Immunogenicity & Biotherapeutic Development

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BioAnalytical Sciences,
Genentech, Inc.

ProImmune Mastering Immunogenicity Conference,
Sep 12 2011, Boston

Outline

- Biotherapeutics & the immunogenicity barrier
- Immunogenicity in product development
- Immunogenicity in process development
- Immunogenicity prediction tools & technologies

Biotherapeutics in Development

Sorted by Product Type

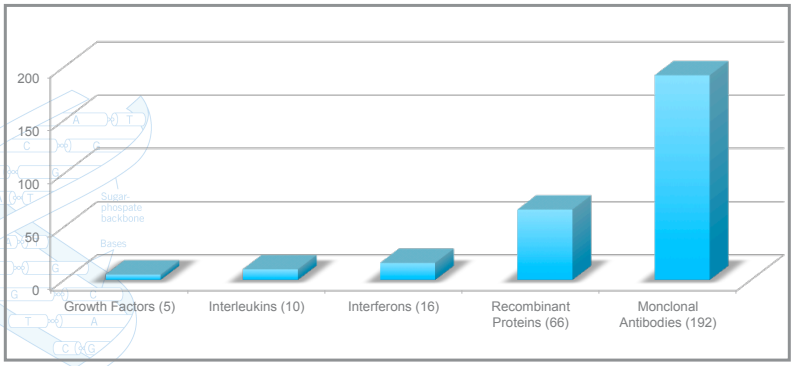

Chart based on data from "Medicines in Development - Biotechnology 2008 " PhRMA Survey
Data are still current, according to PhRMA website,

http://www.phrma.org/medicines_in_development_for_biotechnology

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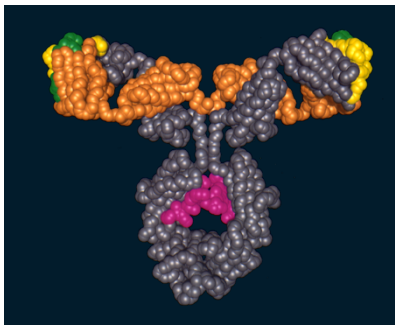
Biotherapeutics are large, complex molecules



Aspirin
180 Daltons
0 amino acids

Interferon-alpha
19,625 Daltons
~165 amino acids

Antibody (IgG)
~150,000 Daltons
~1,300 amino acids

From: Steffen Gross (Paul Ehrlich Institut), PDA Workshop on MAbs, June, 2011

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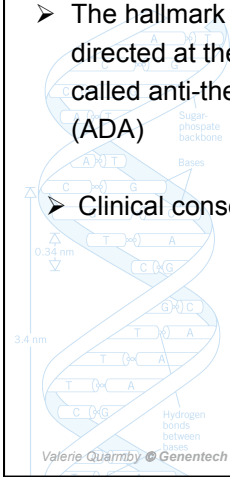
Immunogenicity in Biotherapeutic Development

- Immunogenicity refers to the production of an unwanted immune response directed at a biotherapeutic.
- The hallmark of immunogenicity is the presence of host antibodies directed at the biotherapeutic in the circulation. These are typically called anti-therapeutic antibodies (ATA) or anti-drug antibodies (ADA)
- Clinical consequences vary.



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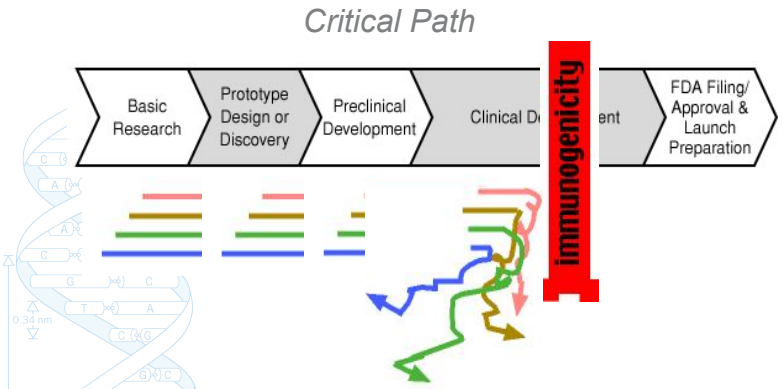
Impact of Immunogenicity on Biotherapeutic Development

