Manufacturing Challenges and Strategies for Bispecific Antibody

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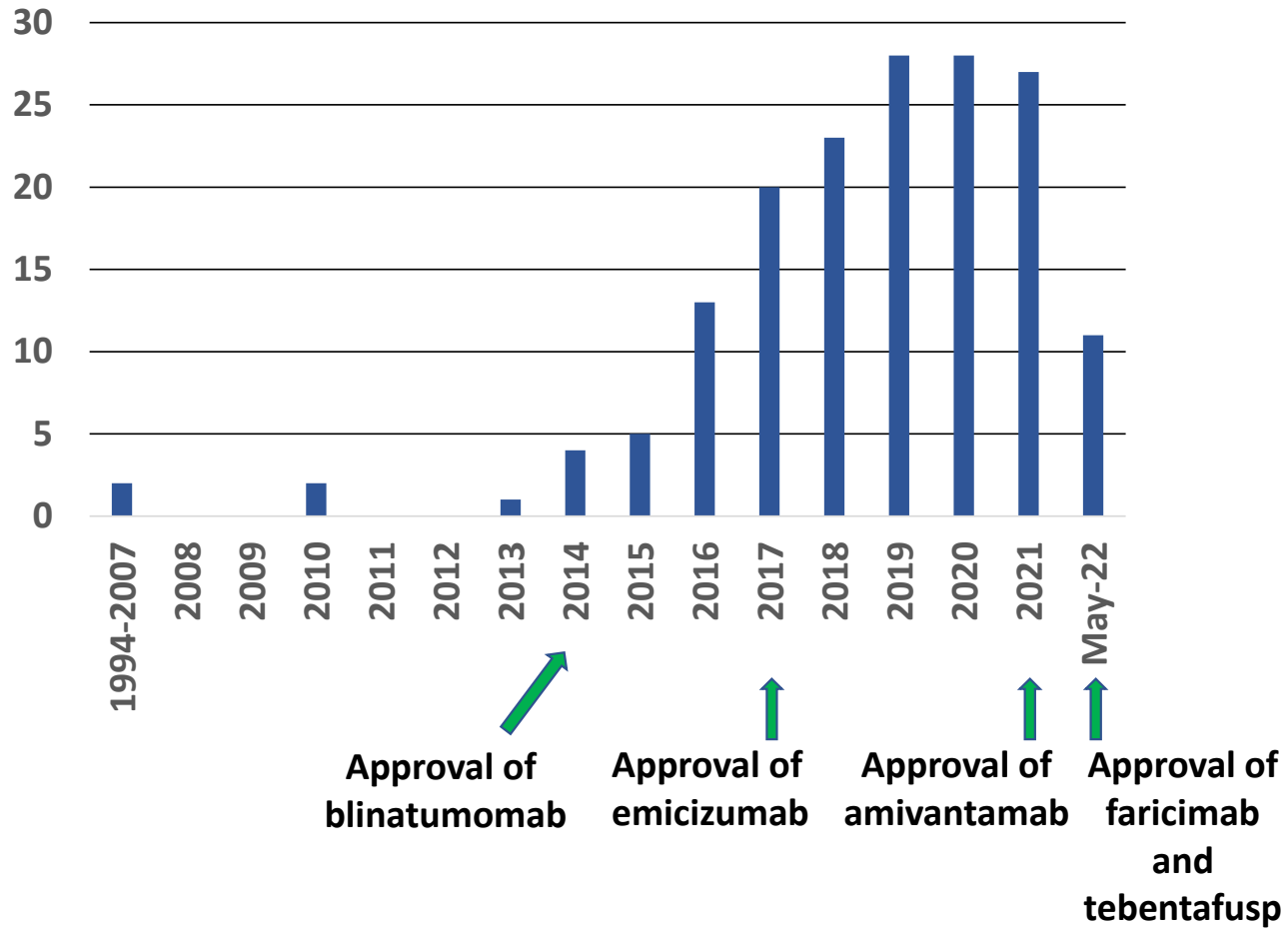
Disclaimer:

