17	2,913.65 ± 722.45
T_{max} (h) ± SD	0.51 ± 0.3	0.22 ± 0.14	0.10 ± 0.07
$t_{1/2}$ (h) ± SD	9.62 ± 0.91	9.51 ± 1.44	9.50 ± 0.98
Cl (mL/h) ± SD	3.53 ± 0.39	3.72 ± 0.68	6.15 ± 0.75
Vd (mL) ± SD	48.95 ± 6.43	50.07 ± 4.70	84.91 ± 17.79

$AUC_{(0\text{-}t)}$, area under the plasma concentration curve vs. time between 0 and last detected concentration; $AUC_{(0\text{-}\infty)}$, area under the plasma concentration curve vs. time between 0 and infinity; C_{max}, maximum concentration; T_{max}, time to reach C_{max}; $t_{1/2}$, biological half-life; Cl, clearance; Vd, distribution volume.

The main pharmacokinetic parameters are summarized in Table 3 and Figure 2. These results demonstrate bioequivalence between ApTOLL 0.1 mg/kg slow infusion vs. APTOLL 0.1 mg/kg bolus injection, since the ratio and 90% confidence interval (CI) for C_{max} (94.73% and 80.04%–112.12%) and AUC (95.82% and 83.15%–110.15%) were within the limits recommended by European Medicines Agency (EMA) guidelines.[9] Nevertheless, the comparison between ApTOLL 0.1 mg/kg and 0.2 mg/kg showed no bioequivalence.

For this reason, we conducted a theoretical comparison between the results obtained with the 14 mg (around 0.2 mg/kg) dose in the ApTOLL-FIH-01 trial (infusion) and the APTABOLUS trial (bolus injection). The pharmacokinetic parameters suggest that, even though it is a theoretical approach, pharmacokinetic parameters are comparable using both routes of administration. As with the 0.1 mg/kg dose, C_{max} (100.5% ratio and 81.18%–124.41% CI 90%), and AUC (85.2% ratio and 71.70%–101.28% CI 90%) would be within the recommended limits.

DISCUSSION

The application of ApTOLL to improve functional outcome after diseases with a high inflammatory component has so far been demonstrated in animal studies[1–5] and confirmed in clinical trials, both in healthy subjects (ApTOLL-FIH-01)[6] and stroke patients (APRIL trial).[7] The APRIL trial demonstrated that ApTOLL at 0.2 mg/kg infusion (30 min) was able to reduce mortality, infarct volume, and disability, improving patient quality of life in the long term.[7] To avoid the possibility of having any issue with the current route of administration of ApTOLL (30 min infusion) in the context of critical acute stroke units, where "time is brain," it has been proposed to administer ApTOLL in a faster way. For that reason, here we conducted a new phase I clinical trial, APTABOLUS, to compare ApTOLL safety and pharmacokinetics of different routes of administration (bolus injection vs. slow infusion) in healthy subjects.

Results obtained in this study confirm the safety of the drug, which has already been demonstrated in several clinical and preclinical contexts. ApTOLL, whether administered as an infusion or as a bolus, does not induce any AEs related to its administration, and no serious AEs have been reported in healthy subjects so far, with headache the most commonly AE identified. These results are in accordance with the fact that ApTOLL is an unmodified aptamer. Even though aptamers have caused increases in plasma coagulation,[10,11] hematological alterations[12] and complement activation,[13] those alterations have always been reported to be related to the target nature or after the administration of modified aptamers (i.e., PEGylation). However, unmodified aptamers, as is the case with ApTOLL, are considered really safe molecules.[14,15] In this work, absence of safety issues after ApTOLL administration is confirmed. Also, in order to confirm the absence of specific alterations described for modified aptamers, we carefully assessed coagulation and complement reactions, and no coagulation, hematology, or complement activation were reported at any study period.

In addition, the results obtained in this trial showed that, as in the previous ApTOLL-FIH-01 study, ApTOLL shows a half-life in plasma of 9.5 h, with C_{max} detected immediately after the injection and rapid clearance during the following hours and almost undetectable 48 h after administration. Importantly, the pharmacokinetic profile detected in the three periods of administration (infusion and bolus and lower/higher doses) showed the same behavior. These results demonstrate bioequivalence between ApTOLL 0.1 mg/kg slow infusion vs. APTOLL 0.1 mg/kg bolus injection since the 90% CI for C_{max} and AUC was within the limits recommended by EMA guidelines.[9] Nevertheless, the comparison between ApTOLL 0.1 mg/kg and 0.2 mg/kg showed no bioequivalence, as expected, because there are no linear pharmacokinetics, as shown in the FIH study. For this reason, a theoretical comparison between ApTOLL 0.2 mg/kg as a bolus injection and slow infusion was conducted, using the results reported in the ApTOLL-FIH-01 clinical trial. Results of this analysis also suggested a bioequivalence of both ways of administration. ApTOLL has been designed for acute indications to reduce the acute inflammatory response after the insult, avoiding interference with the reparative

