The Rollercoaster Between Free Drug Tolerance and Target Interference in the Development of an Antidrug Antibody Assay

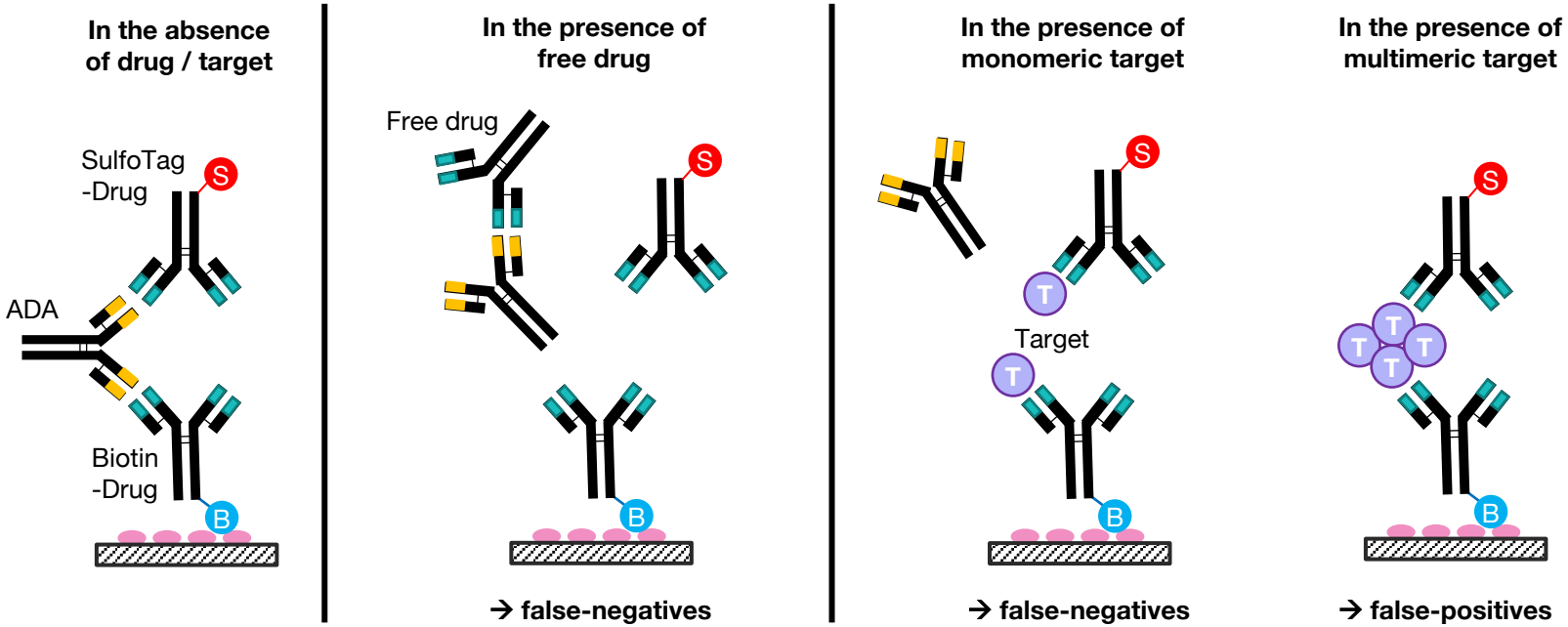
Lysie Champion

EBF 22-Nov-2024

Case Study

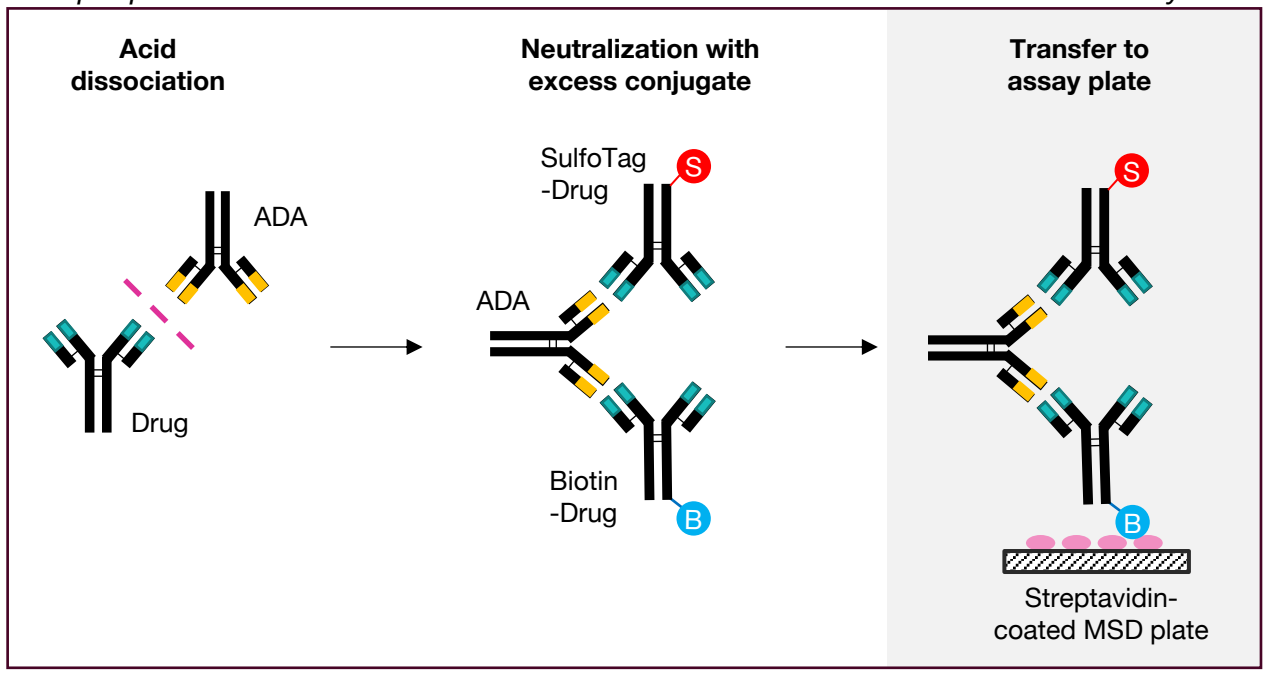
- Development of an antidrug antibody (ADA) assay against a biotherapeutic mAb in human serum
- Assay sensitivity: ≤ 100 ng/mL ADA/PC in the presence of interferences
  - Positive control (PC): mAb
  - Targeted drug tolerance based on C_{trough}: 600 µg/mL at 100 ng/mL PC
  - Targeted target tolerance: 100 ng/mL target (present in monomeric and multimeric form)