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

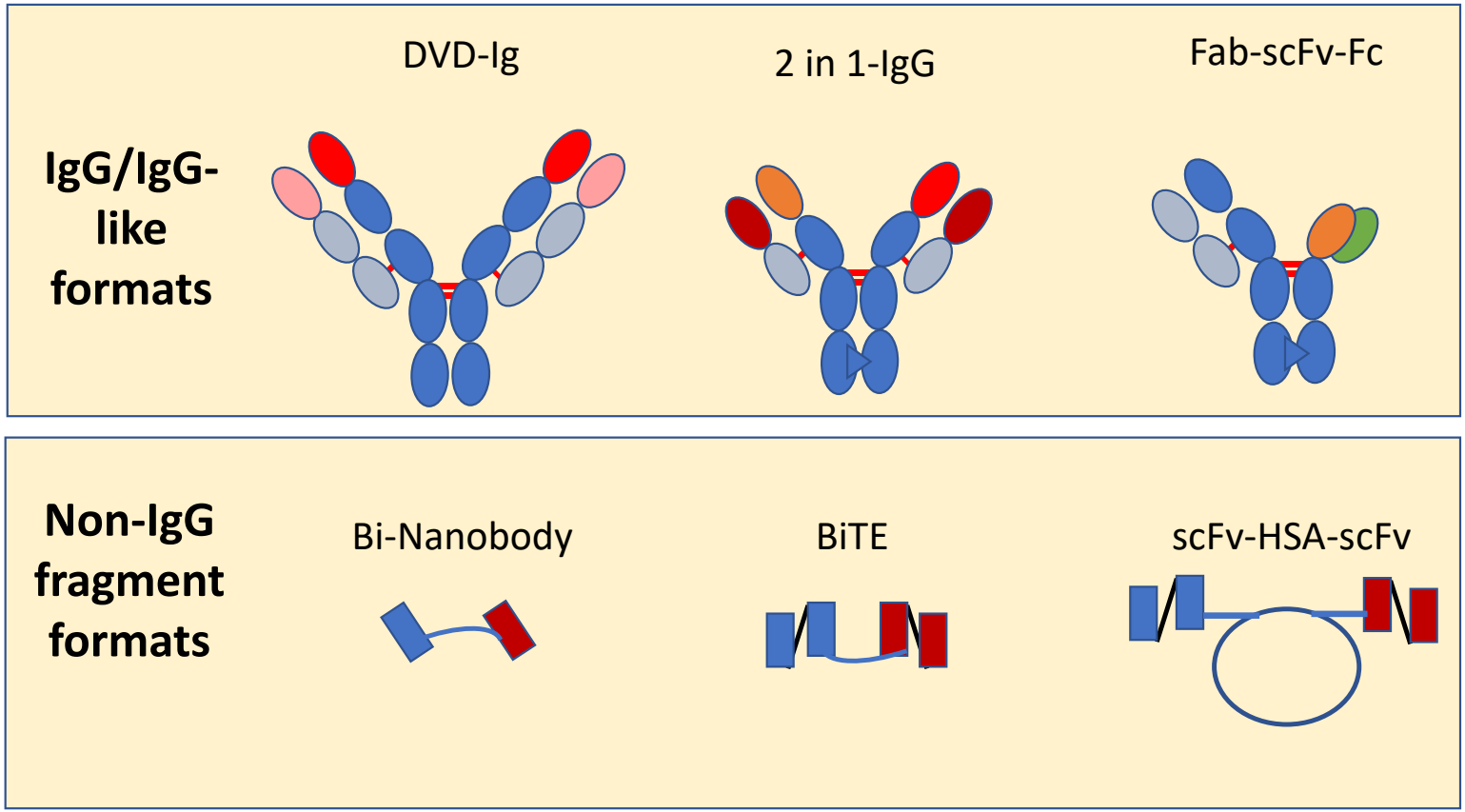


FDA-approved bispecific antibodies

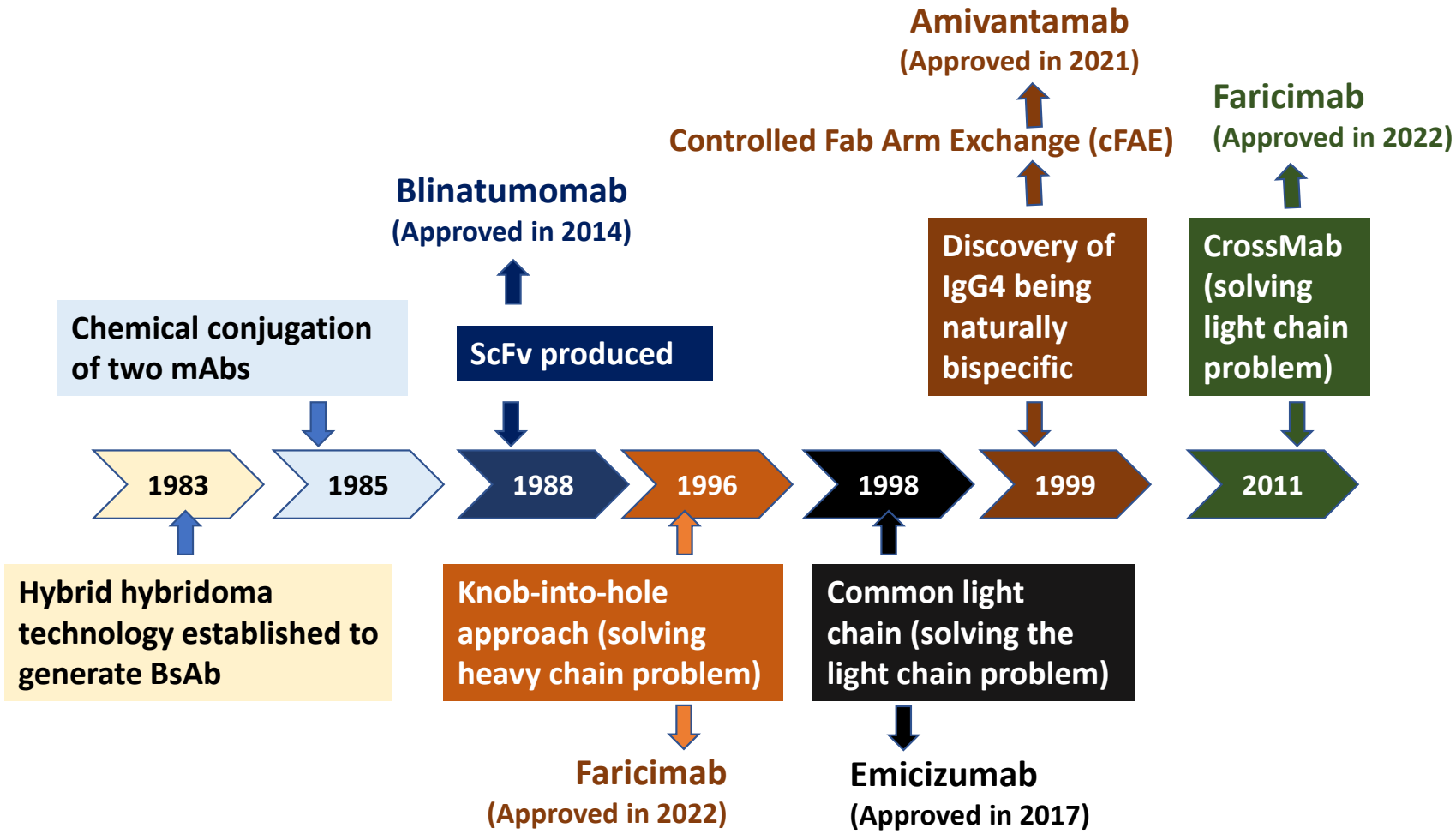
	Blinatumomab	Emicizumab	Amivantamab	Faricimab	Tebentafusp
Target	CD3 and CD19	FIXa and FX	EGFR and c-Met	Ang-2 and VEGF-A	glycoprotein 100 (gp100) and CD3
Indication	Acute lymphocytic leukemia (ALL)	Hemophilia A	Non-small cell lung cancer	Wet age-related macular	Uveal melanoma
Format	BiTE	Heterodimeric asymmetric IgG4/k	IgG like_duobody	CrossMab/Knob-into-hole (KIH)	T cell engager (ImmTac)

