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Effective presentation of immunogenicity-related data in regulatory submissions
Practical guidance

Paul Chamberlain
NDA Advisory Board, NDA Group

Main learning objectives

WHAT?	Translating regulatory guidance for risk identification, evaluation & mitigation to product- and patient-specific applications
HOW?	Alignment of disciplines & processes
WHEN?	Linkage to stage-gate approach for development, registration & life-cycle management

Main elements

1. Regulatory context
   - Risk-based approach in relation to immunogenicity of biotherapeutics
2. Process
   - Multi-disciplinary team input

     → Immunogenicity Risk Assessment
3. Managing regulatory interactions
   - Planning ahead
   - Dealing with unexpected signals during development

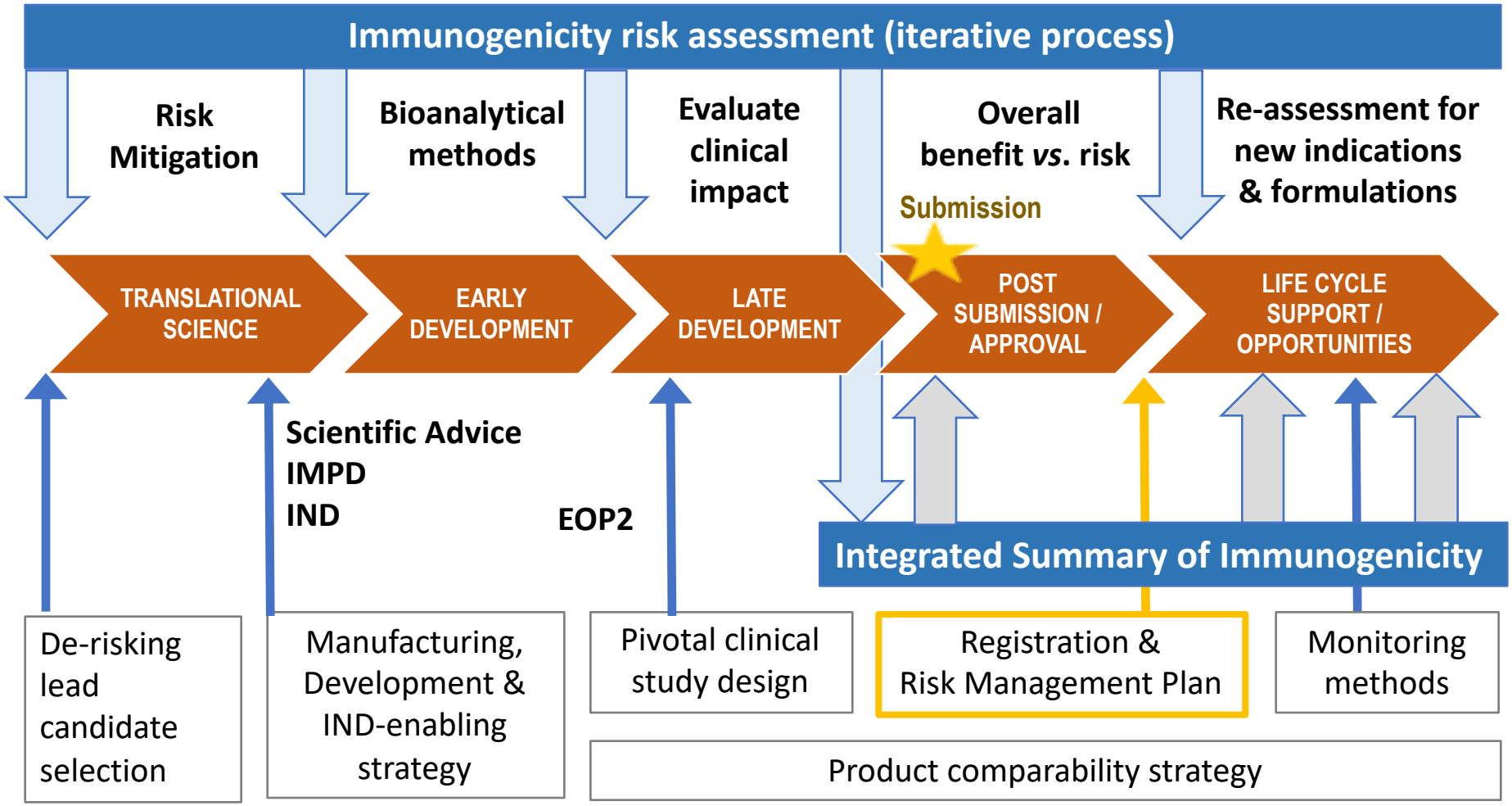
     → Pre-CTA submission / Scientific Advice
4. Documentation
   - Putting it all together for successful regulatory outcomes

     → Integrated Summary of Immunogenicity

Value proposition

- De-risking development
- Facilitation of clinical trial application approval
- Avoiding rate-limiting issues for registration
- Effective life-cycle management
- Focusing resources on what is most important!

Relationship to Product Life-cycle



Part 1:

Regulatory context

Regulatory basis: Risk-based approach

The following guidance documents provide recommendations, rather than legally enforceable requirements:

FDA 2014: Immunogenicity Assessment for Therapeutic Protein Products

EMA 2017: Immunogenicity Assessment of Therapeutic Proteins

FDA 2019: Immunogenicity Testing of Therapeutic Protein Products

USP Chapters 1106 & 1106.1

CLSI document I/LA34-A: Assays for assessment of human allergenicity

Definition

FDA 2014: Immunogenicity Assessment for Therapeutic Protein Products

For the purposes of this guidance, immunogenicity is defined as the **propensity of the therapeutic protein product to generate immune responses to itself and to related proteins or to induce immunologically related adverse clinical events**.

Although this guidance encompasses products used to modulate or modify the immune system, including those that are antigen specific, it does not cover products that are intended to induce a specific immune response to prevent or treat a disease or condition (such as vaccines to prevent infectious diseases) or to enhance the activity of other therapeutic interventions.

Scope

- Therapeutic proteins
- Peptides (synthetic & recombinant)
- Gene therapies
- Cell & tissue-based products
  - Autologous & allogeneic
- Oligonucleotides
- Polysaccharides, e.g. LMW Heparins
- Combination products

Data presentation: When & What?

FDA 2014: Immunogenicity Assessment for Therapeutic Protein Products

Given the variety of factors that can affect immunogenicity, the risk assessment and the control and mitigation strategies will depend on the individual development program and should be considered at the earliest stage and at each subsequent stage of product development.

The extent of immunogenicity safety information required pre-marketing and post-marketing will vary, depending on the potential severity of the consequences of such immune responses and the likelihood of their occurrence.

Data presentation: When & What?

FDA 2014: Immunogenicity Assessment for Therapeutic Protein Products

"*During therapeutic protein product development, elucidation of a specific underlying immunologic mechanism for immunologically related adverse events is encouraged, because this information can facilitate the development of strategies to help mitigate their risk*"

"*In addition to appropriate animal studies, consideration should be given to in vitro and in silico analyses that may supplement animal studies to better or further elucidate risk for immunogenicity.*"

Data presentation: When & What?

EMA 2017: Immunogenicity Assessment of Therapeutic Proteins

"…it is recommended that the applicant will include an integrated summary of immunogenicity in the application, including a risk assessment to support the selected immunogenicity program…"

"This summary with risk assessment can evolve through the lifecycle of the product and may be used to support applications at various steps of product development."

1. Analysis of Risk Factors
2. Risk-based immunogenicity program
3. Immunogenicity results
4. Conclusions on the risk(s) of immunogenicity

Data presentation: When & What?

FDA 2019: Immunogenicity Testing of Therapeutic Protein Products

To facilitate the clinical development of therapeutic biologics, we recommend a life-cycle management approach to immunogenicity through the creation of an integrated immunogenicity summary report that sponsors begin populating early in therapeutic protein product development and update at regular intervals as the individual product clinical program progresses through IND stages into the BLA and even post-approval stages.

1. Immunogenicity Risk Assessment
2. Tiered Bioanalytical Strategy and Assay Validation Summaries
3. Clinical Study Design and Detailed Immunogenicity Sampling Plans
4. Clinical Immunogenicity Data Analysis
5. Conclusions and Risk Evaluation and Mitigation Strategies

Terminology

Shankar G, Arkin S, Cocea L et al. American Association of Pharmaceutical Scientists. Assessment and reporting of the clinical immunogenicity of therapeutic proteins and peptides-harmonized terminology and tactical recommendations. AAPS J. 16(4), 658–673 (2014)

Rup B, Pallardy M, Sikkema D et al. Standardizing terms, definitions and concepts for describing and interpreting unwanted immunogenicity of biopharmaceuticals: recommendations of the Innovative Medicines Initiative ABIRISK consortium. Clin. Exp. Immunol. 181(3), 385–400 (2015)

Industry consensus is consistent with regulatory guidance[#]

[#] FDA 2019: Immunogenicity Testing of Therapeutic Protein Products

Inferences from regulatory guidance

1. Perform immunogenicity risk-assessment early in development process as a multi-disciplinary exercise
2. Relate the risk assessment to:
   - Intrinsic immunogenicity associated with molecular design
   - Method of manufacture & product quality control & stability
   - Subject-related factors (phenotype & genotype)
   - Clinical trial design
   - Risk monitoring & mitigation strategies
3. Update at each stage of clinical development with new information
4. Include the risk assessment in the MAA/BLA dossier to justify adequacy of risk-based immunogenicity evaluation
5. Same format applicable for EMA & FDA submissions
   - Adapt to particular product & therapeutic indication

Part 2:

Process

Guiding principles

- Start with the end in mind
  - Successful CTA's & MAA / BLA submissions
- Address the needs of regulatory assessors
  - Transparency & objectivity
- Create trusting partnership with regulators
  - Regulators want to do their best to help Sponsors
- Team-work
  - Involve all relevant disciplines
- Start early
  - Create risk assessment as part of Lead Candidate Selection stage
  - Update as new information becomes available
- Communication!
  - Demonstrate that you have assessed and understood the pertinent risks, and evaluated / mitigated risks in appropriate manner

Starting out with the end in mind

Immunogenicity Risk Assessment

- Critical analysis of intrinsic & extrinsic risk factors, including molecular design, product quality & patient-related

- Bioanalytical methodology
  - Tiered test strategy
  - Evolution of methods
  - Linkage to clinical studies
  - Summary of assay performance
  - Control of critical reagents
  - Justification of assay cut-points
  - Definition of data outputs
- Clinical study design & sampling strategy

Immune response dynamics (pre-existing & treatment-emergent ADA/NAb incidence & titer) relative to PK, PD, efficacy & safety

- Impact on clinical benefit *vs*. risk for target population and individual subjects
- Implications Risk Management Plan
- Life-cycle management of assays

Integrated Summary of Immunogenicity (ISI)

1. Analysis of risk factors

How might intrinsic & extrinsic factors influence scale of risk?

2. Risk-based program

How were the risks evaluated?
Why were particular methods/ controls selected?
If methodology changed during clinical development, how did this impact the results?
Are the cut-points valid for the target population?

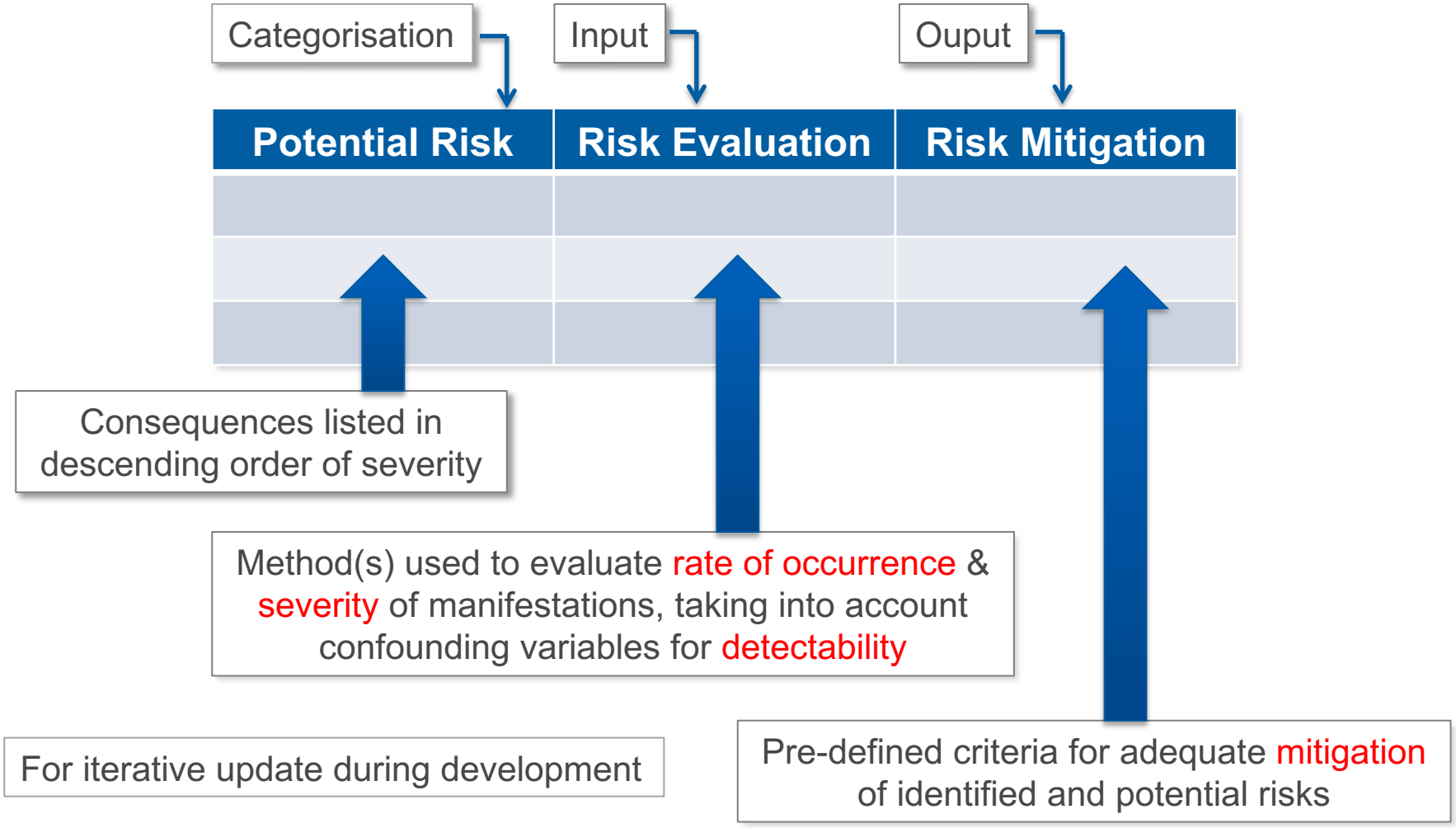
3. Immunogenicity results

ADA response dynamics vs. clinical parameters for individual clinical studies

4. Conclusions

Effect of immunogenicity on safety & efficacy?
Tools for ongoing monitoring?

