Robust Bispecific Process Development and Commercial Manufacturing Platform

Development and Characterization challenges leading to insights for increased understanding and control of Bispecific Production

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Janssen R&D

Confidential Draft : not for distribution
Bispecific Agents in Clinical Trials

- **DuoBody®**
  - Janssen/Genmab

**DuoBody®**

- **Trion Pharma**
  - Removab
  - CD3 x Epcam

**Trion Pharma**

- **Genentech**
- **Regeneron**
- **Xencor**

**Genentech**

- **Roche**

**Roche**

- **Lilly**

**Lilly**

- **AbbVie**
- **Sanofi**
- **Tetravalent bispecific tandem immunoglobulin (TBTI)**

**AbbVie**

- **Chugai**
- **Genentech**

**Chugai**

- **Merrimack**
- **Emergent/Morphosys**

**Merrimack**

- **BMS**
- **DNL-Fab3**

**BMS**

- **DuoBody®**
- **kih IgG common LC**

**DuoBody®**

- **CrossMab**

**CrossMab**

- **Amgen/Micromet**

**Amgen/Micromet**

- **Affimed**
- **Janssen/Macrogenics**

**Affimed**

- **Janssen/Macrogenics**

**Janssen/Macrogenics**

- **Merrimack**

**Merrimack**

- **Immunomedic**
Outline

- DuoBody® platform and cFAE overview
- Kinetic Studies and ΔG
- Mechanism & Manufacturing Insight
- Characterization and Structure Function
- cFAE Model development
Overview Duobody® BsAb Technology

- Bispecific Ab is made by exchange of parental mAbs that contain complementary CH3 mutations
- K409R and F405L are destabilizing mutations in the CH3 interface
- The complementary mutations favor heterodimerization

DuoBody® formation through cFAE is a robust process

- BsAb formation is typically greater than > 90%

- Parental mAbs are generated separately followed by downstream *in vitro* Fab arm exchange

Sum of Parental homodimers ~ 5%
Bispecific Process leverages Janssen mAb platform

Parent 1

Parent 2

USP ~ 90% yield

DSP ~ 70% yield

1 USP
✓ Continuous or Freeze/Thaw
✓ Virus Inactivation/Removal
✓ No cFAE
✓ Similar DSP train (no cFAE)
✓ One set of PTMs and CQAs

2 USP
✓ Freeze/Thaw
✓ Virus Inactivation/Removal
✓ cFAE
✓ Similar DSP train after cFAE
✓ Two sets of PTMs and CQAs
BsAb Impurity Clearance is similar to mAb platform

<table>
<thead>
<tr>
<th>BsAb</th>
<th>mAb 1 %</th>
<th>mAb 2 %</th>
<th>Reducing Agent $\mu$M</th>
<th>HCP ng/mg</th>
<th>DNA pg/mg</th>
<th>Total Virus log Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt; 2.0</td>
<td>&lt;1.0</td>
<td>&lt;2</td>
<td>1</td>
<td>&lt; 2</td>
<td>15.4</td>
</tr>
<tr>
<td>B</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.5</td>
<td>1</td>
<td>&lt; 2</td>
<td>&gt; 18.3</td>
</tr>
<tr>
<td>C</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
<td>&lt;2</td>
<td>3</td>
<td>&lt;2</td>
<td>16.7</td>
</tr>
<tr>
<td>D</td>
<td>&lt;1.0</td>
<td>&lt;1.8</td>
<td>&lt;2</td>
<td>4</td>
<td>&lt; 2</td>
<td>16.3</td>
</tr>
<tr>
<td>E</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
<td>40</td>
<td>&lt; 2</td>
<td>&gt;15.3</td>
</tr>
<tr>
<td>F</td>
<td>&lt;1.8</td>
<td>&lt;2.5</td>
<td>&lt;2</td>
<td>&lt;10</td>
<td>&lt; 2</td>
<td>17.8</td>
</tr>
<tr>
<td>G</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
<td>1</td>
<td>&lt; 2</td>
<td>&gt;21.4</td>
</tr>
</tbody>
</table>
Outline

