Navigating Complexity: A Strategic Framework for Streamlining and Simplifying Antibody Drug Conjugates Development and Potency Assay Lifecycle Management

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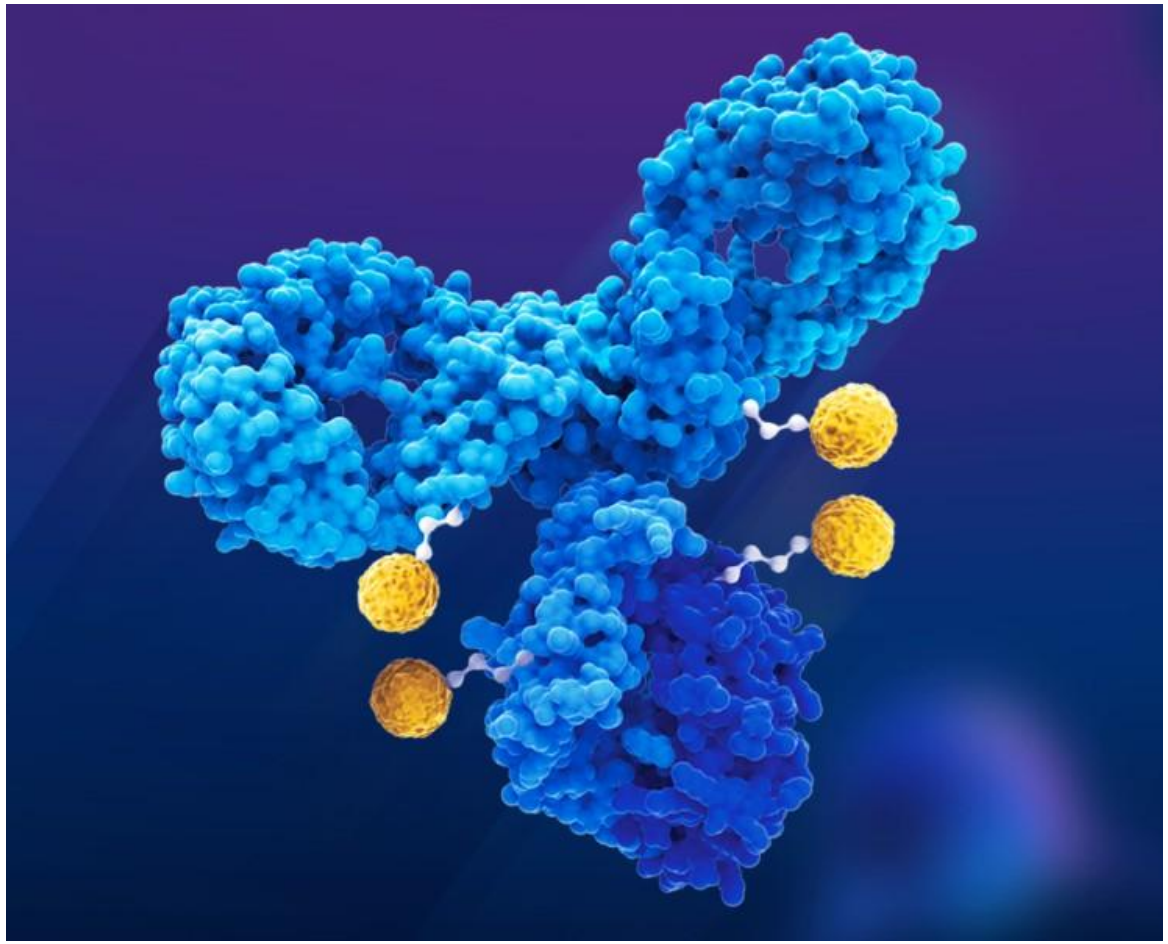
Outline

- ADC as an attractive anti-tumor modality
- ADC-specific challenges for drug development
- Simplifying the analytical development via phase-appropriate potency method studies
- Streamlining and accelerating timelines via applying harmonized approaches across different ADCs
- Conclusions

