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ARTICLE

MB09, a denosumab biosimilar candidate: Biosimilarity demonstration in a phase I study in healthy subjects

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Abstract

This was a Phase I, randomized, double-blinded, three-arm, single-dose, parallel study aimed to demonstrate pharmacokinetic (PK) similarity between MB09 (a denosumab biosimilar candidate) and reference denosumab (XGEVA® from European Union [EU-reference] and United States [US-reference]) in a healthy male population. The primary PK endpoints included: Area under the serum concentration versus time curve from time 0 to the last quantifiable concentration timepoint (AUC_{0-last}); and maximum observed serum concentration (C_{max}). Secondary endpoints included: AUC from time 0 extrapolated to infinity ($AUC_{0-\infty}$), time to reach maximum observed concentration, clearance, terminal phase half-life, pharmacodynamic, safety, and immunogenicity assessments. A total of 255 subjects were randomized (1:1:1) to receive a subcutaneous 35 mg dose of MB09 or reference denosumab. C_{max} was reached after denosumab administration, followed by a decline in the concentration with similar terminal phase half-live across treatment arms. Systemic exposure of MB09 (AUC_{0-last} and C_{max}) was equivalent to the reference denosumab, as the 90% confidence intervals around the geometric least square mean ratios laid within the predefined acceptance limits (80.00%, 125.00%) across all comparisons. Pharmacodynamic parameters, based on the percent of change from baseline in serum C-terminal telopeptide of Type 1 collagen levels, were similar across the three arms. The treatments were considered safe and generally well tolerated, with 92 treatment-emergent adverse events reported (most Grade 2 and 3) and similarly distributed. Immunogenicity was low and similarly distributed. These results provide strong evidence that supports the biosimilarity between MB09 and denosumab reference products.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Denosumab is a bone anti-resorptive drug used to treat osteoporosis and other bone-related disorders. By demonstrating high similarity with the reference

medicine, a biosimilar can largely rely on the proven efficacy and safety of the reference medicine used in real life.

WHAT QUESTION DID THIS STUDY ADDRESS?

By demonstrating that MB09 has a similar pharmacokinetic profile to that of the reference medicine, this study addressed the question of whether the patients can expect the biosimilar to deliver a similar systemic exposure as the reference medicine.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study demonstrated pharmacokinetic similarity, pharmacodynamic, and safety similar profiles between MB09, a new denosumab biosimilar candidate, and the denosumab reference medicines. The demonstration of the pharmacokinetic and pharmacodynamic biosimilarity of MB09 to its reference medicines is a pivotal step that contributes to obtaining the evidence as a whole for the comparative assessments.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Biosimilars reduce the economic burden of health systems worldwide and increase the availability of highly efficacy drugs to patients in low-income countries.

INTRODUCTION

Denosumab is a fully human monoclonal antibody to the receptor activator of nuclear factor kappa-B ligand (RANKL), an osteoclast differentiating factor. The competitive binding of denosumab with RANKL inhibits the activation of the RANK/RANKL/osteoprotegerin signaling pathway and consequently inhibits the osteoclast-mediated bone resorption which leads to decreased bone resorption, increased bone density, and lower risk of bone fracture.

The European Medicines Agency (EMA) first approval of the reference medicine (RM) denosumab – PROLIA® occurred in May 2010 for bone loss indications, and later, a new dosage strength and tradename of the RM – XGEVA® was approved in July 2011, for the prevention of skeletal-related events (pathological fracture, radiation to bone, spinal cord compression or surgery to bone) in adults with bone metastases from solid tumors. Currently, denosumab is indicated for the prevention of skeletal-related events (e.g., bone pain and fractures), secondary to multiple myeloma or bone metastases from solid tumors, giant cell tumor of the bone, hypercalcemia of malignancy, in postmenopausal women as well as men with osteoporosis at high risk of fracture, glucocorticoid-induced osteoporosis, and bone loss.[1,2] In the US (United States), both RMs were approved by the Food and Drug Administration (FDA) in 2010, for the same indications as described above for the EMA's approvals.

Biosimilars have become important therapeutic options, improving patient access to essential treatments across worldwide health systems. mAbxience Research SLU has developed MB09, a denosumab biosimilar candidate, as the active pharmaceutical ingredient of RMs. A biosimilar is a biological medicinal product highly similar to another biological medicine (the RM) already approved by Healthy Regulatory Agencies, in terms of structure, biological activity and efficacy, safety, and immunogenicity profile.[3–5]

Currently, the required comparative assessments in a biosimilar development comprise; quality data (analytical comparative assessments); in vitro and in vivo nonclinical data[3]; and comparative pharmacokinetic (PK), pharmacodynamic (PD), safety, and efficacy studies. MB09 has demonstrated its high similarity to the RM denosumab through an extensive physicochemical and functional characterization that included primary structure, higher order structure, biological activity, and binding affinity to RANKL (data on file). Once these first analytical comparative assessments were successfully established, the next step in the MB09 development was to establish the PK similarity of MB09 to the RMs in a sensitive population.

The main objective of the clinical trial was to assess the biosimilarity of MB09 and the RM (either European-sourced [EU-denosumab] or US-sourced [US-denosumab]), by comparing the PK profiles across the three arms of study in a healthy male population. In addition, secondary objectives aimed to compare the PD, safety, and immunogenicity profiles between MB09 and the RMs.

