Assessing the Safety of Peptide-Related Impurities in Support of Commercial Control Strategy Development

Brian Pack, Michael Hodsdon, Robert Siegel, Laurent Malherbe, Andrea Ferrante, Doug Roepke, Mark Carfagna, and Paul Cornwell

Lilly

Topics of Discussion

- This presentation is a combination of key external opinions with regard to the safety of impurities as they relate to dose level and frequency of dosing and applied those concepts to peptides

- Immunogenicity (IG) of peptide impurities along with unique *in vitro* approach to assess IG risk
- Toxicity of peptide Impurities

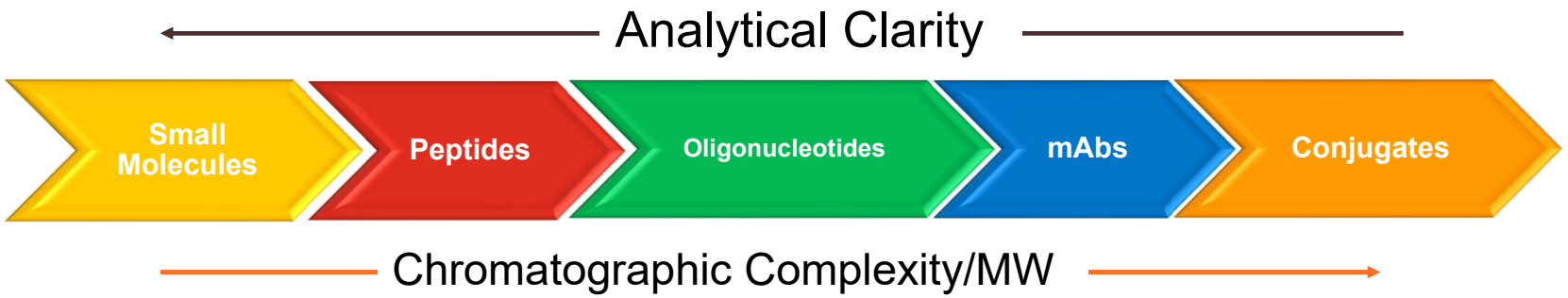
- How these safety threshold concepts can be applied in support of Clinical Trial/Development activities…
  - Specifications
  - GMP impurity profile comparisons
  - "Formal" Comparability Studies

CMC/Analytical Activities

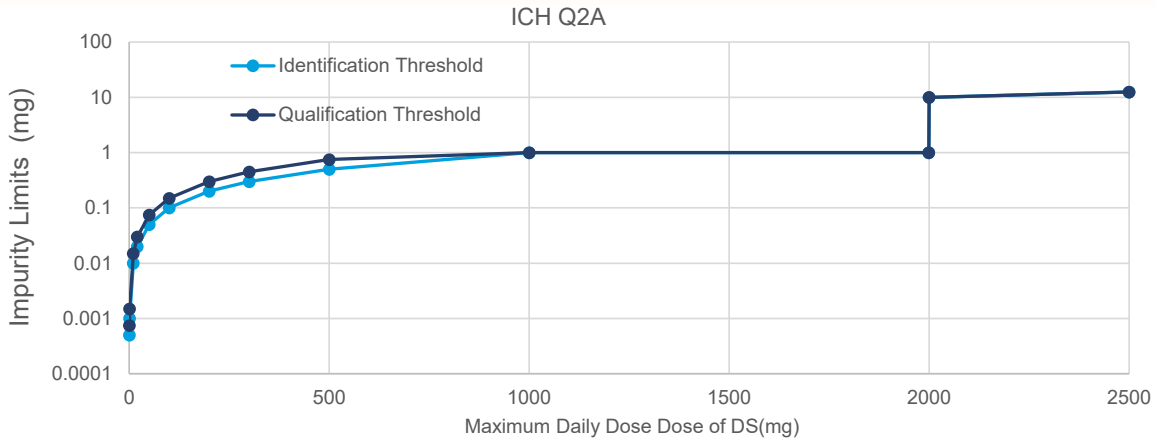
Global patient safety (medical)
Toxicology experts
Immunogenicity experts
Regulatory Scientists

Overarching Problem Statement

- There is no guidance from ICH or FDA on the identification/qualification/comparability thresholds of **peptide impurities** in the drug substance or drug product to support development/clinical trials
  - Commercial limits articulated in Ph Eur <2034> and EMA DRAFT Synthetic Peptide Guidance in preparation
- This lack of guidance can lead to ambiguity when supporting process development, specifications, particularly as to when (how low) to identify impurities, qualify impurities, when are batches comparable, etc.
- We need to think about which guidance could be applicable before applying to a different modality

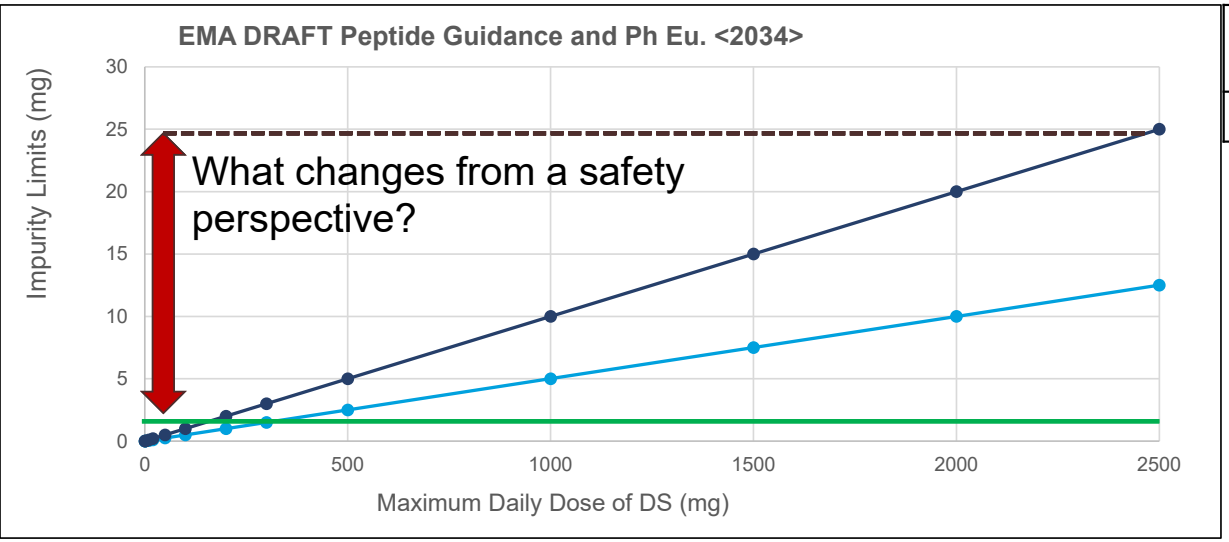


Graphical Representation of Limits for Lifetime **Daily** Dosing



Small Molecule

Maximum Daily Dose[1]	Reporting Threshold[2,3]	Identification Threshold[3]	Qualification Threshold[3]
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%



