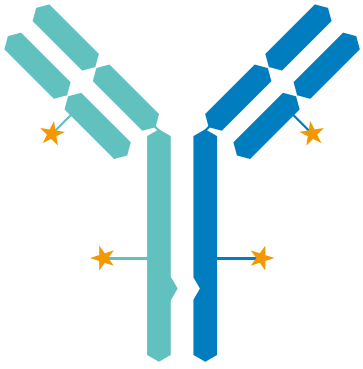
PORTON

CASE STUDIES

Challenges and Advances in CMC Process Development of BsADCs



Introduction

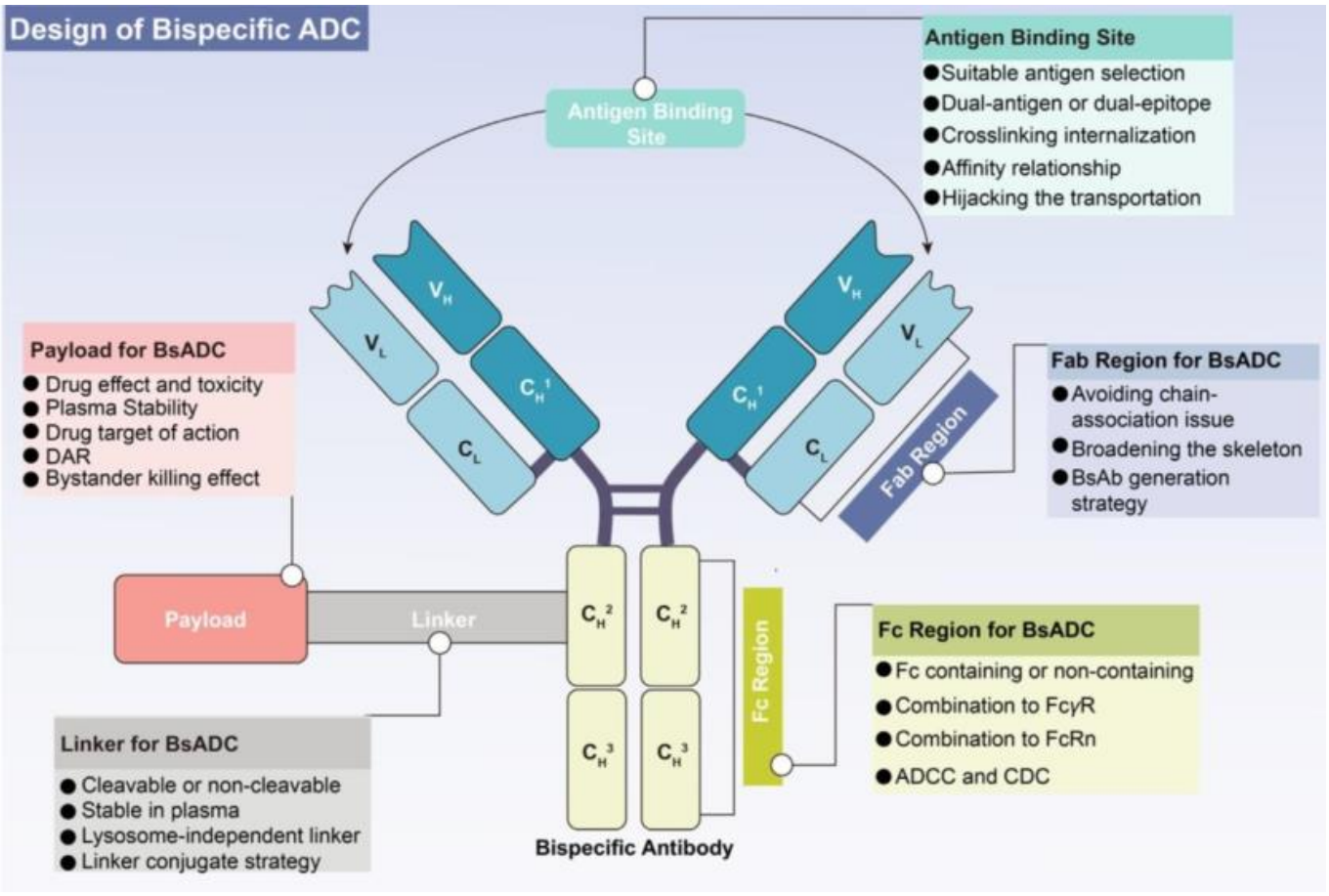
Antibody drug conjugates (ADCs) have seen significant breakthroughs and reached numerous milestones since 2000 when the first ADC was approved by the FDA and have shown great promise for the treatment of cancer. First generation ADCs were designed to use the affinity of an antibody towards a specific cellular target to bring the cytotoxic payload to the tumor, for example. However, some level of off-target toxicity was often seen with many ADCs because other cellular interactions could occur prior to the payload physically reaching its intended target or the payload could cleave off. These events could lead to unintended death of non-cancerous cells.

