Let the Biology guide our choices

Case study : Decoding immunogenicity assay performance for reliable ADA data delivery

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Regulated Bioanalysis & Biosample Operations (RBBO) Chapter

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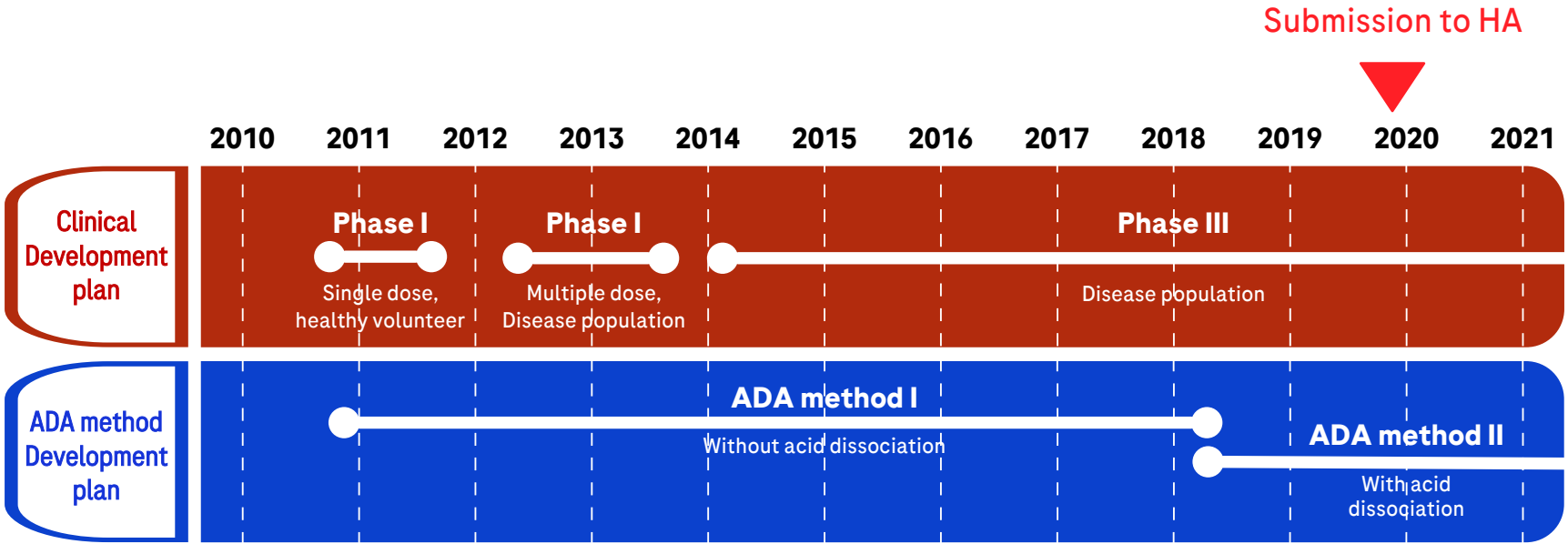
Background

Context



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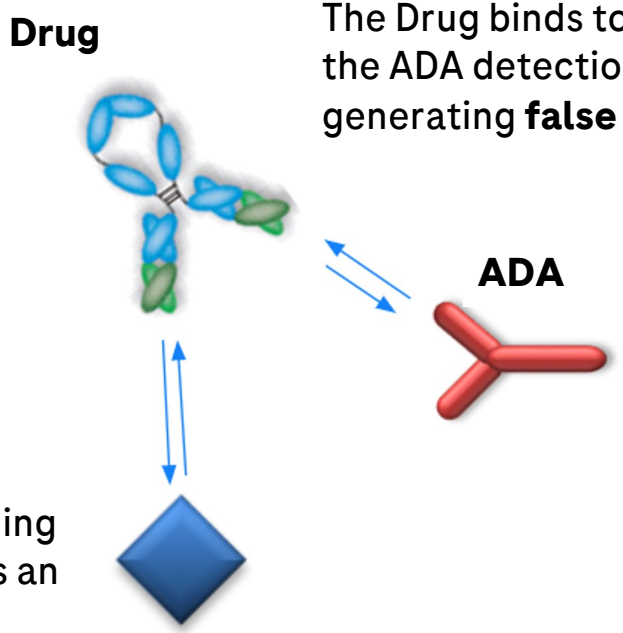
What we know from...

The Biology of our compound

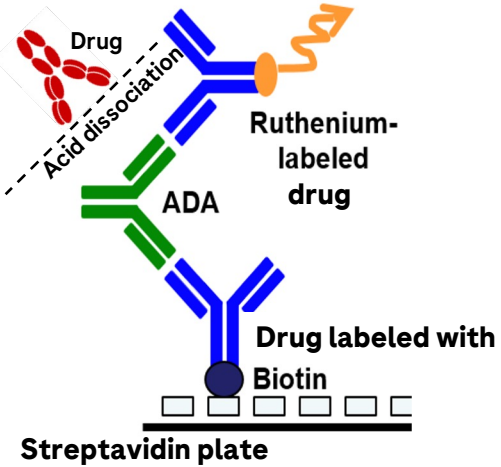


The Drug binds to ADA and can prevent the ADA detection in our method, generating **false negative results**.

The Soluble Target is a dimer. In a bridging ADA assay format, it can be detected as an ADA positive sample, generating **false positive results**.

What we know from...

