Bispecific Nanobodies with enhanced cell specificity
PEGS 2015 Boston
Catelijne Stortelers, 8 May 2015
Forward looking statements

Certain statements, beliefs and opinions in this presentation are forward-looking, which reflect the Company or, as appropriate, the Company directors’ current expectations and projections about future events. By their nature, forward-looking statements involve a number of risks, uncertainties and assumptions that could cause actual results or events to differ materially from those expressed or implied by the forward-looking statements. These risks, uncertainties and assumptions could adversely affect the outcome and financial effects of the plans and events described herein. A multitude of factors including, but not limited to, changes in demand, competition and technology, can cause actual events, performance or results to differ significantly from any anticipated development. Forward looking statements contained in this presentation regarding past trends or activities should not be taken as a representation that such trends or activities will continue in the future. As a result, the Company expressly disclaims any obligation or undertaking to release any update or revisions to any forward-looking statements in this presentation as a result of any change in expectations or any change in events, conditions, assumptions or circumstances on which these forward-looking statements are based. Neither the Company nor its advisers or representatives nor any of its parent or subsidiary undertakings or any such person’s officers or employees guarantees that the assumptions underlying such forward-looking statements are free from errors nor does either accept any responsibility for the future accuracy of the forward-looking statements contained in this presentation or the actual occurrence of the forecasted developments. You should not place undue reliance on forward-looking statements, which speak only as of the date of this presentation.
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
- >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

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

**PARTNERS**

**FINANCIALS**
- €206M in cash at December 31st 2014
What are Nanobodies?

Unique technology
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

Conventional antibodies

- CH1
- CH2
- CH3
- VL
- VH

Heavy chain only antibodies

- CH2
- CH3
- VH

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
## 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>Oral-to-topical</td>
<td>Fc</td>
</tr>
<tr>
<td></td>
<td>Ocular</td>
<td>Albumin binding Nanobody</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Challenging and intractable targets</th>
<th>Cell killing</th>
<th>Manufacturing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanobodies against ion channels and GPCRs</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></td>
<td></td>
</tr>
</tbody>
</table>

Cell-/tissue-homing:
- Cell specificity
- Immune cell recruitment
- Tissue-specific targeting
Nanobodies

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
  - 5-fold more clinical isolates neutralised below LLOD with ALX-0171 compared with palivizumab

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

Number of strains neutralised below lower limit of detection

Improved potency over mAb

Increased strain coverage
Multi-specific targeting of two cytokines

Bi-specific anti-IL-17A/F Nanobody

- Targeting both IL-17A and IL-17F for more effective blocking of the inflammatory response
  - IL-17F forms homodimer and heterodimers with IL-17A
  - IL-17F exerts similar *in vitro* biological activity as IL-17A but is secreted by different cell types

- ALX-0761 blocks both IL-17A and IL-17F
- Binds human serum albumin for improved PK
- Proof of concept in primate CIA model

- ALX-0761 in development by Merck Serono
  - completed Phase I SAD study in healthy volunteers
  - ongoing Phase Ib study in patients with psoriasis
    (results expected in 2015)

---

1 Poster available on Ablynx website: [R&D-pipeline](#)
Bi-specific Nanobodies

Cell specific targeting

• Goal: improve selectivity and reduce toxicity by bispecific Nanobodies targeting 2 membrane receptors on the same cell – *in cis*

• Concept: increase therapeutic window by reducing toxicity to normal cells combining
  – a low affinity antagonist of a functional receptor
  – a cell-specific anchor Nanobody, binder with moderate-high affinity

• Bispecific will potently block receptor function in cell subset-specific way
  – avidity only on cells that co-express both targets upon simultaneous binding
  – no effective receptor blockade on normal cells, reduced toxicity compared to monospecific drugs
Bi-specific Nanobodies

Case studies- enhanced specificity

1. Bispecific CXCR4-CD4 Nanobodies for HIV entry blockade

2. Preferential targeting and blockade of specific T cell subsets

3. Cell-specific blockade of EGFR on colorectal tumours
Bi-specific Nanobodies

Case study 1- CXCR4-CD4 Nanobodies blocking HIV1 entry

• Goal: block HIV gp120 binding to CD4 and CXCR4 on host immune cells
• Viral diversity is extremely high
  – all HIV strains need the primary receptor CD4 and a chemokine co-receptor to enter and infect host CD4+ T cells
  • X4 viruses use CXCR4, syncytium inducing, emerge at late stage
  • R5 viruses use CCR5, non-syncytium inducing, prevalent in early disease

Collaboration with Dr. Dominique Schols, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium
CD4 and CXCR4 Nanobody formats and characterisation

