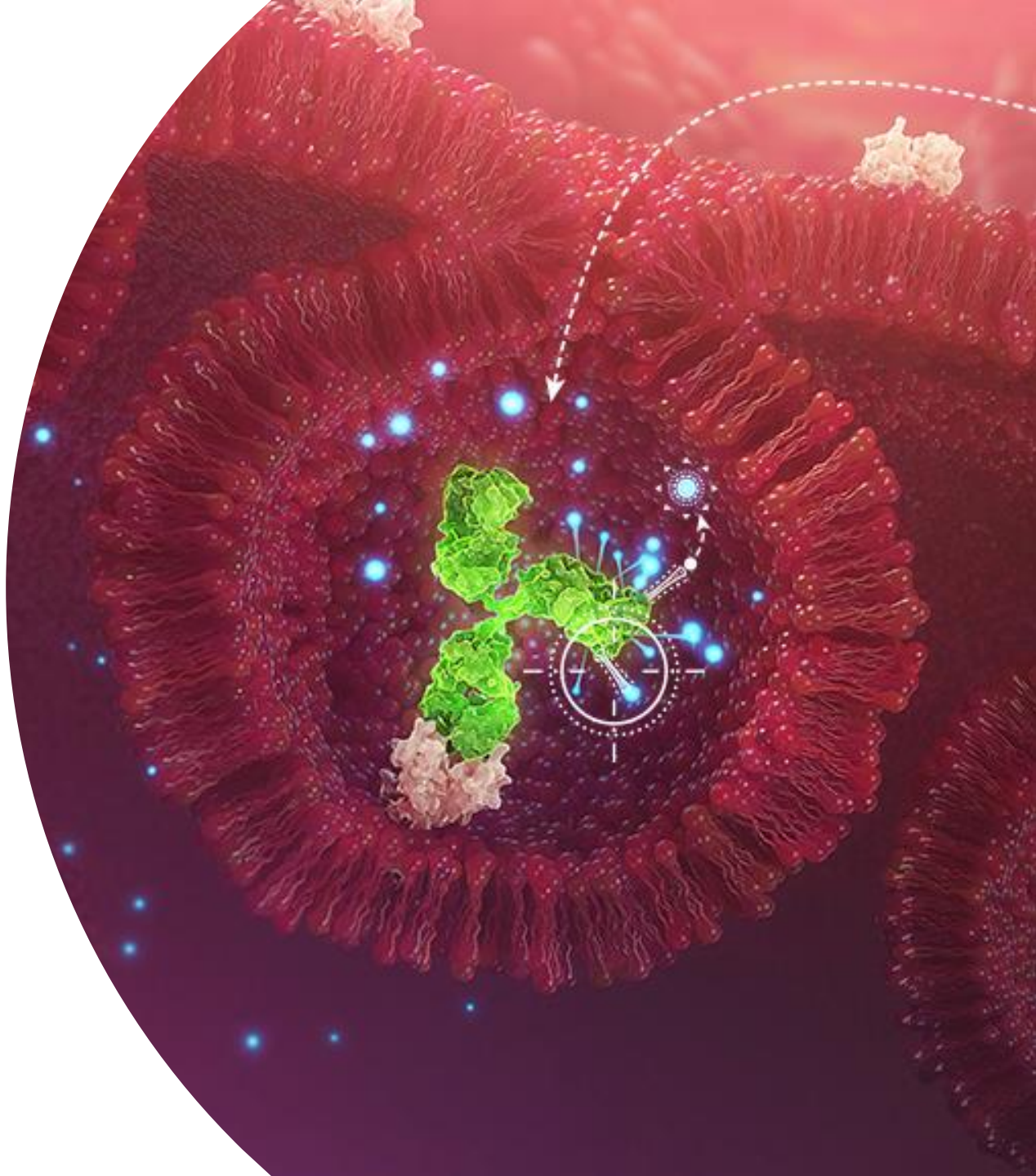
AstraZeneca

Adapting CMC Bioassay Strategies in Response to an Evolving ADC Pipeline

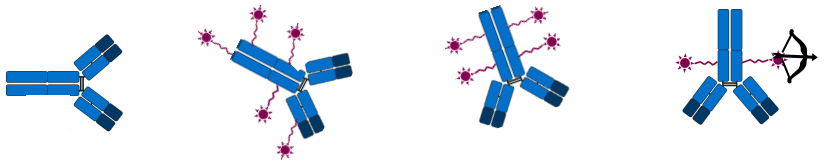
Patrick Hussmann, PhD

16 April 2024



1. Anatomy, MoA and Evolution of ADCs in Brief
2. Current CMC Bioassay Control Strategies
3. Adapting CMC Bioassays: Simplification

Anatomy, MoA and Evolution of ADCs in Brief



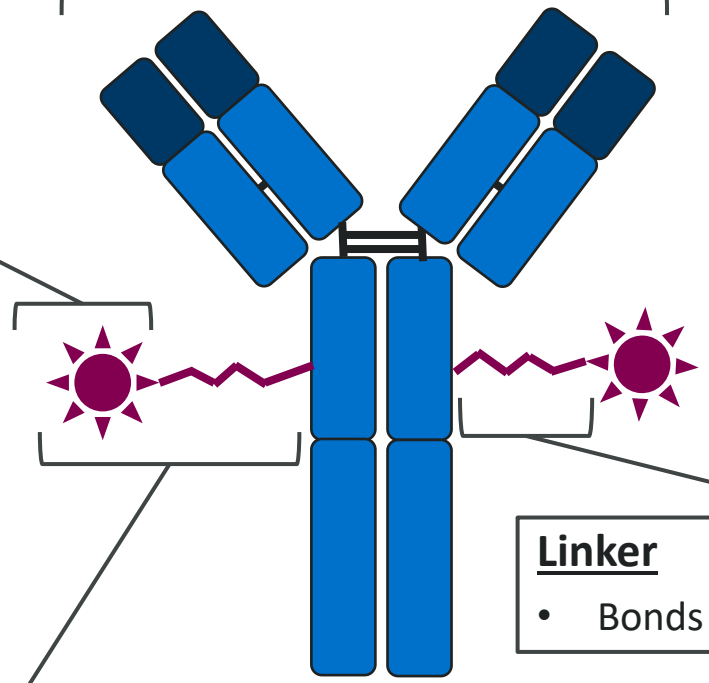
Anatomy of an ADC

Antibody

- IgG1 subtype most common
- Binds tumor-selective, cell-surface antigen
- Targets payload to cancer cells

Warhead

- Cytotoxic small molecules
- Non-specific inducers of cell-death
- Typical targets include DNA, Tubulin, & Topoisomerase