MATERIALS AND METHODS

Study design and study population

This was a Phase I, randomized, double-blind, three-arm, single-dose, parallel study to compare the PK, PD, safety, and immunogenicity profile of MB09 to the RMs (EU- and US-sourced RM) conducted in a single center located in Poland, from February 2022 to March 2023.

Eligible subjects were healthy male subjects and key inclusion criteria included: a BMI between 18.5 and 29.9 kg/m^2, inclusive (total body weight between 60 and 95 kg, inclusive); age (28–55 years); good general health as determined by medical history (clinical laboratory test results, vital sign measurements, 12-lead electrocardiogram (ECG) results; and physical examination findings) at screening and check-in. The main exclusion criteria included: subjects with evidence or history of clinically significant disease; recent infection (within 2 months prior to screening): dental or jaw disease (dental or jaw disease requiring oral surgery, history of osteomyelitis or osteonecrosis of the jaw or significant dental disease or dental neglect, with signs and/or symptoms of infection); or previous treatment with denosumab.

Subjects were randomly assigned by an electronic system in a 1:1:1 ratio to receive either a 35 mg subcutaneous (SC) dose of MB09 or 35 mg SC dose of EU-denosumab or 35 mg SC dose of US-denosumab. Subjects were stratified based on their body weight (60 to <80 kg and 80–95 kg).

The study consisted of a screening period (Days −30 to −2), check-in (Day −1), treatment period (Day 1), follow-up period (Days 2 to 252), and end of study visit (Day 253). On Day −1, subjects were admitted to the clinical research facility. On Day 1, all subjects received a single 35 mg SC dose of the randomly assigned study treatment administered in the upper arm by the blinded clinical unit personnel that were prepared by the unblinded personnel. They remained confined for 2 days and received a standardized diet at the scheduled time of the day. Ambulant follow-up visits were scheduled after discharge. The duration of the study, excluding the screening period, was approximately 36 weeks.

The study was registered with the number NCT05299073 and EudraCT number 2021–003290-54, and approved by the investigator's Independent Ethics Committee (IEC) before implementation. An informed consent form was signed by all the subjects.

Study objectives

The primary objective of the study was to demonstrate the PK similarity between MB09, EU-denosumab, and US-denosumab based on the area under the serum concentration versus time curve from time 0 to the last quantifiable concentration timepoint (AUC_{0-last}) and the maximum observed serum concentration (C_{max}).

The secondary objectives included the evaluation and comparison of the PD, safety, and immunogenicity profiles between the three study treatments.

Pharmacokinetic evaluation

Serum samples were obtained at predose, 8, 16, and 24 h after SC administration as well as at Days 3, 4, 6, 8, 11, 15, 22, 29, 43, 57, 71, 85, 99, 113, 141, 169, 197, 225, and 253 days after administration. For all the sampling points (except 8 and 16 h postdose timepoints), an overnight fasting of at least 10 h was compulsory.

Denosumab serum concentrations were measured using a validated Meso Scale Discovery Electrochemiluminescence (MSD-ECL) assay. In this assay, electrochemiluminescent-compatible plates are coated overnight with human RANKL protein. Sample denosumab (MB09) binds to the RANKL-coated wells, and sulfo tag-conjugated antidenosumab antibodies are used to detect any bound antibodies and compared to a standard curve. A validation experiment was conducted following the current state-of-the-art.[6–10] The method is applicable to the quantitation of MB09 within a nominal range of 20.0–800 ng/mL.

Besides primary PK assessments, the following parameters were also determined as secondary endpoints: Area under the serum concentration versus time curve from time 0 extrapolated to infinity ($AUC_{0-\infty}$), time to reach maximum observed concentration (T_{max}), apparent total body clearance (CL/F), elimination rate constant during terminal phase (K_{el}), terminal phase half-life ($t_{1/2}$, calculated as $t_{1/2} = \ln 2/\text{Kel}$), apparent volume of distribution during the terminal phase (Vz/F). All PK parameters were calculated by noncompartmental analysis (NCA) with Phoenix WinNonlin Version 8.3 (Certara USA Inc., Princeton, NJ, USA).

Pharmacodynamic evaluation

Serum C-terminal telopeptide of Type 1 collagen (sCTX) parameters were calculated as PD endpoint. Serum samples were obtained at same timepoints as the PK sampling. The concentration of sCTX was determined using a validated enzyme-linked immunosorbent assay (ELISA) method within a nominal range from 70.0 to 1130 pg/mL. In this assay, calibrators, quality controls, endogenous serum controls, assay blank, and unknown samples are

added to a streptavidin-coated plate, followed by the addition of an antibody solution (composed by biotinylated antibody and a horseradish peroxidase, HRP). The biotinylated antibody and HRP-conjugated antibody form a complex between the sCTX present, which binds to streptavidin. After incubation, the plate is washed and a tetramethylbenzidine (TMB) solution is added to the plate. After incubating, washing, and adding TMB a colorimetric signal proportional to the amount of sCTX is generated. The validation process was conducted as per guidelines.[6,7,11]

A pharmacodynamic evaluation was assessed using two different approaches: absolute values and relative change from baseline. The absolute PD parameters were calculated as follows: area under the effect–time curve from time 0 to the last quantifiable sCTX concentration timepoint ($AUEC_{0-last}$); area under the effect–time curve from time 0 to Day 253 ($AUEC_{0-253}$); the minimum observed serum concentration (C_{min}); and the time of occurrence of the C_{min} (T_{min}). The relative PD parameters were calculated as a percent of change from baseline (%CfB): area under the % inhibition curve from time 0 to the last quantifiable sCTX concentration timepoint ($AUIC_{0-last}$); area under the % inhibition curve from time 0 to Day 253 ($AUIC_{0-253}$); the maximum % inhibition (I_{max}); and its time of occurrence (TI_{max}). All parameters were calculated using NCA with Phoenix WinNonlin Version 8.3 (Certara USA Inc., Princeton, NJ, USA).

