Challenges of developing an ADA assay for Bispecific antibody therapeutic and further ADA Characterisation

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Overview

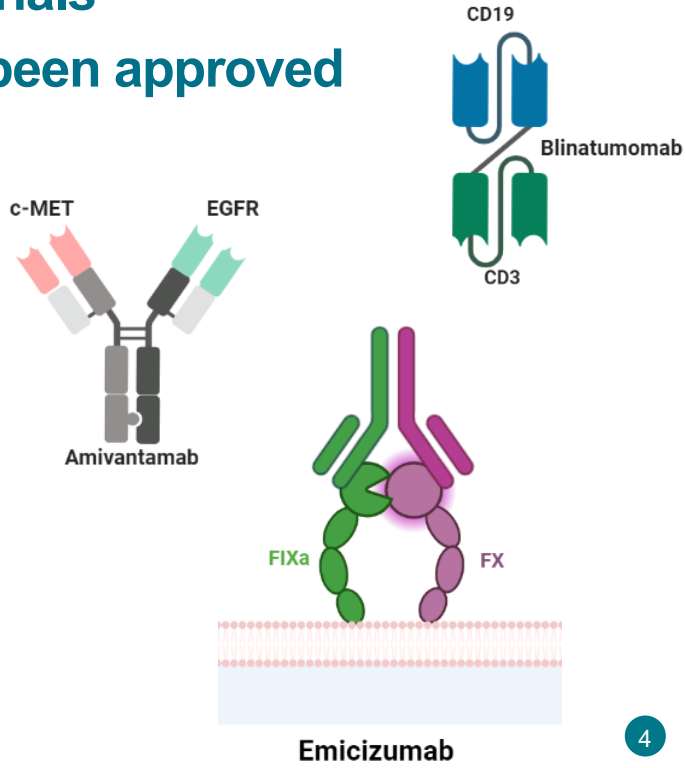
- Introduction
- Bispecific ADA assay approach
- Assay development tool kit
- Single vs multiple domain comparison
- Case study
- Other consideration

Introduction

- **Bispecific refer to compounds that binds to two sites (epitopes)**
- **Introduced in 1960 by Nisonoff et al, but the idea had to wait until 1975 for the invention of hybridoma**
- **There are mainly two type:**
  - IgG Like
  - Non-IgG Like
    - Bites (bispecific T-cell engager)
    - DART (Dual-affinity Re-targeting Antibody)

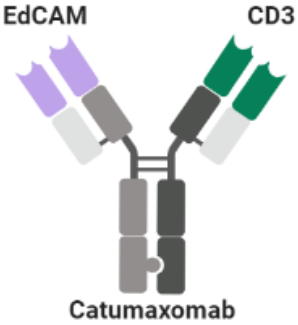
BsAbs potential

- **Over 100 BsAbs at various stage of clinical trials**
- **3 BsAbs with different mode of action have been approved**
- **Cis (same cell)**
  - Amivantamab used for non-small cell lung cancer
- **Trans (two different cells)**
  - Blinatumomab used for acute lymphoblastic leukemia
- **Endogenous target**
  - Emicizumab a FVIII replacement for Haemophilia A

