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ORIGINAL ARTICLE

A randomized, double blind, single dose, comparative study of the pharmacokinetics, safety and immunogenicity of MB02 (bevacizumab biosimilar) and reference bevacizumab in healthy male volunteers

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Aims: The pharmacokinetic (PK) similarity between MB02, a proposed bevacizumab biosimilar, and reference bevacizumab approved from the USA (US-bevacizumab) and European Union (EU-bevacizumab) was evaluated. Safety and immunogenicity were also assessed.

Methods: In this phase 1, randomized, double blind, single dose, parallel group study, 114 healthy male volunteers were randomized 1:1:1 to receive a 3 mg/kg intravenous dose of MB02, US-bevacizumab or EU-bevacizumab, and evaluated for 100 days. PK similarity between MB02 and reference bevacizumab was determined using the standard bioequivalence criteria (0.80–1.25) for the area under the serum concentration–time curve from time 0 extrapolated to infinity ($AUC_{(0-\infty)}$) and the maximum observed serum concentration (C_{max}).

Results: Baseline demographics were similar across treatment groups. All study drugs exhibited similar PK profile. The 90% confidence interval for the geometric lead square means ratios for the primary parameters $AUC_{(0-\infty)}$ and C_{max} for MB02, US-bevacizumab and EU-bevacizumab were fully contained within the pre-defined bioequivalence limits for the 3 pairwise comparisons: $AUC_{(0-\infty)}$ (MB02: US-bevacizumab 0.998 [0.944 to 1.05]; MB02:EU-bevacizumab 1.07 [1.00 to 1.14]; and US-bevacizumab:EU-bevacizumab 0.934 [0.884 to 0.988]) and C_{max} (MB02: US-bevacizumab 0.983 [0.897 to 1.08]; MB02:EU-bevacizumab 1.06 [0.976 to 1.16]; and; US-bevacizumab: EU-bevacizumab 0.926 [0.851 to 1.01]). Treatment emergent adverse events were reported in 87 subjects (76.3%), most being mild and with comparable incidence among treatment groups. Thirty-three subjects (28.9%) reported 56 possibly related treatment emergent adverse events with comparable incidence across treatments, the most frequent being headache (10.5%) and fatigue (3.5%). Anti-drug antibody incidence was low and similar between treatment groups.

The authors confirm that the Principal Investigator for this paper is Dr Angela Sinn and that she had direct clinical responsibility for healthy volunteers.

Conclusions: This study demonstrates the PK similarity and bioequivalence of MB02 to the reference bevacizumab, whether approved from USA or EU. The safety and immunogenicity profile of MB02 was shown also to be similar to the bevacizumab reference product (NCT 04238663).

KEYWORDS
bevacizumab, biosimilars, MB02, pharmacokinetics, safety

1 | INTRODUCTION

Bevacizumab is a recombinant humanized immunoglobulin G1 monoclonal antibody that inhibits angiogenesis by binding to vascular endothelial growth factor (VEGF) and preventing its interaction with VEGF receptors on the surface of endothelial cells.[1,2]

Bevacizumab (Avastin®) was initially approved for treatment of metastatic colorectal cancer by the US Food and Drug Administration in 2004 and by the European Medicines Agency in 2005; since then, a wide range of oncology indications has been approved worldwide.[3,4]

This monoclonal antibody has been used in clinical practice for >15 years, being 1 of the first targeted therapies and the first approved angiogenesis inhibitor. Bevacizumab can be considered the most extensively characterized antiangiogenic treatment; it has demonstrated clinical benefits in terms of prolongation of progression-free and overall survival for patients with advanced cancer.[5] Currently, the access to this treatment option is limited by the high cost of the treatment; the arrival of bevacizumab biosimilars may mitigate cost barriers for patients and increase access to an important therapy in oncology.[5]

In the development of biosimilars, guidance issued by the Food and Drug Administration and the European Medicines Agency specifies that biosimilars should be highly similar to the reference product with respect to quality attributes, notwithstanding minor differences in clinically inactive components. In addition, there should be no meaningful clinical differences with respect to the safety, purity and potency.[3,4] This begins with demonstrating analytical and bio-functional similarity to the reference product. The next step involves demonstrating that the pharmacological profile, including pharmacokinetic (PK) and pharmacodynamic activity, is comparable between the proposed biosimilar and the reference product. The final step is demonstrating clinical similarity with respect to efficacy, safety and immunogenicity in a sensitive population at the same approved dosage and route of administration as the reference product.

MB02 is a proposed biosimilar to the reference product bevacizumab and it has been developed by mAbxience Research SL following the recommendations of the existing guidelines for biosimilar products.[6–9] MB02 has demonstrated its high similarity to the reference bevacizumab through an extensive physicochemical and functional characterization, which included primary structure, higher order structure, biological activity and binding affinity to VEGF. Once biosimilarity in vitro between MB02 and the reference bevacizumab has been established, the next step in the MB02 development is to complete the evaluation of similarity with a comparative assessment of the PK similarity in a sensitive population.

The objective of this phase I study was to provide evidence for PK similarity (bioequivalence), of the proposed biosimilar MB02, as part of the comparability exercise, by comparing the PK profiles of MB02 with reference bevacizumab (USA licensed [US-bevacizumab]) or European approved [EU-bevacizumab]) in a population of healthy male subjects. The study also aims to demonstrate a similar safety and immunogenicity profile of MB02 to reference bevacizumab in this population of subjects.

What is already known about this subject

- Bevacizumab is a recombinant humanized monoclonal antibody that inhibits angiogenesis contributing to a reduction of the tumour growth and progression.
- By demonstrating high similarity with the reference medicine, a biosimilar can largely rely on the efficacy and safety experience gained with the reference medicine.

What this study adds

- This study demonstrates pharmacokinetic similarity in healthy male volunteers between MB02 and the reference bevacizumab, as well as comparable safety and immunogenicity profiles.
- The demonstration of the pharmacokinetic equivalence of MB02 to its reference product is a pivotal step that contributes to obtain the totality of evidence for biosimilarity.