Input / output of risk assessment



Potential Risks → Hierarchy of Concerns

Potential consequences (in order of decreasing severity)
Induction of anti-drug antibodies that cross-react with endogenous counterparts
Allergic-type hypersensitivity/anaphylaxis
Complement activation-related pseudo-allergy
Immune complex-related hypersensitivity
Reduced pharmacodynamic response/efficacy
Altered pharmacokinetics
Compromise of subsequent treatment with related products
Uncertain long-term clinical impact

Adapted from: Rosenberg AS. Immunogenicity of biological therapeutics: a hierarchy of concerns. Dev. Biol. (Basel) 112, 15–21 (2003)

Risk factors to address

Category	Properties
Intrinsic factors	• Structural and functional properties of product that could contribute to intrinsic immunogenic potential
Systems biology	• Abundance and uniqueness/redundancy of function of endogenous counterparts of the drug product • Location and function of target
Conditions of use	• Clinical dosing regimen, including route of administration, level and frequency of dosing
Patient-related	• Characteristics of the target population, including immune competence, prior exposure to the drug product or to related products and genetic factors that may influence immune recognition/responsiveness
Product quality	• Manufacturing process and rigor of product quality control • Extent of analytical methods • Formulation and drug product stability • Comparability of clinical versus commercial product

Refs: FDA 2014 & EMA 2017

Systems Biology

- Tissue compartmentalization
  - Differential capacities of immune tolerance
  - Differential mass-balance of interacting ligands
  - Relevance of systemic indices of immune response
- Temporal separation of 1° *vs*. 2° pharmacodynamics
- Endogenous inhibitors
  - α2-macroglobulin, MMP's
- Abundance (& location) of endogenous counterparts
- Pre-existing cross-reactive antibodies and T-lymphocytes
- Extent of immune tolerance / autoimmune status

Potentially confounding variables

Variable
• Genotypic & phenotypic variability of target population
• Pre-existing, cross-reactive antibodies
• Comorbidities and concomitant medications
• Levels of product-related variants and process-derived impurities
• Performance of bioanalytical methods

Discuss in relation to:

- Clinical study design
- Extent of analytical characterisation
- Performance of bioanalytical methods

Example of Risk Assessment output

Potential Risk	Risk Evaluation	Risk Mitigation
Allergic-type hypersensitivity/ anaphylaxis	Preclinical: • Comparative ex-vivo basophil activation testing (healthy humans vs. atopic subjects) Clinical: • Monitoring of timing and severity of clinical symptoms of infusion-related reactions relative to pre-existing and treatment-emergent ADA with cross-reactivity to non-human glycans (additional specificity tier incorporated in ADA testing scheme) • Measurement of serum tryptase • Follow-up investigation of IgE ADA & ex-vivo basophil activation test in subjects with potential immune-mediated AE's in Phase III study	1. Molecular design to minimize nonhuman glycans associated with expressed protein 2. Absence of ex-vivo basophil activation in naive or treated subjects 3. Negligible serum tryptase in treated patients 4. No subjects fulfilling NIAID FAAN criteria for anaphylaxis 5. No severe systemic hypersensitivity reactions reported in clinical program 6. AE's not related to drug-specific IgE

Main learning point

- Primary objective is to engage multi-disciplinary team to:
  - Identify pertinent risks factors
  - Propose evaluation / monitoring methodology
  - Assign risk mitigation measures / criteria
- Output provides an explicit alignment of the evaluation and mitigation actions with each pertinent risk
- Prospective exercise to inform decision-making

Not an exercise to categorize risk as “low / medium / high” to fit into a pre-defined (more or less rigorous) scheme

Immunogenicity Risk Assessment for CTA dossier

1. Intrinsic immunogenic potential
2. Systems biology
3. Subject-related factors
    i. Immunological competence of the subject
    ii. Prior sensitization / history of allergy
    iii. Genetic factors
    iv. Extent of immune tolerance to structurally-related endogenous factors
    v. Co-morbidities associated with disease state
4. Product Quality
5. Non-clinical evaluation (*in vitro* & *in vivo*)
6. Conditions of use
7. Strategy for effective risk evaluation & mitigation
    i. Tabular summary aligning potential risks to proposed evaluation & mitigation measures
    ii. Bioanalytical strategy
        - Hierarchical test scheme
        - Proposed assay formats & controls
        - Parameters validated / to be validated
        - Potential utility of biomarkers of PD response
    iii. Clinical sampling scheme (including follow-up)

Format & Location

There is no standard or obligatory format

Use the CTD format / headings for IND & IMPD

Locate Immunogenicity Risk Assessment as Section 2.7.2.4 (or 5.3.5)

How to use the output of risk assessment

- Internal company reference document to be updated during product development
- Source document for regulatory submissions:
  - Clinical Trial Application (CTA)
  - Briefing Package for Scientific Advice
  - Marketing Authorisation dossier

    ➜ as 1st section of Integrated Summary of Immunogenicity

Why include risk assessment in MAA / BLA dossier?

Surely, the results of the clinical studies provide solid evidence of impact of immunogenicity on overall clinical benefit vs. risk?

Risk assessment helps to explain:

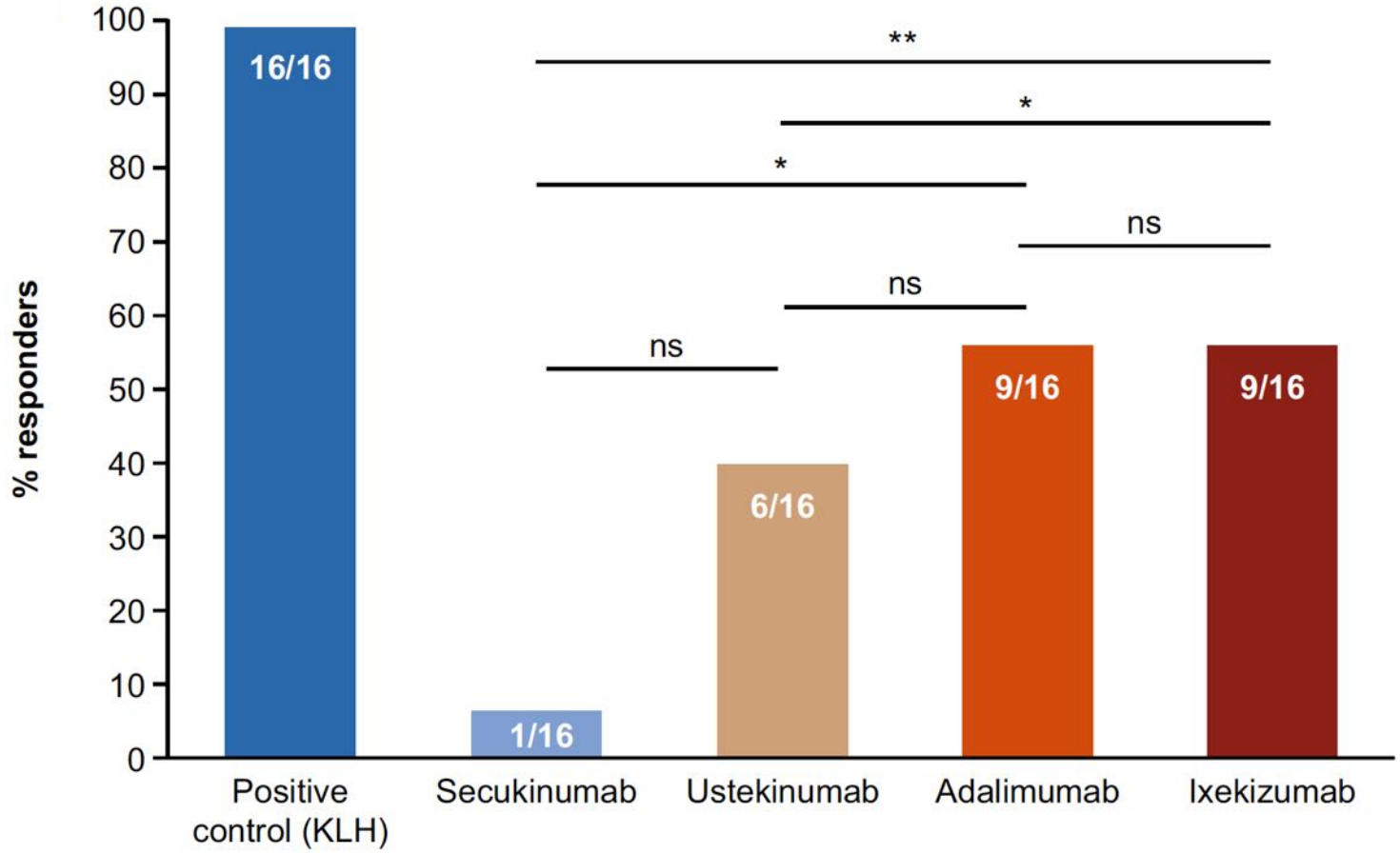
- Scale of risk of inducing a T-dependent immune response in target population(s)
- Incremental risks associated with molecular design or expression system
- Effectiveness of control of pertinent product quality variables
- Justification of improvements to manufacturing process or formulation
- Why some subjects respond in a different manner
- *Etc*.

"*Understanding risks helps to control risks*"

In vitro T-cell stimulation: responder frequency

Spindeldreher S et al. Dermatol Ther 2018, 8, 57-68

Comparison of the frequencies of donors responding to the mAbs in T-cell assay

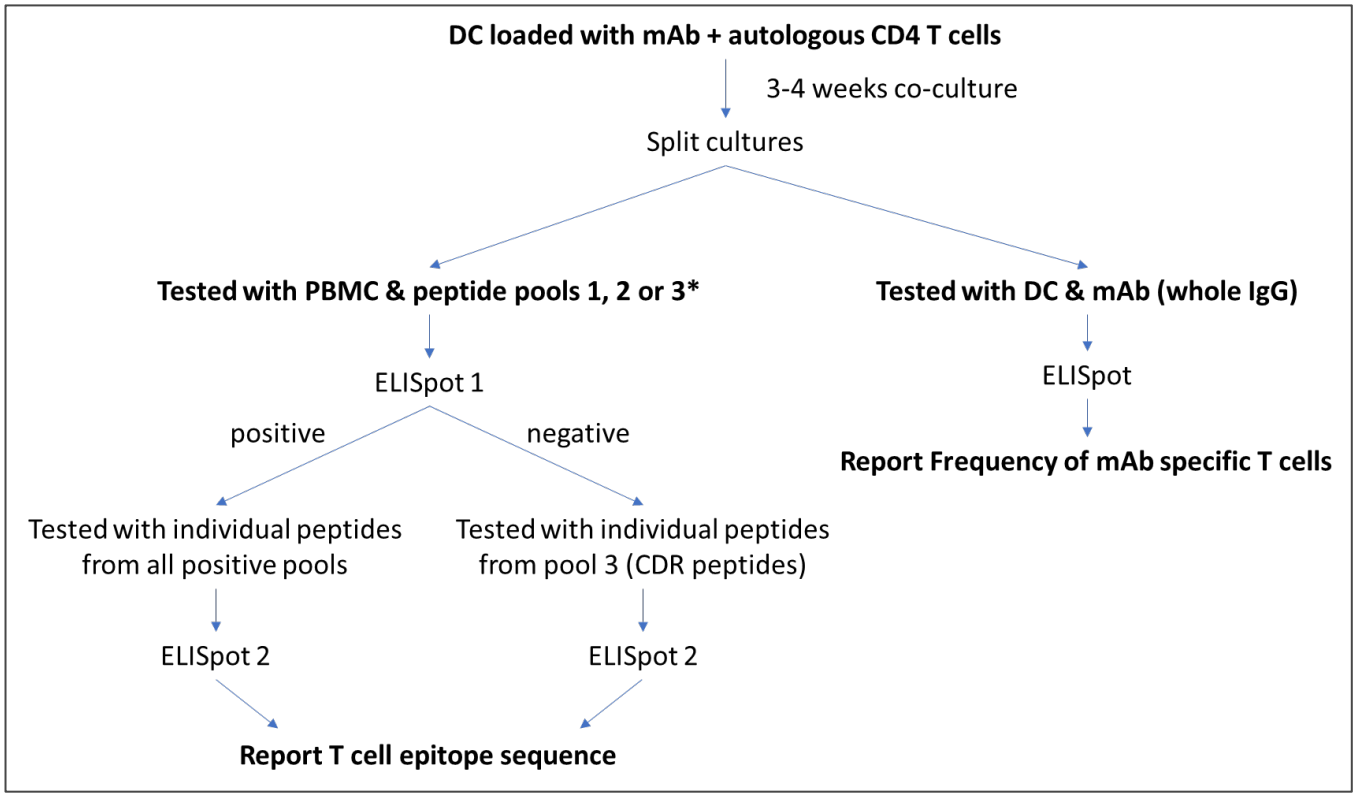


In vitro T-cell epitope mapping

Spindeldreher S et al. Dermatol Ther 2020, 8, 57-68

Reactive T-cell lines re-stimulated with:

- Pool 1 & 2 = peptides presented by DC & identified by MAPPs
- Pool 3 = CDR peptides



- 27 T-cell lines from 15 different donors were derived from ixekizumab; specific T cell epitope epitope was identified for 19 of these cell lines; overlapping with CDR sequences
  - Epitopes contain aa residues introduced during derivation from parental clone
- 2 T-cell lines from 2 donors for secukinumab; T-cell epitopes could not be identified

Question: Risk assessment at lead candidate selection stage

Your R&D team has identified an scFv antibody with high *in vitro* potency for inhibition of a pro-inflammatory cytokine implicated in the aetiology of an autoimmune disease.

