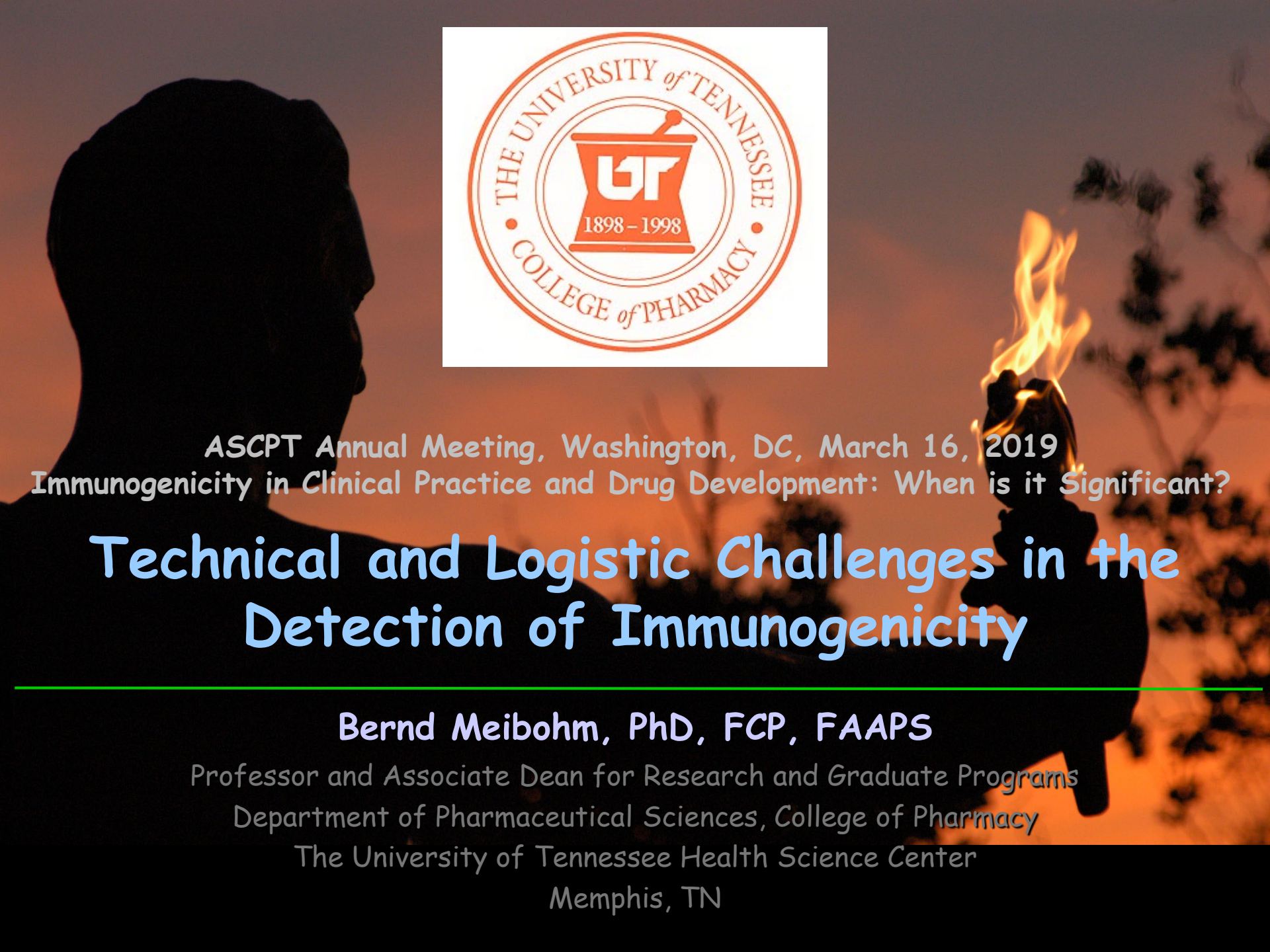


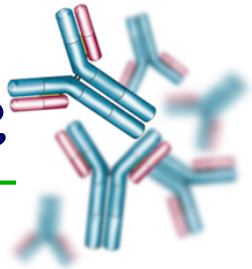
ASCPT Annual Meeting, Washington, DC, March 16, 2019
Immunogenicity in Clinical Practice and Drug Development: When is it Significant?

Technical and Logistic Challenges in the Detection of Immunogenicity

Bernd Meibohm, PhD, FCP, FAAPS

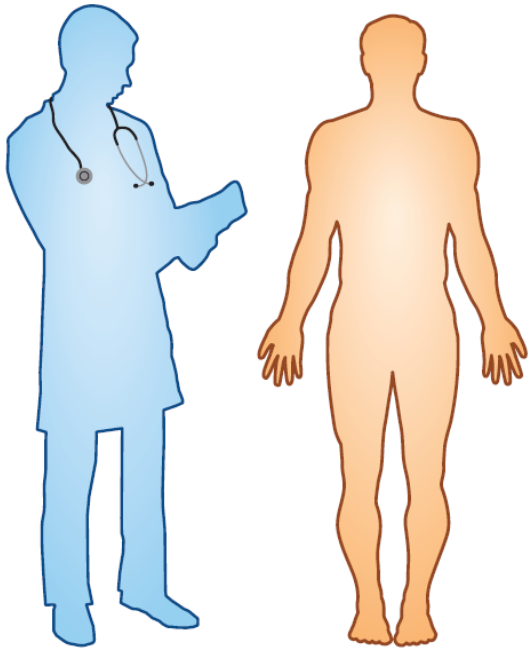
Professor and Associate Dean for Research and Graduate Programs
Department of Pharmaceutical Sciences, College of Pharmacy
The University of Tennessee Health Science Center
Memphis, TN

Immunogenicity & Clinical Relevance



What attributes of ADA immune response have the potential to be clinically relevant?

- Preexisting ADA
- Treatment-induced ADA
- Treatment-boosted ADA
- ADA level/titer
- Antibody isotype
- ADA cross-reactivity with an endogenous component and related biological drug
- ADA duration (persistence)
- Time to ADA onset
- Neutralizing ADA
- Drug-clearing ADA response
- Drug-sustaining ADA response
- For multi domain molecules, the domain specificity of ADA



What types of immunogenicity-related adverse clinical consequences are possible?