- DuoBody® platform and cFAE overview
- Kinetic Studies and $\Delta G$
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Kinetic mechanism of cFAE: 4 different steps

1- Reduction

2- Dissociation

3- Association

4- Oxidation

Goulet et al., Kinetic mechanism of controlled Fab-arm exchange for the formation of bispecific immunoglobulin G1 antibodies, JBC, 2018
Complete kinetic description of cFAE

Fluorescence resonance energy transfer (FRET)*

Association of parental mAbs has a negative ΔG value

\[ \Delta G = -RT \ln \frac{K_{A}K_{B}}{K_{A}^{2}} \]

\[ \Delta G = -27.7 \text{ kJ/mol} \]

H bond equivalent

\(~5-13 \text{ KJ/mol}\)

Goulet. et. all, Kinetic mechanism of controlled Fab-arm exchange for the formation of bispecific immunoglobulin G1 antibodies, JBC, 2018
Tm and Enthalpies of Fabs Arms in BsAbs are conserved

*Fab domains maintain their unique folding upon BsAb formation*

*A unique structure is maintained irrespective of bispecific Ab*

*Mass spec data, peptide mapping, free thiol, bioactivity and other data confirm correct assembly BsAb*
Parameters controlling cFAE: pH, reducing agent concentration, temperature, time

HIC HPLC assay @ T= 5 h
IgG1 BsAb
Reduction step: pH is key in hinge reduction chemistry

Thiolate attacks disulfide bond

H_iN - R - S^- + LH - S - S - HL ↔ LH - S^- + H_2N - R - S - S - HL

Reducing agent

mAb

Fab

Reducing Agent - Fab

Henderson-Hasselbalch Equation

\[
\text{pH} \frac{[S^-]}{[SH]} = 10^{(pH - pKa)}
\]

<table>
<thead>
<tr>
<th>pH</th>
<th>[S^-]/[SH]</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.02</td>
</tr>
<tr>
<td>7</td>
<td>0.2</td>
</tr>
<tr>
<td>7.4</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**cFAE is a robust process – Minimal impact of HMWS (Dimer) on BsAb formation**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Expected Dimer (Da)</th>
<th>Measured Dimer (Da)</th>
<th>DOE Run 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent A</td>
<td>290,936</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Parent B</td>
<td>294,112</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Bispecific</td>
<td>292,524</td>
<td>292,555</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run #</th>
<th>Parent A HMWS</th>
<th>Parent B HMWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15%</td>
<td>Native (&lt;5%)*</td>
</tr>
<tr>
<td>2</td>
<td>Native (&lt;5%)*</td>
<td>15%</td>
</tr>
<tr>
<td>3</td>
<td>15%</td>
<td>15%</td>
</tr>
<tr>
<td>4</td>
<td>Native (&lt;5%)*</td>
<td>Native (&lt;5%)*</td>
</tr>
</tbody>
</table>

Minimal impact of HMWS (Dimer) on BsAb formation

- Parental HMWS entering the cFAE reaction were not observed in the product of the cFAE.
- Parental mAbs containing HMWS entering the cFAE reaction resulted in the formation of both bispecific monomer and bispecific dimer, suggesting parental dimer can reduce and later recombine as bispecific dimer without dissociating into monomer.
- The cFAE is a robust process that has minimal impact from the presence of HMWS on the formation of intact formation of bispecific.
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Insights in cFAE mechanism to enable control: Oxygen is not limiting at manufacturing scale

Air saturation or depleted oxygen during cFAE yields > 90% NR-cSDS BsAb

Oxygen is not limiting at scale = More options

- Multiple pathways to achieve disulfide formation of a DuoBody® BsAb
- Oxygen and free metals are not critical for DuoBody® bispecific Ab formation
- Robust manufacturing with wider bispecific design space.
- Wider design space increases the ability to fit different manufacturing plant configurations providing flexibility to the Janssen Supply Chain (JSC) enhancing our ability to deliver new drugs to patients
Outline

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Key Challenges for DuoBody® Characterization

Bispecfics have similar properties as parental antibodies.