Safety evaluation

All subjects were monitored for safety throughout the study duration up to the end of the study visit. A treatment-emergent adverse event (TEAE) was defined as any untoward medical occurrence or clinical investigation in a subject after the study treatment was administered.

Safety and tolerability endpoints included monitoring and recording of adverse events (AEs), clinical laboratory tests (hematology, coagulation, serum chemistry, and urinalysis), vital sign measurements, 12-lead ECG results, and targeted physical examination. For all safety assessments, the investigator determined whether the results were clinically significant. All AEs were coded by preferred term (PT) and system organ class (SOC) using Medical Dictionary for Regulatory Activities (MedDRA) Version 25.1.

All AEs' relationship to study treatment was evaluated by the investigator as not related, unlikely related, possibly related, probably related, or related. AEs considered not related and unlikely related were mapped to not related and the other three categories to related. All AEs were graded for intensity according to the common terminology criteria for adverse events (CTCAE), Version 5.0—November 2017. All AEs not outlined in the CTCAE that occurred in a subject were assessed (Graded) for intensity and then classified into 1 of 5 categories, Grade 1 (Mild), Grade 2 (Moderate), Grade 3 (Severe), Grade 4 (Life-threatening), and Grade 5 (Death). A serious TEAE (SAE) was defined as any event that either: resulted in death, was life-threatening, caused hospitalization or prolongation of a previous one, resulted in disability, or caused a congenital anomaly.

Immunogenicity evaluation

For each subject, a total of seven blood samples were collected for detection of anti-drug antibodies (ADA) and neutralizing antibodies (nAb), at predose and at Days 11, 43, 99, 169, 225, and 253. Denosumab antibodies were assessed using a validated MSD-ELC assay in a three-tiered approach. Cut point values (CPVs) were determined using upper prediction intervals. As specified in the SOP, 95% screening, 99% confirmation 99.9% titration upper prediction intervals were determined based on the nontransformed data. The CPVs were established in 1.14, 1.19, and 1.26 for screening, confirmation, and titration tiers. Neutralizing antibodies were investigated only if a confirmed ADA-positive result. The validation was developed as per the current state of the art.[12–14]

Statistical methods

In this study, the sample size was based on a statistical power calculation for the biosimilarity demonstration between MB09, the biosimilar candidate, and the reference denosumab (both sources). Based on previous PK studies with the reference denosumab, a coefficient of variation (CV) value of 33% was estimated for AUC parameters.[15,16]

Assuming a ratio of AUC and C_{max} between 0.95 and 1.05, 68 evaluable subjects per arm (204 evaluable subjects in total) were required to provide at least 90% power to conclude biosimilarity of MB09 to the RMs (both sources). Thus, assuming a 20% dropout rate, approximately 255 subjects were planned to be enrolled in this study.

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

No algorithm for imputation of missing data was employed. Concentration below limit of quantification (BLQ) detected was treated as zero at the individual timepoints for the calculation of summary statistics; for calculation of parameters, BLQ values were treated as zero with the two exceptions: BLQ value between 2 quantifiable concentrations

were set as missing and concentrations BLQ were treated as ½ of the low limit for C_{min} estimation. This approach for C_{min} provides a conservative estimate that likely approximates the true concentration, which could be considered better than assuming it to be zero or considering the data as missing, and so introducing a bias in the study.

An analysis of variance (ANOVA) model with treatment and stratification factors (i.e., body weight) as fixed effects was performed on the natural log-transformed values of C_{max}, AUC_{0-last}, and $AUC_{0-\infty}$ to assess the biosimilarity between MB09 versus the RMs (both sources), as well as to compare both referenced RMs to each other.

PK similarity was concluded if the 90% confidence interval (CI) for the test to reference ratios of the geometric least square means (GLSM) for C_{max}, AUC_{0-last}, and $AUC_{0-\infty}$ was entirely contained within the [80.00%, 125.00%] interval.

The nonparametric method Wilcoxon signed-rank test was used to examine median differences in T_{max} comparisons. The Hodges–Lehmann estimate and its 90% CI were calculated for the median difference between treatments.

Regarding PD analysis, an ANCOVA model with treatment and body weight as fixed effects and log-transformed predose sCTX concentrations fitted as a covariate was performed on the natural log-transformed values of $AUEC_{0-253}$ and $AUIC_{0-253}$ to assess the PD similarity between MB09 and EU-/US-denosumab, as well as between both RMs. Biosimilarity in PD biomarker was reported as the test-to-reference ratio of GLSM and its corresponding 90% CI for $AUEC_{0-253}$ and $AUIC_{0-253}$. The proportion of subjects experiencing at least 1 TEAE was compared among the treatments using Fisher's exact test at a 0.05 significance level. As AEs from the same body system may be correlated, and there may also be correlations between AEs from different body systems, Bonferroni correction was applied to the resulting *p*-values from Fisher's test to control the family-wise error rate.

