The Immunogenicity of Protein Therapeutics: time to get personal?

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"It's far more important to know what person the disease has than what disease the person has."

-Hippocrates (460 BC-370 BC)

Disclaimer

The findings and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy

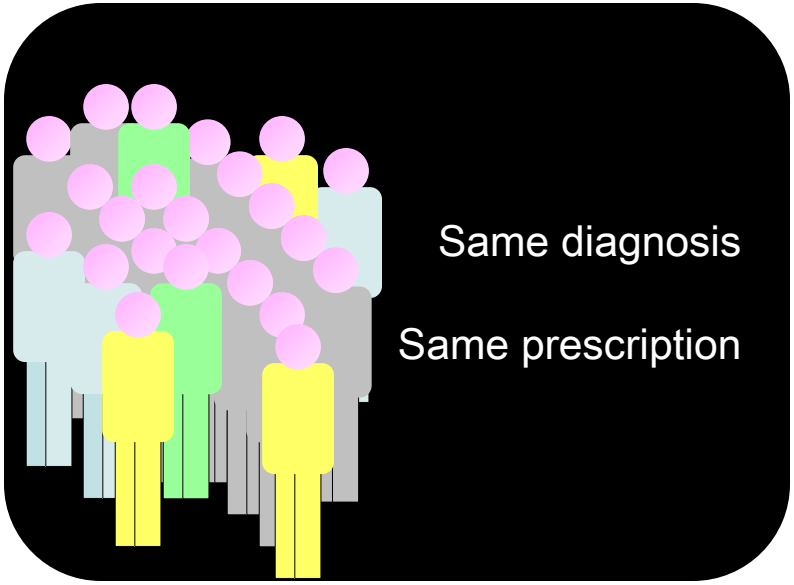
What is Personalized Medicine?

Personalized medicine refers to the tailoring of medical treatment to the individual characteristics of each patient. It does not literally mean the creation of drugs or medical devices that are unique to a patient but rather the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment. Preventive or therapeutic interventions can then be concentrated on those who will benefit, sparing expense and side effects for those who will not."

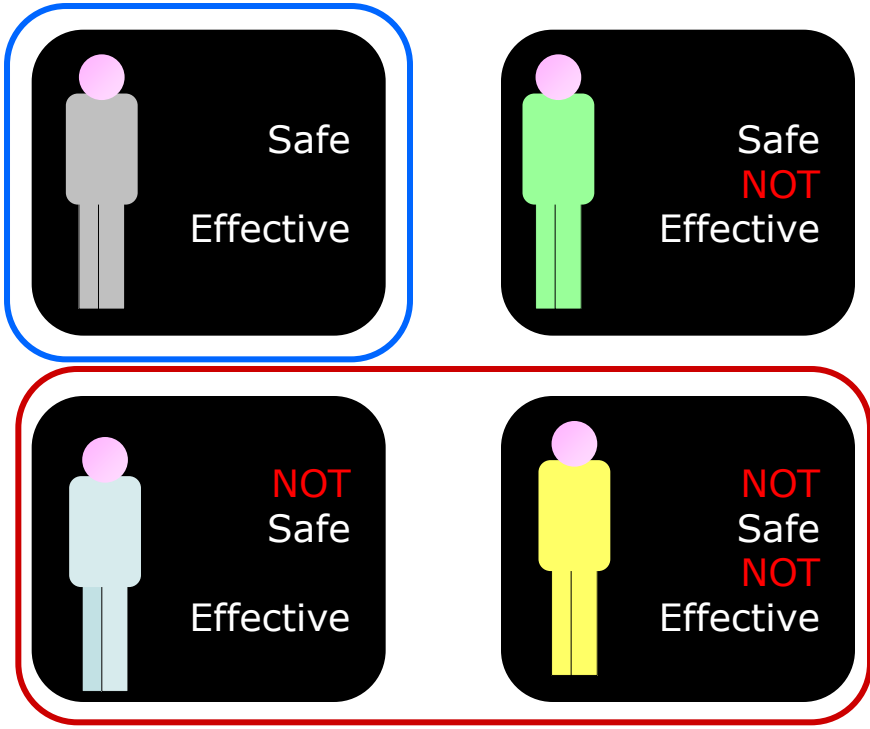
- President's Council of Advisors on Science and Technology (PCAST) "Priorities for Personalized Medicine" September 2008

Getting personal with your medicines

Medical practice based on
population responses



In an individual the prescription can
elicit one of four responses



Drug may be dangerous for
these individuals

Desirable outcome

Sauna, Kimchi-Sarfaty, Ambudkar & Gottesman (2007) Pharmacogenomics 8: 527

Why do people vary in their responses to prescribed medications?

The majority of human sequence variation is due to single nucleotide polymorphisms (SNPs)

SNPs are sites in the human genome where individuals differ in their DNA sequence by a single nucleotide base

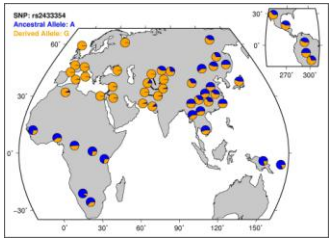
Completion of the Human Genome Project showed that single nucleotide changes constitute the most common type of genetic variation in the human population

A DNA variation which occurs in at least 1% of the population is referred to as a polymorphism

The Single Nucleotide Polymorphisms (SNPs)
Why are they important?

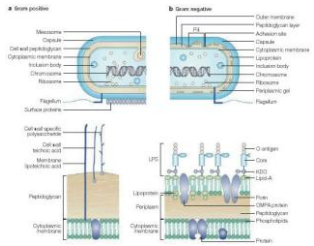
Determining the genetic basis of disease risk

Genotyping



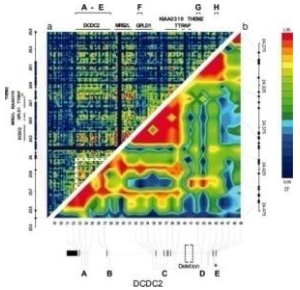
Population diversity & history

SNPs



Susceptibility to pathogens and environmental toxins

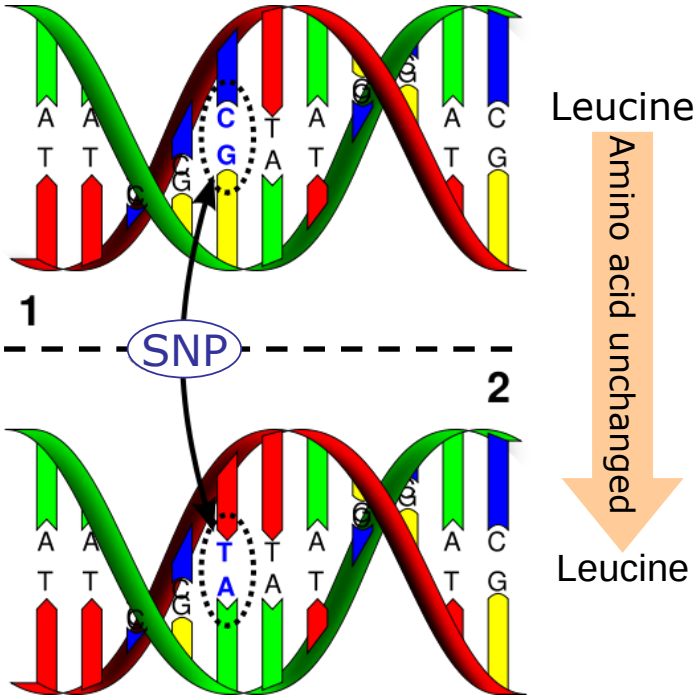
Drug toxicity & efficacy



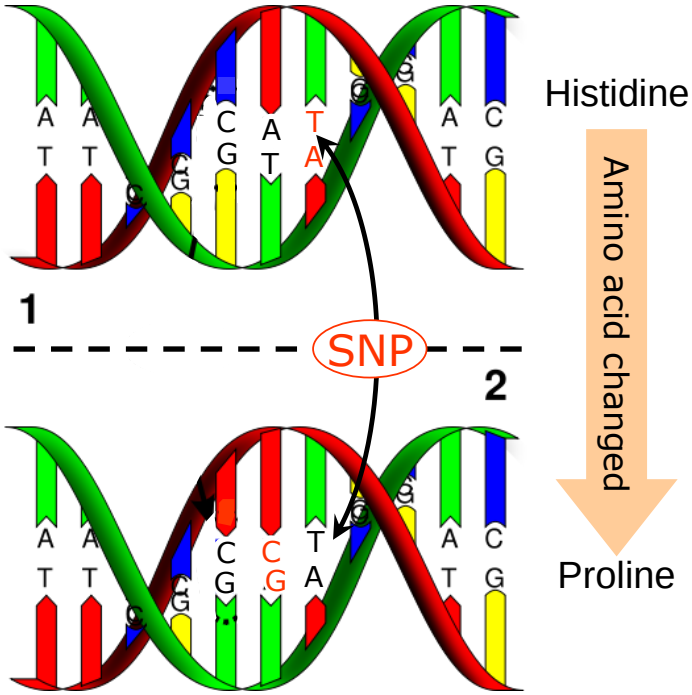
Genetic association studies

Sauna, Kimchi-Sarfaty, Ambudkar & Gottesman (2007) Cancer Res 67: 9609

Synonymous & non-synonymous SNPs



Synonymous SNP:
Amino acid is not altered



Non-synonymous SNP:
Amino acid is altered

Recent studies suggest that both synonymous and non-synonymous SNPs can cause changes in protein expression, conformation and function

