Evaluation of Immunogenicity in Biotherapeutics

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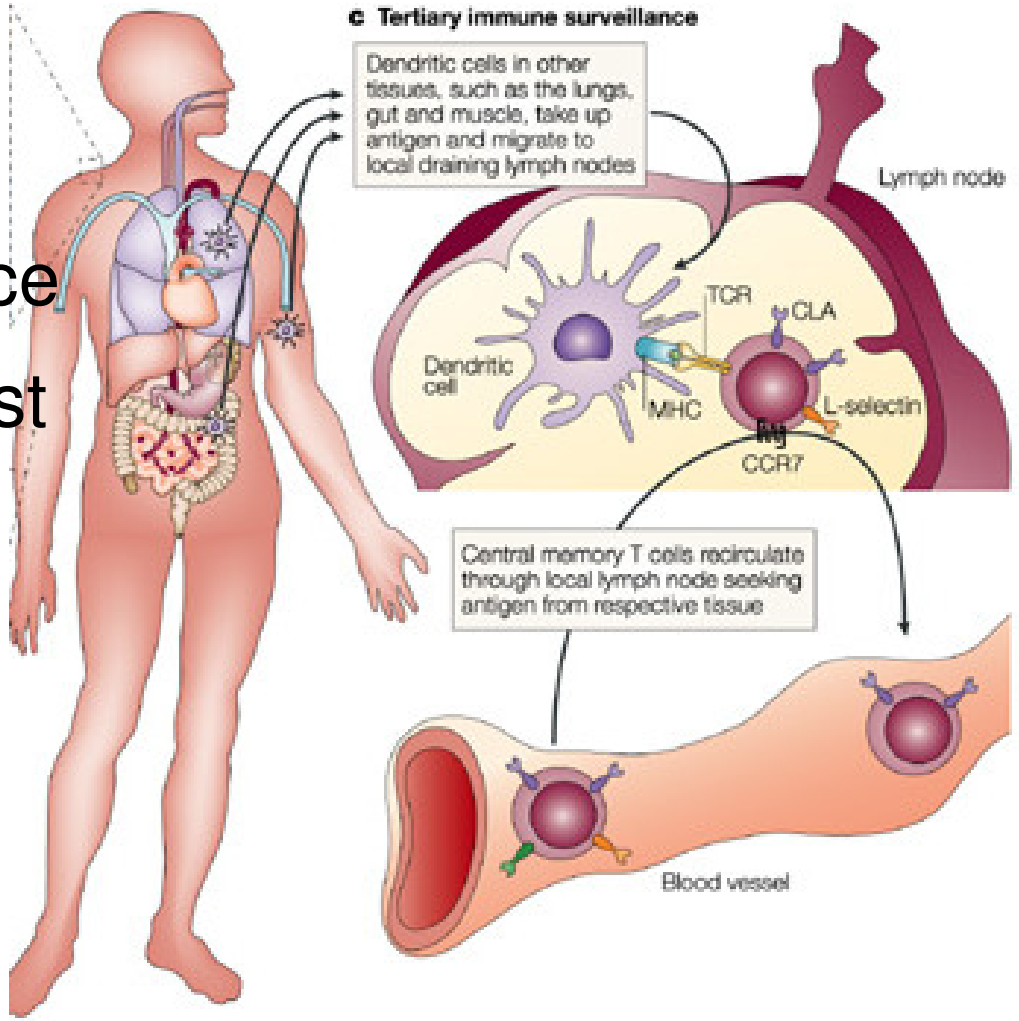
Biowaivers and Biosimilars – 2014
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Hyderabad, India

