Analyzing and Decreasing the Immunogenicity Potential of Biotherapeutics using *in silico* Approaches

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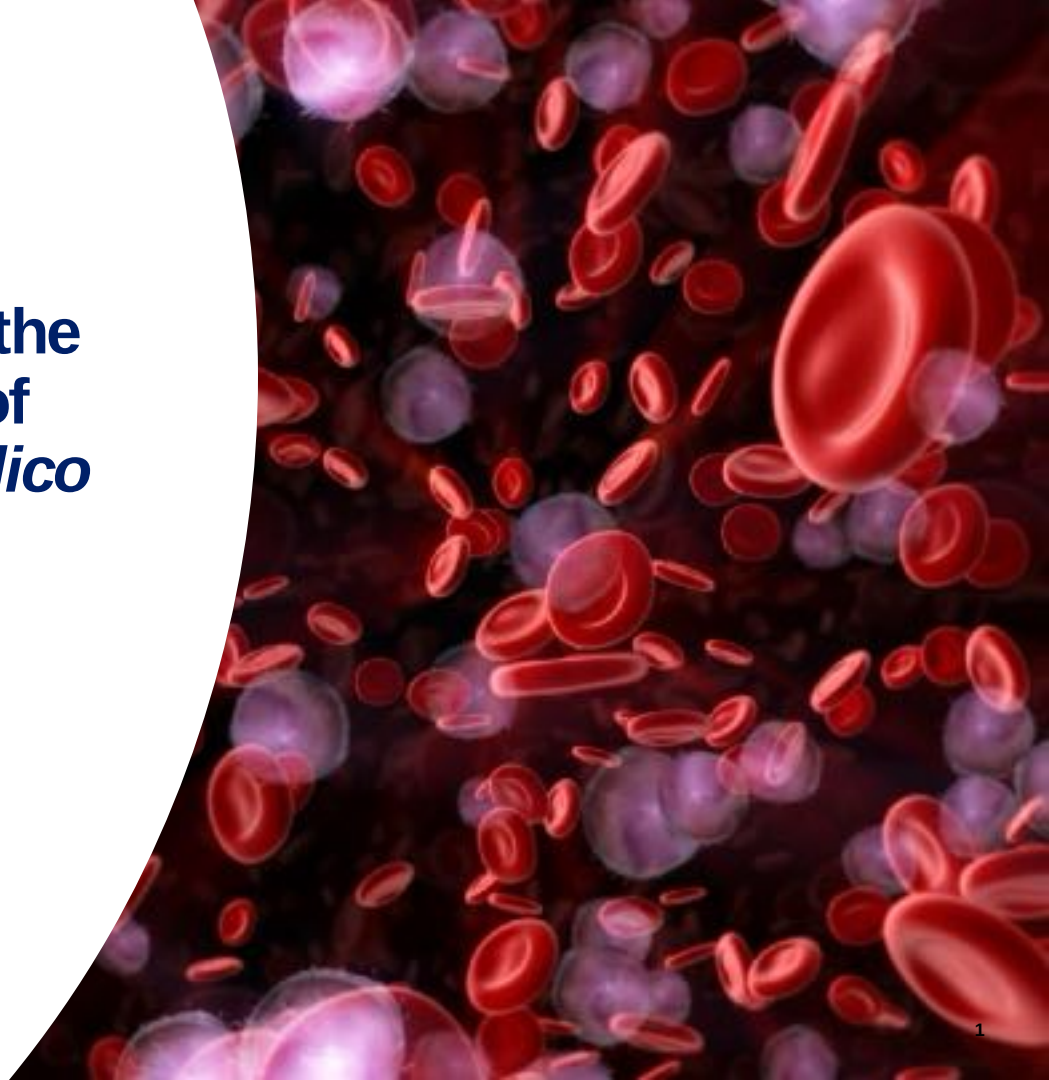

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in silico Immunogenicity Profiling at Novartis

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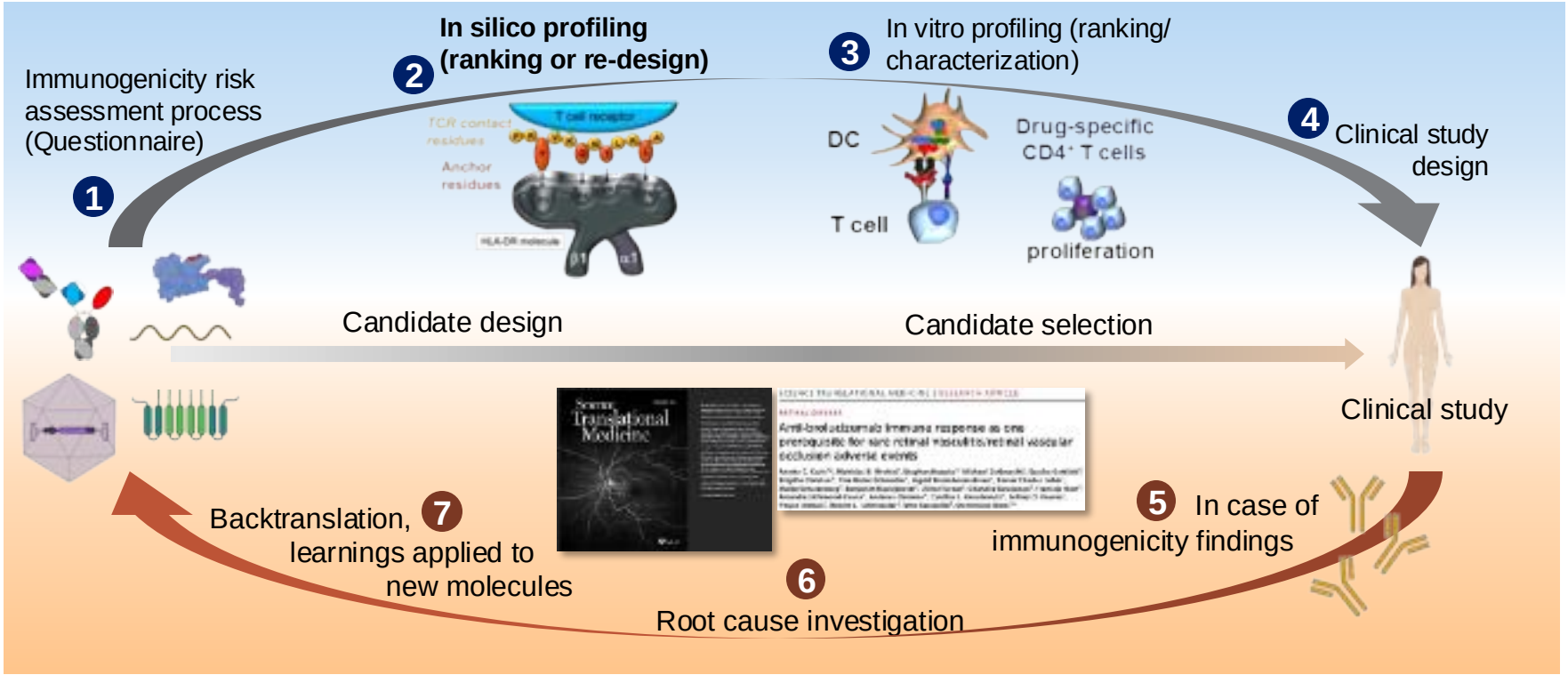
in silico Immunogenicity Profiling - advantages and limitations