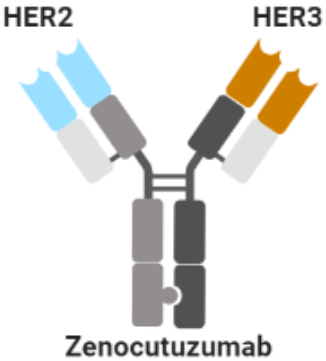


Example of BsAbs half-life

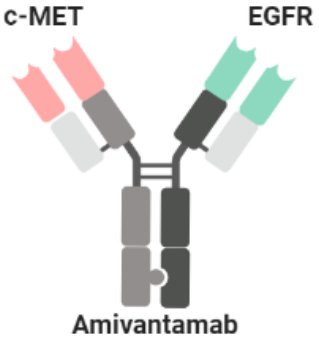
$t^{1}/_{2}$: 2.11 hrs



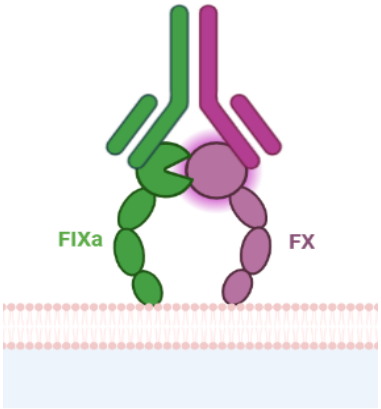
$t^{1}/_{2}$: 2.5 days



$t^{1}/_{2}$: 4.6 days



$t^{1}/_{2}$: 11.3 days



Emicizumab

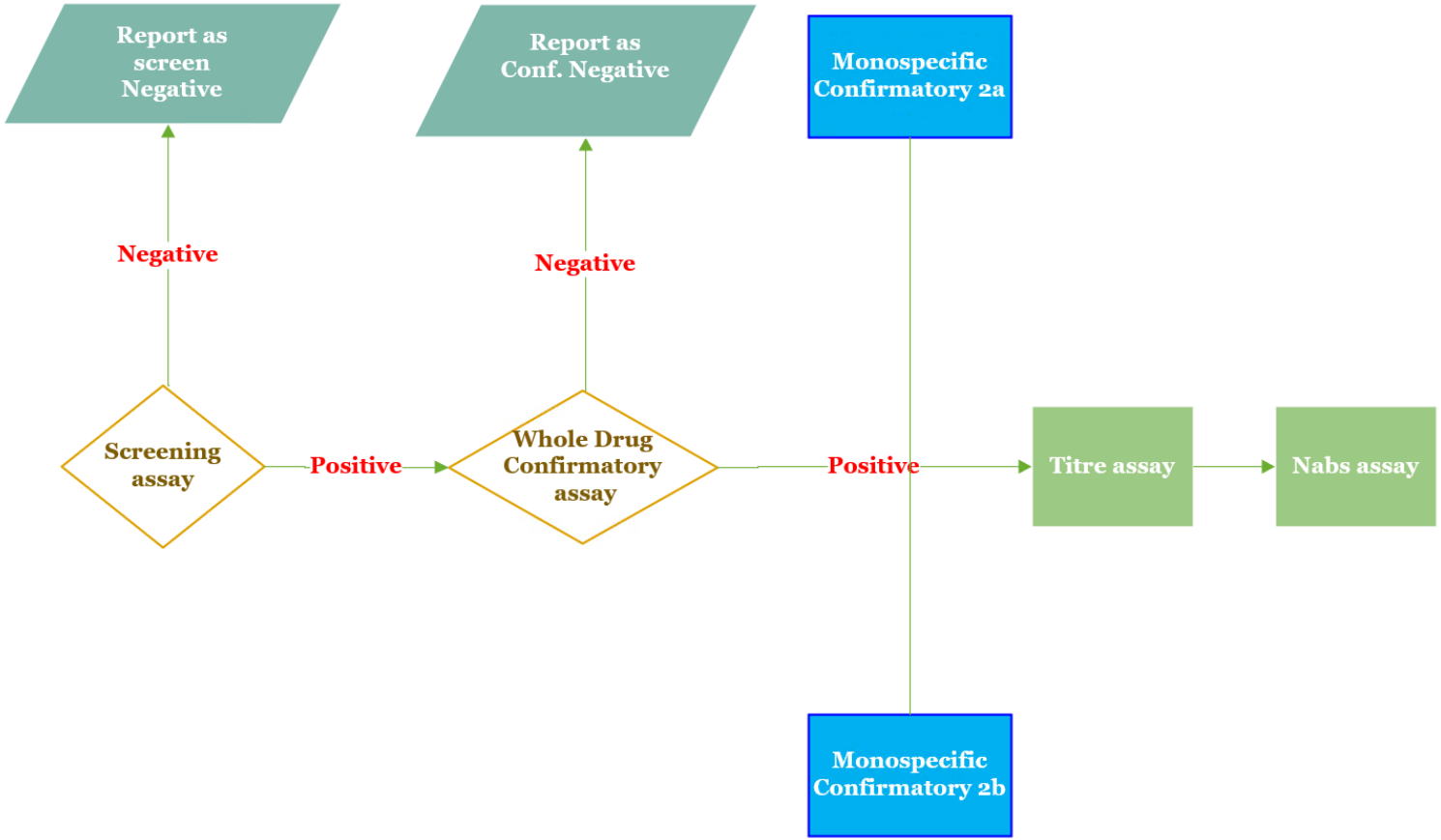
$t^{1}/_{2}$: 4-5 weeks

Guideline

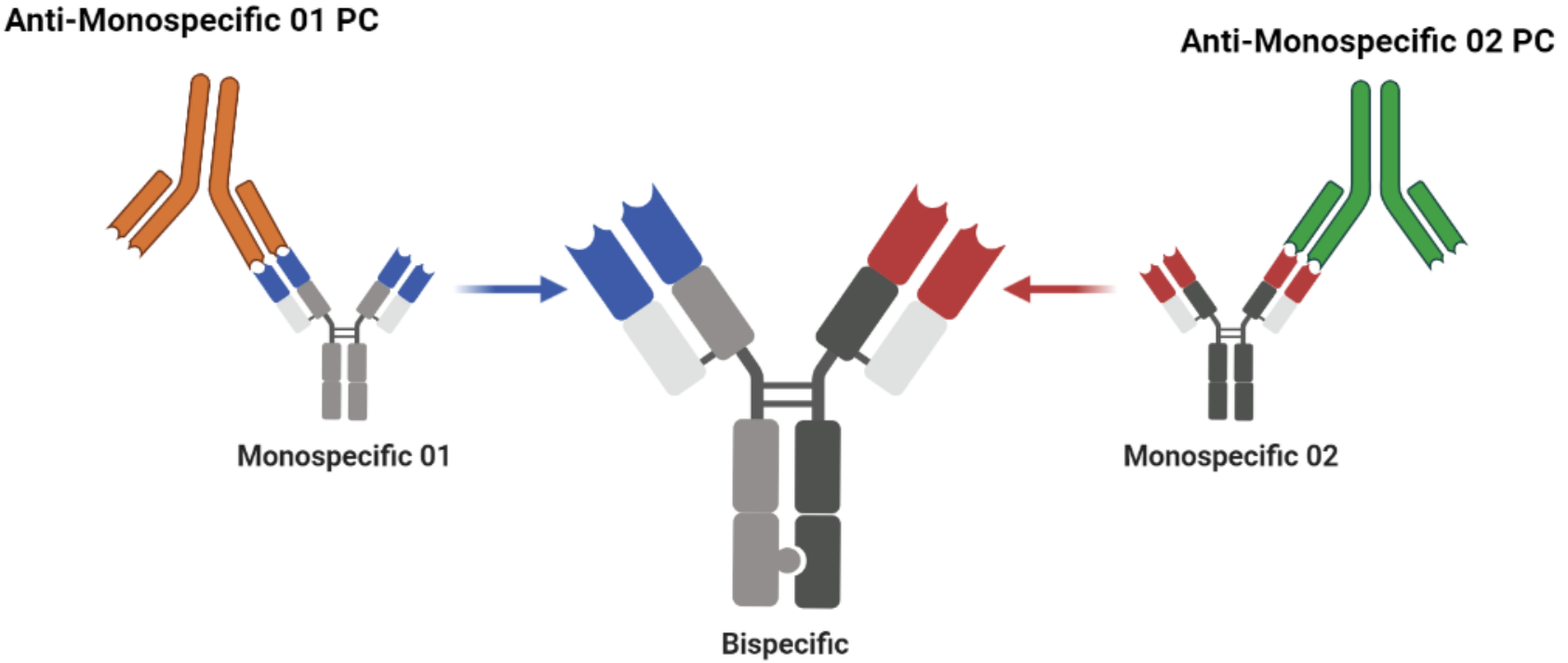
- **FDA 2019 Guidance**
  - **Section IV.A.3 states; 'An immune response to one domain may inhibit a specific function while leaving other intact. FDA recommends that sponsors direct initial screening and confirmatory tests against the whole therapeutic protein product.**
  - **Examination of immune responses to therapeutic protein products with multiple functional domains, such as bispecifics and ADCs may require development of multiple assays to measure immune responses to different domains of the molecules.**

- **EMA 2017 Guideline**
  - **Section 7.4; 'A strategy based on the competitive inhibition principle of the confirmatory assay to dissect the specificities of the antibodies to individual moieties may be used.**