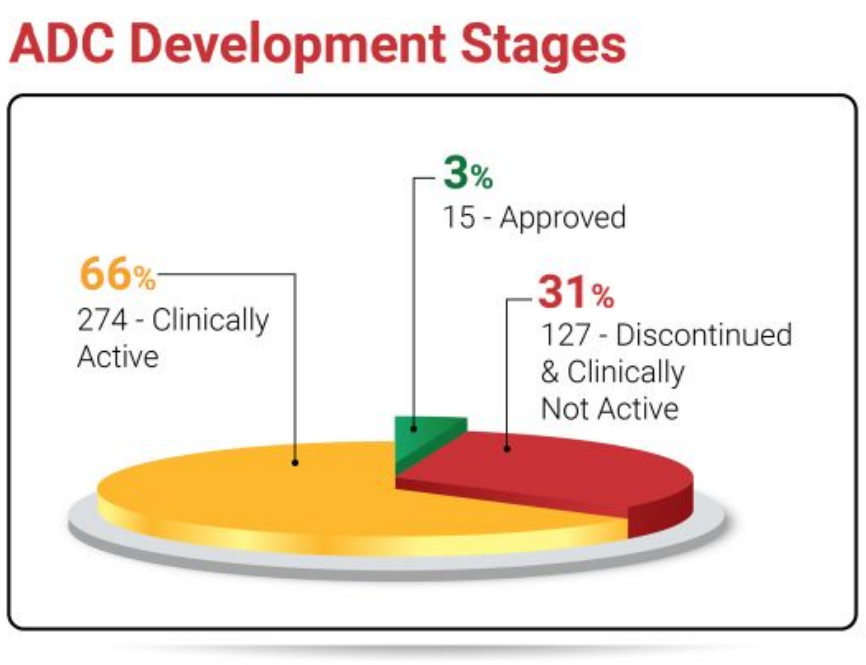


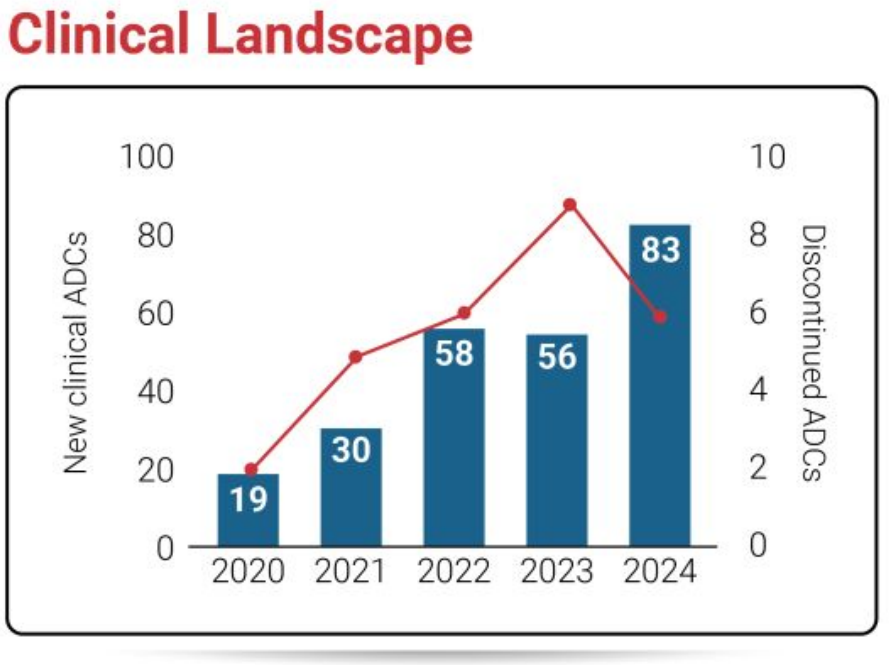
Magic Cece

MedChemExpress LLC, , Weekly Scientific Article, 20Feb2023, LinkedIn.com

ADC as a rapidly developing class of anti-tumor therapy drugs

- As of to date, 15+ ADC have been approved by FDA, and over 270 ADCs are currently in various stages of clinical and commercial development.