- Confirm DuoBody identity and purity
  - High resolution MS analysis

- Confirm DuoBody structural integrity
  - NR peptide map for disulfide linkage analysis
  - Fab and Fc integrity analysis
  - Free thiol analysis
  - Thermal stability analysis
BsAb Identity and Purity by UPLC - High Resolution MS

**BsAb UPLC-Intact Mass Analysis**

Relative Quantitation of Glycoforms by Intact Mass Analysis

**UPLC-Reduced MS Analysis Verifies Chain Composition**

UPLC-Intact Mass Analysis Quantifies Residual Parentals and Other Impurities

Comparative glycoform profiles were observed among DuoBody and parentals

- Quickly identifies all impurities in one assay
- Quantifies relative abundance of all impurities by UV & MS

**BsAb Process Intermediates**

<table>
<thead>
<tr>
<th>BsAb Process Intermediate</th>
<th>LC mAb 2 (23238 Da)</th>
<th>Intact BsAb (46929 Da)</th>
<th>Intact BsAb- LC mAb1 (24991 Da)</th>
<th>Intact mAb 2 (148882 Da)</th>
<th>Intact mAb 1 (147877 Da)</th>
<th>LC mAb 1 (22328 Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB</td>
<td>0.2%</td>
<td>99.5%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.3%</td>
</tr>
<tr>
<td>1xDPBS</td>
<td>0.2%</td>
<td>95.0%</td>
<td>ND</td>
<td>2.6%</td>
<td>1.9%</td>
<td>0.3%</td>
</tr>
<tr>
<td>FAE VIN</td>
<td>0.3%</td>
<td>90.8%</td>
<td>ND</td>
<td>4.9%</td>
<td>3.4%</td>
<td>0.5%</td>
</tr>
<tr>
<td>FAE</td>
<td>0.4%</td>
<td>92.0%</td>
<td>ND</td>
<td>4.1%</td>
<td>2.8%</td>
<td>0.7%</td>
</tr>
<tr>
<td>UF/D1 DV_6</td>
<td>0.6%</td>
<td>90.8%</td>
<td>ND</td>
<td>4.6%</td>
<td>3.2%</td>
<td>0.8%</td>
</tr>
<tr>
<td>UF/D1 DV_8</td>
<td>0.7%</td>
<td>87.4%</td>
<td>3.1%</td>
<td>4.6%</td>
<td>3.1%</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

**Jingjie Mo**
Structural Integrity Characterization

- **Non-reduced Peptide Map** – confirm disulfide bond linkage and detect disulfide scrambling
- **Fab/Fc Mass Analysis** – detect potential LC swapping
- **Free thiol analysis** – measure free thiol content
- **DSC** – evaluate thermal stability
BsAb Structural Integrity Characterization

All 10 Disulfide Bonds Were Confirmed

- High mass accuracy MS and MS/MS give unambiguous peak assignments

Mass Analysis of Fab, Fc shows no evidence of LC Swapping

Jingjie Mo
Free Thiol Analysis of BsAb and IgG1 Parentals

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Free SH/Protein (mol/mol)</th>
<th>SH%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BsAb, Lot M1D03-14</td>
<td>0.26</td>
<td>0.8%</td>
</tr>
<tr>
<td>mAb 2, Lot M14D012</td>
<td>0.13</td>
<td>0.4%</td>
</tr>
<tr>
<td>mAb 1, Lot M1D23-14</td>
<td>0.19</td>
<td>0.6%</td>
</tr>
</tbody>
</table>