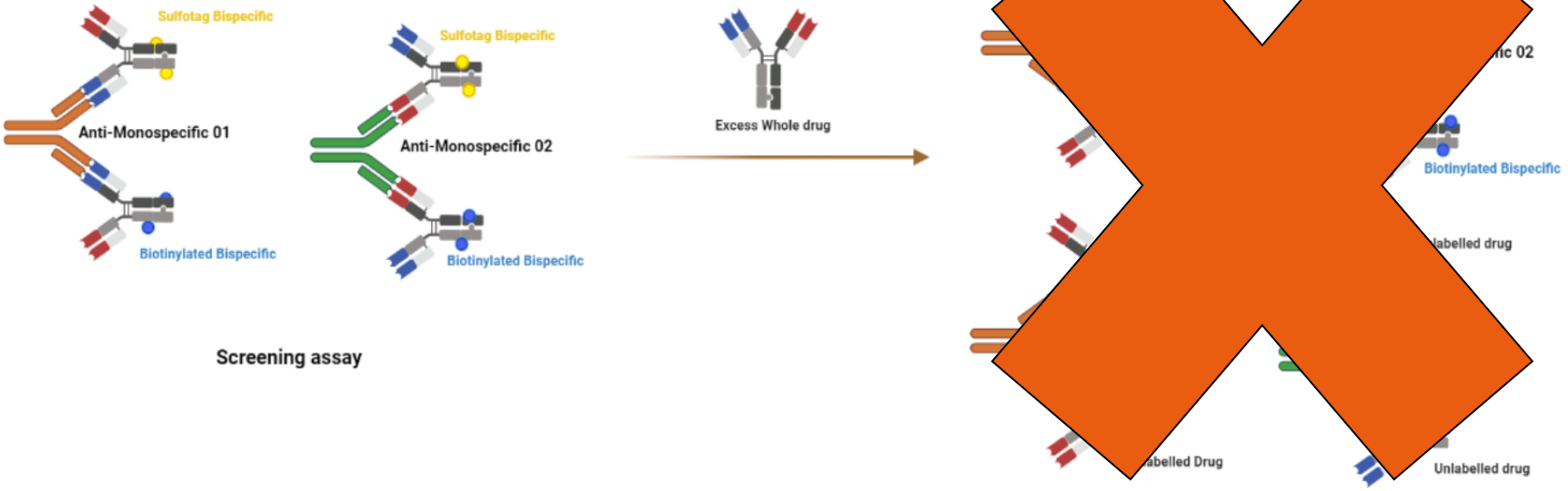
Bispecific ADA tiers approach



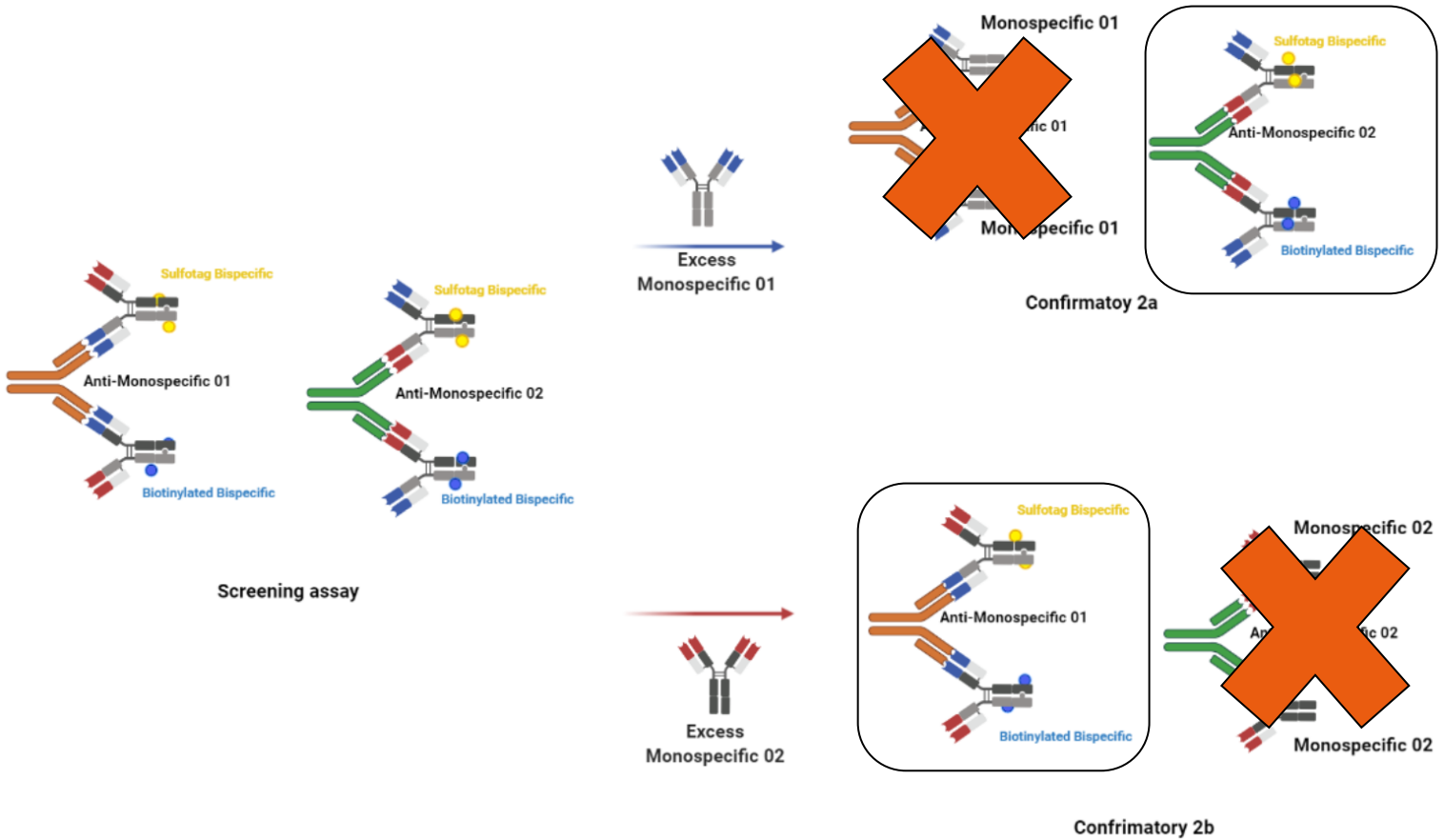
Assay development tool kit



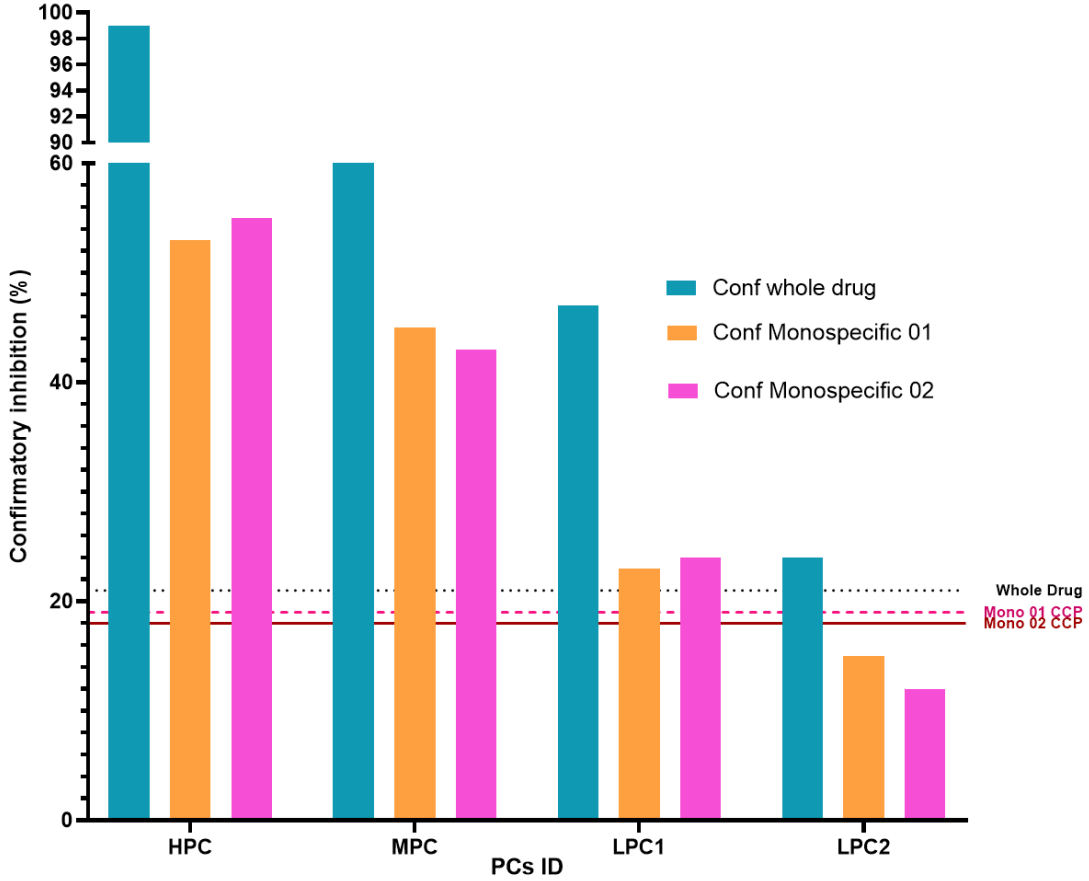
Screening and confirmatory assay Format



Screening and confirmatory assay format



Confirmatory assay sensitivity



Single vs bispecific (plates)