Agenda

What? Why? & How

- Introduction
- Regulatory Guidance
- Immunogenicity Test



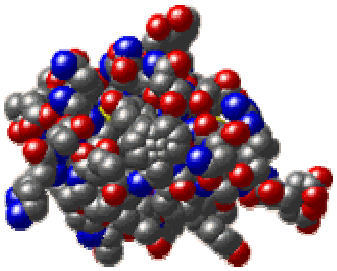


Therapies



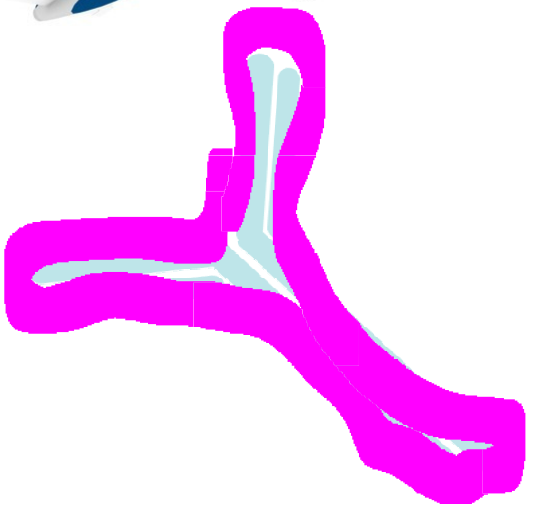
Aspirin
(Small Molecules)
Mw: 181 Da





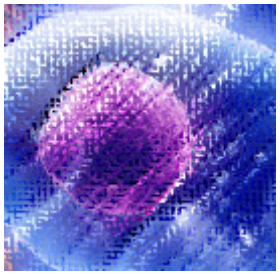
Insulin or EPO or IFN
(Biomolecules)
~ 5800 Da





Monoclonal Antibodies
(Biomolecules)
$C_{3264}H_{5002}N_{840}O_{998}S_{20}$
~150,000 Da





Nanotechnology
&
Cell based therapies

Non-biologics
complex molecules

Generics Vs Biologics/Biosimilars

Small Therapeutic Molecules (Generics)	Biologics / Biosimilars
Low molecular weight	High molecular weight
Species-independent	Species-Specific
Specific mechanisms	Pleiotropic mechanisms
Production – Chemical Synthesis	Production - living cells (prokaryote/Eukaryote)
Easy to analyze generic product by atomic structure	Difficult to impossible to analyze generic product by atomic structure
Pharmacokinetic studies are usually performed by one bioanalytical method (mostly LC/MS)	Several bioanalytical assays are necessary
Non-immunogenic	**Usually immunogenic**

Immunogenicity

Immunogenicity is the ability of a particular substance, such as an antigen or epitope, to provoke an

- Humoral &/or Cell mediated

immune response in the body of a human or animal

Wanted Immunogenicity:

- Vaccine provokes an immune response against the pathogen (virus, bacteria...) aiming at protecting the organism

Unwanted immunogenicity:

- The organism mounts an immune response against a therapeutic antigen (ex. recombinant protein, or monoclonal antibody) which can induce adverse effects

US FDA: It is propensity of the therapeutic protein product to generate immune response to itself and to related proteins or to induce immunologically related adverse clinical events.

Immune system – Immunogenicity



Innate Immunity

B Cell activation

Adaptive Immunity
T Cell activation

Anti-Drug Antibodies (ADA)

Antibody Dependent Cell-mediated Cytotoxicity (ADCC)

Unwanted Immune response against Biologics/Biosimilars

Complement Dependent Cytotoxicity (CDC)

Cell mediated Cytotoxicity

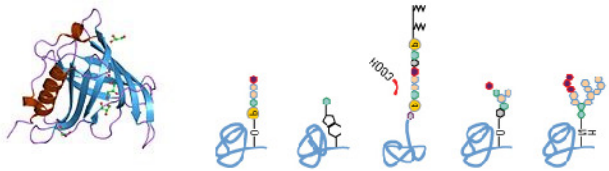
Hypersensitivity

Platelet and Neutrophil activation

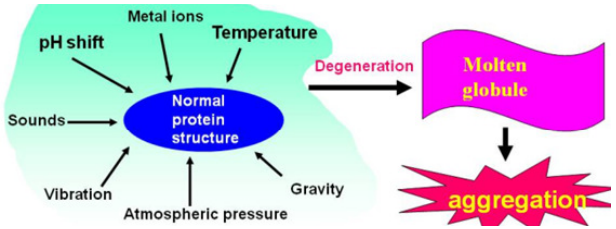
Cytokine Strom

Product and Process Specific Factors Influencing Immunogenicity

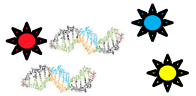
- Product origin
- Sequence variants,
- Structural format – Fusion Protein, bi-specific, multi-specific
- PTM – Oxidation, Deamidation, Aldehyde Modification, Deamination, Glycosylation, Pegylation etc



