Purpose  The aim of this study was to compare first global biosimilar denosumab for the prevention of skeletal-related events (SREs) in patients with bone metastases from solid tumors.

Methods  It was a randomized, double-blind, comparative clinical study. Total of 136 patients of solid tumor were dosed (i.e., 102 subjects in study arm and 34 subjects in the reference arm) with initial double-blind period of 24 weeks (primary efficacy) followed by open-label phase till week 36. Primary endpoint was the incidence of first on-study SRE including hypercalcemia of malignancy with co-primary endpoint of median time to first on-study SRE. Secondary endpoints included mean number and time to first and subsequent on-study SREs (week 12, 24, 36), incidence/proportion of patients with first and subsequent on-study SREs (week 24, 36), change from baseline in nuclear bone scan, quality of life assessment, pharmacokinetics, pharmacodynamic, and safety.

Results  In biosimilar study arm, 06 (5.83%) patients suffered SRE from baseline to week 24 compared with 02 (5.71%) patients in reference arm with one (0.97%) patient showing pathological fracture in study arm and one (2.86%) patient having spinal cord compression in reference arm. There was no statistically significant difference in median time to first SRE, mean number of SRE/patient in both arms and improvement in bone repair on nuclear scan at 12, 24 and 36 weeks. Though the study arm showed better health-related quality of life (HRQoL), mean change in HRQoL was statistically not different in both the arms. Pharmacodynamics, serum bone-specific alkaline phosphatase, pharmacokinetic and safety evaluation did not show any statistical difference between arms.

Conclusion  There was no clinically meaningful difference in the biosimilar denosumab and reference product after detailed efficacy and safety evaluation.

Keywords
► denosumab
► HRQoL
► SRE
Introduction

Bone metastases are common in patients with advanced solid tumors\(^1\) and they affect 68% of patients with prostate cancer (>90% of patients with metastatic castration-resistant disease), 73% with breast cancer, and 36% with lung cancer. Bone metastases can lead to debilitating bone complications, known as skeletal-related events (SREs), which include pathologic fractures, spinal cord compression, hypercalcemia, and the need for radiation or surgery to the bone.\(^2\)–\(^4\) Bone complications can cause pain, reduce patient quality of life (QoL), and can be associated with an increased risk of death.\(^5\)

Bisphosphonates (e.g., zoledronic acid, pamidronate disodium, ibandronate, and clodronate) and denosumab are approved for the prevention of bone complications in patients with advanced malignancies involving bone.\(^6\)–\(^7\)

Denosumab is a fully human, monoclonal antibody against the receptor activator of nuclear factor kappa B (RANK) ligand (RANKL). By disrupting RANK signaling, denosumab prevents the fusion and activation of osteoclasts, thus reducing bone resorption.\(^8\) Bisphosphonates and denosumab reduce the incidence of bone complications in patients with bone metastases from solid tumors, with denosumab showing superiority compared with zoledronic acid in patients with breast and prostate cancer.\(^9\) The subcutaneous administration and its efficacy make it an attractive first-line therapy and reasonable alternative to parenteral bisphosphonates, in particular in patients with advanced renal insufficiency. Denosumab has become a novel therapeutic option for the treatment of benign and malignant bone diseases that combines efficacy with a high degree of safety and comfortable administration.\(^10\)

Biosimilar denosumab needs to proceed by guidelines allowing demonstrating that proposed biosimilar is highly similar to the reference product and that no clinically meaningful differences exist between the biosimilar and reference product in terms of safety, purity, and potency.\(^11\) Biosimilars offer access to an alternative treatment option and compliance for the chronically ill subjects.\(^12\)

The study drug, biosimilar denosumab, was developed as biosimilar to innovator denosumab with comprehensive establishment of physicochemical and biological similarity. The aim of the present study was to establish clinical biosimilarity of the biosimilar denosumab with the reference denosumab product in terms of efficacy and safety for prevention of SREs in patients with bone metastases from solid tumors.

