Immunogenicity of Protein Products

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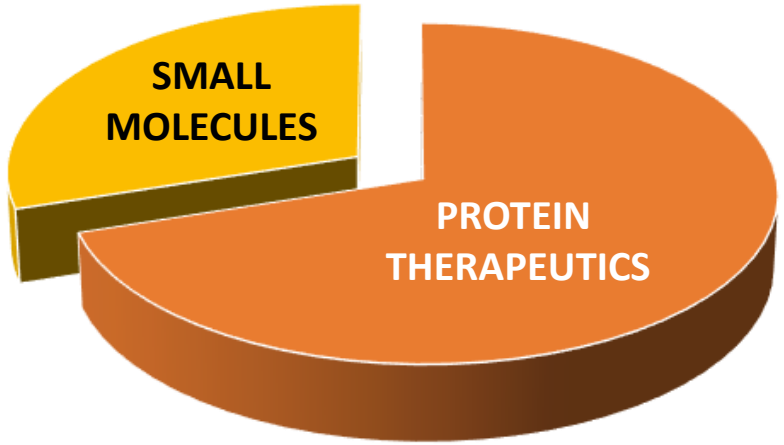
July 18, 2021

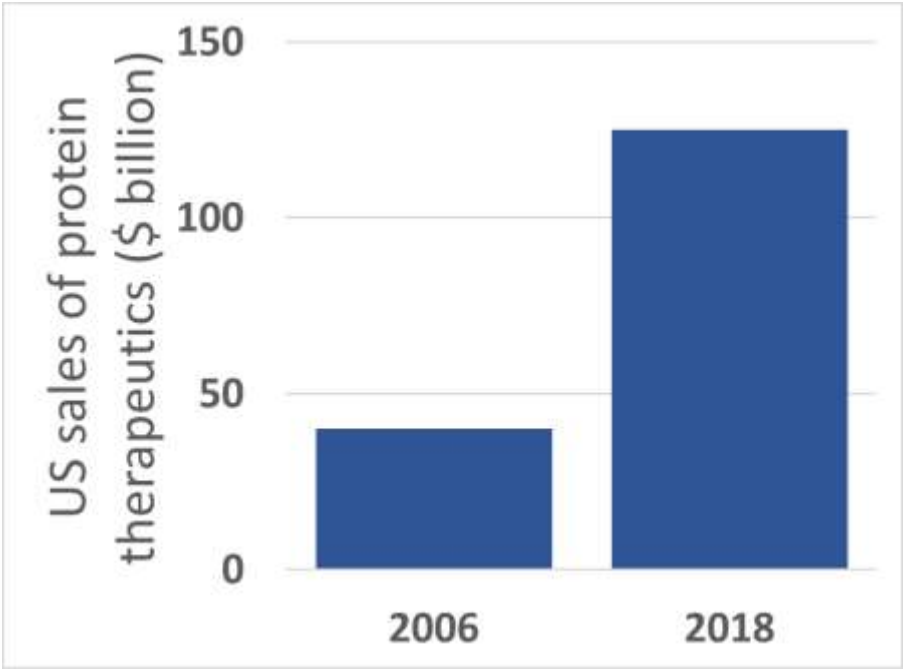
Outline

- The importance of proteins used in therapeutic applications
- Immunogenicity an important issue in the licensure and clinical use of proteins
- Immune responses to therapeutic proteins: scientific background
- The genetic and molecular determinants of immune responses to therapeutic proteins
- The non-clinical assessment of immunogenicity risk: the assays
- The utility of non-clinical assessments of immunogenicity: A case study
- Circumventing immunogenicity: Reengineering a protein - “de-immunization”
- Circumventing immunogenicity: Tolerizing a patient by exploiting Fc interactions with Fcγreceptor

Protein therapeutics in the clinic

TOP 10 BEST SELLING DRUGS





MORE THAN JUST THESE NUMBERS; PROTEIN THERAPEUTICS:

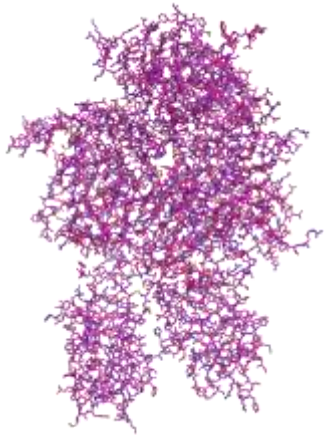
Address unmet medical needs

Provide cures or permit the management of complex diseases

Improve the quality of life

Protein therapeutics: Large & complex

Factor VIII used to treat hemophilia A



Ibuprofen used to treat pain

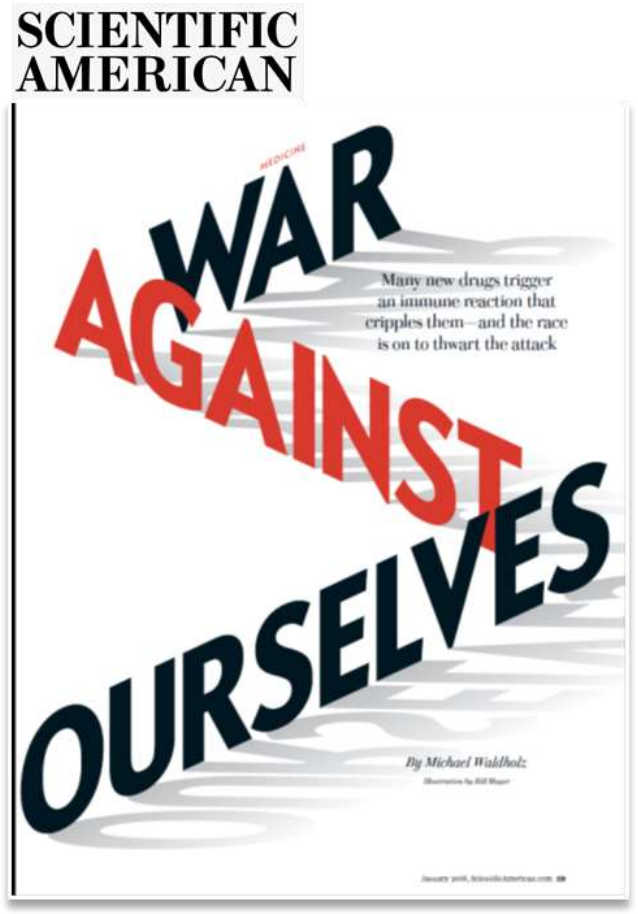
	Small molecule	Biologic
Batch records	<10	>250
Product quality tests	<100	>2,500
Critical process steps	<100	>5,000
Process data entries	<4,000	>60,000

UNIQUE CHALLENGES

Kimchi-Sarfaty, Schiller, Katagiri, Khan, Yanover & Sauna ***Trends Pharmacol Sci.*** 34: 534-548, 2013

Protein therapeutics can be perceived as foreign

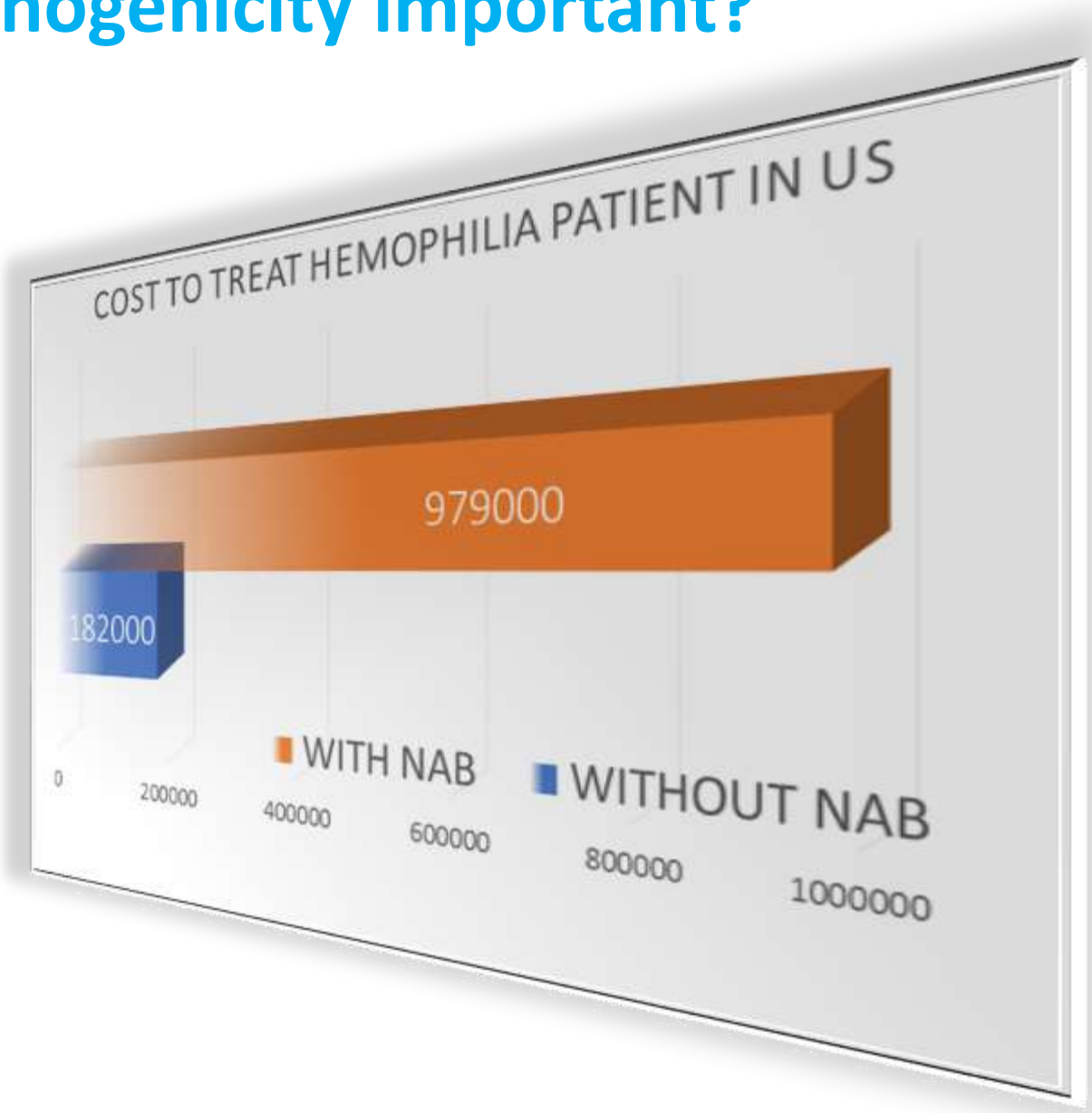
- Unlike small molecules protein therapeutics can elicit immune responses
- The phenomenon is called immunogenicity