Recent Advances in ADC, Jan-2025, https://njbio.com/antibody-drug-conjugates/

Challenges with development of ADC

Clinical examples:

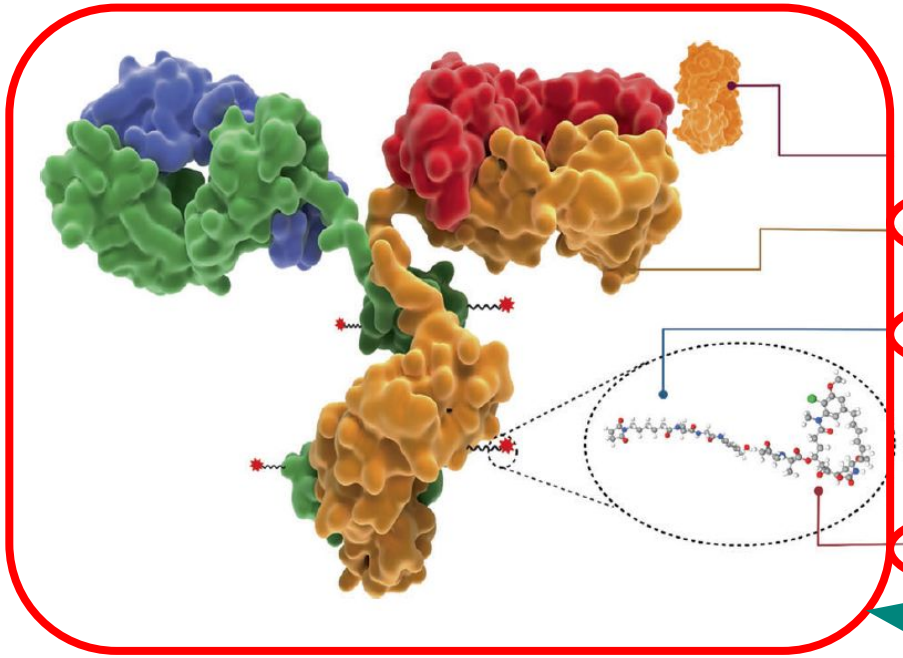
- Complex pharmacokinetic profiles (separate effects from each of the ADC molecular components)
- On-target toxicity (binding to the targets in healthy cells)
- Off-target toxicity (non-specific binding to Fc receptors, passive diffusion across the cell membrane, etc.)
- The bystander toxicity (cleavage of the payload and diffusion of free drug into neighboring healthy cells)
- Not penetrating tumor tissues
- Drug resistance

Design examples

- Antibody:
  - ✔ Selective over normal tissue
  - ✔ Internalizing readily
  - ✔ Low immunogenicity
  - ✔ Long plasma half-life
- Linkers (cleavable and non-cleavable)
  - ✔ Stable
  - ✔ Not inducing ADC aggregation
  - ✔ Not affecting payload potency
- Cytotoxic Drug
  - ✔ Selective for tumor type
  - ✔ High potency (EC50 in low nM or pM range)

1. Recent Advances in ADC, 2025, https://njbio.com/antibody-drug-conjugates/
2. Fu, Z. et al, 2022, Signal transduction and targeted therapy, 7(1), 93

Analytical Challenges



Key functions

Target antigen	Recognition of target cancer cells
Antibody	Guidance system for cytotoxic drugs
Linker	Bridge between antibody and drugs and to control the release of drugs inside cancer cells
Cytotoxic drug	Warhead for destroying cancer cells

Analytical Control Panel #1

Analytical Control Panel #2

Analytical Control Panel #3

Analytical Control Panel #4

Fu, Z. et al, 2022, Signal transduction and targeted therapy, 7(1), 93

Antibody and Antibody Drug Conjugate represent two distinctly different challenges for establishment of control strategy by potency methods

Strategy for selection of most suitable potency method

Regulatory expectations:
- Potency assay should **reflect the MoA,** which should ideally be **related to the clinical response**
- Demonstrated sensitivity to relevant CQAs
- Should be stability indicating
- Well controlled and easy to perform in QC lab