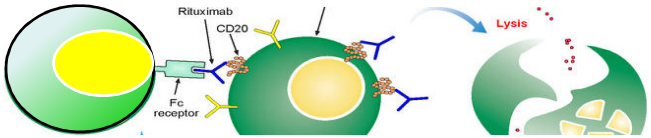
- Aggregates – Size, Reversibility/Dissociation, Confirmation, Chemic Modification, Morphology



- Impurities – HCP, LPS, Protein A/G, Media proteins, CpG, etc



- Properties of the protein – Immuno-modulatory



- Formulation components

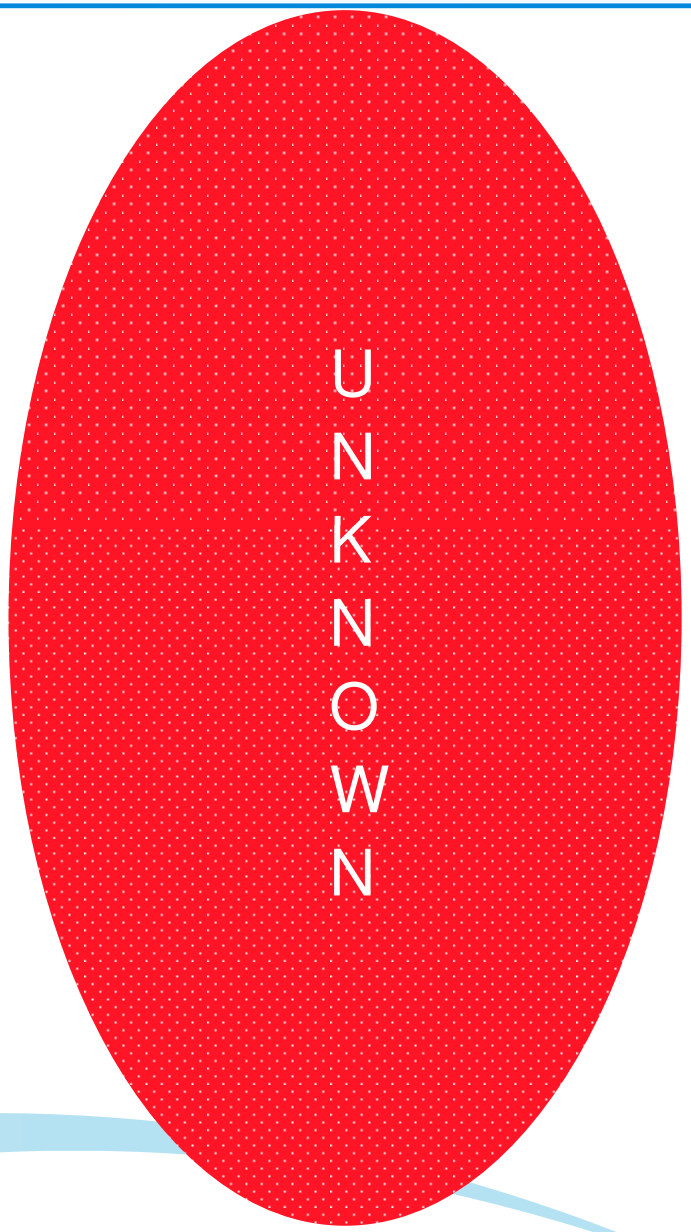


- Container Closure

Patient Specific Factors Influencing Immunogenicity

- Patient features
  - Age
  - Gender
  - Genetic makeup
  - Immune Status
  - Nature of Disease
  - Pre-existing Antibodies
  - Prior sensitization / History of Allergy
  - Tolerance to endogenous proteins
- Target expression
- Dose
- Route (intradermal > inhalation > subcutaneous > intraperitoneal > intramuscular > intravenous)
- Frequency
- Duration
- Contaminant medication

Factors influencing Immunogenicity



Clinical Effects of Immunogenicity

- Benign, non-significant to serious, life-threatening depending on the therapeutic
- Efficacy—Alter PK/PD
  - Reduction of the clinical response and alter bio-distribution
- Safety
  - Safety issues can occur even when there is no loss of efficacy
  - Neutralize biologic effects and **<u>compromise further therapy</u>** (factor VIII, IFNα2a, GM-CSF)
  - Cross-react with native protein and induce adverse symptoms (Epo, Megakaryocyte growth and development factor (MGDF))
- Acute consequences
  - Infusion site reactions (ISR), anaphylactic reactions
- Non-acute consequences
  - Delayed-type hypersensitivity/immune complexes
  - Cross-reactivity with an endogenous counterpart

