Antibody-based Multipurpose Microbicides

Antibodies as a Platform Technology for Multipurpose Microbicides: Specificity and Versatility

Kevin J Whaley
Antibodies as Platform Technology for Multipurpose Microbicides

- Commercially available for multiple indications (safety and versatility)
- Multiple mucosal mechanisms (e.g. block binding to receptor, mucus trapping, agglutination/aggregation)
- Antibodies are immune correlates of protection in many vaccines, including the RV144 HIV vaccine trial (efficacy 31.2%, IgG not IgA)
<table>
<thead>
<tr>
<th>Strategy</th>
<th>Description</th>
<th>How mucosal antibodies could defeat the microbial strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>molecular mimicry</td>
<td>microbial antigens similar to host antigens resulting in an absence of an effective immune response (e.g. Treponema pallidum)</td>
<td>use non-inflammatory monoclonals topically against the microbial antigen</td>
</tr>
<tr>
<td>intracellular or “Trojan Horse”</td>
<td>microbe resides in a host cell thereby evading detection by the immune system (e.g. cell-associated HIV)</td>
<td>use monoclonals against the cell vector</td>
</tr>
<tr>
<td>induction of ineffective antibodies</td>
<td>microbe exposes non-critical antigens to the immune system, so an ineffective antibody response results (e.g. T. pallidum, Chlamydia trachomatis)</td>
<td>use only monoclonals with proven efficacy</td>
</tr>
<tr>
<td>soluble antigen competes up antibody</td>
<td>microbe releases soluble antigen which adsorbs antibody (e.g. cell-free HIV)</td>
<td>choose monoclonals against an antigen that is not released; or, use a polyvalent monoclonal to selectively target multivalent antigen on intact microbes</td>
</tr>
<tr>
<td>protease production</td>
<td>microbe produces an IgA1 protease (e.g. Neisseria gonorrhoeae)</td>
<td>use protease resistant SIgA, engineer out the sensitive site, or include a neutralizing antibody to IgA1 protease</td>
</tr>
<tr>
<td>antigenic variation</td>
<td>microbe mutates immunodominant antigen (e.g. HIV, N. gonorrhoeae)</td>
<td>use monoclonals against less immunodominant but more conserved antigens</td>
</tr>
</tbody>
</table>

(Adapted from Whaley and Zeitlin, Annales de l’Institut Pasteur 2001)
Critical Path

(FDA, 2004)
mAbs to gp120 and gp41

(Koff and Berkley, NEJM 2010)
HIV Neutralizing mAbs

(HIV Neutralizing mAbs)

**Table A**

<table>
<thead>
<tr>
<th>Clade</th>
<th>No. of viruses</th>
<th>b12</th>
<th>2G12</th>
<th>2F5</th>
<th>4E10</th>
<th>PG9</th>
<th>PG16</th>
<th>PGC14</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>27</td>
<td>6.98</td>
<td>17.10</td>
<td>5.70</td>
<td>6.20</td>
<td>0.16</td>
<td>0.11</td>
<td>41.59</td>
</tr>
<tr>
<td>B</td>
<td>31</td>
<td>0.80</td>
<td>0.82</td>
<td>2.41</td>
<td>5.22</td>
<td>0.43</td>
<td>0.70</td>
<td>21.88</td>
</tr>
<tr>
<td>C</td>
<td>27</td>
<td>6.46</td>
<td>2.93</td>
<td>31.51</td>
<td>2.97</td>
<td>0.22</td>
<td>0.25</td>
<td>11.97</td>
</tr>
<tr>
<td>D</td>
<td>25</td>
<td>1.47</td>
<td>7.71</td>
<td>3.17</td>
<td>4.60</td>
<td>0.10</td>
<td>0.02</td>
<td>38.57</td>
</tr>
<tr>
<td>CRF01_AE</td>
<td>10</td>
<td>21.53</td>
<td>&gt;50</td>
<td>0.26</td>
<td>0.51</td>
<td>0.08</td>
<td>0.03</td>
<td>&gt;50</td>
</tr>
<tr>
<td>CRF_AG</td>
<td>10</td>
<td>10.40</td>
<td>0.95</td>
<td>0.64</td>
<td>1.42</td>
<td>0.80</td>
<td>0.03</td>
<td>45.10</td>
</tr>
<tr>
<td>G</td>
<td>15</td>
<td>3.07</td>
<td>31.03</td>
<td>1.24</td>
<td>1.44</td>
<td>0.29</td>
<td>1.21</td>
<td>&gt;50</td>
</tr>
<tr>
<td>F</td>
<td>15</td>
<td>&gt;50</td>
<td>9.23</td>
<td>1.78</td>
<td>2.30</td>
<td>0.09</td>
<td>0.08</td>
<td>25.71</td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25.99</td>
</tr>
</tbody>
</table>