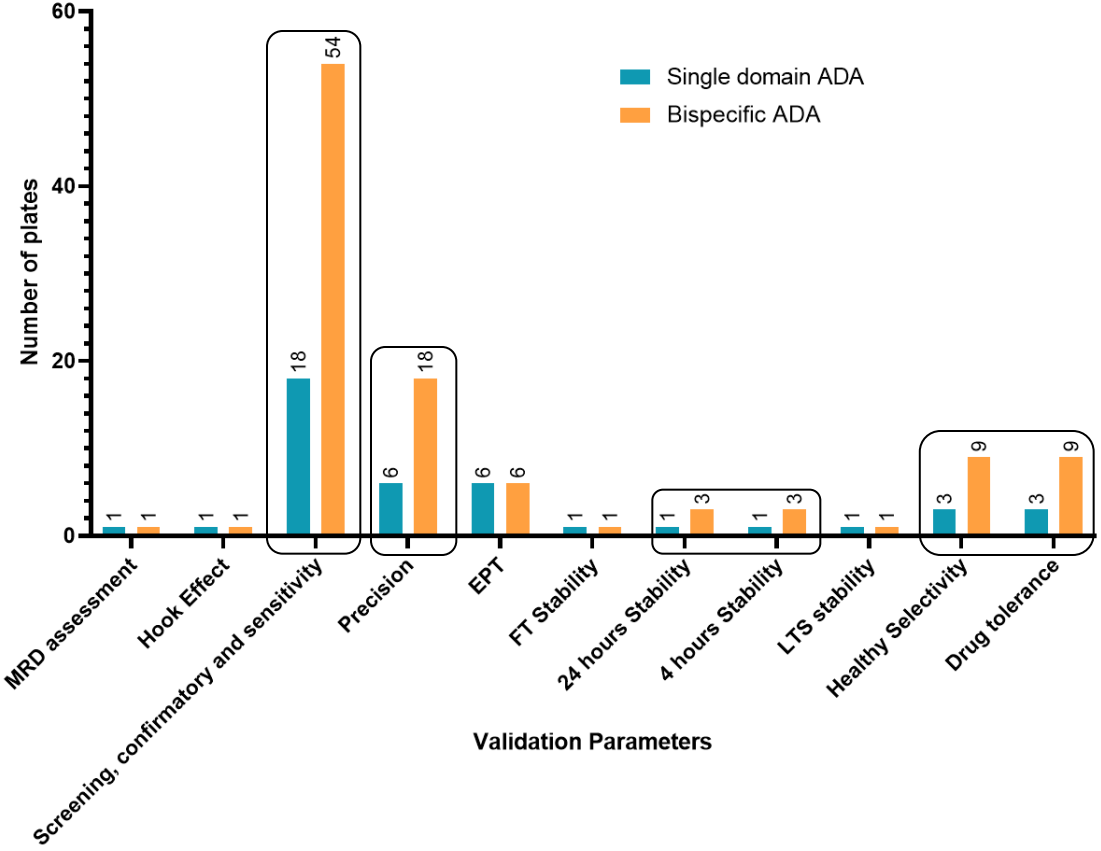
- **Total number of plates:**

- Single domain assay:

~42 plates

- Bispecific domain assay:

~106 plates



Single vs bispecific (days)

