Nanobodies for Healthcare Applications

Prof. Dr. Serge Muyldermans

Vrije Universiteit Brussel
Outline of today’s presentation

1. The discovery: Heavy chain antibodies in camelids and what are Nanobodies
2. Advantages of Nanobodies over other designed target-binding fragments
3. Engineering of Nanobodies to broaden their utility
4. Applications of Nanobodies in diagnosis and therapy
NANOBODIES: Single domain Ab fragments for healthcare applications

1. The discovery: Heavy chain antibodies in camelids and what are Nanobodies
2. Advantages of Nanobodies over other designed target-binding fragments
3. Engineering of Nanobodies to broaden their utility
4. Applications of Nanobodies in diagnosis and therapy
Camelid antibodies

Classical antibody (IgG1)

Camel Heavy-Chain antibody (IgG2 & IgG3)

Single domain antigen binding fragment (15 kDa)
Monomeric
Prolate particle:
Diameter 2.4 nm
Height 4 nm

Hamers et al., Nature, 1993
VH and VHH differences

Vu et al., Mol. Immunol., 1997
NANOBODIES: Single domain Ab fragments for healthcare applications

1. The discovery: Heavy chain antibodies in camelids and what are Nanobodies
2. Advantages of Nanobodies over other designed target-binding fragments
3. Engineering of Nanobodies to broaden their utility
4. Applications of Nanobodies in diagnosis and therapy
Animalarium: Dubaï, Tunisia, Peru
Selection of antigen-specific VHH

- Collect blood
- Isolate lymphocytes
- Extract mRNA
- RT-PCR
- Make library of $\approx 10^7$ transformants
- Select Ag-specific VHHs by panning
- Produce soluble antigen-specific VHH

Immunize camel

Ghahroudi et al., FEBS Letters, 1997
Lauwereys et al., EMBO J., 1998
Antigen-binding fragments of Abs

Classical Ab & its fragments:

Heavy-chain Abs: (camel or shark)

Nanobody (Ablynx)

Scrambling of affinity matured VH-VL pairs

$10^6 \rightarrow 10^{12}$

No scrambling of Ag-specific domain as only one gene fragment is amplified

$10^6 = 10^6$
## Nb properties versus scFv and Fab

<table>
<thead>
<tr>
<th>Property</th>
<th>Nb</th>
<th>scFv</th>
<th>Fab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency to identify Ag binders</td>
<td>&gt;</td>
<td>=</td>
<td>Fab</td>
</tr>
<tr>
<td>Expression yields</td>
<td>&gt;</td>
<td>=</td>
<td>Fab</td>
</tr>
<tr>
<td>Stability</td>
<td>&gt;</td>
<td>Fab</td>
<td>&gt; scFv</td>
</tr>
<tr>
<td>Solubility</td>
<td>&gt;</td>
<td>Fab</td>
<td>&gt; scFv</td>
</tr>
<tr>
<td>Antigen specificity</td>
<td>=</td>
<td>Fab</td>
<td>= scFv</td>
</tr>
<tr>
<td>Affinity for the Ag</td>
<td>=</td>
<td>Fab</td>
<td>= scFv</td>
</tr>
<tr>
<td>Targeting unique epitopes</td>
<td>≠</td>
<td>scFv</td>
<td>= Fab</td>
</tr>
<tr>
<td>Easiness to tailor</td>
<td>&gt;</td>
<td>scFv</td>
<td>= Fab</td>
</tr>
</tbody>
</table>
NANOBODIES: Single domain Ab fragments for healthcare applications

1. The discovery: Heavy chain antibodies in camelids and what are Nanobodies
2. Advantages of Nanobodies over other designed target-binding fragments
3. Engineering of Nanobodies to broaden their utility
4. Applications of Nanobodies in diagnosis and therapy
Supportive Nb engineering

Reporter tool

Human in vivo imaging

Capturing tool

Nanobodies for research, diagnosis & therapy

Nanobodies for research, diagnosis & therapy

Saerens et al., J.Mol.Biol., 2008
Saerens et al., J.Mol.Biol., 2005
Tailoring into pluripotent constructs

