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)
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

- Safe Effective
- Safe NOT Effective
- NOT Safe Effective
- NOT Safe NOT Effective

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?
Synonymous & non-synonymous SNPs

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

## Personalized medicine: making it into product labels

<table>
<thead>
<tr>
<th>THERAPY</th>
<th>BIOMARKER/TEST</th>
<th>INDICATION</th>
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</thead>
<tbody>
<tr>
<td>Herceptin® (trastuzumab)</td>
<td>HER-2/neu receptor</td>
<td>Breast cancer: &quot;...for the treatment of patients with metastatic breast cancer whose tumors over-express the HER2 protein and who have received one or more chemotherapy regimens for their metastatic disease.&quot;</td>
</tr>
<tr>
<td>Tykerb® (lapatinib)</td>
<td></td>
<td></td>
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<tr>
<td>Pharmaceutical and surgical prevention options and surveillance</td>
<td>BRCA 1,2</td>
<td>Breast cancer: Guides surveillance and preventative treatment based on susceptibility risk for breast and ovarian cancer.</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Astaora Breast Cancer Index™ (PAX8, I1A/BR)</td>
<td>Breast cancer: Calculates a combined risk analysis for recurrence after tamoxifen treatment for ER-positive, node-negative breast cancer.</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>MammaPrint®</td>
<td>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.</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Mammostrat™</td>
<td>Breast cancer: Assesses risk of distant metastasis in a 70 gene expression profile.</td>
</tr>
<tr>
<td>Coumadin® (warfarin)</td>
<td>CYP2C9</td>
<td>Cardiovascular disease: &quot;an increased bleeding risk for patients carrying either the CYP2C9<em>2 or CYP2C9</em>3 alleles.&quot;</td>
</tr>
<tr>
<td>Coumadin® (warfarin)</td>
<td>VKORC1</td>
<td>Cardiovascular disease: &quot;Certain single nucleotide polymorphisms in the VKORC1 gene (especially the +1636G&gt;A allele) have been associated with lower dose requirements for warfarin.&quot;</td>
</tr>
<tr>
<td>Coumadin® (warfarin)</td>
<td>PGx Predict™; Warfarin</td>
<td>Cardiovascular disease: Determines CYP2C9 and VKORC1 genotypes to predict likelihood of adverse events with warfarin therapy.</td>
</tr>
<tr>
<td>Coumadin® (warfarin)</td>
<td>Protein C deficiencies</td>
<td>Cardiovascular disease: Hereditary or acquired deficiencies of protein C or its cofactor, protein S, has been associated with tissue necrosis following warfarin administration.</td>
</tr>
<tr>
<td>Pharmacological and lifestyle prevention options</td>
<td>Familial 5-gene profile</td>
<td>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.</td>
</tr>
<tr>
<td>Statins</td>
<td>PhysioType SINM</td>
<td>Cardiovascular disease: Predicts risk of statin-induced myopathy, based on a patient's combinatorial genotype for 50 genes.</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>LDL-R</td>
<td>Cardiovascular disease: &quot;Doses should be individualized according to the recommended goal of therapy. Homozygous Familial Hypercholesterolemia (10-80mg/dl) and heterozygous (10-25mg/dl).&quot;</td>
</tr>
<tr>
<td>Camptothecin® (irinotecan)</td>
<td>UGT1A1</td>
<td>Colon cancer: &quot;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.&quot;</td>
</tr>
<tr>
<td>Erbitux® (cetuximab)</td>
<td>EGFR expression</td>
<td>Colon cancer: &quot;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.</td>
</tr>
<tr>
<td>Gefitinib</td>
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<tr>
<td>Vectibix® (panitumab)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erbitux® (cetuximab)</td>
<td>KRAS</td>
<td>Colon cancer: Certain KRAS mutations lead to unresponsiveness to the drug.</td>
</tr>
<tr>
<td>Gefitinib</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vectibix® (panitumab)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erbitux® (cetuximab) and</td>
<td>Target GMT™</td>
<td>Colon cancer: Provides information of the expression of key molecular targets—KRAS, TS, and TOP2A—to guide therapy.</td>
</tr>
<tr>
<td>Vectibix® (panitumab) and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorouracil and Camptothecian</td>
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</table>
| 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. |
| Ziacon® (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..."
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 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 is processed into overlapping peptides (15 mer)

