Toxicity and Immunogenicity Considerations for Oligonucleotide-Related Impurities: The Impact on Control Strategy Development

Brian Pack, Paul Cornwell, Robert Siegel, Scott May, Laurent Malherbe, Andrea Ferrante, Doug Roepke, Mark Carfagna, Rohit Tiwari, and Allison Wolf

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 oligonucleotides

- Immunogenicity of oligonucleotide impurities
- Toxicity of oligonucleotide Impurities
  - Rationale to support that 1 mg/day impurity exposure, frequency of dosing and molecule weight of oligonucleotide is a safe and conservative means to calculate unspecified impurity limits

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

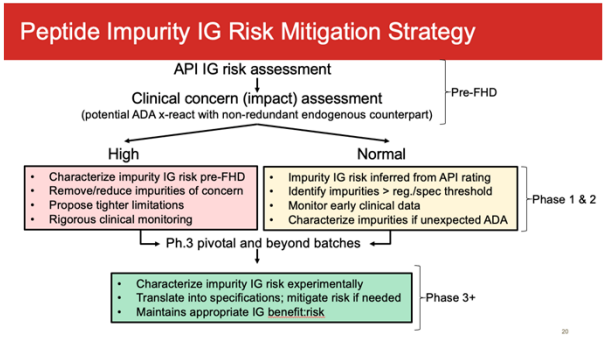
} CMC/Analytical Activities {

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

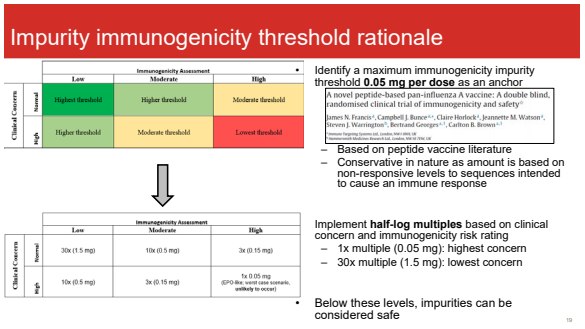
Introduction

- Reminder of the peptide impurity rationale I presented earlier

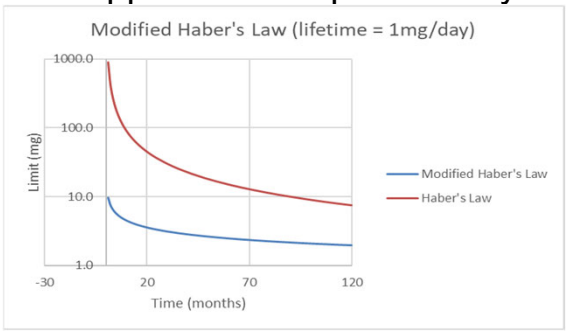
Define the Clinical Risk



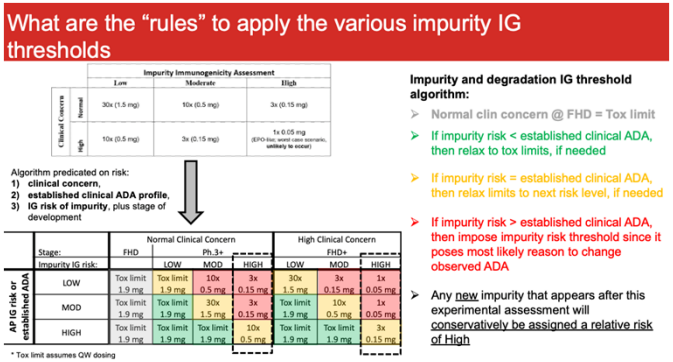
Defined the most conservative IG risk-based rationale on multiples



Outlined maximum safe levels tox can support based upon Harvey



Define safe levels based upon clinical, immunogenicity, tox, and dose along with a fairly complex mitigation strategy that involves in vitro assessments



Published Commercial Limits

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%

Capaldi *et al*. Levels:
Identification threshold = 1.0%
Qualification threshold = 1.5%

?

Peptides Ph Eur <2034>

DRAFT Guideline on the Development and Manufacture of Synthetic Peptides

Reporting threshold	Identification threshold	Qualification threshold
> 0.1 per cent	> 0.5 per cent	> 1.0 per cent

Certain Generic peptides (**no clinical data Required**)

Organic Small Molecule Impurities

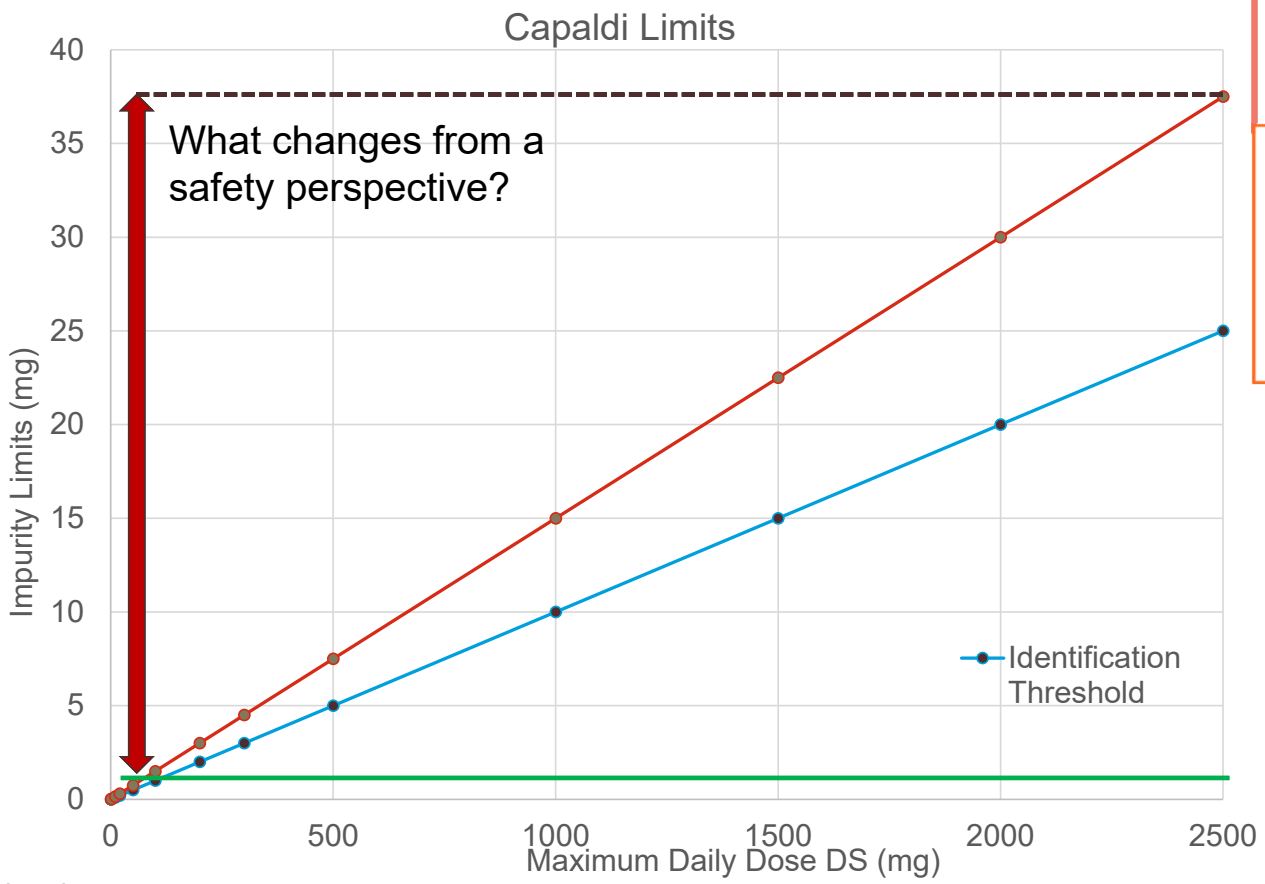
GTIs (low ppm)