(Information included in this slide is all sourced from published papers)

Classification of BsAb



Technologies used to generate BsAbs



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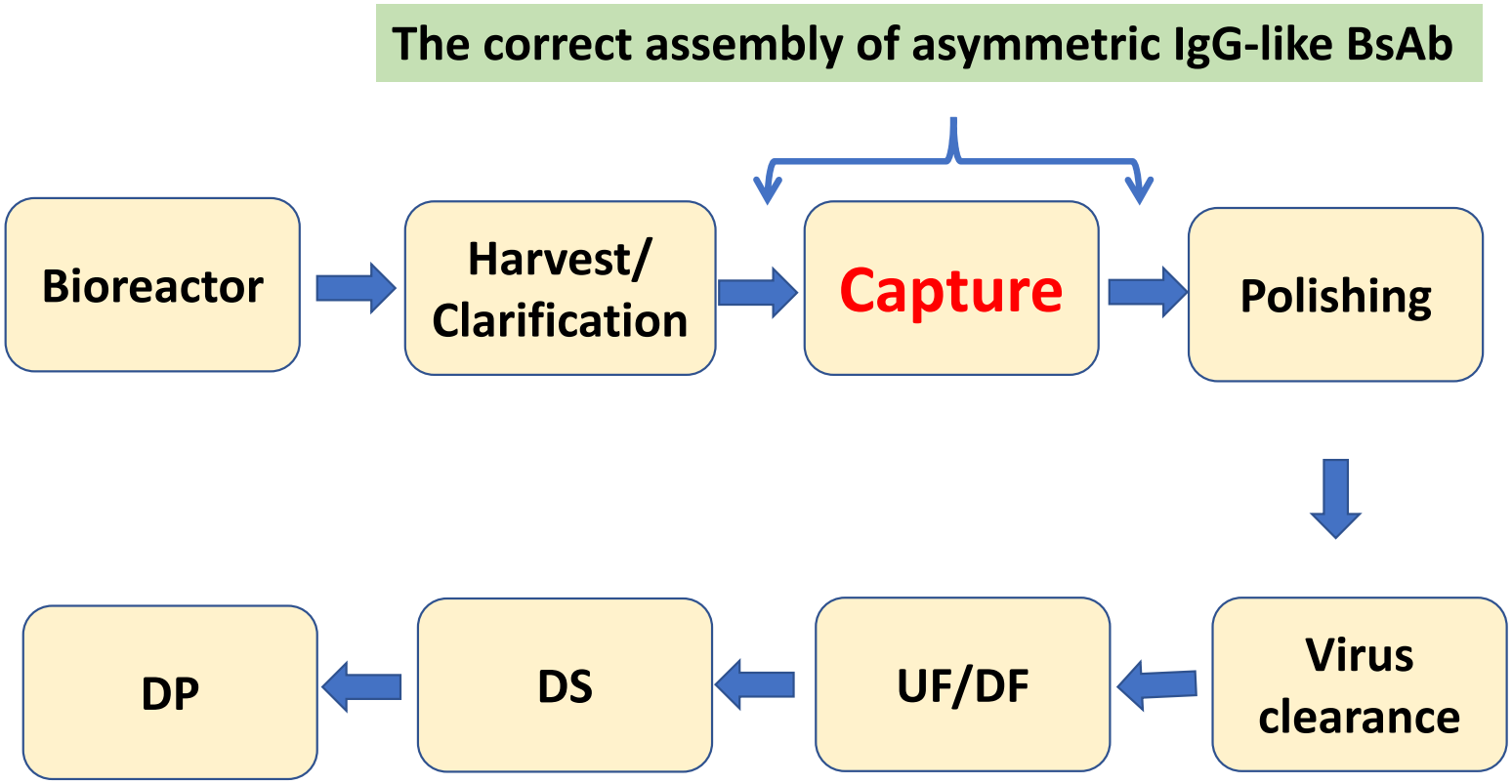
The general schematic for a BsAb manufacturing process