The ADA Assay Validation



Homogeneous Bridging Assay

Soluble Target interference Assessment

Validation Soluble Target tolerance In absence of PC		Drug (ng/mL)		
		0	10000	20000
Soluble target (ng/mL)	0	Neg	Neg	Neg
	40	Neg	Neg	Neg
	200	Neg	Neg	Neg
	600	Pos	Neg	Neg
	1000	Pos	Neg	Neg
	5000	Pos	Pos	Pos

Expected result

Drug interference Assessment

Validation Drug tolerance In presence of 100 ng/mL PC		Drug (ng/mL)		
		0	10000	>20000
Soluble target (ng/mL)	0	Pos	Pos	Pos
	40	Pos	Pos	Pos
	200	Pos	Pos	Pos
	600	Pos	Pos	Pos
	1000	Pos	Pos	Pos
	5000	Pos	Pos	Pos

Interference

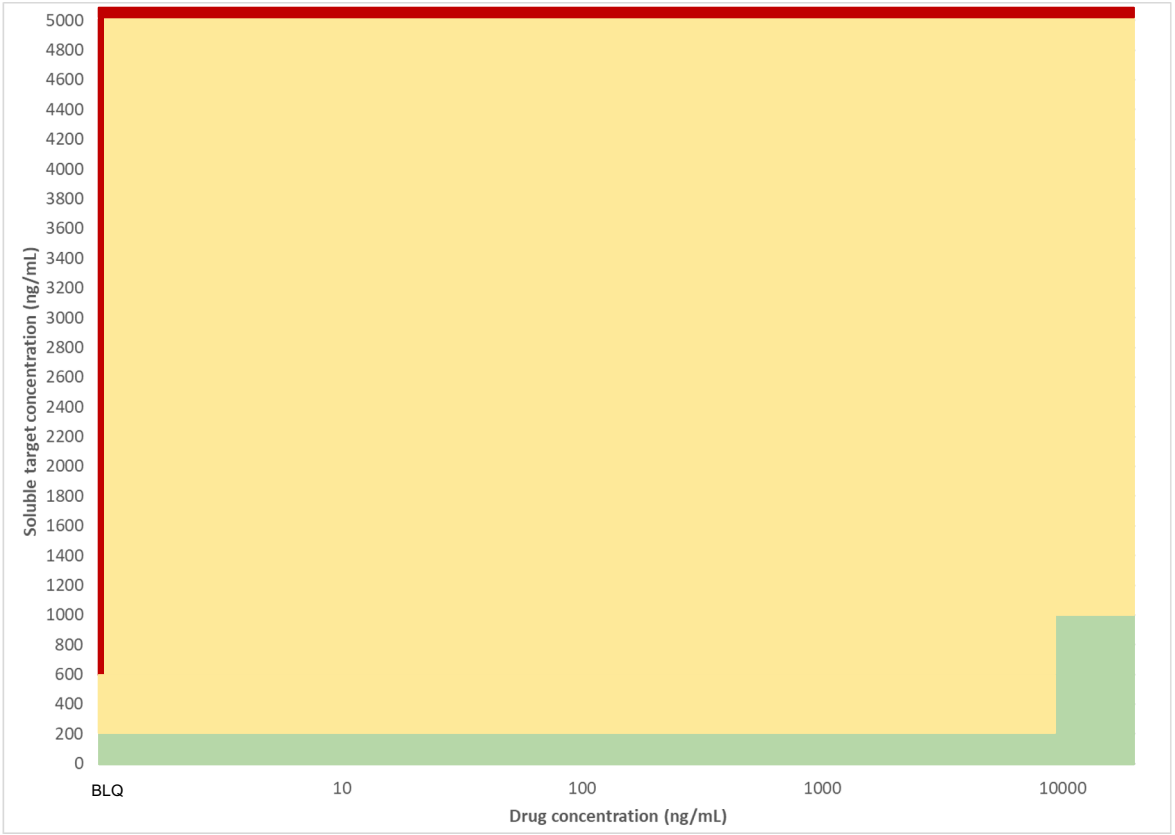
What we know from…

The ADA Assay Validation - Visualization

Zone without target and drug interference

Zone with soluble target interference : **False positive results**

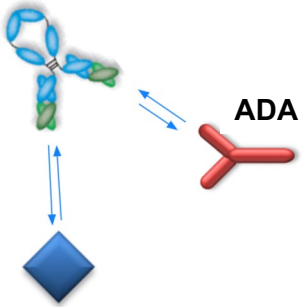
Zone with potential risk of soluble target interference



