Immunogenicity Risk Assessment: Enabling Quality by Design in Therapeutic Protein Discovery and Development

Tim Hickling, PhD, Quasor

Working towards a Quantitative Assessment of Risk

Agenda

01	IRA for business decision making
02	Reintroducing ‘Quality by Design’
03	‘Early IRA’
04	Profile optimisation using NCIRA toolkit
05	Holistic immunogenicity prediction

The IRA - When and Why?

Every stage of discovery and development benefits from an IRA

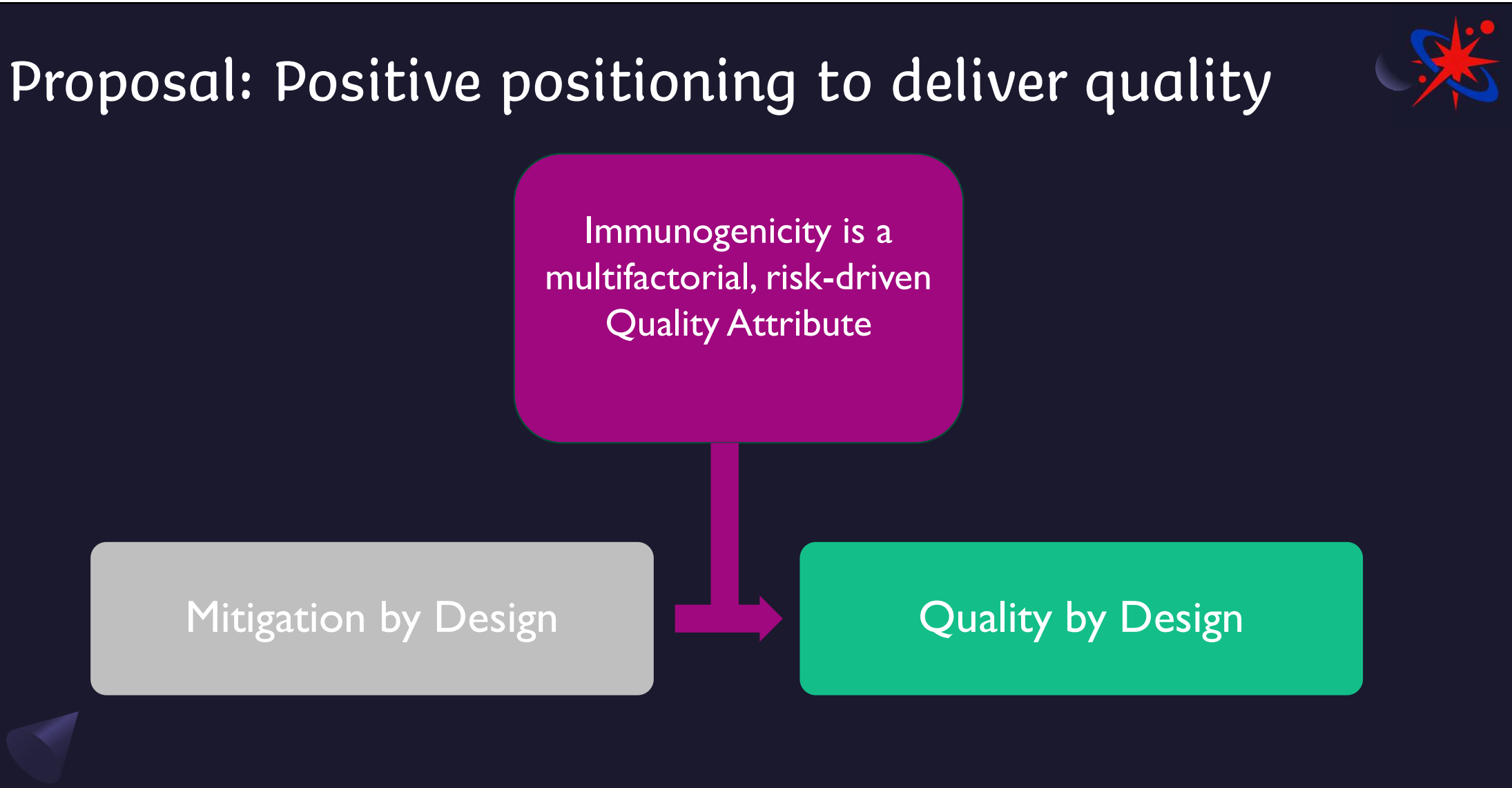
Waiting for a 'regulatory need' is a missed opportunity for making medicines

