Nanobodies® – A Versatile Advanced Therapeutic Platform

G Van Heeke
April 21, 2015
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Ablynx

Corporate snapshot

CORPORATE
- Drug discovery and development company in Ghent, Belgium
- >300 employees

TECHNOLOGY
- Pioneer in next generation biological drugs – Nanobodies®
- >500 granted and pending patents

PRODUCTS
- >30 programmes – six at the clinical development stage
- Three clinical proof-of-concepts (POC)
- 2 wholly-owned products in later stage clinical development (Phase III & Phase II)
- >10 new clinical programmes anticipated over the next 3 years

PARTNERS
- AbbVie, Boehringer Ingelheim, Eddingpharm, Merck & Co, Merck Serono and Novartis

FINANCIALS
- €206M in cash at December 31st 2014
Nanobodies

Derived from heavy-chain only antibodies

- *Camelid* heavy-chain only antibodies are stable and fully functional
- Nanobodies represent the next generation of antibody-derived biologics

Ablynx’s Nanobody
- small
- robust
- sequence homology comparable to humanised/human mAbs
- easily linked together
- nano- to picomolar affinities
- intractable targets
- multiple administration routes
- manufacturing in microbial cells
Ablynx’s platform
Rapid generation of high quality biologics

Immunise llamas with antigen or use synthetic library

Wide range of highly diverse Nanobodies with 0.1-10nM affinities

Formatted* Nanobodies ready for in vivo testing

Cloning and production in microbial systems

~12-18 months

*Glycine-serine linkers from C-terminus to N-terminus
### Nanobody platform

**Competitive advantages**

<table>
<thead>
<tr>
<th>Mix and match</th>
<th>Alternative delivery routes</th>
<th>Customised half-life extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeting different pathways at once with a single Nanobody construct, e.g. multiple checkpoint inhibitors</td>
<td>Inhalation</td>
<td>Weeks/days/hours</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fc</td>
</tr>
<tr>
<td></td>
<td>Needle-free</td>
<td>Albumin-binding Nanobody</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Challenging and intractable targets</th>
<th>Alternative delivery routes</th>
<th>Cell killing</th>
<th>Manufacturing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanobodies against ion channels and GPCRs</td>
<td>Oral-to-topical</td>
<td>Nanobody-drug conjugates</td>
<td>High-yield, high-concentration, low-viscosity, microbial production</td>
</tr>
<tr>
<td>Nanobodies can reach conserved cryptic epitopes</td>
<td>Ocular</td>
<td>Ag-1</td>
<td></td>
</tr>
</tbody>
</table>

**Cell- /tissue-homing**

- Cell specificity
- Immune cell recruitment
- Tissue-specific targeting

**Cell killing**

- Ag-1
- Ag-1
- Ag-1
RSV infection in infants
High unmet medical need

- Leading cause of infant hospitalisation and primary viral cause of infant death
  - ~300,000 children* (< 5 years) hospitalised per year in 7 major markets\(^1,2\)
  - 1.9 million outpatient visits per year for infants under 1 year of age
  - increased medical cost in the first year following RSV infection\(^3\)
  - prolonged wheezing and increased risk for asthma development\(^4\)

- No widely accepted drug available to treat RSV infections
  - Synagis\(^\text{®}\) used as prophylaxis in high-risk pre-term infants only

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* Extrapolation based on estimated US prevalence
Respiratory syncytial virus (RSV)
Generation of Nanobodies to the F-protein

• Glycoprotein F trimer
  – essential for viral entry/fusion of viral and host membranes
  – highly conserved
  – several neutralisable regions / epitopes

RSV F-protein
(pre-fusion)

McLellan et al. 2013 Science
Anti-RSV Nanobody ALX-0171

Multi-valent formatting to improve potency

- Tri-valent anti-RSV (ALX-0171)
  - improve activity and strain coverage by multi-valency
  - superior virus neutralisation as compared to palivizumab

![Graph showing OD450-520 nm vs Concentration (M) for ALX-0171 and palivizumab with a 7,000-fold improvement in potency over mAb]
## Anti-RSV Nanobody ALX-0171

### Increased strain coverage

- **Tri-valent anti-RSV (ALX-0171)**
  - 5-fold more clinical isolates neutralised below LLOD with ALX-0171 compared with palivizumab

<table>
<thead>
<tr>
<th></th>
<th>A-strain</th>
<th>B-strain</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>32</td>
<td>29</td>
<td>61</td>
</tr>
<tr>
<td>palivizumab</td>
<td>0 (0%)</td>
<td>11 (38%)</td>
<td>11 (18%)</td>
</tr>
<tr>
<td>ALX-0171</td>
<td>30 (94%)</td>
<td>23 (79%)</td>
<td>53 (87%)</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Number of strains neutralised below LLOD

**Increased strain coverage**
Neonatal lamb model*

Study design

- Lambs develop lower respiratory tract infection which is associated with general malaise and specific lung pathology (comparable to infants)
- Treatment at peak of viral load on day 3 post infection (symptoms and lung pathology are already clearly present)
- Lambs develop clinical symptoms such as wheezing (comparable to infants)

