BMWP/BWP Workshop on Immunogenicity Assessment of Therapeutic Proteins

Rational Design of Less Immunogenic Biotherapeutics

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Current strategies to minimize the immunogenicity of biotherapeutics

- ☑ Develop new methods to detect potential immunogenicity before clinical trials
- ☑ Develop better assays to quantify immune responses in patients

- ☒ Develop approaches to reduce immunogenicity of protein therapeutics including product and process related impurities while maintaining structure, function and stability

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Rational Design of Less Immunogenic Biotherapeutics
Outline:

- Current strategies for minimizing immunogenicity
- Immune recognition of therapeutic proteins
- Initial approaches to reduce immunogenicity
- Current approaches to reduce immunogenicty
  - Protein
  - Impurities/Excipients
- Confirmation of immunogenicity reduction
- Introduction of "improved" molecule into clinical development

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Immune recognition of therapeutic proteins

Blocking any of these recognition events can reduce incidence of clinical immunogenicity

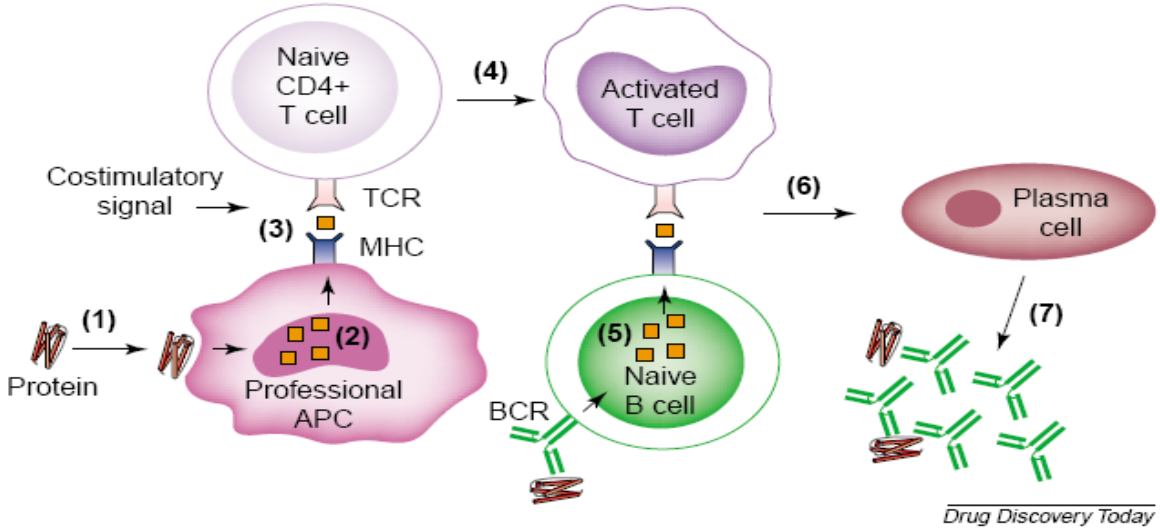

Figure 1. The process of raising an immune response to a protein drug involves several recognition events and signals: (1) protein uptake by antigen presenting cells (APCs), (2) protein antigen processing, (3) interaction between APCs and T cells, including formation of peptide–major histocompatibility complex (MHC)–T-cell receptor (TCR) complexes in the presence of costimulatory signals such as those from infection or inflammation, (4) T-cell maturation, (5) interaction between T cells and B cells, including protein binding to B-cell receptors and formation of peptide–MHC–TCR complexes, (6) B-cell maturation, including isotype switching and affinity maturation, and (7) antibody binding to the protein drug. Blocking any of the above steps can reduce the immunogenicity of protein therapeutics.

(Chirino et al. 2004)

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Initial approaches to reduce immunogenicity of biotherapeutics

- Replacement of proteins derived from non-human sources with human sequences
- Pegylation of proteins
- Humanization of monoclonal antibodies
- Improvements in manufacturing processes to minimize impurities

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Current approaches to reduce immunogenicity of biotherapeutics

- "Rational" Pegylation
  - Serious immunogenicity has occurred despite pegylation
    - MGDF [N-terminal pegylation] elicited cross-neutralizing antibodies
    - Pre existing immunity to PEG has been demonstrated in some humans
  - Rational design approaches select the PEG attachment sites that provide the best balance between reducing immunogenicity, improving PK and maintaining activity

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Current approaches to reduce immunogenicity of biotherapeutics

