Development of ALX-0171, an inhaled Nanobody® for the treatment of respiratory syncytial virus infection in infants

Erik Depla

Human Antibodies and Hybridomas
31 March – 2 April 2014, Vienna, Austria
## Company highlights

### Corporate
- Drug discovery and development company - Ghent, Belgium
- NYSE Euronext Brussels (ABLX)
- 49M shares outstanding (52M fully diluted)
- >300 employees

### Technology
- Pioneer in next generation biologics – Nanobodies®
- >500 granted and pending patents

### Products
- ~30 programmes – seven in clinical development
- Two clinical proof-of-concepts
- >800 healthy volunteers and patients treated with Nanobodies

### Partners
- AbbVie, Boehringer Ingelheim, Eddingpharm, Merck & Co, Merck Serono and Novartis
- >€320M in non-dilutive cash received to date

### Financials
- €200M in cash at 31st December 2013
- €20M net cash burn for the full year 2013
Nanobodies – proven single variable domain approach

Camelidae family has both forms

Conventional antibody
- Heavy and light chains
- Both chains required for antigen binding and stability
- Large size and relatively low formatting flexibility
- Administered through injection

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

Ablynx’s Nanobody®
- Small (1/10 size of a mAb)
- Flexible formatting
- Highly potent, robust and stable
- Broad target applicability
- Multiple administration routes
- Ease of manufacture
- Speed of discovery
Nanobody discovery process – the power of evolution

1. **Immunize llama with antigen**
   - Draw blood 6–12 weeks later

2. **Conventional antibodies**
   - Select Nanobodies of interest
   - Manufacture in micro-organisms
   - Format Nanobody to achieve desired properties
   - plus half-life extension (HLE)

3. **Ablynx’s Nanobody®**
   - Clinical trials
   - Select Nanobodies of interest
Nanobodies – uniqueness and competitive advantages

- Broad target applicability, including challenging targets such as GPCRs and ion channels
- Flexible formatting: multivalent, multi-specific, bi-paratopic
  Nanobodies in the clinic
- Robustness allows for alternative delivery such as nebulisation
- Half-life engineering technology to achieve desired properties (acute vs chronic diseases) ($T_{1/2}$ from 2h to 20 days)
- Excellent manufacturing (yeast and bacteria), high concentration formulations and low viscosity (excellent syringeability)
Anti-RSV Nanobody - ALX-0171

Nanobodies® - Inspired by nature
ALX-0171 – Nanobody for treatment of RSV infection

Large unmet need for RSV-specific treatment
• 1.9 million outpatient visits for infants <1 year
• 0.3 million hospitalised infants <5 years
• adults and elderly with cardiopulmonary disease
• transplant patients

No widely accepted anti-viral treatment available

ALX-0171 a potential first-in-class RSV therapeutic
• treatment of infants presenting as out-patients
• treatment of hospitalised patients
• designed to be potent, safe, and optimally delivered to the site of infection
ALX-0171 – superior \textit{in vitro} efficacy

- ALX-0171 binds F (fusion) protein, a conserved viral target on the envelope
- Trivalent format improves potency and strain coverage over palivizumab

\begin{align*}
\text{A serotype} & \quad \text{RSV-LM-2B(1/30)} \\
\text{B serotype} & \quad \text{RSV-B-1(1/27)}
\end{align*}

ALX-0171*: single amino acid variant of ALX-0171 with equal potency \textit{in vitro}
ALX-0171 designed for superiority - *in vitro* potency against recent clinical RSV isolates

- Plaque reduction assay to evaluate *in vitro* neutralization*:
  - against 61 recent clinical isolates (1990-2010)
  - target concentration of test item 40 µg/mL