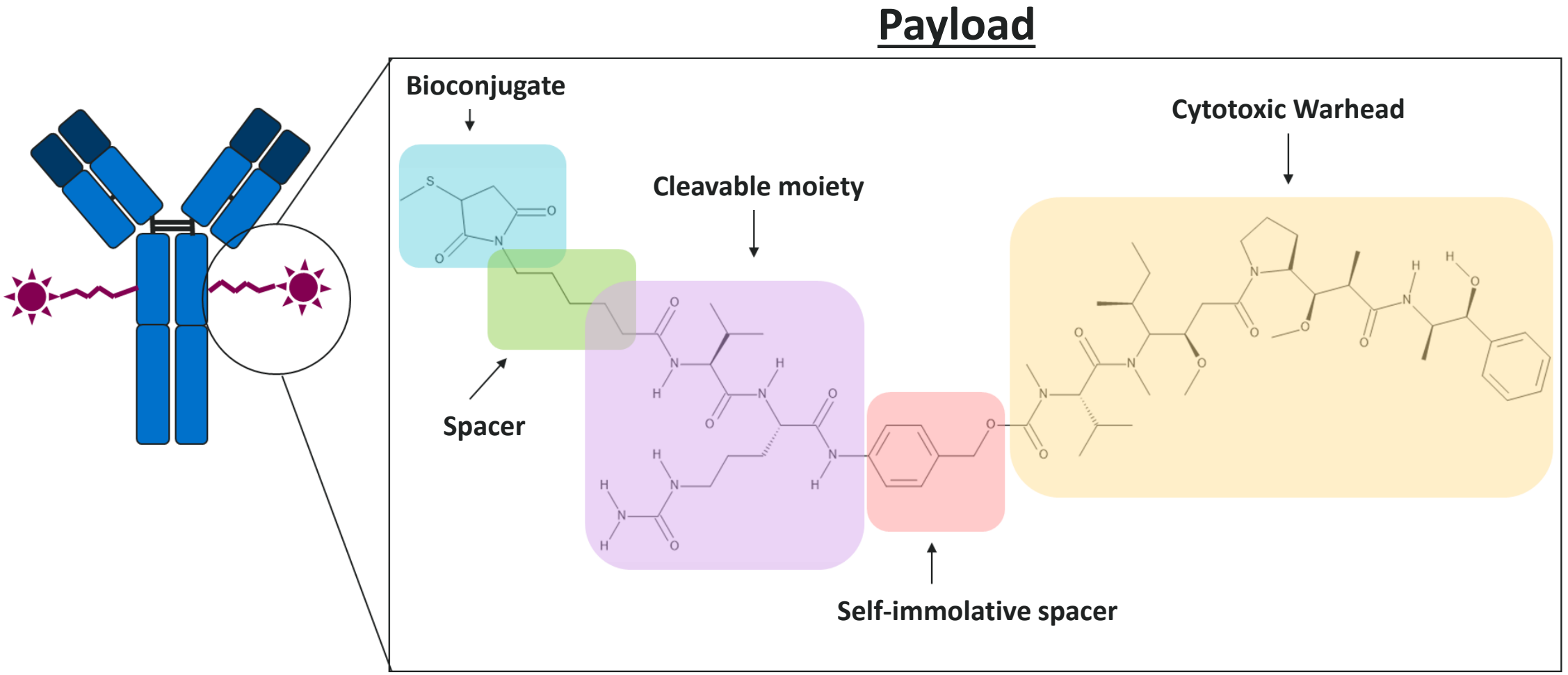
Linker

- Bonds warhead to antibody

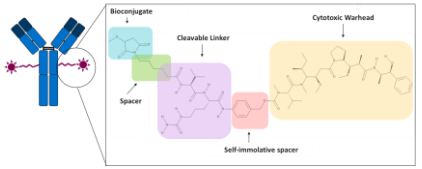
Payload

- Warhead + Linker

Anatomy of an ADC

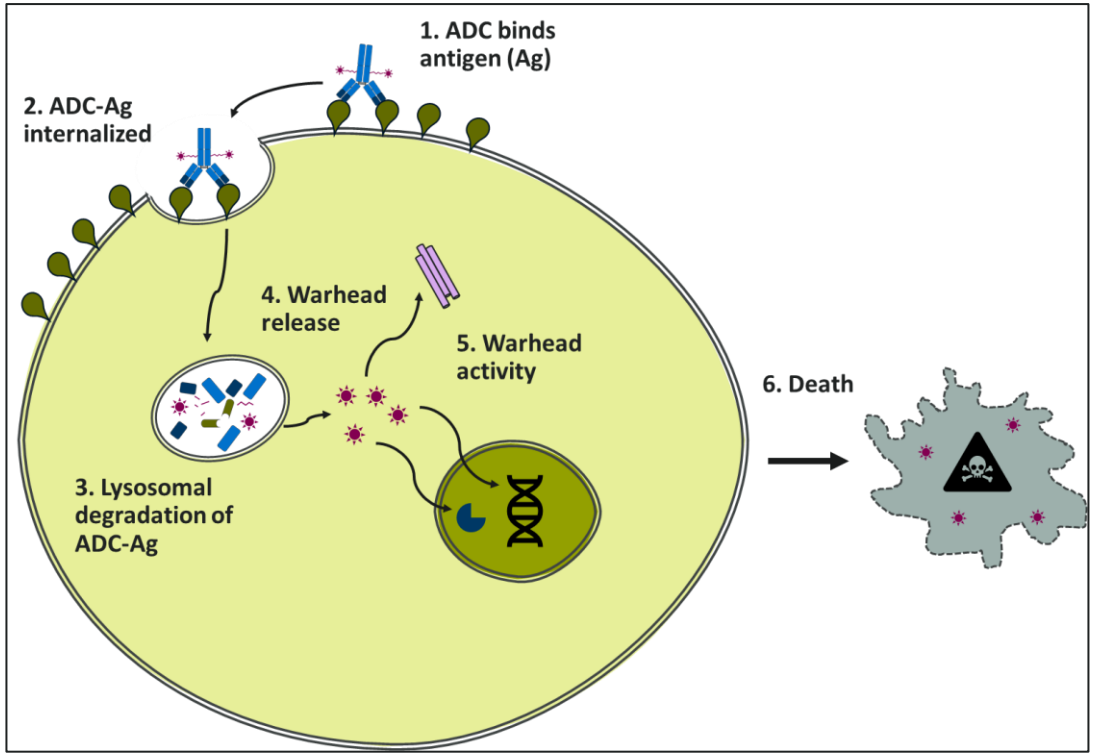


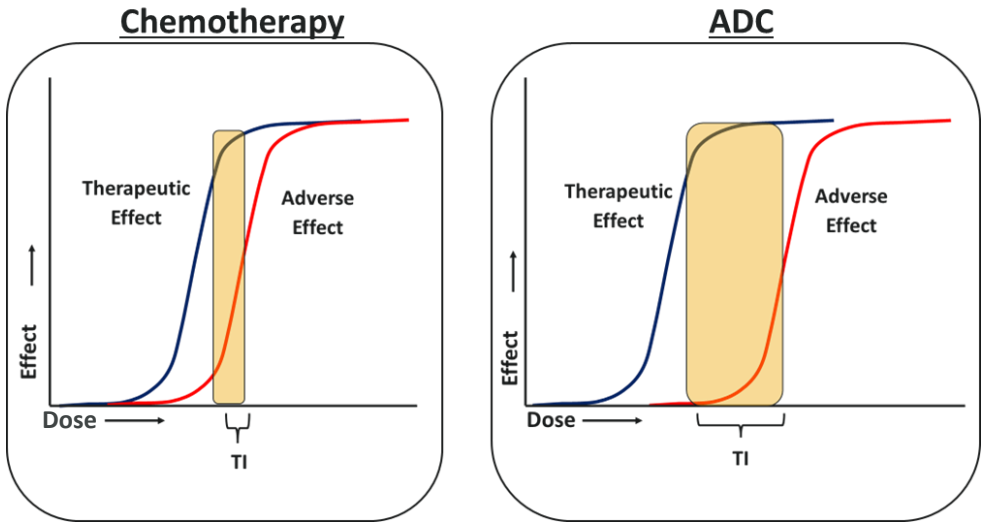
Mechanism of Action



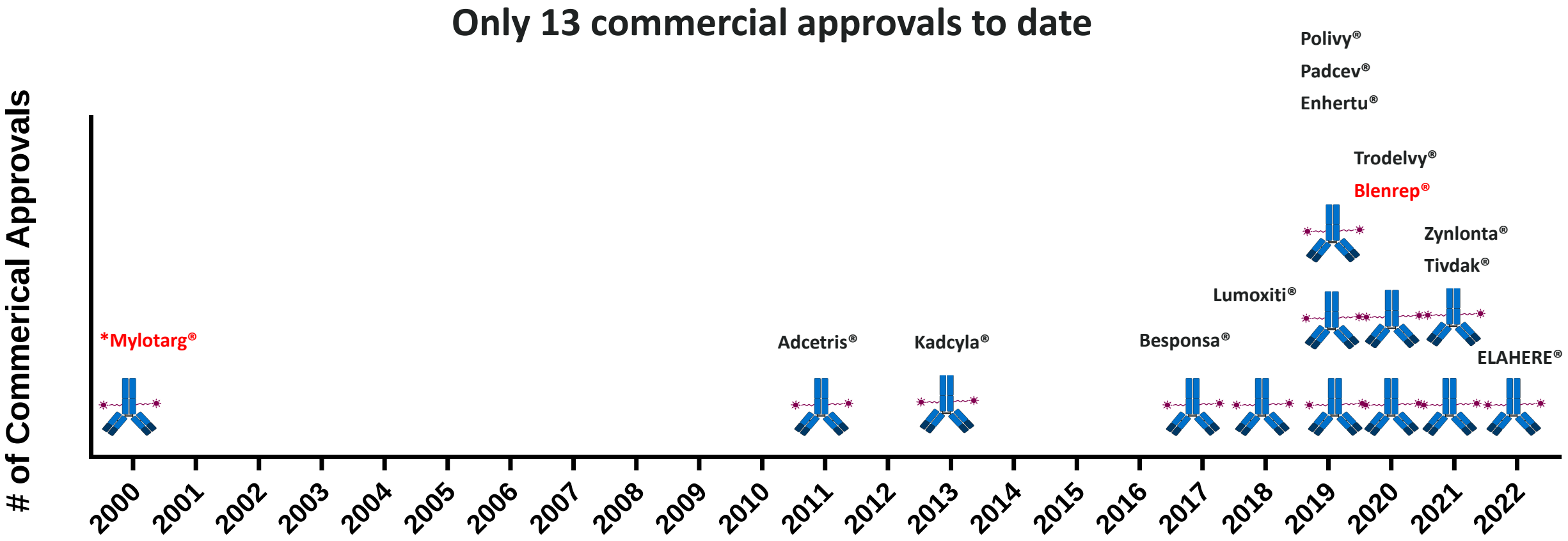
Primary MoA is payload delivery and warhead-mediated cell death

- Selectivity of antigen expression and specificity of mAb binding directs ADC to cancer cells
- Linkers should be stable in circulation and release warhead specifically when inside cancer cells
- Goal is to increase TI of non-specific chemotherapy agents by reducing systemic exposure





Timeline of Commercial Approvals



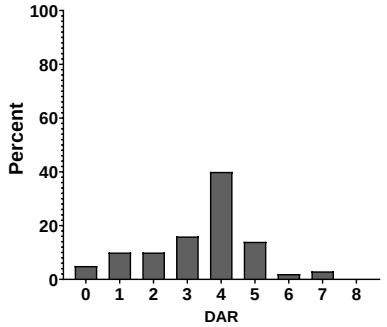
Red = withdrawn

*re-approved in 2017

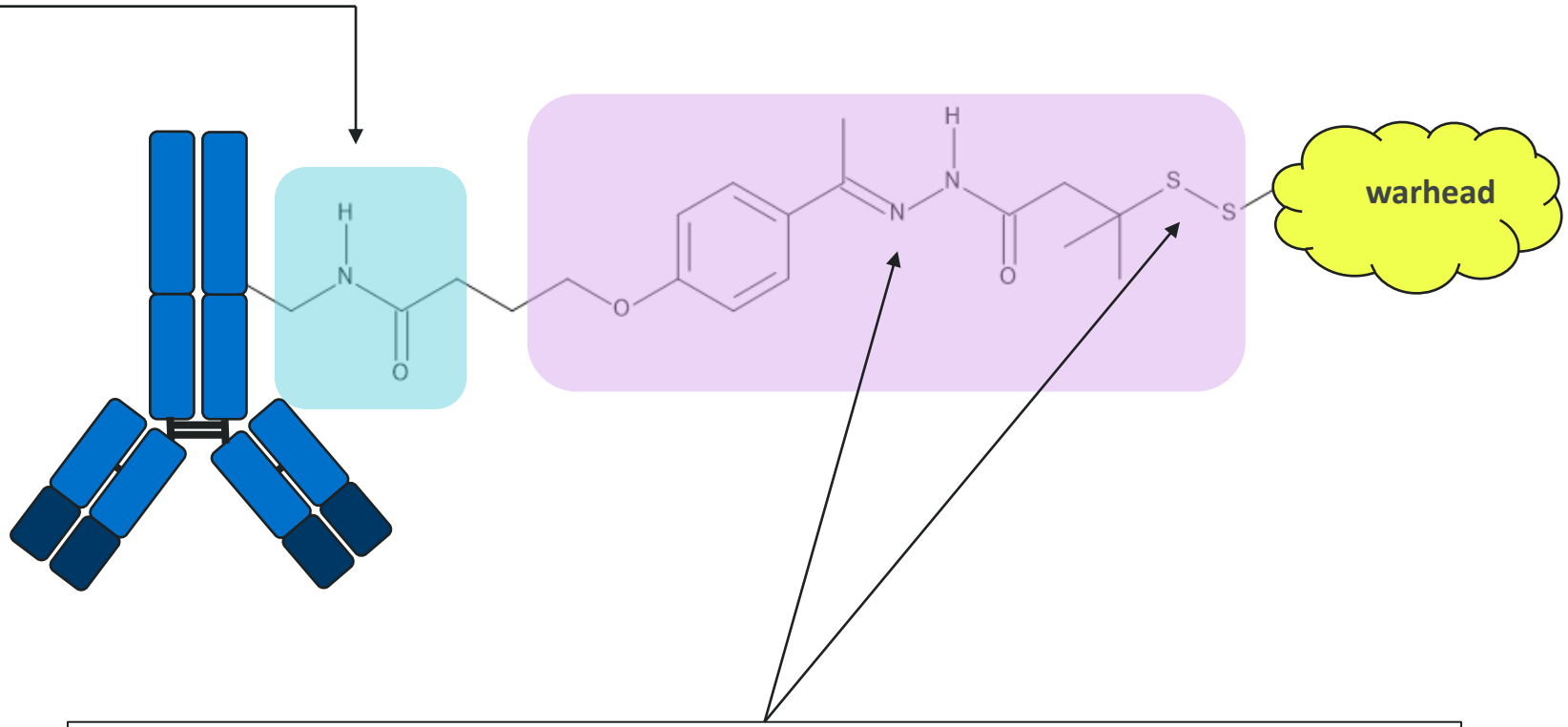
Early ADCs demonstrated narrower than expected TI…

Conjugation at lysine residues

- Poor control of DAR



- Varying drug distribution
- Potential impact to binding



Mylotarg® DAR = 2.5:

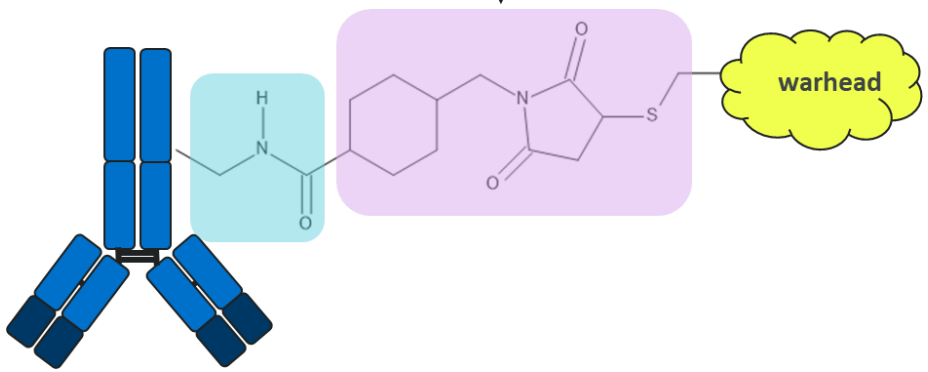
~50% DAR 0

~50% mixture of DAR 4 and/or 5

Chemically cleavable linker (hydrazone and disulfide bond)

- Hydrazone: hydrolysis under the acidic environment of the lysosome
  → Following hydrolysis, S-S reduction by glutathione
- **Issue:** Unexpected instability within circulation

2nd generation ADCs: New linker designs increased clinical success

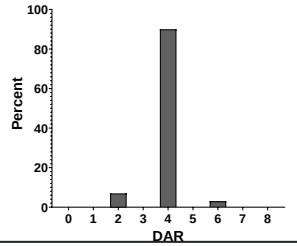


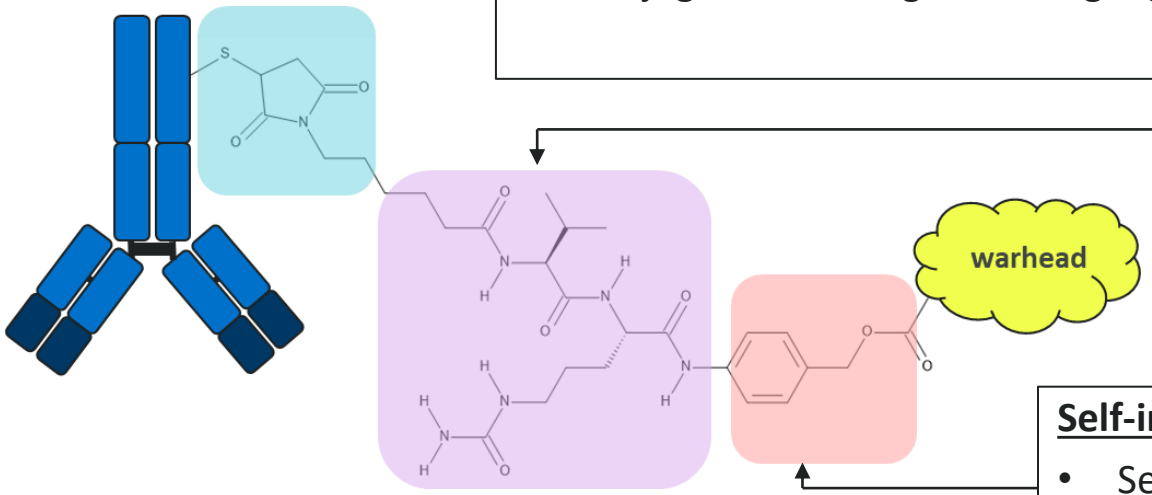
Non-cleavable linker (e.g. Kadcyla®)

- Requires complete lysosomal digestion before release
- Increased stability within circulation

Conjugation at inter-chain disulfide bonds (cysteine)

- Better control of DAR and distribution
- Conjugation in antigen binding region unlikely





Enzyme-cleavable linker (e.g. Adcetris®)

- Dipeptide and cathepsin B → specific to lysosome

Self-immolative spacer

- Self-reactive following cathepsin B digestion for complete release
- Full release of warhead → better enables bystander effect

Next generation ADCs and beyond….