- Format: combine functional antagonists with specific epitope
  - anti-CXCR4 (281F12)
    - inhibitor of HIV1 entry
    - poor ligand blocker (retain normal CXCR4 function)
  - anti-CD4 (3F11)
    - inhibits gp120-CD4 interaction
    - no effect on TCR function
- each in N / C-terminal position
- flexible GGGS-linker of 3 distinct lengths (9, 25, 35)

<table>
<thead>
<tr>
<th>Target</th>
<th>Nb</th>
<th>Cell binding</th>
<th>HIV-1 infection</th>
<th>CXCR4 function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MOLM-13 EC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>T cells EC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>MT-4 - NL4.3 IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
</tr>
<tr>
<td>CD4</td>
<td>3F11</td>
<td>0.7</td>
<td>0.76</td>
<td>34.7</td>
</tr>
<tr>
<td>CXCR4</td>
<td>281F12</td>
<td>5.2</td>
<td>7</td>
<td>22.7</td>
</tr>
</tbody>
</table>

Collaboration with Prof. Dr. Dominique Schols, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium
CXCR4-CD4 Nanobodies synergistically enhance HIV1 neutralization

• Synergistic improvement in X4 HIV-1 blockade of CXCR4-CD4 bi-specific Nanobody over monovalent Nanobodies
  – up to 320-fold enhancement with bi-specific
  – only 2-fold enhancement with combination of monovalents in solution (1:1) over monospecific
  – linking of the Nanobodies is essential for strong enhancement
  – orientation influences degree of synergy

Collaboration with Dr. Dominique Schols, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium
Bi-specific Nanobodies also improve CXCR4 potency on double positive cells

- Effect of bi-specific format on CXCR4 function:
  - CXCR4 Nanobody has only poor potency (>100 nM) on cells that lack CD4
  - in presence of anchor the potency of CXCR4 Nanobody improves 150-fold
  - orientation: loss in affinity of CXCR4 Nanobody in C-terminal position
CXCR4-CD4 Nanobodies retain potency on dual tropic and CCR5 tropic HIV1 strains

- Bi-specific Nanobodies have picomolar potency on CXCR4 (X4) and dual tropic CXCR4/CCR5 (X4/R5) HIV strains
- On CCR5 tropic isolates bi-specific retains low nM potency!
  - improvement over CD4 Nanobody through attachment of CXCR4 anchor

Collaboration with Dr. Dominique Schols, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium
Bi-specific Nanobodies retain nM potency even on resistant HIV strains

- Potency of bi-specific Nanobody maintained even HIV strains resistant to monotherapy
  - both CXCR4 and CD4 resistant strains

Bispecific is relatively insensitive to resistance to one of the targets

Collaboration with Dr. Dominique Schols, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium
Bi-specific Nanobodies

Case study 2- preferential targeting of T cell subsets

- Goal: Preferential targeting & blockade of specific T cell subsets
- Functional arm: low affinity antagonistic Nanobodies against
  - T<sub>H</sub>1 subset specific receptor: IL-12Rβ2
  - T<sub>H</sub>17 subset specific receptor: IL-23R
  - shared dimerisation partner: IL-12Rβ1
- Anchor arm: anti-CD4 (3F11)
  - no effect on TCR function
- Format: N-and C-terminal orientations, flexible 35GS-linker

<table>
<thead>
<tr>
<th>Nb</th>
<th>SPR (ILR-Fc)</th>
<th>Ligand competition ELISA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k&lt;sub&gt;a&lt;/sub&gt; (1/Ms)</td>
<td>k&lt;sub&gt;d&lt;/sub&gt; (1/s)</td>
<td>K&lt;sub&gt;D&lt;/sub&gt; (M)</td>
</tr>
<tr>
<td>IL12Rb2 #2</td>
<td>2.7E+05</td>
<td>1.8E-03</td>
<td>6.9E-09</td>
</tr>
<tr>
<td>IL12Rb1 #31</td>
<td>7.2E+05</td>
<td>1.7E-02</td>
<td>2.3E-08</td>
</tr>
<tr>
<td>IL23R #20</td>
<td>3.0E+06</td>
<td>2.3E-01</td>
<td>7.8E-08</td>
</tr>
</tbody>
</table>
Bi-specific Nanobodies bind preferentially to CD4+ T cells

- All bi-specifics strongly bind to CD4+ but not to CD8+ T cells in heterogeneous T cell pool
  - Monovalent IL-12Rβ1/2 Nbs equally bind to CD8+ and CD4+ T cells
Improved inhibition of T\textsubscript{H1} cell function with bi-specific Nanobodies

- Inhibition of IL-12 mediated release of T\textsubscript{H1} cytokine IFN\textgamma in T cells
  - >1000-fold improvement through attachment of CD4 anchor
  - effect seen only with Nanobodies that block T\textsubscript{H1} function (IL-12R\beta1 and \beta2)
Improved inhibition of $T_{H17}$ cell function with bi-specific Nanobodies

