Immunogenicity Risk in Peptide Therapeutics: Navigating Complexity, Prediction tools, and Bioanalytical Strategies

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Pharmacokinetics, Dynamics, Metabolism and Bioanalytics

Regulated Bioanalytics PK & ADA

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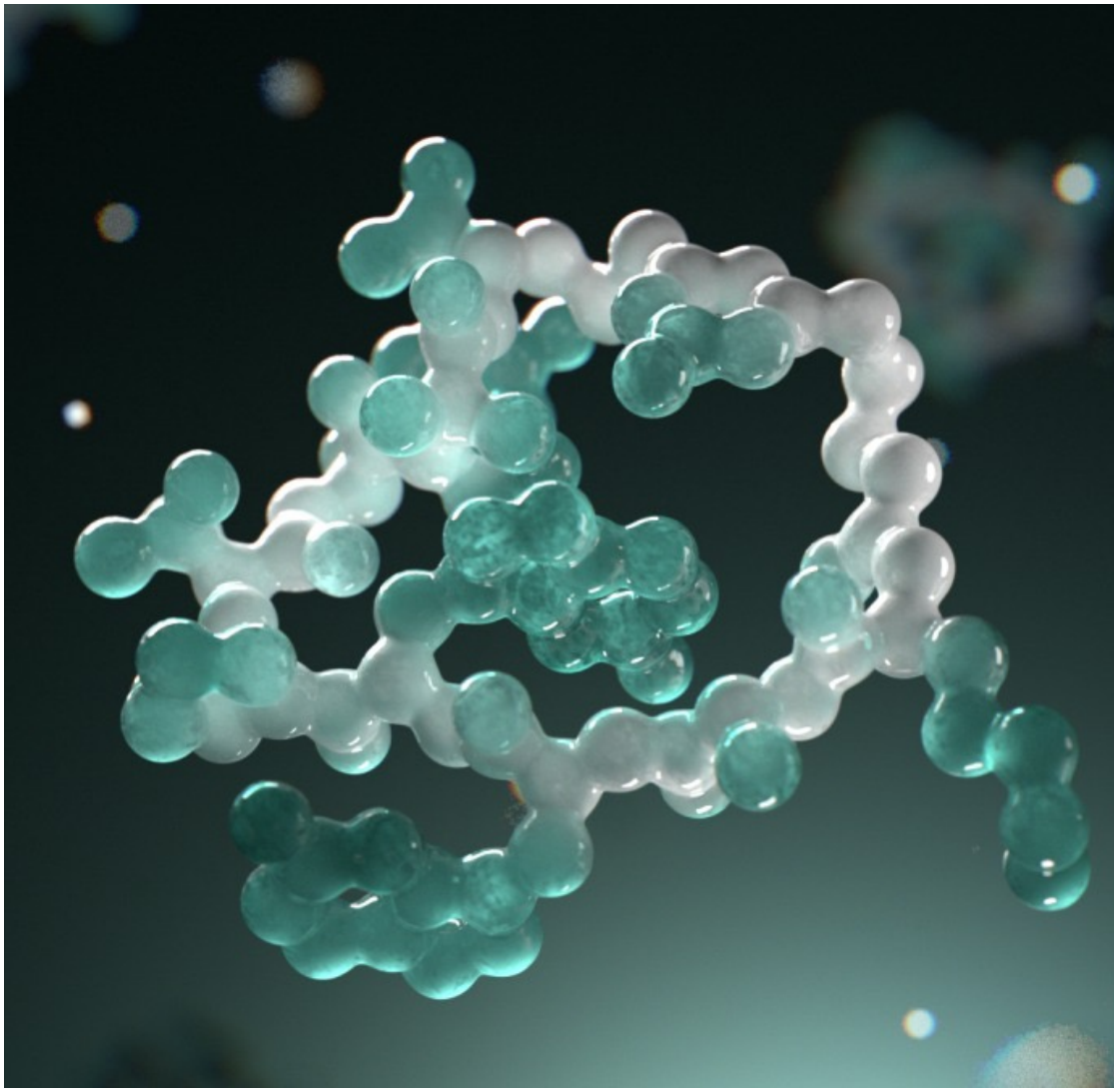


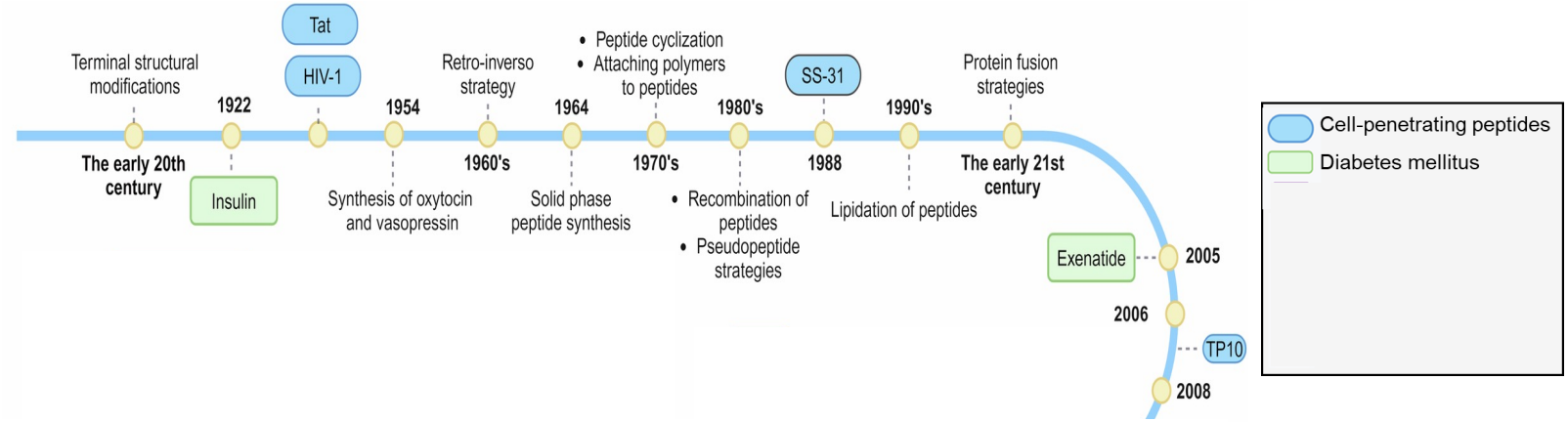
European Immunogenicity Platform

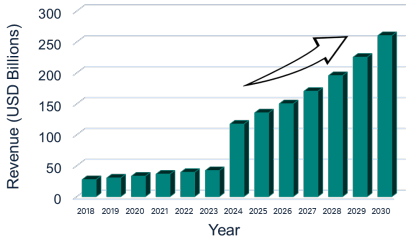
March 2026

Outline

1. Introduction to the relevance of therapeutic peptide drugs
2. Complexity of peptide modalities
3. Immunogenicity risk assessment
4. Non-clinical prediction methods
5. Bioanalytical strategies to assess immunogenicity