2 | MATERIALS AND METHODS

2.1 | Nomenclature classification

Bevacizumab (rhuMAb-VEGF), acts by binding to and blocking the activity of VEGF, thereby impeding angiogenesis and inhibiting tumour growth. This factor belongs to the family of ligands according

to the International Union of Basic and Clinical Pharmacology and the British Pharmacological Society (IUPHAR/BPS) Guide to Pharmacology nomenclature classification.[10,11]

2.2 | Study population

Eligible participants were healthy male subjects of any race aged between 18 and 55 years, with a total body weight between 60 and 95 kg and a body mass index between 18.5 and 29.9 kg/m^2, in good health in general, determined by no clinically significant findings from medical history, physical examination, 12-lead electrocardiogram (ECG), vital sign measurements and clinical laboratory evaluations (haematology, coagulation, urinalysis and clinical chemistry). Key exclusion criteria included a known history of clinically significant essential hypertension, orthostatic hypotension, fainting spells or blackouts for any reason, cardiac failure or history of thromboembolic events, having received any other antibody or protein targeting VEGF or the VEGF receptor and with current/previous evidence or history of clinically significant disease, as well as significant hypersensitivity, intolerance or allergy to any drug compound.

2.3 | Study design

This was a Phase 1, randomized, double blind, single dose, parallel group study performed in 1 centre located in Berlin, Germany, between September 2019 and March 2020 (NCT04238663). To comply with regulatory requirements a 3-treatment group study was designed that compared MB02 to locally approved reference bevacizumab in the main regulatory regions (the USA and Europe).[3,4] The study was conducted in compliance with the ethical principles of the Declaration of Helsinki, International Council for Harmonization Good Clinical Practice Guideline (E6), and local regulatory requirements. All subjects provided written informed consent before any screening procedures were done according to local ethical committee regulations.

Subjects were admitted to the Early Phase Clinical Unit (EPCU) on day −1, the day before dose was administered, and remained at the EPCU until discharge on day 8. Prior to dosing on day 1, eligible subjects were stratified into 2 groups based on weight (stratum 1: ≥60 to <77.5 kg and stratum 2: ≥77.5 to ≤95.0 kg) then randomly assigned according to a computer-generated randomization schedule to 1 of the 3 treatment groups in a 1:1:1 ratio (Figure 1). Except for the pharmacist, who was in charge to prepare the medication, all subjects involved into the study including, investigators, nurses or sponsor were blinded to the assigned treatment.

On day 1, subjects were randomized to receive a single intravenous (IV) infusion (3 mg/kg) over 90 min of MB02, US-bevacizumab or EU-bevacizumab (3 mg/kg). After discharge on day 8, subjects returned on days 10, 14, 21, 28, 42, 56, 78 and 100 for nonresidential visits for the collection of PK/immunogenicity samples (when applicable) and safety assessments.

2.4 | Study objectives and endpoints

The primary objective of this study was to establish bioequivalence between MB02, US-bevacizumab and EU-bevacizumab by comparing the primary PK endpoints: area under the serum concentration–time curve from time 0 extrapolated to infinity ($AUC_{(0-\infty)}$); and maximum observed serum concentration (C_{max}). Derived PK parameters not covered by the primary endpoint include the following: the time of maximum observed serum concentration (t_{max}); the AUC from time 0 to the time of the last observable concentration ($AUC_{(0-t)}$); total body clearance of drug after IV administration (CL); serum terminal elimination half-life ($t_{1/2}$); percentage of AUC that is due to extrapolation from the last quantifiable concentration to infinity ($\%AUC_{extrap}$); elimination rate constant of the terminal phase (kel); volume of distribution during the terminal phase after IV administration (V_z); and volume of distribution at steady-state after IV administration (V_{ss}). Safety and immunogenicity profiles for MB02, US-bevacizumab and EU-bevacizumab were also evaluated and compared as secondary endpoints.

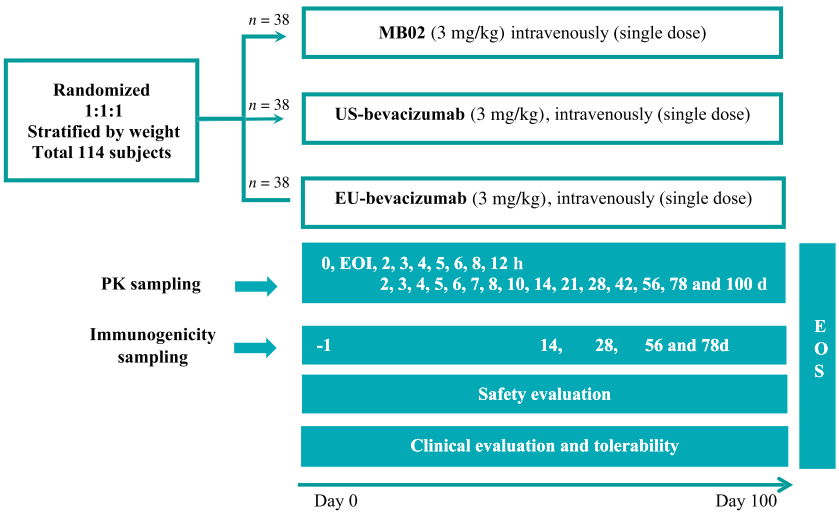

FIGURE 1 Study design. US = US licensed; EU = European approved; EOS = end of study; EOI = end of infusion; PK= pharmacokinetic; *n* = number of subjects in the analysis population

2.5 | PK evaluation

Blood samples were collected by venepuncture or cannulation at time 0 (pre-dose), end of infusion, 2, 3, 4, 5, 6, 8 and 12 hours as well as days 2, 3, 4, 5, 6, 7, 8, 10, 14, 21, 28, 42, 56, 78 and 100 after the start of infusion for the measurement of serum concentrations of bevacizumab. All PK endpoints were determined from the individual serum bevacizumab concentration–time profiles obtained following single dosing by noncompartmental analysis using the validated software programme, Phoenix WinNonlin (Certara USA, Inc. Version 8.1).