They are asking for your advice about whether there could be particular immunogenicity-related risks associated with this candidate molecule.

Even at this very early stage, are you in a position to provide suggestions about the identification, evaluation and mitigation of immunogenicity-related risks?

Risk assessment: Stage 1

Some points to consider:

- How has the scFv been derived?
- Methodology to evaluate risks associated with non-human germline amino acid sequences?
- Could fragmentation of the IgG molecule expose cryptic epitopes?
  - How might this be tested?
  - How could risk be mitigated?
- Expression profile and function of target?
  - Could expression of target with immune effector cells be influential?
- Need for rigorous control of product-related variants & process-derived impurities if the scFv is expressed in *E.coli*

Question: Impact of PEGylation?

Your R&D team understand that it will be necessary to consider a half-life extension strategy to achieve adequate exposure.

The most straightforward and cost-effective strategy would be to conjugate the scFv with a GMP-grade 20 kDa PEG reagent.

The R&D team are seeking your advice about whether PEGylation of the scFV might modify immunogenicity risk profile of the investigational product?

How do you respond?

Impact of PEGylation

Decreased level of risk associated with:

- C-terminal-directed PEGylation might decrease risk by reducing steric hindrance of binding of pre-existing antibodies to cryptic epitope exposed at C-terminus of svFv molecule?
- PEGylation might improve solubility of the scFV in the drug product formulation and decrease risk of aggregation?
- Dose-sparing effect might reduce amount of non-human germline CDR sequences available for stimulation of adaptive immune response?

Increased level of risk associated with:

- PEG moiety could bind to pre-existing PEG-reactive antibodies, which might enhance clearance and/or interfere with binding of the scFv to the target?

Overall, from the immunogenicity perspective, PEGylation might actually reduce risk?

Exercise: Risk assessment pre-Phase 1

Gene therapy product risk assessment

You are proposing to administer a gene therapy product consisting of a transgene to express a protein that is deficient in the population to be treated, to be delivered via a AAV8 vector

The Phase 1 clinical study revealed a suspected CD8+-mediated cytotoxic effect (elevated liver enzymes in systemic circulation)

Your regulatory team is not sure about how to anticipate expectations of regulatory assessors in the CTA and is seeking your advice.

What do you advise?

Points to consider

- Potential consequences of immune responses to:
  - transgene
  - AAV vector
- Extent of data to be included in CTA:
  - Risk assessment
  - Bioanalytical strategy to monitor immune responses
  - Risk mitigation measures to include in clinical trial protocol

PROCESS:

1. Identify potential risks
2. Propose actions to evaluate risks
3. Define risk mitigation measures

Risk assessment Step 1: Identify potential risks

Potential risk
1. Treatment-emergent immune response *vs*. expressed transgene protein with capacity to cross-react with, and neutralize activity of, endogenous counterpart
2. Cellular immune response *vs*. AAV8 translates into reduced duration of expression of transgene protein *and/or* cytotoxicity in target tissue
3. Pre-existing antibodies reactive with components of AAV8 vector could reduce tissue transduction efficiency
4. Treatment-emergent humoral response *vs*. AAV8 translates into reduced duration of expression of transgene protein
5. Pre-existing neutralizing antibodies to expressed transgene protein could reduce efficacy

Start by identifying attributes of an immune response to the treatment that could lead to negative outcomes, in descending order of severity

Risk assessment Step 2: Align actions to evaluate risk

Potential risk	Risk evaluation
1. Treatment-emergent immune response *vs*. expressed transgene protein with capacity to cross-react with, and neutralize activity of, endogenous counterpart	• ADA assay to detect *Protein X* at baseline & 2, 4, 8, 24 & 48 weeks post-dose • Test cross-reactivity *vs*. endogenous protein • Test neutralization of *Protein X* activity *in vitro*
2. Cellular immune response *vs*. AAV8 translates into reduced duration of expression of transgene protein *and/or* cytotoxicity in target tissue	• *Ex-vivo* stimulation (IFNγ ELISpot) of human PBMCs collected at 8- & 48 weeks post-dose • Correlate AAV8-specific CD8+ signals *vs*. liver enzymes etc.
3. Pre-existing antibodies reactive with components of AAV8 vector could reduce tissue transduction efficiency	• ELISA using AAV8 capsid to detect AAV8-reactive antibodies in baseline samples • Correlate *vs*. *Protein X* level / activity
4. Treatment-emergent humoral response *vs*. AAV8 translates into reduced duration of expression of transgene protein	• ELISA using AAV8 capsid to detect AAV8-reactive antibodies in post-dose samples • Correlate *vs*. *Protein X* level / activity
5. Pre-existing neutralizing antibodies to expressed transgene protein could reduce efficacy	• Test anti-*Protein X* antibody positive baseline samples in activity assay *in vitro* • Correlate *vs*. efficacy endpoints

Risk assessment Step 3: Risk mitigation

In this case, the following measures might be considered as contributing to mitigation of risks associated with immunogenicity:

- Vector engineering to minimise residual vector-derived immunogenic sequences
- Verification of fidelity of transgene expression for native sequence protein
- Demonstrate effective quality control of cell banks and drug product
- Exclude subjects with pre-existing liver dysfunction
- Exclude subjects treated previously with AAV-vectored products
- Pre-screening of subjects to enable exclusion of those with pre-existing antibodies above a pre-defined anti-*Protein X* or anti-AAV8 titer
- Prednisolone short-course therapy allowed for subjects with elevated liver enzymes / suspicion of treatment-related hepatoxicity
- Dose-escalation stopping criteria
- Long-term (up to 5 years) follow-up monitoring of anti-Protein X and anti-AAV8 immune responses

Go / No Go decision for progression to Phase 3

The program manager would like to understand how **immunogenicity-related** data to be generated in the Phase 2 study should be interpreted as part of the GO / NO GO decision to proceed to Phase 3

What do you advise?

Go / No Go decision for progression to Phase 3

- Can the target tissue be transduced effectively in the presence of pre-existing antibodies?
- Can durable efficacy be achieved despite induction of a treatment-emergent immune response?
- Is it possible to moderate the treatment-emergent CD8+-mediated hepatoxicity by prednisolone short-course therapy?
- Is there a favourable benefit *vs.* risk balance for a majority of treated subjects?
- Is there an unmet medical need?

Team advised to proceed into Phase 3 studies on the basis of an affirmative response to all of the above question

Exercise: Process

Your clinical study manager advises you that immunogenicity-related endpoints have not yet been defined in the Statistical Analysis Plan for the Phase 3 study of your gene therapy product

Also, it is not clear if the Data Transfer Agreement with the Bioanalytical CRO will capture all of the requisite data fields

Because comprehensive reporting of immune response parameters in the Clinical Study Report is regarded to represent a critical element, your clinical team are requesting your advice about how to proceed?

What do you advise?

Thoughts about data management process

- CSR can provide summary of ADA results & brief narrative
  - Bioanalytical Report included as Appendix
- Use ISI to provide additional granularity & interpretation
  - Relationship of bioanalytical signals to clinical endpoints
- If needed, can define a separate secondary analysis for ISI
  - "ISI SAP"
- **Helpful to have raw data from ADA / NAb testing in Excel spreadsheet format**
  - Enables sorting by ADA titer *etc*.
  - Provides useful data QC check-point to identify errors prior to submission
  - Raw data often requested during GCP audit

ADA assay data granularity

Item
Patient ID/code
Assay run no.
Assay run date
Age
Gender
Treatment group
Sampling time point

Corresponding low QC value in screening assay (RLU)
Corresponding high QC value in screening assay (RLU)
Corresponding negative control value in screening assay (RLU)
Plate-specific screening assay cut-point
Screening assay result (RLU)
Screening assay assignment: positive/negative

Low QC value in confirmatory assay (RLU)
High QC value in confirmatory assay (RLU)
Negative control value in confirmatory assay (RLU)
Confirmatory assay result (RLU)
Percent inhibition in the confirmatory assay
Confirmatory assay assignment: positive/negative
Titer of confirmed positive samples
Concentration of on-board drug at time of sampling

Include sufficient data granularity to enable reviewer to perform an independent analysis

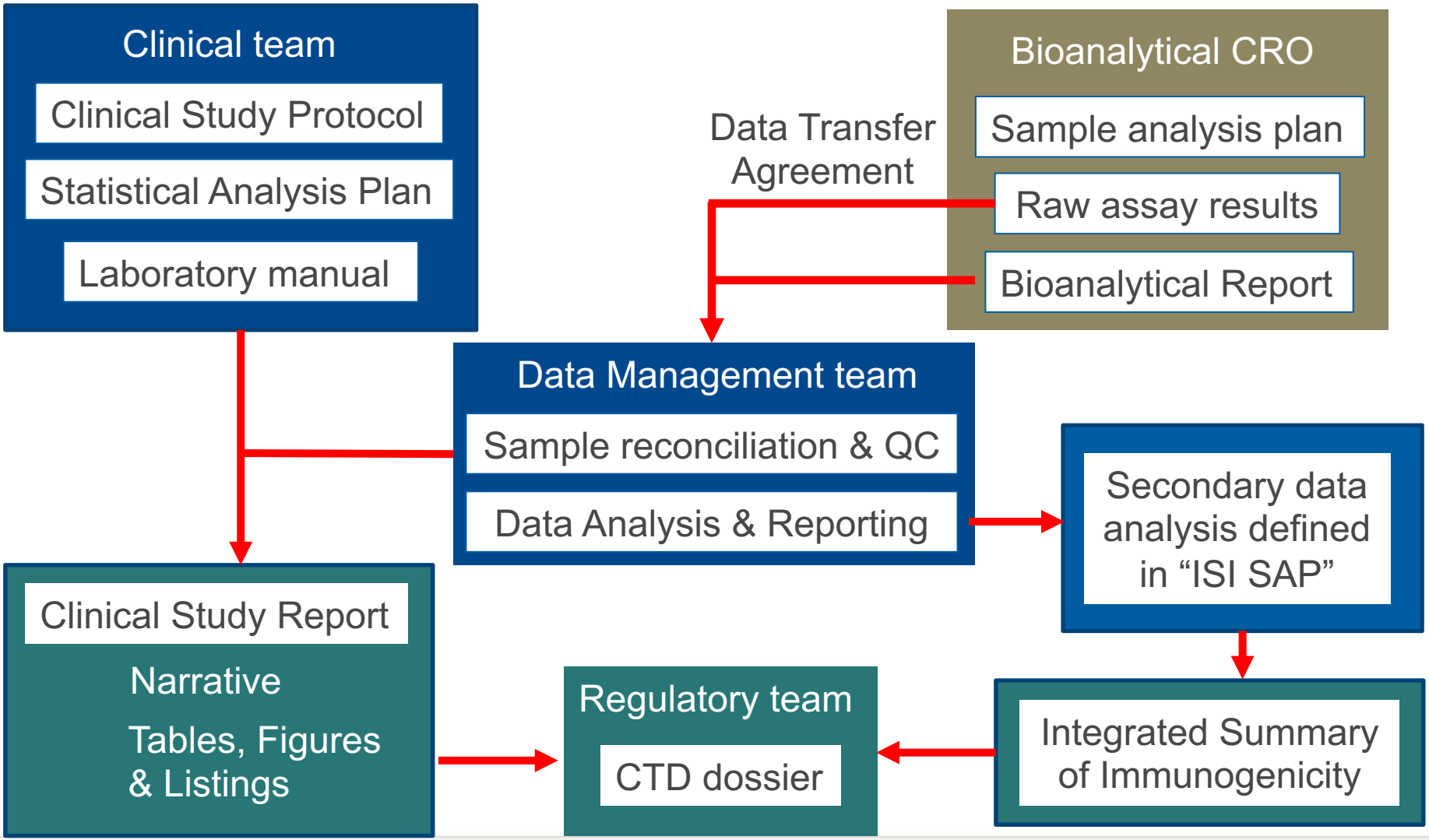
Ideally in excel spreadsheet format

Appendix to:

- CSR or
- Sample analysis report or
- ISI

Plan into data transfer agreement with CRO and assign treatment ID following data lock & un-blinding

Process flow: Input *vs.* Output definition



Part 3:

Managing regulatory interactions

Guiding principles

- Start with the end in mind
  - Successful CTA's & MAA / BLA submissions
- **Address the needs of regulatory assessors**
  - **Transparency & objectivity**
- **Create trusting partnership with regulators**
  - **Regulators want to do their best to help Sponsors**
- Team-work
  - Involve all relevant disciplines
- Start early
  - Create risk assessment as part of Lead Candidate Selection stage
  - Update as new information becomes available
- **Communication!**
  - **Demonstrate that you have assessed and understood the pertinent risks, and evaluated / mitigated risks in appropriate manner**

Exercise: Regulatory Interaction (Pre-Phase 1)

Investigational Medicinal Product = **Fusion protein** with capacity to link cytolytic T-lymphocytes to tumour-associated antigen

CTA-enabling GLP toxicology study results:

Observed infusion-related reactions immediately following 4th weekly dose (Day 21) in most animals in pre-clinical GLP toxicology study in cynomolgus macaques; severe in some animals in highest dose group

Histopathology results show changes in lung, liver and kidneys that are consistent **with immune complex-mediated hypersensitivity reactions**

ADA titers show only a modest increase at each dose administration – but assay sensitivity may be compromised by drug interference

What is your next step?

Which response?

A Request a pre-CTA submission meeting to share results with concerned regulatory agencies to seek their advice on how to proceed

B Focus on building non-clinical weight-of-evidence to support immune complex-mediated causality: IHC detection of co-localization of drug + cyno IgM / IgG + C3; CIC assay. Present these data in IND/IMPD to justify lack of relevance for clinical benefit-risk assessment

C In addition to B, but perform an additional GLP repeat-dose toxicology study in cynomolgus macaques with only 3 doses and additional immuno-phenotyping and haematology endpoints to exclude a pharmacological contribution to the findings that are believed to be related to ADA formation; include these data in IND/IMPD

D As B & C, but request a pre-CTA submission meeting to reach agreement on dose justification and risk mitigation measures for FTIH study; include Immunogenicity Risk Assessment in IND/IMPD.