Acute adverse events:

- Type-I hypersensitivity
- Injection-site reaction or infusion reaction

Non-acute adverse events:

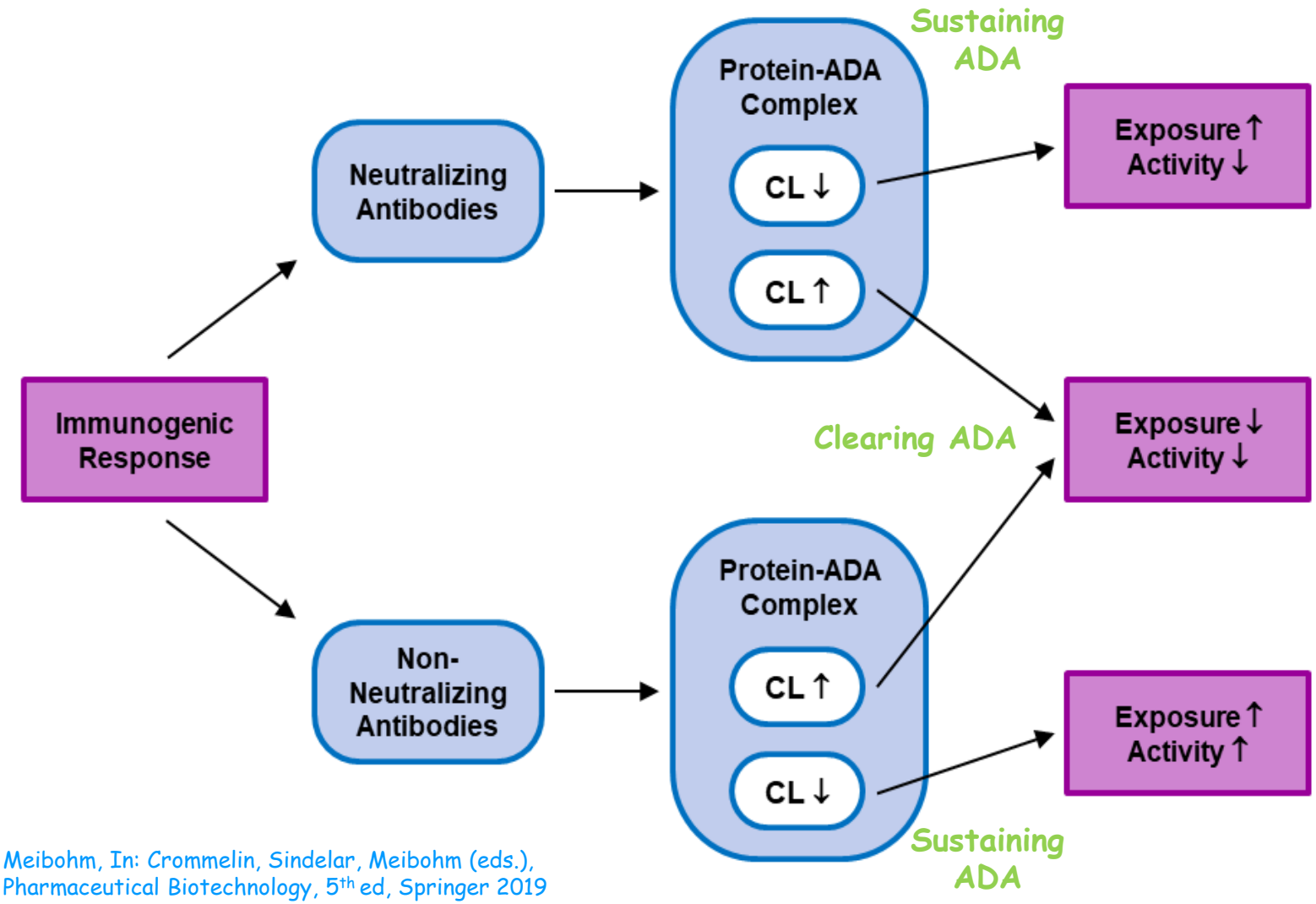
- Type-III hypersensitivity
- Worsening of disease
- Increased drug toxicity
- Partial response (attenuated efficacy)
- Primary loss of response
- Secondary loss of response

Marina Corral Spence/Nature Publishing Group

Shankar et al, Nature Biotechnol 2015, 33, 334-6

Anti-Drug Antibody (ADA) Formation

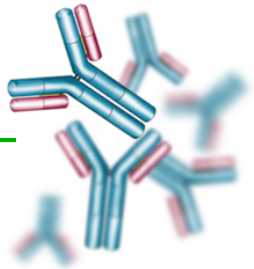
Possible Effect on PK and PD



Meibohm, In: Crommelin, Sindelar, Meibohm (eds.), Pharmaceutical Biotechnology, 5th ed, Springer 2019

ADA Assessment

Multi-Tiered Approach



- Determining the incidence, magnitude and impact of an immune response

Tier 1 - Screening

Test specimens — Including baseline samples for pre-existing ADA

Screening Assay

IgG IgM (IgA IgE)

Inconclusive — Drug concentration > drug tolerance

Negative specimens

Positive specimens

Tier 2 - Confirmation

Confirmatory Assay

Confirmed positive specimens

Tier 3 - Characterization

Characterization Assays

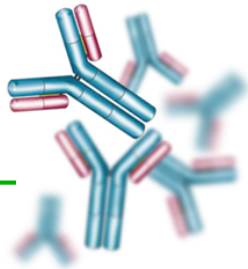
Isotyping
Domain/epitope mapping
Cross-reactivity

Neutralizing Assay

Titer Assay

Correlation with PK, PD and biomarker measures

Immunogenicity Assessment



FDA Guidance

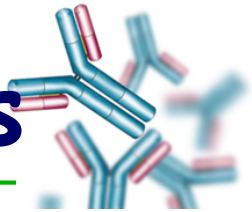
Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2019
Pharmaceutical Quality/CMC

Immunogenicity Incidence Rates



Antibody	Technology	Immunosuppression? Antigen	Immunosuppression? Comedication	Immunogenicity incidence	Reference
OKT®3	Mu	Yes	Yes	80%	Hooks et al. [1991]
Zevalin™	MuRC	Yes	Yes	3.8%	PI
Bexxar®	MuRC	Yes	Yes	10–70%	PI
ReoPro®	Ch	No	No	5.8% (1 dose) 25% (2+doses)	PI, Techeng et al. [2001]
Rituxan®	Ch	Yes	No	1.1%	PI
Simulect®	Ch	Yes	Yes	1.2–3.5% (2 doses)	PI
Remicade®	Ch	Yes	Yes	10–61%	PI, Baert et al. [2003]
Erbitux™	Ch	No	Yes	5%[1]	PI
Zenapax®	Hz	Yes	Yes	8.4%	PI
Synagis®	Hz	No	No	0.7–1.8%	PI
Herceptin®	Hz	No	Yes	0.1%	PI
Mylotarg™	HzTC	Yes	Yes	0% HAHA 2 pts. HATA	PI
Campath®	Hz	Yes	No	1.9% CLL patients 63% RA patients	PI, Weinblatt et al. [1995]
Xolair®	Hz	No	No	<0.1%	PI
Raptiva™	Hz	Yes	No	6.3%	PI
Avastin™	Hz	No	Yes	ND[2]	PI
Humira™	HuPD	Yes	Yes	1% with MTX 12 % monotherapy	PI

