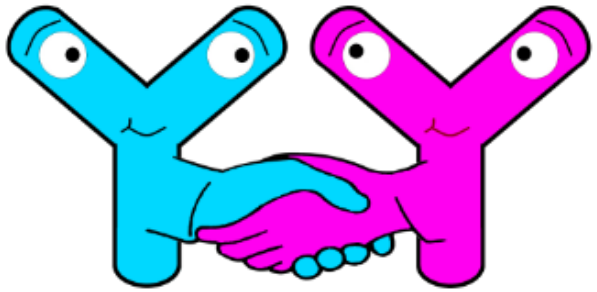
Potency Assay Strategy for a Fixed-Dose Combination Product

Cecile Avenal, CASSS Bioassays 2021



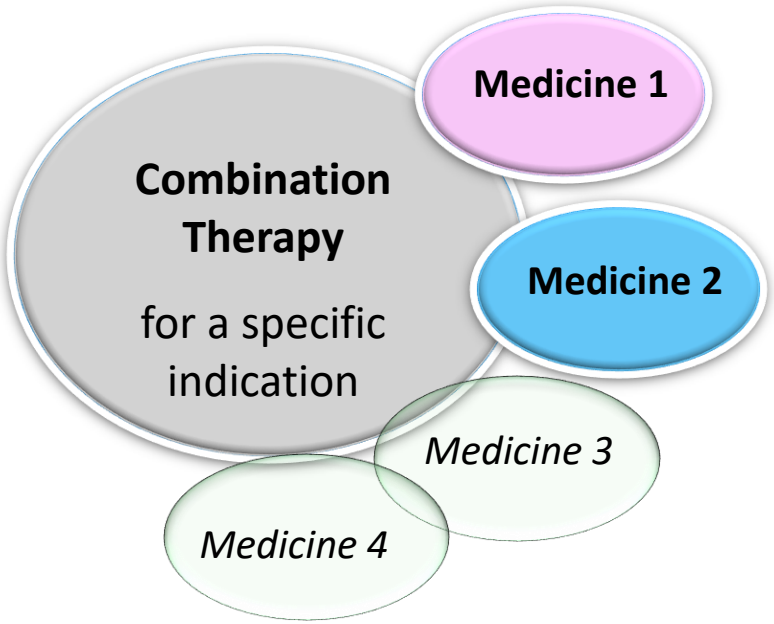
Outline

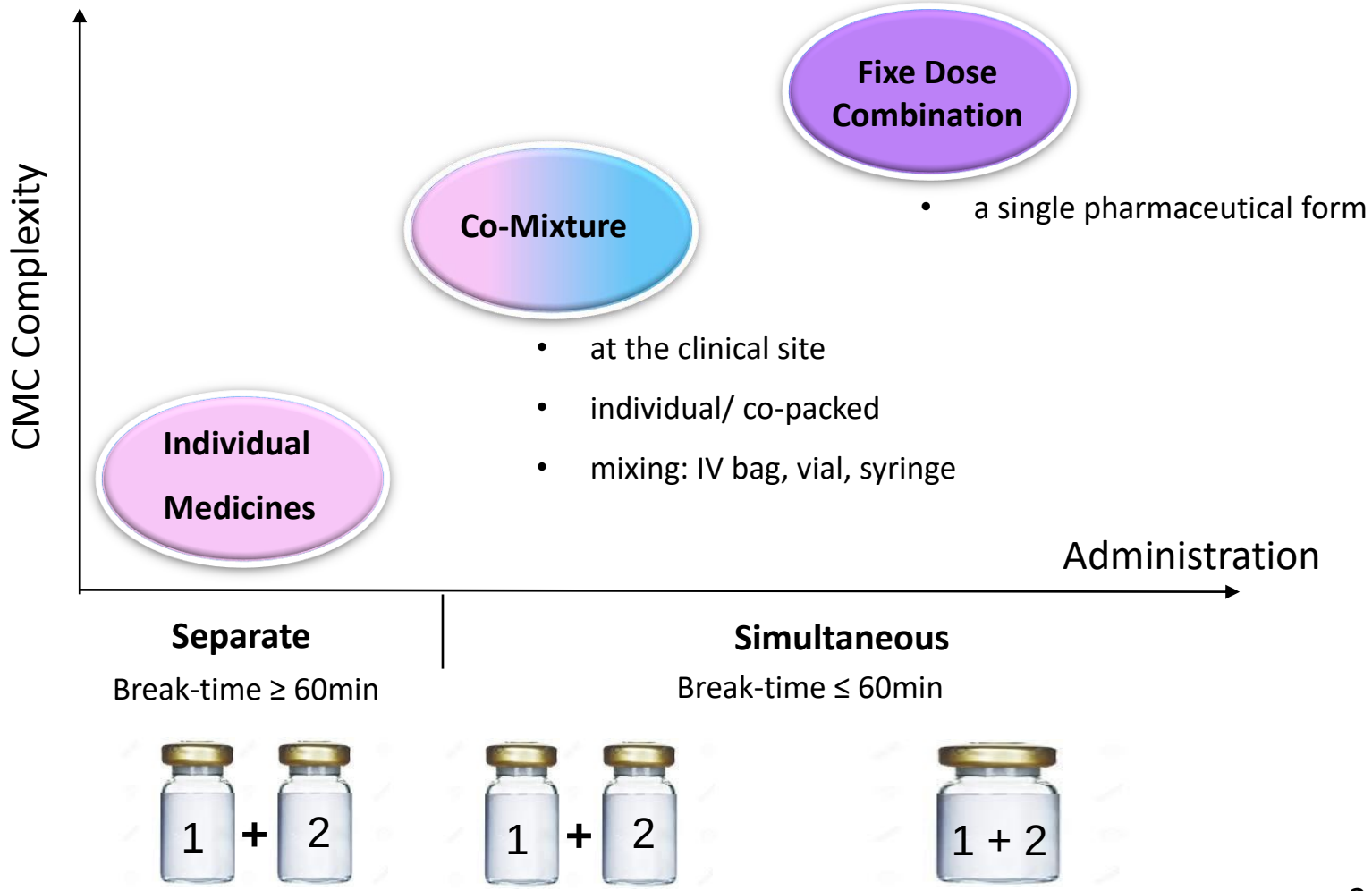
Fixed Dose Combination *vs* Combination Therapy