Direct Binding assay by ELISA:

- Reflects the initial binding of ADC to the antigen via antibody component
- ELISA is not capable to detect ADC internalization and tumor killing activity
- For some CQAs, ELISA is not sensitive to molecular and structural modifications
- Determined potency could be non-specifically impacted by presence of conjugated drug
- Several Biological Critical Reagents (BCRs) must be managed

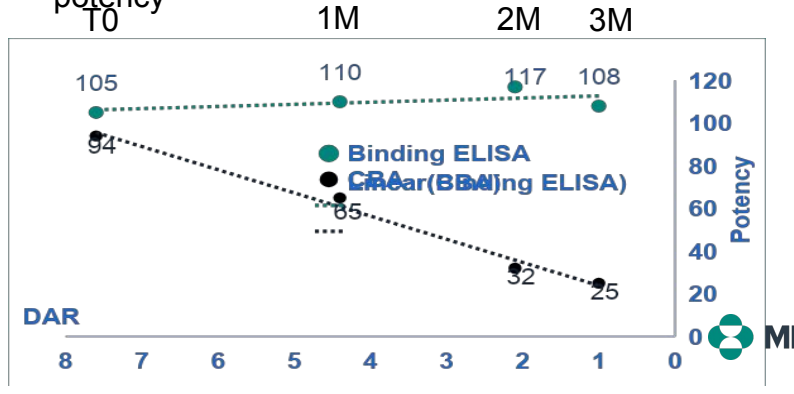
Cytotoxicity by CBA:

- Reflective of MOA to combine effects from the antigen binding and tumor killing by payload
- The cytotoxicity effect is highly specific and directly proportional to the DAR
- Stability indicating
- Simplified BCR management (cells only in majority of the assays)
- QC friendly and usually well-controlled

Impact of changes in CDR on potency of ADC

	ELISA	CBA
AAPH H0	125	120
AAPH H6	91	73
AAPH H24	23	26
40C H1	122	123
40C H24	128	112
0X light	104	100

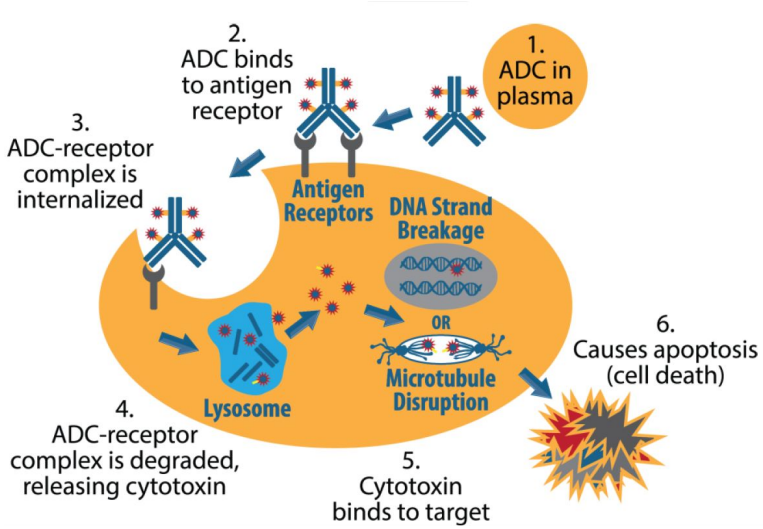
Impact of varying DAR on stability of ADC by potency



Implementation of CBA in the early development phase

Implementing CBA in the earlier phase helps make pivotal data-driven decisions much faster down the road!