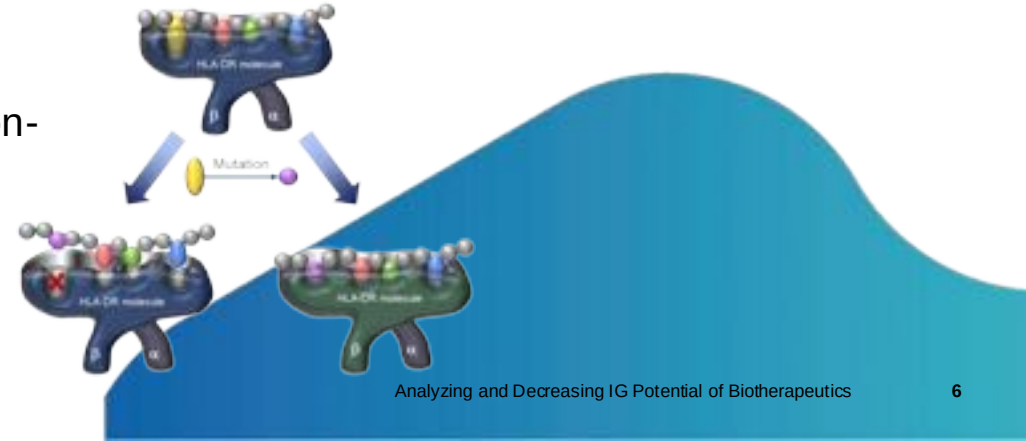
- IG profiling should be started as timely as possible in the biotherapeutic development process to inform necessary de-immunization approaches early on and to avoid resource spending on candidates with a high inherent IG potential in later stages.
- Oftentimes, this is only possible using in silico tools, since in early drug development, high-quality candidate material is not available in the quantities necessary for most in vitro assays.
- Additionally, high cost and long timelines of in vitro assays are also factors that can be hurdles for pharma and biotech companies alike.
- Limiting factors are still the prediction accuracy, especially for B cell epitopes, and that additional aspects like aggregation, PTMs, change in structure upon grafting and endolysosomal processing can't be assessed.

Immunogenicity strategy for biotherapeutics



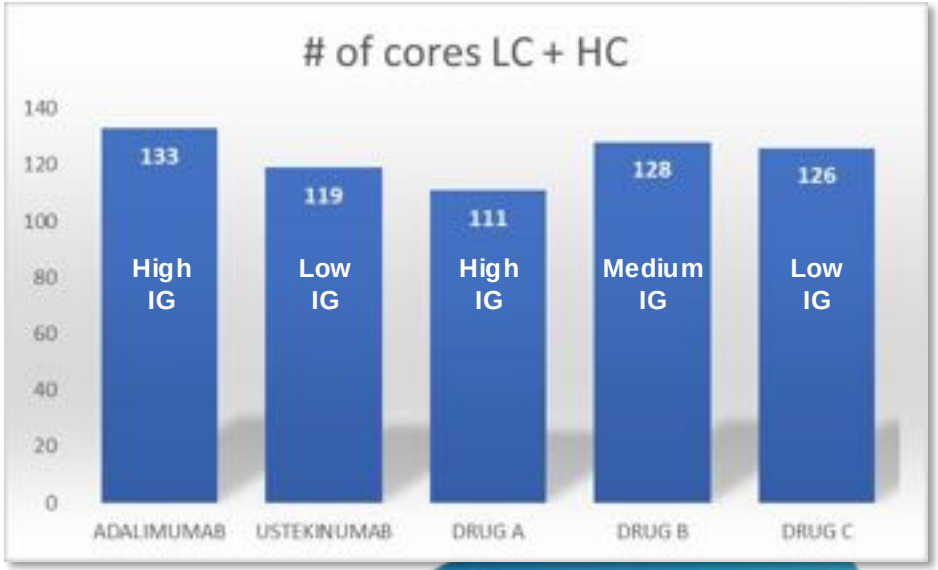
in silico Immunogenicity Profiling at Novartis

- **Prediction of HLA class II binding hotspots based on a PSSM**
  - **IG profiling of large candidate sets** early in the development process based on hotspots and CDR overlap
  - **De-immunization of hotspots** via sequences randomization to find non-binders that are confirmed via MAPPs assay



How we started – Candidate ranking based on # of cores

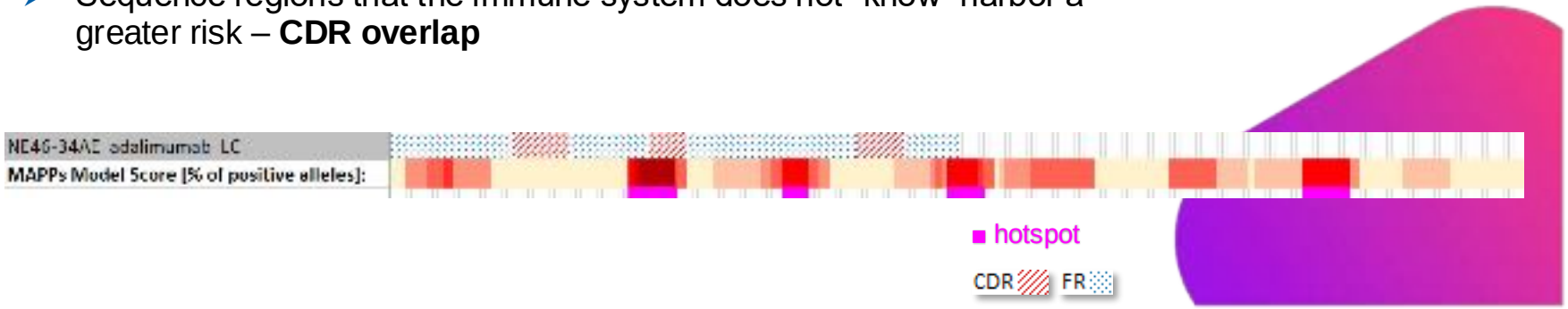
- **The simplest way to rank candidates is just using the # of predicted binding peptide cores in each sequence**
- **But this is only a very high-level analysis, which offers no option to address sequence specific questions!**
  - Like generation of neo-epitopes by introduction of Fc modifications, non-natural junctions, etc.



High / Low IG = relatively high / low clinical IG rate (% of ADA)

How we improved – Introduction of a weigthing matrix

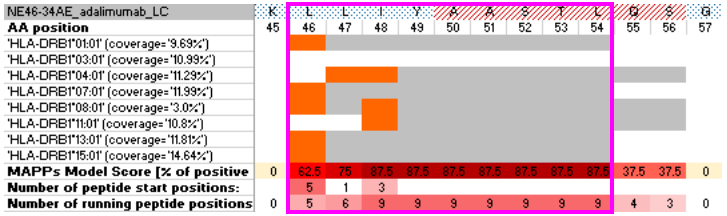
- **Only considering the # of predicted peptides is not enough to rank candidates properly!**
- **The quality of the hits is even more important and enables a better differentiation between candidates.**
  - Highly presented sequence regions harbor a greater risk – **hotspot ranking**
  - Sequence regions that the immune system does not “know” harbor a greater risk – **CDR overlap**