- **100-fold reduction in viral titers**

<table>
<thead>
<tr>
<th>serotype</th>
<th>n</th>
<th>palivizumab</th>
<th>ALX-0171</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-strain</td>
<td>31</td>
<td>27 (87%)</td>
<td>31 (100%)</td>
<td>0.11</td>
</tr>
<tr>
<td>B-strain</td>
<td>30</td>
<td>26 (86%)</td>
<td>28 (93%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>53 (87%)</td>
<td>59 (97%)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

- ALX-0171 reduced all strains by at least 100-fold, except for 2 B-strains

*Pedro Piedra, Baylor College of Medicine*
ALX-0171 designed for superiority - \textit{in vitro} potency against recent clinical RSV isolates

- Plaque reduction assay to evaluate \textit{in vitro} neutralization*:
  - against 61 recent clinical isolates (1990-2010)
  - target concentration of test item 40 µg/mL

- \textbf{Reduction viral titre below detection limit (< 5 pfu/ml)}
  - on average ≈ 10,000 fold reduction

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<tr>
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<th>palivizumab</th>
<th>ALX-0171</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-strain</td>
<td>31</td>
<td>1 (3%)</td>
<td>29 (93%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B-strain</td>
<td>30</td>
<td>11 (37%)</td>
<td>22 (73%)</td>
<td>0.009</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>12 (20%)</td>
<td>51 (84%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

- ALX-0171 neutralizes 84% of isolates and is superior to palivizumab

*Pedro Piedra, Baylor College of Medicine
ALX-0171 – therapeutic effect in cotton rat model

ALX-0171 administered by intranasal instillation significantly reduces viral replication in lung even when administered two days post infection.
Cotton rat safety study reveals beneficial effect on lung inflammation markers

Goal: assessing safety after RSV infection and reinfection

- ALX-0171 delivered by intratracheal instillation as mimic of the clinical route

No safety signals
- e.g. exaggerated inflammation, histopathology

ALX-0171 has a beneficial effect on:
- body weight development
- lung inflammation
Neonatal lamb model* – study design

4 groups:
- non infected/placebo
- non-infected/ALX-0171
- RSV infected/placebo
- RSV infected/ALX-0171

Challenge with RSV-M37 clinical isolate

Therapeutic treatment starts at viral peak (d3)
- coincides with increase in lung pathology and first symptoms

Nebulisation of ALX-0171 / placebo

*Mark Ackerman, Iowa State University
Reduced viral titre and lung lesions in lamb model

ALX-0171 treatment results in
- strong reduction of viral titres in broncho-alveolar lavage fluid (BAL)
- strong reduction of gross viral lung lesions (% involved lung tissue) - co-incides with strong reduction F-protein expression
- a clear effect on general health status was observed - weakness, depression, lethargy, drooping of ears, and not eating

![Graphs showing viral titers and viral lesions](image-url)
Extensive preclinical safety package

- Excellent local and systemic tolerability
- No adverse effects on respiratory parameters (respiration rate, tidal volume and minute volume)
- No exacerbation of pathogenesis during RSV infection
- No inflammation after virus-rechallenge due to drug-virus complexes

<table>
<thead>
<tr>
<th>Study</th>
<th>species</th>
<th>route</th>
<th>goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease model (non-GLP)</td>
<td>cotton rat</td>
<td>intra-tracheal</td>
<td>assess NB safety in RSV-infected cotton rat evaluate possible exacerbations</td>
</tr>
<tr>
<td>14-day toxicity study</td>
<td>rat (adult)</td>
<td>inhalation</td>
<td>assess tolerability and safety after inhalation</td>
</tr>
<tr>
<td>Single dose safety pharmacology</td>
<td>rat</td>
<td>inhalation</td>
<td>assess effect on breathing pattern</td>
</tr>
<tr>
<td>14-day toxicity study</td>
<td>rat</td>
<td>i.v.</td>
<td>assess systemic tolerability and safety</td>
</tr>
<tr>
<td>Single dose safety pharmacology</td>
<td>dog</td>
<td>i.v.</td>
<td>assess parameters after high systemic exposure</td>
</tr>
<tr>
<td>BCOP</td>
<td>bovine</td>
<td>local</td>
<td>assess effect of ocular exposure</td>
</tr>
</tbody>
</table>
ALX-0171 – successful delivery to infant lung model

