Control Strategies for Potency

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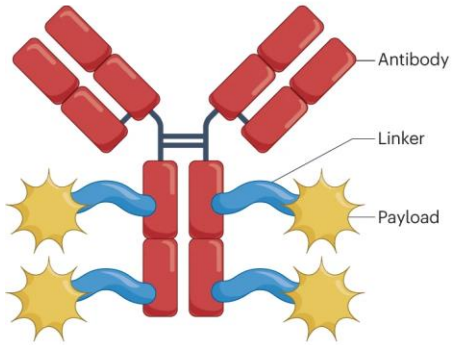
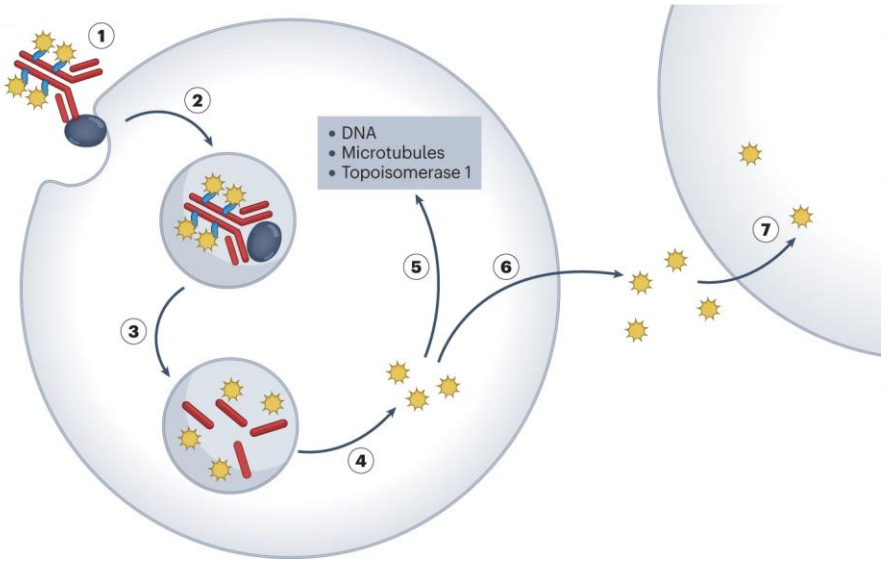
Outline

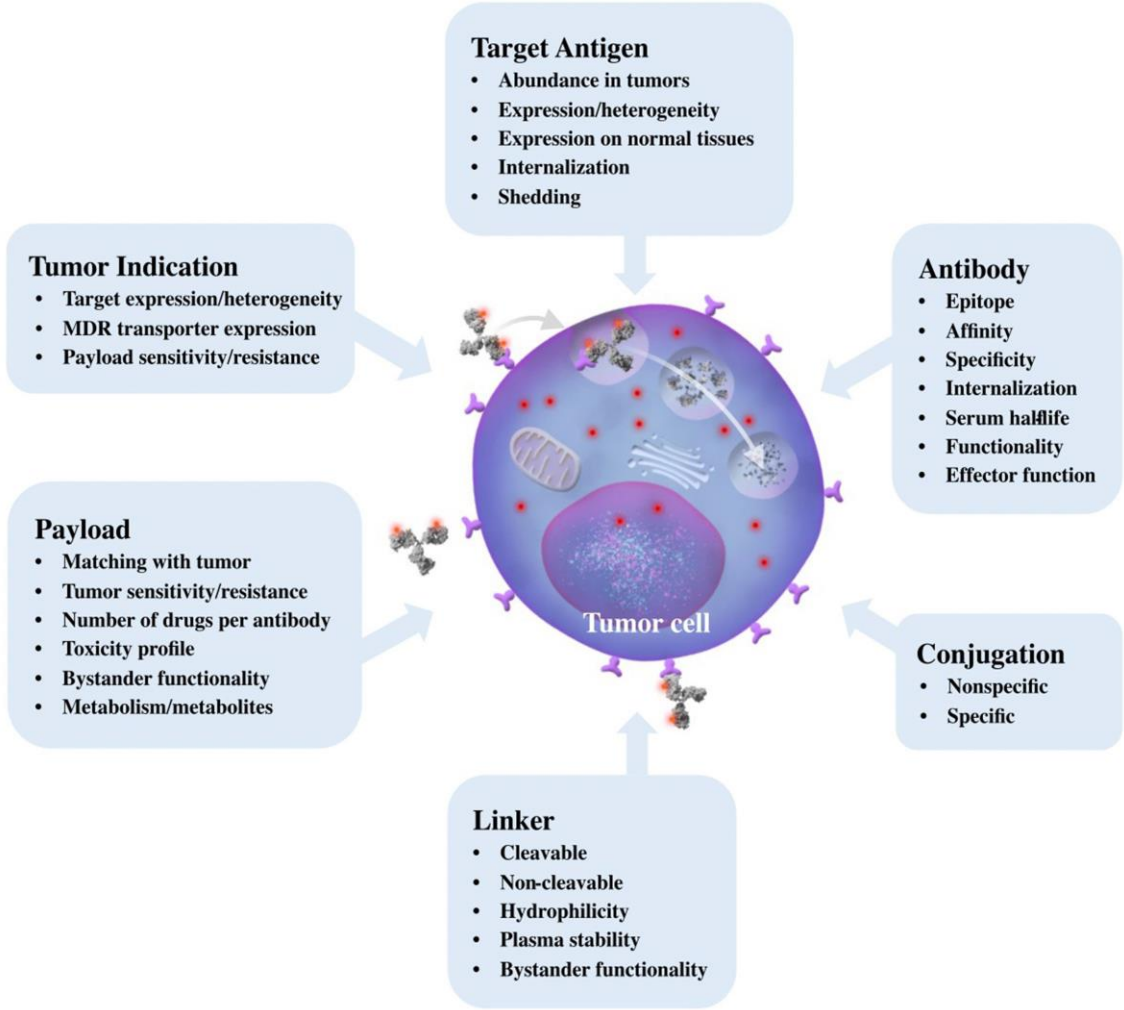
- ❑ ADC introduction
- ❑ Analytical control for ADC and regulatory expectations
- ❑ Potency strategy for ADC
- ❑ Case studies for potency assay standardization and simplification