Case Study

- FDC of 2 MABs
- MAB1/MAB2 Mode of Action
- Bioassay Assay Strategy
- DP Potency Assay Development and Limitations
- DP Assay Selection: Suitability of the ELISAs and Limitations
- Overall Control System for Bioactivity

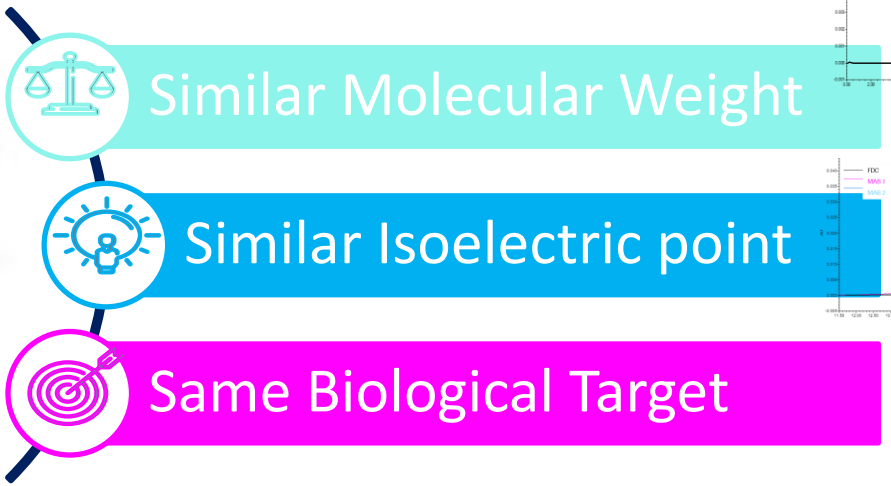
Fixed Dose Combination *vs* Combination Therapy