Nitrosamines (ppb)

ANDA applicants should identify each peptide-related impurity that is 0.10 percent of the drug substance or greater and show that the proposed generic synthetic peptide does not contain any new specified peptide-related impurity that is more than 0.5 percent of the drug substance

The decision on the acceptable thresholds and when we implement these ***commercial requirements*** have an impact on development.

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



Capaldi *et al*. Levels:
Identification threshold = 1.0%
Qualification threshold = 1.5%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Issues in Development

Impurities in Oligonucleotide Drug Substances and Drug Products

Daniel Capaldi,[1] Andy Teasdale,[2] Scott Henry,[1] Nadim Akhtar,[2] Cathaline den Besten,[3] Samantha Gao-Sheridan,[4] Matthias Kretschmer,[4] Neal Sharpe,[5] Ben Andrews,[6] Brigitte Burm,[7] and Jeffrey Foy[8]

Rationale to support:

1 mg/day of an adjusted oligonucleotide impurity daily exposure is a safe and conservative means to calculate unspecified/specified impurity limits

Clinically Relevant Specifications

Marketing Application Goal is to establish Clinically Relevant Specifications that take into consideration the clinical impact of variations in the critical quality attributes (CQA) and process parameters assuring a consistent safety and efficacy profile

Sandra Suarez Sharp: What are clinically relevant dissolution specifications? (fda.report)

ICH Q3A (R2) Impurities in new drug substances - Scientific guideline

- The level of any impurity present in a new drug substance that has been adequately tested in safety and/or clinical studies would be considered qualified.
  - Impurities that are also significant metabolites present in animal and/or human studies are generally considered qualified.

  } Clinical Relevance

  - Higher or lower thresholds for qualification of impurities can be appropriate for some individual drugs based on scientific rationale and level of concern,

  } Higher Levels Possible

- Acceptance criteria should be set no higher than the level that can be justified by safety data, and should be consistent with the level achievable by the manufacturing process and the analytical capability.

} Process and analytical capability

The Regulatory Challenge

- **Health Authority Feedback:**
  - We request that you establish an **any unspecified impurity limit at 0.5%**
  - You are requested to confirm that the levels of all impurities observed in the clinical batches to be used in this clinical trial will be supported by toxicological studies.

- We are proposing to leverage

Higher or lower thresholds for qualification of impurities can be appropriate for some individual drugs based on scientific rationale and level of concern. ICH Q2A

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

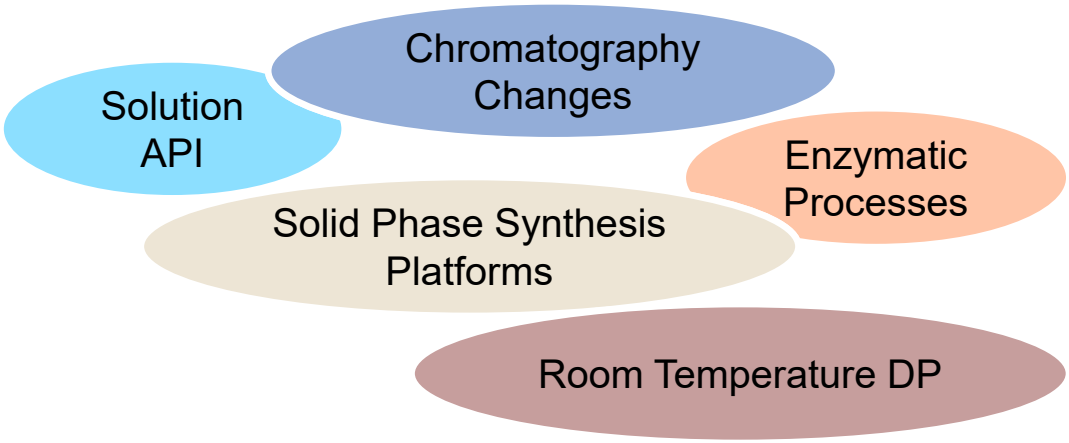
Mayur et al. https://doi.org/10.1016/j.yrtph.2021.104895

1 mg/day impurity exposure, frequency of dosing and molecule weight of oligonucleotide is a safe and conservative means to calculate unspecified impurity limits