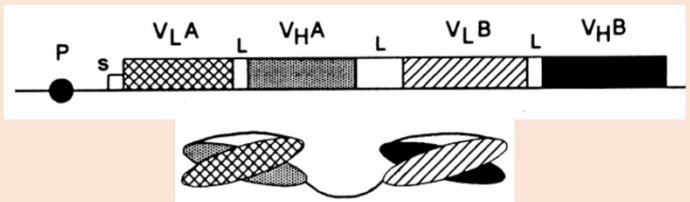
Modified from https://www.adcreview.com/articles/doi-10-14229jadc-2014-6-6-001/

Manufacturing challenges

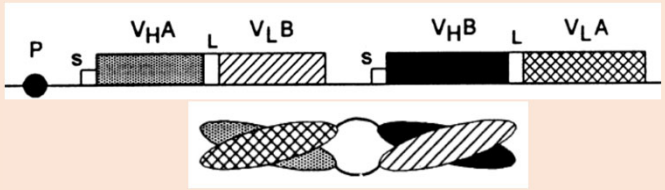
- The more complex composition (e.g., 3–4 chains of BsAb)
- Stable expression system and production yield
- **The correct assembly of asymmetric IgG-like BsAb**
- Absence of undesired side products
- Stability of drug

BsAb manufacturing challenges

Purification of non-IgG fragment BsAbs



Bispecific (scFv)2



Diabodies

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

Regulatory Considerations

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

CMC comment

- 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:

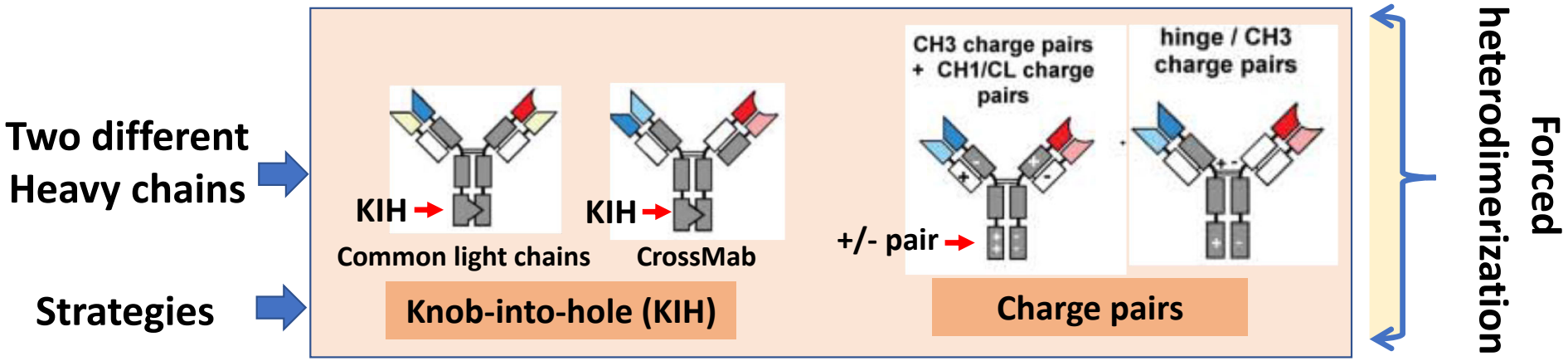
- Quantitative specification for the aggregates are not provided for release and stability. Note that aggregates may have different potency as compared to monomers. The acceptance criteria of “report results”, or ≤ 10% are inadequate to control for the potential HMW species
- Main peak by SEC-HPLC with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

