Denosumab Biosimilar

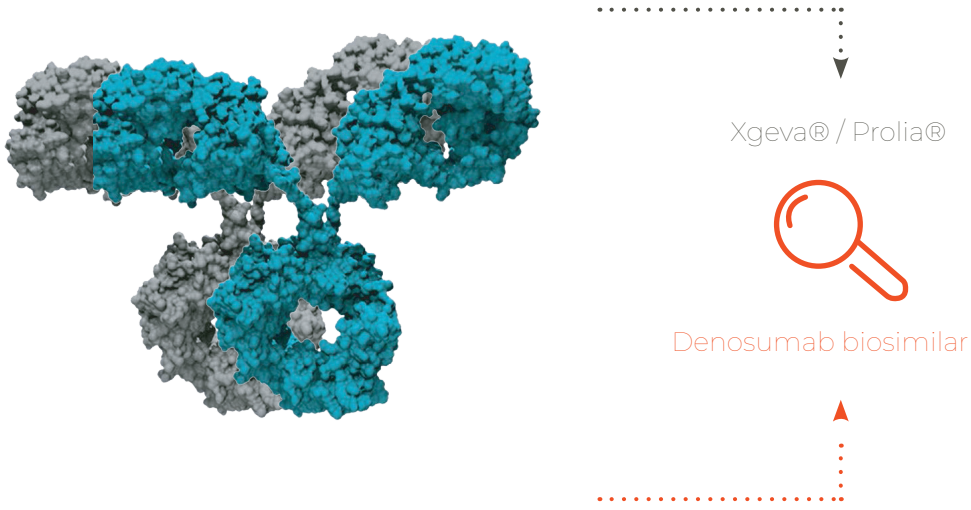
IN VITRO STUDIES: ANALYTICAL SIMILARITY

2.1. PROVEN ANALYTICAL SIMILARITY

A biosimilar medicine is a biological medicine which is highly similar to an existing approved biological product.[1] Similarity studies are needed to generate evidence substantiating the similar nature, in terms of **quality, safety and efficacy** of the biosimilar and the Reference Product (RP)[4].

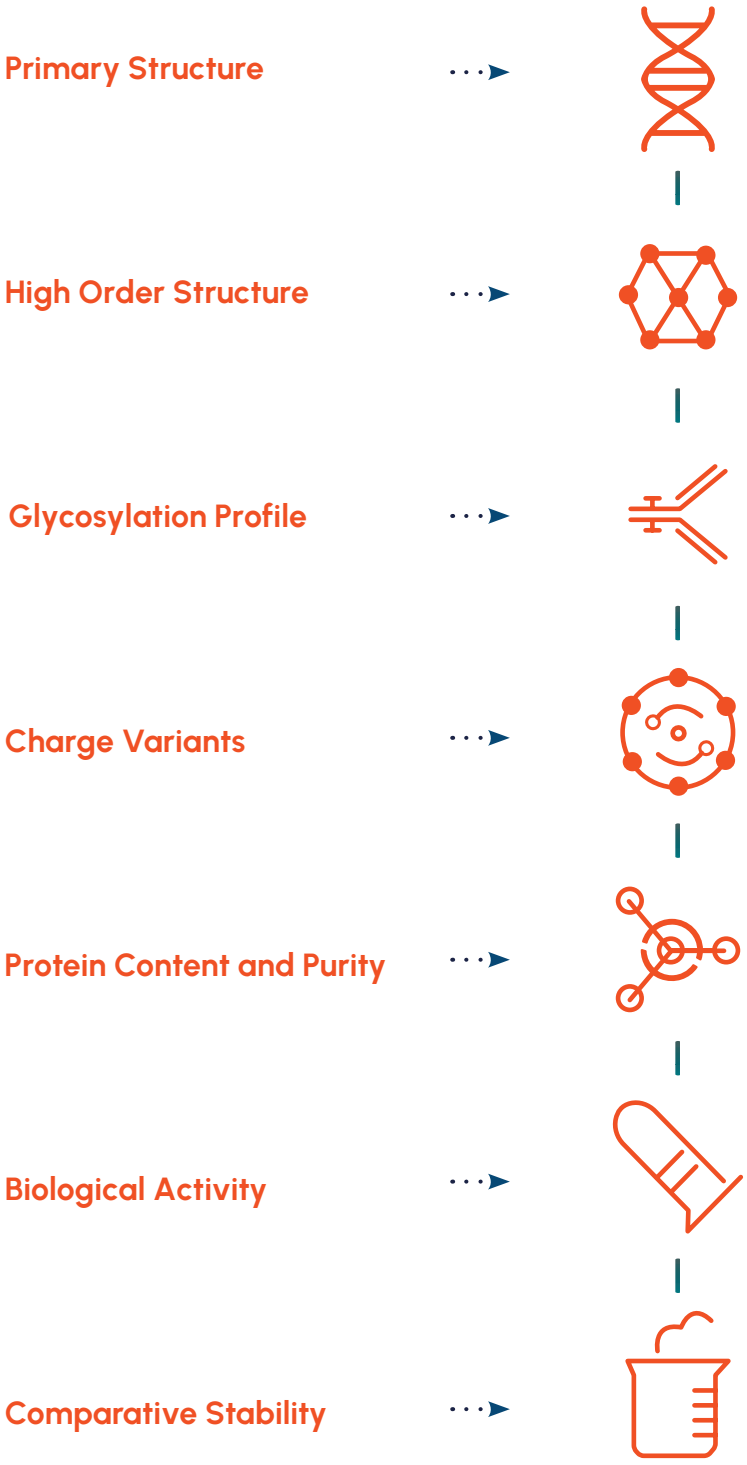
According to regulatory guidelines, all quality attributes of the biosimilar product are not expected to be identical to the RP, especially considering that these drugs are typically **large, complex molecules with tertiary and quaternary structures.** Large-sized molecules with complex high-order structures present significant analytical challenges, making exact replication practically impossible. Therefore, the **analytical data** submitted must be comprehensive and robust enough to allow for definitive conclusions regarding the **physicochemical and biological similarity** between the RP and the biosimilar[4,11].