Materials and Methods

This was a prospective, multicenter, randomized, double-blind, two-arm, parallel group, active control, comparative clinical study to evaluate efficacy and safety of biosimilar denosumab (study drug) against reference denosumab (reference drug) for the prevention of SREs in patients with bone metastases from solid tumors. The study was conducted with informed patient consent and in compliance with the ethical principles that originated in the Declaration of Helsinki and International Committee on Harmonization of Good Clinical Practice (ICH-GCP), protocol, Drug Controller General of India (DCGI), and Schedule Y regulations with the registration number CTRI/2017/10/010093.

In this study, a total of 138 subjects were randomized (103 subjects in the study arm and 35 subjects in the reference arm) and 136 subjects were dosed with the study medication, that is, 102 subjects in the study arm and 34 subjects in the reference arm. A sample size of 136 subjects in a 3:1 ratio was based on the regulatory requirement and assuming the power of 80% and effect size, that is, incidence of first-on-study SREs associated with bone metastases/lesions and median time for first-on-study SREs associated with bone metastases/lesions. The sample size calculation was based on assumption of equivalence trial. Male and female patients of ≥18 to 65 years of age with bone metastases from solid tumors and radiographic evidence of at least one bone metastasis, adequate organ function, and Eastern Cooperative Oncology Group Performance Status ≤2 were considered for screening in this study. Patients with multiple myeloma and patients who had disorders associated with abnormal bone metabolism including uncontrolled hyper- or hypothyroidism or Paget’s disease and patients currently receiving therapy with chronic systemic corticosteroid administration, or received calcitonin, parathyroid hormone-related peptides, mithramycin, strontium ranelate, or gallium nitrate within 8 weeks of selection were excluded. Patient with severe renal impairment (creatinine clearance <30 mL/min or need dialysis); patients with severe, untreated hypocalcemia; patients with hormone treatments, patients with planned radiation therapy or surgery to bone; patients with current or previous osteonecrosis or osteomyelitis of the jaw; patients with significant, nonreversible, active pulmonary disease; and patients with any concurrent severe and/or uncontrolled medical conditions were excluded. History of serious infection, which caused hospitalization within 6 months prior, was also considered in exclusion criteria.

Subjects were randomly assigned to study and reference arm in a 3:1 ratio. The randomization schedule was generated by the statistician. Randomization was managed centrally. Subject identification number was a unique number containing site number and patient number. For pharmacokinetic (PK) population, the randomization took in to account the 1:1 ratio of test and reference arm. Treatment assignment for individual subjects remained double-blind until after the study data was cleaned and the database locked as per the statistical analysis plan. It was recommended that all subjects are given at least 500 mg calcium and 400 IU vitamin D daily as per investigator’s discretion, unless hypercalcemia was present.

To maintain blinding, an unblinded person was employed in the study during the blinding activities who maintained the blinding records and codes for medications and was responsible for release of medications and maintaining the logs. The unblinded person was not involved in any other aspect of the study and was instructed not to provide the patients, physicians, or study personnel with any information about the contents of the vial. An unblinded statistician maintained the actual randomization details to maintain the
blinding. The treatment period consisted of initial double-blind period of 24 weeks followed by open-label phase till week 36. The primary efficacy assessment was performed at week 24. Patients continued to receive study medication as per their randomization arm till week 36 in open label phase.

Primary endpoint was the incidence of first on-study SRE including hypercalcemia of malignancy associated with bone metastases/lesions in patients with bone metastases from solid tumors receiving denosumab till week 24. The coprimary endpoint was the median time to first on-study SRE including hypercalcemia of malignancy associated with bone metastases/lesions in patients with bone metastases from solid tumors receiving denosumab.