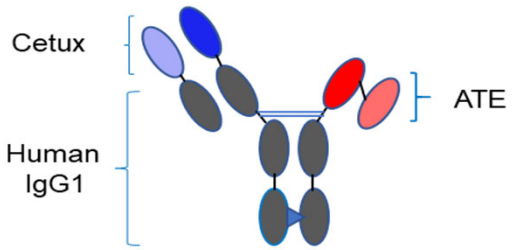


Modified from Ulrich Brinkmann & Roland E. Kontermann (2017) The making of bispecific antibodies, mAbs, 9:2, 182-212,

Regulatory Considerations

- 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

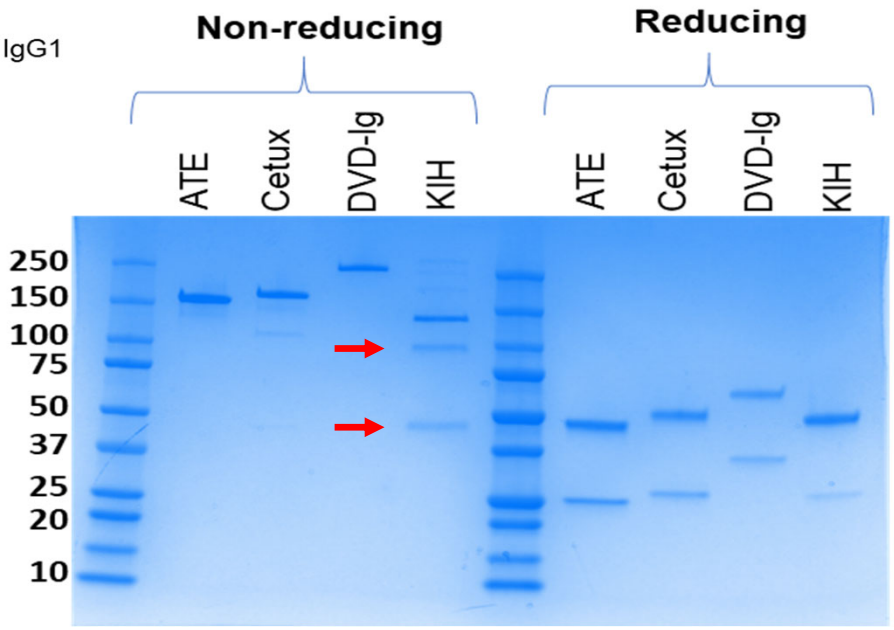
Potential unpaired or incorrectly paired heavy chains found in the asymmetric BsAb with KIH format detected by non-reduced SDS-PAGE



Knob-in-hole (KIH) format

Plasmids:

