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Immunogenicity Considerations for Biologics

Meena Subramanyam, Ph.D.
Senior Director
Clinical Science and Technology
Biogen Idec, Inc.

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What is Immunogenicity

- ❖ Development of an **unwanted** immune response to the administered drug
 - ❖ Anti-drug antibodies
 - ❖ T cell responses; cytokine release
 - ❖ Complement activation

Immunogenicity

- ❖ SAFETY concern
- ❖ Link between immunogenicity and clinical effects critical
 - ❖ impact on safety and product efficacy in the target indication and population
- ❖ Monitorable?
 - ❖ Assays, sample collection time points
- ❖ Risk Manageable?
 - ❖ action plan if warranted

Potential Clinical Sequelae

Safety Impact

- ❖ Cross reactivity with endogenous proteins
- ❖ Allergic reactions
- ❖ Immune complexes-complement activation

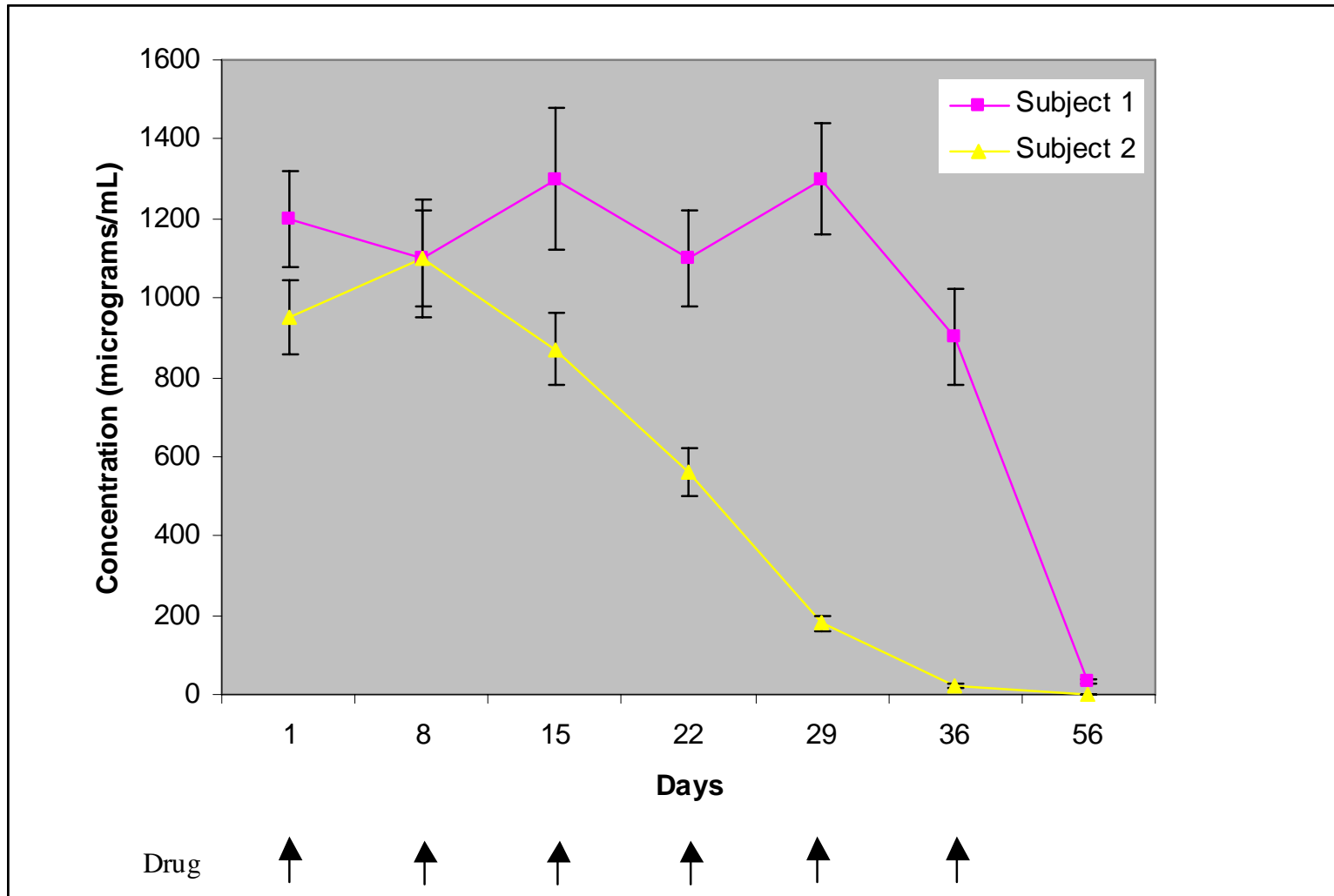
Exposure/Efficacy Impact

- ❖ Neutralizing and/or clearing antibodies
- ❖ Enhanced drug clearance
- ❖ Drug accumulation

