Immunogenicity of Therapeutic Proteins

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Causes of Immunogenicity

- Sequence differences between therapeutic protein and endogenous protein
- Structural alterations
  - Aggregation
  - Oxidation
  - Deamidation and degradation
  - Conformational changes
- Storage conditions
- Production/purification
- Formulation
- Route, dose and frequency of administration
- Immune status of patient
- Genetic background

Immunogenicity Prediction

- May play a role in future drug development
- Could be useful in early drug development and in design of second-generation products
- Could significantly reduce development costs
- The story is still building

Animal Models

- At this time, animal models cannot predict immunogenicity in humans
- Factors limiting predictive value
  - Immune system differences between humans, other primates, and other mammals
  - Lack of 100% homology between human therapeutic protein and non-human endogenous protein

Animal Models for Differential Immunogenicity

- Animal models can be useful for comparing immunogenicity of 2 similar products
  - Parent and second generation product
  - Original therapeutic and product after process changes have been made

NOTE: This will still not necessarily reflect what happens in humans, but may provide advance warning if comparator has different immunogenicity profile from original

How Do T-Cells Boost an Immune Response?

- Initial immune response is typically IgM, low affinity, and low concentration
- T-cell help is needed for class switching and affinity maturation
- High affinity mature antibodies of the IgG class are more likely to neutralize effects of therapeutic proteins

Clinical Trials

- Immunogenicity is best determined through controlled clinical trials
  - Studies need to be powered to detect immunogenicity
  - Duration should be at least 6 months to 1 year
  - Ab samples taken at time when circulating drug has cleared
  - Assays should be robust, sensitive, specific, and validated
  - Binding and neutralizing Abs should both be measured

Significance of Ab Results

- Factors effecting interpretation of results
  - magnitude of response (titer)
  - duration of response (continuous or sporadic)
  - correlation with AE
  - correlation with change in PK (sustaining or clearing)
  - biologically neutralizing antibodies

Relevance of Ab Response

- Examine relevance by patient
  - determine effect of immune response on each patient
- Assess impact of immune response in patients on the project
  - track rate of antigenicity
  - track magnitude of immune response
  - track rate of neutralizing antibody formation