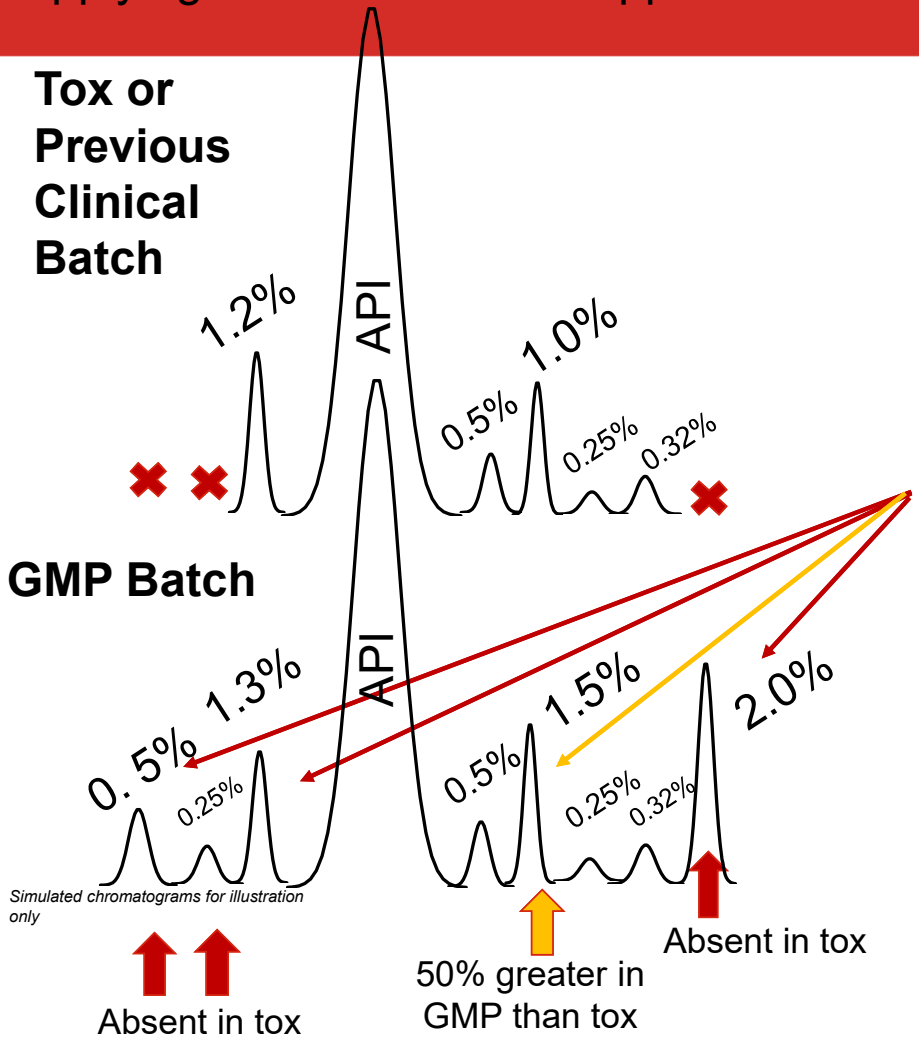
Why would we need to support higher but safe levels of impurities?

- Early in development
  - Small scale manufactures
  - SAD/MAD studies typically go to a much higher exposure than the planned dose.
    - How can you support impurity levels?

- Later in Development
  - Manufacturing site changes
  - Manufacturing Scale Changes
  - Manufacturing Process Changes

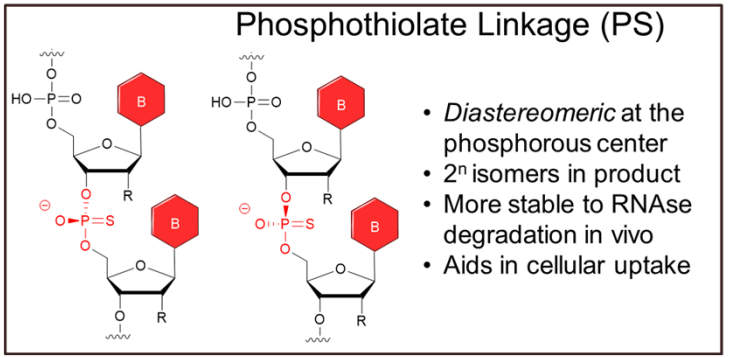


Applying a Science-Based Approach



Whenever we see a chromatographic peak we must remember that is never 1 impurity!

– Based upon diastereomers, it is 2^n isomers of every impurity (n-1, n+1, deletions, etc.)



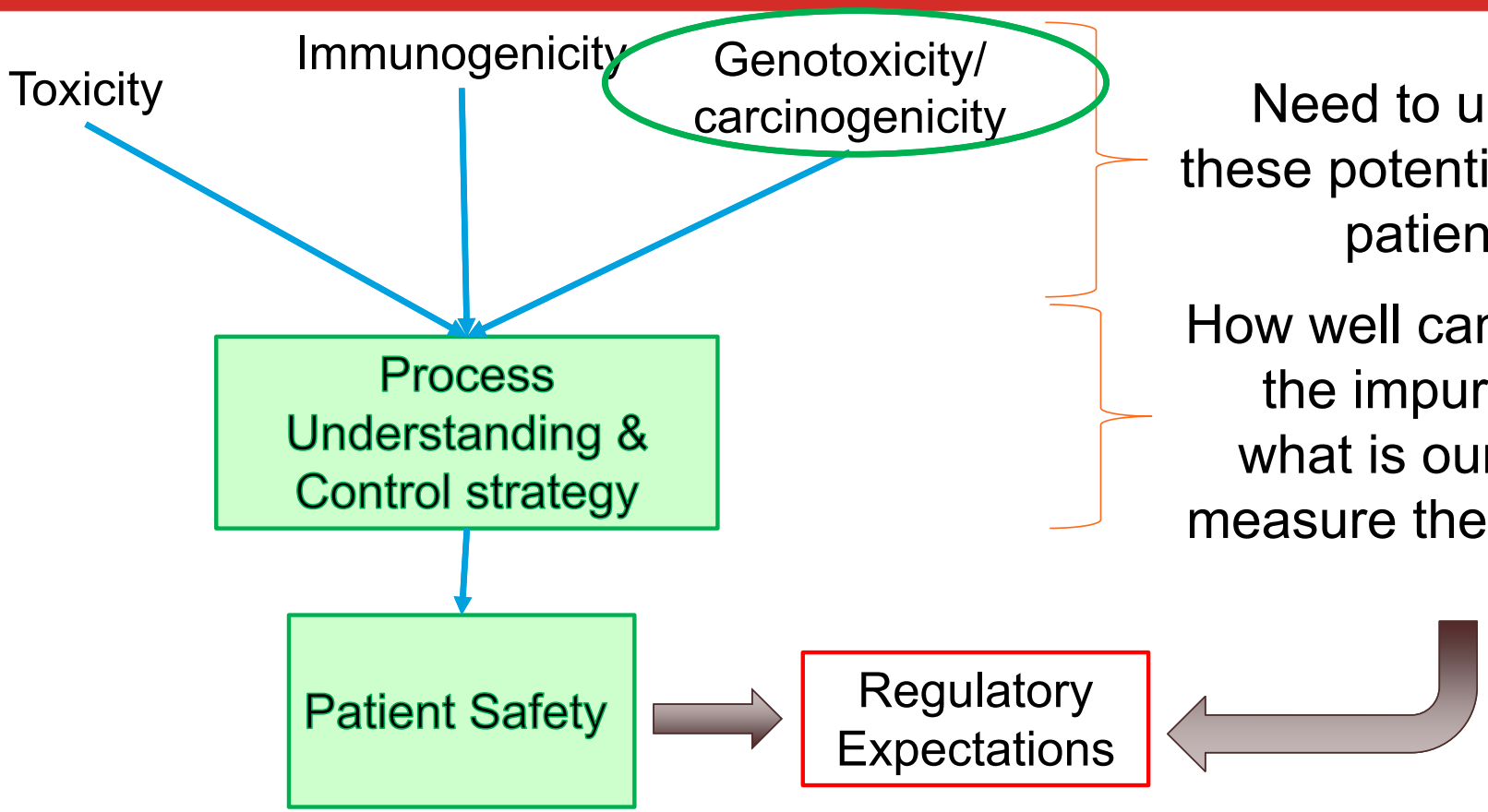
The rest of this presentation will be focused on:

"Are these impurities safe even if they have never been in a toxicology study, or were there at a lower level?"

"What is the risk of immunogenicity associated with that oligonucleotide impurity?"

"Can higher qualification thresholds be supported throughout development based upon literature precedent?"

Why Do We Report/Identify and Potentially Qualify Impurities?

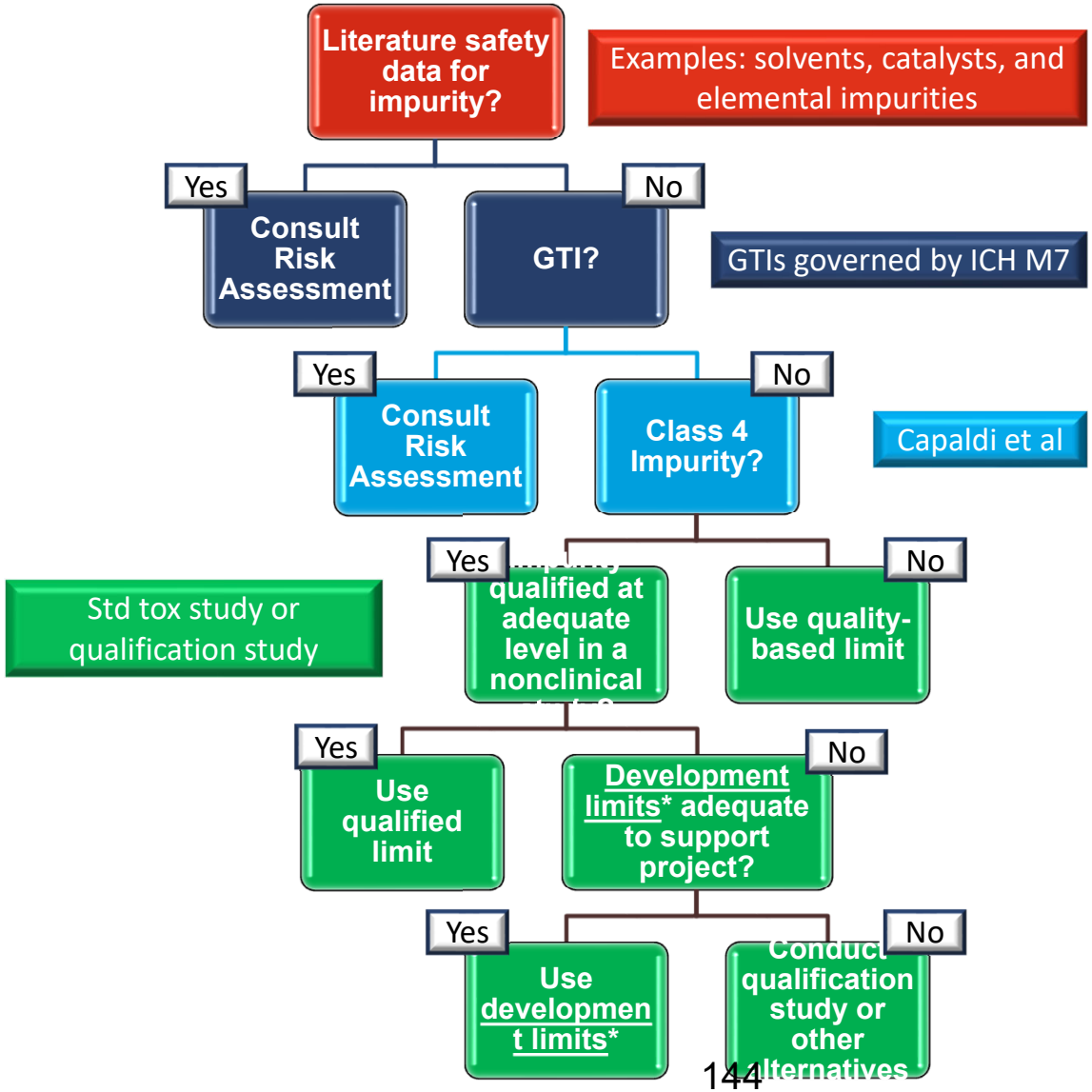


Need to understand these potential sources of patient risks

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

Oligonucleotide Impurity Qualification Decision Tree

*Development limits are developed on next few slides



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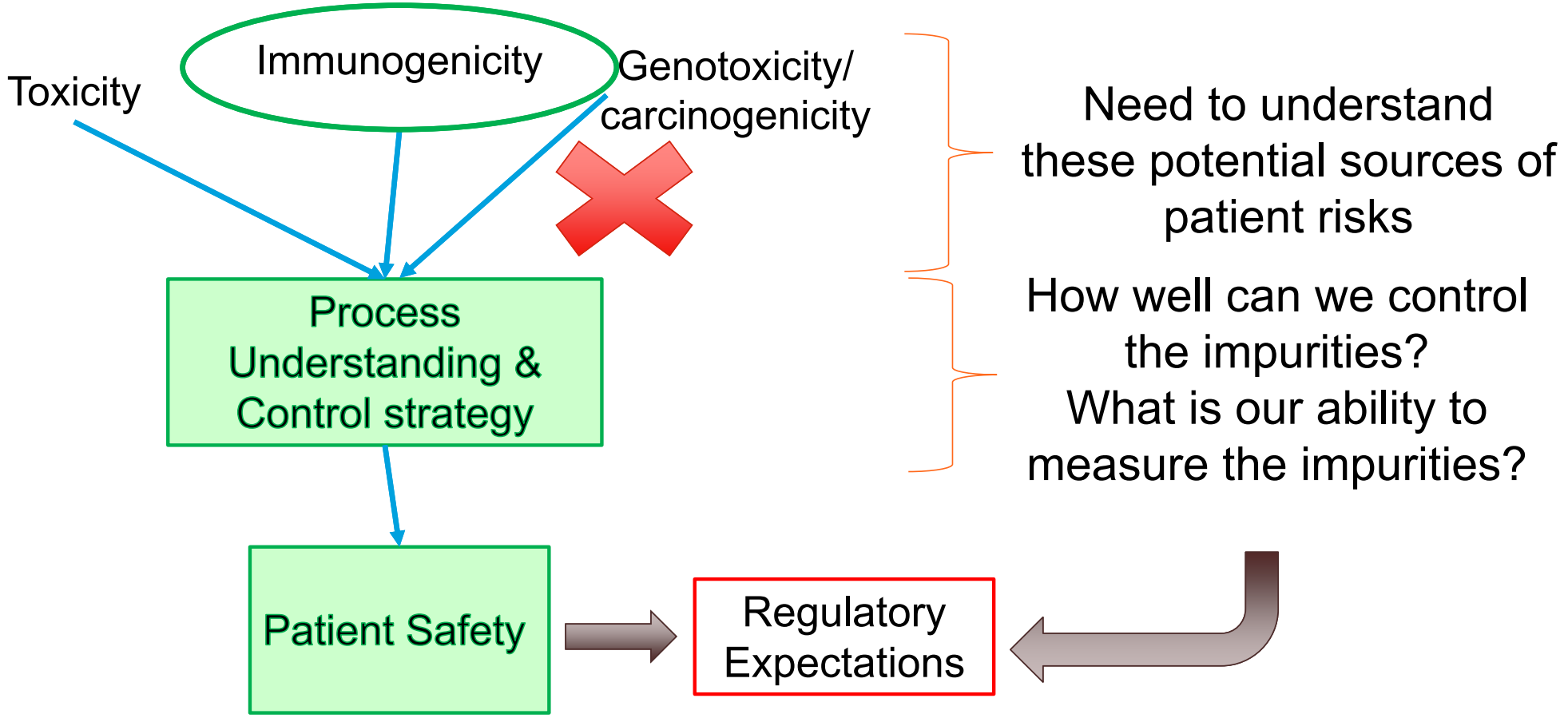


Immunogenicity

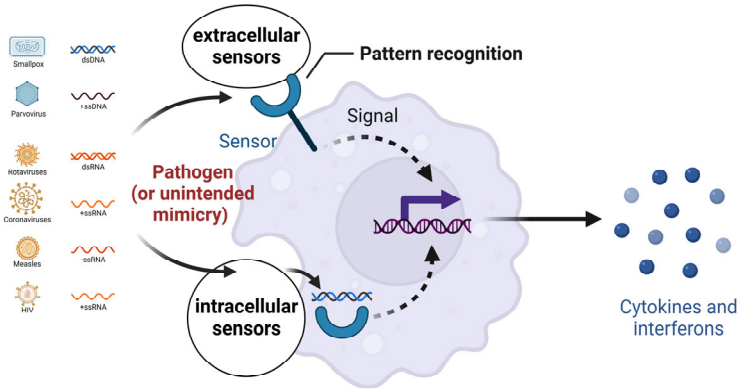
A complex process with different concerns for different types of molecules

Innate Immune System:

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