Immunogenicity: Regulatory Guidance

Testing for unwanted immunogenicity is integral to product development (clinical & post-marketing phase) for ensuring:

- Clinical safety and/or efficacy of a bio-therapeutic
- Chronic use of bio-therapeutics
- Product comparability

US-FDA Guidance:

- Guidance for Industry Immunogenicity Assessment for Therapeutic Protein Product Draft Guidance Aug 2014

EMA Guideline

- Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins EMA/CHMP/BMWP/14327/2006
- Guideline on Immunogenicity Assessment of monoclonal antibodies (in consultation) EMA/CHMP/BMWP/86289/2010

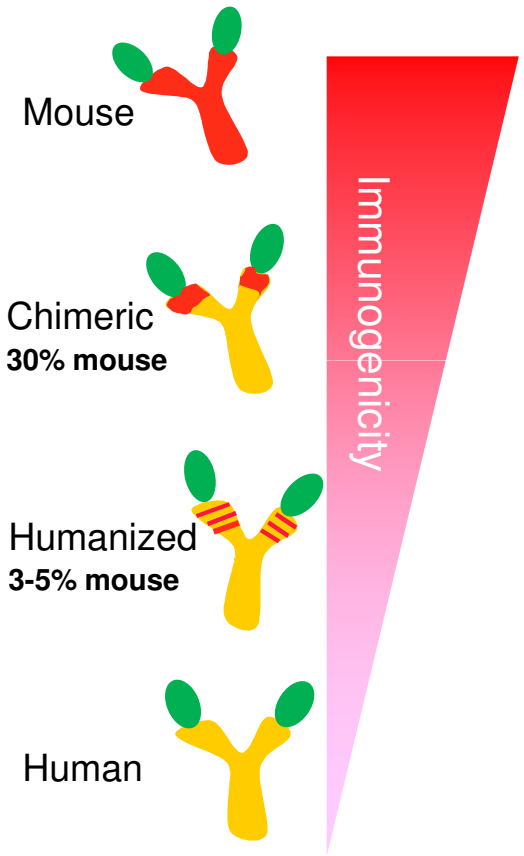
Immunogenicity testing Guidelines

- Recommendations for routine monitoring of changes in clinical response and linking immunologic findings to clinical events
  - Immunogenicity as part of all clinical trials
  - Evaluate all patients and not in a symptom-driven manner
  - Standardize sampling schedule as much as possible
- Specifically analyze adverse events for linkage to an unwanted immune response
- Provide guidance on how prescriber should handle patient in case unwanted immune response occurs (increase dose/discontinue, etc.)

Challenges of Immunogenicity

- Prediction of immunogenicity
  - *In silico* and T cell methods are promising but need further developments
  - Impossible to predict
    - incidence
    - characteristics of immune response
    - clinical consequence and significance of immunogenicity
- Determination of immunogenicity
  - Human clinical data needed—**cannot be replaced by use of animal or *in vitro* or *in silico* tools**
- Requirement of well design studies
- Need for highly sensitive and specific assays