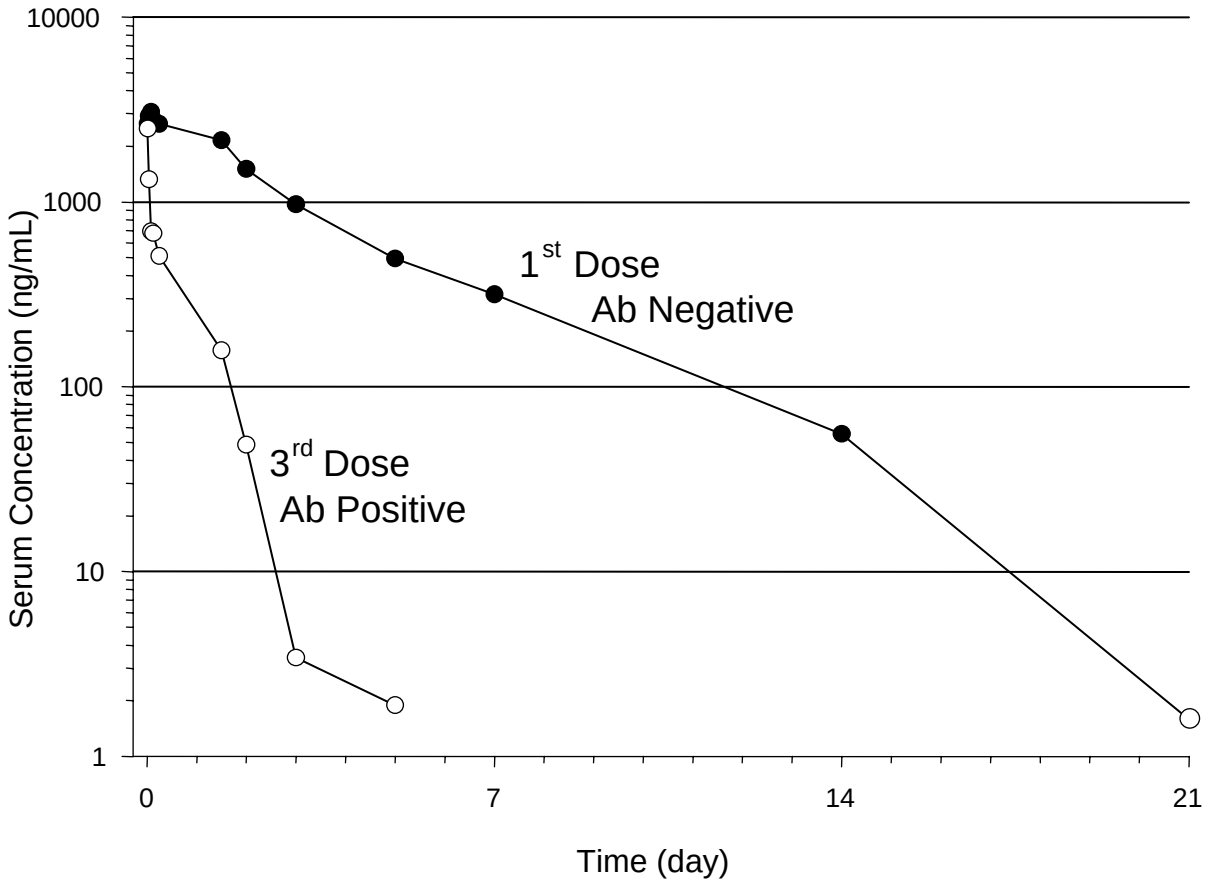
Antibody Significance

Antibody Response = all antibodies generated in a patient in response to a drug

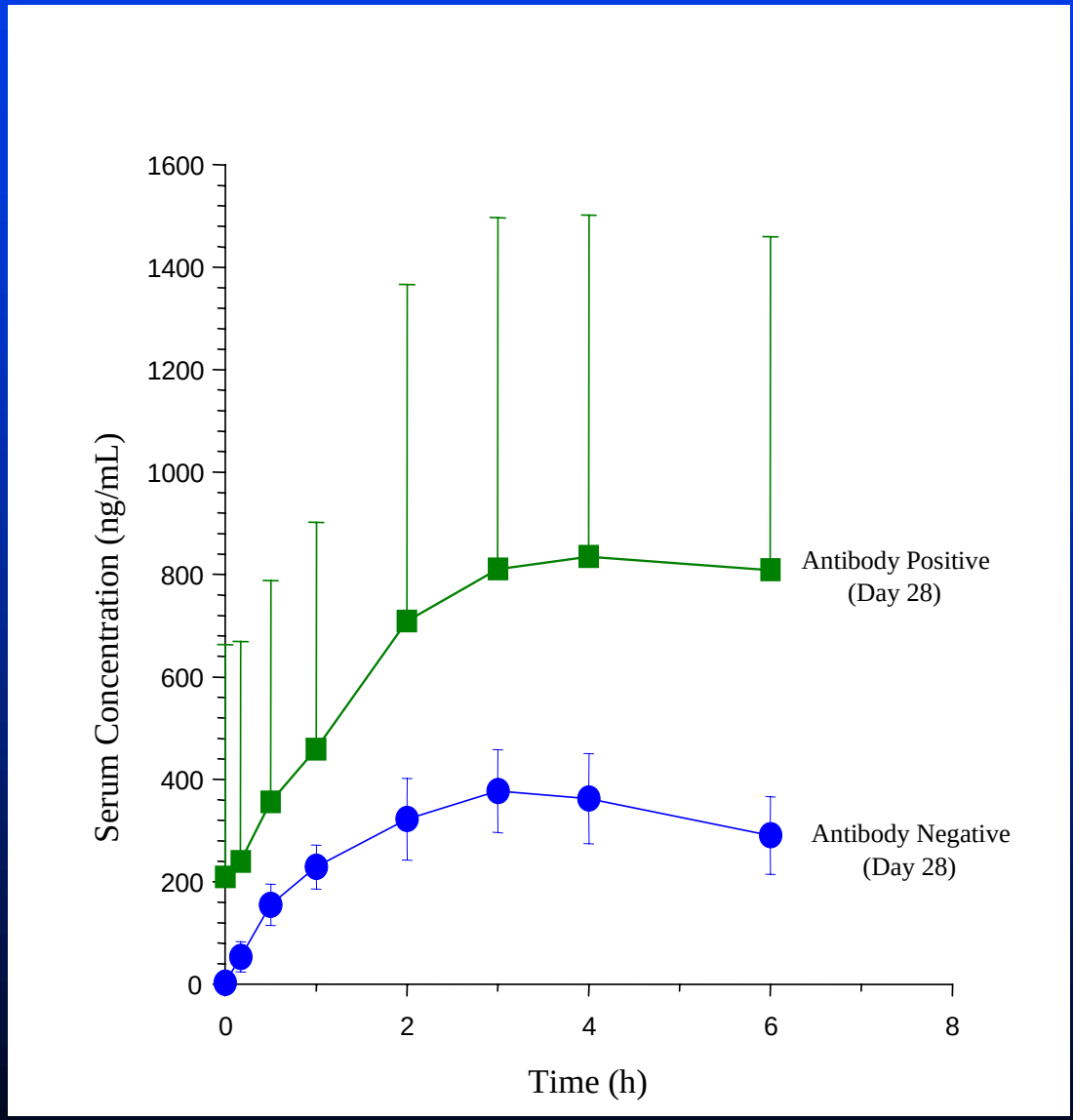
Clinically Relevant Ab =

1) Clearing Ab
2) Sustaining Ab
3) Neutralizing Ab
4) Allergic rxn
5) Cross-reacting w/endogenous protein

