bioanalytical sciences

ADA Assay Life-Cycle Management During Clinical Development

A Case Study

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Outline

- Asset Background/Program Overview
- Immunogenicity Risk Assessment
- Evaluation of 1st Generation ADA Assay
- Development of 2nd & 3rd Generation ADA Assays
- Summary of 3rd Generation ADA Assay
- Integrated Immunogenicity Summary

Asset Background/Program Overview

- ***Biologic:***

  Fully human mAb

- ***Administration:***

  IV multiple dose

- ***Therapeutic area:***

  Oncology (multiple indications)

Immunogenicity Risk Assessment

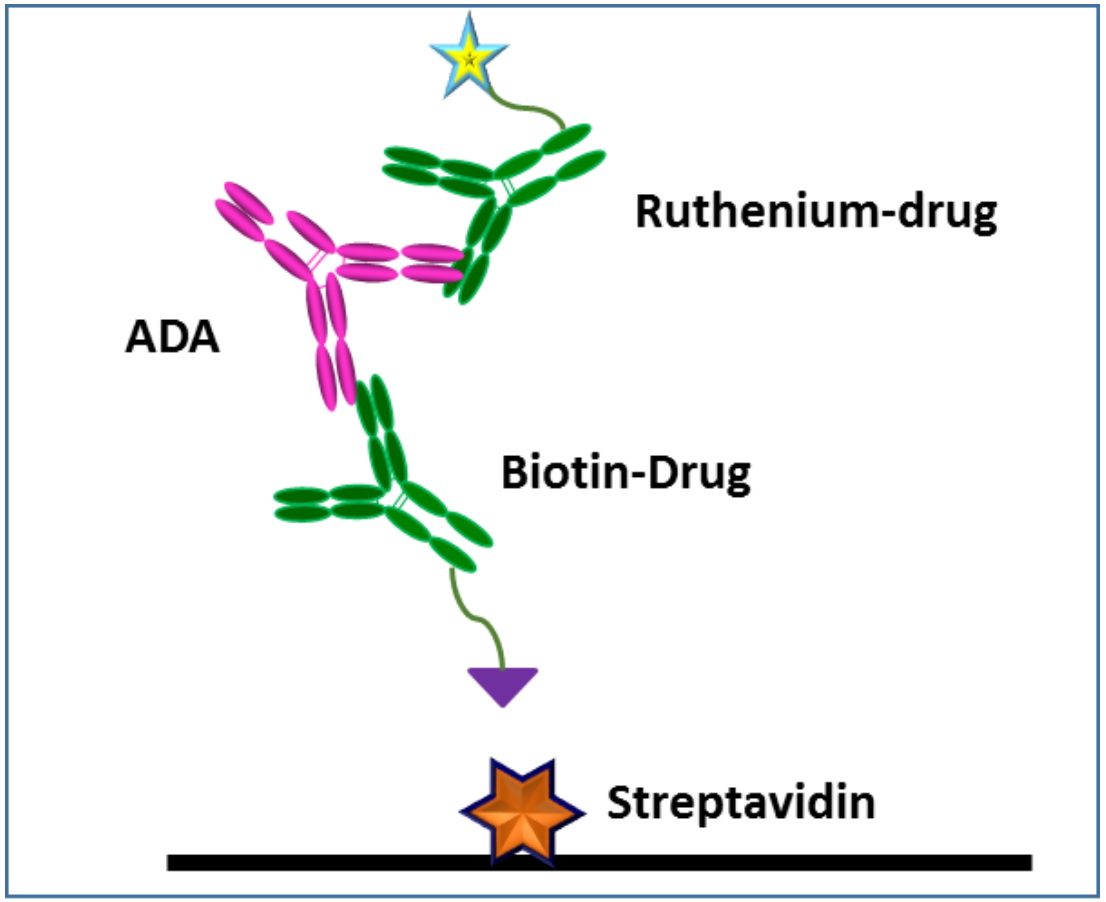
Degree of Foreigness	Low		Medium		High
"Endogenous" version	Low		Medium		High
Target	Soluble				Cell Surface Receptor
Production	Mammalian				Bacterial
Impurities	Low				High
Aggregation	Low		Medium		High
Dosing Frequency	Single	Multiple		Chronic	Intermittent
Dosing amount	Very High				Low -Average
Route of Administration	Oral	i.v.	i.p	s.c.	Inhaled
Clearance ($t_{1/2}$)	Fast				Slow
Patient Immune Status	Suppressed		Normal		Activated

Immunogenicity Risk Assessment

- ***Assay suitability:***
  The ability to detect biologically meaningful levels of ADA in the presence of competing amounts of the biologic.
- ***ADA Assays for Early Clinical Development:***
  - **FIH (Ph1b)**
    - **ADA assay used a screen tier with**
      cut-point (CP) = 1.5*negative control
    - **Data reported as "potentially positive" or negative**
  - **Study A (Ph1b/2a)**
    - **ADA assay used 3-tiered testing (1st generation assay)**
    - **Tier 1 CP and Tier 2 Confirmatory CP established with disease state individuals**