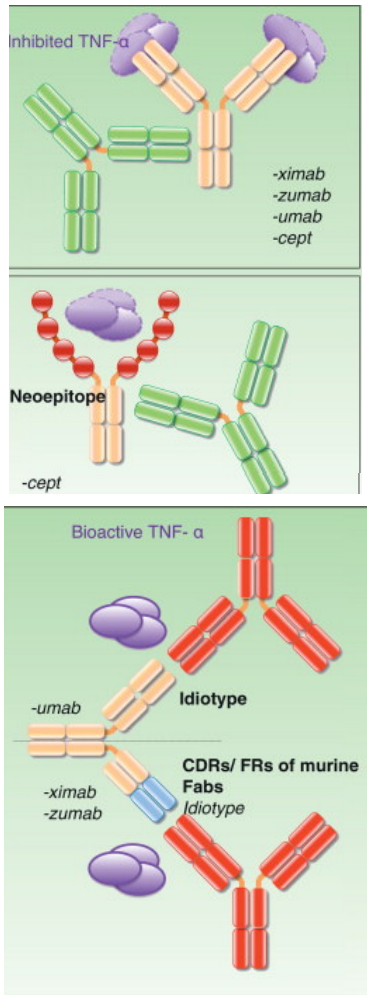
Immunogenicity Testing



Analysis of Immunogenic response

- Antibody response to Biologics (ADA)
  - Binding antibodies to Biologics
  - Neutralizing antibodies to Biologics
- Immunological response
  - Immune cell subset analysis
  - Functional analysis of T cells, B cells, NK cells, Monocytes/Macrophages, Neutrophils
  - T cell receptor repertoire analysis (TCR Vβ analysis)
  - B cell receptor repertoire analysis
- Host Genetic Factors
  - HLA
  - Rheumatoid factor

Immunogenicity Testing:



Multi-tiered approach

Anti-Drug Antibody (ADA) Testing

Binding Antibody Testing (Screening – confirmation and subtyping)

- Enzyme-Linked Immunosorbent Assay (ELISA)
  - Standard sandwich ELISA
  - Bridging ELISA
  - Electrochemiluminescence (ECL)
- Optical Sensor-based
  - Surface Plasmon Resonance (SPR; Bicore, ProteOn XPR36)

Neutralizing Antibody (NAb) Testing with Blocking Assays

- Related to mechanism of action of biologics
  - Receptor Binding
  - Cell based functional assays

ADA Assay Development

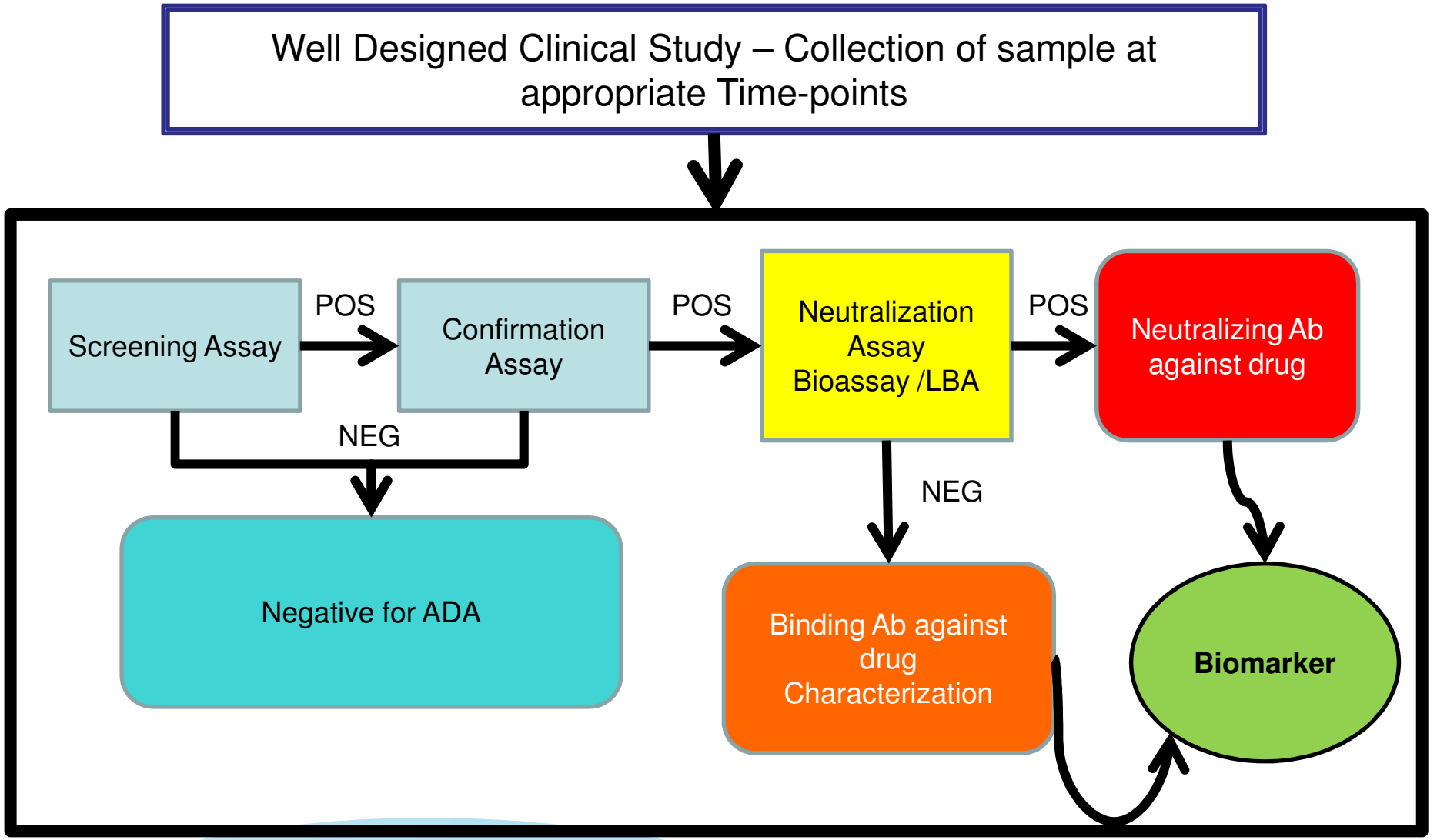
- Positive control antibody
- Sensitivity
- Cut point
- Specificity
- Precision
- Drug tolerance
- Acid-base neutralization/dissociation steps
- Dilution
- Robustness
- Positional variation
- Confirmatory assays