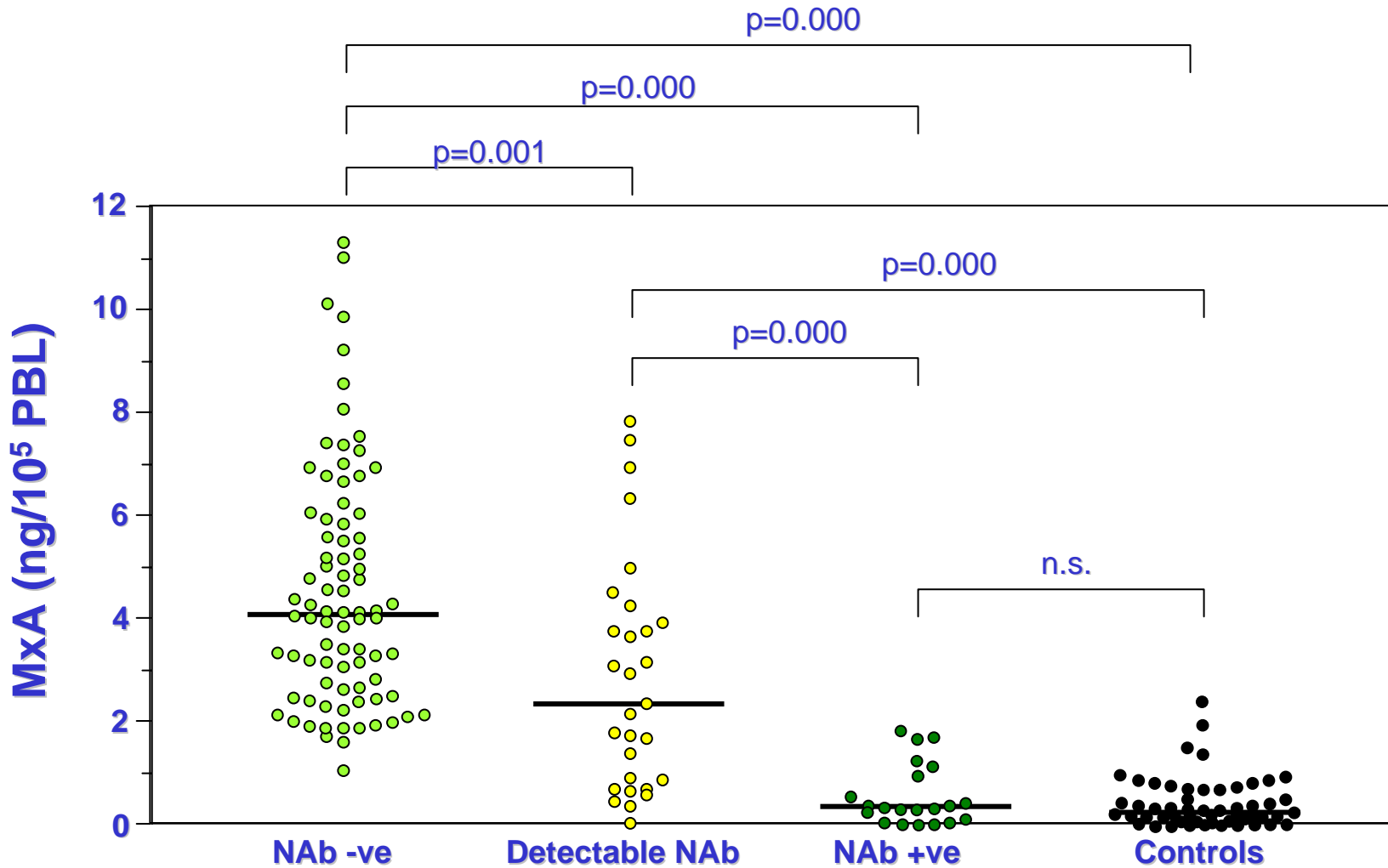
Immunogenicity Response

- ❖ Transient
- ❖ Sustaining
 - ❖ Immune complexes
 - ❖ Rapid drug clearance
 - ❖ Neutralizing Ab
 - ❖ Loss of efficacy
 - ❖ Neutralization of endogenous protein

Clearing Antibody



Neutralizing Antibodies



Deisenhammer, et al, Neurol. 52: 1239 (1999)

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Immunogenicity Concerns

Huub Schellekens, Clinical Therapeutics, Vol. 24, No.11, 2002

Salmon Calcitonin	Paget's disease, Hypercalcemia of malignancy, post-meno osteoporosis,	Abs in 40-70% of pt. Treatment related resistance
Lenercept (TNFr fusion protein)	Multiple Sclerosis	Abs in 88% of treated patients; enhanced drug clearance
GM-CSF	Some Cancers	Abs in 95% of pts. Altered PK and efficacy
IFN alpha2a	Hep C, some cancers	Nabs in 25% pts. Loss of efficacy
IFN beta 1a	Multiple Sclerosis, some cancers	Abs more common in SC Vs. IM administration. Impacts efficacy
IL-2	Some Cancers	BAbs in 100% of pts, Nabs in <50%. Benign
Axokine (modified ciliary NTF)	Obesity Drug	Nabs in >70% of subjects

Causes of Immunogenicity

- ❖ Sequence differences
- ❖ Structural alterations
 - ❖ Aggregation, Oxidation, Deamidation and degradation
 - ❖ Glycosylation differences
 - ❖ Conformational changes
- ❖ Storage conditions
- ❖ Production/purification
- ❖ Formulation
- ❖ Route, dose and frequency of administration
- ❖ Immune status of patient
 - ❖ MHC type
- ❖ Genetic background

Immunogenicity

- ❖ Increased recognition of:
 - ❖ Complexity of issues
 - ❖ Comparability/Similarity discussions
- ❖ ICH S6 Guidance; Note for Guidance on Comparability of Medicinal Products
- ❖ EMEA Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins
- ❖ Evolving technology & Standardization Issues

Risk Assessment of Issues

- ❖ Drug Posology
 - ❖ Acute, Chronic, episodic
- ❖ Half-life of drugs
- ❖ Physical Product
 - ❖ Antigenic potential
- ❖ Patient Characteristics
- ❖ Posology
- ❖ Disease-related

Risk-Based Testing

- ❖ Breadth of immunogenicity (testing) dependent on
 - ❖ Likelihood of an immune response
 - ❖ Likely clinical consequences of an immune response
- ❖ As the concern around immunogenicity increases, the level of testing should also increase
 - ❖ Samples tested more frequently
 - ❖ More thorough characterization

Immunogenic Response

- ❖ Polyclonal - Transient, Persistent
- ❖ Complexed with drug
- ❖ Changes over time - Binding to Neutralizing
 - ❖ concentration
 - ❖ relative affinities
 - ❖ epitope specificity
 - ❖ isotype class
- ❖ Measuring analyte in the presence of
 - ❖ Soluble receptors
 - ❖ Pre-existing antibodies
 - ❖ Rheumatoid factors
 - ❖ Drug!

Immunogenicity in Non-Clinical Studies

- ❖ Safety Studies - NHP species of choice in most cases. Study duration, Drug target homology, functional homology across species? - Use surrogate?

Avonex

- ❖ ADA in rhesus in 2 weeks. Clinical Experience – less than 5% neutralizing Abs in RRMS

Enbrel

- ❖ ADA in 83% to 100% in tox studies in mice, rat, rabbit, monkeys. Clinical experience - less than 5% incidence– RA, PsA.

MGDF

- ❖ NHP - NADA decreased platelet counts. Similar response in Clinic.

Extrapolation? → Clinical testing is required

Implementation Strategy

- ❖ Well-designed clinical trials
 - ❖ Adequate sample size
 - ❖ Duration should be at least 6 months to 1 year
 - ❖ Sampling times - circulating drug
 - ❖ Assays - robust, sensitive, specific, validated
 - ❖ Should facilitate appropriate characterization in the disease matrix with proposed dose and schedule
 - ❖ Non-neutralizing and neutralizing Abs should be measured

Assay Implementation

**Transition
to Dev
stage**

**Pilot Studies
IND enabling**

Clinical Phase I

Clinical Phase II/III

Screening,
confirmation assay
development, *Nab* if
needed in non-clin
matrices

Screening, Confirmation
Neutralizing assay, other
characterization assay
development in
human/disease matrices

Screening, confirmation
assay (*Nab*) validation for
GLP study support

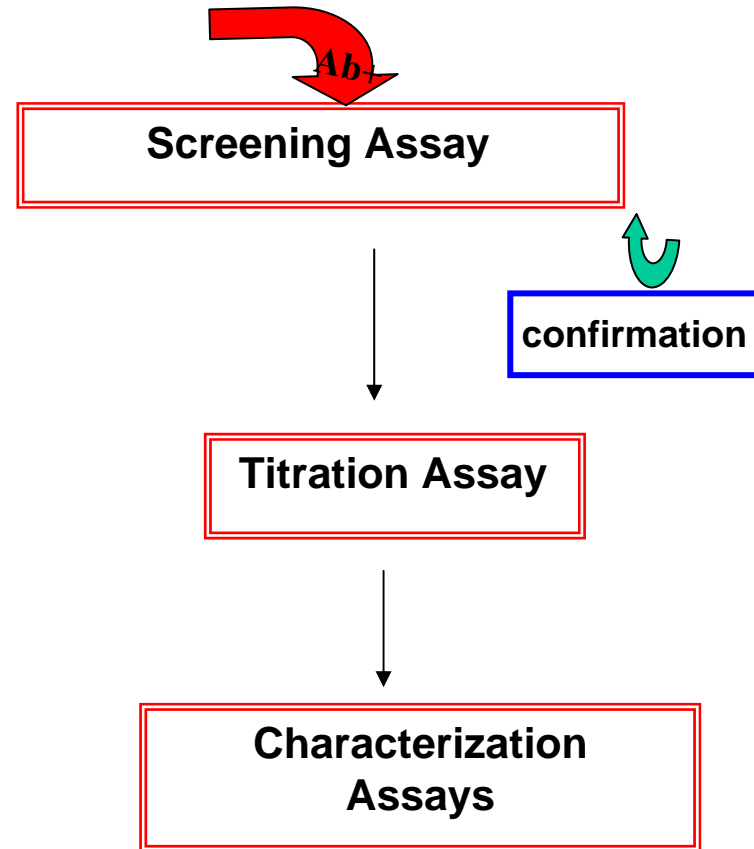
Partial/Full Validation of
all human methods