New conjugation chemistries

- Site-specific: Inserted cysteines or unnatural AA's
- Branched linkers: [Anami et al., (2017) Ang Chem Inter 56:733-737]
- Non-covalent conjugations: [Gupta et al., (2019) Nat Biom Engin 3:917-929]

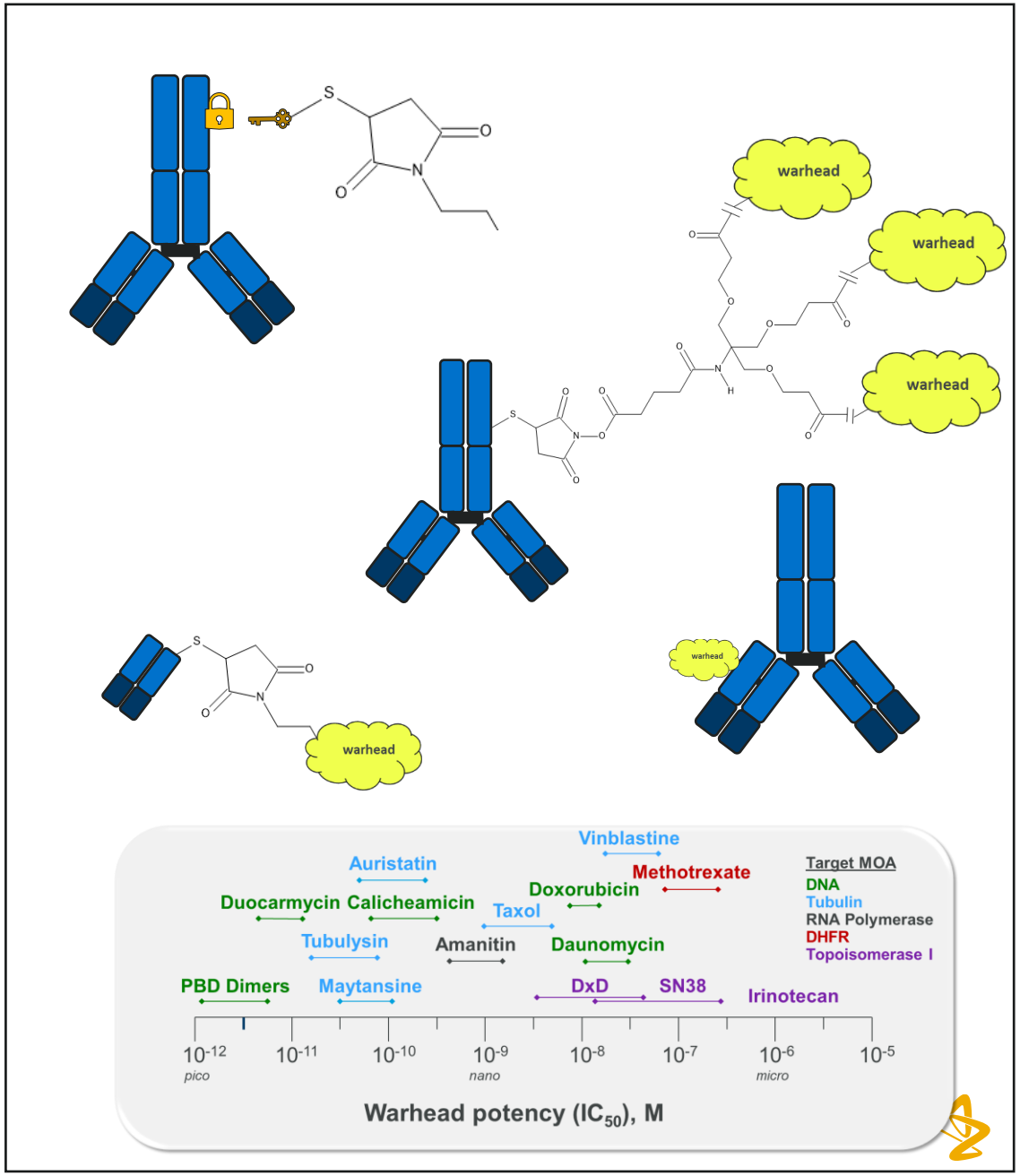
Advanced linker-released mechanisms

Non-traditional antibody formats

Growing toolbox of warheads

- Greater diversity of MoAs
- Non-cytotox MoAs (immune activators/engagers)
- Multi-warhead conjugates

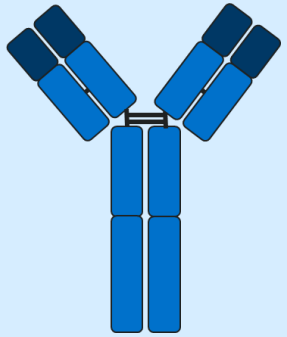
More selective target antigens for cancer or tumor microenvironment



Current CMC Bioassay Control Strategies

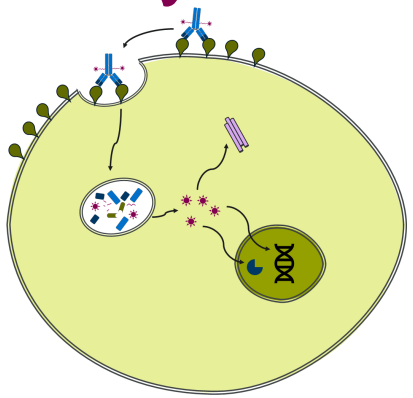
Standard GMP potency assays for ADCs: Lot-release and Stability

mAb intermediate



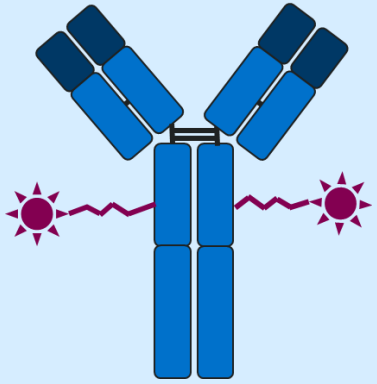
1) Target-antigen binding

- Cell or non-cell based
- Ensures expected potency before conjugation
- Common methods: Indirect or competitive ELISAs
- Common readouts: Fluorescence or colorimetric



ADC

(Drug Substance and Product)



2) Target-antigen binding

- Identical assay as mAb intermediate
- Ensures conjugation does not impact target binding
- Request removal from spec at marketing application

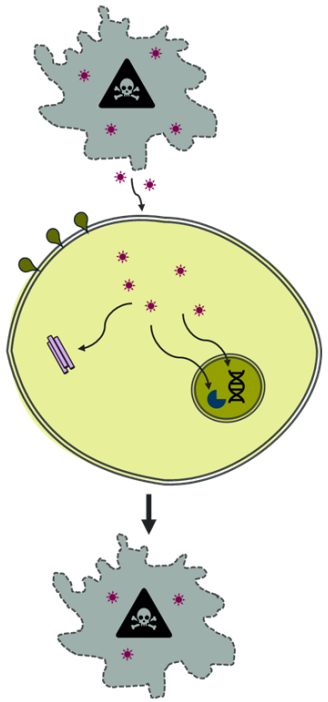
3) Cytotoxicity assay

- Cell-based
- Common endpoints: ATP production, membrane integrity, caspase activity
- Common readout: Luminescence or colorimetric

ADCs can possess secondary MoAs and other biological activities

CMC control not required

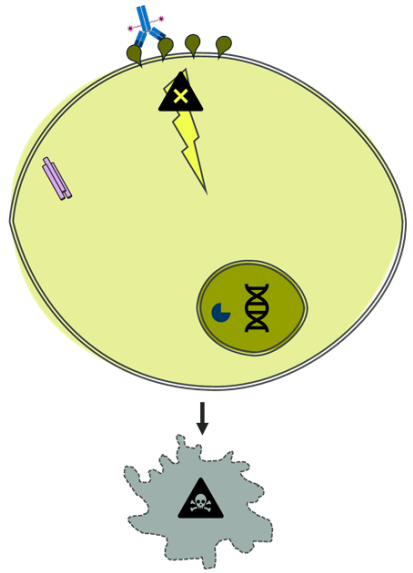
1. Bystander effect



- Can contribute to overall efficacy
- Downstream of CMC control
- Lot-release cytotoxicity assay likely captures this mechanism

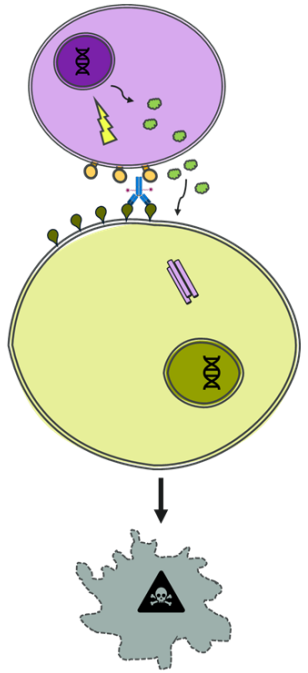
CMC control may be required

2. Antigen signaling inhibition



- Can contribute to overall clinical efficacy
- Lot-release binding assays inherently control for this mechanism.
- Cytotoxicity assay may capture mechanism too

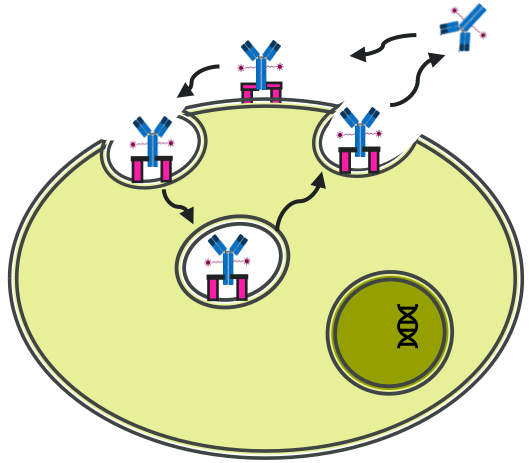
3. Fc-effector functions



- May contribute to overall clinical efficacy
- If relevant, additional assays required for characterization