- Harsh conditions of nebulisation require robust, highly stable biologic

- ALX-0171 nebulised without significant molecular changes or loss in potency
  - nebulisers with mesh principle used (small and silent)
  - up to 95% of filled volume is rapidly nebulised

- Demonstration of delivery to infant lower respiratory tract in SAINT model(1)
  - results with 10 mg ALX-0171 dose:
    - filling volume: 0.2 mL
    - nebulisation time: ≈ 44 seconds
    - nebulised: > 90% (> 9.0 mg)
      - in respiratory tract: ≈ 4.5 mg (≈ 50%)
      - in lower respiratory tract: ≈ 1.0 mg (≈ 12%)

(1) Journal of aerosol medicine, Vol 14, Number 4, 2001; Janssens et al
ALX-0171 – Phase I design

**Randomisation**

- Male Healthy Volunteers

**SAD (double-blinded) inhalation**

- 32 subjects

  - ALX-0171 2.1mg
  - ALX-0171 7mg
  - ALX-0171 21mg
  - ALX-0171 70mg
  - ALX-0171 140mg
  - ALX-0171 210mg

- Placebo

**MD (double-blinded) inhalation (bid 5 days)**

- 12 subjects

  - ALX-0171 – 140mg
  - ALX-0171 – 210mg

- 4 subjects

  - Placebo

**Objectives**

- Determine safety and tolerability
- Evaluate lung function (spirometry and DLCO)
- Evaluate dose limiting toxicity and determine maximum tolerated dose
- Evaluate PK (plasma)
- Evaluate immunogenicity (systemic and local)
ALX-0171 – Phase I results

- Well-tolerated and no dose-limiting toxicity
  - no SAEs occurred
  - no trends and no dose-related TEAEs
  - no clinically significant findings or trends in clinical/laboratory parameters, vital signs, ECGs, physical examinations

- No clinically significant findings or trends in lung function
  - lung auscultations or lung function test parameters (spirometry and DLCO)
  - no trends in exhaled NO

- No treatment emergent immunogenicity observed

- Opportunity for once daily dosing
  - estimate based on plasma PK: pulmonary average half-life of ≈ 20h
ALX-0171
ongoing clinical safety and PK studies

✔️ A specific safety study in adults to evaluate potential risk of bronchoconstriction
  • bronchoconstriction has not been observed in the first-in-man study
  • concern in relation to any inhaled therapy in the infant population
  • study in adults with hyper-responsive airways will further evaluate the potential occurrence of bronchoconstriction
    - following single escalating doses as well as repeated inhalation of ALX-0171
  • if indicated, the prevention or reversion of bronchoconstriction, involving the administration of a standard bronchodilator will be assessed

✔️ A local and systemic PK study in healthy adult volunteers
  • to confirm half-life in the lung upon single and multiple dosing using a once daily regimen
    - plasma, urine and BALF sampling to determine PK profile
  • data will allow to model, in combination with preclinical in vitro and in vivo data, an efficacious dosing scheme for a first-in-infant study
ALX-0171 Summary

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Designed to be POTENT

• high *in vitro* antiviral activity against recent clinical isolates
• efficacy demonstrated in *in vivo* cotton rat and lamb model

Designed with SAFETY in mind

• biologic targeting the virus: intrinsic low risk for off-target effects
• extensive preclinical package demonstrating good tolerability
• well tolerated in first in man study

Designed for OPTIMAL DELIVERY

• nebulisation → fast onset of action and high concentration at infection site
• demonstrated significant deposition in infant lung (SAINT model)

First in infant study planned for the winter season 2014-2015

• following completion of 2 additional phase 1 studies in adults
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