Leveraging Biotransformation Data to Refine Bioprocesses and Derisk PQAs

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CASSS CMC Forum – Washington DC
27 Jan 2025

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Outline

- Biotransformation
  - Definition
  - Tools
  - Cross-functional dependencies
  - Workflow
- CDR succinimide formation in a monoclonal antibody
- KR clipping & Trp mannosylation in a fusion protein
- Benefits of Biotransformation

Biotransformation Definition:

For therapeutic proteins, traditional metabolism studies are not conducted, primarily because proteins are in general broken down to peptides and amino acids (endogenous molecules). As such biotransformation studies of therapeutic proteins are not required as part of submission packages by regulatory agencies on a routine basis (ICH, 2012)

Encompasses such modifications as:

1. Amide and glycosidic bond hydrolysis: clipping of amino acid sequence or glycan. Or any other clipping event.
2. Amino acid modification: Deamidation, oxidation, isomerization, sulfation,
3. Disulfide bond reduction or shuffling (IgG2)
4. ADC biotransformation is mostly focused on linker cleavage and payload metabolism.

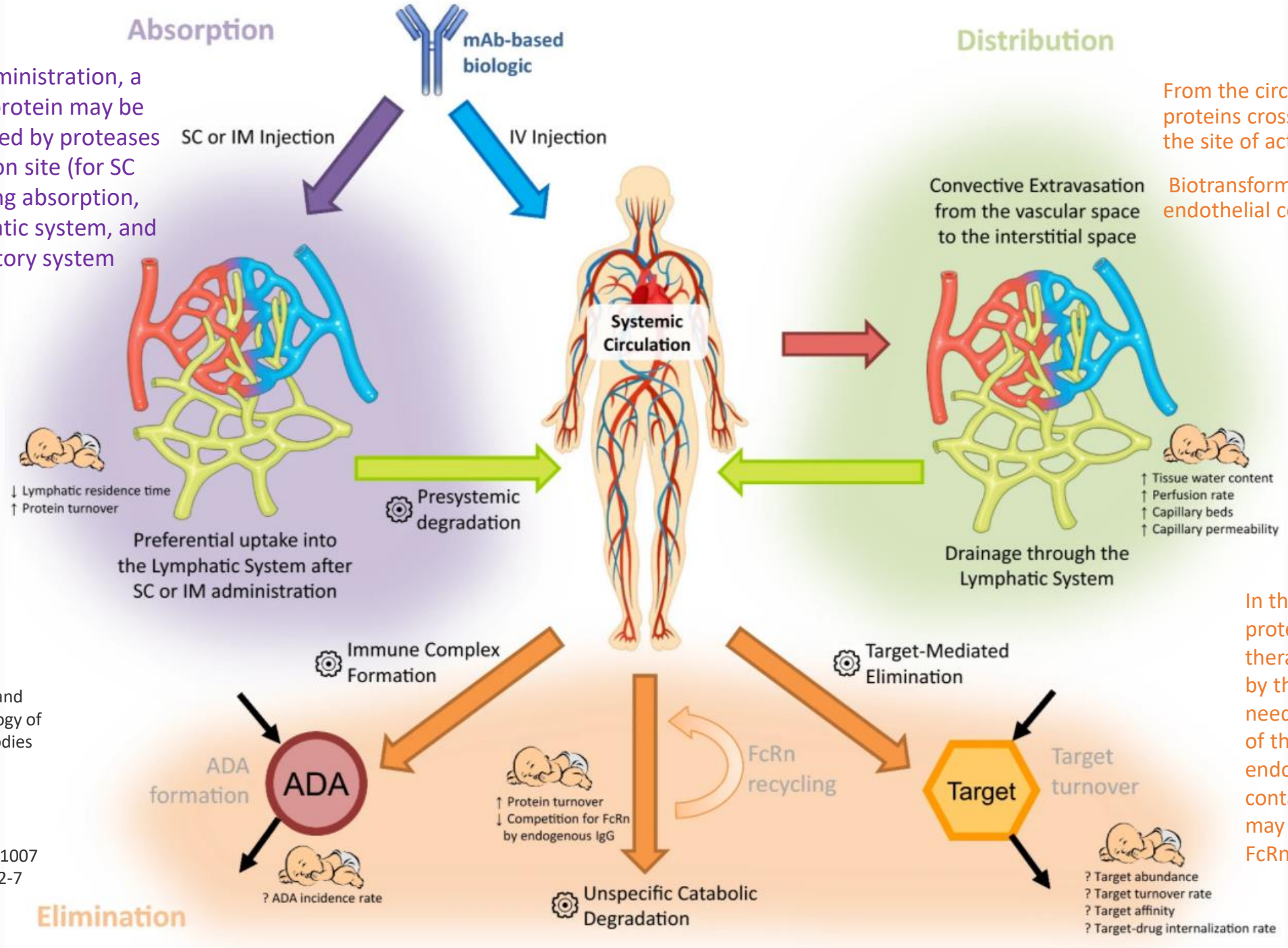
Following administration, a therapeutic protein may be biotransformed by proteases at the injection site (for SC dosing), during absorption, in the lymphatic system, and in the circulatory system

From the circulation, therapeutic proteins cross the vascular wall to reach the site of action in the target tissue.

Biotransformation might also occur by endothelial cells of different organs.

The most likely location for proteases contributing to the formation of circulating biotransformation products is the extracellular space, and the proteases are either soluble (e.g., in blood and lymph) or membrane-associated.