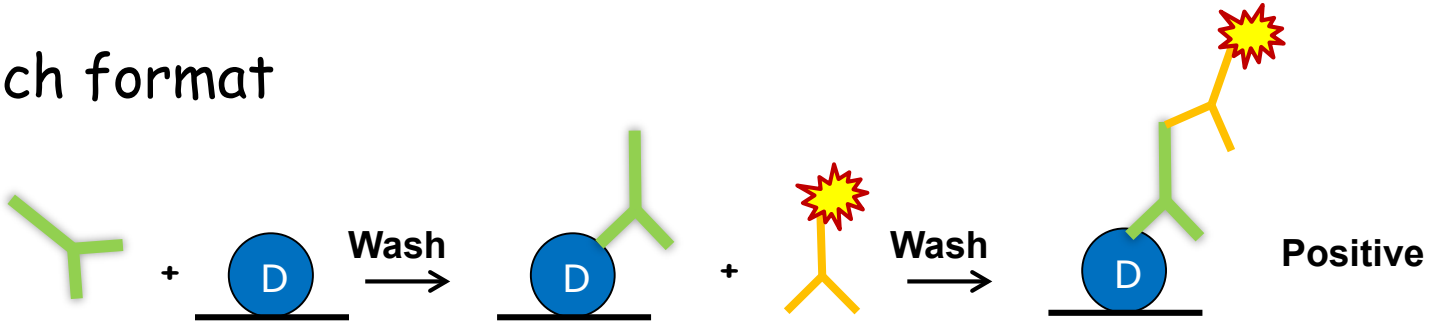
Data are from the product prescribing information (PI) or other references as indicated. Mu: murine; MuRC: murine radioconjugate; Ch: chimeric; Hz: humanized; HzTC: humanized toxin conjugate; HuPD: human phage-display derived. MTX: methotrexate. The immunosupression columns indicate whether the antibody-antigen interaction is immunosuppressive and if immunosuppressive therapies are generally given concurrently with the antibody.

Roskos et al., Drug Develop Res 2004, 61, 108-20

Immunogenicity Assessment

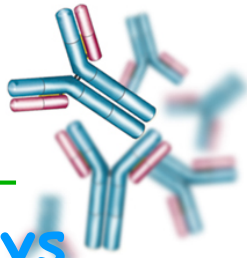
Typical ADA Assay Formats

Sandwich format



Bridging format

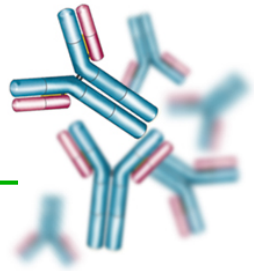
Immunogenicity Assessment



Major Differences between PK and ADA Assays

Drug/Biomarker Assay	ADA Assay
LCMS or Ligand binding assay	Ligand binding assay
Measured against identical, unique, known molecule	Measured against a 'family'/mixture of unknown, species-different molecules with certain common properties
Positive controls/calibration standards available	No definitive positive controls available
Quantitative assessment	Only qualitative or semi-/quasi-quantitative assessment (titer)
Robust towards interferences	Specificity, sensitivity and tolerance against interfering substances are unique for each assay and different for each drug and on different assay platforms