Serum bevacizumab concentrations, EU-bevacizumab, US-bevacizumab and MB02, were determined using a validated quantitative enzyme-linked immunosorbent assay method.

Briefly, VEGF was coated on a 96-well microtitre plate, and then blocked using a nonspecific protein. MB02 was used to prepare standards and quality controls, this was then added to designated sample wells. The assay was visualized by the subsequent additions of anti-human IgG1-HRP and a chromogenic substrate (Tetramethylbenzidine), and the product of this reaction was detected with a spectrophotometer (450 nm detection and 630 nm reference wavelengths). The concentration of US-bevacizumab, EU-bevacizumab, or MB02 in samples was then back-calculated from a MB02 calibration curve.

Samples, standards and controls were required to be subjected to a minimum required dilution of 1 in 10 in low cross buffer prior to analysis. Calibration curve fit: 4-PL weighted (1/Y2). The assay measured free drug concentrations in human serum, and the lower limit of quantification for the method was 400.00 ng/mL.

Acceptable interassay precision (IAP) and interassay accuracy (IAA) were calculated from quality controls (quality controls, lower limit of quantification, low, medium and high quality control sample and upper limit of quantification) in 12 validation runs for MB02 (IAP ≤ 6.2% and IAA ≤ +13.1%), and in 6 validation runs (each) for US-bevacizumab (IAP ≤ 8.7% and IAA ≤ +3.5%) and EU-bevacizumab (IAP ≤ 7.6% and IAA ≤ +6.3%). The performance of the method during the sample analysis study was also acceptable (IAP ≤ 13.5% and IAA ≤ +1.1%). Incurred sample reanalysis demonstrated reproducibility of drug concentrations in study samples.

2.6 | Safety evaluation

Subjects were monitored for treatment emergent adverse events (TEAEs) throughout the study. Subjects who had an unresolved TEAE were followed up until the TEAE or its sequelae resolved or stabilized per the investigator assessment. AEs were coded using the Medical Dictionary for Regulatory Activities (version 22.0), graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0) and were assessed for severity and relationship to the study drug treatment.

Other safety assessments included clinical laboratory tests (haematology, biochemistry, coagulation and urinalysis), vital signs, 12-lead ECG and physical examination. All patient-reported AEs were analysed in the safety population.

2.7 | Immunogenicity evaluation

The immunogenicity measurements of MB02, US-bevacizumab and EU-bevacizumab were determined in serum. The blood samples were collected by venepuncture or cannulation at the day −1 and postdose samples for anti-drug antibodies (ADA) and neutralizing antibodies (NAb), collected at days 14, 28, 56, 78 to determine the incidence of treatment-induced antibodies.

A validated semi-quantitative immunoassay was used for the detection, confirmation and titration of anti-MB02 and anti-bevacizumab antibodies in human serum samples collected from study subjects.

The immune response was evaluated by a 3-tiered approach, which comprised an immunogenicity assay for the screening, confirmation, and titration. All samples were subjected to an initial screening assay (Tier 1), and those falling above a specific predetermined screening cut-point were tested in the confirmation assay (Tier 2). Samples that confirmed positive in the confirmatory assay were deemed positive and further analysed in the titre tier (Tier 3), and for the presence of neutralizing antibodies.

The ADA assay used a ADA bridging format with acid dissociation. The ADA/drug complexes were acid dissociated to release any anti-bevacizumab antibodies complexed with free drug, which were then neutralized with neutralization buffer containing VEGF R1 to mitigate VEGF interference, and captured with biotinylated and sulfo-tagged MB02-labelled material. The antibody–bridge complexes were bound to a streptavidin-coated plate, and the chemiluminescent signal was read on a Meso Scale Discovery (MSD; electrochemiluminescence) platform. Assay sensitivity was 20.0 ng/mL (without drug, low positive control) with a drug tolerance of 200.0 μg/mL at 100.0 ng/mL ADA. The overall IAP for positive control samples was ≤12.4%.

A validated qualitative ligand binding assay was used to detect neutralizing anti-MB02/bevacizumab antibodies in human serum using streptavidin magnetic beads and read on the MSD platform. The signal produced was inversely proportional to the concentration of neutralizing anti-MB02/bevacizumab antibodies present.

2.8 | Statistical methods

In the assessment of bioequivalence, MB02 was the test treatment and US-bevacizumab and EU-bevacizumab were the reference treatments. The PK parameters ($AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and C_{max}) were log transformed (base e) prior to analysis and were analysed using an analysis of covariance (ANCOVA) model,[12] with body weight as covariate to account for potential body weight differences, which is known to be a PK altering factor. Estimates of adjusted mean difference and the confidence intervals (CIs) for the differences obtained from the variance model were exponentiated to provide estimates of the ratio of adjusted geometric means and the CIs for the ratios. PK similarity for MB02 was considered demonstrated to US-bevacizumab and EU-bevacizumab if the 90% CIs for the test-to-reference ratios for the primary endpoints ($AUC_{(0-\infty)}$ and C_{max}) were within the bioequivalence interval of 0.80–1.25.[3,4]

