# Rethinking Immunogenicity Testing Paradigm:
## Leveraging risk assessment outputs to support clinical development

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# Risk Assessment, Identification and Implementation

- Risk Factors
- Stages of Development
- Tools and Implementation

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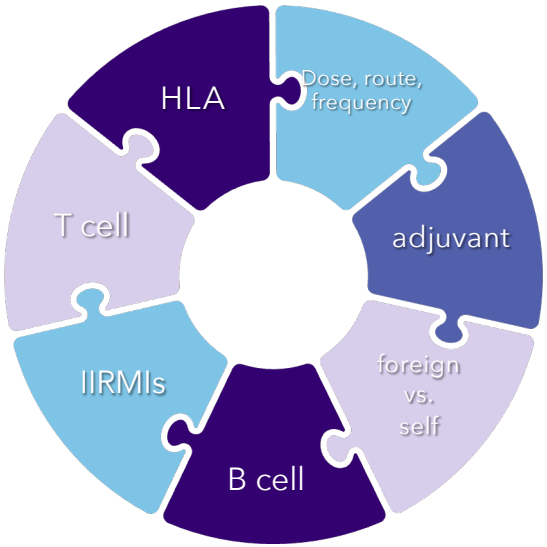
# Various Factors Impact Observed Immunogenicity




**Product**

AA sequence
Foreignness
Structure
Post-translational modifications
Aggregation
Impurities
Formulation buffers
Misfolding
Particulates
Degradation
Leachates
Excipients
Target/ MOA – i.e. target engagement with immune cells

**Treatment**

Dose
Route
Frequency
Duration

**Patient**

Genetic background
HLA
Disease/Immune history
Co-medication

- A comprehensive risk assessment considers all the above factors during early to late stage
- Risks should be addressed at each stage using a mitigation strategy
- A final risk categorization should be concluded and summarized in an Immunogenicity Risk Assessment (IRA) section of IND filing
- Risk categorization can inform development of risk-based bioanalytical strategy for clinical immunogenicity

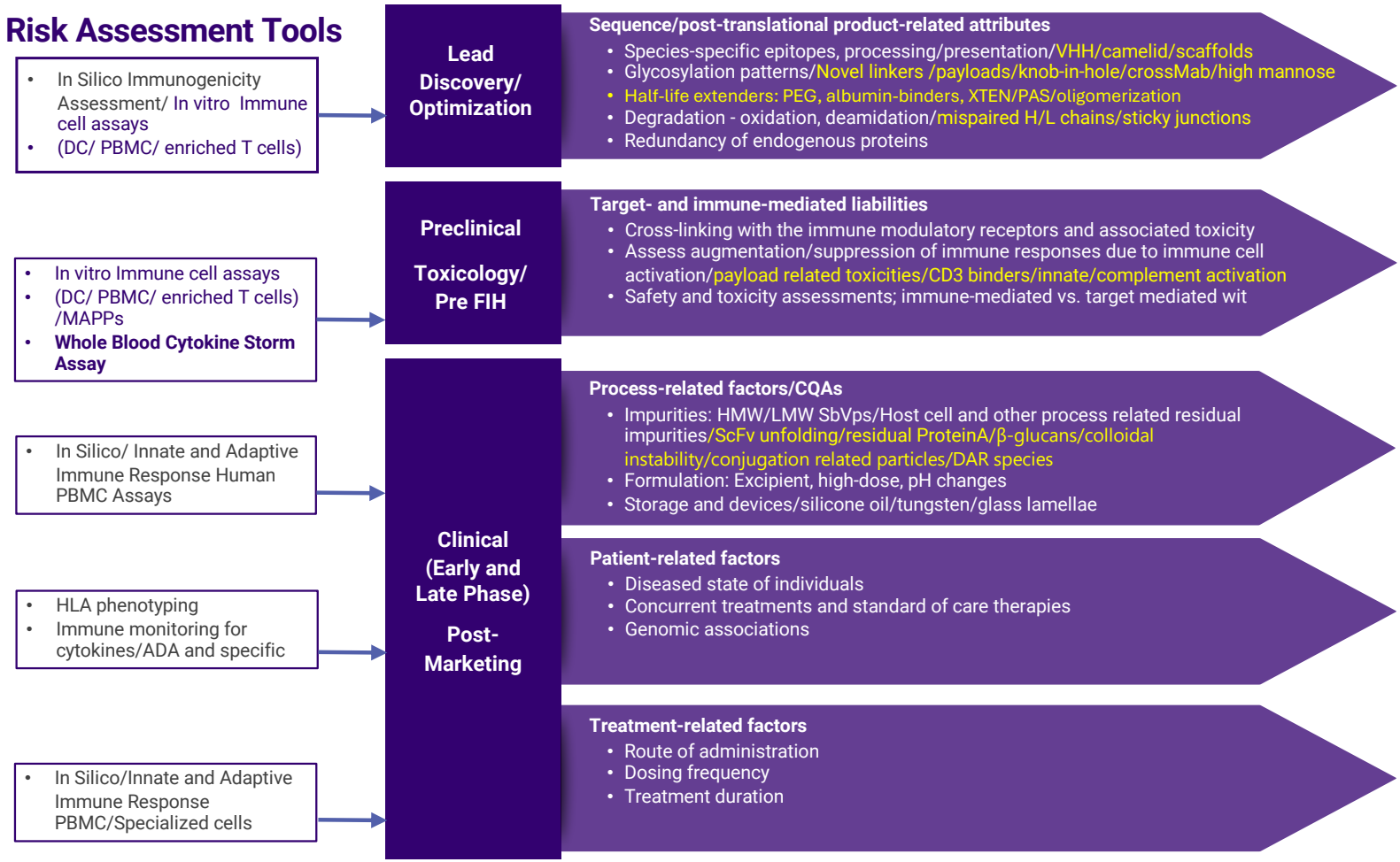
# When immunogenicity should be assessed throughout development