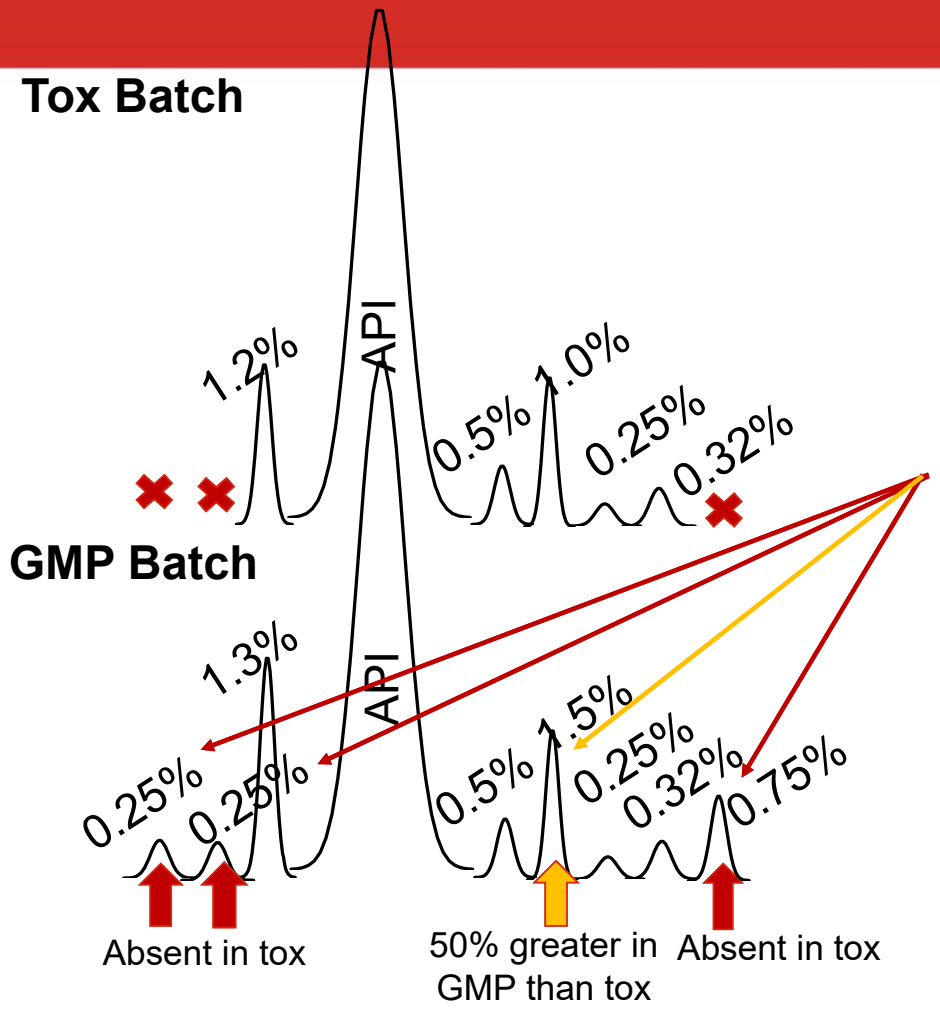
Peptide

Reporting Threshold	Identification Threshold	Qualification Threshold
>0.1 %	>0.5%	>1.0%

Rationale to support:

1 mg/day of an adjusted peptide-related impurity **daily** exposure is a safe and conservative means to calculate unspecified impurity limits

Where a Scientific Rationale Can Help



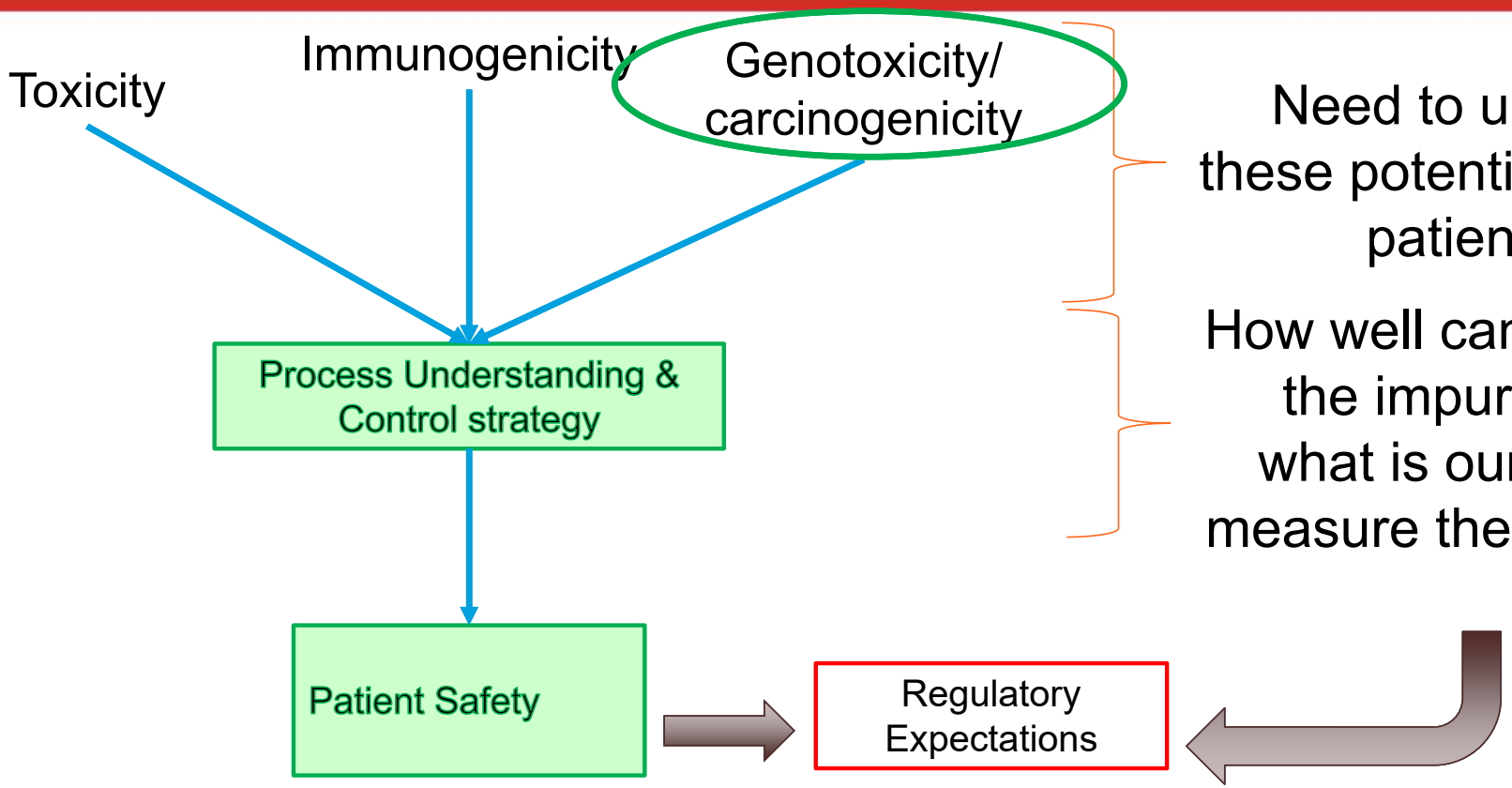
Simulated chromatograms for illustration only

The rest of this presentation will be focused on:

> *"Are these impurities safe even if they have never been in a toxicology study, or were there at a lower level?"*
> *and "What is the risk of immunogenicity associated with that peptide impurity?"*

- **We are providing the safety rationale that includes both toxicity and immunogenicity assessments to address these concerns**

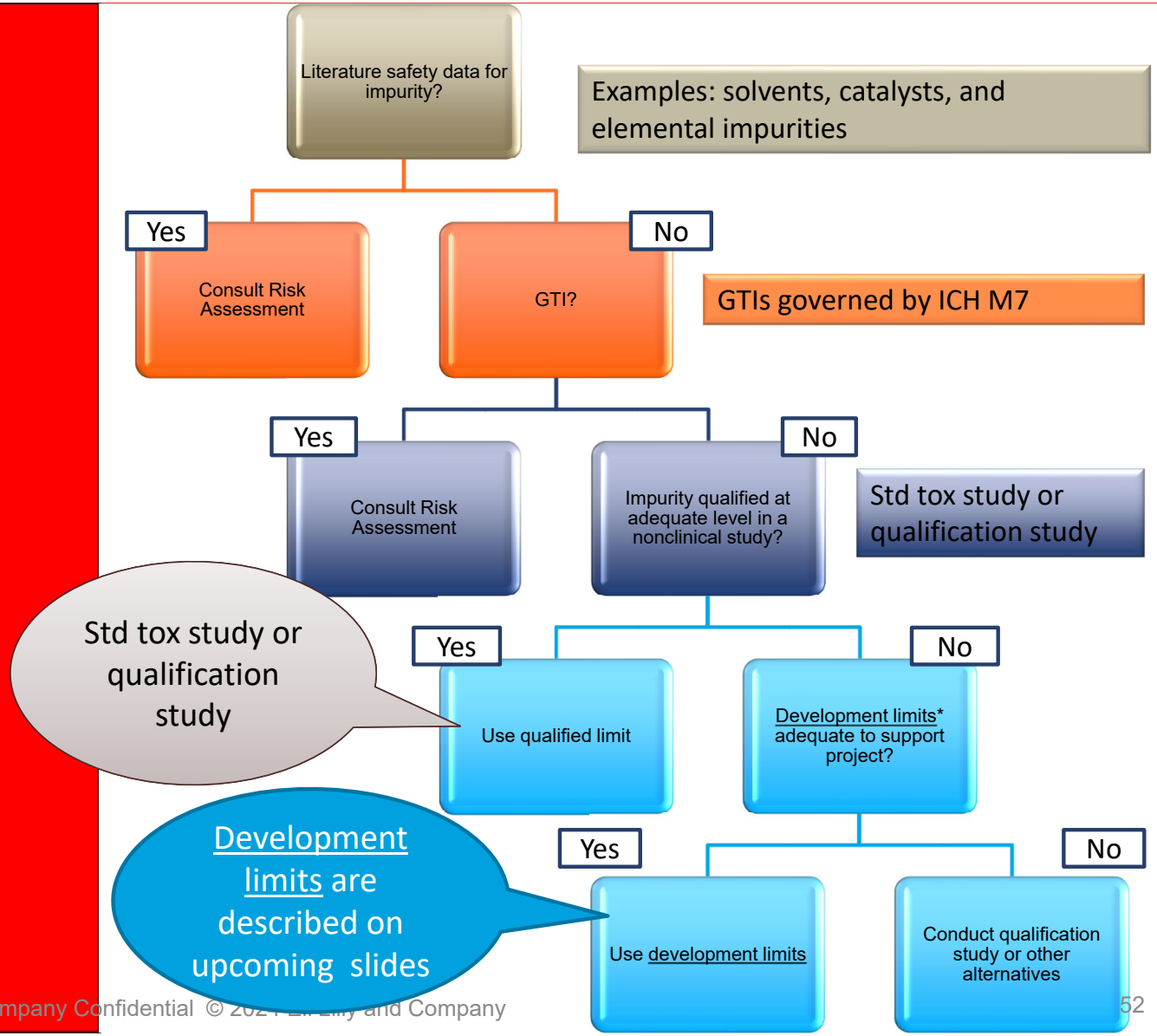
Why Do We Report/Identify and Potentially Qualify Impurities?



Need to understand these potential sources of patient risks

How well can we control the impurities and what is our ability to measure the impurities?

Peptide Impurity Qualification Decision Tree



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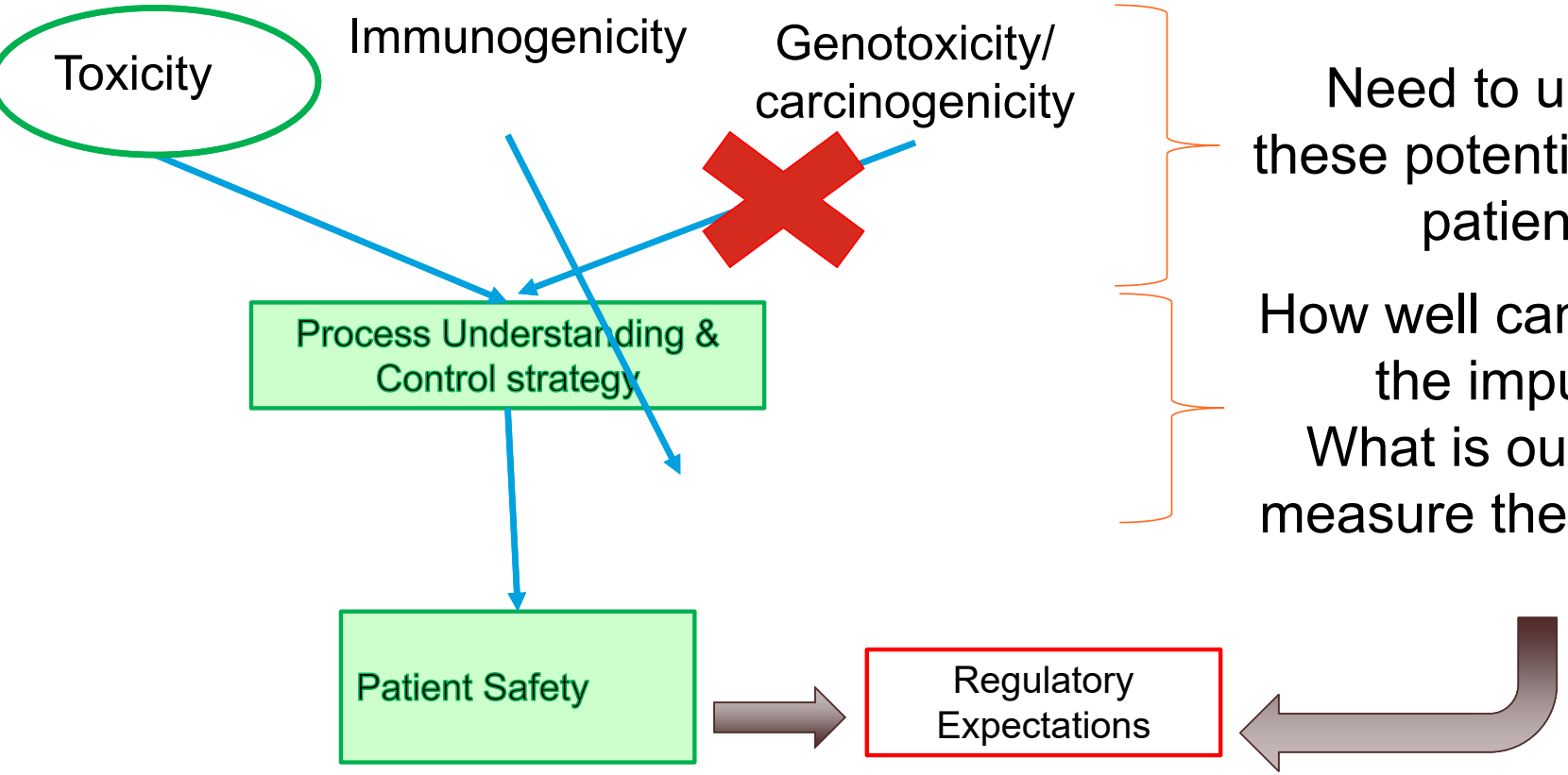