Secondary endpoints included time to first and subsequent on-study SREs associated with bone metastases/lesions (till week 12, 24 and 36); incidence/proportion of patients with first and subsequent on-study SREs associated with bone metastases/lesions (till week 24 and 36); mean number of on-study SREs/patient associated with bone metastases/lesions at week 12, 24, and week 36; assessment of bone repair; change from baseline in nuclear bone scan at week 12, 24, and week 36; QoL assessment—HRQoL, assessed by change from baseline at week 12, 24 and 36; PK parameters (Cₘₐₓ, AUC₀₋ₜ and other PK parameter) assessed for single dose and multiple dose (at steady state) of denosumab. Pharmacodynamic assessment included percentage change from baseline to week 4, 8, 12, 24, and 36 in urinary N-telopeptide/creatinine (uNTx/Cr) in patients with bone metastases from solid tumors receiving denosumab. Evaluation of safety included physical examination, adverse events, abnormal hematology and biochemical laboratory parameters, vital signs, and immunogenicity testing done at baseline, 12, 24, and 36 weeks (Fig. 1).

Results

In this study, a total of 138 subjects (103 subjects in the biosimilar study arm and 35 subjects in the reference arm) were randomized across 15 centers and were included in intent to treat (ITT) population. Total of 136 randomized subjects received at least one dose of the study medication (102 subjects in the study arm and 34 subjects in the reference arm) and were included in safety/modified ITT population. The per-protocol population for this study included 135 subjects, that is, 101 subjects in study arm and 34 subjects in reference arm. A total of 94 subjects completed double-blind phase (week 24) that included 74 (71.84%) subjects in study arm and 20 (57.14%) subjects in reference arm. A total of 78 (56.52%) subjects discontinued from the study before completion of the study that included 54 (52.43%) subjects in study arm and 24 (68.57%) subjects in reference arm. Out of 103 subjects included in the ITT population from study arm, 71 (68.93%) patients were female and 32 (31.07%) were male. The mean age of these subjects

Fig. 1 Study flowchart.
was 50.45 years, mean height was 157.23 cm, mean weight was 56.82 kg, and mean body mass index (BMI) was 23.00 kg/m². Out of 35 subjects included in ITT population from reference arm, 16 (45.71%) patients were female and 19 (54.29%) were male. The mean age of these subjects was 52.63 years, mean height was 159.76 cm, mean weight was 62.26 kg, and mean BMI was 24.40 kg/m².

**Efficacy Analysis**

In primary efficacy analysis, in biosimilar study arm, 06 (5.83%) patients suffered SRE from baseline to week 24 compared with 02 (5.71%) patients in reference arm. The difference in the incidence of first on-study SRE from baseline to week 24 was statistically not significant between the two treatment arms (p > 0.05) (Table 1). No additional SREs were reported after week 24 and the number of SREs remained same in each group at week 36. Out of 06 patients in study arm, 5 (4.85%) patients were from radiotherapy to bone subgroup and 1 (0.97%) patient suffered from pathological fracture of the bone. Out of the 2 (5.71%) patients in reference arm, 1 (2.86%) patient was from radiotherapy group and 1 (2.86%) patient suffered from spinal cord compression. No patient suffered from hypercalcemia of malignancy in both the groups.

In study arm, the median time to first SRE baseline to week 36 was 4.09 months as compared with 5.90 months in reference arm. The difference in median time to first SRE from baseline was statistically not significant between the two treatment arms (p > 0.05).

In the secondary efficacy analysis, the median time to first SRE from baseline till week 12 and week 24 and 36 was 1.35 and 4.09 months, respectively, in study arm. In reference arm, no SRE was reported till week 12, while the median time to SRE from baseline till week 24 and 36 was 5.90 months. The difference in median time to first SRE from baseline till week 24 was statistically not significant between the two treatment arms (Table 2). There were no patients who reported subsequent SRE in this study.

In study arm, the mean number of on-study SRE/patient was 6 (5.83%) compared with 2 (5.71%) in reference arm till week 36 with no statistically significant difference (p > 0.05).