ADA Assay Challenges



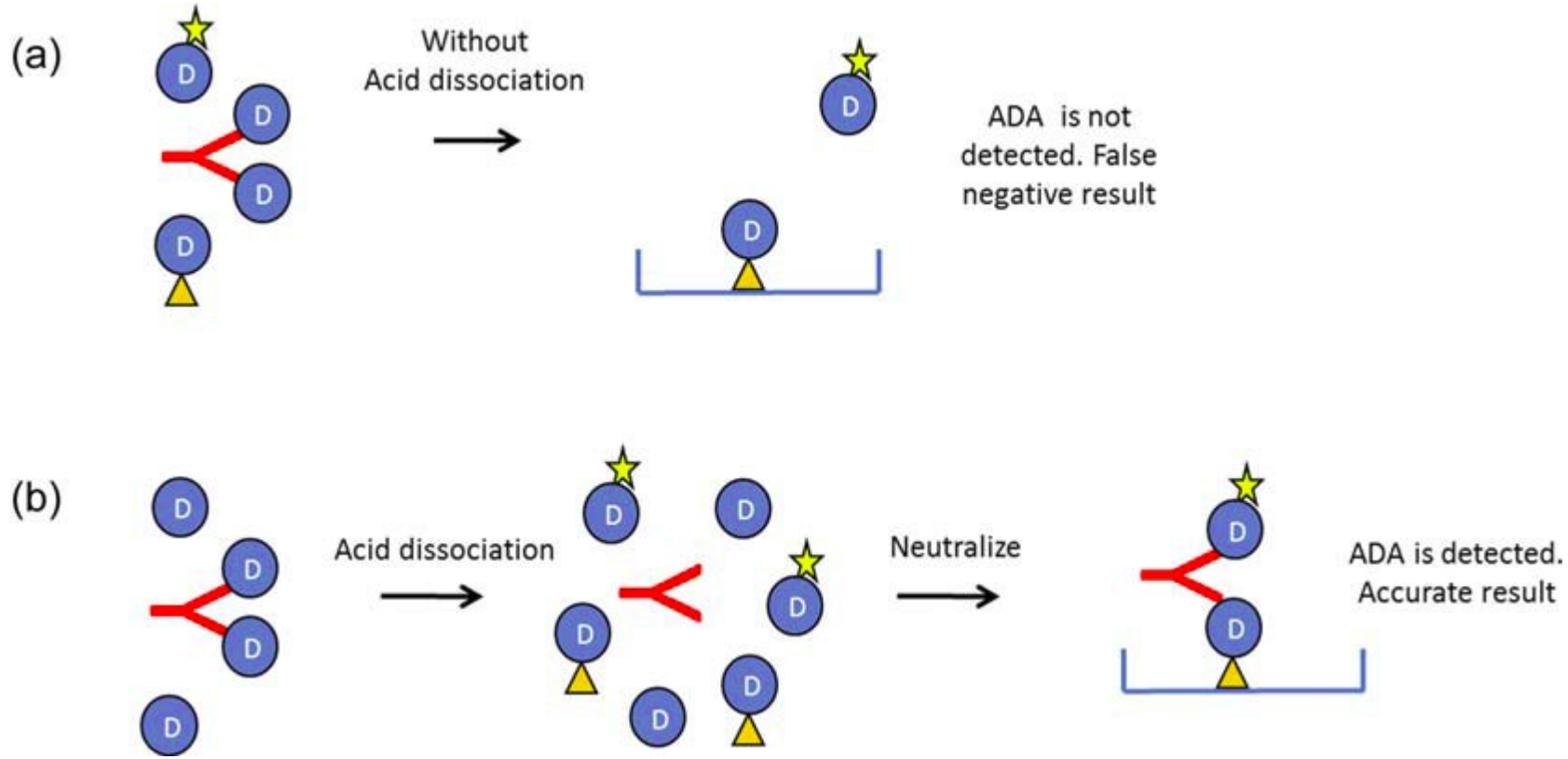
Lack of Suitable Positive Controls

- No positive controls commercially available
- 'Surrogate' ADAs usually generated with a human therapeutic protein in animals (sheep, goat)
  - ✓ Most often polyclonal
  - ✓ Species difference with regard to affinity, epitope specificity
  - ✓ Different epitope binding as human protein is foreign for animals
- Later programs might use affinity purified patient positive controls
- Reagent continuity is challenging
- Semi-quantitative assessments
  - ✓ Even 'titer assessments' are only quasi-quantitative approaches
  - ✓ Quasi-units or titers (dilution steps)