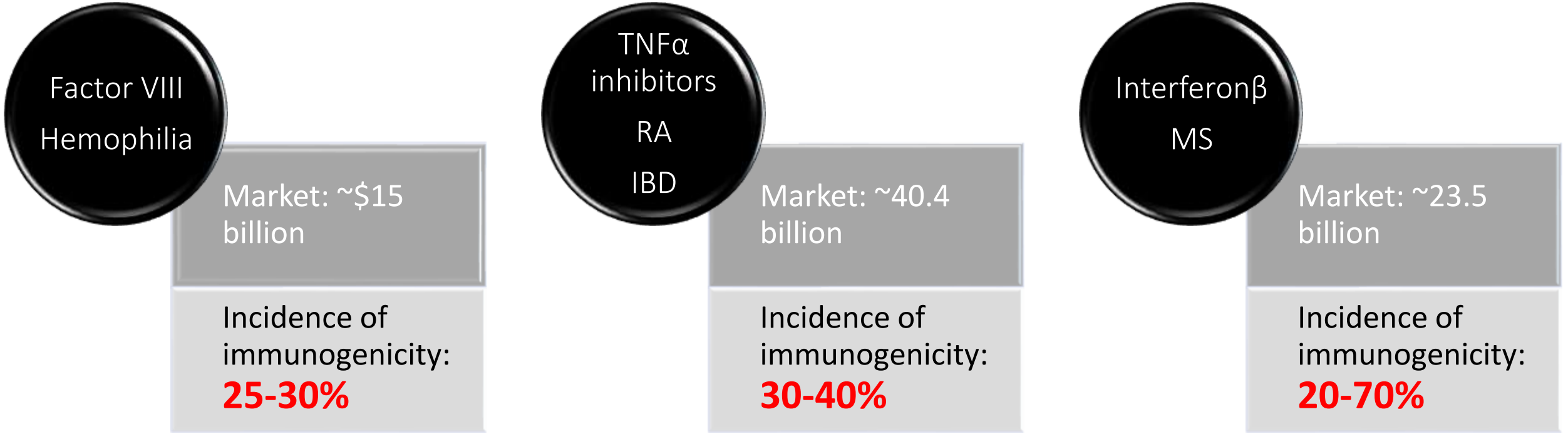
- When anti-drug antibodies (ADAs) develop, these may or may not affect the activity of the drug
- Antibodies that affect drug activity by binding to active protein domains are called neutralizing ADAs (NABs)
- Non-neutralizing antibodies are not necessarily benign as they can affect the PK/PD (activity) profile and causing loss of tissue targeting
- ADAs can also cross-react with endogenous proteins or elicit anaphylactic reactions

Why is immunogenicity important?

1. Higher treatment costs
2. Fewer options for patients
3. Higher development costs



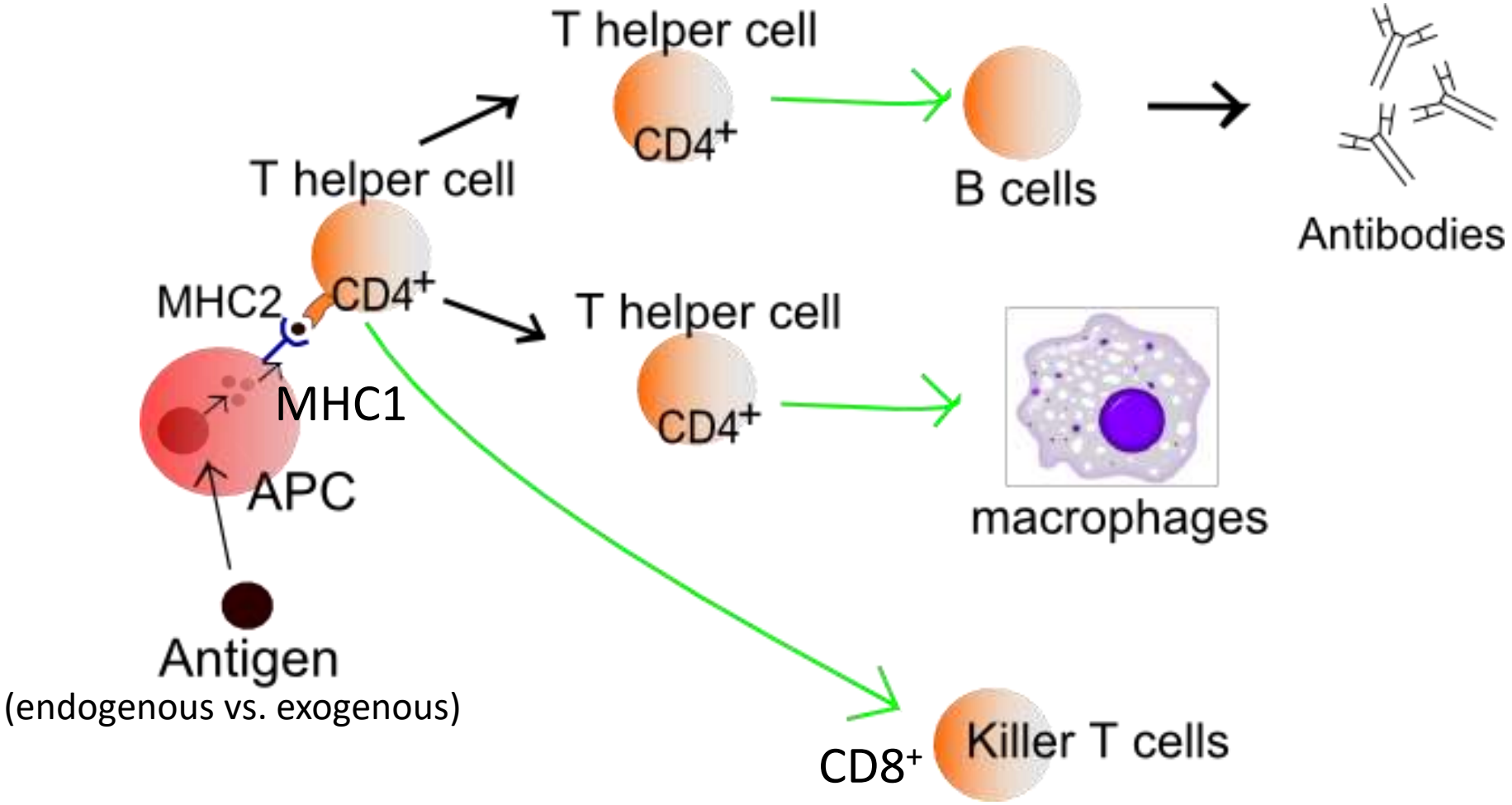
Immunogenicity is not rare



Sauna, Lagasse, Pedras-Vasconcelos, Golding, & Rosenberg ***Trends in Biotechnology***. 36: 1068-84, 2018