Development

Validation

Assay Development

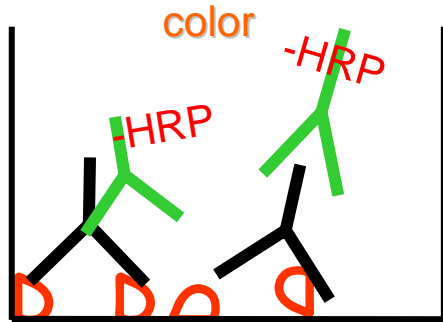
- ❖ Screening Assay
- ❖ Confirmation Assay
 - ❖ Confirmation of positive response with excess drug
- ❖ Titer Determination
- ❖ Characterization Assays
 - ❖ Neutralizing/Blocking
 - ❖ Isotype Determination
 - ❖ Complement fixing ability
 - ❖ Epitope Mapping (anti-frame-work, anti-CDR)
 - ❖ Determination of relative binding affinity






Immunoassay Platforms for Detecting Antibodies

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

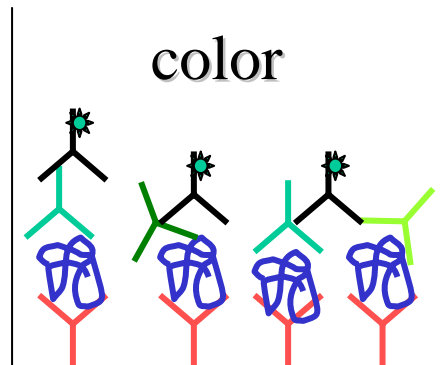
ELISA Formats







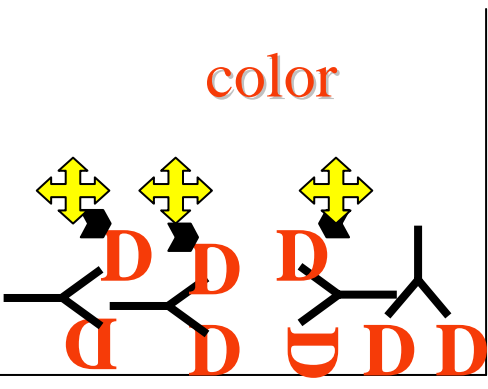
-  -HRP Labeled secondary reagent
-  Human anti-drug Ab
-  Drug "capture"





Direct

Sandwich - Indirect



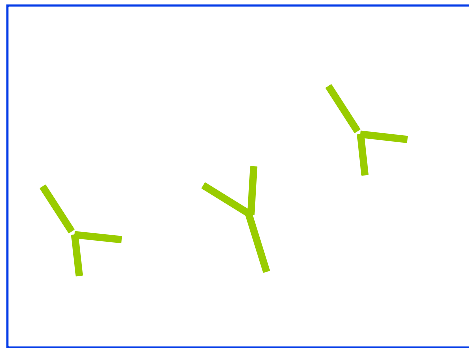
-  Labeled secondary reagent
-  Anti-Drug
-  Drug
-  Anti-drug capture



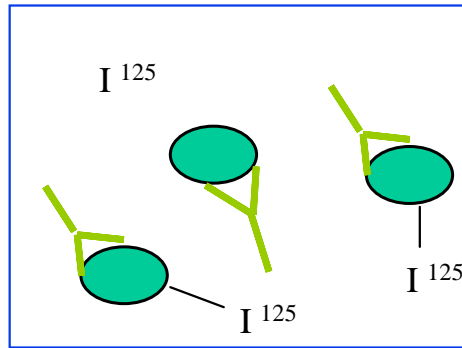
-  SA-HRP
-  Biotin-drug
-  Human anti-drug antibody
-  Drug "capture"