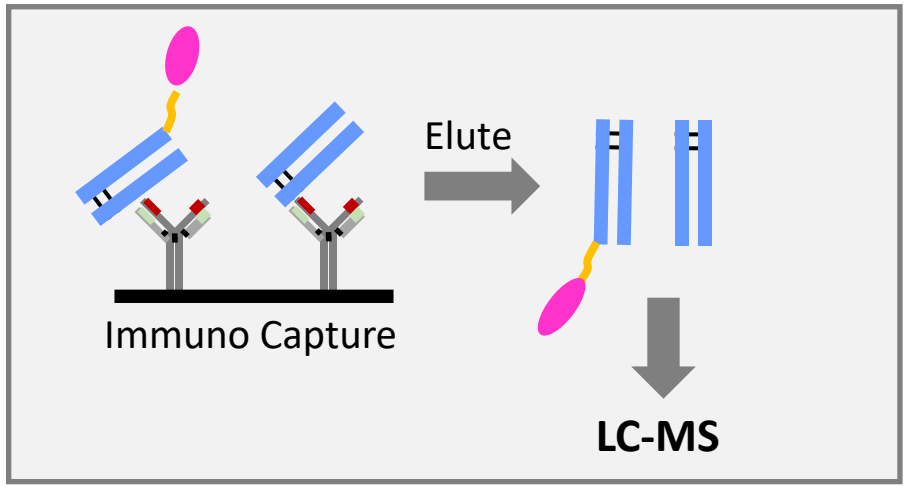
In the case of intracellular proteases, the protein therapeutic needs to be taken up by the cell, and the product needs to be transferred back out of the cell. From early endosomes, biotransformed Fc-containing protein therapeutics may be released into blood by FcRn recycling.



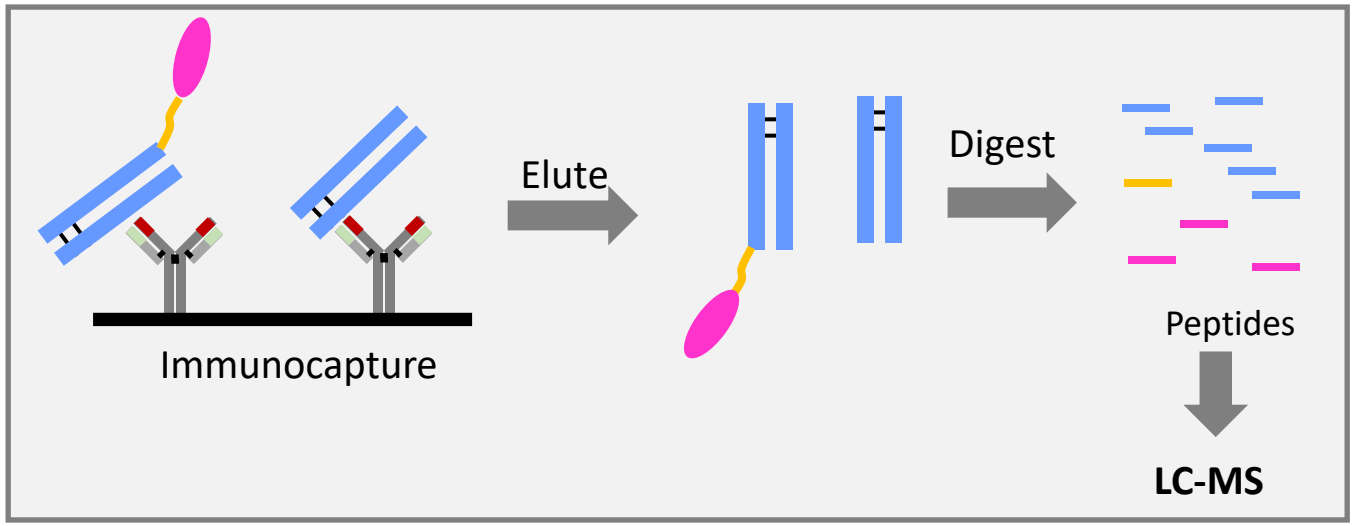
Temrikar, Z.H., Suryawanshi, S. & Meibohm, B. Pharmacokinetics and Clinical Pharmacology of Monoclonal Antibodies in Pediatric Patients. *Pediatr Drugs* **22,** 199–216 (2020). https://doi.org/10.1007/s40272-020-00382-7