Evaluation of 1st Generation ADA Assays

- **Format: bridging ECL**
- **3-Tiered testing for ongoing studies :**
  - **Screening assay: based on a statistically defined cut point (CP) at 95%**
  - **Confirmatory/Specificity assay: based on a statistically defined specificity/confirmation CP (CCP) at 99%** *(latest FDA guidance 99.9%)*
  - **Titer: the reciprocal of the largest dilution which produces a response above a predefined CP**



Evaluation of 1st Generation ADA Assay

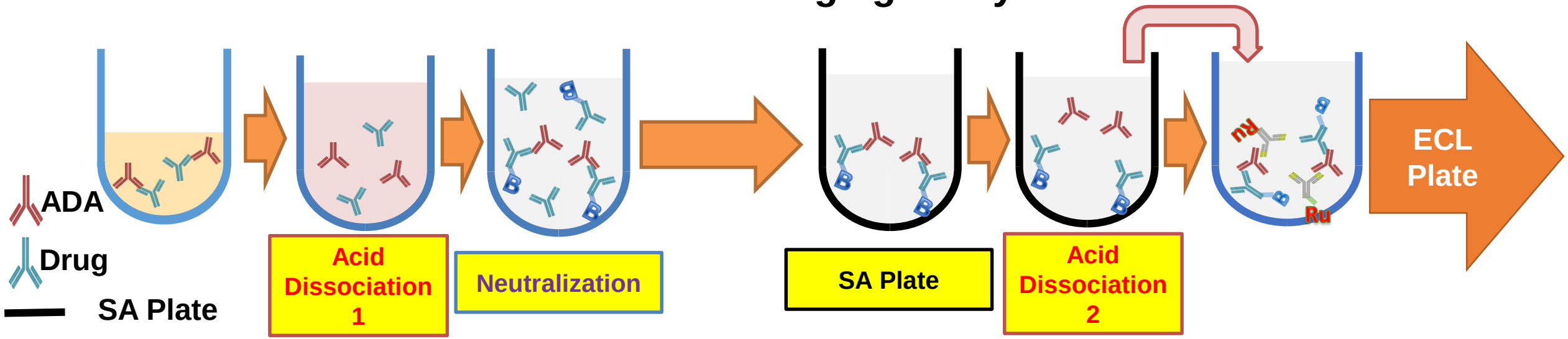
- **Gaps in IMG assessment:**
  - **Poor Drug Tolerance (DT, 1µg/mL drug at 250 ng/mL ADA)**
    - **C_{min} at steady state as high as 189 µg/mL of drug**
    - **[drug] at 100-day Follow Up greater than assay DT**
- **Clinical relevance:**
  - **No apparent drop in Cmin or Cmax at steady state**
  - **No apparent association of immunogenicity and infusion related reactions**

Evaluation of 1st Generation ADA Assay

- **Mitigation provided to clinical team:**
  - **Develop 2nd generation ADA assay with targeted DT ~ 150 µg/mL drug**
  - **Place all ADA analysis on-hold while improved assay is developed**
    - **Samples from on-going study A would be analyzed / reanalyzed using the new method**

Development of 2nd ADA Assay

- **2nd Generation:**
  - **Solid Phase Extraction / Acid Dissociation (SPEAD)**
    - **capture of biotinylated drug/ADA complexes on streptavidin solid phase**
    - **dissociation of ADA from solid phase by acid dissociation**
    - **Detection of ADA in ECL bridging assay**



Development of 2nd Generation ADA Assay

ADA Sensitivity (ng/mL)	[Drug] Tolerance (μg/mL)	
	1st Generation ECL	2nd Generation SPEAD
250	1	12.5
2000	10	60

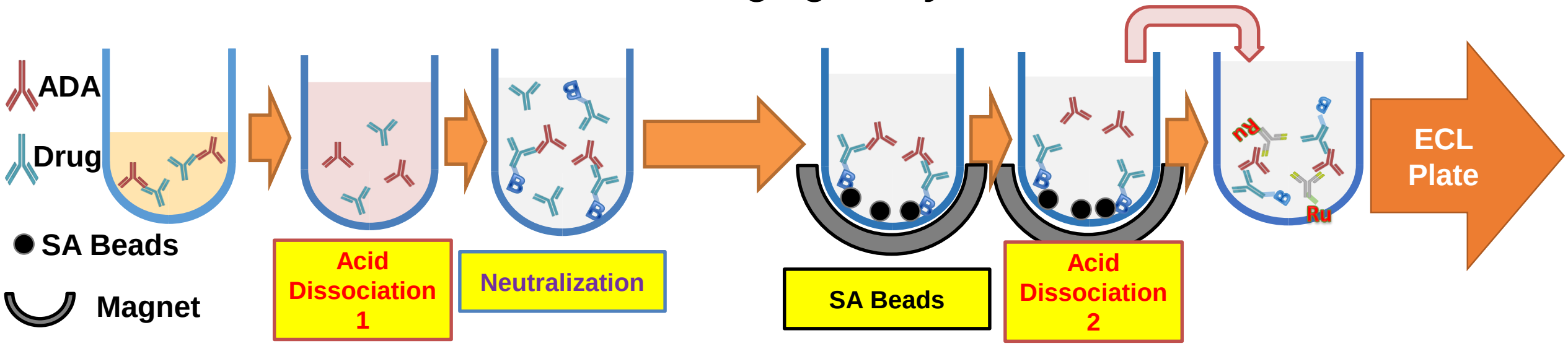
- **2nd Generation:**
  - **Used for ADA analyses for 2 studies (A and B), as team needed immunogenicity data for (Annual Investigator Brochure & IND report filing)**
  - **Marked improvement in DT over original assay, but not sufficient to address requirements**