Hotspot ranking

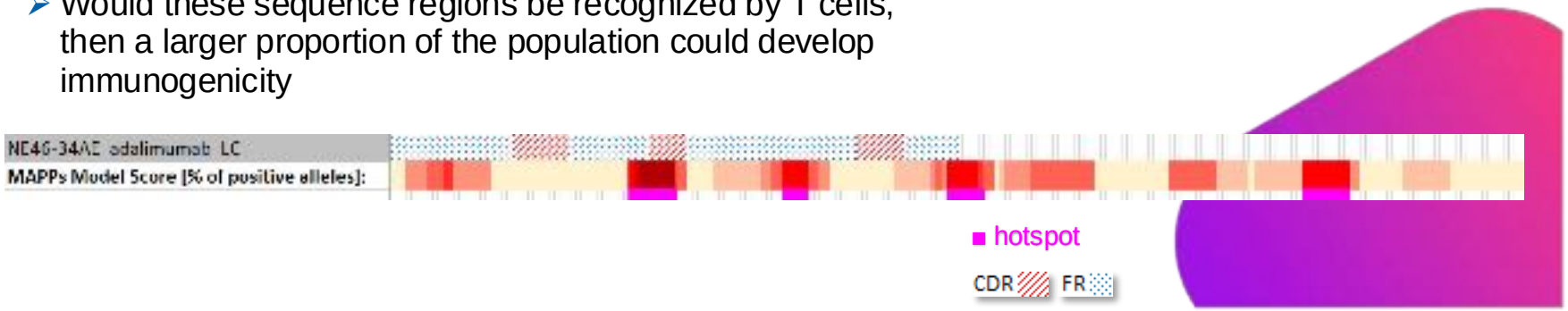
What are hotspots?

- Hotspots are sequence regions that show peptide binding to at least 5 out the 8 most frequent European HLA class II alleles



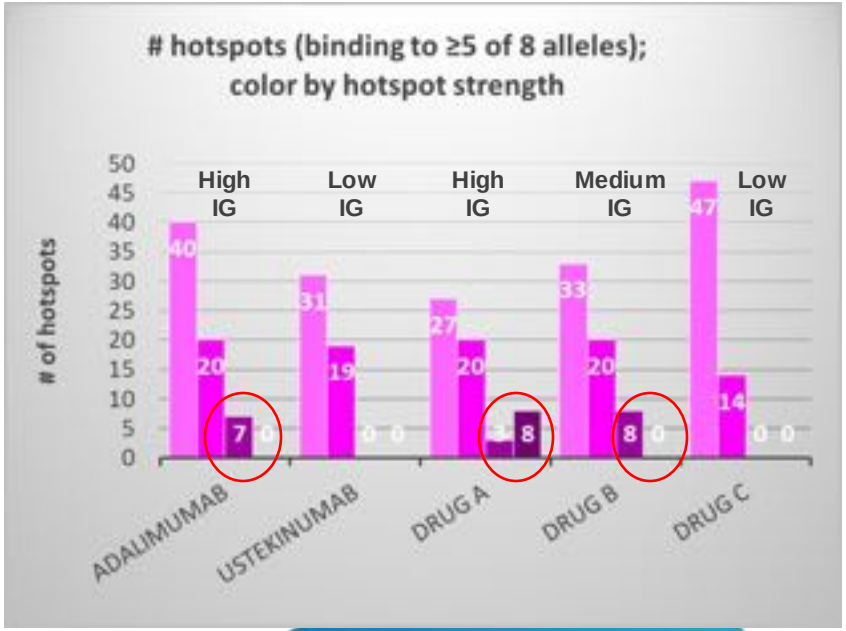
Hypothesis behind the hotspot ranking

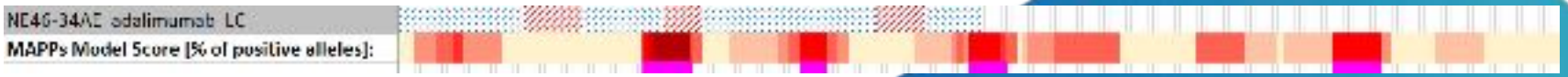
- Would these sequence regions be recognized by T cells, then a larger proportion of the population could develop immunogenicity



Candidate ranking based on # of hotspots

- Hotspot ranking seems to contribute to candidate differentiation and shows a better correlation to known IG rates than only counting binding cores
- But can we do more?

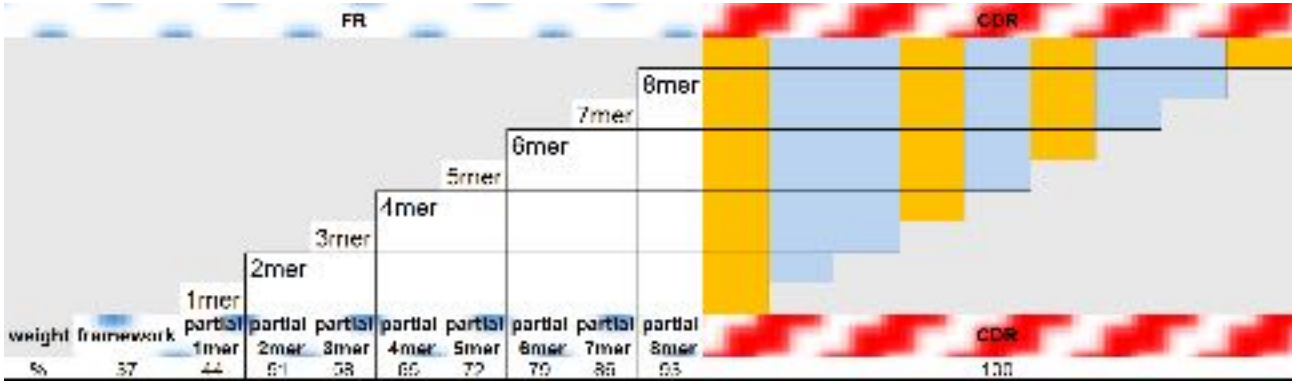




CDR overlap ranking

❑ Hypothesis behind the CDR overlap ranking

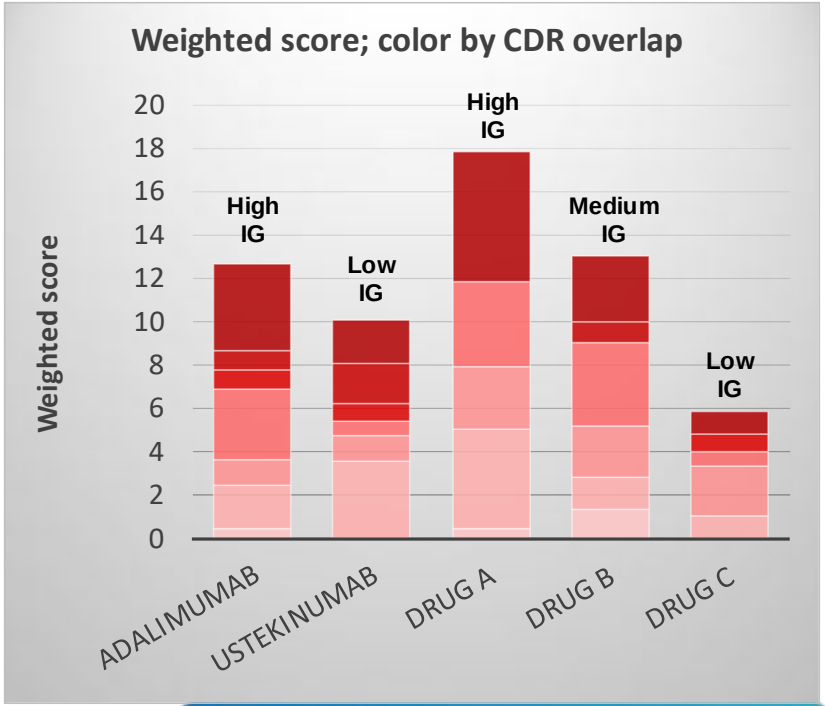
- ➢ T cell frequencies against foreign sequence regions (CDRs) are expected to be higher than for conserved human sequences in the framework (FR)
  - ➢ Introduction of a “weighting system”