Bivalent:
Conrath et al., JBC 2001

Bispecific:
Conrath et al., JBC 2001

Pentavalent:
Zhang et al., JMB 2004

Decavalent/bispecific:
Stone et al., J Imm Meth 2007

Immuno-enzyme (ADEPT):
Cortez-Retamozo et al., Can Res 2004

Immuno-toxin:
Baral et al., Nat Med 2006

Chromobody:
Rothbauer et al., Nat Meth 2006

HCAb:
Hmila et al., Mol Immunol 2008

Scorpion (bispecific + Fc effector function)
NANOBODIES: Single domain Ab fragments for healthcare applications

1. The discovery: Heavy chain antibodies in camelids and what are Nanobodies
2. Advantages of Nanobodies over other designed target-binding fragments
3. Engineering of Nanobodies to broaden their utility
4. Applications of Nanobodies in diagnosis and therapy
Nbs for non invasive tumor imaging

Robustness and solubility: VHH > scFv

Most important for imaging: **Contrast** (tumor load/blood or muscle ratio)
Experimental setup

10-12d (tumor size ≈ 250-300 mm³)

subcutaneous injection of 2×10⁶ HER2 positive tumor cells or 2×10⁶ HER2 negative tumor cells in hind limb of athymic nu/nu mice

Intravenous injection of ⁹⁹mTc-labeled Nanobody®

Imaging

SPECT

Micro CT
In-vivo non invasive imaging

~40 Nbs against Her-2

Select best binder for non-invasive imaging without overlap with Trastuzumab

Produce under GMP and evaluate in breast cancer patients
**Lead anti-HER2 Nb: 2Rs15d**

<table>
<thead>
<tr>
<th>Time p.i. SKOV3 xenograft</th>
<th>1h p.i.</th>
<th>6h p.i.</th>
<th>2h p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2Rs15d</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Trastuzumab</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fab from Trastuzumab</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Tumor uptake**

<table>
<thead>
<tr>
<th>SPECT/CT (%IA/cm³)</th>
<th>4.68 ± 0.64</th>
<th>4.19 ± 0.47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex vivo (%IA/g)</td>
<td>1h p.i.</td>
<td>6h p.i.</td>
</tr>
</tbody>
</table>

**Tumor-to-blood**

<table>
<thead>
<tr>
<th>Ex vivo</th>
<th>16.42 ± 3.64</th>
<th>0.8</th>
<th>0.2</th>
</tr>
</thead>
</table>

**Tumor-to-muscle**

<table>
<thead>
<tr>
<th>SPECT/CT</th>
<th>20.89 ± 8.80</th>
<th>2.5 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex vivo</td>
<td>49.62 ± 11.82</td>
<td>2.5 nM</td>
</tr>
</tbody>
</table>

**Tₘ** | 79°C |

**Lysines in CDRs** | no |

**Kₐ (Biacore)** | 2.7 nM |

**Competition**

| Trastuzumab | no |
| Pertuzumab  | no |

**References**

- Ilse Vaneycken et al., *FASEB J*, 2011
- Orlova et al., 2009
- Tang et al., 2005
Nbs against scorpion toxin

Scorpion in Tunisia:
Androctonus australis hector

Extract venom
(SEC over Sephadex-G50, followed Mono-S FPLC and C-8 reverse phase HPLC to purify AahI’ and AahII (LD50 in Swiss mouse ≈ 3 ng for i.c.v. and 250 ng for s.c.)

Immunise dromedary with AahI’ or AahII enriched fractions and identify Nbs against AahI’ or against AahII
Construction of bispecific Nbs

NbAahI’-F12: neutralises AahI’

NbAahII-10: neutralises AahII

Bispecific Nb-F12-10: targets AahI’ and AahII

Sc injection of 1.5 LD50 of venom (AahI’ + AahII) followed by iv injection of bispecific Nb
Acknowledgments

**Postdocs**
- Hassanzadeh Gholamreza (NSF),
- Devoogdt Nick
- Vincke Cécile

**Non-VIB collaborations**
- **CVRL (Dubai, UAE) & ABLYNX**
- K. Andersson (Uppsala) & D. Altschuh (Strasbourg, QSAR)
- M. Brüggemann (Cambridge, camel-mouse)
- C. Cambillau (CNRS,Marseilles)
- K. Andersson (Uppsala) & D. Altschuh (Strasbourg, QSAR)
- M. Brüggemann (Cambridge, camel-mouse)
- C. Cambillau (CNRS,Marseilles)
- C. Dobson (Oxf. & Cam.) & A. Matagne (Ulg, folding)
- F. Frederix, (IMEC) M. Sara (Vienna, Biosensors)
- **M. El Ayeb & B. Bouhaouala (Institut Pasteur Tunis)**
- H. Leonhardt & U. Rothbauer (U. München)
- M. Przybylski (U. Constan, peptibodies)
- E. Pays (ULB, Gosselies)
- **A. Bossuyt & T. Lahoutte (UZBrussel)**

