BioProcess International
SPECIAL REPORT

# Delivering on the Promise of **Bispecifics**

## State-of-the-Art Bispecific Antibody Development

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An off-the-shelf therapy that can be used immediately, rather than one that must be manufactured individually for each patient (such as chimeric antigen receptor T-cell therapies, or CAR-Ts), can be a life-saving treatment for patients who encounter difficult-to-treat or rapidly progressing diseases, including many cancers. The promise of bispecific antibodies (bsAbs) stems from their off-the-shelf nature and ability to bind to two or more different targets or epitopes, thereby performing multiple functions. Although many bsAbs in development fundamentally maintain the heavy- and light-chain structure of standard antibodies, advances in protein engineering have allowed for development of smaller versions, such as bispecific T-cell engagers (BiTEs) and dual-affinity retargeting (DART) antibodies. Additionally, more complex variants also have been developed with additional binding domains that have been appended to the immunoglobulin G (IgG) heavy or light chain.

The first bsAb, Removab (catumaxomab, approved in 2009), was withdrawn in 2017. Four approved bispecific products are on the market now:

**Blinatumomab** (2014), a BiTe that recognizes CD19 and CD3 for the treatment of acute lymphoblastic leukemia (ALL)

**Emicizumab** (2017), a humanized bsAb that recognizes Factor IXa and Factor X for the treatment of hemophilia

**Amivantamab** (2021), a DuoBody bsAb that targets EGFR/c-Met for the treatment of non–small-cell lung cancer

**Faricimab** (2022), a CrossMab bsAb that recognizes VEGF-A/Ang-2 for the tretatment of diabetic macular edema and wet or neovascular age-related macular degeneration.

About 160 bsAbs currently are in clinical trials, accounting for nearly 20% of the clinical antibody pipeline and spurring significant enthusiasm and investment in this class of drugs across a breadth of therapeutic indications (**1**).

## Reconciling the Complex Designs and Expressability of Bispecific Antibodies

bsAbs are complex, nonnatural, heterodimeric proteins. A bsAb typically comprises two different heavy chains and two different light chains. The primary challenge in developing a bsAb is pairing the chains so that the bsAb assumes appropriate asymmetry for the two different Fv regions (the two different binding domains). Promiscuous pairing of heavy and light chains expressed by a single cell theoretically can result in 10 different molecules, with only one molecule being the intended bsAb. Forced pairing by introducing such point mutations as knobs-into-holes in the Fc region has helped maximize heterodimer formation, but identifying clonal cell lines that express high levels of bsAb heterodimers remains challenging. Furthermore, as bispecific designs increase in sophistication and other domains are added, correct protein folding and transport through the secretory pathway will become increasingly difficult.

Given the complexity of bsAbs, a cell-line development platform for these proteins must contain three key features to be sufficiently robust for generating bsAbs:

**Stable and high expression** of bsAbs often entails the simultaneous high-level expression of two to four different proteins.

**Straightforward early screening** for clones that produce the most intact heterodimers typically requires a panel of cells that have been transfected with a matrix of heavy- and light-chain ratios.

**A robust cell line** must cope with secretory stressors from translating, folding, and pairing such complex, nonnatural proteins that can lead to insufficient productivity.

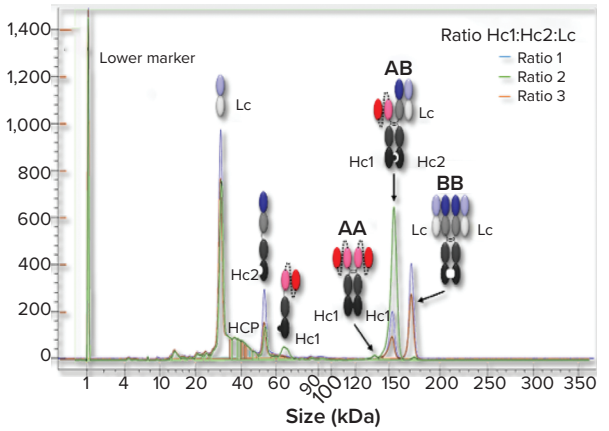
The Selexis SUREtechnology platform provides customizable solutions across the spectrum of cell-line development needs. This platform produces industry-leading titers across a range of modalities (2–7 g/L for MAbs and > 1.5 g/L for bsAbs in fed-batch or shake flask; bioreactor >10 g/L). Furthermore, the platform features sophisticated capabilities that can overcome the many possible challenges associated with producing complex and difficult-to-express proteins, such as bsAbs. The SUREtechnology platform encompasses the three major elements required for optimal bsAb production:

**Selexis SGEs (Selexis genetic elements),** unique epigenetic DNA-based elements that control the dynamic organization of chromatin across all mammalian cells, enhance transcription by shielding the integrated transgene from the silencing effects of the surrounding chromatin.