➔ Cross comparison across assays and platforms is INAPPROPRIATE

ADA Assay Challenges

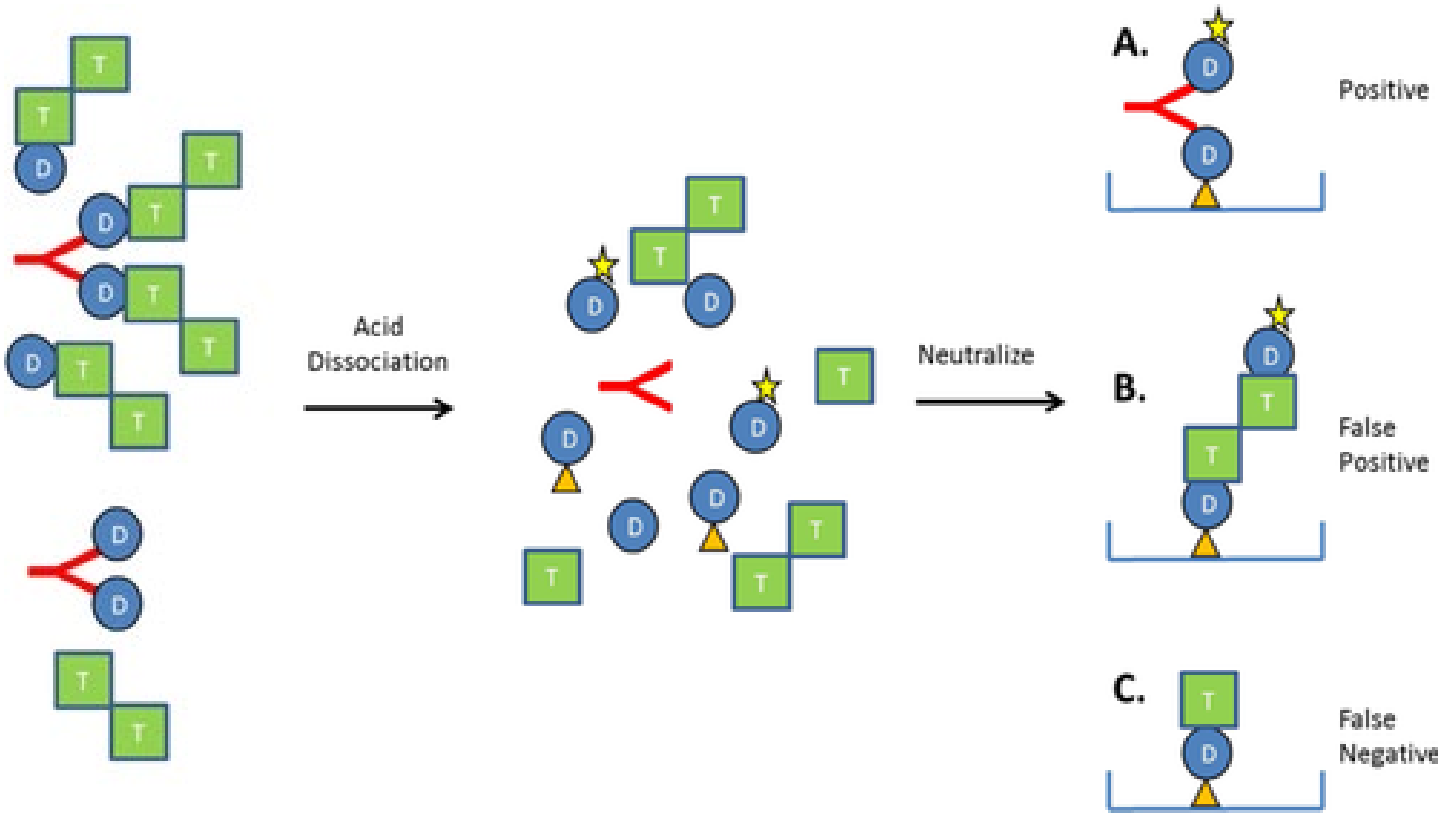
Drug Tolerance – Acid Dissociation



Gunn et al., Clin Exp Immunol 2016, 184, 137-146

ADA Assay Challenges

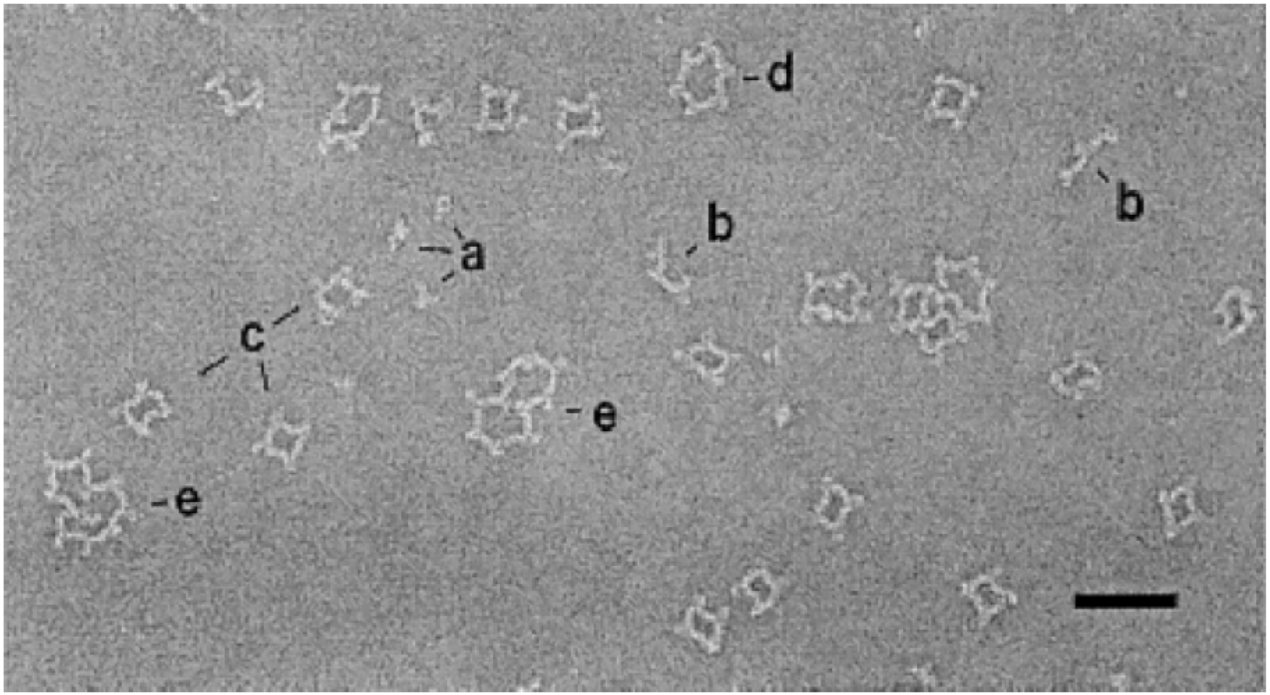
Soluble Target Interference



Gunn et al., Clin Exp Immunol 2016, 184, 137-146
Zhong et al., AAPS J 2017, 19, 1564-75

Immune Complex Formation

TS1 and its monoclonal anti-idiotype, αTS1



Electron micrograph of TS1/αTS1 immune complexes (0.1 mg/mL) 1:1 mixed, incubated for 20 min, and diluted 10-fold just prior to mounting and staining

- The electron micrograph shows
  a. unreacted molecules
  b. chains of three
  c. rings of four
  d. a ring of six
  e. a ring of 10

Johansson et al., Cancer 2002, 94, 1306-13

Immunogenicity Interpretation

Complicating Factors in Clinical Assessment

- Heterogeneous response: Polyclonal and relatively unspecific
  - ✓ May be against one or multiple different epitopes
  - ✓ May vary greatly in affinity: High vs. low affinity
  - ✓ Antibody response = all antibodies generated in a patient in response to a drug
    - o Clearing Ab vs. Sustaining Ab vs. Neutralizing Ab
  - ✓ One patient may form multiple different antibodies in response to a drug; different patients may have different responses
  - ✓ Effect of sustaining vs. clearing antibodies is largely determined by the formed ICs and the size of protein therapeutic
- Subjects/patients may have anti-Abs before first exposure
  - ✓ Sampling prior to first exposure is crucial

Monoclonal Antibody Biosimilars

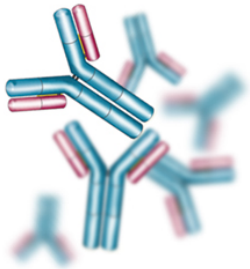


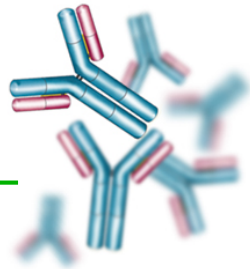
Table 1 | **Selected key products for which monoclonal antibody biosimilars are in development**

Product	Molecule	Number of biosimilars in development	Notable late-stage manufacturers	2014 sales (US$ billions)	Originator	US patent expiry*	EU patent expiry*
Immunology							
Enbrel	Etanercept‡	27	Merck/Samsung Bioepis, Coherus, Sandoz	8.5	Amgen/Pfizer	2028§	2015
Humira	Adalimumab	24	Amgen, Sandoz	12.5	AbbVie	2016	2018
Remicade	Infliximab	13	Celltrion, Hospira	9.2	Johnson & Johnson/Merck	2018	2015
Oncology							
Avastin	Bevacizumab	22	Amgen, Oncobiologics	7.0	Genentech/ Roche	2019	2022
Herceptin	Trastuzumab	37	Actavis/Amgen/Synthon, Biocad, Biocon/Mylan	6.8	Genentech/ Roche	2019	2014
Rituxan	Rituximab	44	Sandoz, Boehringer Ingelheim	8.7	Biogen/ Genentech/ Roche	2018	2013

*The date given is based on the expected expiry of patents protecting the original molecule. ‡Etanercept is a fusion protein, composed of the tumour necrosis factor (TNF) receptor fused to the immunoglobulin G1 Fc domain. §The patent on Enbrel was originally set to expire in 2012, but Amgen received an additional 17 years of patent protection owing to a patent dispute; sources include company financial records, the Generics and Biosimilars Initiative (GABI), BioProcess International and BioPharm International. In the United States, the 'molecule' patents protecting the active ingredient etanercept have all expired aside from US8063182 and US8163522 members from priority CH331989 (1989-09-12) owned by Roche (exclusively licensed to Amgen), which are set to expire in 2028 and 2029, respectively.

