Detection of low affinity anti-drug antibodies and improved drug tolerance in immunogenicity testing by Octet® biolayer interferometry

Jian Li
Biologics Clinical Pharmacology,
Centocor Research and Development, Inc

Background

- ADA immunogenicity assessment is a critical component in demonstrating the safety and efficacy profile of a therapeutic biological drug.
- The bridging immunoassay (typically EIA or ECL-based) is widely used to measure ADA, however, these methods may generate false negative results when
  - Test samples contain the therapeutic
  - ADA is of low affinity
- CNTO X is a therapeutic human IgG1 that neutralizes a cytokine.
- FortéBIO's Octet System utilizes biolayer interferometry (BLI) to perform a label-free analysis of protein binding interactions in real time.

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Proof of Concept Study:
ADA Detection Using Octet QK

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Comparison of Assay Sensitivity

	Anti-CNTO X Ab	K_D (nM)	Sensitivity (ng/mL)		
			ELISA	ECLIA	Octet-QK
	Cyno Poly IgG	NA	6	1	130
Higher affinity	mAb 7473	5.3	195	49	1000
	mAb 8110	6.0	24	12	500
	mAb 2825	6.2	781	391	1000
	mAb 5583	6.5	195	391	1000
	mAb 539	6.6	781	781	1000
	mAb 8584	9.2	391	49	250
	mAb 5984	11.6	391	391	1000
	mAb 7942	12.2	6250	1563	5000
	mAb 9698	14.0	3125	1563	1000
Lower affinity	mAb 1773	38.9	6250	781	500
	mAb 7679	50.0	6250	3125	2000
	mAb 2960	65.8	12500	6250	2000
	mAb 5968	80.7	781	1563	2000

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Comparison of ADA Detection in Context of the Current Regulatory Expectations of Assay Sensitivity

Ability to Detect 9 Higher Affinity ADAs

Method	Sensitivity Target ≤ 250 ng/mL	≤ 500 ng/mL	≤ 1000 ng/mL
ELISA	3/9	5/9	7/9
ECLIA	3/9	6/9	7/9
Octet-QK	1/9	2/9	8/9

Ability to Detect 4 Lower Affinity ADAs

Method	Sensitivity Target ≤ 250 ng/mL	≤ 500 ng/mL	≤ 1000 ng/mL	≤ 2000 ng/mL
ELISA	0/4	0/4	1/4	1/4
ECLIA	0/4	0/4	1/4	2/4
Octet-QK	0/4	1/4	1/4	4/4

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Comparison of ADA Detection in the Presence of Interference Drug

ADA mAb #	K_D (nM)	ADA (µg/mL)	Concentration of CNTO X tolerated					
			ELISA		ECLIA		Octet-QK	
			CNTO X (µg/mL)	*Molar Excess**	CNTO X (µg/mL)	*Molar Excess**	CNTO X (µg/mL)	*Molar Excess**
5583	6.5	0.1	1	*10*	1	*10*	10	*100*
8584	9.2	0.1	1	*10*	10	*100*	10	*100*
7679	50.0	0.5	0.1	*0.2*	1	*2*	10	*20*
2960	65.8	1.25	0.01	*0.008*	10	*8*	100	*80*

* Moles of CNTO X / Moles of ADA

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Summary of Octet QK data

- Within the recommended sensitivity range for clinical ADA methods (≤ 500ng/mL), the ELISA and MSD offered better higher affinity ADA detection capability.
- Within the recommended sensitivity range for non-clinical ADA methods (≤ 1000ng/mL), the Octet was superior.
- For the detection of low affinity ADA, Octet offered a significant advantage.
- Overall ranking of drug tolerance for the three methods was:

Octet > MSD > ELISA.

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Detection of ADA in Samples from A Preclinical Study Using Octet RED

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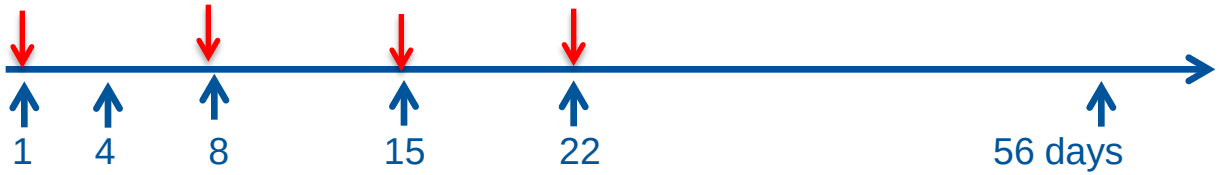
Preclinical Cynomolgus Monkey Toxicity Study Design