Structure, MoA and key factors of ADCs





Dumontet, C et al. Nat Rev Drug Discov 22, 641–661 (2023).
https://doi.org/10.1038/s41573-023-00709-2



Maecker H et al. MAbs. 2023 Jan-Dec;15(1):2229101.
doi: 10.1080/19420862.2023.2229101.

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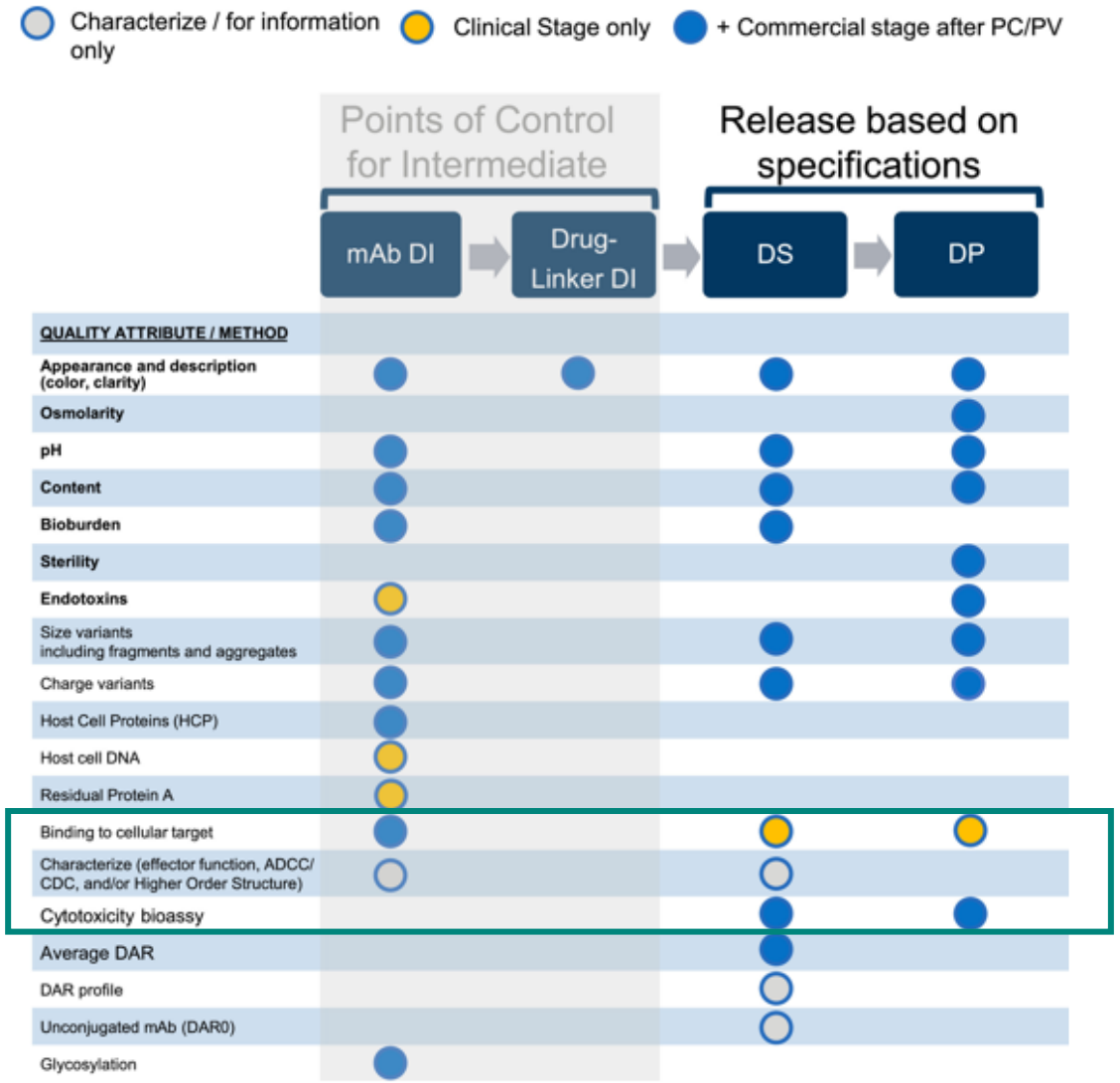
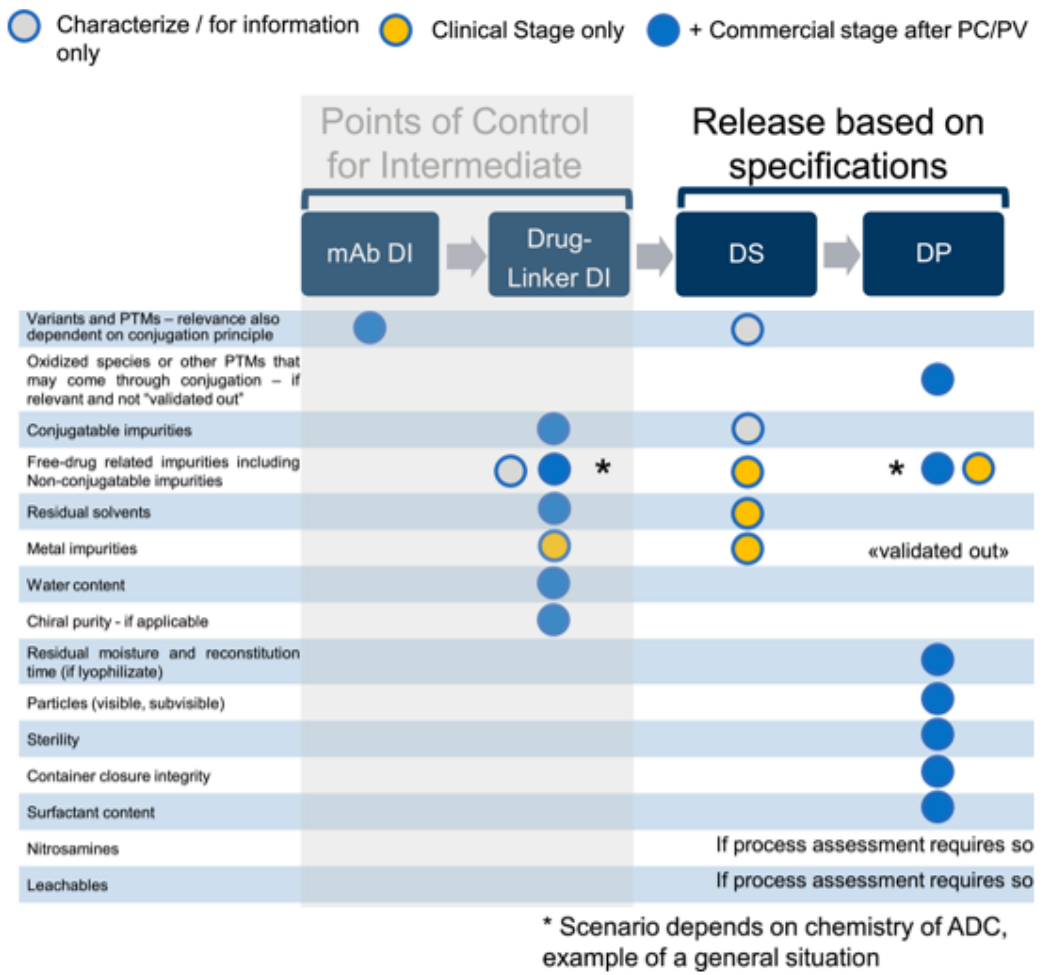
Analytical control for ADC





Bechtold-Peters K et al. J Pharm Sci. 2023 Dec;112(12):2965-2980.
doi: 10.1016/j.xphs.2023.09.007

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Regulatory expectations for potency assays

❖**21 CFR 601.2 & FDC Act:** "All biological products regulated under section 351 of the PHS Act must meet prescribed requirements of safety, purity and potency for Biologic License Application (BLA) approval."

❖**21 CFR 610.1** "No lot of any licensed product shall be released by the manufacturer prior to the completion of tests for conformity with standards applicable to such product," which include tests for **potency, sterility, purity, and identity."**

❖**Potency (21 CFR 600.3(s)):** "the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result."