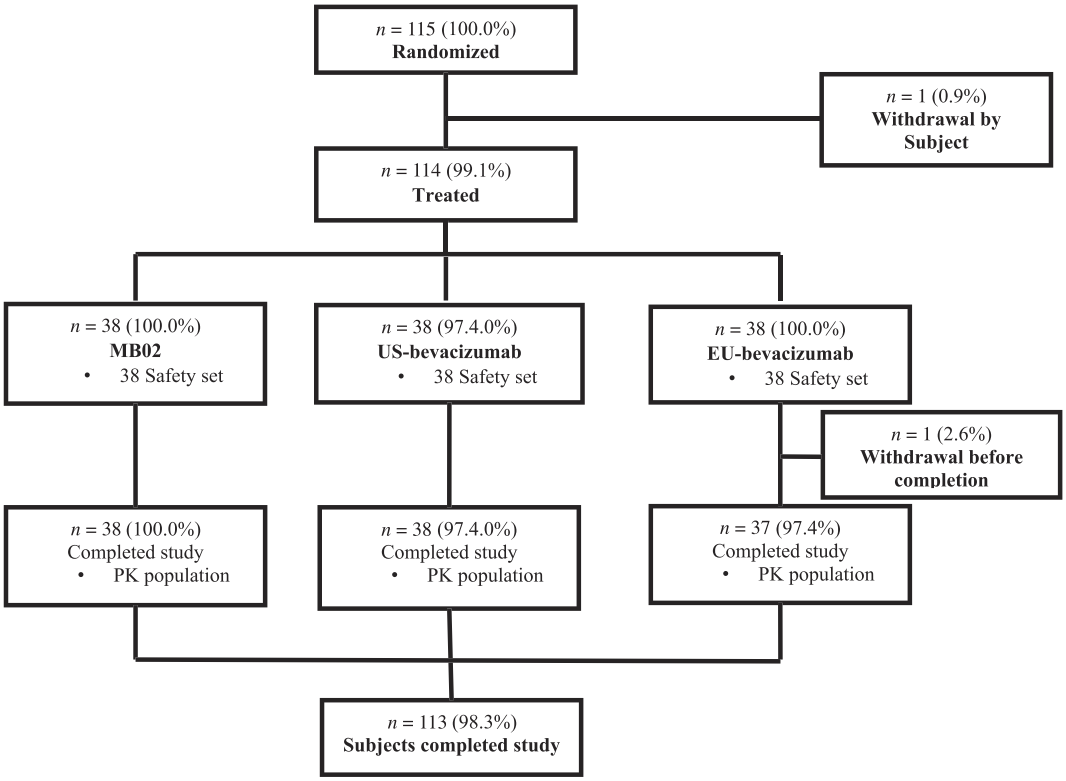

FIGURE 2 Study participant flow (Pharmacokinetic population). US = US licensed; EU = European approved; PK= pharmacokinetic, *n* = number of subjects in the analysis population. Percentages are based on the number of subjects (*n*) in the randomized population

TABLE 1 Summary of demographic characteristics by treatment arm and overall (safety population)

Characteristics	MB02 *N* = 38 (%)	US-bevacizumab *N* = 38 (%)	EU-bevacizumab *N* = 38 (%)	Overall *N* = 114 (%)
Race, *n* (%)				
White	36 (94.7)	37 (97.4)	35 (92.1)	108 (94.7)
Asian	1 (2.6)	1 (2.6)	2 (5.3)	4 (3.5)
Other	1 (2.6)	0	1 (2.6)	2 (1.8)
Mixed: White/black	0	0	1 (2.6)	1 (0.9)
Mother: German; father: African	1 (2.6)	0	0	1 (0.9)
Ethnicity, *n* (%)				
Hispanic or Latino	0	1 (2.6)	1 (2.6)	2 (1.8)
Not Hispanic or Latino	38 (100.0)	37 (97.4)	37 (97.4)	112 (98.2)
Age (y)				
Mean (SD)	39.4 (10.18)	38.3 (9.64)	41.6 (11.10)	39.8 (10.33)
Height (cm)				
Mean (SD)	180.1 (7.25)	178.6 (5.97)	179.5 (5.17)	179.4 (6.16)
Weight (kg)				
Mean (SD)	79.38 (9.56)	79.10 (9.29)	79.36 (9.03)	79.28 (9.21)
BMI (kg/m^2)				
Mean (SD)	24.48 (2.72)	24.80 (2.71)	24.59 (2.32)	24.62 (2.57)

BMI = body mass index; *N* = number of subjects in the analysis population; *n* = number of subjects within the category; SD = standard deviation. BMI (kg/m^2) = weight (kg)/(height [m])^2. Percentages were based on the number of subjects (*N*) in the safety population.

