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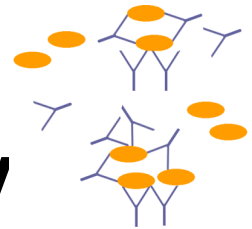
# Detection of Immune Complex Formation in Non-Clinical Studies and Implications for Clinical Risk Assessment

Steven J Swanson, Ph.D.

Executive Director, Medical Sciences/Clinical Immunology

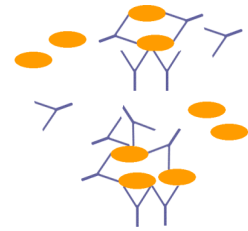
[swanson@amgen.com](mailto:swanson@amgen.com)

# Immune Complex Related Pathology



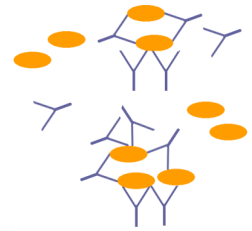
- **Have been observed in non clinical studies**
- **Risk factors for acute effects include:**
  - **mAb therapeutics (due to their large MW)**
  - **IV administration (due to large amount of drug entering the circulation within a short time)**

# Circulating Immune Complex Assays



- **Traditional assays have not met the need**
- **Variable results**
- **Lack of specificity**
- **Immune complexes comprise a diverse population and may have variable stability**
- **New methods are evolving**

# Hypersensitivity reactions (HSRs)



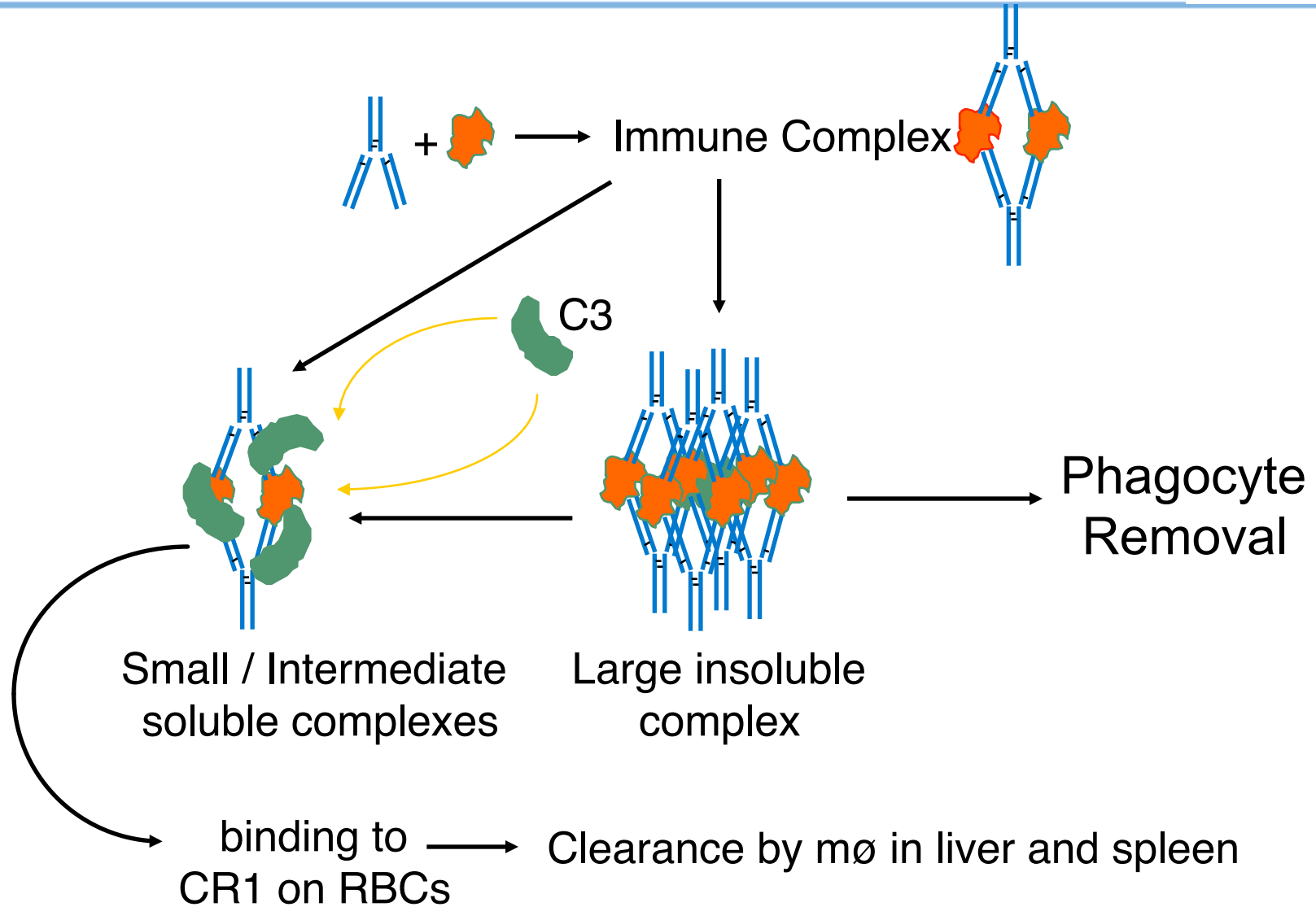
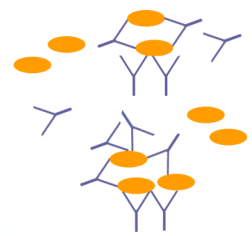
Inappropriate or damaging immune and inflammatory response that is harmful to the host

Reactions initiated by Ab and drug-ADA complexes are referred to as “Immediate” Hypersensitivity and manifest in minutes to hours after “antibody drug”

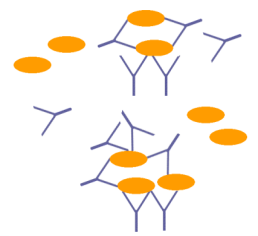
Types

- Type I – Immediate type; IgE mediated
- Type II – IgG or IgM antibody-mediated cell cytotoxicity (ADCC) or complement-mediated lysis of cells (CDC)
- **Type III – IgG mediated immune complex reactions resulting in formation, deposition and complement activation with local tissue destruction**
- Type IV – Delayed-type; Th cell mediated

