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Effective Strategies for Successful Development and Manufacturing of Multi-Specific Antibodies and Proteins

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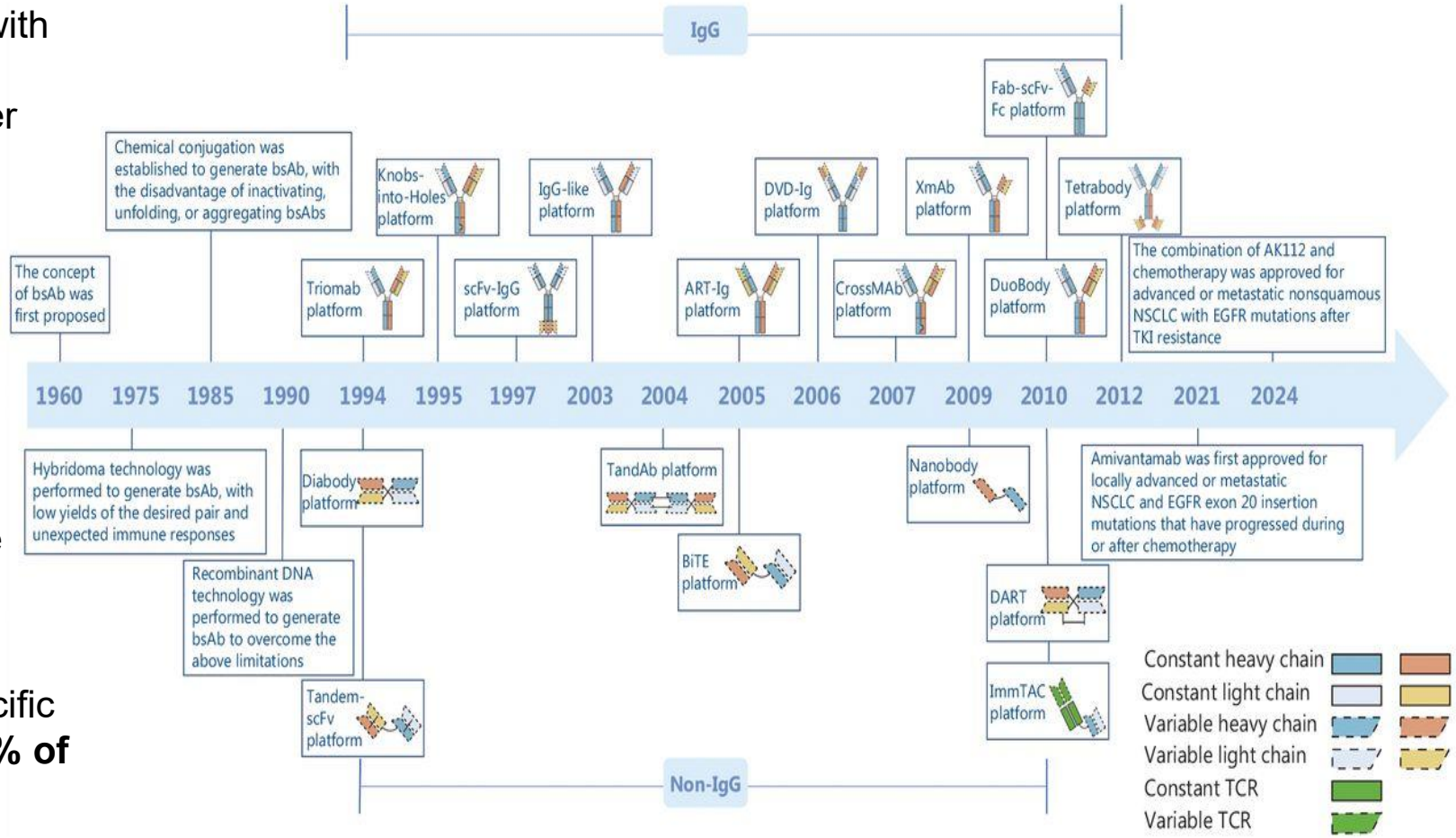
CMC Strategy Forum, Jan 26, 2026

Introduction

- **Biologics innovation is surging**, with rapid advancements in bispecific antibodies, fusion proteins, and other complex modalities, spanning oncology, immunology, infectious diseases and other emerging areas.
- **Commercial successes** such as Amgen's Blincyto and Roche's Hemlibra demonstrate bispecific therapeutics' clinical and market.
- **Over 200 bispecific molecules** are currently reported in preclinical and clinical development.
- **Market projections suggest** bispecific antibodies could represent up to **20% of the monoclonal antibody market** within the next 5 years.



1. Labrijn AF et al (2023) *Nature Reviews Drug Discovery*, 22, 5
2. Goebeler ME et al (2024) *Nature Reviews Clinical Oncology,* 21, 539
3. Wen J et al (2025) *Cancer Biology & Medicine*, Apr 07 on line

Continue Expediting BsAb Development and Manufacturing DNA-to-IND in as Little as 6 Months

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Bi- and Multi-Specifics in WuXi Bio

501 Bi- and Multi-Specifics in the clinical stage

143 terminated/withdraw

299 Phase I/II

39 Phase III

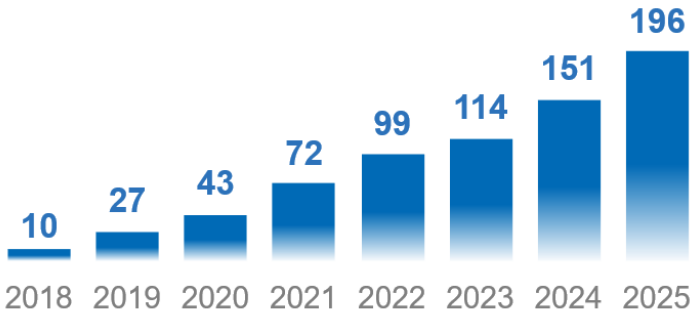
20 Commercial

The data is from a review paper by Roche, see attached

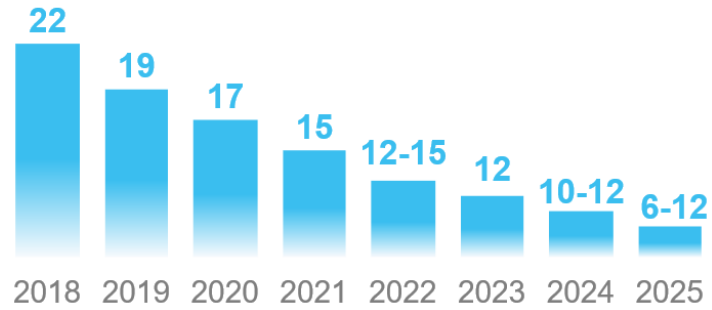
PDF

Roche Review paper

BsAb Projects Increased by Year



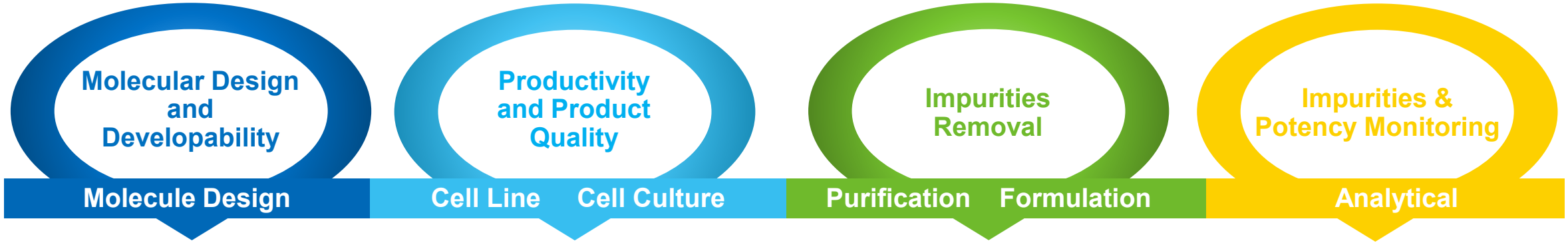
BsAb CMC Timeline from DNA to IND (Months)