Immune responses to therapeutic proteins: The scientific background

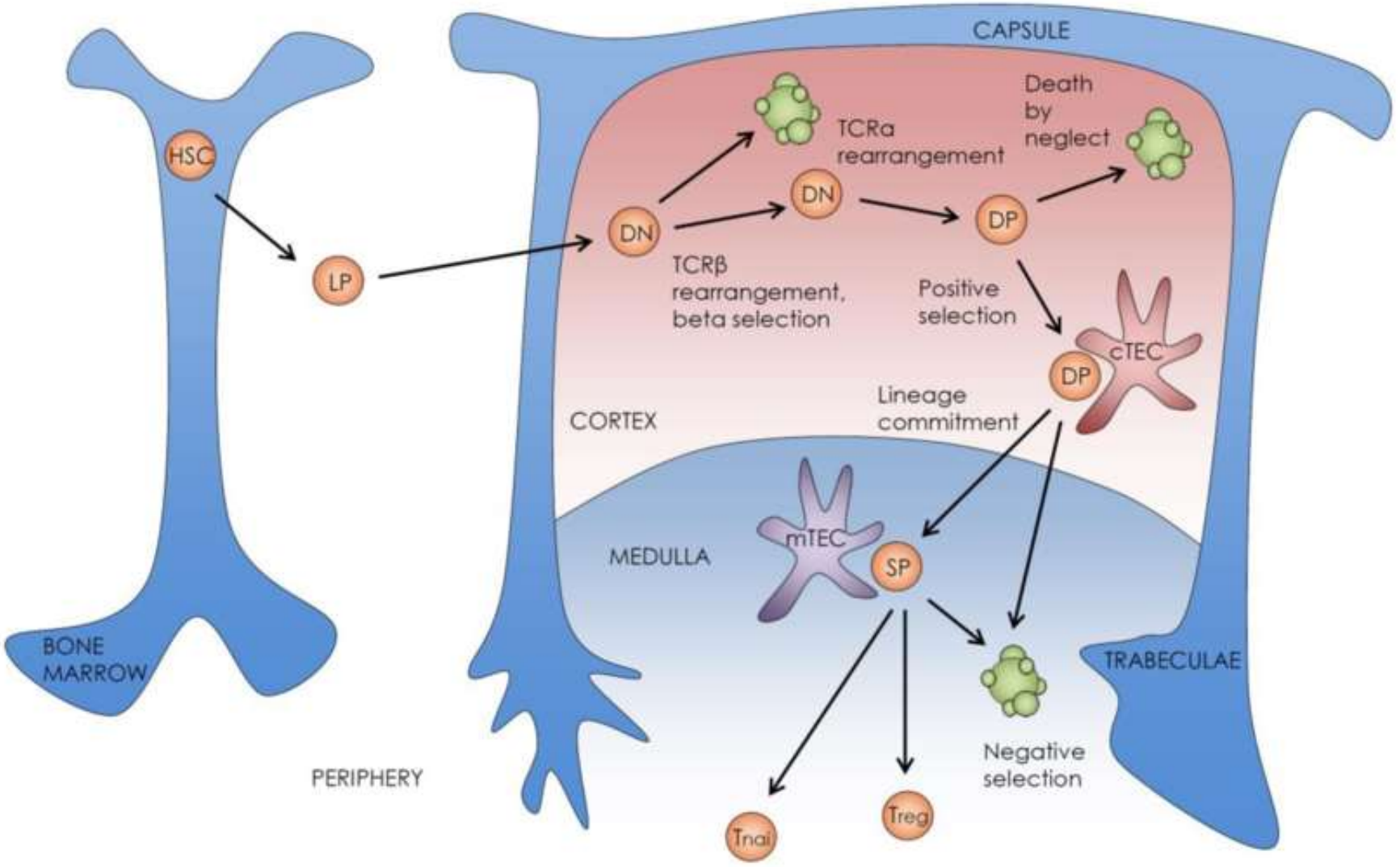
Adaptive Immune Response



T Cell Development

Bone Marrow

Thymus



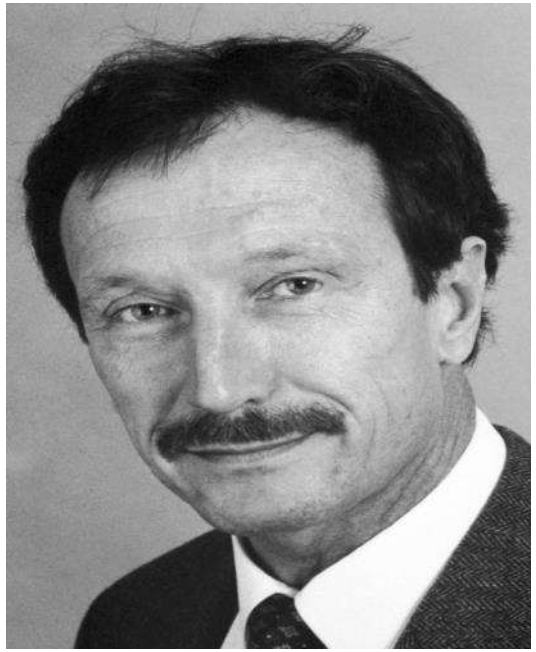
Tolerance

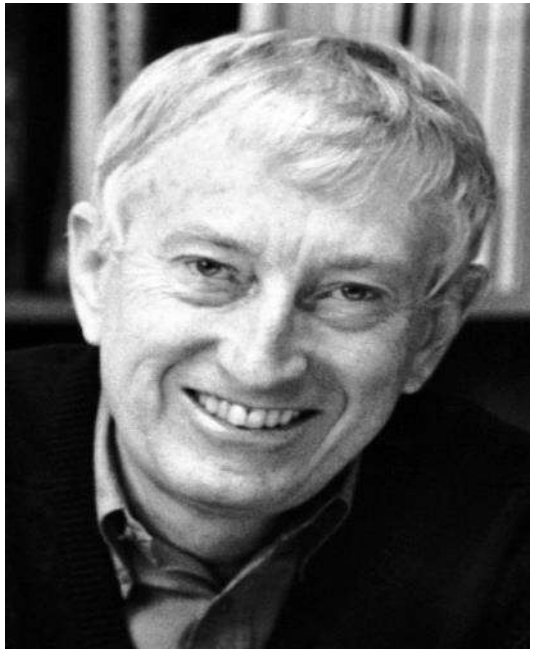
- Self-reactive Effector T cells (T eff.) deleted in thymus
  - Antigen must be expressed
  - In severe deficiency disease – no protein – no tolerance (CRIM –ve)
  - Some patients lack functional protein but produce mutated protein that can induce tolerance (CRIM +ve)
- Self-reactive T regs induced in thymus
  - Can suppress T eff. In periphery
- T eff. can be rendered anergic if only one signal delivered
- T regs can also be induced in the periphery

The discovery of MHC restriction

Rolf M. Zinkernagel and Peter C. Doherty

Nobel Prize winners -1996





FIRST MILESTONE

Function of MHC molecules

LCMV CTL in mice were MHC restricted (1970s).

“The MHC system did not evolve to confuse transplant surgeons”