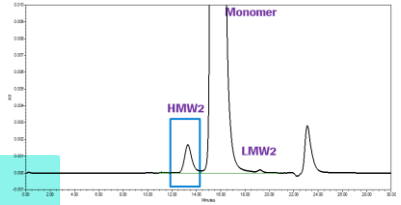


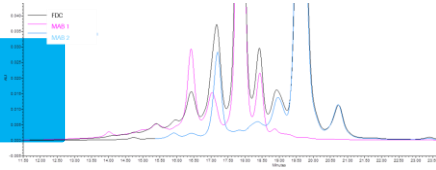


Case Study: A Fixed Dose Combination of 2 MABs

FDC { Loading Dose / Maintenance Dose

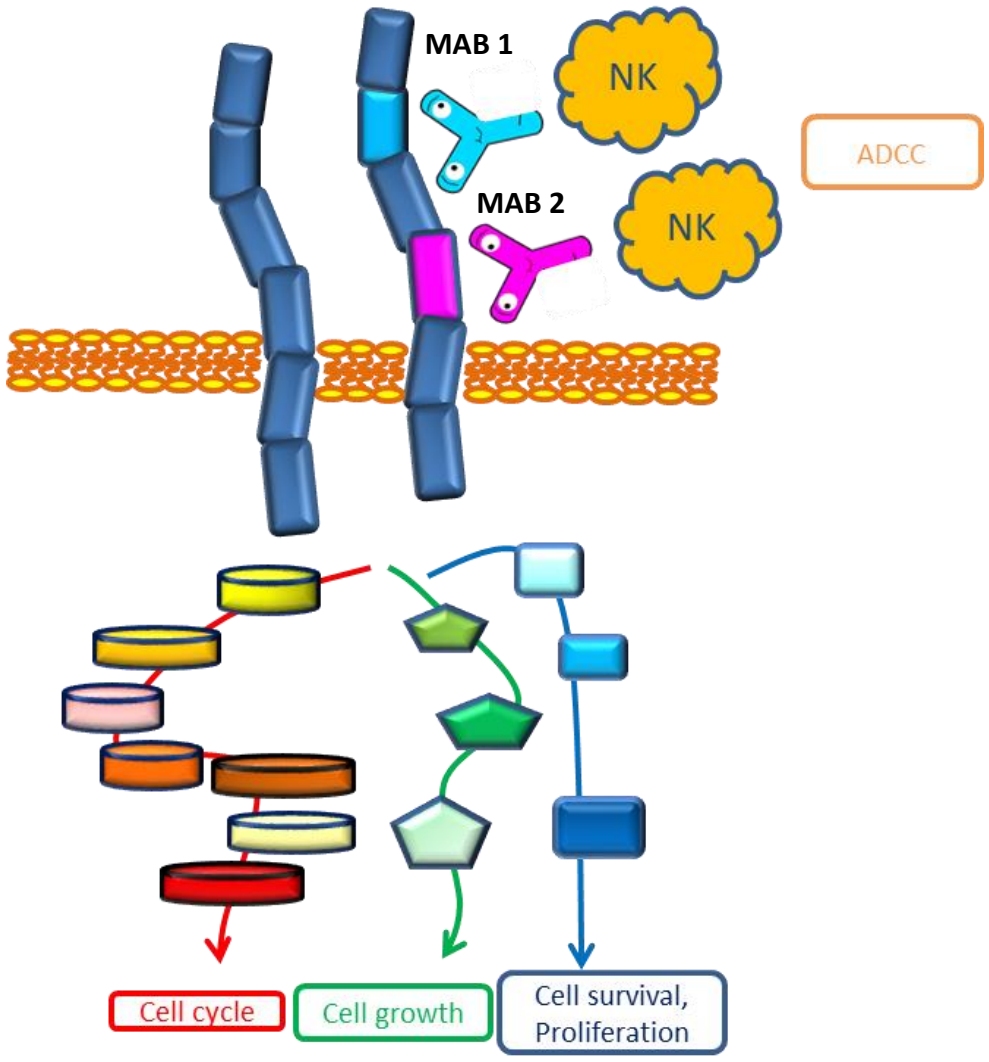






- 2 DS → 1 DP
- 3 RS for bioassay purposes
- Different bioassays at the DS and DP levels

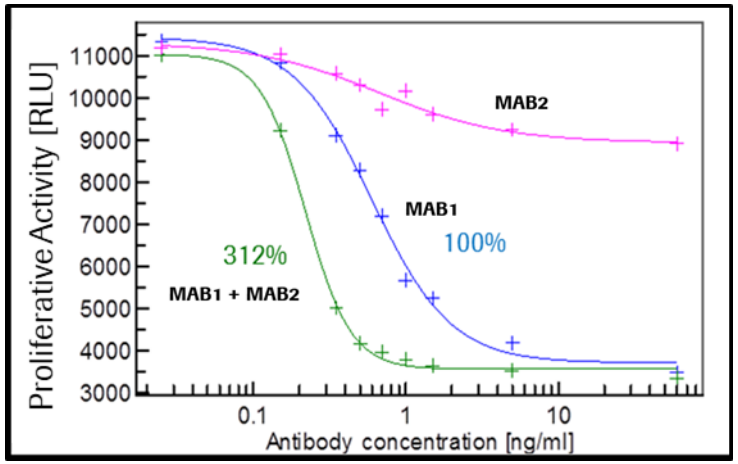
Case Study: MAB1/MAB2 Mode of Action



- Same receptor, but **distinct and non-overlapping epitopes** without competing with each other.
- **Complementary mechanisms** for disrupting the receptor signaling, resulting in an augmented **anti-proliferative activity** (APA, *in vitro* and *in vivo)* when MAB1 and MAB2 are administered in combination:

In vitro

APA1



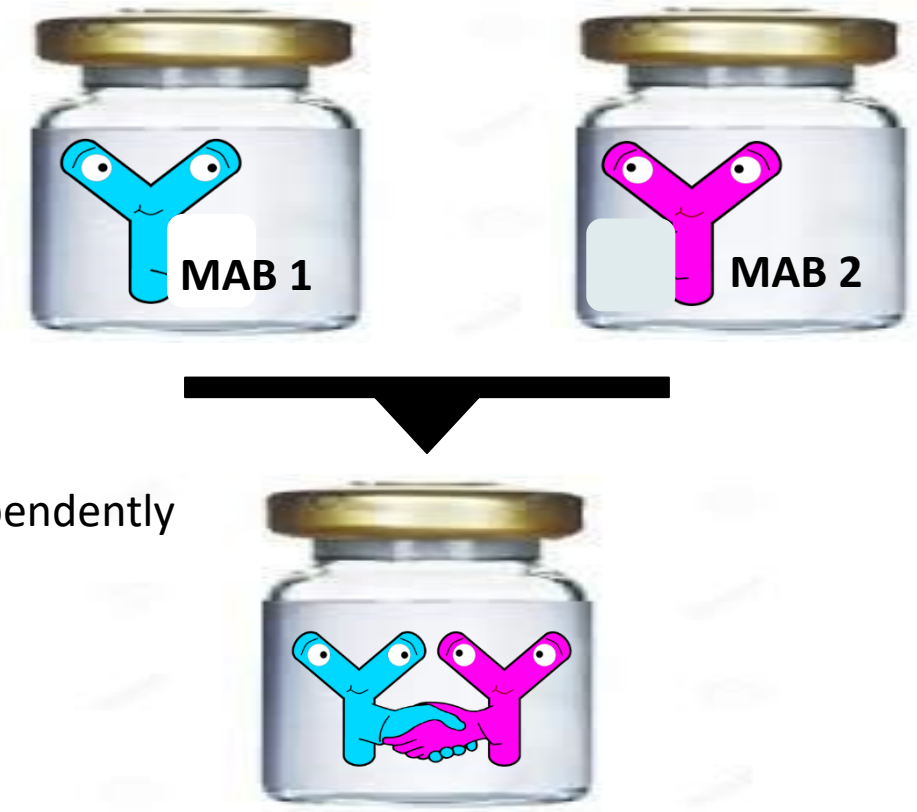
- MAB1 and MAB2 IgG1 framework provides for potent activation of **ADCC**