Clinical Impact	Clinical Outcome
Safety	➢ Hypersensitivity or anaphylactic reactions ➢ Neutralize activity of endogenous counterpart with unique function causing deficiency syndrome ➢ Immune complex formation
Efficacy	➢ Neutralize activity of therapeutic protein ➢ Increase or decrease efficacy by extending or curtailing half life ➢ Increase or decrease efficacy by changing bio-distribution
Pharmacokinetics	➢ Extend, or curtail half life ➢ Alter biodistribution ➢ PK changes may dictate changes in dosing
None	➢ No discernible impact

Adapted from Susan Kirshner, FDA, AAPS NBC, 2010

The Immunogenicity "Barrier"



Susan Kirshner, Division of Therapeutic Proteins, Office of Biotechnology Products, CDER/FDA "Assessment of Immunogenicity of Biological Therapeutics" (2009) AAPS National Biotechnology Conference.

Impact of Immunogenicity on Biotherapeutic Development

- Immunogenicity of biological products is a high profile concern for industry and for regulatory authorities
  - Immunogenicity may impact safety and/or efficacy
  - FDA & EMA require that immunogenicity of biotherapeutics be evaluated
  - Development of biotherapeutics for chronic use is increasing the need to understand potential implications of immunogenicity
  - Immunogenicity strategies and data are essential components of Target Product Profiles, INDs, BLAs, & USPIs

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Immunogenicity has many implications….

Product Development:

- Protein Design
- Preclinical Development
- Clinical Development

Process Development:

- Production system selection
- Cell culture & recovery system design
- Formulation Development
- Packaging & Container Closure Selection
- Commercial Manufacturing

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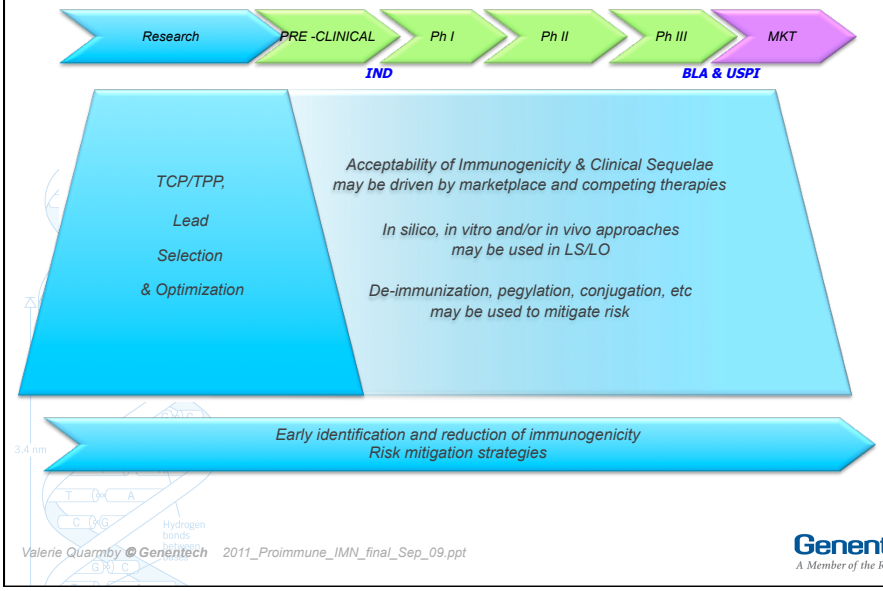
Immunogenicity in Biotherapeutic Product Development



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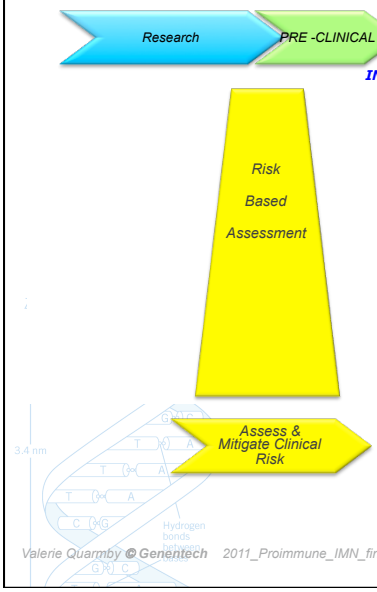
Immunogenicity in Biotherapeutic Product Development