Candidate ranking based on CDR overlap

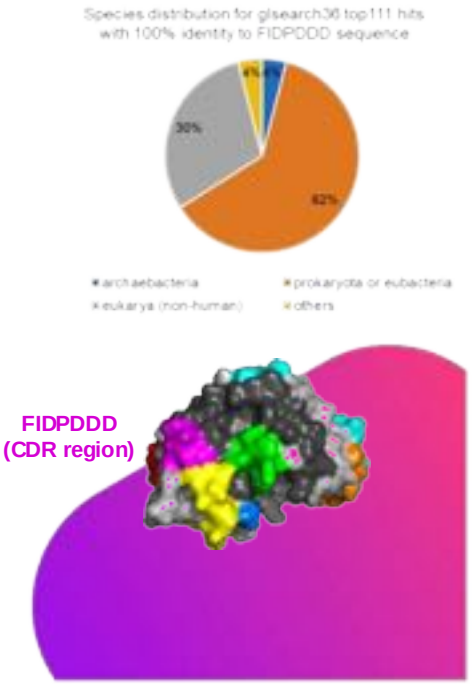
- ❑ **Taking the CDR overlap into account, an even better differentiation between the candidates is possible!**

- ➢ **Are there additional parameters that we can include in our assessment to improve candidate ranking?**



Additional parameters to improve candidate ranking

- Most in silico tools used in biotherapeutic development are predicting peptide binding to HLA class II molecules (e.g. NetMHCIIpan), frequently with the option to apply a weighting matrix, based on the **hypothesis that self-peptides and germline sequences have a lower IG potential.**
- Based on our experience during root cause analysis of adverse events in the clinic, we started to explore additional options to improve this weighting matrix. We could show that **biotherapeutic sequences can bear analogues to pathogen sequences**, which theoretically may result in a **high number of memory T cells** that are cross-specific to the biotherapeutic, as well as a **high prevalence of pre-existing anti-drug antibodies**.



Picture created with BioRender.com

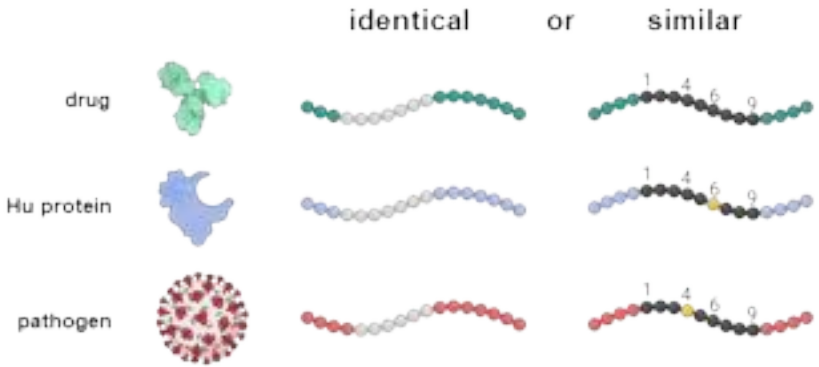
New *in silico* immunogenicity profiling approach based on biotherapeutic / pathogen analogy

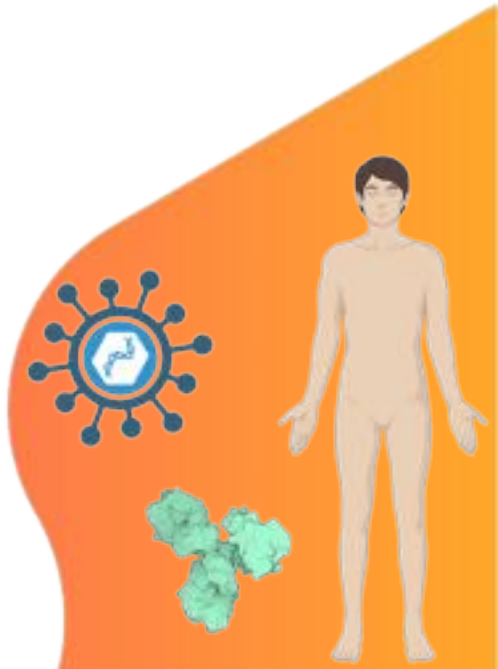
Hypothesis:

Biotherapeutic sequences can bear analogues to pathogen sequences.

These potential cross-reactive T & B cell epitopes may induce a strong immunogenicity response in a large proportion of the patient population.

New tools for the identification of:



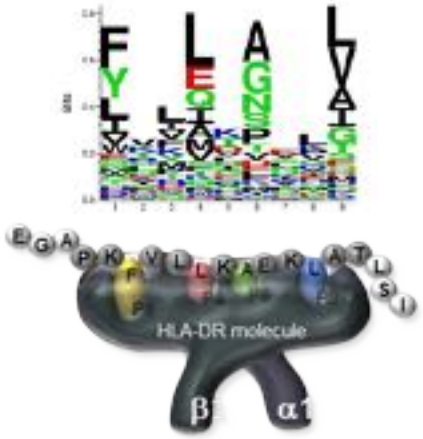


in silico toolbox

iSHAPe

(in silico HLA aggretope prediction)

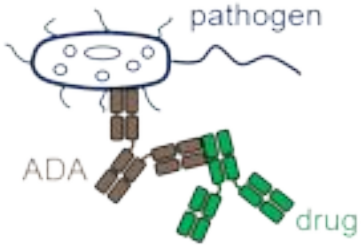
Predictive model for HLA class II binding peptides (potential T cell epitopes)



AP-BLAST

(Antigen vs Pathogen Blast)

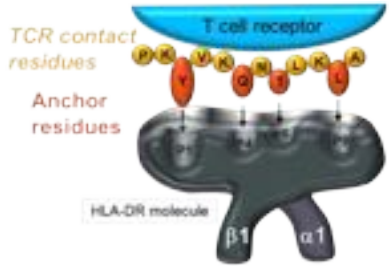
Alignment of drug sequence with other proteins to find analogous sequences which could lead to ADA cross-reactivity



MASE

(MASE: MAPPs Aggretope Similarity Evaluation)

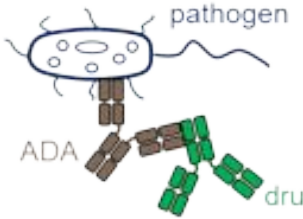
Predictive model for HLA class II binding peptides that could be recognized by cross-reactive T cells



Different anchors but peptide "looks" the same to the T cell

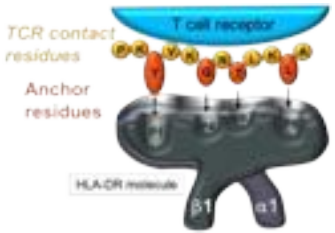
PKYVKQNTLKLA
PKYVKHNTLKLA
PKYVKQNTLKIA

New *in silico* immunogenicity profiling approach based on biotherapeutic / pathogen analogy