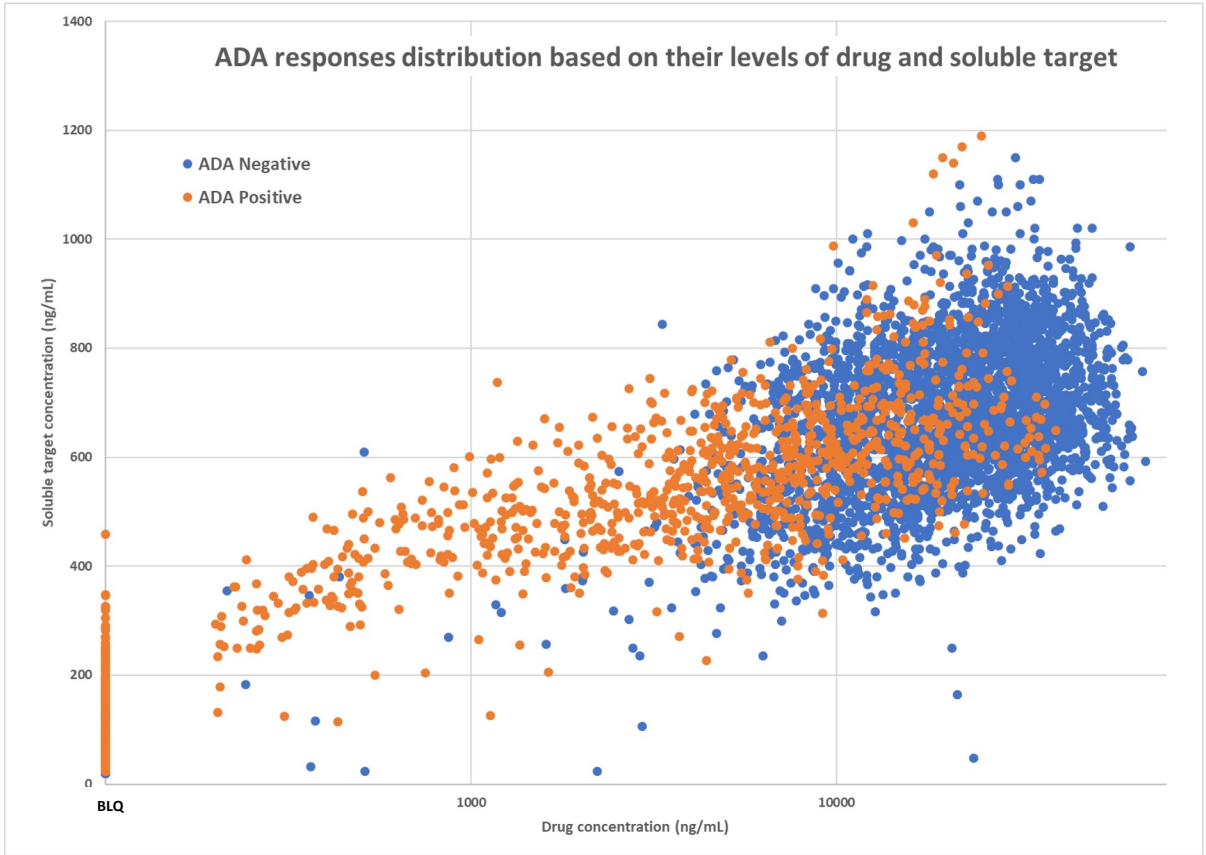
What we know from…

The analysis of Phase III clinical samples

Drug levels **up to 70000 ng/mL**

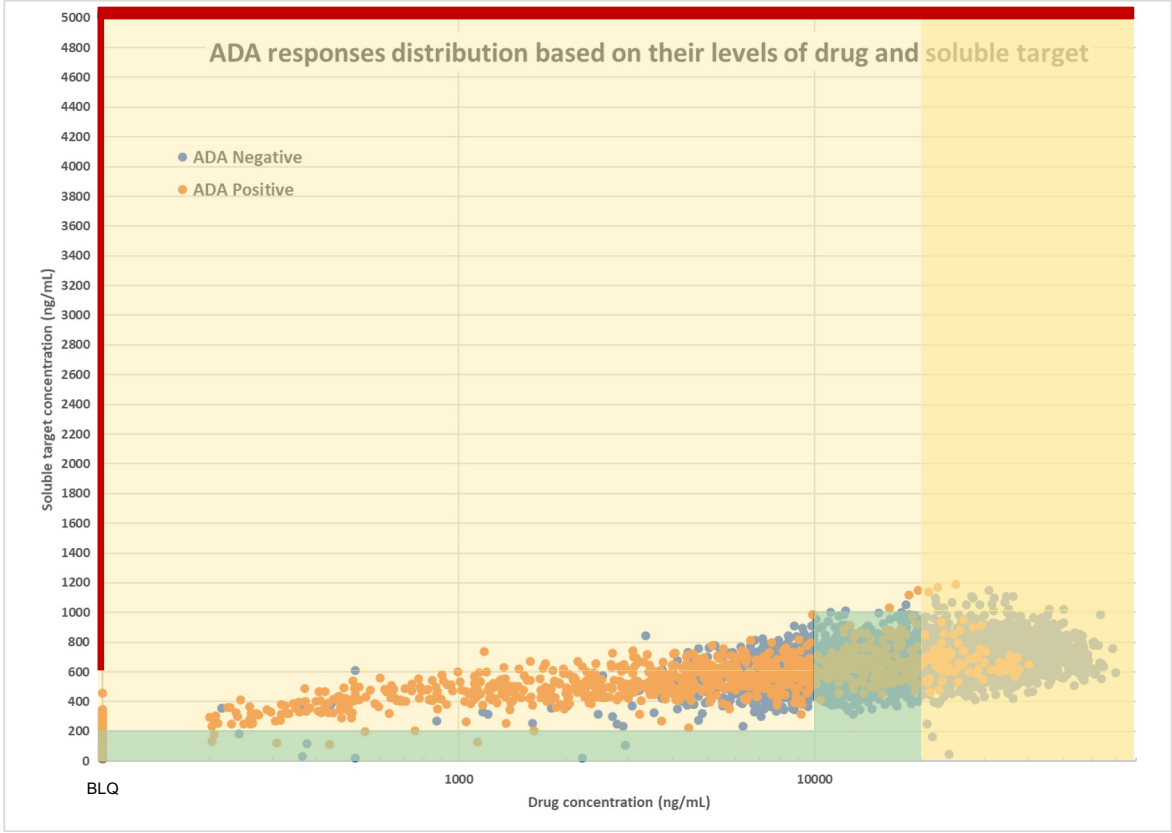


Soluble Target levels **between 20 to 1200 ng/mL**



What we know from…

Combining our learnings



Zone without target and drug interference

Zone with soluble target interference :
False positive results

Zone with potential risk of soluble target interference:
Risk of False positive results