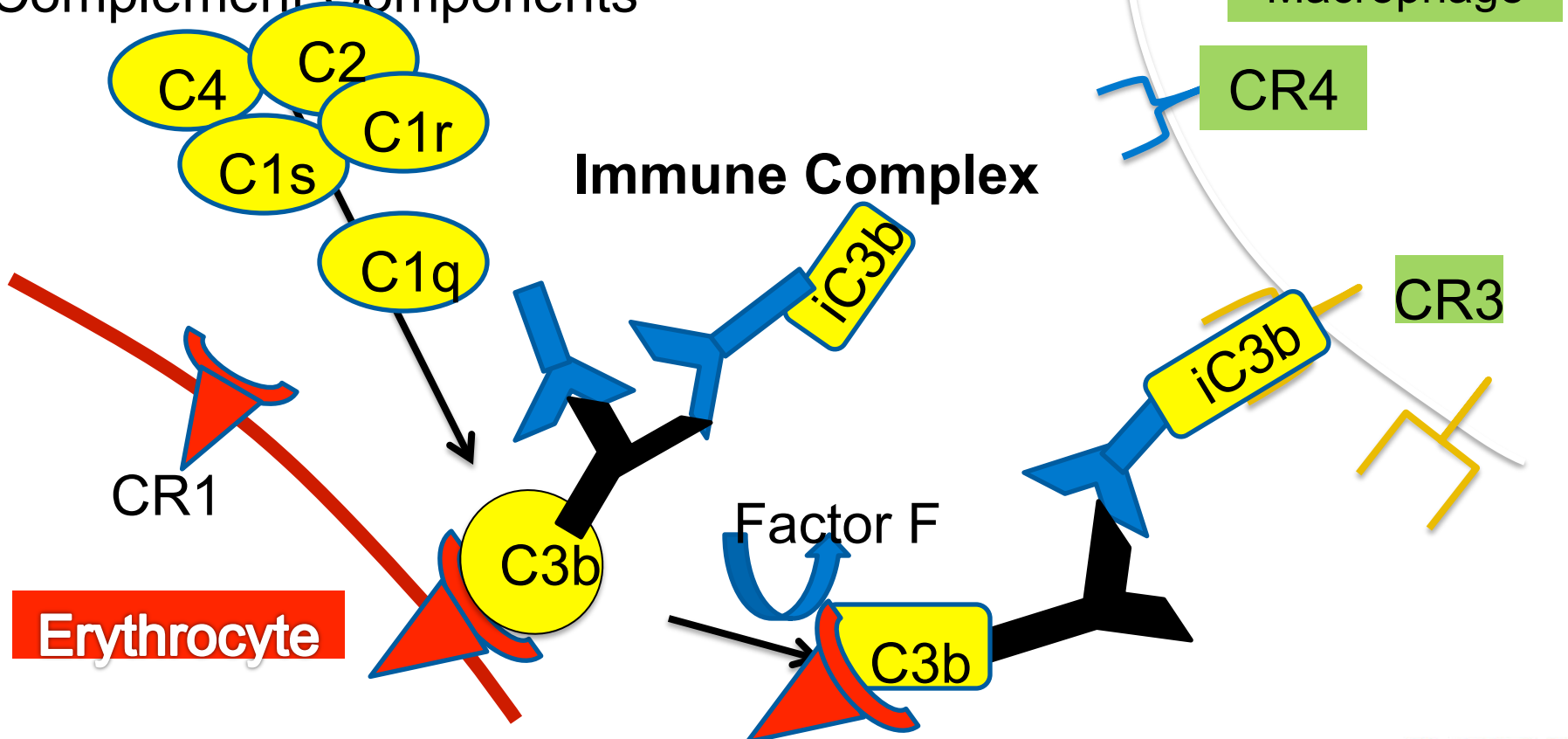
# Formation and clearance of immune complex