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Immunogenicity Risk Assessment

Higher Risk	Lower Risk
Existence of endogenous version	No endogenous version
Unique activity	Redundant activity
Sole therapy	Other therapies
Non Life threatening disease	Life threatening disease
Repetitive treatment	Single dose treatment
Replacement therapy	Non replacement therapy
Non - immunosuppressed subjects	Immunosuppressed subjects
Immunogenicity of the administration route: intradermal > inhalation > subcutaneous > intraperitoneal > intramuscular > intravenous	

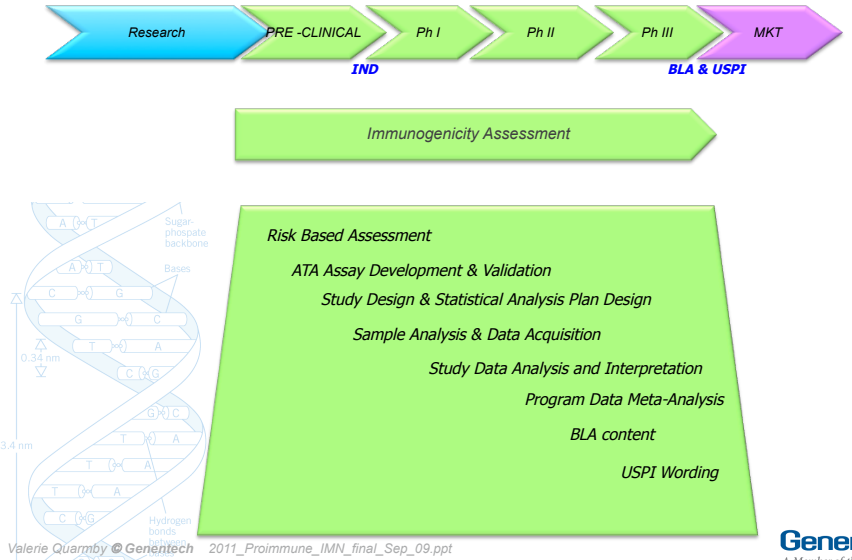
Recommendations on Risk-Based Strategies for Detection and Characterization of Antibodies against Biotechnology Products
Koren et al (2008) JIM

Risk Based Approach to Immunogenicity Concerns of Therapeutic Protein Products
Rosenberg & Worobec (2004 & 2005) BioPharm International

Consequences of Immunogenicity Risk Assessment

Type of Therapeutic	Perceived Risk	Frequency of ATA Sample Collection	ATA Sample Analysis Strategy
Recombinant Endogenous Protein w/ Non-Redundant Critical Endogenous Homolog	High	More frequent during all phases of clinical development	Consider whether real-time analysis/data would impact patient treatment
Recombinant Endogenous Proteins, Proteins with unique structure, Some recombinant Mabs	Medium	More frequent during phases I & II, may be less frequent during phase III	Batch analysis, occasionally real-time analysis may be needed.
Some recombinant MAbs	Low	Same as for Medium Risk	Batch analysis