## Discovery to commercialization

**There are various immunogenicity risk factors that should be addressed throughout development phases.**

**Risk factors vary with modality of interest.**

**There are tools available to help characterize these factors at each stage.**

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# Key Steps in In Silico Immunogenicity Risk Assessment

## Workflow with ISPRI Toolkit Algorithms

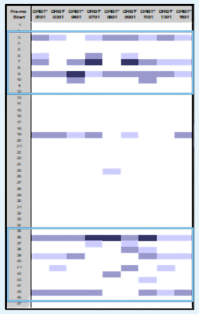
### Identify & quantify HLA ligands

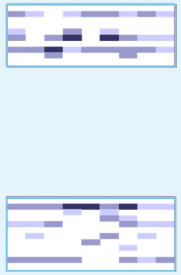
**EpiMatrix™**

GLOBAL REGIONAL

Class II (CD4)

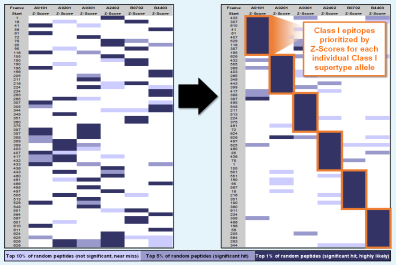
ClustiMer™





Class I (CD8)

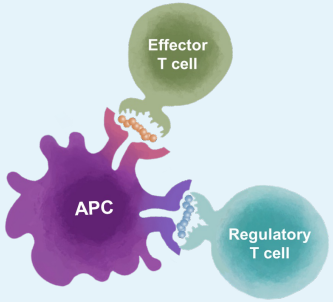
Staircase Report

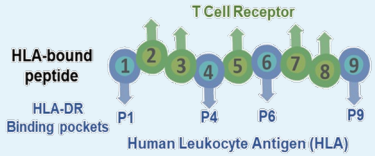



Epitope Density

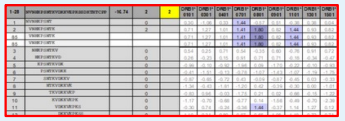
### Adjust for the humanness of epitopes

**JanusMatrix™**



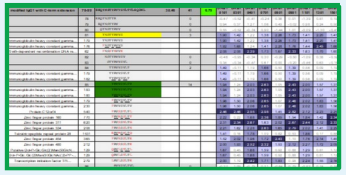




**Foreign** - Immunogenic



vs

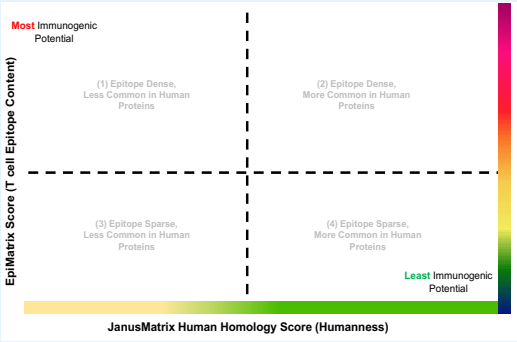
**Human Homology** - Tolerance



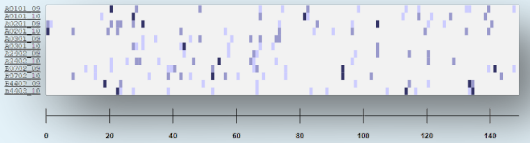
### Evaluation of immunogenic potential

**Bringing the concepts together**

Quadrant Plot




Foreign Epitope Map




**Consideration of Clinical Factors**

Clinical Context Analysis

# In Vitro Assay Overview

| | Cytokine Storm Assay | Innate Assay | PBMC Assay | DC:PBMC Assay | Bystander Activation Assay | MAPPS Assay | Specialized APC Assay |
|---|---|---|---|---|---|---|---|
| **Purpose** | Assess potential stimulation of excessive cytokine release | Assess cytokine release from innate receptor engagement | Assess innate and/or adaptive phase immune response | Probe deeper mechanistic pathways – where PBMCs fall short | Assess immune modulation potential by monitoring Teff suppression | Profile antigen processing and presentation | SC injection site risk modeling |
| **Modalities** | Peptides, proteins, complex biologics, etc. | Immune-cell engagers, modulators, impurities, viral vectors, nucleic acids | Peptides, proteins, complex biologics, impurities | Molecules with known immunomodulatory MOA | Molecules with immunomodulator MOA TBD | Peptides, proteins, complex biologics | All proteins and peptides / formulations / SC route of administration |
| **Input** | Whole blood | Whole blood, PBMC, monocytes, DCs, monocytic cell lines | PBMC | DC/PBMC co-culture | PBMC | PBMC | Specialized APCs such as Langerhans cells |
| **Incubation** | 1 day | 1 day | 1-7 days | 7-10 days | 3-7 days | 7-10 days | 7-10 days |
| **Readout** | Multiplexed cytokine analysis | Multiplexed cytokine analysis | Multiplexed cytokine analysis, T cell proliferation/ ELISPOT | Multiplexed cytokine analysis, T cell proliferation/ ELISPOT | T cell proliferation | LC-MS/MS | Multiplexed cytokine analysis, T cell proliferation |

MABS 2022, VOL. 14, NO. 1, e1993522

# Scoring of clinical immunogenicity risk factors

| Factor | Relative Ranking of Risk (low to high) |
|---|---|
| Primary amino acid sequence | Non-activating sequences < T cell activating sequences |
| Modality | mAb < fusion protein < endogenous protein replacement |
| Mechanism of action | Immune antagonist < Immune agonist |
| Population | Immune suppressed or co-dosed with immunosuppressant < autoimmune |
| Quality attributes | Monomeric proteins < oligomers or aggregates non-modified proteins < degraded or modified proteins |
| Host cell proteins | Mammalian CHO < bacterial/yeast/viral |

Factors were ranked from lower to higher risk with the higher rank associated with more likelihood for being immunogenic.