- Improving solution properties
  - "rational solubility engineering" to identify mutations that will minimize aggregation
- Removing antibody epitopes
  - Modification of crucial residues in an antibody epitope can reduce binding of existing antibodies
    - Replacement of hydrophobic and charged residues with polar residues
    - Removal of B-cell epitopes

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Current approaches to reduce immunogenicity of biotherapeutics

- Immune system generates tremendous diversity
  - Individual antibody repertoire ~10^8
- Physiologically relevant antibody and T-cell epitopes can be identified
- However, it is nearly impossible to remove all potential antibody epitopes or T-cell epitopes

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Current approaches to reduce immunogenicity of biotherapeutics

- MHC molecules are highly polymorphic
  - Individuals only express a handful of MHC molecules
    - DRB1 (especially polymorphic); DRB3/4/5, DQA1, DQB1, DPA1 and DPB1 (moderately polymorphic);DRA (essentially monomorphic)
  - The complete class II MHC genotype >90% of the diverse US population can be accounted for with ~100 of the most prevalent heterodimer combinations

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Current approaches to reduce immunogenicity of biotherapeutics

- Individuals vary in their ability to mount an immune response to a given protein sequence based on their HLA genotype
- Understanding dependency of MHC haplotype
  - May aid in design and monitoring of clinical trials
  - May aid in reducing immunogenicity of biotherapeutics

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Current approaches to reduce immunogenicity of biotherapeutics

- Identifying and removing Class II MHC agretopes
  - Methods for detecting MHC-binding agretopes were initially developed to identify cytotoxic T-lymphocyte (CD8+) and T-helper (CD4+) epitopes for vaccine development
    - Minimizing immunogenicity, however, requires removing all the high affinity agretopes that are recognized by prevalent Class II alleles, including DP, DQ, and DR alleles

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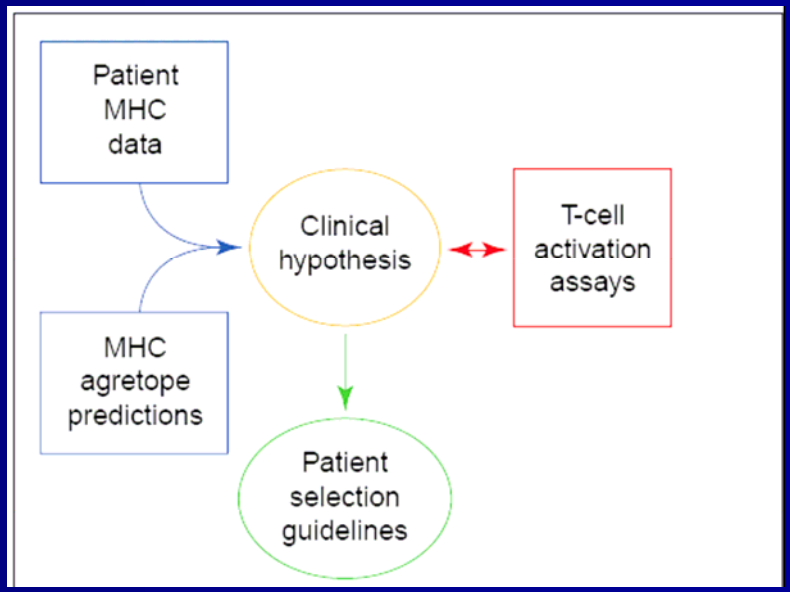
Identifying and removing Class II MHC agretopes

- Patient data and MHC prediction algorithms used to generate hypothesis relating allele to clinical outcomes
- Hypothesis validated by T-cell activation assays
- Results used to identify patients likely or unlikely to raise harmful immune responses



(Chirino et al. 2004)

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Current approaches to reduce immunogenicity of biotherapeutics

- Removal of MHC agretopes may be easier than antibody epitope removal
  - Factors affecting binding are better defined
  - Diversity of binding sites is much smaller
  - MHC molecules and binding specificities are static throughout an individual's lifetime
- Several computational methods can be used to predict MHC agretopes

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Companies that have supplied immungenicity reductions services and technologies