LC-MS Assay Formats

Intact LC-MS

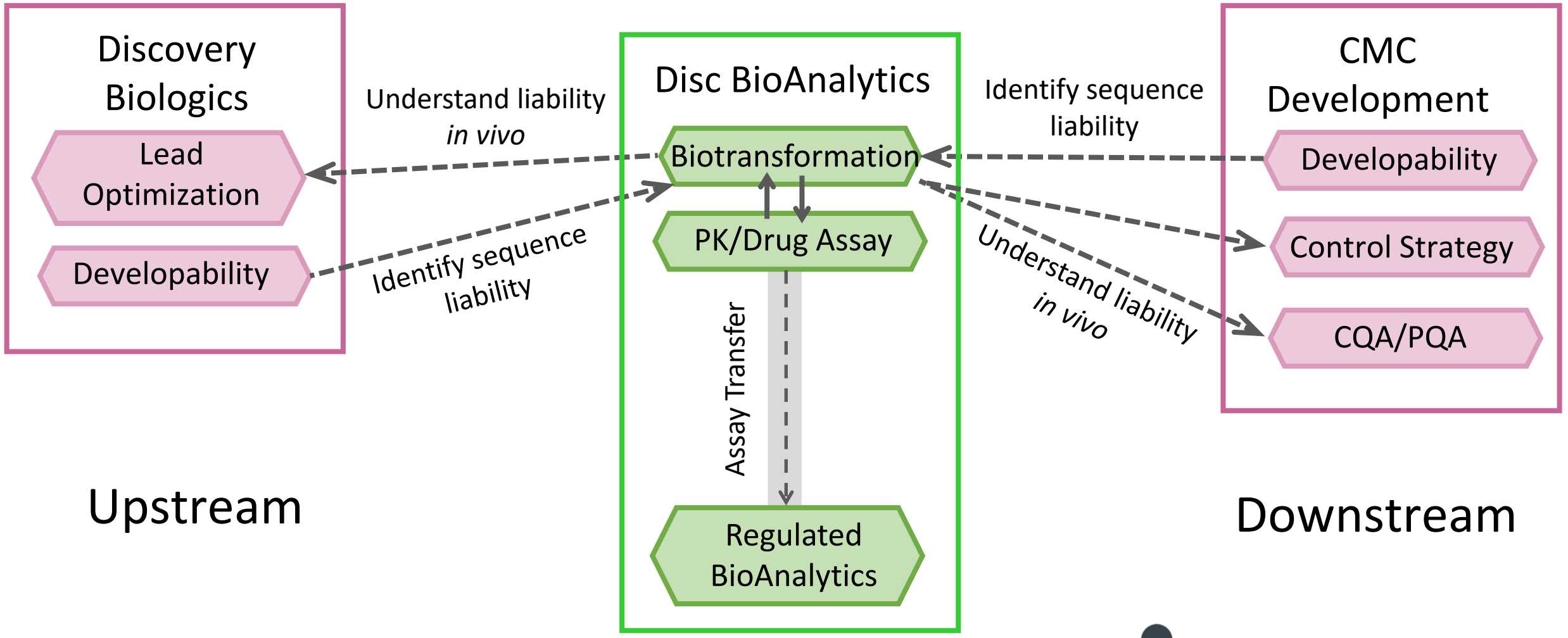


Peptide Based LC-MS



Assay Name	Application			Platform	Throughput	Typical LLOQ (ug/mL)	Note
	Clipping	Small PTM (deamidation)	Large PTM (oxidation etc)				
Intact Mass	✓		Maybe (MS resolution Subunit/Reduced vs Intact)	LC-MS (TOF)	15min/sample	0.5-5	Semi-quantitative Subject to heterogeneity
Peptide based PRM Quant	✓	✓	✓	LC-MS PRM (QE+)	1 hr/sample	0.02-0.2	Subject to digestion issue

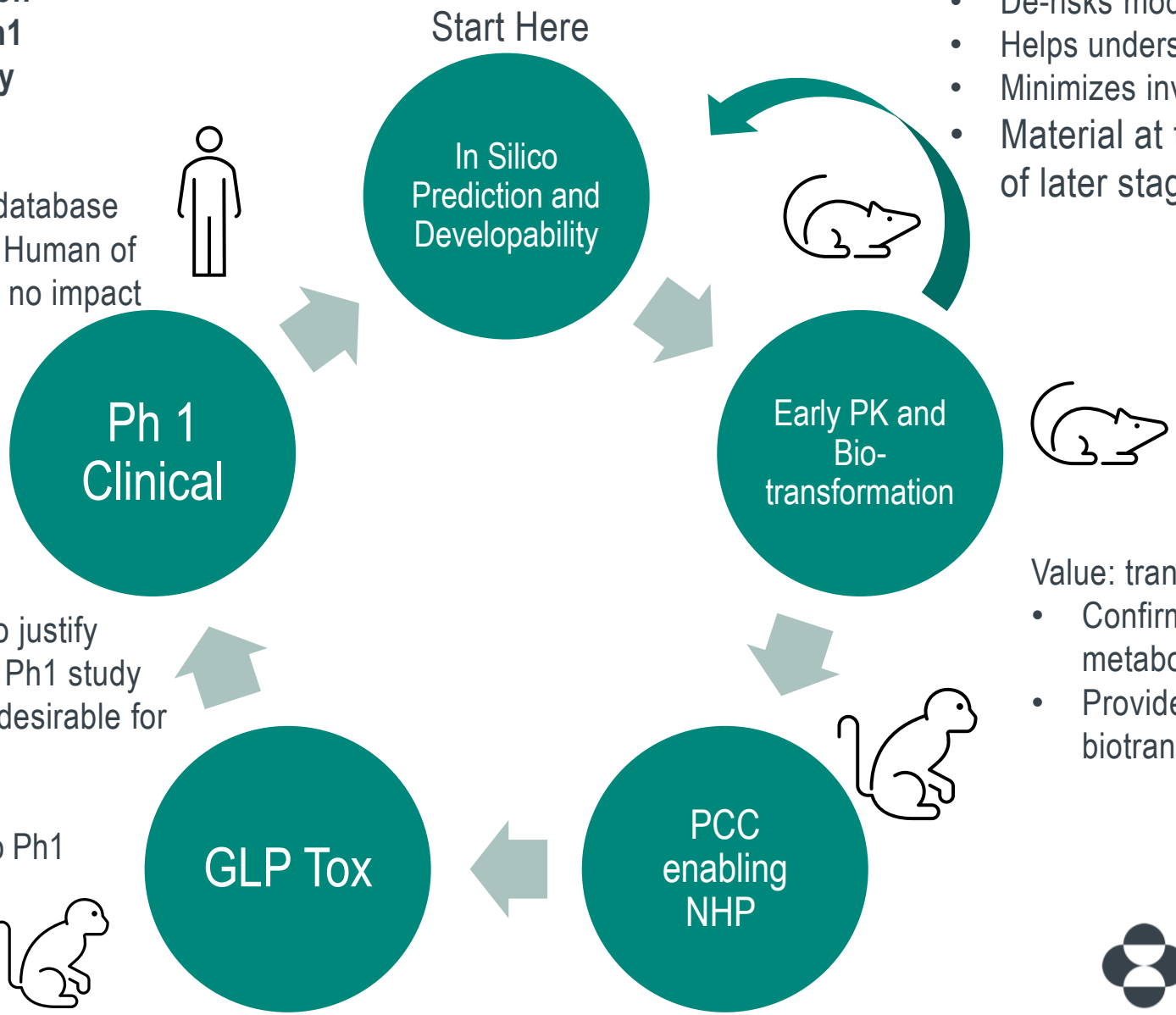
How Biotransformation Connects To Other Activities?