Upda & Million, Nat Rev Drug Discov 2016, 15, 13-4

Example: Adalimumab (I)



- Recombinant human IgG1 monoclonal antibody specific for TNF-α
  - ✓ Created using phage display technology resulting in an antibody with human derived heavy and light chains variable regions and human IgG1:κ constant regions
  - ✓ produced by recombinant DNA technology in a mammalian cell expression
- Prescribing Information HUMIRA 2002

Immunogenicity

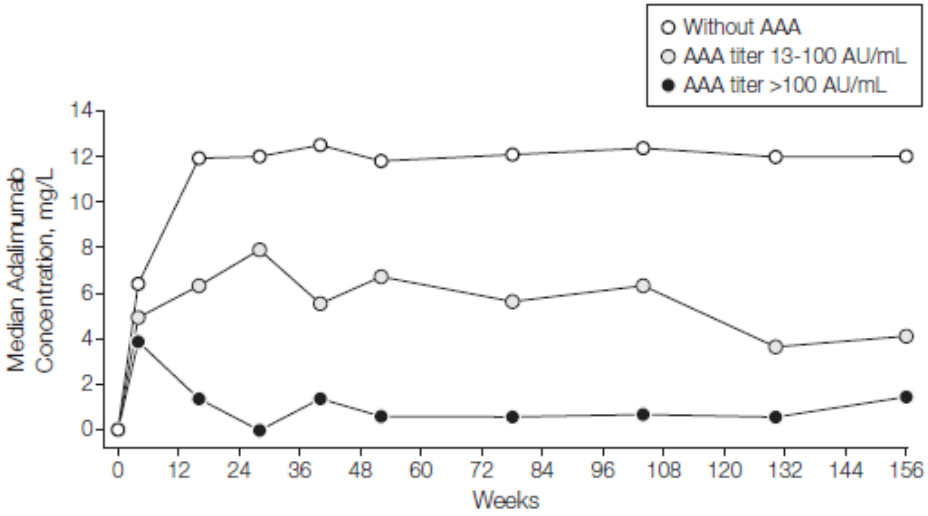
Patients in Studies I, II, and III were tested at multiple time points for antibodies to adalimumab during the 6 to 12 month period. Approximately 5% (58 of 1,062) of adult rheumatoid arthritis patients receiving HUMIRA developed low-titer antibodies to adalimumab at least once during treatment, which were neutralizing *in vitro*. Patients treated with concomitant MTX had a lower rate of antibody development than patients on HUMIRA monotherapy (1% versus 12%). No apparent correlation of antibody development to adverse events was observed. With monotherapy, patients receiving

Example: Adalimumab (II)

Clinical Reports 2007-11

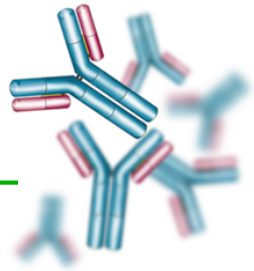
- Bartelds et al.,
  Ann Rheum Dis 2007, 66, 921-6
  - ✓ Active RA patients (n=121)
  - ✓ Either HUMIRA monotherapy or with DMARD (including MT
  - ✓ ADA incidence week 28:
    - o Humira: 17%

- Bartelds et al.,
  JAMA 2011, 305, 1460-8
  - ✓ Active RA patients (n=272)
  - ✓ Either HUMIRA monotherapy or with DMARD (including MT)
  - ✓ ADA incidence week 28:
    - o Humira: 19%
  - ✓ ADA incidence week 156:
    - o Humira: 28%



Week	0	4	16	28	40	52	78	104	130	156
No. of patients										
Without AAA	196	187	177	164	145	139	131	118	107	93
AAA 13-100 AU/ml	45	43	42	37	34	34	28	24	19	17
AAA >100 AU/ml	31	31	28	27	22	19	16	14	11	8

Example: Adalimumab (III)



Current Prescribing Information

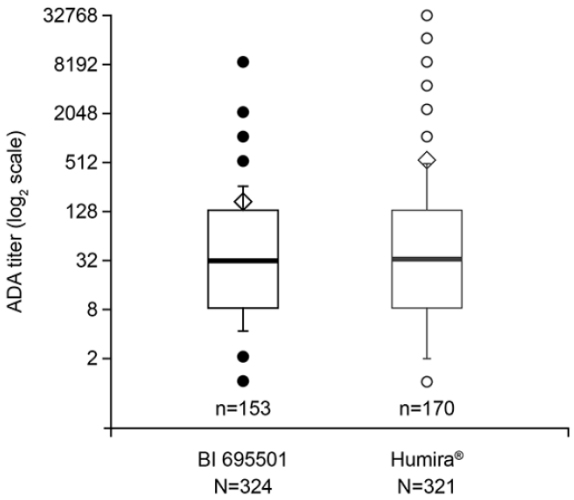
- RA
  - ✓ Approximately 5% (58 of 1062) of adult RA patients receiving HUMIRA developed low-titer antibodies to adalimumab at least once during treatment, which were neutralizing in vitro.
  - ✓ Patients treated with concomitant methotrexate (MTX) had a lower rate of antibody development than patients on HUMIRA monotherapy (1% versus 12%).
- JIA
  - ✓ In patients with polyarticular JIA who were 4 to 17 years of age, adalimumab antibodies were identified in 16% of HUMIRA-treated patients.
  - ✓ In patients receiving concomitant MTX, the incidence was 6% compared to 26% with HUMIRA monotherapy.
- AS
  - ✓ In patients with AS, the rate of development of antibodies to adalimumab in HUMIRA-treated patients was comparable to patients with RA
- PsA
  - ✓ In patients with PsA, the rate of antibody development in patients receiving HUMIRA monotherapy was comparable to patients with RA; however, in patients receiving concomitant MTX the rate was 7% compared to 1% in RA
- CD
  - ✓ In adult patients with CD, the rate of antibody development was 3%

Example: Adalimumab (IV)

Adalimumab Biosimilars vs. Humira

- BI695501
  - ✓ Moderate-to-severe RA patients (n=593): BI695501 vs. Humira (US)
  - ✓ Stable MTX background therapy: 15-25 mg/week
  - ✓ ADA incidence week 24:
    - o BI695501: 47.5% (~50% neutralizing)
    - o Humira: 53.0% (~50% neutralizing)

~50%



- SB5
  - ✓ Moderate-to-severe RA patients (n=508): SB5 vs. Humira
  - ✓ Stable MTX background therapy: 10-25 mg/week
  - ✓ ADA incidence week 24:
    - o SB5: 32.4% (~50% neutralizing)
    - o Humira: 31.4% (~50% neutralizing)

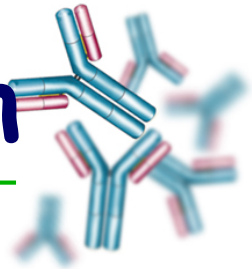
~30%

- ABP501
  - ✓ Moderate-to-severe RA patients (n=494): ABP501 vs. Humira
  - ✓ Stable MTX background therapy: average 16.6-16.9 mg/week
  - ✓ ADA incidence week 26:
    - o ABP501: 38.3% (~24% neutralizing)
    - o Humira: 38.2% (~29% neutralizing)