Sauna & Kimchi-Sarfaty (2011) Nature Reviews Genetics, In Press

Personalized medicine: making it into product labels

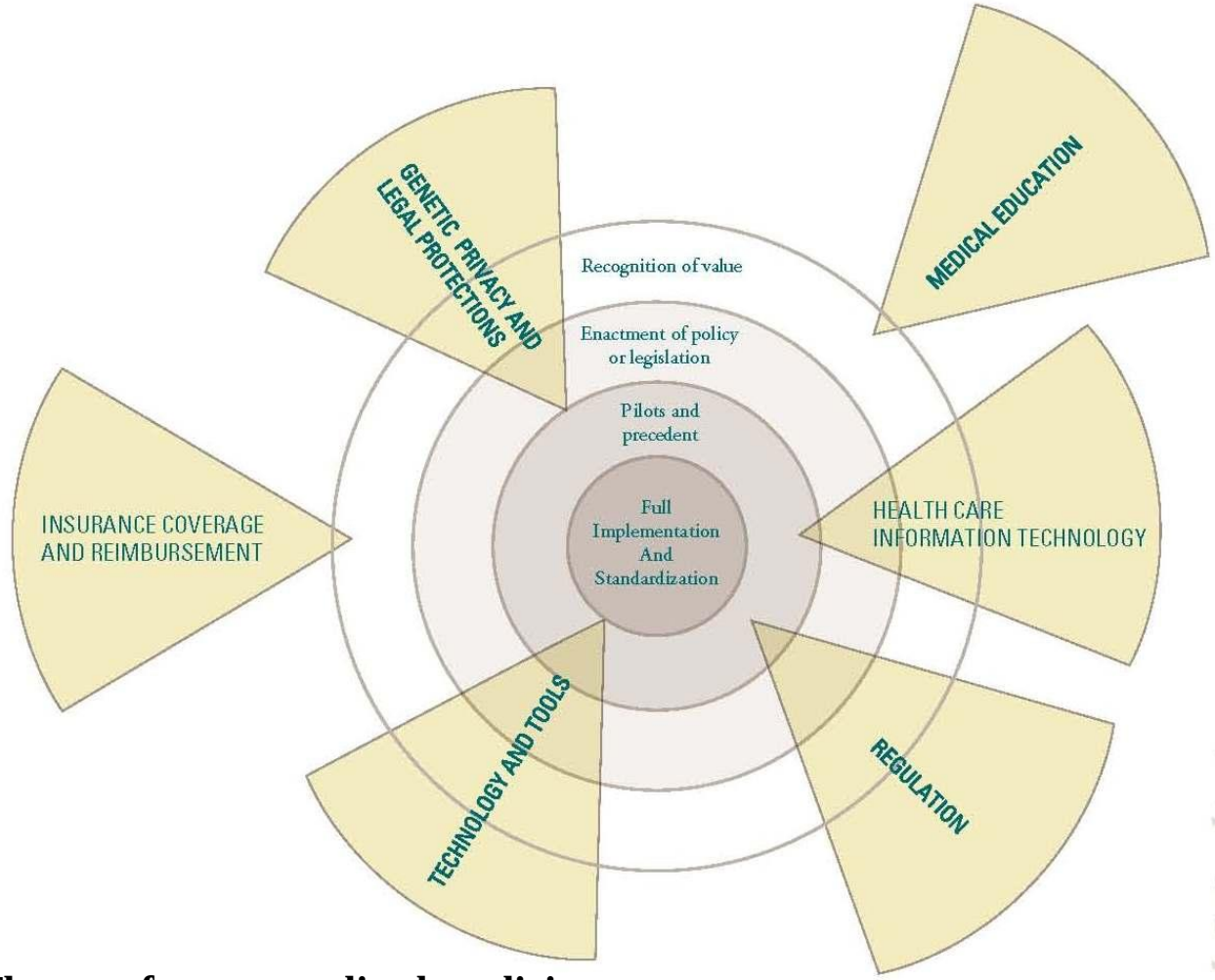
THERAPY	BIOMARKER/TEST	INDICATION
Herceptin® (trastuzumab) Tykerb® (lapatinib)	HER-2/neu receptor	**Breast cancer:** "…for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have received one or more chemotherapy regimens for their metastatic disease."
Pharmaceutical and surgical prevention options and surveillance	*BRCA 1,2*	**Breast cancer:** Guides surveillance and preventive treatment based on susceptibility risk for breast and ovarian cancer.
Tamoxifen	Aviara Breast Cancer Index℠ (*HOXB13, IL17BR*)	**Breast cancer:** Calculates a combined risk analysis for recurrence after tamoxifen treatment for ER-positive, node-negative breast cancer.
Chemotherapy	Mammostrat®	**Breast cancer:** Prognostic immunohistochemistry (IHC) test used for postmenopausal, node negative, estrogen receptor expressing breast cancer patients who will receive hormonal therapy and are considering adjuvant chemotherapy.
Chemotherapy	MammaPrint®	**Breast cancer:** Assesses risk of distant metastasis in a 70 gene expression profile.
Coumadin® (warfarin)	*CYP2C9*	**Cardiovascular disease:** "an increased bleeding risk for patients carrying either the *CYP2C9*2* or *CYP2C9*3* alleles."
Coumadin® (warfarin)	*VKORC1*	**Cardiovascular disease:** "Certain single nucleotide polymorphisms in the *VKORC1* gene (especially the -1639G>A allele) have been associated with lower dose requirements for warfarin."
Coumadin® (warfarin)	PGx Predict™: Warfarin	**Cardiovascular disease:** Determines *CYP2C9* and *VKORC1* genotypes to predict likelihood of adverse events with warfarin therapy.
Coumadin® (warfarin)	Protein C deficiencies	**Cardiovascular disease:** Hereditary or acquired deficiencies of protein C or its cofactor, protein S, has been associated with tissue necrosis following warfarin administration.
Pharmaceutical and lifestyle prevention options	Familion® 5-gene profile	**Cardiovascular disease:** Guides prevention and drug selection for patients with inherited cardiac channelopathies such as Long QT Syndrome (LQTS), which can lead to cardiac rhythm abnormalities.
Statins	PhyzioType SINM	**Cardiovascular disease:** Predicts risk of statin-induced neuro-myopathy, based on a patient's combinatorial genotype for 50 genes.
Atorvastatin	*LDLR*	**Cardiovascular disease:** "Doses should be individualized according to the recommended goal of therapy. Homozygous Familial Hypercholestremia (10-80mg/day)and heterozygous (10-20mg/day)."
Camptosar® (irinotecan)	*UGTIA1*	**Colon cancer:** "Variations in the *UGT1A1* gene can influence a patient's ability to break down irinotecan, which can lead to increased blood levels of the drug and a higher risk of side effects."
Erbitux® (cetuximab) Gefitinib Vectibix® (panitumab)	*EGFR* expression	**Colon cancer:** "Patients enrolled in the clinical studies were required to have…evidence of positive *EGFR* expression using the DakoCytomation *EGFR* pharmDx™ test kit." *EGFR* positive individuals are more likely to respond to the drug than those with reduced *EGFR* expression.
Erbitux® (cetuximab) Gefitinib Vectibix® (panitumab)	*KRAS*	**Colon cancer:** Certain *KRAS* mutations lead to unresponsiveness to the drug.
Erbitux® (cetuximab) and Vectibix® (panitumab) Fluorouracil Camptosar® (irinotecan)	Target GI™	**Colon cancer:** Provides information of the expression of key molecular targets—*KRAS*, *TS*, and *TOPO1*—to guide therapy.
Tegretol (carbamazepine)	*HLA-B*1502*	**Epilepsy and bipolar disorder:** Serious dermatologic reactions are associated with the *HLA-B*1502* allele in patients treated with carbamazepine. "Prior to initiating Tegretol therapy, testing for *HLA-B*1502* should be performed in patients with ancestry in populations in which *HLA-B*1502* may be present."
Immunosuppressive drugs	AlloMap® gene profile	**Heart transplantation:** Monitors patient's immune response to heart transplant to guide immunosuppressive therapy.
Ziagen® (abacavir)	*HLA-B*5701*	**HIV:** "Patients who carry the *HLA-B*5701* allele are at high risk for experiencing a hypersensitivity reaction to abacavir. Prior to initiating therapy with abacavir, screening for the *HLA-B*5701* allele is recommended."
Selzentry® (maraviroc)	CCR5 receptor (1)	**HIV:** "Selzentry, in combination with other antiretroviral agents, is indicated for treatment experienced adult patients infected with only CCR5-tropic HIV-1 detectable..."

REQUIRED

RECOMMENDED

INFORMATION ONLY

The implementation of personalized medicine



The implementation of personalized medicine requires a confluence of several sectors.
Concentric circles and range represent stages of implementation for each sector.
Full implementation of personalized medicine can only be achieved when all sectors converge toward the center.