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How well can we control the impurities?
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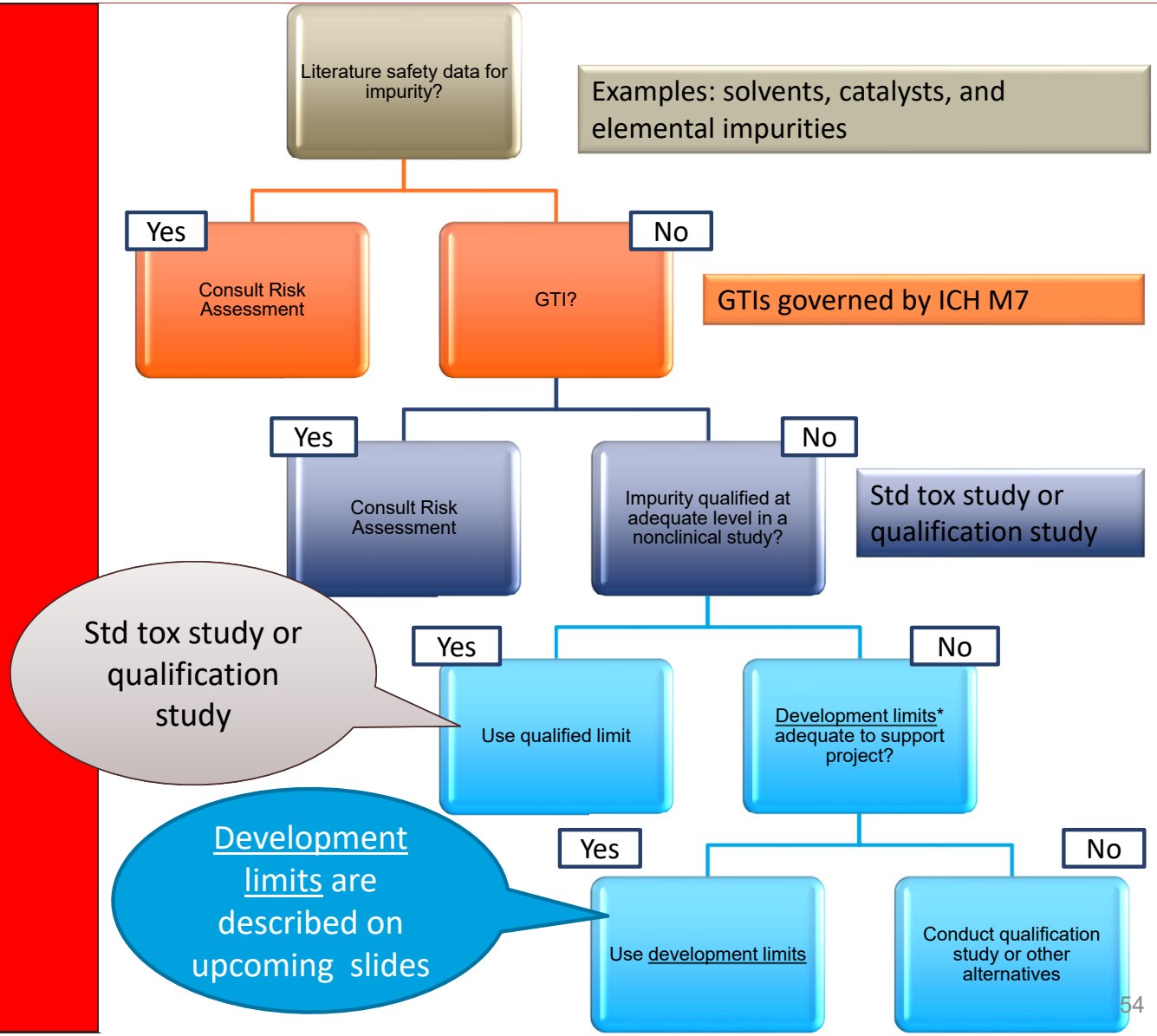


Justification That 1mg/day is Safe

A Wealth of Literature Evidence Exists in support of 1mg/day !

- 1 mg selected to align with ICH Q3A/B limit
- Cramer et al, 1978, Three classes of impurities
  - **Class I low toxicity**, Class II moderate toxicity and Class III high toxicity (mutagens)
  - Most DS and DP-related impurities are likely to by **Cramer class I**
- Munro 1996
  - Analyzed over 600 chemicals with over 2900 NOEL endpoints
  - Established that **≤1.8 mg/day** is not of toxicological concern for Cramer class I chemicals
    - Includes a 100x safety factor to the 5th percentile NOEL
- Kroes 2004
  - 730 compound database
  - Applies same logic as Munro 1996 – supports 1.8 mg/day limit
- Munro 2008
  - Describes use cases for the limits derived in Munro 1996
- Tluczkiewicz 2011
  - Added additional databases to the Munro 1996 analysis
  - Refined limit to **1.9 mg/day** for Cramer class I chemicals
- Graham 2021
  - Analyzed 168 DS intermediates/starting materials – very similar to typical DS impurities
  - None at NOAEL <1 mg/day

Patient Safety

- Much of the literature supports 1.8-1.9 mg /day
- Mayur et al. **23 IQ Consortium DruSafe member companies** : Out of a total of 92 Impurity Qualification studies performed, unique toxicities attributed to the impurities were not observed for any of the studies
- Small molecules are expected to have more off-target/unpredictable effects than derivatives of peptides
- Will apply a non-linear adjustment to account for dosing frequency

