Immunosignatures: a Platform Technology for Diagnosis and Discovery

RUSNANO

Stephen Albert Johnston
Center for Innovations in Medicine
HealthTell
Russian American Anti Cancer Center
Current *Center for Innovations In Medicine* Projects

<table>
<thead>
<tr>
<th>OBJECTIVE</th>
<th>INVENTION</th>
<th>COMPANY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health Monitoring/</td>
<td>Immunosignatures</td>
<td>HealthTell, Inc</td>
</tr>
<tr>
<td>Early Diagnosis</td>
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<tr>
<td>Universal Preventative</td>
<td>Frameshift Antigens</td>
<td>Calviri, LLC</td>
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<tr>
<td>Cancer Vaccine</td>
<td></td>
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<tr>
<td>NextGen Antibiotics, Anti-Virals</td>
<td>Synbodies</td>
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</tbody>
</table>
Forbes Magazine, 1/19/2012

U.S. Healthcare Hits $3 Trillion

National Healthcare Expenditure – or NHE.
... NHE for 2012 is probably closer to $2.7 trillion but there’s this nagging bookkeeping accrual of about $300 billion where we (narrowly) avoided those darn pesky SGR cuts to Medicare. ... That puts the real NHE at about $3 trillion for 2012 (+ about 4% for each year forward – as far as the eye can see). As one economist said – we don’t have a debt problem in this country – we have a healthcare problem.

http://www.forbes.com/sites/danmunro/2012/01/19/u-s-healthcare-hits-3-trillion/

$3 trillion is ~19% of the GDP for the US
Developed & under-developed countries:

- 71% of cancer deaths
- 5,053,872 deaths/yr.

Developed (high-income/industrialized):

- 29% of cancer deaths
- 2,054,897 deaths/yr.

Total cancer deaths/yr:

- 7,108,769 deaths/yr.

WHO: Imminent global cancer 'disaster'
“The total economic impact of premature death and disability from cancer worldwide was $895 billion in 2008.”
Anne Weston, picture of “How to Cure Cancer”, Time magazine web, June, 2013
“When the stars come together cancer doesn’t stand a chance”
Positron Emission Therapy

Development: $200B (1000 centers)
Cost/Year: $50B
Cost/Treatment: ~$30,000
Physicists: 10,000
1. Tumor DNA and/or RNA from ONE Individual is Sequenced

2. Analysis of Sequence Indicates the Right Drug to Use

3. Treatments often >$100,000 US
Post-Symptomatic Medicine
To Pre-Symptomatic Health

2009 GNP $14.7T
2009 Health Care Costs $2.5T

Per capita health expenditure ~$8000
Median adjusted gross income in 2007 ~$33,000*
Median federal taxes per capita in 2007 ~$1,000
Total Medicare expenditures in 2004 ~$3B
Medicare expenditure per capita ~$1000

http://www.kff.org/insurance/upload/7692_02.pdf
Transition from Post- to Pre-Symptomatic Medicine Requires System to Continuously Monitor Health of Well People

Specifications Required:

- Comprehensive
- Sensitive – Early Detection
- Simple
- Inexpensive
- Specificity – What is Wrong?
Can Not Do Early Detection of Disease

Blood Dilution Problem

$u_T(t) = f_{PL,T} N_T(t)$

$u_H(t) = f_{PL,H} N_H(t)$

$10^4$ Improvement in Detection Needed

Sci Transl Med 3, 109ra116 (2011);
Sharon S. Hori, et al.
Detection Strategies and Limitations
Mathematical Model Identifies
Blood Biomarker-Based Early Cancer
The Immune System Detects and Amplifies Signal

- $10^8$ to $10^9$ antibodies exist in serum
- A single reactive B cell encounters antigen and is activated
- Produces 5,000 to 20,000 antibodies per minute
- Divides every 70 hours
- Signal is amplified $\sim 10^{11}$ times in one week
Immunosignatures: A universal, simple and cheap platform for disease diagnosis

CIM10K: 10,000 non-natural sequence peptides

Sykes et al. 2013 Trends Biotechnol. 31(1):45-51
Population-Based Comprehensive Health Monitoring

Central Analysis

Toward a World Without Patients
Immunosignature Process

Array of 10K-350K, Addressable, Non-Natural Sequence Space Peptides
Immunosignature Process

Add diluted blood
Immunosignature Process
Immunosignature Process

- One array for all samples, human and nonhuman
- Very small quantity of blood required
- Scalability and low cost array fabrication
Problem: How to Display Ab Diversity