RESULTS

Study subjects

A total of 257 subjects were enrolled and randomized (85 in the MB09 arm, 86 in the EU-denosumab, and 86 in the US-denosumab), but 255 subjects were treated (1 subject in EU-denosumab arm withdrew consent before dosing; 1 subject in the US-denosumab arm was discontinued due to an AE before dosing). Of the 255 treated subjects, 254 subjects completed the study as one subject in the US-denosumab arm was lost to follow-up after day 71 post-dosing (Figure 1).

All subjects were male, as per protocol, aged between 28 and 55 years, and BMI between 18.8 and 29.9 kg/m^2, inclusive. All of them were White and non-Hispanic or Latino. Demographic characteristics were similar across the three treatment arms (Table S1).

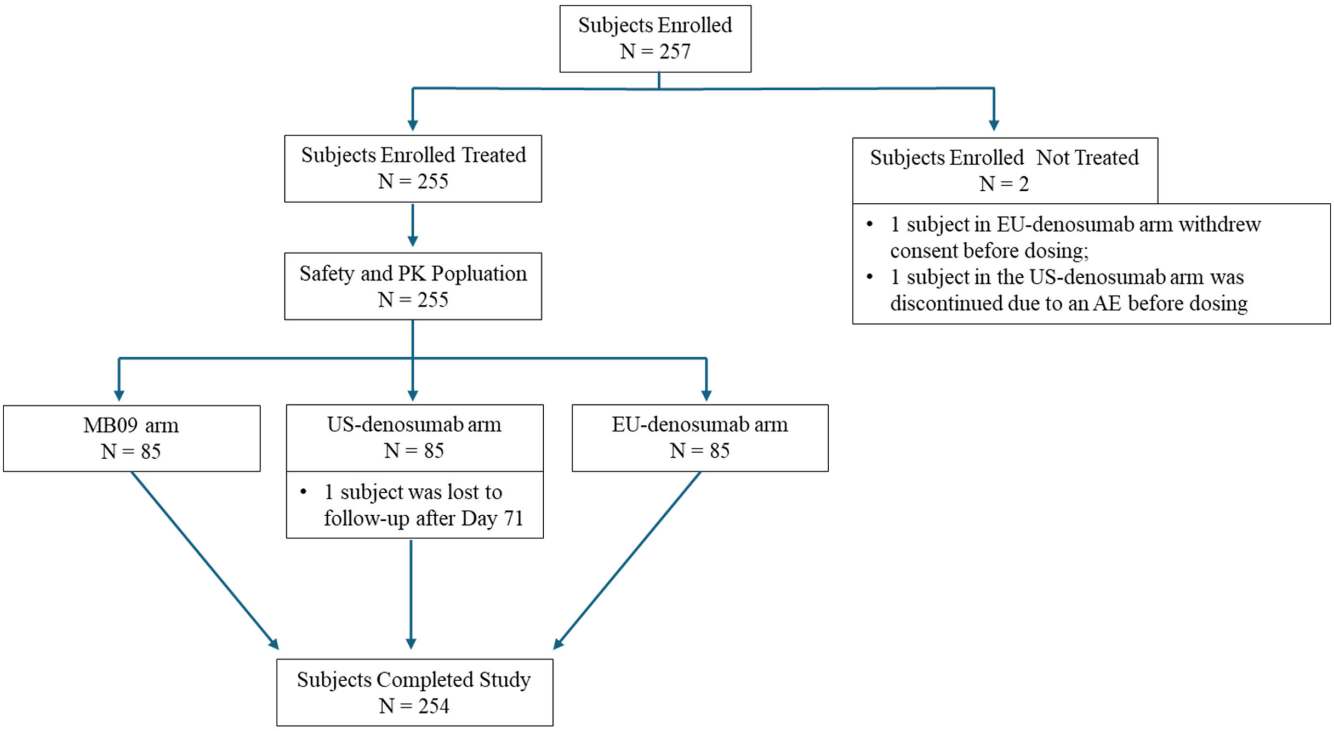

FIGURE 1 Subject disposition flowchart.

Pharmacokinetics

Following single SC doses of study treatments, C_{max} was attained approximately 10 days postdose in all treatment arms, before declining in a mono-exponential manner.

Thereafter, serum concentrations of denosumab declined with similar terminal phase half-live ($t_{1/2}$), with geometric means ranging from 12.1 to 12.5 days across all treatment arms. On Day 141, the concentration of denosumab in 85.7% of overall subjects was below the limit of quantification (Figure 2a,b). Between-subject variability (geometric CV) in the overall extent of systemic exposure to denosumab (C_{max}, AUC_{0-last}, $AUC_{0-\infty}$) was low in all treatment arms ranging from 22.0% to 27.4% (Table S2). The coefficient of determination for Kel and all associated parameters (AUC0-∞, $t_{1/2}$, CL/F, V_z/F) were >0.8 for all the subjects.