❖**Potency Tests (21 CFR 610.10):** "tests for potency shall consist of either ***in vitro* or *in vivo* tests, or both,** which have been specifically designed for each product so as **to indicate its potency in a manner adequate to satisfy the interpretation of potency** given by the definition in § 600.3(s) of this chapter."

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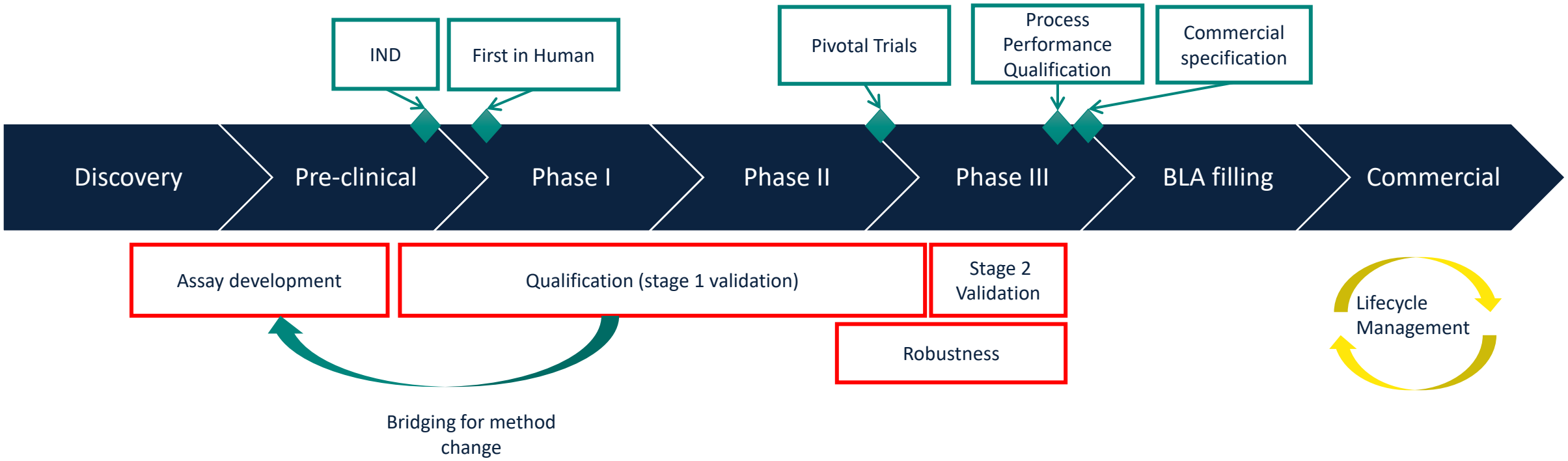
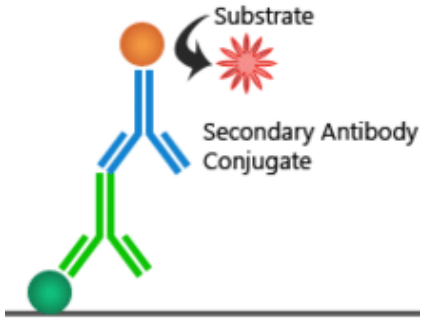
Life cycle of a potency assay