Antibody Diversity

$10^9$

Different Ab/person

$10^{19}$ Peptide Sequence Space

In $3 \times 10^5$ peptides
Nature Does Not Always Know Best

- Life occupies an infinitesimally small part of potential sequence space

- Therefore there are many other sequences that could be useful

- Peptides on array are chosen to evenly sample random sequence space ($3.5 \times 10^5 / 10^{21}$ possibilities)

Consequences: Same set of peptides can be used for any diagnosis
Super-fine resolution of antibody diversity
Monoclonal Antibodies Bind Distinct Patterns on the Array

Stafford et al. Mol Cell Proteomics 2012. 11(4):M111.011593
Information from Each Feature

Intensity
(#Ab/spot)

Isotypes
IgG (4)
IgM
IgE
IgA

Feature On Array

Amino Acid Sequence
(Repeating Motifs)
Peptide Microarray Vs ELISA

ELISA vs CIM10K Percent Maximal Signal

Sera 1:1,634,800
Secondary Alone
Identifying The Immunosignature

10,000 Peptides

\[ p < 1 \times 10^{-6} \]

& Fold Change

\~\ 100 informative peptides

Train a Machine Learning Algorithm
Performance is Tested on a Second Group

Disease  Control
Features of Immunosignatures

Same chip used for all diseases, all species

Detects all antibodies: sugars, non-linear, modifications

Historical sera samples work

No sample preparation

10-100x more sensitive than ELISA
One Chip, Many Tests

- West Nile
- Type 1 Diabetes
- Breast Cancer
Dry Blood Works as Well as Fresh Blood
Dried vs. Undried Whole Blood Immunosignature Correlations

0 Week Dried vs. Undried Whole Blood

1 Week Dried vs. Undried Whole Blood

3 Week Dried vs. Undried Whole Blood

Storage conditions: 25°C

\[ R^2 = 0.919 \]

\[ R^2 = 0.916 \]

\[ R^2 = 0.843 \]
17 amino acids long, random sequence, and all amino acids except C are used. Two copies of the library are printed on a glass slide (~1200 peptides/cm²). Mass spectra available for all peptides spotted on the arrays.
Fabrication Approach

- UV photolithography
- All chemistry performed using coater/developer
- Large number of cycles makes the process challenging
Material in this presentation contains ASU and HealthTell Intellectual Property and is Confidential
Cost analysis includes:

- **Labor cost**
  - Technicians need to run the tools

- **Yield & QA cost**
  - Labor + reagents
  - Each batch sample set is analyzed

- **Chemical/biochemical reagents**
  - Surface prep
  - Litho & resist steps
  - Amino-acid coupling

- **Materials**
  - Wafer
  - Mask set

- **Facility cost**
  - Rent, utility, & chemical disposal

- **Tool maintenance**
- **Packaging**
Array Production Breakdown: Wafer to Individual Spot

~50-Fold Improvement Over CIM10K Printed Arrays
OneTest™
Comprehensive Health Monitoring
Valley Fever (Coccidiomycosis)

- About 30,000 reported cases annually
- Particularly prevalent in the Sonoran desert
- While most cases are mild, it can be life-threatening
- Flu-like symptoms

Coccidioides immitis spherule with endospores.

Coccidiomycosis

Areas in which coccidioidomycosis is endemic
- Green areas: endemic
- Yellow areas: uncertain

The Morphology of Coccidioides

- Septate Mycelium
- Free Arthrospores
- Free Endospores
- Immature Spherules
- Rupturing Spherule
- Endosporulating Spherule (Mature)
- "SAPROPHYTIC CYCLE"
- "PARASITIC CYCLE"
Classic Train/Test Example: Valley Fever

- 10,000 peptides on original array
- 120 patients screened and analyzed
- 100 most informative peptides selected and resynthesized
- Diagnostic array printed

John Galgiani
Univ. of Arizona
Outperforms Existing Diagnostic

- 90 blinded samples from patients presenting at the clinic
- Zero false positives (100% specificity)
- Zero false negatives (100% sensitivity)

All Patients with Valley Fever Presented with Zero CF Titers, but were later shown to have the disease
Breast Cancer Test/Train using geographically distinct cohorts

Healthy

Breast Cancer

[Graph A: Heatmap showing differences between Healthy and Breast Cancer samples]

