Plant rabies immunoglobulin (P-RIG)

PLANT-BASED VACCINES, ANTIBODIES & BIOLOGICS
8-10 June 2015, University of Lausanne, Switzerland

Markus Sack, RWTH Aachen University, Institute of Molecular Biotechnology
sack@molbiotech.rwth-aachen.de, markus.sack@ime.fraunhofer.de
Rabies

- Zoonotic disease transmitted mainly by canines and by bats
- 100% fatal once symptoms occur
- Rabies is a vaccine preventable disease
- Passive immunization with rabies immunoglobulin (RIG) prevents death when given early enough (Post-exposure prophylaxis)
- >15 million people receive post-exposure prophylaxis every year
- About 4 million people receive RIG

BUT !!!

- More than 25,000 die every year from rabies
- 95% of human deaths occur in Africa and Asia
- 40% of infected people are children under the age of 15

People die from rabies due to market failure and limited availability; HRIG costs are too high making the drug unavailable to those who need it!
Different region – different solutions
Aims and Rationales

Rationales

- Exploit advantages of plant-based production systems
  - High intrinsic safety, free of human pathogens, no animal components
  - Eukaryotic host
  - Lower entry barriers
  - More simple supply logistics
  - Rapid, robust, easy, flexible, low cost

Aims

- Cocktail of three highly potent human antibodies
- Market price for Plant-RIG of less than $10 per dose of 3000 IU
- Capacity for global supply
Workflow overview

- Isolation of B-cells from vaccinated individuals
- Generation of stable hybrid cell line
- Identification of 4 highly potent mAbs
- V-Genes rescued from 6 mAbs
- Generation of pTRA plant expression vectors
- Small scale Agro-Infiltration
- Production of >20 mg for all 6 Abs
- Protein-A Affinity Chromatography
- Preliminary affinity analysis by SPR
- Yield and cost analysis
Highly potent human rabies antibodies

- Vaccination of Indian volunteers with rabies vaccine
- B-cells converted to IgG-secreting hybrid cells
  - mAb secretion is genetically stabilized
- Genuine human mAbs with exceptional potency and affinity (efficacy)
- These antibodies have already existed in humans (safety)
- Two cell fusions produced 6 product candidates (feasibility)
Potency

- Stable monoclonal hybrid cell lines were screened by ELISA
- mAbs with high reactivity were purified and analysed

<table>
<thead>
<tr>
<th>Monoclonal Antibody Titer</th>
<th>IU/ml (at 0.9 mg/ml)</th>
<th>IU/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>4C12</td>
<td>162</td>
<td>180</td>
</tr>
<tr>
<td>4H3</td>
<td>1458</td>
<td>1620</td>
</tr>
<tr>
<td>7A2</td>
<td>1458</td>
<td>1620</td>
</tr>
<tr>
<td>7E8</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>8C5</td>
<td>4374</td>
<td>4860</td>
</tr>
<tr>
<td>10H5</td>
<td>2916</td>
<td>3240</td>
</tr>
</tbody>
</table>

- 4H3, 8C5 and 10H5 are non-competing
- 8C5 and 10H5 have excellent potency
Neutralization of Indian street rabies viruses

- 20 IU ml\(^{-1}\) (RFFIT) of each antibody
- 15 µL of antibody:virus (1:1) mixture was injected into mouse brain

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CVS</th>
<th>PV</th>
<th>IKA-R81_04_D</th>
<th>IMA-R146_04_Bu_f</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>R17D6</td>
<td>30</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>Hybridoma</td>
<td>R16C9</td>
<td>70</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Scott Dessain’s human mAbs</td>
<td>4H3</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10H5</td>
<td>100</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>8C5</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Controls</td>
<td>ERIG</td>
<td>ND</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>HRIG</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Unpublished data; personal communication