MOA of ADC



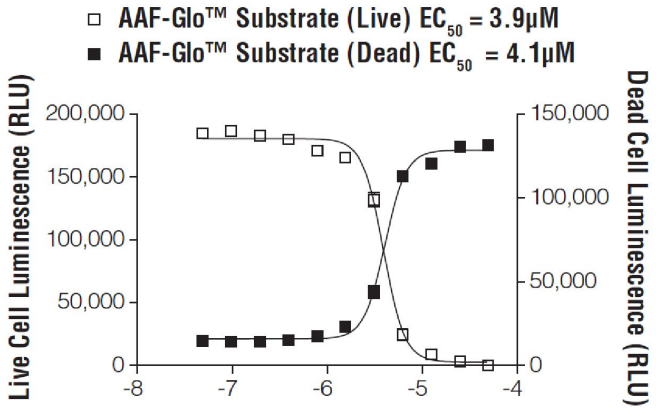
Recent Advances in ADC, January 2025, https://njbio.com/antibody-drug-conjugates/

Current approach for CBA development at Merck

- ❑ Utilize tumor cells identified from screening or engineered cell lines over-expressing target antigen.
- ❑ Measure decrease in cell viability (e.g., by CellTiter-Glo) upon increase of ADC

Platform approach actively pursued

- ❑ **One** parenteral cell line, modified for **specific antigen expression**
- ❑ Measure **cell death** (e.g., by Promega kit for cell cytotoxicity measurement) upon increase of ADC concentration (**activation** curves)



Adapted from Promega Corp.

Additional features for simplification of CBA development:

- The same assay format (plate maps, number of plates, sample positions, etc.)
- Start from applying platform SST (assay and sample) acceptance criteria for assessment of RS and TS curves similarity
- Validated Softmax template only minorly modified for a new product

Method Validation Strategy

We aim to connect method qualification (non-GMP) with early- and late-phase validations of ELISA and CBA for testing of mAB and ADC

New method qualification **prior to** validation:
- Conducted in the development lab
- Platform DOE
- Assay format (# of plates per reportable potency) confirmed statistically

Early-phase ("Stage 1") validation:
- Statistical DOE **linked** to method qualification with considering sufficient power
- Platform acceptance criteria
- Assay format is validated;
- Results are included into assay performance monitoring

Late-phase ("Stage 2") validation
- Statistical DOE based on desired number of data points for each target potency sample
- Data from method qualification **and** Stage 1 validation are leveraged in the DOE and justification of acceptance criteria (AC)
- AC are aligned with **late-phase** and **intended commercial** potency specifications

- The same strategy is applied to both ELISA and CBA methods
- Validations are performed per ICH Q2(R2) guidelines with incorporation of potency-specific recommendations from USP<1033>
- The same statistical DOEs (slightly different between ELISA and CBA) are used across different mAb/ADCs
- This approach leads to reduced risk of validation failures and increased benefit for investigation of failures

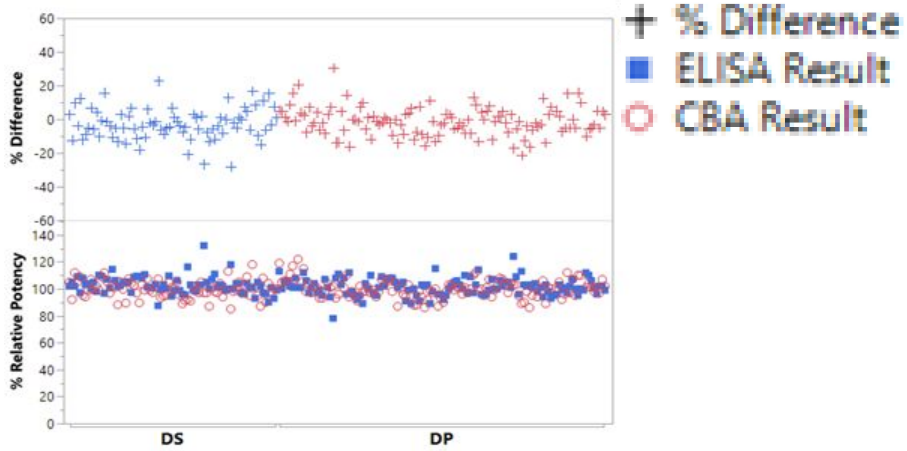
Comparability of CBA and ELISA results

To demonstrate, that CBA is more suitable method to control potency of ADC, **data-driven justification is produced**