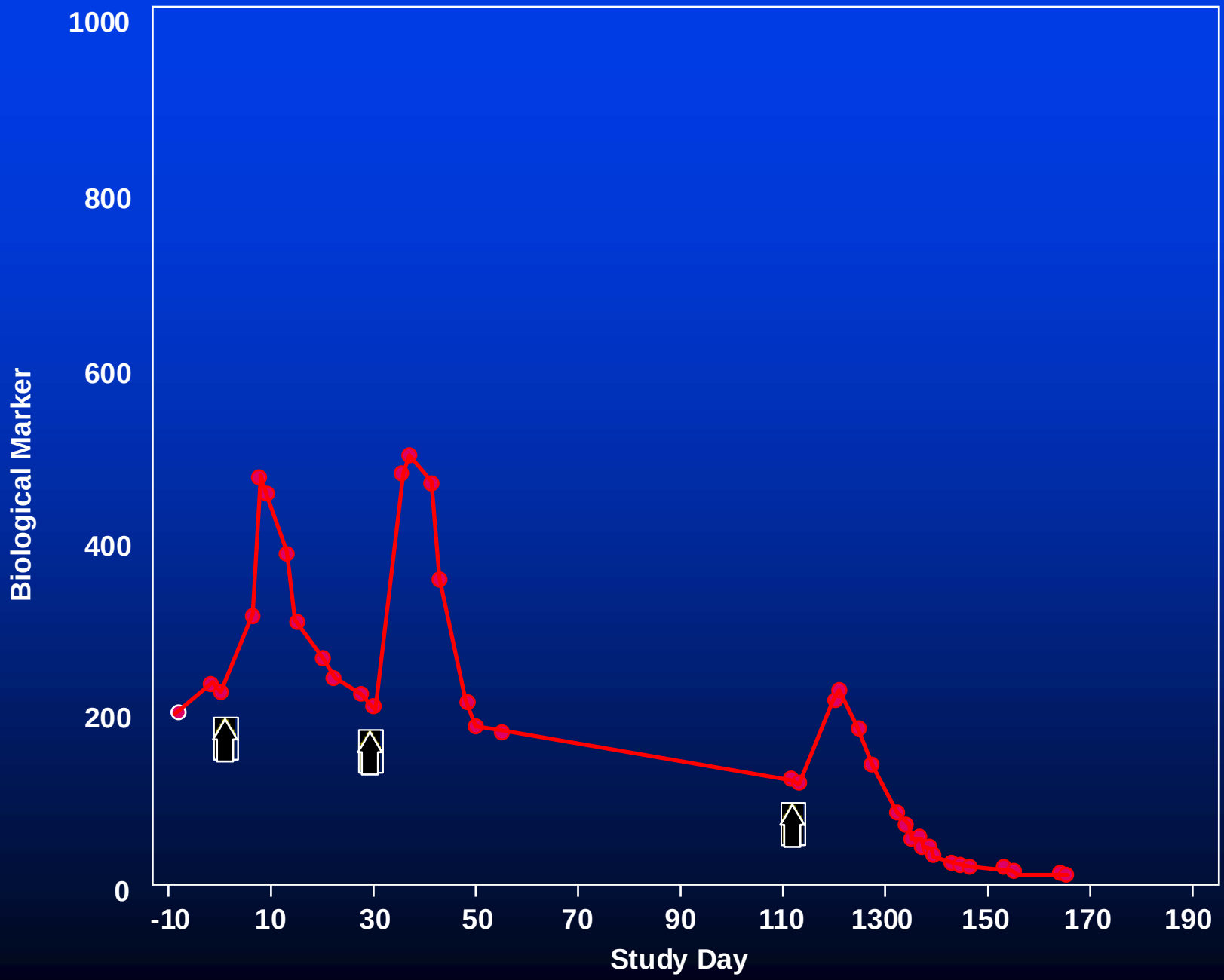
"Clearing" Antibody



"Sustaining" Antibody



Drug Induces Neutralizing Antibody to Drug and to Endogenous Protein



Strategies for Immunogenicity Testing

- Assess impact of antibody response on preclinical and clinical development of therapeutic proteins
- Need to test for presence of binding as well as neutralizing antibodies
- Beneficial to characterize antibodies detected

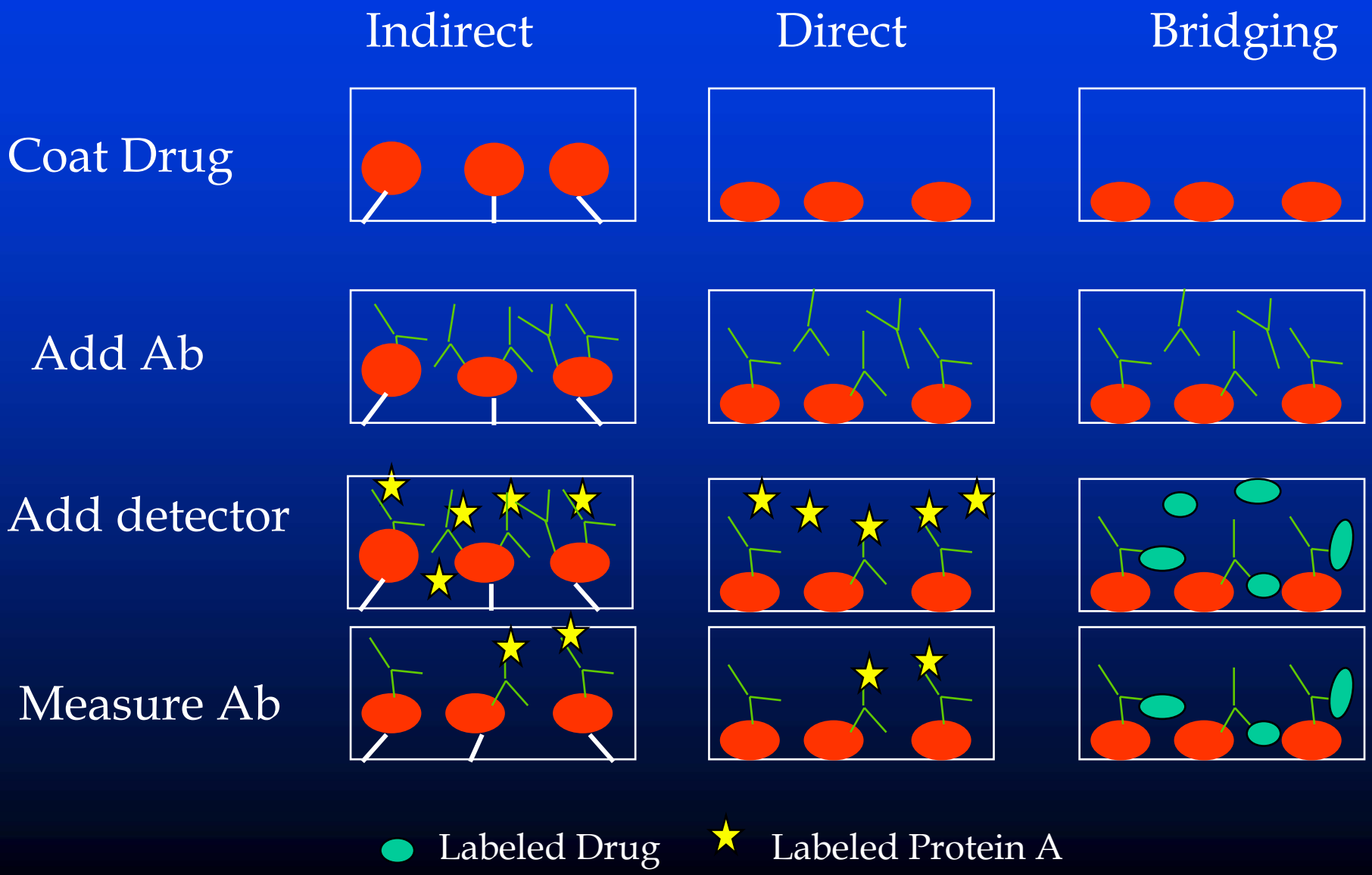
How Should Abs be Tested?