To minimize these unintended side effects and subsequently improve the clinical efficacy of new therapies beyond conventional monoclonal antibodies, researchers have been searching for more selective binders to the target. To achieve this, peptides, nanobodies, fusion proteins, and bispecific antibodies (BsAbs) have been evaluated to enhance specificity and selectivity in delivering cytotoxic payloads to tumor cells.

These newer types of ADCs include bispecific ADCs (BsADCs), conditionally active ADCs, immune-stimulating ADCs, protein-degrader ADCs, and dual-drug ADCs. These drugs all employ the targeting power of the antibody to ferry the cytotoxic payload safely through the body to the tumor cell to increase the effectiveness of the treatment while minimizing or even eliminating side-effects and other off-target toxicities.

Immune-stimulating ADCs (ISACs) and protein-degrader ADCs (PDACs) attempt to treat cancer through different modes of action than traditional ADCs. Bispecific ADCs and dual-drug ADCs are more like traditional ADCs but address the problems of drug resistance and tumor heterogeneity by enhancing selectivity and activity. This report will focus on the design, development, and conjugation methods for BsADCs and their characteristics.

Figure 1: The concept and design considerations of a BsADC [1]



BsADC

The traditional ADC has a cytotoxic payload connected via a linker to a targeting antibody. As a systemic treatment, the ADC must travel through the body, eventually reaching its target site to deliver its payload. However, interactions with various healthy cells before reaching the tumor cell can cause side-effects or limit the effectiveness of the therapy.

To address this, the components of an ADC are carefully chosen. The role of the payload is to kill a tumor cell. The linker is designed to tether the payload to the antibody which is designed or chosen to bring the payload directly to the target. Once the ADC reaches the target, the linker is cleaved and releases the payload into the tumor cell, causing its death. The targeting specificity of the antibody is the primary reason why ADCs have shown somewhat reduced side effects than traditional chemotherapy. However, there is typically a level of off-target toxicity due to unintended interactions with healthy cells.

To improve this, a BsADC uses an antibody to target tumor cells but attempts to improve the overall efficacy by targeting two different antigen binding sites on those cells. This enables the BsADC to simultaneously seek out two different targets or antigens on the same tumor cell to improve the selectivity of the payload toward its intended target. The synergistic effect of having two different binding sites on a single cell allows more precise targeting of the tumor cells and further enhancing drug tumor selectivity and endocytosis efficiency and to compensate for low target expression and heterogeneous patient response. In addition, by precisely controlling the drug-antibody ratio ("DAR") and the conjugation method, the safety and efficacy of the new treatments can be further optimized.