**Table B**

<table>
<thead>
<tr>
<th>Clade</th>
<th>No. of viruses</th>
<th>b12</th>
<th>2G12</th>
<th>2F5</th>
<th>4E10</th>
<th>PG9</th>
<th>PG16</th>
<th>PGC14</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>27</td>
<td>30</td>
<td>37</td>
<td>74</td>
<td>96</td>
<td>85</td>
<td>85</td>
<td>11</td>
</tr>
<tr>
<td>B</td>
<td>31</td>
<td>58</td>
<td>71</td>
<td>68</td>
<td>97</td>
<td>74</td>
<td>74</td>
<td>29</td>
</tr>
<tr>
<td>C</td>
<td>27</td>
<td>33</td>
<td>11</td>
<td>7</td>
<td>96</td>
<td>78</td>
<td>78</td>
<td>19</td>
</tr>
<tr>
<td>D</td>
<td>25</td>
<td>48</td>
<td>24</td>
<td>56</td>
<td>96</td>
<td>76</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td>CRF01_AE</td>
<td>10</td>
<td>30</td>
<td>0</td>
<td>89</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CRF_AG</td>
<td>10</td>
<td>30</td>
<td>50</td>
<td>80</td>
<td>100</td>
<td>80</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>G</td>
<td>15</td>
<td>13</td>
<td>20</td>
<td>80</td>
<td>100</td>
<td>87</td>
<td>73</td>
<td>7</td>
</tr>
<tr>
<td>F</td>
<td>15</td>
<td>0</td>
<td>21</td>
<td>87</td>
<td>100</td>
<td>67</td>
<td>64</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

(Walker...Burton, Science, 2009)
Combining mAbs: Neutralization coverage of a panel of 208 global HIV-1 isolates (190 for VRC-PG04) by MAbs targeting independent epitopes on the Env glycoprotein.

Preventing Vaginal Transmission in Macaques: 2F5 and 4E10 Mabs

(Hessel...Burton, J Virol. 2010)
Preventing Vaginal Transmission in Macaques: b12 mAb

Burton...Moore, PNAS 2011
Preventing Vaginal Transmission in Macaques: TriMab (2F5, 4E10, 2G12) Gel

Fig. 7 Efficacy of TriMab (2F5, 4h10 and 2G12 combination) in cynomolgus macaques. A: The 3 antibodies have been formulated in 1.5% HEC gel and the mix of 20mg of each antibody (2ml) have be deposited in the vagina of 6 Depo-Provera treated macaques. Vaginal secretions have been collected using WeckCek sponges at different time points and antibodies titrated by ELISA (we have previously verified in a pilot study that repeated sample do not affect dosage of antibodies). B: Plasma viral load (PVL) in control Depo-Provera treated macaques challenged with 3-10 AlD50 of SHIV162P3; Animals have been either treated with blank gel as placebo or not treated. C: PVL in animals treated with TriMab gel (1h or 4h before challenge) and challenged with SHIV162P3.