**SUREvariant Screening technology** assesses up to 250 CHO-M cell transfectant pools that express a panel of proteins, including various ratios of bsAb heavy and light chains.

**SURE CHO-Mplus libraries,** a sophisticated solution for secretion bottlenecks, are based on genomic and transcriptomic analyses of the Selexis CHO-M cell line, enabling fine-tuned secretion based on the specific secretory needs of difficult-to-express proteins.

**Figure 1:** (LEFT) Screen of the purity profiles of generated bsAb pools by µCE-SDS; (RIGHT) scalability of production of bsAb from a small-scale vessel to a large production process




| Vessel | Titer (g/L)[a] | Purity (%)[b] AA | AB | BB |
|---|---|---|---|---|
| Spin Tube | 4.9 | 1.4 | 92.7 | 5.9 |
| Shake Flask | 4.2 | 0.7 | 95.5 | 3.8 |
| Ambr15 | 5.6 | 0.6 | 96.5 | 2.9 |
| Ambr250 | 4.9 | 1.0 | 93.5 | 4.9 |
| Bioreactor | 4.9 | 1.1 | 97.6 | 1.6 |

[a] total protein HPLC titer (homodimers + heterodimer); representative of up to six different clone samples

[b] homodimer (AA, BB) and heterodimer (AB) purity determined by µCE-SDS; representative of up to four clone sample repeats

**Figure 2:** Twelve-month timeline depicting key milestones from SKI transfection to bulk drug substance (BDS)

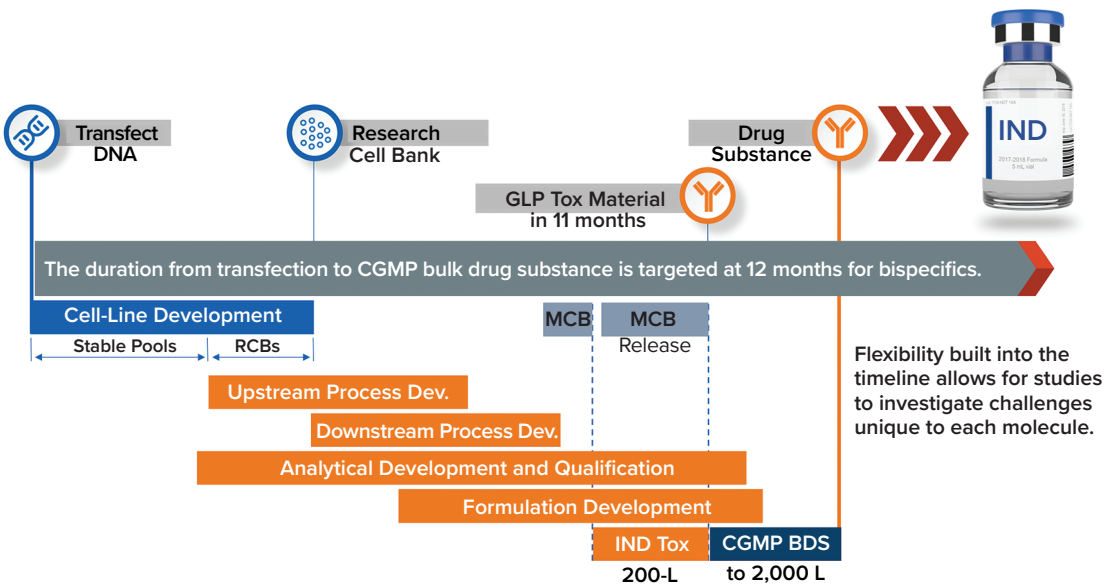



Figure 1 depicts a representative Selexis bispecific development campaign. The bsAb had an scFv-Fc moiety associated with a Fab-Fc. Using Selexis SGEs to express all chains, scientists assessed a range of scFv-Fc:Fab-Fc chain ratios using SUREvariant Screening technology, allowing them to fine-tune production and heterodimer content. By optimizing the chain-to-chain ratio, Selexis generated a research cell bank (RCB) that produced the bsAb at levels of approximately 5 g/L, with a stable heterodimeric composition above 92% on various production scales and for >60 generations (data not shown).