Systemic exposure of MB09 (AUC_{0-last}, $AUC_{0-\infty}$, and C_{max}) was similar to that observed for EU-denosumab and US-denosumab; the 90% CIs around the GLSM ratio fell within the predefined acceptance limits [80.00%, 125.00%] in all instances. The GLMS ratios [90% CI] of MB09/EU-denosumab for AUC_{0-last}, $AUC_{0-\infty}$, and C_{max} were 105.93% (99.54%, 112.73%), 105.92% (99.51%, 112.76%), and 105.13% (98.86%, 111.80%), respectively. The GLSM ratios [90% CI] of MB09/US-denosumab for AUC_{0-last}, $AUC_{0-\infty}$ and C_{max} were 108.87% (102.30%, 115.86%), 108.62% (102.03%, 115.62%), and 104.75% (98.50%, 111.40%), respectively. Systemic exposure was also similar between both RMs; the 90% CIs around the GLSM ratios laid within the predefined acceptance limits [80.00%, 125.00%]. The GLSM ratios [90% CI] of EU-denosumab/US-denosumab for AUC_{0-last}, $AUC_{0-\infty}$ and C_{max} were 102.77% (96.58%, 109.37%), 102.54% (96.33%, 109.15%), and 99.64% (93.69%, 105.96%), respectively (Table 1).

The nonparametric assessment of T_{max} indicated that there was no statistical difference in the time to attain maximum serum concentrations of denosumab between all three treatment groups.

Pharmacodynamics

Baseline levels of sCTX were similar across all arms, with higher between-subject variability noted in the MB09 arm. Baseline arithmetic mean and CV were 610 pg/mL (91.6%) for MB09, 533 pg/mL (41.5%) for EU-denosumab arm, and 567 pg/mL (44.9%) for US-denosumab arm.

Following single SC doses of MB09, EU-denosumab, and US-denosumab, sCTX concentrations decreased steadily during the first days and remained suppressed for the remainder of the sampling period (Figure 3a,b).

The C_{min}/I_{max} was first attained (T_{min}/TI_{max}) at approximately 3 days postdose in all groups (Table S3). Change from baseline sCTX values showed I_{max} of more than 90% attained at approximately 3 days postdose. At the end of the study, a sCTX basal values recovery rate of 54%, 69%, and 64% was reached, which corresponds to serum levels of 331 pg/mL (CV 62.1%) for MB09, 368 pg/mL (CV 49.7%) for EU-denosumab, and 363 pg/mL (CV 61.1%) for US-denosumab.

The observed between-subject variability (geometric CV) in absolute sCTX levels was high across all treatment groups, ranging between 45.8% and 61.5% for

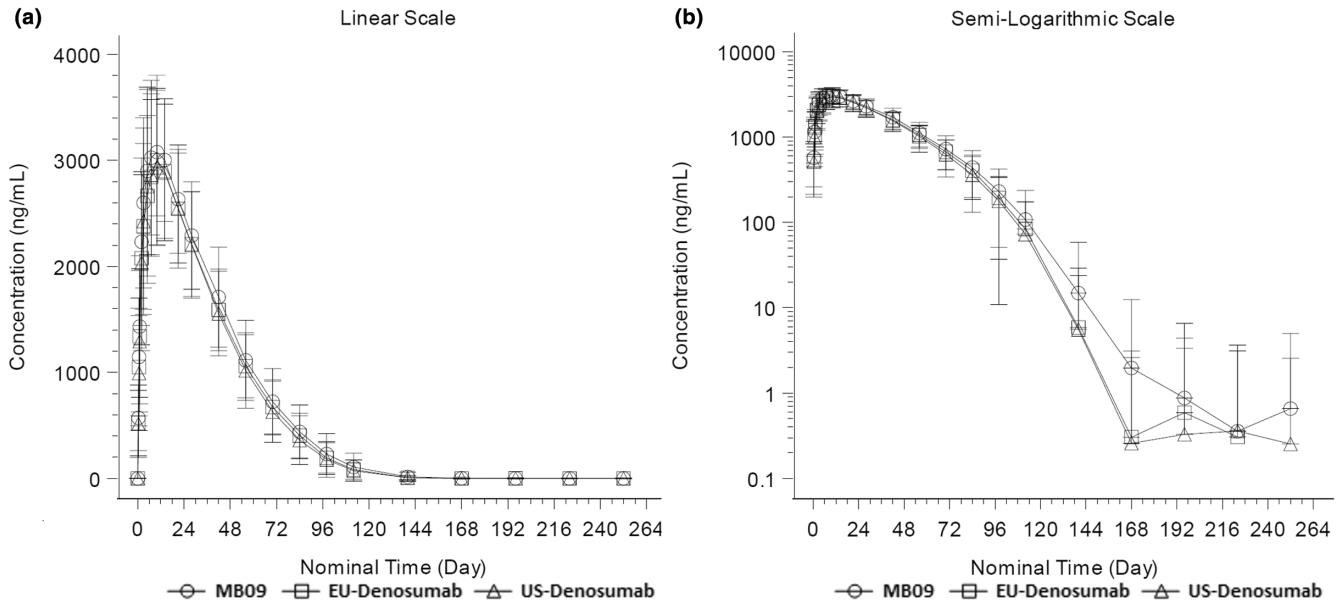

FIGURE 2 (a) Mean (±SD) denosumab serum concentrations versus time following single subcutaneous administration in linear scale. (b) Mean (±SD) denosumab serum concentrations versus time following single subcutaneous administration in semi-logarithmic scale.
Note: All values below LLOQ (20.0 ng/mL) were taken as zero for the calculation of summary statistics.