IMMUNOLOGY TODAY

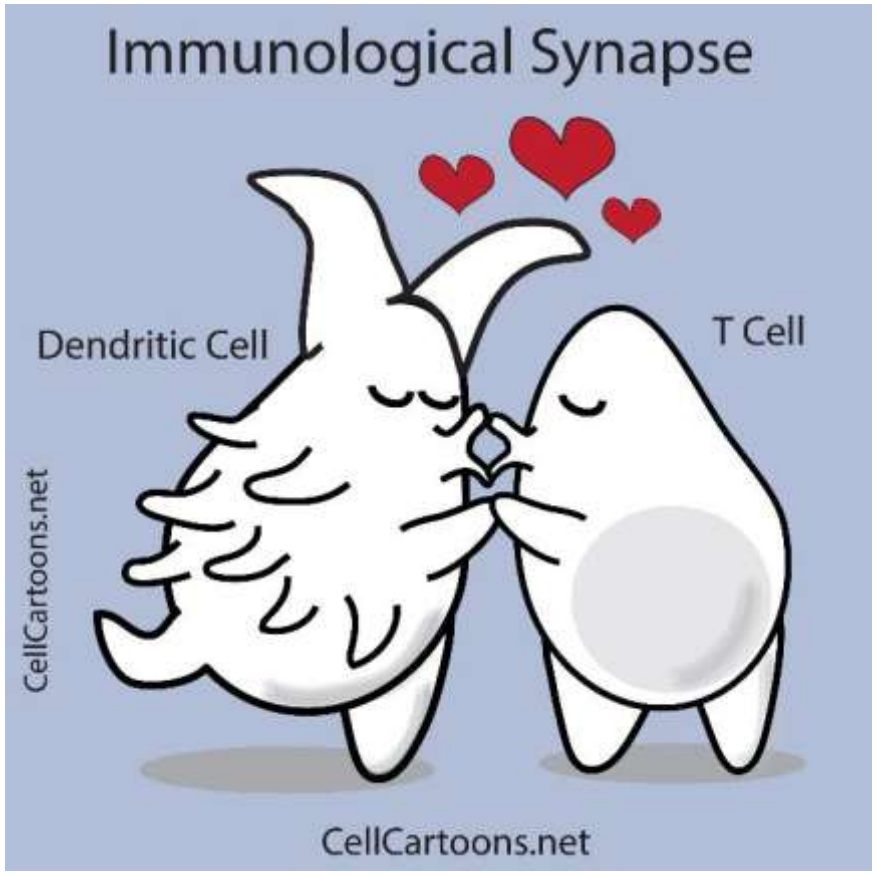
JANUARY 1997

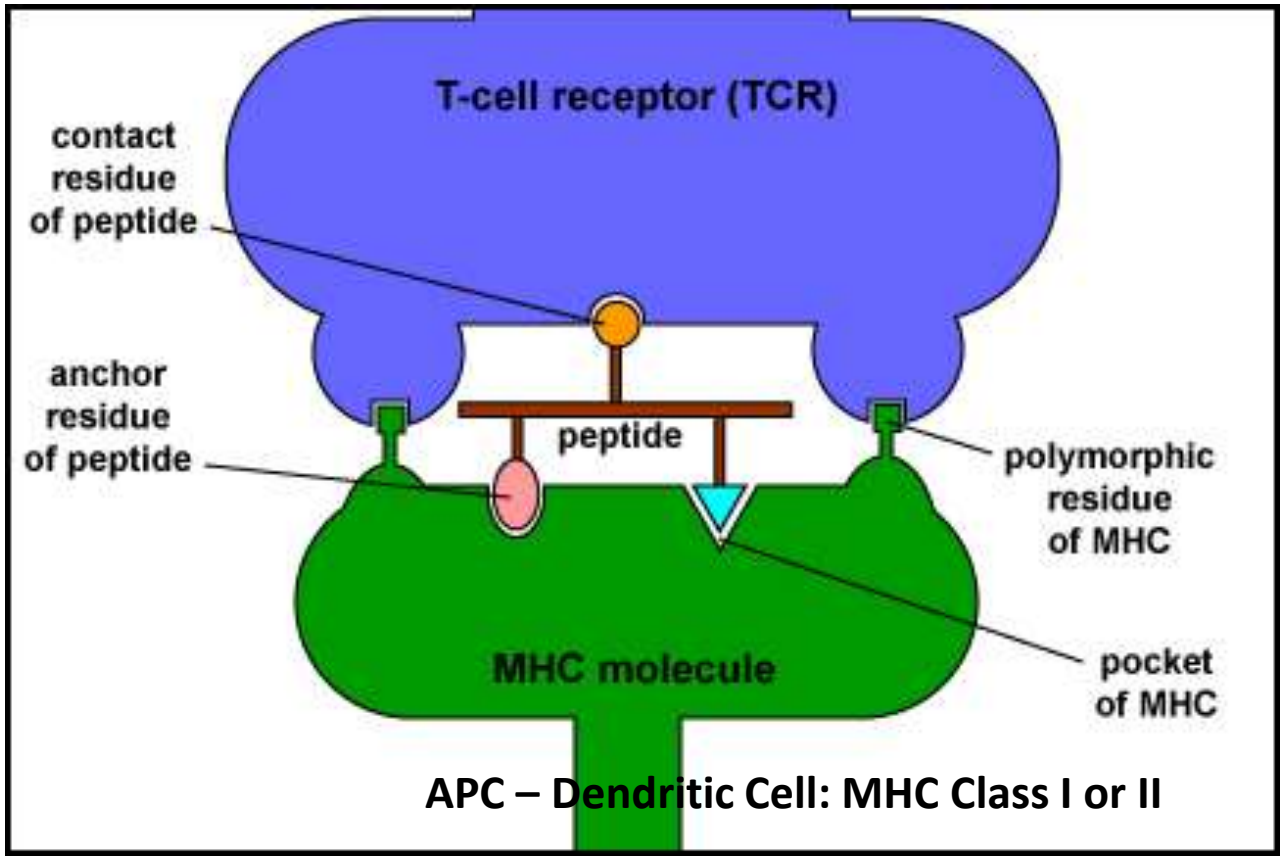
14 Vol. 18 No. 1

Structural Basis: APC/T cell Interaction: MHC + Peptide + TCR

Second Milestone: Discovery and characterization of the T cell receptor (TCR)

James Allison 1982





APC – Dendritic Cell: MHC Class I or II

The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes.

A Sette, A Vitiello, B Reherman, P Fowler, R Nayersina, W M Kast, C J Melief, C Oseroff, L Yuan, J Ruppert, J Sidney, M F del Guercio, S Southwood, R T Kubo, R W Chesnut, H M Grey and F V Chisari

J Immunol 1994; 153:5586-5592; ;
http://www.jimmunol.org/content/153/12/5586

The Immune Epitope Database and Analysis Resource in Epitope Discovery and Synthetic Vaccine Design

Ward Fleri, Sinu Paul, Sandeep Kumar Dhanda, Swapnil Mahajan, Xiaojun Xu, Bjoern Peters and Alessandro Sette*

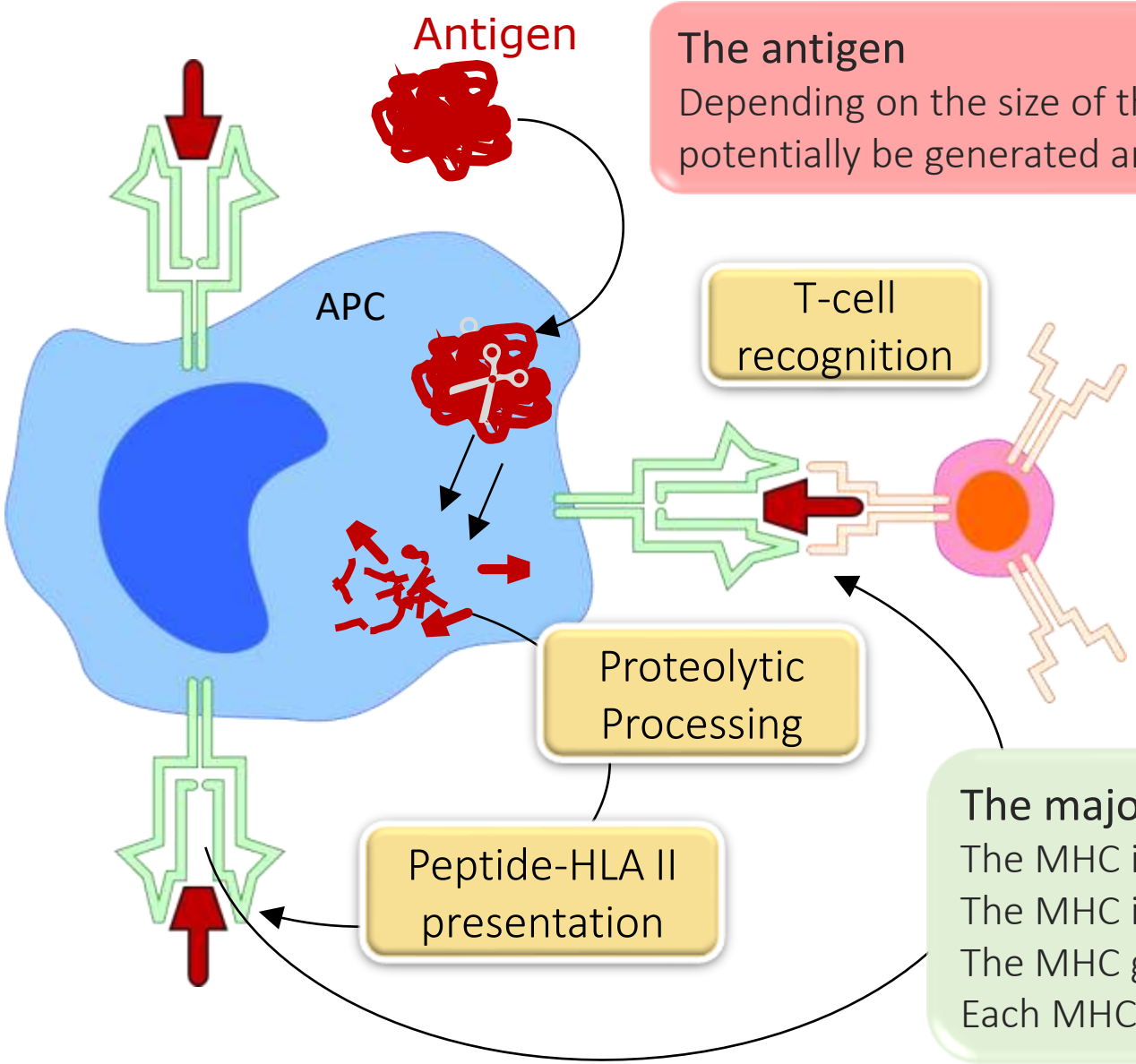
Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

The IEDB was established in 2004, and over the past 10 years our team has manually curated almost 16,000 published manuscripts and processed 200 direct submissions. As a result more than 120,000 epitopes are now freely and easily accessible to the scientific community.

