ALX-0141, an anti-RANK-L targeting Nanobody®, increases bone mass in cynomolgus monkeys

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The study (study no. 518127 – ‘A 26 Week Toxicology Study of ALX-0141 Administered Subcutaneously Every Two Weeks to Cynomolgus Monkeys, with a 26 Week Recovery Period) was sponsored by Ablynx nv
The study assessed safety, PK and PD effects of chronic sc treatment with ALX-0141 in nonhuman primates.

This talk is focused on the pharmacological effect of chronic sc ALX-0141 treatment on bone.
**Nanobodies**

*Camelidae* family has both forms

**Conventional antibody**
- Heavy and light chains
- Both chains required for antigen binding and stability

**Heavy-chain antibody**
- Only heavy chains
- Full antigen binding capacity and very stable

**Ablynx’s Nanobody®**

Based on the smallest functional fragment of a naturally occurring heavy-chain antibody
**ALX-0141 description**

- **Mode of action**: target is RANK-L → specific binding and blocking leads to
  - decrease in osteoclast differentiation, activation and survival and thus
  - decreased bone resorption
- **Possible indications**: (post-menopausal) osteoporosis, cancer-related bone disease, RA
ALX-0141 description

- Highly potent through bivalent formatting: avid binding with high affinity to human target
- Binding to serum albumin prolongs T1/2
- Highly stable compound
  - formulated at high concentrations facilitates administration via sc injection
- Expected favourable immunogenicity and safety
  - no Fc
  - no evidence of high Mw forms
  - humanised and optimised sequence
- Manufactured at high titres in microbial production system
- ALX-0141 has successfully completed a Phase I study in healthy postmenopausal women
Study design

Cynomolgus monkey 3 – 3.5 years old at start of dosing

A single sc administration every 2 weeks during 26 weeks

- Vehicle
- ALX-0141 5 mg/kg
- ALX-0141 50 mg/kg
- ALX-0141 100 mg/kg

4m+4f/group sacrificed after 26-weeks treatment period
+ 2m+2f/group in vehicle and high dose group sacrificed after 26-weeks recovery period
### Study design: bone turnover markers

<table>
<thead>
<tr>
<th>Category</th>
<th>Marker Type</th>
<th>Test Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary bone turnover markers</strong></td>
<td>Bone resorption marker</td>
<td>Collagen type I C-terminal telopeptide (CTx) Nordic Bioscience Diagnostic; Serum CrossLaps ELISA</td>
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<tr>
<td></td>
<td>Bone resorption marker</td>
<td>Tartrate-resistant acid phosphatase isoform 5b (TRAP5b) IDS BoneTRAP® Assay (immunoassay)</td>
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<tr>
<td></td>
<td>Bone formation marker</td>
<td>N-terminal propeptide of type I procollagen (P1NP) Orion Diagnostica UniQ PINP RIA</td>
</tr>
<tr>
<td><strong>Selected serum biochemistry</strong></td>
<td>Total alkaline phosphatase (ALP)</td>
<td>Roche P Module Clinical Chemistry Analyser using Roche Test Kit, IFCC Methods</td>
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<tr>
<td></td>
<td>Calcium</td>
<td>Roche P Module Clinical Chemistry Analyser using Roche Test Kit, IFCC Methods</td>
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<td></td>
<td>PTH</td>
<td>Architect Intact PTH Reagent kit (8K25) <em>In vitro</em> chemiluminescent Microparticle Immunoassay</td>
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<td><strong>Bone evaluation</strong></td>
<td>On excised femur and lumbar vertebral body</td>
<td>Dual Energy X-Ray Absorptiometry (DXA) Hologic Discovery A</td>
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<tr>
<td></td>
<td>Peripheral Quantitative Computed Tomography (pQCT)</td>
<td>XCT Research SA</td>
</tr>
</tbody>
</table>
Results: bone resorption and formation markers

Treatment with ALX-0141 showed expected effect on bone resorption and formation markers.

Note: only male data are shown; Animal 10m was excluded from the mean high dose group value as this animal showed deviating PK/PD profiles (immunogenicity)
Results: bone densitometry values by DXA of lumbar spine (L4) - males

Treatment with ALX-0141 increased BMC and BMD in all dose levels

Note: only male data is shown, dose levels in mg/kg
BMC: bone mineral content
BMD: bone mineral density
*Significantly different from control group p≤0.05
No statistical analysis performed on data of 2 male recovery animals
Results: bone densitometry values by pQCT of lumbar spine (L4) - males

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Results: bone densitometry images by pQCT of lumbar spine (L4) - males

Treatment with ALX-0141 markedly increased bone mass in vertebral body of lumbar spine and femur (data not shown)
Conclusions

• Chronic sc treatment of cynomolgus monkeys with ALX-0141 resulted in expected pharmacology
  ➢ suppression of bone turnover markers
  ➢ positive effects on bone mass with increase in BMC and BMD at lumbar spine and femur

• Effects on the bone turnover markers are reversible after treatment cessation for all recovery animals

• Similar effects were seen in all animals, but to a lesser extent in females

• In male animals, a long-lasting effect during the recovery period could be observed
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