The denosumab biosimilar is a large-sized molecule with **high order** structures, that has been developed in accordance with quality by design principles, consistently utilizing the EU and US reference products **Xgeva® and Prolia®** as RPs. The denosumab biosimilar demonstrated to be highly similar to the RP by using state-of-the-art orthogonal analytical methods. **No meaningful differences** in the quality attributes related with efficacy, pharmacokinetics (PK), safety and immunogenicity were found in the **in vitro analytical similarity exercise.**



As per regulatory recommendations, an exhaustive **analytical similarity study** was carried out. RP's and denosumab biosimilar lots were analysed for primary structure, molecular conformation, glycosylation, charge variants, protein content, purity and biological activity[4,11].

In addition, **comparative stress and stability studies** with the RP were carried out. From the in-depth comparative characterization of denosumab biosimilar and the RP, the **following conclusions** were reached:



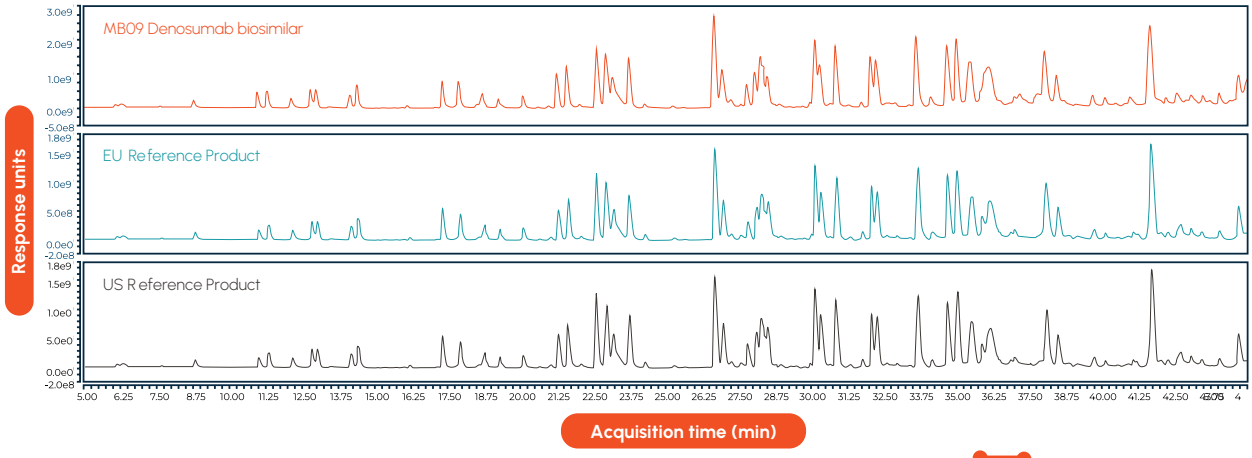
2.2. PROVEN ANALYTICAL SIMILARITY

Same amino acid sequence confirmed for denosumab biosimilar and RP.