The most common BsADCs in development are based on the human IgG1and are divided into two types:

- Dual-epitope BsADC which target different epitopes of the same antigen, such as MEDI4276 and ZW49.
- Dual-antigen BsADC target two different antigens, such as AZD9592, M1231, and BL-BO1D1.[2]

Figure 2: Structures of ZW49 and MEDI4276 [dual-epitope BsADCs]

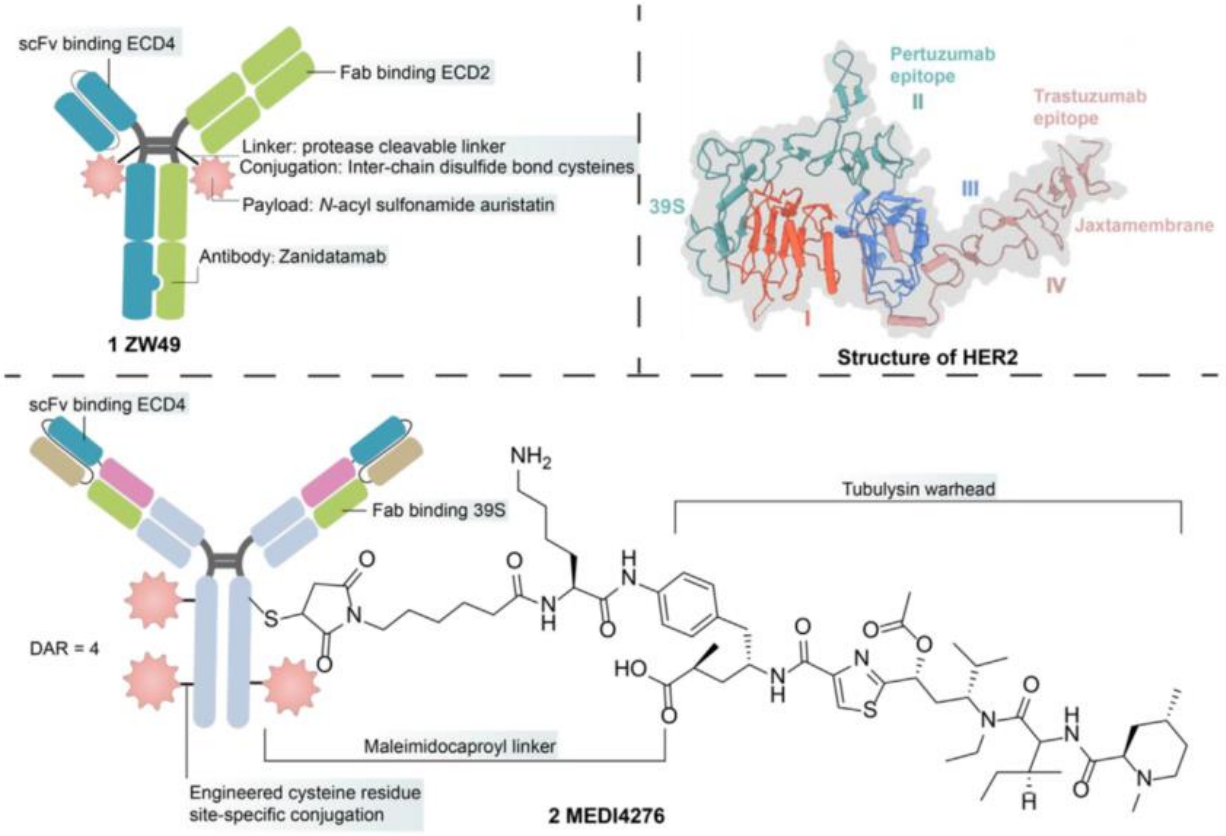
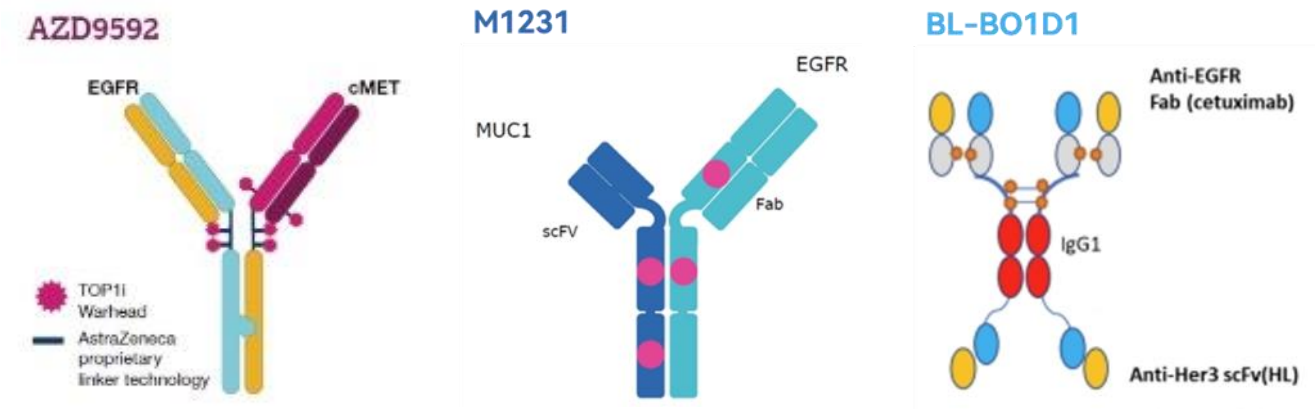