Therapeutic peptide development has resurged





Source: Grand View Research

Generic peptides

From Xiao W et al Signal Translation and Targeted Therapy (2025)

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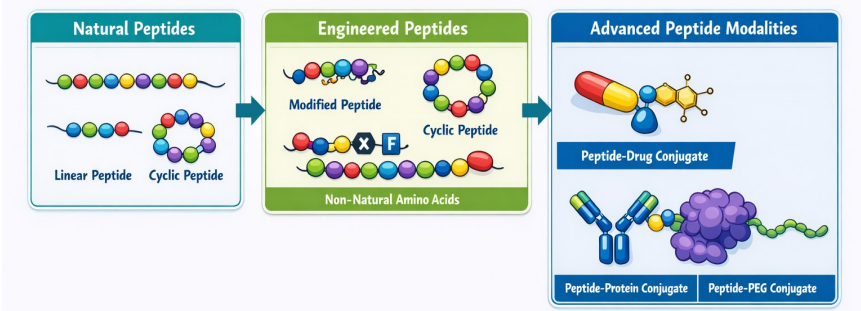
Advances in peptide synthesis

- Natural sources
- Liquid-phase and solid-phase chemical synthesis
- rDNA technology

SPPS

- Scaled-up
- Stability
- Enhanced therapeutic performance

- Non-natural amino acids (NNAA)
- Cyclization, stappling
- Pegylation
- Conjugation (drug, protein)



Strategies to improve peptide drugs

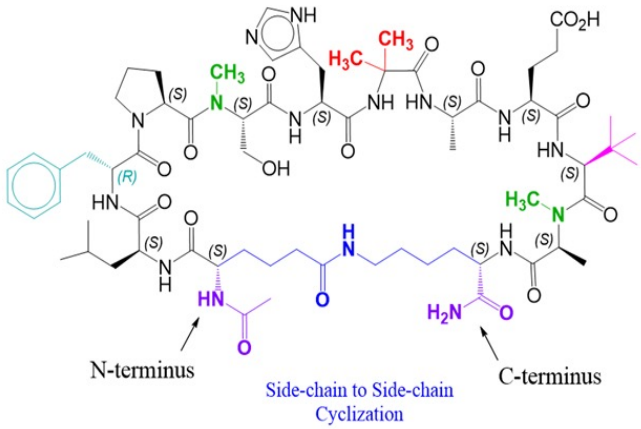
Challenges of peptides as drugs

- **Poor metabolic stability/rapid clearance** – enzymatic degradation, fast renal clearance
- **Poor oral bioavailability** – degraded by peptidases and proteases in GI tract
- **Low permeability** – needed for oral dosing and to prosecute intracellular targets
- **Solubility** – depends on amino acid composition, secondary structure, pH and pI
- **Chemical stability** – sensitivity to hydrolysis, oxidation, deamidation
- **Physical Stability** – risk of self-association, aggregation , fibrillation (can affect biological activity)
- **High cost of goods** compared to small molecules

Strategies to extend the half-life of peptides

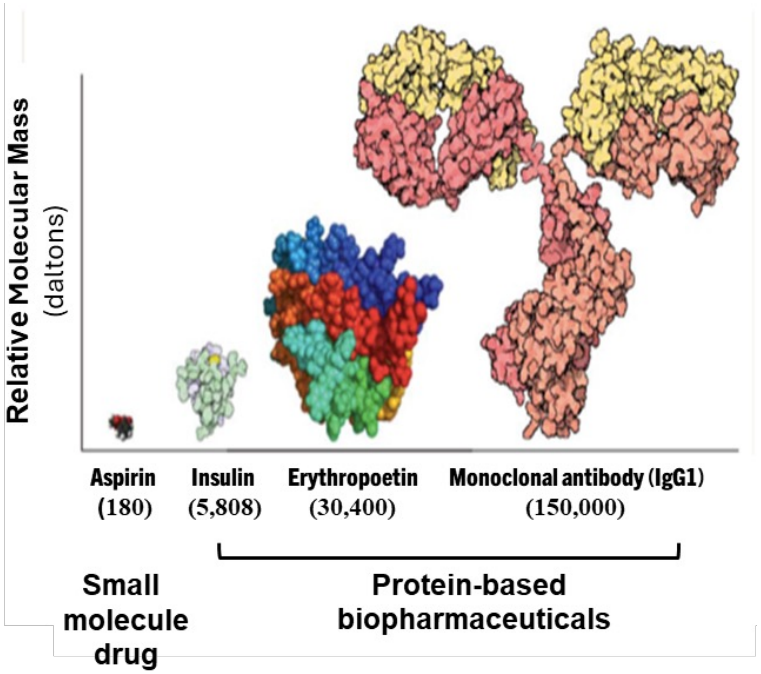
Chemical modifications: **improve metabolic/ proteolytic stability**

- Backbone modifications – cyclization; N-methylation; α,α-disubstitution
- Side chain substitutions – unnatural side chains
- D-amino acids – not recognized by endogenous proteases and peptidases
- Termini capping – Acylation and Amidation



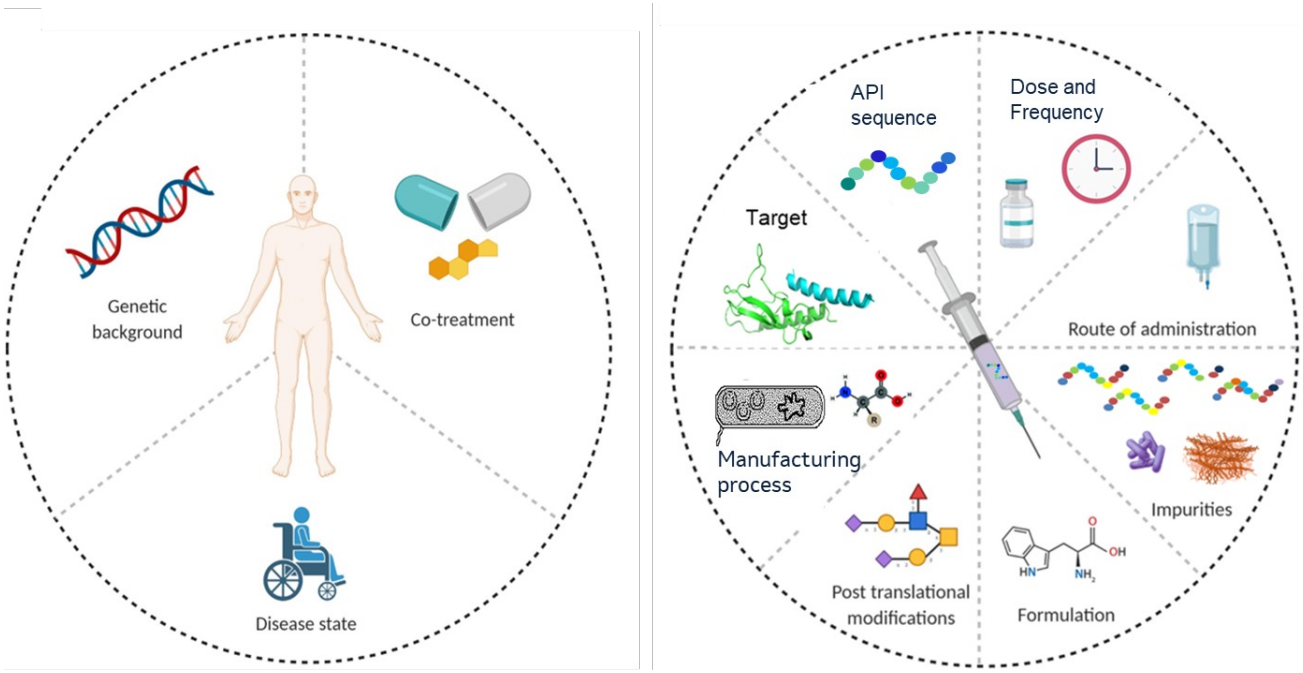
Courtesy of Jennifer Hanisak

Increasing complexity may impact product quality attributes and clinically relevant immunogenicity