The target amino acid (AA) sequence of the biosimilar must be confirmed to be the same as for the RP and is expected to be the same as for the RP.[4]

Each peak observed corresponds to a part of the monoclonal antibody (peptide) after its digestion by trypsin. The detection of the same peptides by mass spectrometry confirms the **same AA sequence between denosumab biosimilar and RP.** Orthogonal mass spectrometry methods confirm the **same primary structure for denosumab biosimilar and RP.**

Reduced peptide mapping



2.3. HIGH ORDER STRUCTURE (HOS)

Circular dichroism and Fluorescence

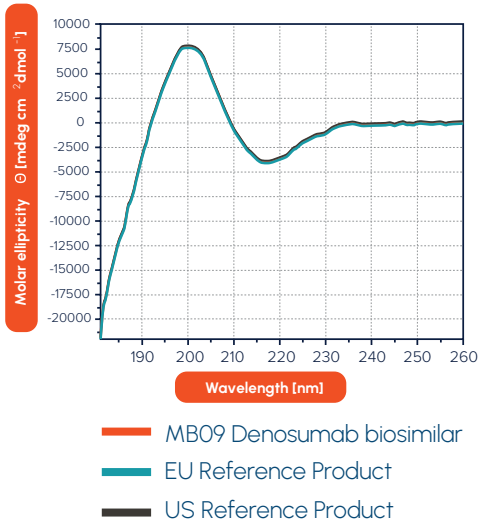
Same amino acid sequence confirmed for denosumab biosimilar and RP.

Multiple structural characterization assays have confirmed similar molecular conformation between denosumab biosimilar and the RP.

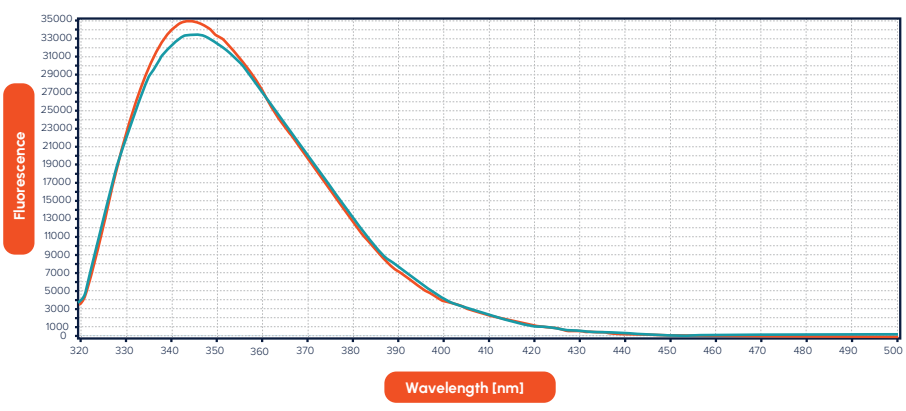
Profiles obtained from circular dichroism (CD) and fluorescence confirm **similar secondary and tertiary structure between denosumab biosimilar and RP.**

Secondary structure

Circular dichroism



Fluorescence

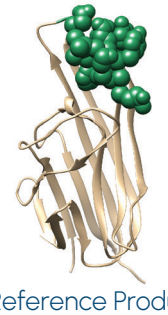


2.3. HIGH ORDER STRUCTURE (HOS)

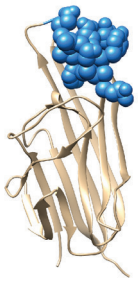
Epitope mapping

Similar epitope mapping behaviour between denosumab biosimilar and RP.

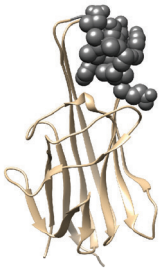
An epitope or antigenic determinant is defined as "the portion of an antigen that makes contact with a particular antibody". Denosumab biosimilar and RP showed binding to the **same epitope on human RANKL protein** evaluated by HDX-MS techniques (Hydrogen Deuterium eXchange Mass Spectrometry).