**PhD students**
- Nguyen Trong
- Jens Devos
- Sam Massa

**Scientists joining Ablynx**
- M. Lauwereys, K. Silence,
- T. Laeremans, H. Revets

**VIB collaborations**
- Prof. L. Wyns (ULTR),
- Prof. P. De Baetselier (CIMM)
Artilodactyla

Tylopoda  Camelidae

Suiformes

Ruminantia

Camelus dromedarius
Camelus bactrianus
Lama glama
Lama guanoco
Lama alpaca
Lama vicugna

Suidae
Hyppopotamidae
Tayassuidae

Tragulidae
Cervidae
Giraffidae
Antilocapridae
Bovidae

Antilopinae
Cephalophinae
Hippotraginae
Bovinae
Caprinae
Purification of Nbs

Nb expressed in *E. coli*
Extracted from periplasm,
Immobilized Metal Affinity Chromatography,
Size Exclusion Chromatography

UV-280
Conductivity
pH

Nanobodies for research, diagnosis & therapy
2-4-12 Slide 24

Ben Abderrazek et al., *Biochem. J.*, 2009
## Selecting the lead compound

<table>
<thead>
<tr>
<th>Radotracer</th>
<th>%ID/g lesion</th>
<th>Lesion:control</th>
<th>Lesion :blood</th>
<th>Lesion:Heart</th>
<th>Kp mVCAM1 (nmol/L)</th>
<th>Kp hVCAM1 (nmol/L)</th>
<th>Production yield (mg/L)</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAbVCAM1-1</td>
<td>0.87±0.08*</td>
<td>2.15±0.20*</td>
<td>0.74±0.10*</td>
<td>2.65±0.23*</td>
<td>8.3±1.2</td>
<td>ND</td>
<td>2.0</td>
<td>72.3±0.1</td>
</tr>
<tr>
<td></td>
<td>#9</td>
<td>#9</td>
<td>#10</td>
<td>#9</td>
<td></td>
<td></td>
<td></td>
<td>#2</td>
</tr>
<tr>
<td>cAbVCAM1-2</td>
<td>2.15±0.29*</td>
<td>2.90±0.45*</td>
<td>3.37±0.32*</td>
<td>5.55±0.58*</td>
<td>0.3±0.0</td>
<td>ND</td>
<td>5.0</td>
<td>62.3±0.1</td>
</tr>
<tr>
<td></td>
<td>#6</td>
<td>#6</td>
<td>#5</td>
<td>#7</td>
<td></td>
<td></td>
<td></td>
<td>#6</td>
</tr>
<tr>
<td>cAbVCAM1-3</td>
<td>2.95±0.16*</td>
<td>4.07±0.56*</td>
<td>5.06±0.30*</td>
<td>7.40±0.91*</td>
<td>2.4±0.1</td>
<td>9.1±0.9</td>
<td>6.8</td>
<td>59.7±0.1</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>#3</td>
<td>#1</td>
<td>#3</td>
<td></td>
<td></td>
<td></td>
<td>#9</td>
</tr>
<tr>
<td>cAbVCAM1-4</td>
<td>2.21±0.59*</td>
<td>3.20±0.74*</td>
<td>1.41±0.29*</td>
<td>1.96±0.56*</td>
<td>0.2±0.0</td>
<td>ND</td>
<td>6.8</td>
<td>59.4±0.1</td>
</tr>
<tr>
<td></td>
<td>#5</td>
<td>#5</td>
<td>#9</td>
<td>#10</td>
<td></td>
<td></td>
<td></td>
<td>#10</td>
</tr>
<tr>
<td>cAbVCAM1-5</td>
<td>2.53±0.08*</td>
<td>4.95±0.65*</td>
<td>4.32±0.48*</td>
<td>8.30±1.11*</td>
<td>2.0±0.0</td>
<td>6.5±0.7</td>
<td>10.5</td>
<td>&gt;87</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>#1</td>
<td>#2</td>
<td>#1</td>
<td></td>
<td></td>
<td></td>
<td>#1</td>
</tr>
<tr>
<td>cAbVCAM1-6</td>
<td>0.73±0.08</td>
<td>4.57±0.93*</td>
<td>1.85±0.37*</td>
<td>4.98±0.75*</td>
<td>5.2±0.6</td>
<td>ND</td>
<td>3.0</td>
<td>72.0±0.1</td>
</tr>
<tr>
<td></td>
<td>#10</td>
<td>#2</td>
<td>#8</td>
<td>#8</td>
<td></td>
<td></td>
<td></td>
<td>#6</td>
</tr>
<tr>
<td>cAbVCAM1-7</td>
<td>1.27±0.25*</td>
<td>2.88±0.65*</td>
<td>4.02±1.05*</td>
<td>5.98±0.96*</td>
<td>2.6±1.2</td>
<td>ND</td>
<td>6.9</td>
<td>60.9±0.3</td>
</tr>
<tr>
<td></td>
<td>#8</td>
<td>#7</td>
<td>#3</td>
<td>#4</td>
<td></td>
<td></td>
<td></td>
<td>#2</td>
</tr>
<tr>
<td>cAbVCAM1-8</td>
<td>2.48±0.46*</td>
<td>1.40±0.10*</td>
<td>3.66±0.10*</td>
<td>7.71±0.38*</td>
<td>13.2±0.3</td>
<td>1.4±0.5</td>
<td>1.5</td>
<td>61.5±0.1</td>
</tr>
<tr>
<td></td>
<td>#4</td>
<td>#10</td>
<td>#4</td>
<td>#2</td>
<td></td>
<td></td>
<td></td>
<td>#8</td>
</tr>
<tr>
<td>cAbVCAM1-9</td>
<td>2.99±0.07*</td>
<td>2.19±0.60*</td>
<td>2.51±0.03*</td>
<td>5.69±0.36*</td>
<td>0.9±0.2</td>
<td>5.3±0.7</td>
<td>6.8</td>
<td>66.8±0.2</td>
</tr>
<tr>
<td></td>
<td>#1</td>
<td>#8</td>
<td>#6</td>
<td>#6</td>
<td></td>
<td></td>
<td></td>
<td>#4</td>
</tr>
<tr>
<td>cAbVCAM1-10</td>
<td>1.93±0.14*</td>
<td>3.47±0.67*</td>
<td>2.01±0.14*</td>
<td>5.76±0.56*</td>
<td>ND</td>
<td>13.4±7.0</td>
<td>6.8</td>
<td>63.4±0.2</td>
</tr>
<tr>
<td></td>
<td>#7</td>
<td>#4</td>
<td>#7</td>
<td>#5</td>
<td></td>
<td></td>
<td></td>
<td>#8</td>
</tr>
<tr>
<td>cAbBcll10</td>
<td>0.68±0.06</td>
<td>1.66±0.28</td>
<td>1.57±0.09</td>
<td>4.00±0.14</td>
<td>ND</td>
<td>ND</td>
<td>5.0</td>
<td>77.5±0.2</td>
</tr>
</tbody>
</table>