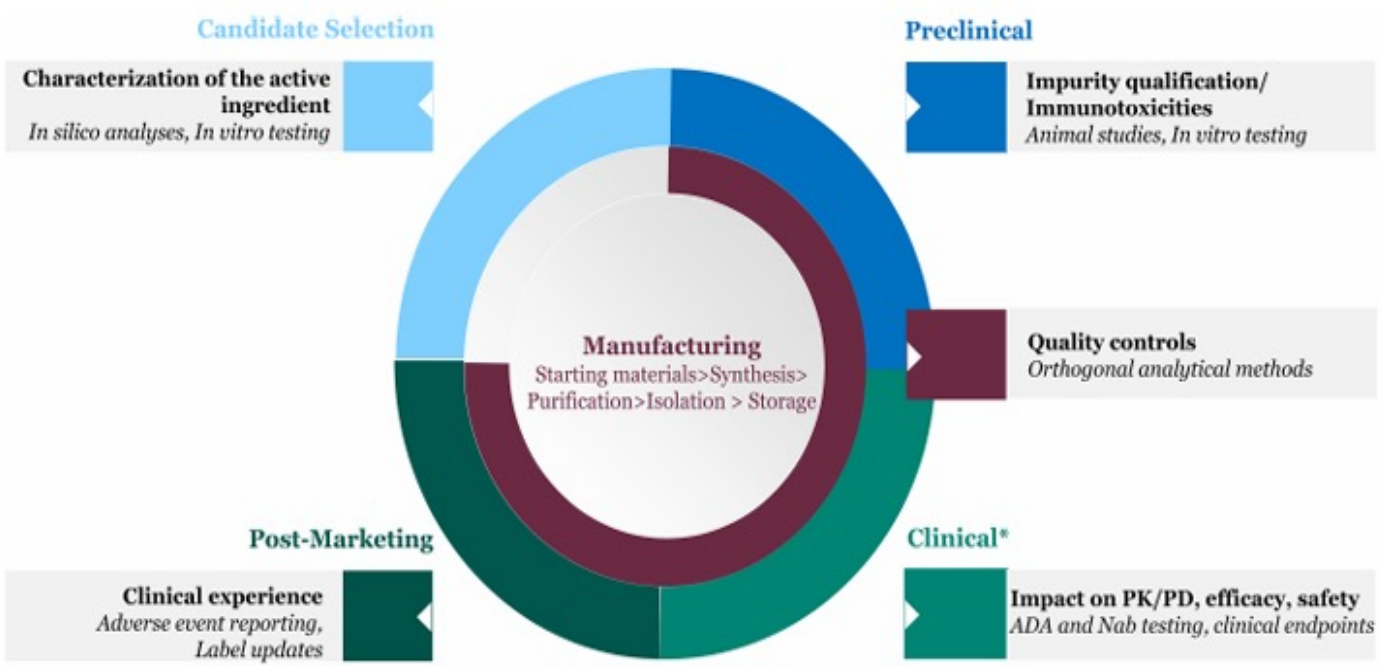
Factors	Small molecule drugs	Large molecule drugs
Molecular Weight	Low	High
Structure	Well-defined	Complex
Synthesis	Chemical process	Cell culture systems and biotechnology processes
Characterization	Complete	Challenges due to molecular composition and heterogeneity
Stability	High	Sensitive to external conditions
Immunogenicity	Rare	Common

Patient- and product-related factors impacting immunogenicity risk



Adapted from Vaisman-Mentesh in Front. Immunol (2020)

Regulatory guideline approaches to peptide immunogenicity risk assessment

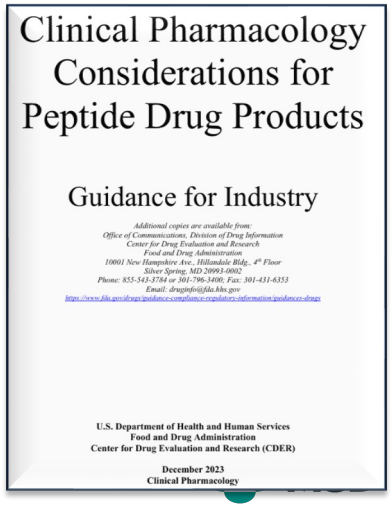


From: Puig M and Shubow S. Front. Imm. 2025


EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

1 12 October 2023
2 EMA/CHMP/CVMP/QWP/387541/2023
3 Committee for Medicinal Products for Human Use (CHMP)
4 Committee for Veterinary Medicinal Products (CVMP)

5 Guideline on the Development and Manufacture of
6 Synthetic Peptides
7 Draft


Clinical Pharmacology Considerations for Peptide Drug Products

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

December 2023
Clinical Pharmacology

Non-clinical strategies to predict immunogenicity risk

- Market experience
- *In silico* and *in vitro* tools
- Pre-clinical immunogenicity translatability