Bridging

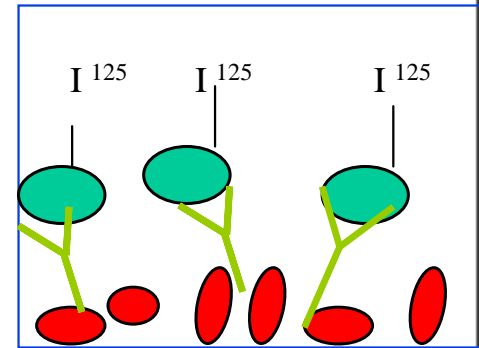
Radioimmuno Precipitation Assay



Dilute sample

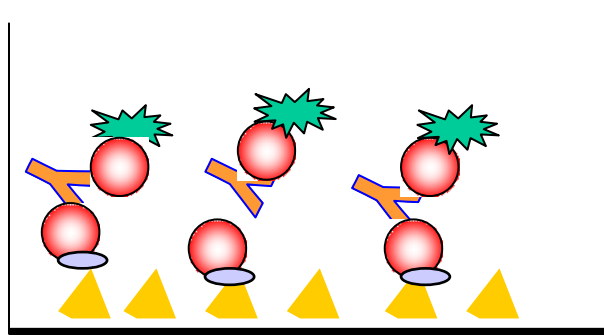






Add radioactive-labeled drug

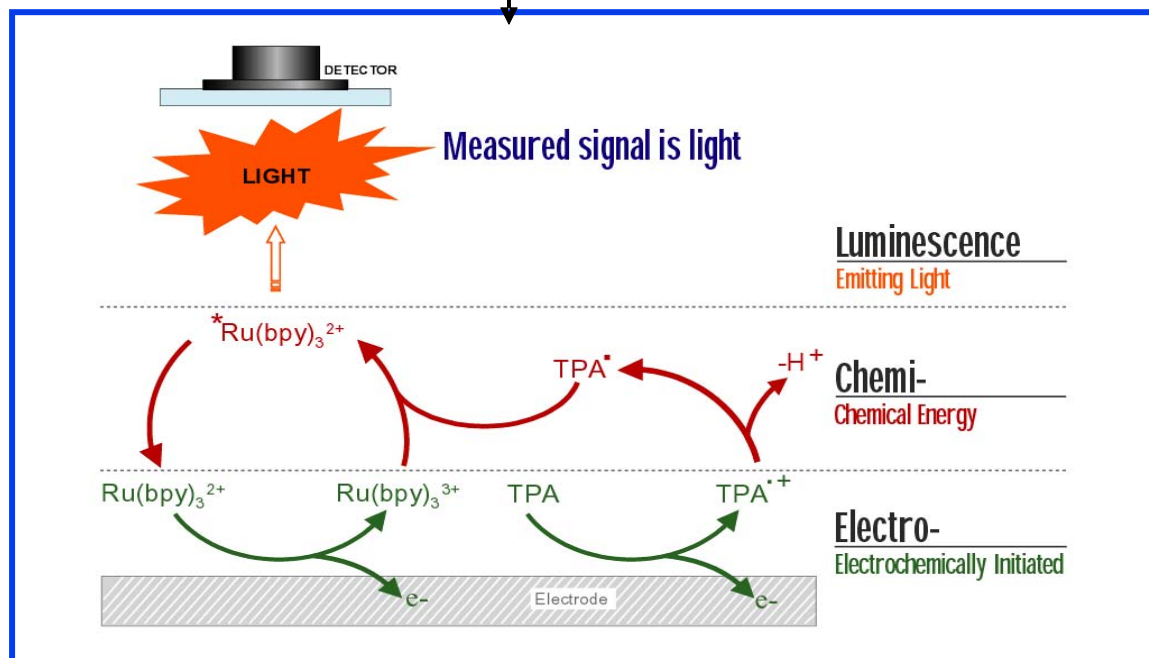


Add Protein A, precipitate Ab, and measure labeled drug

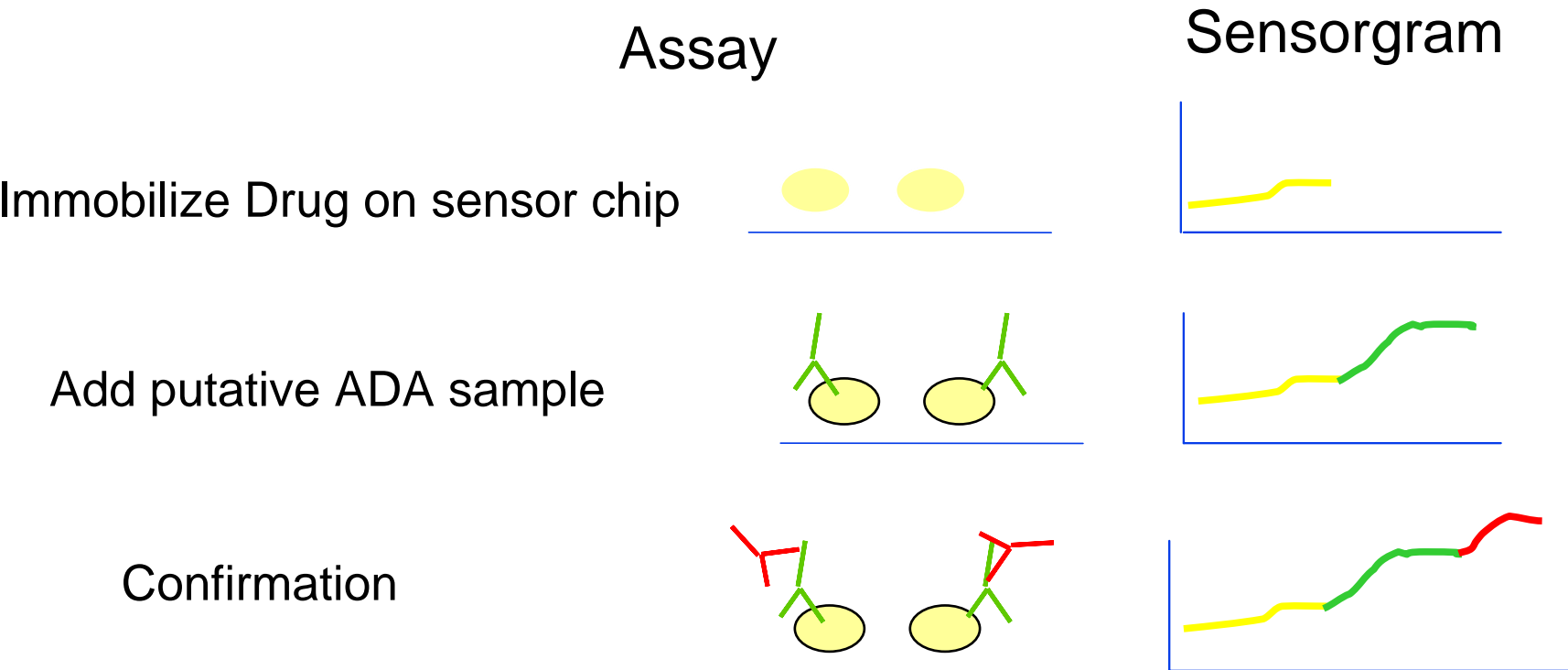
Electrochemiluminescence Assay (ECL)



-  Biotin Drug
-  Ruthenium Drug
-  Anti-drug Ab
-  Streptavidin



Surface Plasmon Resonance (Biacore)



Comparison of Screening Assay Formats

Parameters	Standard Elisa	Bridging Elisa	RIPA	ECL	Biacore
Phase	Solid	Solid	Solution	Solution	Solution
Cost	Inexpensive	Inexpensive	Moderate	Moderate	Expensive
Reagent availability	Readily Available	Readily Available	Radio-label	Single vendor, 2 labeled agents needed	Single vendor
Throughput	High	High	Moderate	High	Low
Epitope Modification	Possible	Possible	Possible	Possible	Possible
Low affinity Abs	May	Favors IgM; monovalent IgG binding	Yes	Yes	High, early immune response
Multiple isotypes	Conjugate-dependent	Yes	Dependent on ppt agent	Yes	Yes
Species specificity	Conjugate-dependent	Yes	Dependent on ppt agent	Yes	Yes
Humanized Abs	F(ab) ₂ coat needed	Yes	Yes	Yes	Yes

Neutralizing Antibody Assays

- ❖ Bioassay or blocking assays used to determine ability ADA to neutralize biological effect of the drug.
- ❖ Possible derivative of potency assay.
- ❖ Assay format depends on the drug target and known signaling pathways used by the target molecule. Most appropriate signal is that associated with disease pathology or drug MOA.
- ❖ Cell-based neutralizing Ab (Nab) assays may provide information on whether ADA might interfere with *in vivo* drug activity

Assay Development Challenge

- ❖ Measuring a drug-specific polyclonal antibody response in a complex serum matrix containing about 10-15 mg/mL Igs
- ❖ Measuring analyte in the presence of
 - ❖ Soluble receptors
 - ❖ Pre-existing antibodies
 - ❖ Rheumatoid factors
 - ❖ Drug!

Analyte Challenge

- ❖ Response is polyclonal
- ❖ Could be complexed with drug
- ❖ Response changes over time
 - ❖ concentration
 - ❖ relative affinities
 - ❖ epitope specificity
 - ❖ isotype class

Assay Controls - Positive

- ❖ Standardization difficult – a challenge for biosimilars
- ❖ Control may not match study population
- ❖ From multiple surrogate species
- ❖ Polyclonal preferred
- ❖ Types
 - ❖ mAb
 - ❖ Polyclonal Ab (affinity purified)
 - ❖ High, medium, low affinity
 - ❖ Blocking/neutralizing

Assay Sensitivity Impacted By Cut-Point

- ❖ Level of response above or below which a sample is defined to be negative or positive.
 - ❖ Threshold for determination of the presence or absence of ADA
- ❖ Statistically derived (parametric, non-parametric, or alternate).
- ❖ An ideal balance has to be achieved between minimum dilution, sensitivity and the cut point.
- ❖ Assess assay response variability using samples from healthy donors as well as drug naïve disease subjects

Gupta, S. et al. J. Immunol. Methods (2007), doi:10.1016/j.jim.2006.12.004

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Reporting Results

- ❖ Reportable Results from ADA Assays
 - ❖ Positive/negative
 - ❖ Titer
 - ❖ Concentration (positive control equivalents; mass/mL)
- ❖ Reportable results are quasi-quantitative
 - ❖ Can be expressed in terms of amount of drug activity neutralized by ADA containing serum for a more quantitative read.
- ❖ Relative to pre-dose values

Clinical Sequelae of Nabs

Antibody-Mediated PRCA

- ❖ Cases of antibody-mediated pure red cell aplasia in patients treated with EPO alpha reported in NEJM in 2002.
- ❖ These patients had neutralizing antibodies against erythropoietin
 - ❖ EPO resistance and transfusion dependence developed.
- ❖ Focused attention on the analytical procedures used for detecting and characterizing antibodies against ESAs

Thrombopoietin (TPO)

- ❖ Central regulator of megakaryo cytopoiesis leading to platelet production
- ❖ rHuTPO and PEG-MGDF (non-glycosylated) help alleviate thrombocytopenia associated with non-myeloablative chemotherapy.
- ❖ Nabs to PEG-MGDF led to **thrombocytopenia** and neutralized endogenous TPO in healthy volunteers and in cancer patients.
 - ❖ Abs mostly IgG with preponderance of IgG4. No IgM
 - ❖ Antibodies recognized epitopes in the binding region preventing circulating endogenous TPO from binding to the receptor.