TABLE 1 Summary of statistical comparisons of PK parameters across treatment arms.

Parameter (units)	Comparison	%Ratio GLSM	90% CI of the %Ratio GLSM
C_{max} (ng/mL)	MB09/EU-Denosumab	105.13	(98.86, 111.80)
	MB09/US-Denosumab	104.75	(98.50, 111.40)
	EU-Denosumab/ US-Denosumab	99.64	(93.69, 105.96)
AUC_{0-last} (day*ng/mL)	MB09/EU-Denosumab	105.93	(99.54, 112.73)
	MB09/US-Denosumab	108.87	(102.30, 115.86)
	EU-Denosumab/ US-Denosumab	102.77	(96.58, 109.37)
$AUC_{0-\infty}$ (day*ng/mL)	MB09/EU-Denosumab	105.92	(99.51, 112.76)
	MB09/US-Denosumab	108.62	(102.03, 115.62)
	EU-Denosumab/ US-Denosumab	102.54	(96.33, 109.15)

Note: An ANOVA model was fitted to the natural log transformed PK parameters with treatment and stratification factors (body weight) as fixed effects.

Abbreviations: AUC_{0-last}, Area under concentration–time curve from time 0 to the last quantifiable concentration; $AUC_{0-\infty}$, Area under concentration–time curve from time 0 extrapolated to infinity; CI, confidence interval; C_{max}, Maximum observed concentration; GLSM, geometric least squares mean; *N*, Number of subjects in treatment.

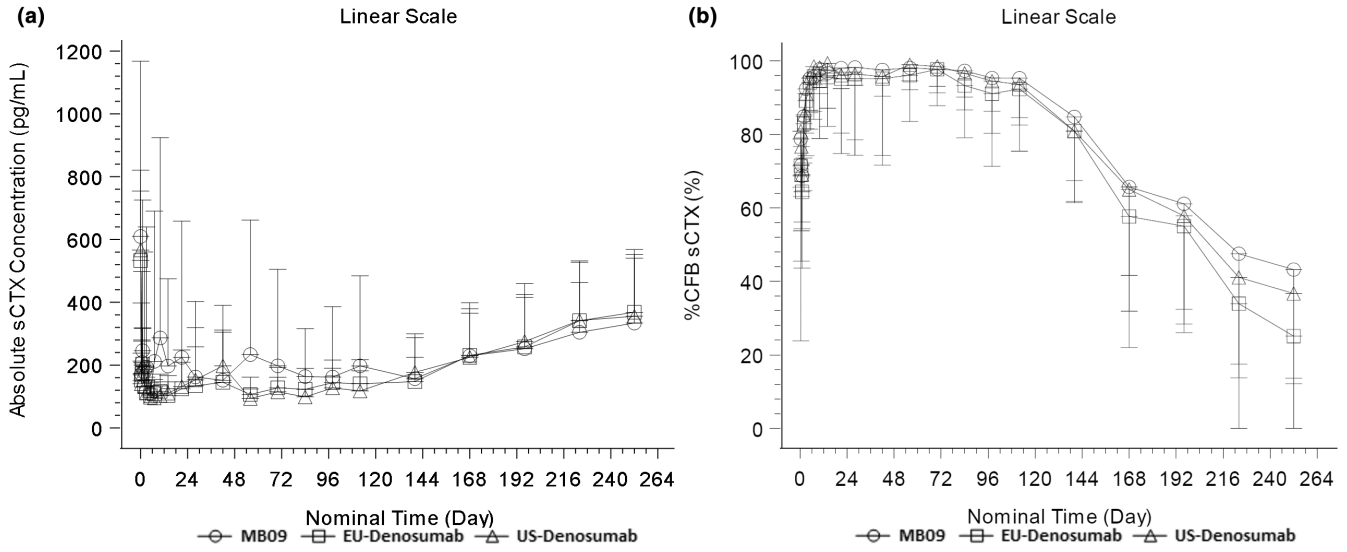

FIGURE 3 (a) Mean (±SD) absolute serum CTX concentrations versus time following single subcutaneous administration. (b) Suppression over time (Mean [±SD] percent change from baseline serum CTX concentrations versus time following single subcutaneous administration). *Note*: %CfB (percentage change from baseline) calculated as ([Predose concentration—concentration at timepoint]/Predose concentration)*100.

$AUEC_{0-last}$. and between 54.1% and 81.1% for $AUEC_{0-253}$. Consequently, none of the three pairwise comparisons, the 90% confidence intervals around the GLSM ratios, fell into the predefined acceptance limits [80.00%, 125.00%] (Table 2). On the other hand, the observed geometric CV in change from baseline sCTX levels, as measured by $AUIC_{0-last}$ and $AUIC_{0-253}$, was low across all treatment arms (between 16.2% and 19.5% and between 15.8% and 19.8%, respectively). Following dosing, the $AUIC_{0-253}$ were similar within all three treatment groups, with just a 2% to 4% difference observed. Furthermore, for the three pairwise comparisons, the 90% confidence intervals around the GLSM ratios fell within the predefined acceptance limits [80.00%, 125.00%] (Table 2).