Case Study: Bioassay Assay Strategy

- **At the DS level,** each MAB independently

APA 1 (on target cell line 1, mid-expressing)

ADCC (on target cell line 2)



APA 2 (on target cell line 2, high-expressing)

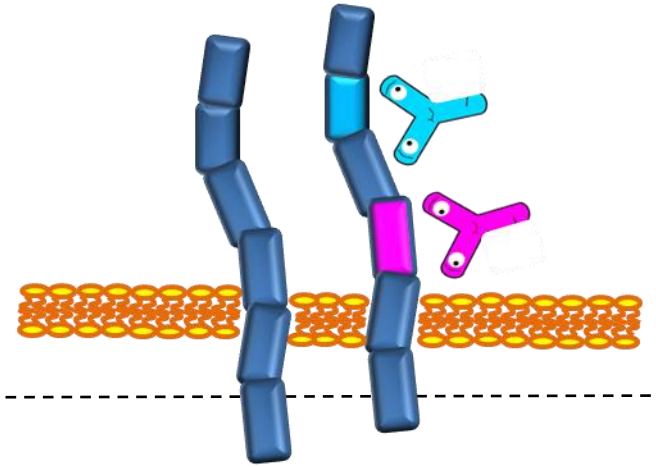
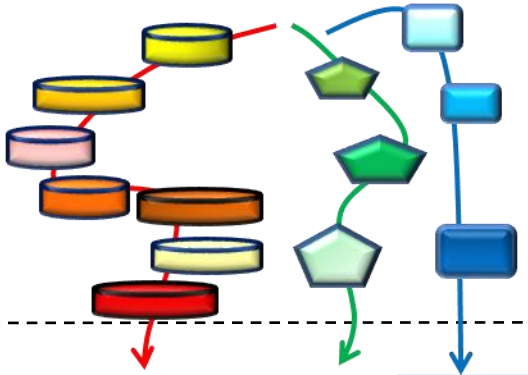
ADCC (on target cell line 2)

- **At the DP level,** each MAB independently (Potency) *vs* potency of the FDC (extended)

FDC { **Loading Dose**, **Maintenance Dose**

→ **APA and/or ADCC ?**

Case Study: DP Potency Assay Development
Anti-Proliferative Activity APA

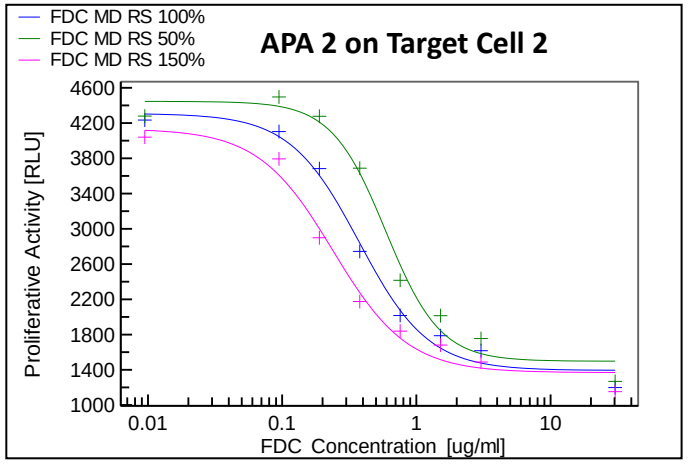
Biological Events		Possible Assays	Assay Suitability
Early: Binding/Dimerization		- ELISA - Dimerization assay	**Yes** **No**
Middle: Phosphorylation events		- Reporter Gene Assay - Kinase Receptor Phosphorylation Assay	**No** **No**
Late: Impact on cells		- Anti-Proliferation Assay	**Yes, but...**

Case Study: DP Potency Assay Development
Anti-Proliferative Activity (APA) Assay Limitations

- **DS APA Assays**

2 assays using different target cell lines available at the DS level.