- **Project start – set expectations for challenges and give opportunities for mitigation by design**
- **Selecting hits – positions *in silico* analysis to pick the leads**
- **Lead development – enables criteria for defining what will be good enough in cellular assays**
- **Candidate selection – ranking of leads to choose the optimal candidate profile**
- Phase I – Bioanalytical strategy to understand onset and magnitude of immune response (not just ADA)
- Phase II – Identify process and treatment opportunities to manage risk profile
- Phase III – Optimize testing strategy to understand immunogenicity's impact on Benefit:Risk profile
- Phase IV/market – Additional study or PV data to maintain accurate dosing and safety information

Product optimisation makes good commercial sense

IRA used to define the effort needed to optimize immunogenicity profile

Proposal: Positive positioning to deliver quality



QbD framework fits early immunogenicity mitigation

Quality Target Product Profile (QTPP)

i. Predictable immunogenicity profile across populations
ii. No clinically meaningful impact on PK/PD and no loss of efficacy
iii. No hypersensitivity or immune mediated toxicity

Continuous improvement during product lifecycle

Critical Quality Attributes (CQAs) of DS/DP

i. Developability
ii. Sequence liabilities
iii. Post translational modifications
iv. Impurities
v. Contaminants

✓ Immunogenicity tools and studies include prior knowledge, risk assessment, mechanistic models, design of experiments (DoE) and data analysis, and process analytical technology (PAT).

Developability for biologics is good for reducing immunogenicity risk

Table 1. The developability assays evaluated in the competition dataset (adapted from [6])

Biophysical or Biological Property Measured	Developability Assay	Readout	Developability Relevance
Expression yield	Titer	mg/L	Composite measure of antibody secretion, folding and correct chain association
Temperature induced domain unfolding	nanoDSF, DSF-SYPRO	Tm2	Indicative of real-time and accelerated storage stability
Surface hydrophobicity	HIC	Retention Time (RT)	High RT indicative of increased risk of aggregation and nonspecific binding
Self-association	AC-SINS	Δλmax	Propensity for aggregation, poor colloidal stability and increased viscosity
Binding to CHO lysate	Polyreactivity	PR Score	Propensity for nonspecific off-target binding to non-antigen species. Potential impact to pharmacokinetics (PK)

van Niekerk L *et al* 2026 - **Ginkgo Datapoints Antibody Developability Competition**

'…available datasets are too small and heterogeneous to support robust, assay-spanning prediction.'

Mapping Immunogenicity risks onto current developability measurements

- Expression yield – higher production can reduce contaminants, whilst increasing impurities
- Temperature stability – possible surrogate for unfolding rate in APCs
- Hydrophobicity and self association – aggregation risk
- CHO lysate binding – non-specific binding for adjuvant effect

Adding sequence risk assessment and mitigation to developability saves the need for deimmunization

Earliest risk assessment can be made with three simple questions:

- What's the target?
- Who are the patients?
- What modality will be used?

Answers to these questions may hide complexity, so a critical thinking approach is needed

Project specific details captured from the start gives the opportunity for earlier action

1	**Idea to Candidate**			
		Answer	Risk for induction	Risk for impact
	Target			
1.1	What is the target or targets?			
1.2	Is the target involved in immunity?			
1.3	Is the target present on immune cells?			
1.4	Does the target form higher order structures			
1.5	What is the proposed mechanism of action?			
1.6	Is the MoA likely to modulate the immune response?			
1.7	For non-Ig proteins, is there an endogenous counterpart to the molecule?			
1.8	What are the similar therapies in development and what is known about their immunogenicity risk?			