Table 1. Companies supplying immunogenicity reduction services and technologies

Company[a]	Immunogenicity reduction strategy	Epitope/agretope identification and validation strategy	Epitope/agretope modification and removal strategy
Biovation	MHC II agretope removal	Threading[b]/*in vitro* MHC binding/*Ex vivo* T-cell proliferation	Substitutions in single and combined agretope variants by iterative site-directed mutagenesis
Epimmune	MHC II agretope removal	*In vitro* MHC binding	Iterative site-directed mutagenesis
EpiVax	MHC II agretope removal	Quantitative matrix[c]	Substitution suggestions based on quantitative matrix results
Genencor	MHC II agretope removal	Multi-donor T-cell proliferation	Iterative site-directed mutagenesis/ alanine scanning
Nektar	Antibody epitope blocking (PEG)	Not applicable	Advanced PEGylation
Novozymes	Antibody epitope removal	Search of proprietary epitope database	Directed evolution
Xencor	MHC II agretope removal/Antibody epitope removal/antibody epitope blocking (PEG)/solubility improvement	Quantitative matrix/ multi-donor, *in vitro* protein-primed T-cell activation	Rational, structure-based design (PDA® technology)

(Chirino et al. 2004)

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Epimmune now [IDM Pharma]
Genencor now [Danisco Genencor]

Current approaches to reduce immunogenicity of biotherapeutics

- Mutagenesis approaches to produce variant sequences that do not interact with MHC
  - Alanine screening
  - Random mutagenesis
- Exon shuffling
  - Creation of novel human hybrid proteins
- Rational protein design approaches
  - Identify immunogenic regions
    - Replace with less immunogenic sequences
  - Product stabilization
    - Specific sequence changes

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Rational Design of Less Immunogenic Biotherapeutics

Validation ≠ Predictive Value

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Predictive Value of Preclinical Studies?

- Inherent limitation of preclinical studies
  - Test article is expected to be representative of the clinical material
    - Often times lots are which may be "less pure" to study "worse case scenarios" including justification of acceptable ranges for product specifications
  - Studies designed to mimic clinical regimen may be "immunizing" to animals
  - Alternative routes of administration may be needed to define a toxic dose

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Predictive Value of Preclinical Studies?

- Development of homologous proteins for products with unique species specificity
  - Useful for establishing POC
  - May be less useful for establishing safety
    - Level of process-related impurities may differ, in addition to potential differences in potency, pharmacology, protein aggregation, post-translational modification, formulation, container closer, stability etc.
      - Requires major commitment of additional resources
  - Homologous proteins may be immunogenic in animals
    - Not relevant to extrapolation to humans

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Predictive Value of Preclinical Studies?

- Preclinical safety evaluation generally performed in "normal animals" rather than disease state
  - Lack of similar host and disease factors to intended clinical population
  - Pre-medication strategies have been used in human to reduce potential for immune response
- Excipients
  - Inclusion of human serum albumin as a stabilizer can impact results/interpretation of preclinical data
    - Use of homologous albumin considered in toxicity evaluations
  - Assessment of comparability needed
    - Removal of HSA
    - Exchange plasma derived vs. recombinant

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Predictive Value of Preclinical Studies?

- Traditional animal models
  - Poor predictive value in general
    - Timing of assessments and sensitivity of assays are important
    - Inverse dose-relationship observed for some proteins
    - May over predict fully humanized molecules
    - NHP may exhibit species specific responses
      - Identification of NHP MHC alleles ongoing
      - NHP with defined MHC haplotypes are being bred for vaccine studies
    - Use of deliberately or "hyperimmunized" animals for predicting anaphylaxis –questionable utility
      - Standard anaphylaxis models are not relevant
    - Induction of immune tolerance –questionable relevance to a specific molecule in a clinical setting
      - Mechanism of tolerance is species specific

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Predictive Value of Preclinical Studies?

- Traditional animal models
  - Serious reactions following repeat dose testing of human proteins with mice and dogs more common
    - Lower predictive value in mice and dogs compared to NHP
  - Chimpanzees are not useful due to status as endangered species, high cost and low numbers
- Importantly humans are *often* not predictive of humans
  - Idiosyncratic responses
  - Phase I and II may not predict Phase III responses
  - Phase Is + Phase IIs + Phase IIIs may not predict Phase IV/post marketing immune responses

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Confirmation of immunogenicity reduction

- Traditional animal models
  - Useful for assessing relative immunogenicity
    - NHP more useful for distinguishing minor changes and as a measure of antigen presentation due to aggregation
    - Rodents are useful for assessing significant process changes
    - Dose, ROA and regimen and sampling times are important considerations
  - More useful when proteins exhibit strong immune response (e.g. PEG-rhMGDF, rTPO, immunotoxin conjugates, bacterial derived proteins etc.)
    - Immune complex disease is rare in animals

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Confirmation of immunogenicity reduction