Effect of Anti-Natalizumab (Tysabri) Antibodies on Trough Serum Concentration

Humanized anti-VLA4 mAb (a4 subunit specific). Approved for RRMS.

Antibody Status	Mean Serum Natalizumab Concentration (µg/mL)			
	Week 12	Week 24	Week 36	Week 120
Persistent Positive	1.3	BLQ*	1.4&	2.9-7.9**
Transient Positive	1.3	6.4#	17.5	~20
Negative	14.9	21.2	24.3	~23

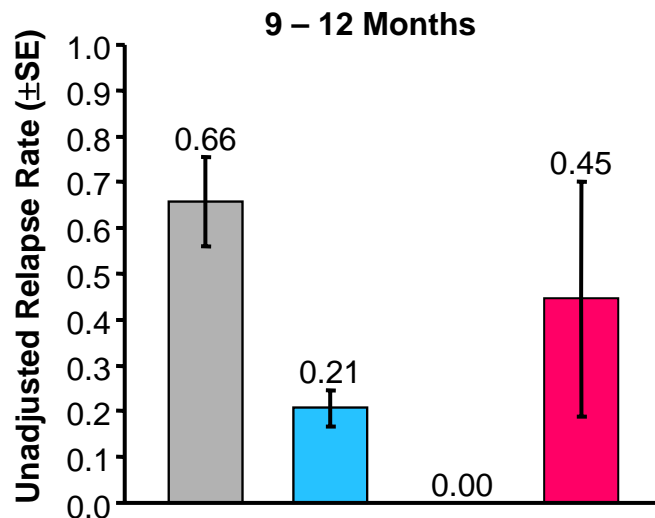
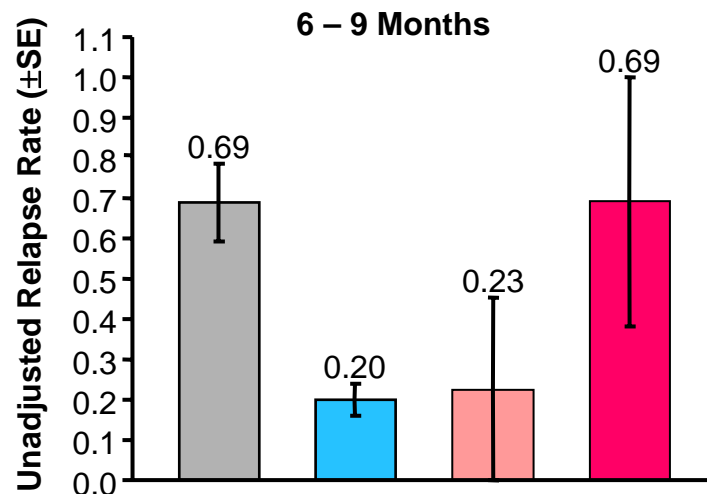
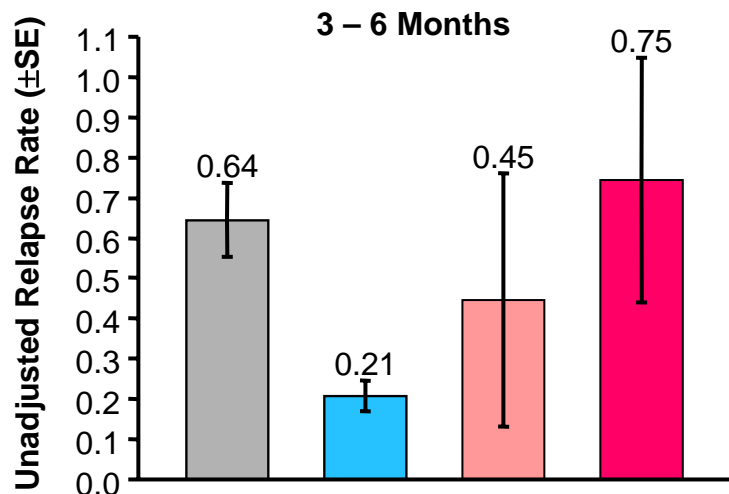
*Below Limit of Quantitation;

2 out of 15 transient antibody positive subjects tested BLQ at week 24

& 16 out of 20 persistent antibody positive subjects tested BLQ at week 36

** 50-70% persistent antibody positive subjects tested BLQ at week 120

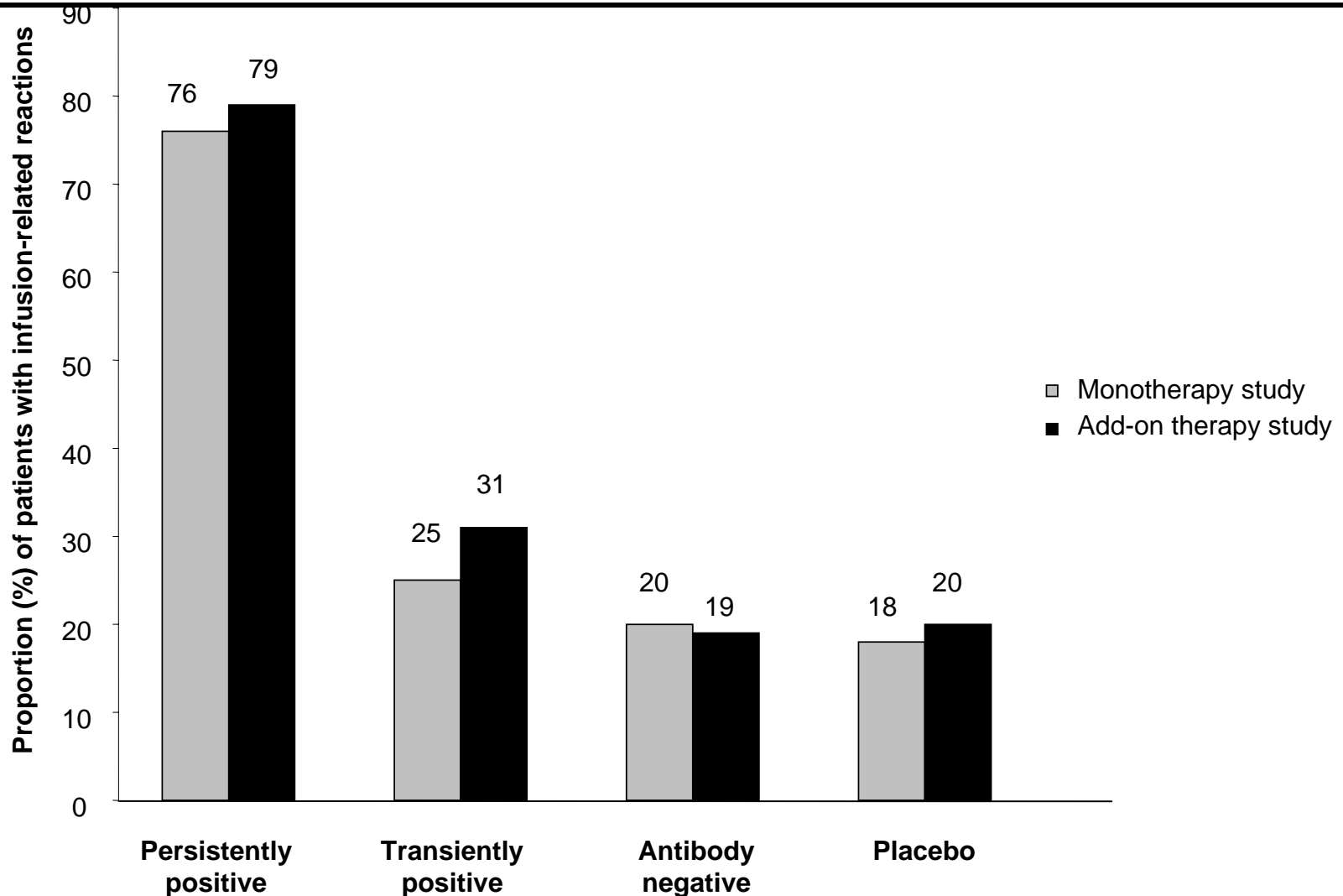
Effect of Anti-Natalizumab Antibodies on Relapse Rate (efficacy measure)



