Manufacturing Challenges and Strategies for Bispecific Antibody

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Disclaimer:

The information presented here reflects the views of the presenter and should not be construed to represent FDA’s views or policies.
Bispecific antibody (BsAb) IND submissions have grown significantly in recent years.

- Approval of blinatumomab
- Approval of emicizumab
- Approval of amivantamab
- Approval of faricimab and tebentafusp
## FDA-approved bispecific antibodies

<table>
<thead>
<tr>
<th></th>
<th>Blinatumomab</th>
<th>Emicizumab</th>
<th>Amivantamab</th>
<th>Faricimab</th>
<th>Tebentafusp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td>CD3 and CD19</td>
<td>FIXa and FX</td>
<td>EGFR and c-Met</td>
<td>Ang-2 and VEGF-A</td>
<td>glycoprotein 100 (gp100) and CD3</td>
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<tr>
<td><strong>Indication</strong></td>
<td>Acute lymphocytic leukemia (ALL)</td>
<td>Hemophilia A</td>
<td>Non-small cell lung cancer</td>
<td>Wet age-related macular</td>
<td>Uveal melanoma</td>
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<tr>
<td><strong>Format</strong></td>
<td>BiTE</td>
<td>Heterodimeric asymmetric IgG4/k</td>
<td>IgG like_duobody</td>
<td>CrossMab/Knob-into-hole (KIH)</td>
<td>T cell engager (ImmTac)</td>
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(Information included in this slide is all sourced from published papers)
### Classification of BsAb

<table>
<thead>
<tr>
<th>IgG/IgG-like formats</th>
<th>DVD-Ig</th>
<th>2 in 1-IgG</th>
<th>Fab-scFv-Fc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi-Nanobody</td>
<td>BiTE</td>
<td>scFv-HSA-scFv</td>
<td></td>
</tr>
</tbody>
</table>

Non-IgG fragment formats
Technologies used to generate BsAbs

- Hybrid hybridoma technology established to generate BsAb
  - Chemical conjugation of two mAbs
    - 1983
    - 1985
- 1988
- 1996
- 1998
- 1999
- 2011

- Blinatumomab (Approved in 2014)
  - ScFv produced
  - Knob-into-hole approach (solving heavy chain problem)
- Controlled Fab Arm Exchange (cFAE)
- Amivantamab (Approved in 2021)
  - Discovery of IgG4 being naturally bispecific
- Faricimab (Approved in 2022)
  - CrossMab (solving light chain problem)
- Faricimab (Approved in 2022)
  - Emicizumab (Approved in 2017)
  - Common light chain (solving the light chain problem)

(Information included in this slide is all sourced from published papers)
The general schematic for a BsAb manufacturing process

The correct assembly of asymmetric IgG-like BsAb

Bioreactor → Harvest/Clarification → Capture → Polishing

→ DP ← DS ← UF/DF ← Virus clearance

Modified from https://www.adcreview.com/articles/doi-10-14229jadc-2014-6-6-001/'
Manufacturing challenges

• The more complex composition (e.g., 3–4 chains of BsAb)
• Stable expression system and production yield
• The correct assembly of asymmetric IgG-like BsAb
• Absence of undesired side products
• Stability of drug
BsAb manufacturing challenges
Purification of non-IgG fragment BsAbs

The capture step for Fc-less BsAbs

1) Histidine tag _ metal affinity chromatography
2) The variable region of the kappa light chain _ Protein L affinity chromatography
3) Ion exchange (IEX) or hydrophobic interaction
4) Based on our research experience, BsAb with BiTE format targeting EGFR and PD-L1 is not soluble when expressed in bacteria

• Product-related Impurities: HMW/aggregation and LMW/fragmentation
• Process-related Impurities: e.g., protein L, zinc, and imidazole

Regulatory Considerations
Prior to the initiation of phase III studies, the sponsor should either formally validate the removal of Zn\(^{2+}\), methotrexate and imidazole by your purification process or incorporate lot release assays for these impurities into your drug substance lot release program.
CMC comments
Product related impurities (non-IgG formats)

Aggregates:
• Quantitative specification for the aggregates are not provided for release and stability. Note that aggregates may have different potency as compared to monomers. The acceptance criteria of “report results”, or ≤ 10% are inadequate to control for the potential HMW species
• Main peak by SEC-HPLC with appropriate acceptance criteria should be included as part of release and stability testing
CMC comments

Fragmentation:

- The levels of fragmentation should be monitored and maintained during manufacturing process and manufacture changes as monospecific fragments may have reduced or no potency.

- Quantitative specification for the low molecular weight fragments should be set for release and stability.

