The Frontiers of Precision Medicine:
You Are There!

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Getting to Precision Medicine: Biomarkers Are the Driving Force

Vision of 21st Century Medicine: Greater Efficiency and Efficacy

- Better understanding of the biology of disease
- Diagnosis based on molecular characterization of disease
- Rational treatment using molecularly targeted agents
- Connection of research and clinical practice in seamless feedback loop

ALL OF THESE ARE BIOMARKER-DRIVEN
Biomarkers and the Laboratory

Biomarker: A *measurable* characteristic used as an indicator of a biological state or condition

Usually a protein or a set of proteins measured in cells, tissue, blood but may be any class of biomolecule – DNA, RNA, miRNA, other
Uses of Biomarkers in Precision Medicine

- Early detection, surveillance
- Prognosis, prediction
- Choice of treatment
- Monitoring of treatment
- Monitoring of disease
- Drug development – clinical trials:
  - patient selection
  - efficacy, toxicity
  - surrogate endpoints
Biomarkers: Many Are Reported, Few Are Qualified

Estimated number of papers documenting thousands of claimed biomarkers

150,000

Estimated number of biomarkers routinely used in the clinic

100

Source: Poste G. Nature 469, 156-157 13 Jan 2011
Consequences for Product Development - Massive Attrition, Long Duration, High Costs

The average drug developed by a major pharmaceutical company now costs at least $5 billion, and it can be as much as $11 billion.

- The Truly Staggering Cost of Inventing New Drugs.
  Matthew Herper, Forbes 2/20/12
- The Cost of Creating a New Drug Now $5 Billion, Pushing Big Pharma to Change.
  Matthew Herper, Forbes 8/11/13

5-10,000:1 chance of success  12 Years  ~ US$ 1.6 B

Time and attrition are both directly related to lack of validated biomarkers of efficacy and toxicity
Sad Status of Protein-Based Biomarkers

- Few biomarker candidates are being approved for clinical use by FDA/EMA
- Approval rate is steadily declining rate

![Graph showing the number of new protein analytes approved by year of FDA approval.](source-image-url)

- Biggest problem is non-reproducibility across labs and studies

Source: Based on data from FDA and Plasma Proteome Institute
Amgen attempts to verify results of 53 landmark studies in oncology and hematology; Only 6 (11%) could be reproduced.
How Widespread Are Failures to Reproduce Published Biomedical Science?

- Mass spec diagnostic for ovarian cancer – results due to experimental artifact and bias – control and experimental groups run separately (Lancet, 2002)
- Five of 7 largest molecular epidemiology cancer studies did not classify patients better than chance (JNCI, 96:2004)
- Microarray drug sensitivity signatures – from cell lines – to predict patient response (named one of top100 breakthroughs in 2006) could not be reproduced in large clinical trial in 2009 (Nature Medicine, 2006)
- Of 18 published microarray studies, only 2 were reproducible (Science, 2011)
- Bayer scientists can reproduce only 20-25% of 67 key published experiments and halts 2/3 of its target validation projects as a result (Nature Reviews Drug Discovery 10, 712 doi:10.1038/nrd3439-c1, 2011)
- Amgen’s team of 100 scientists could reproduce only 11% of 53 seminal studies published on reported drug targets or toxicity (Nature 483, 531-533 doi:10.1038/483531a, 2012)
Reproducibility Rate of 10-30% in Academic Biomedical Science

- For biomedical businesses relying on academic discovery to drive product development (like pharma), flipping a coin would be superior to reading *Science* or *Nature* in making business decisions.

- US government spends nearly $31 billion in science funding through the NIH every year, mainly for research grants to academic scientists
  
  - 10% reproducibility rate ➔ 90% of this money ($28 billion) is wasted

- Wasted money, wasted time, lost opportunities

- Pollution of the biomedical literature by bad studies and bad data:
  
  - What do we really know? What can we really trust?

- Why should patients and the public believe in what we do?
Irreproducibility in Biomedical (Biomarker) Research: A Crisis in Confidence (Public View)

Trouble at the lab

Scientists like to think of science as self-correcting. To an ala

Lies, Damned Lies, and Medical Science

Much of what medical researchers conclude if their studies is misleading, exaggerated, or flat-out wrong. So why are doctors—to a striking extent—still drawing upon misinformation in their everyday practice? Dr. John Ioannidis has spent his career challenging his peers by exposing their bad science.