Gupta et al 2007; Journal of Immunological Methods 321 p1-18
Gupta et al 2011; Journal of Pharmaceutical and Biomedical Analysis, 55 p878-888

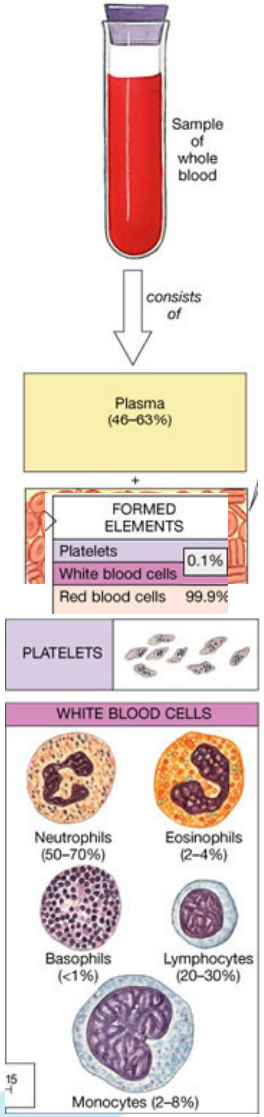
Key parameters - Optimization of Nab Bioassay

- Temperature (growth, media for seeding & stimulation)
- Time of cell growth prior to stimulation
- Time of stimulation
- Confluency of cells
- Therapeutic drug/serum incubation time/concentration etc
- Assay read out method
- Incubation time with assay read out reagents
- Type & Brand of 96-well plates
- Plate sealers/evaporation controls
- Media composition (FBS, Defined Media, antibiotics, etc; e.g. Cells may require FBS for survival, but FBS may interfere with the assay outcome)
- Plate Layout

Strategy to assess Immunogenicity



Roadways to evaluate Immunogenicity for novel biologics @ QIS

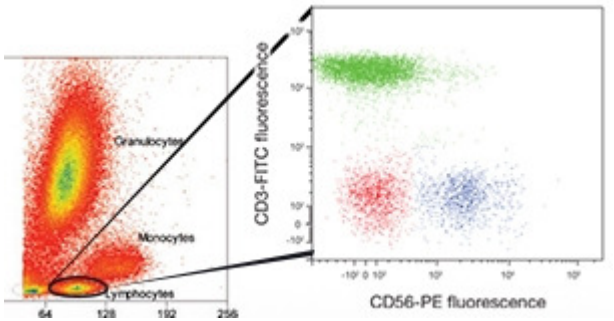


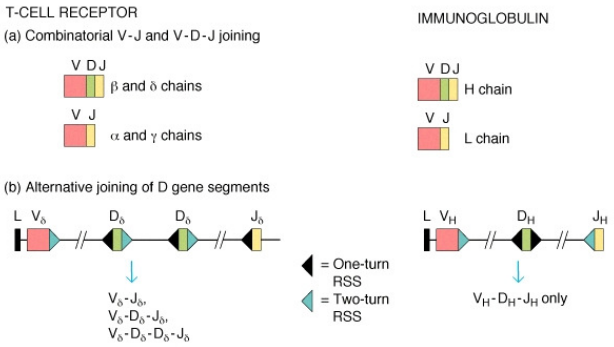
Ex vivo studies in samples from healthy donors and patients

