EIP Immunogenicity Risk Assessment (IRA) for Tailored Mitigation and Monitoring of Biotherapeutics – Deep Dive

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on behalf of the EIP IRA Working Group

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EIP European Immunogenicity Platform

Disclaimer

This presentation represents the view of the EIP working group and is not necessarily reflective of the specific views of any member company.

Agenda

EIP recommendations on Immunogenicity Risk Assessment for a tailored mitigation and monitoring strategy of Biotherapeutics:

- Literature Overview & Gaps
- Immunogenicity Risk Factors
- Assignment of Overall Risk Level incl. points considered in EIP discussion
- Tailored risk-based clinical immunogenicity monitoring and sampling strategy
- Immunogenicity risk assessment impact on clinical mitigation beyond the bioanalytical monitoring
- Impact of IRA during development
- **Interactive session on assignment of overall risk level and definition of tailored bioanalytical strategy**

EIP recommendations for Immunogenicity Risk Assessment

EIP Immunogenicity Strategy Working Group: Consensus of **26 authors from 23 companies** incl. feedback from FDA Reviewer

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Immunogenicity risk assessment for tailored mitigation and monitoring of biotherapeutics during development: recommendations from the European Immunogenicity Platform

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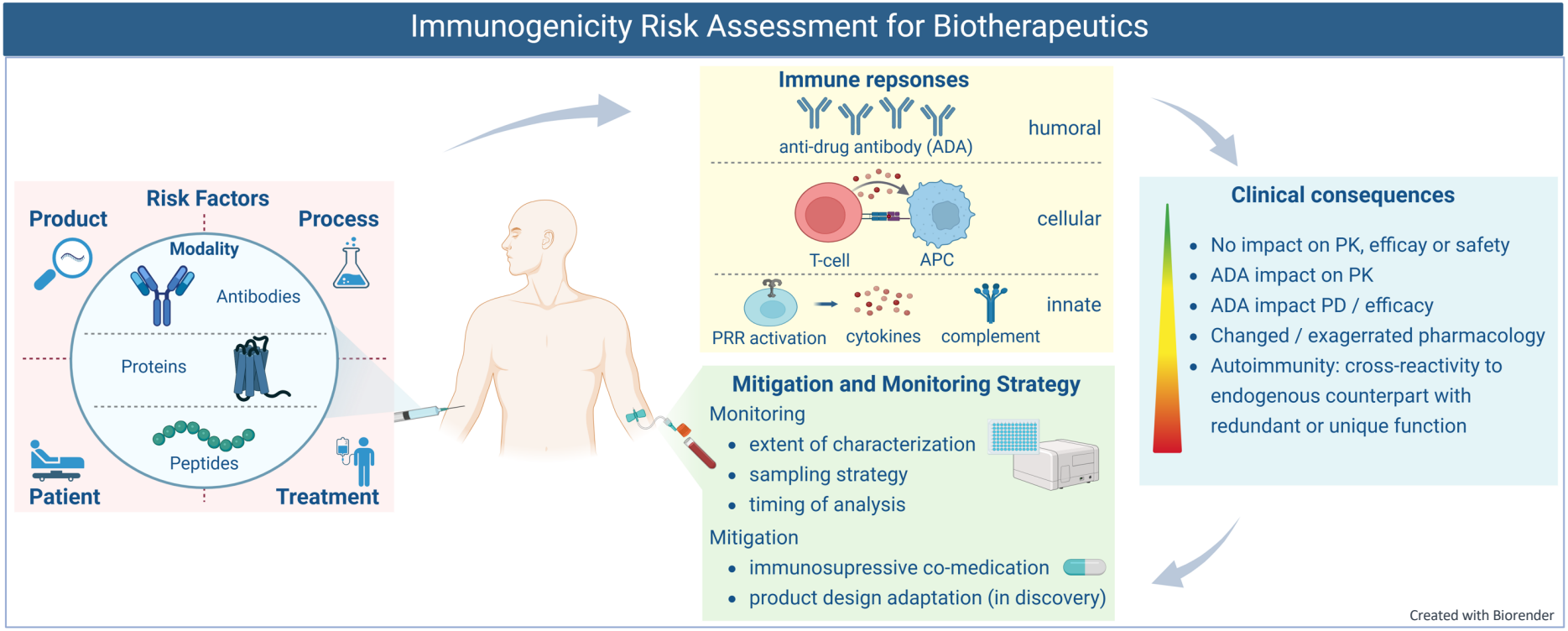
Immunogenicity Risk Assessment Process



Immunogenicity Risk Assessment enables to define a tailored mitigation & monitoring strategy to ensure safe and efficacious biotherapeutics reach patients

Regulatory Guidance for IRA of Biotherapeutics

FDA: Immunogenicity Assessment for Therapeutic Protein Products (2014, FDA-2013-D-0092)

- Comprehensive **overview of immunogenicity risk factors**
- **Consequences of clinical immunogenicity** on efficacy & safety incl. hypersensitivity

FDA: Immunogenicity Testing of Therapeutic Protein Products (2019, FDA-2009-D-0539)

- **Inclusion of immunogenicity risk assessment expected in IND submission**
- **Multi-tiered testing strategy**
- **Method validation** and method development

EMA: Guideline on Immunogenicity assessment of therapeutic proteins (2017, EMEA/CHMP/BMWP/14327/2006 Rev 1)

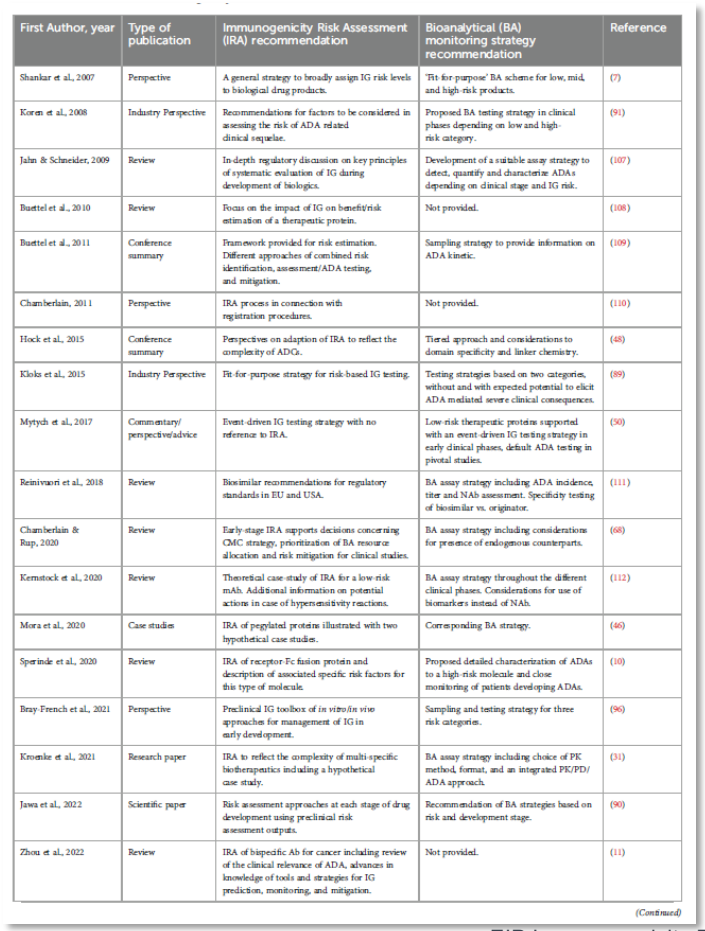
- **Multidisciplinary** evaluation of immunogenicity risk factors incl. clinical consequences
- Nonclinical assessment of immunogenicity
- Assay development and sampling strategy
- Integrated summary of immunogenicity

EMA: Guideline on immunogenicity assessment of monoclonal antibodies for in vivo clinical use (2012, EMA/CHMP/BMWP/86289/2010)

- **Assay recommendations**
- **Immunogenicity risk assessment** recommendations incl. relevant risk factors
- Integrated summary of immunogenicity

Immunogenicity Risk Assessment is required by health authorities (HA)