Biotransformation Workflow

- **Ensure biotransformation analysis is written in Ph1 protocols as exploratory analysis.**

- Builds a comprehensive database from mouse, to NHP and Human of modifications which have no impact on exposure and safety



Enables choosing the seq with least liabilities

- Enables lead design, selection and optimization for intended pharmacological effects,
- De-risks modifications (for non-mutable residues)
- Helps understand MOA
- Minimizes investigation work in development
- Material at this stage not fully representative of later stage material

Value: translational

- Confirms de-risked PTMs in a higher, less metabolic species
- Provide early relevant data to justify PCQS biotrans work in Ph1 study

Value:

- Provide relevant data to justify PCQS biotrans work in Ph1 study
- Higher GLP-tox doses desirable for low level modifications

- Material grade closest to Ph1 material

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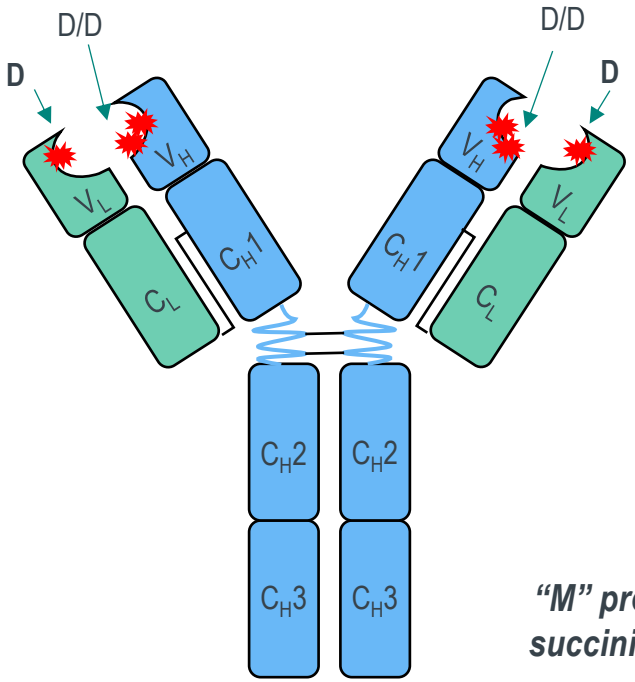
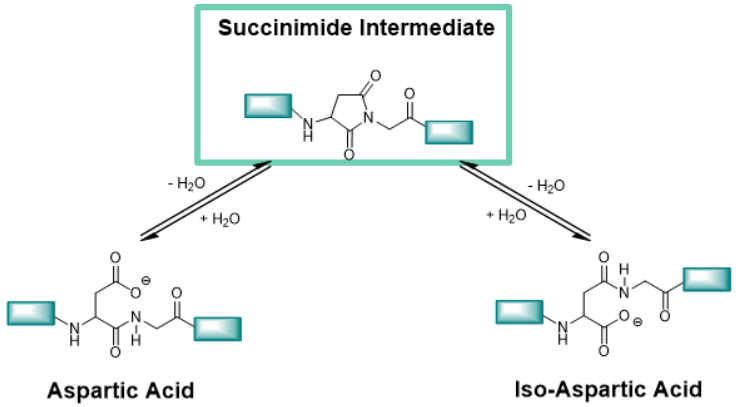
Overview of Monoclonal Ab “M”



“M” predominantly accumulates as the succinimide form instead of isomerized



Monoclonal Ab “M” Overview

- IgG4 monoclonal antibody
- Currently in Phase 2 development

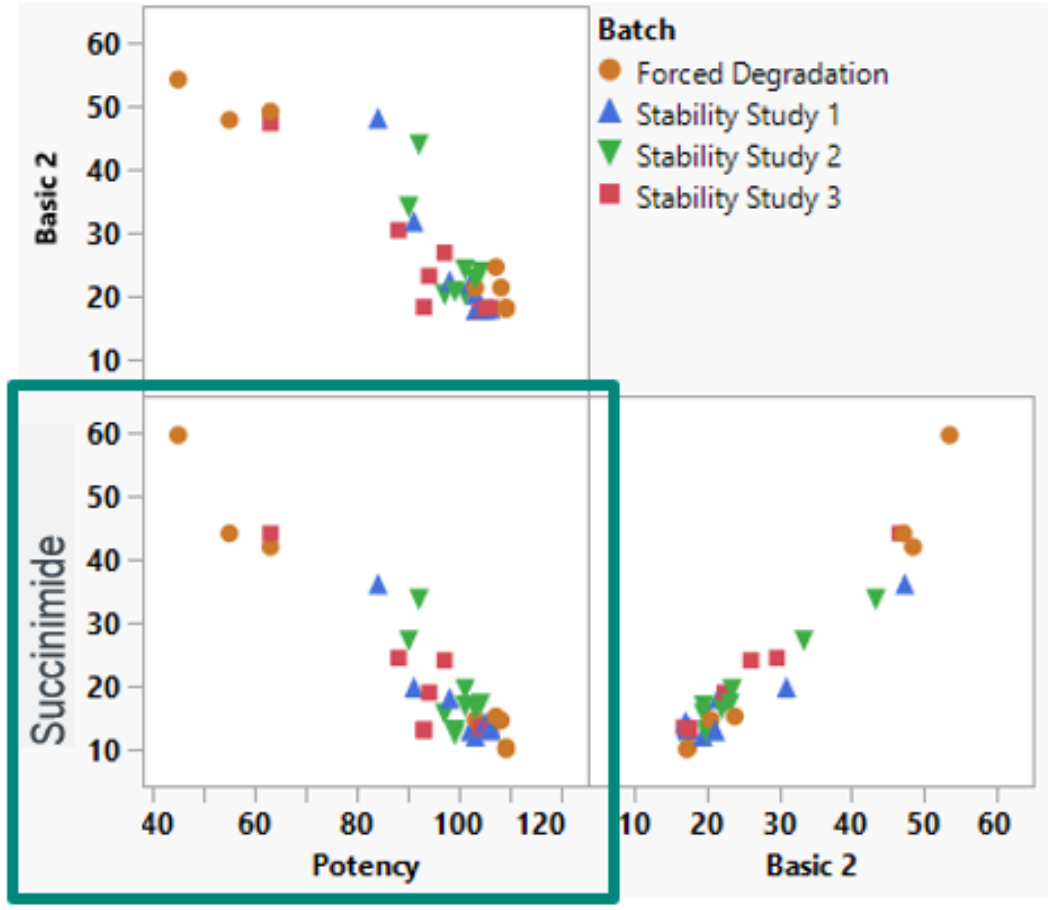
Succinimide: A Key Product Quality Attribute (PQA)

