Conflict of interest statement

• **Integrated Diagnostics**—blood protein diagnostics company employing targeted proteomics

• **Indi Molecular**—a company developing peptide-based, protein-capture agents for in vitro and in vivo diagnostics and as well as therapeutics

• **Arivale**—a scientific wellness company directed at consumers
The grand challenge for biology and medicine: Deciphering biological complexity
6 Blind Men and an Elephant
I Participated in Six Paradigm Changes in Biology Dealing with Complexity

1. 1970: Brought engineering to biology
2. The Human Genome Project
3. Cross-disciplinary biology
4. Systems biology
5. Systems medicine / Emergence of proactive P4 medicine
6. Bringing P4 medicine to Providence and the US Healthcare System

1970
2016

I Participated in Six Paradigm Changes in Biology Dealing with Complexity
What is Systems (P4) Medicine?

Predictive
Preventive
Personalized
Participatory
Systems features of big data: dealing with biological complexity

- Global analyses of all components—DNA, RNA, protein, etc.
- Dynamics of systems (networks)—temporal and spatial
- Integration of different data types from the system
- Large data sets reflect two types of noise—biological and technical
Dense, Dynamic Personal Data Clouds

These data clouds should be the foundation for Precision Medicine
Systems (P4) Medicine
The Network of Networks

ORGAN NETWORKS
CELLULAR NETWORKS
MOLECULAR NETWORKS
GENES

INDIVIDUAL

SOCIAL NETWORKS
Sequential Disease-Perturbation of the Four Major Networks of Prion-Induced Neurodegeneration in Mice

Prion accumulation

Glia Activation

Synaptic Degeneration

Neuronal Cell Death

Clinical Signs

Disease Transition

0 wk 7 wk

18~20 wk 22 wk

Analyzed with 10 Brain Transcriptomes across the 22 weeks of disease progression
100 Brain-Specific Blood Proteins Reflect Key Networks (SRM assays)

- **Nerve growth factor signaling**: APLP1, SNAP25, LG1, NAC M1, CLSTN2
- **Synaptic vesicle transport**: KINESIN, MAP1B, SYT3, CTNN1
- **Calcium mediated signaling**: CAMKII, PCLO, GRIA4, GLUR3, NSF, ANK2, ENO2, DOCK3, SCG3
- **Synaptic Transmission**: CAMKII, PCL0, GRIA4, GLUR3, NSF, ANK2, ENO2, DOCK3, SCG3
- **Neurogenesis**: L1CAM, CTF1, ARF3, ANK3, MAP3K12, CTN NA2, KIF3A, GFA P, CNTN1, ENC1, CRMP2, SYNAPSIN1
- **Cell surface receptor signaling**: NEUROMOD, ULIN, HUC, CAMKII, RIN3, SYNAPSIN1, RG S4, PEA15, RASGRF1, NR1
- **Cellular differentiation**: TAU, MAP2, CAMKII, EPHA5, UCHL1, NCA M1
- **Anatomical structure development**: MAP1A, SPTBN, SPTBN4, FOX G1, EPHA5, N CAM2, ELAVL3
- **GPCR signaling**: GNAO1, GNA13, GABBR1, GLUR1, GR1 A1

**Key Networks (SRM assays)**
- GNAO1, GNA13, GABBR1, GLUR1, GR1 A1
- NEUROMOD, ULIN, HUC, CAMKII, RIN3, SYNAPSIN1, RG S4, PEA15, RASGRF1, NR1
- TAU, MAP2, CAMKII, EPHA5, UCHL1, NCA M1
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- L1CAM, CTF1, ARF3, ANK3, MAP3K12, CTN NA2, KIF3A, GFA P, CNTN1, ENC1, CRMP2, SYNAPSIN1

**Networks and Proteins**
- Nerve growth factor signaling
- Synaptic vesicle transport
- Calcium mediated signaling
- Synaptic Transmission
- Neurogenesis
- Cell surface receptor signaling
- Cellular differentiation
- Anatomical structure development

**Brain-Specific Blood Proteins**
- Reflect Key Networks (SRM assays)

**Institute for Systems Biology**
Revolutionizing Science. Enhancing Life.
Dynamics mouse models for glioblastoma

Terry van Dyke NCI and Burak Kutlu and Rhishi Bargaje ISB

18 disease-perturbed networks explain most of the pathophysiology of glioblastoma

Significant disease-perturbed network changes at 1 week—months before clinical signs (progression over 4 months)
Mouse Glioblastomas 18 disease—perturbed networks

Temporal dynamics of 10 Hallmarks of Cancer

1. p53 signaling altered in 87%: Activated oncogenes, TP53 amplification in 7%, senescence, mutation, homozygous deletion in 35%