Exercise: Regulatory Interaction (Phase 1)

Phase 1 clinical study for novel multi-domain therapeutic protein

- Pre-existing antibody detected in most subjects
- Strong treatment-boosted ADA response detected at day 28 post-IV admin in SAD period of Phase 1 study
- At higher dose levels, reports of flushing and urticaria in acute phase following dosing
- MAD period of Phase 1 study planned (& approved), but not yet commenced

How do you deal with this?

Which response?

A	Proceed as planned with MAD period of Phase 1 study
B	Voluntarily suspend the Phase 1 study. Convene Safety Review Board to review dose levels planned for MAD period in relation to observations in SAD period; revise protocol to reflect a more cautious dose-escalation approach and re-submit to Agency for approval
C	Voluntarily suspend the Phase 1 study. Assess intrinsic immunogenic potential in relation to extrinsic factors for incremental risk. Request a meeting with concerned regulatory agencies to discuss risk measures to be applied as part of a revised protocol for the MAD period.
D	Because you suspect that product aggregates formed during dilution of the drug product into the solution for intravenous infusion may have contributed to enhanced immunogenicity, submit an amendment to the CTA to enable use of an alternative diluent in the MAD period.
E	Other – but please suggest actions to follow

Exercise: Regulatory Interactions (Phase 1)

As preceding case example, but with additional information from product quality investigation:

Elevated levels of **process-derived impurities** detected by orthogonal analytical techniques in DP batch used in Phase 1

How does this impact your regulatory strategy?

Exercise: Regulatory Interactions (Phase 1)

A Develop and qualify an assay to detect ADA to host cell-derived proteins to run in tandem with the ADA assay for the therapeutic protein in the MAD period of the Phase 1 study. Submit this as part of the IND/IMPD to support a revised clinical study protocol

B Modify the down-stream process to add chromatographic steps to reduce the level of process-derived impurities; revise IND/IMPD and seek approval from Agency to proceed into MAD period of Phase 1 study with new drug product batch from revised process.

C As B, but request meeting with Agency to discuss findings and to seek endorsement for risk mitigation provisions proposed for MAD period.

D Delay the program to enable additional evaluation of the biologically relevant levels of the particular process-derived impurities that were identified in the product quality investigation. Amend the manufacturing process and DS/DP specifications accordingly. Request pre-CTA meeting with Agency to discuss conditions for recommencing Phase 1 study

Question

- ***What should be included in a Briefing Book for Pre-CTA meeting?***

Meeting package for pre-CTA meeting

- Product quality investigation
  - Analytical sensitivity?
  - Identity of process-derived impurities?
  - **Biologically-relevant level?**
- Mechanistic aspects
  - Balance of intrinsic immunogenic potential *vs*. extrinsic factors for incremental risk
  - Weight-of-evidence to support **immune complex-mediated** causality for AE's
- Clinical impact
  - Relationship of pre-existing ADA titer to treatment-boosted ADA titer
  - Impact of pre-existing & treatment-boosted ADA titer on PK / PD
  - Coincidence of elevated ADA titer to timing / incidence and severity of adverse events
- **Proposed risk mitigation**
  - **Improved analysis / control of risk factors for incremental immunogenicity**
  - **Reduced dose levels and rate of IV infusion**
  - **Monitoring for complement activation**

Exercise: Regulatory Interactions (EOP2)

Meeting Package for EOP2 meeting already sent to Agency, including proposed protocol for Phase 3 clinical study in indication ***X***

Unexpected case report of anaphylaxis in Phase 2 study for indication ***Y***

- For both indication ***X*** and indication ***Y***, immediate hypersensitivity reactions of mild or moderate severity observed in most subjects at 1st dose
- No ADA detected pre- or post-treatment
- Mechanism not identified

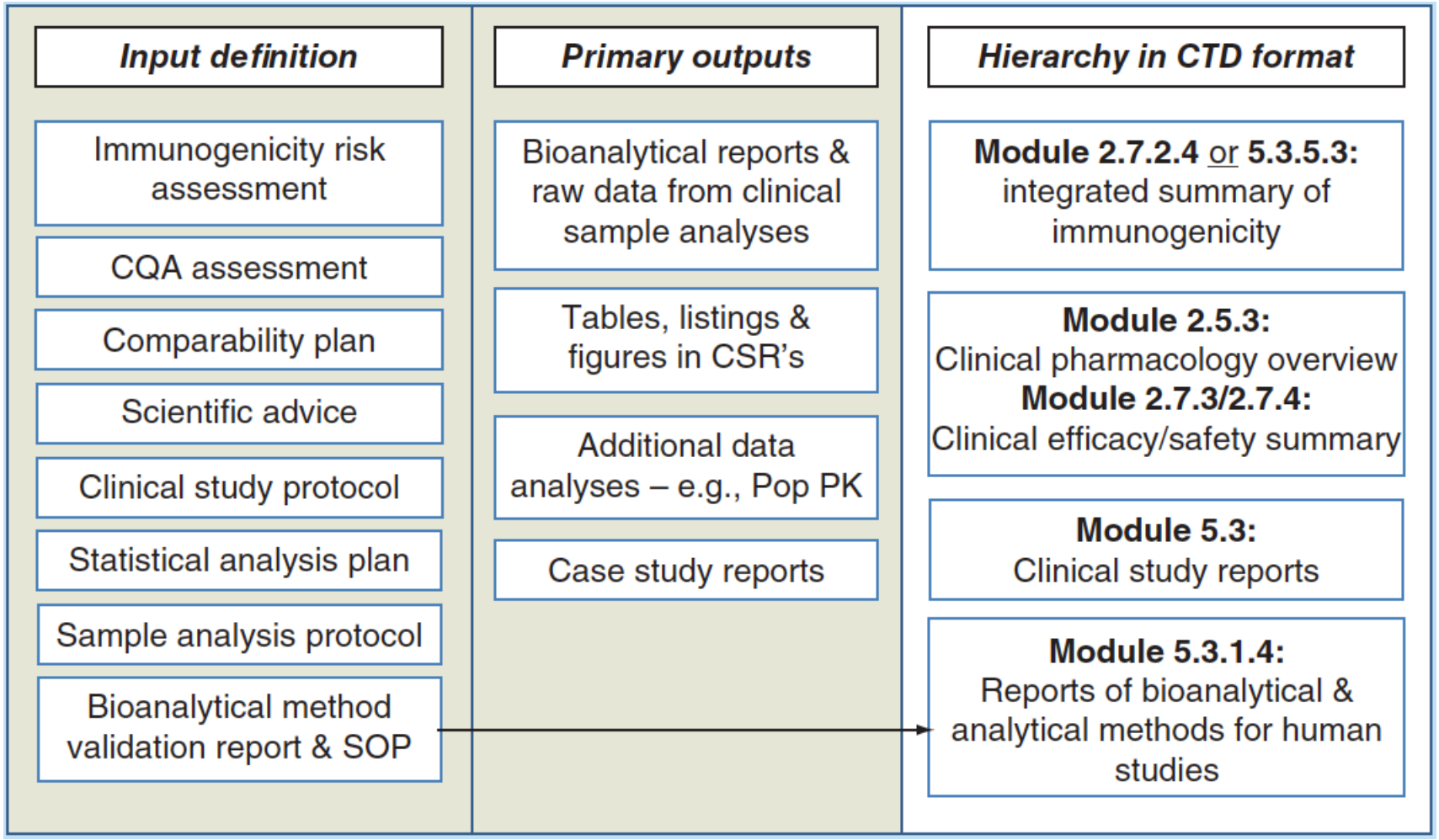
How do you deal with this?

Which response?

A	Postpone the planned EOP2 meeting for indication *X* to enable internal company discussion about how to proceed
B	As the Meeting Package has already been submitted, simply report the SAE using the usual expedited mechanism and proceed with the EOP2 meeting for indication *X*
C	In addition to expedited reporting of SAE, advise the Agency Project Manager in writing of SAE reported in study for indication *Y*
D	As C, but also modify proposed risk mitigation provisions for Phase 3 study in indication *X* & include these in slides to be presented in EOP2 meeting for indication *X*
E	Other – but please suggest actions to follow

Part 4:

Documentation



Integrated Summary of Immunogenicity (ISI)

- Based on regulatory guidance for risk-based approach
- EMA 2017 and FDA 2019 guidance provide consistent advice to enable a **common format / content**
- "Integrated" refers to the process of combining information from different disciplines, rather than aggregating data from different clinical studies
  - Intrinsic & extrinsic risk factors
  - Methodological approach
    - Bioanalytical
    - Clinical study design
  - Results by each clinical study
  - Interpretation of impact on benefit-risk based on weight of evidence
  - Linkage to Risk Management Plan

Benefits both Applicant & Regulatory Assessors

EMA 2017: Section 10 – Integrated Summary of Immunogenicity

Analysis of risk factors

1. Previous experience of the product/product class
2. Physicochemical and structural aspects
3. Does the route and/or the mode of administration raise concerns
4. Patient- and disease-related factors

Risk-based program

5. Assay strategy
6. Approach to immunogenicity in clinical trials
7. Impact of the risk assessment on the immunogenicity program

Immunogenicity results

8. Immunogenicity in clinical trials (relative immunogenicity in case of manufacturing changes and biosimilars)

Conclusions on the risk(s) of immunogenicity

9. Impact of the immunogenicity on the benefit/risk
10. Tools to manage the risk

Suggested minimum content, to be adapted according to product

FDA 2019 guidance

- As per Jan, 2019 FDA Guidance “Immunogenicity Testing of Therapeutic Protein Products- Developing and Validating Assays for anti-Drug Antibody Detection” (section VIII. Documentation)
  - ISIs are requested for all new 351(a) and 351(k) BLA submissions.
    - Provide brief summaries of the immunogenicity results in relevant places in eCTD section 2.7. Clinical Summary and the full report in section 5.3.5.3 Reports of Analysis of Data from More than One Study
      - Will receive IR if absent at filling.
    - Harmonizes with EMA guidelines

Slide prepared by João A. Pedras-Vasconcelos, PhD
Presented at CHI Immunogenicity Summit Short-course, Oct 2019

FDA recommendations#

- New and ongoing INDs are suggested to include ISI with stage appropriate information.
  - Regular updates as clinical program progresses
  - for novel biologics ISI recommendations may be sent as pre-IND meeting comments
    - Include Immunogenicity Risk Assessment with initial IND

Slide prepared by João A. Pedras-Vasconcelos, PhD
Presented at CHI Immunogenicity Summit Short-course, Oct 2019

FDA recommendations#

- Recommend the use of a “living” integrated immunogenicity summary document that sponsors would begin populating early in product development , and would update as clinical program progresses through IND stages into BLA and post-approval
  1. Immunogenicity risk assessment
  2. Tiered bioanalytical strategy and assay validation summaries (with stage- appropriate information)
  3. Clinical study design and detailed immunogenicity sampling plans
  4. Clinical immunogenicity data analysis
  5. Conclusions and Risk Evaluation and Mitigation Strategies (REMS)
     a) Include post-marketing/Life-Cycle management plans

Slide prepared by João A. Pedras-Vasconcelos, PhD
Presented at CHI Immunogenicity Summit Short-course, Oct 2019

Model format for ISI

Effective presentation of immunogenicity risk assessments and related data in regulatory dossiers

Paul Chamberlain*,[1]
[1]NDA Advisory Services, Ltd, Grove House, Guildford Road, Leatherhead, Surrey KT22 9DF, UK
*Author for correspondence: Tel.: +33 5 62 96 34 37; paul.chamberlain@ndareg.com

The purpose of this article is to provide practical advice about how to present immunogenicity-related information in regulatory dossiers, with a particular focus on a model for an Integrated Summary of Immunogenicity to be submitted in the marketing authorization application for novel biopharmaceutical products in ICH regions (EU, USA and Japan). A format that links the analysis of potential risk factors to a justification of the methodology applied for risk evaluation and conclusions for risk mitigation is presented as a model that can be adapted according to the weight of evidence to be submitted in support of the assessment of impact on overall clinical benefit versus risk for the particular situation.

Bioanalysis (2019) 11(17), 1581–1592

Model template to be adapted to product

Bioanalysis (2019) 11(17), 1581–1592

Table 2. A model template for the Integrated Summary of Immunogenicity

Executive summary	• **Key messaging**
1. Introduction	• Brief overview of content of extent of data contributing to immunogenicity assessment, including discussion of comparability of drug product to be commercialized relative to material used during clinical development
2. Analysis of risk factors for *PRODUCT X*	
2.1. Risk assessment: identification of potential risk factors	• Concise discussion of intrinsic and extrinsic risk factors in relation to product attributes, proposed therapeutic use & existing knowledge
3. Suitability of bioanalytical methods	
3.1. Overview of methods	• Tabular summary of methods applied by clinical study with cross-references to supporting documentation • Schematic diagram of hierarchical test scheme
3.2. ADA assay for screening, confirmatory, specificity and titration	• Clear explanation of each of the following topics to guide the reviewer through the process of demonstrating suitability of the method, including its evolution during the risk-based program
3.2.1. Synopsis of method development	• Design of method in relation to drug and target interference, including evaluation of sample pre-treatment steps and choice of minimum required dilution
4. Results of clinical evaluation	
4.1. Extent of clinical database	• Tabular summary of number of treated subjects and treatment regimen by study
5 Overall conclusions for clinical immunogenicity evaluation and risk mitigation	
• Discuss consistency of observations across studies regarding the relationship of the treatment-related immune response to safety and efficacy at the population level and for individual subjects, for example, those developing relatively high ADA titers or antibodies cross-reactive with endogenous counterpart • Justify adequacy of proposed risk management plan, including product labeling, and describe any ongoing or planned postapproval studies with potential to address uncertainty about impact of immunogenicity on overall benefit versus risk	
6 References	
APPENDICES:	

Bioanalytical section of ISI

- Consolidate information presented in individual reports included in 5.3.1.4
- Rationale for choice of methods
  - Format / pre-treatment steps / MRD
- Explain how evolution of methodology relates to specific clinical studies
  - What was changed and why?
  - How did this affect assay performance?
  - Clear cross-references to supporting documents
- Opportunity to justify choice of positive and negative controls
- Drug tolerance *vs*. actual drug concentrations
- Clarification / justification of statistical approach for assay cut-points for different populations used in clinical program
- Control of critical reagents

Method Validation Reports often lack essential information for the naive reviewer!