Duration Adjustment

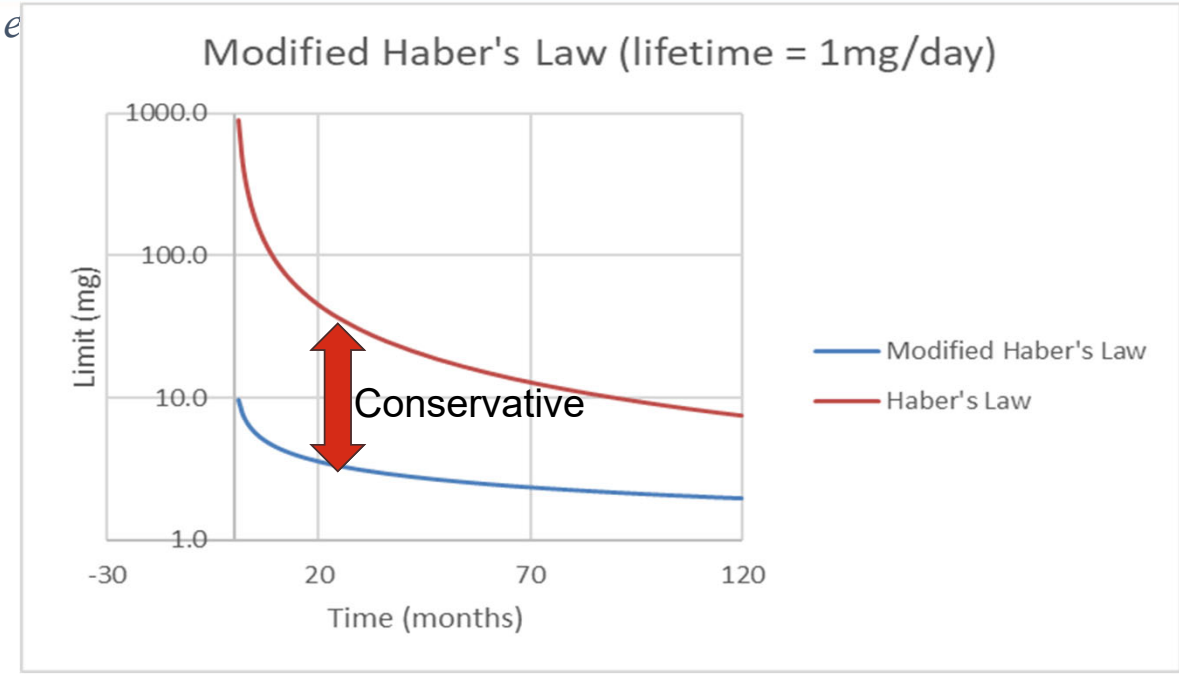
A conservative version of Haber's Law (Harvey et al 2017).

Haber's Law: $c \times t = c' \times t'$

Modified Haber's Law: $c' = \sqrt[3]{\frac{c^3 \times t}{t'}}$

c = acceptable impurity limit for duration t
c' = acceptable impurity limit for duration t'

For peptide related impurities, **c = 1 mg/day** and t = 75 years or 27375 days.



- More conservative than the linear less-than-lifetime concept used in ICH M7 for the Assessment and Control of DNA Reactive Impurities to Limit Carcinogenic Risk!
- ICH M7(R2) "In the case of intermittent dosing, the acceptable daily intake should be based on the total number of dosing days instead of the time interval over which the doses were administered...."

Individual Peptide Impurity Limits

Toxicology Supported Limits

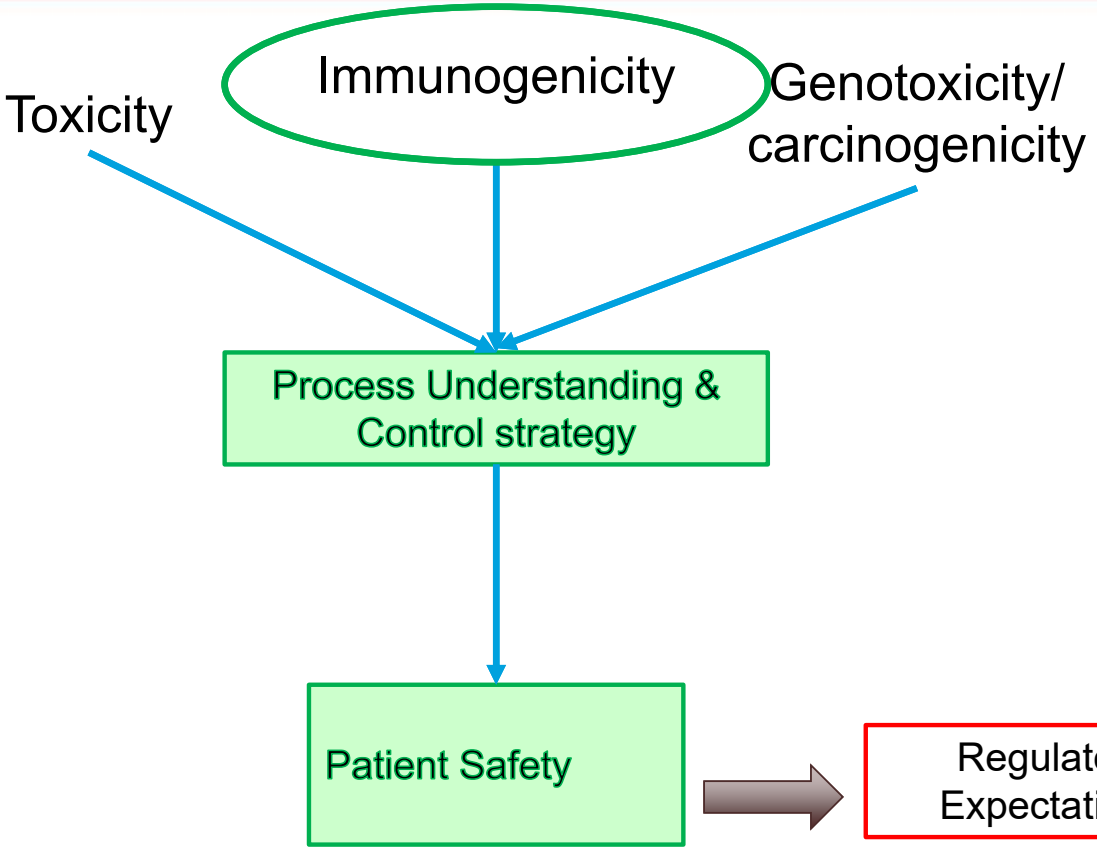
Frequency	Limit
Daily	1 mg/dose
Weekly	1.9 mg/dose
Monthly	3.1 mg/dose
Quarterly	4.5 mg/dose
Twice yearly	5.7 mg/dose
Once yearly	7.1 mg/dose

- Assumes that 1 mg/day for a lifetime is safe
- Applies modified Haber's Law to provide conservative adjustment for less-than-lifetime exposure due to intermittent dosing (Harvey et al)

- Conservative because it does not account for large molecular weight of peptides (however, we could consider this adjustment)

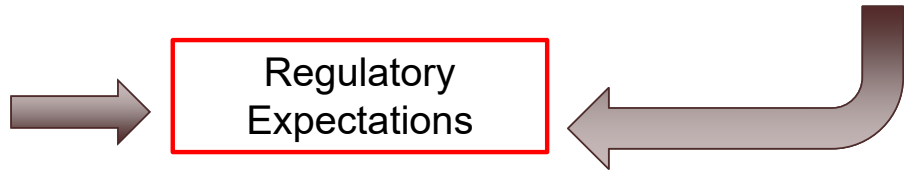
		Safety Threshold (%)			
	Impurity Limit (mg)	Therapeutic Dose (1 mg)	Therapeutic Dose (10 mg)	Therapeutic Dose (50mg)	Therapeutic Dose (100 mg)
Daily	1.0	100.0	10.0	2.0	1.0
Weekly	1.9	191.3	19.1	3.8	1.9
Monthly	3.1	310.7	31.1	6.2	3.1

Why Do We Report/Identify and Potentially Qualify Impurities?