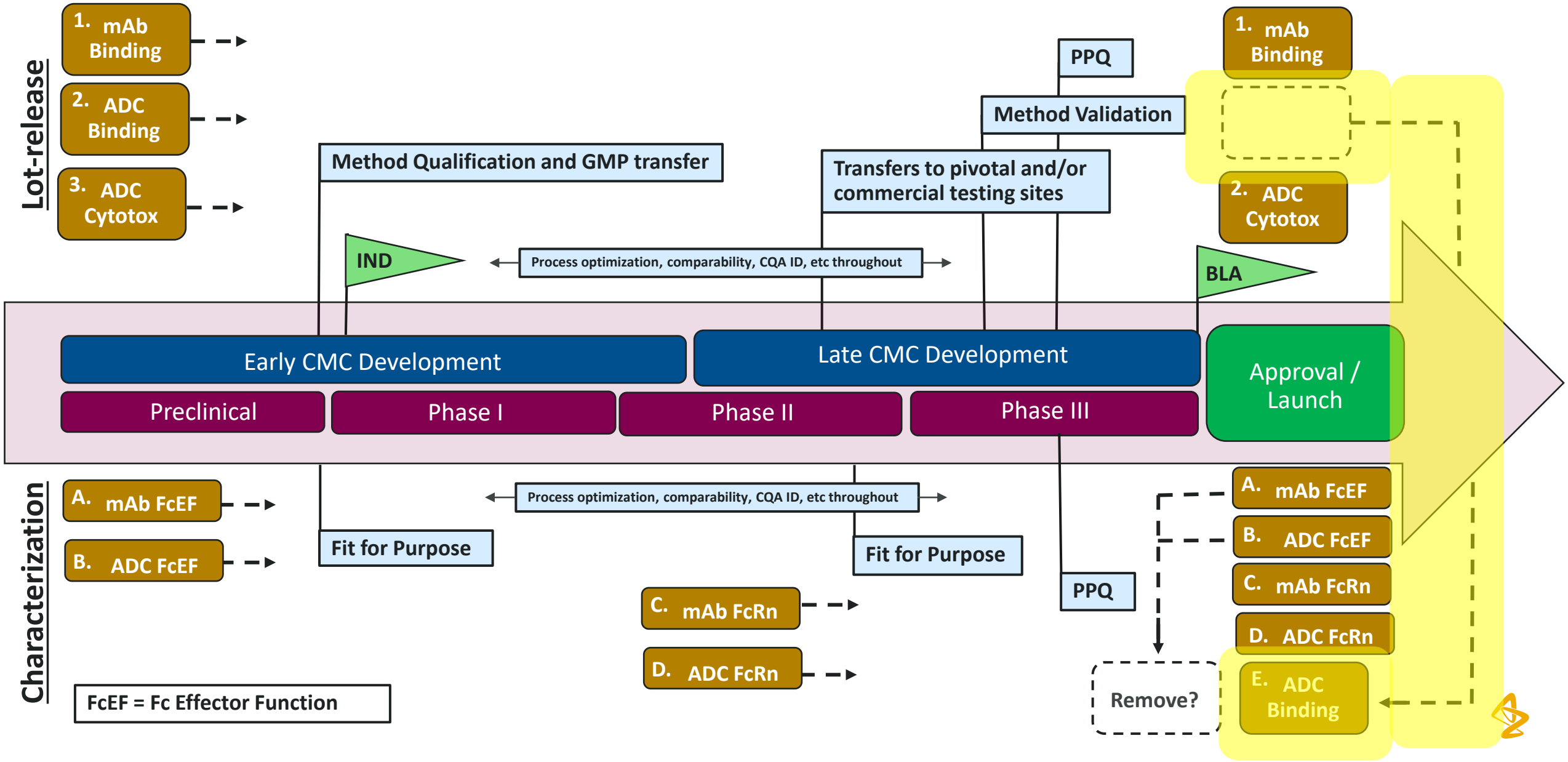
CMC control required

4. FcRn Binding



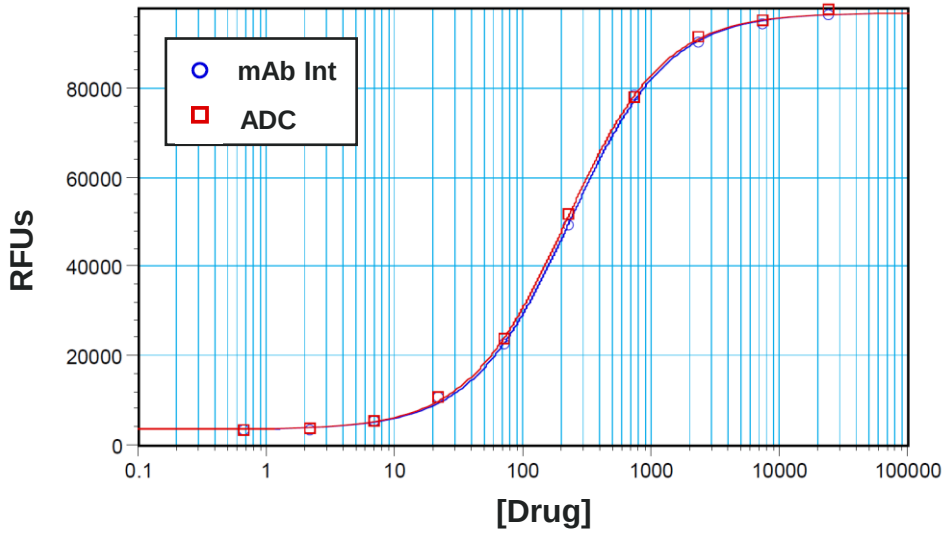
- Surrogate for ensuring consistent PK
- FcRn binding assay required for characterization

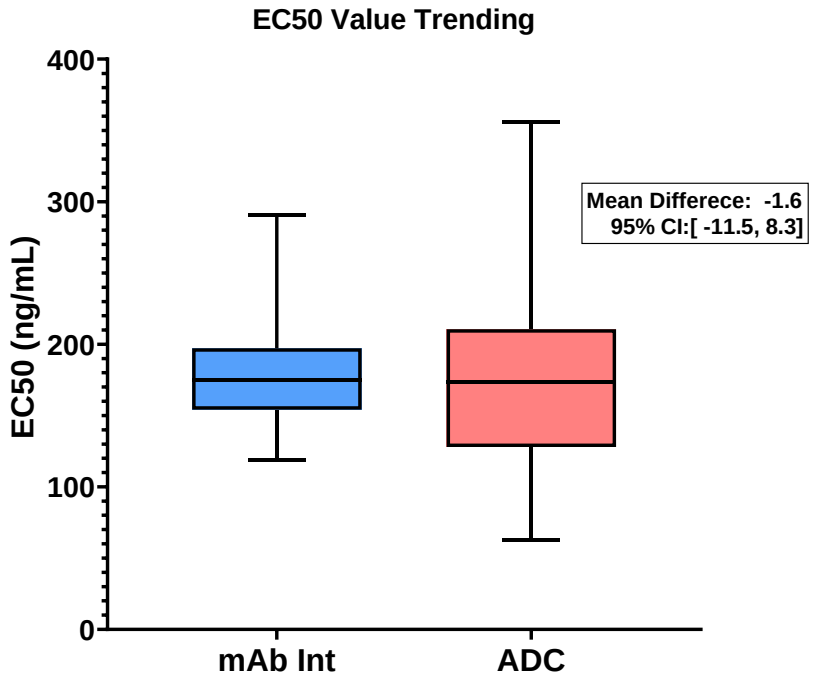
Current Bioassay Control Strategy



Justifications for removing the ADC binding assay from specification

1. **Conjugation does not impact target binding**
   - Head-to-head comparison in assay
   - Comparison of trending data

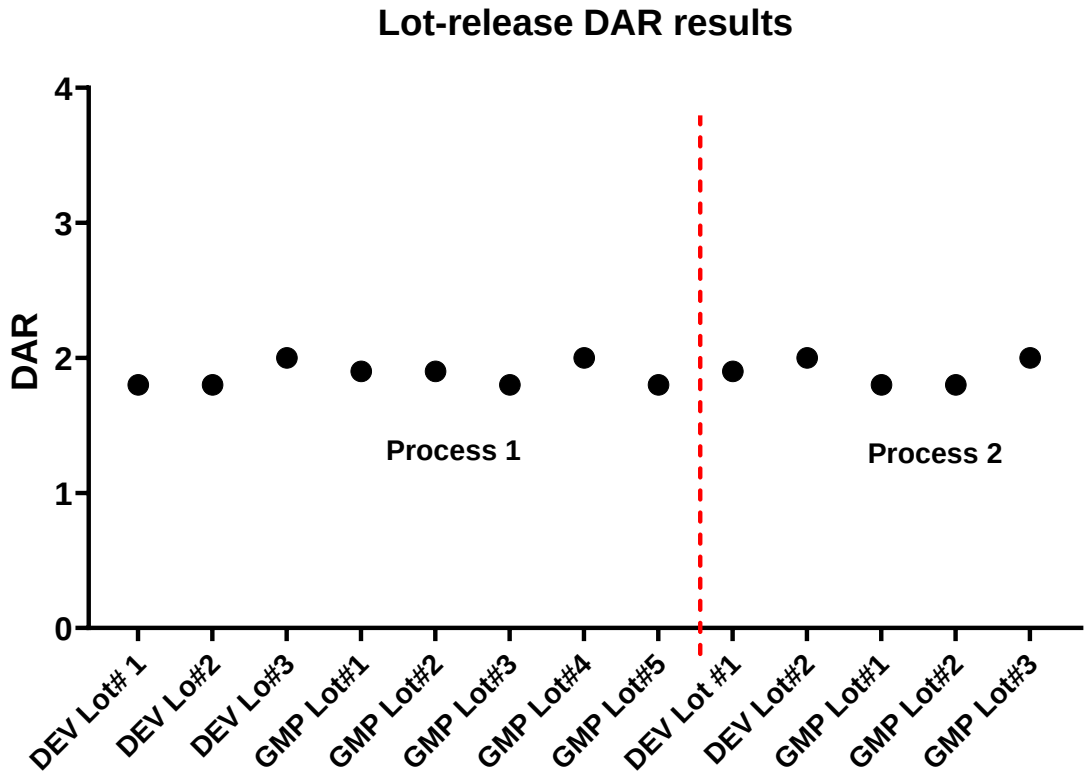




Justifications for removing the ADC binding assay from specification

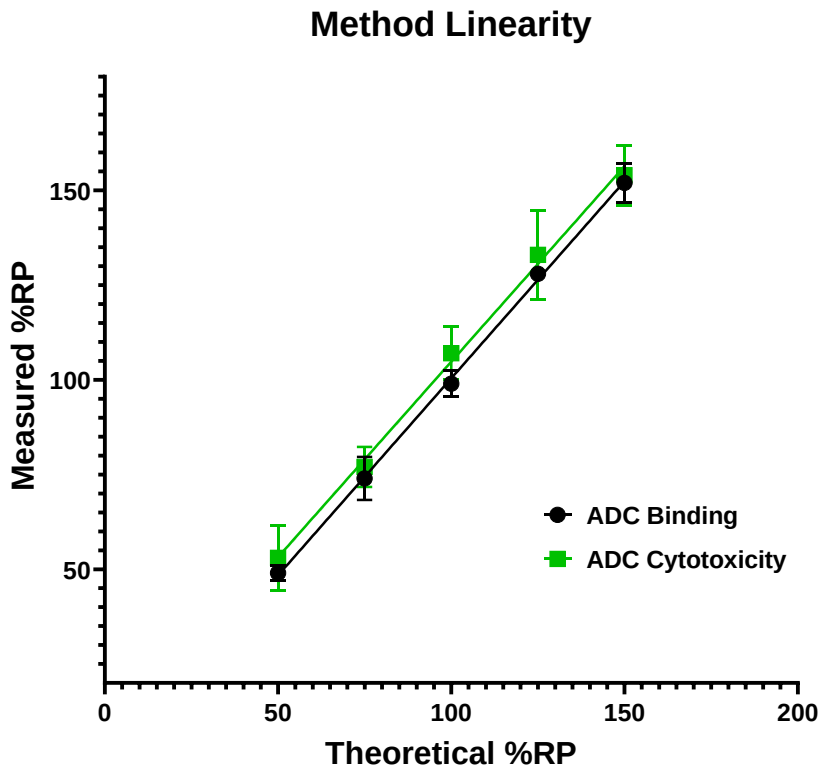
1. **Conjugation does not impact target binding**

2. **Conjugation process is well-controlled**
   - Charting of DAR



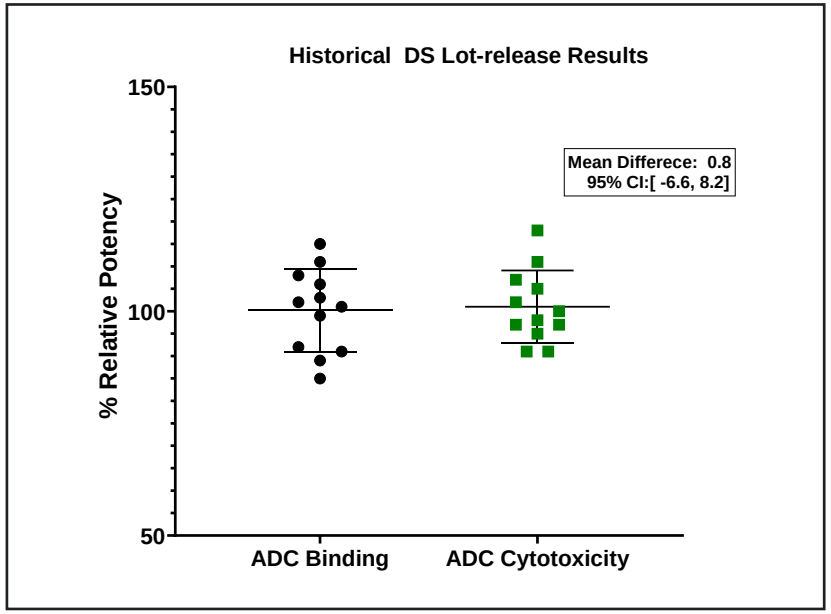
Justifications for removing the ADC binding assay from specification

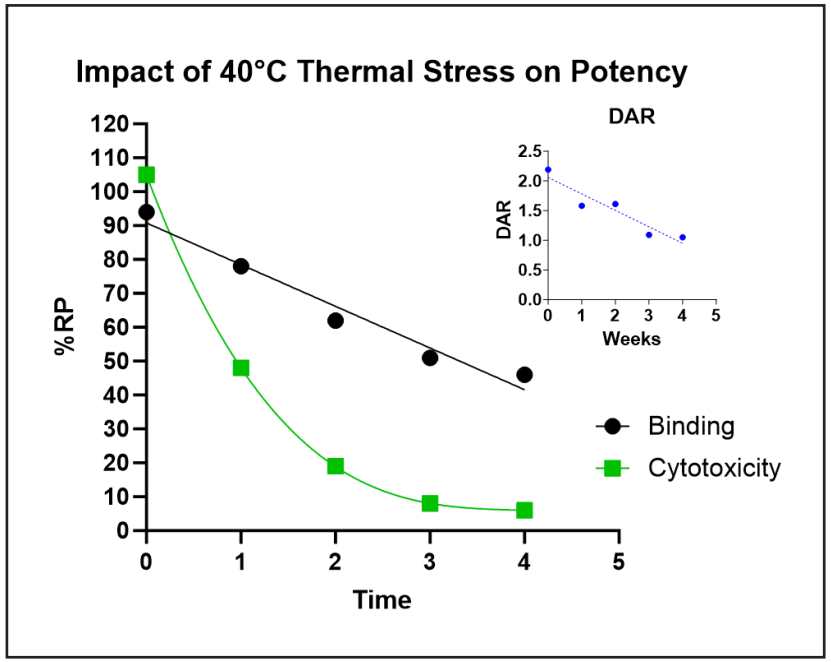
1. **Conjugation does not impact target binding**
2. **Conjugation process is controlled**
3. **Performance of ADC cytotoxicity and binding assays are comparable**
   - Accuracies and linearity are comparable
   - Leverage qualification, validation, and trending data