Potency strategy for ADCs

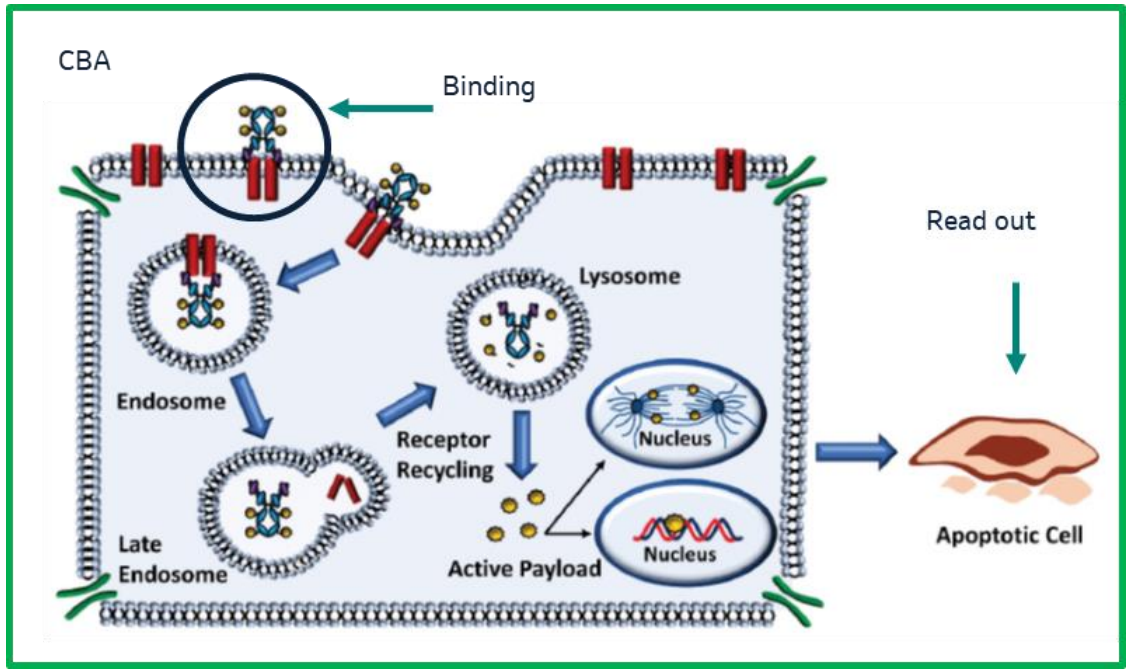
Release and Stability

- ❑ mAb drug Intermediate (DI): Binding ELISA



- ❑ Drug-Linker DI: no potency assay needed

- ❑ ADC DS and DP:
  - ✓ Cell Based Assay (CBA, Cytotoxicity)
  - X Binding ELISA is optional



Characterization

- ❑ Antigen affinity
- ❑ Fc Effector function

Dean AQ et al. MAbs. 2021 Jan-Dec;13(1):1951427.
doi: 10.1080/19420862.2021.1951427 & doi: 10.1080/19420862.2021.1966993.

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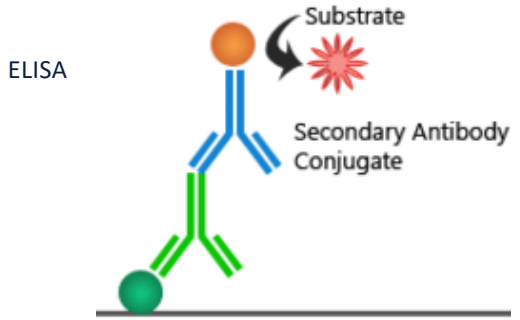
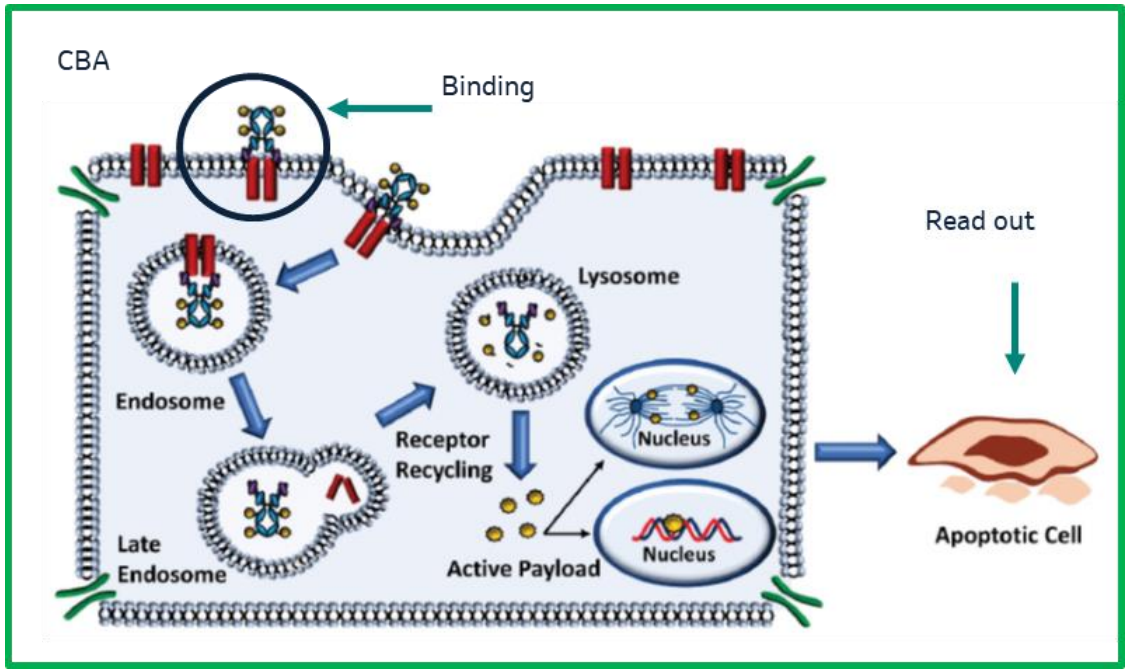
Case study: Remove the binding ELISA from release and stability?

Target DAR	Measured DAR	CBA	ELISA	Antigen affinity by Biacore
DAR 0 (mAb)	0.0	0	213	100
DAR 2	2.4	21	149	97
DAR 4	3.9	43	124	101
DAR 6	6.1	72	104	99
DAR 7	7.1	84	103	101
DAR 8	7.7	92	100	95





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doi: 10.1080/19420862.2021.1951427 & doi: 10.1080/19420862.2021.1966993.