The case for personalized medicine

The pharmacogenetic basis of immunogenicity: Coagulation Factor VIII as a case study

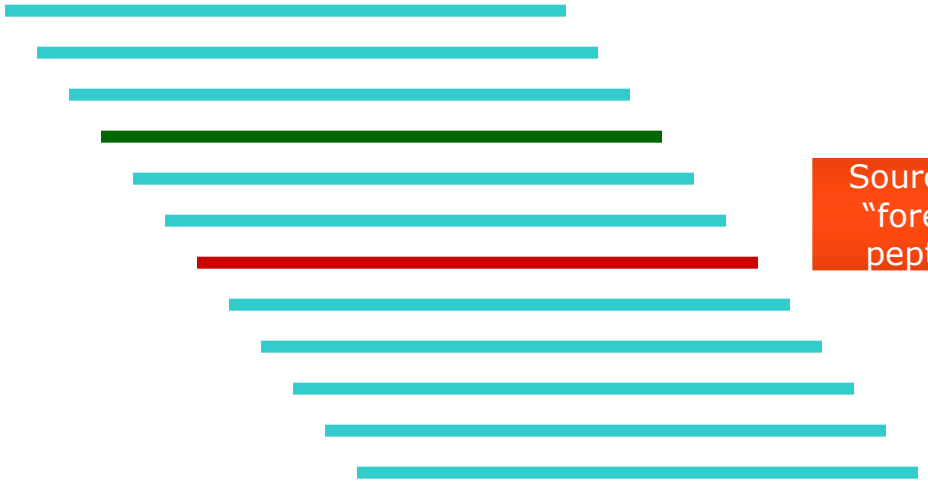
The development of inhibitory antibodies to FVIII: Why sequence matters

The endogenous FVIII of the hemophilia A patient provides the tolerance

Comparing the sequences of the infused FVIII with the endogenous ("self") sequence can identify foreign peptides

FVIII drug

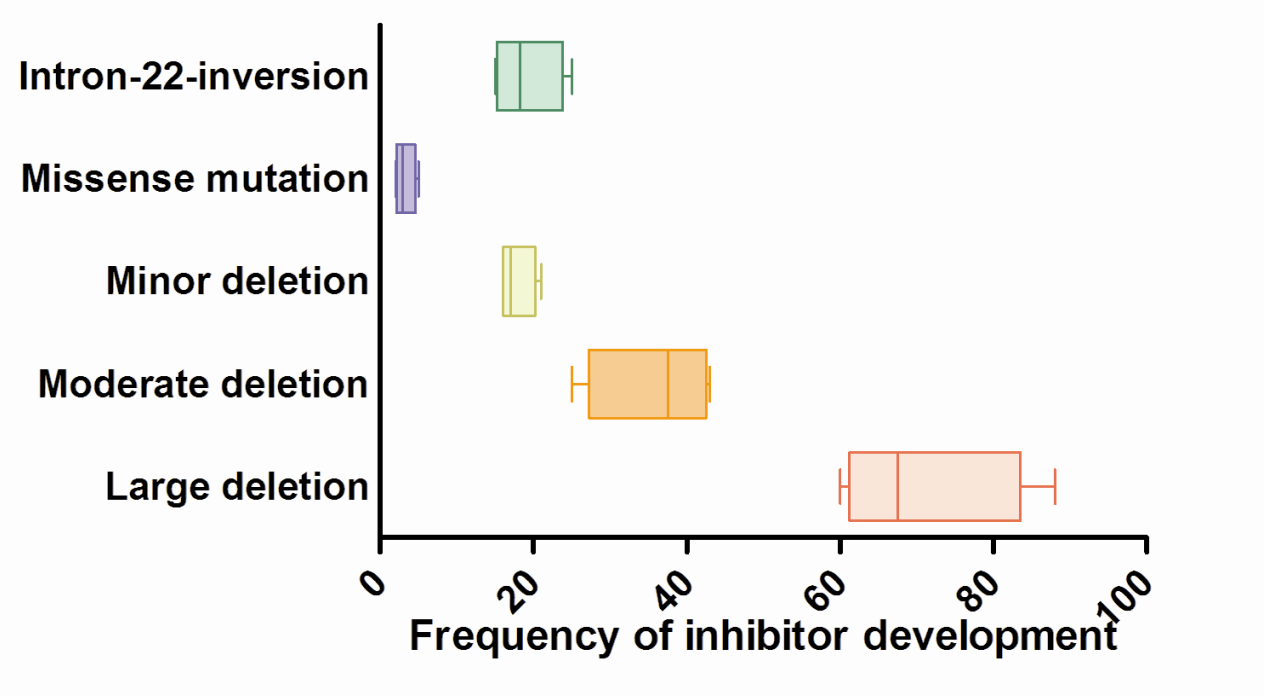
FVIII is processed into overlapping peptides (15 mer)



Sources of "foreign" peptides

1) Genetic mutations in "self" protein that cause hemophilia A
2) SNPs in "self" protein
3) Untranslated "self" protein

Genetic mutations & the development of inhibitors



In general:
The greater the extent of the genetic mutation
The lower the detectable levels of factors VIII
The higher the levels of both binding and neutralizing antibodies

SNPs & inhibitor development

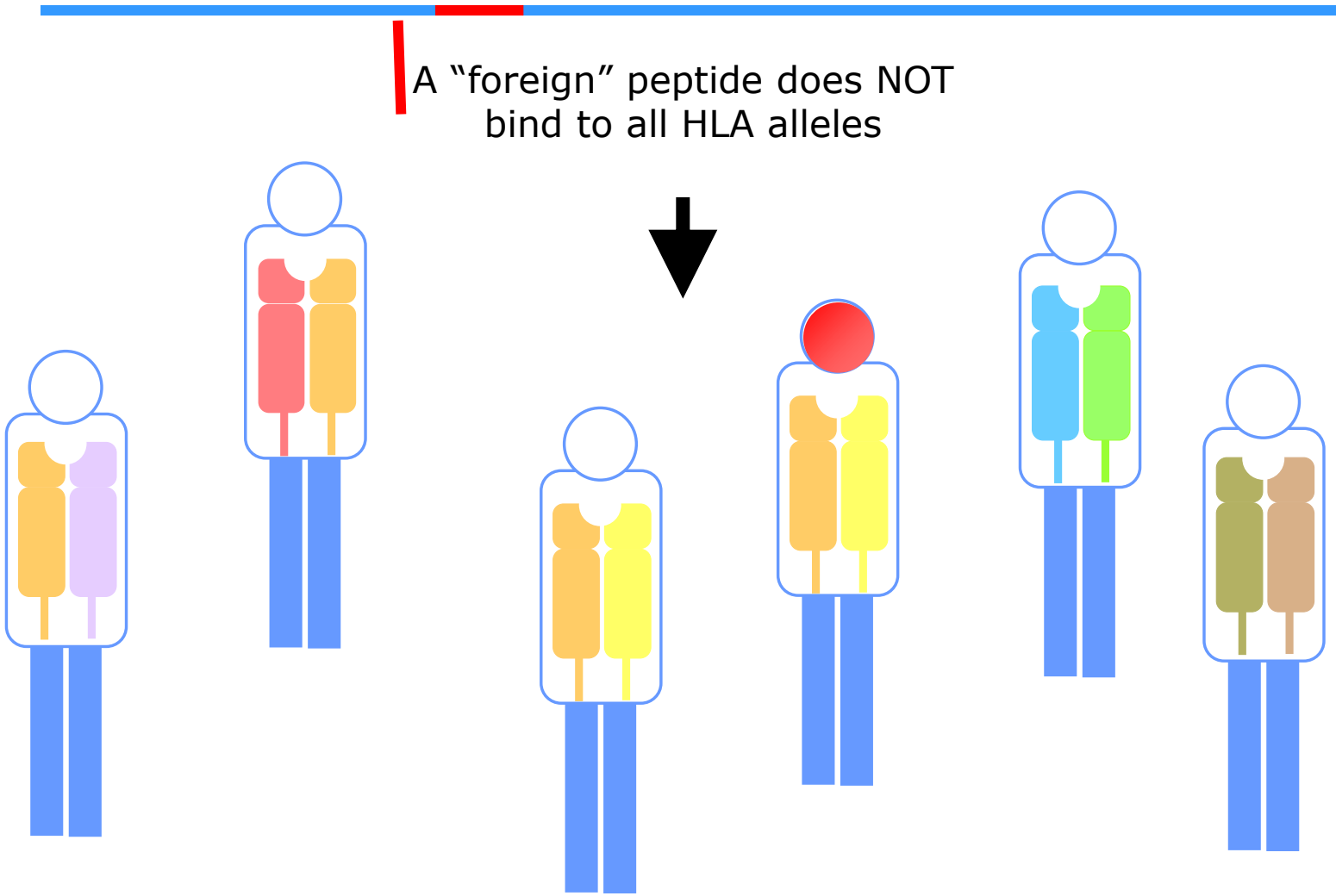
(N. Engl J Med 2009, 360: 1618-1627)

Recombinant FVIII products

Fully or partially matched

SNP or Haplotype	f_{white} (healthy)	f_{black} (healthy)	f_{black} (patient)	% inhibitors (patient)
H1	0.926	0.354	0.236	22.22
H2 D1241E	0.074	0.374	0.513	20.51
H3 D1241E M2238V	0.000	0.222	0.223	41.18
H4 D1241E R484H	0.000	0.040	0.026	100.00
H5 M2238V	0.000	0.010	0.000	Mismatched
H6 D1241E R776G	0.000	0.000	0.000	