Non-Clinical & Clinical Immunogenicity Assessment



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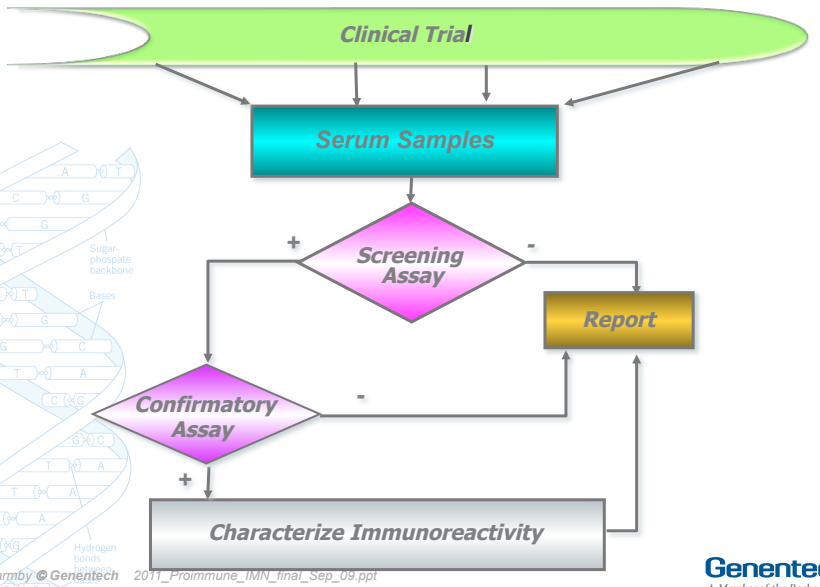
Immunogenicity – Tiered Testing Strategy



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Interpreting Impact of Immunogenicity Data in Context

Status	ATA	PK/PD	Safety	Efficacy	Interpretation
Optimal	Yes /No*	No	No	No	ATA not detected, no apparent S & E concerns with respect to immunogenicity ATA detected but no clinically relevant FX on PK/PD/S/E
Acceptable	Yes	Yes	No	No	ATA present but minimal FX on PK/PD No clinically significant S or E concerns regarding immunogenicity
Tolerable [Benefit > Risk]	Yes	Yes	No	Yes	ATA present and has FX on PK/PD No efficacy impact or impact can be managed with dose adjustments or changes in frequency Safety concerns regarding immunogenicity are none or minimal & can be managed with premedication or symptomatic treatment
	Yes	Yes	Yes	No	
No Go [Risk > Benefit]	Yes	Yes/No	Yes/No	Yes/No	ATA present and confers limits on efficacy ATA present and confers limits on safety

** FDA will question assay methods if no ATA responses detected*

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USPI summarizes Immunogenicity Data

USPI will reflect ATA incidence, neutralizing ability, and clinical significance

Immunogenicity

As with all therapeutic proteins, there is potential for immunogenicity. The immunogenicity of Vectibix™ has been evaluated using two different screening immunoassays for the detection of anti-panitumumab antibodies: an acid dissociation bridging enzyme linked immunosorbent assay (ELISA) (detecting high-affinity antibodies) and a Biacore® biosensor immunoassay (detecting both high- and low-affinity antibodies). The incidence of binding antibodies to panitumumab (excluding predose and transient positive patients), as detected by the acid dissociation ELISA, was 2/612 (< 1%) and as detected by the Biacore® assay was 25/610 (4.1%).

For patients whose sera tested positive in screening immunoassays, an in vitro biological assay was performed to detect neutralizing antibodies. Excluding predose and transient positive patients, eight of the 604 patients (1.3%) with postdose samples and 1/350 (< 1%) of the patients with follow-up samples tested positive for neutralizing antibodies.

There was no evidence of altered pharmacokinetic profile or toxicity profile between patients who developed antibodies to panitumumab as detected by screening immunoassays and those who did not.

Vectibix USPI

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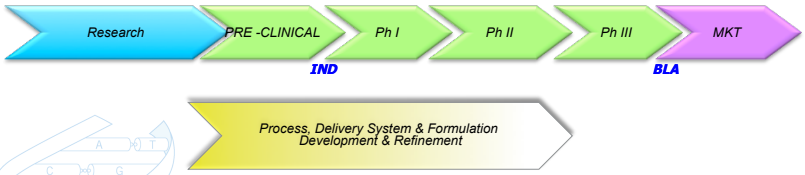
Immunogenicity in Biotherapeutic Process Development



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Immunogenicity in Biotherapeutic Process Development

The FDA Office of Biotechnology Products has implemented a Quality by Design (QbD) pilot program.

FDA & EMA have launched a program for parallel assessment of QbD applications.

Extensive activity in FDA & across industry to establish QbD practices.