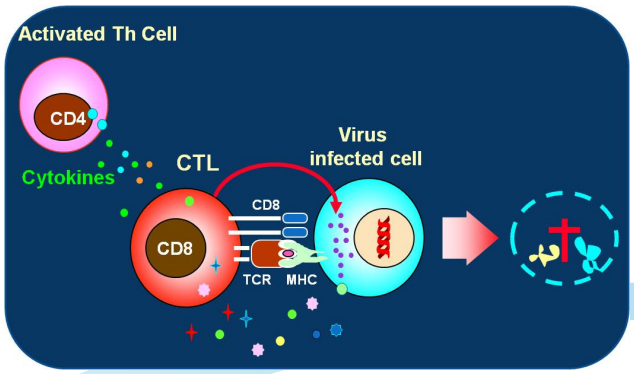
Database of ~30,000 healthy donors

- Interaction between therapeutic proteins and serum proteins
- Platelet activation studies
- Whole blood activation studies
- Macrophages and DC activation through Pattern recognition receptors such as TLRs
- HLA-binding studies
- Gene array studies
- 3D-Culture systems

Immunogenicity / Immunotoxicity Studies







Static Studies

- Immunoglobulin, Complement, Acute Phase Protein Levels
- Cytokine Evaluations
- Histamines
- Immunophenotyping: Peripheral Blood

Functional studies

- T cell - Dependent Antibody Response
- Natural Killer Cell Assays
- Mitogen/Antigen Proliferation
- Hemolytic Complement assays
- Immune cell subset activation studies
- Phagocytosis or Oxidative Burst potential
- Functional analysis of T cells, B cells, NK cells, Monocytes/Macrophages, Neutrophils
- T cell receptor repertoire analysis (TCR Vβ analysis)
- B cell receptor repertoire analysis
- Evaluation of thymic function (TREC assay)