The development of inhibitory antibodies to FVIII: Why the HLA type of the patient matters



Even a single amino acid change in the foreign protein can have severe immunological consequences

Ann Hematol (2010) 89:607–612
DOI 10.1007/s00277-009-0887-3

ORIGINAL ARTICLE

Acquired haemophilia caused by non-haemophilic factor VIII gene variants

Andreas Tiede · Roswith Eisert · Andreas Czwalinna · Wolfgang Miesbach · Inge Scharrer · Arnold Ganser

Individuals with a rare mutation E2204K in association with HLA-DRB1*0101
Or
The SNP D1241E in association with HAL-DRB1*0301
Developed inhibitor antibodies (and thus acquired hemophilia) following massive transfusion

Mismatched peptides from these regions bind their respective MHC Class II proteins with very high affinity

Binding of FVIII peptides to specific HLA alleles comparing computational and experimental results

COMPUTATIONAL

15 mer overlapping peptides representing either the entire protein sequence or a particular area of interest are computationally generated

For each peptide binding to a specific HLA is estimated using three or more unrelated predictors

For each predictor a percentile rank is generated for each 15 mer peptide

The median of the top three percentile ranks is used for each peptide-HLA allele complex

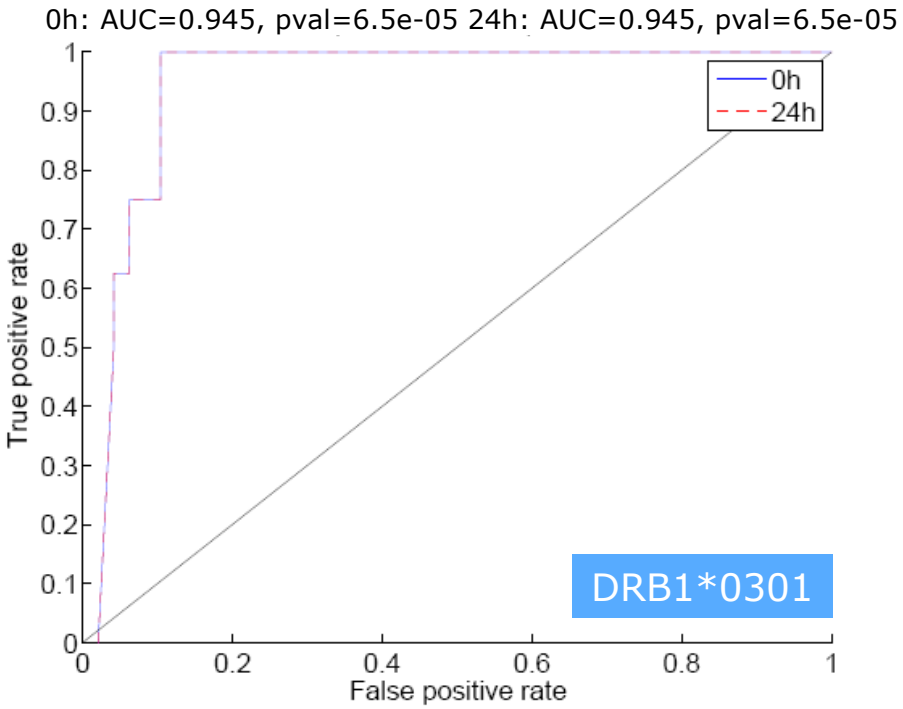
EXPERIMENTAL

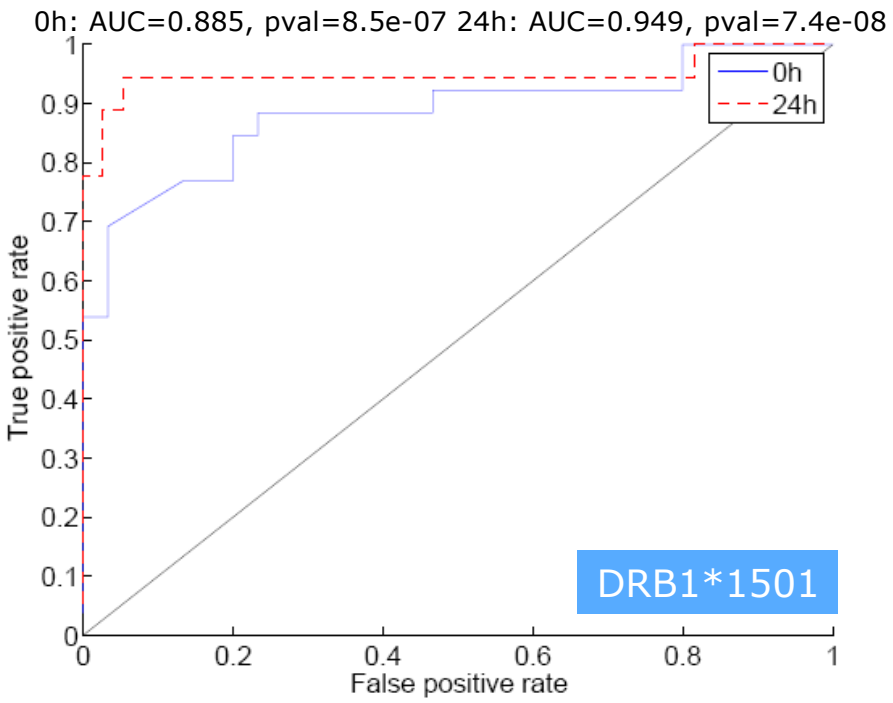
15 mer overlapping peptides representing areas of interest in the protein are synthesized

Binding of individual peptides to specific HLA alleles was determined was determined using the Class II Reveal™ binding and stability assays

Both affinity and stability of the complex were estimated compared to positive and negative controls

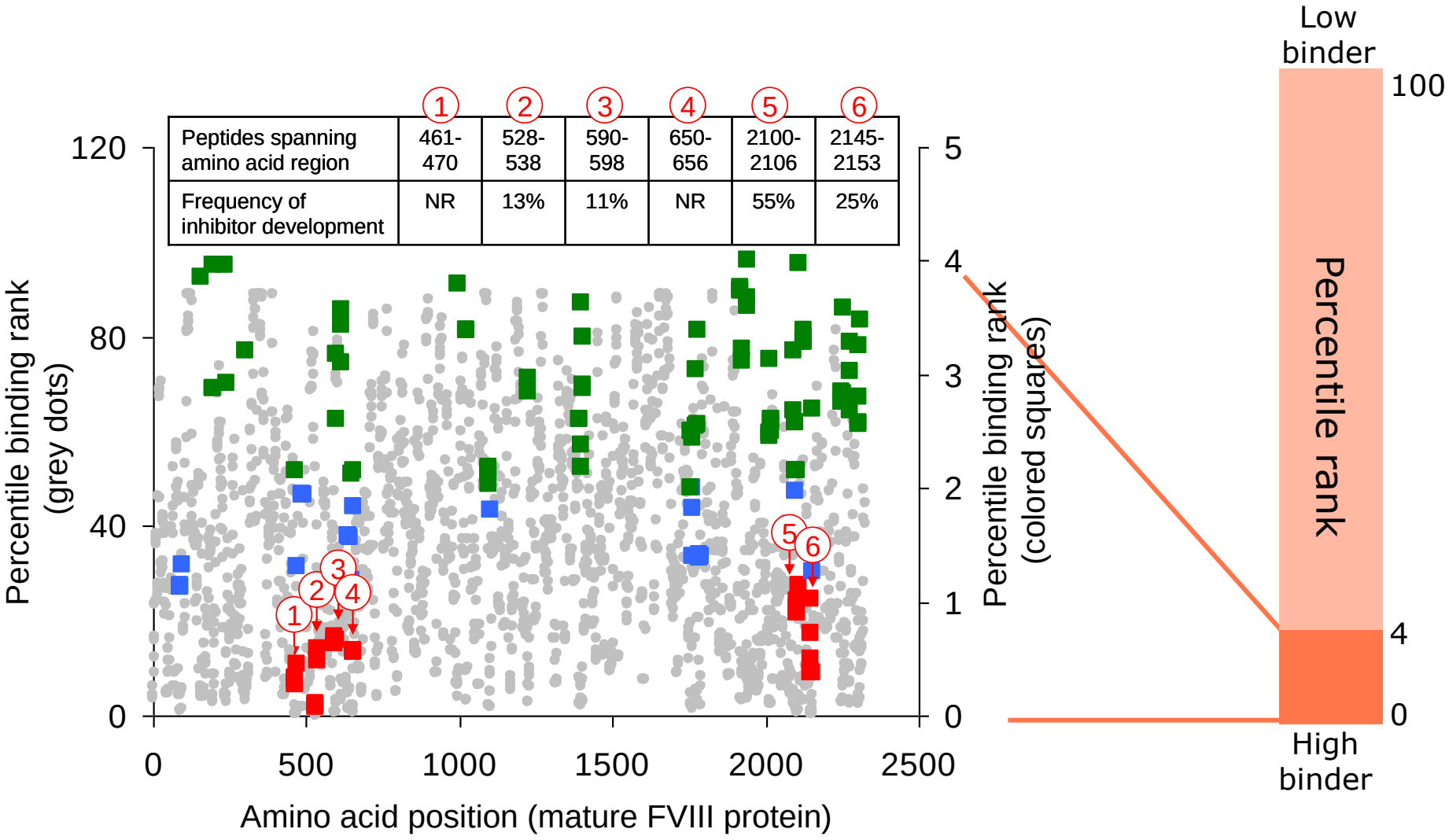
How good are computational predictions of peptide binding to specific HLA alleles?