*mAb: monoclonal antibody; CHO: Chinese Hamster Ovary

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# Risk-based bioanalytical strategies for clinical studies

| Risk | Bioanalytical Strategy | Clinical Strategy |
|---|---|---|
| **Low** | • Minimal sampling during early and late phase studies<br>• Collect and hold approach<br>• ADA assessment with a binding ADA assay<br>• Monitor for impact on exposure, efficacy, and safety<br>• NAb assay; leverage the PKPD strategy and early clinical data to trigger additional NAb assessments | Low/none |
| **Intermediate** | • Frequent sampling in early studies<br>• Less sampling in later development<br>• ADA assessment with a binding ADA assay<br>• NAb assay developed for Phase 3<br>• Monitor for impact on exposure, efficacy, and safety | Monitor for endogenous protein neutralization and any impact due to functionality |
| **High** | • Frequent sampling through all stages<br>• Rapid turnaround of sample analysis to get ADA related information in real-time for managing any adverse events<br>• ADA assessment with a binding ADA assay<br>• Characterize ADA response: cell based NAb assays, domain mapping, isotyping<br>• Monitor for impact on exposure, efficacy, and safety | Monitor for endogenous protein neutralization and any impact due to functionality |

ADA: antidrug antibody; NAb: neutralizing antidrug antibody, PK: Pharmacokinetics; PD: Pharmacodynamics

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# Immunogenicity Risk Assessment

## Key components of a risk assessment strategy document

| Section | Topic | Rationale |
|---|---|---|
| Brief background | Structure of the drug (therapeutic protein, mAb, nanobody, bispecific etc.) | Provide context for understanding risk of immunogenicity. |
| | Target and indication | |
| Risk assessment | Sequence-based risk | Identify amino acid sequences which are likely to bind and activate a T-cell response. |
| | Mechanism of action-based risk | Discuss if drug mechanism of action may result in immune stimulation or suppression. |
| | Population-based risk | Discuss if specific populations are likely to have different ADA incidences. Potential HLA genotype associations with immunogenicity. |
| | Quality attribute-based risk | Discuss if there are any product structural variants, process related impurities, or formulation changes that could influence immunogenicity. |
| | Description of estimated half-life, dosing paradigm, and sampling scheme | Knowledge of drug concentrations over time informs how long drug is present to initiate an immune response and also when drug concentrations may be high enough to interfere with ADA assay. |
| Immunogenicity Strategy | Description of how rapidly the immunogenicity data would be shared . | Discuss plans for frequent monitoring vs. infrequent monitoring vs. storage of samples until end of trial. |
| | Description of bioanalytical assays | Describe assays for binding ADA, domain specificity, and or isotyping. |
| | Titer vs signal/noise | Describe magnitude of immune response |
| | Description and timing of NAb assay | Discuss plans for NAb assay. |
| Clinical Results | Immunogenicity results from clinic (if available) | Provide clinically observed ADA incidence and effects. |
| Conclusion | Summary | Summary of most relevant concerns and conclusion. |

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V. Jawa et al. / Journal of Pharmaceutical Sciences 111 (2022) 960–969

# A Risk-Based Framework for Interpreting Immunogenicity Signals

### Top — Highest Risk

Loss of efficacy or serious immune-mediated safety events

- neutralizing antibodies
- hypersensitivity reactions
- anaphylaxis

### Middle — Moderate Risk

Changes in drug exposure or pharmacology

- altered pharmacokinetics
- reduced target engagement

### Lower — Limited Risk

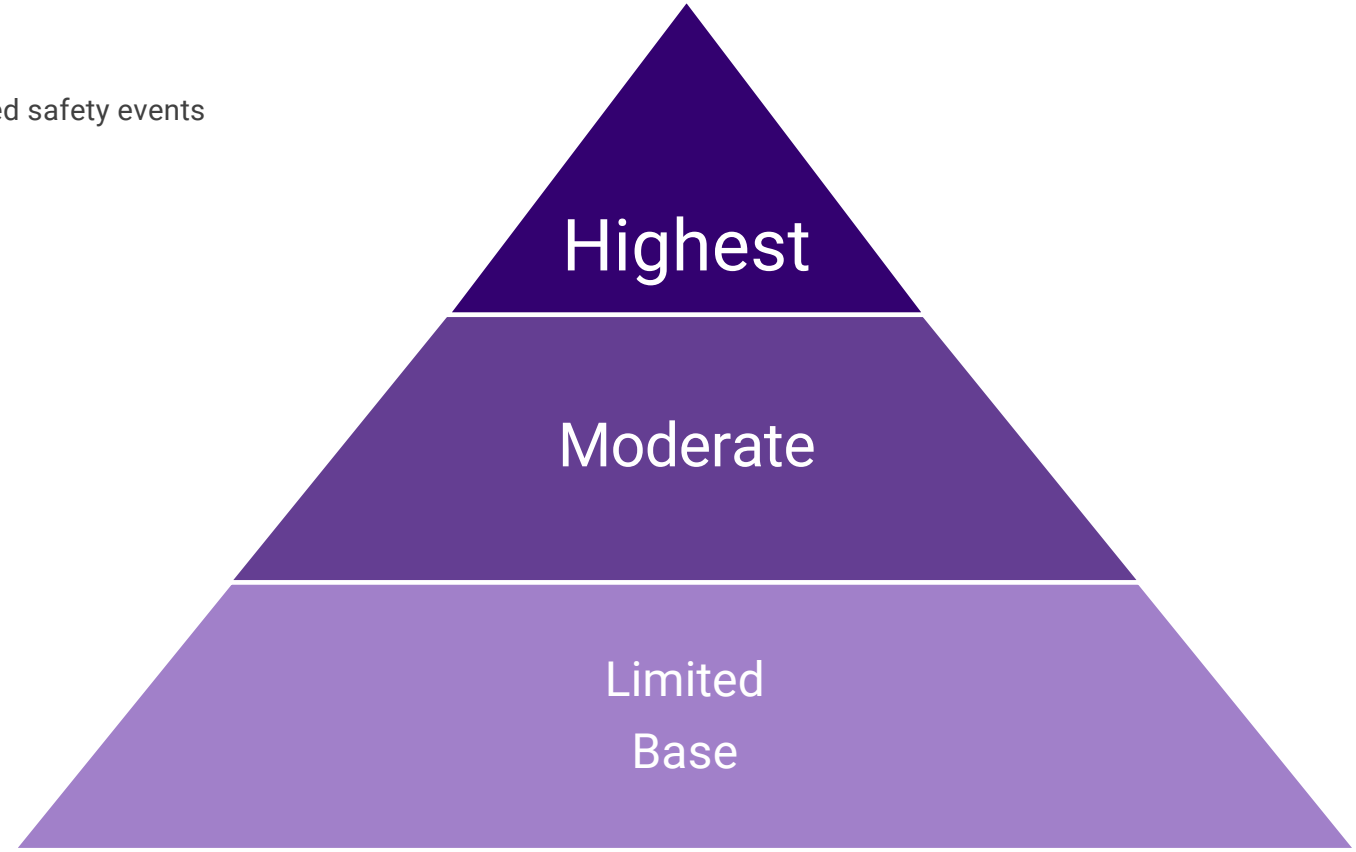
Presence of ADA without functional impact