Literature Overview & Gap Analysis



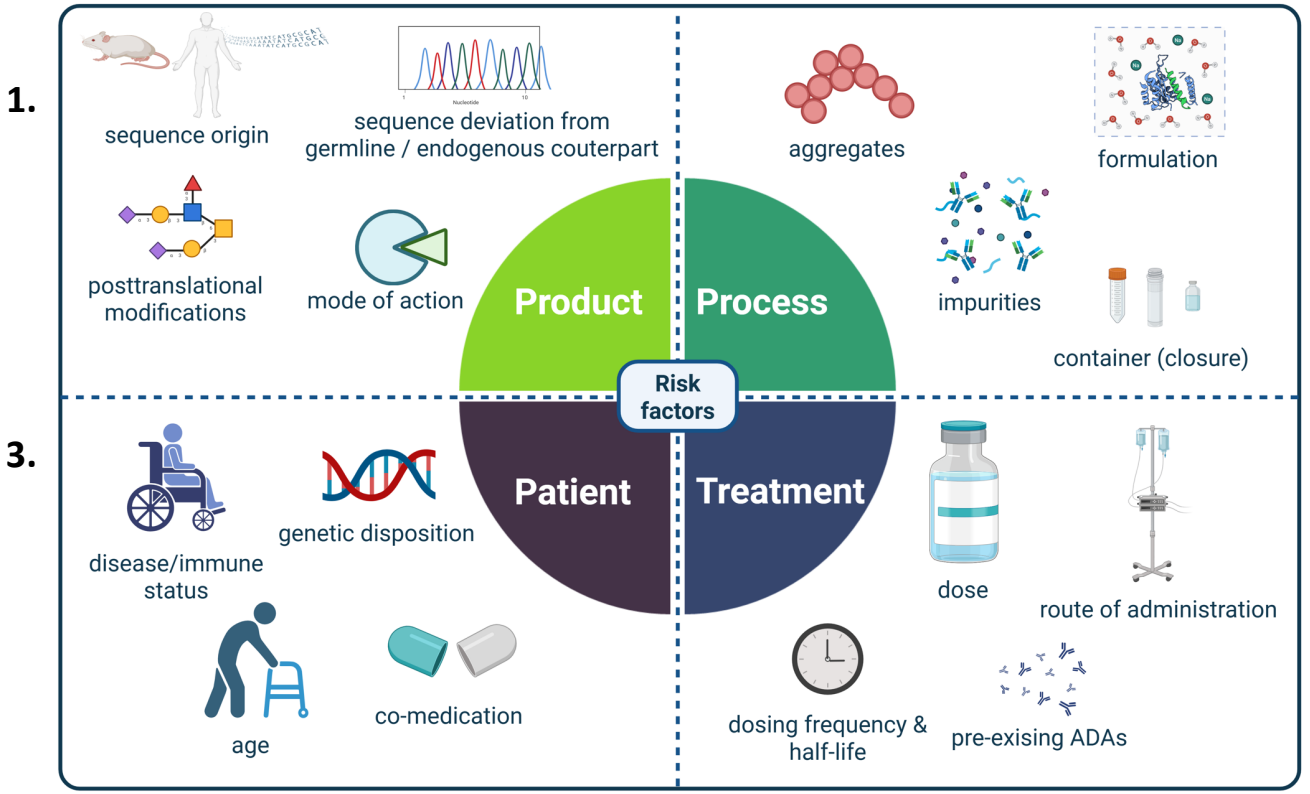
First Author, year	Type of publication	Immunogenicity Risk Assessment (IRA) recommendation	Bioanalytical (BA) monitoring strategy recommendation	Reference
Shankar et al., 2007	Perspective	A general strategy to broadly assign IG risk levels to biological drug products.	'Fit-for-purpose' BA scheme for low, mid, and high-risk products.	(7)
Koren et al., 2008	Industry Perspective	Recommendations for factors to be considered in assessing the risk of ADA related clinical sequelae.	Proposed BA testing strategy in clinical phases depending on low and high-risk category.	(91)
Jahn & Schneider, 2009	Review	In-depth regulatory discussion on key principles of systematic evaluation of IG during development of biologics.	Development of a suitable assay strategy to detect, quantify and characterize ADAs depending on clinical stage and IG risk.	(107)
Buettel et al., 2010	Review	Focus on the impact of IG on benefit/risk estimation of a therapeutic protein.	Not provided.	(108)
Buettel et al., 2011	Conference summary	Framework provided for risk estimation. Different approaches of combined risk identification, assessment/ADA testing, and mitigation.	Sampling strategy to provide information on ADA kinetic.	(109)
Chamberlain, 2011	Perspective	IRA process in connection with registration procedures.	Not provided.	(110)
Hock et al., 2015	Conference summary	Perspectives on adaption of IRA to reflect the complexity of ADCs.	Tiered approach and considerations to domain specificity and linker chemistry.	(48)
Kloks et al., 2015	Industry Perspective	Fit-for-purpose strategy for risk-based IG testing.	Testing strategies based on two categories, without and with expected potential to elicit ADA mediated severe clinical consequences.	(89)
Mytych et al., 2017	Commentary/ perspective/advice	Event-driven IG testing strategy with no reference to IRA.	Low risk therapeutic proteins supported with an event-driven IG testing strategy in early clinical phases, default ADA testing in pivotal studies.	(50)
Reinivuori et al., 2018	Review	Biosimilar recommendations for regulatory standards in EU and USA.	BA assay strategy including ADA incidence, titer and NAb assessment. Specificity testing of biosimilar vs. originator.	(111)
Chamberlain & Rup, 2020	Review	Early-stage IRA supports decisions concerning CMC strategy, prioritization of BA resource allocation and risk mitigation for clinical studies.	BA assay strategy including considerations for presence of endogenous counterparts.	(68)
Kernstock et al., 2020	Review	Theoretical case study of IRA for a low-risk mAb. Additional information on potential actions in case of hypersensitivity reactions.	BA assay strategy throughout the different clinical phases. Considerations for use of biomarkers instead of NAb.	(112)
Mora et al., 2020	Case studies	IRA of pegylated proteins illustrated with two hypothetical case studies.	Corresponding BA strategy.	(46)
Sperinde et al., 2020	Review	IRA of receptor-Fc fusion protein and description of associated specific risk factors for this type of molecule.	Proposed detailed characterization of ADAs to a high-risk molecule and close monitoring of patients developing ADAs.	(10)
Bray-French et al., 2021	Perspective	Preclinical IG toolbox of *in vitro/in vivo* approaches for management of IG in early development.	Sampling and testing strategy for three risk categories.	(96)
Kroenke et al., 2021	Research paper	IRA to reflect the complexity of multi-specific biotherapeutics including a hypothetical case study.	BA assay strategy including choice of PK method, format, and an integrated PK/PD/ADA approach.	(31)
Jawa et al., 2022	Scientific paper	Risk assessment approaches at each stage of drug development using preclinical risk assessment outputs.	Recommendation of BA strategies based on risk and development stage.	(90)
Zhou et al., 2022	Review	IRA of bispecific Ab for cancer including review of the clinical relevance of ADA, advances in knowledge of tools and strategies for IG prediction, monitoring, and mitigation.	Not provided.	(11)

(Continued)

Though extensive literature is available

- there is a **lack of a harmonized framework** on the immunogenicity risk assessment and its use throughout different development stages
- **no updated and comprehensive list** of immunogenicity risk factors
- **no** harmonized **guidance** on how to assess the **overall immunogenicity risk level** exists
- unclear whether **business risk aspects** are taken into consideration in the IRA
- Lack of **risk-tailored bioanalytical strategy**

Immunogenicity Risk Identification



EIP Recommendation: Rating of each risk factors using only two categories low or high

1. Product-related risks & consequences

- Sequence liabilities are the major contributor to immunogenicity risk
  - Human, partially human, non-human
  - Sequence homology to endogenous counterpart incl. polymorphisms
- Mode of action
  - suppressing vs. activating the immune system
  - soluble multimeric targets
  - membrane-bound targets
    - ADA-mediated crosslinking w. enhanced pharmacology
    - expressed on antigen presenting cells
  - Effector functions
- Targeted modification e.g. pegylation