- Method Development starting point:
  - standard bridging assay with acid dissociation
- 3-tiered approach

Potential Interferences Using the Bridging Assay Format



Standard Bridging Assay With Acid Dissociation

Sample pre-treatment *ADA detection assay*



Evaluation of drug tolerance

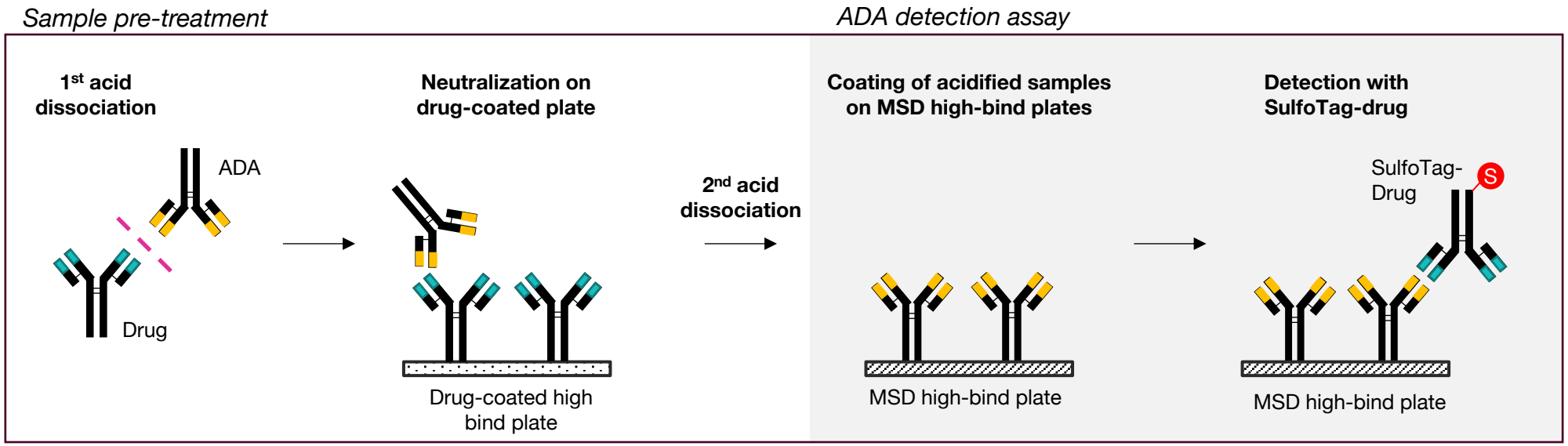
Drug [µg/mL]	Response [S:N ratio]	
	100 ng/mL PC	3000 ng/mL PC
0	6.67	87.37
30	1.33	7.21
60	1.10	3.76
120	0.98	1.65
250	0.96	1.17
500	0.93	0.96

Reactive samples are shown in green.
Preliminary CF=1.04. MRD 60.