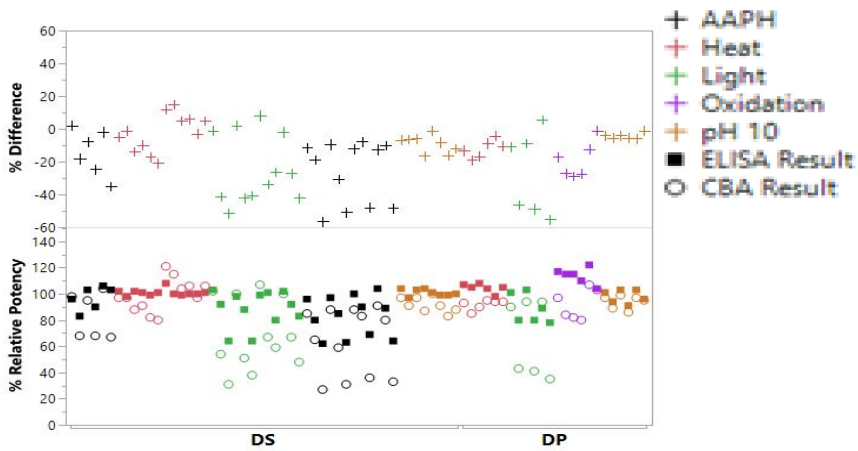
- ❑ When ELISA and CBA methods are implemented from the beginning, results from **long-term** analytical testing are accumulated
- ❑ With the comprehensive information from extensive clinical studies, understanding of MOA is facilitated to benefit the selection
- ❑ The difference between ELISA and CBA potency results is analyzed statistically to assess and demonstrate:
  a. Correlation between potency results from the **cell toxicity and antigen binding** using release and intended storage stability data
  b. Impact and sensitivity to changes in CQAs determined by CBA and ELISA

Example

Comparison of release and stability potency results produced by ELISA and CBA



Comparison of forced degradation potency results produced by ELISA and CBA



NOTE: Binding ELISA is not fully abandoned but retained throughout the product life cycle management as part of extended characterization in various product and process studies (e.g., process comparability, Reference Standard program, etc.)

Strategy for Biological Critical Reagents (BCR)

Implement **dual** system for BCR management

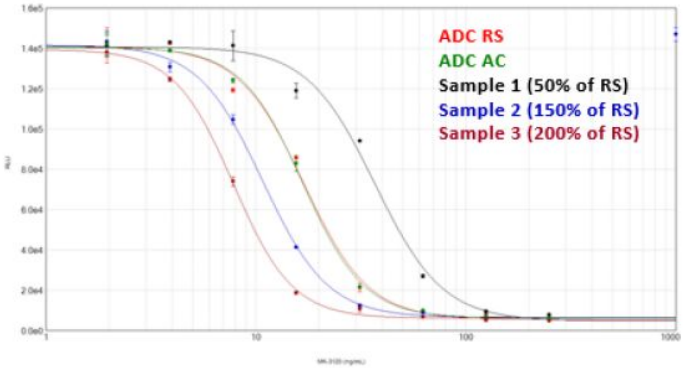
BCRs for mAB testing only

- ❑ ELISA reagents (coating ligand and detection Ab at the minimum)
- ❑ Interim mAb RS □Primary mAb RS□ Primary mAb RS + Secondary mAb RS
- ❑ mAB quality Control Sample