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

Sources of "foreign" peptides
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

<table>
<thead>
<tr>
<th>SNP or Haplotype</th>
<th>$f_{\text{white}}$ (healthy)</th>
<th>$f_{\text{black}}$ (healthy)</th>
<th>$f_{\text{black}}$ (patient)</th>
<th>% inhibitors (patient)</th>
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</thead>
<tbody>
<tr>
<td>H1</td>
<td>0.926</td>
<td>0.354</td>
<td>0.236</td>
<td>22.22</td>
</tr>
<tr>
<td>H2 D1241E</td>
<td>0.074</td>
<td>0.374</td>
<td>0.513</td>
<td>20.51</td>
</tr>
<tr>
<td>H3 D1241E M2238V</td>
<td>0.000</td>
<td>0.222</td>
<td>0.223</td>
<td>41.18</td>
</tr>
<tr>
<td>H4 D1241E R484H</td>
<td>0.000</td>
<td>0.040</td>
<td>0.026</td>
<td>100.00</td>
</tr>
<tr>
<td>H5 M2238V</td>
<td>0.000</td>
<td>0.010</td>
<td>0.000</td>
<td>Mismatched</td>
</tr>
<tr>
<td>H6 D1241E R776G</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>
The development of inhibitory antibodies to FVIII:
Why the HLA type of the patient matters

A “foreign” peptide does NOT bind to all HLA alleles
Even a single amino acid change in the foreign protein can have severe immunological consequences

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 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?

0h: AUC=0.945, pval=6.5e-05
24h: AUC=0.945, pval=6.5e-05
0h: AUC=0.885, pval=8.5e-07
24h: AUC=0.949, pval=7.4e-08

DRB1*0301

DRB1*1501
Computational predictions of peptide binding & historical clinical data

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

Peptides spanning amino acid region

<table>
<thead>
<tr>
<th>Peptides spanning</th>
<th>461-470</th>
<th>528-538</th>
<th>590-598</th>
<th>650-666</th>
<th>2100-2106</th>
<th>2145-2153</th>
</tr>
</thead>
</table>

Frequency of inhibitor development

<table>
<thead>
<tr>
<th>Frequency of inhibitor development</th>
<th>NR</th>
<th>13%</th>
<th>11%</th>
<th>NR</th>
<th>55%</th>
<th>25%</th>
</tr>
</thead>
</table>

A immunogenicity score: correlation with historical clinical data

1) Determine regions of sequence mismatch between the endogenous and infused FVIIIIs (“foreign peptides”)

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

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).

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

Full-length FVIII: A1 A2 B Cu$^{2+}$ A3 C1 C2

FVIII$_B$: C2

FVIII fusion transcript: A1 A2 B Cu$^{2+}$ A3 C1

FVIII$_B$: C2
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

**Counts**

**Unpermeabilized, normal**

**Permeabilized, normal**

**Isotype controls**

**Anti-FVIII Abs**

**Unpermeabilized, patient**

**Permeabilized, patient**

**Isotype controls**

**Anti-FVIII Abs**

**Fluorescence**
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

Normal liver

Secondary antibody alone

Liver of HA patient with I22I

Ab41188

ESH8
SNPs, sequence variation & immunogenicity

**FVIII (endogenous)**

```
H
```

“self” protein

**FVIII (infused)**

```
R
```

“foreign” protein

Sequence variation necessary but not sufficient to elicit an immune response

(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
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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Chava Kimchi-Sarfaty
Basil Golding
Nisha Jain

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