Zone with potential risk of drug interference:
Risk of False negative results

We have gathered some information, but there are still significant gaps

What we know from…

Combining our learnings

Defining what we don't know…

At sample level (4290 samples evaluated)

True Positive n (%) 426 (10)	**(Risk of) False Positive n (%)** 683 (16)
(Risk of) False Negative n (%) 1721 (49)	**True Negative n (%)** 1460 (25)

Only 44 % of ADA data reported are reliable

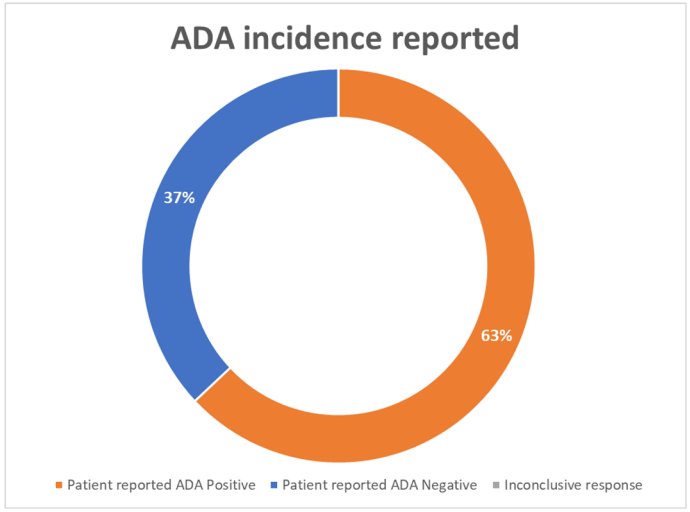
For 56% of ADA data reported, there is a risk of drug and/or target interference

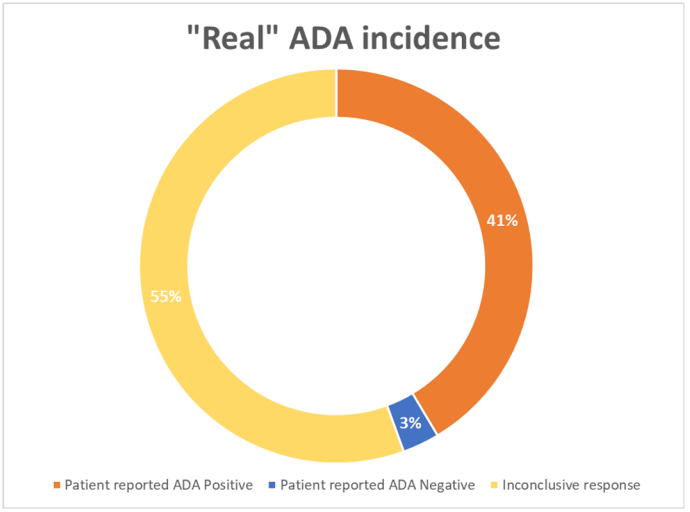
What we know from…

Combining our learnings

Defining what we don't know…

At patient level (166 ADA profiles evaluated)





Only 45 % of tested patients have reliable ADA results

For 55% tested patients, we can't be sure of their ADA profiles

What we know from…

Combining our learnings : Conclusion

Health Authorities expressed concern over the potential risk of false negatives (due to drug interference) at low ADA levels, but no queries regarding soluble target interference risk.
By combining our learnings, there is a huge uncertainty about real ADA incidence :

> 50% of treated patients have inconclusive ADA response, which could potentially impact the overall ADA data interpretation.

Nevertheless :

- The safety profile of the drug is comparable, irrespective of ADA status.
- Between 10-15% of patients demonstrate sustained loss of exposure.
- Clinical data demonstrate target engagement even in the ADA-affected population.

Our ADA Assay is only partially decoded
What else can it teaches us ?