CNTO X administration (SC or IV)



Blood was collected immediately prior to administration of drug as well as on days 4 and 56

	Control	CNTO X 20mg/kg	CNTO X 100mg/kg	CNTO X 100mg/kg
Route	SC	SC	SC	IV
Monkeys	3	3	3	3

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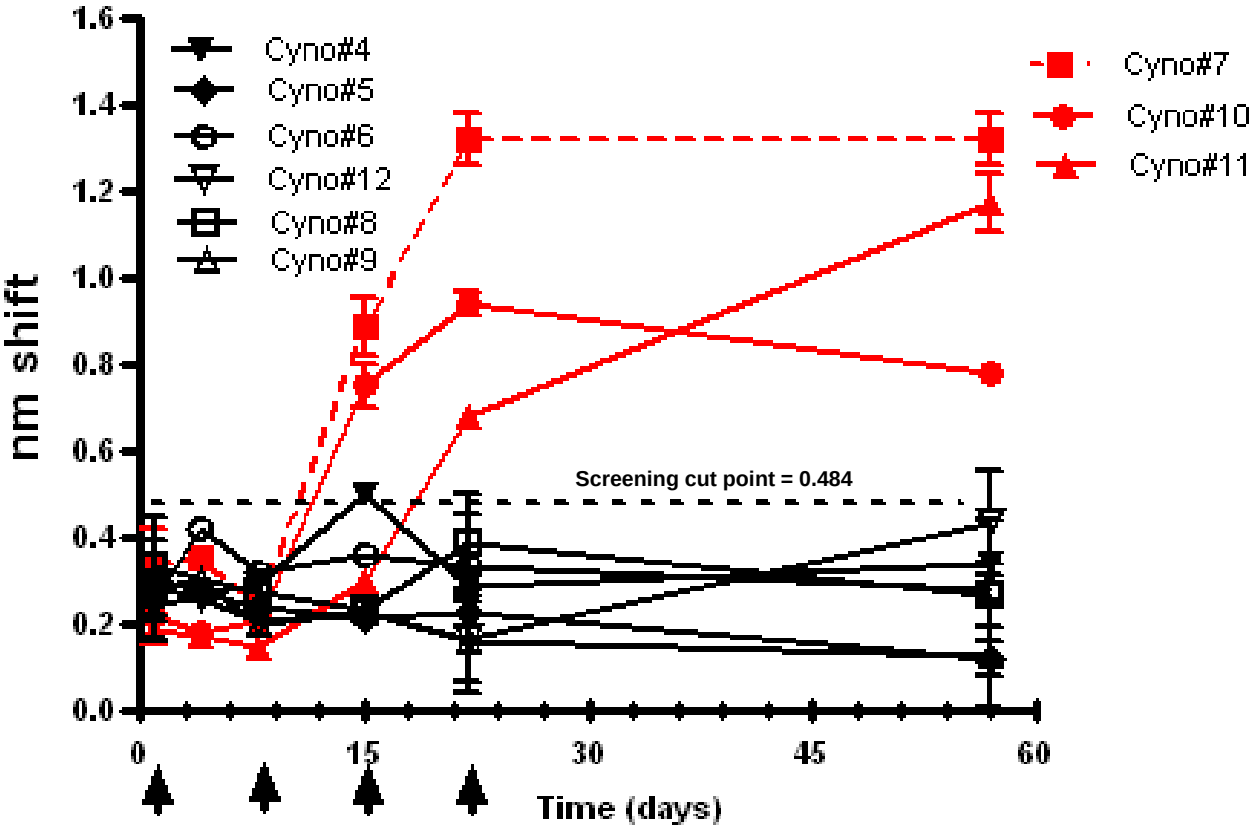
Using Octet RED, ADA Was Detected In 3 Of 9 Monkeys Receiving Weekly Doses Of CNTO X