The assessment of bone repair was done based on the nuclear bone scan that was conducted on screening, week 12, week 24, and week 36 visit. In the study arm, 55/77 (71.43%) subjects had improvement in bone repair at 12 weeks as compared with 17/21 (80.95%) patients in reference arm. At week 24, in study arm 44/63 (69.84%) patients were assessed to have improvement in the bone scan as compared with 13/20 (65.00%) patients in reference arm. The difference in both arms was considered as not statistically significant. At week 36, in study arm 32/40 (80.00%) patients were assessed to have improvement in the bone scan as compared with 2/5 (40.00%) patients in reference arm. The difference in both arms was considered as not statistically significant.

The mean change in HRQoL was −1.54 in study arm as compared with 2.32 in reference arm at week 12. The difference between the two treatment arms was statistically significant (p < 0.05). The mean change in HRQoL was −4.62 in study arm as compared with −1.45 in reference arm at week 24. The difference between the two treatment arms was statistically significant (p < 0.05). The mean change in HRQoL was 0.58 in study arm as compared with −6.15 in reference arm at week 36. The difference between the two treatment arms was statistically not significant (p > 0.05).

The pharmacodynamic assessment showed that at week 12, the median (range) % change in uNTX from baseline in study arm was −6.78% compared with 40.00% in reference arm. At week 24, the median (range) % change in uNTX from baseline in study arm was −16.87% compared with −6.78% in reference arm.

### Table 1 Incidence of first on-study skeletal-related event including hypercalcemia of malignancy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study (n = 103)</th>
<th>Reference (n = 35)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal-related events</td>
<td>6 (5.83%)</td>
<td>2 (5.71%)</td>
<td>0.9806</td>
</tr>
<tr>
<td>Radiotherapy to bone</td>
<td>5 (4.85%)</td>
<td>1 (2.86%)</td>
<td>0.6167</td>
</tr>
<tr>
<td>Pathological fractures</td>
<td>1 (0.97%)</td>
<td>0 (0.00%)</td>
<td>NE</td>
</tr>
<tr>
<td>Spinal cord compression</td>
<td>0 (0.00%)</td>
<td>1 (2.86%)</td>
<td>NE</td>
</tr>
<tr>
<td>Hypercalcemia of malignancy</td>
<td>0 (0.00%)</td>
<td>0 (0.00%)</td>
<td>NE</td>
</tr>
<tr>
<td>Surgery to bone</td>
<td>0 (0.00%)</td>
<td>0 (0.00%)</td>
<td>NE</td>
</tr>
</tbody>
</table>

### Table 2 Median time to first SRE (ITT population)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of subjects</th>
<th>Median time (months)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study arm (n = 103)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects without SRE</td>
<td>97 (94.13%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Subjects with SRE</td>
<td>6 (5.83%)</td>
<td>4.09</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Reference arm (n = 35)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects without SRE</td>
<td>33 (94.29%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Subjects with SRE</td>
<td>2 (5.71%)</td>
<td>5.9</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Abbreviations: ITT, intent to treat; ND, not documented for evaluation period; SRE, skeletal-related event.
Table 3: Change from baseline in nuclear bone scan (PP population)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study arm (N = 102)</th>
<th>Reference arm (N = 34)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment of bone repair</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Week 12</td>
<td>55/77 (71.43%)</td>
<td>17/21 (80.95%)</td>
<td>0.341</td>
</tr>
<tr>
<td>Week 24</td>
<td>44/63 (69.84%)</td>
<td>13/20 (65.00%)</td>
<td>0.690</td>
</tr>
<tr>
<td>Week 36</td>
<td>32/40 (80.00%)</td>
<td>2/5 (40.00%)</td>
<td>0.079</td>
</tr>
</tbody>
</table>

Abbreviation: PP, per-protocol.