Development of 3rd Generation ADA Assay

- **Mitigation provided to clinical team:**
  - **Continue development of ADA assay (3rd generation)**
    - Further improve DT
    - Establish CPs for multiple tumor types
  - **All new studies' sample analysis remained on-hold**

Development of 3rd Generation ADA Assay

- **3rd Generation:**
  - **Bead Extraction / Acid Dissociation (BEAD)**
    - **capture of biotinylated drug/ADA complexes on magnetic streptavidin beads**
    - **dissociation of ADA from beads by acid dissociation**
    - **Detection of ADA in ECL bridging assay**



Drug Tolerance Comparison Across the ADA Assays

[Pos. Control] (ng/mL)	[Drug] Tolerance (μg/mL)		
	1st Generation ECL	2nd Generation SPEAD	3nd Generation BEAD
250	1	12.5	800
2000	10	60	1000*
*: highest concentration tested			

3rd generation ADA assay implemented throughout the program moving forward

3rd Generation Assay: Combined Disease State Cut-points

- Comparison of RLU's for Cancer Population 1, 2 & 3

Cancer Population	n*	Mean (SD) RLU	Minimum, Maximum RLU
Population 1	20	173 (34)	131, 250
Population 2	20	187 (57)	116, 310
Population 3	20	160 (32)	108, 229

*Assay results averaged over analyst for each donor sample. Individual outliers were excluded prior to averaging within each donor sample.

- **No mean difference in RLUs (p=0.15) supports the calculation of a combined CP**

3rd Generation Assay: Combined Disease State Cut-points

- Comparisons of Percent Inhibitions for the 3 donor populations

Cancer Population	n*	Mean (SD) % Inhibition	Minimum, Maximum % Inhibition
Population 1	20	45.6 (8.4)	29.2, 63.4
Population 2	20	42.0 (13.2)	21.1, 67.7
Population 3	20	37.7 (7.9)	21.9, 52.4

*Assay results averaged over analyst for each donor sample. Individual outliers were excluded prior to averaging within each donor sample.

- **No overall mean difference in % Inhibition (p=0.06) supports the calculation of a combined CCP**

3rd Generation Assay: Combined CP In –Study Assessment

Populations 1, 2 & 3	n*	Mean (SD) RLU and Sample to NC ratio	Min. – Max. RLU and Sample to NC ratio
In-study	388	103 (22) 1.02 (0.18)	63 – 175 0.67 – 1.57
Pre-Study	55	116 (23) 1.00 (0.19)	88 – 187 0.76 – 1.62

*Outliers excluded

- **No overall mean difference in RLUs for the populations in -study (p=0.55) supports the calculation of a combined CP**
- **High degree of overlap from validation data to in- study data**

Integrated Immunogenicity Summary

Study	Assay ID	# Subjects	ADA positive at Baseline	ADA positive Rel. to Baseline
			n (%)	
B	2nd Gen	243	7 (2.9)	21 (8.6)
C	3rd Gen	109	8 (7.3)	21 (19.3)
D	3rd Gen	181	9 (5.0)	13 (7.2)
E	3rd Gen	251	18 (7.2)	43 (17.1)
F	3rd Gen	101	11 (10.9)	12 (11.9)
G	3rd Gen	107	3 (2.8)	6 (5.6)
H	3rd Gen	288	10 (3.5)	33 (11.5)
Combined (Studies B-G)	3rd Gen	1037	59 (5.7)	128 (12.3)
Validation	3rd Gen	60	3 (5)	not applicable

Integrated Immunogenicity Summary

Immunogenicity has no clinically meaningful effect on the safety, efficacy, or pharmacokinetics, of the drug

- ***Safety:*** ***No observed association between ADA status and hypersensitivity or infusion site reactions.***
- ***Efficacy:*** ***ADA was not associated with loss of efficacy***
- ***Pharmacokinetics:*** ***The presence of ADA did not significantly alter PK.***

Summary

- ***Through the course of the program, ADA methodology evolved with the primary focus to improve drug tolerance***
- ***Mitigation consisted of re-analysis of in-study samples during Ph1, banking of samples and implementation of fit-for purpose validated assay***

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