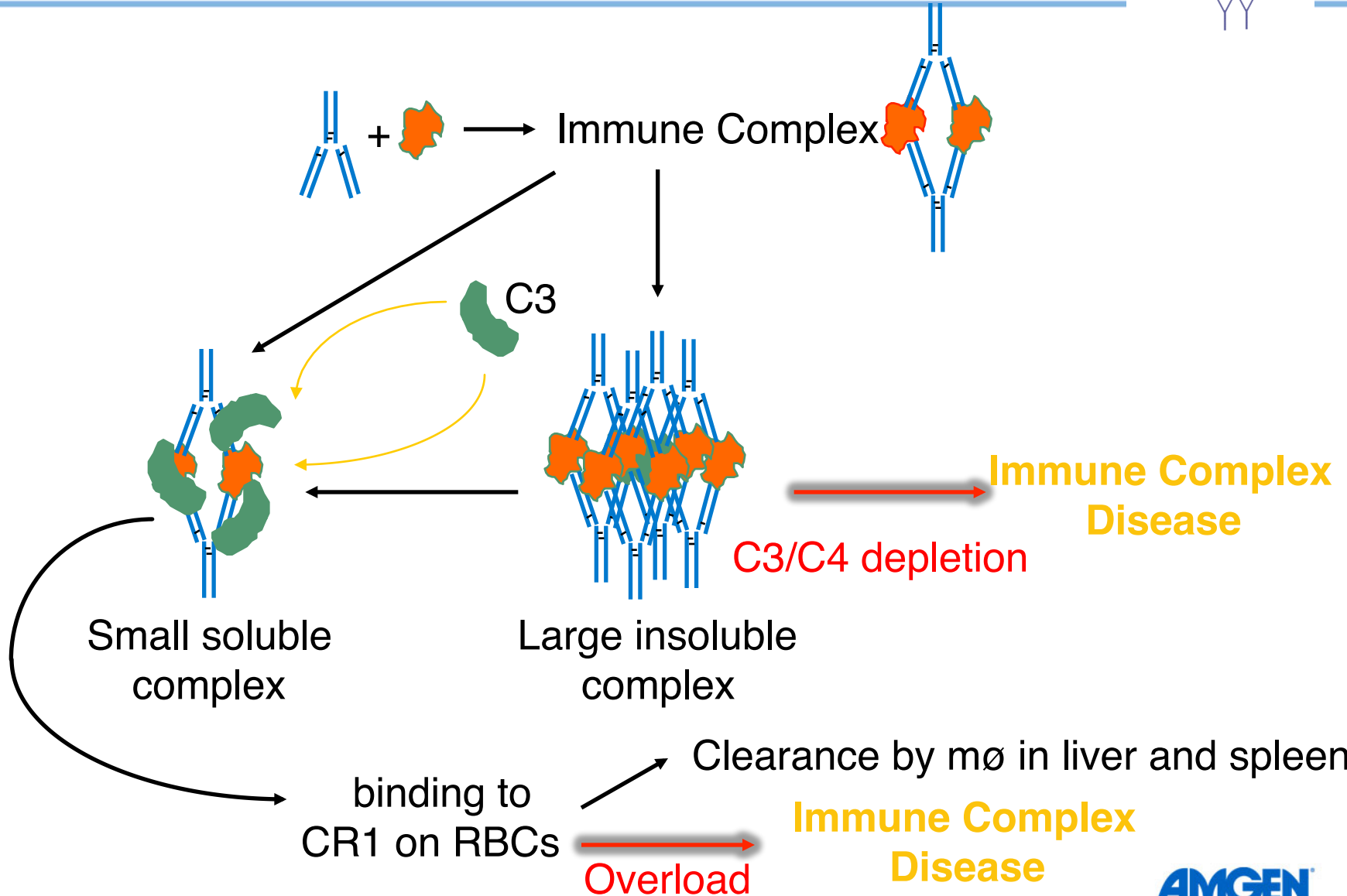
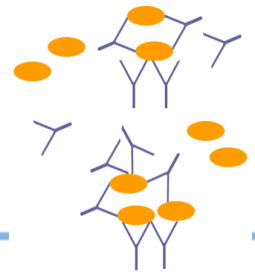
# CICs are transferred from RBCs to liver macrophages



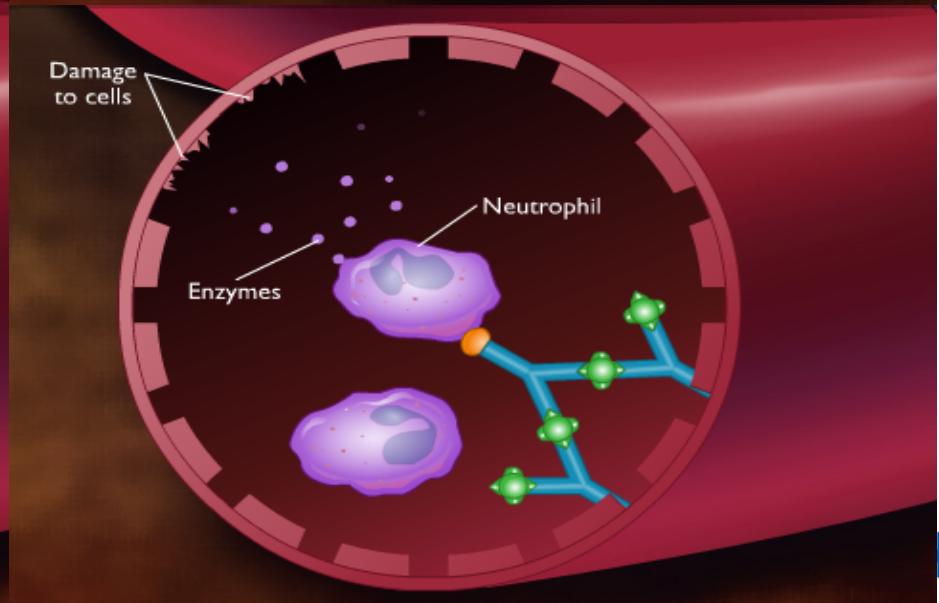