→ Suitable to control the <u>overall DP APA</u>

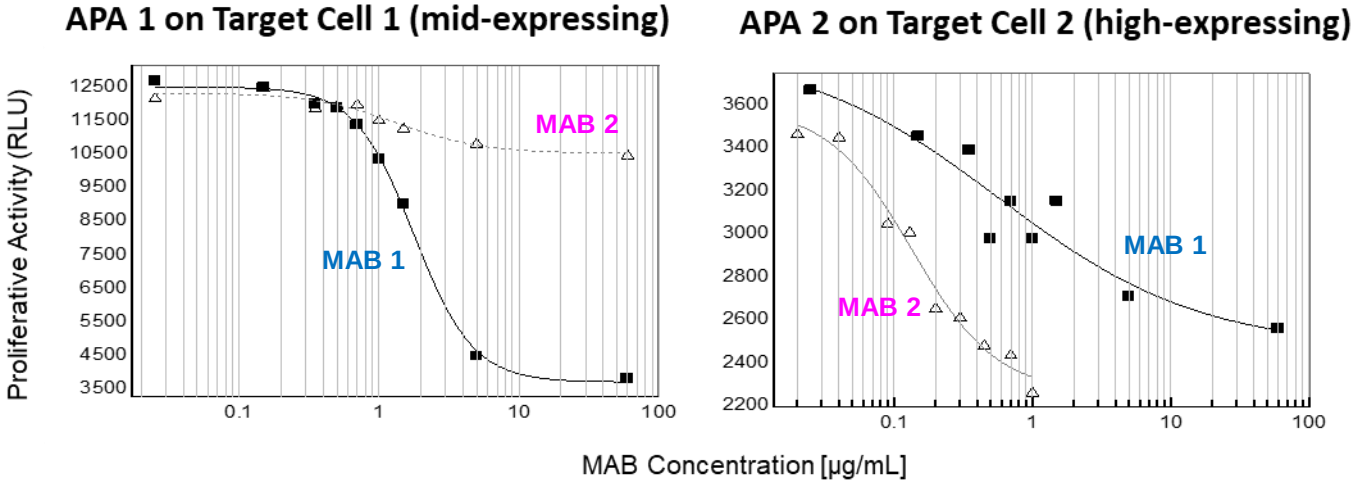


- **... But 1) Selective Sensitivity**

For one or the other MAB in the DP, but not for both MABs.