- transient antibodies
- low titer responses

### Base — Minimal Concern

Analytical detection without clinical signal

- low-level ADA detection
- trace host cell proteins

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Immunogenicity findings can be categorized by **increasing levels of clinical concern**.

# Why ADA Incidence Alone Is a Poor Predictor of Clinical Risk

## Two contrasting scenarios:

| Scenario | ADA Incidence | Clinical Impact |
|---|---|---|
| Drug A | 40% | No effect on PK, efficacy, or safety |
| Drug B | 5% | Neutralizing antibodies causing loss of efficacy |

ADA Incidence Does Not Equal Clinical Impact
ADA incidence is often reported as the primary immunogenicity metric, but incidence alone does not predict clinical risk.

### Factors that determine clinical relevance

*Not all antibodies are equal. Important characteristics include:*
Neutralizing vs non-neutralizing antibodies
•Antibody persistence
•Antibody titers
•Impact on pharmacokinetics (drug clearance)
•Association with hypersensitivity reactions

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# Perspective on the 3-Tiered Paradigm

Lauren Stevenson

CSO

Immunologix

# Spoiler Alert: It's all biomarkers!

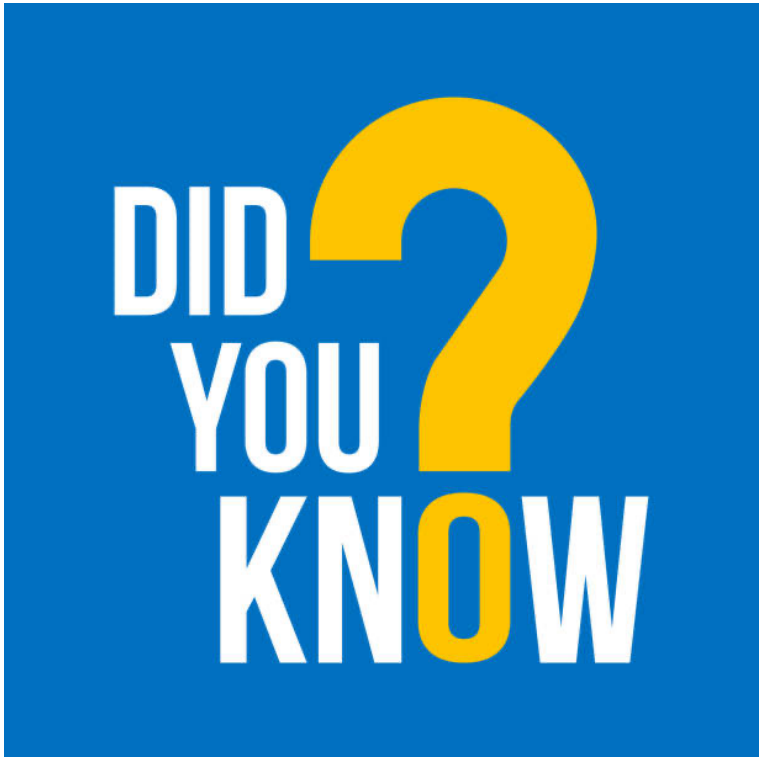
- According to BeST a biomarker is a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or *biological responses to an exposure or intervention*, including therapeutic interventions
- Immunogenicity is a biological response to a therapeutic intervention
- PK (measurement of drug concentration) is a biomarker of drug administration
- Context of Use (COU) needs to be understood in each case to ensure the assay meets the needs of the program (e.g. PK assay sensitivity is dictated by the drug program and specific study needs – it is not dictated by guidance)
- Similarly, one-size-fits-all guidance cannot adequately address the varied COUs for immunogenicity testing

# Perspective on the 3-tiered paradigm – in context

- In the beginning…when biotherapeutics were new… routine, prospective ADA testing was often limited or reactive
  - Only explored if unexplained loss of efficacy or other clinical impact was observed

- Immunogenicity gained notoriety when alarming safety events occurred with what we now understand to be a high-risk scenario (EPO)
  - An *unusual spike* in cases of pure red cell aplasia (PRCA) caused by Nab that eliminated both exogenous and endogenous EPO activity

# Historical Perspective




- From 1989 - 1998, PRCA was extremely rare in EPO-treated patients with only a handful of cases worldwide (1/10,000 patient years)
- But from 1998 - 2002 hundreds of cases were reported, particularly in chronic kidney disease patients
- Investigations concluded the cause of increased immunogenicity to be due to formulation and drug handling changes