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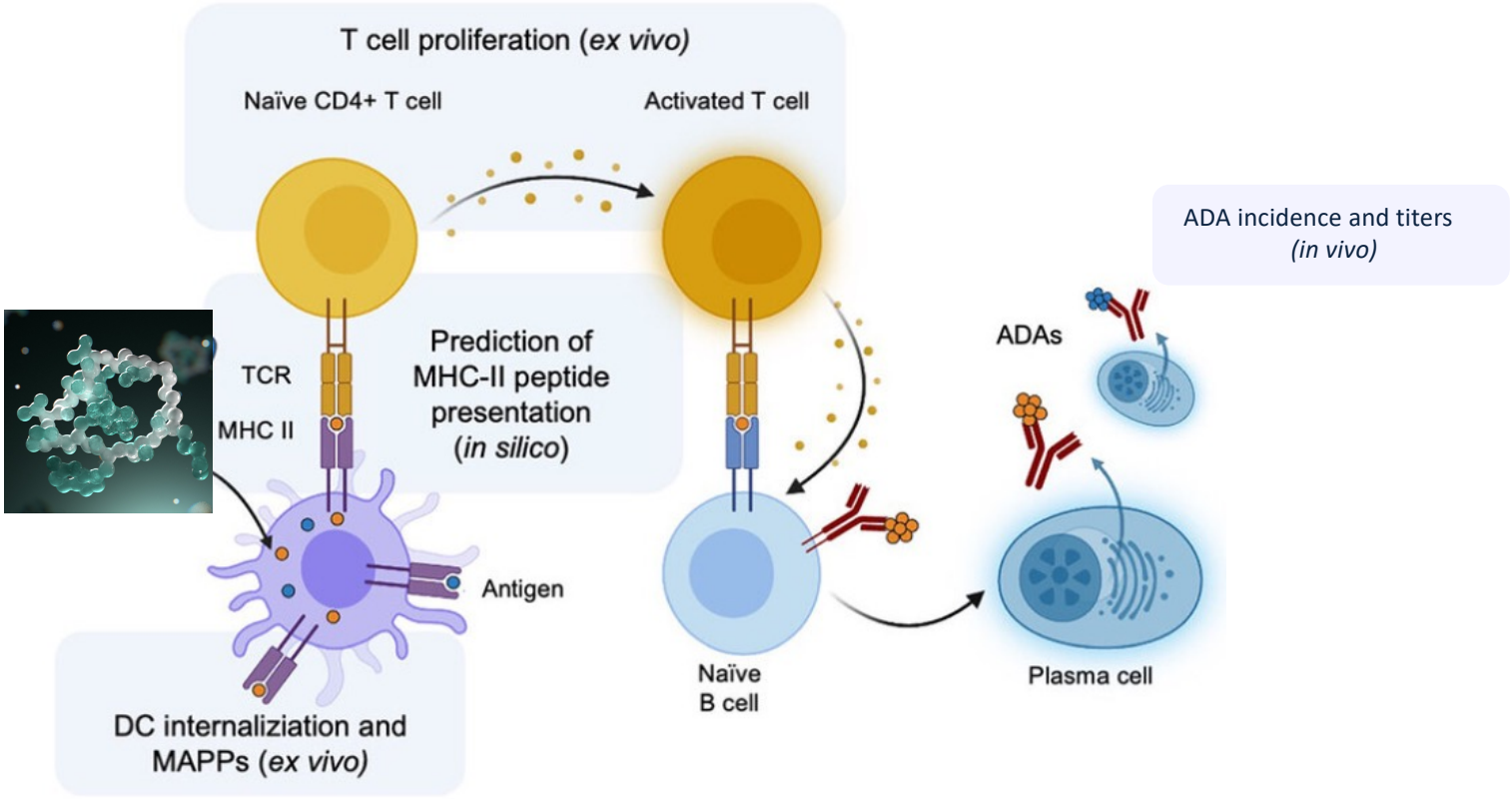
Market experience shows mostly no ADA assessment or reporting

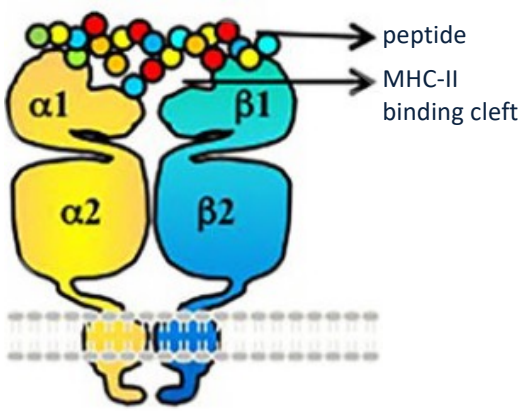
Type	Molecular Description AA-amino acids; (N-natural; S- synthetic)	Route	Indication	# products	Clinical Immunogenicity
Cyclic	8-16 (N &S)	**Oral**	Organ transplant rejection; infections; autoimmunity; diabetes, acromegaly	6	ADA not assessed; no immunogenicity described in label
	4-12 (S)	IV	Cancer, hepatorenal syndrome, acute coronary syndrome; hepatorenal syndrome	5	ADA not assessed; no immunogenicity described in label
	7-14 (S)	SC	Cancer, hormonal, vasodilatory shock	6	ADA not assessed; no immunogenicity described in label except for one (11% GEP-NETand <4% acromegaly).
	25 (S)	IT	Severe and chronic pain	1	No ADA formation reported in the label
	15 & 30; covalently **linked to PEG**	SC	Generalized myasthenia; paroxysmal nocturnal hemoglobinuria	2	ADA (23%) (including NAb (3%)) and anti-PEG Ab detected (9.3%); no clinical impact or unclear
	6 -13 **(semi-synthetic) lipo- or glycopeptides;**	IV	Anti-bacterial or fungal	9	No ADA formation reported in the label
Linear	31 (S)	**Oral**	Diabetes	1	Very low immunogenicity (0.5% ADA), not clinically relevant.
	8-39 (S)	IV (3), SC (7)	Cardiovascular, Diabetes, obesity, infectious diseases, endocrine	10	Not reported for 8 aa; >30-80% ADA, NAb & endogenous counterpart reactivity, w no clinical effect; anaphylaxis in peginesatide (withdrawn) and hypersensitivity in taspoglutide (discontinued)

Non-clinical strategies to predict immunogenicity risk



Adapted from https://app.biorender.com/biorender-templates

In silico prediction of immunogenicity for NNAA-containing cyclic peptides remains challenging



MHC-II presentation

- Peptides are typically 13–25 AA long with a 9 AA core binding motif.
- Peptides must be able to adopt an extended linear conformation compatible with the MHC II groove
- Undergo endosomal processing or direct binding

Cyclization

- Confers resistance to linearization by protease digestion
- Increased rigidity

NNAA

- Resistant to endosomal processing
- D-AA change the orientation of peptide side chains and prevents proper accommodation of anchor residues in HLA pocket, decreasing HLA binding and T-cell stimulation(1)
- Weaker peptide immunogenicity reported in animal models when D-AA were introduced(2-5)
- Unlikely to mimic endogenous peptide sequences

***In silico* prediction tools**

- Training datasets are biased towards linear peptides with natural AA
- Attempts to predict binding of NNAA rely on proxy substitutions (6), empirical corrections, or docking, each with known limitations.

1. Azam et al. Front Immunol 2021
2. Maillere et al. Mol Immunol 1995
3. Dintzis et al. Proteins 1993
4. Jaton & Sela. J Biol Chem 1968
5. King et al. J Immunol 1994
6. Mattei AE, et al. Front Drug Discov 2022

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In vitro data are needed to validate *in silico* prediction

1. **MHC-Associated Peptide Proteomics (MAPPs)**
   - **Aim:** expose dendritic cells or monocytes to the peptide (often over a day or two), then isolating the HLA-DR molecules and identify the sequence of bound peptides, including drug.
   - **Advantage**: capturing antigen processing and presentation in a physiologically relevant way (with real human APCs).
   - **Challenges** for cyclic peptides with NNAA: may require linearization; binding does not necessary inform of T-cell activation.