- Many different formats available
- No “perfect” assay currently exists

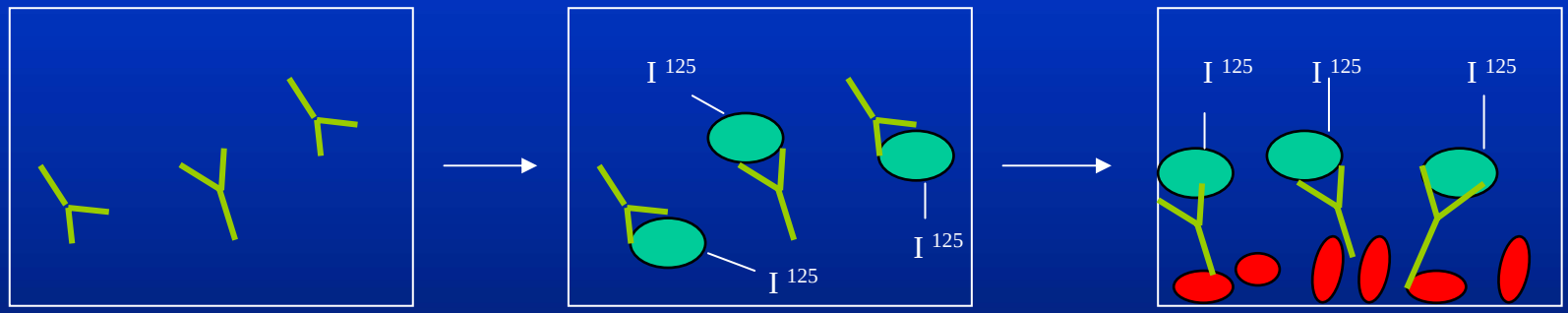
Immunoassay Platforms for Detecting Antibodies

- ELISA
  - Bridging format
  - Direct format
  - Indirect format
- Radioimmune precipitation
- Surface plasmon resonance
- Electrochemiluminescence