- Integrating data allows for an assessment of risk for inducing ADA or for the impact of those ADA.
- Confidence in the assessment increases with information and certainty of each answer

As project progresses, additional data helps to refine the assessment and supports ongoing decision making

	Product	Answer	Risk for induction	Risk for impact
1.9	Non-human content			
1.10	For human proteins, which SNPs are reported?			
1.11	In silico post-translational modifications			
1.12	What is the risk associated with pre-existing ADA?			
1.13	Screening for pre-ADA indicated (e.g. novel format)?			
1.14	In vitro risk assessment			
	Assays performed and outcomes:			
	- in silico			
	- DC activation/internalization assay			
	- T cell assay(s) - peptide or whole protein			
	- MAPPs			
1.15	Additional mechanistic studies (e.g. in vivo model)			

- During discovery and lead optimisation phases, assessments for multiple molecules will be needed.
- Data availability depends on immunogenicity workflow
- Comparative assessments are more realistic than setting thresholds that anticipate clinical outcomes.

Product risks occur due to choices made from initial idea...

IRA application during discovery offers opportunity to change course on immunogenicity

1. Target
   - Many biologics antagonise receptor-ligand interactions. An immunogenicity target risk assessment can inform choice of whether to target the receptor or the ligand.
   - EIP recommendation mentions increased risk for targets on APCs, however, target presence on other immune cells can also increase risk
   - Lack of target redundancy might pose a safety risk

Target Risk
Agonists targeting the immune system

Risk Rank	Class	Mechanistic immunogenicity risk rationale	Example	Clinical impact (excluding safety)
1	Receptor agonists on immune cells	Strong APC/T-cell activation, cytokine release, costimulation, often engineered/multimeric formats	Cibisatamab (TCE)	Loss of exposure and efficacy
2	Engineered cytokine super-agonists	Enhanced receptor clustering, altered PK, non-native epitopes, strong immune activation	PD-1-IL2 variants	Loss of exposure and efficacy
3	Cytokine receptor Blockers (antagonists)	Bind receptors on immune cells but usually dampen signaling; standard IgG scaffolds	Satralizumab	Loss of exposure and efficacy in subset of patients
4	Cytokine-neutralizing antibodies (antagonists)	Neutralize soluble cytokines, often reduce inflammation; human/humanized IgG;	Adalimumab	Loss of exposure and efficacy in subset of patients
5	Endogenous hormone agonists	High homology to endogenous proteins, non-immune targets, chronic dosing → tolerance	Liraglutide	Limited clinical impact

Receptor vs ligand

IRA application during discovery offers opportunity to change course on immunogenicity

Receptor targeting risks	Ligand targeting risks
Target often on activated immune cells	Reduce immune cell activation
Drug binding can enhance activation	
Enhance drug uptake and presentation	Drug presentation can be mitigated, e.g. single arm Ab

Receptor	Ligand
IL4R – AMG317 (Ph2), Dupixent	IL4 (none) and IL13 - Lebrikizumab
IL21R – ATR107 (Ph 1)	IL21 - NNC0114 (Ph2)
TNFSF – targets membrane bound and soluble – differential epitope targeting?	