Safety

Among the 255 treated subjects, 63 (24.7%) subjects reported a total of 92 TEAEs. The majority of the TEAEs

TABLE 2 Pharmacodynamic statistical analysis (serum CTX).

Parameter (units)	Comparison	%Ratio GLSM	90% CI of the %Ratio GLSM
$AUEC_{0–253}$ (day*pg/mL)	MB09/EU Denosumab	84.71	(64.79, 110.74)
	MB09/US Denosumab	82.64	(65.28, 104.62)
	EU Denosumab/US Denosumab	97.56	(75.45, 126.15)
$AUIC_{0–253}$ (day*%)	MB09/EU Denosumab	103.68	(99.30, 108.24)
	MB09/US Denosumab	101.69	(97.42, 106.15)
	EU Denosumab/US Denosumab	98.08	(93.95, 102.41)

Note: An ANCOVA model was fitted to the natural log transformed PD parameters with treatment and stratification factors (body weight) as fixed effects. For AUEC, the log-transformed pre-dose CTX concentration (i.e., baseline) was also fitted as a covariate.

Abbreviations: $AUEC_{0–253}$, Area under the effect-time curve from time 0 to Day 253 (without baseline adjustment); $AUIC_{0–253}$, Area under the % inhibition curve from time 0 to Day 253 (using percent change from baseline data); CI, confidence interval; GLSM, geometric least squares mean.

TABLE 3 Overview of treatment-emergent adverse events.

TEAEs	MB09 (*N* = 85), *n* (%) [E]	EU-sourced denosumab (*N* = 85), *n* (%) [E]	US-sourced denosumab (*N* = 85), *n* (%) [E]	Overall (*N* = 255), *n* (%) [E]
Any TEAE	18 (21.2) [29]	28 (32.9) [40]	17 (20.0) [23]	63 (24.7) [92]
Any grade 1 TEAE	4 (4.7) [5]	5 (5.9) [6]	3 (3.5) [3]	12 (4.7) [14]
Any grade 2 TEAE	10 (11.8) [11]	14 (16.5) [17]	11 (12.9) [16]	35 (13.7) [44]
Any grade 3 or higher TEAE	9 (10.6) [13]	13 (15.3) [17]	4 (4.7) [4]	26 (10.2) [34]
Any treatment-related TEAE	1 (1.2) [1]	1 (1.2) [2]	1 (1.2) [1]	3 (1.2) [4]
Any SAE	2 (2.4) [2]	0 [0]	0 [0]	2 (0.8) [2]
Most common (>1%) TEAEs by preferred term				
Blood creatine phosphokinase increased	6 (7.1) [9]	9 (10.6) [11]	2 (2.4) [2]	17 (6.7) [22]
Nasopharyngitis	3 (3.5) [3]	2 (2.4) [2]	2 (2.4) [3]	7 (2.7) [8]
Blood triglycerides increased	1 (1.2) [1]	3 (3.5) [4]	1 (1.2) [1]	5 (2.0) [6]
Urinary tract infection	0 [0]	1 (1.2) [1]	3 (3.5) [4]	4 (1.6) [5]
Headache	2 (2.4) [3]	1 (1.2) [1]	1 (1.2) [1]	4 (1.6) [5]
Syncope	0 [0]	2 (2.4) [2]	1 (1.2) [1]	3 (1.2) [3]

Note: At each level of subject summarization, a subject is counted once if the subject reported one or more events. n represents the number of subjects at each level of summarization.

Abbreviations: [E], represents the number of events at each level of summarization; "*N*", represents the number of subjects in each arm; "*n*", represents the number of subjects with TEAE; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

were of Grade 2 and Grade 3 in severity. During the study, three (1.2%) subjects were reported with four TEAEs (one subject in each arm), which were considered as related to the study treatment. Except for one, all reported TEAEs were resolved by the end of the study.

The most reported TEAEs were blood creatine phosphokinase (CPK) increased (17 [6.7%] subjects), nasopharyngitis (7 [2.7%] subjects), and blood triglycerides increased (5 [2%] subjects). CPK increased is also the most common TEAE in MB09 and EU-denosumab arms and urinary tract infection is the most common TEAE in US-denosumab arm (Table 3).

The study treatment-related TEAEs included Grade 1 headache (MB09 arm), 2 episodes of Grade 1 arthralgia (EU-Denosumab arm), and Grade 2 papular rash (US-denosumab arm). Two SAEs, osteoma (Grade 3) and depression (Grade 3) which led to hospitalization, were reported in two subjects in the MB09 arm, both resolved and were considered as unrelated to the study treatment. There were no deaths during the study. No

subjects were discontinued from the study due to a TEAE (Table 3).

There were some numerically small differences between treatment arms; however, there was no statistically significant difference between the treatment arms with respect to the number of subjects reported with at least a TEAE, as determined by Fisher exact test and adjusted according to the Bonferroni correction method.

Besides those laboratory-related reported TEAEs, the results from mean hematology, serum chemistry, urinalysis and coagulation tests, vital sign measurements, and ECG parameters observed after dosing were generally similar to baseline.