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

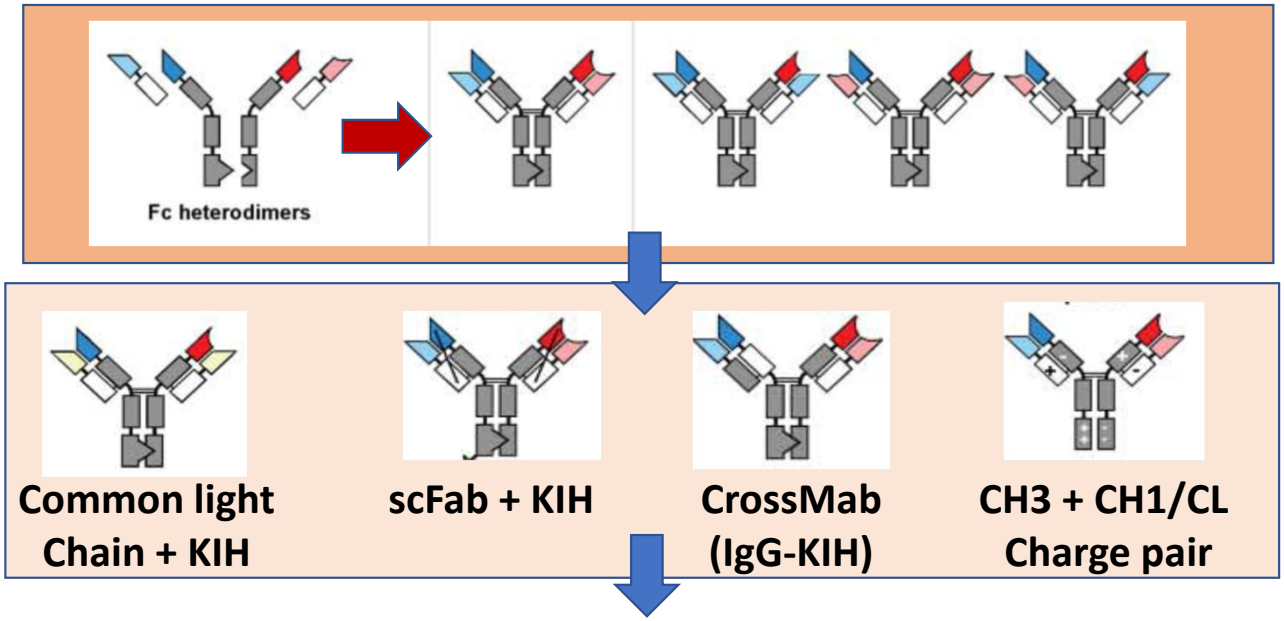


Nishant Mohan et al. Comparative characterization of different molecular formats of bispecific antibodies targeting EGFR and PD-L1. 2022, Manuscript under revision

Unpublished data from Wu lab in OBP at FDA

Solving the light chain problem in asymmetric BsAb

Two different light chains



Strategies

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

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**

Post-assembly approaches to purify BsAbs

Mutations in the Fc region facilitate purification

Fc*, Fc

Fc*-Fc*, Fc-Fc*, Fc-Fc

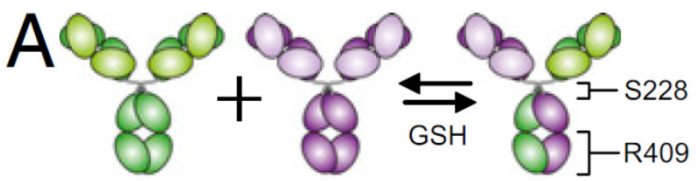
1) Fc* chain: with mutations, for example: H435R and Y436W in CH3 domain of an IgG1
2) Fc chain: no mutations

Regulatory considerations

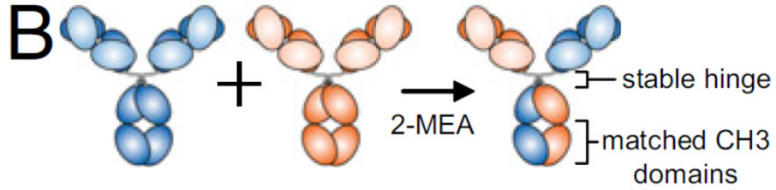
- **The Fc* sequence allows fractionated elution of BsAb from Protein A column**
- **Protein A column used in both capture and polishing**
- **Variable region Protein A binding, resin selection, selective elution optimization**
- **Impurities e.g., Fc-Fc, should be removed during manufacturing or controlled**