Product risks occur due to choices made from initial idea…

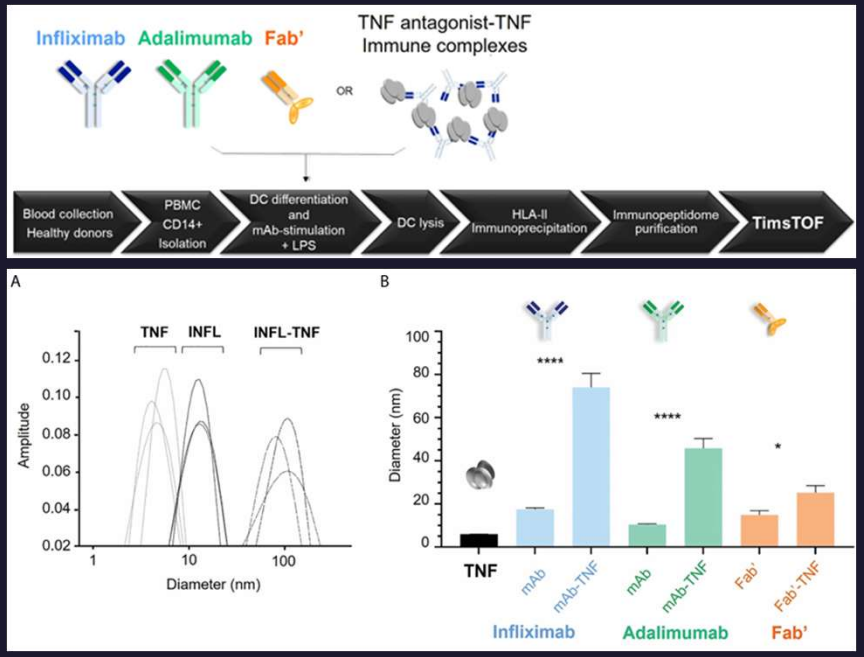
IRA application during discovery offers opportunity to change course on immunogenicity

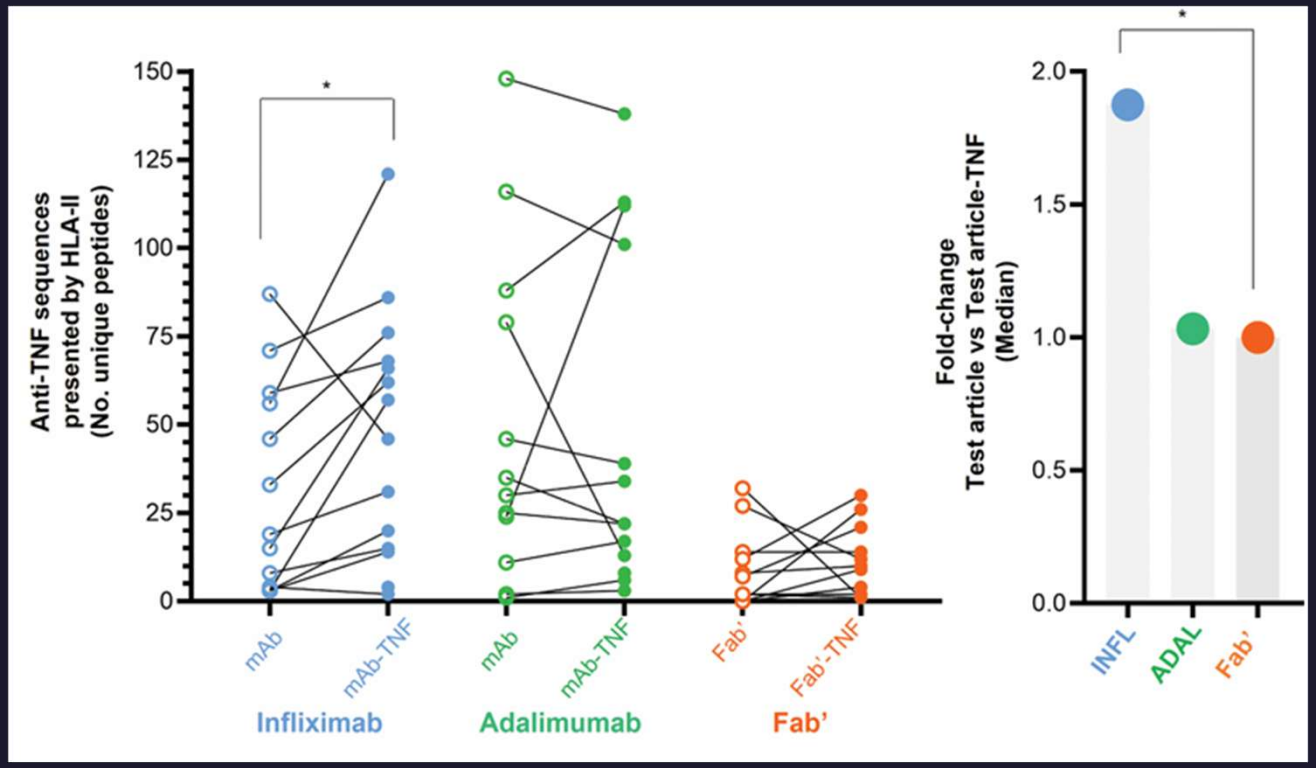
2. Mechanism of action (MoA)