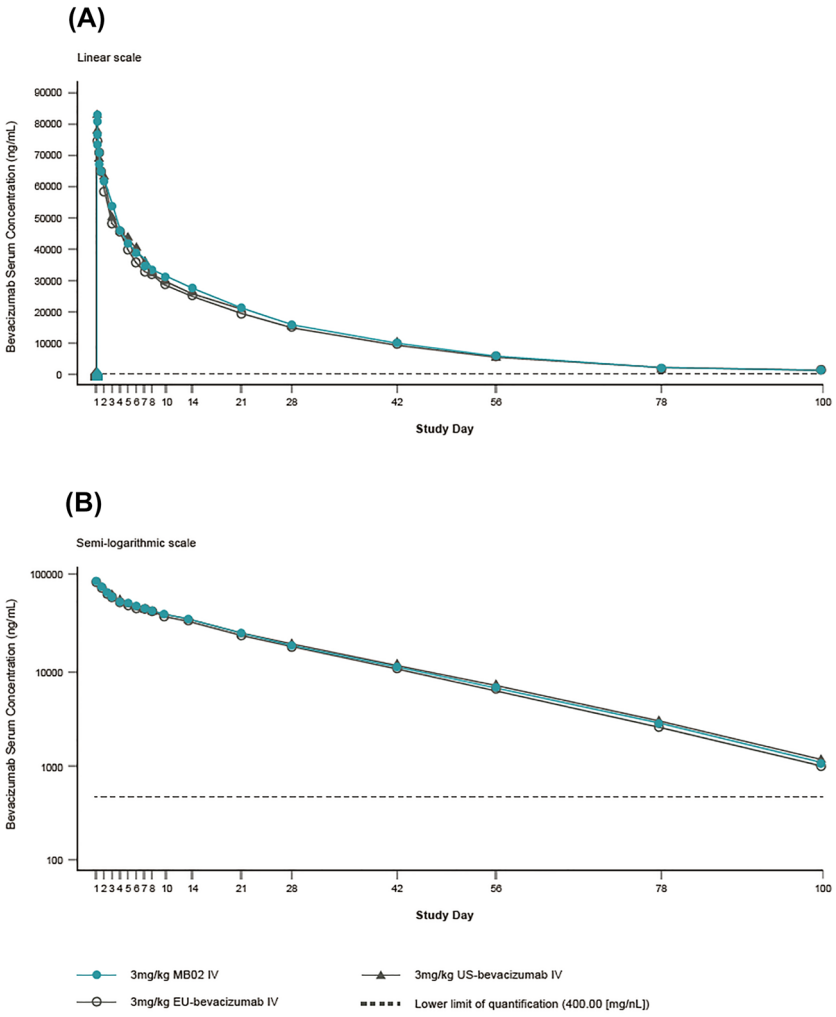

FIGURE 3 Arithmetic mean serum concentration profiles of bevacizumab (across all days) (pharmacokinetic population). Mean serum concentrations versus nominal times on linear (A) and semilogarithmic scale (B) of MB02, EU-bevacizumab, and US-bevacizumab (across all days). US = US licensed, EU = European approved, IV = intravenous

TABLE 2 Summary pharmacokinetic parameters of bevacizumab (pharmacokinetic population)

Parameter	3 mg/kg MB02 IV *n* = 38 (%)	3 mg/kg US-bevacizumab IV *n* = 38 (%)	3 mg/kg EU-bevacizumab IV *n* = 38 (%)
$AUC_{(0\text{-}t)}$ (h ng/mL)	29 900 000 (15.6)	29 900 000 (12.5)	27 900 000 (16.0)[a]
$AUC_{(0\text{-}\infty)}$ (h ng/mL)	30 700 000 (16.2)	30 700 000 (12.9)	28 800 000 (16.4)
$\%AUC_{extrap}$ (%)	2.6 (1.45)	2.8 (1.5)	3.3 (4.6)
C_{max} (ng/mL)	86 100 (24.8)	87 500 (24.9)	81 100 (20.0)
t_{max} (h)	4.0 (1.5; 8.0)	4.0 (1.5, 12.0)	4.0 (1.5, 11.9)
$t_{1/2}$ (h)	443 (16.9)	458 (16.1)	444 (14.5)
k_{el} (1/h)	0.00157 (16.9)	0.00151 (16.1)	0.00156 (14.5)
CL (L/h)	0.00770 (18.1)	0.00766 (16.2)	0.00823 (18.5)
V_z (L)	4.92 (15.6)	5.07 (16.7)	5.28 (18.2)
V_{ss} (L)	4.76 (15.8)	4.87 (16.0)	5.11 (17.9)

AUC = area under the serum concentration–time curve; $AUC_{(0\text{-}t)}$ = AUC from time zero to the time of last quantifiable concentration; $AUC_{(0\text{-}\infty)}$ = AUC from time zero extrapolated to infinity; CL = total body clearance of drug after intravenous administration; C_{max} = maximum observed serum concentration; CV = coefficient of variation; IV = intravenous; kel = elimination rate constant of the terminal phase; t½ = apparent serum terminal elimination half-life; t_{max} = time of maximum observed serum concentration; V_{ss} = volume of distribution at steady state after intravenous administration; V_z = volume of distribution during the terminal elimination phase after intravenous administration; $\%AUC_{extrap}$ = percentage of AUC that is due to extrapolation from the last quantifiable concentration to infinity.

Note: Geometric mean (geometric CV%) results are presented unless otherwise indicated.

Arithmetic mean (SD) is presented for $\%AUC_{extrap}$.

Median (range) is presented for t_{max}.

[a]*n* = 37.

A sample size of 90 subjects provided at least 90% probability of concluding PK similarity for all pairwise comparisons in terms of $AUC_{(0-\infty)}$ and C_{max} using a percent coefficient of variation (CV%) of 25% in both PK parameters for the similarity objective if the true ratio was ≤1.05. This estimate is based on information available on bevacizumab in the published literature.[13] Final sample size was increased to 114 subjects (38 subjects per treatment group) considering ≤5% loss of data due to premature discontinuation.

All PK parameters, as well as secondary safety and immunogenicity parameters were analysed descriptively. For overall incidence of AEs, a variable exploratory statistical treatment comparison was carried out. Additionally, the influence of ADA on PK was also analysed.

The per-protocol analysis set, which included all randomized subjects who received the full dose of the assigned study medication and who did not have major protocol deviations, was used as the population for PK analysis. The safety analysis set included all enrolled subjects who received the study medication.

All statistical analyses were performed using the SAS® Version 9.4. (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

3.1 | Study subjects or participants

A total of 115 healthy male volunteers were enrolled and randomly assigned to 1 of 3 treatment arms: MB02 (38 subjects); US-bevacizumab (39 subjects); and EU-bevacizumab (38 subjects). One subject withdrew before receiving treatment, and therefore 114 subjects received study treatment and 113 subjects completed the study (Figure 2). One (2.6%) EU-bevacizumab subject received study treatment and was included in the safety and PK populations but was lost to follow-up on day 56 and considered not to have completed the study.

Demographic characteristics were well balanced and comparable between the treatment groups. The majority (108 [94.7%]) of subjects were white. The mean age, weight and body mass index were similar for all subjects across all treatment groups: MB02 (39.4 y; 79.38 kg; 24.48 kg/m^2); US-bevacizumab (38.3 y; 79.10 kg; 24.80 kg/m^2); and EU-bevacizumab (41.6 y; 79.36 kg; 24.59 kg/m^2; Table 1).

3.2 | PK

Arithmetic mean serum concentration profiles of bevacizumab following dosing with MB02, US-bevacizumab or EU-bevacizumab across all days were similar (Figure 3). Following IV infusion dosing of MB02, US- or EU-bevacizumab to healthy subjects, median peak serum bevacizumab concentrations (t_{max}) were delayed and occurred at 4.0 hours post the start of infusion. No trend was noted for t_{max} between treatments with similar median values and overlapping ranges. After reaching C_{max}, serum bevacizumab concentrations slowly declined in a biphasic manner for subjects administered with MB02, US- or EU- bevacizumab. All PK parameters were comparable between the treatment groups (Table 2).