- **43** Papers published related to BsAb development

Notes:
[1] Data as of Dec. 31, 2025
[2] Bi- & multi-specific antibody projects Included both WuXiBody™ projects and non-WuXiBody™ projects; and included 24 multispecific antibody projects

Key Challenges and CMC Solutions for BsAbs



- WuXiBody™ designed to facilitate BsAb byproduct separation
- Low-toxicity CD3 for T cell engager and SDArBody™ technologies

- WuXia™ cell line families and optimized cell line development strategy for BsAbs
- WuXiUP™ continuous bioprocessing and WuXiUI™ ultra-Intensified fed-batch platforms enhance BsAb productivity and product quality

- Purification roadmap for product-related impurities based on BsAb formats
- Extensive experience on detrimental HCP removal
- Comprehensive formulation development

Sophisticated analytical approaches for impurity detection and potency assay development

A Diverse Host Cell Family Provides Flexibility

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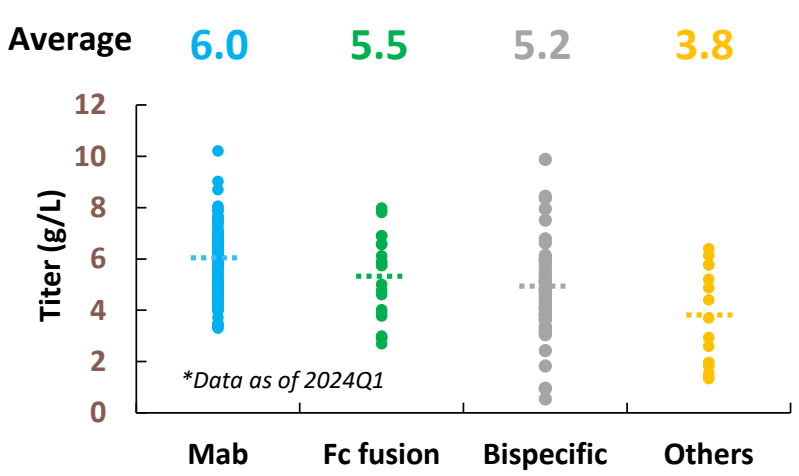
WuXia™ Cell Line Development Platform

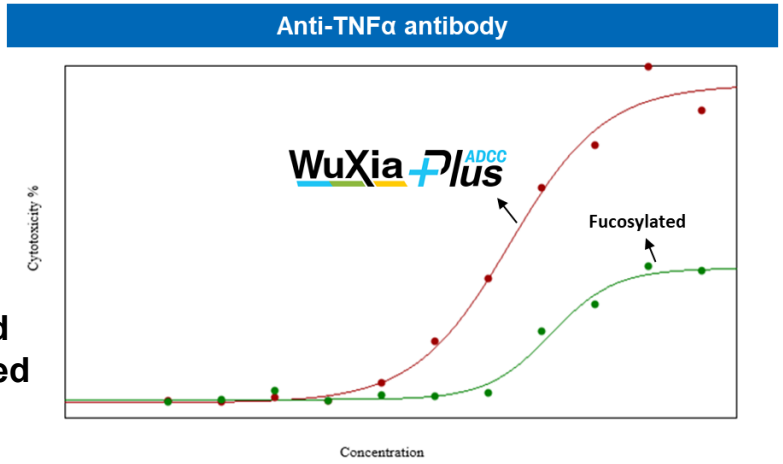


CHO-K1

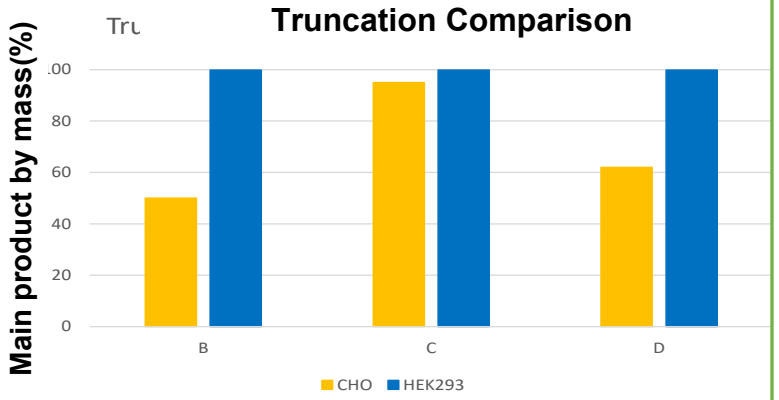
Acceleration: **9–10** weeks from DNA to MCB creation
Productivity: Average BsAb titer of **5.2 g/L** (mAb 6 g/L)
Quality: **No** sequence variants at genetic level
Stability: **> 90 % top 3** clones, **100% final clone** stable

ADCC-Enhanced

Ab produced showed significantly enhanced ADCC activity



HEK293



Flexible Host Choices

CHO-K1	CHO-K1 GS	HEK293	WuXia^{ADCC} PLUS	Customized Host	Alternative Host	Client Host

Cell Culture Strategy for Productivity and PQ

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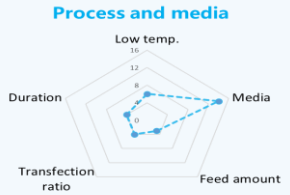
Cell Line

- Host cell line design
- Transfection ratio effect
- Clone selection weighting titer and quality

Design-of-Experiments

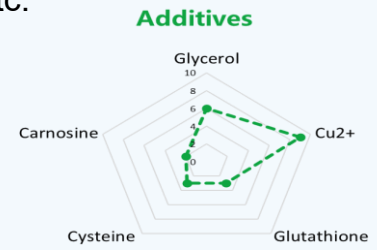
Culture Process

Temperature, pH, feeding strategy, osmolarity, cell media, duration, etc.



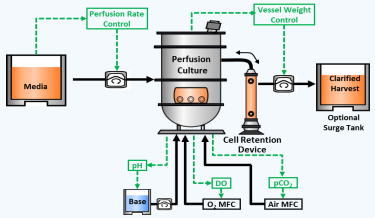
Additives

Trace metal ion, amino acids, nucleoside related, growth factor like, antioxidant reagent etc.