# Unfortunately, a Slippery Slope Fallacy was born…

Although the initial risk was narrow and well defined…

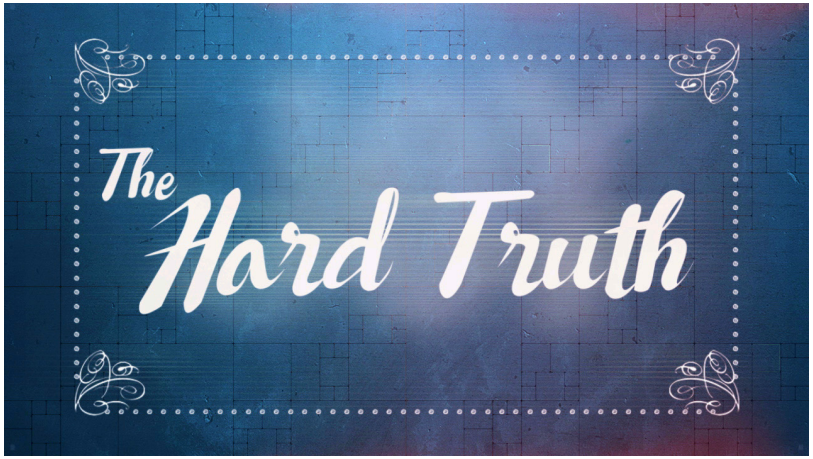
We invoked a slippery slope fallacy

- Reality: Severe immunogenicity happened once with a high-risk product under high-risk circumstances
- What if… severe immunogenicity could happen in all biologics?
- Slippery slope conclusion: We must test all biologics the same way to play it safe!


This faulty logical leap ignored all the specifics that defined the risk

# What happened?

- Mandatory, blanket immunogenicity testing became the norm, regardless of true risk profile of the biologic = NOT RISK-BASED
- The current testing paradigm was born out of an understandable, but fear-driven reaction to safety events caused by a high-risk product in very specific circumstances



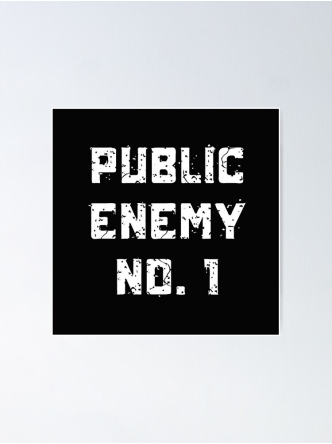

The current paradigm was NOT designed to build an understanding of clinically relevant immunogenicity for all biotherapeutics…

# More bad news…

Although the current paradigm was built with the intention to ‘not miss anything’ its design is flawed…because we did not work from First Principles

- We did not consider the underlying principle that immunogenicity responses are biological responses = biomarkers
- We therefore did not leverage what could have been learned if we’d looked to successfully implemented safety biomarkers, diagnostic biomarkers, PD biomarkers…
- We simply looked where we believed how to measure immune response magnitudes was already understood (vaccine titers)

# Reasoning by Analogy missed COU




Reasoning by analogy is dangerous because it feels like we are leveraging our prior knowledge and experience, but it tempts us to not think it through…

Where did it land us?
Three tiers of analysis = slow & expensive (and not FFP!)
BECAUSE…Context of use for drug development not addressed!


Titers are designed to measure large responses that do not require fine precision while COU for drug development requires:

- Understanding of low-level responses, especially for high-risk molecules as well as…
- Characterization of the full landscape of response development & magnitude that allows correlations with clinical impact to be determined

# Cut points create a false binary flaw

- Cut points cause us to ignore and discard data
  - By making a binary call of positive or negative, and focusing only on ‘positives’ we lose biological context and any/all insights the totality of screening data could tell us
- Cut point censored data sets confound study data interpretation and communication
  - Prevents analysis of full response profiles - distinguishing biological variability from low-level, irrelevant responses from meaningful ADA development
- Cut points create misalignment with clinical risk
  - Inflated incidence does not correlate with clinical impact and fuels organizational angst
  - Ability to correlate response magnitude with clinical impact is compromised

The stark truth is:

Cut points cause us to discard nuance, inflate incidence, and mislead ourselves, our stakeholders, clinicians, and regulators

# Fear led to foundational flaws

Fear caused our failure to think like scientists

We abandoned first principles and instead…

- **Slippery Slope Fallacy** got risk assessment wrong (all biologics ≠ high risk)
- **Reasoning by Analogy** got the testing paradigm wrong (titers are not fit-for-purpose)
- **False Binary Flaw** of CPs blinds us to contextual data that inform clinical relevance
- **Context of Use for drug development is not being met**

## So Now what?

3-tiered paradigm

# Risk-based strategies revisited

# The Starting Point: Context of Use

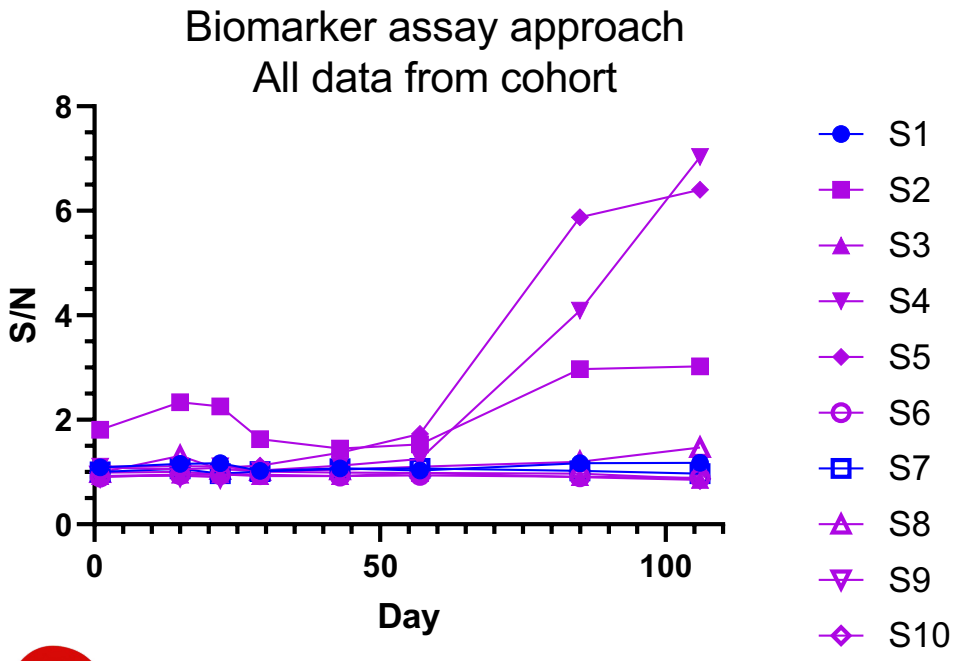
- The broad COU for drug development is to identify and understand *clinically relevant immunogenicity* (not ALL potentially detectable responses)
- The specifics of COU will be dictated case by case – defining clinically relevant ADA for a specific therapeutic program = discovering a new biomarker
- Ask yourself: What is clinically relevant for my program?
  - Is there a safety risk from low-level responses?
  - What risks are associated with high level responses?
  - Consider exactly what data set will add value (ie. inform program decisions, study interpretation, patient safety & efficacy) versus data that will add no value or confound interpretation
- Then design your assay (not 3 tiers of assays) to meet COU
  - What sensitivity and drug tolerance is appropriate to reliably detect clinically relevant responses?
  - What range of responses need to be captured?
  - Other product specific considerations…
- And then use all the data!