Need to understand these potential sources of patient risks

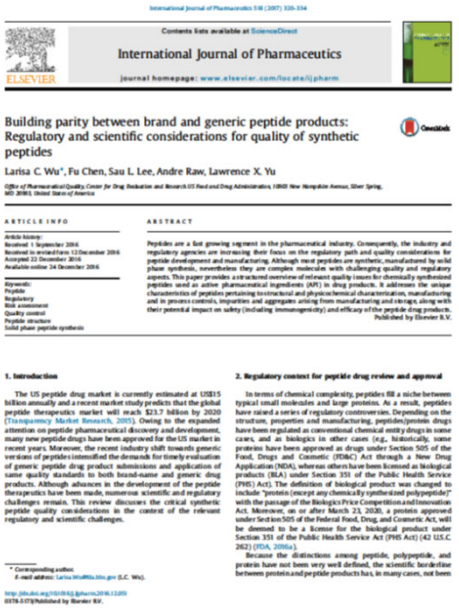
How well can we control the impurities?
What is our ability to measure the impurities?



Immunogenicity of Peptides

Immunogenicity is mentioned 25 times in the cited manuscript: “Safety concerns including peptide immunogenicity may be due not only to the peptide itself, but also to the impurities and contaminants that are arising from the manufacturing process and storage.”

Larisa C. Wu, Fu Chen, Sau L. Lee, Andre Raw, Lawrence X. Yu Office of Pharmaceutical Quality, Center for Drug Evaluation and Research US Food and Drug Administration*


International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Building parity between brand and generic peptide products: Regulatory and scientific considerations for quality of synthetic peptides

Larisa C. Wu*, Fu Chen, Sau L. Lee, Andre Raw, Lawrence X. Yu

Immunogenicity (IG) is the biggest concern with new impurities

- standard nonclinical toxicology models are considered to be unreliable for predicting human immunogenicity
- Unaware of literature that shows process or product-related impurity (excluding HMW aggregates) as cause for IG
  - Tungsten leachates from needle caused erythropoetin aggregation

Assess this risk early!

Immunogenicity

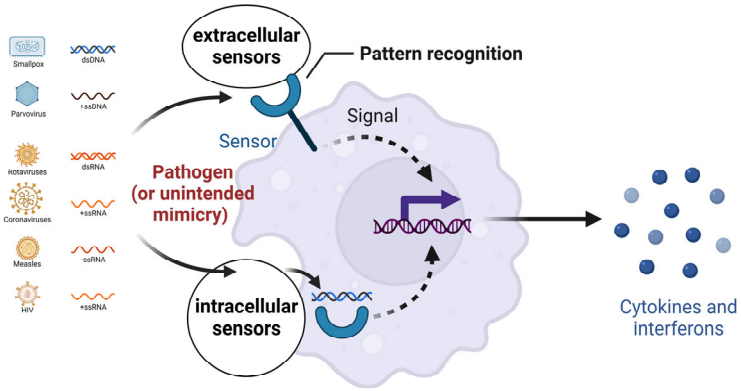
A complex process with different concerns for different types of molecules

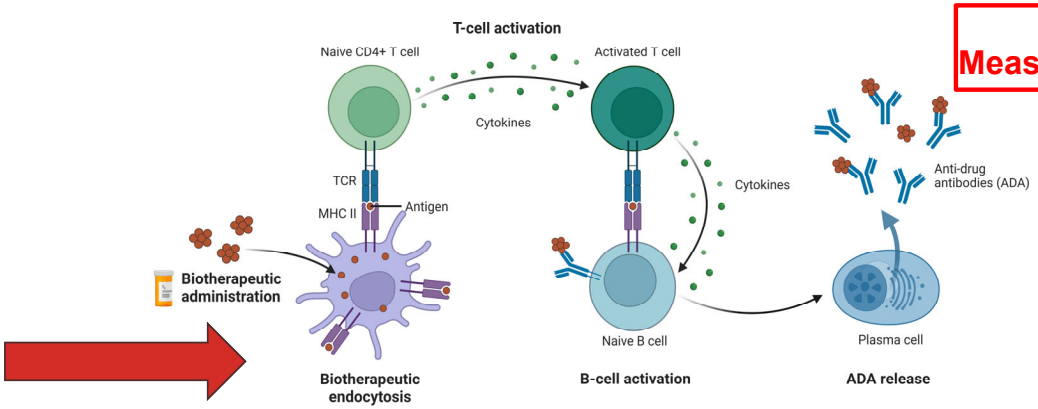
Innate Immune System:

- **Generalized 1st line of defense against infection (time to onset < 48 hrs)**
- **Inflammation / fever / malaise**

Adaptive Immune System:

- **Specific response if innate system is insufficient (time to onset ~ 2 weeks)**
- **Lasting immunity**