False positive rate

= Index of reliability of applied assay cut points

Statistic	Result
Total number of samples screened	1992
Number of samples screened Positive	218
Number of sample confirmed Positive	196
Number samples confirmed Negative	22
Screening False Positive Rate [a]	10.1 %
FPER [b, c]	1.1 %

[a] Screening False Positive rate = (No. of samples confirmed negative / No. of samples screened positive) x 100

[b] FPER = [(# of samples screened positive - # of samples confirmed positive) / Total sample #] x 100

[c] Confirmatory cut point was based on a 1% false positive rate

Presenting clinical results

Overview of clinical studies performed

Tabular summary of number of treated subjects & treatment regimen by study

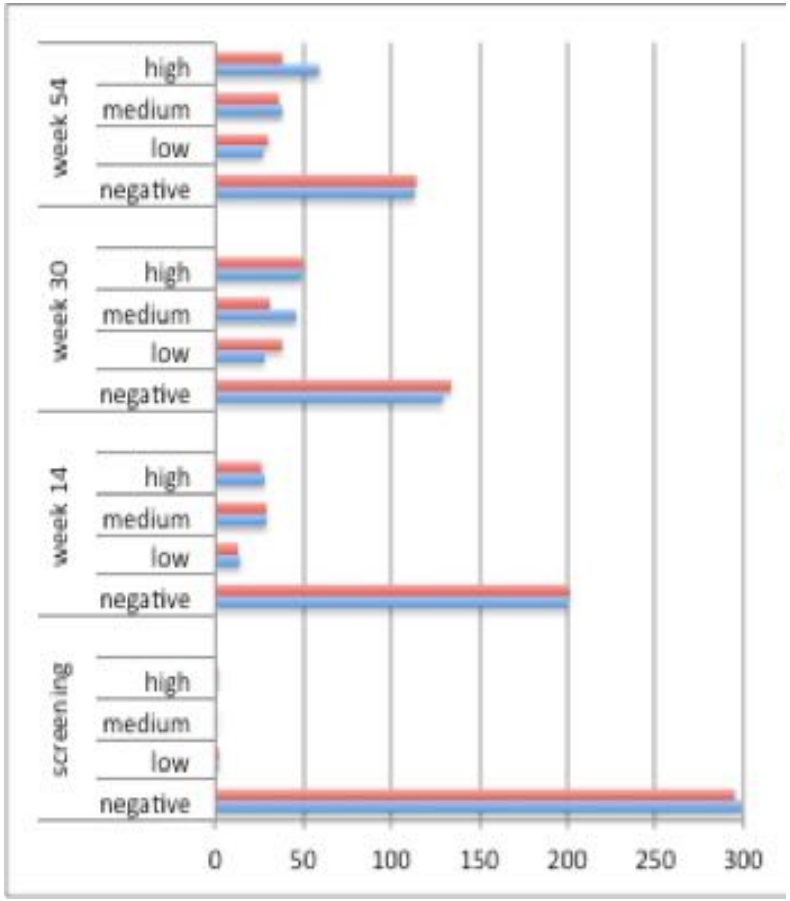
Summary by study#

- Diagram of study design & ADA & drug conc. sample time-points
- Drug product batches / presentations used
- Sample handling / missing samples
- Concomitant immune-suppressive medications
- ADA & nAb assay results
- ADA *vs*. PK / drug levels
- ADA / nAb *vs*. efficacy
- Immune-mediated TEAE's
- Conclusions

In order of weight of evidence, i.e. starting with pivotal clinical studies

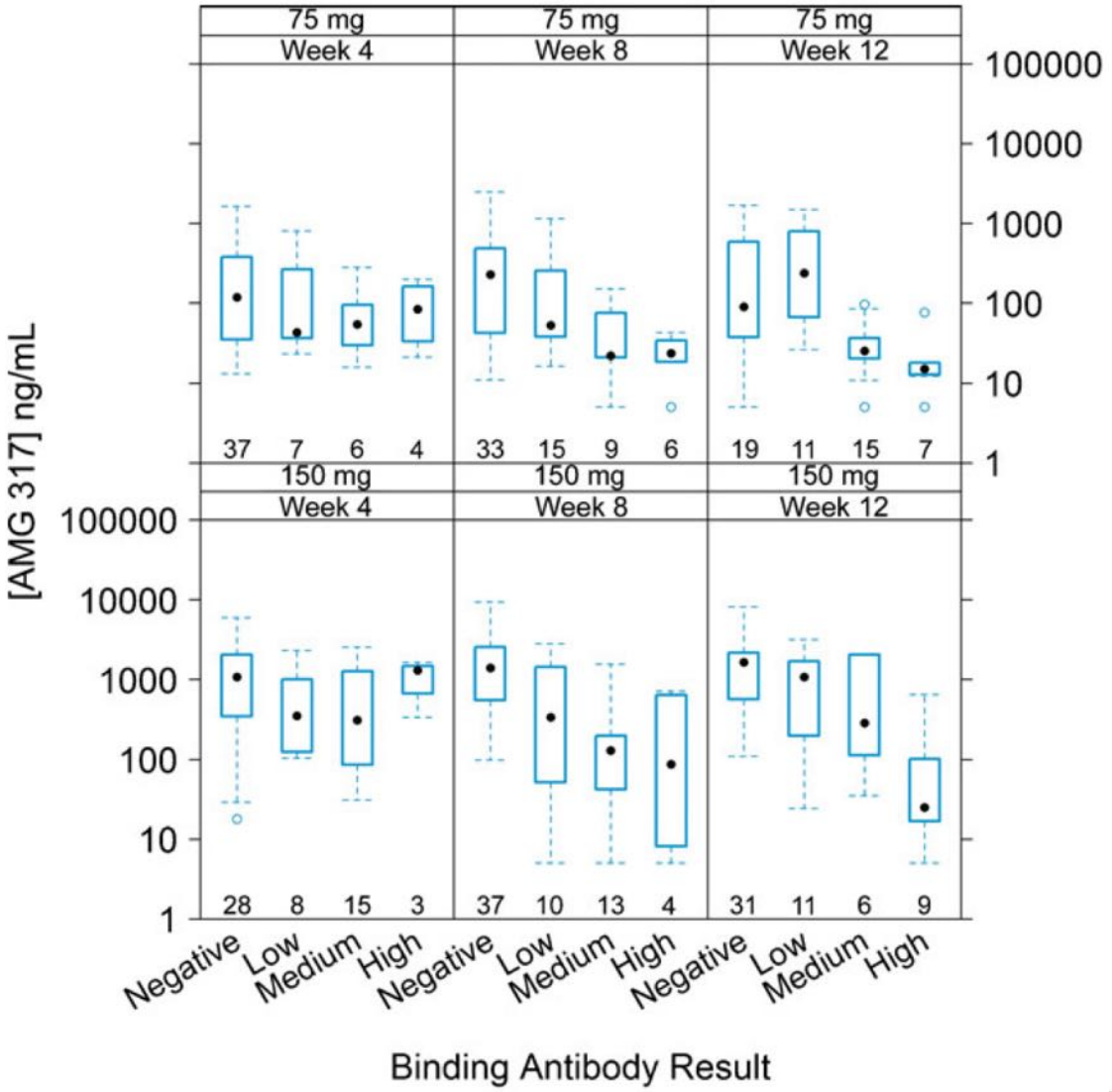
ADA / NAb response dynamics

Category	Treatment Group *X* (N)	Treatment Group *Y* (N)
ADA prevalence (any ADA positive, baseline or post-baseline)		
n (%) Median of maximum titer Min - Max		
Treatment-emergent ADA positive (ADA incidence)		
n (%) Median of maximum titer Min - Max		
ADA positive post-baseline and positive at baseline		
n (%) Median of maximum titer Min - Max		
Treatment-induced ADA (ADA positive post-baseline only)		
n (%) Median of maximum titer Min - Max		
ADA positive at baseline only		
n (%) Median of maximum titer Min - Max		
Treatment-boosted ADA		
n (%) Median of maximum titer Min - Max		
Persistent positive		
n (%) Median of maximum titer Min - Max		
Transient positive		
n (%) Median of maximum titer Min - Max		
NAb positive at any visit		
n (%) Median of maximum titer Min - Max		

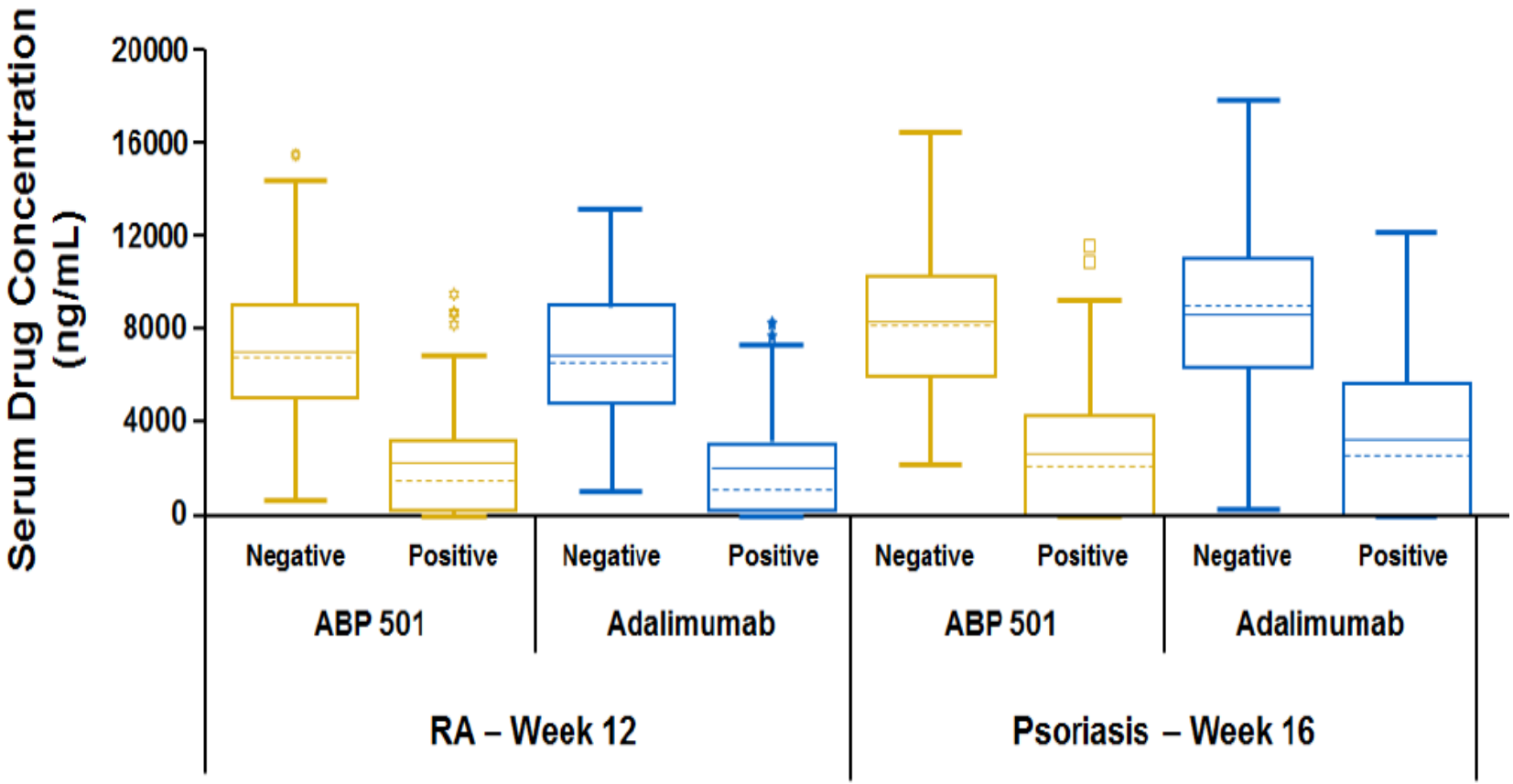


PK *vs*. ADA titer quartile

Zhou L et al; AAPS Journal 2013, 15 (1), 30-40



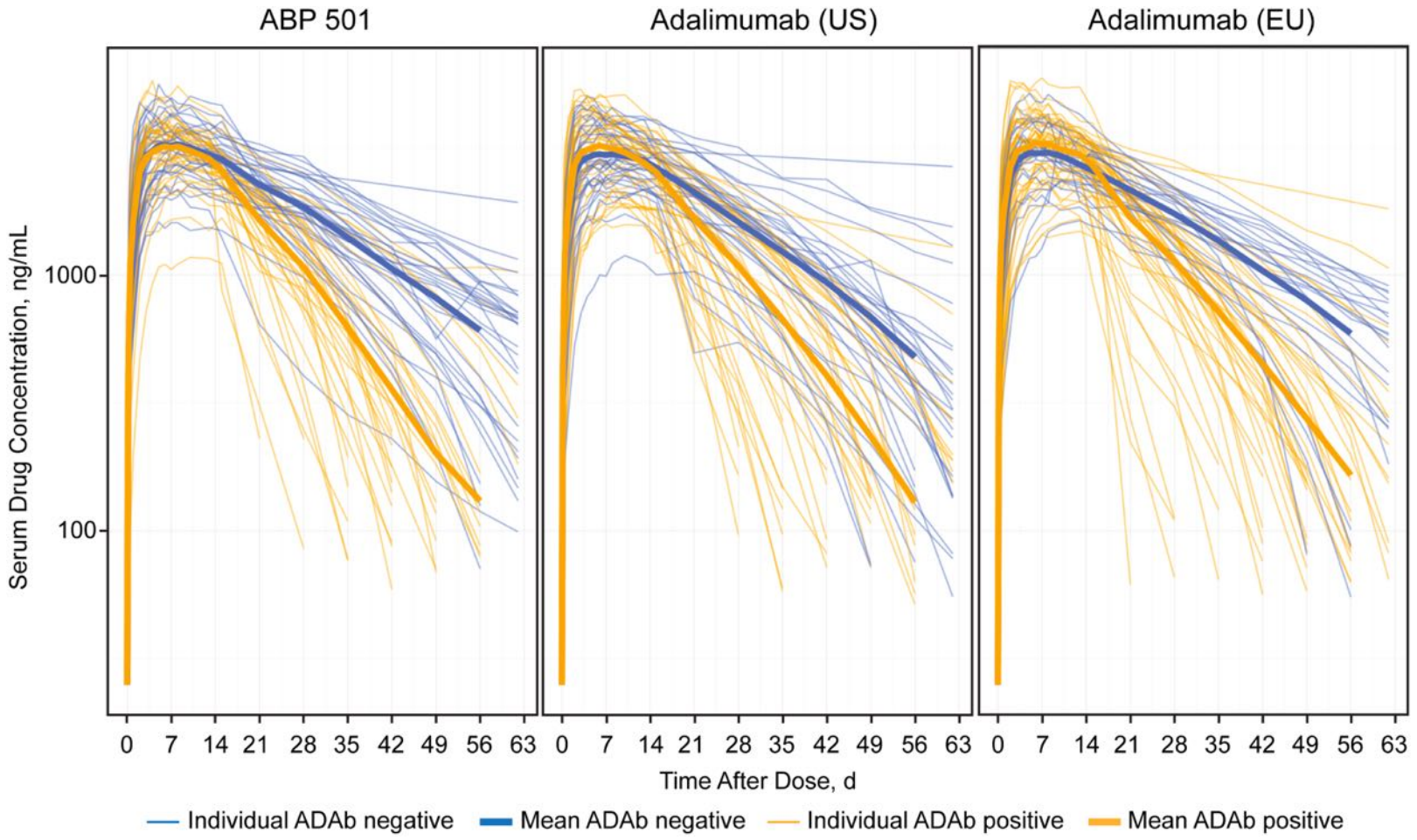
Serum trough concentration by ADA status