(LeGrand, 2010)
MABGEL I
A phase I randomised controlled trial of a triple anti-HIV-1 monoclonal antibody vaginal microbicide

Georgina Morris, Rebecca Wiggins, Sarah Woodhall, Carol Taylor, Brigitta Vcelar, Martin Bland, Charles Lacey;
Centre for Immunology and Infection, Hull York Medical School, & Department of Health Sciences, University of York, & Polymun Scientific, Vienna
MABGEL Results: Safety Analyses

Mean numbers of AEs assigned as at least possibly related to gel

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number in group</th>
<th>Mean number of events</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>9</td>
<td>2.67</td>
<td>2.24</td>
</tr>
<tr>
<td>Low dose</td>
<td>9</td>
<td>2.11</td>
<td>1.69</td>
</tr>
<tr>
<td>High dose</td>
<td>10</td>
<td>1.80</td>
<td>1.40</td>
</tr>
</tbody>
</table>

There were no statistically significant differences in AEs between the groups  \( P = 0.6 \) (negative binomial regression).

(CROI 2011, courtesy of Morris and Lacey)
MABGEL Pharmacokinetic results

• There were statistically significant differences between placebo, low dose and high dose group median values at each time point except C2G12 36 hrs post-12th dose

• Clear differences between the high and low dose MAb groups were seen but did not reach statistical significance due to small sample sizes

• MAb levels in serum at low or background levels in all groups, with no statistically significant differences between the arms

(CROI 2011, courtesy of Morris and Lacey)
First-in-human safety trial of Nicotiana-derived 2G12

(EUDRACT No. 2009-011820-68)

- Study design: A double-blind, placebo-controlled, dose escalation trial in 11 women
- Administration of a single vaginal dose of Nicotiana-derived G12
- Doses: 7, 14 and 28 mg
- Objectives: safety evaluation (clinical safety tests, local reactions, adverse events)
- Evaluate survival of Nicotiana-derived G12 in vaginal secretions and entry into circulation

(Courtesy of Julian Ma, 2012)
2G12-N: Preliminary results and indications

- All volunteers completed the trial protocol
- No significant changes in clinical tests or clinical inspection
- No clinically significant changes on vaginal examination

At all doses:

- Nicotiana-derived G12 is safe and well tolerated
- No clinical changes of concern
- Final report available Q2 2012

(Courtesy of Julian Ma, 2012)
Summary of Greenhouse Production of Nicotiana-derived 2G12

- Total production time is 6 weeks
  - 3 weeks for plantlet growth (transgenic 2G12)
  - 3 weeks for biomass production
- Pesticide free production
- 250Kg Nicotiana tissue/ batch / 250 m²
- Average yield – 10g MAb /batch
- 20 batches / year are possible at this pilot scale

(Courtesy of Julian Ma, 2012)
Antibody-based Multipurpose Microbicides

Nicotiana-based Manufacturing
A Technology Platform for
Multipurpose Microbicides:
Speed, Cost, Scale, Versatility
SWOT Analysis

Courtesy of Gleba (Icon Genetics), 2012

**Transient Plant Production:**

**Strengths**
- Manufacturing speed
- Fast gene to protein
- Production yield
- Low COGs
- Safety benefits
- Low regulatory risk

**Weaknesses**
- No approved product
- No clear guidelines

**Opportunities**
- Reduce COGs
- Increase R&D&M speed
- Increase flexibility

**Threats**
- Regulatory burden

---

*Images of various organisms and cell types are used to illustrate the SWOT analysis.*
IgG glycosylation

core glycans – plants and mammals:

plant glycans:

mammalian glycans:

IgG Asn297
# Glycosylation of h-13F6

<table>
<thead>
<tr>
<th></th>
<th>Aglycosylated</th>
<th>M5</th>
<th>GnM</th>
<th>GnMF(^6)</th>
<th>G0</th>
<th>G1</th>
<th>G2</th>
<th>GnGn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rituxan</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td><strong>h-13F6(_{CHO})</strong></td>
<td>6</td>
<td>11</td>
<td>35</td>
<td>27</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>h-13F6(_{ΔXF})</strong></td>
<td>5</td>
<td>5</td>
<td>35</td>
<td>27</td>
<td>9</td>
<td></td>
<td></td>
<td>90</td>
</tr>
<tr>
<td><strong>h-13F6(_{agly})</strong></td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **GlcNAc**
- **Galactose**
- **Mannose**
- **core α1,6Fucose**
Antibody IPCP: mapp66 mAbs