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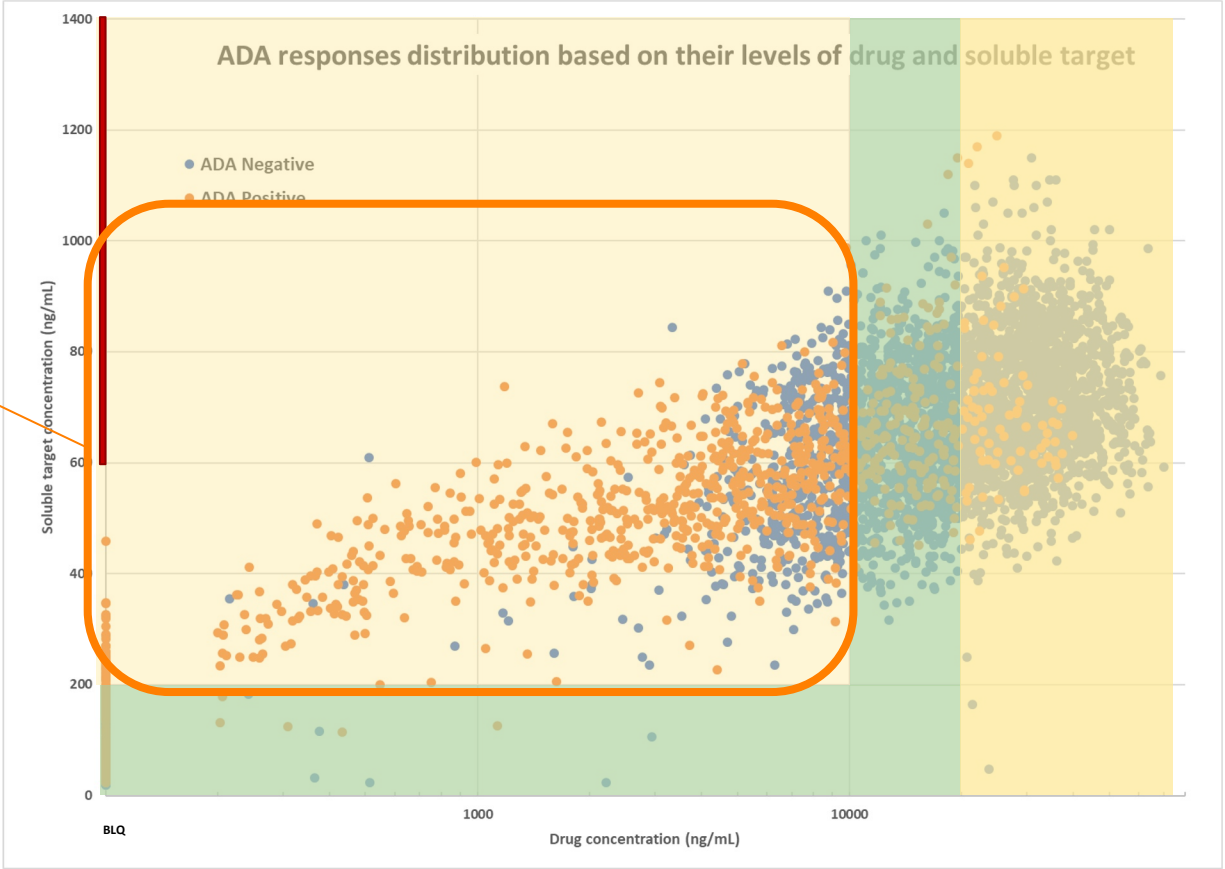
Decoding our ADA assay

Identifying our gaps

In this area, does the soluble target interfere in our assay ?



Decoding our ADA assay

Covering gaps

Additional soluble target interference assessment by combining drug/target concentration in the area of interest:

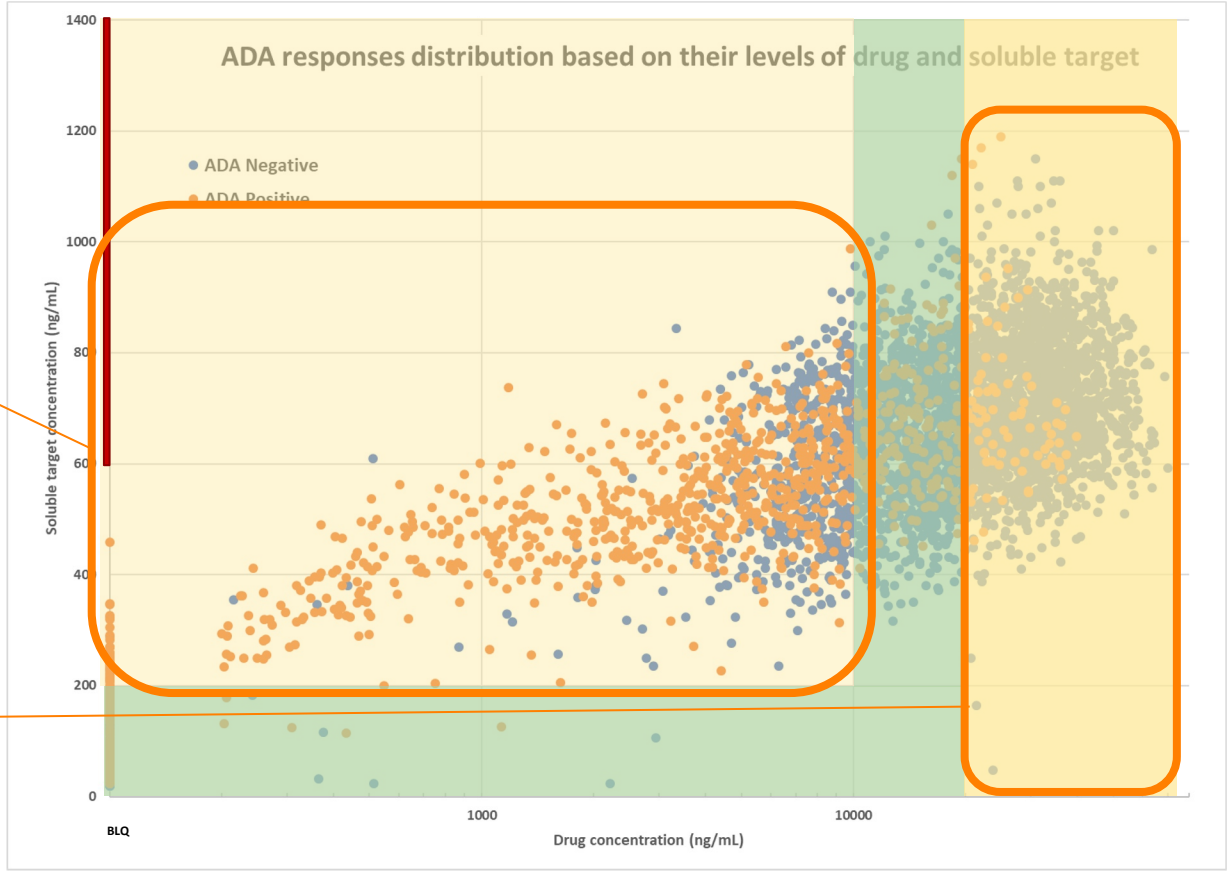
in absence of PC		Drug levels (ng/mL)						
		0	200	500	1000	2000	5000	10000
Soluble Target levels (ng/mL)	0	X	X	X	X	X	X	X
	100	X	X	X	X	X	X	X
	200	X	X	X	X	X	X	X
	400	X	X	X	X	X	X	X
	600	X	X	X	X	X	X	X
	1000	X	X	X	X	X	X	X