Reproduced without changes from Amgen Briefing Document for Arthritis Advisory Committee meeting held on 12 July 2016

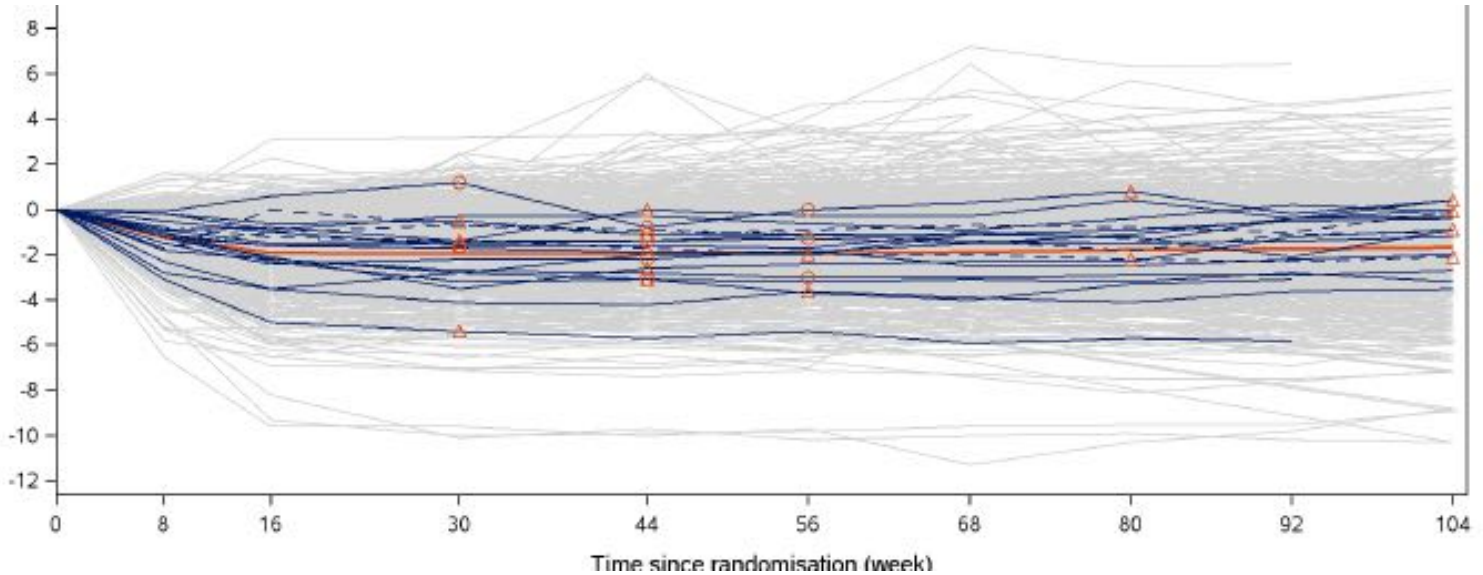
PK: Spaghetti plots

Kaur P, et al. Ann Rheum Dis 2017;76:526–533



Individual PK profiles depicting longer t1/2 in ADAb-negative subjects for all three test products: ABP 501, adalimumab (USA) and adalimumab (EU). ADAb, antidrug antibody

PK vs. ADA: Spaghetti plot



Blue = subjects with confirmed ADA positive

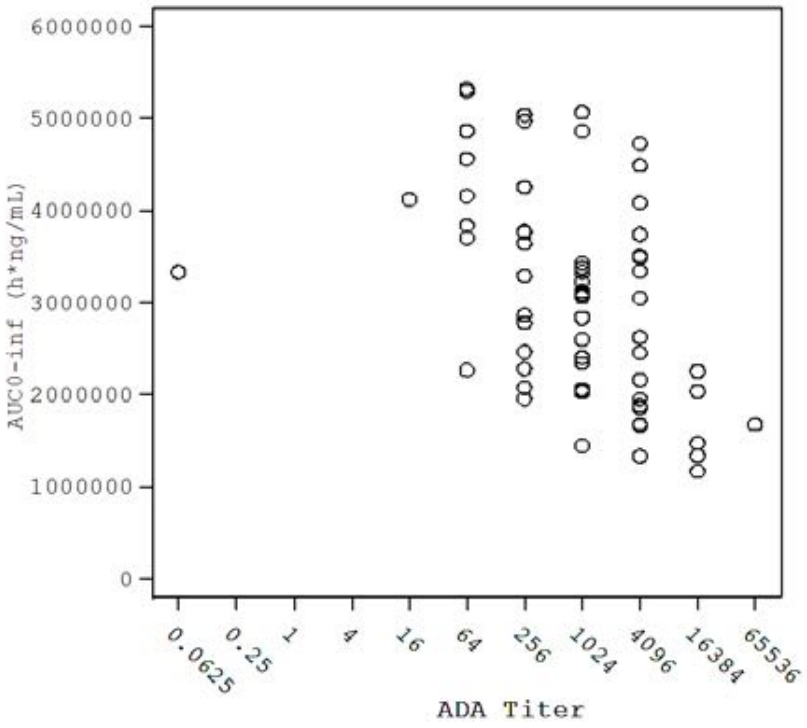
Grey = ADA negative subjects

Red line = arithmetic mean for ADA negative subjects

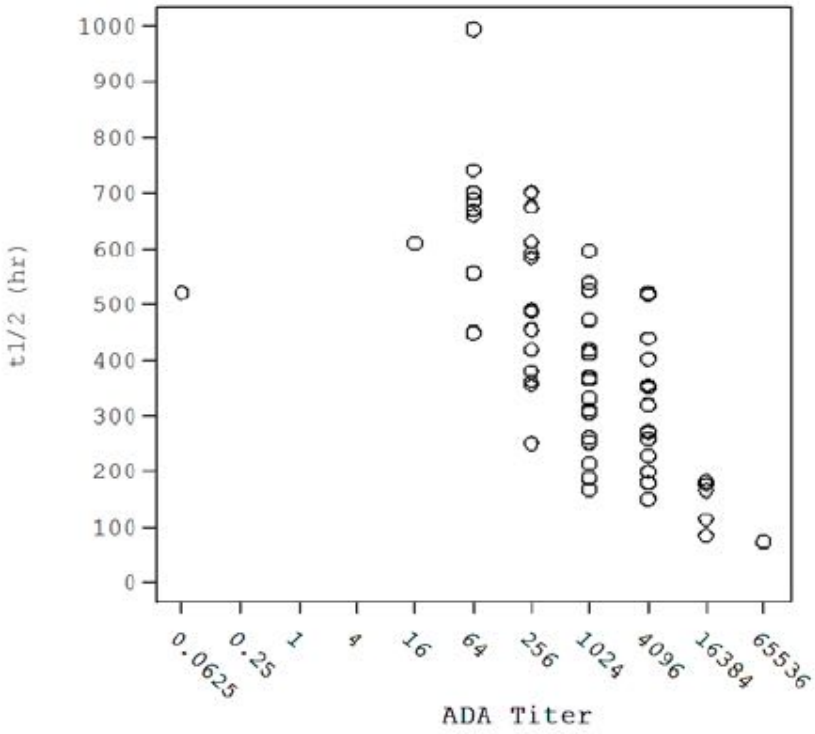
Spaghetti plot can be very effective to illustrate the overlap of the sub-populations