2. **Naïve T-cell proliferation or cytokine release-based assays:**
   - **Aim:** PBMCs or DC:T-cells from multiple donors are incubated with the peptide for about a determined length of time to achieve T-cell activation.
   - **Advantages**: adds functionality to antigen presentation
   - **Challenges** for cyclic peptides with NNAA: may require linearization, uptake/processing, assay sensitivity limitations.

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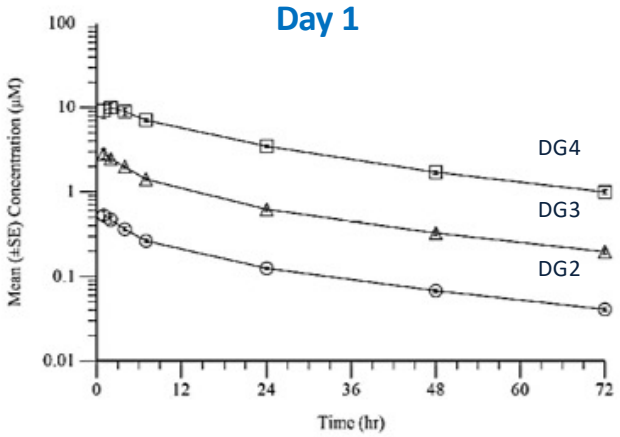
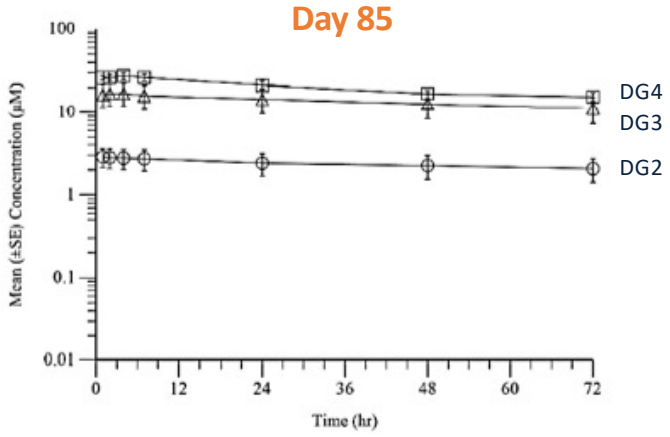
Pre-clinical immunogenicity translatability
Case Study: When drug exposure appears altered in NHP

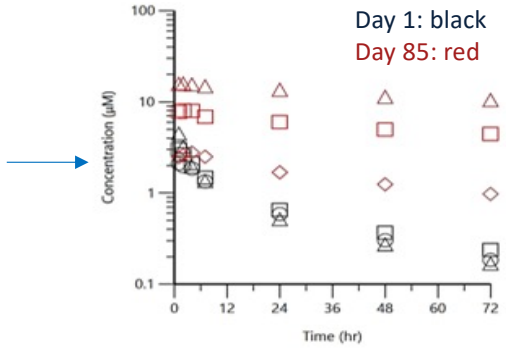
Therapeutic-A

Dose Group (DG)1-vehicle
DG2<DG3<DG4





- Day 85: very different exposure profiles (Cmax, AUC)
- Similar observation in rat study
- Variability on individual animal level noted



- PK assay?
- Therapeutic-A batch?
- Tissue-depot effect?
- Soluble target engagement?
- Unknown binding/ release effect?
- ADA?

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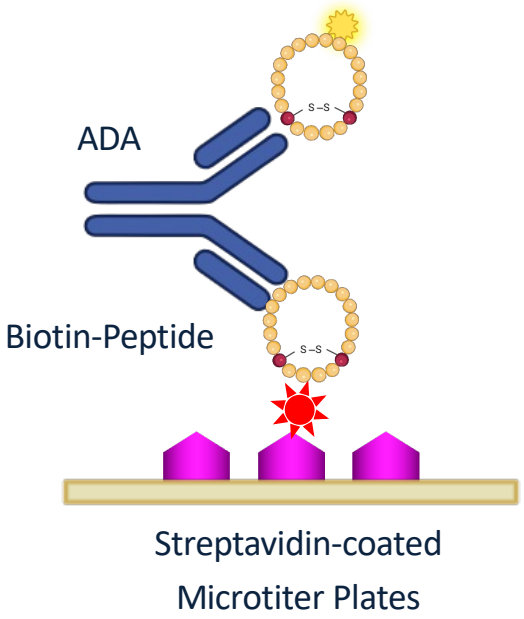
Bioanalytical strategy for clinical ADA testing