ELISA Platforms



Radioimmune Precipitation Assay



Dilute sample

Add radioactive-labeled drug

Add Protein A, precipitate Ab, and measure labeled drug

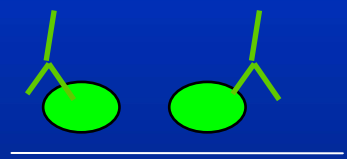
BIAcore Assay

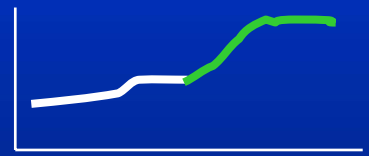
Event

Sensorgram

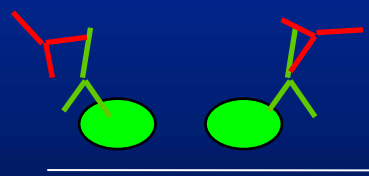
Immobilize Drug

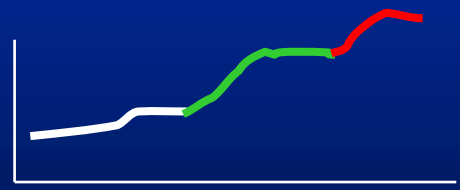
Add Sample



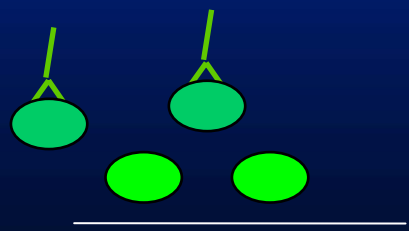


Confirm binding is antibody

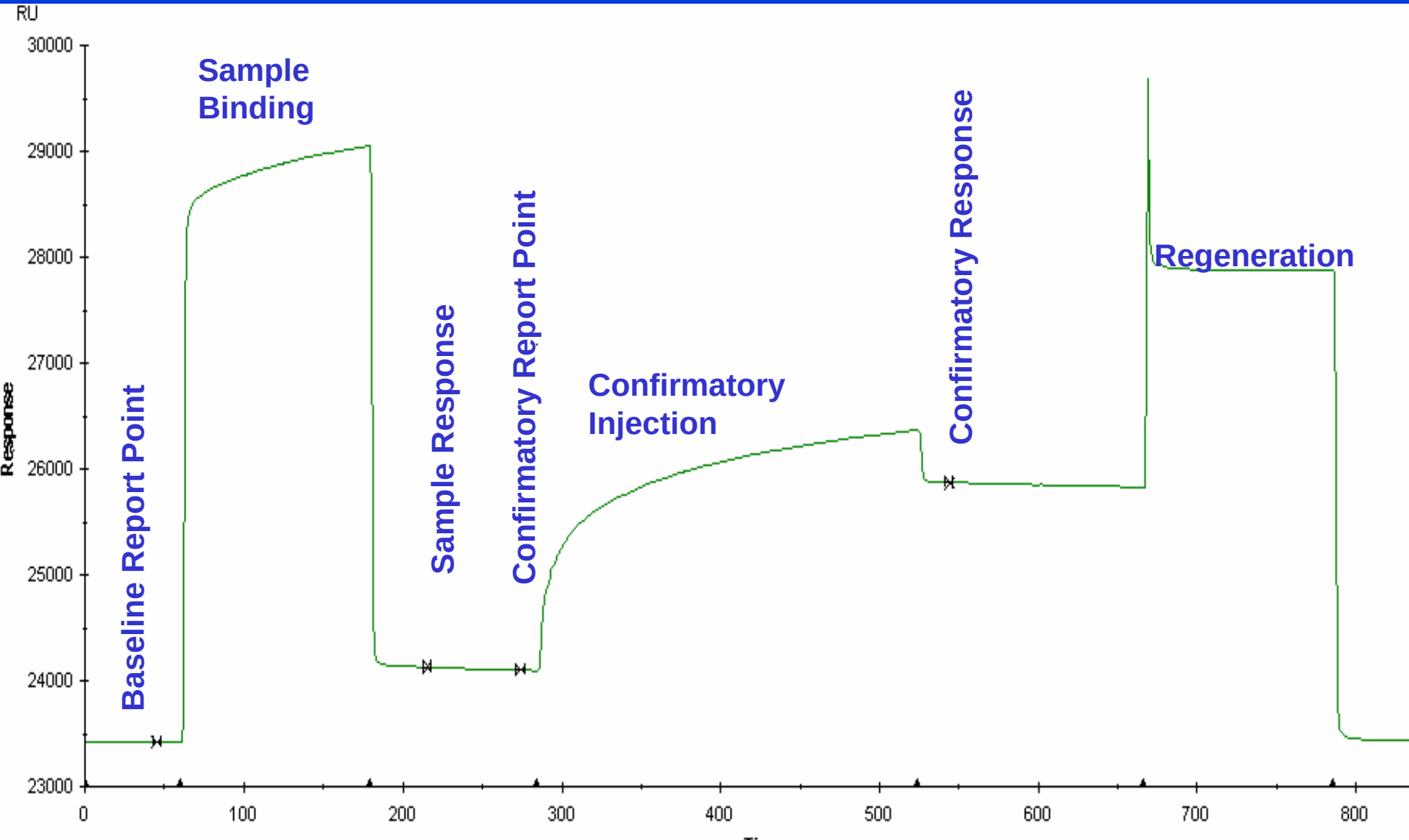