- HSV8-N, VRC01-N, 4E10-N
- IgG1, IgG2, IgG3, IgG4, IgA, S-IgA
- GnGn, Aglycosylated, +fucose/xylose, GnGn+galactose, GnGnNaNa
Mapp66 Upstream

Plasmid Vector

- mAbs held in Apoplast

Agrobacterium Strain Development

Infiltration Chamber

Plant AgroInfiltration
Large scale manufacturing in *Nicotiana* at KBP

- More than 1 acre of indoor controlled growth space

- Recently completed Blue Angel project for DARPA: 10 M doses of H1N1 vaccine under GMP in 1 month
Study: PAVEG 894, VRC01 produced in N. benthamiana

Assays: Neutralization in TZM-bl cells

Virus stocks: Derived by transfection in 293T cells. Stock IDs shown in table.

Report Date: March 8, 2012

<table>
<thead>
<tr>
<th>Virus</th>
<th>Tier</th>
<th>Stock ID</th>
<th>Mapp66/VRC01 Lot#12V001 QC#6738-1</th>
<th>VRC01 (in house control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF162.LS</td>
<td>1</td>
<td>1825</td>
<td>0.34</td>
<td>0.43</td>
</tr>
<tr>
<td>6535.3</td>
<td>2</td>
<td>4439</td>
<td>1.76</td>
<td>1.59</td>
</tr>
<tr>
<td>QH0692.42</td>
<td>2</td>
<td>2189</td>
<td>1.72</td>
<td>1.32</td>
</tr>
<tr>
<td>PVO.4</td>
<td>2</td>
<td>845</td>
<td>0.38</td>
<td>0.44</td>
</tr>
<tr>
<td>TRO.11</td>
<td>2</td>
<td>847</td>
<td>0.33</td>
<td>0.34</td>
</tr>
<tr>
<td>RHPA4259.7</td>
<td>2</td>
<td>855</td>
<td>&lt;0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>AC10.0.29</td>
<td>2</td>
<td>1798</td>
<td>2.35</td>
<td>1.82</td>
</tr>
<tr>
<td>THRO4156.18</td>
<td>2</td>
<td>949</td>
<td>3.9</td>
<td>2.95</td>
</tr>
<tr>
<td>REJO4541.67</td>
<td>2</td>
<td>792</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>TRJO4551.58</td>
<td>2</td>
<td>963</td>
<td>0.1</td>
<td>0.08</td>
</tr>
<tr>
<td>WITO4160.33</td>
<td>2</td>
<td>851</td>
<td>0.1</td>
<td>0.11</td>
</tr>
<tr>
<td>CAAN5342.A2</td>
<td>2</td>
<td>858</td>
<td>3.54</td>
<td>2.51</td>
</tr>
</tbody>
</table>

(courtesy of David Montefiori, 2012)
Table 3. Cost and time comparisons for mAb manufacturing systems

<table>
<thead>
<tr>
<th>Manufacturing system</th>
<th>Time to Phase 1 cGMP supply</th>
<th>Cost to Phase 1 cGMP supply</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian cell culture (CHO, NS0, PER.C6)</td>
<td>18 months</td>
<td>$5–6 M</td>
</tr>
<tr>
<td>Transient Nicotiana (magnICON, Geneware)</td>
<td>6–12 months</td>
<td>$0.5–0.8 M</td>
</tr>
</tbody>
</table>

Based upon quotes from Lonza (Slough, UK) and Kentucky BioProcessing (Owensboro, KY).