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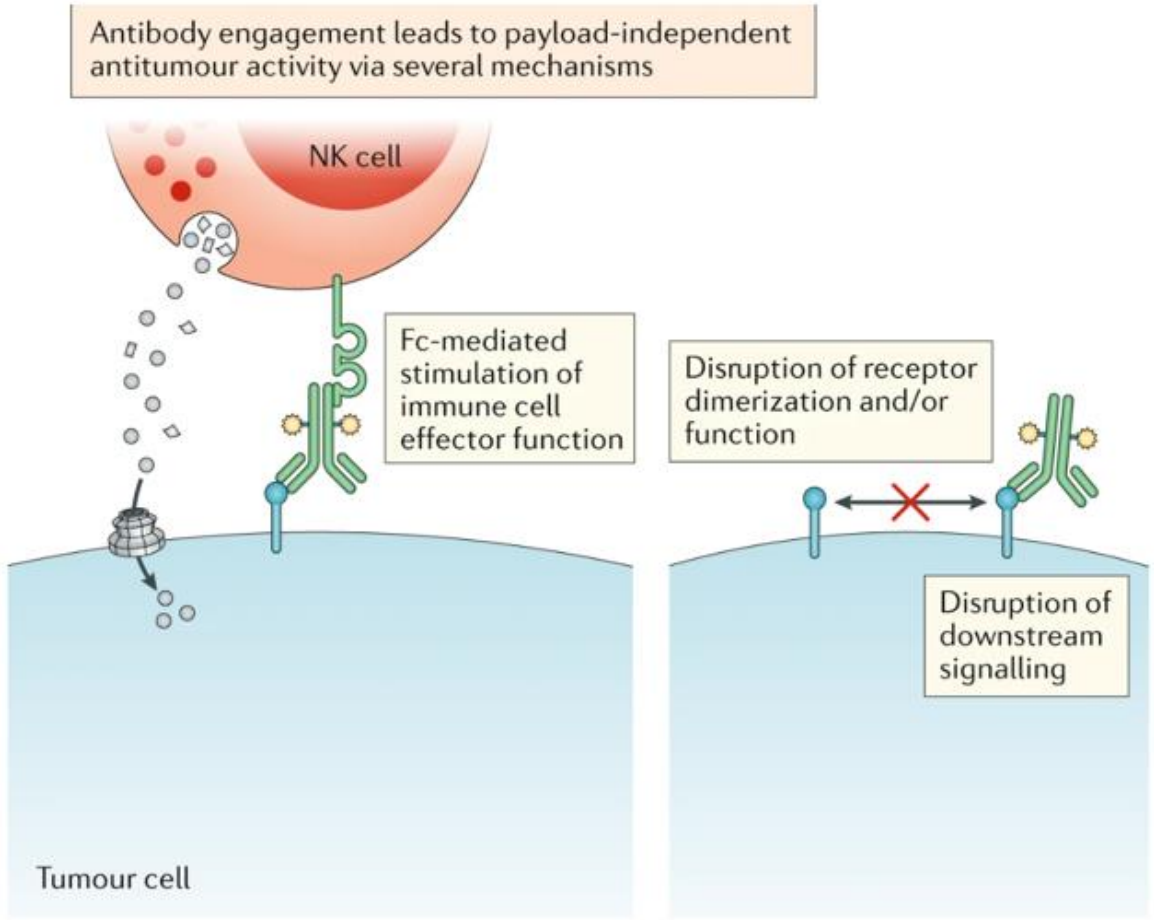
Case study: Fc effector function characterization



Target DAR	Measured DAR	%Relative Affinity			
		FcRn	FcγRI	FcγRIIIa	C1q
DAR 0 (mAb)	0.0	100	100	100	100
DAR 2	2.4	103	112	64	82
DAR 4	3.9	113	106	54	68
DAR 6	6.1	121	112	29	NA
DAR 7	7.1	125	100	22	NA
DAR 8	7.7	131	104	19	NA

Drago JZ et al. Nat Rev Clin Oncol. 2021 Jun;18(6):327-344.
doi: 10.1038/s41571-021-00470-8.

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Problem statement for ADC cell-based assay

1. Traditional CBA development has a long lead time
2. Long assay incubation time for ADC (3-7 days post ADC treatment)
3. Some human tumor cell lines are difficult to grow and/or modify

How can we accelerate ADC CBA development?