Figure 3: Structures of AZD9592, M1231, and BL-BO1D1 [Dual-antigen BsADCs]



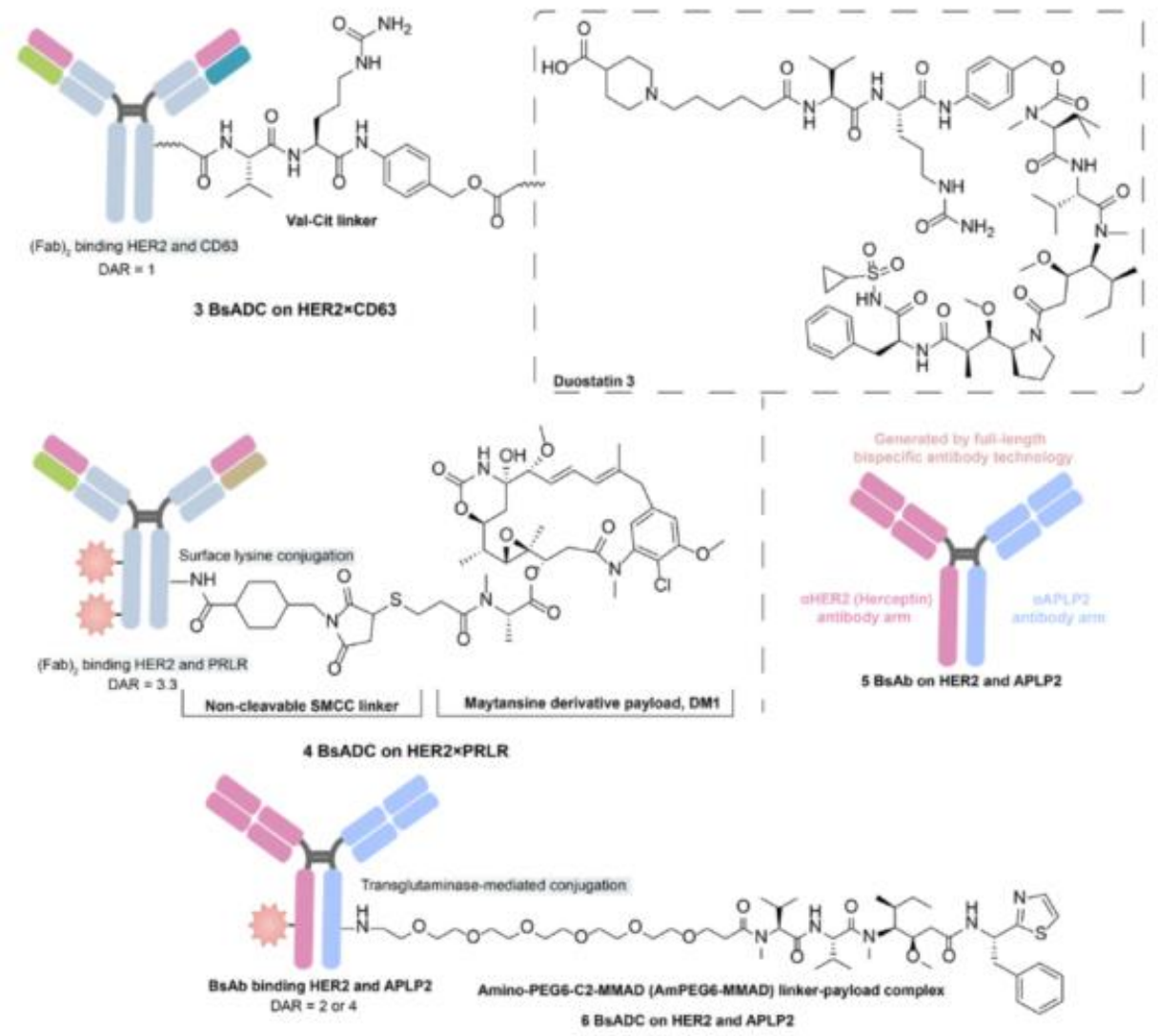
Progress in Development of BsADCs

At present, BsADCs have made significant progress in the field of anti-tumor therapy, especially in clinical efficacy and supported by numerous commercial alliances. Based on the latest information and historical records, we can see several key development deals made and milestones met in the BsADC arena:

- At the end of 2023, the BsADC BL-B01D1, developed by BioKin Pharmaceutical was exclusively licensed outside of China to BMS with a total transaction value up to $8.3B USD.
- In March 2024, Hansoh Pharma and Biotheus further expanded their cooperation. Biotheus licensed their independently developed anti EGFR/cMET bispecific antibody PM1080/HS-20117 to Hansoh for up to $690M USD in upfront and milestone payments.
- In August 2024, Merck made an initial payment of $37.5M and chose to exercise its rights outside of China for SKB571, a BsADC from Kelun-Biotech.
- There are three BsADCs on HER2 in clinical phase III trials worldwide:
  - TQB2102 (developed by CTTQ Pharma)
  - JSKN-003 (developed by Alphamab Oncology)
  - KM501 (developed by Xuanzhu Biopharm).
- At the 2024 AACR conference, several domestic companies including Innovent, VelaVigo, Alphamab Oncology, Profound Therapeutics, and Hangzhou DAC biotechnology jointly announced their research results on BsADCs, covering multiple targets such as B7-H3, EGFR, Trop2, c-Met, Her2, PTK7, and MUC1.[1]