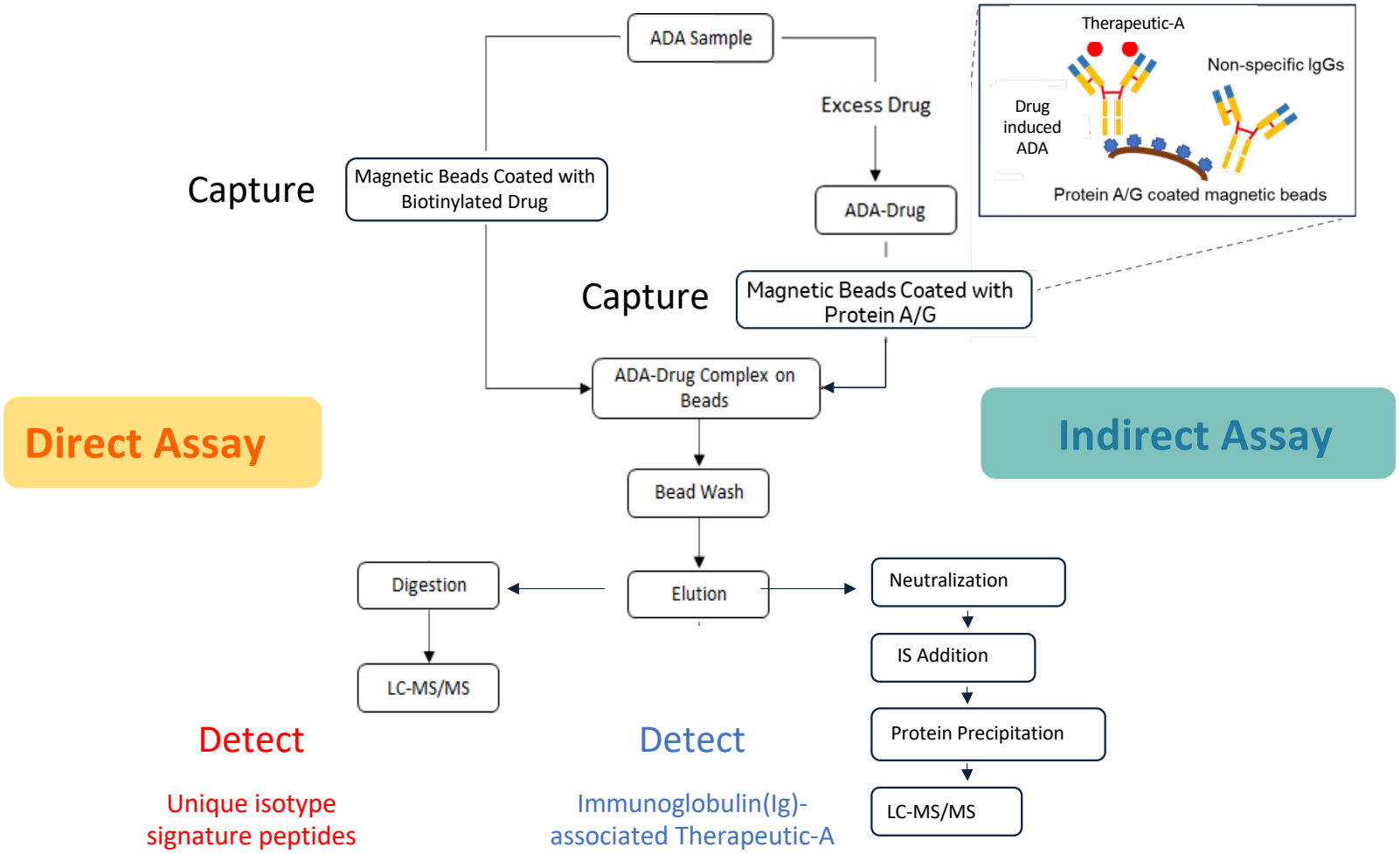


How can one label a small cyclic peptide without blocking some of the epitopes recognized by ADA?

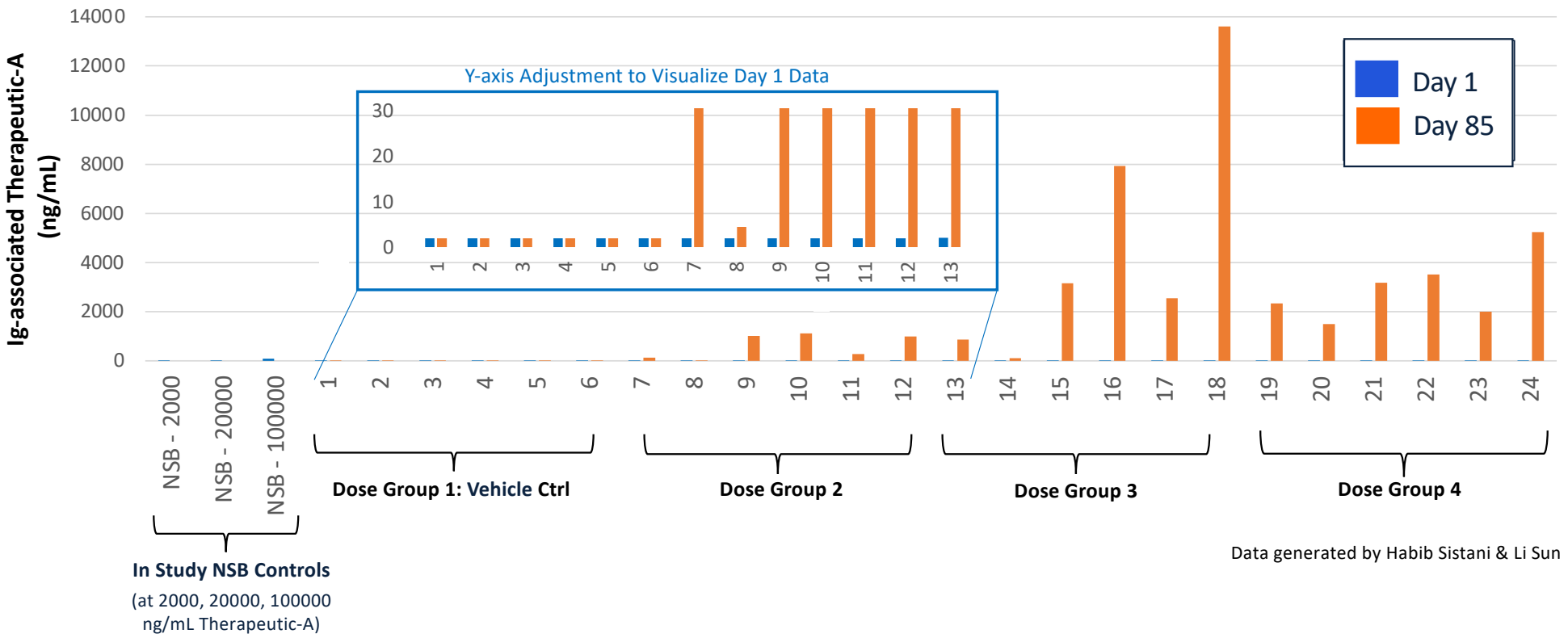
❑ Challenges related to critical reagent generation

- Critical reagents:
  - Finding conjugation site(s) not interfering on binding to target
  - Special synthesis for biotin- and sulfo-Tag conjugation with the help of Protein Chemistry
- PC generation:
  - Generate peptide-KLH, -biotin

Schematic of ADA semi-quantitative assay by IP-LC-MS/MS



Indirect LC-MS/MS revealed presence of Ig-associated Therapeutic-A at day 85



Data generated by Habib Sistani & Li Sun

- Non-specific binding (NSB) samples contained Therapeutic-A spiked at or above highest level observed in study: background signal only
- Ig-associated Therapeutic-A concentrations for all of dose group 1 subjects (Vehicle Ctrl Grp): < LLOQ (2 ng/mL)
- Ig-associated Therapeutic-A concentrations for DG2-DG4 at day 85: significant levels, individual animal variability