- Can be exerted on targets in different ways, e.g. soluble versus transmembrane TNF engagement by TNFi (Ubah OC *et al* 2025)
- Choice of epitope can mitigate the risk of complex formation
- Fc modifications can alter immune cell interactions with consequences for induction or impact of ADA on safety (case-by-case)

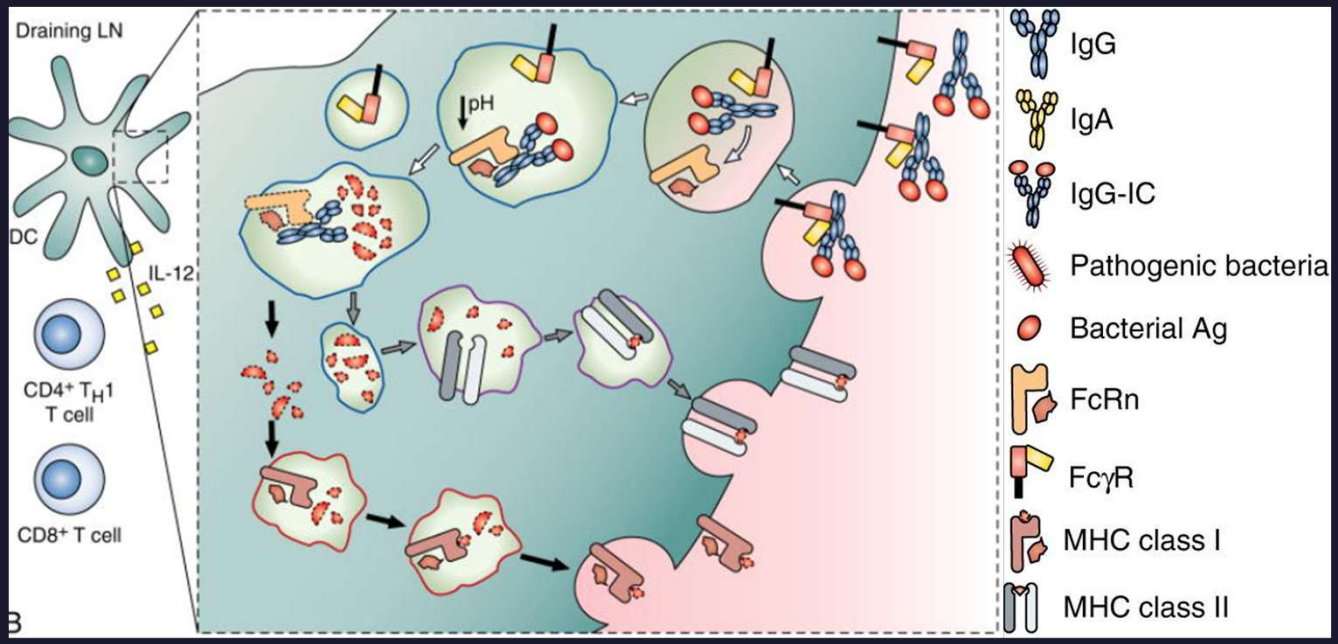
Format risk: drug-target complex formation
Example 1 - Trimeric soluble target