Risk type	Risk Factor	Risk evaluation		Potential IG consequence(s)	Reference
		Low risk	Flag for higher risk		
Product	Sequence origin and degree of sequence foreignness	Fully human (germline) sequence with no polymorphism	Partially human (e.g. chimeric; homology in CDR to bacterial sequence; humanized; human sequence with polymorphism)	ADAs due to recognition of MHC-presented peptides as foreign	(5, 12, 16, 20, 81)
			Multi-specifics (higher number of foreign CDRs)		(11, 31)
			Neoepitopes in fusion molecules or conjugates		(10, 11)
			Cryptic epitopes		(11)
			Non-human (animal, bacterial, viral)	In addition to ADAs, risk for hypersensitivity reactions	(2, 5)
	MoA	Immunosuppressive No immune modulation	Immune stimulatory, synergistic or agonistic	ADAs in case of immunostimulatory/ synergistic biotherapeutics. For agonistic MoA risk of ADA-mediated cross-linking of cell-surface receptors potentially leading to exaggerated pharmacology	(2, 5)
	MoA: Target	Soluble, monomeric	Soluble, multimeric	Immune complex formation between drug and soluble multimeric target leading to high incidence (>80%) of ADAs and loss of PK/ PD, efficacy	(29, 30)
		Membrane protein no impact of receptor cross-linking on pharmacology	Membrane protein impact of receptor cross-linking on pharmacology	Altered/exaggerated pharmacology by ADA-mediated cross-linking of membrane receptors with safety consequences	(7, 32, 33)
			Membrane protein on APCs	Receptor-mediated uptake into APCs enabling MHC peptide presentation leading to ADAs	(21, 25, 41)
	MoA: Effector function (ADCC, ADCP, CDC)	No ADCC, ADCP, CDC Effector function leading to strong B-cell depletion	ADCC, ADCP, CDC without strong B-cell depletion	Exaggerated pharmacology, induction of necrosis creating an inflammatory environment and activation of adaptive immunity. However, afucosylated mAbs with enhanced ADCC in cancer show low IG but higher IRR incidences	(34–36, 38–40, 47)
	Targeted chemical modification	No targeted modification	PEGylation	PE anti-PEG Abs potentially leading to accelerated blood clearance, complement activation-related pseudo allergy (CARPA) or in very rare cases to anaphylaxis mediated by anti-PEG IgE Abs	(45, 46, 114)
			Fusion with linker and/or chemical structure e.g. toxophore or chelator etc.	Toxicity due to uptake of ADA-drug complexes into non-target tissue ADAs against hapten-like structures (vc-MMAE, DM1/4 or calicheamicin) reported only in very rare cases with no impact on PK, PD, efficacy, safety	(43, 47, 48, 115)
	Homology of biotherapeutic to endogenous counterpart	Endogenous counterpart with redundant function	Full or partial sequence homology to endogenous counterpart Endogenous counterpart with unique function	ADAs cross-reactivity to endogenous counterpart potentially resulting in autoimmunity phenotype	(2, 5)

2. Process-related risks & consequences

Established IG risk factors include

- Aggregates
- Impurities
- Container closure
- Chemical modifications
- Glycosylation
- Formulation

- Advances in manufacturing and analytics improved drug quality control limiting impact of process-related impurities on IG for IV/SC formulations
- Concerns shifted to later development when manufacturing process is changed (scale up, facility change etc.)
- If any manufacturing change results in product quality attributes exceeding their established limits an IRA is required

Risk type	Risk Factor	Risk evaluation		Potential IG consequence(s)	Reference
		Low risk	Flag for higher risk		
Process	Aggregates	Low aggregate number (within process specific CQA limits)	Higher aggregate number (outside process specific CQA limits)	ADAs due to aggregate-mediated cross-linking of B-cell receptors, uptake into antigen presenting cells or triggering of immunostimulatory danger signals. However, a high threshold of aggregates needed (beyond that typically seen in marketed products)	(2, 49, 116)
	Level of impurities (e.g. host-cell proteins, lipids, DNA, bacterial contaminants)	Low (within process specific CQA limits)	Higher (outside process specific CQA limits) Presence of microbial impurities or impurities with homology to endogenous proteins	HCPs may be immunogenic, biologically or enzymatically active mediating •adjuvant activity of innate immune responses leading to ADA formation •hypersensitivity reactions when of microbial origin •proteolytic drug or excipient degradation resulting in ADAs •anti-HCP antibody formation potentially cross-reacting to homologous endogenous counterparts or remain without any consequences	(2, 49, 117–120)
	Leachables from containers or closures	Low (within process specific CQA limits)	Higher (outside process specific CQA limits) for tungsten, glass and metal particles, silicon oil	Leachable-induced ADAs due to protein denaturation, aggregation, modification	(2, 49)
	Degree of chemical modifications	Low (within process specific CQA limits)	Higher (outside process specific CQA limits)	Chemical modification (e.g. oxidation, deamidation, hydrolysis, isomerization) inducing drug aggregation resulting in ADAs	(2, 49, 121)
	Glycosylation patterns	Fully human	Partially or non-human (e.g. galactose-alpha-1,3-galactose, N-glycolylneuraminic acid (NGNA), high mannose content)	Non-human glycosylation triggering innate/adaptive immune responses Glycosylation pattern (e.g. high mannose content) enhancing uptake into APCs, leading to ADAs PE-ADAs leading to accelerated drug clearance, loss of efficacy or hypersensitivity (e.g. serum sickness) or anaphylaxis in case of anti-IgE abs	(122–125)
	Formulation	No adjuvant effect	Adjuvant effect not known or expected e.g. for polysorbate 20 or 80 Formulation impacting drug stability	Hypersensitivity including anaphylaxis Formulation-mediated adjuvant activity of innate immune responses resulting in ADAs Formulation-mediated aggregation or degradation leading to ADAs	(2, 49, 52, 53, 126)

3. Patient- & treatment-related risks & consequences

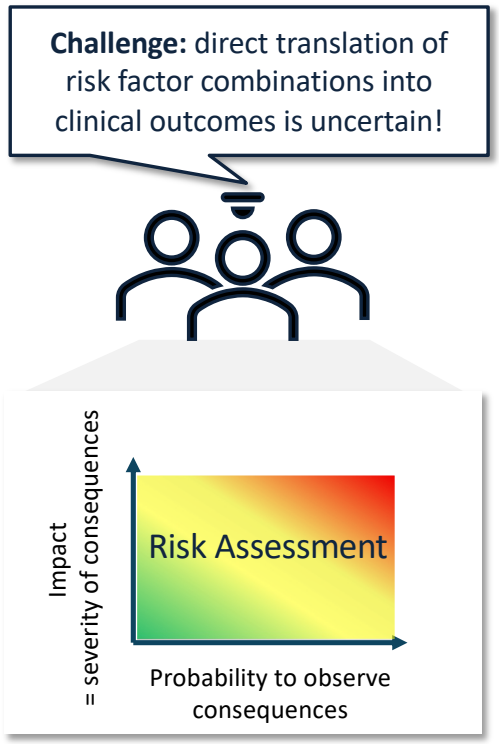
- Patient immune status
- Endogenous counterpart high abundance vs. null mutation / low expression and prior treatment experience with similar therapy
- History of allergy
- Pre-existing ADA against small antibody formats, PEG
- Route of administration
  - IV / SC similar risk for mAbs only!
- Dosing frequency (single dose vs. chronic dose)