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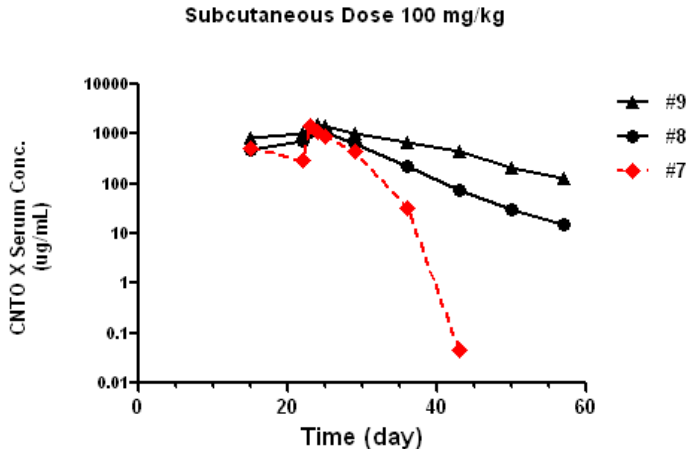
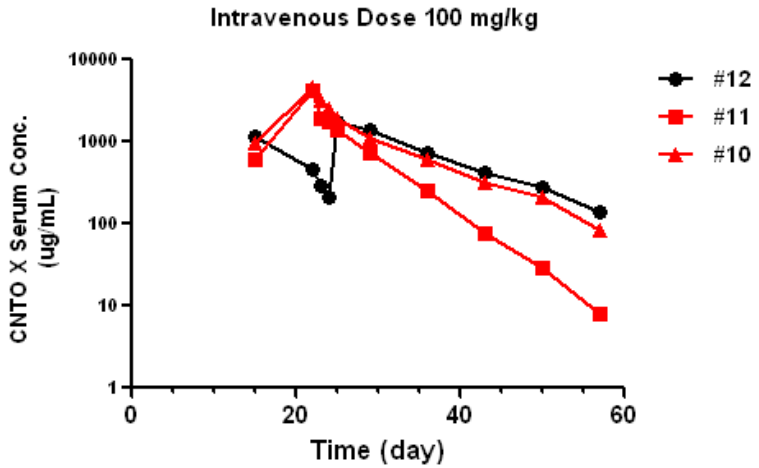
Individual Serum Concentration-Time Profiles of CNTO X Following Multiple Doses Of 100 mg/kg

A



B



Deborah Kwok's data

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Comparison Of ADA Detection By ECLIA And Octet RED Verses Circulating Serum CNTO X Concentration

Monkey	Dose Group	Study Day	CNTO X (µg/mL)	ADA detection by ECLIA		ADA detection by Octet-RED	
				Value†	Result	Value†	Result
7	100 mg/kg, SC	1	**<0.02	0.83	Neg	0.70	Neg
		4	799.03	0.61	Neg	0.73	Neg
		8	552.33	0.57	Neg	0.51	Neg
		15	537.09	0.59	Neg	1.80	**Pos**
		22	257.92	0.50	Neg	2.70	**Pos**
		57	**<0.02	1.70	**Pos**	5.90	**Pos**
10	100 mg/kg, IV	1	<0.02**	0.28	Neg	0.46	Neg
		4	1083.73	0.59	Neg	0.38	Neg
		8	738.62	0.59	Neg	0.44	Neg
		15	951.89	0.58	Neg	1.60	**Pos**
		22	941.08	0.40	Neg	1.90	**Pos**
		57	82.50	0.30	Neg	1.80	**Pos**
11	100 mg/kg, IV	1	**<0.02	0.92	Neg	0.38	Neg
		4	965.86	0.62	Neg	0.36	Neg
		8	547.90	0.60	Neg	0.31	Neg
		15	615.99	0.60	Neg	0.60	Neg
		22	731.33	0.50	Neg	1.40	**Pos**
		57	8.02	0.50	Neg	2.40	**Pos**

† Normalized value = assay result /assay cut point, so that values > 1 indicate positive ADA.
** Result was less than the LLOQ of the serum CNTO X concentration assay.

^Abu Siddique's data

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Confirmation of Octet RED ADA Screening Result in Cynomolgus Monkey Study Of CNTO X

Monkey	Dose Group	Study Day	% Inhibition of ADA signal (Mean±SD) by CNTO X	% Inhibition of ADA signal (Mean±SD) by Isotype matched human IgG
#1	Vehicle	22	4.5± 5.7	3.8± 4.2
#12	CNTO X 100 mg/kg, IV	57	28.1± 32.5	10.7± 26.3
#7	CNTO X 100 mg/kg, SC	15	65.4± 2.3	ND*
		22	62.9± 3.3	3.6± 2.1
		57	80.9± 2.2	12.8± 0.9
#10	CNTO X 100 mg/kg, IV	15	67.7± 0.2	ND*
		22	76.6±2.2	6± 3.1
		57	86.1± 1.9	2.4± 0.9
#11	CNTO X 100 mg/kg, IV	22	79.6± 2.5	7.6± 4.4
		57	96.2± 1.9	1.9± 5.5

*ND: not done
Specificity cut point = 56.8%

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A Simple Model Depicting Possible Molecular Interactions

Conclusion

- Octet appears to be a promising technology platform for the detection of lower affinity ADA, and particularly suitable for the detection of ADA when drug persists at levels that cause negative interference in bridging immunoassays.

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Key Contributors

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John Proctor

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