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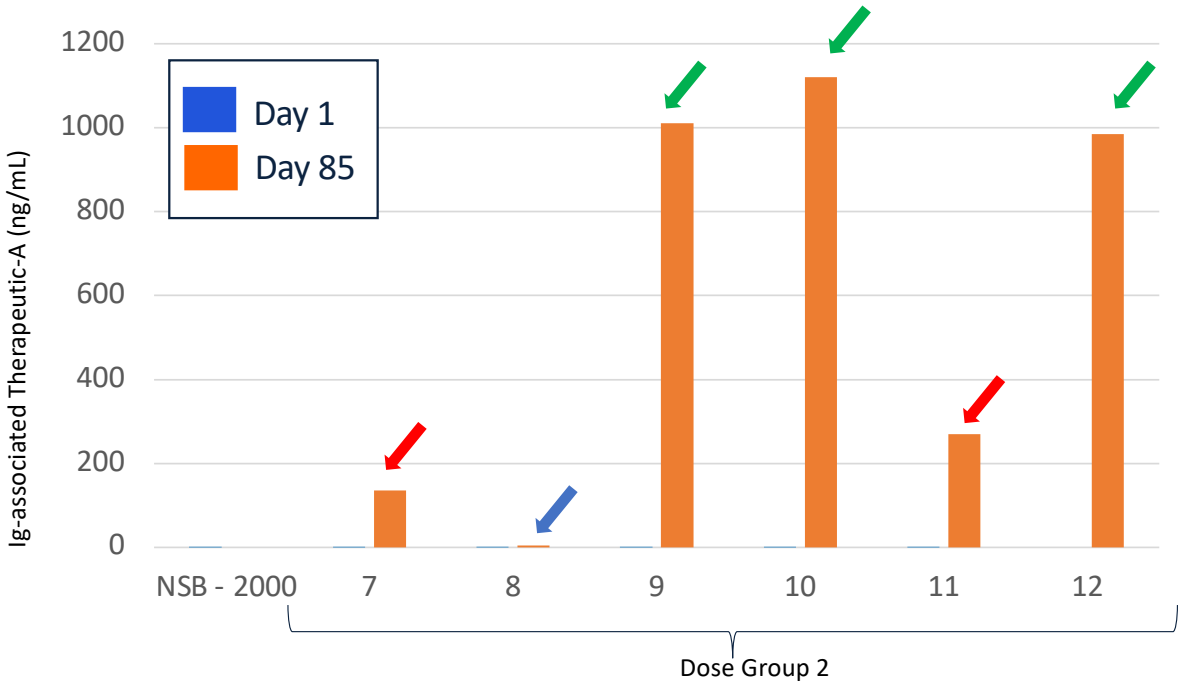
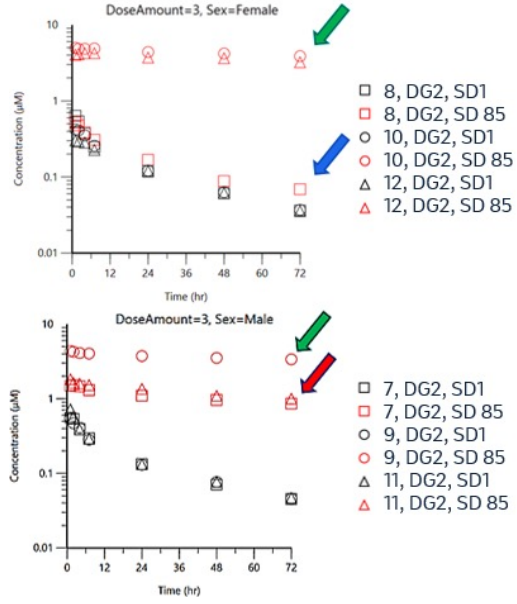
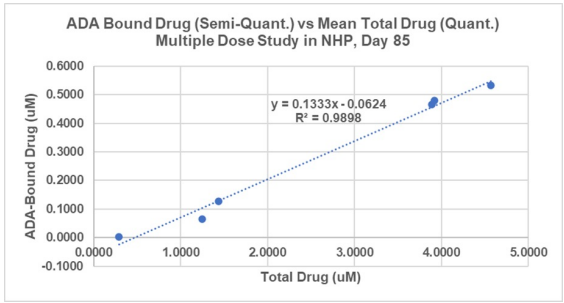
Ig-associated Therapeutic-A correlate with NHP exposure profiles



Strong correlation between PK data and data from the indirect LC-MS/MS method was observed across all dose groups.

Exposure Profiles for Dose Group 2

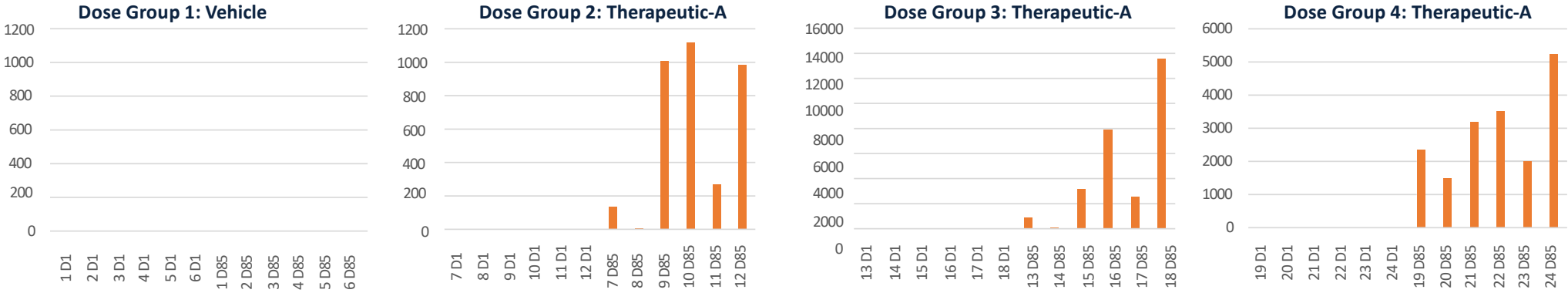




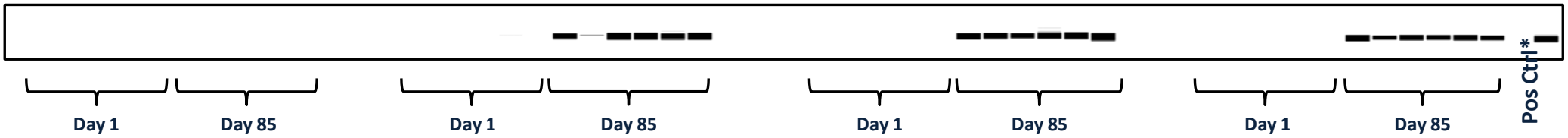
Data generated by Habib Sistani & Li Sun

Confirmation of LC-MS/MS derived data by an orthogonal method strengthened evidence of ADA formation

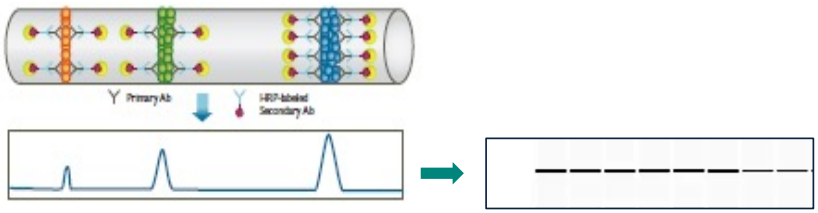
A (Indirect LC-MS/MS)