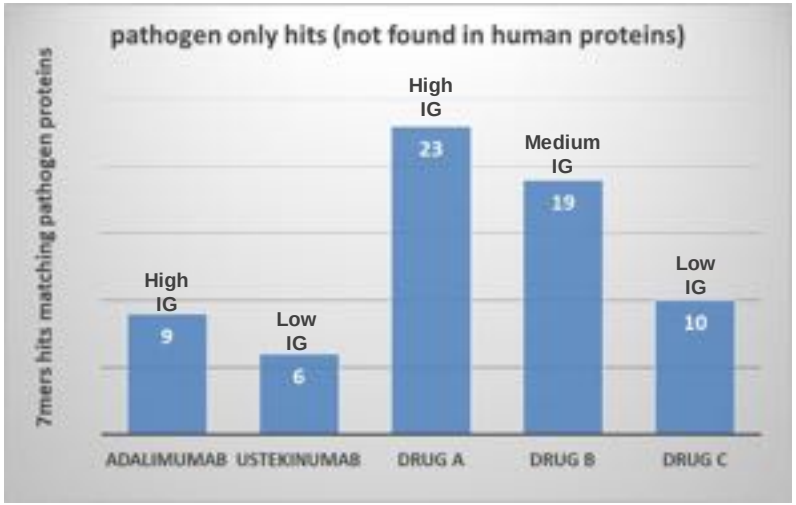
AP-BLAST

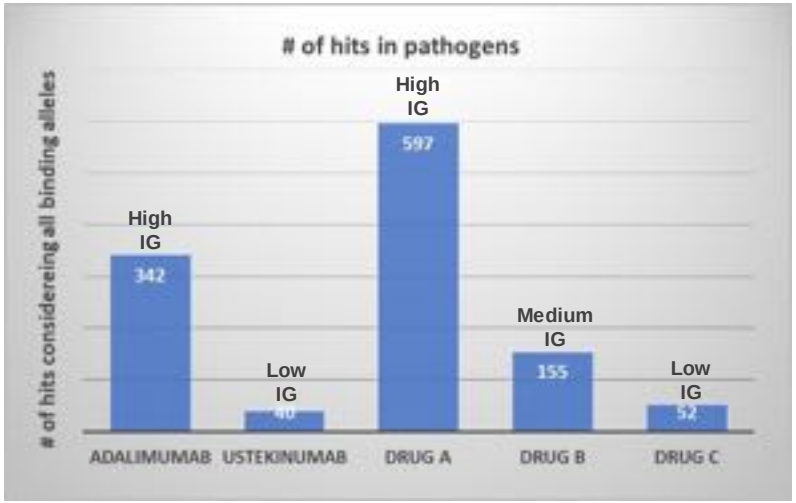
(Antigen vs Pathogen Blast)



MASE

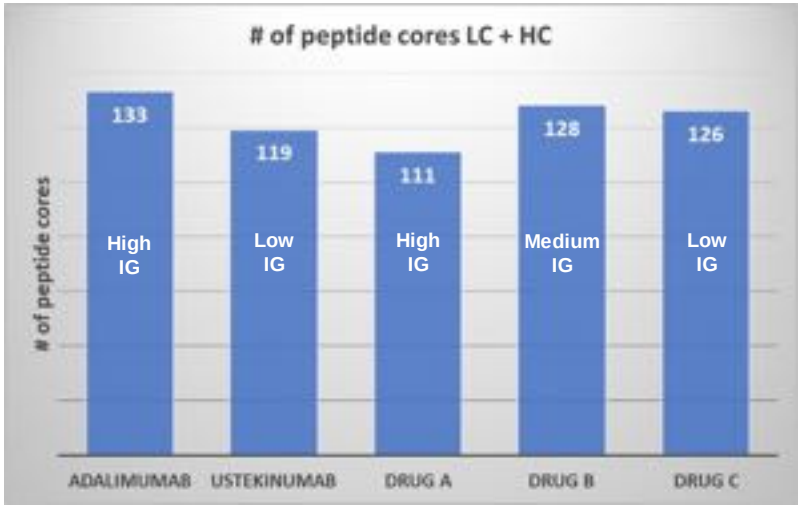
(MASE: MAPPs Aggretope Similarity Evaluation)