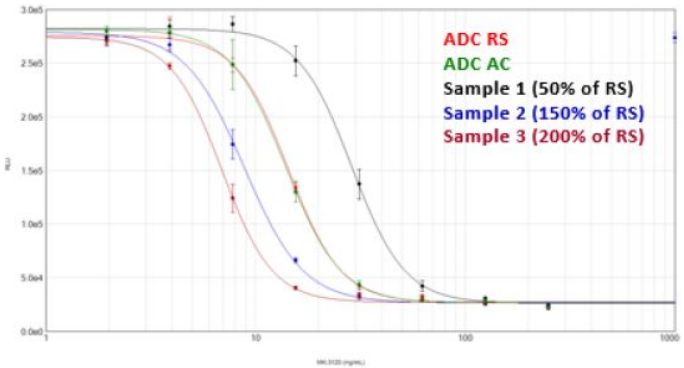
BCRs for ADC testing only

- ❑ Interim ADC RS □Primary ADC RS□ Primary ADC RS + Secondary ADC RS
- ❑ ADC quality Control Sample
- ❑ Cell Line: Master and Working Cell Banks, produced and tested for viral clearance, mycoplasma, functionality.
  - continuous culturing and Ready-to-Use (RTU) cells
  - qualified as BCR during late-phase method validation

ADC CBA - Continuous Culture

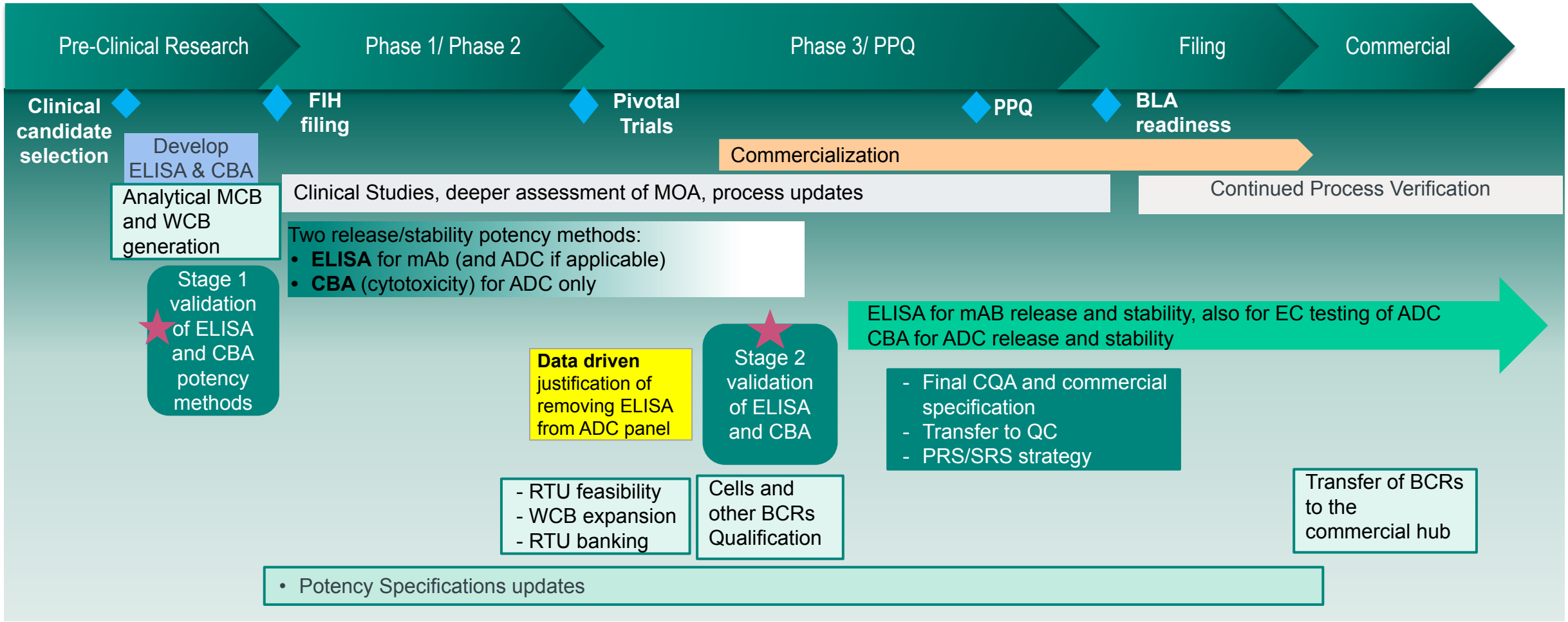


ADC CBA - RTU cells



ADC CBA - Continuous Culture				ADC CBA - RTU			
Target Potency	GeoMean %RP	%GSD	Recovery	Target Potency	GeoMean %RP	%GSD	Recovery
50% of RS	47	2	94%	50% of RS	49	2	98%
150% of RS	147	5	98%	150% of RS	159	3	106%
200% of RS	210	5	105%	200% of RS	202	3	101%