From 6 to 12 months, full efficacy restored in “transient” antibody-positive patients, but not in “persistent” antibody-positive patients

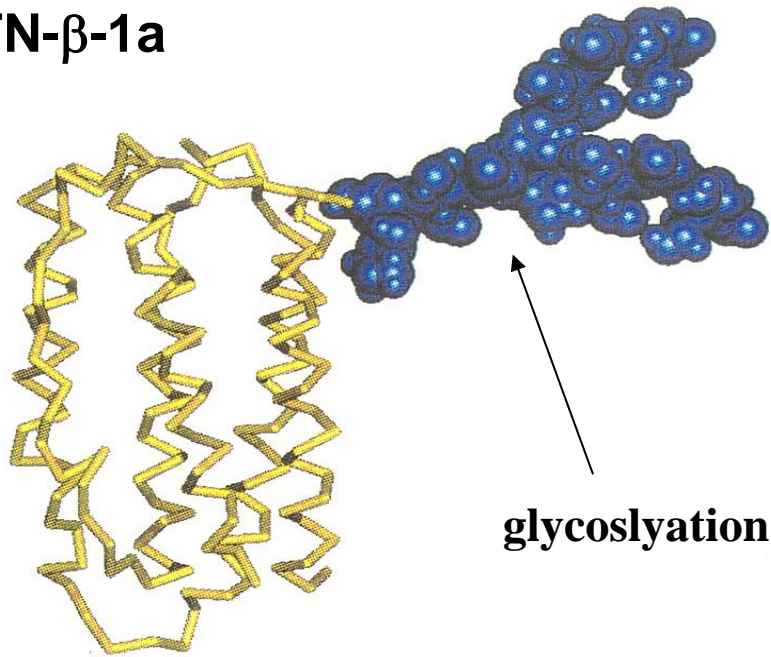
- Placebo n=315
- Antibody Negative n=569
- Transient Positive n=19
- Persistent Positive n=37