Camel antibody as lead compound
29-01-11 Slide 25
Detection of breast cancer by FDG-PET

- low specificity
- poor molecular information
Problem with breastcancer

- Cancer is one of the most important causes of death
- Of all cancers in women, breast cancer has highest incidence rate

WHAT IS THE PROBLEM?

- Tumors are very heterogeneous: 30% of cells from breast tumors express the proto-oncogen HER2\(^+\) (\textit{neu} of \textit{c-erbB-2}) at their cell surface
- Prognosis for patients with HER2 positive breastcancer is very bad
- However, .... 2 therapeutic monoclonal antibodies are available (Trastuzumab en Pertuzumab)
- Treatment with Trastuzumab or Pertuzumab is of high risk because of life-treathening side effects; it is very expensive and only effective in 30% of patients.
The solution: in vivo imaging

- An efficient screening of HER2 expression to identify patients eligible for Traztuzumab/Pertuzumab treatment is required to increase possibly the efficacy of the outcome.

- Classic biopsy is possible but it is invasive, painful, complicated by metastasis and unreliable due to heterogeneity of the tumors.

- In vivo imaging with radio-labeled monoclonal antibodies (Trastuzumab of Pertuzumab) could be an option **BUT** this approach is **NOT** feasible because of their high liver uptake and long retention time in blood (t½ of 2-3 weeks due to large MW and presence of Fc).