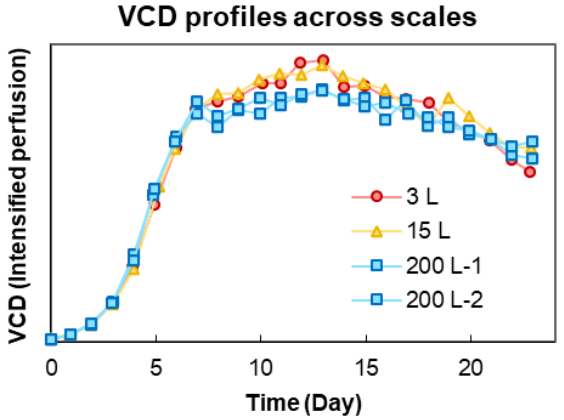


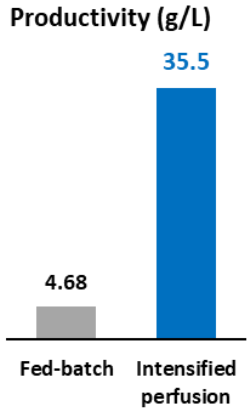
Culture Mode

Fed-batch (FB), intensified FB, perfusion, WuXiUI, and WuXiUP continuous processing platform

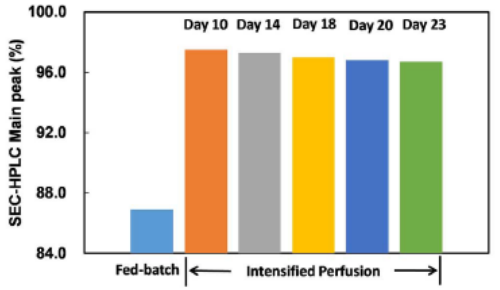


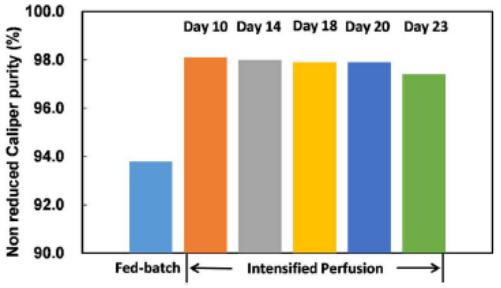
Robust Process Performance with High Yield





High Product Quality

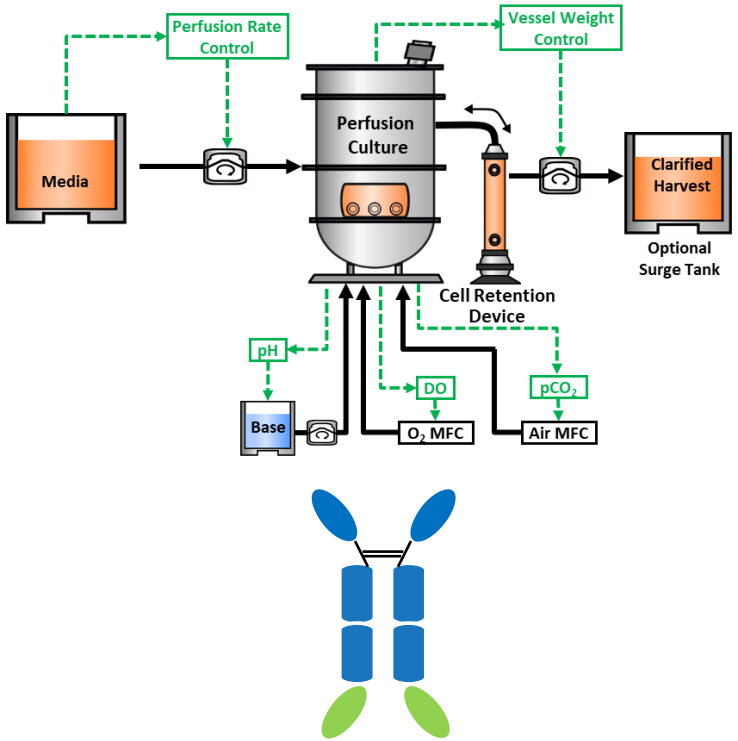


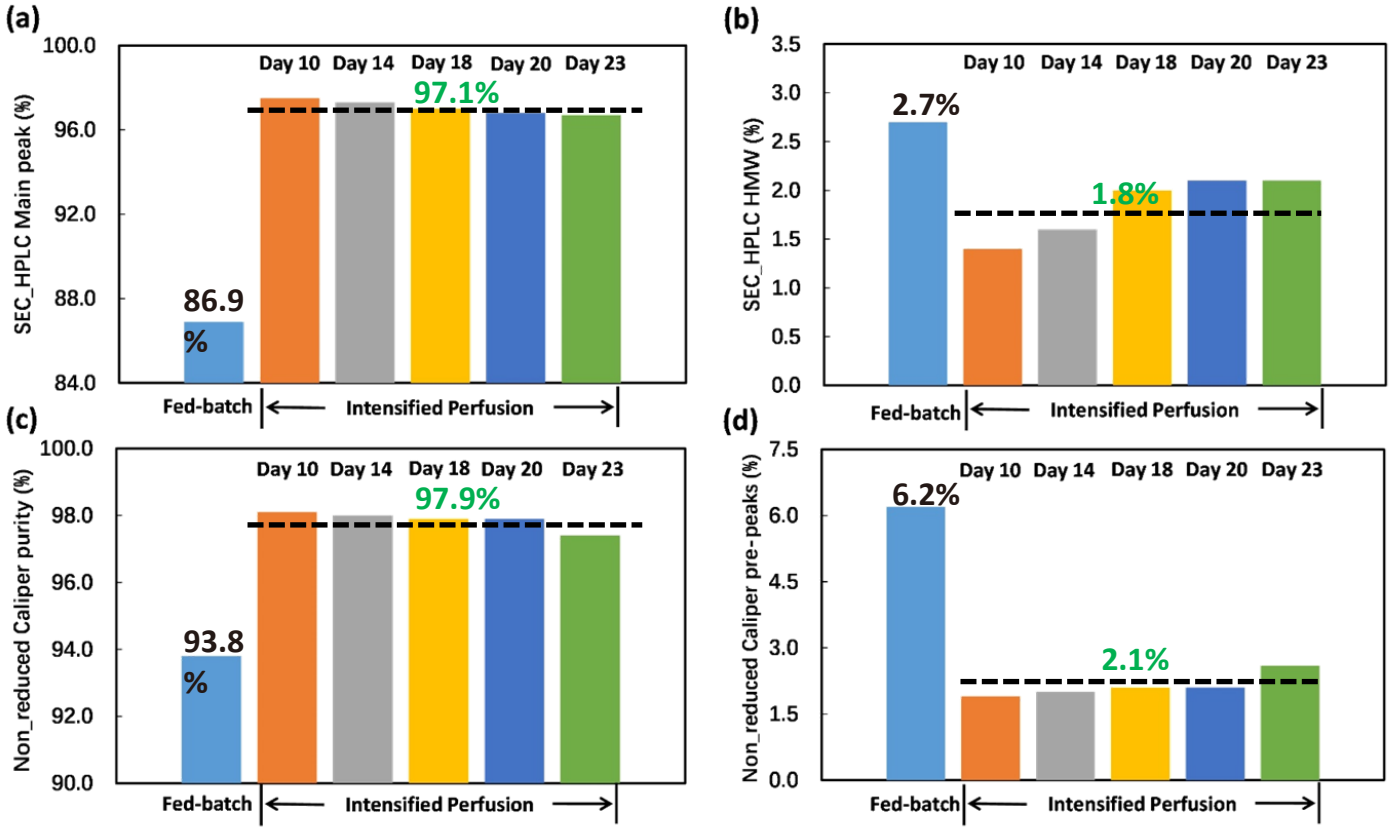


Qin Y, Ma R, Li Y, Li Y, Chen G, Zhou W. ***Productivity and quality improvement for a symmetric bispecific antibody through the application of intensified perfusion cell culture****. Antib Ther. 2022; 5:111-120.*

CASE STUDY 1:

Intensified Perfusion Culture – Control Aggregation and Fragmentation of BsAbs





Compared with FB, WuXiUP achieved:

- 95% SEC monomer (1-step ProA purification), aggregation decreased from 2.7% to 1.4–2.1%
- Fragmentation decreased from 6.2% to 1.9%-2.6% (Caliper-NR)

Antibody Therapeutics, 2022, 5.2: 111-120.

Tools to Effectively Remove Diverse Byproducts

Affinity Chromatography

Optimize the AC to enhance the initial quality of products for complex projects.