Effect of Anti-Natalizumab Antibodies on Infusion-Related Reactions

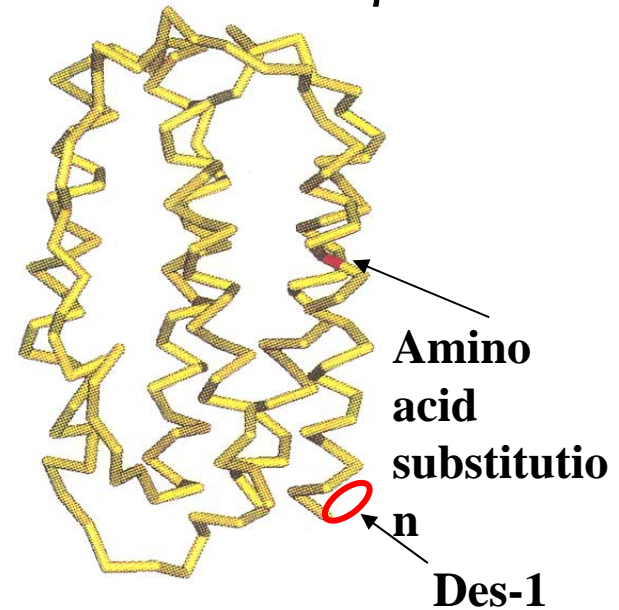


Interferon beta Multiple Products

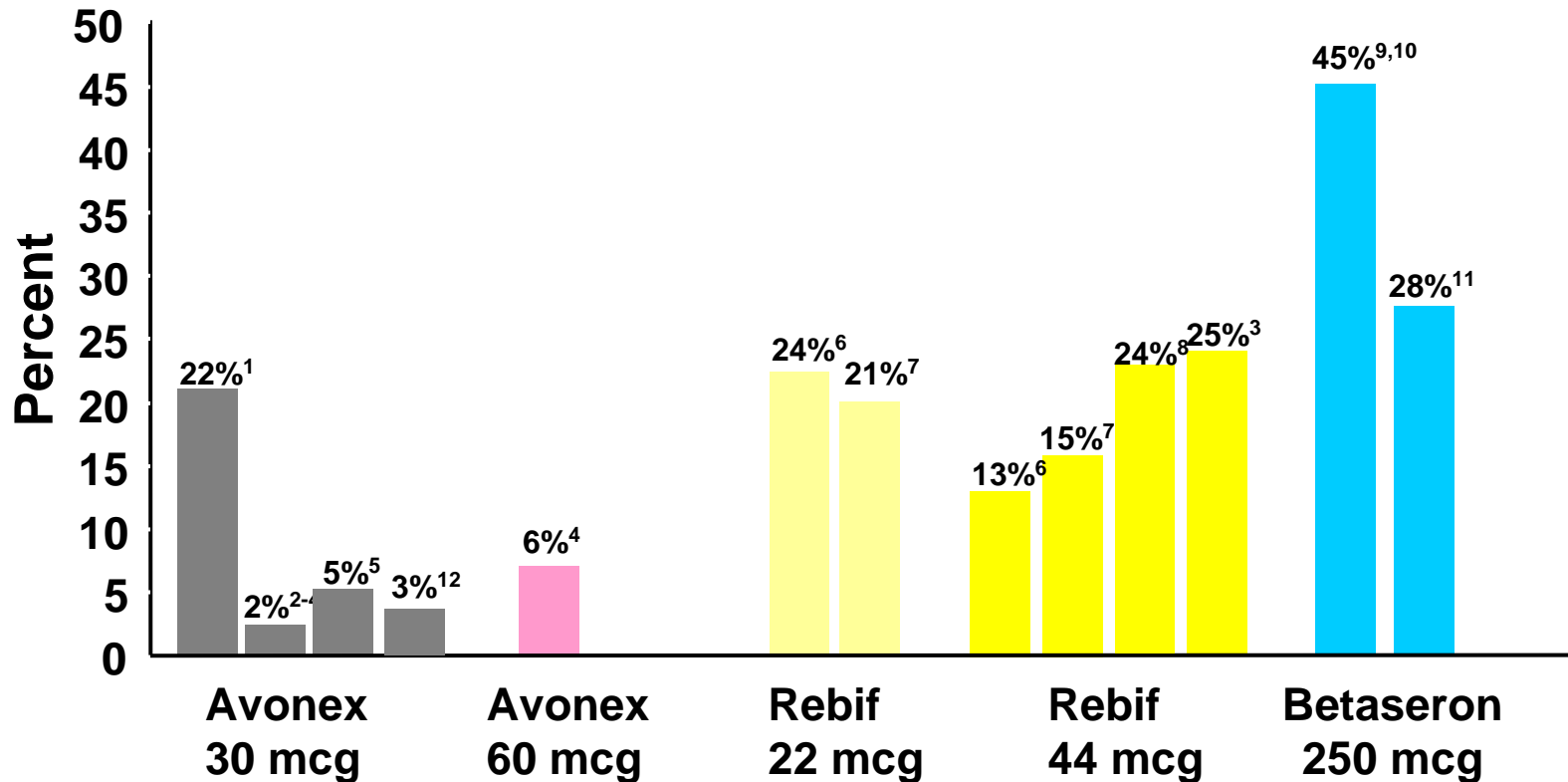
IFN- β -1a



IFN- β -1b



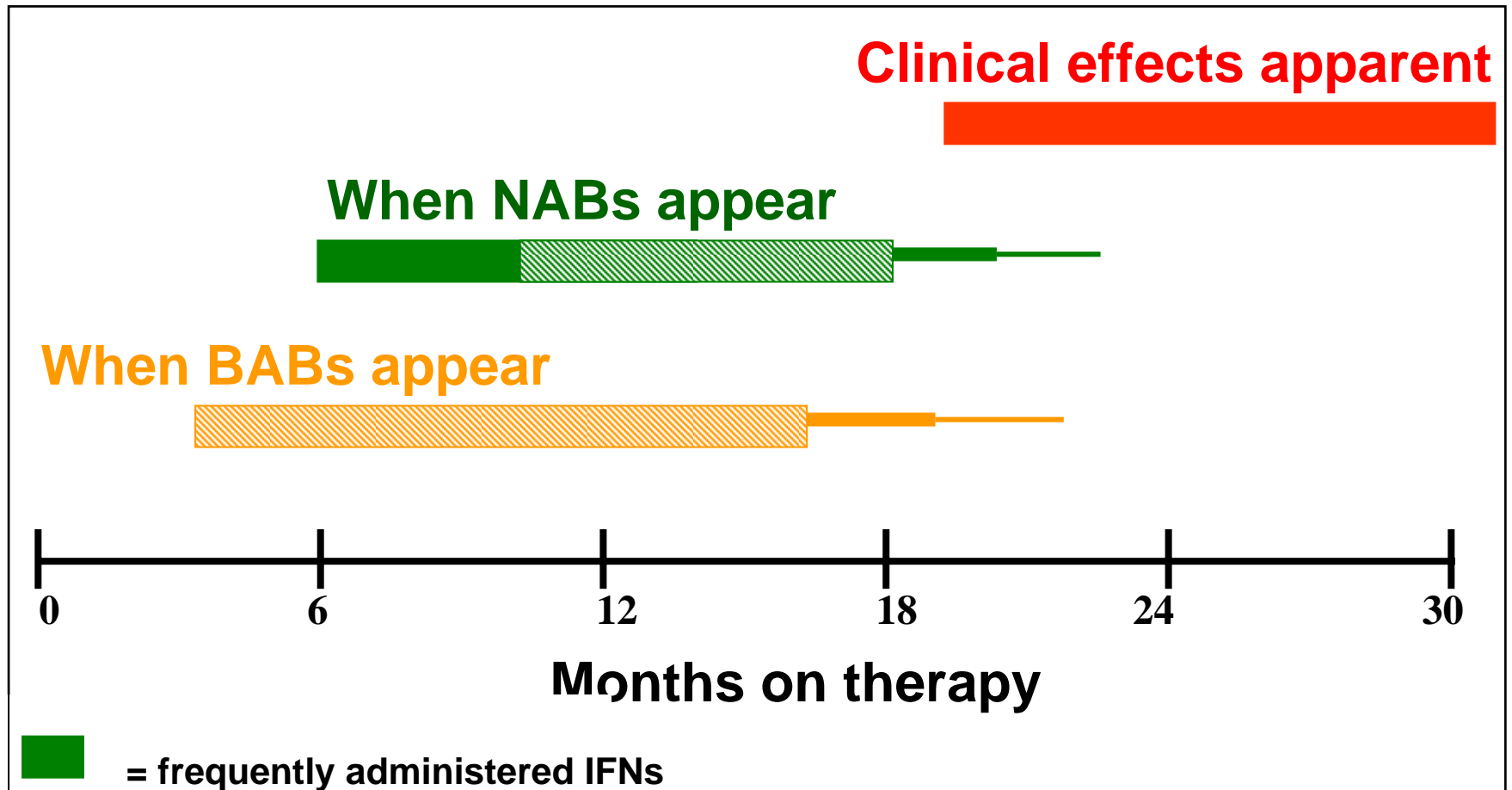
Incidence of IFN- β Nabs in Clinical Trials



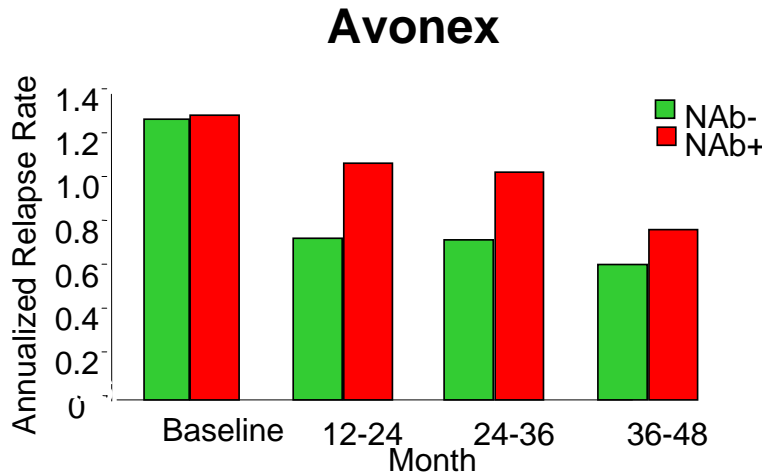
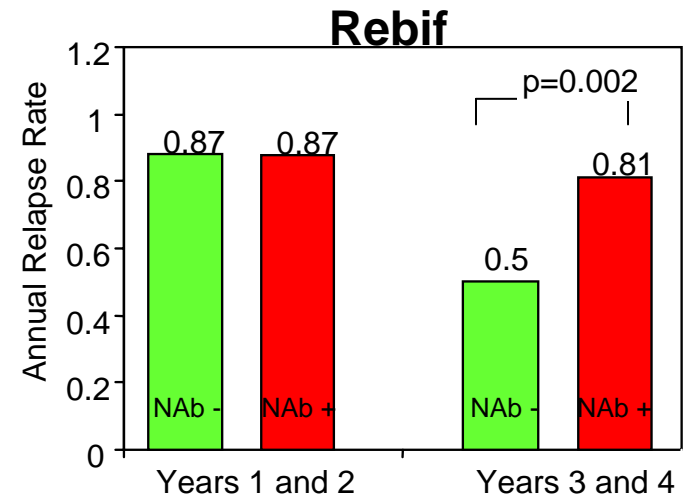
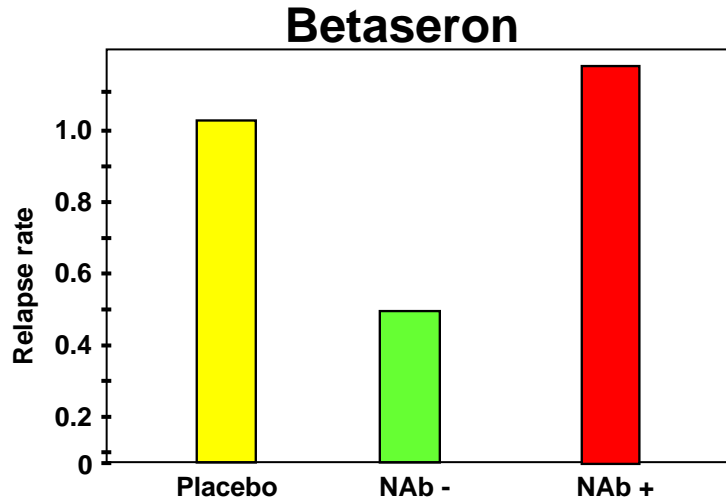
Note: Not all studies were designed for direct comparison among products.