- Inhibition of IL-23 mediated release of $T_{H17}$ cytokine IL-17 in PBMCs
  - >1700-fold improvement through attachment of CD4 anchor
  - effect seen only with Nanobodies that block $T_{h17}$ function (IL-12Rb1 and IL-23R)

Preferential blockade of T cell subsets achieved with bispecific Nanobodies
Bi-specific Nanobodies

Case study 3- enhanced cell specificity EGFR-CEA in CRC

• Goal: Inhibition of EGFR function only on tumour cells, but not on normal cells
  – prevent toxicity (skin rash), liver uptake observed with anti-EGFR therapies

• Functional arm: EGF receptor (ErbB-1, Her1)
  – over-expressed in cancers of epithelial origin, correlates with poor prognosis
  – also expressed on normal cells

• Tumour specific antigen: CEACAM5
  – marker for GI tract cancers, breast, and lung cancers
  – 60 times higher in tumour than in healthy tissues

• Co-expressed in gastric and CR cancer
  – CEA > EGFR expression

Expression levels EGFR and CEA assessed by IHC in 280 patients (15 scores)

EGFR and CEA Nanobody formats and characterisation

- Format:
  - Anti-EGFR Nanobody 7D12 variants with range in affinity
    - dematured based on co-crystal structure with EGFR ectodomain
    - binding to EGFR domain III, overlapping with Cetuximab epitope
  - Anti-CEACAM5 Nanobody NbCEA5 variants with 2 different affinities
    - $K_D$ 0.5 nM and 3 nM
  - Orientation: EGFR-CEA, single flexible 35GS linker

<table>
<thead>
<tr>
<th>Nb</th>
<th>SPR sol. hEGFR</th>
<th>EGF displacement HeLa cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$ka$ (1/Ms)</td>
<td>$kd$ (1/s)</td>
</tr>
<tr>
<td>EGFR#1</td>
<td>2.1E+05</td>
<td>2.4E-02</td>
</tr>
<tr>
<td>EGFR#11</td>
<td>2.2E+05</td>
<td>3.7E-02</td>
</tr>
<tr>
<td>EGFR#33</td>
<td>2.2E+05</td>
<td>5.4E-02</td>
</tr>
<tr>
<td>EGFR#32</td>
<td>2.7E+05</td>
<td>2.3E-01</td>
</tr>
</tbody>
</table>

Model based on Schultz et al. 2013, Structure
Bi-specific Nanobodies show enhanced potency against tumours

- Selective blockade of EGFR on CEA⁺ tumour cells over normal cells
  - mono- and bispecific formats poor blockers on EGFR⁺ normal cells
  - up to 80 fold potency enhancement with bi-specific Nanobody on CEA⁺/EGFR⁺ tumour cells compared to mono-specific Nanobody
  - enhancement dependent on CEA/EGFR target ratio
Bi-specific Nanobodies

Conclusions

• Bispecific Nbs to CXCR4-CD4 synergistically enhance potency to HIV1 viruses
  – bi-specifics significantly more potent (160-320) fold than monovalent Nanobodies alone or when single Nanobodies are used in combination
  – bi-specifics retain potency even in viruses resistant to monotherapy, and CCR5-tropic strains

• Bi-specific enhancement in binding and functionality also demonstrated with multiple other Nanobody pairs
  – non-related receptors (GPCR, RTK, cytokine receptors, GPI-linked proteins,..)
  – CD4/IL-12R and CD4/IL-23R bi-specific Nanobodies show selective binding and functional activity on CD4+ T\textsubscript{H1} and T\textsubscript{H17} cells, respectively
  – EGFR/CEA bi-specific Nanobodies have improved EGFR potency on double positive tumour cells versus EGFR-expressing normal cells
  – enhancements of 10 to 2,000-fold typically seen

• Ease of Nanobody formatting and construction of bi-specifics allows rapid development of biologics with dramatically improved cell selectivity and potency
Acknowledgements

- Rega Institute for Medical Research, University of Leuven, Belgium
  - Dominique Schols, Sandra Claes, Thomas D’huys, Geoffrey Ferir

- Ablynx team
  - Hugo Soares, Ines Cabrito, Maria Gonzalez-Pajuelo, Philippe Van Rompaey, Miguel Conde, Jorn Audiens & Peter Vanlandschoot
  - Annelies Roobrouck, Femke Calle, Tom Verhaeghe, Lien Leutenez, Lies Dekeyzer, Valerie Ryheul, Andreia Correia, Heidi Rommelaere, Robbe Tanghe, Stephanie Staelens, Carlo Boutton, Erika Morizzo & Tony de Fougerolles