Baseline in study arm was −11.20% compared with −15.39% in reference arm. The proportion of patients achieving the uNTx level <50 nM BCE/mM (nanomol bone collagen equivalent/millimol) was 14 (53.85%) in study arm compared with 12 (50.00%) in the reference arm. The difference in the both arms was considered to be clinically nonsignificant. At week 36, the median (range) % change in uNTX from baseline in study arm was −29.33% compared with 55.11% in reference arm. The proportion of patients achieving the uNTX level <50 nM BCE/mM was 9 (34.62%) in study arm compared with 2 (8.33%) in the reference arm. The difference in the both arms was considered to be clinically nonsignificant.

In the PK assessment, the mean Cmax and AUC0-t was 15.92 μg/mL and 7,393.07 μg x h/mL in study arm as compared with 14.35 μg/mL and 6,471.16 μg x h/mL, respectively, in reference arm. The median T<sub>max</sub> observed for study and reference arms was 201.49 and 230.89 hours, respectively. The coefficient of variation for C<sub>max</sub> and AUC<sub>0-t</sub> was 33.45 and 37.80%, respectively, in study arm compared with 30.39 and 32.09%, respectively, in reference arm. The ratios of the mean of the ln-transformed data (T/R ratio) and 90% confidence interval for lnC<sub>max</sub> and lnAUC<sub>0-t</sub> were 109.64 and 112.07%, respectively, for denosumab. The comparable PK parameter signifies the comparable rate and extent of absorption of study and reference products. The observed T<sub>R</sub>/T<sub>R</sub> ratio for AUC and C<sub>max</sub> during this study is within 80 to 125% range. The upper limit of 90% confidence interval for AUC and C<sub>max</sub> was marginally higher than the limit for bioequivalence (80–125%).

Safety Analysis

In this study, a total of 197 adverse events were reported in the study arm and 30 were reported in the reference arm. Out of these, 162 events in study arm and 28 events in reference arm were treatment emergent adverse events. There were 61 (59.80%) subjects in study arm and 17 (50.00%) subjects in the reference arm with at least one treatment emergent adverse event. There were 07 (6.86%) subjects in study arm and 02 (5.88%) subjects in the reference arm with at least one treatment emergent adverse events related to study medication. There were five (4.90%) subjects in study arm and one (2.94%) subjects in the reference arm with at least one treatment emergent severe adverse event. There were a total of nine serious adverse events reported in the study. Out of these, eight (7.84%) events reported in study arm and one (2.94%) in the reference arm. In this study, five (4.90%) death cases were reported in study arm and one (2.94) death in reference arm. In the study arm, the most common reported adverse events were reported in general disorders and administration site conditions (21; 20.59%), followed by musculoskeletal and connective tissue disorders (19; 18.63%), gastrointestinal disorders (18; 17.65%), metabolism and nutrition disorders (11; 10.78%), nervous system disorders (9; 8.82%), blood and lymphatic system disorders (7; 6.86%), infections and infestations (7; 6.86%), investigations (6; 5.88%), renal and urinary disorders (1; 0.98%), skin and subcutaneous tissue disorders (4; 3.92%), neoplasms benign, malignant and unspecified (3; 2.94%), cardiac disorders (1; 0.98%), ear and labyrinth disorders (1; 0.98%), eye disorders (1; 0.98%), injury, poisoning and procedural complications (2; 1.96%), psychiatric disorders (1; 0.98%); and vascular disorders (1; 0.98%). The most common reported adverse events in general disorders and administration site conditions were chest pain, injection site reactions, pyrexia, and pain and disease progression.