These numerous recent collaborations suggest that BsADCs are indeed becoming high value targets due to the potential for improved efficacy.

Figure 4: BsADC design for HER2 target[1]



Bispecific Antibodies for BsADC

The bispecific antibodies usually require design of two antigen binding sites and with stability of the antibody itself. At present, there are few antibody molecules available for constructing bispecific ADCs, and the targets are mainly concentrated in HER2, HER3, EGFR, MUC1, etc. In addition, bispecific antibodies themselves may involve complex engineering modifications, such as Knob-into-hole, CrossMab technology, etc. Their development and manufacturing have many challenges, such as correct pairing, removal of homodimers, yield and quality, etc. Therefore, a powerful and reliable expression platform is needed.

To accelerate the BsADC design process, Porton has an efficient CHO cell expression system for exploring the optimal transfection ratio through vector design which can minimize the mispairing rate. The purity of the target molecule can also be improved by developing purification strategies to remove homodimers. For example, for bispecific molecules containing light chain (LC), heavy chain (HC), and ScFv-Fc, three plasmids were constructed for LC, HC, and ScFv-Fc, respectively. By adjusting different transfection ratios to reduce mismatches, the results showed that increasing the ScFv-Fc ratio appropriately could reduce the proportion of homodimers and half antibodies, affording higher purity for the target bispecific antibodies.