Pre-Clinical & Clinical Immunogenicity Assessment – Novel Biologics

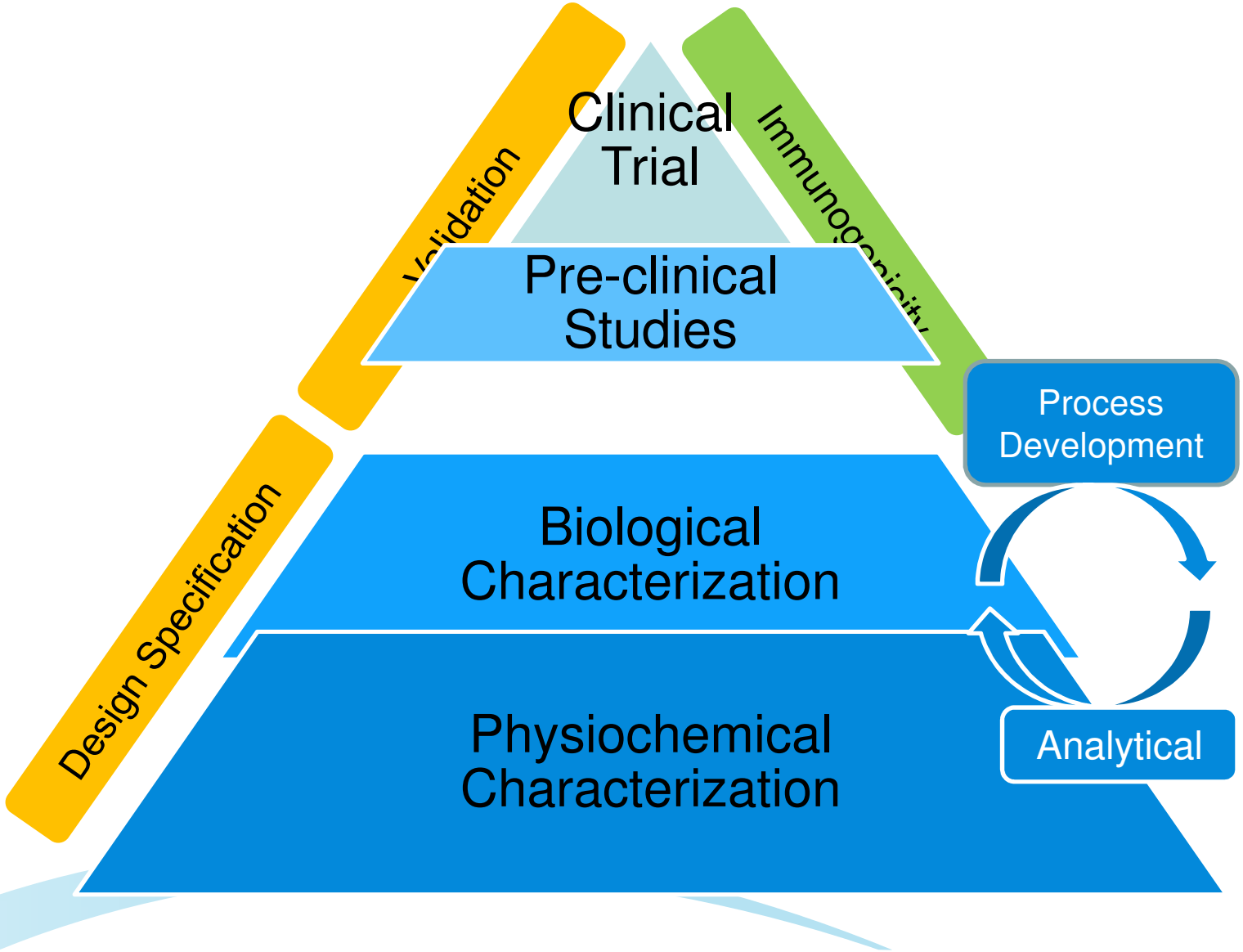


In silico Assessment

Immunogenicity Assessment

- Risk Based Assessment
- Sensitive ADA Assay Development & Validation
- ADA characterization (Incidence, Titer, Ig Isotype, Nab/Titer, Other)
- Study Design & Statistical Analysis Plan Design
- Sample Analysis & Data Acquisition
- Study Data Analysis and Interpretation
- Impact assessment (PK/PD/Safety/Efficacy)
- Program Data Meta-Analysis

Immunogenicity in Biosimilar Development

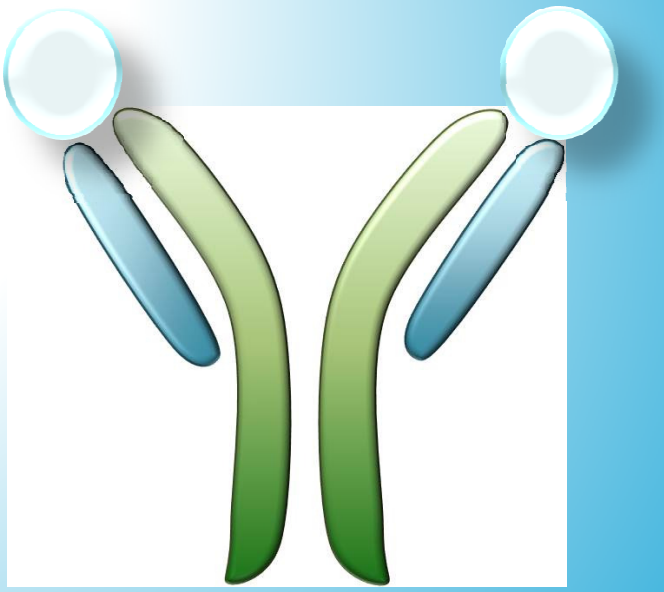


Concluding thoughts Immunogenicity

- Immunogenicity is a key matric of Product Safety and Quality
- Complex and Challenging
- Time need to develop and validate – start early
- Think about control antibodies and cut point samples early in advance
- Evaluate Product/Process/Patient specific factors
- Being wrong may have serious consequences – always cautious, don't be shy to turn the stones
- There is always room for improvement – use modern but correct techniques
- Use – why, when, where & How
- Innovators has lot of information use it smartly and wisely
- Remember – a one size fits all approach is not applicable in Immunogenicity assay development
- Sharing improves the knowledge
- Keep it Simple and Sweet (KISS)

accutest

Quantimmune Solutions



Thank You!

Scientist and Regulatory Agencies Across the World

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