In reference arm, the most common reported adverse events were in blood and lymphatic system disorders (3; 8.82%), general disorders and administration site conditions (3; 8.82%), infections and infestations (3; 8.82%), investigations (3; 8.82%) followed by gastrointestinal disorders (2; 5.88%), skin and subcutaneous tissue disorders (2; 5.88%), renal and urinary disorders (2; 5.88%), metabolism and nutrition disorders (1; 2.94%), musculoskeletal and connective tissue disorders (3; 8.82%), nervous system disorders (1; 2.94%), and respiratory, thoracic, and mediastinal disorders (1; 2.94%). The most common reported adverse events in reference arm were anemia, neutropenia, chest pain, asthma, injection site erythema, urinary tract infection, tooth abscess, and septic shock.
Discussion

Metastatic bone disease is most commonly seen with specific cancer types, notably those arising from the breast, prostate, lung, and kidney, as well as multiple myeloma. Preventing the onset of skeletal lesions through the inhibition of key steps of the “BM cascade” might more significantly impact both patient QoL and survival and has become the major target of several research groups.

Denosumab is a fully human anti-RANK ligand (RANK-L) antibody that prevents the interaction of this cytokine with its receptor, thereby suppressing osteoclast maturation and function. Besides the key role played in osteoclastogenesis, other functions have been attributed to RANK/RANK-L axis, including the modulation of immune response and the regulation of progesterone-induced mammary carcinogenesis. Moreover, evidence has recently described a significant reduction of circulating tumor cell count in advanced breast cancer patients receiving denosumab compared with those who did not receive the anti-RANK-L antibody ($p = 0.03$), suggesting a potential inhibitory effect of this agent against tumor cell intravasation.

Denosumab has demonstrated superiority to bisphosphonates in preventing bone complications in patients with solid tumors. Second, in an integrated analysis of three phase III clinical studies in patients with bone metastases from solid tumors, denosumab was more effective than zoledronic acid at extending the time to significant increases in pain and use of strong opioids. Finally, use of intravenous (IV) bisphosphonates requires routine renal monitoring and dose adjustments, complicating the care of these patients. In contrast, denosumab is not excreted by the kidneys and does not require renal monitoring, which may make optimum compliance and persistence easier to achieve.

Patients initiated on denosumab following a bone metastasis diagnosis had higher medication persistence, longer time to discontinuation, improved compliance, and lower switch rates compared with discrete-choice studies have suggested that patients and physicians prefer the 4-weekly subcutaneous injection of denosumab to the 3 to 4-weekly IV infusion that patients and physicians prefer the 4-weekly subcutaneous injection of denosumab to the 3 to 4-weekly IV infusion of bisphosphonates with those initiated on a bisphosphonate. Median time to first and subsequent bone complications was shorter for late versus early initiators of either agent, thereby supporting clinical recommendations to introduce bone-protecting therapy at the time of bone metastasis diagnosis to provide optimal patient care and maximize patient QoL.

In the present study, there was a complete establishment of clinical similarity of the biosimilar with the reference denosumab with respect to primary efficacy endpoint of “incidence of first on-study SRE till week 24” and the secondary efficacy endpoints related to first and subsequent on-study SREs associated with bone metastases/lesions, change from baseline in nuclear bone scan, QoL assessment, HRQoL, PK parameters ($C_{\text{max}}$, $AUC_{0-\text{t}}$, and other PK parameter) and pharmacodynamic assessment. The incidence of adverse events was comparable between two treatment arms supporting the similarity in safety profile.

There were no apparent immunologically mediated safety or efficacy concerns reported in this study. The study results have established that there is no statistically significant difference between the biosimilar denosumab and reference product in the evaluation of PK, pharmacodynamics, efficacy, safety, and immunogenicity.

Conclusion

The comprehensive evaluation in the study proves the clinical comparability of the biosimilar denosumab and reference product. There was no clinically meaningful difference in the biosimilar denosumab and reference product and this first biosimilar of denosumab can be interchangeable to the reference innovator product. Therefore, based on this comparability, it is proposed that study denosumab biosimilar may be considered as a viable alternative to reference product in the prevention of skeletal-related events in patients with bone metastases from solid tumors.

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