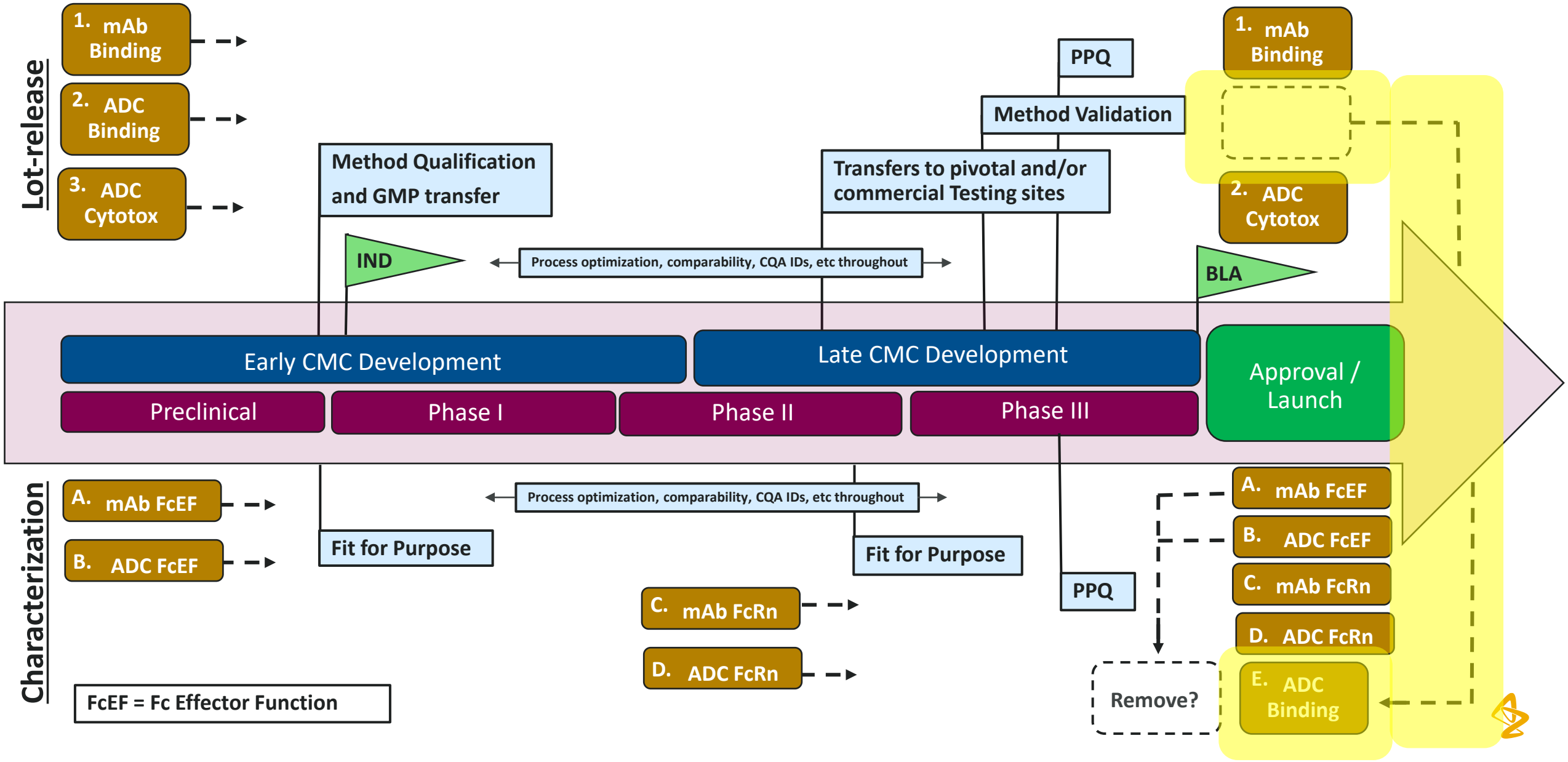
Justifications for removing the ADC binding assay from specification

1. **Conjugation does not impact target binding**
2. **Conjugation process is controlled**
3. **Performance of ADC cytotoxicity and binding assays are comparable**
4. **“Linkage” between ADC cytotoxicity and binding assays**
   - Historical lot-release data is comparable.
   - Stability testing trends are comparable, or cytotoxicity assay is more sensitive at detecting degradation.

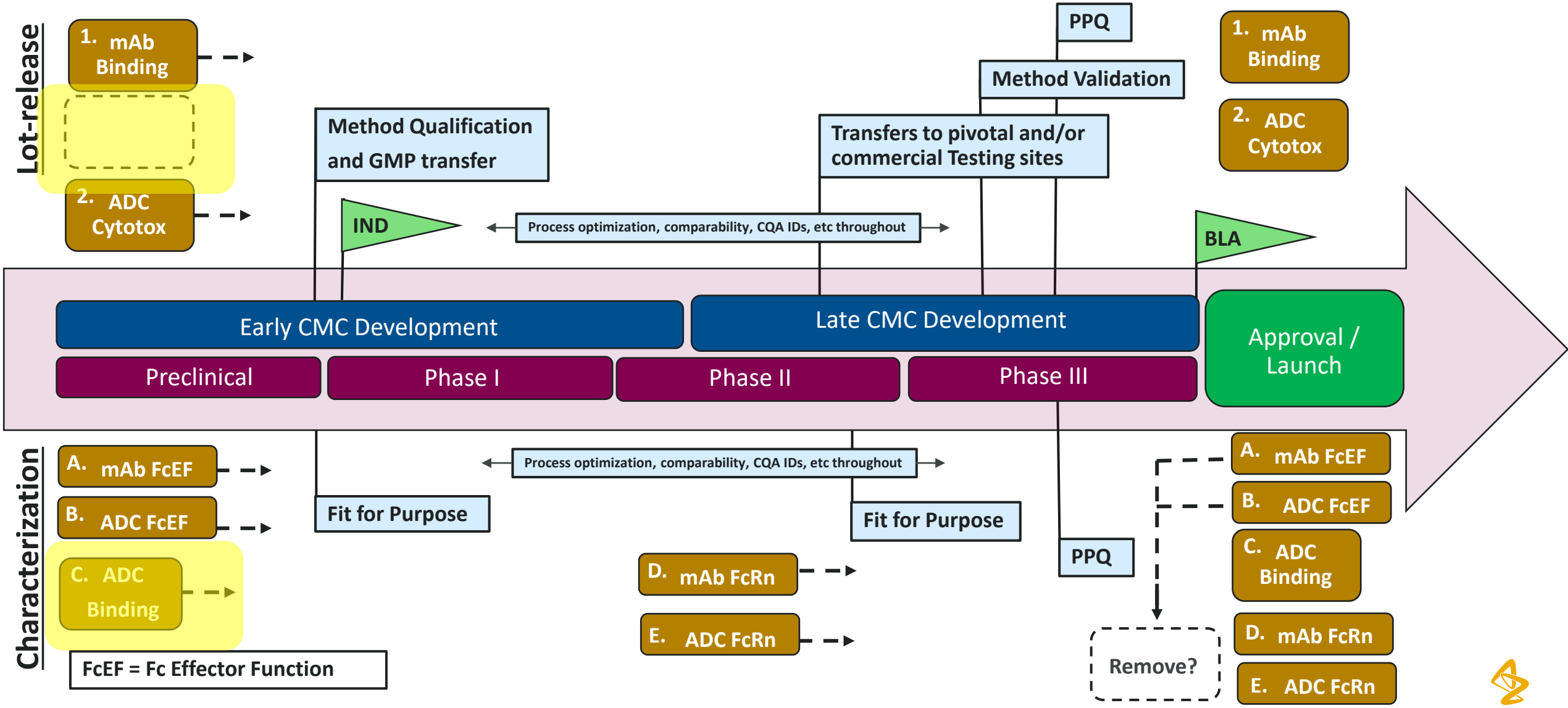




Current Bioassay Control Strategy Through Drug Development



Considering the evolution of ADCs, is the ADC binding assay redundant from the get-go?



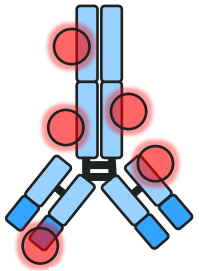
Adapting CMC Bioassays: Simplification

Is the ADC binding assay redundant?

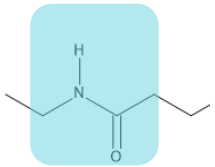
Purpose of target-antigen binding is to ensure conjugation does not impact target-antigen binding; however…

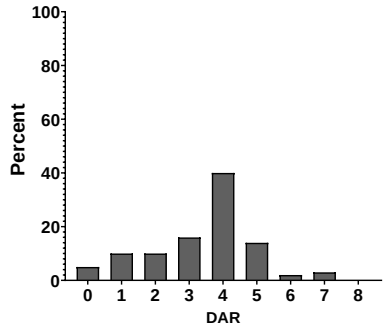
1. **Conjugation of modern ADCs is more controlled, and specific to sites located away from antigen binding regions**
   - Impact to target antigen binding is inherently minimized within new conjugation strategies

1st Generation

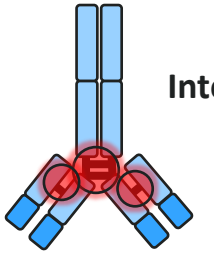


Lysine

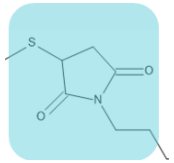




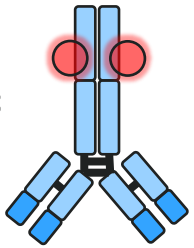
2nd and Next Generation

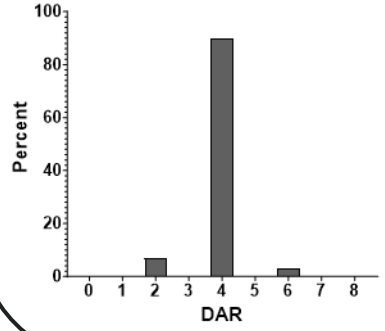


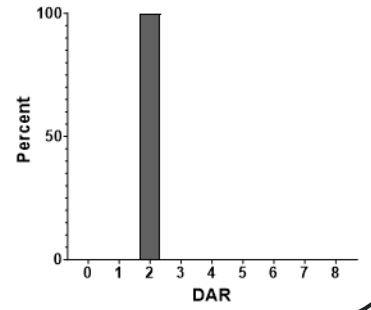
Interchain S-S



Site Specific



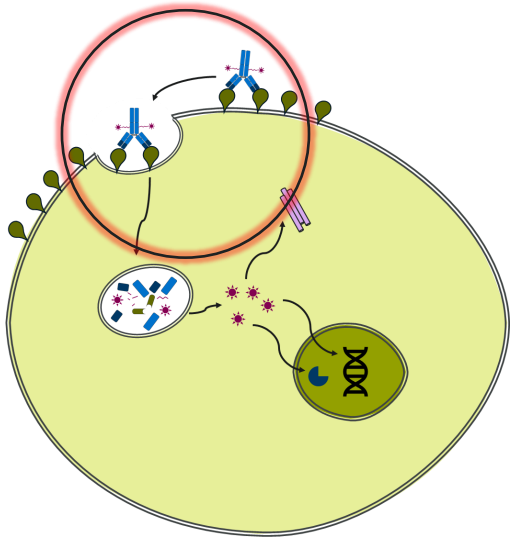




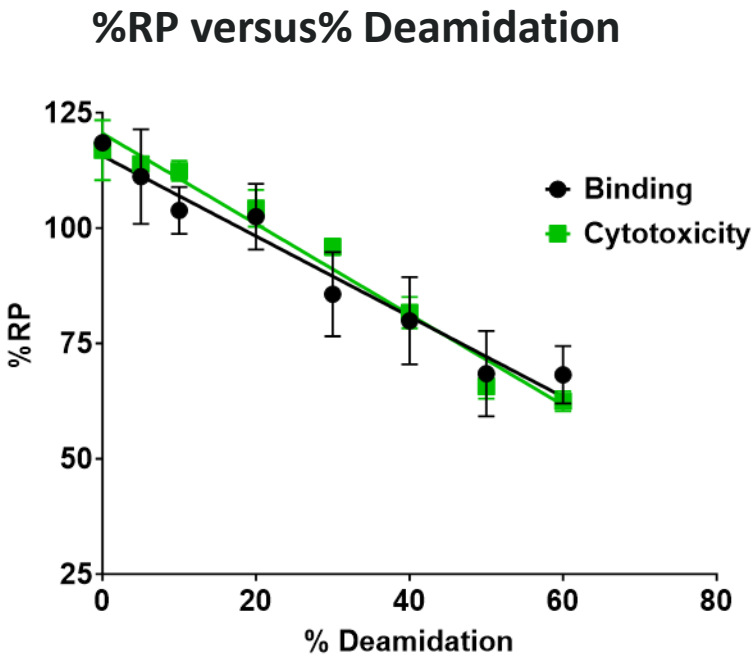
Is the ADC binding assay redundant?