- Formation of succinimide at aspartic acid residues in CDRs: LC D is key site
- Occurs upon thermal stress and stability
- Correlated with loss of potency

Impact on Drug Product (DP)

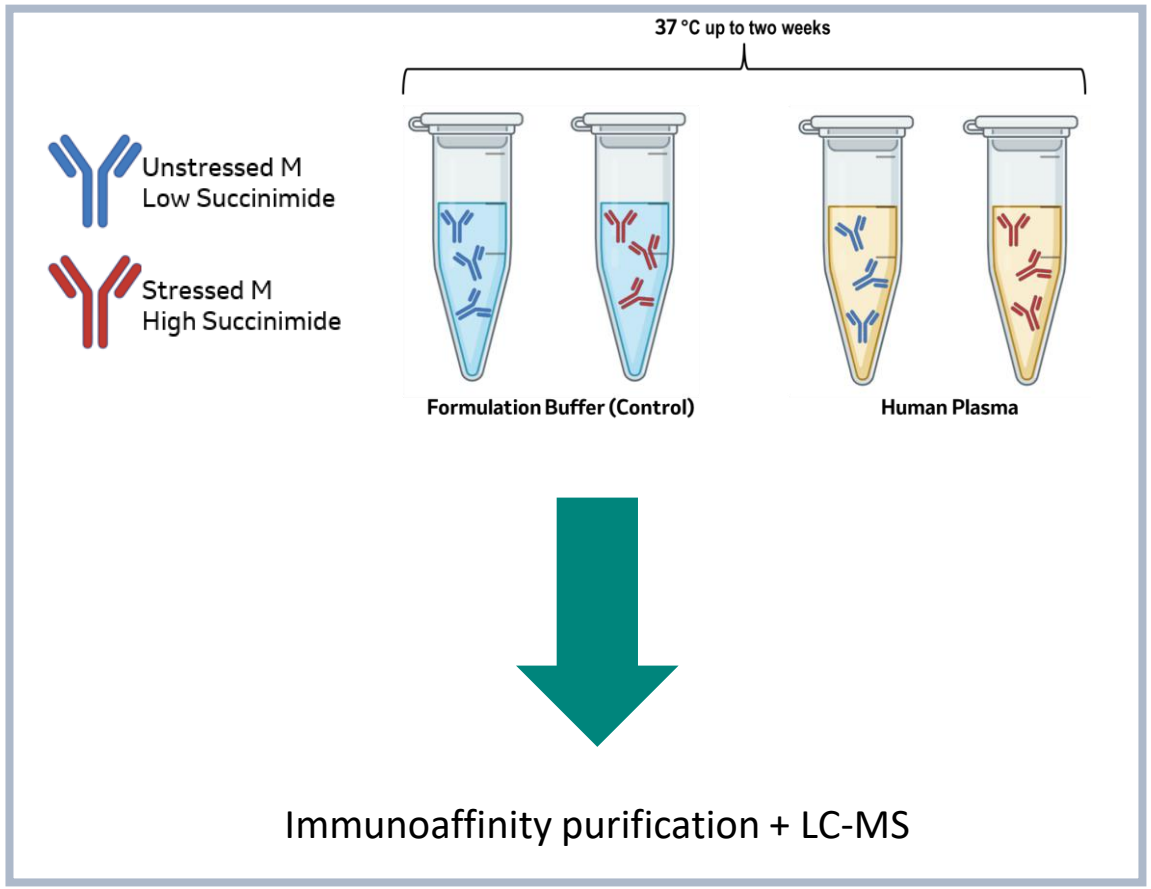
- Succinimide formation increases over the shelf life
- Necessitated Frozen DP/Lyo DP for Ph1/Ph2 studies respectively