\(\text{n=10 per treatment; ND: not done}\)
Analysis of rescued V-genes

<table>
<thead>
<tr>
<th>Isotype</th>
<th>ID</th>
<th>$V_H$</th>
<th>$D_H$</th>
<th>$J_H$</th>
<th>$V_L$</th>
<th>$J_L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\kappa$</td>
<td>10H5</td>
<td>IGHV3-30*18</td>
<td>IGHD3-22*01</td>
<td>IGLJ3*02</td>
<td>IGKV1-17*02</td>
<td>IGKJ4*01</td>
</tr>
<tr>
<td></td>
<td>7A2</td>
<td>IGHV3-15*07</td>
<td>IGHD3-3*01</td>
<td>IGHJ6*02</td>
<td>IGKV3-20*01</td>
<td>IGKJ2*01</td>
</tr>
<tr>
<td></td>
<td>7E8</td>
<td>IGHV4-4*02</td>
<td>GHD6-25*01</td>
<td>IGHJ4*02</td>
<td>IGKV3-15*01</td>
<td>IGKJ5*01</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>8C5</td>
<td>IGHV3-43D*01</td>
<td>IGHD6-19*01</td>
<td>IGHJ4*02</td>
<td>IGLV3-21*02</td>
<td>IGLJ3*02</td>
</tr>
<tr>
<td></td>
<td>4C12</td>
<td>IGHV4-61*01</td>
<td>IGHD3-22*01</td>
<td>IGHJ1*01</td>
<td>IGLV1-44*01</td>
<td>IGLJ3*02</td>
</tr>
<tr>
<td></td>
<td>4H3</td>
<td>IGHV1-2*02</td>
<td>IGHD3-10*01</td>
<td>IGHJ4*02</td>
<td>IGLV1-44*01</td>
<td>IGLJ3*02</td>
</tr>
</tbody>
</table>
### V-gene somatic mutations

<table>
<thead>
<tr>
<th>Isotype</th>
<th>ID</th>
<th>Potency [IU/mg]</th>
<th>$V_H$</th>
<th>$J_H$</th>
<th>$V_L$</th>
<th>$J_L$</th>
<th>$\Sigma$</th>
<th>%</th>
<th>theo. pl</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\kappa$</td>
<td>10H5</td>
<td>3240</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>15</td>
<td>6.6</td>
<td>8.30</td>
</tr>
<tr>
<td></td>
<td>7A2</td>
<td>1620</td>
<td>18</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>31</td>
<td>13.0</td>
<td>7.83</td>
</tr>
<tr>
<td></td>
<td>7E8</td>
<td>7</td>
<td>11</td>
<td>1</td>
<td>11</td>
<td>0</td>
<td>23</td>
<td>10.2</td>
<td>9.10</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>8C5</td>
<td>4860</td>
<td>12</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>22</td>
<td>9.5</td>
<td>5.31</td>
</tr>
<tr>
<td></td>
<td>4C12</td>
<td>180</td>
<td>6</td>
<td>2</td>
<td>11</td>
<td>1</td>
<td>20</td>
<td>8.4</td>
<td>5.74</td>
</tr>
<tr>
<td></td>
<td>4H3</td>
<td>1620</td>
<td>17</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>23</td>
<td>9.9</td>
<td>7.79</td>
</tr>
</tbody>
</table>

- 8C5, 4H3 and 10H5 recognize different epitopes
- theo. pl was calculated for Fv
High *in-planta* antibody accumulation

Nicotiana benthamiana

Transient Gene Expression (TGE)

5 days after infiltration

single leaf assay

$N = 3$

800 – 1400 mg/kg

Antibody accumulation [µg/g]

Antibody

- 8C5
- 4H3
- 4C12
- 7E8
- 10H5
- 7A2

IgG1 $\lambda$

IgG1 $\kappa$
Purification from small scale expression
### Yield and recovery after protein A purification

<table>
<thead>
<tr>
<th>ID</th>
<th>Leaf biomass [g]</th>
<th>Accumulation [μg/g]</th>
<th>Yield [mg]</th>
<th>Recovery [%]</th>
<th>Potency [IU/mg]</th>
<th>#Doses @1500 IU</th>
<th>#Doses/50 kg batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>8C5</td>
<td>153</td>
<td>540</td>
<td>40</td>
<td>49</td>
<td>4860</td>
<td>130</td>
<td>42,480</td>
</tr>
<tr>
<td>10H5</td>
<td>51</td>
<td>530</td>
<td>15</td>
<td>58</td>
<td>3240</td>
<td>33</td>
<td>32,350</td>
</tr>
<tr>
<td>7A2</td>
<td>87</td>
<td>465</td>
<td>16</td>
<td>40</td>
<td>1620</td>
<td>17</td>
<td>9,770</td>
</tr>
<tr>
<td>4H3</td>
<td>202</td>
<td>420</td>
<td>30</td>
<td>35</td>
<td>1620</td>
<td>32</td>
<td>7,920</td>
</tr>
</tbody>
</table>

- **One leaf**: 800 – 3,000 IU
  - 1 dose
  - 13 – 40 doses

- **One plant**: 40 – 120 kIU
  - 13 – 40 doses

- **100 m² research greenhouse**: 20 – 60 Mio. IU
  - 6,700 – 20,000 doses

- **5,000 m² / 54,000 ft² commercial greenhouse**: 1,000 – 3,000 Mio. IU
  - 0.3 – 1 Mio. doses
Further yield increase?