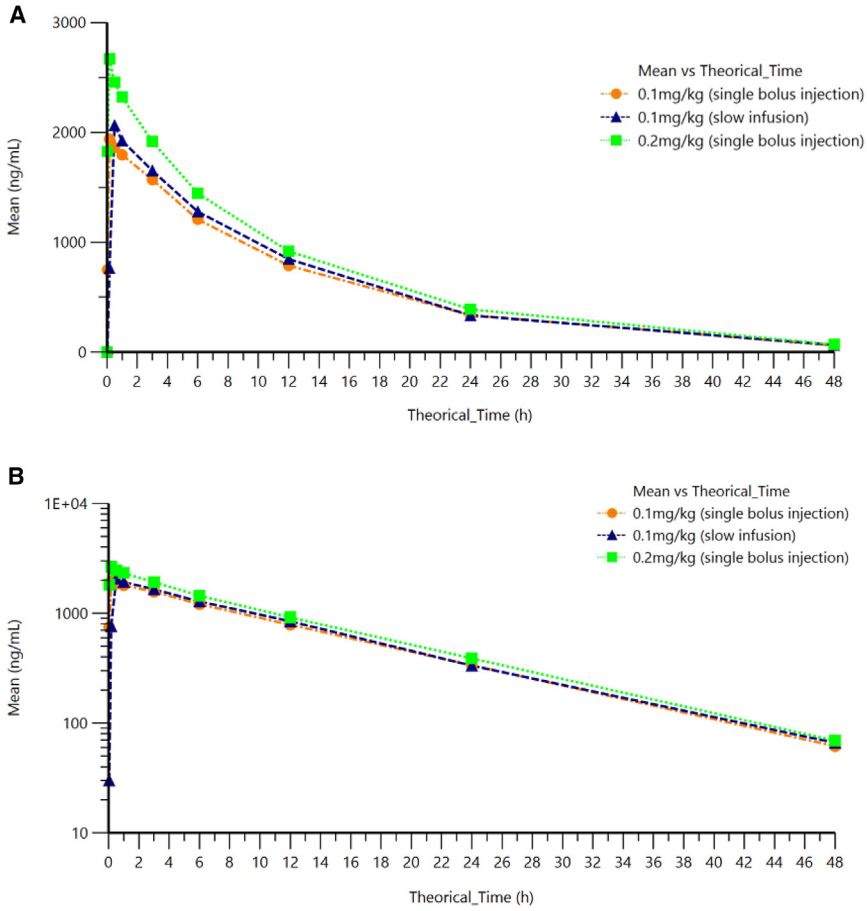

Figure 2. ApTOLL plasma concentrations vs. time after administration in the APTABOLUS clinical trial

Shown are means of the concentrations in lineal and semilogarithmic scale from dosing to 48 h after dosing. (A) Linear plot of plasma concentrations (ordinate) vs. time (abscissa). (B) Semilogarithmic plot of plasma concentrations (ordinate) vs. time (abscissa).

and proliferative phase of the inflammation. In this context, ApTOLL, administered as a slow infusion, has demonstrated a beneficial effect, reducing inflammation in patients after ischemic stroke. The results obtained in the APTABOLUS clinical trial support that ApTOLL shows bioequivalence using infusion or bolus at the 0.1 mg/kg dose. Additionally, our results also suggest that bioequivalence is also possible with the 0.2 mg/kg dose with both routes of administration when compared with the previous data reported in the ApTOLL-FIH-01 clinical trial.

Altogether, the results obtained in the present trial support that a change of the route of administration of ApTOLL from the current slow i.v. infusion to the proposed i.v. bolus injection could be done safely and while preserving the exceptional pharmacokinetic properties for stroke treatment. This would accelerate the management of patients in the context of the critical acute stroke units, facilitating the dosing and improving the clinical practice and patient well-being.

MATERIALS AND METHODS

Study design

This was an open-label phase I clinical trial to compare safety and tolerability between i.v. slow infusion (30 min) and bolus injection (1–3 min) of ApTOLL in healthy subjects (study code: APTABOLUS).

The study was registered on EudraCT (EudraCT: 2021-006871-40) and ClinicalTrials.gov (ClinicalTrials.gov: NCT05569720).

Before starting the trial, all documentation was submitted to the medical ethics committee (La Princesa Hospital, Madrid, Spain) 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 on Good Clinical Practice (CPMP/ICH/135/95), and the current applicable Spanish legislation regarding clinical trials.

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

The primary objective of the study was to evaluate the safety and tolerability of two routes of ApTOLL administration: i.v. infusion vs. bolus i.v. injection. As secondary objective, the pharmacokinetic parameters of ApTOLL $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}\infty)}$ (AUC vs. time between 0 and infinity), C_{max}, T_{max}, $t_{1/2}$ (biological half-life), Cl (clearance), and Vd (distribution volume) were evaluated.