Decoding our ADA assay

Identifying our gaps

In this area, does the soluble target interfere in our assay ?

In this area, does our assay maintain sufficient drug tolerance in the presence of low ADA levels?



Decoding our ADA assay

Covering gaps

Additional drug interference assessment by combining drug/target concentration in the area of interest:

In presence of 100 ng/mL PC		Drug levels (ng/mL)						
		0	20000	30000	40000	50000	60000	70000
Soluble Target levels (ng/mL)	0	X	X	X	X	X	X	X
	800	X	X	X	X	X	X	X
	900	X	X	X	X	X	X	X
	1000	X	X	X	X	X	X	X
	1200	X	X	X	X	X	X	X

Decoding our ADA assay

Data outcomes

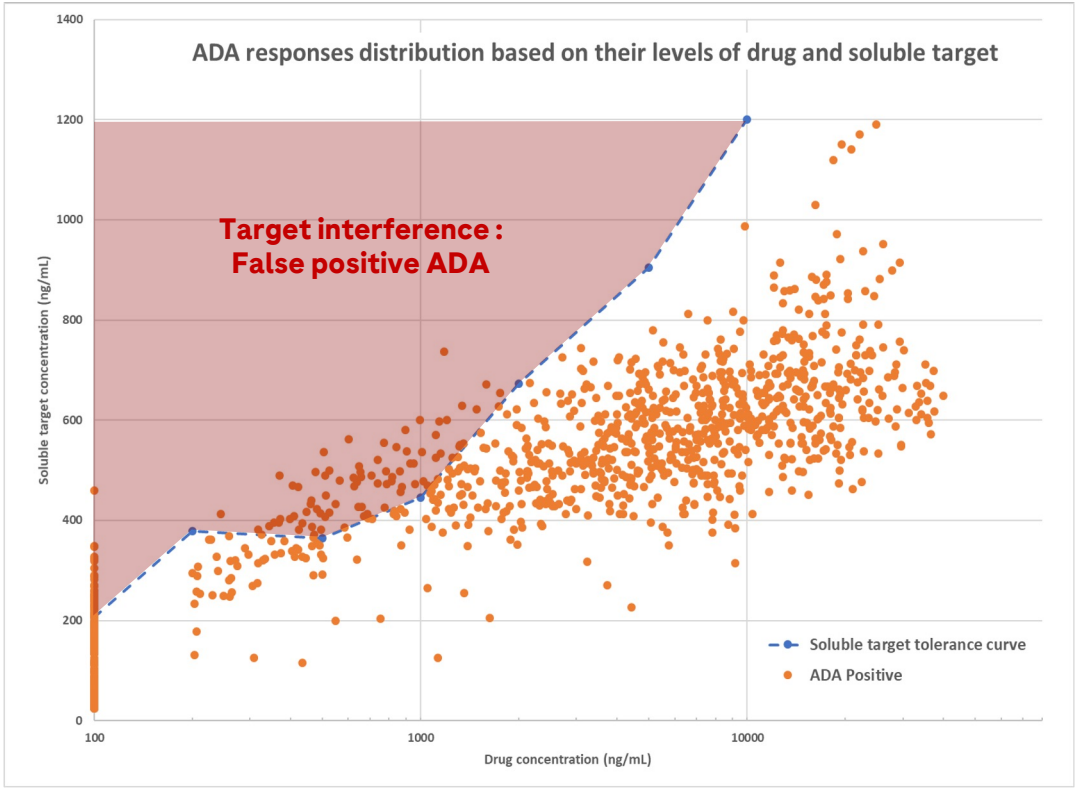
- **Determining the soluble target tolerance curve:**
  For each level of drug tested, the target tolerance level is calculated via the intercept method.

In absence of PC	Drug concentration (ng/mL)						
	0	200	500	1000	2000	5000	10000
Soluble Target tolerance (ng/mL)	206	378	364	446	673	905	>1200