THIRD MILESTONE
Algorithms for identifying potential T cell epitopes based on peptide-MHC binding affinity

The genetic and molecular determinants of immune responses to therapeutic proteins

Immunogenicity: The players and their complexity



The antigen

Depending on the size of the antigen 100s or 1000s of peptides can potentially be generated and presented to the immune system

The protein processing machinery

The antigen (protein) has to be processed into peptide Fragments. Not every possible peptide can be generated

The major histocompatibility complex (MHC) - HLA

The MHC is <u>polygenic</u>: every individual contains several MHC genes
The MHC is <u>polymorphic</u>: The population has variants of each gene
The MHC genes are the most polymorphic genes in the human genome
Each MHC molecule binds different peptides with different affinities

Biomarkers used for in-silico prediction of immunogenicity

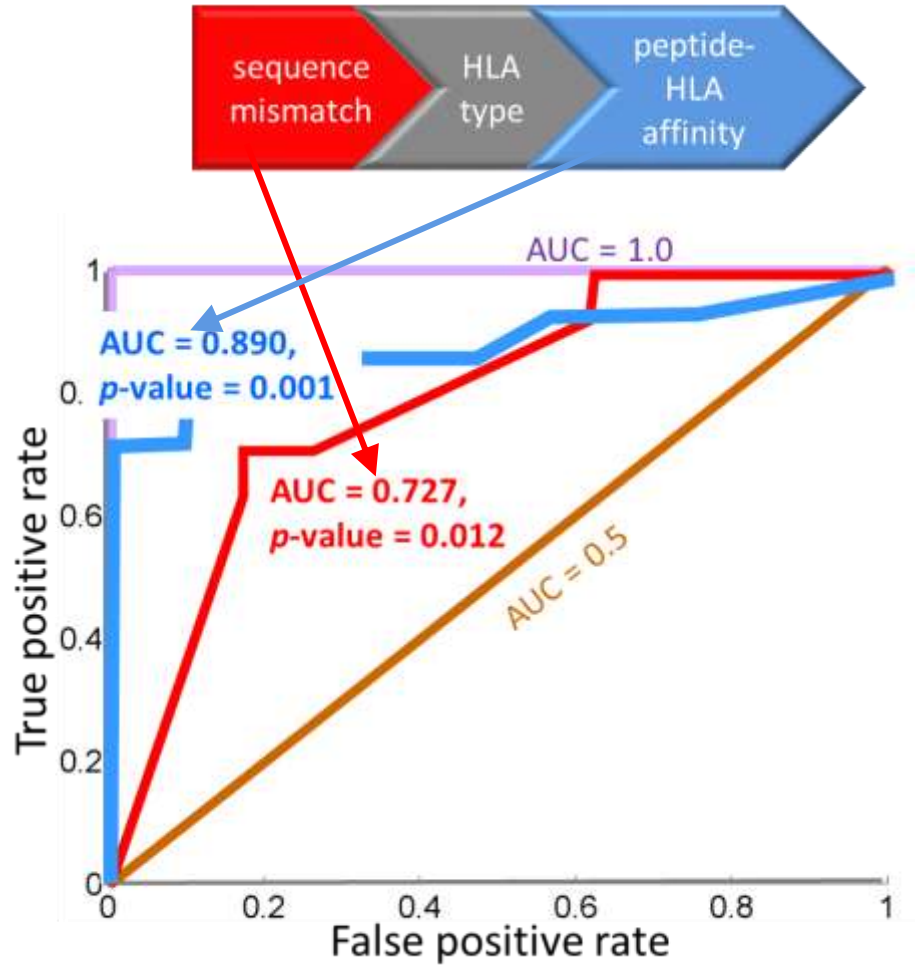
- Sequence mismatch
  - Sequence of product vs. sequence of patient base on gene sequencing
- MHC (HLA) peptide affinity
  - Affinity binding between patient MHC and product peptide

Translating rules based on clinical observations into useful biomarkers: Can in-silico algorithms predict immunogenicity?

Potential biomarkers:

- Greater sequence mismatch between endogenous FVIII and infused therapeutic FVIII
- Affinity of “foreign peptides” for the patients HLA

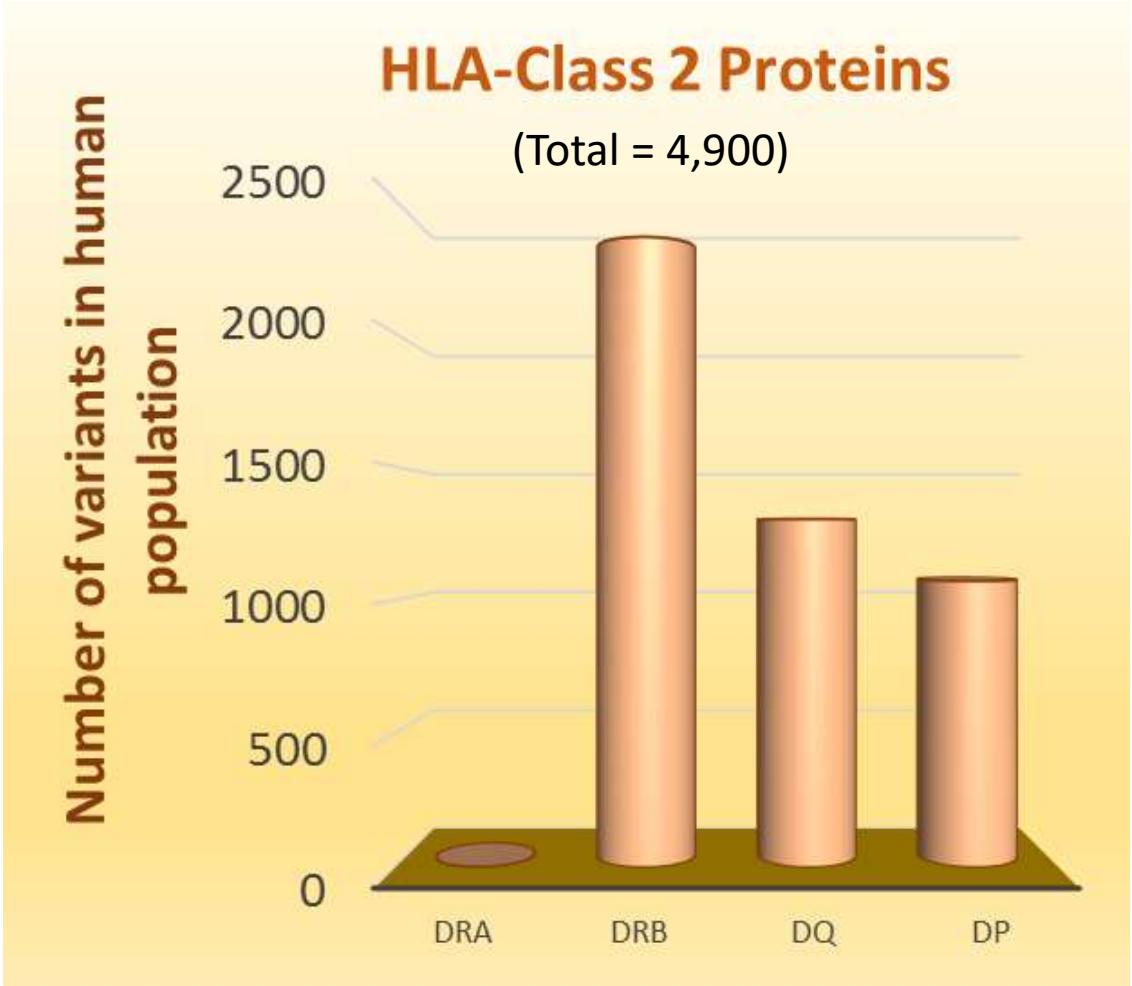
HOW PREDICTIVE ARE THESE BIOMARKERS IN A COHORT OF HEMOPHILIAC PATIENTS?

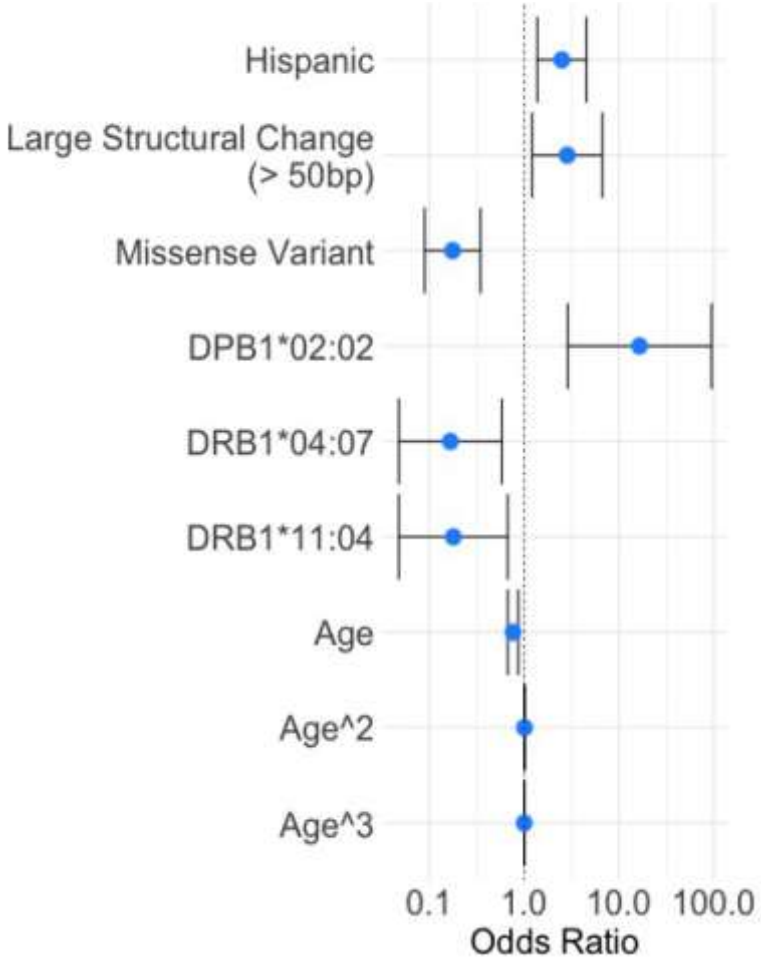


Pandey, Yanover, Miller-Jenkins, Garfield, Moses, Rydz, Simhadri, Kimchi-Sarfaty, Lillicrap, Przytycka, Pierce, Howard & Sauna ***Nature Med.*** 19: 1318-1324