Volunteers were allocated to only one group of 6 volunteers. The study was divided into three admission days with 1 week of washout between them, allowing drug clearance, based on the pharmacokinetic results obtained in previous clinical trials.[6,7] During the first 2 admission days, the volunteers received a dose of ApTOLL of 0.1 mg/kg administered i.v.ly as a slow infusion (30 min) in the first period and as a single i.v. bolus injection (1–3 min) in the second period. On the third and last admission day, subjects received a dose of 0.2 mg/kg of ApTOLL administered as a single i.v. bolus injection (1–3 min). Subjects received ApTOLL at the appropriate concentration by dilution of the aptamer in water for injection, followed by dilution in saline buffer (50 mL). All subjects were confined at the clinical trials unit of the Hospital Universitario de La Princesa from the day before drug administration (days 0, 7, and 14) until 24 h after drug administration.

The main inclusion criteria were as follows: male or female subjects (women without a 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 and BMI between 18.5 and 30.0 kg/m^2) with clinical history and physical examination with values within normality (including vital signs and ECG), and no clinically significant abnormalities in hematology, coagulation, biochemistry, serology and urine tests.

Dose selection

Doses of 0.1 mg/kg and 0.2 mg/kg were chosen for this study, considering the doses administered to AIS patients in the APRIL trial.[7]

Physical examination and vital signs

Blood pressure, ECG recordings, and heart rate (HR) were obtained during screening and the safety follow-up visit and at each period of the study. Tympanic temperature was measured at the screening, in each period, and at the follow-up visit.

AEs

During the study, all untoward events were recorded, including AEs after study drug administration that affected the study participants regardless of their relationship to the study medication. These untoward events were described temporarily and coded according to the latest available version of MedDRA (Medical Dictionary for Regulatory Activities) (at the time of coding/reporting).

Blood and urine analyses

Blood and urine samples from subjects were obtained at screening and at follow-up visits.

Hemogram, biochemistry, and urine were analyzed at screening and on day 3 of each period. Serology (HIV, hepatitis B, and hepatitis C) was done at the screening visit. A urine drug abuse test (cannabinoids, cocaine, opiates, and amphetamines) was performed at screening and at follow-up.

Additionally, for deeper control of the aptamer's safety, CK, C-reactive protein, coagulation (PT and aPTT), and complement factors (C3, C4, and CH50) were determined at screening and on day 3 of each period.

Pharmacokinetic determinations

For the pharmacokinetic analysis, plasma concentrations of ApTOLL were determined at the following times: 0.00 h (pre-dose) and 0.033, 0.166, 0.5, 1, 3, 6, 12, 24, and 48 h after drug administration. Blood samples were collected in 4 mL EDTA tubes by direct venipuncture. A different venous access point was used for drug administration and blood sampling. Within 30 min of being collected, samples were centrifuged at 1,900 × *g* for 10 min at 2°C–8°C to obtain the plasma. Plasma samples were stored at −80°C ± 15°C until analysis.

ApTOLL plasma concentrations were measured using a validated (according to good laboratory practices and EMA bioanalytical method validation guidelines) dual hybridization assay at Axolabs (Kulmbach, Germany). $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}\infty)}$, C_{max}, T_{max}, $t_{1/2}$, Cl, and Vd were calculated by WinNonLin.

Statistical methods

There were no withdrawals, and all subjects completed the study. All of them were considered valid for the assessment of safety and pharmacokinetics.

For the analysis of the bioequivalence of the different ways of ApTOLL administration (slow infusion vs. bolus injection), the primary endpoint was $AUC_{0\text{-}t}$ and C_{max} calculated from the plasma concentrations of ApTOLL. The T_{max} of ApTOLL was also taken into account. The total $AUC_{0\text{-}t}$ was calculated between the previous time and the first with detectable concentrations and the last with detectable concentrations, calculated using the linear trapezoidal method. A non-compartmental model was used to calculate the Vd, the half-life, and the drug's Cl. C_{max} and T_{max} were obtained directly from the plasma concentration information.

The statistical analysis was done using Microsoft Excel and WinNonLin Professional Edition (current version; Pharsight, Cary, NC, USA).

Finally, the Spanish Pharmacovigilance System algorithm was used to evaluate the relationship between the AEs and the treatment (causality determination).[16]

DATA AND CODE AVAILABILITY

Data supporting this study are included within the article and/or supplemental information.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.H.-J. and F.A.S.; methodology, S.M.-V., F.A.S., D.O., M. Román, and M.H.-J.; investigation S.M.-V., G.M.-A., D.O., D.P.R., M.R., S.L.-B., and M.H.-J.; formal analysis, G.M.-A.; writing – original draft, M.H.-J.; writing – review and editing, M.H.-J. and F.A.S.; resources, F.A.S. and D.O.; supervision D.O., F.A.S., M. Ribó, and M.H.J.

DECLARATION OF INTERESTS

M.H.-J. and D.P.R. are employees of aptaTargets S.L. M. Ribó receives payment from Philips as co-principal investigator of the WE TRUST study and has a consulting agreement with Medtronic, Stryker, Cerenovus, CVAid, Methinks, Anaconda Biomed, and 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, Farmalíder, 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 applications WO2015197706 A1, WO2020/230108 A1, and WO2020/230109 A1 and their extensions to different countries.

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