→ Good assay sensitivity, but drug tolerance not reached

→ More complex sample pre-treatments required

Affinity Capture Elution (ACE)



Evaluation of sensitivity and precision

Run	Response [S:N ratio]	
	100 ng/mL PC	3000 ng/mL PC
1	1.23	5.20
2	0.98	1.14

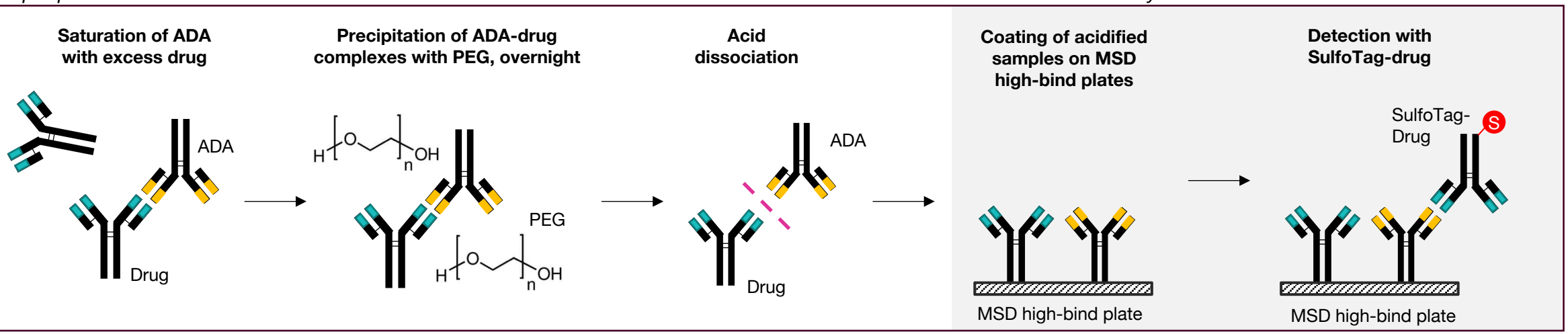
→ poor assay sensitivity and reproducibility

→ Assay format not suitable

Precipitation and Acid Dissociation (PandA)

Sample pre-treatment

ADA detection assay



Evaluation of sensitivity and precision

Run	Response [S:N ratio]	
	100 ng/mL PC	3000 ng/mL PC
1	1.45	5.50
2	1.06	2.41

→ poor assay sensitivity and reproducibility

→ To exclude acid sensitivity of the selected PC, six different PCs have been tested, none leading to an improvement of assay performance

→ Assay format not suitable

Go Back to Bridging Assay With Acid Dissociation….

… evaluating MRD > 100 (MRD150)

Drug tolerance achieved up to 400 µg/mL

Drug [µg/mL]	Response [S:N ratio]
	100 ng/mL PC
0	2.38
63	1.48
400	1.08

Positive target interference at 100 ng/mL target

Target [ng/mL]	Response [S:N ratio]
	NC (0 ng/mL PC)
0	1.00
100	1.68
400	3.46

Preliminary CF=1.03 (at MRD150)

Go Back to Bridging Assay With Acid Dissociation….

… evaluating MRD > 100 (MRD150)