For all pairwise comparisons of MB02 *vs.* US-bevacizumab, MB02 *vs.* EU-bevacizumab and US-bevacizumab *vs.* EU-bevacizumab, the 90% CI for the geometric least squares (LS) means ratios for the primary PK parameters ($AUC_{(0-\infty)}$ and C_{max}) were fully contained within the predefined bioequivalence limits of 0.80–1.25. The 90% CIs for the geometric LS means ratio of the secondary endpoint, $AUC_{(0-t)}$, were also fully contained within the predefined bioequivalence limits of 0.80–1.25. Between-subject variability for $AUC_{(0-\infty)}$ and C_{max} was low (<25%) for MB02, US-bevacizumab and EU-bevacizumab treatments with geometric CV% ranging from 12.8 to 24.8% (Table 3). Between-subject variability for the secondary PK parameter $AUC_{(0-t)}$ was also low (<16%) for MB02, US-bevacizumab and EU-bevacizumab treatments with geometric CV% ranging from 12.4 to 16.0% (Table 2).

3.3 | Safety

Overall, 199 TEAEs were reported in 87 (76.3%) subjects. The overall incidence of TEAEs was higher in the EU-bevacizumab group (72 TEAEs) than in the MB02 group (60 TEAEs) and in the US-bevacizumab group (67 TEAEs); while the proportion of subjects

TABLE 3 Statistical analysis of the primary pharmacokinetic parameters of bevacizumab: MB02 *vs.* EU-bevacizumab, MB02 *vs.* US-bevacizumab, EU-bevacizumab *vs.* US-bevacizumab (pharmacokinetic population)

	Ratio of geometric least square means (90% CI)		
Comparison	**C_{max} (ng/mL)**	**$AUC_{(0-t)}$ (h ng/mL)**	**$AUC_{(0-\infty)}$ (h ng/mL)**
MB02: US-bevacizumab	0.983 (0.897–1.08)	1.00 (0.948–1.05)	0.998 (0.944–1.05)
MB02: EU-bevacizumab	1.06 (0.976–1-16)	1.07 (1.01–1.14)	1.07 (1.00–1.14)
US-bevacizumab: EU-bevacizumab	0.926 (0.851–1.01)	0.931 (0.882–0.982)	0.934 (0.884–0.988)

AUC = area under the serum concentration–time curve; $AUC_{(0-\infty)}$ = AUC from time 0 to infinity; CI = confidence interval; C_{max} = maximum observed serum concentration. The PK parameters were log transformed (base e) before analysis and analysed using an ANCOVA model. The model included treatment as a fixed effect and body weight as a covariate. The ratio and corresponding CIs were back transformed from the difference and CIs calculated on the loge scale.

TABLE 4 Overview of adverse events (safety population)

Adverse event by PT	MB02 $N = 38$ n (%) E	US-bevacizumab $N = 38$ n (%) E	EU-bevacizumab $N = 38$ n (%) E	Overall $N = 114$ n (%) E
Any TEAEs	30 (78.9) 60	32 (84.2) 67	25 (65.8) 72	87 (76.3) 199
Diarrhea	0	3 (7.9) 3	2 (5.3) 2	5 (4.4) 5
Fatigue	1 (2.6) 2	2 (5.3) 2	1 (2.6) 1	4 (3.5) 5
Nasopharyngitis	14 (36.8) 17	13 (34.2) 14	12 (31.6) 12	39 (34.2) 43
Rhinitis	4 (10.5) 4	0	0	4 (3.5) 4
Pulpitis dental	2 (5.3) 2	2 (5.3) 2	1 (2.6) 1	5 (4.4) 5
Blood creatine Phosphokinase Increased	3 (7.9) 3	0	5 (13.2) 5	8 (7.0) 8
Aspartate Aminotransferase Increased	1 (2.6) 1	0	2 (5.3) 2	3 (2.6) 3
Back pain	2 (5.3) 2	1 (2.6) 1	6 (15.8) 6	9 (7.9) 9
Myalgia	2 (5.3) 2	1 (2.6) 1	0	3 (2.6) 3
Arthralgia	1 (2.6) 1	1 (2.6) 1	2 (5.3) 3	4 (3.5) 5
Headache	5 (13.2) 6	6 (15.8) 8	9 (23.7) 14	20 (17.5) 28
Sleep disorder	0	0	2 (5.3) 2	2 (1.8) 2
Oropharyngeal pain	3 (7.9) 3	1 (2.6) 1	0	4 (3.5) 4
Cough	2 (5.3) 2	0	0	2 (1.8) 2
Epistaxis	0	1 (2.6) 1	2 (5.3) 4	3 (2.6) 5
Rhinorrhoea	0	2 (5.3) 2	0	2 (1.8) 2
Dry skin	0	2 (5.3) 2	0	2 (1.8) 2
TEAE severity				
Mild	20 (52.6) 46	23 (60.5) 55	10 (26.3) 48	53 (46.5) 149
Moderate	10 (26.3) 14	9 (23.7) 12	14 (36.8) 23	33 (28.9) 49
Severe	0	0	1 (2.6) 1	1 (0.9) 1
TEAE causality				
Not related	16 (42.1) 38	16 (42.1) 34	12 (31.6) 44	44 (38.6) 116
Unlikely related	4 (10.5) 8	4 (10.5) 13	2 (5.3) 6	10 (8.8) 27
Possibly related	10 (26.3) 14	12 (31.6) 20	11 (28.9) 22	33 (28.9) 56
Probably related	0	0	0	0
Related	0	0	0	0

AE = adverse event; E = number of TEAEs; N = number of subjects in the analysis population; n = number of subjects with event; TEAE = treatment emergent adverse event; PT = preferred term. Percentages were based on the number of subjects in the safety population. If a subject had multiple events with different severity (or causality), then the subject was counted only once at the worst severity (or causality) for the number of subjects (N).

with any TEAE in the EU-bevacizumab group (25 subjects; 65.8%) was slightly lower than in the MB02 (30 subjects; 78.9%) and US-bevacizumab (32 patients; 84.2%) groups. Nevertheless, the differences in the proportion of subjects reporting TEAEs in the treatment groups (MB02 *vs*. US-bevacizumab [$P = .554$] and MB02 *vs*. EU-bevacizumab [$P = .200$] were not statistically significant (Table 4).