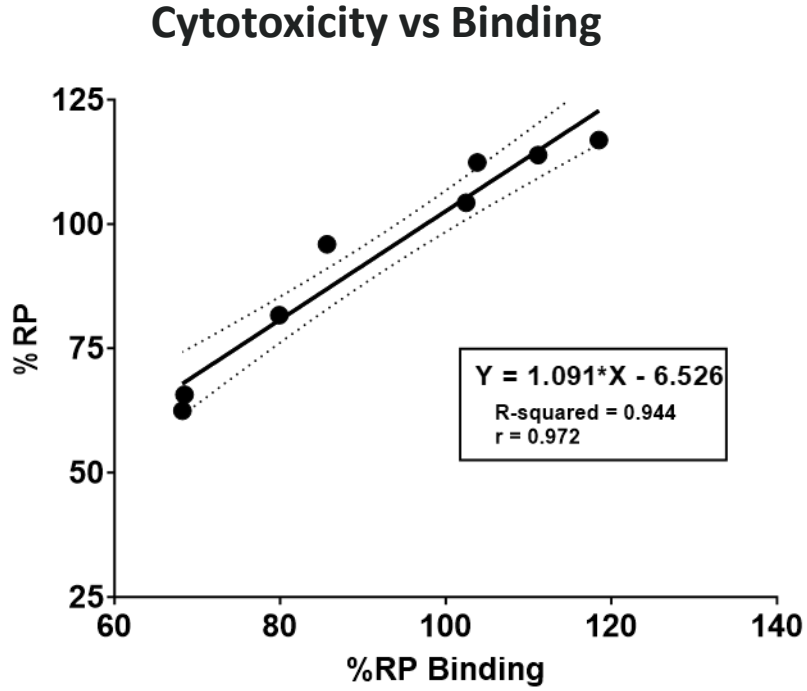
Purpose of target-antigen binding is to ensure conjugation does not impact target-antigen binding; however…

1. Conjugation of modern ADCs is more controlled, and specific to sites located away from antigen binding regions
2. **ADC cytotoxicity assay is fully MoA-reflective, and is dependent on target antigen binding**
   - Cytotoxicity assay is intrinsically sensitive to changes in ADC binding affinity, as well as payload/DAR



Impact of N102 Deamidation to ADC-A Biological Activities





Cao et al., (2023) Antibodies, 12(4):68

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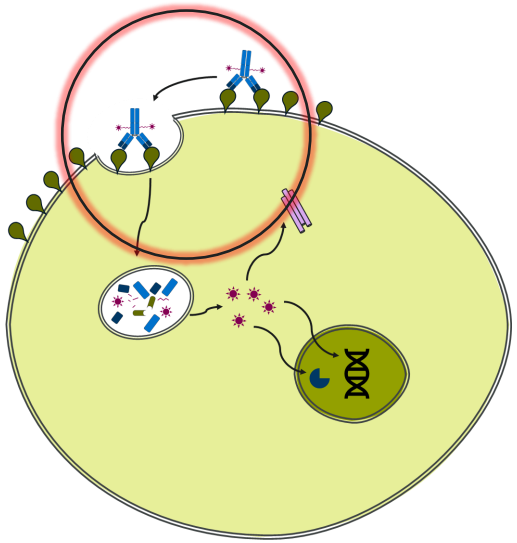
2. **ADC cytotoxicity assay is fully MoA-reflective, and is dependent on target antigen binding**
   - Changes in binding affinity impact ability/rate of ADC internalization, and thus payload delivery



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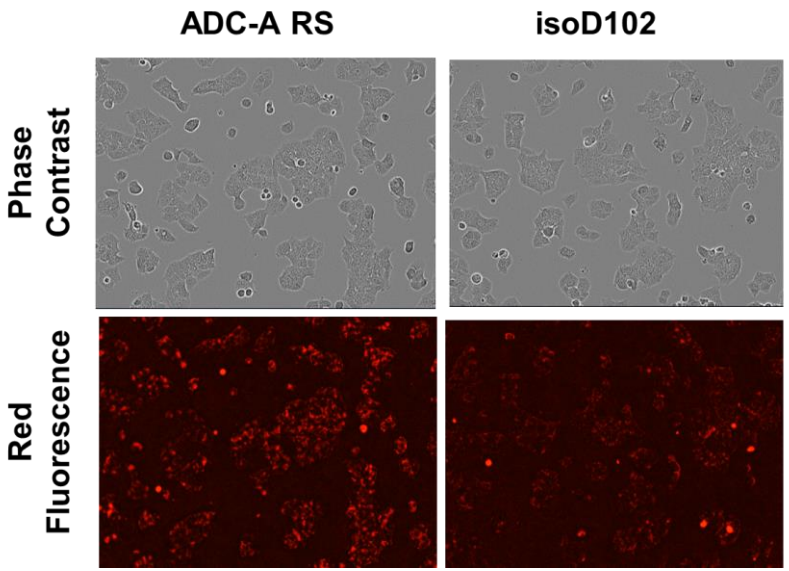

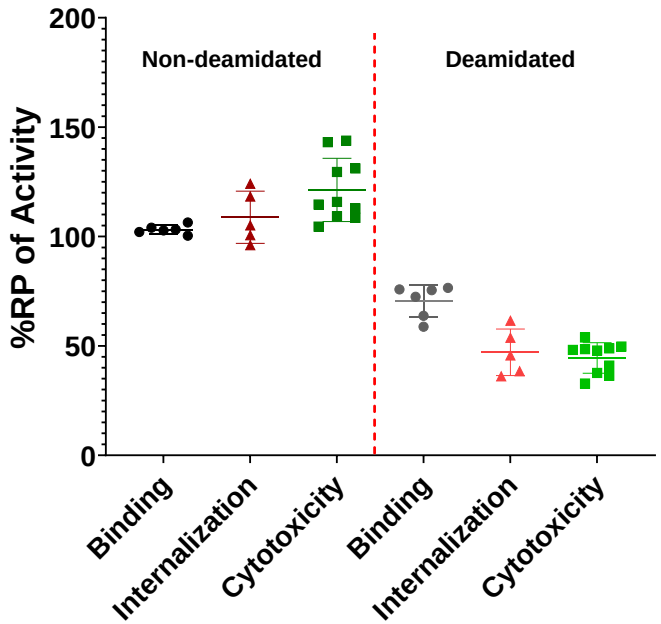

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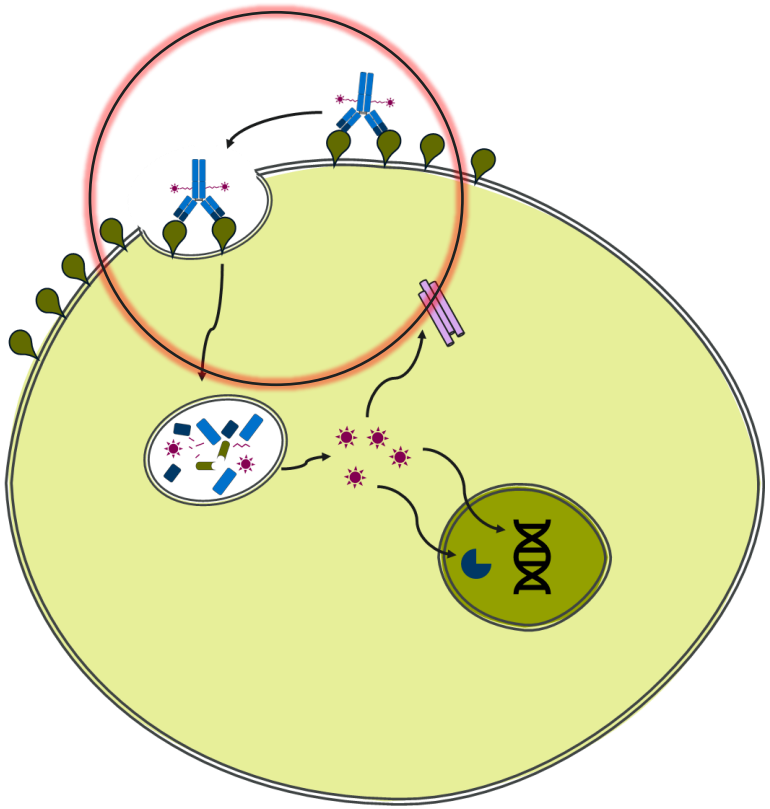
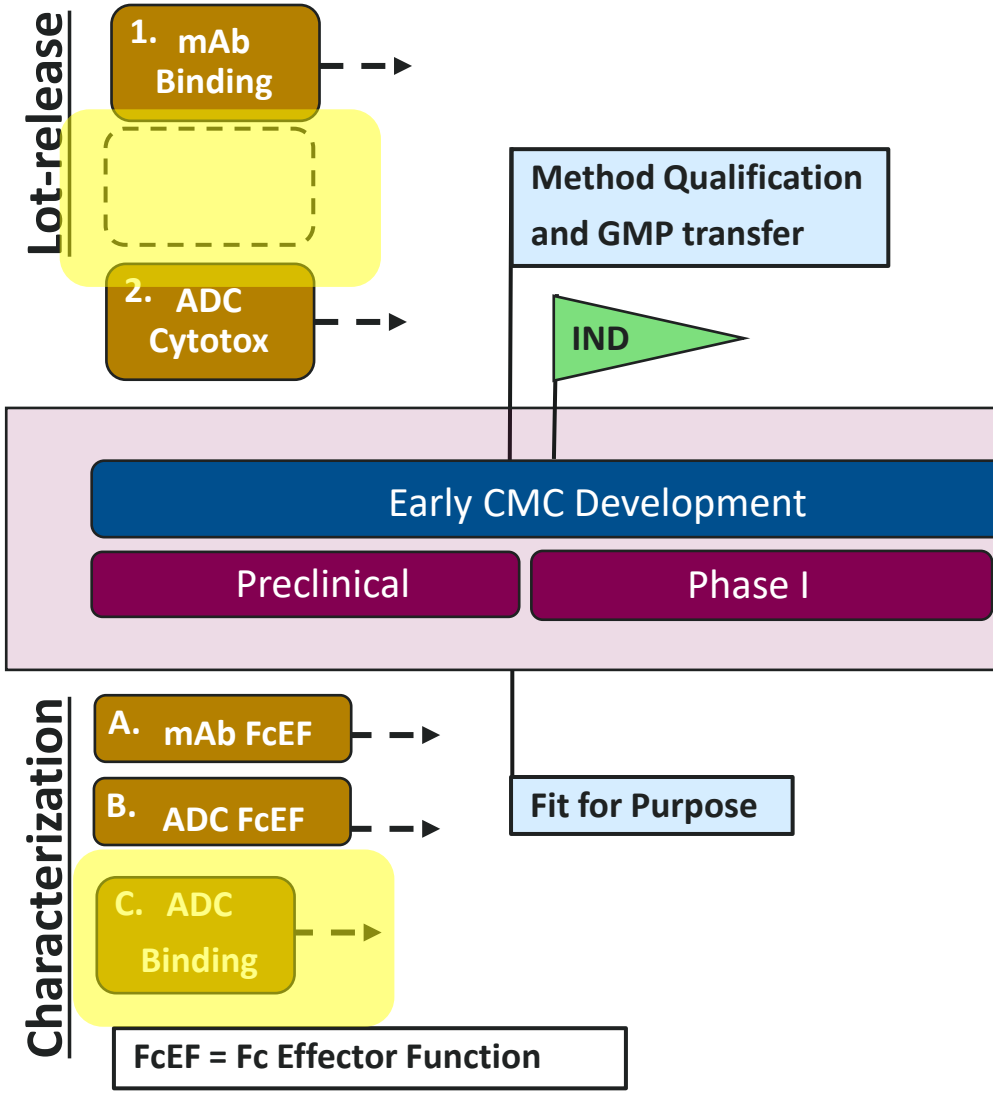
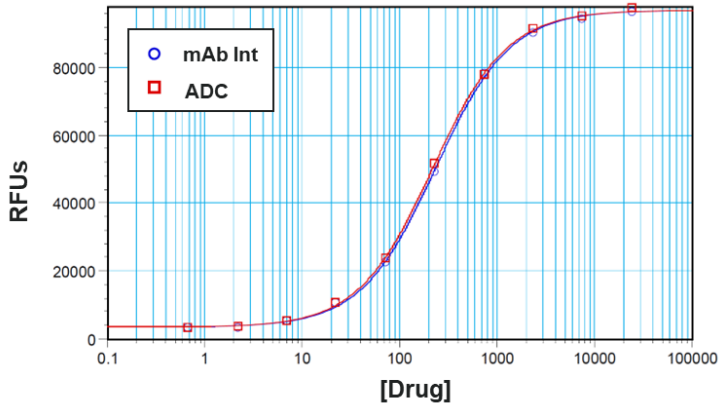