- Smaller sized (MW<50 kDa and without Fc), HER2-specific probes – such as Nanobodies – are required for successful in vivo imaging.
Screening of best anti HER2 Nb

1. Selection on HER2, screen via ELISA

2. Screening on HER2 positive cells via FACS

Ilse Vaneycken et al., FASEB J, 2011
Screening of best anti HER2 Nb

1. Selection on HER2, screen via ELISA
2. Screening on HER2 + cells via FACS
3. Screening on affinity

2Rs15d, $K_D = 2.7$ nM

4. Screening on epitope

Ilse Vaneycken et al., FASEB J, 2011
Screening of best anti HER2 Nb

1. Selection on HER2, screen via ELISA
2. Screening on HER2 + cells via FACS
3. Screening on affinity
4. Screening on epitope
5. Screening on stability

Thermostability via Circular Dichroism

6. Screening on labeling and retention of affinity/specificity

Thermostability:

\[ T_{m} = 79^\circ C \]

Thermostability:

\[ T_{m} = 79^\circ C \]

Camel antibody as lead compound

Ilse Vaneycken et al., FASEB J, 2011
biodistribution and tumor targeting of $^{99m}$Tc-Nanobodies

- radioactivity of dissected organs and HER2$^+$ SKOV3 xenograft 1h after i.v. injection
- expressed as percent injected activity per organ weight (%IA/g)

<table>
<thead>
<tr>
<th>Organ</th>
<th>$^{99m}$Tc-1R119b</th>
<th>$^{99m}$Tc-2R5a</th>
<th>$^{99m}$Tc-2Rb17c</th>
<th>$^{99m}$Tc-1R136d</th>
<th>$^{99m}$Tc-2Rb13d</th>
<th>$^{99m}$Tc-1R133a</th>
<th>$^{99m}$Tc-2Rb13c</th>
<th>$^{99m}$Tc-1R135a</th>
<th>$^{99m}$Tc-2Rb3b</th>
<th>$^{99m}$Tc-2Rb18a</th>
<th>$^{99m}$Tc-1R59b</th>
<th>$^{99m}$Tc-ctrl Nanobody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.17 ± 0.02</td>
<td>0.25 ± 0.11</td>
<td>0.28 ± 0.05</td>
<td>0.30 ± 0.09</td>
<td>0.16 ± 0.01</td>
<td>0.22 ± 0.02</td>
<td>0.20 ± 0.08</td>
<td>0.17 ± 0.13</td>
<td>0.92 ± 0.12</td>
<td>0.19 ± 0.02</td>
<td>0.35 ± 0.11</td>
<td>0.42 ± 0.11</td>
</tr>
<tr>
<td>Liver</td>
<td>0.79 ± 0.43</td>
<td>3.05 ± 0.71</td>
<td>2.49 ± 0.60</td>
<td>1.16 ± 0.59</td>
<td>0.72 ± 0.14</td>
<td>0.31 ± 0.11</td>
<td>0.53 ± 0.07</td>
<td>8.60 ± 1.11</td>
<td>3.60 ± 0.91</td>
<td>1.30 ± 0.16</td>
<td>8.39 ± 1.86</td>
<td>2.93 ± 0.24</td>
</tr>
<tr>
<td>Kidney</td>
<td>240.1 ± 76.9</td>
<td>170.7 ± 17.4</td>
<td>130.6 ± 30.2</td>
<td>259.0 ± 12.8</td>
<td>154.7 ± 17.7</td>
<td>107.4 ± 5.6</td>
<td>242.6 ± 21.0</td>
<td>171.7 ± 22.8</td>
<td>127.4 ± 4.2</td>
<td>130.7 ± 19.5</td>
<td>209.4 ± 47.2</td>
<td>99.37 ± 9.87</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.17 ± 0.01</td>
<td>0.15 ± 0.03</td>
<td>0.38 ± 0.06</td>
<td>0.46 ± 0.28</td>
<td>0.21 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.17 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.13 ± 0.05</td>
<td>0.54 ± 0.26</td>
<td>1.18 ± 0.18</td>
<td>0.69 ± 0.18</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.15 ± 0.03</td>
<td>0.06 ± 0.06</td>
<td>0.20 ± 0.02</td>
<td>0.24 ± 0.08</td>
<td>0.09 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td>0.34 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>0.18 ± 0.07</td>
<td>0.17 ± 0.02</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.84 ± 0.49</td>
<td>0.29 ± 0.30</td>
<td>1.24 ± 0.33</td>
<td>1.81 ± 1.23</td>
<td>1.09 ± 0.40</td>
<td>0.11 ± 0.03</td>
<td>0.55 ± 0.12</td>
<td>0.26 ± 0.32</td>
<td>0.37 ± 0.10</td>
<td>0.52 ± 0.26</td>
<td>0.96 ± 0.21</td>
<td>1.54 ± 0.21</td>
</tr>
<tr>
<td>SKOV3</td>
<td>2.17 ± 0.22</td>
<td>0.78 ± 0.50</td>
<td>3.57 ± 0.13</td>
<td>4.00 ± 1.99</td>
<td>4.19 ± 0.47</td>
<td>2.65 ± 0.19</td>
<td>4.44 ± 0.78</td>
<td>2.28 ± 0.24</td>
<td>2.16 ± 0.26</td>
<td>2.62 ± 0.27</td>
<td>2.25 ± 0.30</td>
<td>0.37 ± 0.06</td>
</tr>
<tr>
<td>Tumor</td>
<td>0.30 ± 0.27</td>
<td>0.34 ± 0.26</td>
<td>0.49 ± 0.04</td>
<td>0.42 ± 0.03</td>
<td>0.26 ± 0.21</td>
<td>0.42 ± 0.17</td>
<td>0.34 ± 0.04</td>
<td>0.36 ± 0.16</td>
<td>1.86 ± 0.14</td>
<td>0.48 ± 0.02</td>
<td>0.77 ± 0.10</td>
<td>0.37 ± 0.09</td>
</tr>
<tr>
<td>Blood</td>
<td>14.6 ± 1.4</td>
<td>17.3 ± 6.4</td>
<td>16.2 ± 1.4</td>
<td>49.6 ± 11.8</td>
<td>50.0 ± 47.6</td>
<td>27.4 ± 15.4</td>
<td>42.4 ± 55.5</td>
<td>22.1 ± 4.6</td>
<td>17.1 ± 6.4</td>
<td>14.5 ± 6.9</td>
<td>2.1 ± 0.1</td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td>Tumor/muscle</td>
<td>7.3 ± 0.7</td>
<td>6.4 ± 9.2</td>
<td>7.8 ± 2.3</td>
<td>9.3 ± 3.8</td>
<td>16.4 ± 3.6</td>
<td>7.1 ± 3.1</td>
<td>14.6 ± 5.9</td>
<td>6.3 ± 0.8</td>
<td>1.2 ± 0.8</td>
<td>5.5 ± 0.8</td>
<td>3.0 ± 1.0</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