Adapted streamlined potency strategy for ADC development



NOTE:
More recent pipeline ADCs already implemented CBA for release and stability of ADC DS and DP, while ELISA is only used in mAB testing.
Since direct binding between ADC and its target **is part of MOA**, it is retained in EC studies of both mAB and ADC

Case Study:

- ADC program was granted Breakthrough Therapy for treatment of lung cancer
- Multiple clinical studies are in place
- Program was accelerated under aggressive business timelines

Challenges related to potency strategy:

- ELISA: used for all early-phase testing of both mAb and ADC, assay format was different from that of followed at Merck
- CBA: not QC friendly, lacked desired features (e.g., low sample throughput, long incubation times, etc.)

Tasks:

1. Update ELISA to increase sample throughput
2. Develop a new robust and MOE-reflective CBA to increase sample thruput and ensure QC friendliness
3. Validate both methods as Stage 2
4. Immediately transfer both ELISA and CBA **to multiple** commercial laboratories
5. Justify removal of ELISA from ADC testing panel
6. Develop PRS/SRS qualification strategy

Case Study contd.: Acceleration in action

METHOD DEVELOPMENT

- Minorly updated ELISA to preserve robustness (already performed)
- Re-developed CBA
- Qualified both methods to inform on robust method performance
- Updated existing **validated** Softmax templates for reading and automated data analysis (with “pass/fail” conclusions)

BCR STRATEGY

- Justified selection of BCRs for ELISA
- Identified suitable mAb and ADC DS lots as Reference Standards and Control Samples, respectively.
- Defined PRS/SRS strategy per historical communications with FDA
- Generated sufficient supply of MCB and WCB, and demonstrated suitability of RTU cells

METHOD VALIDATION

- Included 3 designated QC labs into **co-validation**
- Extensive training of new analysts
- Leveraged results from method qualification to inform on robustness and proposed acceptance criteria
- Statistical analysis performed using previously developed templates for faster availability of results

TRANSFER TO QC

- Three QC laboratories participated
- Most experience lab was Sending Unit, executed full DOE (5 target potency levels)
- Receiving Units (2) executed partial DOE (3 target potency levels)
- Assessed Reproducibility, Relative Bias and Equivalency against acceptance criteria aligned with product specifications

Using knowledge of MOA and available historical data from release and stability testing, justified **removal of ELISA from ADC testing panel**

Conclusions

- **ADC potency strategy simplification**
  1) Earlier removal of ELISA testing of ADC on the release and stability panel
     - Early phase generation of CBA and ELISA potency results of ADC can confirm CBA to produce equivalent potencies and stability indicating properties as ELISA
     - ADC binding to target protein monitored by ELISA on the extended characterization panel allows for continual assessment of the direct binding via various product and process studies
     - Reduces Biologics Critical Reagent (BCR) management load
  2) Phase-appropriate implementation of Ready-to-Use (aka Thaw-and-use) cells for flexibility
- **Streamlined and accelerated method validation and transfer strategy**
  1) Stage 1 validation by platform criteria as confirmed by method qualification study
  2) Stage 2 validation against criteria based on method and process variability data obtained from early phase release data and stage 1 validation results, and also informed by expected commercial specifications
  3) Co-validation approach to accelerate assay transfer time

Acknowledgements

MRL AR&D, Cell-Based Sciences:

- Petra Bennington
- Daisy Sun
- Wenyong Tong
- Bo Feng
- Kevin Gurney
- Anka Ehrhardt
- Shara Dellatore

MRL Clinical Statistical Team:

- Monisha Dey
- Patrick Gaffney

MMD, Center for Mathematical Sciences:

- PC (Pieta) Boon
- Chuck Miller
- Eric Talens