- Drug-target complexes can contribute to enhanced presentation on APCs
- IRA can signal early assessment via uptake/presentation assay
- Derisk: MAPPs (Casasola-LaMacchia A 2022)

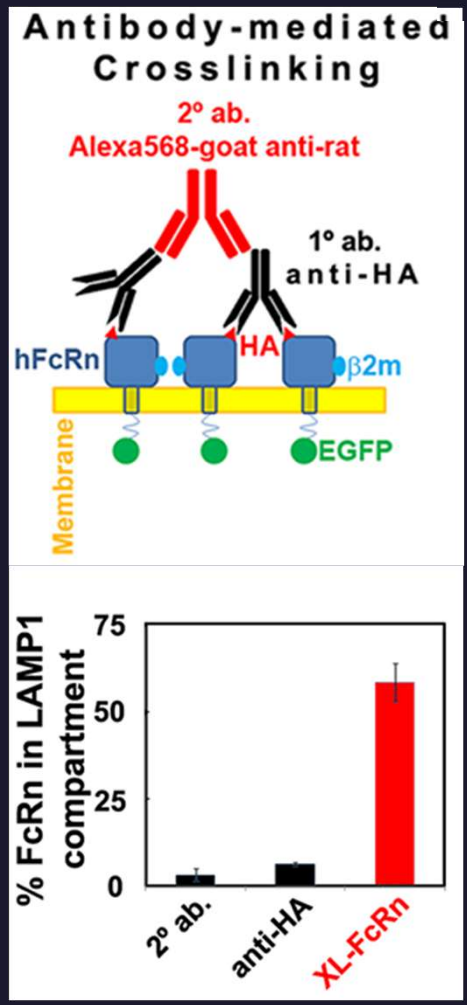




Format risk: drug-target complex formation
Example 2 - FcRn binding



Pyzik M *et al* 2015



Weflen AW *et al* 2013

DC presentation could result from enhanced FcRn binding, if drug-target complexes form

- Assess risk based on biology and modality (e.g. YTE, LS)
- Assays to derisk – MAPPs (Karle AC *et al* 2025) or uptake assay (Siegel M *et al* 2024)

Product risks occur due to choices made from initial idea...

IRA application during discovery offers opportunity to change course on immunogenicity

3. Product

- Sequence foreignness can be assessed early using *in silico* and *in vitro* assays to minimise risk
- Targeted chemical modification
  - Half-life extension – e.g. pre-existing antibodies PEG
  - ADC linkers – hapten potential increased with cleavable payload
- Modelling can be used to identify a risk of high molecular mass species formation

Critical Quality Attributes Developability

Hybridoma antibody campaign

1,000 clones

⇩

Confirmed in vitro target binders

200 clones

⇨

Early assessment		Lead 1	Lead 2
Target recognition	Human		
	Cyno		
	Natural ligand blocking		
Target binding	Affinity		
In silico predictions	Non-Germline Epitopes	4	2
	Oxidation Sites	2	2
	Deamidation Sites	0	0
	Isomerization Sites	2	0
	Total CDRH3 liabilities	2	0
	Viscosity (150mg/ml)		
aSEC	% HMMS		
DSC	Tm1 °C		
	Apparent Fab Tm °C		
iCE	pI		
	% Main Species		
	% Acidic		
	% Basic		
Low pH hold	Δ % HMMS		
Non-specificity	AC-SINS		
	DNA		
	Insulin		

⇨

Expand ~400 de-risked clones

2 rounds of design to address CD4 T cell epitopes and PTM sites sequence liabilities

⇨

10 de-risked leads

selected for further in vitro and in vivo characterization, including expression and purification

⇨

Clinical candidate

exhibits the most favorable critical attributes

CD4 T cell epitopes and PTM sites removal: potential workflow for lead development and candidate selection