- **Half-antibody** removal by optimized wash
- **Aggregates** resolution enhanced by additives to elution buffer: PEG, NaCl
- **LC-missing species, homo-dimer, half-antibody** removal by different binding sites Pro L, KappaSelect, LamdaSelect, CH1-LX

Half-antibody	Aggregates
13.4% → 3.0%	20% → 3-4%

Polish I (AEX/MMA)

Optimized loading and wash conditions for weak binding by-products removal by AEX

- **Homo-dimer** removal by pI difference
- **Fragments** removal by different binding strength

Homo-dimer	Fragments
10% → <1%	27.5% → 5%

Polish II (CEX/MMC/HIC)

Robustness process for by-products level control

- **Homo-dimer, fragments, LC-missing species** removal by optimized wash steps
- **Aggregates, homo-dimer** separated by elution step
- pH/salt dual gradient to explore additional potential conditions for enhancing resolution

SEC-HPLC	CE-SDS
≥ 98.5%	≥ 97%

A Comprehensive Suite of Analytical Techniques That Leverage Diverse Mechanisms Enable Accurate and Efficient Detection

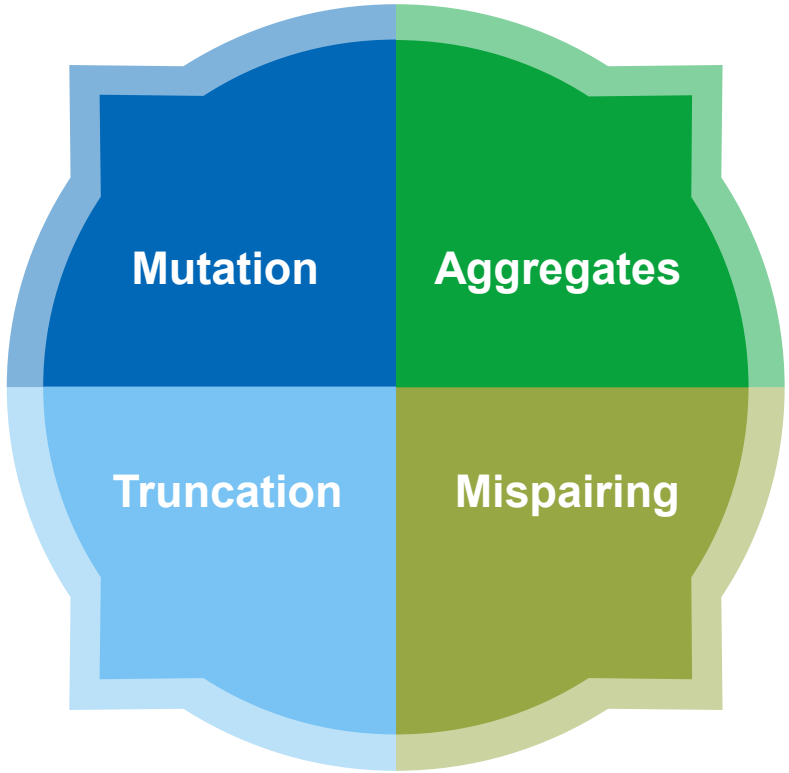
Covering everything from rapid screening to in-depth de-risking

	MASS	Hydrophobicity	Size (Denature)	Size (Native)	Charge Variants
Essential	• Intact MS • Deglycosylated MS • Reduced MS • Deglycosylated Reduced MS	• HIC • RP • HILIC • Reduced RP/HIC	• CE-SDS (NR) • Deglycosylated CE-SDS (NR) • CE-SDS (R) • Deglycosylated CE-SDS (R)	• SEC	• iCIFE • IEX • CZE
Premier	• IgdE/IdeS MS	• IgdE/IdeS HIC • IgdE/IdeS RP	• IgdE/IdeS CE-SDS (NR/R) • IgdE/IdeS Deglycosylated CE-SDS (NR/R)		
Elite	• Sequence coverage • Sequence variants • Non-reduced Peptide Mapping • Cystine Knot ID by LC-ETD/EAD-MS	• 2D HIC RP MS		• SEC MALS • AUC • DLS • Native SEC MS • 2D SEC RP MS	• CIEF-MS • Native IEX MS • 2D IEX RP MS

- State of the art instrument including **> 10** high-resolution mass spec
- High throughput, fast turnaround MS and separation methods with **LOQ < 1%**
- Varieties of **bottom-up** for **orthogonal confirmation** and **deep understanding**
- Further **increased resolution** and localized difference within specific domain by **reduced** and **subunit level analysis**
- Quick and **conclusive identification** of byproducts in separation method guaranteed by **native MS, 2D LC MS and CIEF MS**

Analytical: “Watchful Eyes” throughout Development

Challenges of Product-Related Impurities



DM (R& NR)
R Mapping

SEC
SEC-MALS

Caliper-SDS
CE-SDS
DM, DRM

iCIEF
Caliper SDS
CE-SDS
HIC/ RP
Molecular Mass (DM or IdeS-DM)
NR Mapping

WuXiMassSpec
Center of Excellence

DM: Deglycosylated MS
DRM: Deglycosylated Reduced MS
R mapping: Reduced peptide mapping
NR mapping: Non-reduced peptide mapping

- Understanding the Critical Quality Attributes (CQAs) is especially essential for complex molecules, allowing selecting right analytical methods and developing a robust quality by design testing strategy.
- Structure-function studies can improve CQA understanding and drive advances in CMC support.

Major Challenges Faced During a BsAb CIU study

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T-cell engagers (TCEs) are usually delivered at a low dose due to high potentcy.

01 Complex dose preparation procedure

- Dilution accuracy
- Multi-step dilution, priming or flushing
- particle formation

02 Dose accuracy concerns

- Drug adsorption to contact surfaces
- Hold-up volume in consumables

03 Drug instability

- Incompatible with contact materials
- Changes in solution environment, decrease of surfactant concentration in the solution

04 Low concentration testing challenges

- Protein concentration and purity are the most two challenge analysis

Toolbox to address low dose CIU challenges

① **Optimize dosing preparation approach**

② **Assess and develop analytical methods**

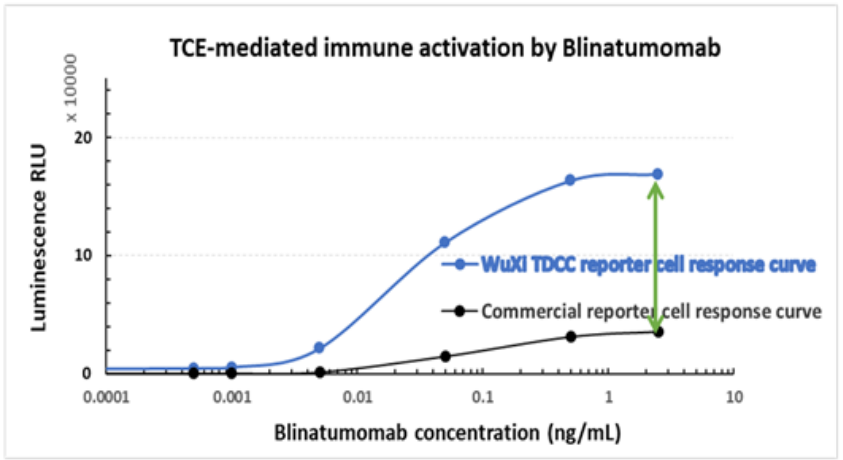
③ **Develop an IVSS (Intravenous solution stabilizer)**

CASE STUDY 2:

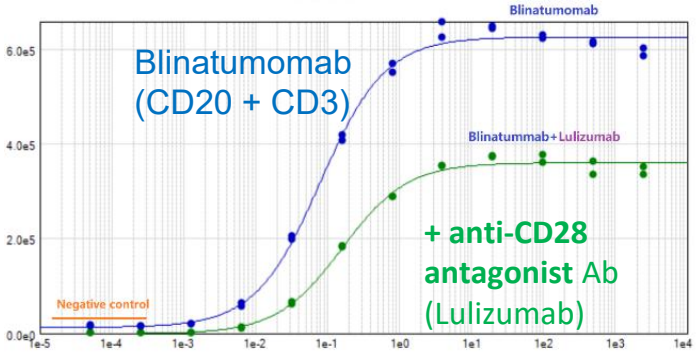
Function Support for TCE-Based Multi-Specific Antibodies: *MOA Reflective WuXi Biologics' TDCC Reporter Cell*

Outperforming commercial cell lines

- Large assay window under commonly used cell density
- Superior signal strength
- Meet QC qualification standards

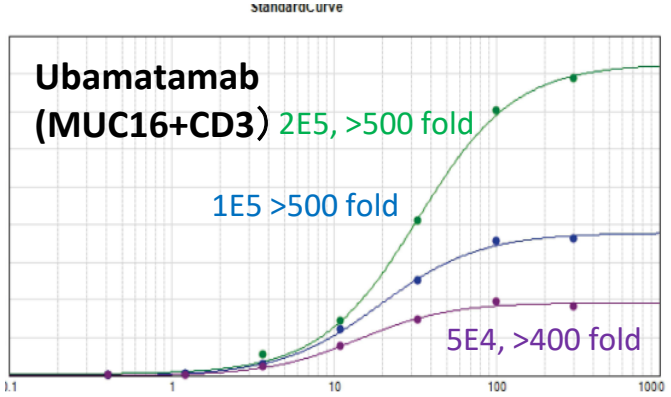


CD3 and CD28 Co-Stimulation MoA: Next-Generation TCE



- Build-in CD28 co-stimulation pathway
- Quantitative response: good for both screening and demonstration of specificity

New Proprietary Application: Cell-Free TDCC format

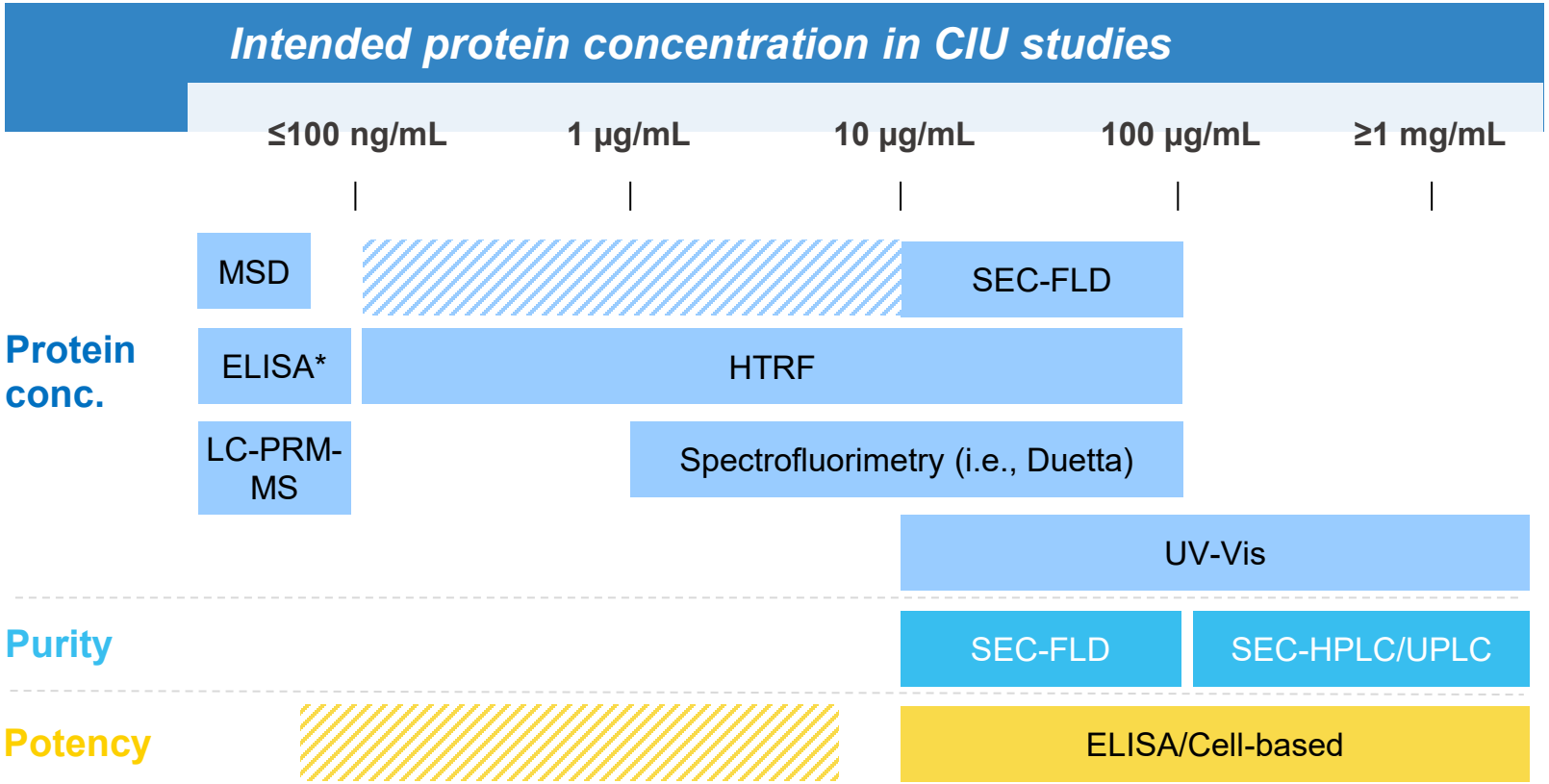


- ELISA + TDCC: friendly to target that is difficult to express on cells
- Stable performance: independent of cell line condition

Innovative TDCC reporter cell line enables robust *in vitro*, cell-based TDCC potency assay for CMC. No reliance on advanced plate readers and proprietary luciferase substrate reagent.

Analytical Techniques for Low-Dose CIU

Comprehensive solution for analytical method development in CIU studies, especially on protein concentration, purity and potency, covering dose ranges from ng to mg.