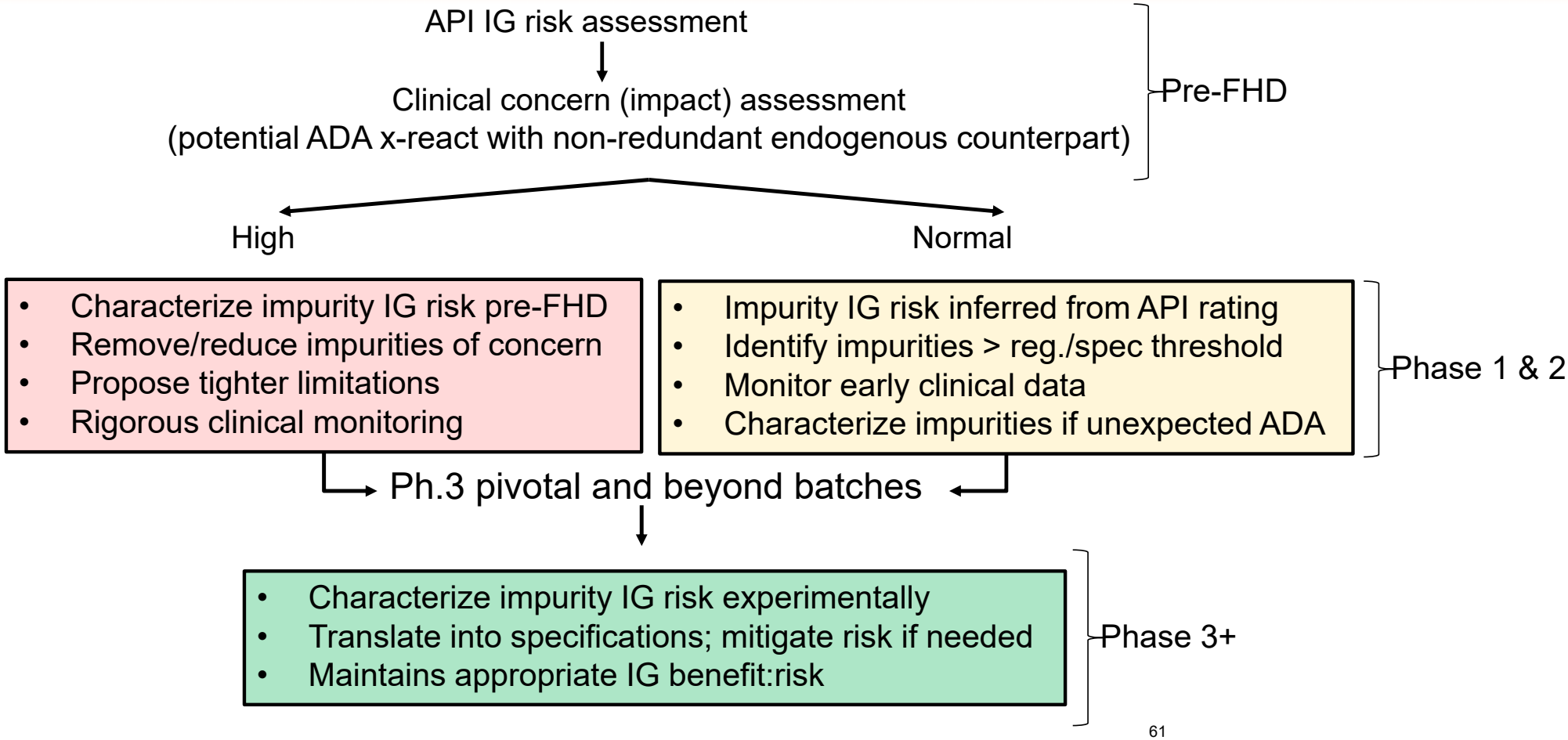


Measured in clinic

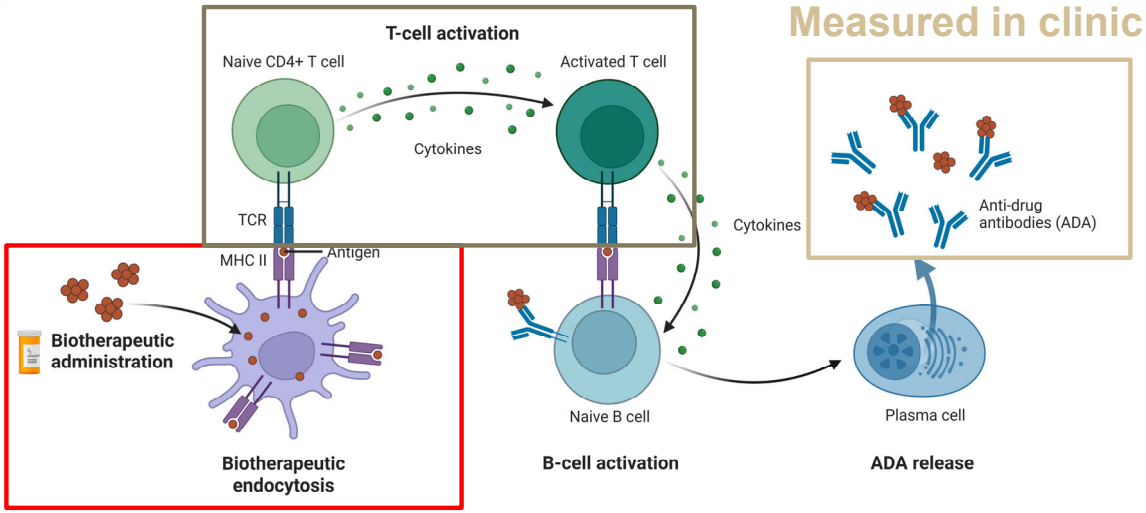
Primarily oligonucleotide concern

Primarily protein concern

Peptide Impurity IG Risk Mitigation Strategy



Peptide Impurities IG Assessment Scheme

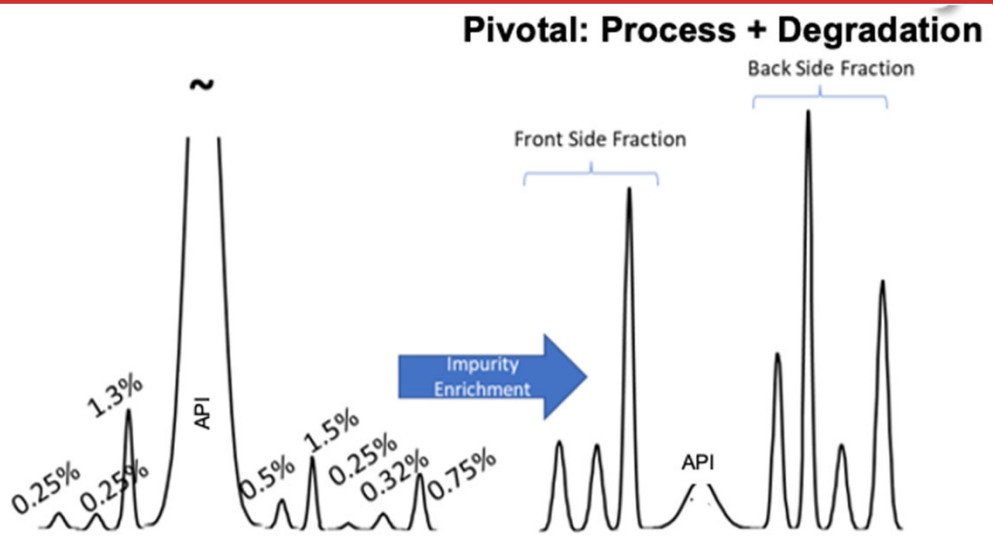


- Leverage *in vitro* assays used for API characterization that best characterize risk for [1]relevant aspects required for treatment-emergent ADA
  - **[2]MAPPs**: determine if regions are presented for T cell surveillance
  - **T cell proliferation assay**: bulk impurities and MAPPs-peptides

[1] literature and regulator guidance are aimed at informing clinical development paradigms for a generic synthetic peptide of a previously approved peptide of recombinant DNA origin instead of guiding internal decision-making processes during the development of originator molecules

[2] MHC-Associated Peptide Proteomics: mass spectrometry method able to determine precise sequences bound by HLA class II molecules for T cell surveillance

How best to characterize peptide impurities for Immunogenicity Risk



- Limited quantities of any given impurity have impact on assay sensitivity
- Generate enriched impurity samples from batches of interest

- Pooled Initial Read: Signal of concern will trigger additional studies to identify and eliminate purities of concern
- Immunogenicity assessment ranking will be used to support comparability thresholds and specified impurity levels, if needed

Impurity immunogenicity threshold rationale

Clinical Concern	Immunogenicity Assessment: Low	Moderate	High
Normal	Highest threshold	Higher threshold	Moderate threshold
High	Higher threshold	Moderate threshold	Lowest threshold

- Identify a maximum immunogenicity impurity threshold **0.05 mg per dose** as an anchor

A novel peptide-based pan-influenza A vaccine: A double blind, randomised clinical trial of immunogenicity and safety☆

James N. Francis[a], Campbell J. Bunce[a,*], Claire Horlock[a], Jeannette M. Watson[a], Steven J. Warrington[b], Bertrand Georges[a,1], Carlton B. Brown[a,1]

[a] Immune Targeting Systems Ltd., London, NW1 0NH, UK
[b] Hammersmith Medicines Research Ltd., London, NW10 7EW, UK