PK *vs*. ADA: Scatter plots

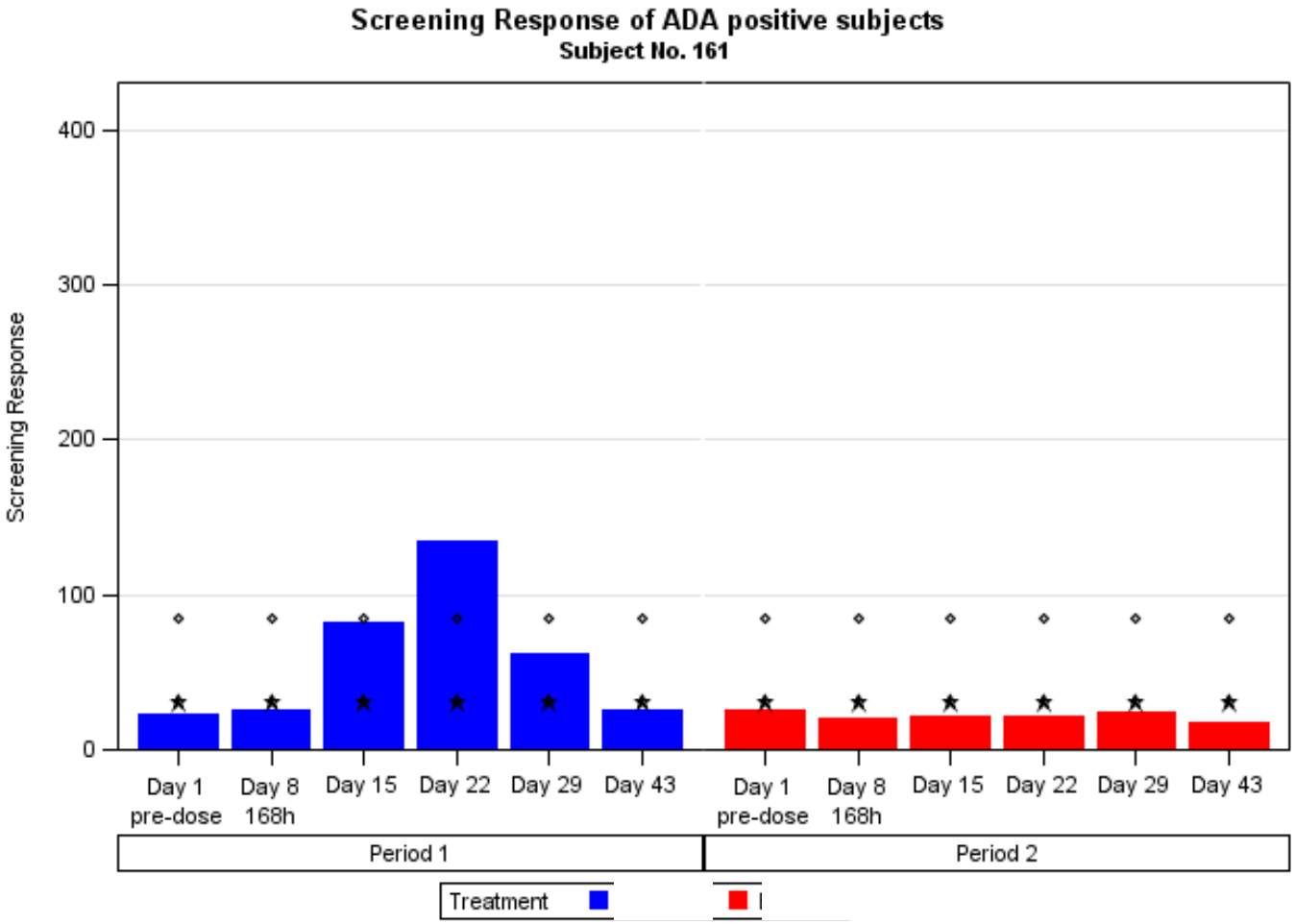
AUC 0-inf *vs*. ADA titer



T1/2 *vs*. ADA titer

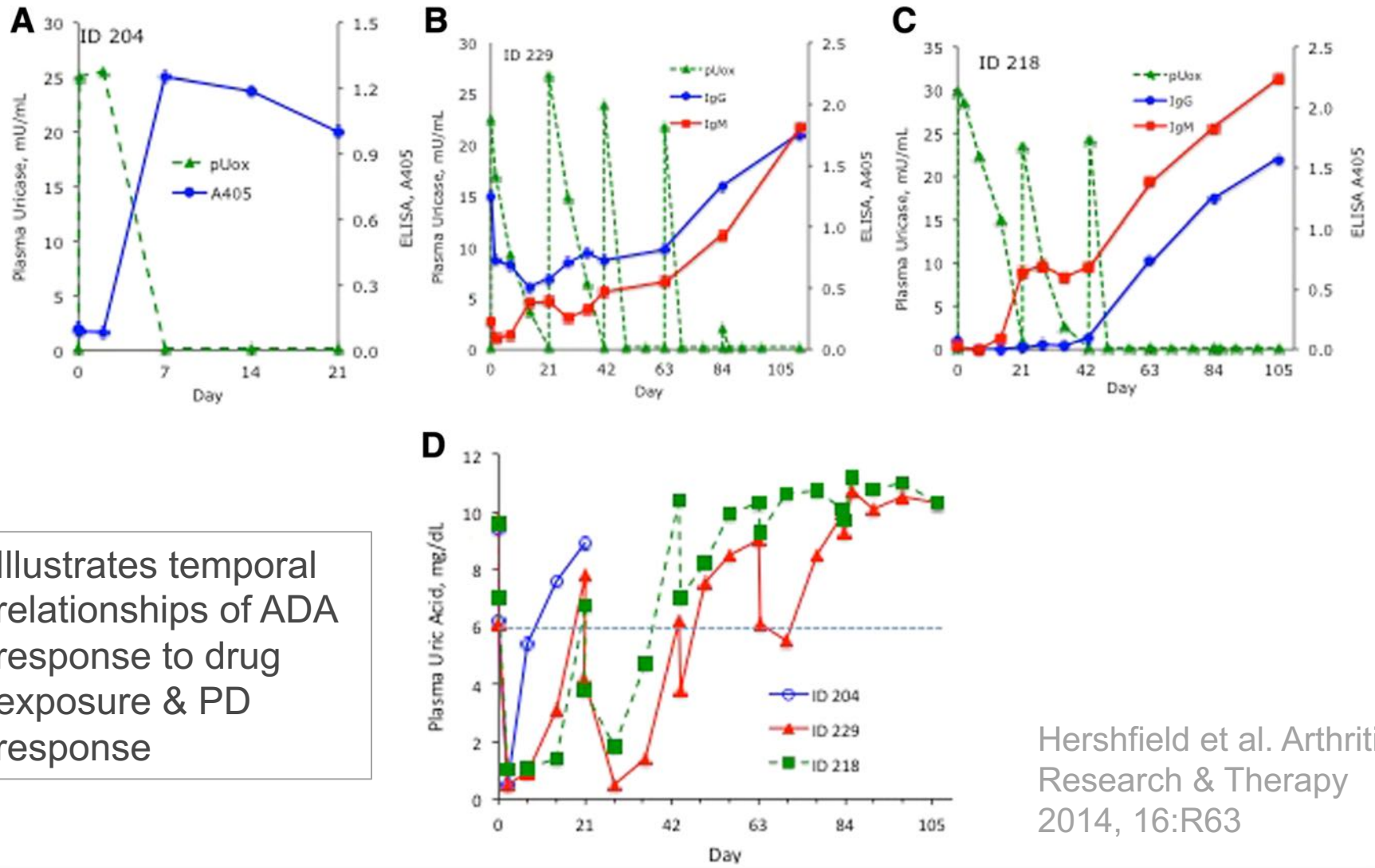


Individual subject profiles: ADA signal *vs*. time



Star display the plate-specific assay cut-point per timepoint, diamond display the plate-specific low QC sample

Individual subject profiles

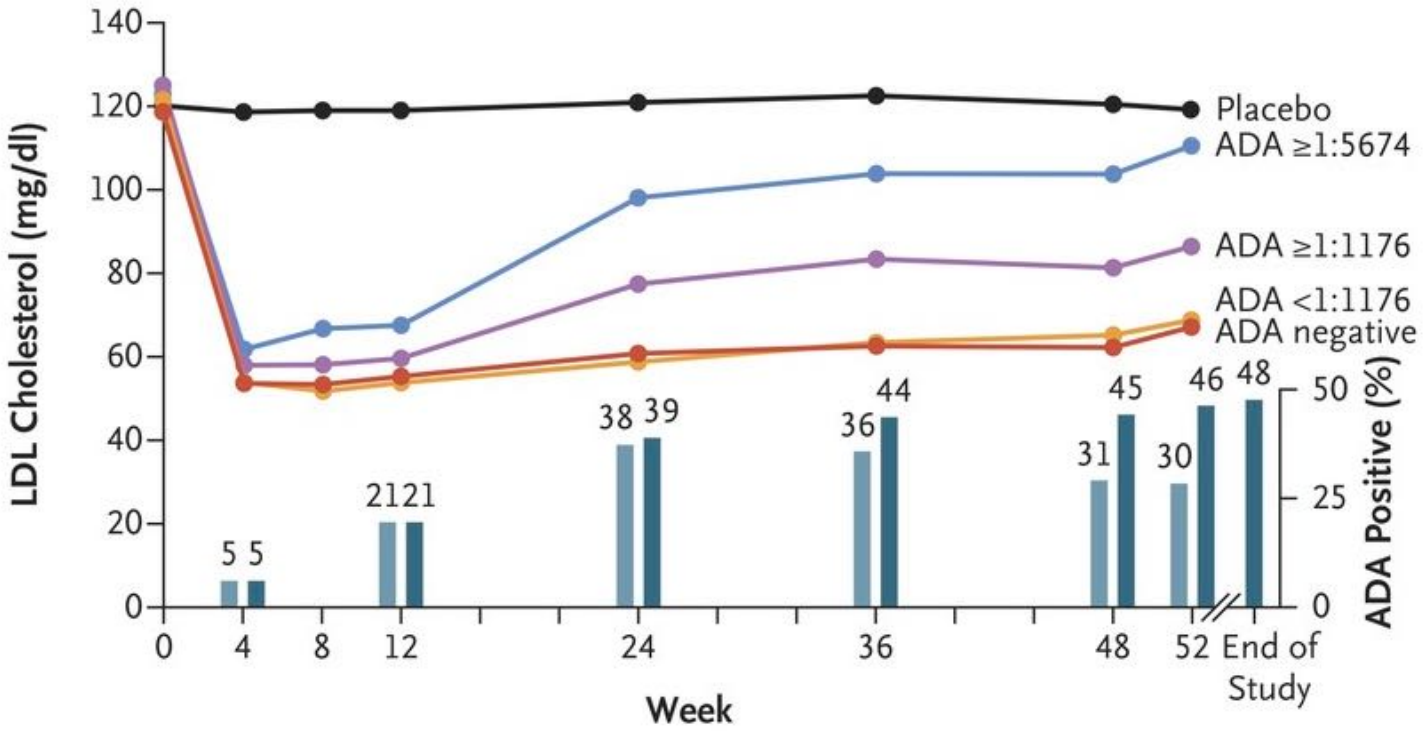


Illustrates temporal relationships of ADA response to drug exposure & PD response

Hershfield et al. Arthritis Research & Therapy 2014, 16:R63

Ridker PM et al. N Engl J Med 2017;376:1517-26

A LDL Cholesterol Level, According to Antidrug Antibody (ADA) Titer

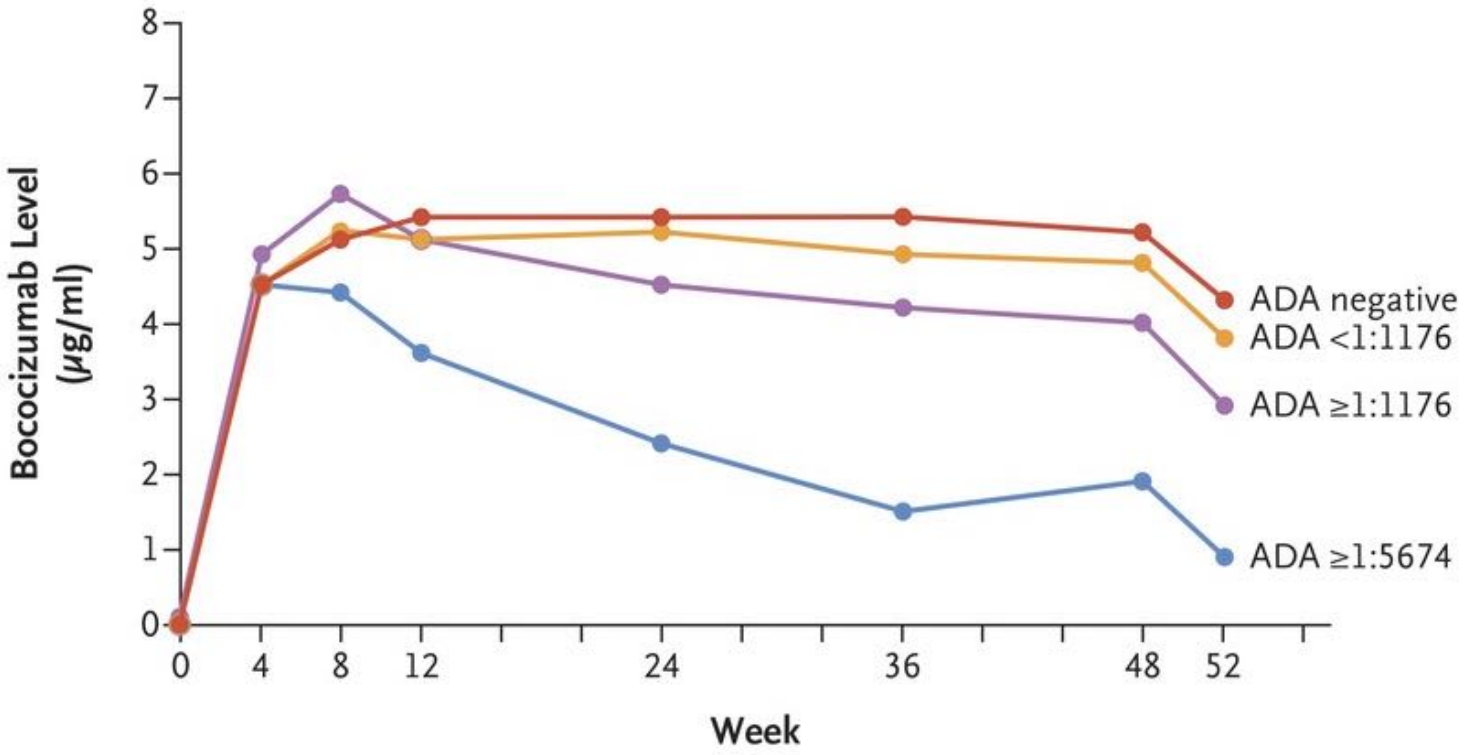


No. at Risk

Placebo	1856	1800	1767	1734	1730	1678	1583	1623
ADA negative	1068	1053	1027	977	979	957	891	921
ADA <1:1176	672	663	658	650	656	645	612	618
ADA ≥1:1176	333	327	328	321	322	313	293	297
ADA ≥1:5674	99	97	96	97	97	94	90	87

Ridker PM et al. N Engl J Med 2017;376:1517-26

B Bococizumab Level, According to ADA Titer

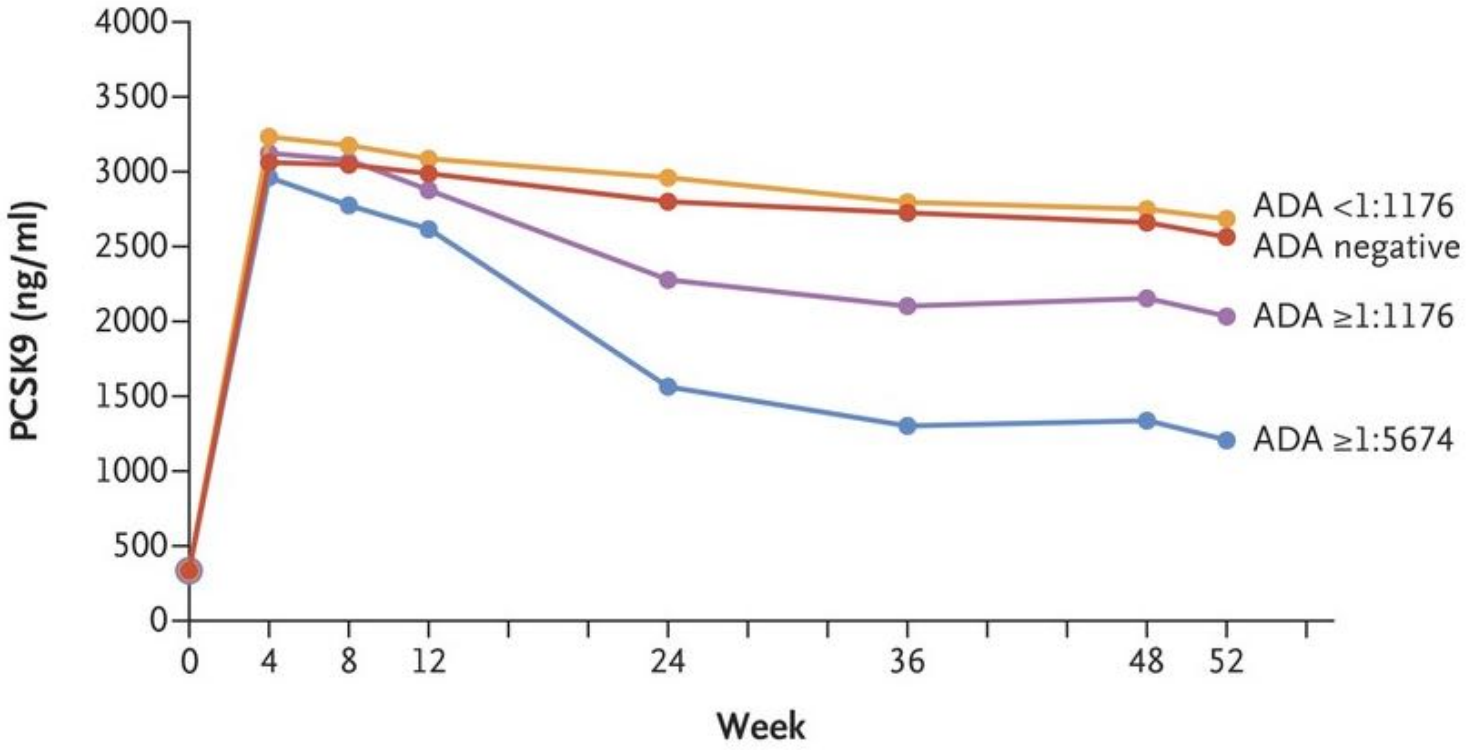


No. at Risk

ADA negative	1014	1025	999	955	950	941	877	908
ADA <1:1176	642	649	642	643	646	635	601	611
ADA ≥1:1176	320	322	320	319	321	311	292	291
ADA ≥1:5674	93	97	94	95	96	92	88	86