EU Reference Product



MB09 Denosumab biosimilar



US Reference Product

2.4. GLYCOSYLATION PROFILE

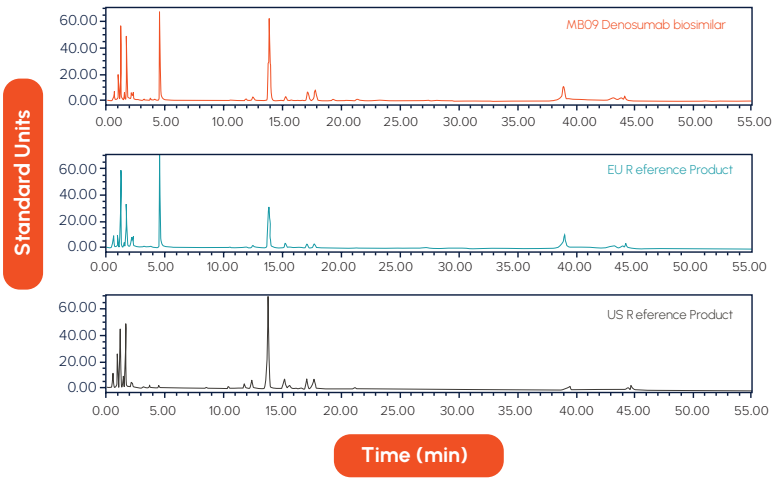
Similar N-glycans identity and distribution between denosumab biosimilar and RP.

Protein glycosylation is an important quality attribute which may impact the product's **immunogenicity, PK, safety** and **efficacy.**

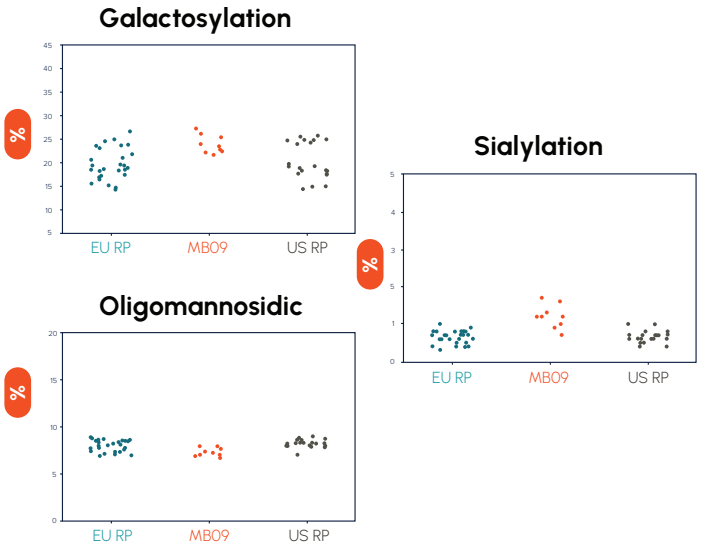
Denosumab biosimilar and RP glycosylation profiles have been characterised confirming minor differences which are not clinically meaningful. Variability in glycosylation depends on the **cell line** and **manufacturing process** which are specific to each product[14].

Denosumab biosimilar manufacturing process was designed to achieve a quality profile close to the RP and the small differences observed were confirmed to not affect the clinical performance of the product.