  - [redacted]
  - Conservative in nature as amount is based on non-responsive levels to sequences intended to cause an immune response

Clinical Concern	Immunogenicity Assessment: Low	Moderate	High
Normal	30x (1.5 mg)	10x (0.5 mg)	3x (0.15 mg)
High	10x (0.5 mg)	3x (0.15 mg)	1x 0.05 mg (EPO-like; worst case scenario, **unlikely to occur**)

- Implement **half-log multiples** based on clinical concern and immunogenicity risk rating
  - 1x multiple (0.05 mg): highest concern
  - 30x multiple (1.5 mg): lowest concern

Below these levels, impurities can be considered safe

What are the “rules” to apply the various impurity IG thresholds

		Impurity Immunogenicity Assessment		
		Low	Moderate	High
Clinical Concern	Normal	30x (1.5 mg)	10x (0.5 mg)	3x (0.15 mg)
	High	10x (0.5 mg)	3x (0.15 mg)	1x 0.05 mg (EPO-like; worst case scenario, **unlikely to occur**)

Algorithm predicated on risk:

1) **clinical concern**,
2) **established clinical ADA profile**,
3) **IG risk of impurity**, plus stage of development

	Stage:	Normal Clinical Concern				High Clinical Concern		
		FHD	Ph.3+			FHD+		
	Impurity IG risk:		LOW	MOD	HIGH	LOW	MOD	HIGH
AP IG risk or established ADA	LOW	Tox limit 1.9 mg	Tox limit 1.9 mg	10x 0.5 mg	3x 0.15 mg	30x 1.5 mg	3x 0.15 mg	1x 0.05 mg
	MOD	Tox limit 1.9 mg	Tox limit 1.9 mg	30x 1.5 mg	3x 0.15 mg	Tox limit 1.9 mg	10x 0.5 mg	1x 0.05 mg
	HIGH	Tox limit 1.9 mg	Tox limit 1.9 mg	Tox limit 1.9 mg	10x 0.5 mg	Tox limit 1.9 mg	Tox limit 1.9 mg	3x 0.15 mg

* Tox limit assumes QW dosing

Impurity and degradation IG threshold algorithm:

- Normal clin concern @ FHD = Tox limit
- If impurity risk < established clinical ADA, then relax to tox limits, if needed
- If impurity risk = established clinical ADA, then relax limits to next risk level, if needed
- If impurity risk > established clinical ADA, then impose impurity risk threshold since it poses most likely reason to change observed ADA
- Any <u>new</u> impurity that appears after this experimental assessment will <u>conservatively be assigned a relative risk of High</u>

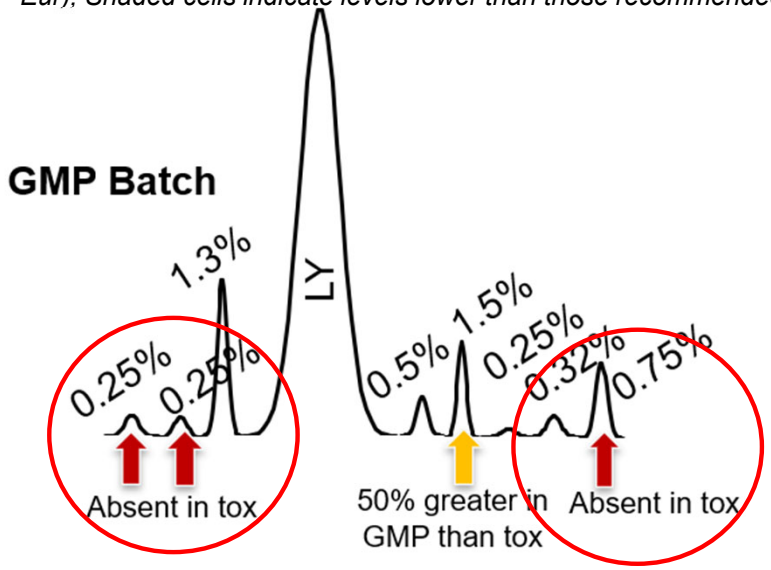
Safety Threshold as a Function of API Dose

Immunogenicity risk is derived from a mass perspective, whereas impurity specifications are % based

Scenario
Normal Clinical Concern
Moderate IG Risk
30 mg weekly dose
Phase 2: NMT 1.5% for any unspecified imp.

Impurity level / LY Dose Level	#Tox limit (1.9 mg)	30x (1.5 mg)	10x (0.5 mg)	3x (0.15 mg)	1x (0.05 mg)
10 mg	~~*19.0%~~	*15.0%	~~*3.33%~~	*1.5%	0.33%
30 mg	*6.33%	*5.0%	*1.67%	0.5%	0.11%
100 mg	~~*1.9%~~	*1.5%	~~0.5%~~	0.15%	0.05%
1000 mg	0.19%	0.15%	0.05%	0.015%	0.005%

**likely defer to lower levels from other established norms, but may be used to support specified impurity specifications (i.e., anything greater than 0.5% as defined by Ph Eur); Shaded cells indicate levels lower than those recommended in the Ph Eur guidance and challenging to achieve from a technical standpoint*



Toxicology Supports 6.33%
Immunogenicity Supports 1.67%
Regulatory Commitment is NMT 1.5%

This material passes 1) specifications, 2) impurity profile comparison and 3) should also be deemed comparable as ICHQ5E is safety focused .

High Molecular Weight Species

- From immunological perspective, protein aggregates are defined very broadly as high MW proteins composed of multimers of natively conformed or denatured monomers resulting in a polymeric structure (Rosenberg, 2006).
- Aggregates of therapeutic peptides/proteins that consist of 10–20 epitopes at a repetitive spacing of approx. 100 Å and a molecular weight greater than 100 kDa is required before an immunogenic signal is delivered to the responding cell (Dintzis et al., 1976).
  - A pattern that mimics pathogens needs to be present to trigger pattern recognition sensors of the innate immune system
- These large (>trimer) highly ordered aggregates are controlled by other processes and are out of scope for impurities discussion.

- **Peptide dimers/trimers**: not sufficient to trigger innate and/or T cell independent B cell activation

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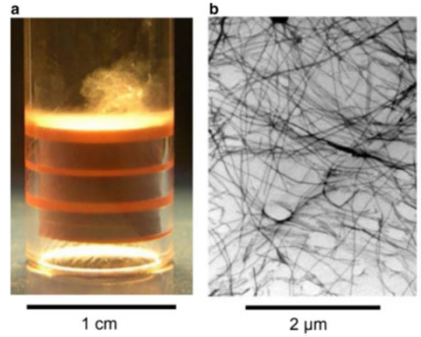

Summary

- This presentation applied a combination of key external opinions with regard to the safety of impurities as it relates to dose level and frequency of dosing

- Risk-based approach is conservative because it
  1) does not account for large molecular weight of peptides (however we could consider this adjustment),
  2) it is not a linear extrapolation of acceptable levels (as is used for GTIs)
  3) it uses 1 mg as opposed to higher limits supported by additional database analysis, and
  4) for the first time, addresses the risk of immunogenicity of impurities relative to the API

- We understand that there is no intrinsic value to impurities and strive to remove them as development proceeds; however, it would be beneficial allow for this science-based argument to support safety limits throughout development