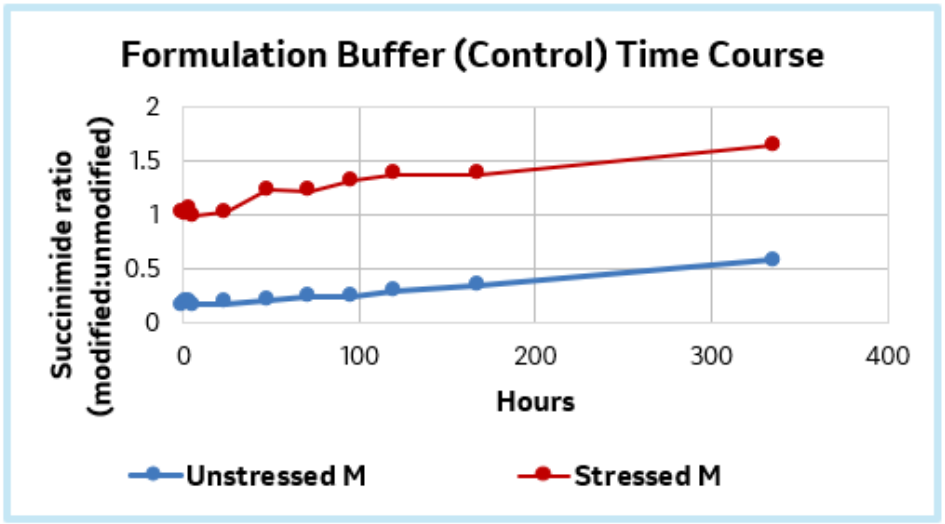
Succinimide Amount is Strongly Correlated with Potency

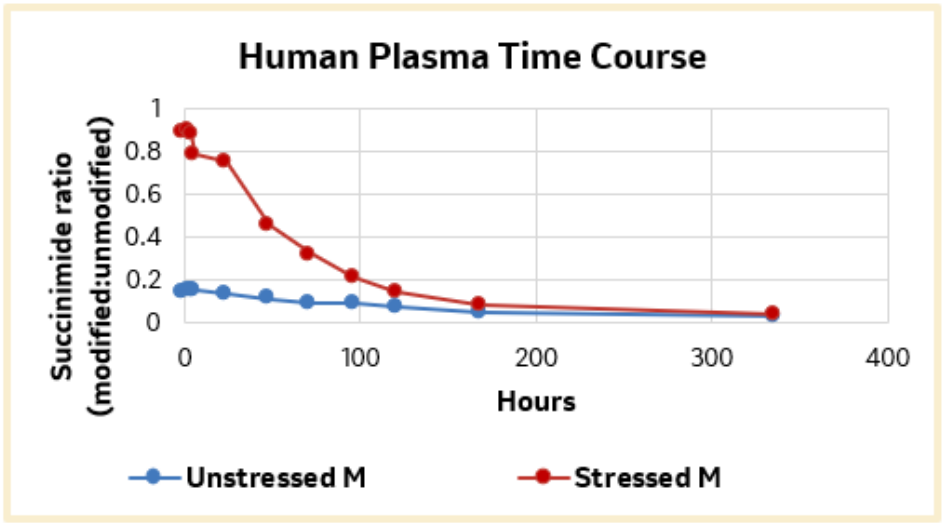


Note: Potency measured by Binding ELISA

In-vitro Biotransformation of Succinimide







In Human plasma, succinimide amount decreases over time to unstressed levels

Biotransformation Findings Implications

- Succinimide liability requires a lyophilized DP (liquid cannot achieve appropriate shelf life)
- Literature suggests that succinimide may be labile in vivo (physiological conditions)
- In-vitro incubations in human plasma shows succinimide levels reduce over time

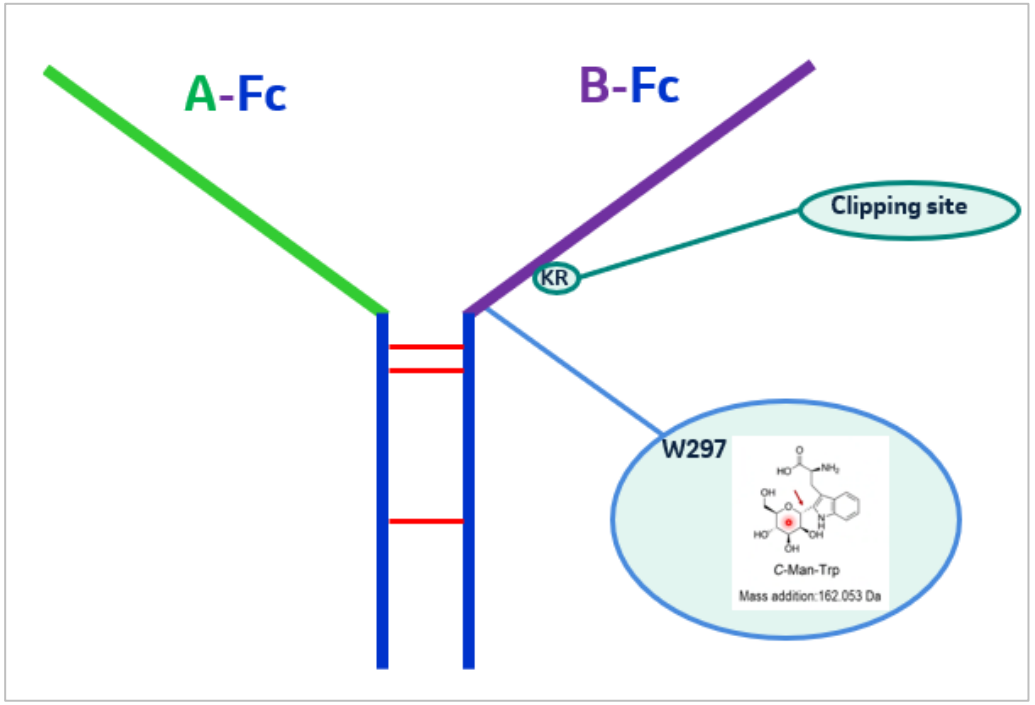
- Potential implications if we could show conversion of succinimide in clinical trial patients:
  - Could de-risk succinimide as a CQA
  - May suggest feasibility of liquid DP (if potency is restored upon administration).