~40%

Cohen et al., Ann Rheum Dis 2018, 77, 914-21
Weinblatt et al., Arthritis Rheumatol 2018, 70, 40-8
Cohen et al., Ann Rheum Dis 2017, 76, 1679-87

Immune Complex Formation



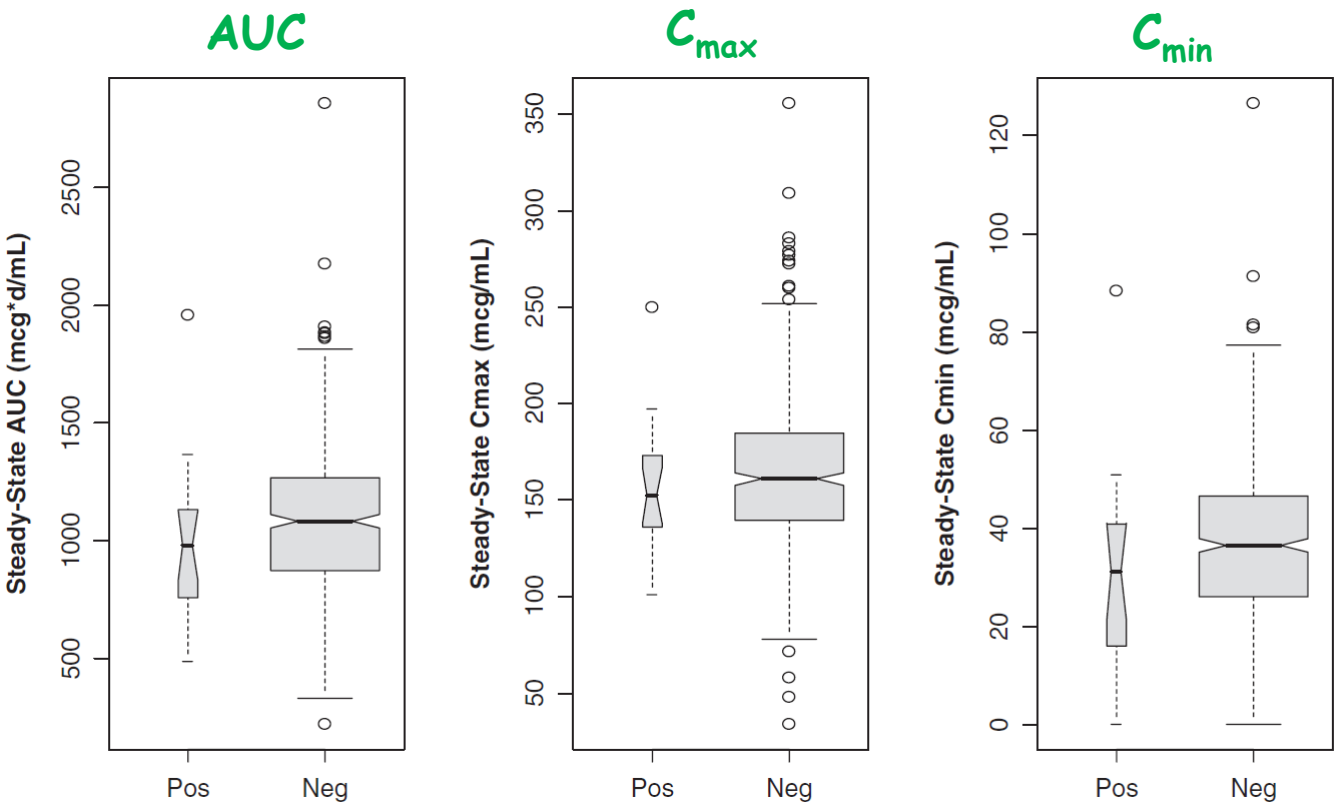
Clearance

- Circulating immune complexes trigger regular endogenous elimination processes
- Uptake and lysosomal degradation by reticulo-endothelial system (phagocytic cells [monocytes and macrophages])
  - ✓ Primarily in liver and spleen
  - ✓ Mediated via Fcγ receptors, primarily FcγRIIb2 (in rat liver sinusoidal endothelial cells)
  - ✓ Human platelets contribute to the clearance of IgG-containing complexes
    - o Express FcγRIIA
    - o Bind IgG complexes and are internalized by circulating phagocytes

Cohen et al., Ann Rheum Dis 2018, 77, 914–21. Ali Mousavi et al., Hepatology 2007, 46, 871-84
Huang et al., Mol Immunol 2011, 48, 691-6