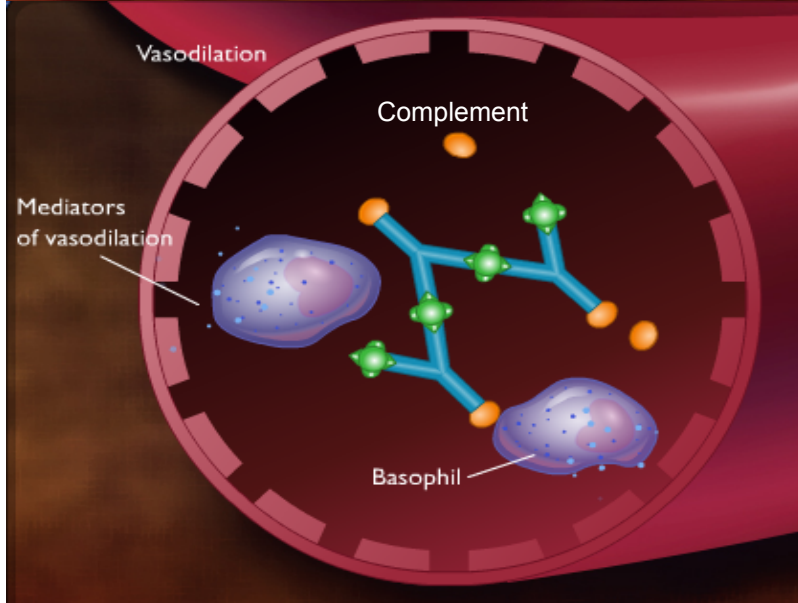
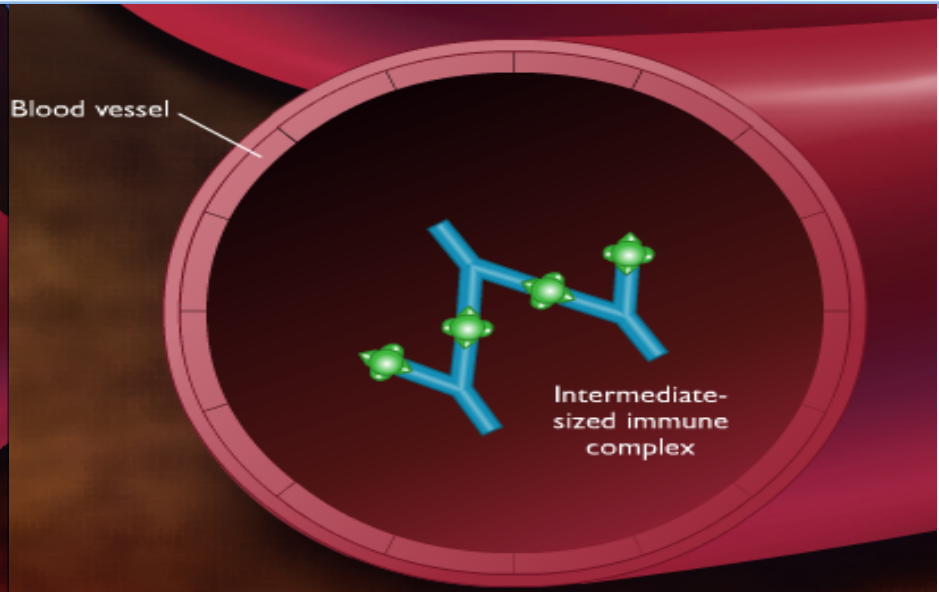
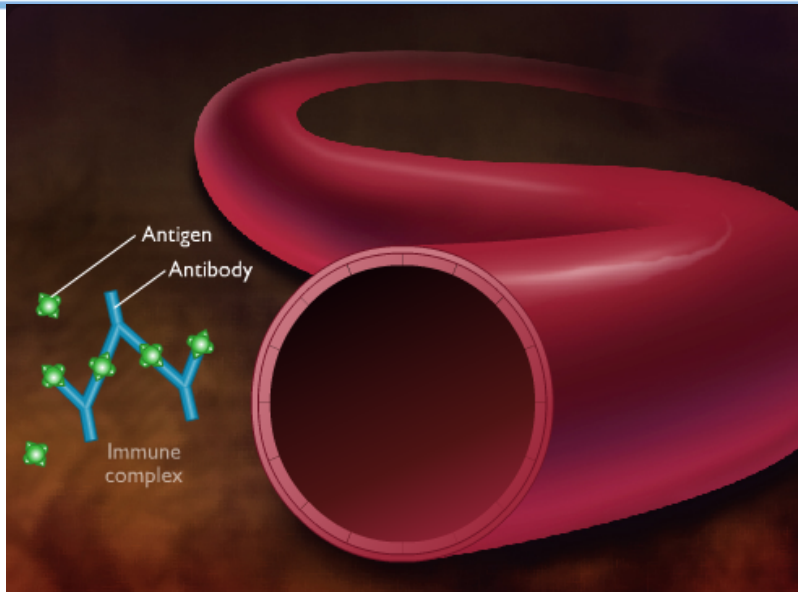
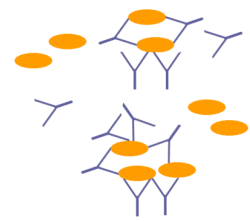
## Complement Components