[Graph B: Scatter plot showing PCA components with Cancer and Control groups]

X-axis: PCA component 1 (57.78% variance)
Y-axis: PCA component 2 (7.401% variance)
Conditions: All - Breast cancer all, Default Inter...
Colored by: Parameter status
Immunosignaturing Brain Tumors

Collaborator: Adrienne C. Scheck, BNI

100% accurate detection training and testing on samples taken years apart using printed arrays

Not only can immunosignaturing detect the brain tumor, it can distinguish accurately between the common types of brain tumors
Disease: ADNI collection of serum samples from Alzheimer’s Disease (AD) and non-AD controls
Feature selection: 1 sided T-test, 50 peptides were selected
Classification: 4 samples were called normal when they were Alzheimer’s (FN)
Sensitivity=89%, NPV=92%, Accuracy=95%, specificity and PPV=100%
Interpretation: AD signature blends gradually into controls with no clearly defined threshold
Towards Comprehensive Testing

15 Diseases Simultaneously Analyzed

Stafford et al. 2014, PNAS in press
## Cross Validation, 15 Diseases

<table>
<thead>
<tr>
<th>disease</th>
<th>accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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</thead>
<tbody>
<tr>
<td>2ndBC</td>
<td>97.8±0.14</td>
<td>69.1±2.82</td>
<td>99.21±0.1</td>
<td>81.05±3.46</td>
<td>98.48±0.11</td>
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<td>Astro</td>
<td>96.93±0.17</td>
<td>90.1±1.3</td>
<td>97.82±0.17</td>
<td>83.79±1.11</td>
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<td>BC</td>
<td>99.51±0.05</td>
<td>99.71±0.2</td>
<td>99.49±0.08</td>
<td>95.45±0.68</td>
<td>99.97±0.02</td>
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<td>BClVa</td>
<td>99.62±0.06</td>
<td>89.85±1.49</td>
<td>100±0</td>
<td>100±0</td>
<td>99.6±0.06</td>
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<tr>
<td>GBM</td>
<td>99.18±0.1</td>
<td>94.33±2</td>
<td>99.25±0.09</td>
<td>62.1±4.24</td>
<td>99.92±0.03</td>
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<tr>
<td>Lung</td>
<td>99.02±0.12</td>
<td>92.37±0.58</td>
<td>99.59±0.09</td>
<td>94.79±1.27</td>
<td>99.35±0.05</td>
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<tr>
<td>MM</td>
<td>98.72±0.11</td>
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<td>85.13±1.13</td>
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<td>ND</td>
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<td>85.45±0.77</td>
<td>99.31±0.1</td>
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<tr>
<td>Oligo</td>
<td>99.65±0.07</td>
<td>92.57±1.95</td>
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<td>95.21±1.19</td>
<td>99.78±0.06</td>
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<tr>
<td>OligoAstro</td>
<td>98.94±0.15</td>
<td>98.45±0.82</td>
<td>98.95±0.12</td>
<td>86.41±1.78</td>
<td>99.91±0.04</td>
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<tr>
<td>Ovarian</td>
<td>99.92±0.03</td>
<td>100±0</td>
<td>99.91±0.03</td>
<td>98.67±0.47</td>
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<tr>
<td>Pancreatitis</td>
<td>99.67±0.05</td>
<td>95.42±1</td>
<td>99.91±0.03</td>
<td>98.5±0.54</td>
<td>99.74±0.05</td>
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<tr>
<td>PC</td>
<td>97.69±0.11</td>
<td>86.61±1.39</td>
<td>98.79±0.08</td>
<td>87.22±1.19</td>
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<tr>
<td>Sarcoma</td>
<td>98.81±0.11</td>
<td>54.15±5.48</td>
<td>99.67±0.07</td>
<td>71.55±5.65</td>
<td>99.12±0.12</td>
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<tr>
<td>VF</td>
<td>99.67±0.08</td>
<td>100±0</td>
<td>99.64±0.09</td>
<td>96.87±0.74</td>
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<tr>
<td>total</td>
<td>98.77±0.04</td>
<td>89.87±1.32</td>
<td>99.33±0.08</td>
<td>88.89±1.59</td>
<td>99.33±0.07</td>
</tr>
</tbody>
</table>
Simultaneous Distinction of 6 Infection and Normal Sera