Drug tolerance achieved up to 400 µg/mL

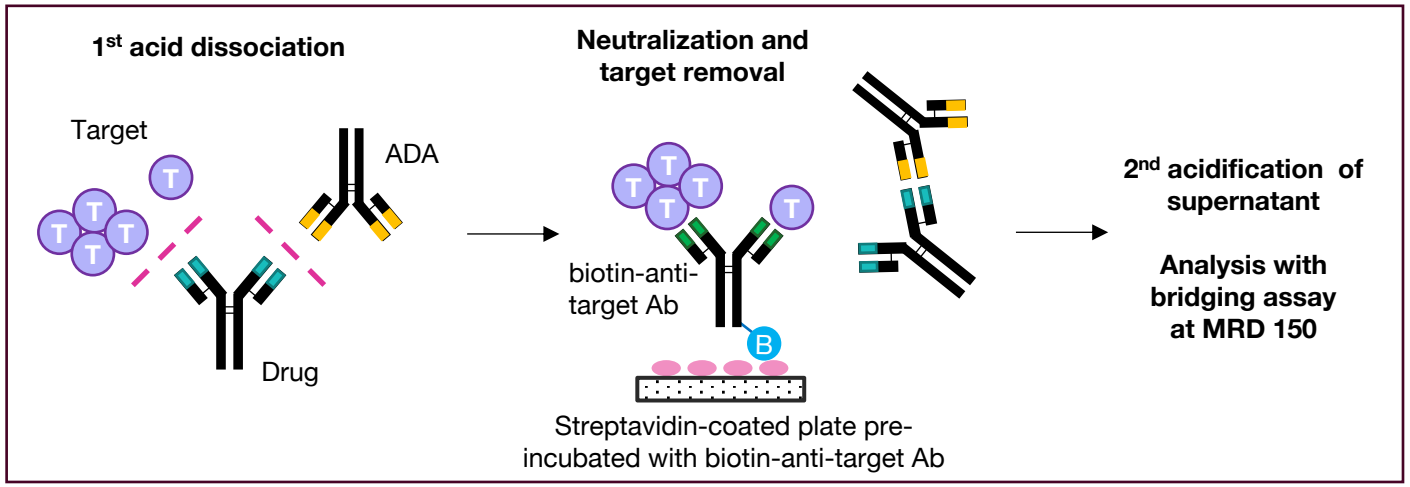
Drug [µg/mL]	Response [S:N ratio]
	100 ng/mL PC
0	2.38
63	1.48
400	1.08

Positive target interference at 100 ng/mL target

Target [ng/mL]	Response [S:N ratio]
	NC (0 ng/mL PC)
0	1.00
100	1.68
400	3.46

Preliminary CF=1.03 (at MRD150)

… evaluating MRD 150 and target removal



No target interference up to 200 ng/mL

Target [ng/mL]	Response [S:N ratio]	
	NC (0)	100 ng/mL PC
0	1.00	1.49
100	0.95	N/AV
200	0.97	N/AV

Preliminary CF=1.08 (at MRD150, with target depletion)

Go Back to Bridging Assay With Acid Dissociation….

… evaluating MRD > 100 (MRD150)

Drug tolerance achieved up to 400 µg/mL

Drug [µg/mL]	Response [S:N ratio]
	100 ng/mL PC
0	2.38
63	1.48
400	1.08

Positive target interference at 100 ng/mL target

Target [ng/mL]	Response [S:N ratio]
	NC (0 ng/mL PC)
0	1.00
100	1.68
400	3.46

Preliminary CF=1.03 (at MRD150)

… evaluating MRD 150 and target removal

Drug tolerance failed… and reduced sensitivity

Drug [µg/mL]	Response [S:N ratio]
	100 ng/mL PC
0	1.48
50	0.97
400	0.98

No target interference up to 200 ng/mL

Target [ng/mL]	Response [S:N ratio]	
	NC (0)	100 ng/mL PC
0	1.00	1.49
100	0.95	N/AV
200	0.97	N/AV

Preliminary CF=1.08 (at MRD150, with target depletion)

→ Assay format not suitable

Standard Approaches Didn’t Work

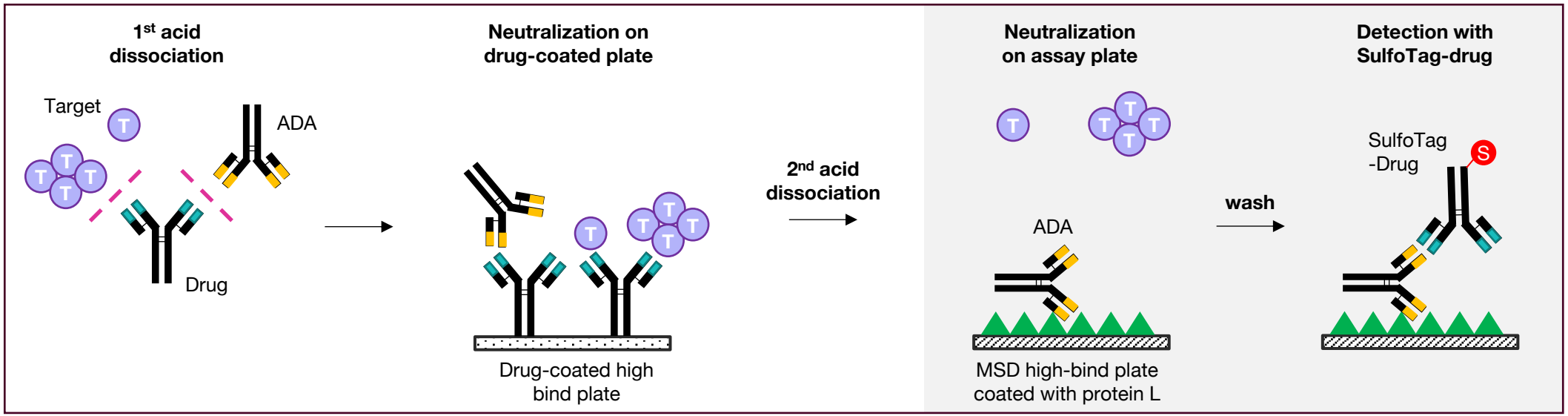
Considerations:

- Direct binding assay prevents positive interference from target
- ADA & drug = Antibodies, Target ≠ Antibody

ADA Capture and Reverse Direct Binding Assay – Set-up 1

Sample pre-treatment: solid-phase capture of ADAs

ADA detection assay: reverse direct binding assay



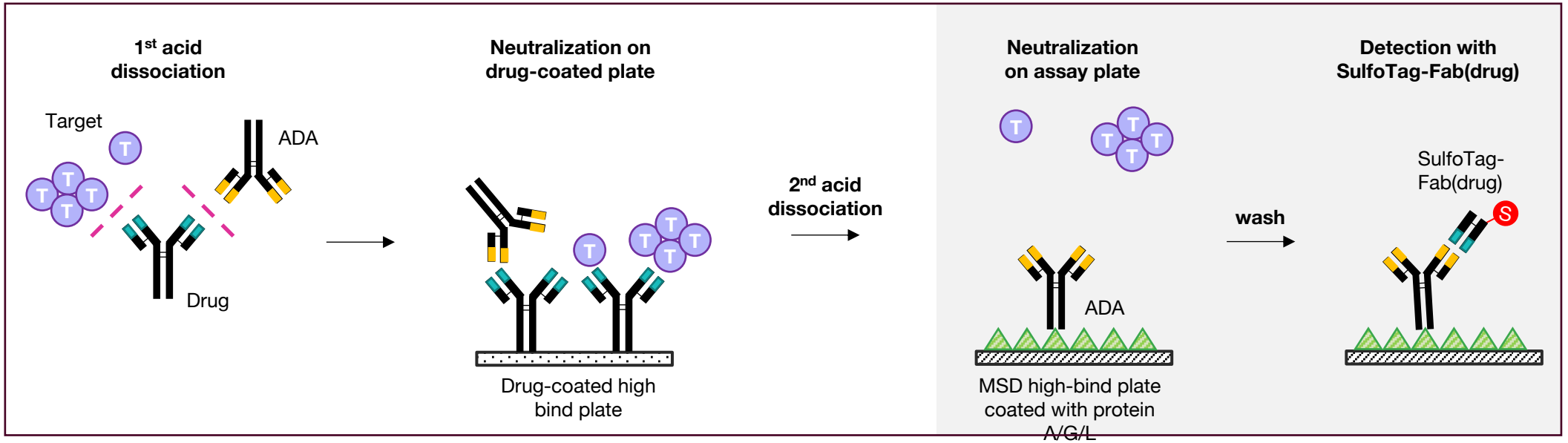
→ Capture only κ-LC ADAs

- ✓ Drug tolerance
- ✓ Target tolerance
- ✓ Sensitivity
- ✗ Not all ADAs captured and/or detected

ADA Capture and Reverse Direct Binding Assay – Set-up 2

Sample pre-treatment: solid-phase capture of ADAs

ADA detection assay: reverse direct binding assay



- Drug tolerance
- Target tolerance
- Sensitivity
- Not all ADAs captured and/or detected

→ Capture a broader range of ADAs

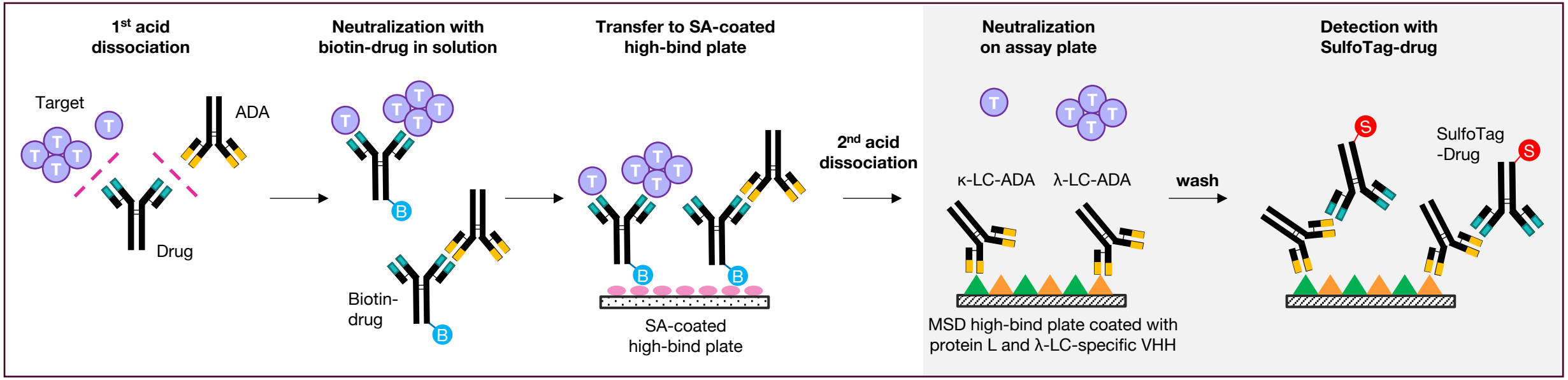
Ig binding properties	Protein A/G/L	
	κ-LC	λ-LC
IgG1, 2, 4	+++	+++
IgG3	+++	+++
IgM	+++	+
IgA	+++	+
IgE	+++	+
IgD	+++	-