By David H. Freedman

Why Most Published Research Findings Are False

John P. A. Ioannidis
Published: August 30, 2005  DOI: 10.1371/journal.pmed.0020124

Abstract

There is increasing concern that most current published research findings are false. The probable number of other studies on the same question, and, importantly, the ratio of true to no relation framework, a research finding is less likely to be true when the studies conducted in a field are and lesser preselection of tested relationships; where there is greater flexibility in designs, definitions, and other interest and prejudice, and when more teams are involved in a scientific field in choosing designs and settings, it is more likely for a research claim to be false than true. Moreover, for mar simply accurate measures of the prevailing bias, in this essay, I discuss the implications of these

“This is one of medicine's dirty secrets: Most results, including those that appear in top-flight peer-reviewed journals, can't be reproduced"
Irreproducibility in Biomedical (Biomarker) Research: Cultural Contributing Factors

In science, irreproducible research is a quiet crisis

- Few scientists attempt to repeat their own studies
- Publications often based on the one time out of multiple attempts that it actually worked
- External validation (by another lab) is extremely rare
- Few, if any analyses, focus on the quality and consistency of the biological materials that are the test subjects
- Shockingly, this is also true in clinical medicine
Quality Data Begins with Quality Analytes

Garbage in…

...Purgamentum init, exit purgamentum.

Diamonds in……

…Garbage out

Modified from Jerry Thomas
Here Today, Gone Tomorrow

In the early 2000s, only about 30 retraction notices appeared annually. This year, the Web of Science is on track to index more than 400 (see 'Rise of the retractions') — even though the total number of papers published has risen by only 44% over the past decade.

You can’t even be sure you know what you thought you did!

- **MISCONDUCT**
  - Self-plagiarism: 11%
  - Honest error: 28%
  - Other: 11%

- **Fabrication or falsification**
  - Plagiarism: 17%
  - Irreproducible results: 17%
White House Takes Notice of Irreproducibility in Science and Seeks Public Input

August 21, 2014

- Federal Register:

- The Office of Science and Technology Policy and the National Economic Council request public comments to provide input into an upcoming update of the *Strategy for American Innovation*.

- “Given recent evidence of the irreproducibility of a surprising number of published scientific findings, how can the Federal Government leverage its role as a significant funder of scientific research to most effectively address the problem?”
Taking Action in the Public Sector

• National Institutes of Health: Rigor and Reproducibility Workshop, 2014
  – Joint meeting with Science and Nature publishing groups
  – Principles and Guidelines for Reporting published
    • “Sufficient information about sample collection must be provided to distinguish between independent biological data points and technical replicates”
  – Refers to rigor in use/description of biological reagents (antibodies), cell lines and animals, but **omits reference to human biological materials**!
  • NOT because it is unimportant
  • Complex for the NIH and publishers to approach because this issue is embedded in the world of clinical medicine and its requirements, standards and legal restrictions
Where is the Variability Causing Non-Reproducibility and How Is it Controlled?

- **Place** where test is done
  - CLIA/CAP laboratory accreditation
- **People** doing the test
  - Education
  - Proficiency testing
  - Licensure
- **Platforms** used for testing
  - CDRH approved devices
- **Processes** followed for testing
  - SOPs
  - Quality management
- **Patient samples** to be tested
  - WILD WEST
Evolution of Biomarker Testing

Unianalyte Tests

Multianalyte Tests

Omics Analyses

Networks and Systems

Raising the Bar for Specimen Quality Even Further!
Biospecimens – A Likely Source of Biomarker Irreproducibility at Every Level

Molecular Data ➔ Diagnosis / Therapy

PERSONALIZED CANCER CARE

Biospecimen Analysis ➔ Biospecimen Collection

QUALITY HERE

Biospecimen Processing and Banking

DETERMINES QUALITY HERE
Pre-analytical Factors Affect Both Molecular Composition and Molecular Quality

Specimen is **viable** and biologically reactive

Molecular composition subject to further alteration/degradation

Factors (examples):
- Antibiotics
- Other drugs
- Type of anesthesia
- Duration of anesthesia
- Arterial clamp time

Factors (examples):
- Time at room temperature
- Temperature of room
- Type of fixative
- Time in fixative
- Rate of freezing
- Size of aliquots

Time 0
Cold Ischemia and Molecular Assay Results

HER2 IHC and FISH in Breast Cancer:
Loss

HER2/CEP17 = 0.98
HER2/CEP17 = 0.29

30 min
2 hr
4 hr delay

Without knowledge about tissue processing methods and assurance of rapid tissue fixation, protein expression data are unreliable, and understanding of pathway activity is impossible.