Chromatogram profiles by HILIC-UPLC-FLR



N-glycan quantification by HILIC-UPLC-FLR



2.5. CHARGE VARIANTS

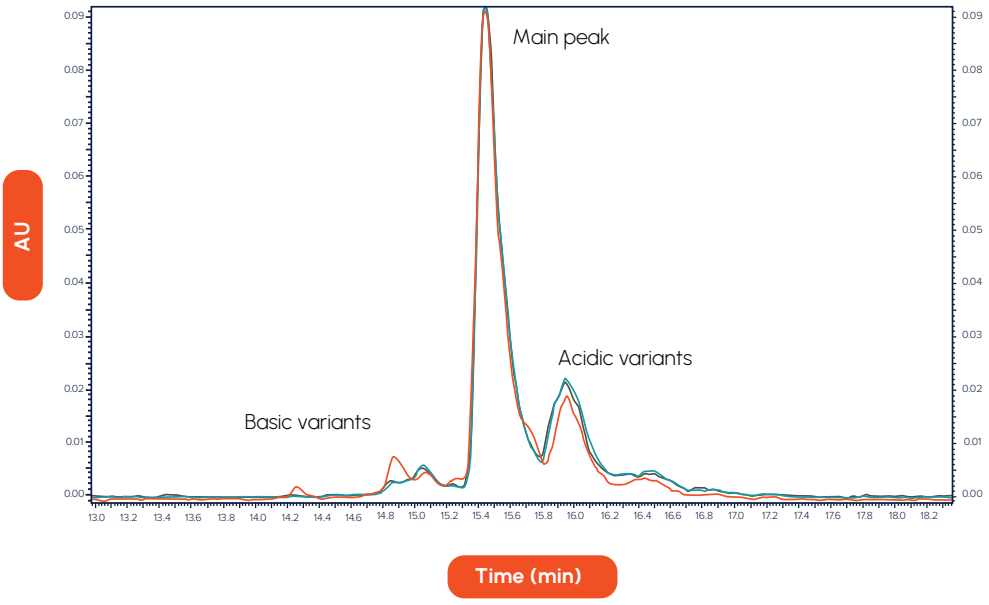
cIEF

Similar charge variants profile between denosumab biosimilar and RP.

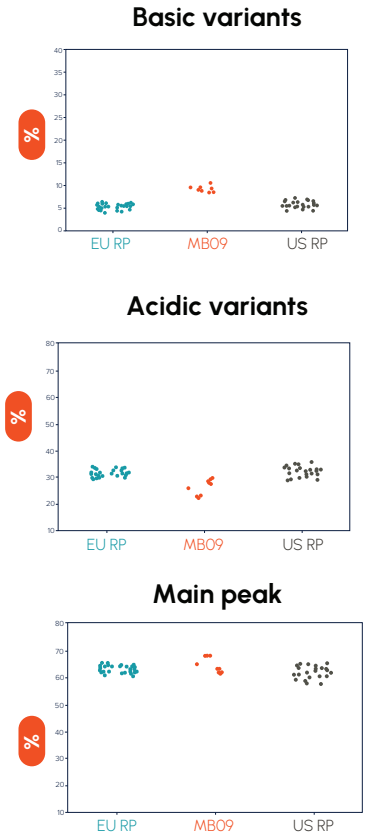
Large proteins exist as multiple charged species, with presence of positively and negatively charged AA. Protein degradation during their shelf life can also create new charges e.g. by oxidation or deamidation of the AA forming the primary sequence. Charge variants must be controlled since they may affect the efficacy and safety of the molecule.

The **distribution of charge variants** is similar in denosumab biosimilar and RP as demonstrated when analyzing by capillary isoelectric focusing. Small differences have been found in the quantification of acidic and basic species which demonstrated not to have impact on the molecule functionality.

cIEF electropherogram profiles



Charge variants quantification for cIEF



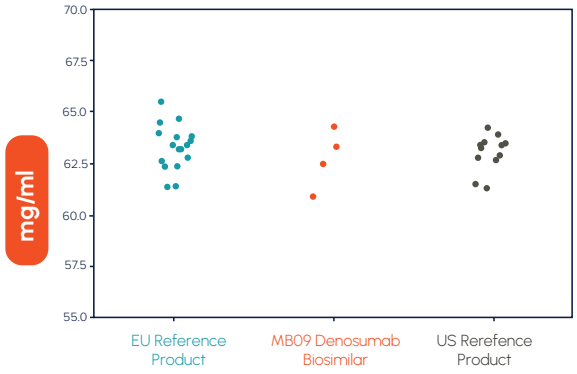
2.6. PROTEIN CONTENT AND PURITY

Denosumab biosimilar and RP have similar protein concentration and purity profile.

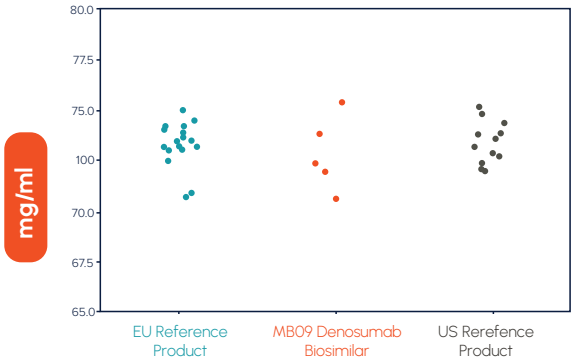
Similar protein concentration was measured for denosumab biosimilar and RP.