Camel antibody as lead compound
29-01-11  Slide 32
Ilise Vaneycken et al., FASEB J, 2011
General strategy to identify binders

Scaffold choice

Stable, recombinant scaffold
Ankyrin, lipocalin, Protein A...

Antibody fragments
Fab, scFv, ..., VHH

Diversification mechanism

Synthetic library: codon randomisation

Immunisation
= affinity maturation
= enrich for Ag-specific clones

Large, diverse bank as source for selections

The larger the banks, the more diversity among individual clones
The higher the success rate for target-specific, high affinity binders
Library size versus affinity

- Colony screening & two-hybrid
- Phage display
- Ribosome display

Affinity (M):
- $10^{-10}$
- $10^{-9}$
- $10^{-8}$
- $10^{-7}$
- $10^{-6}$
- $10^{-5}$

Library size:
- $10^4$
- $10^5$
- $10^6$
- $10^7$
- $10^8$
- $10^9$
- $10^{10}$
- $10^{11}$
- $10^{12}$
- $10^{13}$

- Immune bank
- Synthetic bank (Nanoclonetm)
V-D-J rearrangement produces VHH

~ 50 V_H genes

~ 40 V_HH-genes

(VH3) → 7 subfamilies → Position of Cys in CDR1

CDR1 CDR2 CDR3

IgG1

IgG2

IgG3

D-genes

RSS

Ig-promotor

Leader signal peptide

Nguyen et al., EMBO J., 2000
Molecular imaging: *In vivo* cell staining

Nanobodies for research, diagnosis & therapy

Rothbauer et al., *Nat. Meth.*, 2006
Molecular imaging: *In vivo* cell staining

+ chromobody:
  - anti-cytokeratin 8
  - anti-Lamin

---

Rothbauer et al., *Nat.Meth.*, 2006
Nb specificity + use as intrabody

Rothbauer et al., Mol Cell Prot, 2008