Anti-Drug Antibodies

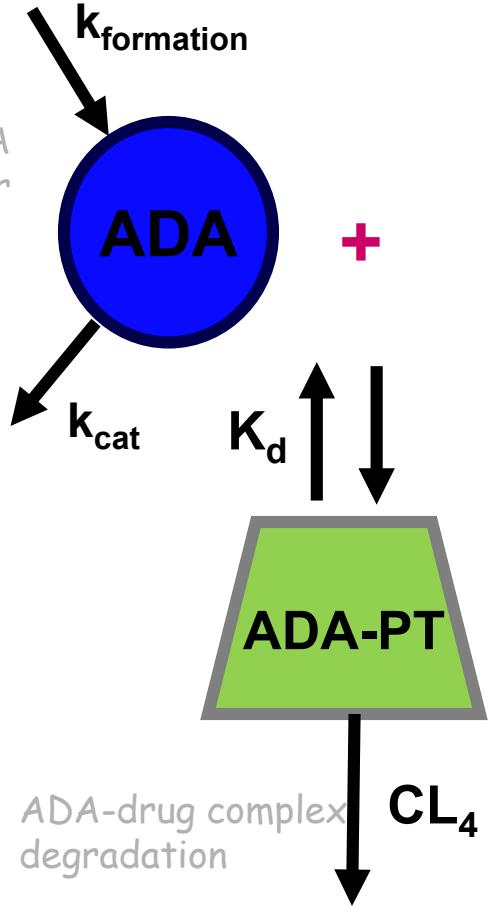
With No Effect on Disposition

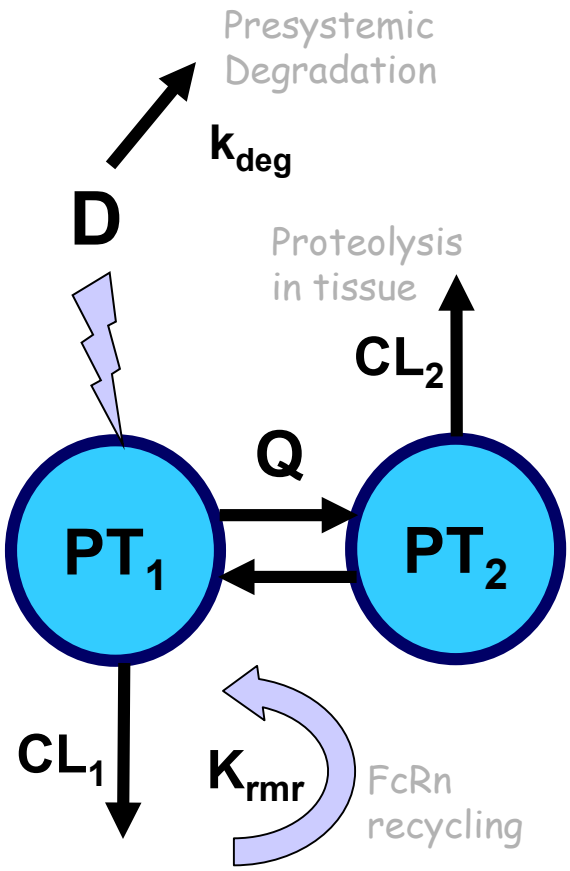


Panitumumab exposure in patients with and without ADA (Median; lower and upper quartiles; 95% confidence intervals; Box widths ~ √n)

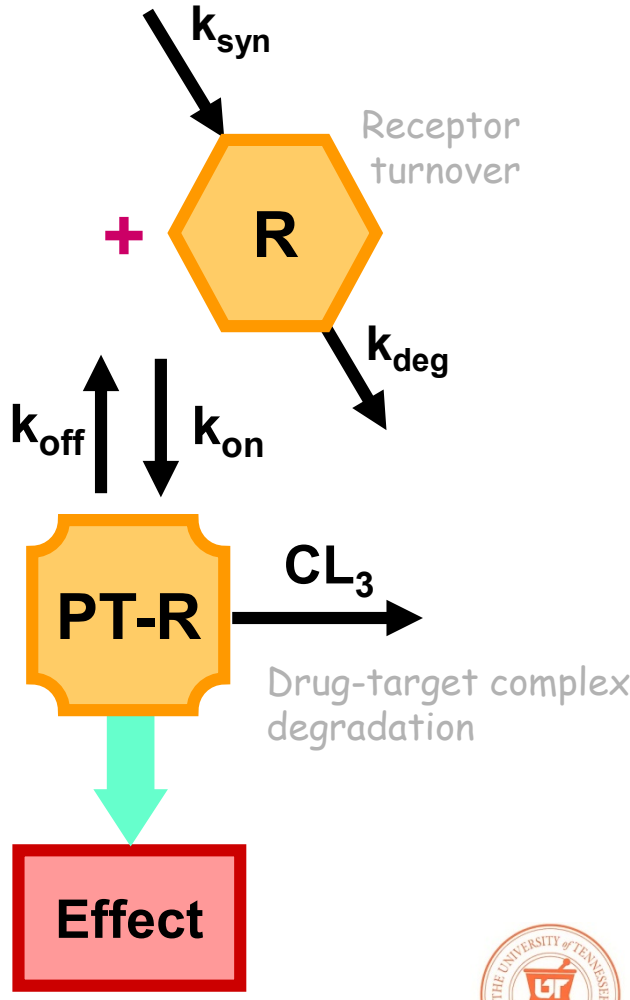
Ma et al., J Clin Pharmacol 2009, 49, 1142-56

ADA immune complex formation & disposition



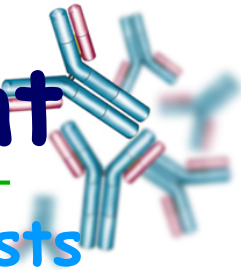


Target-mediated drug disposition



Chirmule, Jawa & Meibohm. AAPS J 2012, 14: 296-302

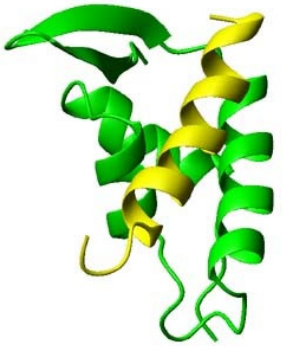
Challenges in ADA Assessment



Take-home Message for Clinical Pharmacologists

- ADA assays are qualitative or semi-quantitative assessments
- Lack of defined, standardized positive controls, the polyclonal nature and between-patient variability of immune response make comparisons between different drugs and different assay platforms impossible
- Drug and target tolerance pose limitations on ADA assays
- Due to heterogeneity in ADA response in different patients semi-quantitative measurements (titer) may not be related to clinical effects
- Incidence and magnitude of ADA response as assessed by ADA assays always needs to be considered in context with its PK (clearing/sustaining) and PD (neutralizing) effects for meaningful clinical interpretation

www.PKPDofProteinTherapeutics.com



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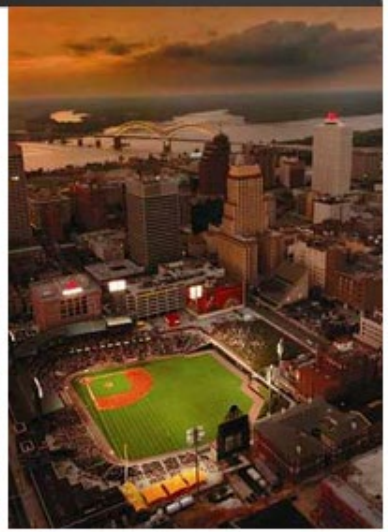
9th Introductory Pharmacometric Training Course

Pharmacokinetics & Pharmacodynamics of Protein Therapeutics

- Concepts and Hands-On Modeling and Simulation -



Course Directors:
Bernd Meibohm, University of Tennessee
Johan Gabrielsson, Swedish University of Agricultural Sciences

The 5-day course will introduce participants to basic principles in the pharmacokinetic and pharmacodynamic evaluation of novel protein therapeutics and provide opportunities for hands-on PK and PK/PD modeling and simulation examples relevant for protein drugs. Topics include target-mediated drug disposition, tissue and tumor penetration, interspecies scaling, first-in human dose selection, immunogenicity, model-based drug development, disease progression modeling, and drug-drug interactions. Hands-on data analysis will be performed individually and in small groups using several software packages.

Location: **University of Tennessee College of Pharmacy, Memphis, TN, USA**

Time: April 1-5, 2019

Last updated: May 30, 2012

Click here First Announcement Flyer

Participants of the 2nd 'PKPD of Protein Therapeutics' pharmacometric training course, April 2012

April 1-5, 2019

y of Tennessee bmeibohm@uthsc.edu

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