Protein concentration by UV

60 mg/1 mL presentation (PFS)



120 mg/1.7 mL presentation (vial)



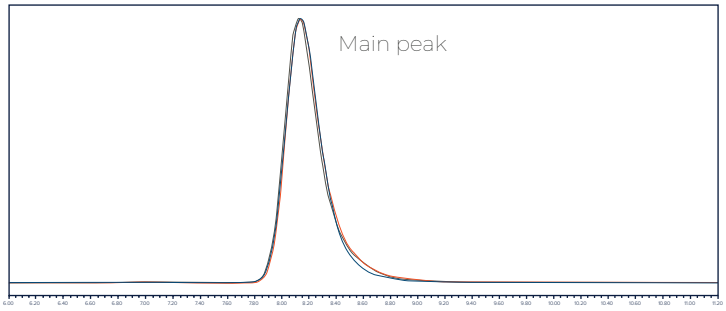
2.6. PROTEIN CONTENT AND PURITY

SE HPLC

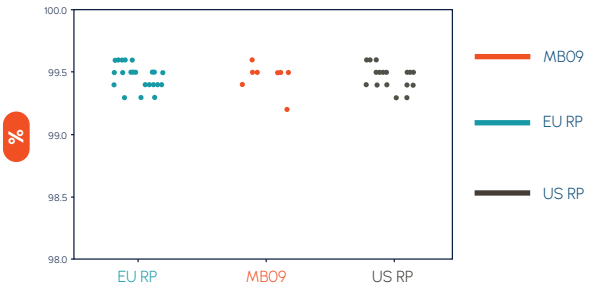
Biotherapeutic monoclonal antibodies are **highly pure molecules**, but aggregates and fragments may potentially affect the biological activity and safety.

Both denosumab biosimilar and RP are highly pure products with **> 99% purity** measured by size exclusion chromatography. Similar low levels of aggregates, such as High Molecular Weight (HMW) species, are present in both products.

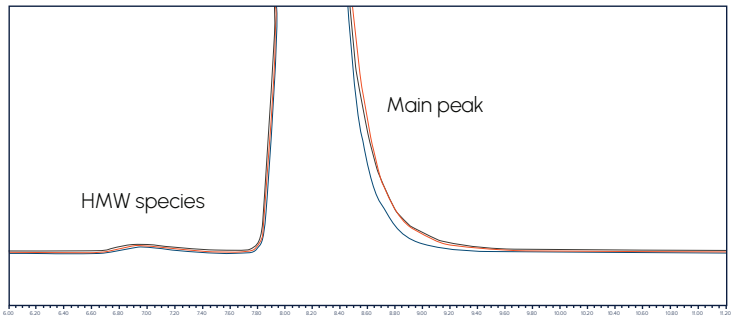
Size exclusion chromatography



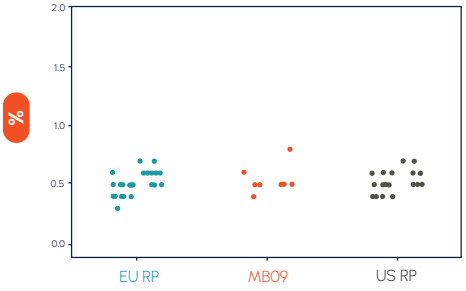
Main peak



Size exclusion chromatography (zoom)



High Molecular Weight (HMW) species



2.7. BIOLOGICAL ACTIVITY

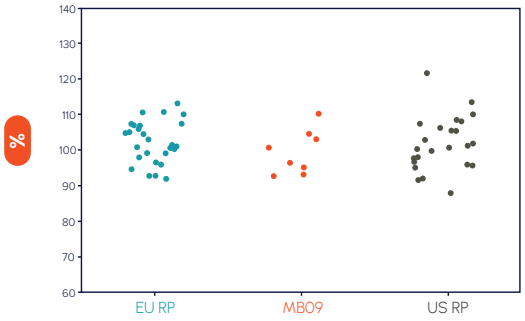
Similar biological activity between denosumab biosimilar and RP.

Denosumab targets the receptor activator of nuclear factor-kappa B ligand (RANKL), a TNF ligand superfamily member that is essential for the formation, activation, and function of osteoclasts.

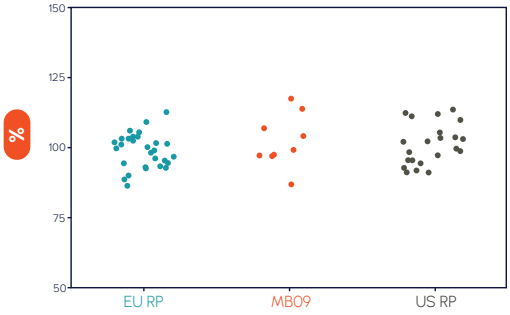
The binding of denosumab biosimilar and RP demonstrates that both products are **similar in terms of relative binding** to RANKL.