# Saturated IC clearance or large complexes can contribute to pathology

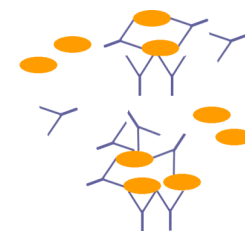


# Immune complexes and vasculitis





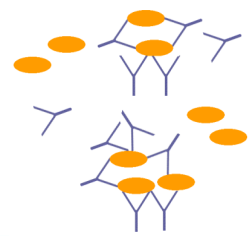
# Non-Clinical Observation When Drug Was Cleared Between Doses



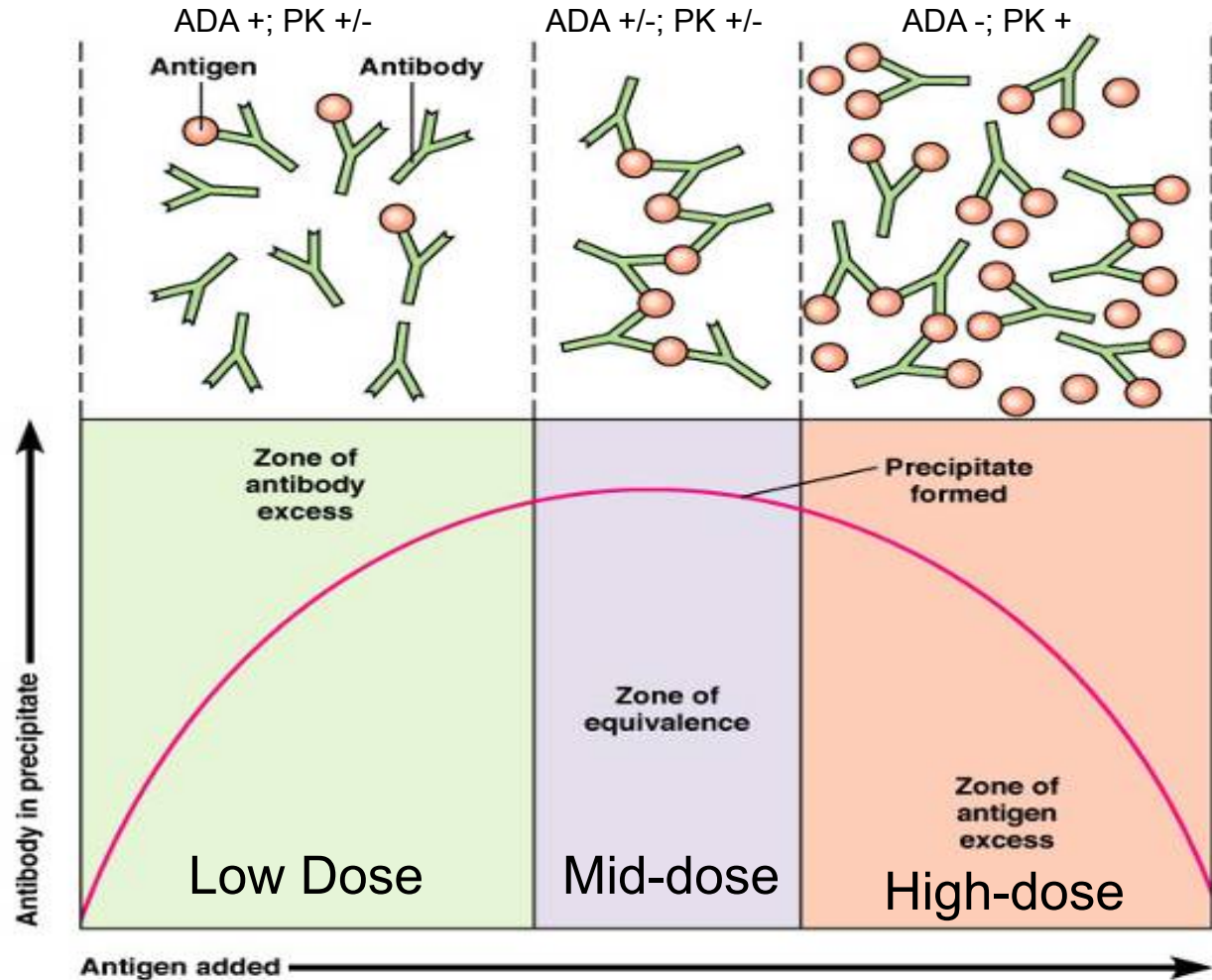
- NHPs administered multiple injections of IgG1/IgG2 human antibodies
- Not a Group effect; individual animals
- Effects noted a short period after dosing (minutes to ~2 days)
- In IV dosed groups (vs SC) at lower (eg 10-50 mg/kg) as opposed to higher doses (i.e., 300 mg/kg)
- Potential clinical findings post-dose:
  - Vomiting, difficulty breathing, weakness/lethargy, death
  - Prominent bleeding or bruising at injection site
  - Petechial hemorrhages
- Clinical pathology findings:
  - Activated platelets +/- change in platelet counts
  - Decreased neutrophils and monocytes
- Affected animal(s) had:
  - High ADAs
  - Below Quantifiable Limits (BQL) drug prior to next dose

**NOTE: Example only, does not always occur**

# ADA/drug Ab complex complicates CI ADA detection and PKDM drug Ab detection

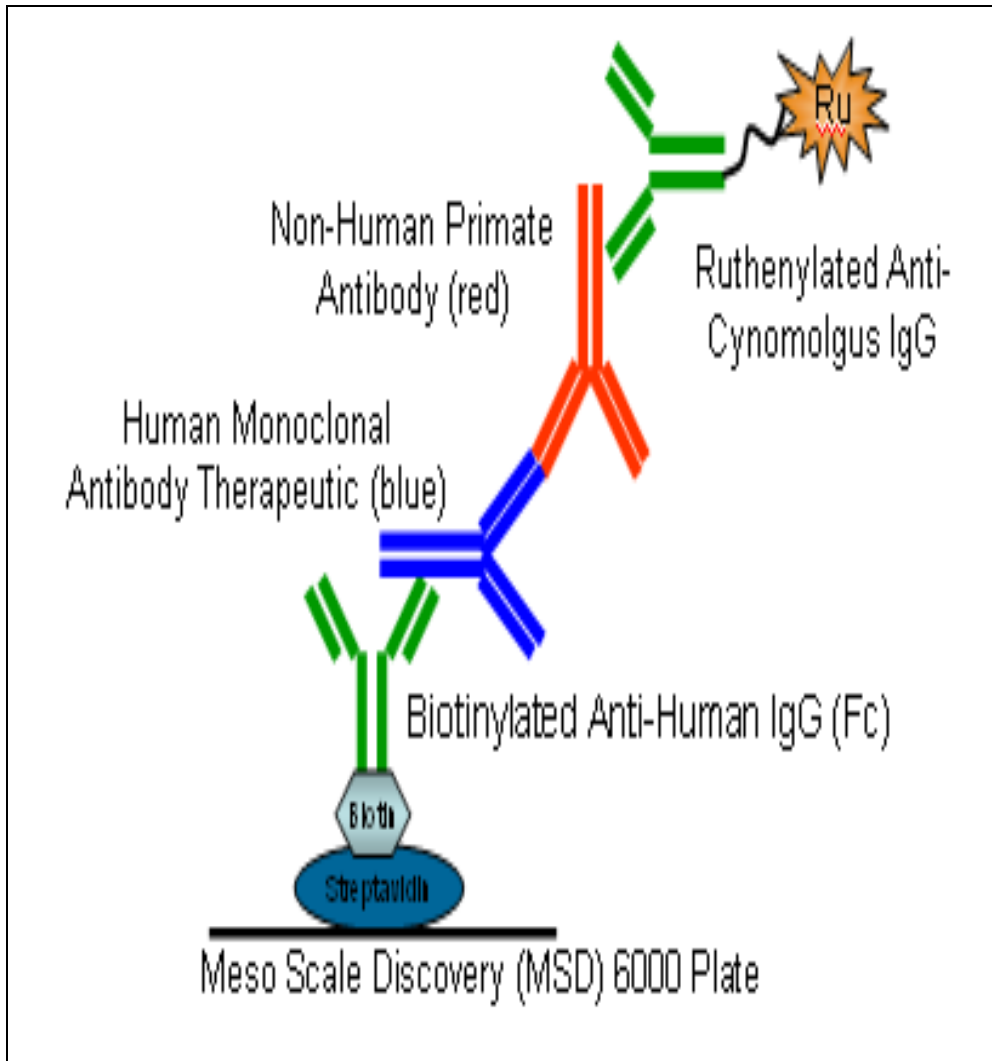
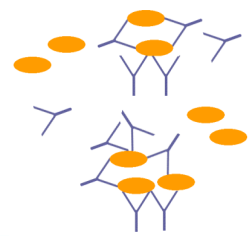