B Capillary Electrophoresis / Peggy Sue Method

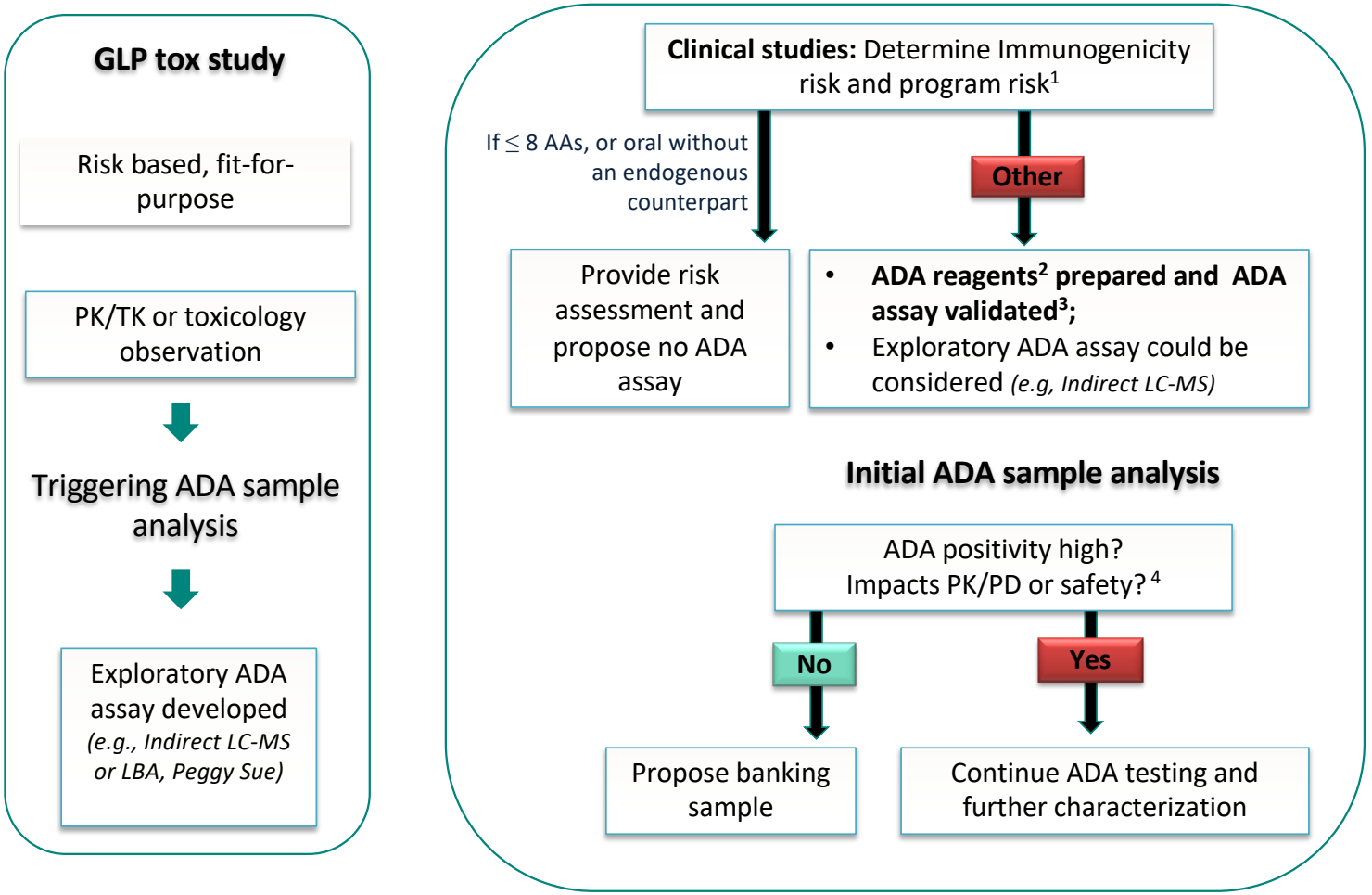


Data generated by Minchao Chen



- Immobilize drug at its isoelectric point position in capillary
- Add sample containing putative binding partner (e.g. ADA)
- Detect drug bound ADA via species-specific anti-IgG antibody
- Record signal -> transform to Western blot type visualization

Potential ADA testing workflow for peptide products



1. **Immunogenicity prediction and risk assessment** (In silico, in vitro); endogenous counterpart? Cleavage resistance of cyclic peptide? MOA, indication, preclinical species, and prior experiences.

2. **Reagents:** PC generation (3-6 months), drug labeling (2-3 months) (both need Chemistry's help)

3. Typically, **bridging ADA assay** using biotin- and SulfoTag-drug as capture and detector, respectively. If not working, then direct binding format or indirect IP-LC/MS.

4. High ADA incidence with no efficacy or safety impact – safe clinical profile; Low ADA incidence does not always correlate with a safe clinical profile (e.g. hypersensitivity, allergic-type reactions).

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Interpretation of ADA positivity and neutralizing activity

Neutralizing Activity= ADA-induced exposure reduction **+** Neutralizing Ab (NAb)

Exposure Level	**Clinical Efficacy Impact**	**Neutralizing Activity** (or adverse reactions)
No change	No impact	Non-neutralizing activity
	Reduced efficacy	**Clinically meaningful neutralizing activity due to NAb**
Reduced	No impact	Neutralizing activity, not clinically meaningful
	Reduced/loss of efficacy	**Clinically meaningful neutralizing activity due to ADA and/or NAb**
Increased	No impact or increased efficacy	No neutralizing activity
	Reduced/loss of efficacy	**Clinically meaningful neutralizing activity due to NAb**
	No impact	Are there any adverse reactions (**safety**) correlated with increased exposure?

1. Partridge MA, Kamen L, Wu B, Solberg H, McNally J, Stevenson L, Gupta S, Liu S, Xu W, Wu Y, White J. **Assessment of Neutralizing Antibody Activity in Clinical Studies: Use of Surrogate Measurements Instead of Stand-alone Assays**. AAPS J. 2025 Aug 13;27(5):132. doi: 10.1208/s12248-025-01118-6. PMID: 40804295.
2. Xu W, Maas B, Roadcap B, Swarup A, Steinmetz T, Luo L, Ichetovkin M, Wood S, Vazvaei-Smith F, Lee AW, Vora K, Helmy R. **Neutralization Activity of Anti-drug Antibodies Against a Biotherapeutic Can Be Predicted from a Comprehensive Pharmacokinetics, Pharmacodynamics, and Anti-drug Antibody Data Analysis**. AAPS J. 2022 Sep 27;24(6):102. doi: 10.1208/s12248-022-00753-7. PMID: 36167856.

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General considerations for peptide immunogenicity reporting in regulatory filings

1. FDA expects an **immunogenicity risk assessment and a tiered ADA testing**, except for:
   - Oral
   - Small peptide with ≤ 8 amino acids
   - Peptide/cyclic peptide drugs with very short-term use (≤14 days) in a hospital setting
   - Peptide/cyclic peptide drugs with no or minimal systemic exposure

2. It's not the ADA positive rate but **clinical impact** what really matters. Peptides with:
   - High ADA positive rate may be acceptable if there is no safety concern and no/low impact on efficacy
   - Low ADA positive rate could be rejected or withdrawn if there is a severe safety concern
   - ADA/NAb cross-reactive with endogenous peptides are seen to be approved if there is no clinically significant impact

3. NAb assay development and validation should be driven by a **risk-based assessment** of clinical impact.

Summary

- **Peptide complexity** may increase immunogenicity considerations
- **Modality-driven design (cyclization, NNAAs)** has shown to lower immunogenicity potential
- **Market experience** indicates peptides are generally associated with low ADA incidence and minimal PK/efficacy impact
- **Immunogenicity prediction tools** such as in silico + in vitro T-cell methods are still limited for complex peptides
- **LC–MS/MS platforms (e.g. indirect assay)** are valuable for early ADA detection impacting PK
- **Risk-based bioanalytical strategy** should guide stage-appropriate assay selection and critical reagent readiness while incorporating orthogonal analytical approaches to help contextualize and evaluate immunogenicity risk.

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Thank you!