Even with the knobs-into-holes technology, the >92% heterodimeric rate was impressive and helped drive the high titers. For most products, Selexis SGEs and SUREvariant Screening platforms are sufficient for generating high-quality cell lines. However, in certain cases, a bsAb can encounter issues with folding or exporting through the secretory pathway. In these situations, Selexis uses SURE CHO-Mplus libraries, a technology that provides effective solutions for the many possible secretion bottlenecks that are associated with difficult-to-express proteins.

To date, Selexis has successfully developed more than 34 cell lines expressing bi- and multispecific antibodies for global partners; over two-thirds of these cell lines are in clinical evaluation.

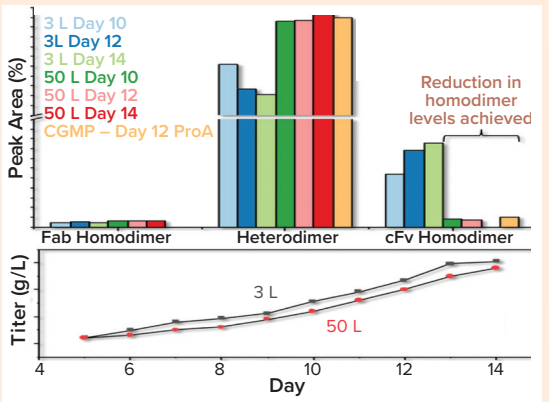
## Advancing bsAbs from the Bench to Clinic

Advancing bsAbs into the clinic requires process development, analytical methods, and scale-up for CGMP manufacturing. The development of a CGMP manufacturing process must take into consideration many factors — including removing impurities, maximizing heterodimer formation, and limiting fragmentation — while ensuring robustness and scalability at production capacities of 200 L to 2,000 L. The processes and analytical tools that are needed to ensure a robust clinical supply also must be examined, as should the ability to support long-term supply needs. Selexis and KBI Biopharma (JSR Life Science companies) have developed an integrated workflow (SKI) for generating clinical bulk drug substance within 12 months (Figure 2). The SKI approach has been used successfully to generate more than 25 bsAb molecules. As a result of such production campaigns, KBI has built extensive experience with this class of molecules.

The accelerated process development and manufacturing timelines are supported by KBI, which begins process development efforts using early transfection pools from Selexis. That allows for weeks of development work before receiving the single-cell clones. Upstream and downstream process development studies occur in parallel with analytical and preformulation development activities, further accelerating the process. KBI routinely achieves titers of 3–6 g/L for bsAbs with heterodimer purities that typically exceed 95% and often are as high as 98%. Cell viability at the time of harvest is >80% for most cell lines, and high production is maintained throughout the process. This ability to achieve a significant production capacity with high heterodimer purity is a unique attribute of the SKI platform.

Process development requires optimization of a myriad of parameters during the manufacturing of biologics. However, a unique issue of bsAb manufacturing is homodimer formation. For example, high scFV homodimer levels were observed in the case of a client's bsAb product early in process development (Figure 3). KBI was able to improve heterodimer formation (lower homodimer formation) through process improvements, which included feeding strategy optimization; temperature downshift triggered by a viable cell count threshold; and production bioreactor duration. With these process parameters, KBI reduced homodimer formation by >85%, converting the scFV homodimers to heterodimers and yielding a titer of ~3 g/L of the heterodimeric product.

**Figure 3:** bsAb homodimer (TOP) and titer (BOTTOM) comparison in 3-L and 50-L production bioreactor scales




Although strategies such as the strategic use of cell lines and upstream development efforts can be used to reduce homodimer formation during manufacturing, it is unreasonable to expect yields of pure heterodimer. Rather, with the right analytical assays to distinguish homo- from heterodimers, purification can then be used to enrich heterodimers to levels greater than 98%.

## A Science-Driven Collaboration

The Selexis/KBI partnership combines a best-in-class cell-line development organization with a science-driven, experienced contract development and manufacturing organization in a highly productive collaboration. Scientists on both sides cooperate to drive product development through extensive sharing of knowledge and experience. This full-scope solution provides outstanding expertise, quality and production efficiencies, and time savings in bsAb generation across a multitude of bispecific formats.

## Reference


**1** Zhang Z, et al. Anticancer Bispecific Antibody R&D Advances: A Study Focusing on Research Trend Worldwide and in China. *J. Hematol. Oncol.* 14(1) 2021: 124; https://doi.org/10.1186/s13045-021-01126-x.



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