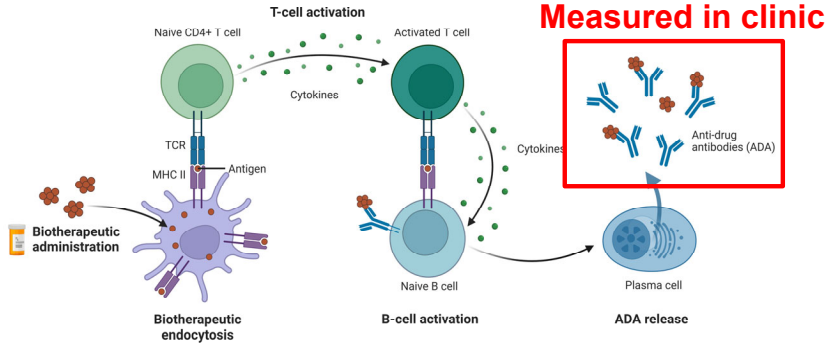
Adaptive Immune System:

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



Primarily oligonucleotide concern

Provides a pro-inflammatory environment which can stimulate activation of adaptive immune system



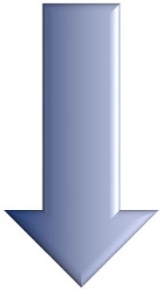
Measured in clinic

Primarily protein concern

The lack of a proteinaceous component limits anti-nucleic acid antibodies to T-cell independent path which has specific requirements.

Immunogenicity of Oligonucleotides

Current anti drug antibody (ADA) data for all the ONs suggest that either ONs pose a low immunogenicity risk without any measurable impact on PK, PD, and safety, or meaningful aspects of immunogenicity have not been measured.



The AAPS Journal (2022) 24: 93
https://doi.org/10.1208/s12248-022-00741-x

REVIEW ARTICLE

Considerations in the Immunogenicity Assessment Strategy for Oligonucleotide Therapeutics (ONTs)