→ Detect only Fab-targeted ADAs

Final ADA Capture and Reverse Direct Binding Assay Format

Sample pre-treatment: "in solution" capture of ADAs

ADA detection assay: reverse direct binding assay



- Drug tolerance – improved through ''in solution" ADA capture
- Target tolerance
- Sensitivity

  Whole range of ADAs captured

Assay Optimization

- Optimization of acid treatment
  - 1st acidification with 150 mM Glycine-HCl pH 1.5
  - 2nd acidification with 300 mM acetic acid
- Titration of reagents
- MRD testing: MRD 60
- Confirmatory assay set-up
  - 25 µg/mL inhibitory drug in detection buffer

Optimization of 1st and 2nd acid treatment

PC [ng/mL]	Drug [µg/mL]	Response [S:N ratio] AA + AA	AA + Gly	**Gly + AA**	Gly + Gly	Comment
100	0	3.84	1.48	**3.51**	1.30	Drug tolerance
100	200	1.51	1.27	**2.06**	1.12	
100	800	1.07	1.04	**1.34**	0.75	
5000	0	150.3	22.2	**106.9**	8.25	High PC

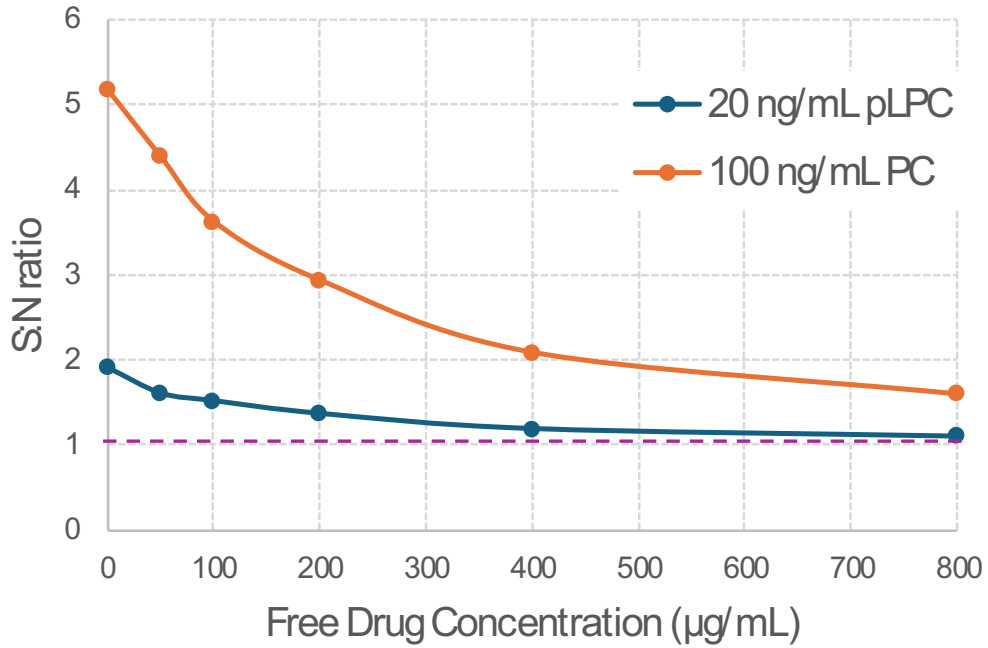
AA: 300 mM Acetic Acid; Gly: 150 mM Glycin-HC pH 1.5; preliminary CF: 1.1

Drug Interference

Targeted drug tolerance = 600 µg/mL

Drug [µg/mL]	Response [S:N ratio]	
	20 ng/mL (pLPC)	100 ng/mL PC
0	1.90	5.17
50	1.60	4.38
100	1.51	3.62
200	1.36	2.93
400	1.18	2.08
800	1.09	1.59

sCF: 1.072; mean NC: 65 RLU



✓ Drug tolerance confirmed up to 800 µg/mL free drug at pLPC (20 ng/mL) and 100 ng/mL PC