Inhibit binding w/drug



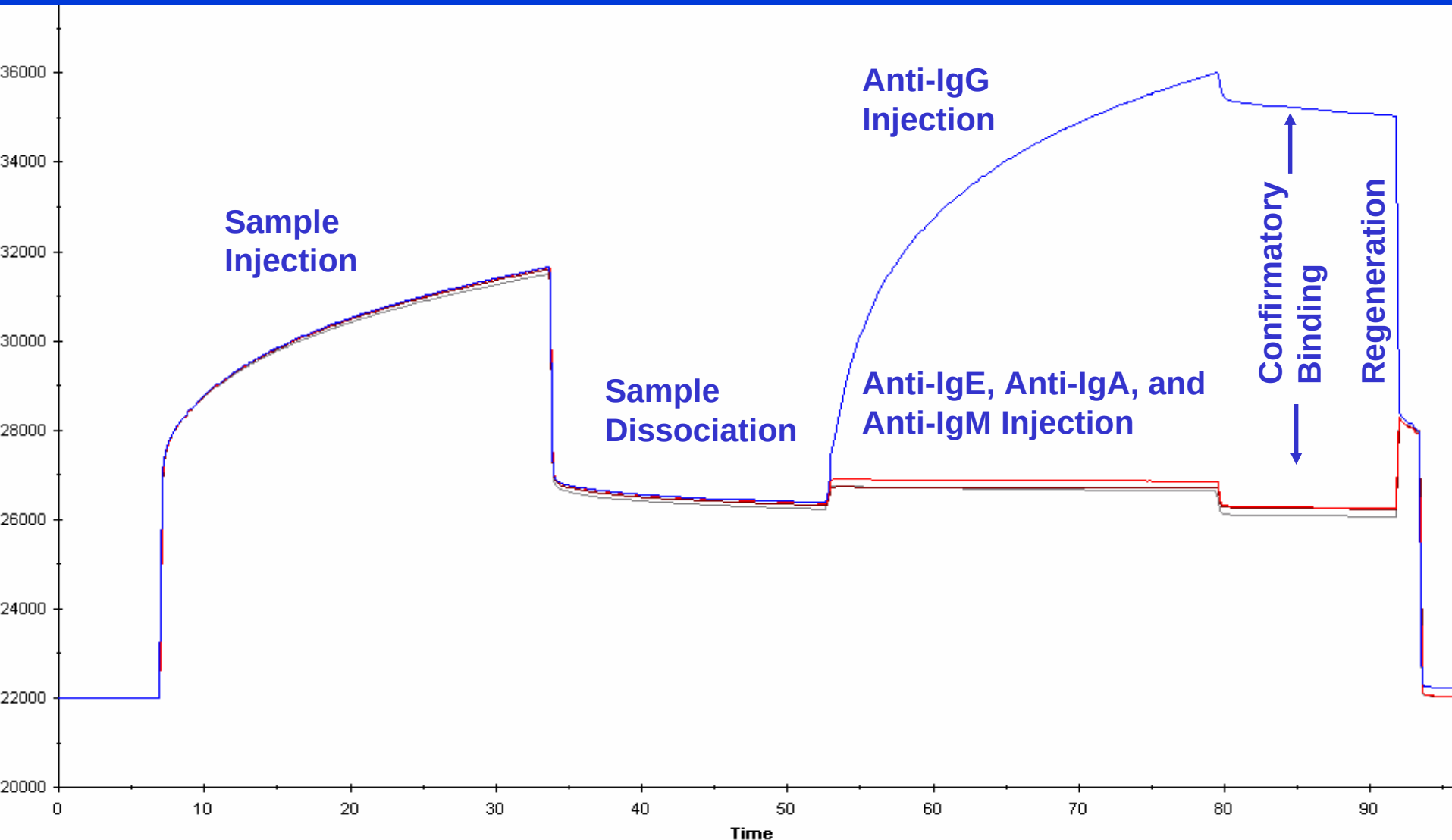
BIAcore Sample Analysis Sensorgram



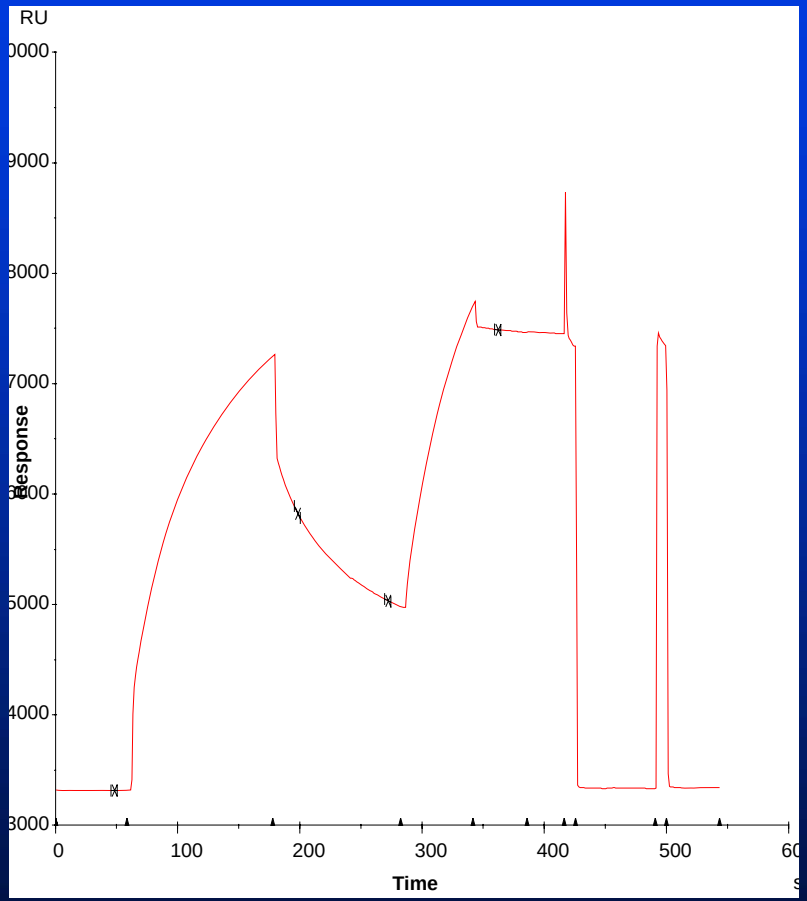
Characterization of Antibodies

- Isotype determination
- Binding inhibition with soluble drug
- Determination of relative binding affinity
- Relative antibody concentration
- Specificity to native and second generation product
- Ability to neutralize in a cell-based system

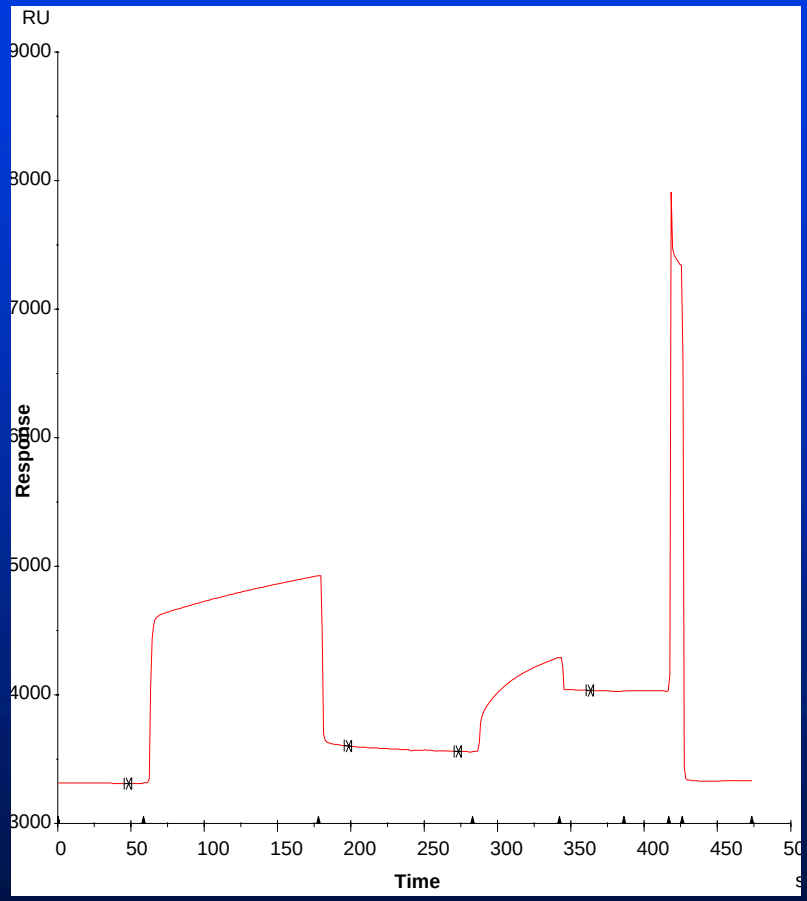
BIAcore: Determination of Antibody Isotype