Decoding our ADA assay

Data outcomes

Soluble target tolerance curve



Decoding our ADA assay

Data outcomes

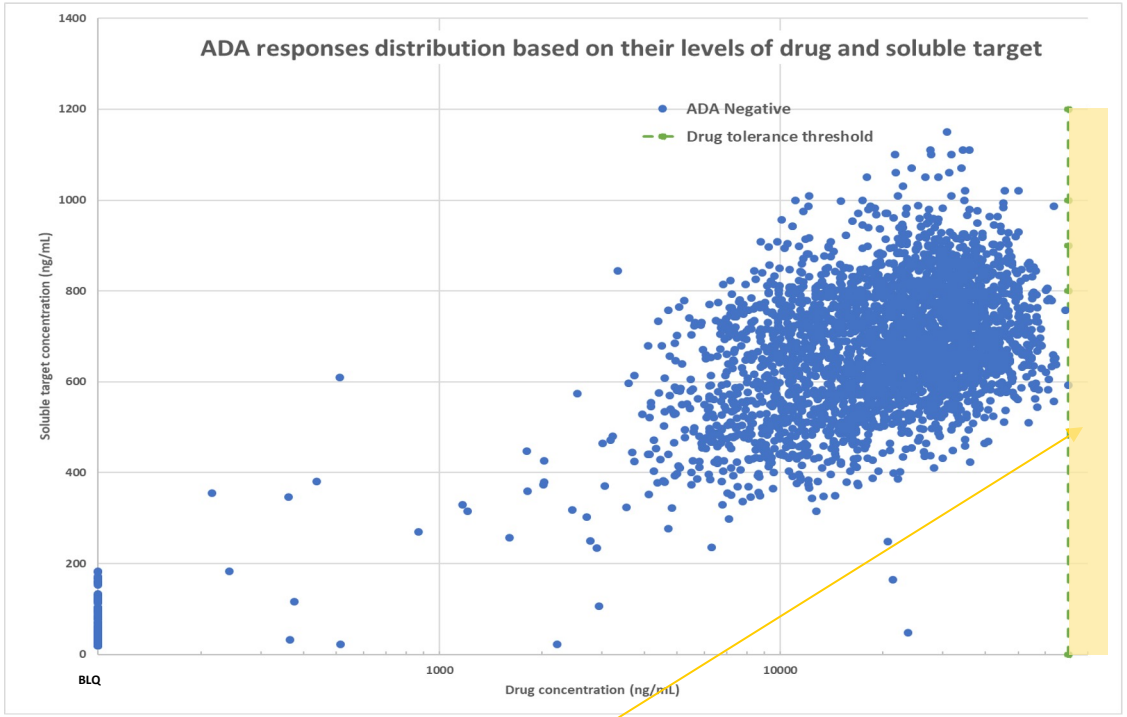
- **Determining the drug tolerance threshold:**

In presence of 100 ng/mL of PC	Soluble target concentration (ng/mL)				
	0	800	900	1000	1200
Drug tolerance (ng/mL)	>70000	>70000	>70000	>70000	>70000

Decoding our ADA assay

Data outcomes

Drug tolerance threshold



For low level of ADA, potential risk of false negative results

Decoding our ADA assay

Data reevaluation

At sample level

True Positive n (%) 991 (23)	False Positive n (%) 118 (3)
(Risk of) False Negative n (%) 1 (0)	True Negative n (%) 3180 (74)

From 44 % to 97% reliable ADA data !

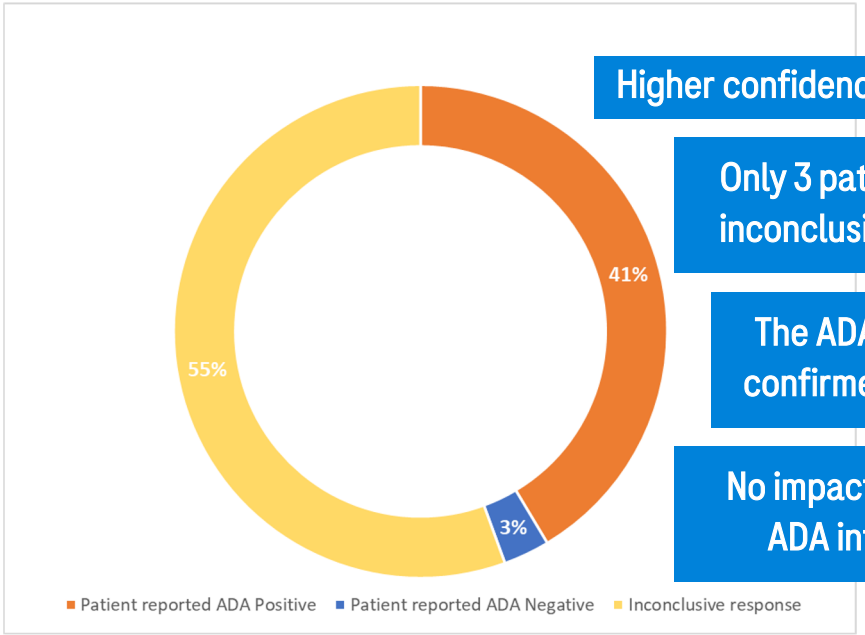
3% of samples analysed are in the “target interference” area