QbD tracks process development parameters & links these to metrics of biological activity, safety & efficacy.

- Immunogenicity is one key metric of product quality

Valerie Quarmby © Genentech 2011_Proimmune_IMN_final_Sep_09.ppt

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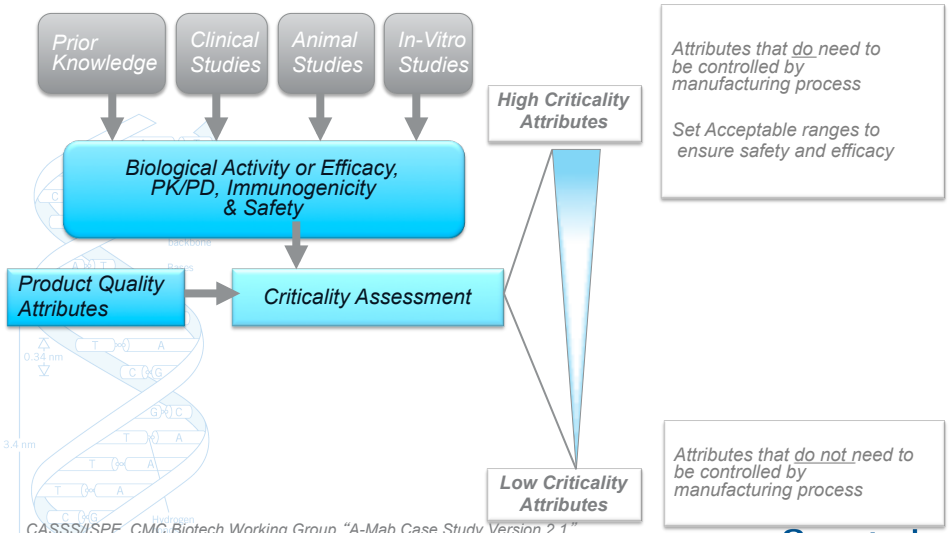
Assessing Criticality of Product Quality Attributes



CASSS/ISPE CMC Biotech Working Group "A-Mab Case Study Version 2.1"

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Typical MAb Product-Variant Critical Quality Attributes

Attribute	Rationale for Categorization
Afucosylated glycans	Biological Activity for MAb with ADCC as MOA
G0, G1, G2	Biological Activity for MAb with CDC as MOA
Gal-α1,3-gal	Safety
Met-oxidation	Biological activity if antigen binding PK if FcRn binding residue
Fragments	Altered PK, biological activity
Soluble Aggregates	Immunogenicity, biological activity
Disulfide variants	Multiple effects
Sequence variant	Residue dependent, multiple effects

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High Criticality Score

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Key Messages…

Immunogenicity is a key metric of product safety.

Immunogenicity is now also a key metric of product quality.

- In the context of biotherapeutic development:
  - What tools & data can be used to assess the risk of immunogenicity prior to first in human studies?

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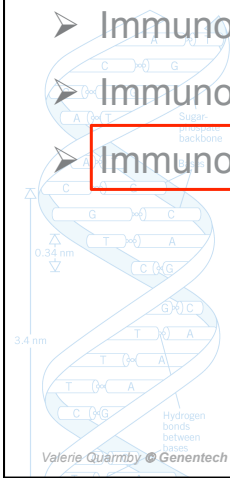

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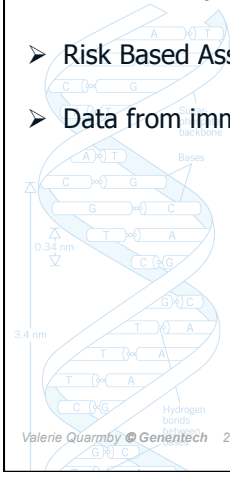
Data to assess immunogenicity risk prior to FIH

- IND-Enabling Analytical Characterization Data
- IND-E Animal Study Data
- Risk Based Assessment
- Data from immunogenicity prediction tools & technologies



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Data to assess immunogenicity risk prior to FIH

IND-Enabling CMC & Analytical characterization data:

SEC, SDS PAGE, LC/MS, IEF, QAAA….

