ALX-0061, an Anti-IL-6R Nanobody® for Use in Rheumatoid Arthritis, Demonstrates a Different In Vitro Profile as Compared to Tocilizumab

Maarten Van Roy, Ariella Van de Sompel, Kristi De Smet, Jasper Jacobs, Tinneke Denayer, Sven Hoefman, Laura Sargentini, Hans Urrichts

Poster
#1948

Affinity of ALX-0061 and TCZ for sIL-6R and mIL-6R

- The sensitive Gyrolab® platform was used to determine Kᵢ of ALX-0061 and TCZ for both IL-6R forms.
- ALX-0061 demonstrated strong binding to the IL-6R.
- The affinity of ALX-0061 for sIL-6R is 2500-fold higher compared to TCZ.
- ALX-0061 showed preferential binding properties for sIL-6R over mIL-6R.
- Presence of HSA had no influence on binding of ALX-0061 (data not shown).

IL-6R pathway

- Due to restricted expression of membrane (m) IL-6R, signaling via the classic signaling pathway is confined to only a small population of cell types.
- IL-6 can activate cells that do not express mIL-6R through a process known as trans-signaling.

Similar potency of ALX-0061 and TCZ on mIL-6R

- Selective inhibition of IL-6 trans-signaling could provide a higher therapeutic efficacy with a better side effect profile.
- Animal studies with IL-6 inhibition show that the classic IL-6 signaling pathway via the mIL-6R has an important physiological function, such as metabolic control in the liver.
- whereas blocking the trans-signaling pathway via sIL-6R is sufficient to prevent or treat IL-6-driven diseases.

Superior potency of ALX-0061 compared to TCZ on sIL-6R

- ALX-0061 and TCZ replaced sIL-6R at a large extent (~70%) from a pre-formed ligand/target complex. The replacement IC₅₀ was comparable to the inhibition IC₅₀ for both compounds.
- In the high-affinity IL-6R/GP130 complex, maximal inhibition is lost for both compounds (~20% after 3hr and ~40% after 24hr incubation).
- While ALX-0061 maintained potent IC₅₀ in the replacement setting, TCZ showed lower potency.

<table>
<thead>
<tr>
<th>Compound</th>
<th>mIL-6R</th>
<th>sIL-6R</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALX-0061</td>
<td>0.19± 0.08 nM</td>
<td>0.61±0.36 nM</td>
</tr>
<tr>
<td>TCZ</td>
<td>9.1± 3.8 nM</td>
<td>46±12 nM</td>
</tr>
</tbody>
</table>

Ligand replacement

- Results:
  - ALX-0061 showed ~10-fold stronger potency compared to TCZ in neutralizing sIL-6R as an sIL-6R/gp130 complex.
  - The IC₅₀ of ALX-0061 remained the same, while the potency of TCZ decreased 2-fold.

Conclusions

- ALX-0061 has single domain binding and preferential sIL-6R engagement with a high affinity for sIL-6R (~2.6 nM).
- Compared to TCZ, ALX-0061 demonstrated superior affinity and potency in multiple in vitro sIL-6R based neutralisation and replacement assays.
- Presence of HSA had no influence on affinity and potency of ALX-0061.

- Potency is confirmed in vivo, using sIL-6R as PD biomarker.
- The difference in target binding profile compared to TCZ may result in:
  - a stronger engagement of the disease-driving target form (sIL-6R) in a physiological situation with both forms (sIL-6R and mIL-6R) present.
  - a different safety profile.
  - a superior benefit-risk profile.

- Results of a phase (I) clinical trial with ALX-0061 in RA patients were indicative of a strong potential for disease modifying activity of ALX-0061 (poster presentation EUP407).

References:
(1) Rose et al. J. Autoimmun. 2015
(2) Caracciolo et al. J. Autoimmun. 2015
(3) de Smet et al. J. Autoimmun. 2015
(4) Van Roy et al. J. Autoimmun. 2015