2. Homozygous deletion, mutation in 49%: MDM4 amplification in 7%

3. Apoptosis

4. Sustaining proliferative signaling

5. Evading growth suppressors

6. Resisting cell death

7. Deregulating cellular energetics

8. Genome instability & mutation

9. Inducing angiogenesis

10. Activating invasion & metastasis

11. Enabling replicative immortality

12. Tumor-promoting inflammation

13. Perturbed networks

- **2006**: Vision – Clearly articulated
- **2008**: Tipping point – Luxembourg partnership--$100 million over 5 years
- **2012**: Proof-of-concept – Systems(P4) medicine pilot wellness project proposed
- **2015**: Precision medicine initiative – Obama’s 2015 Precision Medicine Initiative: dense, dynamic, personalized data clouds
- **2016**: P4 medicine to patients – Affiliation between ISB and Providence Health & Services
Systems-driven Novel and Emerging Technologies

3\textsuperscript{rd} generation DNA sequencing
• Nanopore/nanochannel, single molecule, electronic detection

Nanostring Instrument
• Single molecule nucleic acid analyses, single-cell analyses, protein analyses

Peptide protein-capture agents
• For sensitive protein quantification to replace antibodies

Global mass spectrometry
• Proteome analyses (SWATH)

Targeted mass spectrometry (SRM)
• Analyze 100 proteins from complex samples like blood

Single-cell highly multiplexed omic and phenotypic analyses
• Detecting quantized cell populations and tipping points
Systems-driven Strategies Transforming Healthcare

Follow disease progression from beginning to end—delineating dynamical disease-perturbed networks

• Enable mechanistic insights, diagnosis, therapy & prevention

Transform blood into a window to distinguish health from disease

• Disease diagnostics, assess drug toxicity, assess wellness
• Human examples: lung cancer, PTSD, liver toxicity, liver hepatitis

Longitudinal, individual high-dimensional data clouds (dense, dynamic, personal data clouds)

• Billions of data points, capturing genetic & environmental contributions to wellness and disease

Family genome sequencing—identifying disease genes

• Identify disease, wellness genes and drug-intolerant genes
Making Blood a Window into Wellness and Disease—with a systems-driven targeted mass spectrometry approach

• Paul Kearney—Integrated Diagnostics

• Nathan Price—ISB
Indeterminate Pulmonary Nodules

Is this cancer?

~3 million cases annually in the USA
Systems Approach to Distinguishing Benign from Malignant Lung Cancer Nodules (with Integrated Diagnostics)

- 371 SRM assays for lung cancer tissue/190 detectable in the blood
  - Differentially secreted (normal vs. neoplastic)
  - Differentially shed from cell surface (normal vs. neoplastic)
  - Candidates captured from the literature
- Discovery samples—analyze all 190 detectable proteins
  - 72 cancer vs. 72 benign/from four sites
- Discovery algorithm for “cooperative” proteins
  - Select the 32 (out of 190) best proteins for distinguishing nodules
  - A million random panels of 10 of 32 best proteins were scored
  - Identified 13 proteins that were highly “cooperative”—generally in most effective panels
- Validation study—13-protein panel—identifies 36% of benign nodules
  - 52 cancer vs. 52 benign/from 4 old sites plus 1 new site
  - Identifies 36% of the benign lung nodules
- Integrated Diagnostics commercialize the panel of 13 blood proteins in Q4 2013
- Integrated Diagnostics develops a two-protein blood panel that identifies more than 50% of the benign lung nodules 2016

Red indicates systems-driven approaches.
Systems Driven Blood Targeted Human Blood Proteomics Biomarkers

- Distinguish benign and neoplastic lung nodules—two blood proteins can identify with more than 95% specificity more than 50% benign nodules—saving US healthcare more than $4.5 billion/year

- Preterm birth—distinguish at 19 weeks mothers destined to have preterm birth from those with normal births (Sera Prognostics in Salt Lake City)—in time for actionable therapy

Discovery

- Post traumatic stress disorder (PTSD)—blood proteins that allow one to distinguish from plasma 50 normal Afghanistan soldiers and 50 PTSD Afghanistan soldiers (ISB)

- Glioblastoma—distinguish normal from patients with glioblastoma
Peptide protein-capture agents will replace antibodies

Jim Heath  Caltech
Indi Molecular
Circular 5-mer D amino acid peptides are positioned on a protein and joined together with click chemistry.