Computational predictions of peptide binding & historical clinical data

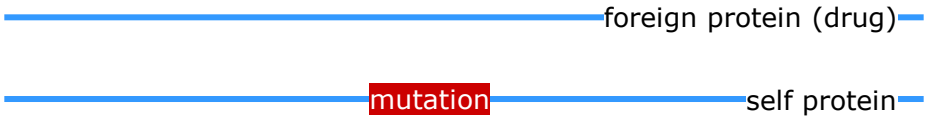
(Haemophilia A Mutation, Structure, Test & Resource Site, HAMSTeRS)



Yanover, Jain, Pierce, Howard & Sauna (2011) Nature Biotechnology, In Press

A immunogenicity score: correlation with historical clinical data

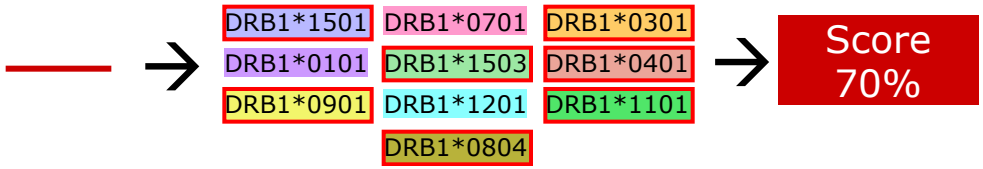
1) Determine regions of sequence mismatch between the endogenous and infused FVIIIs ("foreign peptides")



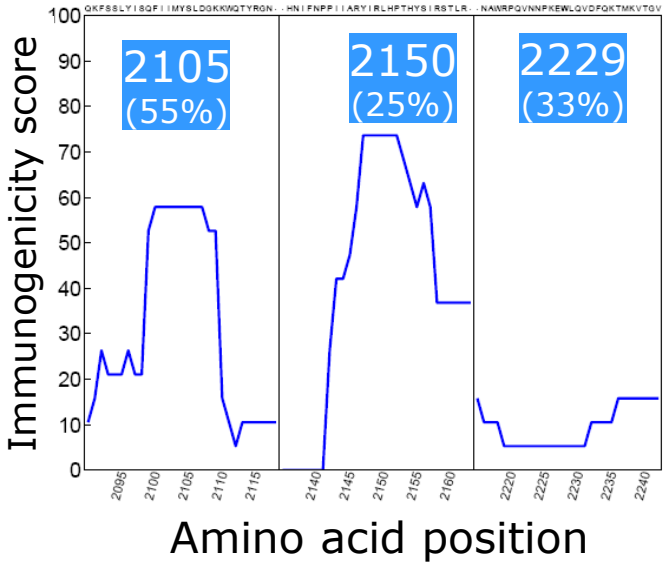
2) Select a set of MHC Class II alleles (representing >80% of population of interest)

N. America	Europe	China	Africa
DRB1*1501	**DRB1*1501**	**DRB1*0901**	**DRB1*0701**
DRB1*0701	**DRB1*0701**	**DRB1*1501**	DRB1*0301
DRB1*0301	DRB1*0301	DRB1*1202	DRB1*1302
DRB1*0101	DRB1*0101	**DRB1*0701**	**DRB1*1503**
DRB1*0401	DRB1*0401	DRB1*1101	DRB1*0804

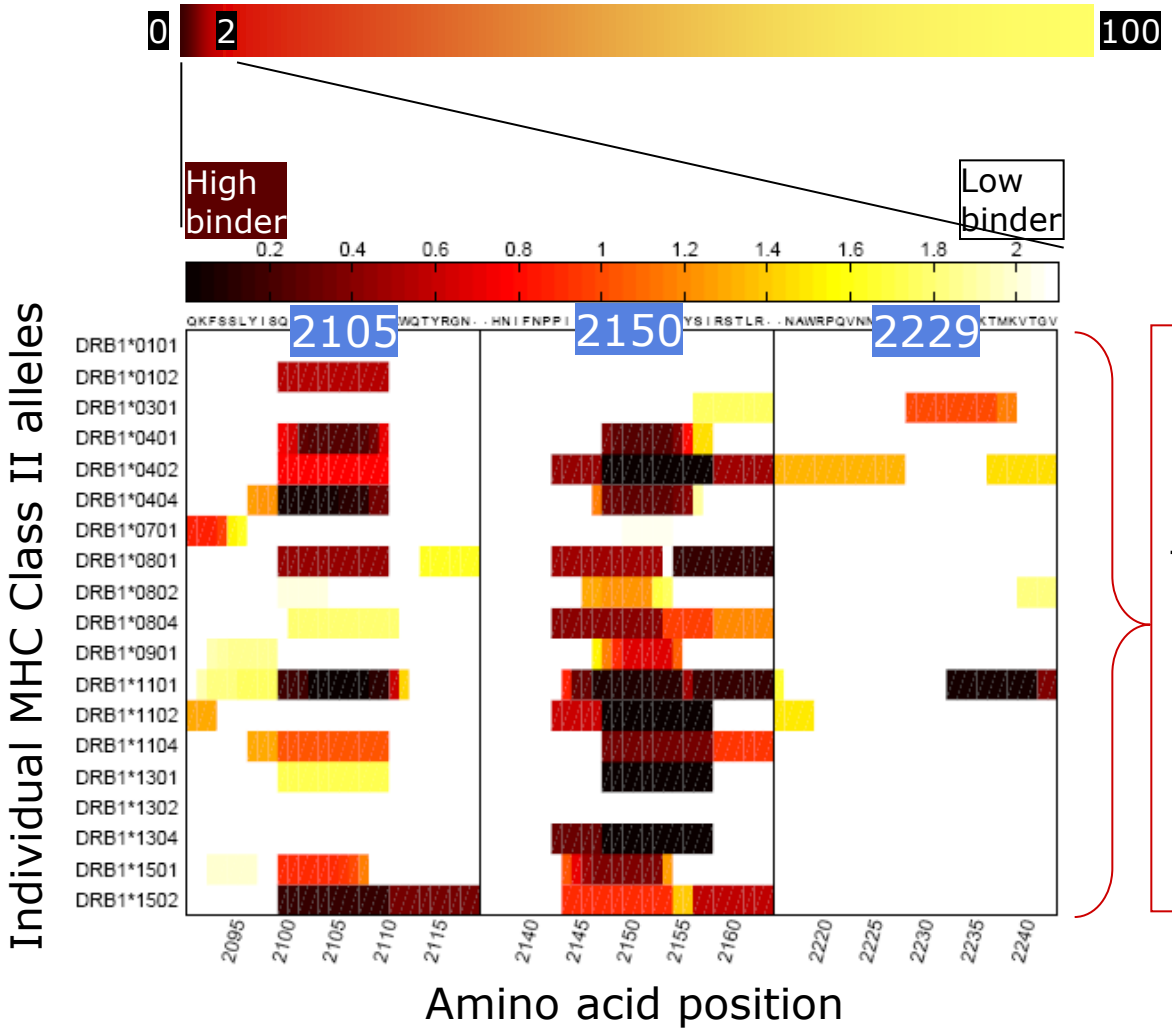
3) Determine immunogenicity score: percent HLA alleles in a set that bind each "foreign peptide" with high affinity (percentile rank <2)



4) Plot immunogenicity scores of all overlapping peptides in regions of sequence mismatch



Computational predictions of peptide binding & historical clinical data: The importance of the HLA repertoire



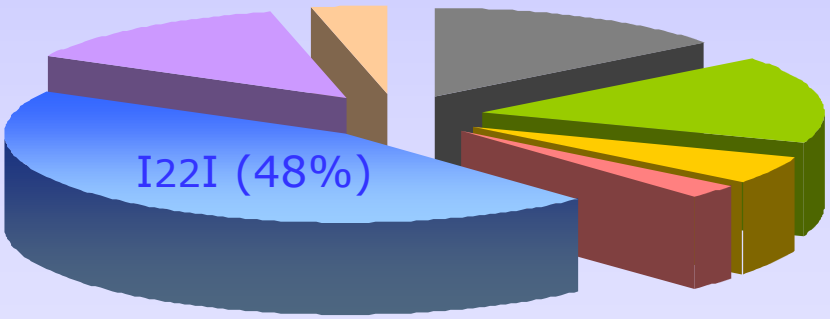
Peptides that incorporate position 2229 bind with high affinity to fewer HLA alleles

These alleles however occur at high frequencies in the North American and European populations (cumulative frequency of 28.5 and 34 percent respectively)