→ APA 1 not sensitive to MAB2

→ APA2 not sensitive to MAB1

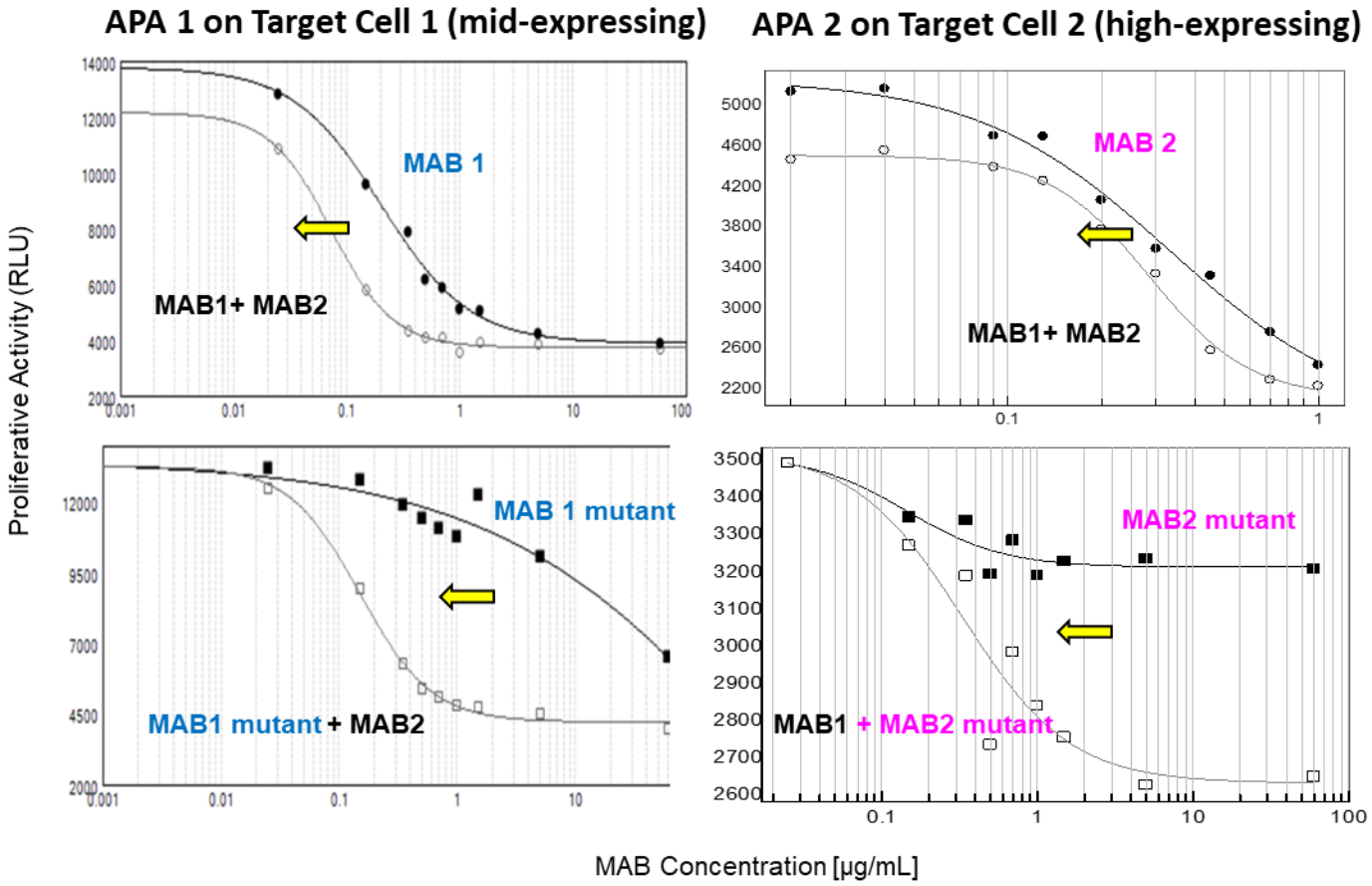


Case Study: DP Potency Assay Development
Anti-Proliferative Activity Assay Limitations

- **… and 2) Masking effect due to the Complementary Effect**

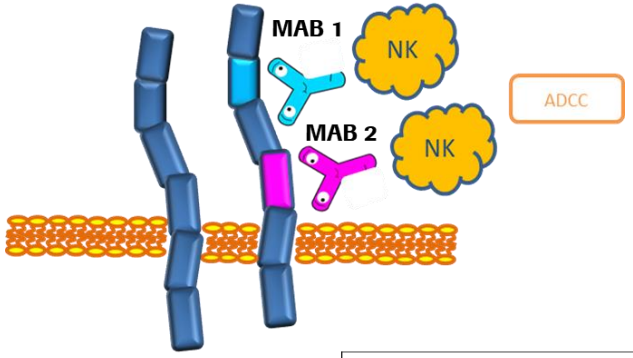
The presence of one MAB influences the response of the other, masking potential quality changes occurring in one or the other MAB.

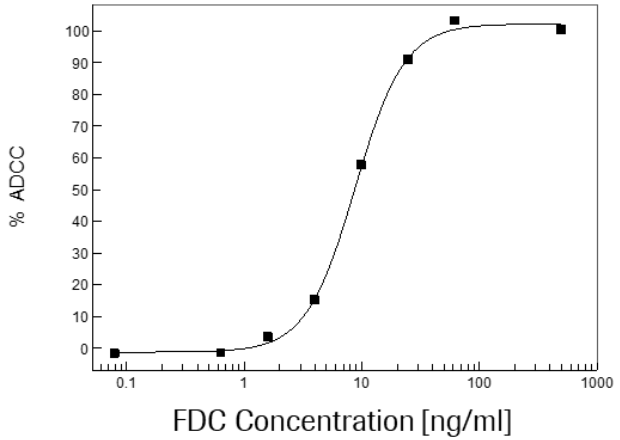
APA is partially restored when combining MAB1 to the MAB2 CDR-affinity mutant (or MAB2 to the MAB1 mutant) demonstrating that substantial quality changes of either MAB in the FDC DP cannot be detected.



→ Anti-Proliferation assays (APA) are not suitable to detect relevant changes in the activity of either MAB in the FDC DP (additional assay to cover the complementary effect only)