Peptides, some small proteins

Can completely define chemically

More complex proteins

Cannot completely define chemically

Microheterogenity

Cannot completely define structurally

HOS data reflect ensemble averages

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Data to assess immunogenicity risk prior to FIH

IND-Enabling Animal Study Data:

- Immunogenicity is not usually a safety endpoint.
- Impact on exposure:
  - Neutralization of activity
  - Effects on PK (↑ or ↓ clearance) and/or PD
- Impact on interpretation of Tox results
  - Anaphylaxis
  - Immune complex disease
  - Crosslinking of antibodies can cause toxicity
- May show impact of neutralizing endogenous homolog
- Not considered predictive for humans

Ponce et al 2009 Reg Tox Pharm 54:164

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Data to assess immunogenicity risk prior to FIH

Risk Based Assessment

Molecule/indication is assigned a higher or lower level of perceived risk, where risk = likelihood x severity

The extent of monitoring and characterization of immune responses is determined on the basis of this risk based assessment

As potential risk increases, more frequent ATA testing and more extensive ATA characterization may be needed.

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Immunogenicity prediction tools & technologies.

- *In silico*
  - Algorithms to screen for potential T cell epitopes (TCE).
  - Identify linear motifs of 9-10 amino acids that bind to HLA MHC Class II molecules.
  - Fast, extensive databases exist.
  - Tend to over-predict potential for immune response relative to in vitro methods.
  - Typically used as part of lead selection/optimization.
  - Can be used retrospectively

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Immunogenicity prediction tools & technologies.

- *In vitro*
  - HLA Binding Assays
  - T Cell assays
    - Peptides or proteins
    - Naïve donors → primary response
    - Treated donors → recall response
    - Secondary signals matter
    - Cell handling matters
  - 3D Culture Systems
- Typically used as part of lead selection/optimization
- Retrospective use of T cell assays feasible if PBMCs available.

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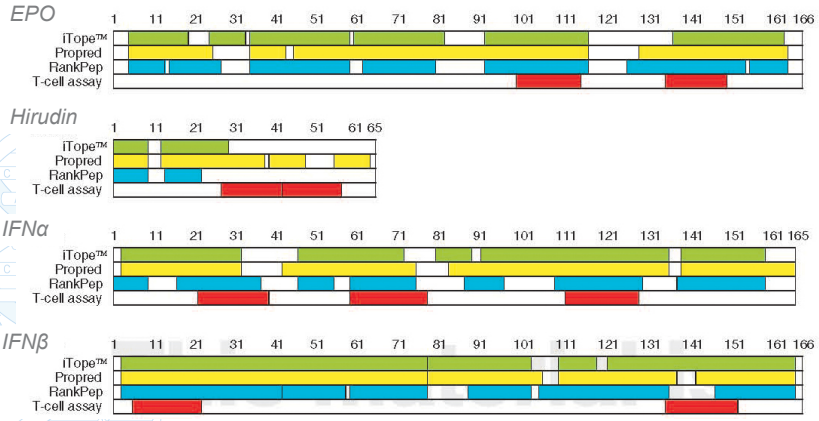
In Silico and In Vitro TCE Data – EPO, Hirudin, IFNα and IFNβ



Perry et al (2008) Drugs R&D 9: 385

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Immunogenicity of FPX: *In Silico, in vitro*, and ATA data

Clinical ATA data drove retrospective *in silico* and *in vitro* analysis of Fusion Protein X

- FPX = two 24 aa peptides attached to huIgG Fc
- 37% (28/76) of patients were ATA positive after a single IV (33%) or SC (40%) dose.
- In vitro T cell analysis done for 4 ATA neg and 11 ATA pos subjects
- in silico analysis revealed high T cell epitope content
  - C-terminal TCE cluster

Koren et al 2007 Clin Immunol 124:26

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Immunogenicity prediction tools & technologies.

- *In vivo*
  - Mouse models for immunogenicity risk assessment
    - wt, Tx Tg mice, HLA Tg, hu-SCID…
    - Respond in context of wholly/partially murine immune system
    - Elegant but expensive & limited throughput
  - Typically used late in lead optimization
  - Have also been used to assess "relative" immunogenicity.