Case study: FP-Fc, leveraging biotransformation data to derisk perceived CMC risks and inform on product quality attribute assessment (PQAA) for a non-platform modality

Molecule

FP-Fc is a heterodimer Fusion Protein including an IgG1 Fc with **mutations to modulate potency, effector function activity and extend half-life.**

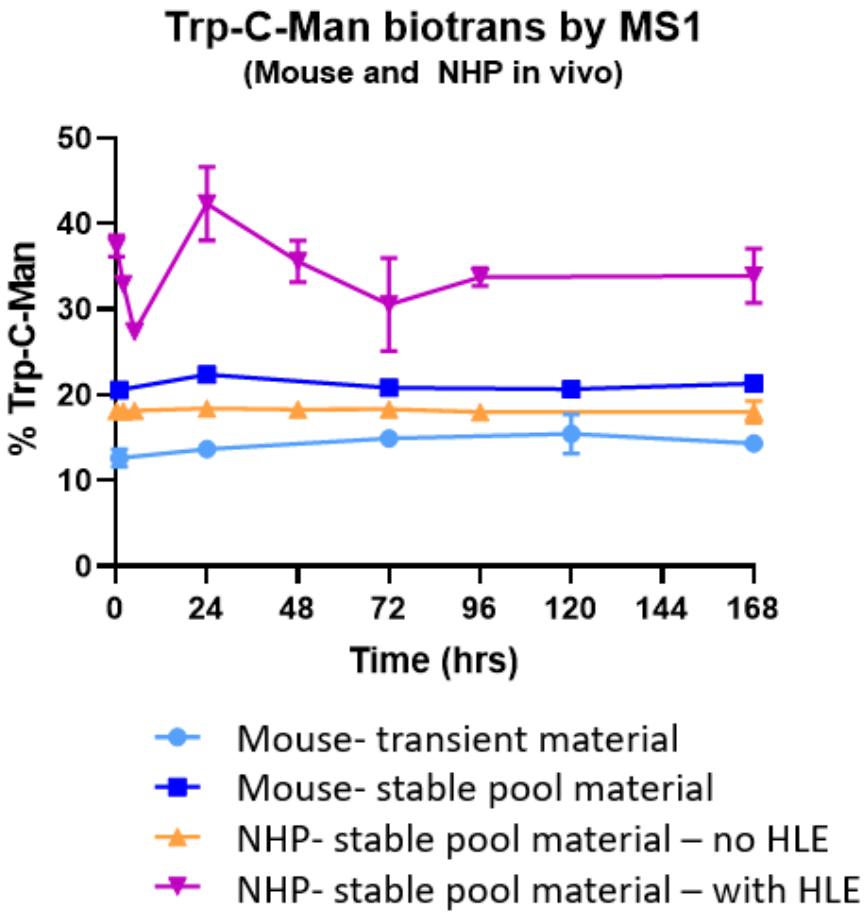
Rationale: Increase therapeutic window through half-life extension using a combination of Fc fusion and potency modulation, allowing less frequent systemic administration

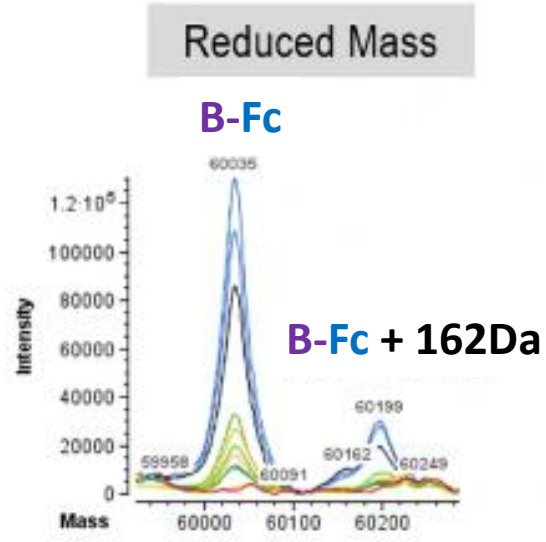


Biotransformation data collected in support of preclinical studies to guide therapeutic profile definition, GLP tox dose selection and the potential FIH dose.

- KR-clipping (enzymatically mediated)
- W97-mannosylation (unusual PTM)

W297-Mannosylation





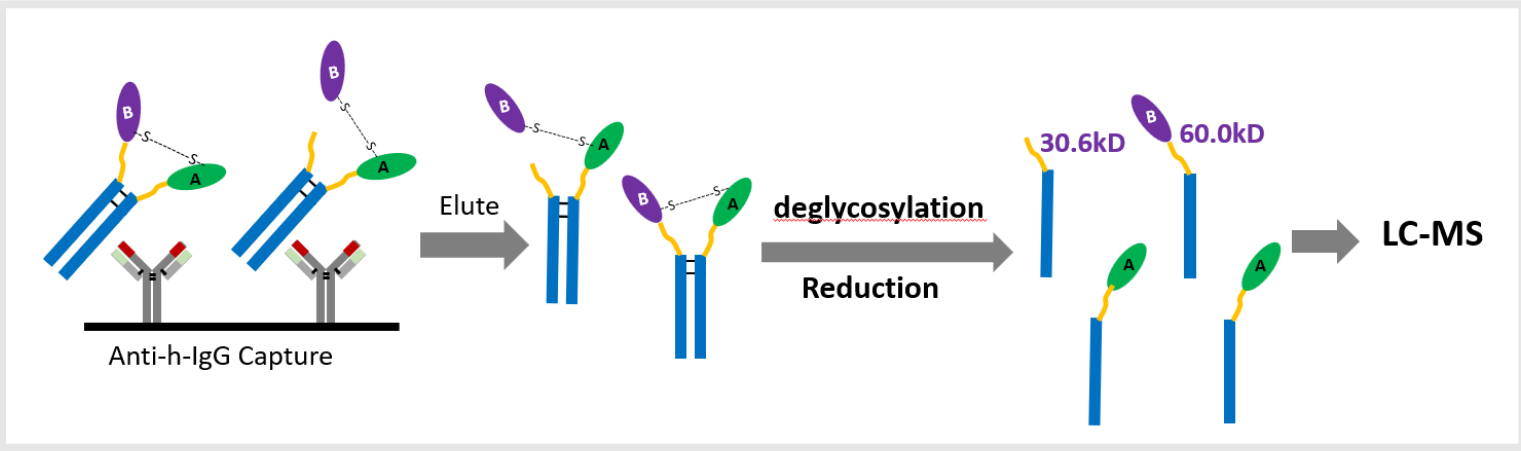
$$\%\,Trp-C-Man = \frac{MS1_{Trp-C-man}}{MS1_{Trp-C-man} + MS1_{unmodified}}$$