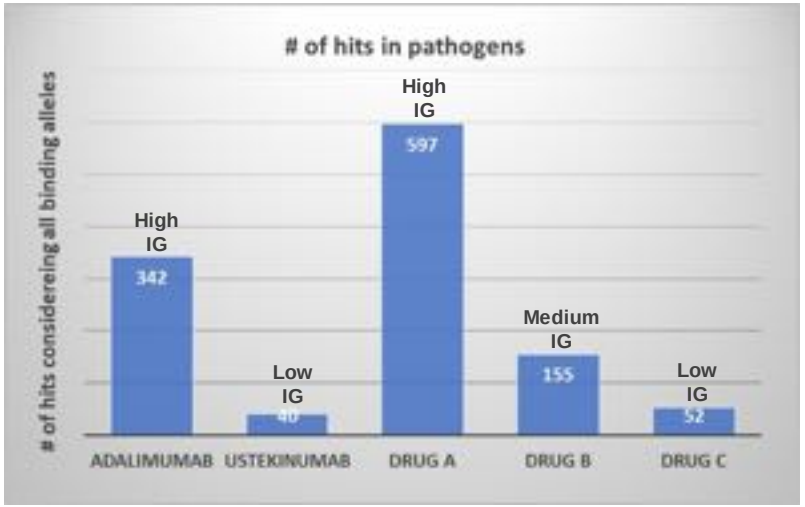




Immunogenicity (IG) Profiling

Comparison between counting cores and biotherapeutic / pathogen analog profiling

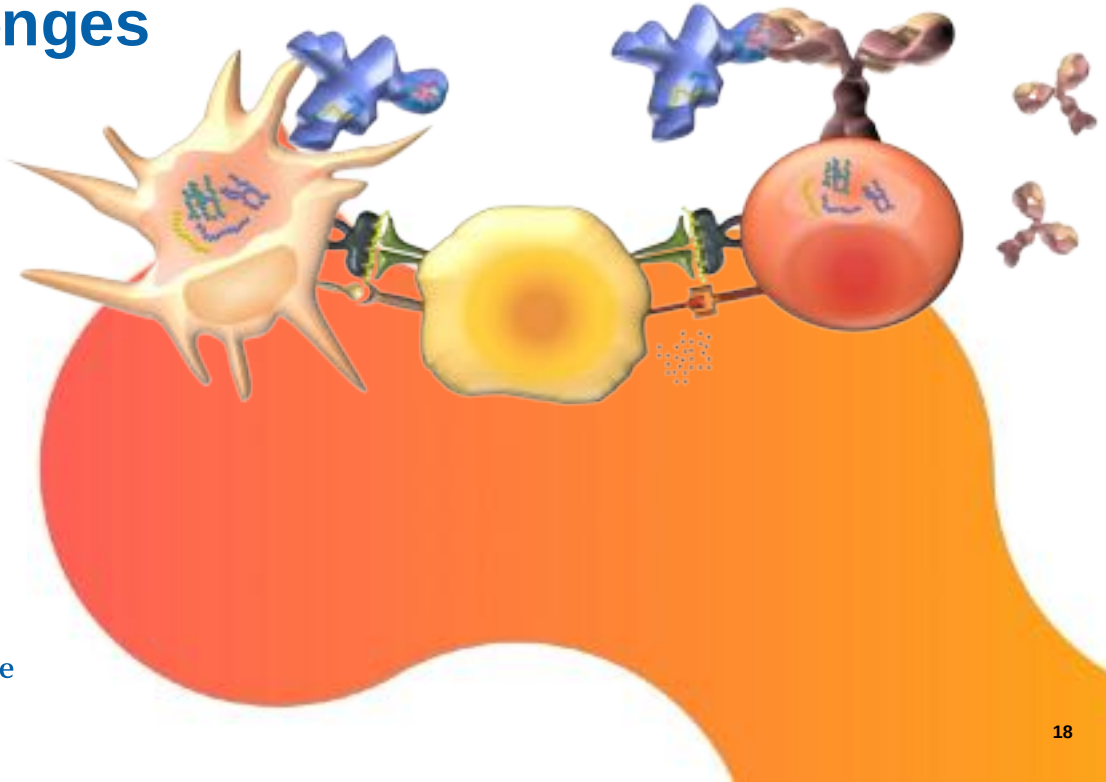




- **Implementation of biotherapeutic / pathogen analog profiling is a clear improvement for candidate ranking and shows better correlation to clinical IG rates!**

Decreasing Immunogenicity Potential of Biotherapeutics

- Strategies and Challenges



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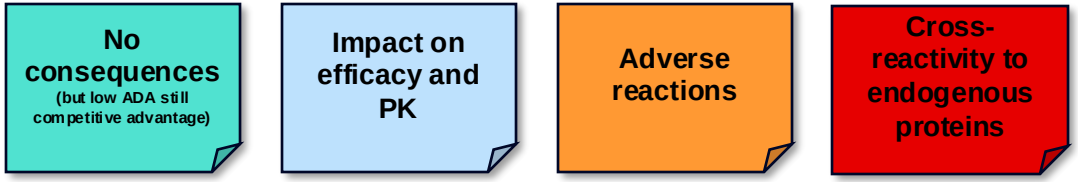
De-immunization – Rationale

Why de-immunize?

- Immunogenicity can have a broad variety of consequences.



- Even in the absence of consequences, a low immunogenicity incidence rate is a clear competitive advantage.
- Adverse reactions and cross-reactivity can sometimes lead to a drug being withdrawn from the market.

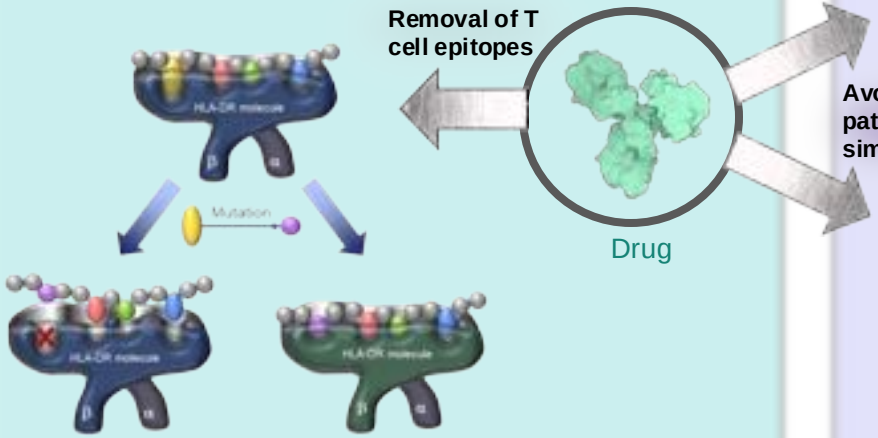
Can we now re-design biotherapeutics to be less immunogenic?

- **The landscape of in silico tools has evolved**. HLA binding evaluations are more sophisticated and can incorporate pathogen sequence similarity assessments!

Decreasing IG potential of biotherapeutics via re-design

De-immunization commonly focuses on T cell epitope removal (T cell epitopes are the pre-requisite for high affinity ADA responses)

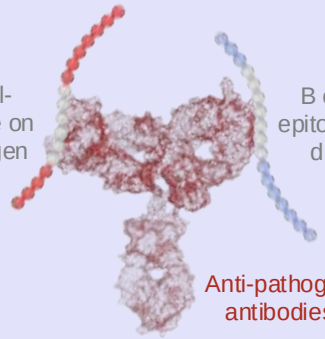
In silico re-design and prevention of neoepitopes



HLA molecules differ in amino acids anchor preferences

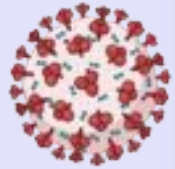
Pictures partially created with BioRender.com

Linear B cell epitopes



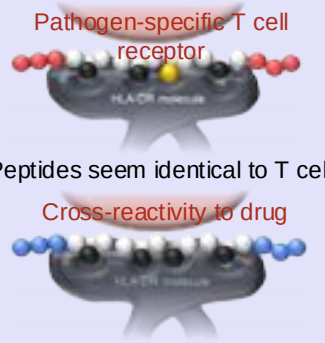
AP-BLAST

Pathogen similarity may lead to immune cross-reactivity



Avoiding pathogen similarity

Linear T cell epitopes



MASE

De-immunization approach depends on project aim

Different approaches can be followed when aiming at reducing T cell immunogenicity depending on:

- How much emphasis is on immunogenicity (balance de-immunization vs stability and affinity)
- How many positions will have to be mutated (defines complexity and dimension of approach)
- Whether affinity maturation runs as parallel independent process or combined with de-immunization (ideally combine but sometimes not possible due to parallel at-risk activities)

➢ **As POC, we started two projects with the aim to reduce immunogenicity via re-design, using different approaches based on the specific aims and requirements of each project.**

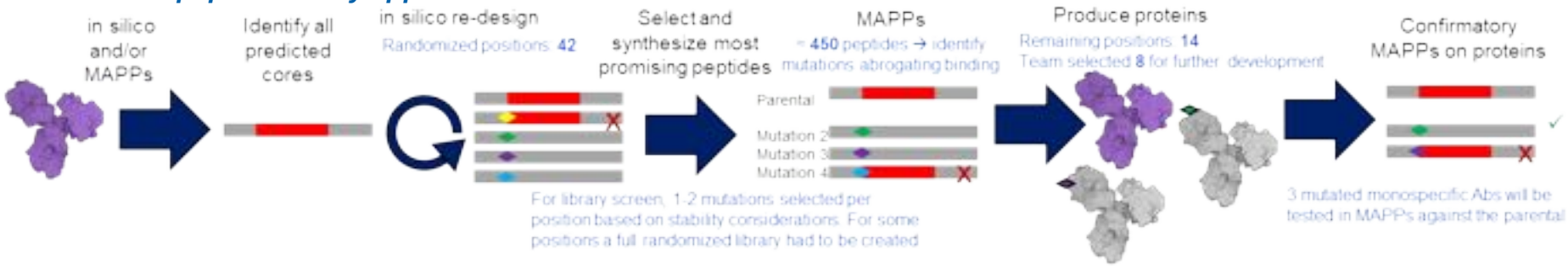
Case study Drug A

- Following FDA approval of Drug A, cases of severe adverse events were reported post-marketing.
- Based on our current understanding, immunogenicity is a prerequisite for the adverse events. As a result, the drug is no longer considered as a first line treatment, leading to significant financial impact.