- Hartmut Juhl, CEO Indivumed
Blood Collection and Plasma Processing: Biomarkers and Circulating Tumor Cells

Blood Draw Procedure

Collection Tubes and Order of draw

Processing Procedure, Temperature and Time

Distribution & Storage

Patient Consent and Preparation

Molecular Analysis
And It’s Getting Far More Challenging

Biospecimens and Analysis of Molecular Pathway/Network Perturbations

Multiplex Assays and Complex Signal Deconvolution Algorithms

Novel Instrumentation, Automation and Large Scale Informatics

Patient Profiling, Rational Rx and Health Monitoring

It all starts with the “Right Stuff”.

Courtesy of G. Poste
The Right Answers Depend on the Right Stuff: Challenges for DNA Sequencing Tests

- Tumor cells are mixed with normal cells
- Tumor content may be enriched by micro-dissection

- Tumors have background of normal wild-type DNA
- Challenge to detect low % mutant alleles

- Tissue fixation damages DNA
- Necrotic cells may not have amplifiable DNA

FFPET = formalin-fixed, paraffin-embedded tissue
Estimating the Changing Aspects of NGS

Is Pathology prepared for what’s coming?
More sample collection upfront; more data analysis downstream!!

From Ken Bloom, MD, GE Healthcare, June 2014
NBDA: Realizing an End-To-End, Standards-Based Approach to Biomarker Development

Standards are needed at every step and across the continuum
Biospecimens Flank End-To-End Biomarker Development

The Continuous Feedback Loop of Quality
NBDA: Understanding The Issues - Building Towards Solutions
Goal:

• Converge (agree) on the pre-analytical steps in the biospecimen lifecycle that MOST compromise the quality of tissue and blood for cutting edge molecular analysis: NGS and proteomics

  – “Top 10 List”

• Identify where the greatest value can be delivered in the control of pre-analytical variation (biggest quality bang for the buck)

  – “Top 5 List”
Think: Pareto Principle (20/80 rule)

For many events 80% of the effects come from 20% of the causes
## Top 5 Lists

### Tissue

1. **Time to stabilization**
   - Cold ischemia time

2. **Method of processing**
   - Section thickness
   - Mass/volume ratio
   - Temperature

3. **Method of stabilization**
   - Type of fixative
   - Time in fixative

4. **Tissue processor variables**
   - Quality of processing fluids
   - Paraffin type
   - Paraffin temperature

5. **Storage conditions**

6. (Metadata to be collected)

### Blood/Serum

1. **Time to processing**

2. **Method of acquisition**
   - Tube type
   - Draw order
   - Draw parameters (needle, vein vs. line)
   - Volume of tube fill

3. **Method of stabilization**
   - Tube type (stabilizer preset or not)
   - Tube inversions

4. **Method of processing**
   - Centrifugation speed/time
   - Temperature

5. **Storage conditions**
   - Freeze/thaw cycles

6. (Metadata to be collected)
Actions In Progress

- Pre-analytics for Precision Medicine Project Team: College of American Pathologists
- Establish performance metrics around the Top 5
  - **DATA-DRIVEN**: Validated from primary literature review and verification of guidelines from CLSI, NCI and ISBER
  - **PRACTICAL**
- Develop a Top 5 for cytology specimens
- Educate pathology workforce (pathologists, pathology assistants, medical laboratory technicians, phlebotomists)
- Implement and enforce performance metrics through the CAP Laboratory Accreditation Program checklists
- Seek new reimbursements codes, if needed
- Seek reinforcement through FDA guidance, research funder requirements
Envisioned Result

Historic transformation of practice with far-reaching impact:

• Variably variable and unknown quality $\rightarrow$ uniform, known quality that is consistent with molecular analysis

• Simultaneous impact on both clinical and research results

• “Convenience samples” for research become fit for purpose!

• A “bar” is established that may be electively raised as needed to meet requirements of specific analysis types/platforms
  
  – There will, at last, BE a bar to raise

  – It’s about time
Specimen Quality Is A Front-loaded Issue

“If you don’t have the time to do it right, when will you have the time to do it over?”

- John Wooden, Coach UCLA
The Ultimate Lab Revolution

Garbage in...

...Garbage out
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