Case study: Targeting simplification & standardization of CBAs

Existing Approach

1. Use a **human tumor cell line** +/- over-expression of target antigen. **Parental cell lines vary for given ADC**

2. Measure live cells using CellTiter-Glo. Inhibition curve

3. Specificity: similar curve with higher EC50 by off-target ADC

Step 1. Switch to **a parental** cell line

Current & A Potential Future Approach

1. Use **one parental** cell line stably over-expressing target antigen.

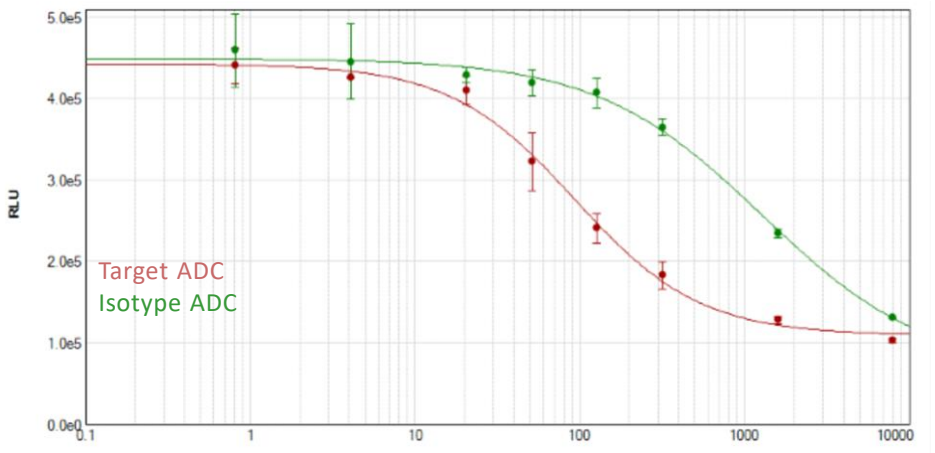
2. Measure live cells using CellTiter-Glo. Inhibition curve

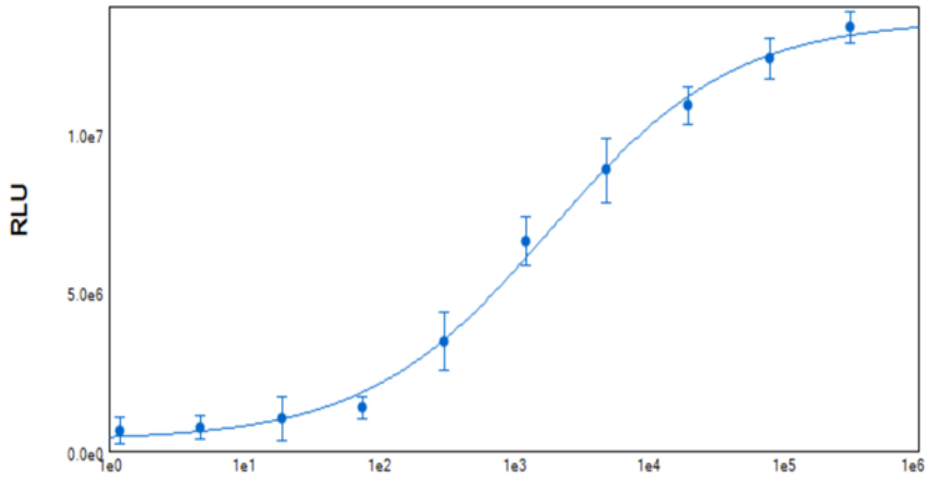
3. Specificity: Improved

Step 2. Measure dead cells instead of live cells

1. Use one parental cell-NanoLuc + target antigen expression

2. Measure **dead cells** using Nano-Glo: **Activation curve**, **Larger Assay window**, more **sensitive** and shorter incubation