Use of immunogenicity *in vitro* assays for PTM risk assessment may differentiate leads (*Zeunik R et al., 2022*)

Critical Quality Attributes
Impurities and contaminants

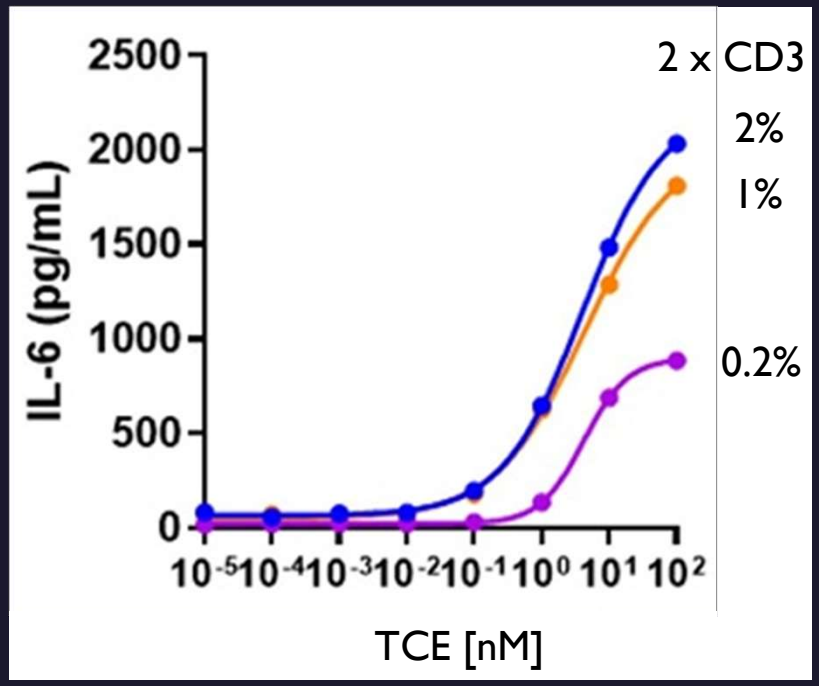
Can be informative for interpreting *in vitro* assay and *in vivo* results

2	**Preclinical Development**			
	Product Quality	Answer	Risk for induction	Risk for impact
2.1	Host cell origin			
2.2	HCP			
2.3	DNA			
2.4	Other contaminants			
2.5	Impurities (product related)			
2.6	HMWS (% and characterization)			
2.7	PTMs including glycosylation			
2.8	Formulation			
2.9	Container			

- Prior to candidate selection, product quality may be lower, or less well characterised
- HMMS usually characterised, other impurities or contaminants or PTMs not usually
- Small changes in product quality might not be detected by *in vitro* assays

Critical Quality Attributes
Impurity example – bispecific mispairing

- Mispairing of TCEs is minimised through specific pairing technologies (Zhang W *et al* 2022)
- Residual mispairing usually quoted as %
- Pharmacology of mispaired product is dose dependent
- NB: Relationship between cytokine release and immunogenicity is complex