"High" and "Low" Affinity Antibodies



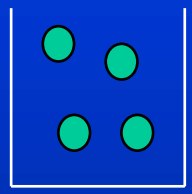
Low Affinity Antibody
(rapidly dissociating)



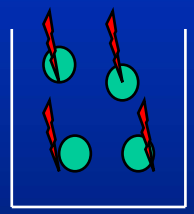
High Affinity Antibody

Clinical Immunology Assay Platforms Bioassay

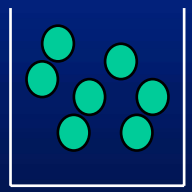
Culture cells



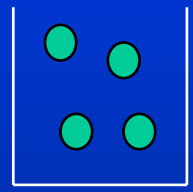
Add drug



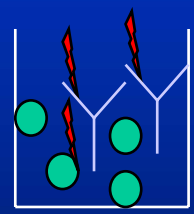
Measure biological response (proliferation)



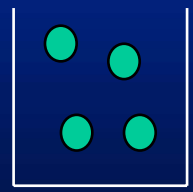
Culture cells



Add drug and Ab sample

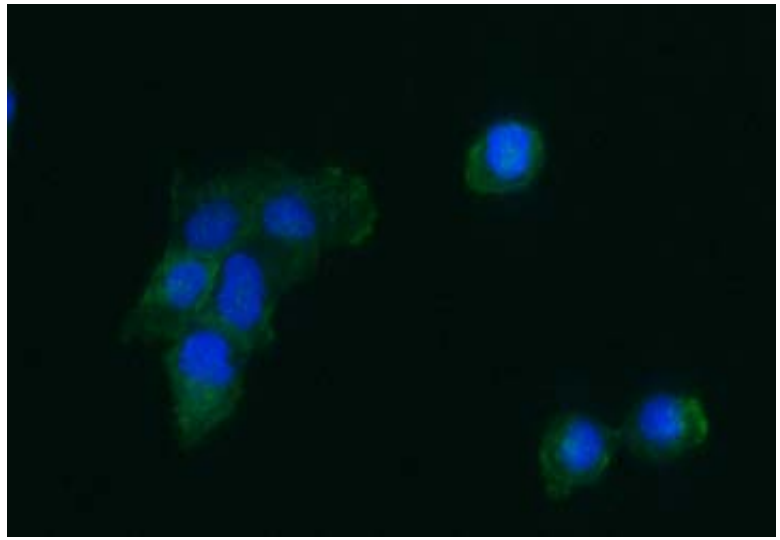


Measure biological response (proliferation)

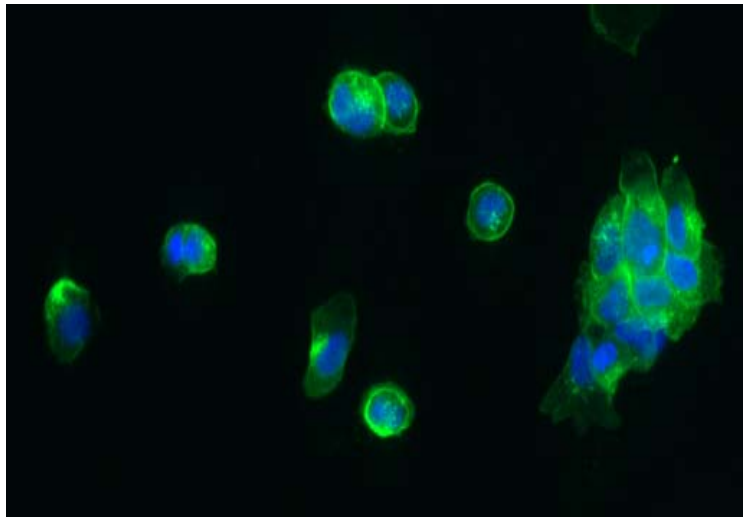


Receptor Tyrosine Kinase Activation

Cell line untreated and treated with a growth factor for 20 minutes. Blue is Hoechst nuclear stain and green represents phosphorylated receptor antibody.