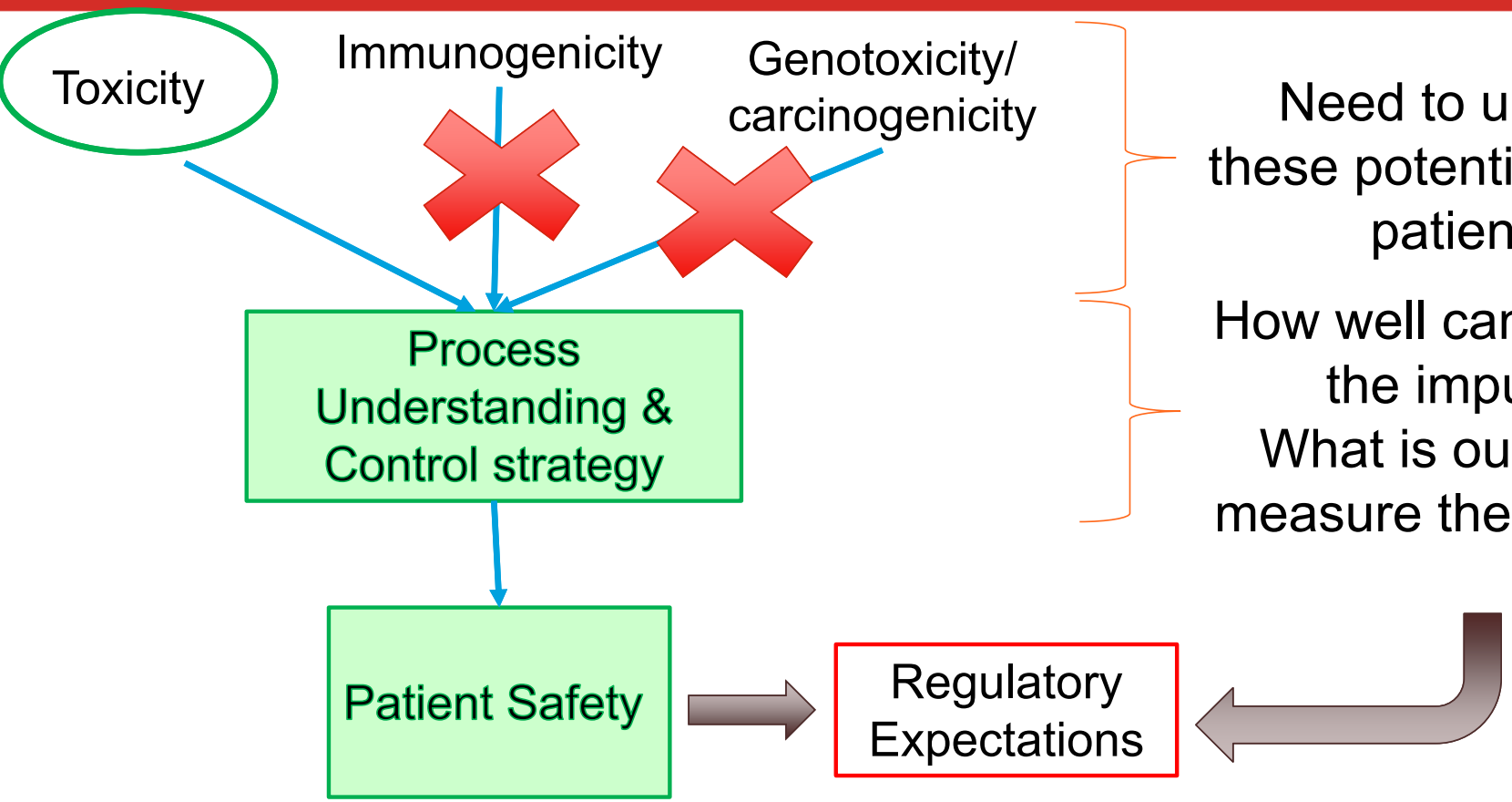
Nazneen Bano[1] · Christopher Ehlinger[1] · Tong-yuan Yang[1] · Michael Swanson[1] · Schantz Allen[1]

Received: 25 May 2022 / Accepted: 2 August 2022 / Published online: 26 August 2022

- Evidence to date suggests low TE-ADA risk for siRNA therapeutics.
  - This risk rating extends to product-related impurities (e.g., n-1 / n+1, adduct impurities do not pose any greater risk than API).

- The generation of TE-ADA to ON, and by analogy impurities, is believed to be low prevalence and, if developed, low clinical risk.
  - Current recommendation is to defer impurity limits to toxicology specification level**s**, monitor clinical immunogenicity and adjust impurity strategy if warranted.

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?

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 be **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

- 1 mg/day of an impurity is still a Conservative Limit
- Much of the literature supports 1.8-1.9 mg /day
- Small molecules are expected to have more off-target/unpredictable effects than derivatives of peptides and oligonucleotides
- Will apply a non-linear adjustment to account for dosing frequency

Duration Adjustment

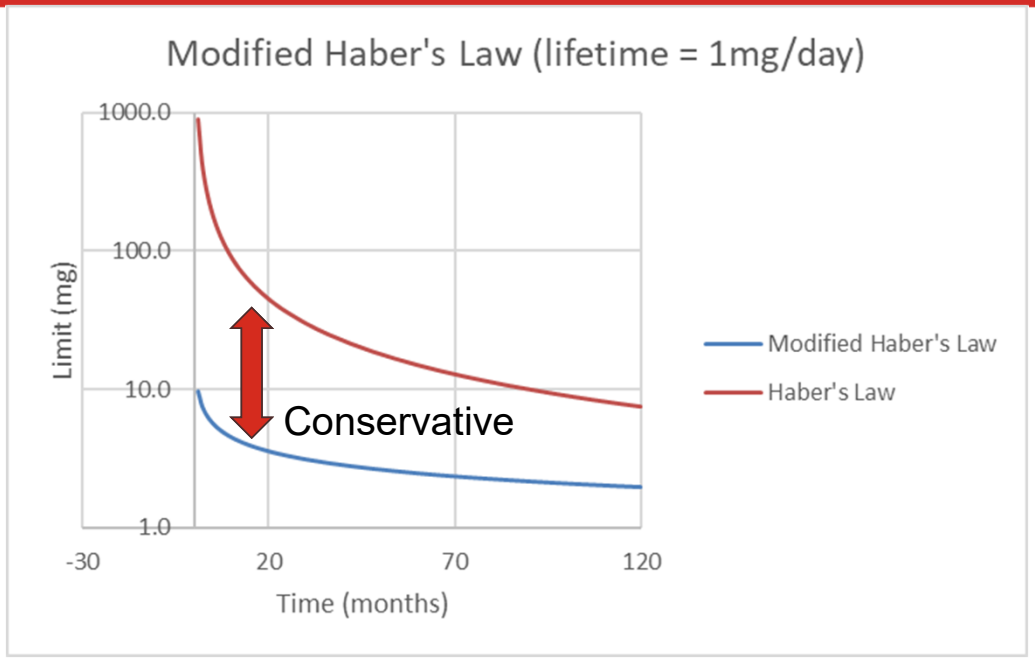
A conservative version of Haber's Law (Harvey et al 2017).

$$\textbf{Haber's Law:} \quad c \times t = c' \times t'$$

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

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

For ON 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 ...."