The majority of TEAEs (149 TEAEs in 53 [46.5%] subjects) were considered mild in intensity, 49 TEAEs in 33 (28.9%) subjects were considered moderate and 1 severe TEAE (an upper respiratory tract infection) was recorded in 1 (0.9%) subject in the EU-bevacizumab treatment group, which was not considered to be treatment related. The most frequently recorded TEAEs were nasopharyngitis and headache, both of which occurred with a similar frequency in all treatment groups.

The largest proportion of TEAEs were not related (116 TEAEs in 44 [38.6%] subjects) or unlikely related (27 TEAEs in 10 [8.8%] subjects) to study treatment. A total of 33 (28.9%) subjects reported 56 TEAEs considered possibly related to the study treatment and incidences were comparable across the 3 treatment groups (26.3%

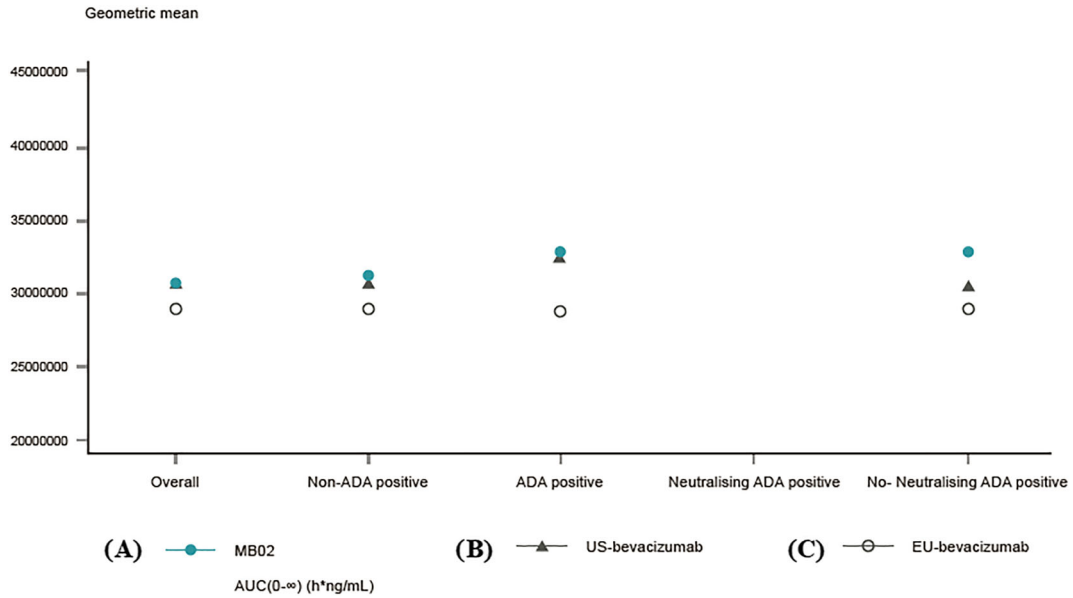

FIGURE 4 Relationship between drug $AUC_{0\text{-inf}}$ and immunogenicity (pharmacokinetic population. (A) MB02: Geometric mean $AUC_{(0\text{-}\infty)}$ (h ∗ ng/mL) values. Overall = 30 700 000, non-ADA positive = 31 000 000, ADA positive = 32 800 000, no-neutralizing ADA positive = 32 800 000. (B) US-bevacizumab: geometric mean $AUC_{(0\text{-}\infty)}$ (h ∗ ng/mL) values overall = 30 700 000, non-ADA positive = 30 700 000, ADA positive = 31 700 000, no-neutralizing ADA positive = 30 500 000. (C) EU-bevacizumab: geometric mean $AUC_{(0\text{-}\infty)}$ (h ∗ ng/mL) values. Overall = 28 800 000, non-ADA positive = 28 800 000, ADA positive = 28 000 000, no-neutralizing ADA positive = 288 000 000

[MB02] *vs.* 31.6% [US-bevacizumab] *vs.* 28.9% [EU-bevacizumab]). Among these treatment-related TEAEs, the most frequently reported included headache (19 TEAEs in 14 [12.3%] subjects), fatigue (5 TEAEs in 4 [3.5%] subjects), epistaxis (5 TEAEs in 3 [2.6%] subjects) and diarrhoea (3 TEAEs in 3 [2.6%] subjects), and were mild and comparable between treatment groups (Table 4). Bevacizumab-related TEAEs (i.e. those commonly reported in the reference bevacizumab product information [3, 4]) were reported in 5 subjects (3 in the US- and 2 in EU-bevacizumab treatment group) and mainly consisted in mild bleeding events (epistaxis [5 TEAEs], haemoptysis [2 TEAEs] and splinter haemorrhage [1 TEAEs]). None of them was reported in the MB02 treatment group.

There were no serious TEAEs and no deaths or discontinuations occurred due to TEAEs in this study.

Clinical laboratory data, vital signs and 12-lead ECG parameters did not show any clinically relevant changes over time and no relevant differences between treatment groups.

3.4 | Immunogenicity

A total of 79 (69.3%) subjects tested negative for treatment-induced ADA at all timepoints. Treatment-induced ADA were developed in a total of 35 (30.7%) subjects with similar distribution between treatment groups (12 [31.5%], 9 [23.6%] and 14 [36.8%] subjects administered MB02, US-bevacizumab or EU-bevacizumab, respectively). Three subjects developed NAb response after treatment, 1 in the MB02 and 2 in the US-bevacizumab group, both being positive in a single timepoint (transient response).