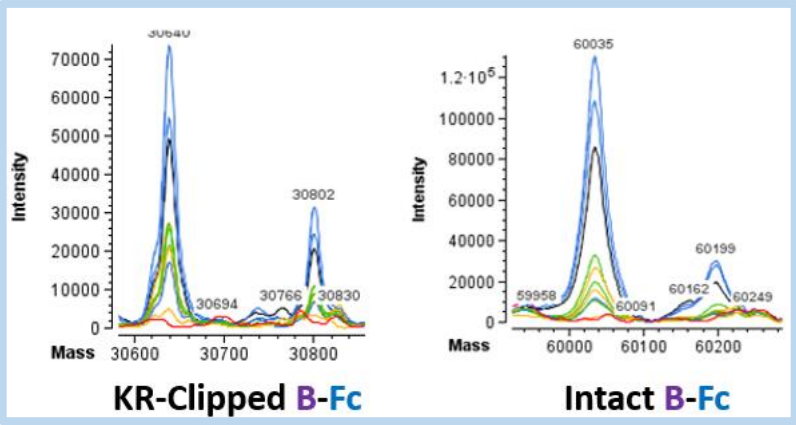
Modification	Chain	Site	PTM Quantitation (%)					
			CC31	CC31	CC31	CC31	HD	HD
			Clone 5	Clone 24	Clone 34	Clone 73	Clone 54	Clone 95
			3F5	14F2	2F10	10F2	31C6	28D11
C-Mannosylation	B-Fc	W297	9.5	8.6	14.3	10.4	18.0	22.2

W297mannosylation = Different levels observed for top 6 clones.

- No impact on clearance and no potency impact is expected, **the team will likely not be considering this attribute in the selection of the top clone**

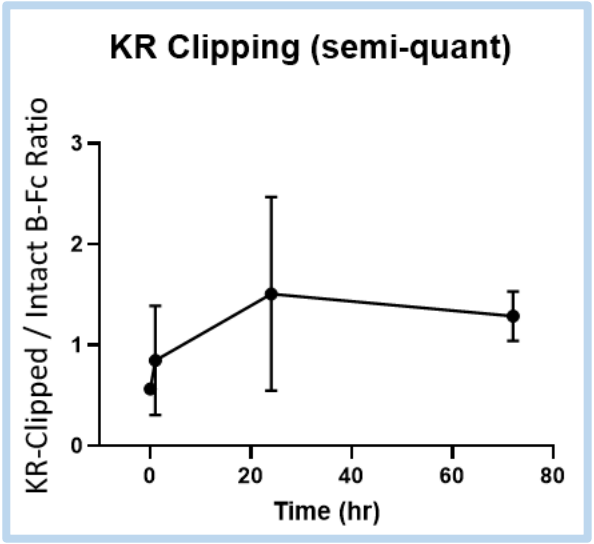
KR-Clipping





KR clipping product = Enzymatically mediated and difficult to remove through downstream purification.

- The ratio between **clipped** B-Fc chain and **intact** B-Fc chain does not increase significantly, indicating no further clipping in vivo.
- **In vivo data may be used to justify a wider specification for LMW impurities**



FP-Fc Leveraging Biotransformation to shape the FIH CMC control strategy

- Leveraging in vivo mouse and NHP data to inform on attribute ranking and development focus areas
    - W97-mannosylation will be tracked through characterization testing with no additional investment for a higher throughput method to support process development
    - The PK impact of the KR clipped product can be derisked and the biological activity will be assessed with representative materials to justify the phase appropriate specification setting

Summary - Benefits of Biotransformation Investments

- Discovery
  - Early understanding of molecular liabilities and influence on PK
  - Mitigate design risk and accelerate design cycle with additional data
  - Inform or derisk final sequence selection (executed on final ~3 sequences max)
  - Create internal databased for PTM impact on PK and incorporate Quality by Design approach in candidate design and selection

- Development
  - Continue to understand impact of product PTMs on PK/PD and support PQA assessment
  - Generate data to support the establishment of Patient centric specifications and process/product comparability
  - Accelerate formulation development and focus QTPP
  - Focused CMC regulatory submissions and address RTQs effectively

Acceleration of drug development as future candidates/programs will be in a better position to leverage data for candidate selection, developability and manufacturability

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Acknowledgments

Alex Pavlon

Fillipos Kesisoglou

Doug Richardson

Christina Shen

Mei Han

Xibei Dang

Zac VanAernum

Petra Benington

Jeffery Chapman

Joe Sergi