Dengue Fever

Valley Fever

*Bordetella pertussis*

Lyme Disease

West Nile Virus

Syphilis

Legutki *et al* Submitted
### 10k vs 330K comparison

#### CIM10K KNN Classification Results using the 160 peptides

<table>
<thead>
<tr>
<th>Sample</th>
<th>Correctly Identified</th>
<th>Incorrectly Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTRA 1</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>DTRA 2</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>DTRA 3</td>
<td>40</td>
<td>0</td>
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<td>DTRA 4</td>
<td>49</td>
<td>0</td>
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<td>DTRA 5</td>
<td>42</td>
<td>1</td>
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<tr>
<td>DTRA 6</td>
<td>41</td>
<td>0</td>
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<tr>
<td>DTRA 7</td>
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<td>0</td>
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<tr>
<td>DTRA 8</td>
<td>46</td>
<td>0</td>
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<tr>
<td>Local Normal</td>
<td>59</td>
<td>0</td>
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<tr>
<td><strong>Total</strong></td>
<td>382</td>
<td>1</td>
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</table>

#### HT330K KNN Classification Results using the 160 peptides

<table>
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<th>Sample</th>
<th>Correctly Identified</th>
<th>Incorrectly Identified</th>
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</thead>
<tbody>
<tr>
<td>DTRA 1</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>DTRA 2</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>DTRA 3</td>
<td>12</td>
<td>0</td>
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<tr>
<td>DTRA 4</td>
<td>11</td>
<td>0</td>
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<tr>
<td>DTRA 5</td>
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<td>DTRA 7</td>
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<td>DTRA 8</td>
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<tr>
<td>Local Normal</td>
<td>6</td>
<td>0</td>
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<tr>
<td><strong>Total</strong></td>
<td>164</td>
<td>0</td>
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</table>

**Minimal P-Value**

- CIM10K: $2.7 \times 10^{-13}$
- HT330K: $6.2 \times 10^{-38}$
Transition from Post- to Pre-Symptomatic Medicine Requires System to Continuously Monitor Health of Well People

Specifications:

- Comprehensive
- Sensitive – Early Detection
- Simple
- Inexpensive
- Specificity – What is Wrong?
Population-Based Comprehensive Health Monitoring

Central Analysis

Direct Mail-in of Samples
With current diagnosis and treatment, the cancer death rate will be 585,720 in 2014.

detected by image or symptoms
start the treatment

Anti-CTLA4 Anti-PD-1 chemotherapy

detected by IMS
start the treatment

closely monitor by IMS
evaluate the treatment

Earlier diagnosis treatment, higher survival

Cancer is eradicated

Vision: Eradicating Cancer by Immunosignature Monitoring of Health
Goal: Complete Annual Wellness Test for Dogs

- A comprehensive assessment of health
- Indicative of Health Status
- Derived from a single, simple to use, low cost test
Which Vaccine is Protective?
The Immunosignature Distinguishes Infected from Mock Infected Mice

73 Peptides Selected Using expression profile mapping.
Killed PR8 Predicted as More Effective Than Seasonal Trivalent Vaccines

- Killed vs Mock: 60, 20, 53
- Live vs Mock: 34, 4, 69
- Live vs Mock: 107, 9, 64
Challenge Results

Legutki and Johnston, 2013, PNAS (Embargoed In Press)
Immunosignature of Seasonal TIV Survivors
Groups Survivors with The Killed PR8 Immunized

38 Peptides Selected using a T test with $p < 0.05$
Benjamani and Hochberg MTC and $>1.3x$ Fold Change

Legutki and Johnston, 2013, PNAS (Embargoed In Press)
TIV Recipients Who Died Lack Specific Reactivity

Whole PR8 Virus ELISA in 2006-2007 TIV Recipients

Legutki and Johnston, 2013, PNAS (Embargoed In Press)
Missing Reactivity Aligns to NA195-219

Survived  Died

Legutki, JB & Johnston, SA: PNAS (Embargoed In Press)
Summary

- Immunosignature Technology is a Universal Diagnostic Platform

- It is Simple, Sensitive and Potentially Inexpensive

- It Also Can Be Employed as a Discovery Tool
Russian-American Collaboration

Altai State University

Professor Andrei Chapoval, Director
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  - Kathryn Sykes
  - David Smith
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  - Matthew Greving

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  - George Poste

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  - Hoda Anton-Culver, UC Irvine
  - Sam Hanash, Fred Hutchinson Cancer Center
  - Adi Gazdar, UT Southwestern
  - Adrienne Scheck, Mayo Clinic
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