Target Interference

Targeted target tolerance = 100 ng/mL

PC [ng/mL]	Target [ng/mL]	Drug [µg/mL]	Response [S:N ratio]	Comment
0	0	0	1.00	NC
0	50	0	1.05	No positive interference
0	100	0	0.98	
0	200	0	1.03	
0	100	400	1.00	
0	100	600	0.98	
100	0	0	9.41	No negative interference
100	100	0	9.48	
100	200	0	10.40	
100	100	200	3.35	
100	100	400	2.52	
100	100	600	2.16	

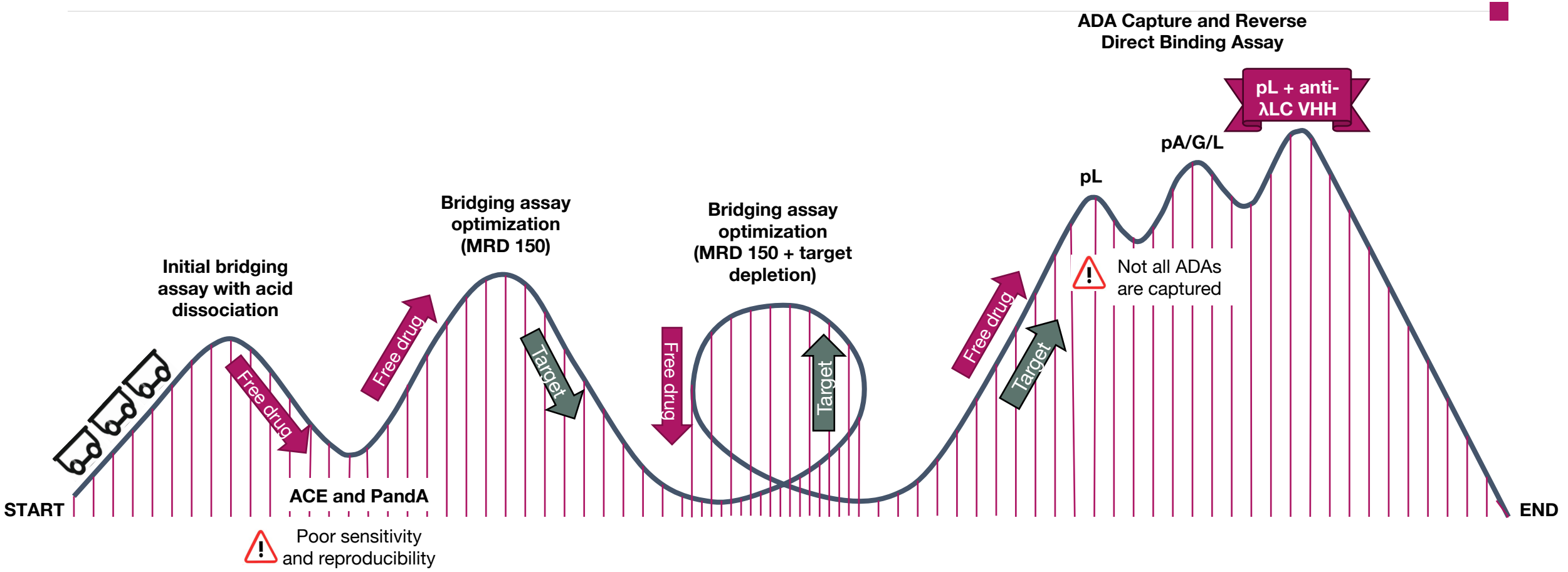
sCF: 1.072

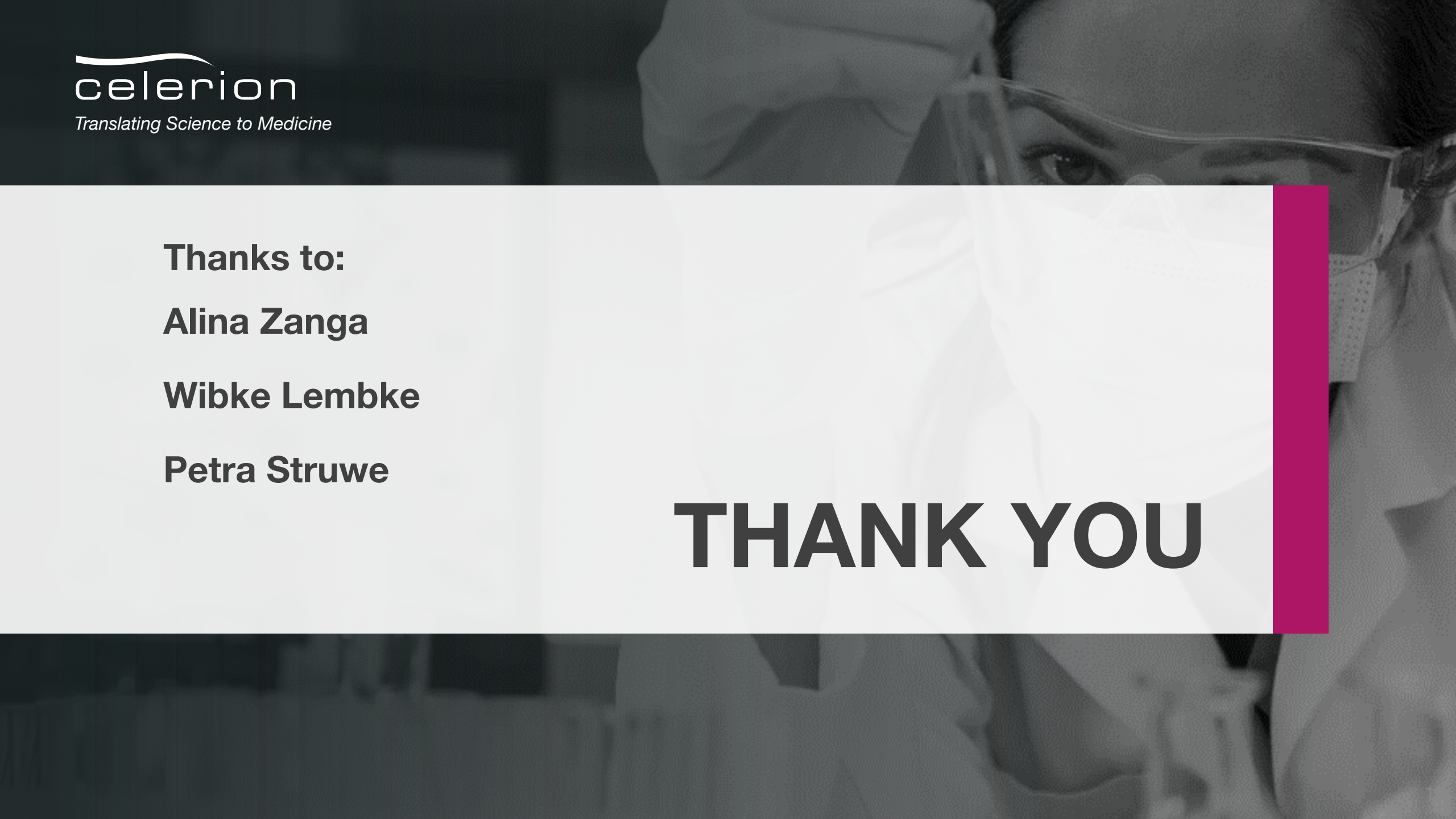
✓ Target tolerance confirmed up to 100 ng/mL target at 0 and 100 ng/mL PC, with or without drug

Assay Confirmation Summary

Method summary		
MRD	60	
PC level	pLPC: 20 ng/mL; MPC: 500 ng/mL; HPC: 2500 ng/mL	
Assay Parameters	**Results**	
CF	Screening: Confirmatory:	1.072 (Robust parametric approach) 21.6%
Sensitivity	Screening: Confirmatory:	4.04 ng/mL 18.18 ng/mL
Hook effect	No hook effect observed (tested up to 284 ug/mL PC)	
Drug tolerance	600 µg/mL drug tolerance confirmed at pLPC and 100 ng/mL PC	
Target tolerance	100 ng/mL target tolerance confirmed at 0 and 100 ng/mL PC	
Precision	Intra- and Inter-assay precision < 20%CV at all levels tested	
Selectivity, healthy	pLPC HPC	10/10 individuals positive 10/10 individuals positive
Selectivity, diseased	Blank pLPC	4/4 individuals negative → no false-positive 4/4 individuals positive → no false-negative

Summary


ADA Capture and Reverse Direct Binding Assay
pL + anti-λLC VHH
pA/G/L
pL
Not all ADAs are captured
Initial bridging assay with acid dissociation
Free drug
Bridging assay optimization (MRD 150)
Free drug
Target
Bridging assay optimization (MRD 150 + target depletion)
Free drug
Target
Free drug
Target
START
ACE and PandA
Poor sensitivity and reproducibility
END



Thanks to:

Alina Zanga

Wibke Lembke

Petra Struwe

THANK YOU