Case Study: DP Potency Assay Development
ADCC Assay Limitations

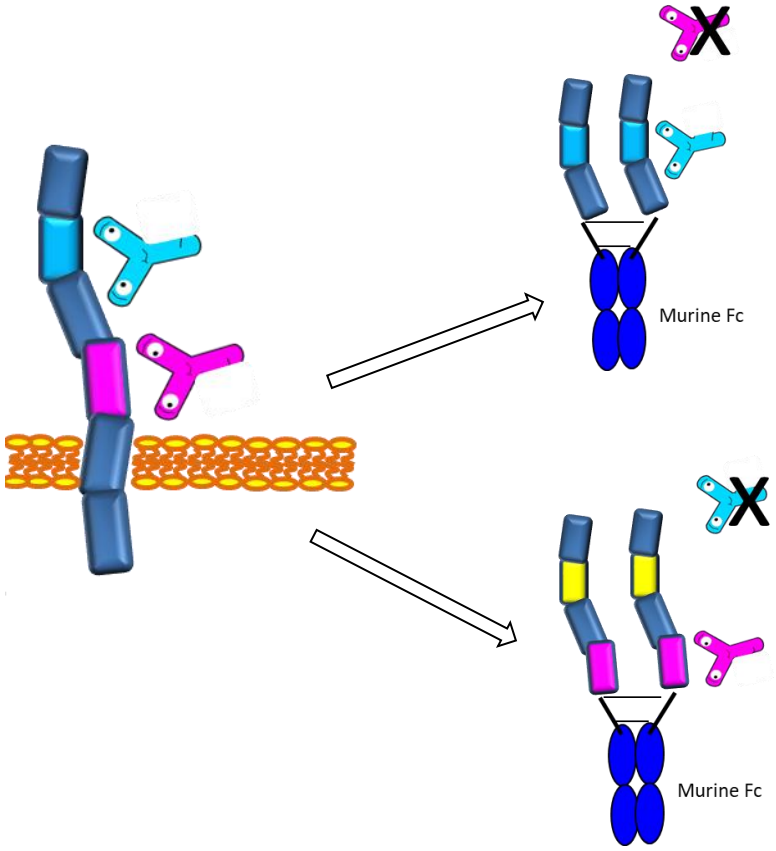




- MAB1 and MAB2 are not specifically Fc-engineered, have similar but non-overlapping afucosylation ranges.
- ADCC is based on dye-release system that cannot distinguish the distinct binding of MAB1 and MAB2 to different epitopes on the target cells.
- No complementary mechanism on ADCC level, but ADCC is believed to be additive.
- ADCC is dependent on antibody load on target cells: maximum FcyR interaction is already enabled and ADCC is expected to reach a saturated level that cannot be further enhanced by the addition of the second MAB.

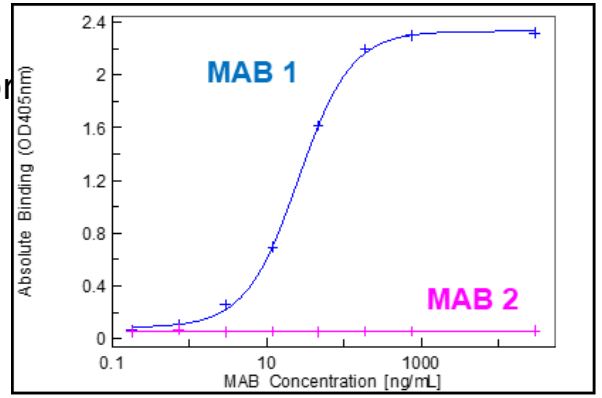
→ **ADCC assay cannot detect changes in the quality of either MAB's effector functions in the FDC DP (Extended Assay only)**

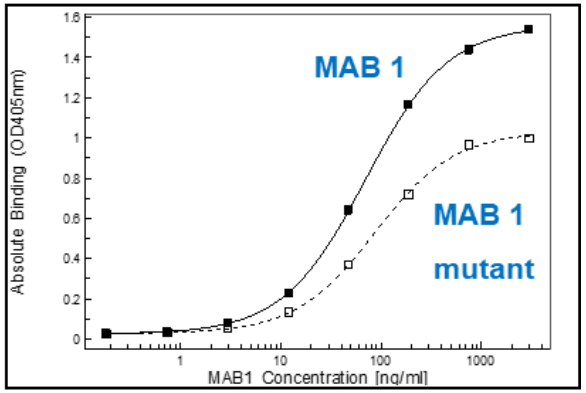
Case Study: DP Assay Selection
Suitability of the ELISAs



Antigen 1 specific for MAB1

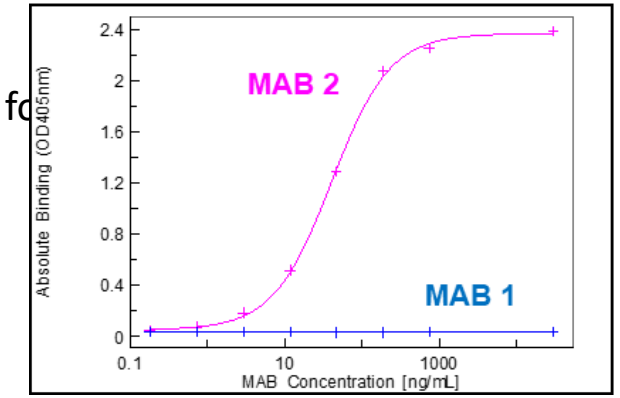
→ **ELISA 1**

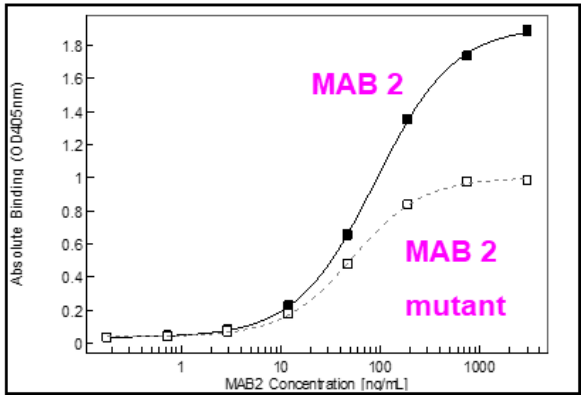




Antigen 2 specific for MAB2

→ **ELISA 2**





→ **ELISA assays are specific for either MAB in the FDC DP and can detect substantial changes occuring in the CDR (CDR-affinity mutants)**

→ **What is the relevance to the *in vivo* MoA?**

Case Study: DP Assay Selection
Suitability of the ELISAs

- **Theoretical Assessment**

Potential molecular changes of the MABs that affect their potency to inhibit target receptor-driven cell growth are already observed at the binding level.

- **Practical Assessment by Comparative Studies**

CDR-affinity Mutants

Charge Variants: well characterized IE-HPLC Fractions of individual MAB in the FDC MD Formulation.

Size Variants: Fractions of FDC, individual MAB in the FDC MD Formulation, stressed vs non stressed, digested samples for LMW forms. Overall Low levels of aggregation/fragmentation. Different tendencies in APA (hypopotent) and ELISAs.