Risk type	Risk Factor	Risk evaluation		Potential IG consequence(s)	Reference
		Low risk	Flag for higher risk		
Patient	Basal patient immune status	Compromised or immunosuppressed	Activated or inflammatory immune system; autoimmune patients	ADAs in patients with activated, inflammatory, or autoimmune status of immune system	(2, 5, 41, 127)
	Genetic status	No classification		Identification of subpopulation at risk based on (retrospective) HLA analysis	(2, 5)
	Age	Elderly	Children/infants	Immunosenescence i.e. natural decline in immune system function with increasing age. However, exposure to a greater variety of antigens over the lifetime may increase the probability for IG	(5, 128, 129)
	Pre-existing anti-drug antibodies	Not detected	Detected	PE-ADAs described against PEG, non-human glycans, recombinant cytokines, growth factors, enzymes, pathogen similarity and new antibody fragment formats (e.g. Fabs, scFVs and VH domains) exposing cryptic or neoepitopes	(15, 32, 33, 56–58)
	History of Allergy	No allergy	Allergy(ies) present	Individuals with allergies have a hyperactive or dysregulated immune system with enhanced predisposition to develop IgE/Th2 immune responses against the drug or excipients	(2)
	Prior treatment with similar therapy w/ o ADAs	Yes	No	ADA cross-reactivity to endogenous counterpart potentially resulting in autoimmunity phenotype	(5)
	Concentration of fully homologous endogenous counterpart	Really high abundance	None (null mutation) or relatively low abundance	For null mutation elevated probability of ADAs. For low abundance proteins weaker robustness of immune tolerance	(5)
Treatment	Dose level	High dose	Low dose	ADA impact on PK/PD more pronounced at lower doses	(2, 130)
	Treatment regimen: dosing frequency, schedule, length of treatment	Single dose	Multiple chronic dosing	Impact of ADAs on PK/PD, efficacy less likely for acute MoA upon fast clearing single dose compared to multiple chronic dosing	(2)
			Intermittent/episodic treatment	Longer drug holidays have been shown to correlate with increased ADA risk. Frequent dosing poses higher IG risk due to 'prime and boost' phenomenon often utilized in vaccine development	(130, 131)
	Route of administration	IV For mAbs only: SC	SC (for non-mAbs) < intramuscular or intradermal or inhaled	ADAs due to favorable uptake of biotherapeutic into APCs for all administration routes other than IV. However, for mAbs in many cases similar ADA incidence observed for SC and IV administration	(2, 60–63)
	Immunomodulating concomitant medication	Immunosuppressive comedication	Immunostimulatory comedication	Immunosuppressive co-medication may reduce whereas immunostimulatory co-medication may enhance ADA incidence	(132, 133)

How to assign an overall risk level prior to start of clinical development?

EIP Discussion on assignment of overall immunogenicity risk level

Challenge: direct translation of risk factor combinations into clinical outcomes is uncertain!



Topic	EIP Discussion Summary
Detailed vs. simplified **rating of individual risk factors**	Highest confidence present for low risk whereas differentiation into moderate vs. high more difficult. Recommendation to use **only low/high categories**, however, no restriction to go beyond 2 categories
Use of **numerical rating** system based on risk factors	**Not implemented** in most companies
Weighting of risk factors against each other	**Weighting depends** on **individual case** incl. modality type, no general recommendation possible
Purpose of individual **risk factor evaluation**	Define (early) mitigation strategies, estimate **consequences** and **probability** of occurrence (based on previous experience and literature data)
Assign risk category based on **safety** or **safety and efficacy** consequences	**Impact of IG on business risk shown in literature** (failure of "low" risk molecules in Phase 1) but both approaches are used

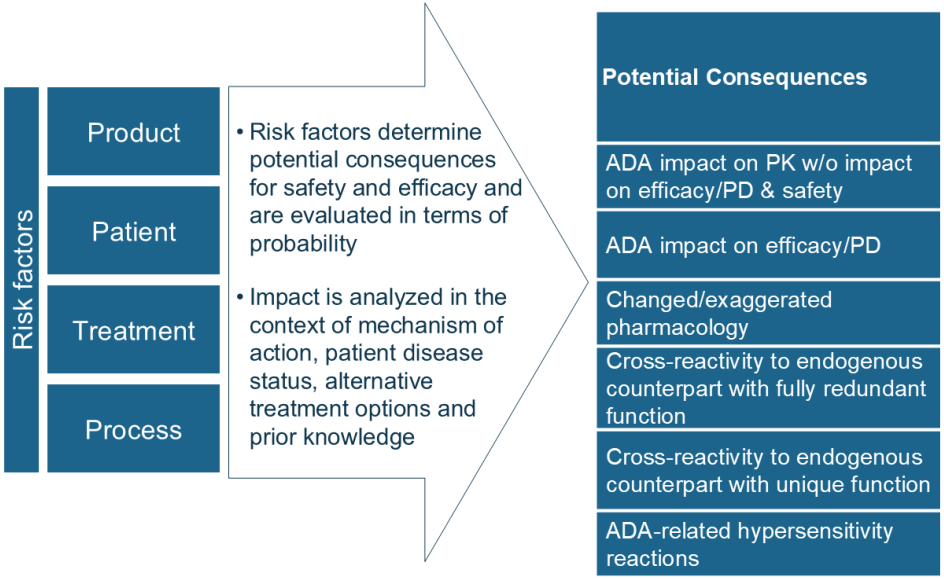
EIP European Immunogenicity Platform

Assignment of Overall Immunogenicity Risk Prior to Start of Clinical Development

IRA before start of clinical development
Risk = Probability x Severity of Consequences

1. Risk identification: probability and potential impact

2. Assignment of overall IG risk level based on estimation of probability for impact on safety and efficacy in context of disease and medication status



Potential Consequences
ADA impact on PK w/o impact on efficacy/PD & safety
ADA impact on efficacy/PD
Changed/exaggerated pharmacology
Cross-reactivity to endogenous counterpart with fully redundant function
Cross-reactivity to endogenous counterpart with unique function
ADA-related hypersensitivity reactions

IRA - Hypersensitivity

- May affect patient safety
- **May not** necessarily be mechanistically linked to ADA

Immune-related AEs
- Acute or delayed hypersensitivity reactions (including anaphylaxis)
- Cytokine release syndrome (often driven by MoA of the therapeutic)
- Consequences may vary widely
- Often unpredictable

→ to be **monitored closely and management strategies available** even if IRA indicates low risk including ad-hoc sampling

→ **Characterization** to elucidate underlying pathophysiology for irAE may identify patient at risk and provide information on mitigation strategies (depending severity and frequency; and not always possible)

Tailored risk-based clinical immunogenicity monitoring and sampling strategy

IRA drives the IG bioanalytical monitoring strategy and defines

1. Assay types
2. Schedule/timing of analysis
3. Sample collection
4. Choices for supporting clinical endpoints for PK, PD, efficacy and safety

Risk based approach

- **Allows for a flexible investment of time, assays and cost in testing strategies**
- **Allows prioritization of essential activities bringing added value and ensure appropriate resource allocation**
- Level of investment for ADA assay development and sample analysis may be staggered based on the IG risk and the clinical development phase
- Engage with HA to aling on overall strategy: IG sampling and testing

Tailored risk-based clinical immunogenicity monitoring and sampling strategy

ADA testing strategy

Risk classification	Phase I			Phase II			Phase III/Pivotal Study		
	Low	**Moderate**	**High**	**Low**	**Moderate**	**High**	**Low**	**Moderate**	**High**
	Assay available	Analyze samples	Analyze samples *fully validated assay*	Analyze samples		Analyze samples *fully validated assay*	Analyze samples *fully validated assay*		

Event-driven IG testing strategy

- Banking samples
- Analyse only when altered PK,PD/efficacy or safety-related event
- Batchwise ADA analysis at study end

Consultation with HA is advisable prior to implementing (eg clarifying as part of IRA, testing/monitoring strategy at start clinical development)

Tailored risk-based clinical immunogenicity monitoring and sampling strategy

	Phase I			Phase II			Phase III/Pivotal Study		
	Low	**Moderate**	**High**	**Low**	**Moderate**	**High**	**Low**	**Moderate**	**High**
ADA testing strategy	Assay available	**Analyze samples**		**Analyze samples**			**Analyze samples**		
Timing of ADA analytics	**Collect and hold** **Analyse in case of unexpected PK/PD or safety AE**	**End of Study (EOS)**	At least at the end of each dose level Close monitoring prior to next drug administration	**EOS**		Batch wise throughout study Close monitoring	**EOS** (recommend to frontload analysis ahead of study end)		Batch wise throughout study Close monitoring
ADA sample collection frequency	**Baseline, end of cycle/dose tier (based on dosing)**	Baseline and end of each cycle/dose tier Selected timepoints at 7-14 days 3-6 weeks EOS	Baseline Onset 7-14 days End of cycle/dose tier For consecutive cycle/dose tier: predose and EOS Frequent sampling Post-study FU sampling required for high risk with serious safety consequences from ADA*	Baseline End of cycle/dose tier Selected timepoints at 7-14 days 3-6 weeks based on regulatory requirements and project needs EOS		Frequent sampling through all stages	**Less frequent sampling than phase I and II**		Frequent sampling through all stages and EOS Post-study FU sampling required for high risk with serious safety consequences from ADA*
	Ad hoc samples in case of SAE as part of safety assessment								