Protein selectively couples only those peptide library elements that fit onto its surface in just the right fashion.
Breakthrough Technology #2: Precise Targeting of Protein Epitopes

- Synthetic epitope derived from AA sequence of target protein and modified to support a CLICK reaction
- Epitope (fragment) serves as a catalytic scaffold for first step in PCC creation: Anchor selection
- Full PCC developed from selected anchor utilizing the native target to catalyze the next reaction
- Not easily achieved by biologics
PCC agent drugs RAS

Data
• reveals ~10:1 selectivity for G12D vs wt
• PCC Agent is cell-penetrant
• PCC Agent is an inhibitor of RAS
• Selectivity for G12D variant unknown
• Xtal structure of KRAS$^{G12D}$ with PCC underway
• Med-chem improvements of PCC underway

RAS inhibition data in pancreatic cancer cell line

Earlier generation related approach: selectively drugging Akt$^{E17K}$

Peptide Protein Capture Agents--Features

• Stable—send to Africa in an envelop
• Sensitive—each monomer a log increase in sensitivity
• Digital—synthesize unlimited quantities
• Reduced cross reactivity--may be precisely directed at epitopes—hence avoids much of the cross-reactivity problems that plague antibodies
• Can be adapted to large-scale production through automation—easy to produce—have more than 50 PPC reagents—never failed

• Functions
  – In vitro diagnosis
  – In vivo diagnosis
  – Therapeutic reagents—possibly lacking cross reactivities

• Prediction—will replace monoclonal antibodies with 10-15 years
The Emergence of P4 Medicine

Converging Megatrends

- Systems Medicine
- Social Networks
- Big Data/Analytics
- Digital Revolution

P4 MEDICINE
**P4 Medicine**

- Proactive
- Individual
- Wellness & disease
- Personalized data clouds
- Personalized data clouds for clinical trials (N=1 experiments)
- Patient activated social networks

**Contemporary Medicine**

- Reactive
- Population
- Only disease
- Averaged patient populations
- Averaged patient populations for clinical trials

Institute for Systems Biology
Imprecision Medicine: Time for N=1 Drug Trials to Stratify Disease Subtypes

For every person in the US that the 10 highest grossing drugs do help (orange), they fail to improve the conditions of between 3 - 24 people (blue).

Conceptual Themes of P4 Medicine

P4 MEDICINE
Predictive, Preventive, Personalized, Participatory

Dense, dynamic, personal data clouds will enable us to:

- Optimize human potential / wellness
- Identify earliest wellness to disease transitions
- Follow disease, response to therapy and return to health
Understanding Scientific Wellness is Key

More than half of all children born today in developed countries can expect to celebrate their 100th birthday.

The 108 Person Wellness Project 2014
Principal Investigators: Lee Hood and Nathan Price
Assays / Measurements

Creating dense, dynamic, personal data clouds

**GENOME**
Whole Genome Sequencing
SNPs Millions

**SELF-TRACKING**
Continual self-tracking & lifestyle monitoring

**LABS**
Detailed lab tests 3x (blood, urine, saliva)
Clinical chem. 150
Metabolites 1700
Proteins 400

**MICROBIOME**
Gut Microbiome 3x

Creating dense, dynamic, personal data clouds.

** graphical representation of the timeline:**
- **April**: Intro
- **May**: Round 1
- **June**: Coaching Sessions
- **July**: Round 2
- **August**: Coaching Sessions
- **September**: Round 3
- **October**: Coaching Sessions
- **November**: Coaching Sessions
- **December**: Coaching Sessions
Wellness Coaching for Participants
A Critical Component of Scientific Wellness

Wellness Coach
Sandi Kaplan, MS, RD

Study Physician
Craig Keebler, MD
Data analysis of the dense, dynamic, personal clouds of the 108 Wellness Pioneers
The Hubble Telescope allows us to probe the dark matter of the universe just as dense and dynamic personal data clouds allow us to probe the dark matter of human biology and disease.
Deriving Insights from Data: New Frontiers of Precision Medicine
Community analysis of the P100 correlation network
Total cholesterol community
We can determine your genetic risk for at least 60 diseases.
Individuals contain different subsets of the 59 GWAS variants that affect cholesterol levels

Each individual harbors a subset of the universe of possible variants that affect a trait.

Although each variant alone has only a small effect, the cumulative effect of an individual’s variant set can add up to significant differences between individuals.
LDL cholesterol in Participants Shows Monotonic Relationship with ‘Genetic Risk’

LDL Cholesterol Levels vs Genetic Risk (59 variants)

- Genetic Risk
- Baseline (No Meds)

Number of participants in this risk range:

- Very Low: 5
- Low: 15
- Medium: 35
- High: 30
- Very High: 10

Genetic risk vs. Disease state

LDL Cholesterol (mg/dL)
GWAS variants have been determined for about 60 diseases and traits