More importantly, the development of ADA or NAb responses were considered to have no effect on PK or safety (Figure 4).

4 | DISCUSSION

MB02 is a biosimilar candidate to the reference product bevacizumab that has been developed following the recommendations of the existing international guidelines.[6–9] Its high similarity to the reference product has been demonstrated through an extensive exercise of physicochemical and functional characterization. A comprehensive comparison of the in vitro pharmacodynamic properties of MB02 and the reference product was conducted as part of the comparability exercise, demonstrating comparable binding affinities to all VEGF isoforms, similar neutralization potencies and similar Fc-related effector functions (binding to C1q and Fcγ receptors). As part of the clinical development programme and in order to provide evidence for PK similarity (bioequivalence), this phase 1 study compared the PK profiles of MB02 with reference bevacizumab (US-licensed or EU-approved) following the administration of a single dose (3 mg/kg IV) in a population of healthy male subjects.

Results from the present study showed a comparable PK profile of MB02 to that of reference bevacizumab whether US-licensed or EU-approved. The selected doses of MB02, US-bevacizumab and EU-bevacizumab were considered bioequivalent in terms of the primary parameters ($AUC_{(0\text{-}\infty)}$ and C_{max}) as the 90% CI for the geometric LS means ratios for both parameters were fully contained within the predefined bioequivalence limits of 0.80–1.25, complying with international guidelines on biosimilarity.[3,4] Although equivalence margins were only defined for the primary endpoints, the 90%

CIs for the geometric LS means ratio of the secondary endpoint, $AUC_{(0\text{-}t)}$, were also fully contained within the predefined bioequivalence limits of 0.80–1.25. Concentration–time profiles for bevacizumab in all 3 treatment groups were characterized by a biphasic decline in serum concentration after reaching C_{max} and consistent with previous reports for reference bevacizumab.[14]

The PK profile of the reference bevacizumab is well understood, with PK data available from numerous clinical studies in patients with solid tumours.[14] However, a large interindividual variation in bevacizumab PK has been reported in cancer patients due to the influence of disease condition. Other parameters such as gender and body weight are also known to affect bevacizumab PK.[15] Therefore, a population of healthy male subjects was considered as the most homogeneous and sensitive 1 to determine PK bioequivalence. In addition, bevacizumab PK is linear between 1 and 10 mg/kg, which allows dosing in healthy volunteers at lower doses than those indicated for therapeutic indications, reducing the risk of adverse events in healthy volunteers while still obtaining informative PK data.[16–18] For this reason, a dose of 3 mg/kg administered in a 90-minute IV infusion was selected for the study as it balanced the safety considerations in healthy volunteers with the requirement to capture the full PK profile.

The selected dose was well tolerated, observing no remarkable differences between MB02 and the reference bevacizumab, whether US-licensed or EU-approved. No SAEs and no TEAEs led to study discontinuation or dose interruptions, and no infusion reactions were recorded. TEAEs were recorded with comparable frequency, severity and causality, and were similar in nature across all treatment groups. Since this study was dimensioned to investigate similarity in C_{max} and $AUC_{(0\text{-}\infty)}$ but not TEAEs, the slight differences observed in TEAEs incidence might be ascribed to randomness or subjects idiosyncrasy. Very few bevacizumab-related events such as bleeding were reported in the study and were all mild in severity. In addition, the safety profile reported for MB02 in this study is in line with that observed in previous studies with other bevacizumab biosimilar drugs in healthy volunteers.[17,18]

Immunogenicity profile was also comparable for all study drugs. A total of 69.3% subjects tested negative for ADA at all timepoints. In those subjects (30.7%) where a positive ADA response was detected, the incidence of ADA was similar in the 3 treatment groups, and no apparent effect on safety or PK profile was seen.

EU-bevacizumab was associated with lower exposure indicators (C_{max}, $AUC_{(0\text{-}\infty)}$) than the 2 other products though the overall incidence of TEAEs was higher for EU-bevacizumab (Table 4). Since this study was dimensioned to investigate similarity in C_{max} and $AUC_{(0\text{-}\infty)}$ but not TEAEs, there is nothing to suggest that the phenomenon is not ascribed to randomness. Therefore, MB02 can be considered bioequivalent to reference bevacizumab in healthy male subjects and there is no particular reason to suspect different safety or immunogenicity profiles between MB02 and bevacizumab sourced in the EU or USA.

The next step in the programme of biosimilar clinical development was to confirm comparable clinical performance of MB02 and the reference bevacizumab, rather than demonstrate patient benefit per se, which has already been demonstrated for the reference bevacizumab in numerous clinical trials and published studies. Due to the absence of pharmacodynamic markers for bevacizumab that can be related to patient outcome, a comparative study designed to demonstrate similar clinical efficacy between MB02, US-bevacizumab and EU-bevacizumab was required to confirm efficacy.[19] The clinical development of MB02 continues with 1 pivotal Phase III clinical study in patients with non-small cell lung cancer, which has been published recently.[20] In the non-small cell lung cancer study, the efficacy and safety MB02 were comparable to the reference bevacizumab.

The results from the present PK study provides strong evidence to support the equivalence between MB02 and US-bevacizumab and MB02 and EU-bevacizumab and contributes to obtain the totality of evidence for biosimilarity as required by international guidelines on biosimilarity. Recently, the European Authorities have granted the approval of MB02 as biosimilar to reference bevacizumab,[21] giving the opportunity to expand and facilitate patient access to this biological treatment.

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COMPETING INTERESTS

A. Sinn from Parexel International GmbH declares no potential financial interest in the subject matter or materials discussed in this manuscript. F. García-Alvarado, V. Gonzalez, C. Huerga and F. Bullo are employees of mAbxience Research S.L.

CONTRIBUTORS

S.A. was involved in the provision of study material and patients and acquisition of data. G.A.F., B.F., G.V. and H.C. did the analysis and/or interpretation of data. All authors wrote the report and/or revise it critically for important intellectual content. All authors approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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