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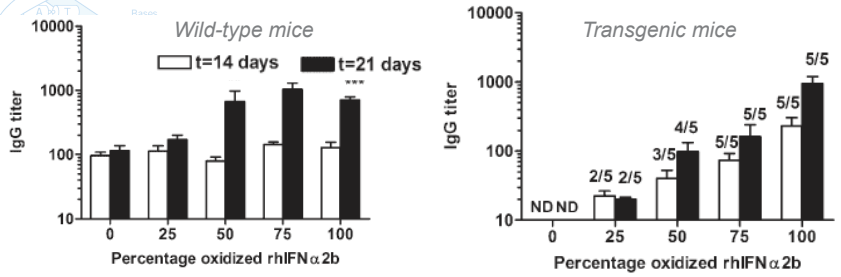
Relative Immunogenicity - rhIFNa in wt and Tg mice

Sample	Monomer* (%)	Dimer* (%)	Trimer* (%)	Oligomer* (%)	Insol. Agg. (%)
Native	99	1	nd	nd	nd
Oxidized	41	11	3	10	34



Hermeling S (2006) J. Pharm Sci

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Immunogenicity Prediction & Product Development

- Multiple tools typically used in "Tiered" analysis
  - Data from any one tool not robust enough to enable go/no go decisions for FIH molecules.
  - Methods complex, data context dependent & hard to interpret.
- More clinical validation data are needed.
  - ATA incidence should be linked to HLA allotypes.
  - FVIII, IFNβ, EPO links are still emerging
  - Clinical trial subjects rarely HLA typed, so retrospective analyses often can't be done.
- Systems that are typically used for lead optimization may have utility for "comparative immunogenicity" in context of process development.

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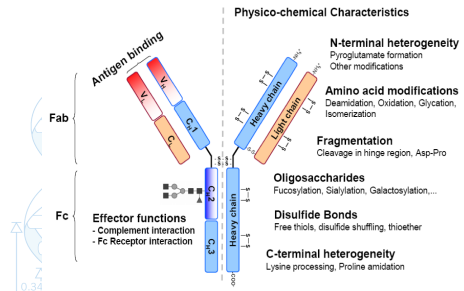
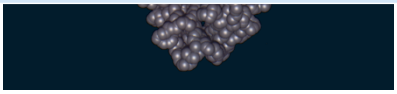
Product Related Variants & Process Related Impurities

Product Related Variants	Process-related impurities
	Endotoxins
	Cell Culture Medium Components
	Host Cell DNA
	Host Cell Proteins
	Protein A
	

L: Steffen Gross (Paul Ehrlich Institut), PDA Workshop on MAbs, June, 2011

R: CASSS /ISPE CMC Biotech Working Group "A-Mab Case Study Version 2.1"

Immunogenicity & Process Development:

Most product related variants assumed to have low immunogenic potential:

- eg deamidation, glycation

Some product related variants & process related impurities could lead to an increased risk of immunogenicity:

- Sequence variants
- Host Cell Proteins
- LPS
- CpG DNA

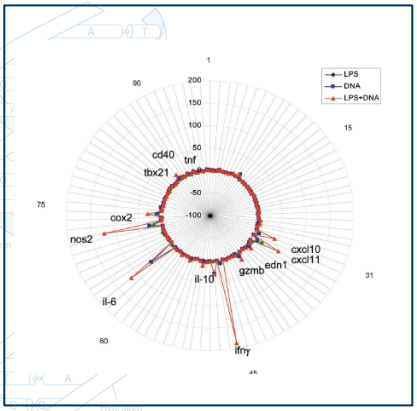
There is concern that some types of protein aggregates could lead to an increased risk of immunogenicity:

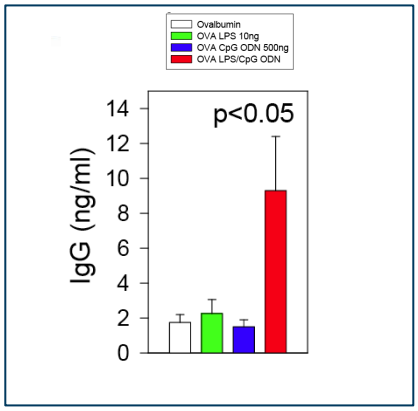
- Subvisible particulates (0.1 – 10 um particle size)
- Soluble aggregates

Can we use some immunogenicity tools for "comparative immunogenicity" assessments in context of process development?