- Transgenic animal models (mice)
  - Have been used to assess relevant immunogenicity
  - Inter-species differences in MHC alleles and T-cell repertoire limit predictability
  - Mouse models to express human MHC molecules are in development
  - KO may be useful to assess consequence of cross-reacting neutralizing antibodies

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Confirmation of immunogenicity reduction

- Human T-cell activation assays
  - Do not address antibody binding or peripheral tolerance
  - Use fully human APCs and T cells
    - Best available model for Ag processing, presentation and recognition by MHC and T-helper cells
    - Response is antigen dependent- not all antigens "work"

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Application of Immunogenicity Assessment
T-cell Epitope Identification

- In *silico* method
  - Epibase® profiling
    - Epitope identification on full sequence
    - Removal of epitopes present in the human germline
    - Critical epitopes identified as the strong and medium binders to DRB1, and the strong binders to DRB3/4/5, DQ and DP

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Case Study: Adalimumab

- 109 RA patients
  - HAHA response
    - 17.6% (19 patients)
  - DQ, DR high resolution typing
    - Not performed since no strong epitopes were identified
  - RA associated HLA allotypes
    - DRB1*0101, DRB1*0401, DRB1*0404

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Case Study: Adalimumab

Epitopes and HAHA response

- The 7 strong epitopes explain 17/19 HAHA+ patients
- Epitopes are directed against the RA associated allotypes

Epitopes	Region	HLA allotypes	HAHA+ patients
1	FwR2-HCDR2	DRB1*0701	1
2	FwR2-HCDR2	DQA1*0201|DQB1*0303	1
		DQA1*0401|DQB1*0402	
		DQA1*0501|DQB1*0301	3
		DRB1*0101	4
		DRB1*0401	7
		DRB1*0405	1
		DRB1*0407	
		DRB1*0901	1
3	FwR3-HCDR3	DRB5*0101	5
4	FwR3-HCDR3	DRB1*0407	
5	FwR3-HCDR3	DRB1*0801	
6	LCDR1	DQA1*0501|DQB1*0201	3
7	FwR3-LCDR3	DRB5*0101	5

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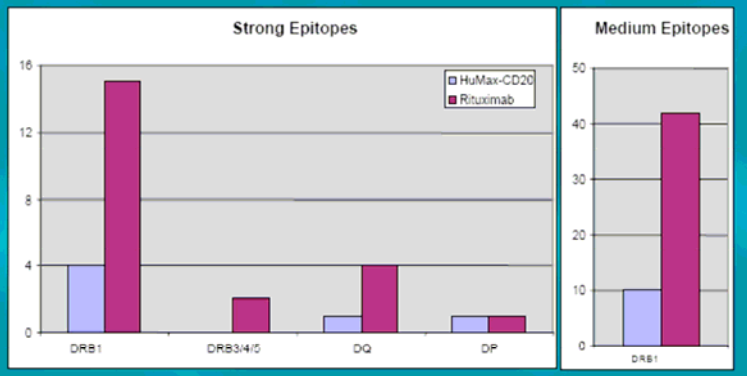
Case Study: Ofatumumab and Rituximab

Immunoprofile Ofatumumab and Rituximab

- **Ofatumumab is very clean in epitopes as compared to rituximab**
- **Ofatumumab contains no epitopes for HLA allotypes associated with RA**



HLA class II gene	RA Risk ratio	Epitopes in rituximab	Epitopes in ofatumumab
DRB1*0401	**1 in 35**	**2 strong**	**No**
DRB1*0404	**1 in 20**	**no**	**no**
DRB1*0101	**1 in 80**	**4 strong**	**no**
0401 and 0404	**1 in 7**	**2 strong**	**no**

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Introduction of "improved" molecule into clinical development

- Consideration of improvement during clinical development
  - Comparability assessment
    - In vitro human T cell assays
    - Animal data?
    - Clinical data?
- Consideration of improvement during Phase IV
  - New molecule?

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Summary

- A number of "deimmunization" strategies are in development to reduce the potential immune risk of human biotherapeutics
- While preclinical studies are limited for predicting immunogenicity in humans, immunogenicity assessment is important for interpreting PK, PD and toxicity, including potential type of antibody response (e.g. sustaining, clearing, neutralizing) in animals as well as in assessing product comparability
- Additional data necessary to validate the various approaches should not preclude their consideration
- Advances made in improving vaccines (e.g. detection of MCH-binding agretopes and "reverse vaccinology") may also provide information to complement the various approaches
- Importantly it is necessary to consider the type of product, the mechanism of action and intended patient population in considering potential immune risks to better inform ultimate risk communication and risk mitigation strategies.

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