*EIP paper, Lotz et al AAPSJ 2022 - on recommendation as when to extend ADA monitoring beyond study end

Tailored risk-based clinical immunogenicity monitoring and sampling strategy

	Phase I			Phase II			Phase III/Pivotal Study		
	Low	**Moderate**	**High**	**Low**	**Moderate**	**High**	**Low**	**Moderate**	**High**
ADA testing strategy	Assay available	**Analyze samples**		**Analyze samples**			**Analyze samples**		
ADA characterisation Neutralization	**Not needed**	Assess the need and **consider reagent generation**	NAb for high risk is **often expected** Consider integrated (active) PK/PD/ADA as potential alternative	**Not needed**	NAb evaluation to be considered for moderate risk, expected for high risk Consider integrated (active) PK/PD/ADA as strategy instead of NAb		Consider integrated (active) PK/PD/ADA as strategy instead of Nab	NAb evaluation **expected for moderate and high risk** Consider integrated (active) PK/PD/ADA as strategy instead of NAb	

- Moderate and high risk - ADA testing to be conducted during clinical development
- **Need for ADA characterisation (NAb assay) is risk based**
  - For Moderate risk therapeutics, NAb may not be required for non-pivotal studies and may be guided **on impact seen on PK, PD/efficacy and safety**
- Availability of active PK and PD endpoints can be used to conclude on the neutralizing impact of ADA (integrated approach)

Tailored risk-based clinical immunogenicity monitoring and sampling strategy

	Phase I			Phase II			Phase III/Pivotal Study		
	Low	**Moderate**	**High**	**Low**	**Moderate**	**High**	**Low**	**Moderate**	**High**
ADA testing strategy	Assay available	**Analyze samples**		**Analyze samples**			**Analyze samples**		
ADA characterisation Neutralization	**Not needed**	Assess the need and **consider reagent generation**	NAb for high risk is **often expected** Consider integrated (active) PK/PD/ADA as potential alternative	**Not needed**	NAb evaluation to be considered for moderate risk, expected for high risk Consider integrated (active) PK/PD/ADA as strategy instead of Nab		Consider integrated (active) PK/PD/ADA as strategy instead of Nab	NAb evaluation **expected for moderate and high risk** Consider integrated (active) PK/PD/ADA as strategy instead of Nab	
Othe domain specificity, cross-reactivity to endogenous counterpart	**Not needed**	Assess the need and consider reagent generation	**Assess need** and selection of relevant assay **if of added value**	Not needed	Assess need and selection of relevant assay based on Phase I data **if of added value**		Assess need and selection of relevant assay based on Phase I/II data **if of added value**		

- Need for domain specificity is risk based and if off added value
- Fit for purpose approach can be followed: for multidomain therapeutics, if one domain is linked to potential safety/efficay, domain characterisation limited to that domain

Tailored risk-based clinical immunogenicity monitoring and sampling strategy - focus on HIGH risk category

	Phase I		
	Low	**Moderate**	**High**
ADA testing strategy	Assay available	**Analyze samples**	
ADA characterisation Neutralization	**Not needed**	Assess the need and **consider reagent generation**	NAb for high risk is **often expected** Consider integrated (active) PK/PD/ADA as potential alternative
Othe domain specificity, cross-reactivity to endogenous counterpart	**Not needed**	Assess the need and consider reagent generation	**Assess need** and selection of relevant assay **if of added value**

Need for IG monitoring with additional ADA characterisation (focus on **risk-based demand**) and more frequent sampling **at early stage (to mitigate risk when occuring)**

Advantage of early stage expanded ADA characterisation is provision of data that may correlate with ADA mediated safety signals and inform on appropriate mitigation and intervention strategies

- Typically, PK/PD testing including safety biomarkers
- Sampling for ADA and/or NAb/domain characterisation

- Valuable if Nab is expected to drive safety consequences

- Valuable if specific domain is driver for the high risk category
- Cross-reactivity to endogenous counterpart valuable if its neutralization is the driver for the high risk
- For ADA- mediated exaggerated pharmacology, tailored testing is required (e.g. cytokine release)

Sampling and testing strategy – additional considerations

Sample use and volume considerations

- Clinical protocol and ICF: allow for residual PK sample use in ADA and additional characterisation methods (NAb or domain) or in case of high risk projects if additional ADA analysis is required
- Pretreatment baseline to have sufficient volume to develop the additional ADA characterisation methods

Testing paradigm

- **S/N instead of titer for ADA response magnitude** – independent of the risk class but suitability is data driven, depending on assay characteristics and dynamic range of the method (**upfront consultation with HA recommended prior to implementation)**

- **No analysis of non-treated placebo study participants**
  - Placebo samples can be used as disease baseline
  - **In case of pre-ADA** (high prevalence), placebo samples can be analysed to assess natural longitudinal fluctuation of pre-existing Ab

- **Large Phase III/pivotal study – low risk**: Collect and analyse for ADA only **in representative subset of study participants,** representative of ethnicity and disease spectrum (consult HA upfront!)

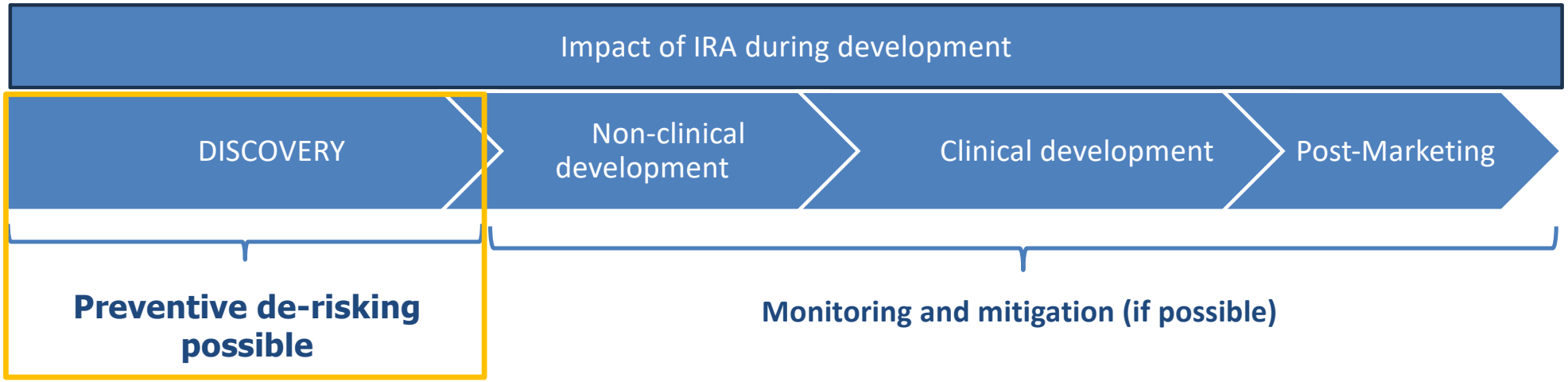
Immunogenicity risk assessment impact on clinical mitigation beyond the bioanalytical monitoring

Clinical mitigation strategies

- Identify and exclude patient at risk for severe safety consequences
- Adjust dosing frequency – avoid intermittent dosing
- Infusion related reactions: slowing rate or lowering dose, versus stop infusion/treatment – with careful evaluation if re-introduction of treatment is possible and immunosuppressive premedication
- If hypersensitivity is observed, ad hoc sampling is recommended to investigate if ADA is the cause (Type I and Type III reactions) and to define appropriate mitigation

Mitigation depending on risk category if impact on efficacy or safety

- **Life-threatening diseases without alternative treatment options:**
  - Discontinuing treatment for patients with ADA impacting efficacy and/or safety may result in disease progression and be fatal
  - Mitigation: tolerance induction strategies (high drug or novel approaches); concomitant immunosuppressive treatment
- **Life-threatening with alternative treatment options**
  - Treatment discontinuation for patients not showing therapy benefit to allow alternative medication



Risk assessment early on allows considering the immunogenicity related risk factors leading to the candidate with best/lowest IG risk profile while maintaining essential pharmacological aspects and characteristics