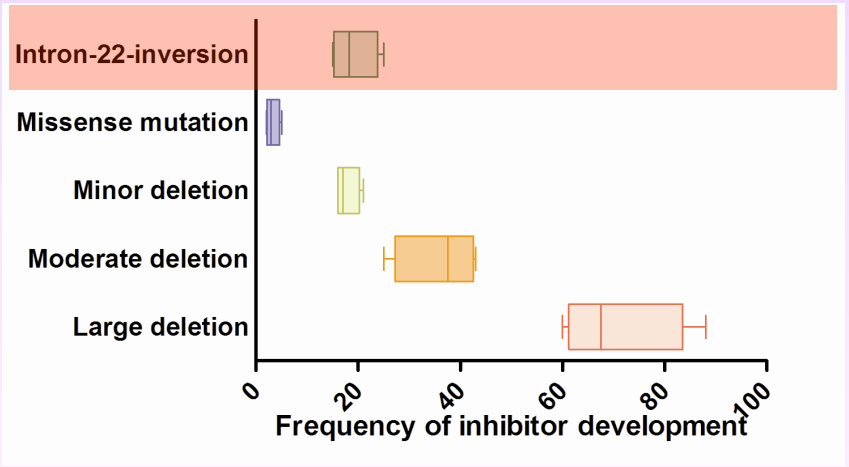
Yanover, Jain, Pierce, Howard & Sauna (2011) Nature Biotechnology, In Press

SNPs in the endogenous F8 of hemophilia A patients as risk-factors for immunogenicity

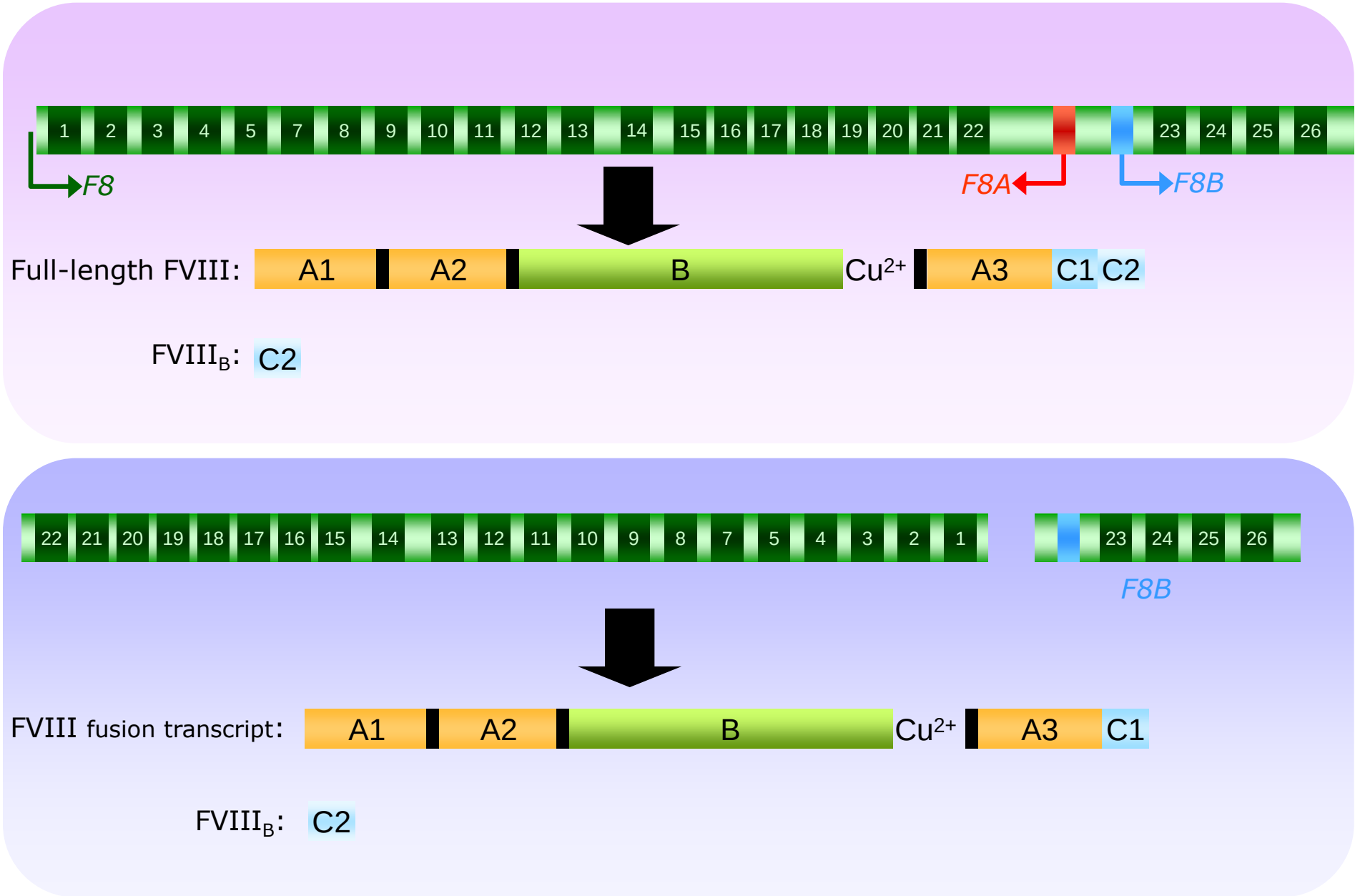
In about half of all severe Hemophilia A patients, the causative mutation is the intron-22 inversion



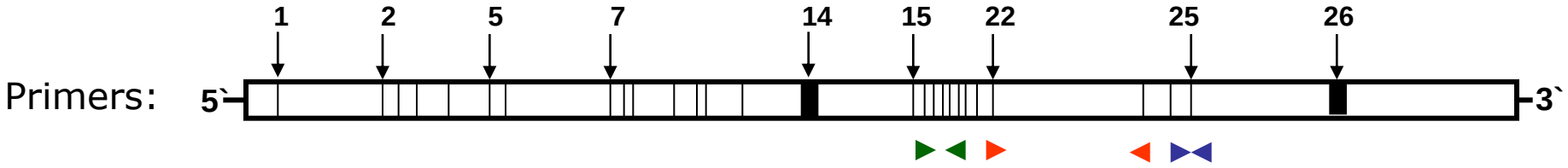
In Hemophilia A with the intron-22 inversion, inhibitors occur at a lower than expected frequency