Capaldi et al. Impurity Classification

TABLE 2. IMPURITY CLASSES AND QUALIFICATION DATA REQUIREMENTS

Impurity class	*Examples*	*Safety assessment required (Y/N)*	*Data type*
Class I Impurities that are also major metabolites (structure and sequence are the same as parent)	Impurities that lack multiple nucleotides from the 3′ or 5′-end of the parent oligonucleotide Impurities formed by incomplete conjugation of (parent) conjugated oligonucleotides Parent single-stranded impurity of double-stranded oligonucleotides	No	Not applicable
Class II Impurities that contain only structural elements found in naturally occurring nucleic acids	Phosphate diester impurity of phosphorothioate diester oligonucleotides 2′, 5′ linked sugar in RNA	No	Not applicable
Class III Impurities that are sequence variants of the parent oligonucleotide	$n-1$ $n+1$ Deaminated impurities	No[a]	Not applicable
Class IV Impurities that contain structural elements not found in the parent oligonucleotide or in naturally occurring nucleic acids	See Table 1 Unidentified impurities	Yes[b]	Nonclinical safety studies

[a]Assumes that at the specification limit, the individual components of the impurity are each present below the qualification threshold.
[b]Safety assessment required if specification limit is higher than the qualification threshold.

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Issues in Development

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All of these impurity classes are likely **Cramer Class I impurities (low toxicity risk)!**

Let's assume that these impurities do need to be qualified…..can a higher qualification level be supported?

1 mg limit adjusted for frequency of dosing and molecular weight

- We Consider all ON Impurities Cramer Class I Impurities
- **Capaldi Class 1-3 impurity limits will be based solely on process capabilities**
- **Capaldi Class 4 Impurity Limits**
  - Applies modified Haber's Law to provide conservative adjustment for less-than-lifetime exposure due to intermittent dosing (Harvey et al) with a 10x adjustment for molecular weight differences-
    - Conservative, as most oligonucleotide products are greater than 5000 Da/strand
  - If impurity > safety threshold still have the option to qualify by traditional toxicology studies

	Impurity Safety Threshold (%)			
	Therapeutic Dose (10 mg)	Therapeutic Dose (100 mg)	Therapeutic Dose (500 mg)	Therapeutic Dose (1000 mg)
Daily	10.0	1.0	0.2	0.1
Weekly	19.1	1.9	0.4	0.2
Monthly	31.1	3.1	0.6	0.3
Quarterly	45.0	4.5	0.7	0.4
Semi annually	56.7	5.7	1.9	0.6
Yearly	71.5	7.1	1.4	0.7

Other elements of control strategy will prohibit such levels (i.e., assay or total impurities).

	Impurity Safety Threshold (%) 10X MW Adjustment			
	Therapeutic Dose (10 mg)	Therapeutic Dose (100 mg)	Therapeutic Dose (500 mg)	Therapeutic Dose (1000 mg)
Daily	100.0	10.0	2.0	1.0
Weekly	191.3	19.1	3.8	1.9
Monthly	310.7	31.1	6.2	3.1
Quarterly	449.8	45.0	9.0	4.5
Semi annually	566.7	56.7	11.3	5.7
Yearly	714.7	71.5	14.3	7.1

Impact to Specification Strategy

Specification Test	Acceptance Criteria
Purity and related impurities antisense/sense strand	Purity ≥ 80.0%-area* or Impurities ≤ 20.0%
	Report impurities ≥ 0.2% by RRT
	Report total impurities (% area)

- Class I, II, III Capaldi et al. impurities limited by consistent process controls in practice-no safety concerns
- Class IV Capaldi et al. Impurities limited by consistent process controls as well in addition to limits on previous slide
- Identification of impurities < the safety threshold should be performed for process understanding and eventual commercial specification support

Project	Dose	DS Safety Limit with MW Adjustment	Single Strand Safety Limits*
Low-dose ON	100 mg twice yearly	57%	*NMT 20%
ON 1	600 mg per quarter	7.5%	15%
ON 2	300 mg twice yearly	19%	*NMT 20%
ON 3	400 mg once yearly	18%	*NMT 20%
High-dose Oligo	1000 mg once monthly	3.1%	6.2%

Values estimated assuming sense strand and anti-sense strand are equal mass (can use the exact MW conversions)

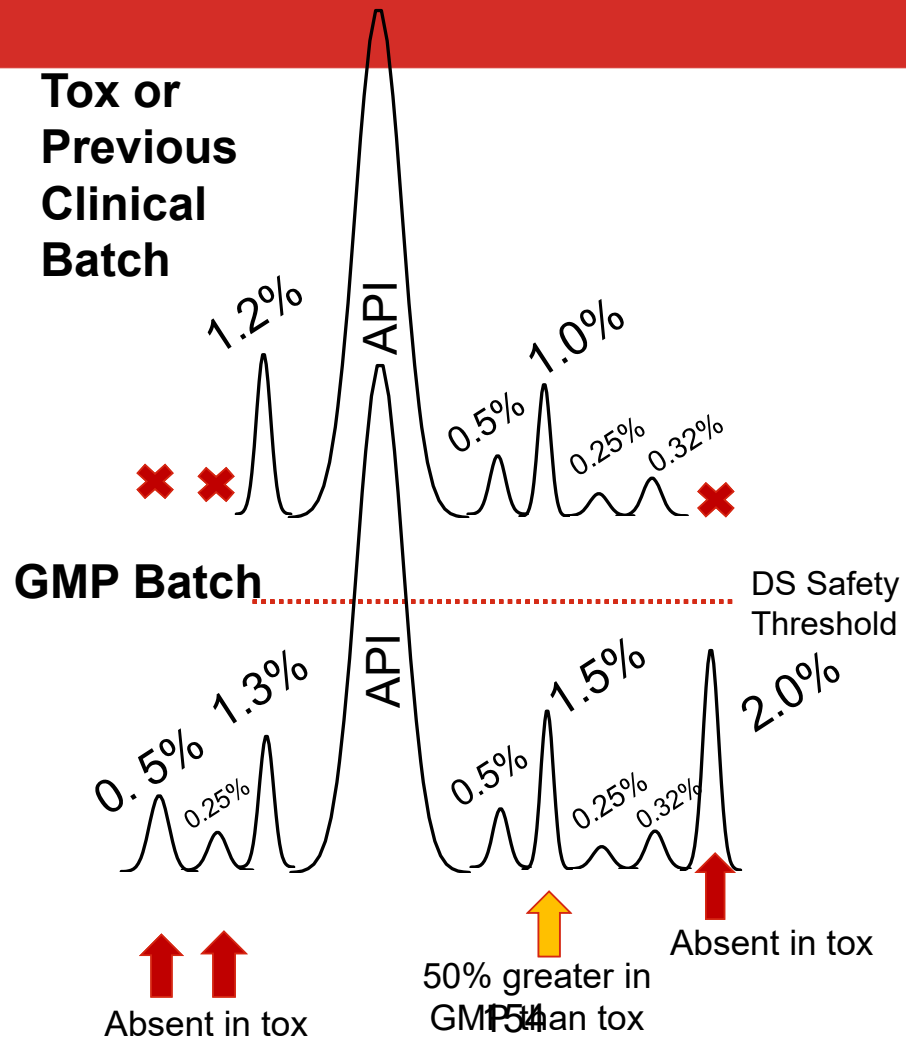
* Will be controlled by typical “total impurity” specification for single strands, in siRNA duplex