<table>
<thead>
<tr>
<th>ADHD</th>
<th>COPD</th>
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<tbody>
<tr>
<td>Alzheimer's disease</td>
<td>Crohn's disease</td>
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<td>Anorexia</td>
<td>Esophageal cancer</td>
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<tr>
<td>Asthma</td>
<td>Gout</td>
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<td>Atrial fibrillation</td>
<td>Grave's disease</td>
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<tr>
<td><strong>Breast cancer</strong></td>
<td>Hematocrit</td>
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<tr>
<td>Bipolar disorder</td>
<td>Hypertension</td>
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<td>Blood pressure</td>
<td>Hypothyroidism</td>
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<td>Bone mineral density</td>
<td>Inflammatory bowel disease</td>
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<td>Inflammation</td>
<td>Iron levels</td>
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<td><strong>Calcium</strong></td>
<td>Lung Cancer</td>
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<td>Cardiovascular disease</td>
<td>Lupus</td>
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<td>Celiac disease</td>
<td>Macular degeneration</td>
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<td>Metabolic syndrome</td>
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<td><strong>Colorectal cancer</strong></td>
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<td>Coronary heart disease</td>
<td>Multiple sclerosis</td>
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<td>Myopia</td>
<td>Obesity</td>
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<td><strong>Ovarian cancer</strong></td>
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<td>Primary biliary cirrhosis</td>
<td>Prostate cancer</td>
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<td>Psoriasis</td>
<td>Rheumatoid arthritis</td>
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<td>Stroke</td>
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<td>Type 1 Diabetes</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Urate levels</td>
</tr>
</tbody>
</table>
State Transitions: Wellness and Disease

• Wellness to greater wellness
• Wellness to less wellness
• Wellness to earliest disease transitions—learning how to achieve early reversal will be the preventive medicine of the future
• Disease progression to therapy (to wellness)—powerful disease stratification and identification of effective therapies
• Transitions due to the individual acting upon actionable possibilities
Initial Clinical Labs Discovery: High Rate of Actionable Clinical Lab Results

- The 108 “well” participants had a high rate of initial abnormal lab results
- **100%** of the participants had actionable recommendations from their blood results

Baseline Blood Results:
- Cardiovascular: 59%
- Inflammation: 68%
- Nutrient Abnormalities: 91%
- Diabetes Risk: 54%
Participant Insights

I can take control of my health with the proper data/coaching.

Your genome does not control your destiny – just your potential.

We are less well than we think. Everyone has multiple actionable possibilities.
Scientific Wellness: Two Integrated Directions

Arivale
- A consumer facing scientific wellness company
- 10,000 individuals in the first 18 months
- Transform how biotech industry operates

ISB-Providence
- Dense, dynamic, personal data clouds
- Research to validate wellness metrics
- Research for better assays
- Improve wellness analytics
- Optimize wellness
- Study wellness to disease transitions
- Study disease progression, response to therapy and transition to wellness
Providence-ISB Affiliation

ISB is the research arm of Providence
LH is chief science officer of Providence
Initiating Four Translational Pillars
**Providence Health & Services**

States served: 7

<table>
<thead>
<tr>
<th>Hospitals</th>
<th>50</th>
</tr>
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<tr>
<td>Physicians</td>
<td>5000</td>
</tr>
<tr>
<td>RNs</td>
<td>36,000</td>
</tr>
<tr>
<td>Unique patients served each year</td>
<td>5 million</td>
</tr>
<tr>
<td>Total Assets</td>
<td>$20 billion</td>
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</tbody>
</table>

*Third largest not-for-profit healthcare system in the US*

*Integrated Medical Electronic Health Records for 30 million patients*
TRANSLATIONAL PILLAR:
Scientific Wellness
TRANSLATIONAL PILLAR:
Breast Cancer Survivor Wellness
TRANSLATIONAL PILLAR:
Alzheimer’s Disease
The Future of Healthcare

- P4 medicine to healthcare
- Precision medicine—employ dense, dynamic data clouds for each individual to allow one to explore interactions between genetics and environment
- Optimize wellness through actionable possibilities
- Reverse disease at its earliest transition point: Prevention
- Scientific Wellness: A lifetime journey
- Healthcare costs dramatically reduced
- Creating a Scientific Wellness industry
- Transformation of biotech, pharma, diagnostic, nutrition industries
- Democratization of healthcare through digitization of medicine
ISB Hundred Person Wellness Project – Team

Project Leadership
- Leroy Hood, MD, PhD PI
- Nathan Price, PhD, Co-PI
- Sean Bell, Business Director

Participant Engagement
- Jennifer Lovejoy, VP Clinical Affairs
- Sandi Kaplan, Wellness Coach
- Craig Keebler, M.D., Study Physician

External Relations
- Gretchen Sorensen, Consultant

Project Management
- Sean Bell, Business Director
- Kristin Brogaard, Project Manager
- Sara Mecca, Project Assistant
- Mary Brunkow, Project Coordinator

Data Analytics
- Nathan Price, Analytics Lead
- Gustavo Glusman, Genomics
- Andrew Magis, Multi-Omics

Medical Advisory Board
- Robert Green, M.D.
- Michael Raff, M.D.
- Sarah Speck, M.D.