BsADCs Conjugation Process

More consideration also needs to be given to the stability of bispecific antibodies during the conjugation process. Through statistical analysis of multiple BsADCs that have entered the clinical stage in terms of conjugation methods, as in traditional monoclonal antibody ADCs, most drugs still use traditional cysteine conjugation methods. Some companies also try to modify conjugation methods, including glycosylation site-specific conjugation, lysine conjugation, and unnatural amino acid conjugation. The following table summarizes the progress of BsADCs that entered the clinical stage so far.

Table 1: BsADCs entered the clinical stage

Drug Name	Target	Company	Conjugation Method	Clinical Stage
JSKN-003	HER2/HER2	Alphamab Oncology	Glycosylation site-specific, DAR4	Clinical Phase III
BL-B01D1	EGFR/HER3	BioKin Pharmaceutical	Cysteine, DAR8	Clinical Phase III
TQB2102	HER2/HER2	CTTQ Pharma	Cysteine, DAR5.8	Clinical Phase III
REGN5093-M114	cMet/cMet	Regeneron Pharmaceuticals	Lysine, DAR3.2	Clinical Phase I/II
MEDI4276	HER2/HER2	Astrazeneca	Cysteine, DAR4	Clinical Phase I
ZW49	HER2/HER2	Zymeworks	Cysteine, DAR2	Clinical Phase I
M1231	MUC1/EGFR	Merck/Sutro	Unnatural amino acids, DAR4	Clinical Phase I
IMGN151	FRα/FRα	ImmunoGen	Lysine, DAR3.5	Clinical Phase I
IBI3001	B7H3/EGFR	Innovent	Glycosylation site-specific, DAR4	Clinical Phase I
DM001	Trop2/EGFR	Doma Biopharmaceutical	DAR8	Clinical Phase I
DM005	EGFR/cMet	Doma Biopharmaceutical	DAR4	Clinical Phase I
DB-1419	PD-L1/B7-H3	Duality Biologics	DAR8	Clinical Phase I
KM501	HER2/HER2	Xuanzhu Biopharm	Cysteine DAR4	Clinical Phase I

To compensate for the poor stability of BsADCs, Porton has developed new conjugation methods (such as glycosylation site-specific conjugation), optimized conjugation reaction parameters, and tuned UF/DF conditions to effectively improve their stability. Porton is committed to providing customers with high-quality and efficient services for constructing these new modalities.

Brief Summary

The development of BsADCs faces high technical barriers and requires continual improvements and innovations to overcome these challenges. In recent years, with the rapid development of biotechnology and deeper understanding of tumor biology, BsADC has very bright prospects in the future. How to effectively develop the manufacturing process and establish quality control of BsADC is vital for development.

Porton offers end-to-end solutions to address these challenges, including:

• Cell Line Development

• Bispecific Antibody Process Development and Manufacturing

• Conjugation Process Development and Manufacturing

• Formulation and Drug Product Process Development

• Analysis and Characterization

These can effectively solve the problems of bispecific antibody expression and quality optimization, conjugation process control, etc. to reduce the development risks in the CMC stage and beyond to help BsADC molecules quickly succeed in clinical and market applications.

Zhenkun Song

Associate Director of Production at the Large Molecule Platform

With 10 years of experience in biomacromolecule drug process scale-up, clinical and commercial production. He has rich experience in the construction of new sites, process scale-up, clinical and commercial manufacturing in the field of antibodies and ADCs.

Aini Wan, Ph.D.

Senior Manager of Cell Line Development and Cell Culture Process Development at the Large Molecule Platform of Porton

7 years of experience in cell line development of biologics, previously worked in Harbour Biomed and WuXi Biologics, with more than 20 cell line development experience, proficient in the whole process of cell line development and upstream development, and rich experience in IND/BLA filing.

REFERENCES

1. Yilin Gu et al. Bispecific antibody drug conjugates: Making 1+1>2.
2. Kyoji Tsuchikama et al. Exploring the next generation of antibody–drug conjugates. Nature reviews Clinical Oncology. Vol. 21, Mar. 2024: 203-223. Doi: 10.1038/s41571-023-00850-2.

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