Link to Impurity Profile Comparison

Project	Dose	DS Safety Limit	Single Strand Safety Limits*
High-dose Oligo	1000 mg once monthly	3.1%	6.2%

* siRNA duplex

- Impurity profile comparisons required as part of current Good Manufacturing Processes (cGMP) in order to understand how current GMP batch compares with those batches previously used in toxicology studies or in clinical studies.
  - Must still pass all specifications
  - All impurities below the DS Safety Threshold; therefore, pass the impurity profile comparison

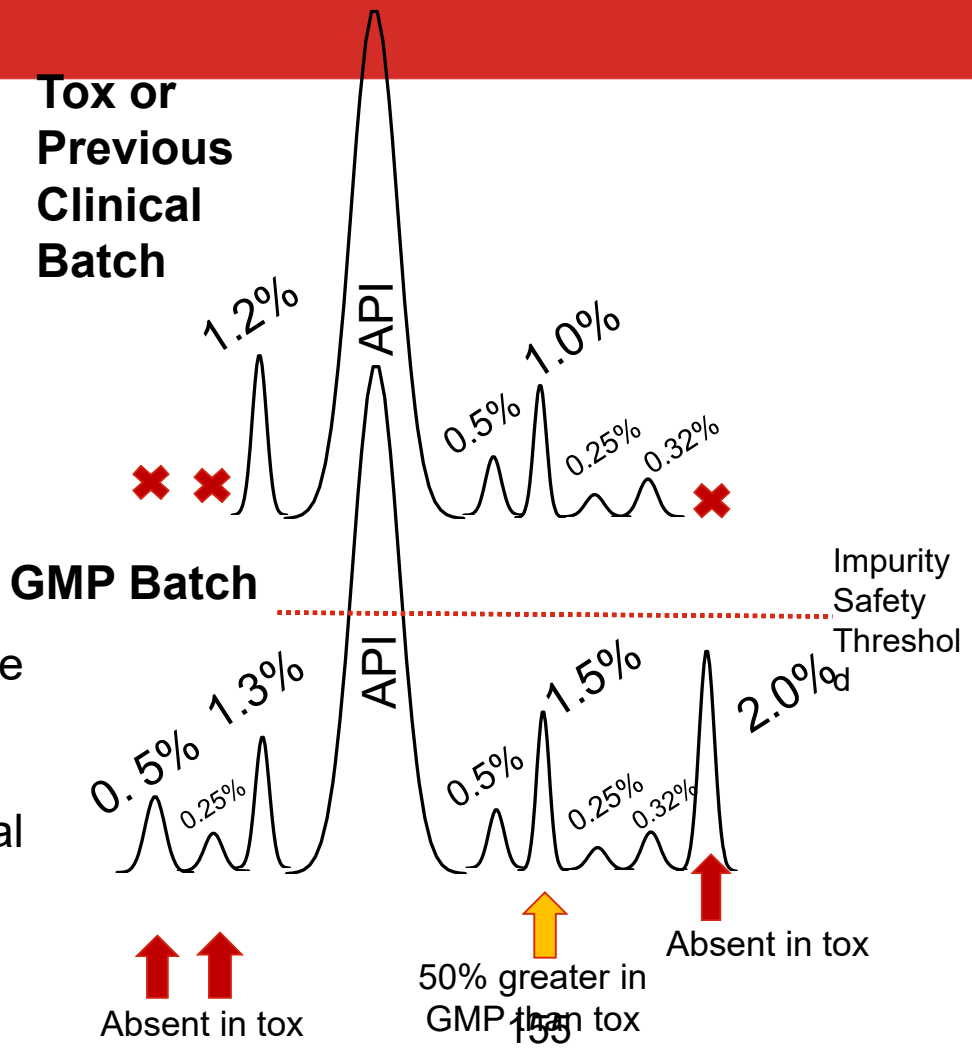


Comparability Assessment

Project	Dose	DS Safety Limit	Single Strand Safety Limits*
High-dose Oligo	1000 mg once monthly	3.1%	6.2%

* siRNA duplex

- Must still pass all specifications
- Additional characterization (beyond specification tests) are typically employed as outlined in Draft guidance
- In this scenario, all impurities below the DS Safety Threshold; therefore, should be considered comparable if all specifications and characterization tests align
- This material should be suitable to enter into a phase 3 study based upon impurity quality profile without qualifying the new impurity at 2.0% in an animal study



Summary

- This presentation applied a combination of key external opinions with regard to the safety of impurities as they relate to dose level and frequency of dosing to oligonucleotides
  - Specifications (qualification)
  - GMP impurity profile comparisons
  - “Formal” Comparability Studies

- This strategy is conservative but illustrates what level of individual impurities can be supported throughout development
  - 1 mg /day of oligonucleotide related impurities is supported by general toxicological principles and a wealth of literature
  - Dose durations / frequency of dosing adjustments already supported in regulatory guidance and we did not propose a linear extrapolation
  - Molecular weight adjustment is proposed only under certain circumstances (e.g., high dose)
  - Unlike proteins, immunogenicity concerns for impurities very low since clinically meaningful ADA has not been observed for oligonucleotides

- At time of regulatory submission, we understand that specifications will be based upon clinical relevance in addition to process and analytical variability, long term specifications and controls required at some level; however, this strategy should be acceptable to support impurity levels throughout clinical development (including Ph 3)