Immunogenicity

After study treatment administration, ADAs assay results were positive in six subjects (1 in MB09 arm, 3 in EU-denosumab, and 2 in the US-denosumab), most of them were transient (except for one subject in the EU arm) and exhibited low titers. None of the ADAs detected had neutralizing capacity. ADA-positive subjects showed no apparent differences in terms of PK, PD, or safety profiles in comparison to ADA-negative subjects.

DISCUSSION

This Phase I clinical trial was designed to establish the similarity between MB09 (denosumab biosimilar candidate) and the references EU- and US-denosumab. Performing a three-arm study in a similarity study, with one arm for the biosimilar candidate and two arms for the RMs, ensures thorough evaluation and regulatory compliance. Some benefits are derived from having three arms: (a) ensuring similarity with both RMs adds robustness to the study findings, (b) the comparison of the two RMs can help to mitigate any biases or confounding factors, and (c) demonstrating similarity to both EMA' and FDA's RMs can streamline conducting future trials with only one of them.

To ensure a homogenous population and to have the sensitivity to detect PK difference, if there were any, only healthy male volunteers were included. This population, together with a selection of eligible criteria and the weight as a stratification factor, ensured the selection of subjects with less potential confounding factors that would lead to a very sensitive setting for similarity evaluation.[4,5]

In addition, and as per guidelines, the more sensitive dose should be chosen for the PK comparative assessments. It is well known that denosumab displays dose-proportional increases in exposure when used at a dose of 60 mg (or 1 mg/kg) and higher; and when it is used at doses lower than 1.0 mg/kg, the exposure is nonlinear. This fact is due to target-mediated disposition that predominates when serum denosumab concentrations drop below approximately 1 μg/mL, and that becomes saturated as concentration increases.[17,18] Therefore, due to the higher prevalence of target-mediated elimination at lower concentrations, the use of a sub-therapeutic dose may be considered more discriminatory to detect PK differences between the biosimilar candidate and the reference product. In addition, a sub-therapeutic dose would limit the exposure and considered safer to be administered in healthy volunteers.

Under these selective homogeneous and sensitive conditions, MB09 demonstrated a similar systemic exposure to both references denosumab, in terms of AUC_{0-last}, $AUC_{0-\infty}$, and C_{max}, as the three pairwise comparisons and 90% confidence intervals around the GLSM ratios fell into the predefined acceptance limits [80.00%, 125.00%]. While the assessment of PK similarity for subcutaneous drugs relies on AUC0-inf, denosumab exhibits a target-mediated disposition and thus the concentration profile does not present a first-order elimination phase, which is required for a reliable estimate of AUC_{0-inf}. Therefore, to demonstrate PK similarity of the denosumab biosimilar candidate MB09, C_{max}, and AUC_{0-last} were selected as co-primary endpoints and AUC_{0-inf} as one of the secondary endpoints.

In terms of PD comparative assessments, sCTX was the selected bone turnover marker as it is well-known and widely used. It is also known that bone turnover markers, such as sCTX, show a large variability and that the several factors that contribute to it include uncontrollable factors (e.g., age, ethnicity) and controllable factors (e.g., fasting/feeding state, and timing relative to circadian rhythms, and exercise).[19] In this study, most controllable factors were taken into account, particularly those related to collection sample conditions. Despite this, the statistical comparison in terms of $AUEC_{0-253}$, the three absolute values pairwise comparisons laid outside the predefined acceptance limits. sCTX absolute values differences at baseline and the different timing in the physiological bone function recovery, are probably behind these results. As this possibility was foreseen, a prespecified analysis standardizing the baseline level was conducted, and PD was analyzed as a percent of change. Another option could have been to increase the study sample size, considering sCTX CV% instead of PK CV%, but this approach was considered unethical as it would increase the exposure of healthy subjects to an investigational product to assess a secondary objective and therefore discarded. Using $AUIC_{0-253}$ for the statistical analysis, the three pairwise comparisons of %CFB

laid completely within the predefined acceptance limits for relative PD similarity.

The select dose was well tolerated and a low number of TEAEs was reported; it was observed a small numerical but nonsignificant statistical difference between treatment arms. No unexpected reactions were reported. Immunogenic response to study treatment was low and similar between treatment arms.

The results are aligned with those previously reported by other clinical trials with denosumab biosimilar candidates in healthy volunteers, despite the different doses tested. Nevertheless, in other studies the ADA response rate was higher, probably related to the high sensitivity of the assay methodology used with no relevant impact on PK, PD, and safety.[20–23]

Beyond demonstrating similarity between MB09 and denosumab (EU- and US-sourced), this study also established the scientific bridge between the two references denosumab allowing the use of a single comparator in the following MB09 efficacy similarity study (NCT05338086).

The results from this PK clinical trial provide strong evidence to support the similarity between MB09 and the reference medicine denosumab (both sources). The entirety of evidence collected for MB09 thus far indicates that MB09 is biosimilar to denosumab.

AUTHOR CONTRIBUTIONS

M.T.K., E.C., and J.Q.P. wrote the manuscript. A.F.I. and J.Q.P. designed the research. M.T.K. performed the research. E.C., A.F.I., and J.Q.P. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

E.C., A.F.I., and J.Q.P. are employees of mAbxience Research S.L.U. All other authors declared no competing interests in this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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