(Whaley, Hiatt, Zeitlin, Human Vaccines, 2011)
Antibody-based Multipurpose Microbicides: NIAID funded IPCP

• Purpose: evaluate safety, efficacy, and mechanisms of antibody-based microbicides

• Principal Investigators: Deborah Anderson (BUMC), Thomas Moench (ReProtect), Kevin Whaley (Mapp), Larry Zeitlin (Mapp), Richard Cone (JHU), Sam Lai (UNC), Francois Villinger (Yerkes Primate Center), Thomas Smith (Auritec), Ken Mayer (Fenway), Susan Cu-Uvin (Brown)

• Methods: in vitro, NHP, clinical
IgG Uptake by Apical Vaginal Epithelial Cells

IgG-Cy3

Negative Control

(Courtesy of Deborah Anderson, M2012)
Retention of HSV-Cy3 mab by vaginal stratum corneum

After 1 hour

After 12 hours

(Courtesy of Deborah Anderson, M2012)
Suppression of HSV-2 Infection in vitro with Anti-HSV Mab

Treatment Groups:
- No treatment
- Anti-HSV Mab
- Anti-HSV Mab Preincubation/Rinse

p<0.01
p<0.05
p<0.05
p<0.01

(Courtesy of Deborah Anderson, M2012)
Vaginal Stratum Corneum Battlefront

Summary

Pathogens (HIV, HSV-2) can enter the SC

Soluble immunological mediators and leukocytes provide SC immune defense

It may be possible to further fortify SC defense with microbicides (eg. plantibodies)

(Courtesy of Deborah Anderson, M2012)
Antibodies and Cell-Associated HIV

- Cell-associated vaginal HIV transmission is highly efficient and not prevented by topically applied 1% tenofovir (Swanson and Garcia-Martinez, CROI 2012)

### Table 5. Antiviral efficacy of m9 in CCR5-dependent cell-to-cell transmission assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (nM)</th>
<th>IC$_{90}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMD3100$^a$</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>TAK779$^b$</td>
<td>3.0</td>
<td>6,420</td>
</tr>
<tr>
<td>enfuvirtide</td>
<td>35</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>m9</td>
<td>0.066</td>
<td>2.3</td>
</tr>
<tr>
<td>4E10</td>
<td>1.4</td>
<td>9.1</td>
</tr>
<tr>
<td>2F5</td>
<td>1.5</td>
<td>6.4</td>
</tr>
</tbody>
</table>

(Zhang...Dimitrov, mAbs 2010)
Antibody-based Contraception

*Figure 2.* Agglutination of human sperm with Mab HC4 produced in *N. benthamiana*. Purified Mab was added to undiluted human semen and observed within 30 seconds via light microscopy.

(Whaley, Hiatt, Zeitlin, Human Vaccines, 2011)
Anti-inflammatory Antibodies

• HIV upregulates inflammatory cytokines that lead to impairment of mucosal epithelial barrier functions. Antibodies to TNF prevented the loss of barrier function (Nazli et al., PLoS 4-10)

• Mapp is producing TNF and IL-6 mAbs.
Anti-Idiotype Vaccines

• FcRn-mediated uptake
• HIV vaccine based on 2F5 anti-Id
• HSV vaccine based on gD-Fc

(Ye...Zhu, Nat. Biotech 2011)
AAV-vectored HIV antibodies and Systemic Protection

(Balazs...Baltimore, Nature 2011)
AAV-vectored HIV Antibodies and Mucosal Protection

(Abdel-Motal...Anderson... Marasco, PLoS One 2011)
Acknowledgements

**Mapp**
Natasha Bohorova
Ognian Bohorov
Andrew Hiatt
Do Kim
Michael Pauly
Jesus Velasco
Larry Zeitlin

**Antibody IPCP**
Deborah Anderson
Thomas Moench
Richard Cone
Francois Villinger
Kenneth Mayer
Larry Zeitlin
Sam Lai
Susan Cu-Uvin

**Icon Genetics (Halle)**
Yuri Gleba
Victor Klimyuk

**BOKU (Vienna)**
Herta Steinkellner
Fredrich Altmann
Richard Strasser
Renate Kunert

**Aridis Pharmaceutical**
Vu Truong
Jeff Anderl

**KBP**
Barry Bratcher
Hugh Haydon
Steve Hume
Ernie Hiatt
Josh Morton
Katrina Whelan
Ashley Johnson

**Funding**
NIAID: U19AI096398