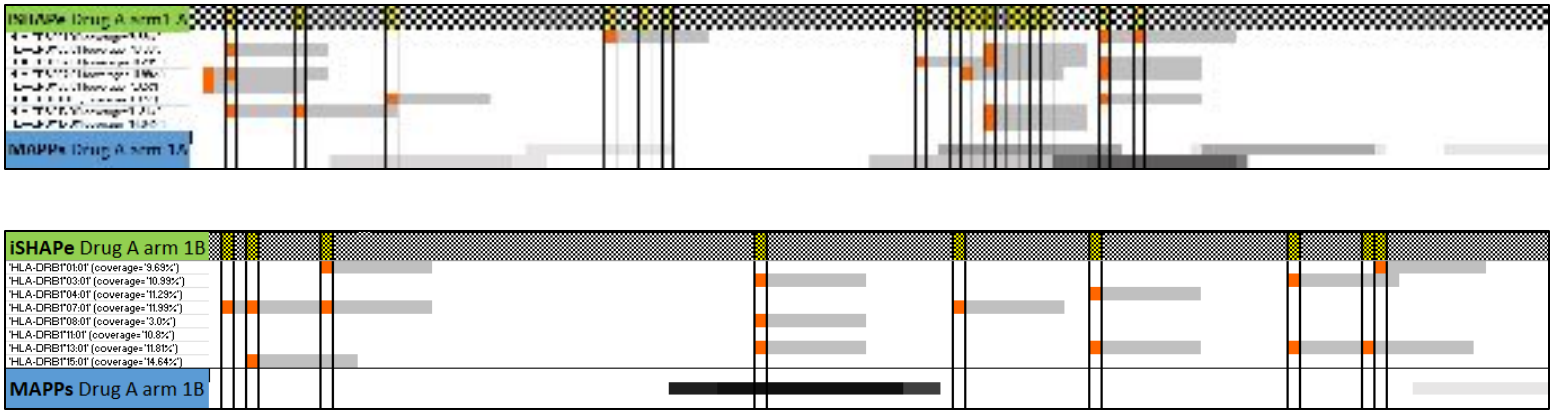
- Consequently, the team decided to generate a follow up molecule with the aim to lower the immunogenicity potential of the new molecule as much as possible.

2 different approaches tested based on project needs

Project A: lower immunogenicity <u>as much as possible</u> while maintaining stability
→ Extensive peptide library approach

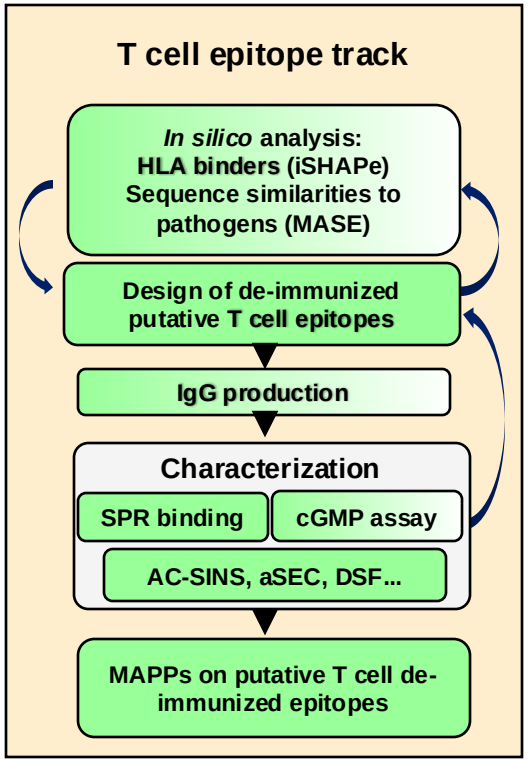


Drug A: Comparison of *in silico* prediction and MAPPs assay



- **We are rather over predictive but sometimes *in silico* predictions miss clusters completely.**
  - Therefore, relying solely on in silico predictions for comprehensive de-immunization approaches is insufficient, and it is important to combine in silico and in vitro assays.
- **Optimized variants are now in production for confirmatory MAPPs assay**

Case study Drug B



Integration of de-immunization in affinity maturation workflow

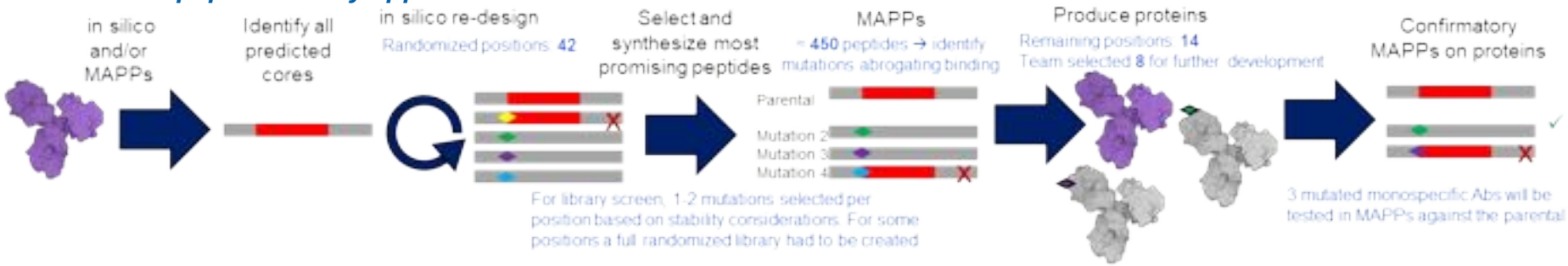
- Elevated ADA levels were observed in drug B FIH.
- The decision was made to mitigate immunogenicity potential in the next-gen drug B project.
- To save time and resources, de-immunization efforts were combined with affinity maturation.
- IG hotspots in LC and HC were identified via in silico IG profiling (iSHAPe and MASE).
- 4 rounds of rational in silico re-design parallel to affinity optimization mainly focusing on the LC CDR2 hotspot.

2 different approaches tested based on project needs

In both cases, extensive design sessions with project team needed to smartly generate structurally sound mutated proteins

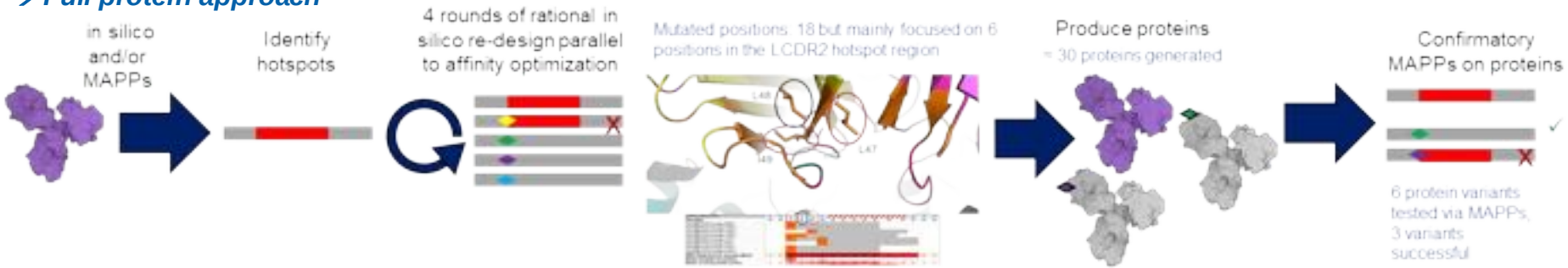
Project A: lower immunogenicity as much as possible while maintaining stability