Big Data: Why computer algorithms are needed

Factor VIII consists of 2332 **amino acids**
= 156 15-mer peptides





ATHN dataset

Joseph R. McGill, Vijaya L. Simhadri and Zuben E. Sauna. Frontiers in Medicine. May 2021 Vol. 8, article 663396

Predicting Immunogenicity Based on MHC and Peptide Sequence: Summary and Conclusions

- Most replacement therapeutic proteins treat rare diseases, e.g., hemophlia A
- Risk-factors for immunogenicity are generally estimated based on studies generally carried out on relatively small cohorts (50-200 patients)
- One example of a large cohort for analysis of genetic risk factors is the MLOF Research Repository which includes pathogenic *F8* variant data for 7,151 hemophilia A patients.
- Using the MLOF dataset we provided estimates of inhibitor-risk as Odds Ratios for anti-drug antibody (ADA) development in a multivariate model considering HLA-DRB1/3/4/5, HLA-DPB1, HLA-DQB1, race, *F8* pathogenic variant type, and age:
    - Participants with 1 HLA variant (DPB*02:02) developed ADAs at a higher rate while participants with 2 HLA variants (DRB1*04:07; DRB1*11:04) developed inhibitors at a lower rate
    - Patients with missense variants developed ADAs at a lower rate and participants with large structural changes (>50bp) developed at a higher rate (both compared to intron 22 inversion).

McGill, Simhadri & Sauna Front. Med. (2021) 8: 663396

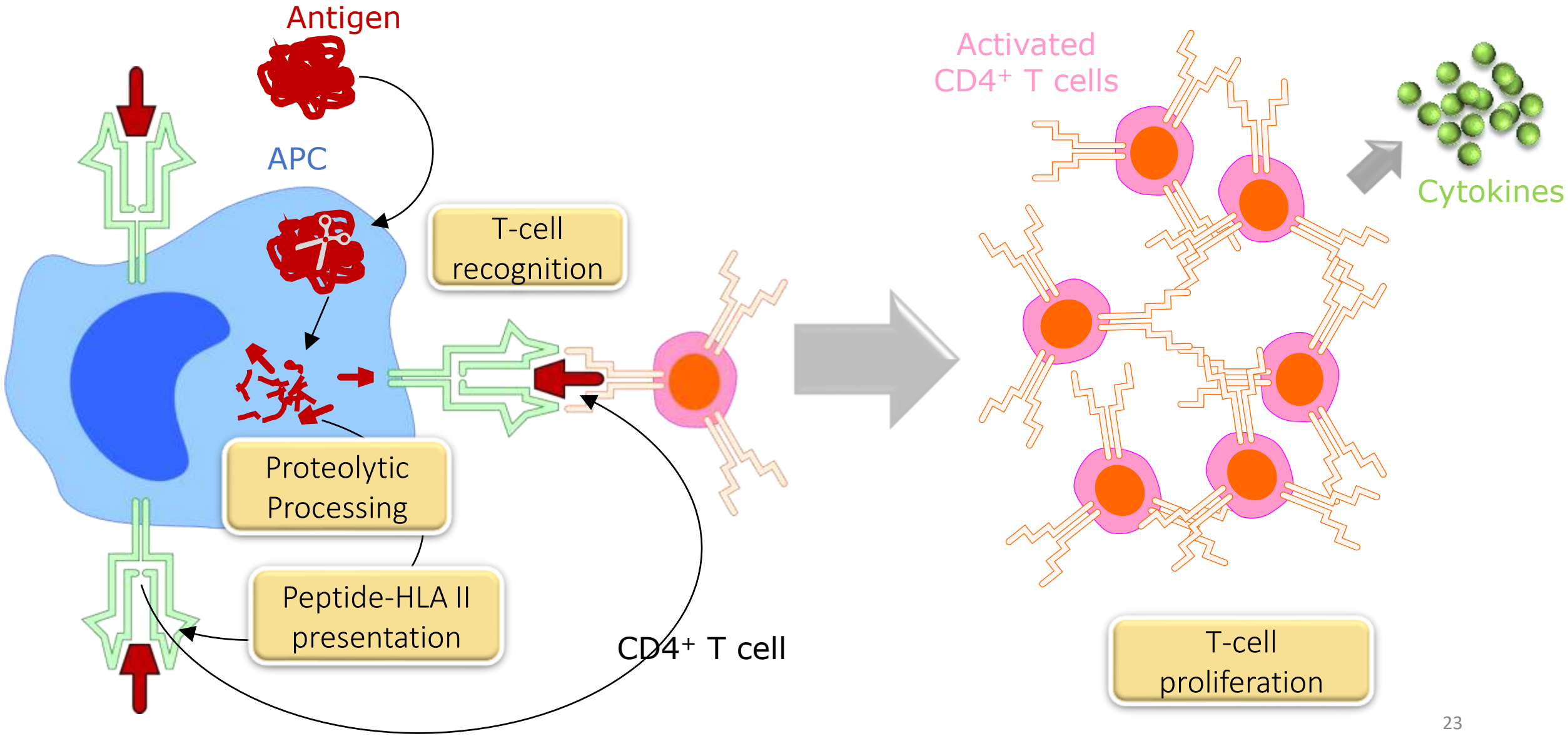
Non-clinical assessments of immunogenicity

Assays for non-clinical assessments of immunogenicity

	Method	Immune Process Probed
Computational/ in silico	HLA- Peptide binding algorithms	Antigen presentation
In vitro	HLA-Peptide binding assay	Antigen presentation
Ex vivo	LC/MS-based MHC associated peptide proteomics (MAPPs)	Antigen processing and presentation
	MHCII tetramer-guided epitope mapping (TGEM)	Antigen recognition
	Protein-specific T cell amplification	Antigen processing, presentation, and recognition
	Human blood-derived cell-based assays (PBMCs/DCs as APCs; T cells as effector cells)	Depends on assay design
Animal model/ in vivo	HLA transgenic mice (humanized immune system)	All

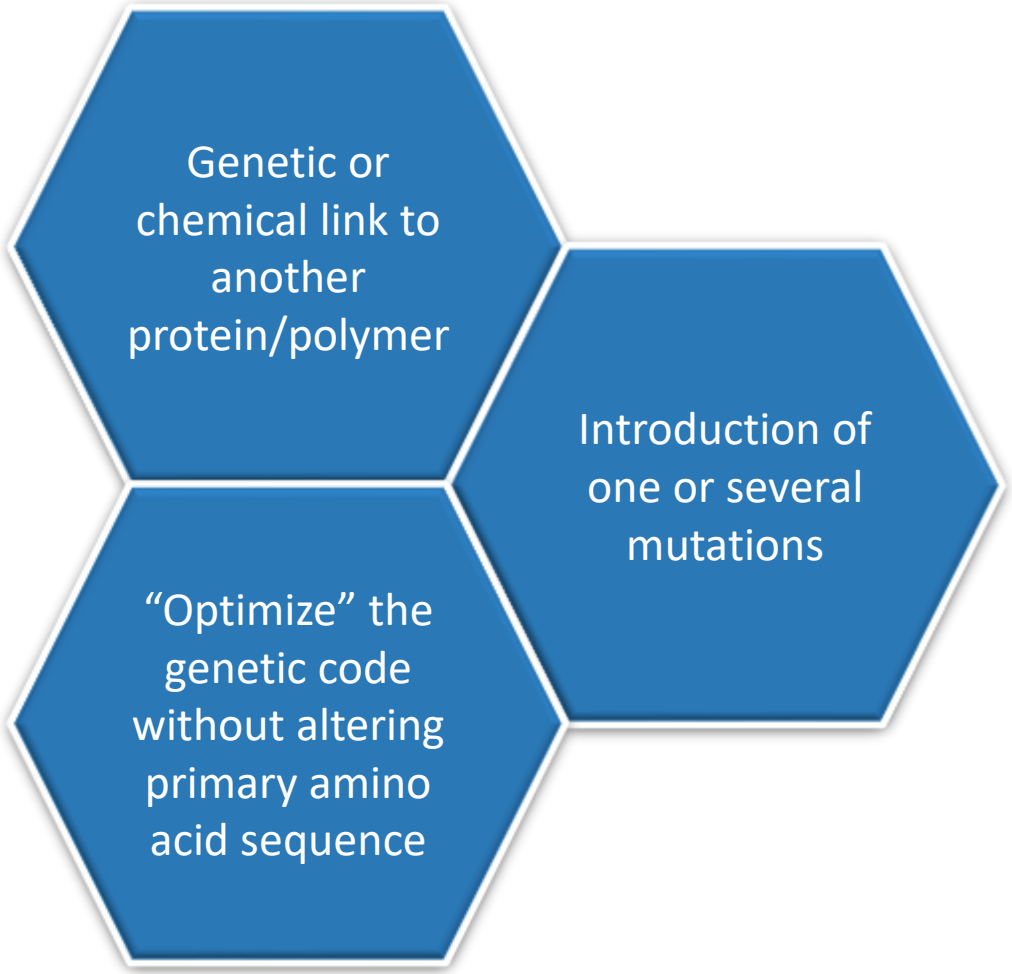
Sauna, Lagasse, Pedras-Vasconcelos, Golding, & Rosenberg ***Trends in Biotechnology***. 36: 1068-84, 2018

Early steps in the immune response to therapeutic proteins



Engineered proteins: Therapeutics by design

There are many ways to engineer proteins to make them better therapeutics → But there are risks!