1. Jacobs et al. *Ann Neurol.* 1996;39:285; 2. Jacobs et al. *N Engl J Med.* 2000;343:898; 3. Panitch et al. *Neurology.* 2002;59:1496;
 4. Clanet et al. *Mult Scler.* 2004;10:139; 5. Avonex package insert; 6. PRISMS Study Group. *Lancet.* 1998;352:1498;
 7. SPECTRIMS Study Group. *Neurology.* 2001;56:1496; 8. Rebif package insert; 9. IFNB Study Group. *Neurology.* 1995;45:1277;
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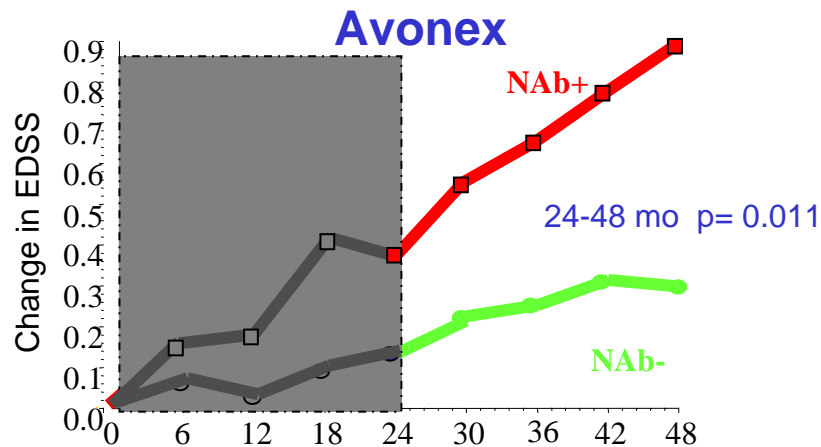
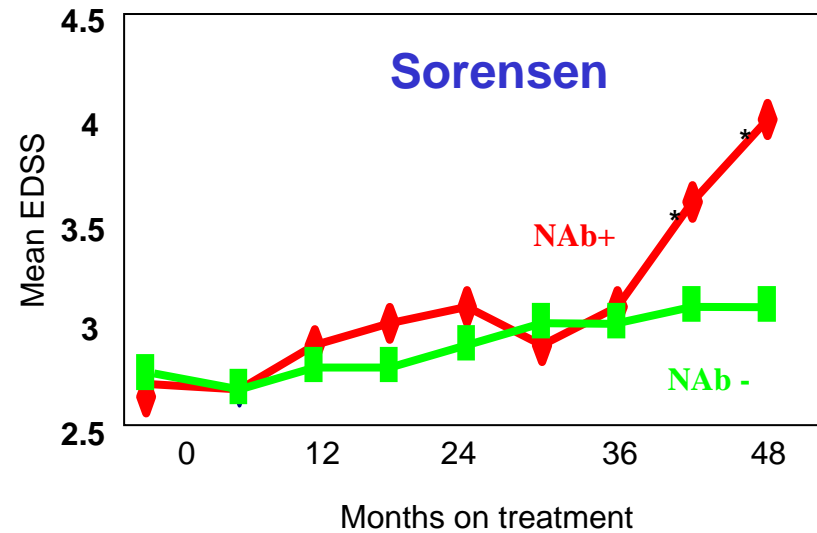
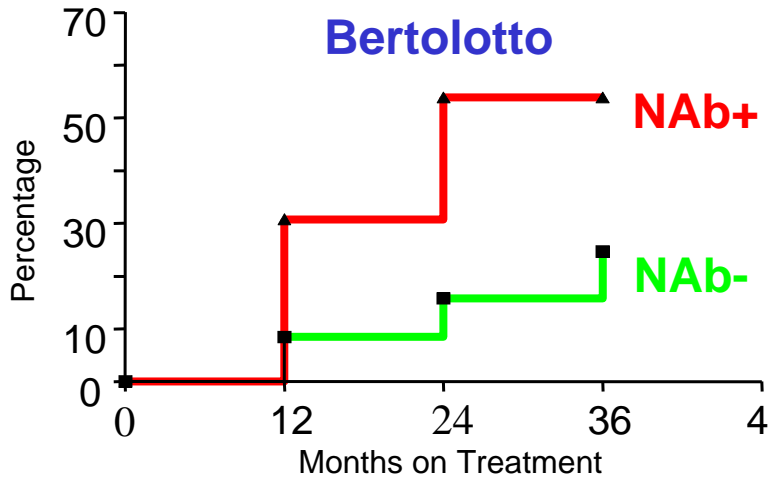
Kinetics of ADA development



Effect on Efficacy: Annualized Relapse Rate



Effect on Efficacy: Disability Progression



Clinical Trial Expectation for Biosimilars

- ❖ Comparative clinical trials adequately powered to detect efficacy and safety differences
- ❖ Immunogenicity plan to state rationale for approach and characterization tests used. Methods should be validated.
- ❖ Neutralizing and non-neutralizing Abs to be identified and correlated to effects on safety and efficacy.

Immunogenicity Testing for Biosimilars

- ❖ Immune system sensitive to distinguish differences
- ❖ Current analytical testing methods may not distinguish subtle differences.
- ❖ Small differences in protein therapeutics can result in distinct immunogenicity profiles
 - ❖ Avonex experience
- ❖ Multiple factors contribute to immunogenicity
- ❖ Clinical trials should be powered (# of patients and Rx duration) to avoid unexpected immunogenicity.

Conclusions

- ❖ Immunogenicity testing is complex
 - ❖ Assays, characterization of response, clinical monitoring and action plan
- ❖ Immunogenicity may change during product life cycle
 - ❖ Key to comparability assessments
- ❖ May impact risk/benefit ratio. Dynamic and evolving area
- ❖ Standardization of approaches and methodologies useful to compare incidence across products of the same class?

Additional References

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Acknowledgements

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- ❖ Regulatory
- ❖ Many Others.....