Tustian et al. mAbs, 8:4, 828-838, DOI: 10.1080/19420862.2016.1160192

Controlled Fab arm exchange (cFAE)



Bispecific IgG4



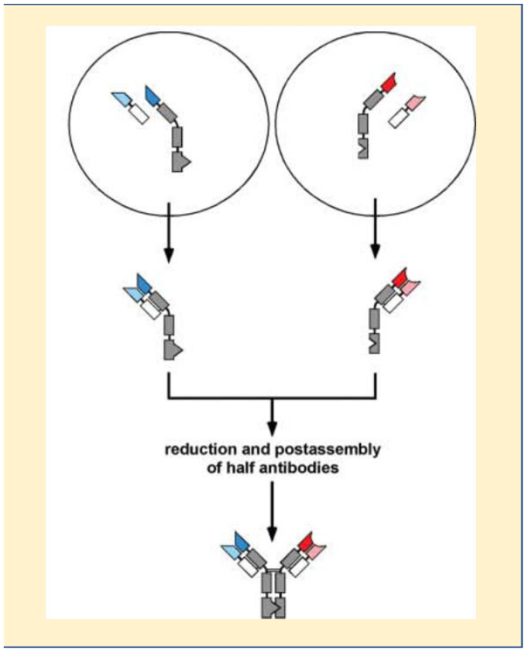
Bispecific IgG1

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

Regulatory considerations

- **Protein A for the parental IgG1 purification**
- **The reduction process: β-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 μg/day**
- **Impurities, e.g., two homodimeric parental antibodies, high molecular weight species (HMWS), and low molecular weight species (LMWS)**

Post-production assembly of half-antibodies



Two separate cell cultures

↓

Two half-antibodies

↓

Post-production Assembly

↓

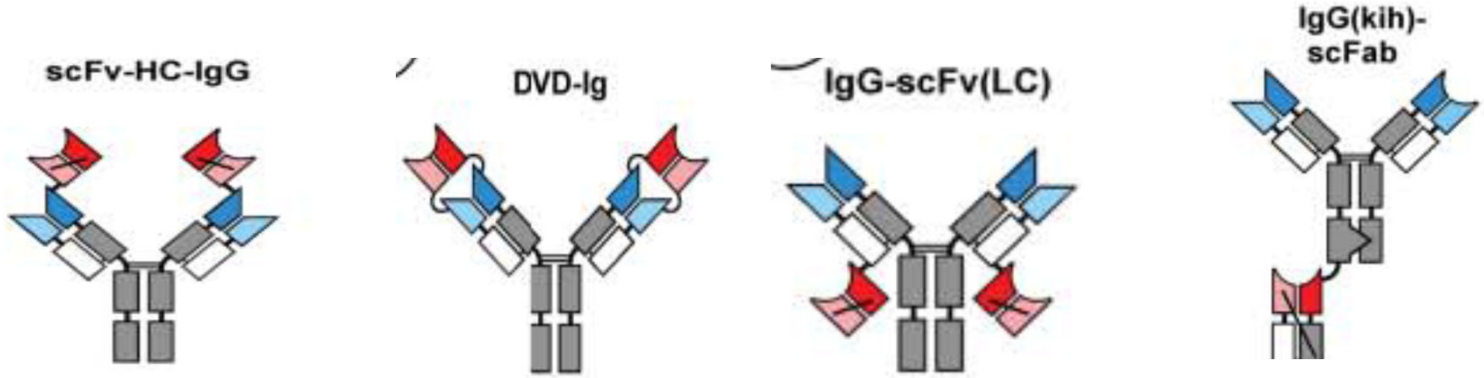
Protein A followed by hydrophobic interaction to remove half-antibodies

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

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

IgG/IgG-like formats with a symmetric architecture

(Appended IgGs)



Regulatory considerations

- 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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