	Relative Potency of MAB1 in FDC DP MD Formulation		Relative Potency of MAB2 in FDC DP MD Formulation	
Sample	by ELISA (%)	by APA (%)	by ELISA (%)	by APA (%)
CDR-affinity Mutants				
MAB1 Mutant	23[a,b]	no dose-response curve	NA	
MAB2 Mutant	NA		10[a,b]	no dose-response curve
IE-HPLC Fractions (non-stressed)				
Peak 1	88	94[b]	75	61
Peak 2	95	84	87	90
Peak 3	93	112	95	114
Peak 4 (MP)	113	107	86	83
Peak 5	109	109	89	109
Peak 6	81	104[b]	102	116
Peak 7 (MP)	NA[c]		101	109
Peak 8			100	105
Peak 9			73	91
Peak 10			71	73

[a] Outside of the 50-150% validated range of the assay
[b] Dose-response curves of sample and reference standard are not similar and therefore not reportable ($n \geq 2$ single plate results).
[c] Peaks 7 to 10 contain only MAB2 isoforms.

→ **ELISA assays reflect the *in vivo* situation, except for the size variants,** that are controlled by biochemical methods on the DP specification.

Case Study: DP Assay Selection
Limitations of the ELISAs

- **ELISA Design / Set-Up:**

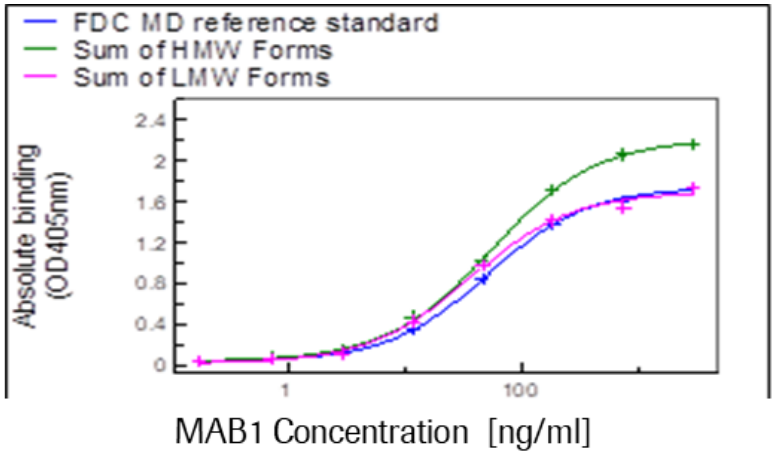
Quantification of the total amount of MABs bound to the antigen by detecting the bound material with a secondary detection antibody specific for the F(ab′)2 portion of human IgG.

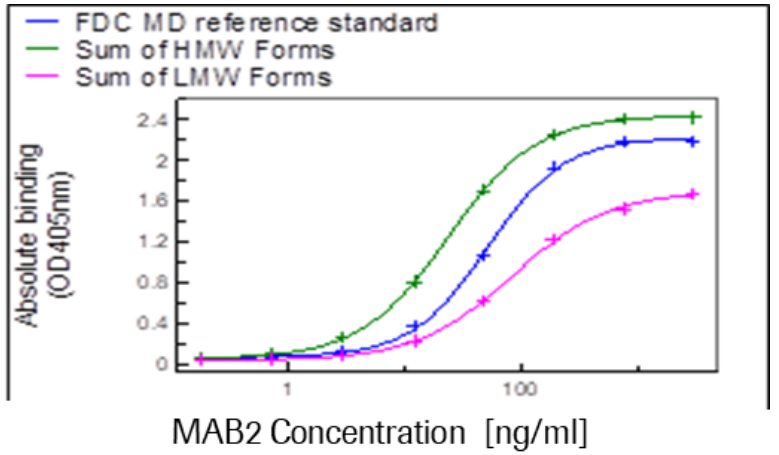
HMW forms (more epitopes to bind the detection antibody)
→ shift of the higher asymptote to a higher signal
→ similarity criteria failure, no reporting of potency

- **Dilutions are based on concentration** (weight per volume) rather than molecular weight:

HMWs forms,
LMW forms (i.e Fab Fragments still binding to the detection antibody)
→ More binding epitopes/mg protein may be present compared to the monomeric form.

- However, based on spike study, no significant impact on binding is expected in the specification range (HMW forms up to 1.3 area % and LMW forms up to 5.3% CPA at end of shelf-life).





Case Study: Overall Control System for Bioactivity

5 Potency Assays

2 Extended Assays

1 Additional Assay

- At the DS level, each MAB independently

APA 1 (on target cell line 1)

ADCC (on target cell line 2)

APA 2 (on target cell line 2)

ADCC (on target cell line 2)

- At the DP level (Compounding), each MAB independently

ELISA 1 (on antigen 1)

ELISA 2 (on antigen 2)

APA 2 (on target cell line 2)

ADCC (on target cell line 2)

FDC { **Loading Dose**, **Maintenance Dose**

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.... THANK YOU FOR YOUR ATTENTION ....

Doing now what patients need next