→ Extensive peptide library approach

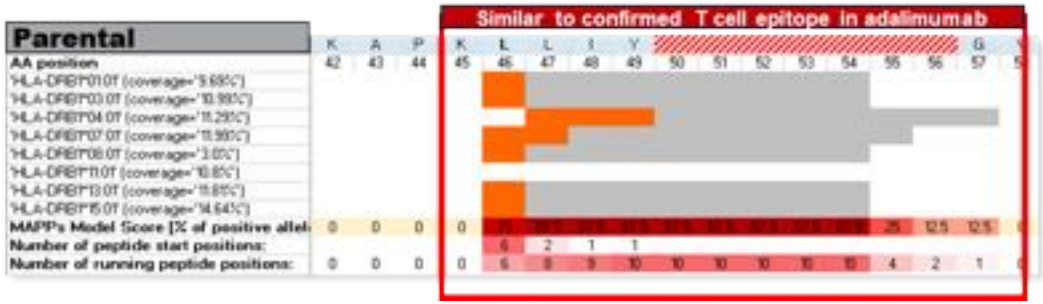


Project B: lower immunogenicity with focus on only few hotspots. Prioritize developability and affinity aspects

→ Full protein approach



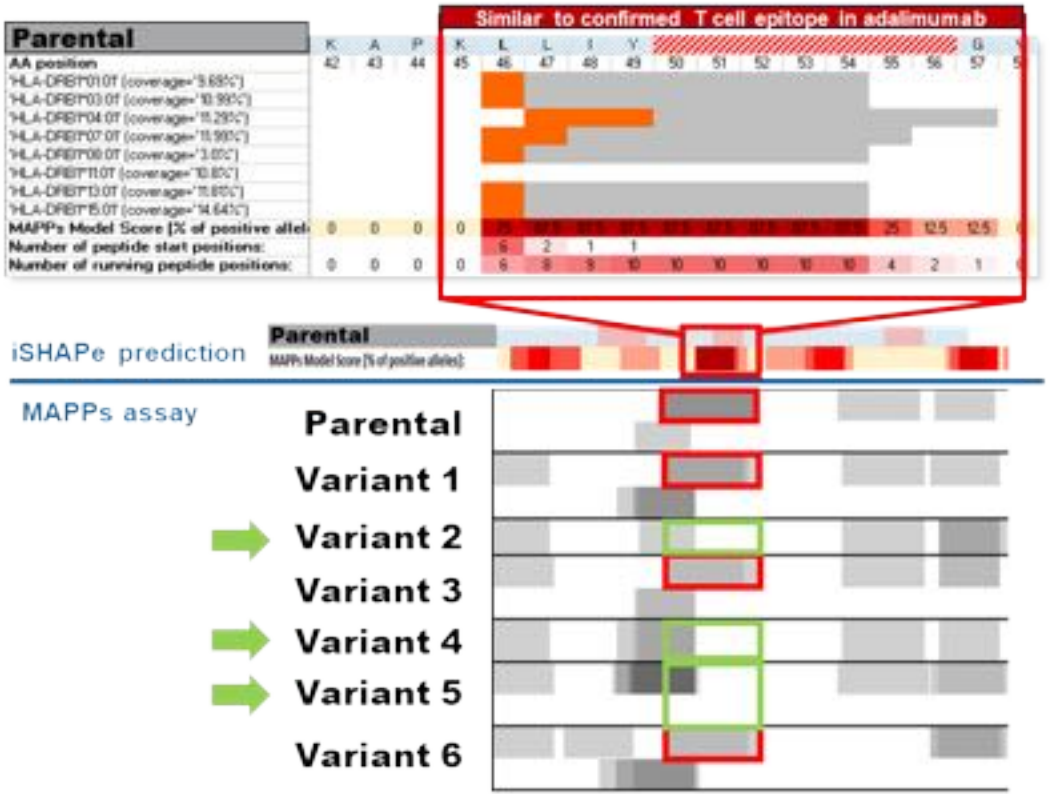
Project B: LC de-immuno variants



- We mainly focused on LC CDR2 due to its high sequence similarity with adalimumab (Humira), which has a high clinical ADA rate and harbors a confirmed T cell epitope in this region.

- All generated variants were compromises between affinity maturation and de-immunization.

Project B: MAPPs assay of LC de-immuno variants



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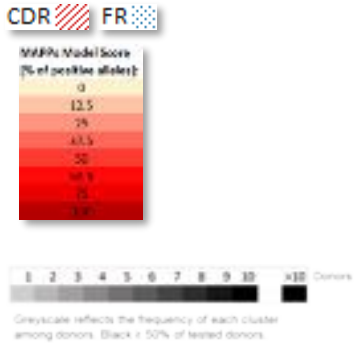

Pros/Cons of the two tested approaches

Pros/Cons of approach	Full protein approach 	Peptide library approach 
Advantages	• Simpler and less costly => faster • no peptide synthesis, saves one MAPPs step • Can be combined with affinity optimization	• Very broad approach - large number of positions can be de-immunized and many variants can be tested, which provides better opportunities to identify suitable mutations • Suitable to randomize clusters that are observed in MAPPs but not predicted in silico
Disadvantages	• Approach limited to a handful of positions (mutants need to be produced as proteins) • Mutation selection relies on in silico predictions only • Higher risk to fail to identify suitable mutations	• Higher complexity and cost and overall effort for peptide synthesis and additional MAPPs assay step • peptide-MAPPs mimics HLA binding not presentation • Insoluble peptides pose a challenge
Sweet spot	• Faster and higher risk approach • Suitable if only few regions need to be modified • Better for «reducing» IG potential than for full de-immunization	• Slower and lower risk approach • Suitable if many regions to be modified • Better if IG is dominating aspect of project

Technical challenges and risks

- **The set of proteins that can be produced for final testing is limited, and sometimes mutations for half-life extension, chain-pairing, and Fc engineering compete with de-immunization mutations in terms of number of produceable/testable variants.**

- **Not all presented hotspots may necessarily have to be removed in case T cells do not recognize them.**
  - A T cell epitope mapping prior to de-immunization would be very resources & time intense
    - For now, we focus on **hotspots overlapping with CDRs**, without having proof upfront that they are recognized by T cells.
    - Potential resource saving and efficiency increase: **In future approaches, embed MASE and GenAI** approach to identify epitopes with higher risk →highest priority for de-immunization.

- **Every surface exposed mutation bears the risk of introducing a B cell neo-epitope.**
  - For now, there are no tools available that can reliably predict this.

Can we truly «de-immunize»? What are key learnings from the two studies?

- **We should consider de-immunization approaches as attempts to <u>decrease</u> the immunogenicity potential of a drug**
  - Developability factors such as stability and affinity need to be balanced with de-immunization → a complete abrogation of immunogenicity is often not achievable

- **The two approaches were very different and designed to optimally address the individual project needs.**
  - We could show that removal of major hotspots is possible with low impact on timeline and cost if combined with development steps like affinity maturation or Fc modification
  - We also recognized that aiming for “de-immunizing as much as possible” should be carefully considered, due to the high timeline and resource requirements involved in a “full de-immunization”

- **Now, an improved approach needs to be identified, considering the advantages and challenges of these two approaches to make it applicable for a variety of projects**

Acknowledgement

Key team members involved in data generation

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Martin Jockel

Guillaume Roellinger

Stephan Koepke

Elodie Riquet

Martine Marchant

Jason Marchese

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Thank you