Reliability Factor:

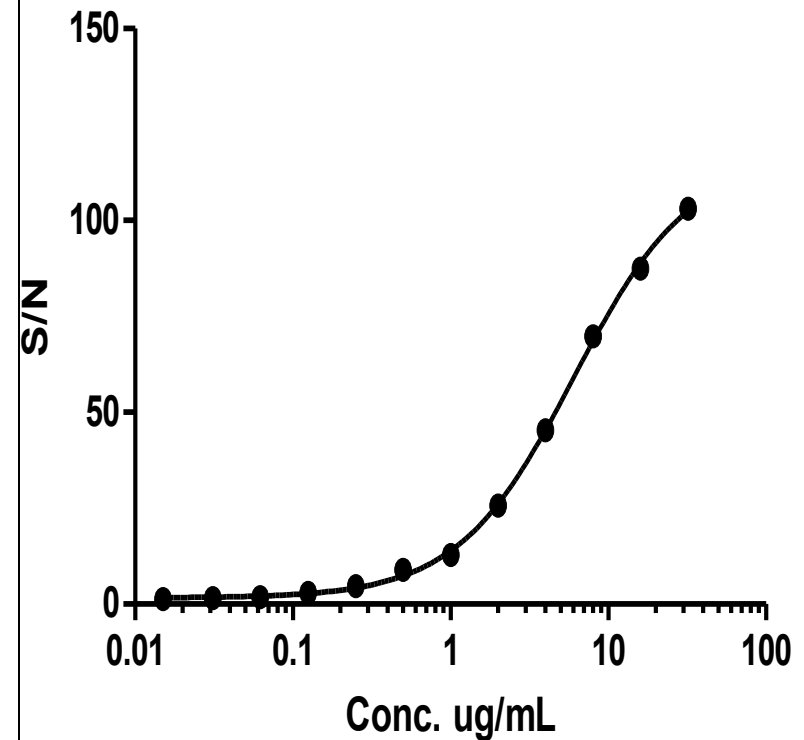


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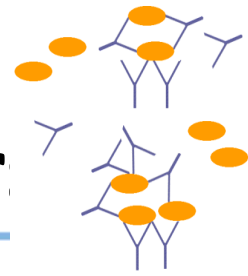
# Immunoassay for huAb drug induced-immune complexes in NHP



CIC Assay Standard Curve using 1:1 Positive Control

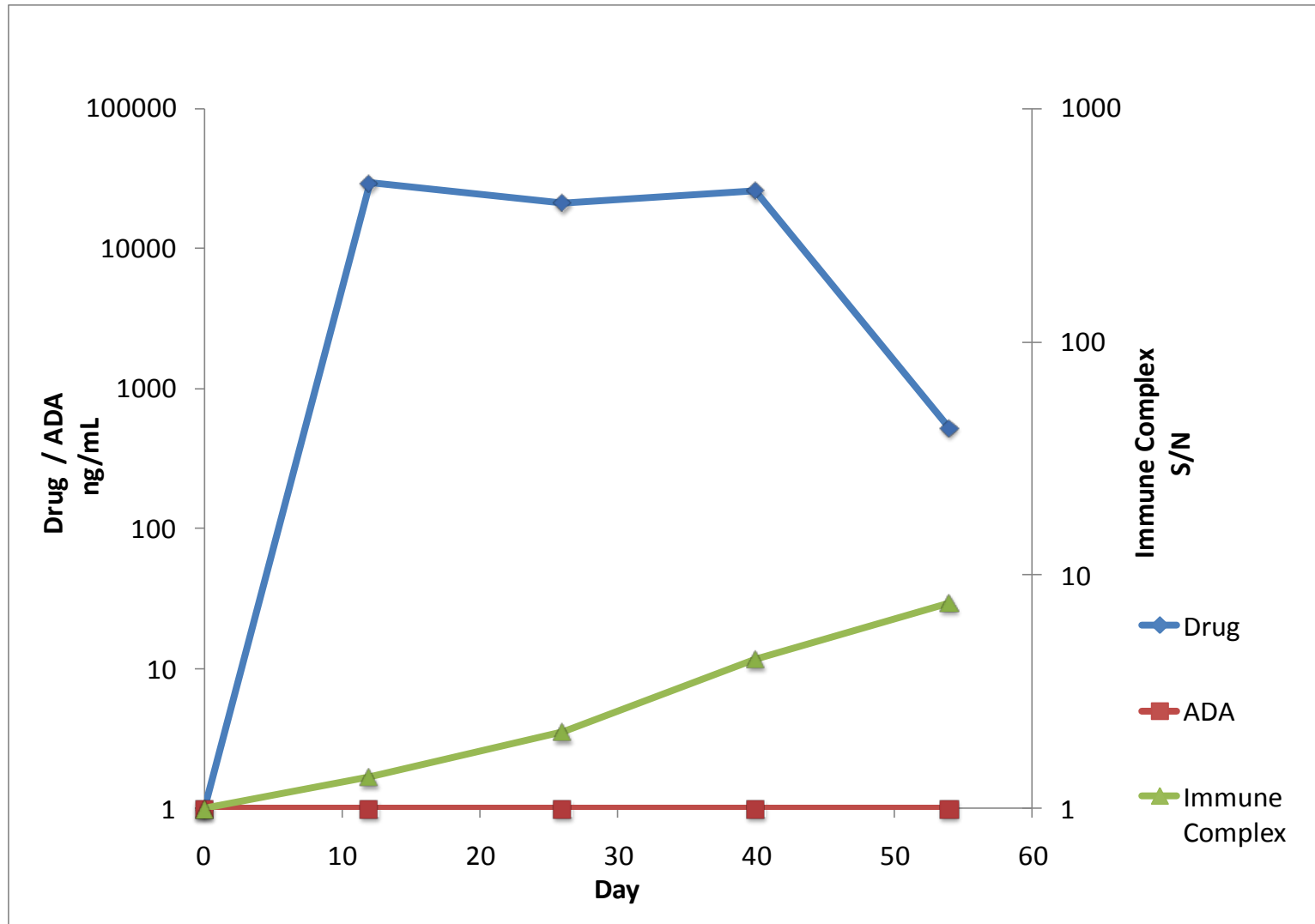
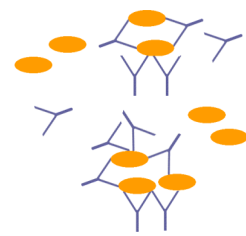