ADC binding assay is redundant to the cytotoxicity assay on lot-release, as ADC-antigen binding and cytotoxicity are inherently connected

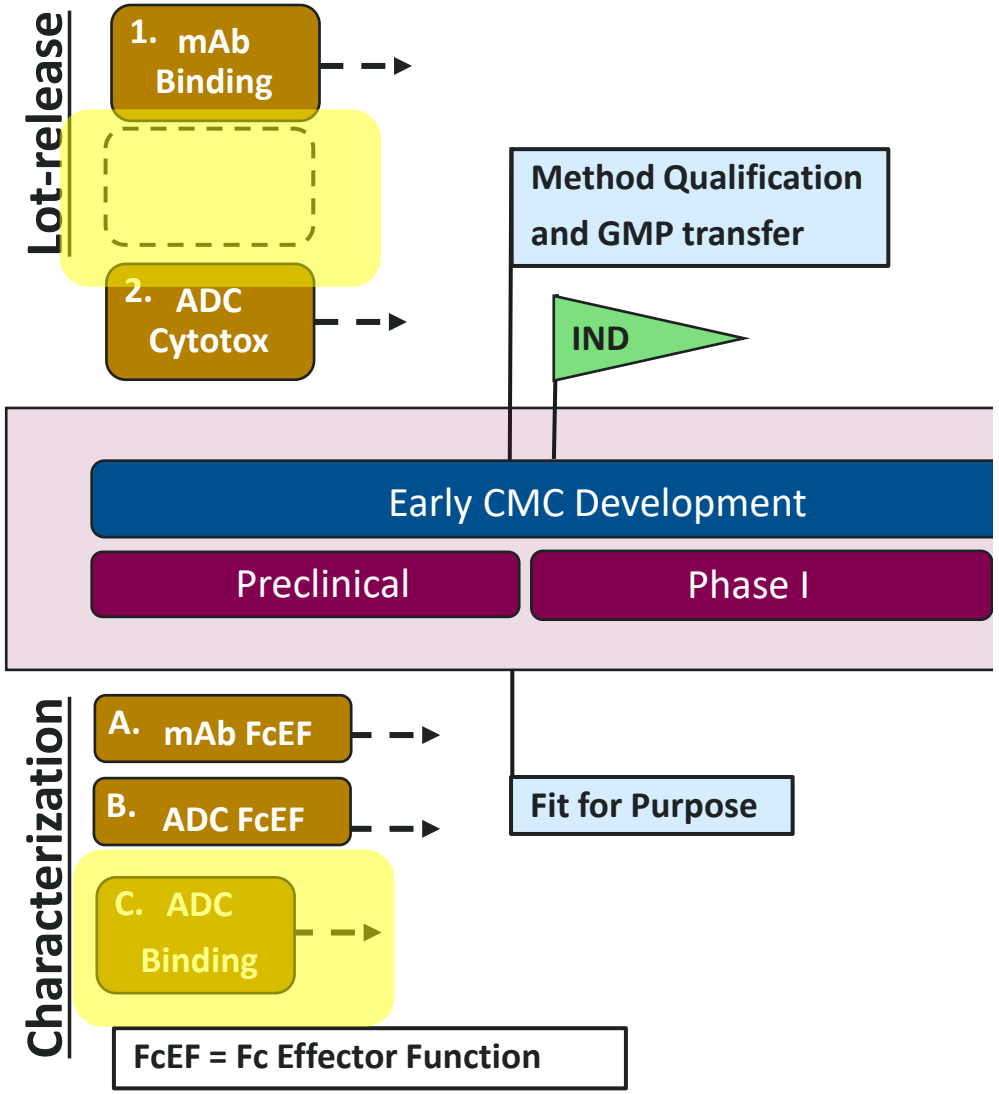
Potential strategy for not including ADC binding on lot-release from get-go



1. Demonstrate conjugation minimally impacts target binding

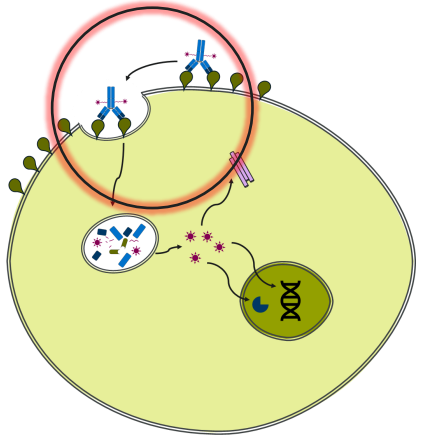


Potential strategy for not including ADC binding on lot-release from get-go



1. Demonstrate conjugation minimally impacts target binding

2. Defend that cytotoxicity assay is fully MoA-reflective and is intrinsically dependent on binding
   - Assay inherently sensitive to changes in antigen binding



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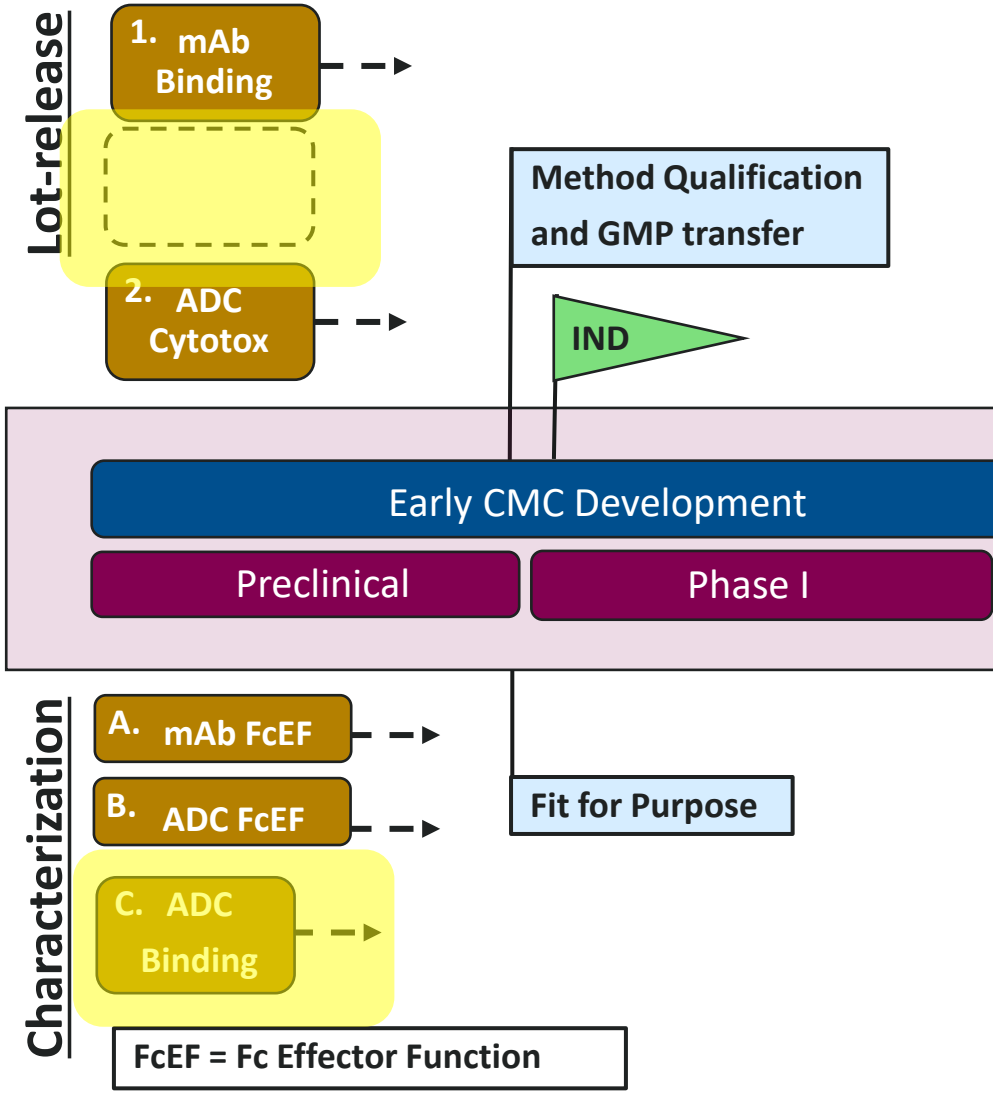

1. **Demonstrate conjugation minimally impacts target binding**

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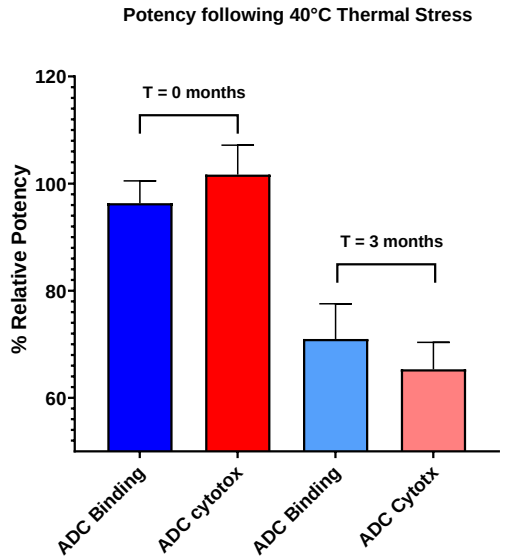
3. **Leverage early data linking binding to cytotoxicity, if needed and if available.**



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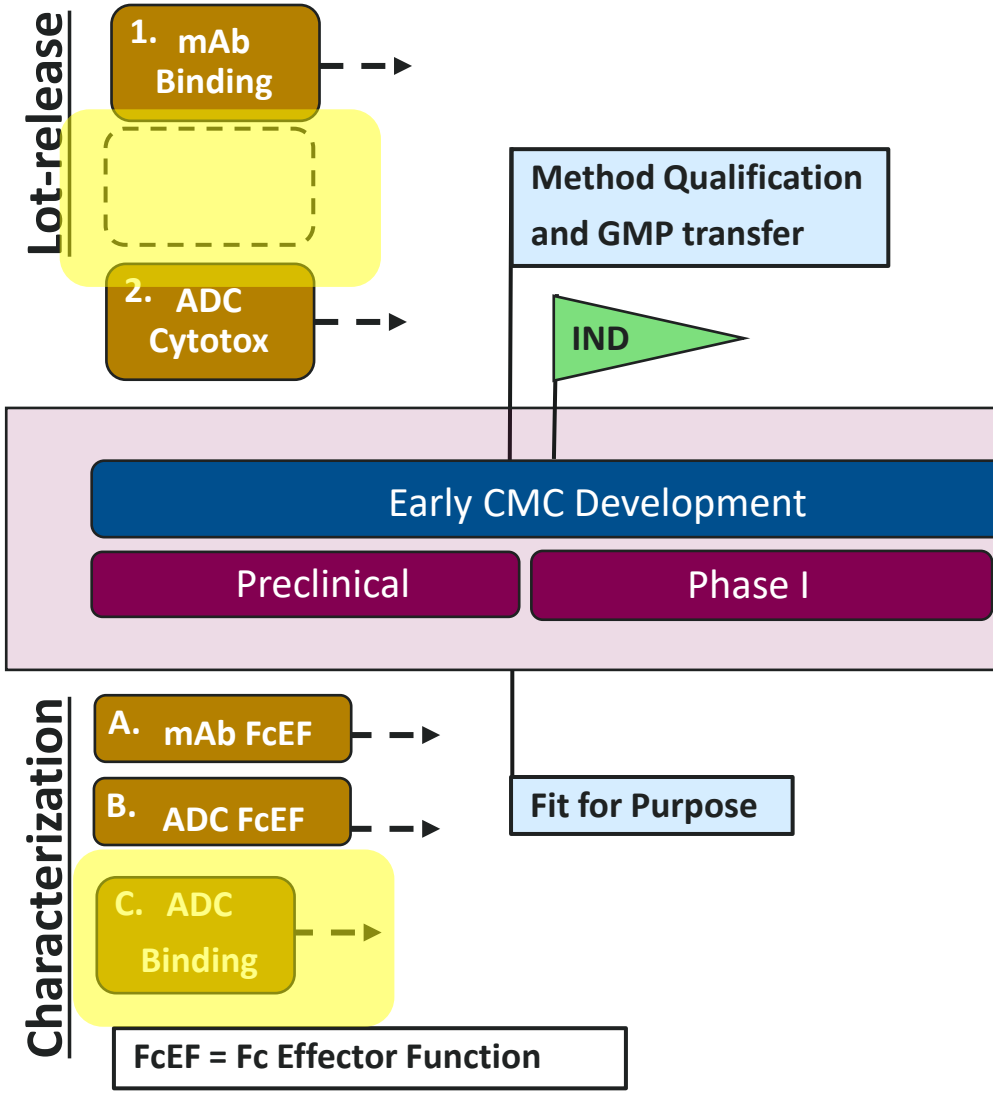