RANKL mediates osteoclasts activation, and therefore, by the neutralization of RANKL, denosumab prevents osteoclasts from activating. denosumab biosimilar and RP showed **comparable relative potency** evaluated by osteoclastogenic assay.

Relative binding by RANKL ELISA

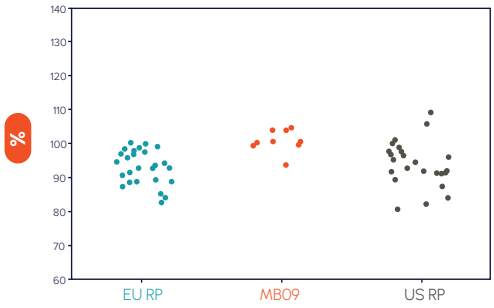


Relative potency by osteoclastogenic assay



Binding to FcRn is responsible for the long half-life of IgGs. denosumab biosimilar and RP showed comparable relative binding to FcRn by ELISA.

FcRn relative binding by ELISA

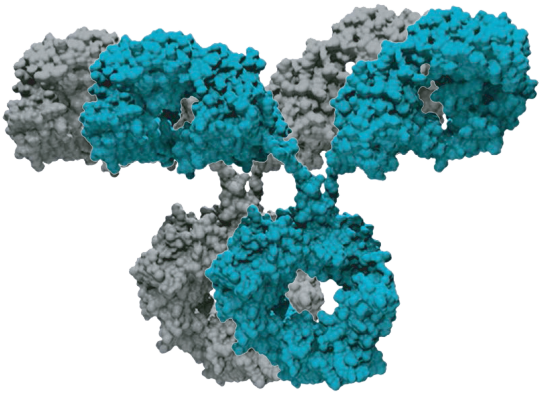


2.8. COMPARATIVE STABILITY

Similar biological stability from comparative stress and stability studies between denosumab biosimilar and RP.

MB09 Denosumab biosimilar and RP stability was compared in studies under accelerated conditions at 25°C, forced degradation conditions such as high temperature (45°C), agitation, oxidative stress, high and low pH and comparative temperature (45°C), agitation, oxidative stress, high and low pH and comparative freeze-thaw studies at -80°C. The two products showed **similar degradation pathways and kinetics.**

Denosumab biosimilar and RP were extensively characterized by orthogonal state-of-the-art assays which demonstrate analytical similarity:



Primary structure. The same AA composition is confirmed in denosumab biosimilar and RP.

High order structure. Similarity between denosumab biosimilar and RP in terms of high order structure was demonstrated.

Glycosylation profile. Similar profile with the same main glycoforms in denosumab biosimilar and RP. Few minor quantitative differences were found which are not clinically meaningful.

Charge variants. The post-translational modifications analysis demonstrated similar profiles with small variability in acidic and basic forms which have no impact on the functionality of the molecule.

Protein content and purity. Similar protein content and high purity of denosumab biosimilar and RP.

Biological activity. The extensive characterization of Fab and Fc related functions showed:

- Similar binding to RANKL by ELISA between denosumab biosimilar and RP.
- Similar relative potency in osteoclastogenic bioassay between denosumab biosimilar and RP.
- Similar binding to FcRn by ELISA between denosumab biosimilar and RP.

Comparative stability. Similar biological stability between denosumab biosimilar and RP from comparative stress and stability studies. Comparative accelerated, forced degradation, and freeze-thaw studies have shown similar behaviour between denosumab biosimilar and RP.

EXCERPT FROM THE REFERENCES

1. **European Medicines Agency.** ICH Q5E Biotechnological/biological products subject to changes in their manufacturing process: comparability of biotechnological/biological products. Step 5. CPMP/ICH/5721/03; 2005. Available from: https://www.ema.europa.eu/en/ich-q5e-biotechnological-biological-products-subject-changes-their-manufacturing-proces s-comparability-biotechnological-biological-products-scientific-guideline

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11. **Wang D, Tang X, Shi Q, et al.** Denosumab in pediatric bone disorders and the role of RANKL blockade: a narrative review. Transl Pediatr. 2023;12(3):470–486. Available from: https://doi.org/10.21037/tp-22-276

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