# CIC Immunoassay Validation Parameter



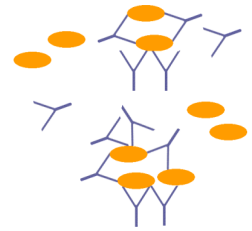
- Sample with  $S/N > 3.35$  = CIC Positive
- Sample dilution: 1:20 ; 1:400 and 1:8000
- Quantitation based on a 1:1 CIC standard
  - Dynamic range : 0.250-10 mcg/ml CIC
  - Precision : 25%
- Sensitivity: 142 ng/ml
- Quantifiable Limit: 250 ng/ml
- Drug tolerance @ QL: 1 mcg/ml IgG2

# Dynamics of Detecting ADA & CIC in Presence of High Serum Drug levels



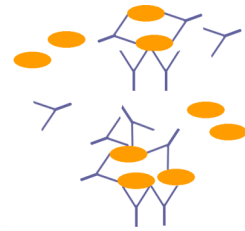
Note: These are representative data for demonstration purposes

# Case Study 1: Immune Complex in Non-Human Primate

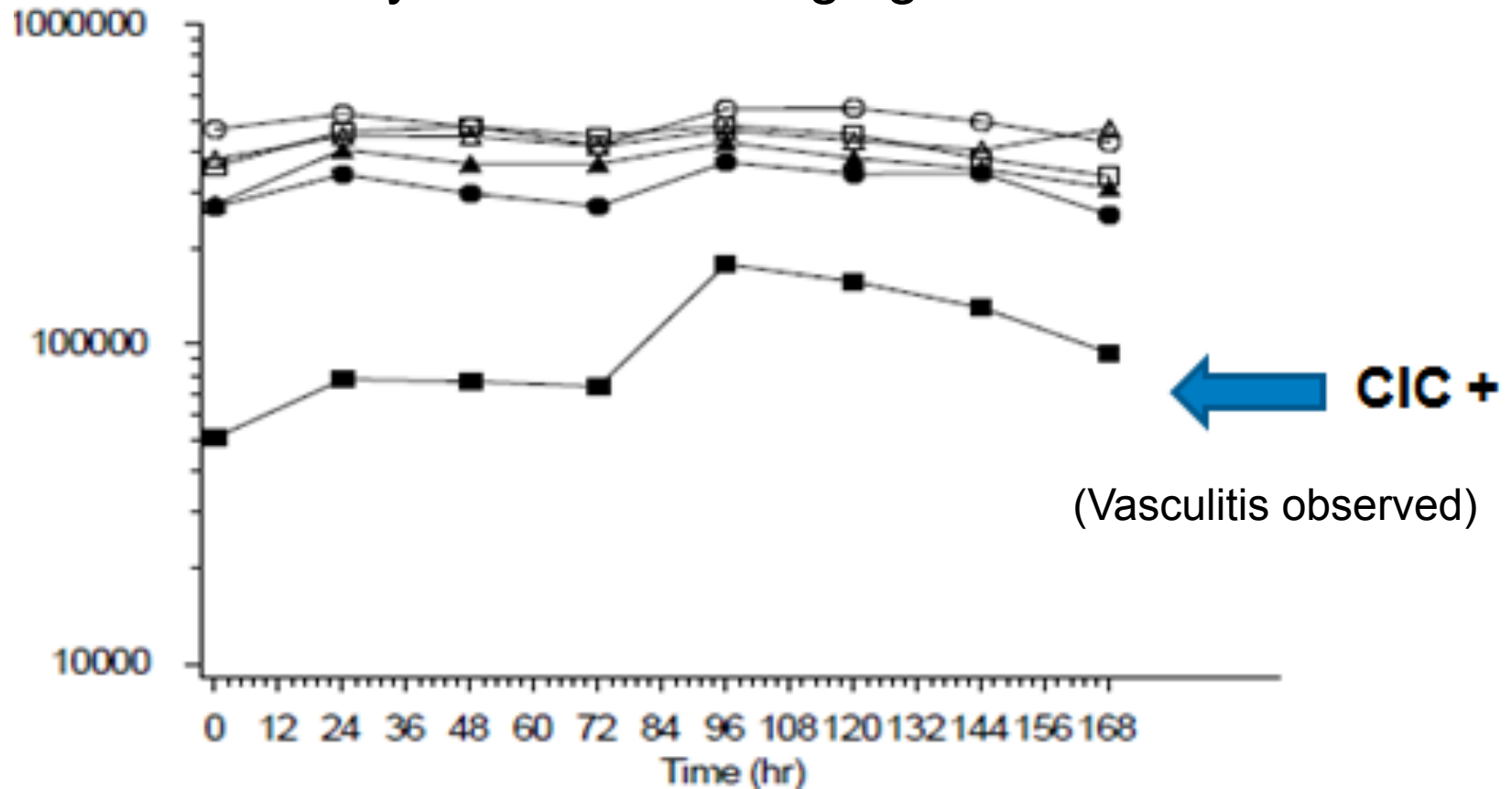


- **Single animal presented on Day 23 of 28 day study with:**
  - Lymphadenopathy
  - Inflammatory leukogram
  - Decreased serum drug concentrations
  - Early euthanasia
- **Other differentials considered: Infectious (TB or atypical Mycobacteria, protozoal—T. cruzi), test-article related effect**
- **Additional data collected: ADA, CIC, special stains for infectious organisms**
- **WOE for immune complex-mediated etiology**
  - No evidence of infection
  - Single animal affected
  - CIC detected when symptomatic, and decreasing drug concentrations
  - Histopathology
    - Chronic active inflammation aorta-coronary artery branch point
    - Pyogranulomatous lymphadenitis