Guidance for Industry

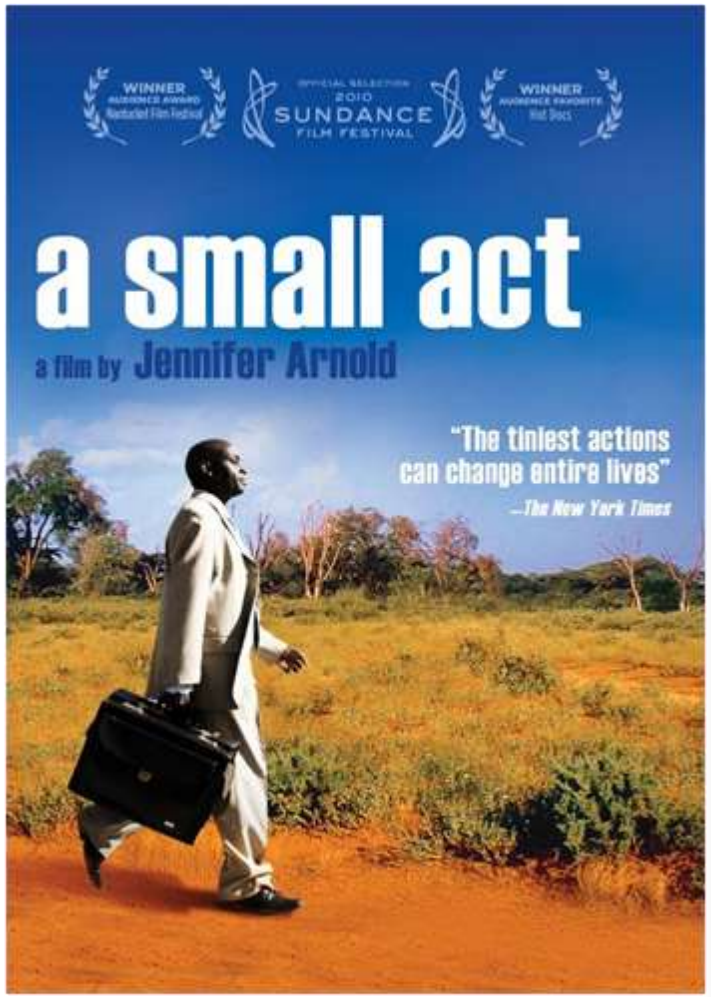
Immunogenicity Assessment for Therapeutic Protein Products

*"Similarly, bioengineered proteins involve the introduction of sequences not normally found in nature and may thus contain **neo-epitopes**."*

Kimchi-Sarfaty, Schiller, Katagiri, Khan, Yanover & Sauna ***Trends Pharmacol Sci.*** 34: 534-548, 2013

Engineered proteins & immunogenicity risk: A case study

A small change in protein sequence but a large consequence vis-à-vis immunogenicity



Factor VIIa

NO reports of anti-FVIIa antibodies in hemophilia patients

FVIIa variant, Vatreptacog alfa
{V158D, E296V, M298Q}

Incidence of anti-FVIIa antibodies = **11.1%**

Company Announcement

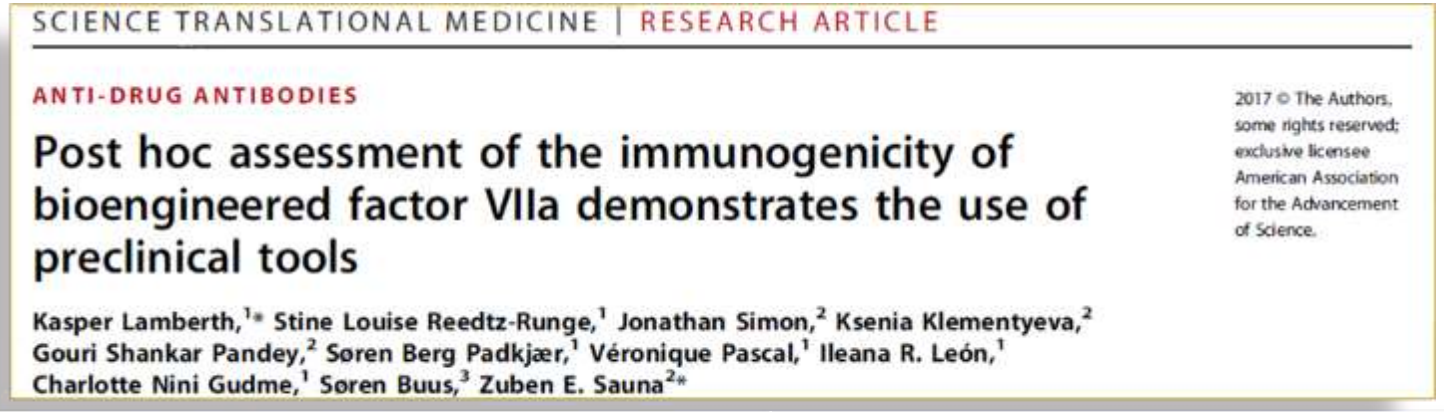
28 September 2012

novo nordisk®

Novo Nordisk discontinues development of vatreptacog alfa following analysis of phase 3 results

Novo Nordisk today announced the decision to discontinue the development of vatreptacog alfa, a fast-acting recombinant factor VIIa analogue for haemophilia patients with inhibitors. The decision follows analysis of the data from the phase 3a trial adept™2. On 9 August, Novo Nordisk announced that a few patients in the trial had developed anti-drug antibodies to vatreptacog alfa, one patient with a potentially neutralising effect.

Non-clinical assessments could have identified risks!



SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

ANTI-DRUG ANTIBODIES

Post hoc assessment of the immunogenicity of bioengineered factor VIIa demonstrates the use of preclinical tools

Kasper Lamberth,[1]* Stine Louise Reedtz-Runge,[1] Jonathan Simon,[2] Ksenia Klementyeva,[2] Gouri Shankar Pandey,[2] Søren Berg Padkjær,[1] Véronique Pascal,[1] Ileana R. León,[1] Charlotte Nini Gudme,[1] Søren Buus,[3] Zuben E. Sauna[2]*

ASSAY/METHOD	RESULTS
Do mutant peptides bind HLA-II molecules with high affinity (in silico)?	Mutant peptides bind with high affinity to some but not all HLA-II variants
Do mutant peptides bind HLA-II molecules with high affinity (in vitro)?	Confirmed in silico findings
Are mutant peptides presented on HLA-II molecules (MAPPs)?	YES
Do mutant peptides that bind with high affinity elicit a T-cell response?	YES
Are there any associations with clinical outcomes?	ADA-positive patients carry HLA-II that bind to mutant peptide with high affinity

Circumventing immunogenicity

De-immunizing Vatreptacog alfa (immunogenic FVIIa variant)

IDENTIFY NEO-EPITOPES INTRODUCED BY BIOENGINEERING

- Wild-type rFVIIa used as a drug for >2 decades; no reports of immunogenicity
- Bioengineered variant of rFVIIa was immunogenic in clinical studies
- Neo-sequence resulted in T-cell epitopes (in silico, in vitro and ex vivo methods)

USE ALGORITHM [RATIONAL IMMUNOGENICITY DETERMINATION (RID)] TO REDESIGN NEO-EPITOPE

Introduce amino acid substitutions to decrease binding affinity to a "*representative*" group of HLA-variants

Avoid amino acid substitutions at conserved positions on the protein sequence

Use EV Mutation to ascertain that amino acid substitutions will not be deleterious to function