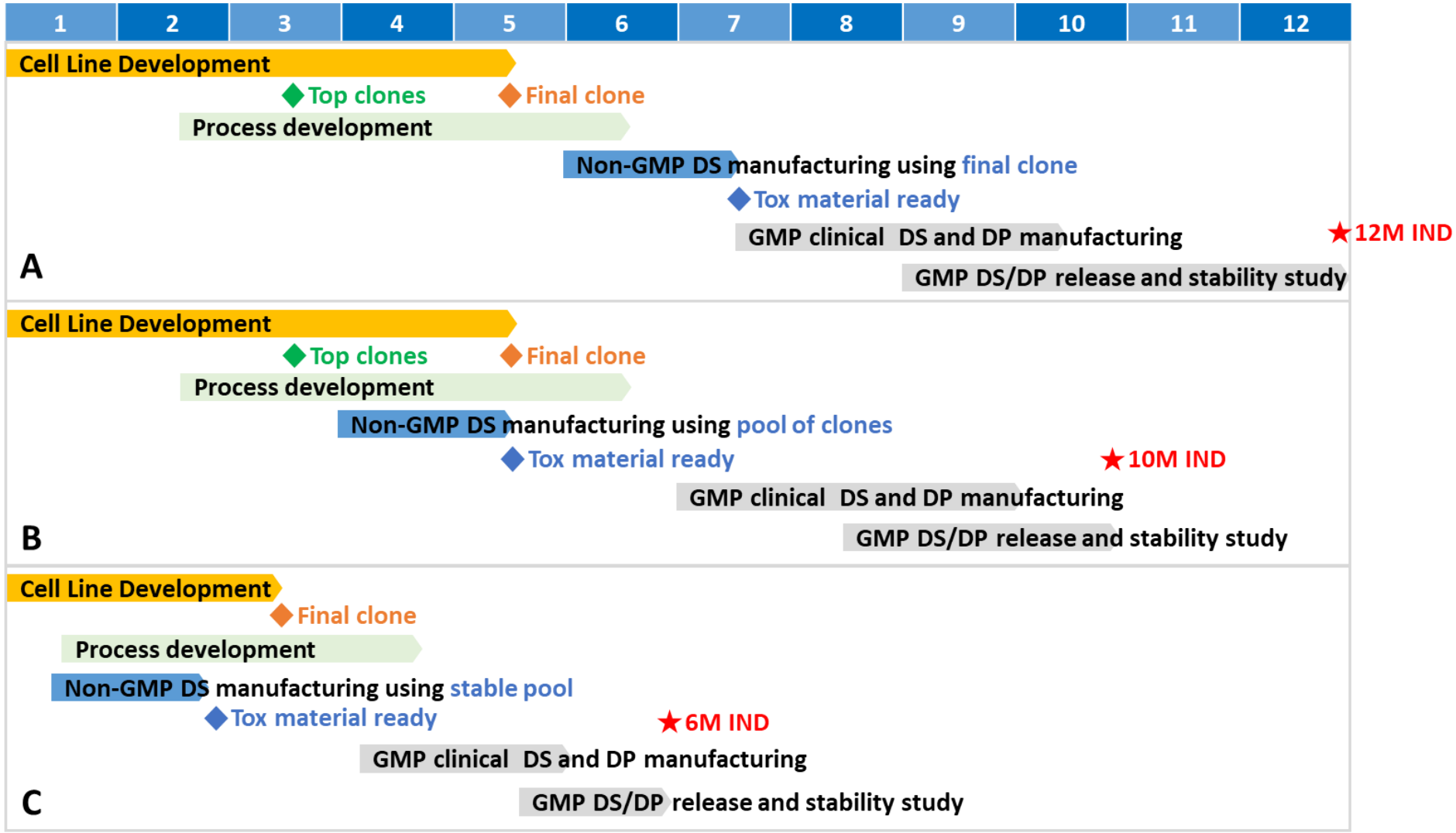


Method	Molecule Type	Actual Detected Protein Concentration
MSD	BsAb,TCE	0.3 ng/mL
ELISA (Residual IgG)	BsAb	80 ng/mL
	Conjugate	3.4 µg/mL
SEC-FLD	mAb	10 µg/mL
	Fusion	40 µg/mL
Spectro-fluorimetry (Duetta)	Multi-specific Antibody	2 µg/mL
	Fusion	4 µg/mL
HTRF	BsAb,TCE	300 ng/mL

* ELISA: Residual IgG method used.

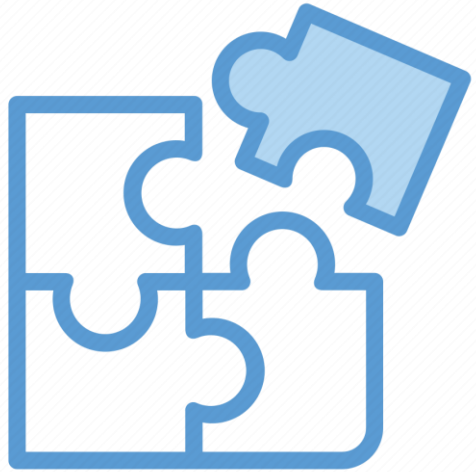
MSD: Meso Scale Discovery, HTRF: Homogeneous Time-Resolved Fluorescence, FLD: Fluorescence detector.

Flexible Development Timeline

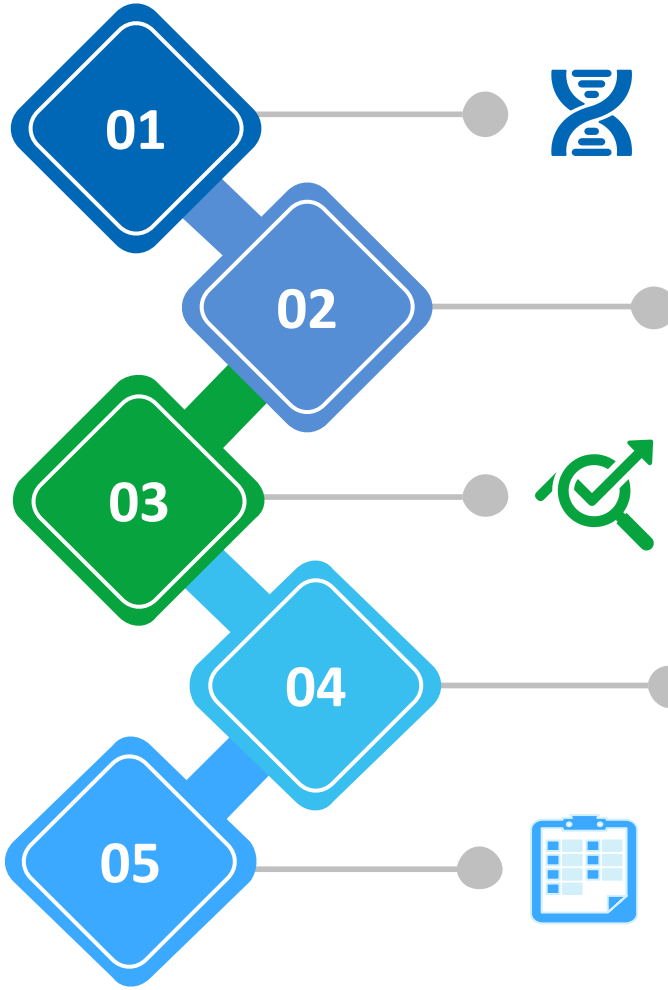


Summary: Accelerating CMC Development of Complex Molecules Is about Smart Integration

- **Collaboration with the research team** to ensure knowledge transfer.
- **Preliminary studies before iCMC** proactively address risks and identify challenging product quality attributes, saving significant effort during formal iCMC development.
- **Analytical capabilities serve as a "watchful eye,",** guiding and informing decision-making across all stages of development.
- **Cell line and cell culture process development** focused on selecting cell lines and designing processes that support both high productivity and good product quality.
- **Tailored purification toolboxes** are optimized for specific molecule designs and supported by high-throughput downstream process development.
- Strategically **adopt WuXi Biologic's established timeline acceleration strategy** and use **gate keepers** to further accelerate CMC timelines.