Dummy data

Critical Quality Attributes
Application of tools early in discovery/development

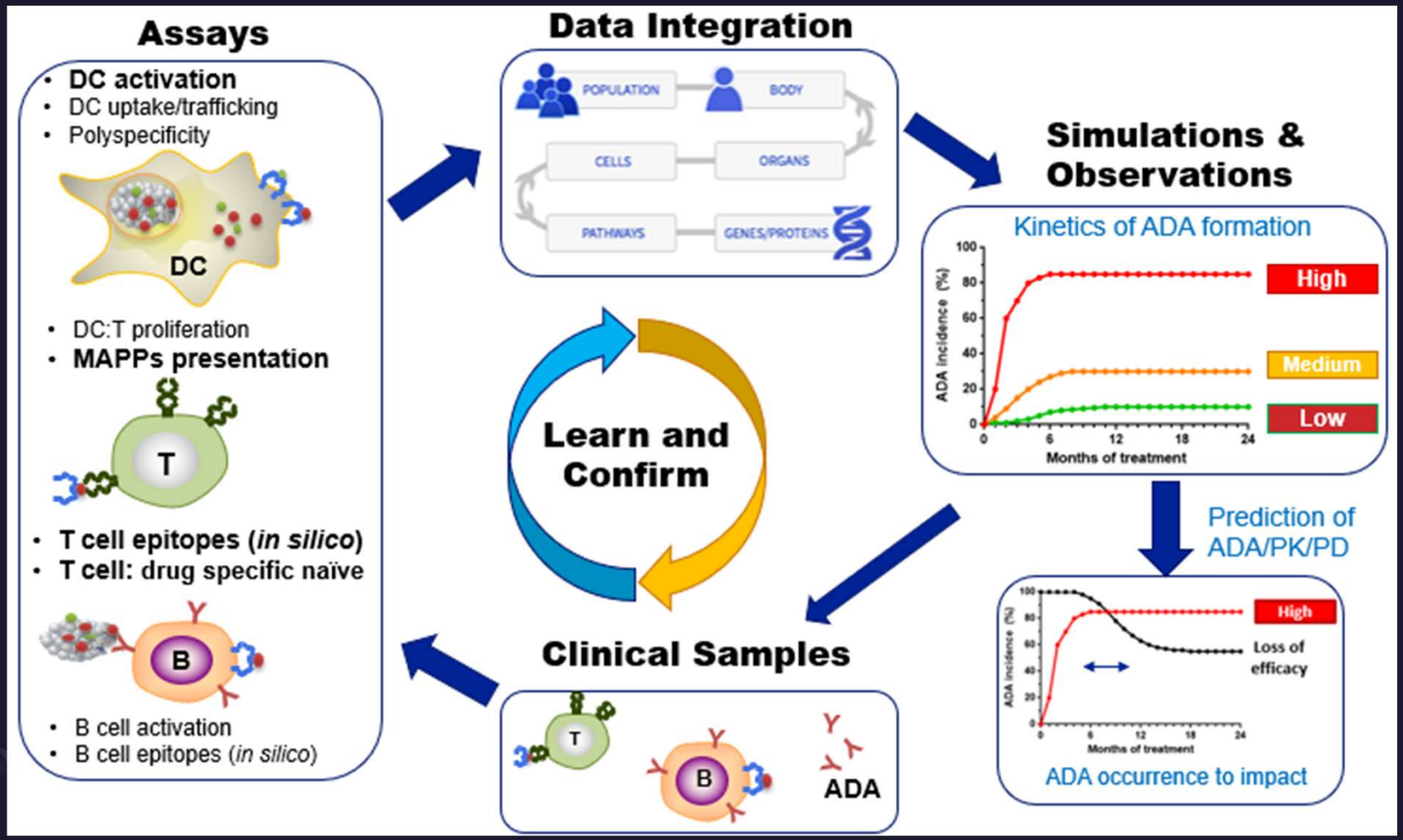
Risk Factor	Screen/derisking
T cell stimulator	*in silico* threshold adjustment
Immune cell target	early use of DC uptake assay to derisk or select low risk target epitope
Half-life extension with target valency	assess presentation risk with tool compound
Process changes	Test *in vitro* to support clinical release and therefore expansion of specification

Not a one-size fits all approach

Strategy based on the expected level of risk (e.g. immune oncology for solid tumours, modified), program development timelines & resources

'Re-testing' with *in vitro* assays may be justified during process changes

Incorporate knowledge from hundreds of clinical programs: Immunogenicity Database (IDC) – Agnihotri S *et al* 2025 (Preprint)



Summary

Mitigation of immunogenicity is challenging

Quality by Design approach to immunogenicity mitigation improves development of safer drugs

Combining QbD mindset and embracing AI/ML tools will accelerate delivery of breakthrough medicines to patients

Acknowledgement: Sophie Tourdot