Synthesize peptides containing computationally designed amino acid substitutions

- T-cell proliferation assay
- ELISpot assay

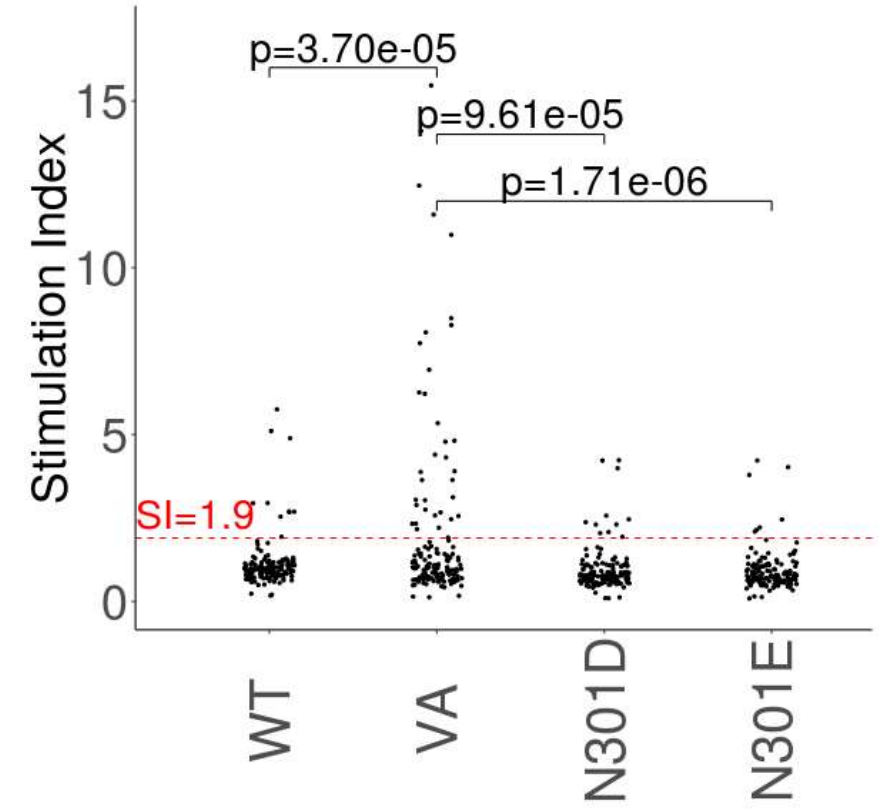
Express & purify proteins predicted to **retain** desired functionality

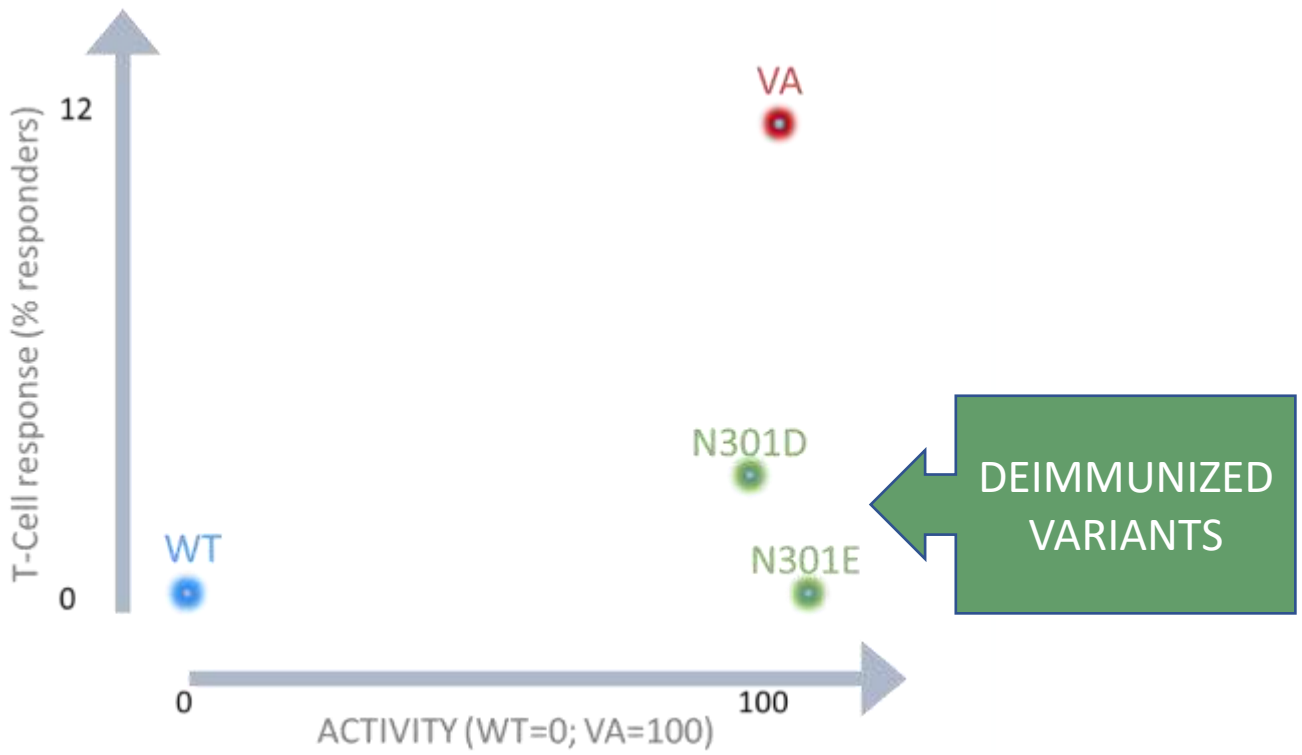
Express & purify proteins predicted to lose desired functionality

Thrombin Generation Assay

How effective is computational deimmunization?

Deimmunized construct has high activity AND low immunogenicity





Jankowski, McGill, Lagassé, Surov, Bembridge, Bunce, Cloake, Fogg, Jankowska, Khan, Marcotrigiano, Ovanesov & Sauna **Blood Adv.** 3: 2668-2678, 2019

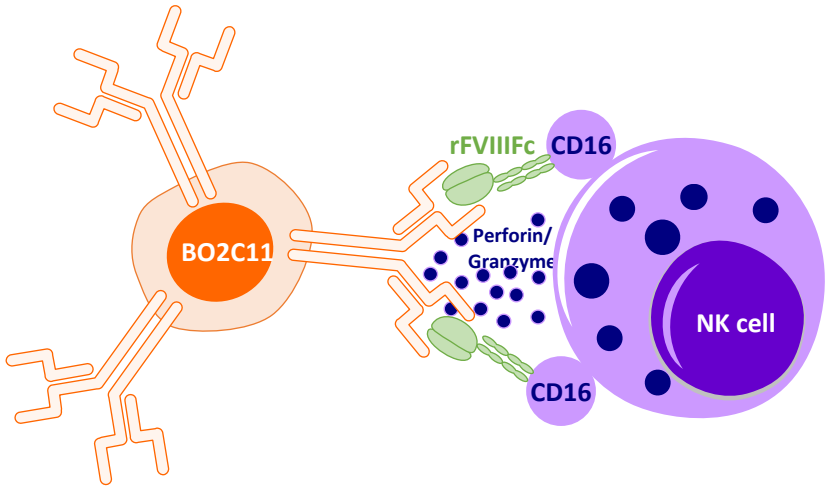
Innovative approach to tolerance induction in hemophiliacs with inhibitors

- Classical approach
  - Repeated high dose Factor VIII taking weeks or months, and very costly

- New approach using Factor FVIII-Fc (FVIII-Fc)
  - FVIII-Fc binds and activates NK cells via CD16 (FcγRIIIa)

rFVIIIFc could promote lysis of FVIII-specific memory B cells via CD16 engagement

FVIII-specific B cell killing: a potential mechanism for rFVIIIFc in ITI regimens?



BO2C11*, a human lymphoblastoid cell line specific for FVIII-C2 (IgG4 kappa) by Epstein-Barr virus–transformed B cells collected from a hemophilia A patient with inhibitors.

BO2C11 could serve as a target cell for FVIII-specific B cell killing assays using rFVIIIFc and $CD16^{+}$ NK cells.

H.A. Daniel Lagassé, Louis B. Hopkins, Wojciech Jankowski, Marc G. Jacquemin, Zuben E. Sauna, Basil Golding. Frontiers in Immunology. In Press. 2021.

*Courtesy of Dr. Marc Jacquemin (University of Leuven, Belgium)

Immunogenicity is a problem: What should the regulatory agencies do about it?

Policy

18 May 2017
EMEA/CHMP/BMWP/14327/2006 Rev 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on Immunogenicity assessment of therapeutic proteins

"However, ongoing consideration should be given to the use of emerging technologies (novel in silico, in vitro and in vivo models), which might be used as tools during development or for a first estimation of risk for clinical immunogenicity. In vitro assays based on innate and adaptive immune cells could be helpful in revealing cell-mediated responses".

Guidance for Industry

Immunogenicity Assessment for Therapeutic Protein Products

"If both appropriate and feasible, HLA mapping studies may help define a subset of the patient population at increased risk."

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

August 2014
Clinical/Medical

"Moreover, mismatches between the sequence of the endogenous protein of the patient and that of the therapeutic protein product caused by naturally occurring polymorphisms are one risk factor for the development of immune responses to the therapeutic protein product."

Summary of Guidance Recommendations in Practical Terms

- Pre-clinical studies
  - *In silico:* algorithms based on MHC class II-peptide binding to identify T cell epitopes
  - *In vitro:* MAPPs, MHC-peptide binding, and T cell activation
  - Assays for binding and neutralizing antibodies
- Post-marketing
  - Availability of assays for binding and neutralizing antibodies and monitoring of patients with these assays
  - Work-up of patients who develop ADAs
- Search for new biomarkers pre- and post-marketing

ACKNOWLEDGEMENTS

FDA

- Z. Sauna
- D. Lagasse
- D. Levin
- W. Jankowski
- J. Mcgill
- K. Klementyeva
- G.S. Pandey
- J. Simon

- C. Kimchi-Sarfaty

Novo Nordisk

- K. Lamberth
- S. L. Reedtz-Yunge
- S.B. Padkjaer
- V. Pascal
- I.R. Leon
- C. N. Gudme

Thank You for Your Attention!!!

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