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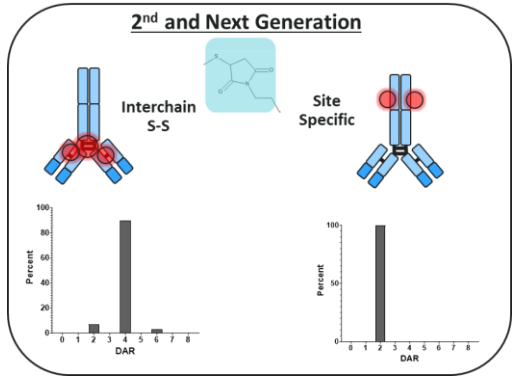
4. **Speak to control of conjugation strategy**



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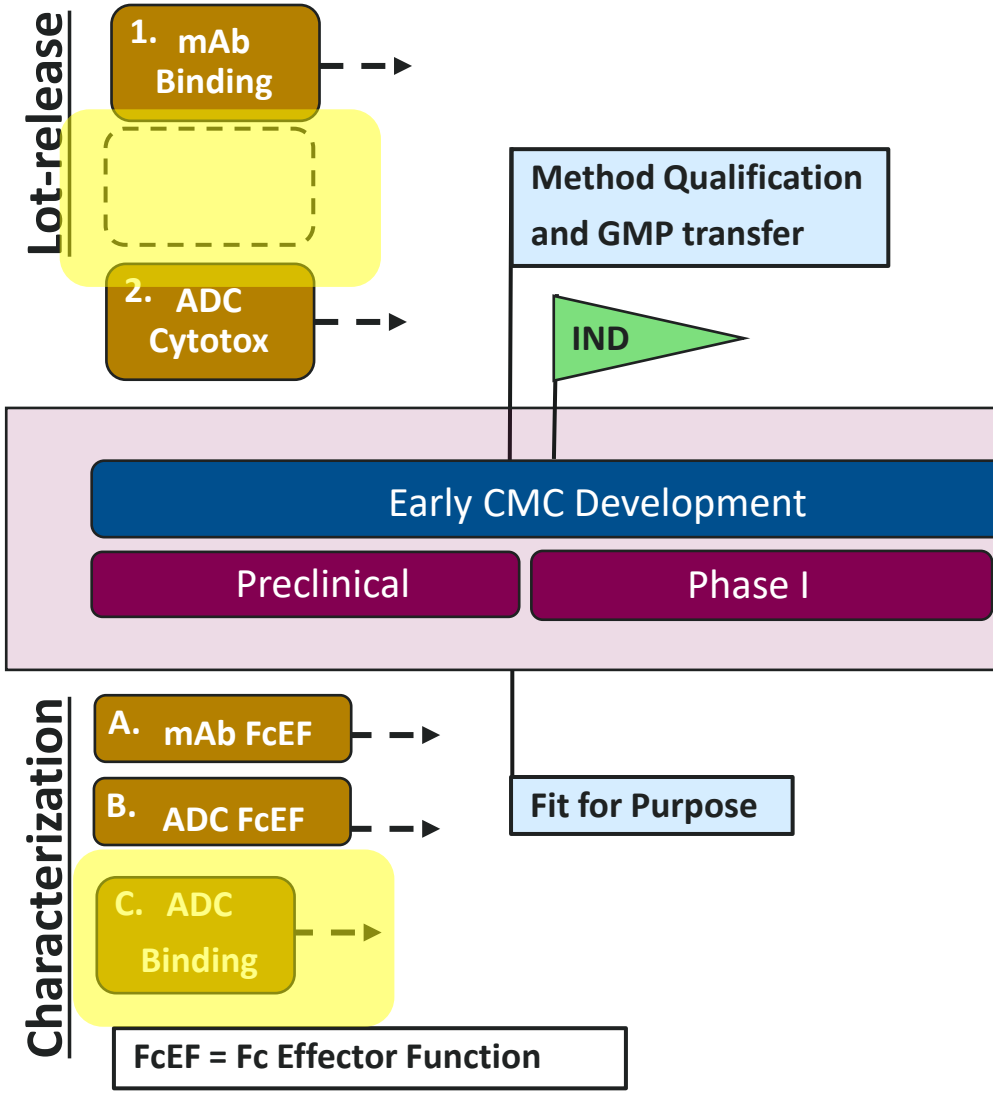

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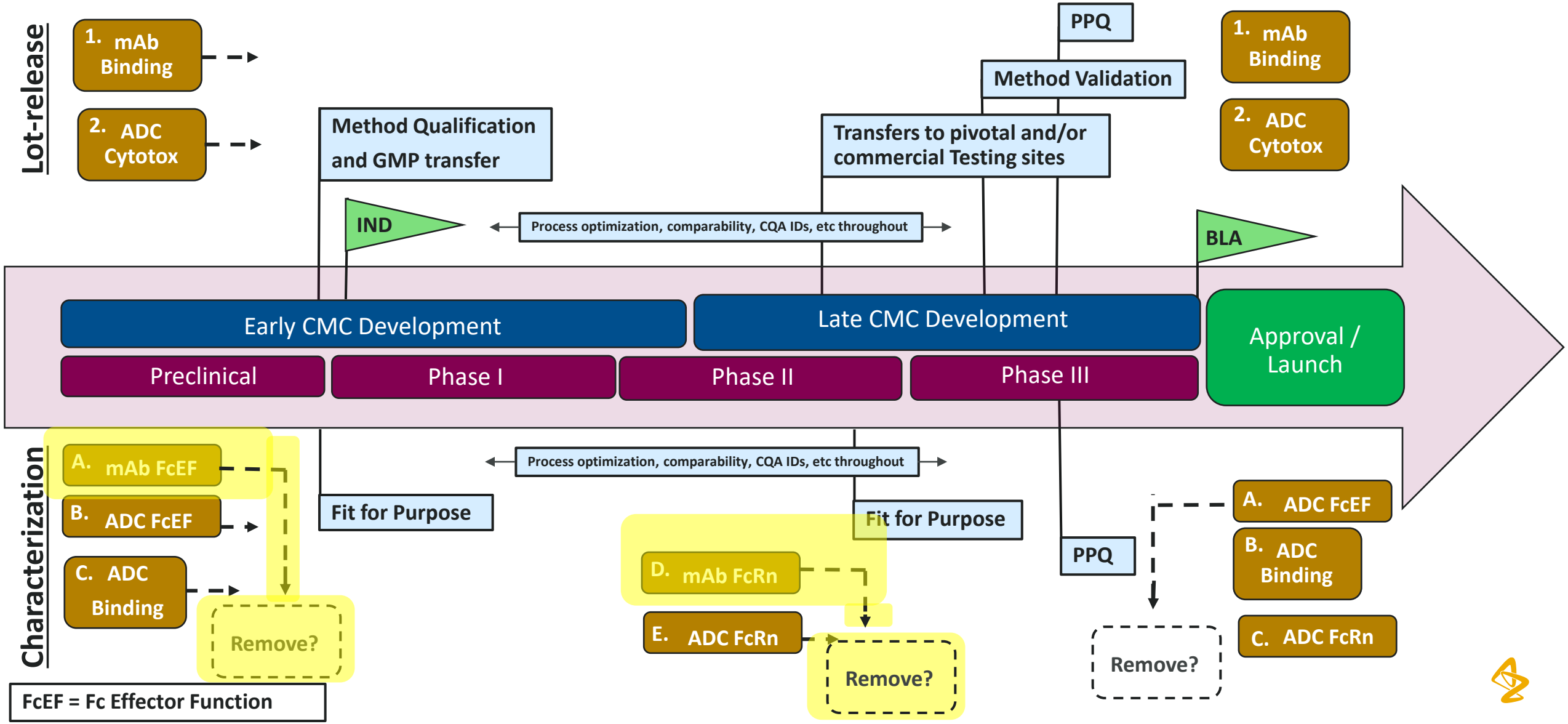
2. Defend that cytotoxicity assay is fully MoA-reflective and is intrinsically dependent on binding
   - Assay inherently sensitive to changes in antigen binding
3. Leverage early data linking binding to cytotoxicity, if needed and if available.
4. Speak to control of conjugation strategy
5. Remind that binding is on control strategy as characterization assay
   - Binding will be measured for future comparability, implementation of new reference standards, etc.

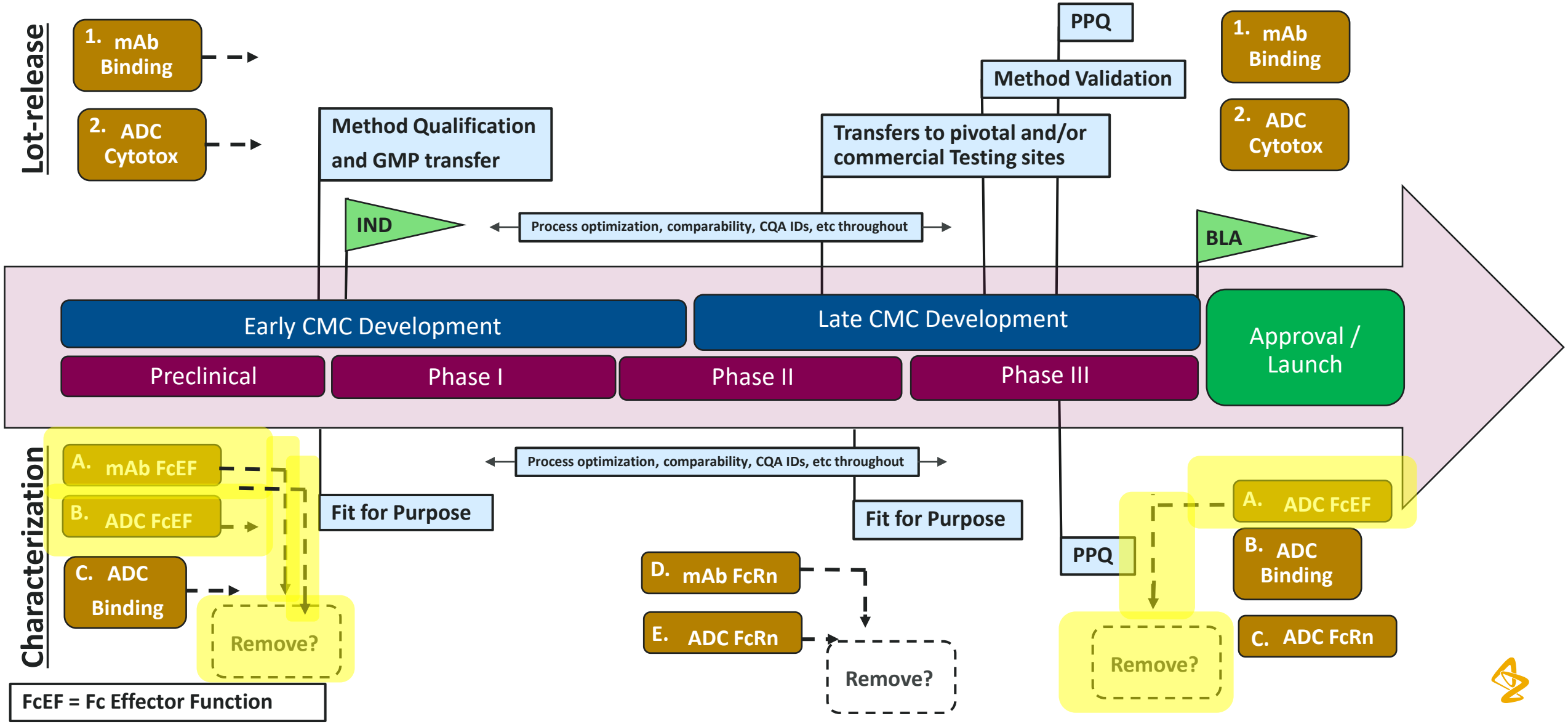
Further areas of simplification?

Do characterization assays/testing for mAb intermediate need to be as extensive as the ADC, when mAb intermediate is never administrated to patients?



Further areas of simplification?

How relevant are in vitro FcEF activities of ADC molecules to in vivo/clinical efficacy?



FcEF = Fc Effector Function

Conclusions

Overall Conclusions

Recent advances in ADC engineering are increasing their success in the clinic

CMC will need to adapt to modern ADCs and increased pipeline. Bioassay is no exception

Current and next generation ADCs may allow areas of simplification to CMC bioassay strategies

- Both ADC binding and cytotoxicity assays on spec. for lot-release may be considered redundant
- Do all characterization assays (e.g. FcRn, FcEFs) for the ADC need to be applied to the mAb intermediate? When does over-characterization of the mAb intermediate contradict its status as an “intermediate”.

Simplification of bioassay strategies affords more time to overcome new challenges with key assays

- Example: Diversity of warheads with different MoAs of inducing cell death, as well as newer payload/cleavable moieties, can make development of lot-release cytotoxicity assays more challenging.

Acknowledgements

BPD-CMC

Michaela Wendeler

Niluka de Mel

Rachel Mowery

Chunlei Wang

Paul Santacroce

Zubair Bhuiyan

Linan Ha

Bioassay Development

Arsala Wallace

Deepa Vedam

Michael Hufker

Erin Clausen

Jaytee Sonawane

Christina Grigoriadou

Scott Umlauf

Research/ADPE

Trinity Perry

Krista Kinneer

Thank you