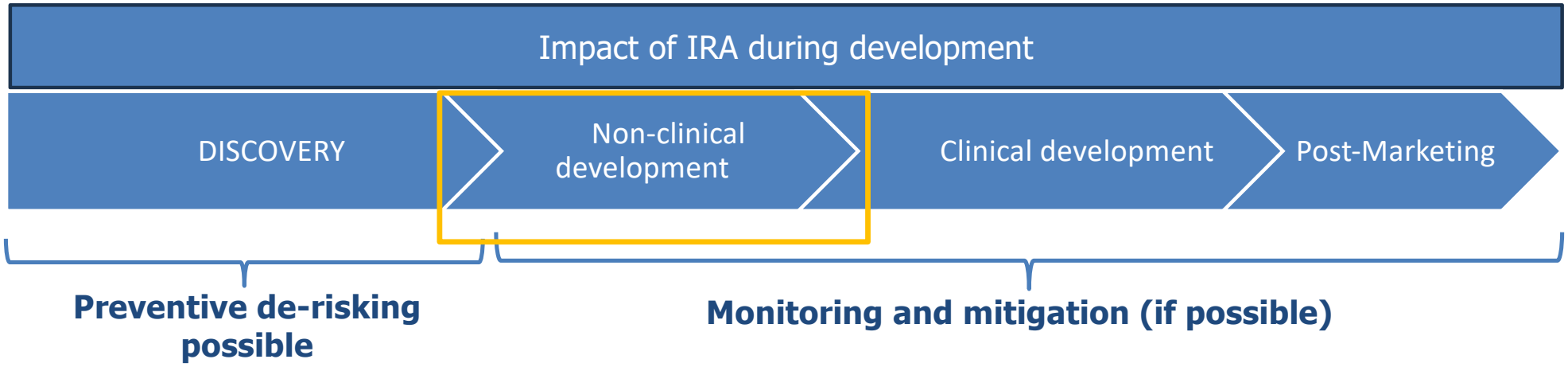
- adaptation of mitigation strategies based on specific risks identified

Derisking via selection of most optimal therapeutic modality:

- Consideration of MoA -risk for altered pharmacology
- Different target binding properties,
- Presence multimeric binding sites,
- FcγR/complement binding
- Half-life extension

Derisking by design and sequence optimization (in silico, in vitro tools) to reduce

- Immunogenic T cell epitopes
- Pre-existing antibody binding
- Immune complex formation
- Product internalisation
- Aggregate formation



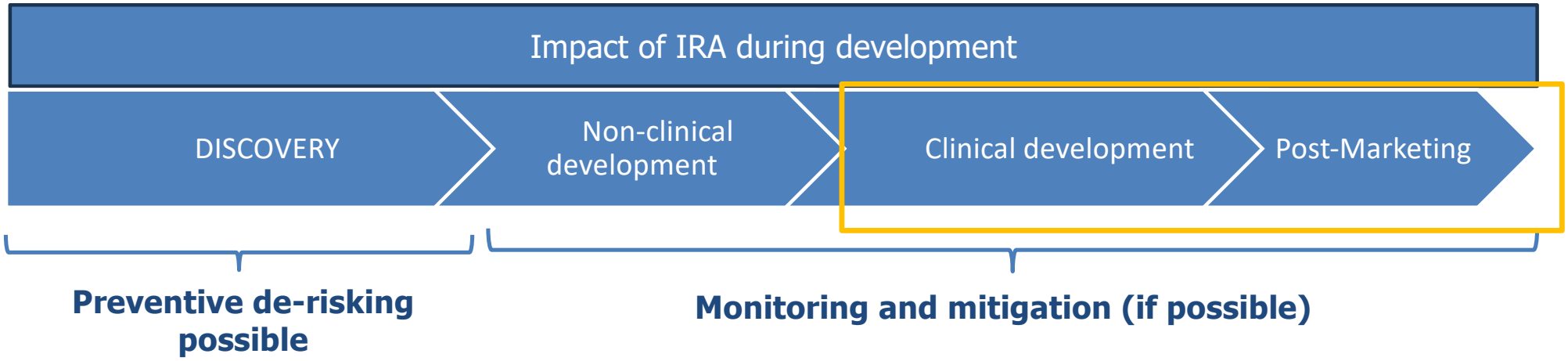
Nonclinical studies

- ADA to support data interpretation (impact exposure, efficacy, iR safety events)
- IRA helps in defining business risk for ADA method development : development at risk ↔ development data driven

- ADA incidence not predictive for immunogenicity incidence in human
- Extrapolation to human of causality of IG related consequence on safety, PK/PD, efficacy if MoA related and target biology/binding characteristics are similar between species → may affect monitoring and mitigation strategy FIH

In support of FIH

- Define a bioanalytical monitoring and mitigation strategy based on the IRA prior to the start of clinical development
- Consultation and agreement on strategy with HA

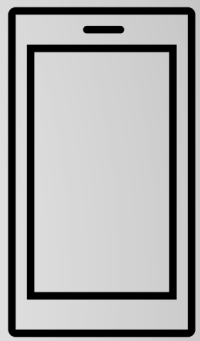


Clinical

Life cycle management approach, with risk, monitoring and mitigation strategy to be adjusted once clinical data is available (risk decreased or increased – e.g. safety implications), and throughout clinical development, with continuation through BLA and after market approval

- Implementation of a tailored clinical bioanalytical strategy and assays
- Iterative adjustments of monitoring and/or mitigation plans based on clinical data
- Interactions with HA (scientific advise as applicable)

Interactive session – please take out your mobiles



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Translate IRA into a tailored bioanalytical strategy

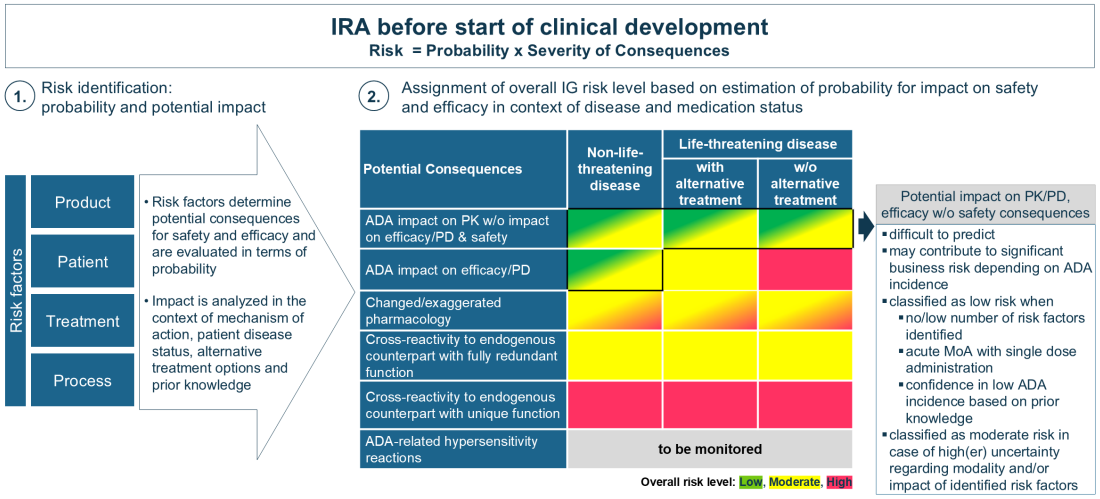

TABLE 3 Immunogenicity monitoring and sampling strategy.