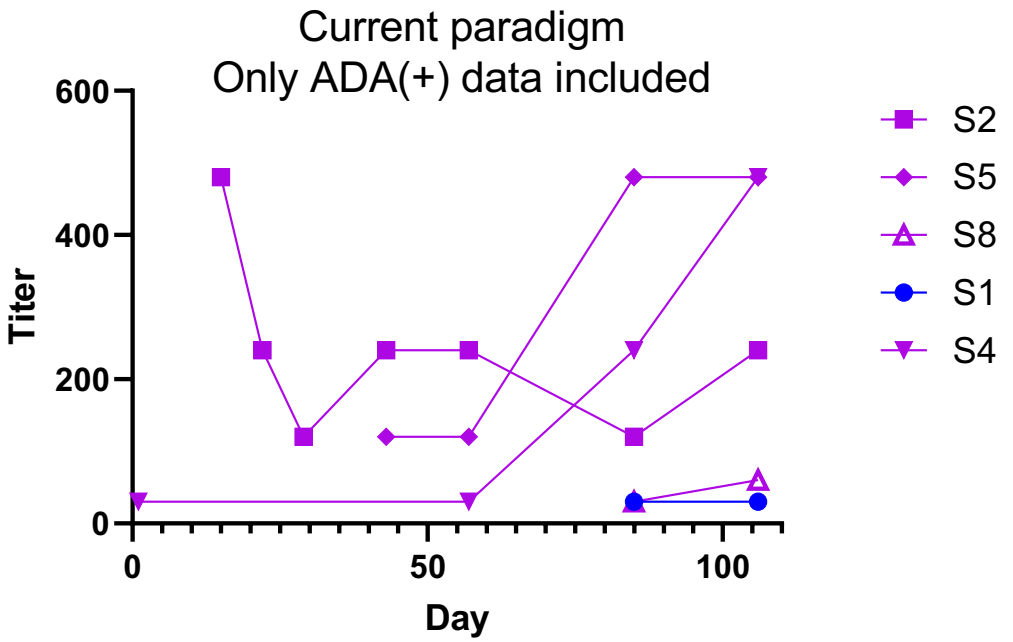
# One tier, many truths

# Tier-free, data rich

- Appropriately designed (single-tier) immunogenicity biomarker assays provide greater insights
  - Biological context – biological variability is distinguished from true biological responses
  - Full profiles provide insight into response development and magnitude
  - Continuous response data (S/N) enhance ability to correlate responses with clinical relevance
  - Complete, transparent data sets are intuitive to interpret and communicate to stakeholders and regulators
- No fear of missing anything – because ALL the data are included
  - Especially important for high risk!

# Which picture is worth 1000 words?




Why would we spend more time, money and resourced to see only a subset of the data?
Why would we not want to see the data in context?
How would we explain our choices to patients?

# Discussion Points

# Discussion Points

- Rethinking assumptions
  - Goal of immunogenicity assessment?
    - To identify and characterize clinically relevant responses…not to identify every molecule of antibody that may be in a samples
  - Titers are not gold standard for understanding the full landscape of response magnitudes (low level responses most important for high risk)
    - Titers are designed to measure magnitude of large responses (good precision not required; e.g 4x)
  - Cut Points do not ensure nothing is missed; nor do they ensure accurate identification of true positives – they routinely cause biological variability to be labeled positive
    - They cause us to discard contextual data that inform the relevance of immunogenicity responses
- Sponsors will increasingly submit data in new formats:
  - For protein therapeutics where alternative datasets will more meaningfully assist in interpreting clinical information
  - For novel modalities where the traditional approach is not appropriate
- Can we move to a true risk-based approach where risk assessments directly inform and justify the bioanalysis plan for immunogenicity in clinic (customized for COU)?

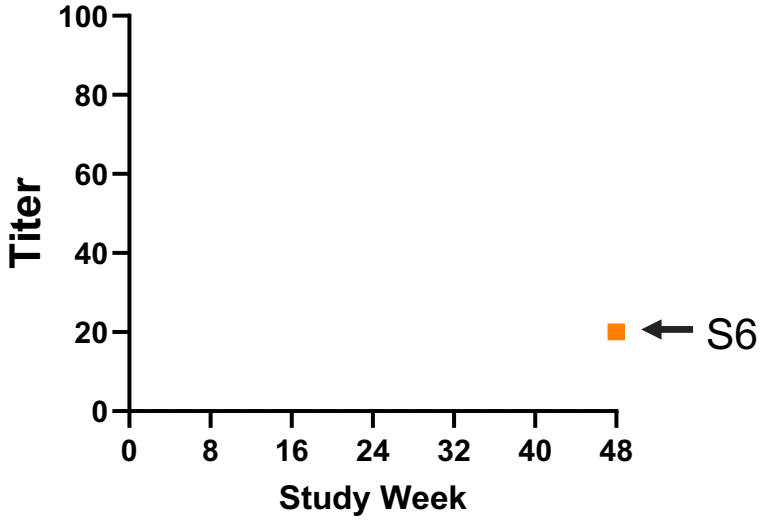
# Thank You!