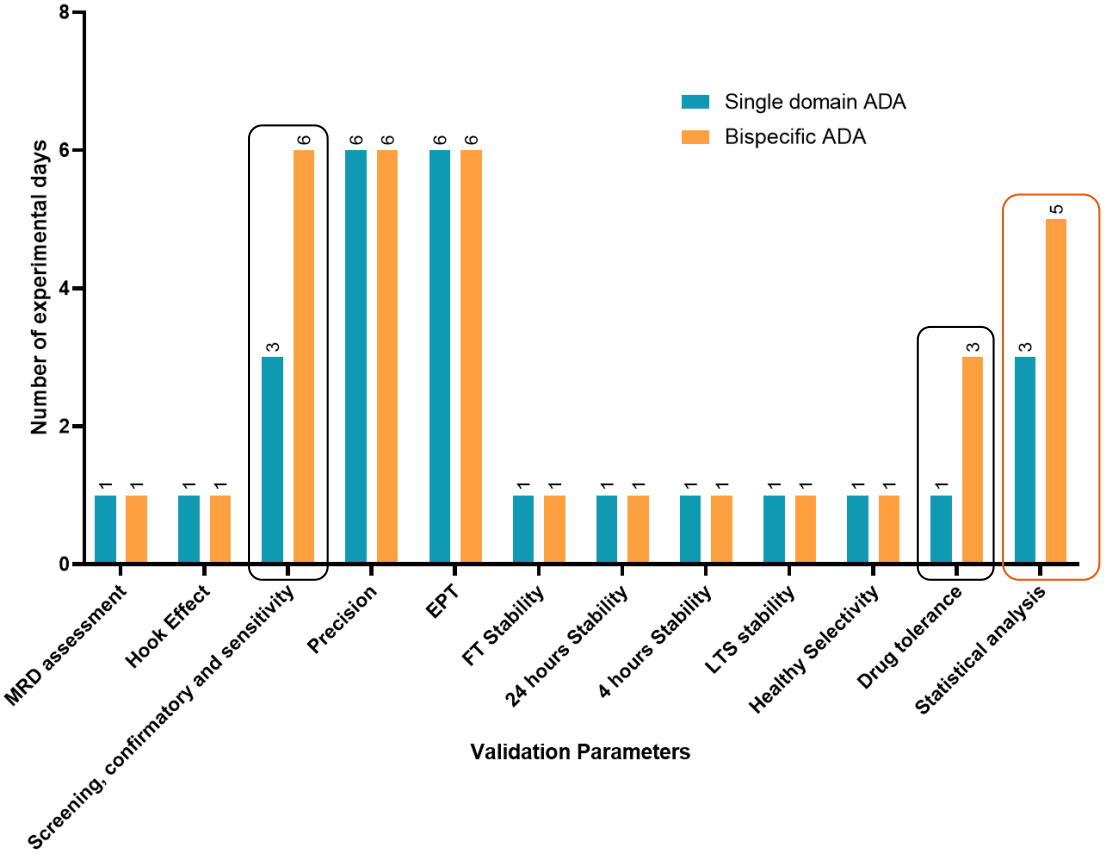
- **Total number of days:**

- Single domain assay:

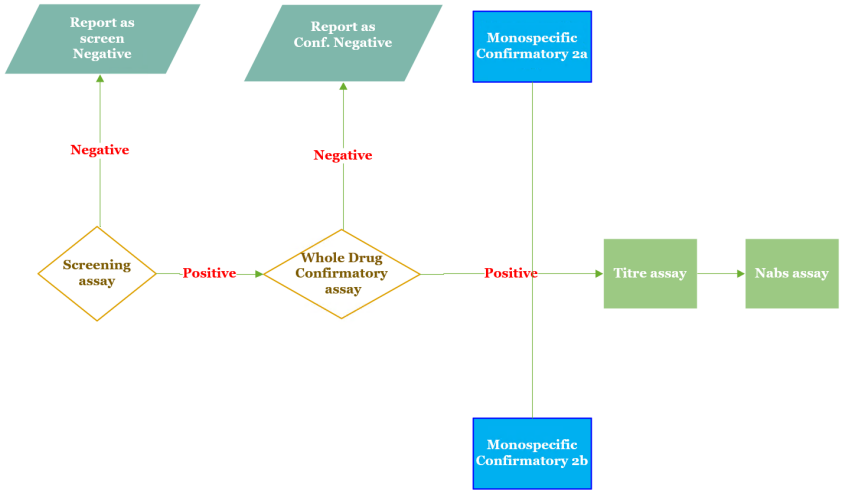
~26 days

- Bispecific domain assay:

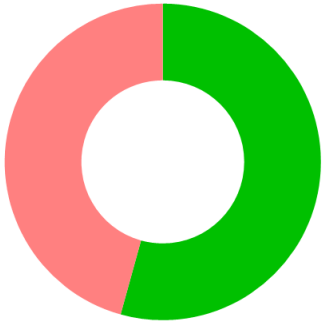
~33 days



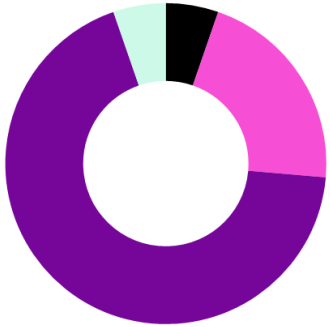
Case study



Screened Negative
Screened Positive

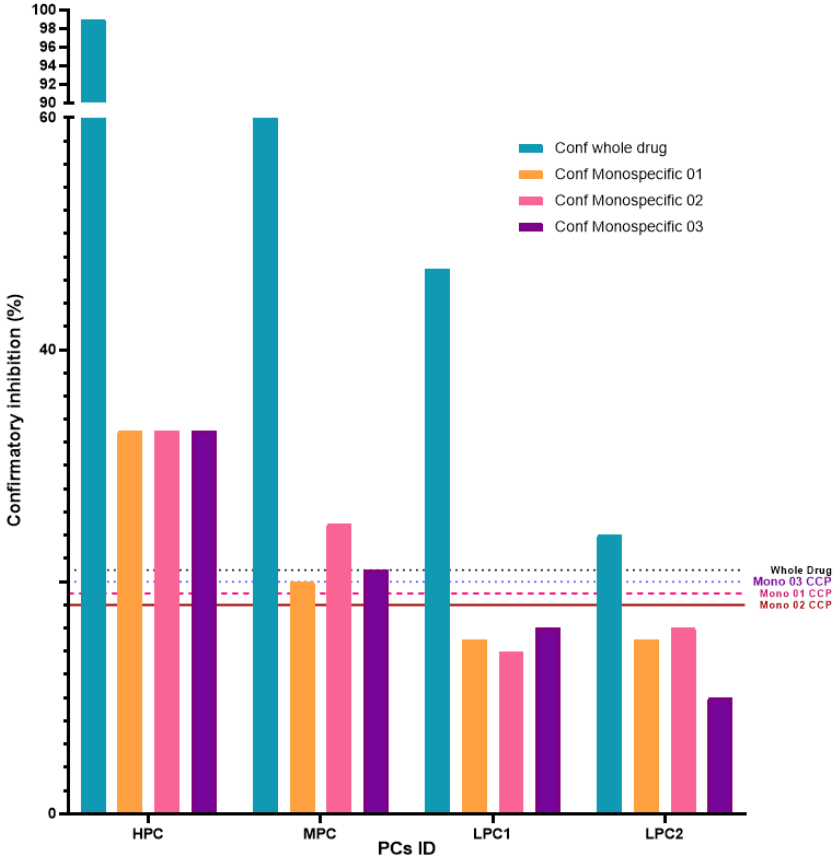


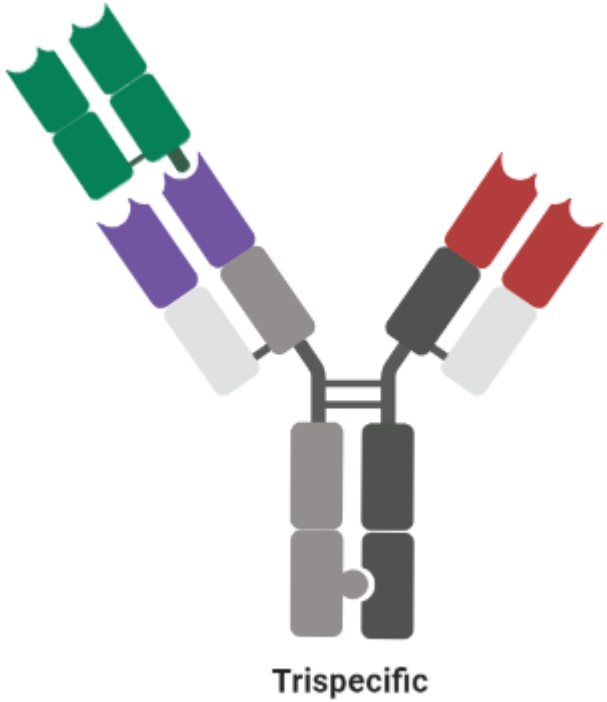
Confirmed positive against whole drug
Confirmed negative against whole drug



Confirmed Positive against both monospecifics
Confirmed Positive Monospecific 02
Confirmed Positive Monospecific 01
Confirmed Negative against both monospecifics

How about trispecifics and more domain





Summary

- Domain specific characterisation is challenging but expected
- Appropriate tool kit
- Single assay format is preferable
- Confirmatory sensitivity of each monospecific drug may be limiting

Acknowledgement and reference

Acknowledgement

- Method Development Team and sample analysis team
- Phil Driver and Deborah McManus
- LGC

Reference

- https://app.biorender.com/

Contact

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Any Questions?