No treatment

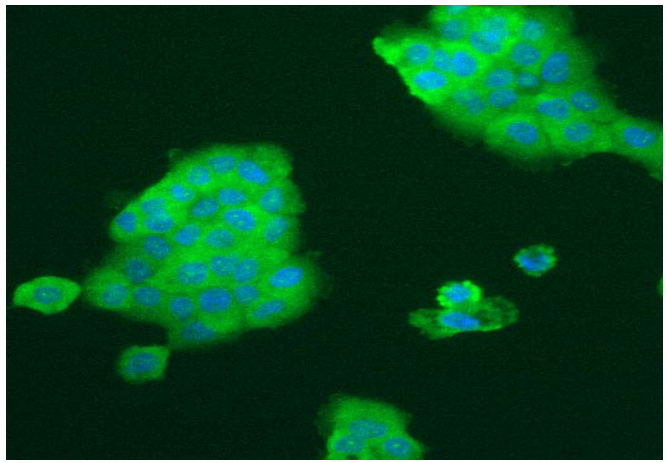


+ Growth Factor

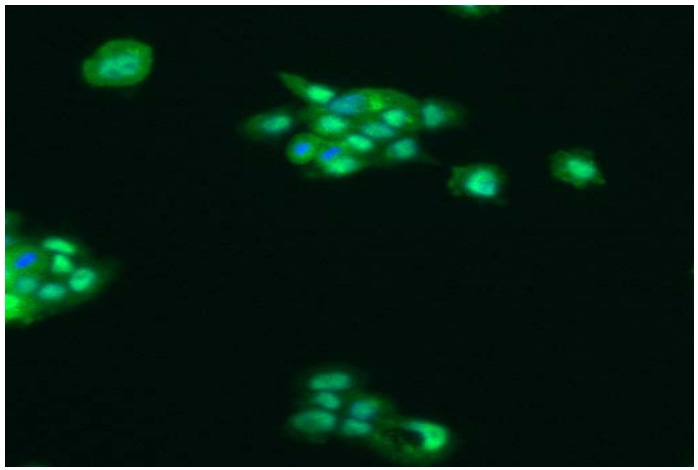
Transcription Factor Activation (STAT-1)

Cell line untreated and treated with a growth factor for 30 minutes. Blue is Hoechst nuclear stain and green represents STAT-1.

In untreated cells, STAT-1 is inactive and remains primarily in the cytoplasm. Upon activation, STAT-1 translocates to the nucleus to mediate gene expression.



No treatment



+ Growth Factor

Neutralizing Antibodies

- Bioassay used to determine ability of the antibody to neutralize a biological effect of the drug in a cell-based system
  - Proliferation assay
  - Cytokine release assay
  - m-RNA measurement
- Bioassays typically more variable and less sensitive than immunoassays

Unique Features of Bioassays

- Determines effect of an antibody in a cell-based system
- Only assay that determines if an antibody can neutralize the biological effect of the drug
- Results must be coupled with a specific immunoassay to verify neutralization is due to antibody

Antibody response to genetically engineered therapeutic proteins

- Historically, many therapeutic proteins have induced Ab formation
- Some of these antibodies are associated with serious adverse effects
- Both biopharmaceutical industry and regulatory agencies continue to partner for a better approach to Ab testing

Antibody Response to Genetically Engineered Therapeutic Proteins

Number of drugs	18	3	6	19	6
% antibody positive patients	<1	1-5	5-10	10-50	>50

Based on 52 proteins reviewed
(Koren et al. Curr. Pharm. Biotech. 3, 349, 2002)

Antibody-Mediated PRCA

- Professor Nicole Casadevall reported in NEJM in 2002 on cases of Ab-mediated pure red cell aplasia in patients treated with ESAs
- These patients had neutralizing antibodies against erythropoietin
- Very few reports in the literature of this phenomenon prior to the Casadevall manuscript
- Focused attention on the analytical procedures used for detecting and characterizing antibodies against ESAs

Summary of Anti-EPO Results

	Immunoassays			
Subject	RIP*	ELISA	BIACORE	Bioassay
001	+	+	+	+
002	+	+	+	+
003	+	+	+	+
004	+	–	+	+
005	+	–	+	+
006	+	+	+	+
007	+	+	+	+
008	+	+	+	+

* RIP = Radioimmune precipitation assay
+ = Positive for anti-rHuEPO antibodies
– = Negative for anti-rHuEPO antibodies

Characterization of PRCA Antibodies

Relative Ab Concentration (mcg/ml)	Ab Dissociation Rate (RU/min)	Predominant Isotype
43.46	10.7	IgG4 (IgG1, 2)
15.86	3.9	IgG4 (IgG1, 3, 2)
14.57	6.2	IgG4 (IgG1, 2, 3)
5.96	1.9	IgG1 (IgG4)
6.57	2.1	IgG4 (IgG1, 2)
8.68	2.1	IgG1 (IgG4, 2)
4.78	1.7	IgG4 (IgG2, 1, 3)
4.10	1.6	IgG4 (IgG1)

Antibody Monitoring Strategy for Clinical Studies

Highly recommended steps:

- A reliable antibody screening assay capable of detecting high and low affinity antibodies must be developed and thoroughly validated
- Sensitivity of 0.5 μg/ml in neat serum or better is necessary
- Neutralizing antibody assay, preferably cell based, should be developed and used to analyze samples positive in the screening assay
- Antibody levels should be determined (concentration or titer)

Antibody Monitoring Strategy for Clinical Studies

Highly recommended steps:

- If applicable, crossreactivity with endogenous molecule must be evaluated
- Drug interference must be evaluated and maximal concentration of tolerated drug established (IC dissociation if needed) especially in high dosing regimens
- Screening should be done at various time points during clinical development and if needed in post-approval studies

Antibody Monitoring Strategy for Clinical Studies

Recommended steps:

- Isotyping
- If applicable, characterization of pre existing antibodies
- Epitope mapping

Antibody Related Issues in Clinical Studies and Post-Approval Stage

Patient safety

- Crossreactivity with endogenous proteins
- Allergic reactions
- Immune complexes-complement activation

Reduced efficacy

- Neutralizing and/or clearing antibodies

Altered PK

- Enhanced drug clearance
- Drug accumulation
- Interference with PK assay

Conclusions

- Recombinant therapeutic proteins can be immunogenic
- Antibodies to therapeutic proteins can cause difficulties in preclinical animal studies and occasionally, serious side effects in humans
- Careful antibody monitoring with appropriate assays is necessary throughout preclinical and clinical development in order to ensure safety and efficacy of therapeutic proteins

Acknowledgements

Amgen’s Clinical Immunology Department