Acknowledgements



WuXi Biologics Development Teams

WuXi Biologics MFG, QC, and QA

WuXi Biologics Project Teams

Partners and Collaborators

WuXi Biologics Management Teams and the Board

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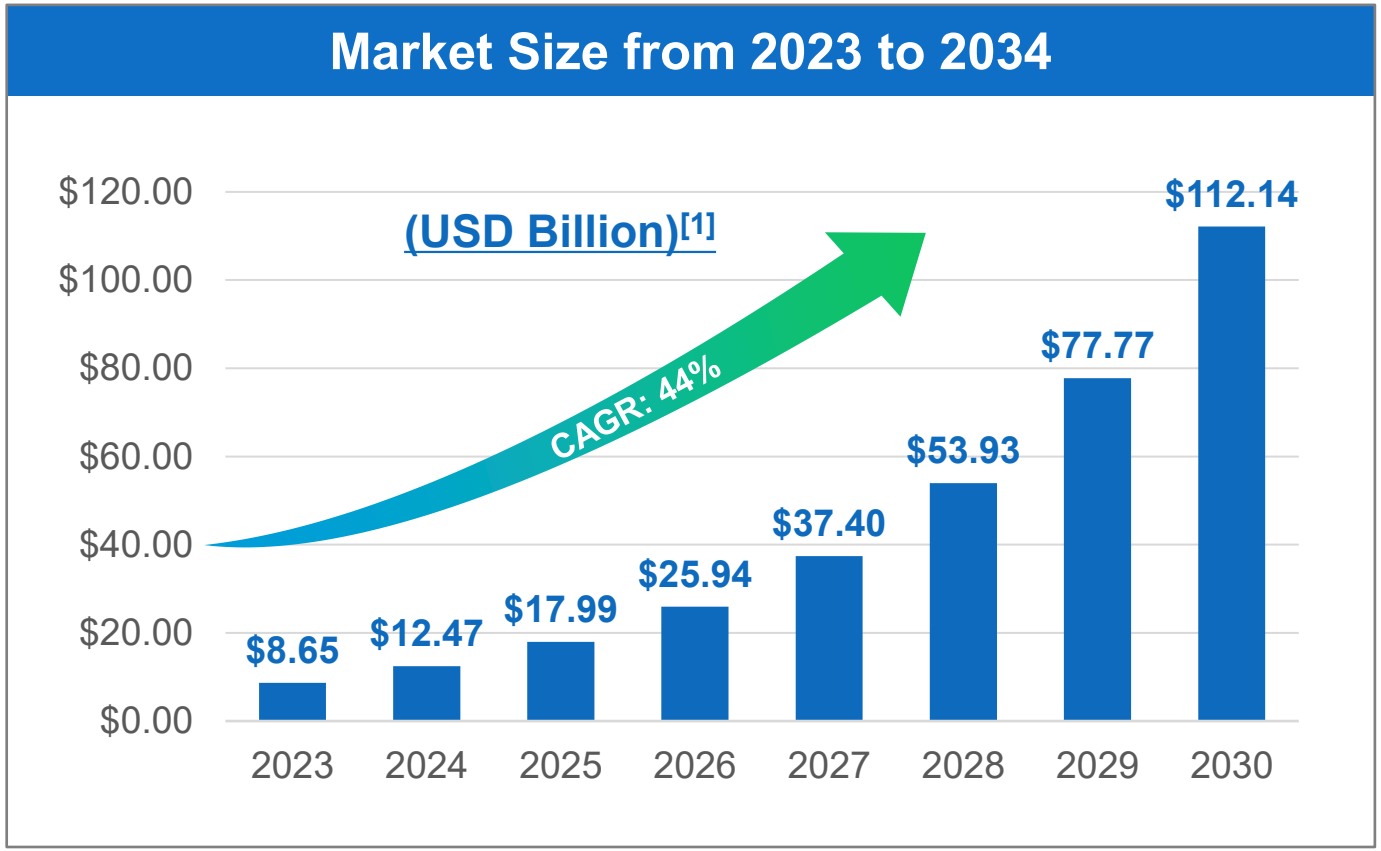
Thank You !

For more information or feedback, contact
Yuan.Chang@wuxibiologics.com

CRDMO
EVERY BIOLOGIC CAN BE MADE

Growing Market Size and Mergers and Acquisition Deals for Multi-Specific Biologics



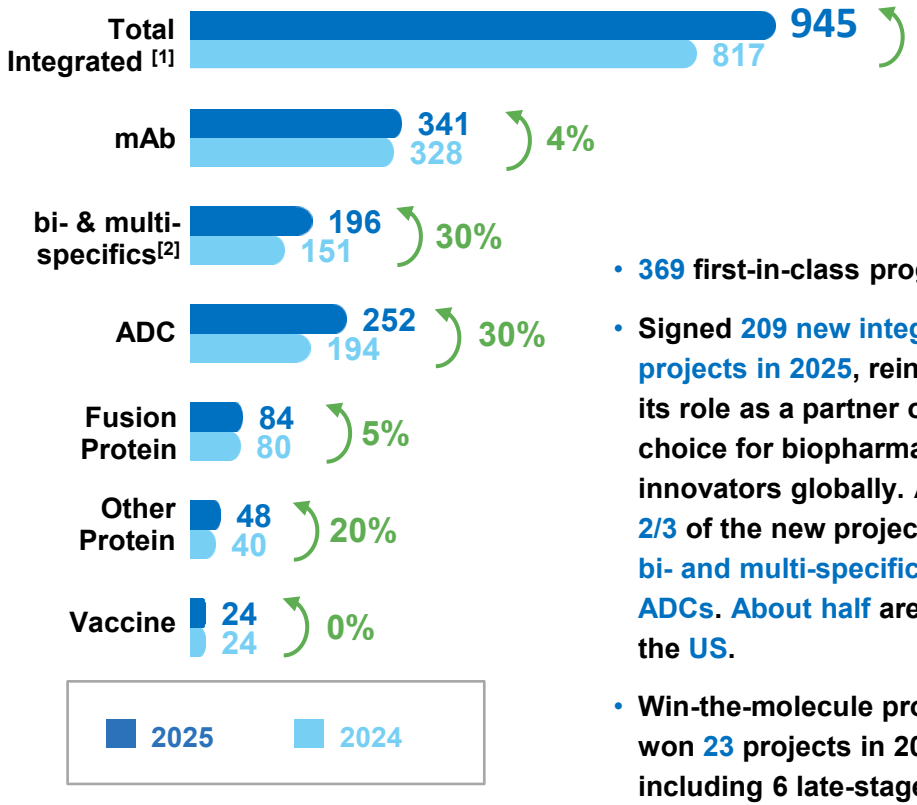
~14
multi-specific antibodies approved by 2023

300+
multi-specific antibodies related clinical trials[2]