*Mark Ackerman, Iowa State University
**ALX-0171**

*In vivo* proof-of-concept achieved

**Mean viral titers in BALF (day 6 post infection)**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>RSV Vehicle</th>
<th>RSV ALX-0171</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log10 FFU/mL BAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

**Lung viral lesions (day 6 post infection)**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>RSV Vehicle</th>
<th>RSV ALX-0171</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean % Involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

**ALX-0171 treatment results in**

- strong reduction of viral titres in bronchoalveolar lavage fluid (BAL)
- strong reduction of gross viral lung lesions (% involved lung tissue)
  - coincides with strong reduction F-protein expression
- a clear effect on general health status
  - weakness, depression, lethargy, drooping of ears, not eating
ALX-0171

Effect on viral lung lesions

- Plum red RSV lesions seen in lungs of RSV-infected lambs on day 6 post-infection
  - present on all lung lobes assessed

Daily inhalation of ALX-0171 markedly reduced gross lung viral lesions
ALX-0171
Highly effective in RSV-infected lambs

Daily inhalation of ALX-0171 markedly reduced symptoms of illness in RSV infected neonatal lambs

- Subjective scoring (0 to 4*) of parameters that measure general health
  - “Malaise” score: weakness, depression, lethargy, drooping of ears, and not eating

* 0 = no clinical signs; 4 = animals down
ALX-0171

Current status and next steps

• Strong therapeutic effect demonstrated in a neonatal animal model for infant RSV infection

• Well tolerated in multiple Phase I studies in adults

• First-in-infant Phase IIa study initiated in Northern Hemisphere; lead-in phase successfully completed and confirmation to proceed with placebo-controlled phase of the study

• Recruitment of Phase IIa study to continue in parts of the southern hemisphere and Asia to complete recruitment in 2015 with results anticipated in H1 2016
CXCR2

• CXC chemokine receptor family (CXCR1-CXCR7)
• Binds multiple ligands incl GROα, IL-8, ENA-78, GROβ, GROγ, GCP-2, NAP-2
• Human vs NHP
  – high sequence diversity at N-terminus, ECL2 and ECL3
• CXCR2 vs CXCR1
  – conserved EL1
CXCR2
Scientific/Therapeutic Rationale

- Airway Epithelium
- Ciliated Epithelial Cells
- Goblet Cell (discharging)
- Goblet cell hyperplasia
- Mucus secretion
- Smooth Muscle
- Contraction
- Migration
- Angiogenesis
- Blood Vessel
- Fibroblast
- myofibroblast
- Microvascular leakage
- VCAM-1 expression
- Chemotaxis
- Neutrophil
- Macrophage
- Eosinophil
- T-cell
- Mast Cell
- Collagen
- Blood Vessel
- Capillary
CXCR2

Generation of Nanobodies

Immunise llamas

Construction of Nb libraries on phage + panning

Binding assays (Phage ELISA, FACS)

*In vitro* functional assays:
- CHO-huCXCR2 GTP\(\gamma\)S/FLIPR
- Isolated and whole blood nphil shape-change
- Nphil chemotaxis

Selectivity vs CXCR1, 4, CCRs

X-reactivity with NHP and rabbit CXCR2

Formatting and humanise

Half-life extension, DAS, epitope mapping

MoA, *in vivo* PK/PD

3x RBL/huCXCR2 and 1x RBL/cyCXCR2

3 rounds of panning against whole cells, cell membranes, peptides

77 % homology with CXCR1
33 % homology with CXCR4

92 % homology with cyCXCR2
73 % homology with rabbitCXCR2
CXCR2 lead Nanobodies

Two classes with distinct properties

- Large panel of Nanobodies identified
- Class 1 (Nb 127D1)
  - bind to 1-19 N-terminal peptide
  - partial but very potent inhibition of GROα-activation
- Class 2 (Nb 163E3)
  - do not bind to 1-19 N-terminal peptide
  - full but less potent inhibition of GROα-binding
- Bind to human and cynomolgus CXCR2
- Nanobodies do not bind CXCR1

CXCR2 Nanobodies
Understanding the MoA

- Schild experiments performed using GRO-α-stimulated [35S]GTPγS binding in the presence of a range of concentrations of
  (a) Class 1 Nb (1-19 binder)
  (b) Class 2 Nb (non-1-19 binder)
  (c) Schild plot for Class 2 binder derived from data shown in (b)
**CXCR2 Nanobodies**

Where do they bind?

- **Class 1**
  - N-terminal peptide
  - linear epitope

- **Class 2**
  - ECL1 and ECL3
  - complex epitope
  - conformationally sensitive

- **Class 1 and 2** recognize non-overlapping epitopes
Nanobody formatting

Biparatopic format yields the required potency and efficacy

Biparatopic is both potent and efficacious
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