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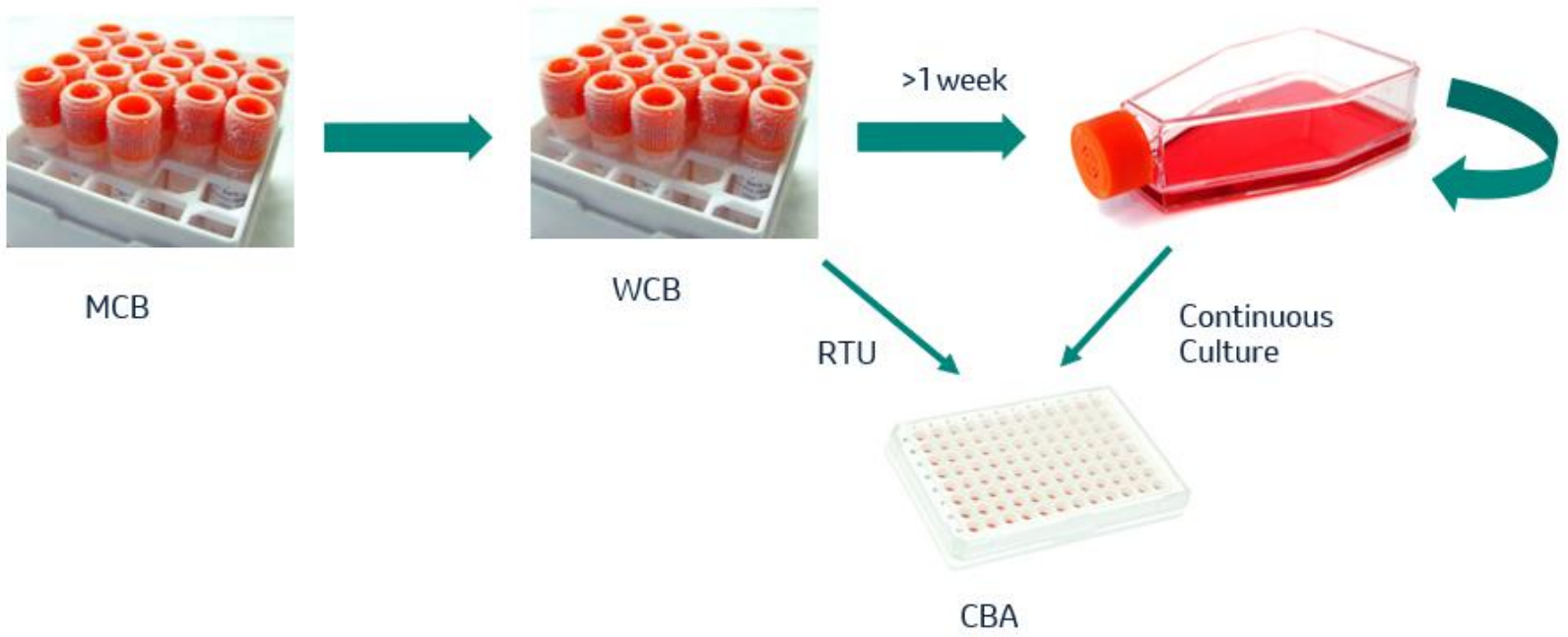
Case study: Targeting simplification & standardization of CBAs

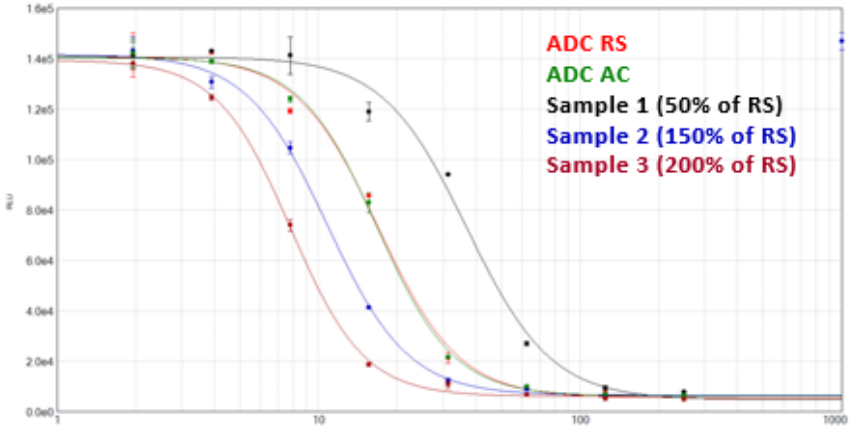
- ❑ Strategy: both continuous culture and RTU in one method
- ❑ RTU advantage: fast, flexible, same lot, same passage#, lower risk of mycoplasma/sterility during culture
- ❑ RTU disadvantage: cost, storage, stability?



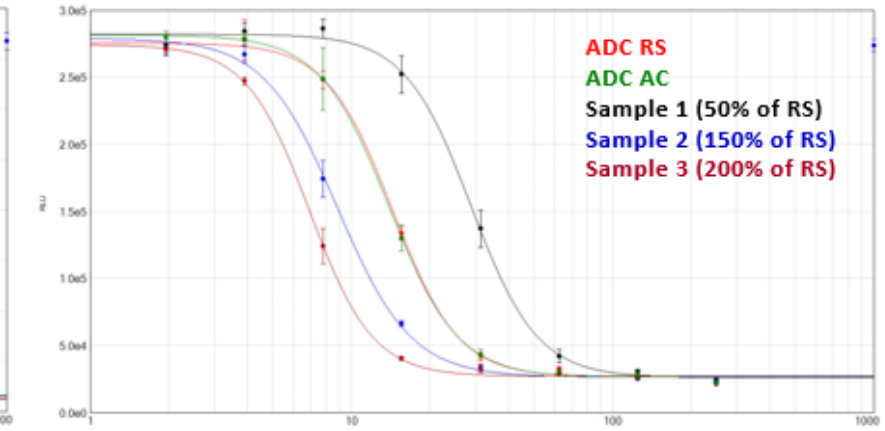
Case study: Targeting simplification & standardization of CBAs

- ❑ Same procedure
- ❑ Same performance
- ❑ Same WCB density

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%

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Summary

- ADC is a promising modality
- The complicated structure raises challenges in analytical control, particularly potency
- Potency CBA could potentially be standardized by streamlining the parental cell lines
- Potency CBA can be further simplified by using RTU