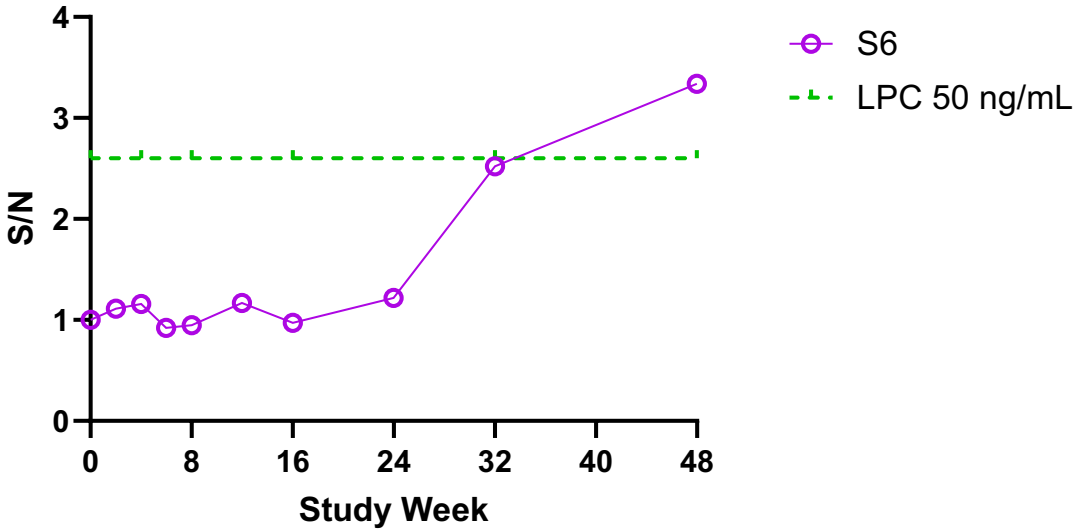
#BeAScientist

# Illustrative Examples

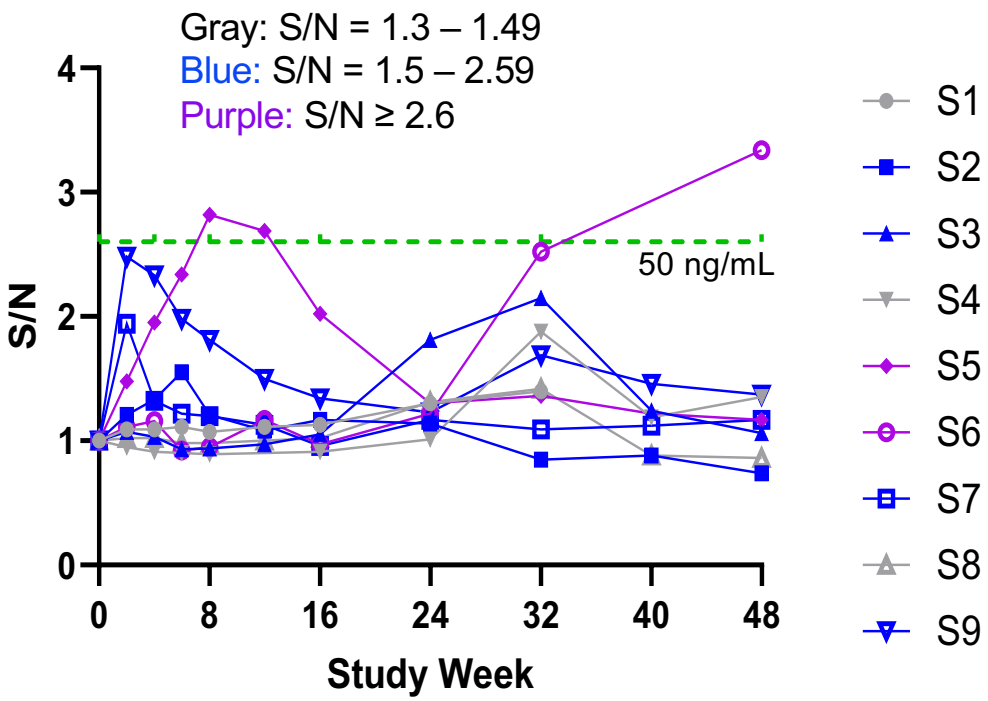
# Single-Tier S/N Analysis – depth and clarity of information

**PH2 IMMUNOGENICITY**



# No CP: ADA profiles with S/N ≥ 1.3 at any time point




## PH2 IMMUNOGENICITY CONCLUSIONS

**Traditional approach:**

- A single positive subject with low titer (MRD) was detected; no clinical impact

**Biomarker approach:**

- 9 patients had S/N responses ≥1.3 at any time point
- Of these, 2 had S/N > the 50 ng/mL PC (>2.6)
- All responses returned to near baseline by EOS except for Patient 6 whose response increased from week 24 to EOS with no impact on PK noted
- Overall, no clinical impact of immunogenicity was observed