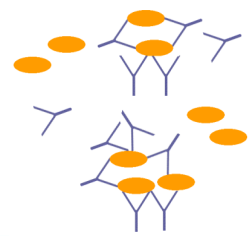
# Case Study 1 (IgG2 mAb): Alteration in PK Levels and Associated Pathology in an ADA Negative Animal



Day 22 – SC- 25 mg/kg 2X/week



# Case Study 2 (IgG2 mAb) : Clinical and Anatomic Pathology Finding of an IC Mediated Hypersensitivity

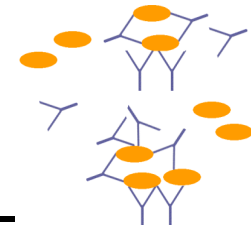


- **Clinical signs:**
  - Decreased activity; decreased use of left hindlimb
  - Ecchymosis/petechiation was observed on all 4 limbs
- **Timing:**
  - Occurred 24 hrs after dose administration
  - Occurred after 23<sup>rd</sup> dose (ie, late in dosing phase) in an individual animal
- **CI / TK:**
  - Binding ADAs observed D57; increasing levels of Abs at D113, D141, D156
  - Positive immune complexes (CIC) on D156
  - Decreased bioactivity (D57, D113) and serum concentration (D57 through D156)
- **Histopathology:**
  - Multifocal vasculitis / thrombosis of small vessels in skin and GI serosa



# Case Study 2: Data

## Evidence of Immune Complex Formation in Animal X



Day	Serum Concentration <sup>1</sup>		Bioactive Drug Level		Antibody Positive Animals in Group 4			Immune Complex Assay	
	Animal X	Grp 4 Mean	Animal X	Grp 4 Mean	Animal X	Animal Y	Animal Z	Animal X	Grp 2 - 4
	(µg/mL)		(µg/mL)		(Signal/Noise Ratio)			(µg/mL)	
57	172	934	149	945	9.08	Neg	Neg		
85	< 2	1010							
113	< 2	978	BQL	958	5739	1.17	Neg		
141	< 2	968			8046	1.32	1.48	Neg	
156	< 2				3077	Neg	Neg	> 32	
162		1040				1.43	1.56		Neg
183		982 <sup>2</sup>		1061		1.51	1.45		

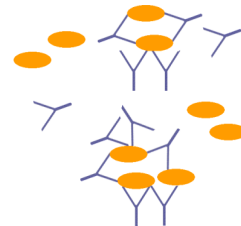
Blank squares indicate blood sample not collected <sup>1</sup> Pre-dose, <sup>2</sup> 7-d post-dose

Serum concentration: 5x to 500x decreased D57 through D156

Bioactivity: 6x to 1000x decreased D57 and D113

ADA: S/N 3000x to 8000x increased D113 through D156

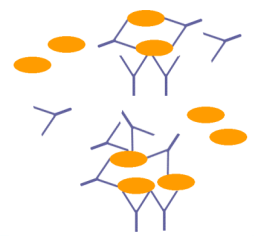
CIC: Positive D156



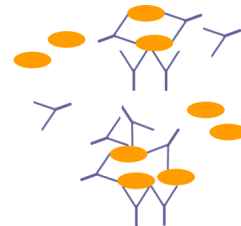
# Impact to the Programs

- **Clinical and pathologic changes consistent with immune complex secondary to ADA formation**
- **Consequences of IC formation were not a direct TA-related effect**
  - ADA formation in NHP not clinically relevant
- **There was no impact to either program**
  - Did not impact the NOAEL or safety margins
  - No impact on timelines or clinical trials progress

# “Triggers” for CIC Assay



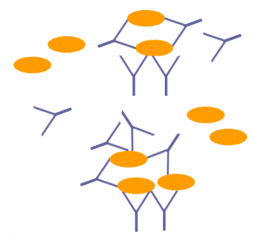
- Unusual PK/PD/pathology findings in animals that test ADA negative (**Case Study 1**)
- Post-dose clinical signs (e.g. fainting, weakness, etc) or clinical/anatomic pathology findings in animals ADA+ on study (**Case Study 2**)
  - IV dosing
  - Predose (trough) drug low or BQL
  - Robust ADA
- To test the assertion **that** immune complexes are the cause of the pathology findings in ADA+ animals (**Case Study 2**)



# ADAs Rarely Cause Safety Issues

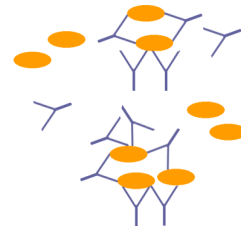
## Our challenge

- **Determine why some human IgGs cause ADA-mediated toxicity**
  - Animals with IC-related effects are CIC+
  - Not all CIC+ animals have adverse events
- **What is the role of route, dose, infusion rates, antibody vs antigen excess, molecule characteristics, etc.?**
- **How does CIC size correlate with adverse effects/pathology?**
- **What does CIC composition tell us?**
  - Detect C3b on CIC
  - Cyno IgG subclass
  - Other serum proteins



## Conclusion

- A validated CIC assay provides direct evidence of circulating immune complexes (human IgG drugs/ cyno IgG ADAs)
- In Case Study 1, CIC results were critical in explaining the alteration in PK levels and associated pathology in an animal that was ADA negative by traditional methods
- In Case Study 2, CIC results supported the clinical and anatomic pathology findings of an immune-complex mediated type III hypersensitivity reaction



# Acknowledgements

- **Clinical Immunology**
  - Dan Mytych
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