"Comparative immunogenicity" Assessment of TLR Agonists

In vitro and in vivo "comparative immunogenicity" assessments using in vitro T cell assays & Balb/c mice suggest that low levels of TLR agonists (LPS and CpG DNA) may synergize to induce or exacerbate antibody responses to foreign proteins in mice.





Verthelyi & Wang 2010 PLoS One 5:e15252

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"Comparative immunogenicity" & IFN β Re-formulation

Rebif (rhuIFN β -1a, SC t.i.w)

- ~ 40% of RRMS patients develop ATAs
- ~ 20 - 30% patients develop neutralizing antibodies (NAbs)
- High ATA rate attributed to formation of IFN /HSA Aggregates

Can Rebif be reformulated to reduce ATA rates and improve injection site tolerability?

in vitro T cell assays, & Balb/c mice were used for comparative immunogenicity assessments to help select a Rebif New Formulation (RNF) with reduced immunogenic potential.

Jaber et al 2007 Drugs RD 8:335

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IFN β Re-formulation - Impact on Immunogenicity in Phase III

	n	ATA (%)	ATA rate change (P values vs RNF)		NAb (%)	NAb rate change (P values vs RNF)	
Rebif (Regard)	374	37.7	<0.05	0.022	27.3	<0.05	0.005
Rebif (Evidence)	336	36.9	<0.05	0.04	21.4	>0.05	0.266
RNF	259	28.6	NA	NA	17.4	NA	NA

Data from G. Giovannoni, et al, Multiple Sclerosis 2009; 15: 219-228

Statistical analysis by Dan Coleman

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Conclusions

Immunogenicity is a key metric of product safety.

Immunogenicity is now also a key metric of product quality.

Immunogenicity assessment in the context of biotherapeutic product & process development is multi-faceted and highly nuanced.

Immunogenicity assessment tools developed for lead identification & optimization may have added utility in the assessment of the "comparative immunogenicity" of product related variants and process related impurities….

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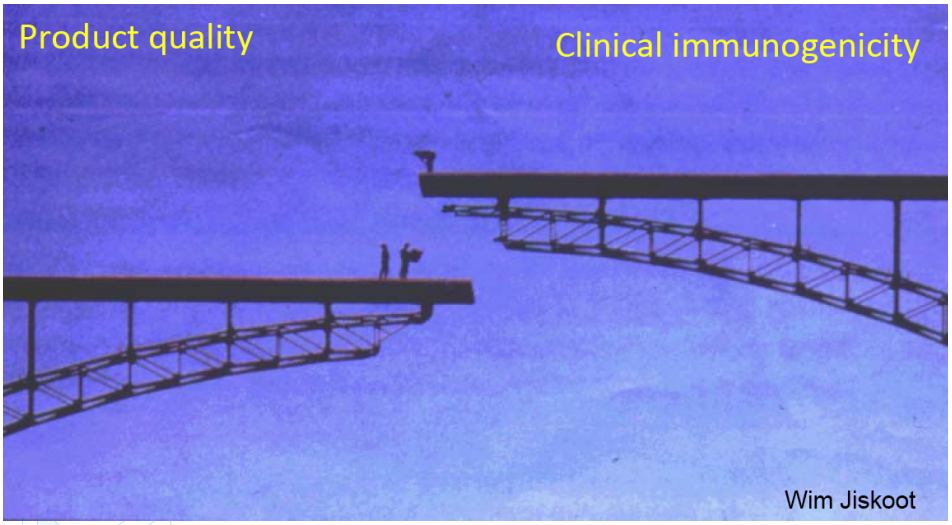
Immunogenicity assessment tools may help us bridge this gap!



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