# Example: Low-risk molecule

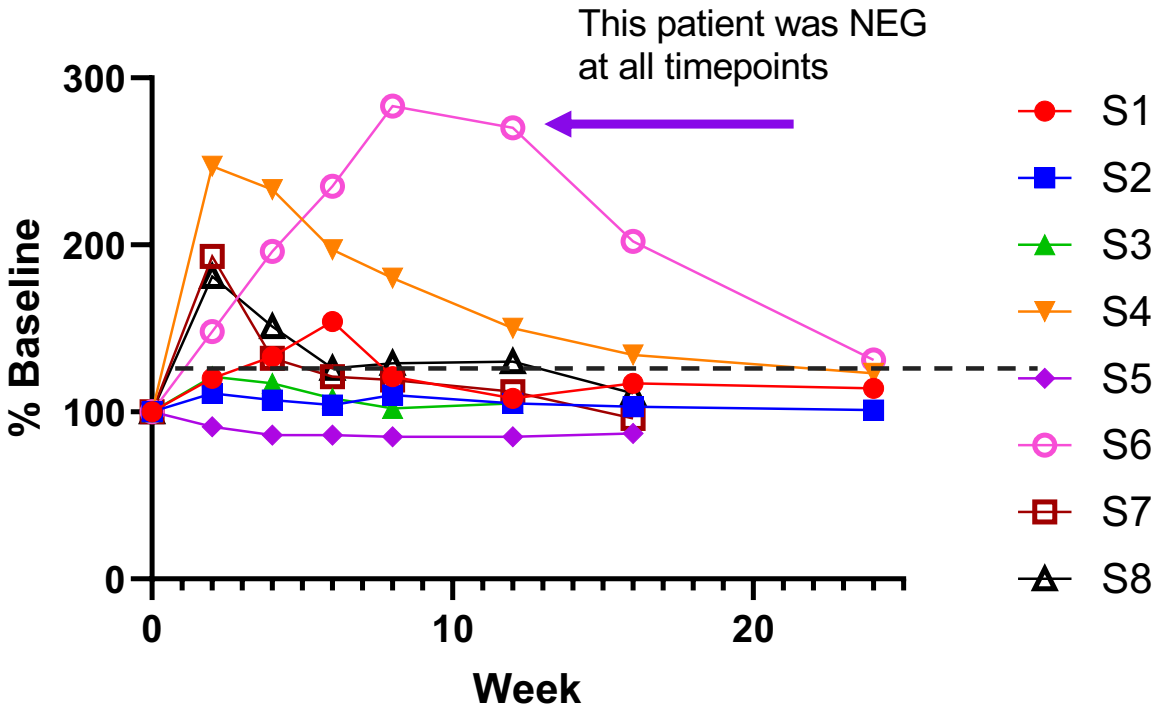
## Clinical Study Context of Use for a low-risk molecule:

- Identify clinically relevant ADA - clinical impact will be on drug exposure
- No safety concerns with low levels of ADA, but impact of high-level ADA should be monitored

## Study Results (3-tiered paradigm): No confirmed positives

- Potential regulator question – was something missed?

# Reviewing all the data – No Cut Point




3-Tiered Paradigm: No confirmed positives…

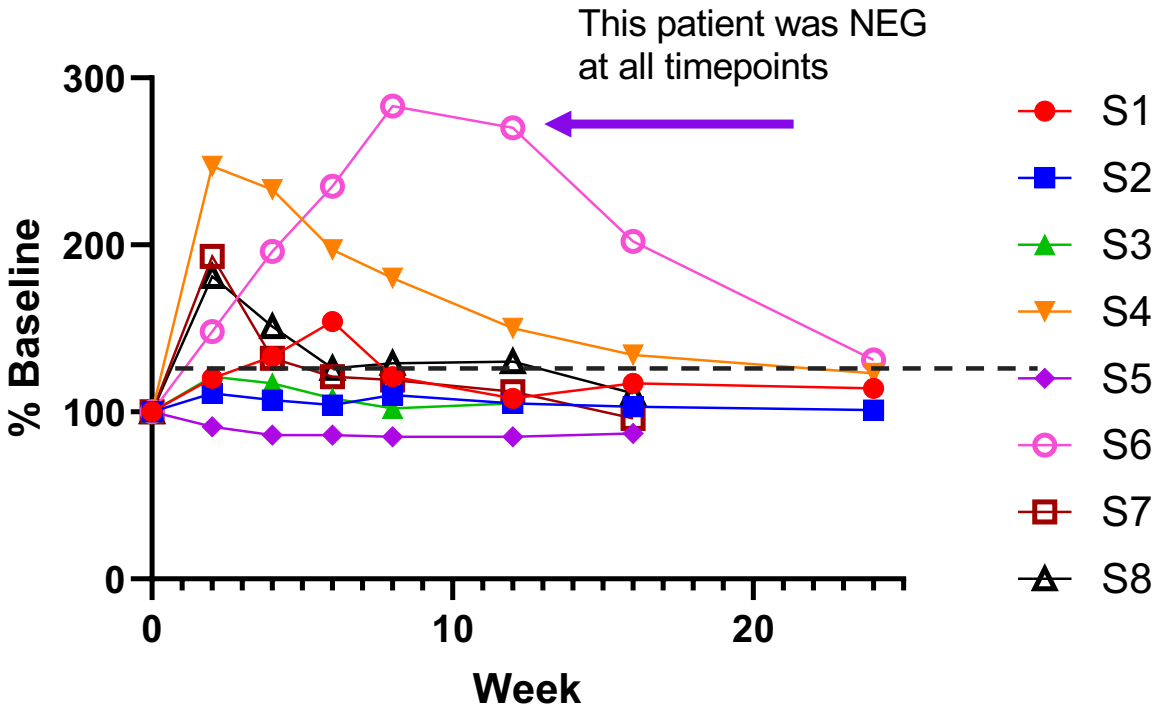
Profiles of all subjects that had ≥ 50% increase in S/N at any time point

Conclusions:

- *Low level response profiles were observed in 5 patients*
- *Maximum response magnitudes were all < response level of the 100 ng/mL PC*
- *Responses returned to near baseline levels by EOS*
- *No clinical impact*

S/N analysis demonstrates that the assay is sensitively detecting low level response profiles. Nothing was missed!

# Consider for a high-risk program…




3-Tiered Paradigm: No confirmed positives…

Wouldn't we want to see all the data and catch any hint of response as early as possible?

**NOTE:**
Cut points cause both false positives and false negatives = real risk