* SH% = mean free SH per protein / 32 * 100%

- Observed free thiol values for BsAb and IgG1 parentals mAb1 and mAb2 were typical for Janssen IgG1 products.
DSC Analysis of BsAb and Parentals in Formulation Buffer

<table>
<thead>
<tr>
<th></th>
<th>BsAb</th>
<th>mAb 1</th>
<th>mAb 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_m1$ (°C)</td>
<td>71.6</td>
<td>72.6</td>
<td>67.9</td>
</tr>
<tr>
<td>$\Delta H$</td>
<td>4.6E+5</td>
<td>7.0E+5</td>
<td>2.1E+5</td>
</tr>
<tr>
<td>$T_m2$ (°C)</td>
<td>81.0</td>
<td>80.7</td>
<td>80.7</td>
</tr>
<tr>
<td>$\Delta H$</td>
<td>2.2E+5</td>
<td>4.4E+5</td>
<td>2.2E+5</td>
</tr>
</tbody>
</table>

- DuoBody inherited $T_m$'s from both parentals.

Jingjie Mo
Structure–function of symmetrically and asymmetrically modified BsAb

Robust assembly of BsAb in presence of Oxidized Fab Arms

Adam Evans et al. mAbs 2019
Structure–function of symmetrically and asymmetrically modified BsAb

- Asymmetrically oxidized BsAb 2 & 3 bind ~ 50% of the FcRn vs the control BsAb1
- Experimental support for 2:1 FcRn:IgG binding ratio
- FcRn can bind independently to either chain.

<table>
<thead>
<tr>
<th>Description</th>
<th>mAb1</th>
<th>mAb2</th>
<th>Final Product</th>
<th>FcRn</th>
<th>FcγRI</th>
<th>FcγRII</th>
<th>FcγRIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>BsAb1</td>
<td>Native</td>
<td>Native</td>
<td>3.8%</td>
<td>5.7%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>BsAb2</td>
<td>Native</td>
<td>Oxidized</td>
<td>3.8%</td>
<td>99.9%</td>
<td>56.4%</td>
<td>44.4%</td>
<td>97.6%</td>
</tr>
<tr>
<td>BsAb3</td>
<td>Oxidized</td>
<td>Native</td>
<td>99.9%</td>
<td>3.0%</td>
<td>57.6%</td>
<td>54.3%</td>
<td>120.2%</td>
</tr>
<tr>
<td>BsAb4</td>
<td>Oxidized</td>
<td>Oxidized</td>
<td>99.9%</td>
<td>99.9%</td>
<td>99.4%</td>
<td>8.6%</td>
<td>128.6%</td>
</tr>
</tbody>
</table>

*Binding is normalized relative to control
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Modeling cFAE Chemistry:

- **Optimizing Process Parameters**
  - Finding the target and MORs
- **Defining a Design Space**
  - Designing DOE studies
- **Limiting the Number of experiments**
  - Avoiding unnecessary experiments
  - Reducing development time
- **Monitoring and Investigating**
  - Using the model to investigate failed batches
Mixed Model
- First principles
- Experimental Data
  - Lab & Manufacturing Scale
- Mechanism of Action
- Kinetic Forms
- Linear/Non-Linear behavior

Interactive web based app uses >25 parameters to predict BsAb reformation of disulfides
Using cFAE model to guide design space selection

Predictive modeling uses over 25 parameters to narrow optimized conditions.
Key Messages

• Controlled Fab arm exchange (cFAE) is a robust process
• Leverage existing mAb platform & yields: 2 cell lines & USP
• cFAE understanding is key to manufacturing control
• BsAb characterization supports correct assembly and Structure/Function
• cFAE modeling optimizes development and design space
• DuoBody® technology is translated into a robust manufacturing platform for Janssen BsAbs
Acknowledgements

- John Knighton, Barry McCarthy, Chuck Goochee, Graham Tulloch
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- Leiden and Cork Manufacturing