Ridker PM et al. N Engl J Med 2017;376:1517-26

C PCSK9 Level, According to ADA Titer

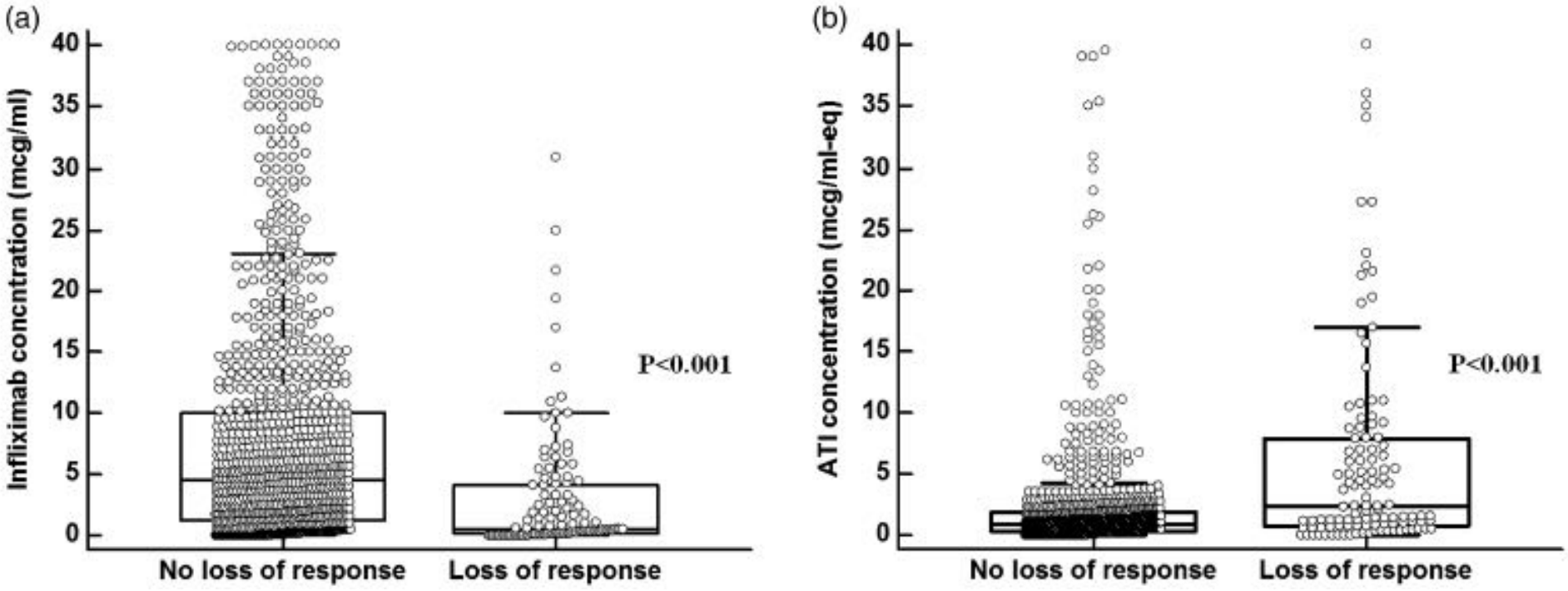


No. at Risk

ADA negative	1041	1023	1002	960	953	943	878	906
ADA <1:1176	659	650	643	643	646	636	602	611
ADA ≥1:1176	324	324	321	320	323	310	291	291
ADA ≥1:5674	94	97	94	96	98	92	88	86

Loss of response to infliximab *vs*. ADA and drug levels

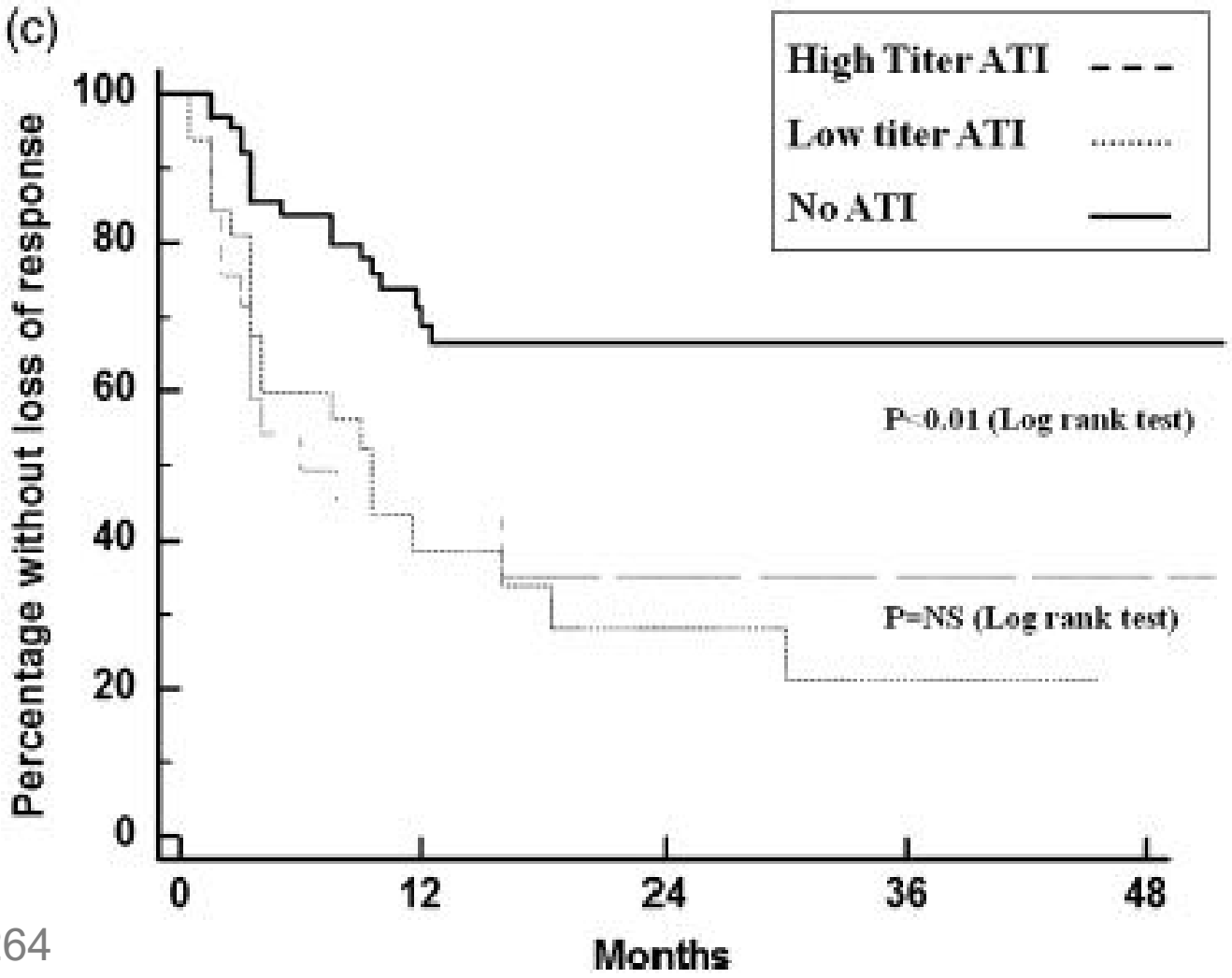
Dot plots illustrate data distribution



Ungar B, et al. Gut 2014;63:1258–1264

Loss of response to infliximab *vs*. ADA Titer category

Impact of ADA on sustainability of efficacy



Ungar B, et al.
Gut 2014;63:1258–1264

ADA specificity

Table 4: Incidence and specificity of ADA signals detected in clinical studies for pegfilgrastim biosimilar candidate, MYL-1401H

Adapted from Waller et al 2017

Category n (%)	MYL-1401H-1001 Healthy volunteers			MYL-1401H-1002 Healthy volunteers		MYL-1401H-3001 Breast cancer	
	MYL-1401H	EU-Neulasta	US-Neulasta	MYL-1401H	EU-Neulasta	MYL-1401H	EU-Neulasta
ADA positive	14 (22.2)	16 (23.5)	21 (30.4)	6 (26.1)	7 (29.2)	0	1 (1.8)
Specificity:							
PEG only	8 (12.7)	13 (19.1)	14 (20.3)	5 (21.7)	6 (25.0)	0	0
G-CSF only	0	0	0	1 (4.3)	0	0	1 (1.8)
PEG & G-CSF	6 (9.5)	4 (5.9)	6 (8.7)	1 (4.3)	2 (8.3)	0	0
Neither	1 (1.6)	2 (2.9)	2 (2.9)	2 (8.7)	2 (8.3)	0	0
NAb positive	2 (3.2)	0	0	0	0	0	0

Waller, C. et al. Blood, 2017, 130(Suppl 1), 3568

Safety endpoints for ISI

- ISS is the main location of the safety data
- Use ISI to summarise relationship of safety signals to
  - Treatment time-course
  - ADA positive *vs*. negative status
  - Coincident ADA titer
- Safety Signals to analyse by incidence & severity
  - All treatment-emergent AE's
  - Drug hypersensitivity & anaphylaxis
  - Infusion-related / injection site reactions
- Other endpoints:
  - Complement activation products, serum tryptase, cytokines etc.
  - Antigen-specific IgE
  - *Ex-vivo* basophil activation
- Discuss individual cases if there is an apparent relationship between ADA titer and severity of safety outcomes

ADA *vs*. Safety signals

Table X: Incidence of AESI in complete safety population up to week 56 by ADA positive *vs*. negative status and ADA titer quartile

Category	Number (% of category total) of subjects with	
	≥ 1 AESI	**0** AESI
Product XYZ, all treatment groups (N=600)	x (Y%)	x (Y%)
Patients with negative ADA result (N=*x*)	x (Y%)	x (Y%)
Patients with positive ADA results (N=*x*)	x (Y%)	x (Y%)
Patients with low (Q1) ADA titer (N=*x*)	x (Y%)	x (Y%)
Patients with high (Q4) ADA titer (N=*x*)	x (Y%)	x (Y%)
Placebo group (N= 200)	x (Y%)	x (Y%)

AESI = Adverse Events of Special Interest

Question-based approach to Risk Management

Table 6. Risk management plan	
Questions	
1.	Are there potential patient sub-populations to whom this product should not be administered?
2.	Under which circumstances might it be necessary to modify or stop treatment?
3.	What monitoring methods should be applied to investigate suspected host immune responses to the product?
4.	Is it necessary to collect additional data, eg from a patient registry, to monitor long-term risks in a wider population?
5.	What are the risks associated with off-label use?

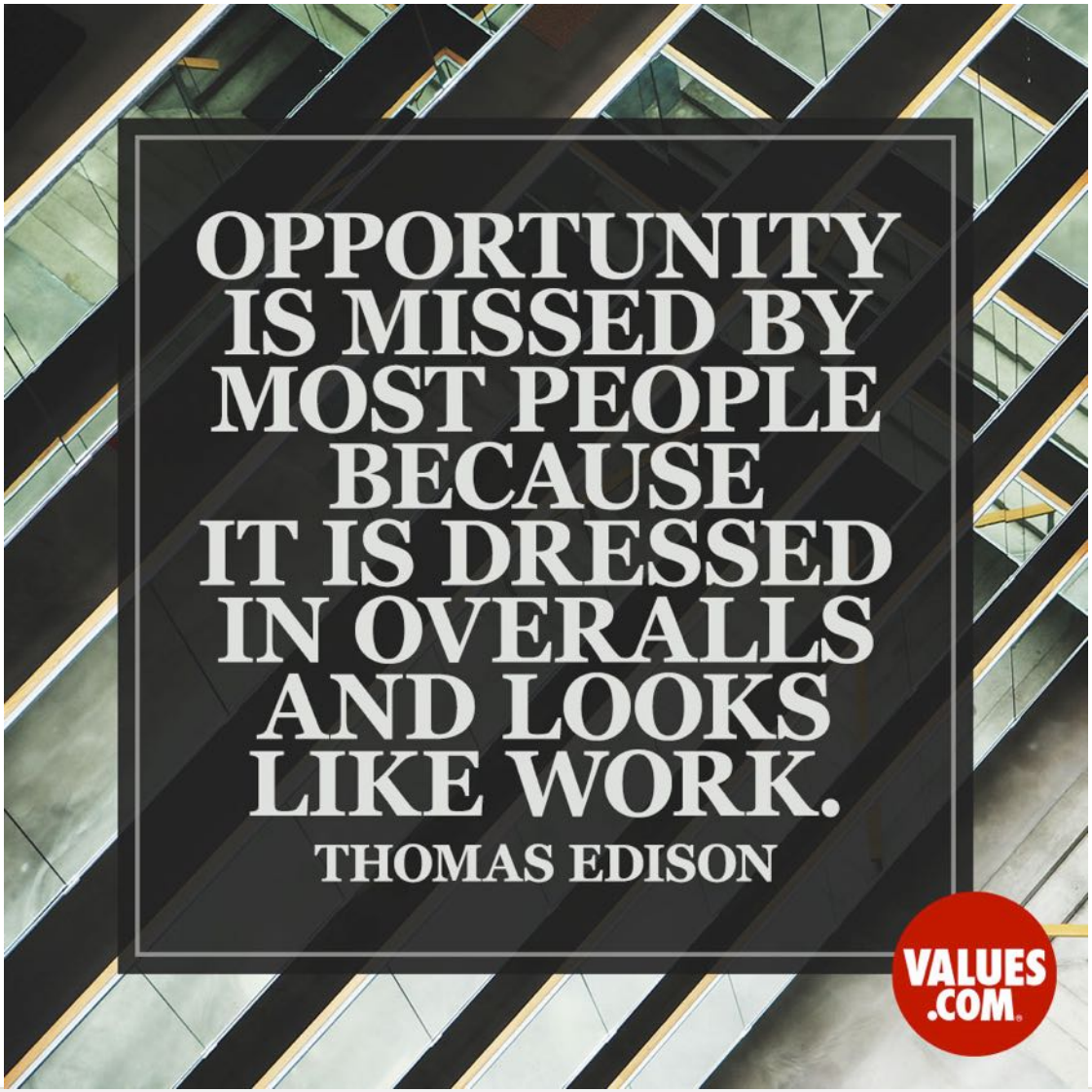
Chamberlain P. Addressing immunogenicity-related risks in an integrated manner. *Regulatory Affairs Pharma*, Jan 2011, 10-15

FDA recommendations#

A RISK-BASED approach is required to balance the potential harm with potential good of a new biotherapeutic throughout clinical development

- Likelihood of developing an immune response
- Risk of immune response to patient
- Are there therapeutic alternatives
- Reversibility of response

Slide prepared by João A. Pedras-Vasconcelos, PhD
Presented at CHI Immunogenicity Summit Short-course, Oct 2019



Home-work

- ***Is your Company exploiting the opportunities presented by the ISI to the fullest extent?***
- ***If not, what recommendations will you take back for discussion with your team?***