	Phase I			Phase II			Phase III/Pivotal study		
Monitoring and Sampling Strategy	Low risk	Moderate risk	High risk	Low risk	Mode-rate risk	High risk	Low risk	Mode-rate risk	High risk
ADA testing strategy	Assay available	Analyte samples	Analyte samples (fully validated assay)	Analyte samples		Analyte samples (fully validated assay)	Analyte samples (fully validated assay)		
ADA characterization: Neutralization	Not needed	Assess the need and consider reagent generation for NAb and additional characterization assay development	NAb for high risk is often expected. Consider integrated (active) PK/PD/ADA as potential alternative	Not needed	NAb evaluation to be considered for moderate risk, expected for high risk Consider integrated (active) PK/PD/ADA strategy instead of NAb		Consider integrated (active) PK/PD/ ADA strategy instead of NAb	NAb evaluation expected for moderate and high risk Consider integrated (active) PK/PD/ADA strategy instead of NAb	
Other: domain specificity, cross-reactivity to endogenous counterpart			Assess need and selection of relevant assay if of added value		Assess need and selection of relevant assay based on Phase I data if of added value		Assess need and selection of relevant assay based on Phase I/II data if of added value		
Timing of ADA analytics	Collect and hold, analyze in case of unexpected PK, PD/ efficacy (if applicable) or safety-related AE	End of study	At least at the end of each dose level Close monitoring prior to next drug administration	End of study		Batch wise throughout study Close monitoring	End of study (recommend to frontload analysis ahead of study end)		Batch wise throughout study Close monitoring
ADA sample collection frequency	Baseline, end of cycle/dose tier (based on dosing)	Baseline, end of cycle/dose tier, selected timepoints at 7–14 days and 3–6 weeks based on regulatory requirements and project needs and EOS	Baseline, onset of ADA response such as Day 7–14 after first exposure, end of cycle/dose tier, for consecutive cycle/ dose tier pre-dose and EOS Frequent sampling through all stages. Post-study follow-up sampling required for high-risk with serious safety consequences from ADA Consider use of other samples, i.e. PK/PD sample for additional ADA analysis In disease population collect higher volume of pre-dose samples (at least 2ml)	Baseline, selected timepoints at 7–14 days and 3–6 weeks based on regulatory requirements and project needs and EOS		Frequent sampling through all stages	Less frequent sampling than Phase I & II		Frequent sampling through all stages and EOS Post-study follow-up sampling required for high-risk with serious safety consequences from ADA
	Ad hoc samples in case of SAE as part of safety assessment								
Overall strategy	Engage with HAs to align on IG sampling and testing								

EOS, end of study (approximately 5 half-lives after last drug exposure (6)).

IRA-based IG monitoring - Example 1

low | moderate | high

Risk Identification

Product-related:

- fully human monoclonal antibody
- low sequence risk after *in silico (*& *in vitro* assessment)
- mode of action:
  - function blocking
  - soluble, monomeric target,
  - no effector function
  - **PD effect within 1-3 days post administration (acute mode of action)**; PD marker available

Process-related:

- All CQAs in typical range for mAbs

Patient-related:

- healthy volunteers in Phase I
- Patients with normal immune status
- No evidence for pre-existing ADA

Treatment-related:

- Single IV administration (acute mode of action)!
  -> **NO SECOND DOSE**

Risk Evaluation

Potential IG consequences:

- Impact on PK possible **without impact on PD**
- No safety consequences (no endogenous counterpart, no exaggerated pharmacology)
- Hypersensitivity possible as with all mAbs, no additional risk identified

IRA-based IG monitoring - Example 1

low | moderate | high

Risk Identification

Product-related:
- fully human monoclonal antibody
- low sequence risk after *in silico* (& *in vitro* assessment)
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Process-related:
- All CQAs in typical range for mAbs

Patient-related:
- healthy volunteers in Phase I
- Patients with normal immune status
- No evidence for pre-existing ADA

Treatment-related:
- Single IV administration (acute mode of action)!
  -> **NO SECOND DOSE**

Risk Evaluation

Potential IG consequences:
- Impact on PK possible **without impact on PD**
- No safety consequences (no endogenous counterpart, no exaggerated pharmacology)
- Hypersensitivity possible as with all mAbs, no additional risk identified

Which IG risk category would you assign to the program?

Low	Moderate	High
0	0	0

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Example 1

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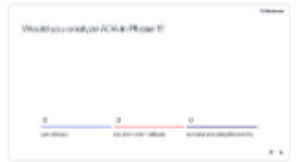


EIP European Immunogenicity Platform

EIP recommendation – Example 1

Which IG risk category would you assign to the program?

Low

Would you analyze ADA in Phase 1?

Collect and hold, analyze in case of unexpected PK, PD/efficacy (if applicable) or safety–related AE

Would you analyze ADA samples with a fully validated method?

A fully validated ADA method is typically implemented in pivotal clinical studies, except for high-risk molecules where full validation may be considered in earlier phases.

Would you implement a NAb assay in Phase 3 (ADA/NAb dev. delayed to PD)?

Consider integrated (active) PK/PD/ADA strategy instead of NAb

Engage with Health Authorities to align on IG sampling and testing

EIP
European Immunogenicity Platform

IRA-based IG monitoring - Example 2

low | moderate | high

Risk Identification

Product-related:

- fully human monoclonal antibody
- **No *in silico* & *in vitro* assessment**
- mode of action:
  - function blocking
  - soluble, monomeric target
  - no effector function
  - **PD marker available**

Process-related:

- All CQAs in typical range for mAbs

Patient-related:

- Healthy volunteers in Phase I
- Patients with normal immune status
- No evidence for pre-existing ADA

Treatment-related:

- **Chronic SC administration (Q3W)**

Risk Evaluation

Potential IG consequences:

- **Impact on PK and PD, efficacy possible**
- No safety consequences (no endogenous counterpart, no exaggerated pharmacology)
- Hypersensitivity possible as with all mAbs, no additional risk identified

IRA-based IG monitoring - Example 2

low | moderate | high

Risk Identification

Product-related:
- fully human monoclonal antibody
- **No *in silico* & *in vitro* assessment**
- mode of action:
  - function blocking
  - soluble, monomeric target
  - no effector function
  - **PD marker available**

Process-related:
- All CQAs in typical range for mAbs

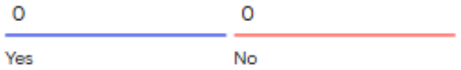
Patient-related:
- Healthy volunteers in Phase I
- Patients with normal immune status
- No evidence for pre-existing ADA

Treatment-related:
- **Chronic SC administration (Q3W)**

Risk Evaluation

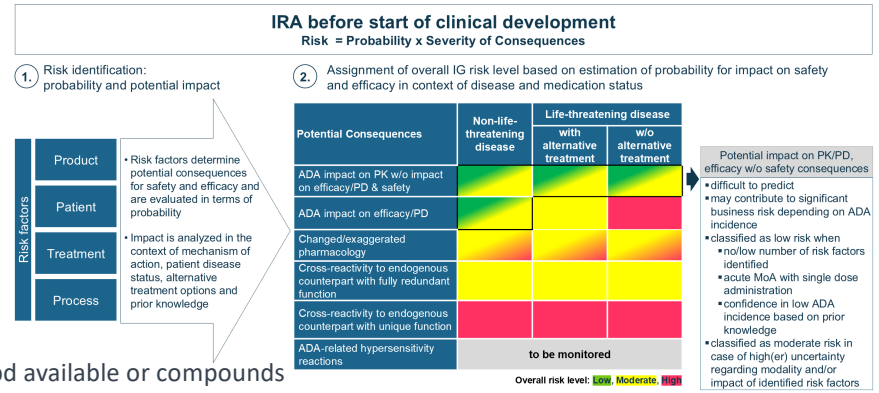
Potential IG consequences:
- **Impact on PK and PD, efficacy possible**
- No safety consequences (no endogenous counterpart, no exaggerated pharmacology)
- Hypersensitivity possible as with all mAbs, no additional risk identified

Example 2: Would you invest in an active PK instead of NAb?