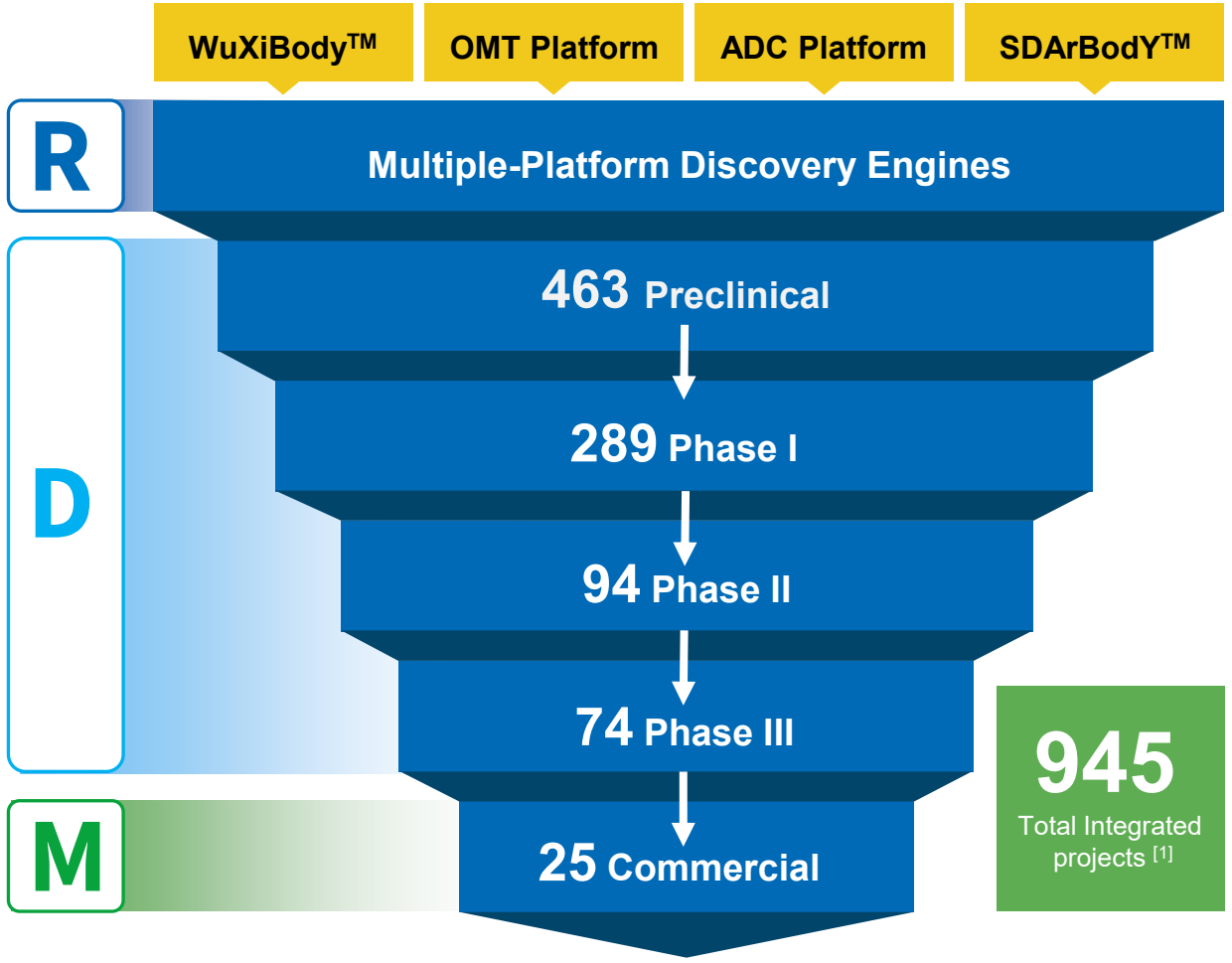
20+
multi-specific antibodies related deals from 2022

[1] Yinghua, Ding. *Bio Process International.* 22(2024), 11–12.
[2] https://synapse-zhihuiya-com/

Rich Pipelines across All Biologics Modalities



- 369 first-in-class programs
- Signed 209 new integrated projects in 2025, reinforcing its role as a partner of choice for biopharma innovators globally. About 2/3 of the new projects are bi- and multi-specifics and ADCs. About half are from the US.
- Win-the-molecule projects：won 23 projects in 2025, including 6 late-stage projects



Notes:

[1] As of Dec 31, 2025, compared with projects number as of Dec 31, 2024

[2] Bispecific Antibody (BsAb) Included both WuXiBody™ projects and non-WuXiBody™ projects

Continue Expediting BsAb Development and Manufacturing DNA-to-IND in As Little As 6 Months

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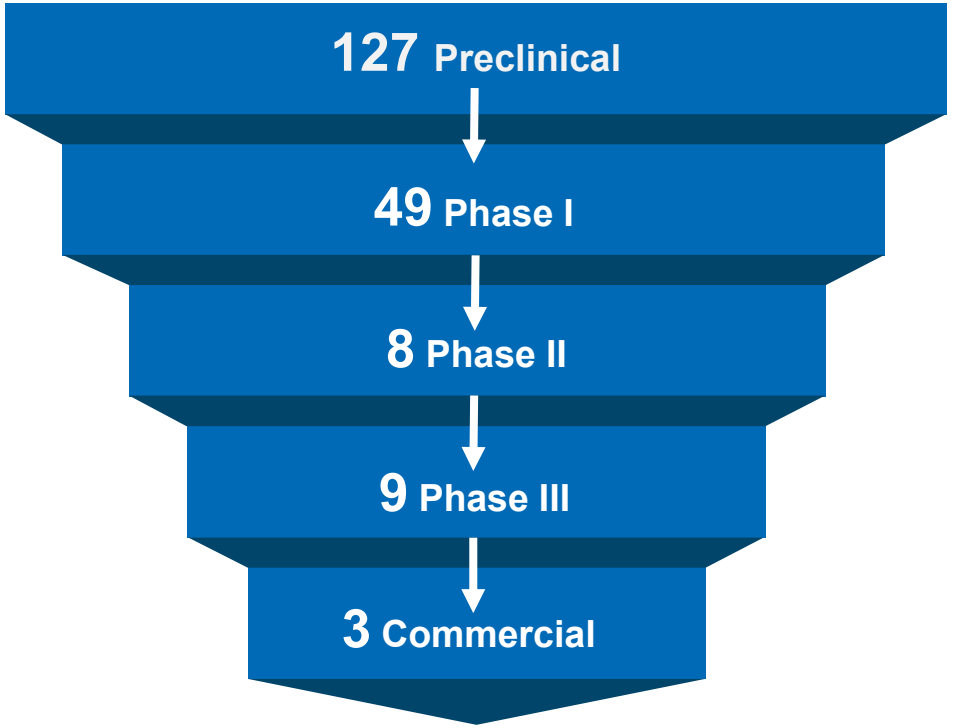
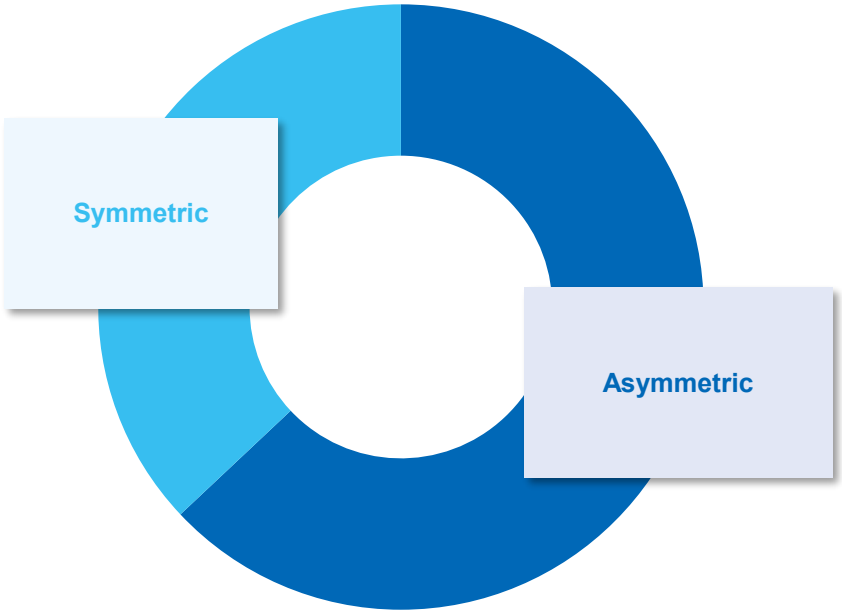
BsAb Project Funnel



- **196 projects** with **80+** different formats
- **39** papers published related to BsAb development

BsAb Project Format Distribution



Notes:
[1] Data as of Jun. 30, 2025
[2] Bispecific Antibody (BsAb) projects Included both WuXiBody™ projects and non-WuXiBody™ projects; and included 15 multispecific antibody projects