- **Protein engineering** of variable domains
- Yields of **up to 2 g/kg** have been observed at lab scale
- Similar to generally accepted commercial viability for CHO

Proof-of-principle for two model antibodies

Classical Agro-infiltration

*N. benthamiana*

Small scale

4 days post infiltration

Without silencing suppressor
SPR binding analysis

Captured plant-derived human Rabies mAb

Solubilized Rabies Glycoprotein

VSV-G prefusion (1)

SPR-based surrogate assay for biological potency

Albertini et al., *Viruses* 2012, 4(1), 117-139; (1)
**Affinity**

8C5: \( K_D = 13 \text{ pM} \)
\( k_{on} = 6.1 \cdot 10^6 \text{ s}^{-1} \text{M}^{-1} \)
\( k_{off} = 7.7 \cdot 10^{-5} \text{ s}^{-1} \)
\( R_{max} = 88 \text{ RU} \)

10H5: \( K_D = 57 \text{ pM} \)
\( k_{on} = 1.7 \cdot 10^6 \text{ s}^{-1} \text{M}^{-1} \)
\( k_{off} = 9.9 \cdot 10^{-5} \text{ s}^{-1} \)
\( R_{max} = 88 \text{ RU} \)

4H3: \( K_D = 23 \text{ pM} \)
\( k_{on} = 3.0 \cdot 10^6 \text{ s}^{-1} \text{M}^{-1} \)
\( k_{off} = 7.0 \cdot 10^{-5} \text{ s}^{-1} \)
\( R_{max} = 80 \text{ RU} \)

7A2: \( K_D = 56 \text{ pM} \)
\( k_{on} = 2.9 \cdot 10^6 \text{ s}^{-1} \text{M}^{-1} \)
\( k_{off} = 1.6 \cdot 10^{-4} \text{ s}^{-1} \)
\( R_{max} = 80 \text{ RU} \)
Plant-RIG vs. E-RIG

**Proposed strategy Plant-RIG**
- Cocktail of 3 mAbs
- 11 batches á 50 kg per year
- 5,500 Plants per year
- 11 x 185 L extract
- 360 Mio. IU total
  - 120 Mio. IU per mAb per year
  - 2x 8C5; 3x 10H5; 6x 4H3
- Less infrastructure required for Plant-RIG

**current E-RIG production (approx.)**
- Polyclonal Fabs
- 15 bleedings per year
- 2,500 L blood
- 1,600 L serum
- 160 Mio. IU total
  - Complex downstream processing
  - Batch-to-batch variation

research greenhouse
100 m² planting area

50 horses
600 m² stables
Several acres grassland

© Fraunhofer
Commercial viability – bottom up cost analysis

Transgenic

Production costs of < $2 per dose appear feasible for a 8C5 - 10H5 – 4H3 cocktail
Further cost reductions may be possible
Cost of one dose of HRIG for a 75 kg person is $1,500 in the US or $250-500 in India
Cost for ERIG is $12 in India

Transient
Commercial viability – top down cost analysis

Investment and Operating costs

- Based on a technoeconomic case study by Tuse et al. 2014 for BChE
- Facility lifetime of 15 years, depreciation over 10 years
  - $100 Mio. of total capital investment for a new facility
  - $15 Mio. in annual operating costs (excluding facility dependent costs)
  - $30 Mio. in annual operating costs (including facility dependent costs)

Capacity

- %Recovery and Dose were adjusted
- Dose requirement for Plant-RIG was set to 40 IU per kg
- Number of batches per year was reduced from 47 to 36 to account for product changeover

40 million x 3,000 IU per year of Plant-RIG at $0.75 per dose
Production cost

- Bottom-up and top-down cost estimates yield similar results
  - $0.75 – $2 per dose of 3,000 IU
  - In line with a sales price of < $10 per dose
- Large scale manufacturing plant has capacity for 40 Million doses
  - Exceeds yearly global demand
- Short response time – lower need for stockpiling

Who needs 40 million doses???

- Multiple Products
  - 10% facility capacity for Plant-RIG
  - 90% facility capacity for other products
Outlook

Manufacturing issues
- Product development cycles to maximize yield
- Evaluation of process performance
- Generation of transgenic tobacco plants and suspension cell lines
- Comparative cost analysis
  - transient vs. stable transgenic
  - whole-plant vs. cell suspension cultures

Efficacy
- Strain coverage
- Dose requirements of Plant-RIG cocktail

⇒ GMP production and clinical development
Acknowledgements

Rainer Fischer
Isabel Doch
Tarek Shanati
Johannes Buyel
Holger Spiegel
Thomas Rademacher
Stefan Rasche
Scott Dessain
Ramdev Puligedda
Rashmi Sharma
Rajan Sriraman
Julian Ma
Audrey Teh
Matthias Schnell