EIP European Immunogenicity Platform

EIP recommendation – Example 2



Which IG risk category would you assign to the program?
Impact on PK/PD efficacy difficult to predict
Low
- when low number of risk factors identified
- confidence in low ADA incidence based on prior knowledge (e.g. information on structurally similar cpd available or compounds targeting same molecule w/o safety concern

Moderate
- In case of high(er) uncertainty regarding modality and/or impact of identified risk factors

Would you analyze ADA in Phase 1?
Low: Collect and hold, analyze in case of unexpected PK, PD/efficacy (if applicable) or safety–related AE
Moderate: Analyze samples

At which frequency would you include sampling for Phase 1?
Low: Baseline, end of cycle/dose tier (based on dosing)
Moderate: Baseline, end of cycle/dose tier, selected timepoints at 7–14 days and 3–6 weeks based on regulatory requirements and project needs and EOS

Would you implement a NAb assay in Phase 2 (total PK, PD available)?
Low: Not needed
Moderate: NAb evaluation to be considered for moderate risk; consider integrated **(active) PK**/PD/ADA strategy instead of NAb

Would you implement a NAb assay in Phase 3 (total PK, PD available)?
Low: Consider integrated (active) PK/PD/ADA strategy instead of NAb
Moderate: NAb evaluation expected for moderate Consider integrated **(active) PK**/PD/ADA strategy instead of NAb

Would you invest in an active PK instead of NAb?
Low & Moderate: Consider integrated **(active) PK**/PD/ADA strategy instead of NAb

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European Immunogenicity Platform

Engage with Health Authorities to align on IG sampling and testing

IRA-based IG monitoring - Example 3

low | moderate | high

Risk Identification

Product-related:

- **IgG-scFv**
- Low risk in *in silico* & *in vitro* assessment
- mode of action:
  - function blocking
  - IgG: soluble and membrane bound target; scFv: soluble, dimeric target
  - no effector function
  - PD marker available

Process-related:

- All CQAs in typical range for mAbs

Patient-related:

- Healthy volunteers in Phase I
- Patients with normal immune status
- **Pre-existing ADA**

Treatment-related:

- Single IV dose in phase I; multiple dose in later trials

Risk Evaluation

Potential IG consequences:

- **Impact on PK and PD, efficacy possible**
- No safety consequences (no endogenous counterpart, no exaggerated pharmacology)
- Hypersensitivity possible as with all mAbs, no additional risk identified

IRA-based IG monitoring - Example 3

low | moderate | high

Risk Identification

Product-related:
- **IgG-scFv**
- Low risk in *in silico* & *in vitro* assessment
- mode of action:
  - function blocking
  - IgG: soluble and membrane bound target; scFv: soluble, dimeric target
  - no effector function
  - PD marker available

Process-related:
- All CQAs in typical range for mAbs

Patient-related:
- Healthy volunteers in Phase I
- Patients with normal immune status
- **Pre-existing ADA**

Treatment-related:
- Single IV dose in phase I; multiple dose in later trials

Risk Evaluation

Potential IG consequences:
- **Impact on PK and PD, efficacy possible**
- No safety consequences (no endogenous counterpart, no exaggerated pharmacology)
- Hypersensitivity possible as with all mAbs, no additional risk identified

Example 3: Would you implement two NAb assays in Phase 2 (total PK, PD available)?

Yes always	Yes, only at high ADA incidences	No, independent of ADA incidence
0	0	0

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Example 1

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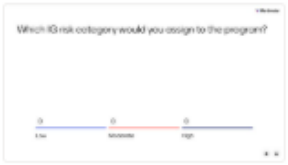
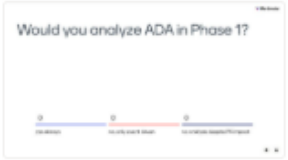
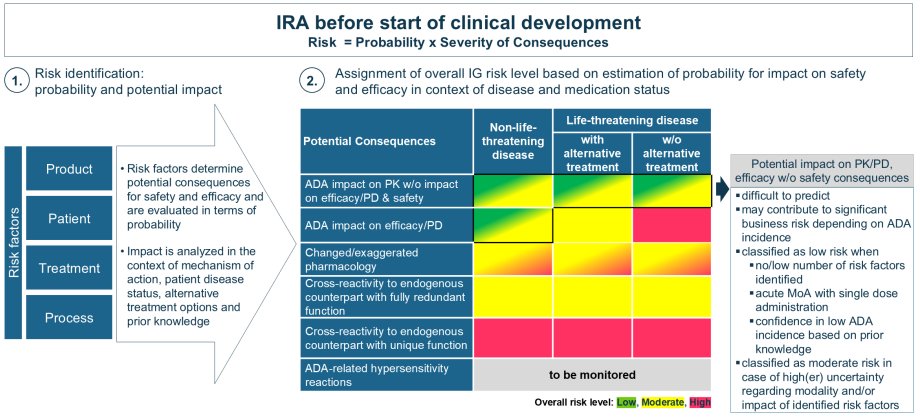




EIP recommendation – Example 3



Which IG risk category would you assign to the program?
Impact on PK/PD efficacy difficult to predict
Low
- when low number of risk factors identified
- confidence in low ADA incidence based on prior knowledge

Moderate
- In case of high(er) uncertainty regarding modality and/or impact of identified risk factors

Would you analyze ADA in Phase 1?
Low: Collect and hold, analyze in case of unexpected PK, PD/efficacy (if applicable) or safety–related AE
Moderate: Analyze samples

Would you implement domain characterization and if yes, when?
Assess need and selection of relevant assay if of added value (based on Phase 1 / based on Phase 1 / 2 data). *New manuscript in preparation.*

Would you implement two NAbs assay in Phase 2?
Low: Not needed
Moderate: NAb evaluation to be considered for moderate risk; consider integrated (active) PK/PD/ADA strategy instead of NAb

Would you implement two NAbs assay in Phase 3?
Low: Consider integrated (active) PK/PD/ADA strategy instead of NAb
Moderate: NAb evaluation expected for moderate; consider integrated **(active) PK**/PD/ADA strategy instead of NAb

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Engage with Health Authorities to align on IG sampling and testing

IRA-based IG monitoring – Example 4

low | moderate | high

Risk Identification

Product-related:

- **Endogenous protein**
- Full sequence homology to endogenous counterpart
- mode of action:
  - **functional redundancy expected, but not proven**
  - **PD marker available**

Process-related:

- All CQAs in typical range for mAbs

Patient-related:

- Patients in Phase I
- Rare disease (non-life-threatening) with normal immune status
- No pre-existing ADA

Treatment-related:

- Single and multiple IV administration in Phase I

Risk Evaluation

Potential IG consequences:

- Impact on PK and PD, efficacy possible
- ADA & NAb cross-reactivity to endogenous counterpart possible
- Hypersensitivity possible as with all biotherapeutics, no additional risk identified

IRA-based IG monitoring – Example 4

low | moderate | high

Risk Identification

Product-related:
- **Endogenous protein**
- Full sequence homology to endogenous counterpart
- mode of action:
  - **functional redundancy expected, but not proven**
  - **PD marker available**

Process-related:
- All CQAs in typical range for mAbs

Patient-related:
- Patients in Phase I
- Rare disease (non-life-threatening) with normal immune status
- No pre-existing ADA

Treatment-related:
- Single and multiple IV administration in Phase I

Risk Evaluation

Potential IG consequences:
- Impact on PK and PD, efficacy possible
- ADA & NAb cross-reactivity to endogenous counterpart possible
- Hypersensitivity possible as with all biotherapeutics, no additional risk identified

Example 4: Would you implement a NAb assay?

No	Yes from Phase 1 onwards	Yes from Phase 2 onwards	Yes from Phase 3 onwards
0	0	0	0

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Example 1

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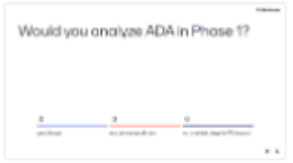

EIP recommendation – Example 4

Which IG risk category would you assign to the program?

(Moderate risk only when data on functional redundancy available)

High risk when no data on functional redundancy available: It is important to underline that consequences of neutralization of an endogenous protein with partial redundant function may not always lead to immediate clinical symptoms (FDA Guidance 2014), emphasizing that a high-risk categorization should be considered in case of uncertainty towards full functional redundancy.

Would you analyze ADA in Phase 1?

ADA: Analyze samples (fully validated assay)

At which frequency would you include sampling for Phase 1?

Baseline, onset of ADA response such as Day 7–14 after first exposure, end of cycle/dose tier, for consecutive cycle/dose tier pre-dose and EOS

Frequent sampling through all stages. Post-study follow-up sampling required for high-risk with serious safety consequences from ADA Consider use of other samples, i.e. PK/PD sample for additional ADA analysis In disease population collect higher volume of pre-dose samples (at least 2ml)

Would you implement a NAb assay and if yes, when?

NAb for high risk is often expected in Phase 1 and is expected in Phase 2 and 3. Consider integrated (active) PK/PD/ADA as potential alternative

Engage with Health Authorities to align on IG sampling and testing

BACKUP

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Immunogenicity Risk Adaptation in Clinical Development

Immunogenicity risk level can be adapted based on incoming clinical data allowing adjustments in IG mitigation and monitoring (healthy volunteers ≠ patients):