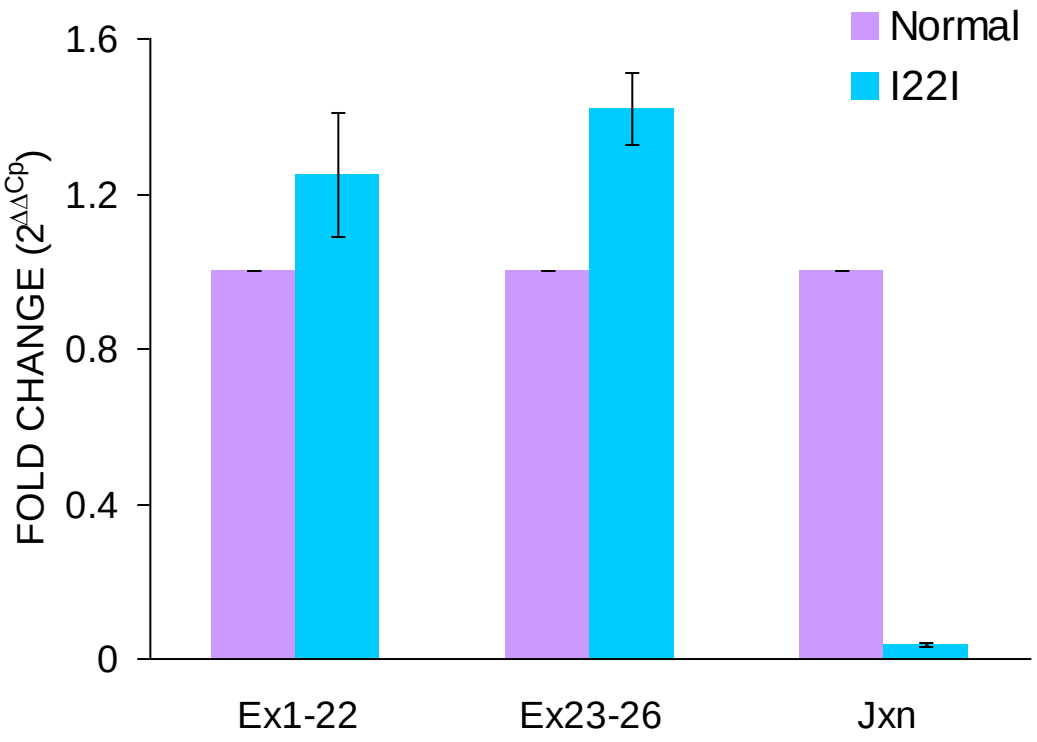


F8 gene structure, the I22I and the synthesis of Factor VIII protein

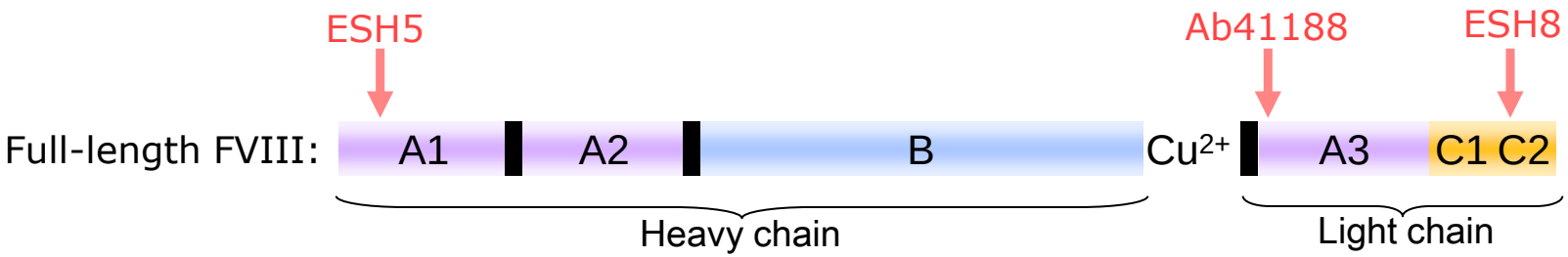


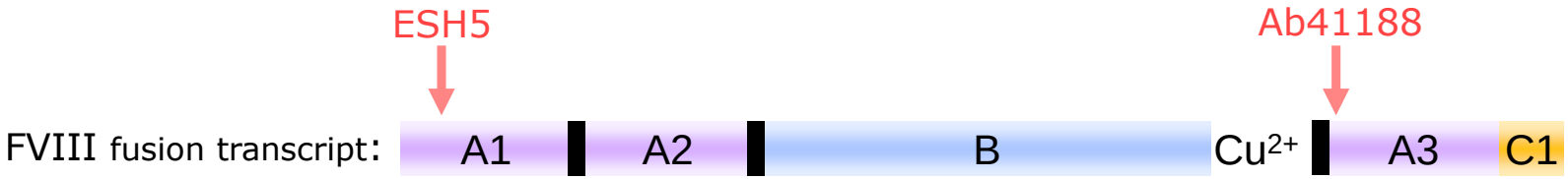
Cells from a normal individual and an hemophilia A patient with the I22I express *F8* mRNA

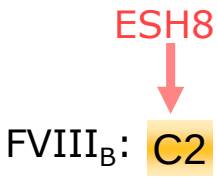




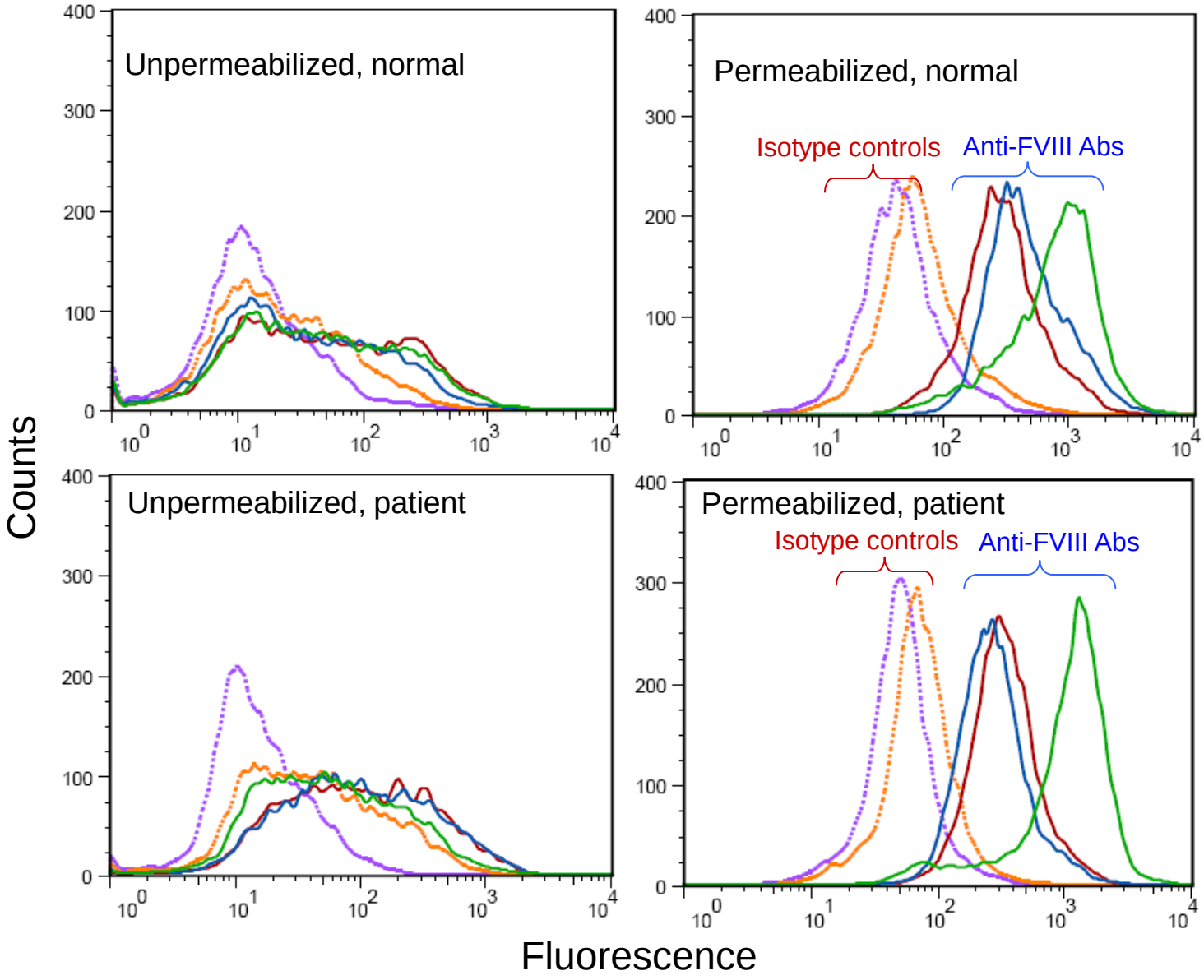
Anti-Factor VIII monoclonal antibodies can detect different regions of the Factor VIII protein