Decoding our ADA assay

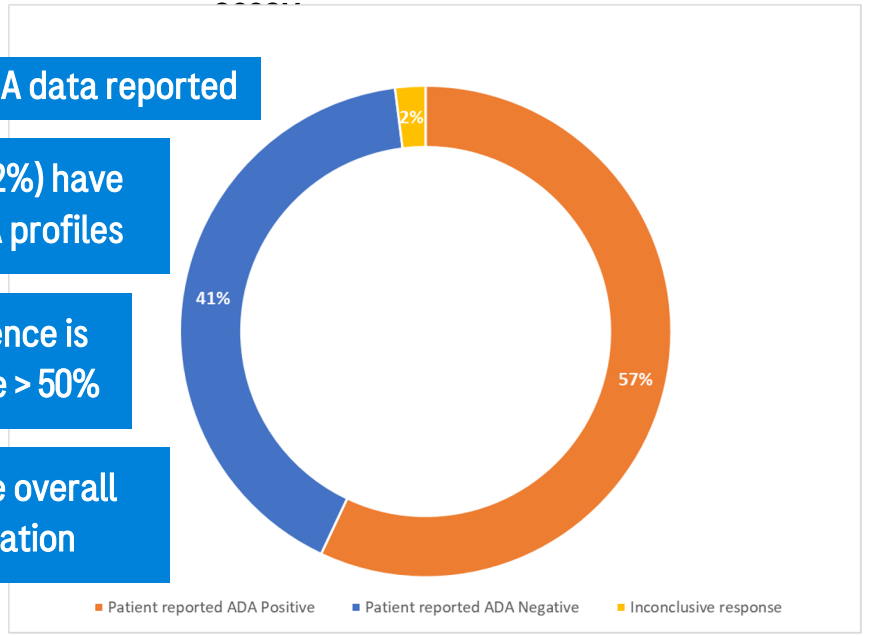
Data reevaluation

At patient level

Before decoding our ADA assay



After decoding our ADA



Higher confidence in ADA data reported

Only 3 patients (2%) have inconclusive ADA profiles

The ADA incidence is confirmed to be > 50%

No impact on the overall ADA interpretation

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Summary : It's not always all about drug interference !

- Don't forget the soluble target when assessing your ADA assay performance *(only if relevant)*!
  - What is the biology of the soluble target ?
  - What is the expected level of soluble target in your studies ?
- Only evaluate what is needed !
  - No need to do too much if not required.
  - Evaluate the drug/target tolerance mapping concept ?

Key takeaway

Anticipate as much as possible all relevant parameters which can impact the development and validation of your ADA assay

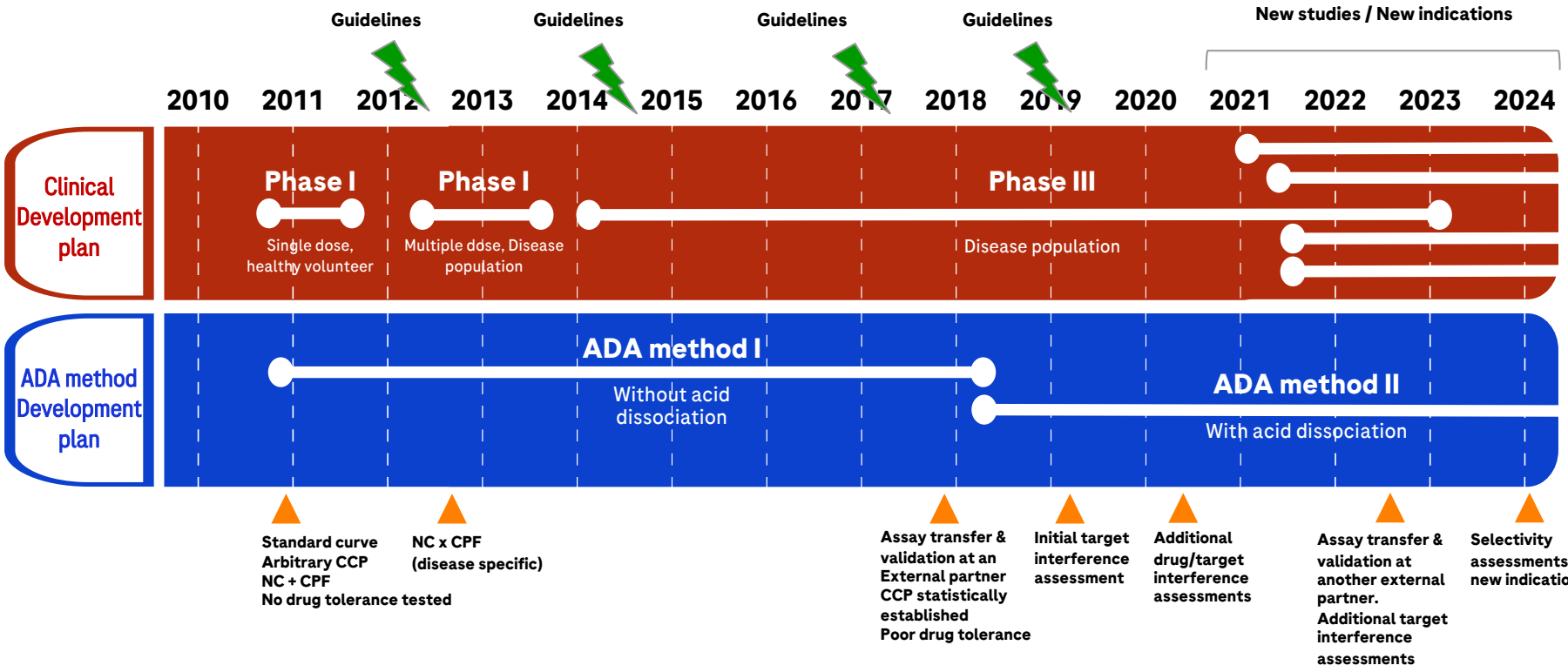
Conclusion

- Our ADA methods are not static; they are dynamic and adaptable tools.
- Throughout their life cycles, numerous factors can shape their performances, including new regulations and biological insights.
- We must continually question our methods' capabilities; they have much to teach us !
- Science should be our guiding compass in making informed choices and decisions.

Key takeaway

Embrace how your ADA methods evolve, use their insights and let the Science guide your decisions throughout their lifecycles

ADA Life cycle



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- My Colleagues from **Chugai Pharmaceutical CO., Ltd** who designed and developed all bioanalytical methods.
- Our **External Partners** who validated the different methods and has been running sample analysis for over a decade.

Doing now what patients need next