- LMW by rCE-SDS should be closely monitored as it appears to be a potential stability indication attribute.
Solving the heavy chain problem in asymmetric IgG/IgG-like BsAb

- Protein A for IgG purification
- Product-related impurities:
  - The HC-LC form of BsAb by CE-SDS is present in the non-reduced samples for KIH format
  - The mis-paired heavy chain homodimers are detected by CE-HPLC for charge pair format
  - Half molecules can be detected by non-reduced CE-SDS for charge pair format
  - As the development proceeds, the safety and activity of product variants will need to be determined
- ADCC activity is not generally interfered by KIH and charge pairs. However, the mutations can be introduced to attenuate Fc effector function, as needed

Modified from Ulrich Brinkmann & Roland E. Kontermann (2017) The making of bispecific antibodies, mAbs, 9:2, 182-212,
Potential unpaired or incorrectly paired heavy chains found in the asymmetric BsAb with KIH format detected by non-reduced SDS-PAGE

Plasmids:
1. Cetux VL + Human IgG KAPPA
2. Cetux VH + Human IgG1
3. ATE VL + ATE VH + Human IgG1

Nishant Mohan et al. Comparative characterization of different molecular formats of bispecific antibodies targeting EGFR and PD-L1. 2022, Manuscript under revision

Unpublished data from Wu lab in OBP at FDA
Solving the light chain problem in asymmetric BsAb

Two different light chains

Strategies

- Common light Chain + KIH
- scFab + KIH
- CrossMab (IgG-KIH)
- CH3 + CH1/CL Charge pair

Regulatory considerations

- Protein A for IgG purification
- The different product variants e.g., CrossMab-specific side product (the crossed LC2 light chain is missing as detected by non-denaturing hydrophobic interaction chromatography (HIC))
- As development proceeds, the different product variants observed as part of the product characterization will need to be analyzed

Modified from Ulrich Brinkmann & Roland E. Kontermann (2017) The making of bispecific antibodies, mAbs, 9:2, 182-212,
Post-assembly approaches to purify BsAbs

Mutations in the Fc region facilitate purification

1) Fc* chain: with mutations, for example: H435R and Y436W in CH3 domain of an IgG1
2) Fc chain: no mutations

- The Fc* sequence allows fractionated elution of BsAb from Protein A column
- Protein A column used in both capture and polishing
- Variable region Protein A binding, resin selection, selective elution optimization
- Impurities e.g., Fc-Fc, should be removed during manufacturing or controlled

Tustian et al. mAbs, 8:4, 828-838, DOI: 10.1080/19420862.2016.1160192
Controlled Fab arm exchange (cFAE)

Labrijn et al. PNAS 26, 2013:26 5145–5150

- Protein A for the parental IgG1 purification
- The reduction process: $\beta$-mercaptoethylamine hydrochloride (2-MEA). 2-MEA impurity is assigned as Class 2 in accordance with ICH M7, and its levels in all clinical drug substance batches should be controlled to below 3 $\mu$g/day
- Impurities, e.g., two homodimeric parental antibodies, high molecular weight species (HMWS), and low molecular weight species (LMWS)

Bispecific IgG4

Bispecific IgG1
Post-production assembly of half-antibodies

Two separate cell cultures

Two half-antibodies

Post-production Assembly

Protein A followed by hydrophobic interaction to remove half-antibodies

- Co-culture of two bacterial cells to produce bispecific antibody
- No ADCC or other Fc-mediated effector functions

Modified from Ulrich Brinkmann & Roland E. Kontermann (2017) The making of bispecific antibodies, mAbs, 9:2, 182-212,
IgG/IgG-like formats with a symmetric architecture (Appended IgGs)

- They are not tri-specific antibodies as CD16a (FcγR IIIa) binding through the Fc region of an antibody is not specific to these molecules
- Symmetric or asymmetric architectures
- Tetravalent or trivalent
- Fc effector function may be silenced dependent on MOAs and clinical indications
- Multiple CD3 binding domains may be found in HMW species of CD3-based appended IgGs, leading to cytokine release. In this case, quantitative specification for HMW species for release and stability testing should be set for IND
- Some of appended BsAbs may induce drug specific immune response and produce anti-drug antibody (ADA)
Linker engineering and general considerations

- The length and composition of the connecting linker can affect correct folding, stability and antigen-binding of the BsAbs
- The linker has to be stable, ideally flexible, and non-immunogenetic
- Fusion of linker to antibody or antibody fragment should not interfere with antigen binding activity
- Various connecting linkers have been utilized to generate BsAbs, such as short alanine linkers (Ala3), hydrophilic linkers, glycine-serine-rich linkers, linkers adopting a helical conformation, and linkers derived from various immunoglobulin and non-immunoglobulin molecules

https://www.tandfonline.com/action/showCitFormats?doi=10.1080/19420862.2016.1268307
Summary

- BsAb IND submissions have grown significantly in recent years
- Five FDA-approved BsAbs, each employed complicated and advanced technologies with different molecular formats
- Differences in molecular formats impact BsAb manufacturing processes, characterization and quality control strategies
- Understanding of the advanced technologies, molecular formats, and their related quality attributes informs optimal science-based regulatory decisions
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