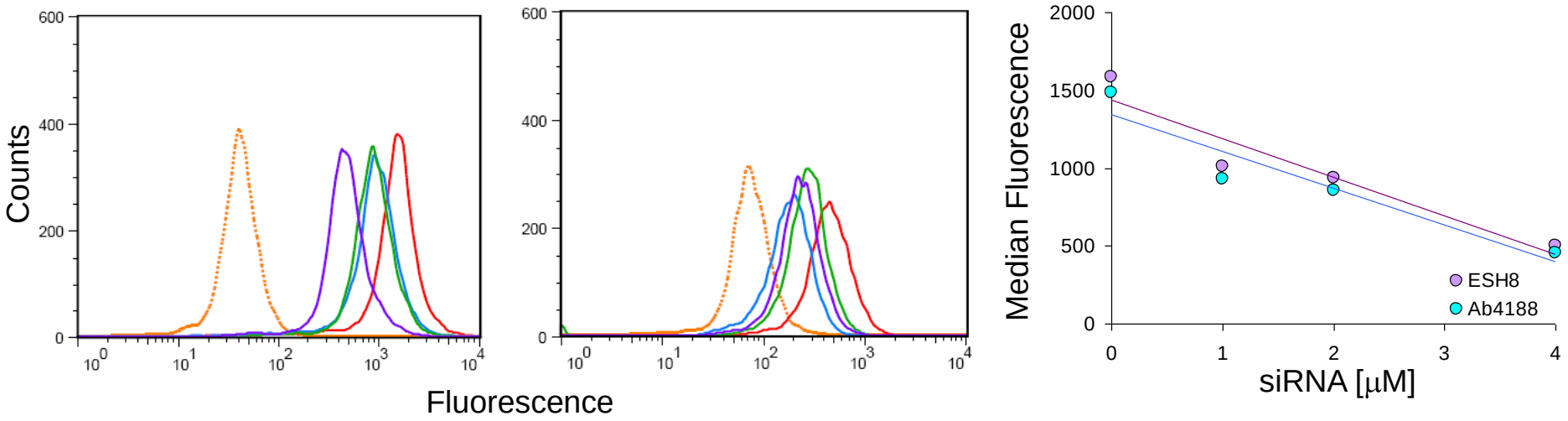




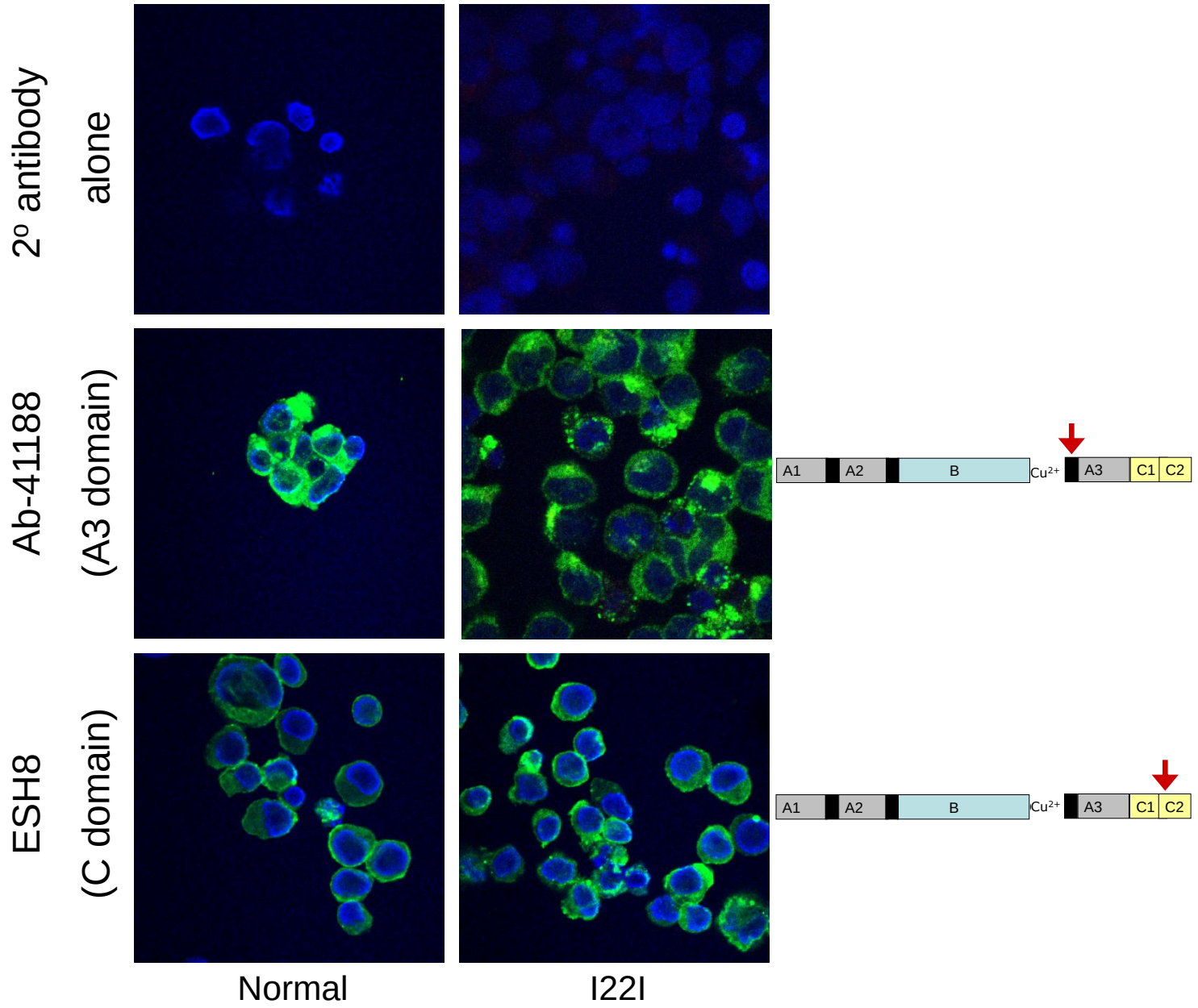
Cells from hemophilia A patient with the I22I express Factor VIII polypeptides



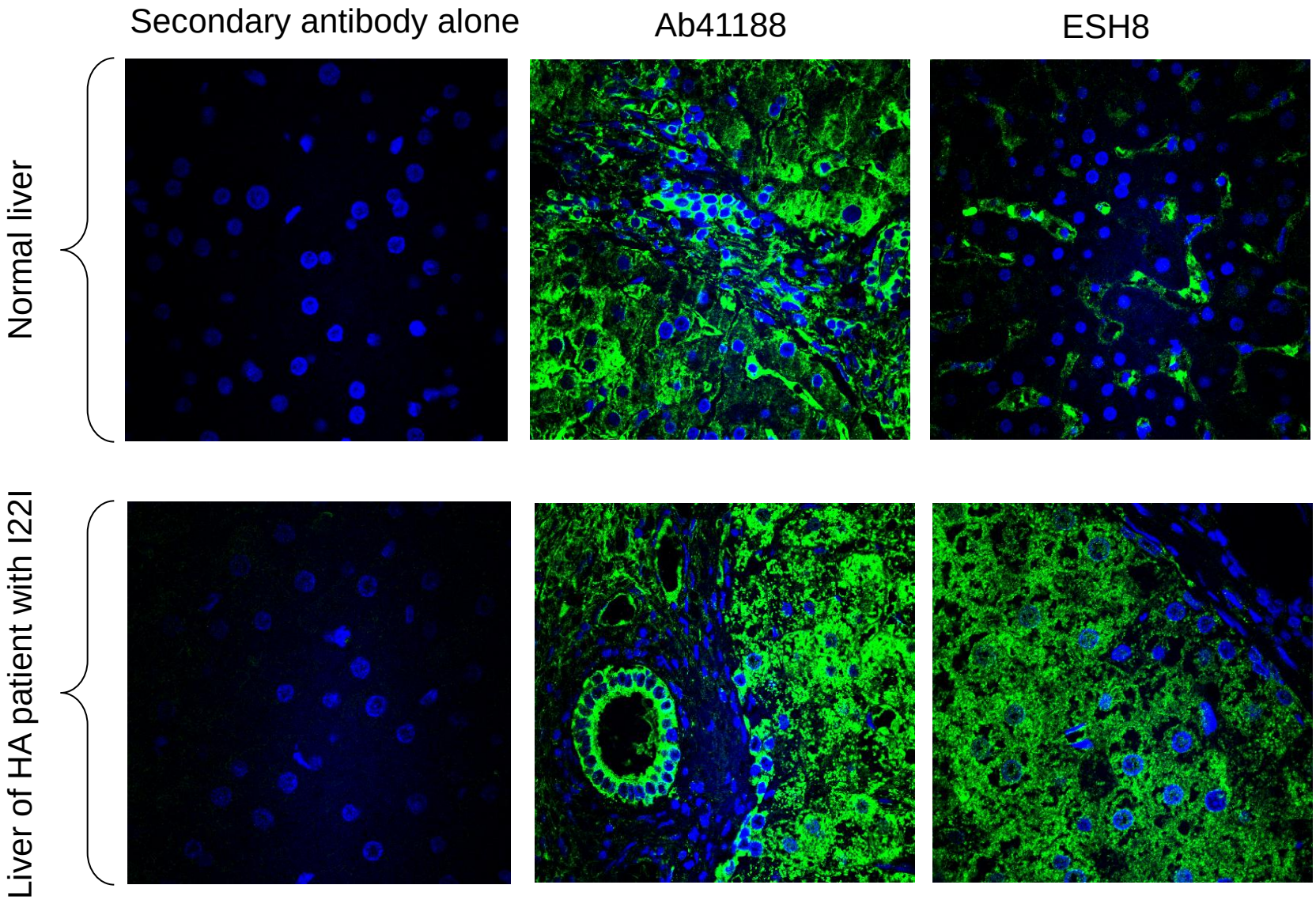
siRNA mediated knockdown results in reduced levels of intracellular Factor VIII



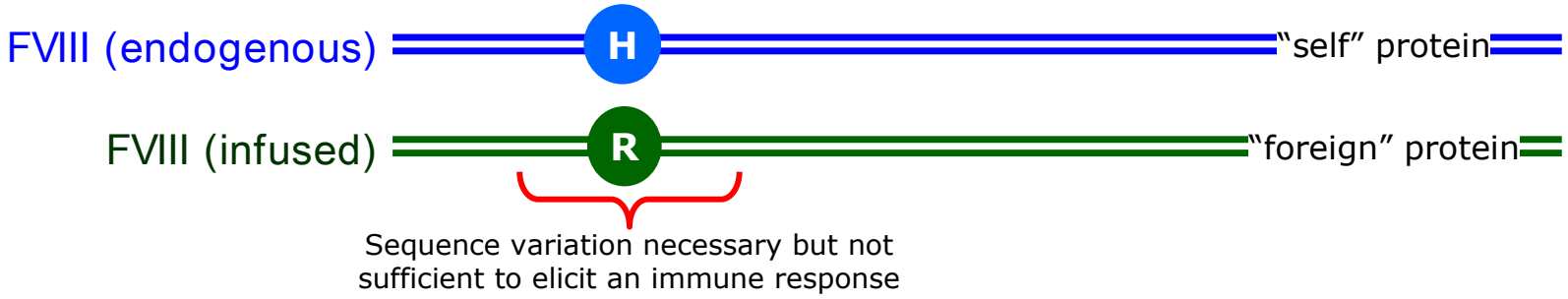
Cells from hemophilia A patient with the I22I express Factor VIII polypeptide (confocal microscopy)



Sections of liver explanted from a hemophilia A patient with the I22I stained with anti-Factor VIII antibodies



SNPs, sequence variation & immunogenicity



(Only about 2% of peptides generated can bind to a MHC molecule)

Do "foreign peptides" bind to MHC Class II Proteins?

(MHC proteins are among the most polymorphic in the human genome)

Which MHC Class II alleles bind to the "foreign peptides"?

What is the distribution of these alleles in the general population and in specific ethnic groups?

Potential for personalized therapy

Summary

- Sequencing of patient's gene and high resolution HLA typing have become quite inexpensive making computational approaches to identifying individuals at risk of developing inhibitory antibodies feasible

- It may be possible to personalize management of a disease with a "matched" (or, less mismatched,) replacement product

- This could potentially reduce the disproportionate frequency of adverse alloimmune events in vulnerable populations (as currently occurs in hemophilia A patients of black-African descent)

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