Precision Medicine and the Biospecimen Quality Imperative

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Northwell Health Grand Rounds
December 8, 2016
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

- 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

100

Estimated number of biomarkers routinely used in the clinic

Source: Poste G. Nature 469, 156-157 13 Jan 2011
Sad Status of Protein-Based Biomarkers

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

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 Research: A Crisis in Confidence (Public View)

Unreliable research

Trouble at the lab

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

Oct 19th 2013 | From the print edition

November 2010

Lies, Damned Lies, and Medical Science

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

By David H. Freedman

November 2010

Why Most Published Research Findings Are False

John P. A. Ioannidis

Published: August 30, 2005 | DOI: 10.1377/journal.pmed.0020124

Abstract

Summary

There is increasing concern that most current published research findings are false. The probability of a 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 an and lesser preselection of tested relationships; where there is greater flexibility in designs, definiti and other interest and prejudice, and when more teams are involved in a scientific field in choose c 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 Research: A Cultural Norm (Researcher View)

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
Quality Data Begins with Quality Analytes

Garbage in...  Purgamentum init, exit purgamentum.

Diamonds in......

...Garbage out

Modified from Jerry Thomas
Here Today, Gone Tomorrow

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

• Public sector: NIH Rigor and Reproducibility Workshop, 2014
  – Joint meeting with Science and Nature publishing groups
  – Refers to rigor in use/description of biological reagents (antibodies), cell lines and animals, but **omits reference to human biological materials**

• Private Sector: The Reproducibility Project
  – Joint venture between Science Exchange and Center for Open Science
  – Independently replicating a subset of research results from 50 high-impact cancer biology studies published from 2010-2012 using the Science Exchange network of expert scientific labs also **omits reference to human biological materials**
Latest Update

• Yesterday, Congress passed the 21st Century Cures Act
• Increases funding for biomedical research and innovation:
  – $4.8 billion for NIH
  – $500 million for FDA
• Increases funding for FDA
• One provision states that the NIH must convene a working group to develop recommendations for increasing the “rigor and reproducibility” of NIH-funded scientific research and develop or update policies within 18 months.
Powerful Tools: Powerful Risks

• Technology development is exponential, not linear

• Analysis technologies become ever faster, better, cheaper

• No technology can spin straw into gold – you must begin with gold!
  
  – “Even our technology cannot save a bad sample.” – Carrie Browning, Illumina

• The technological capacity exists to produce low-quality data from low-quality analytes with unprecedented efficiency

• We now have the ability to get the wrong answers with unprecedented speed
Biospecimen Quality Drives Both Molecular Medicine and Translational Research

Biospecimen Collection →品質訂義→ Biospecimen Handling and Processing → PRECISION MEDICINE → Diagnosis / Therapy → DEVELOPMENT

Biospecimen Analysis

QUALITY HERE

DETERMINES QUALITY HERE
Rigor and Reproducibility for Biomarker Measurement in the Lab: How Is It Assured?

- **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: No requirements to control or document pre-analytics except the ASCO-CAP guidelines for breast cancer
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

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Cold Ischemia and Molecular Assay Results

HER2 IHC and FISH in Breast Cancer:
Loss of Biomarker Signal with Time to Fixation

pMAPK IHC of Colon Cancer:
Gain of Biomarker Signal with Time to Fixation

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

Hartmut Juhl, Indivumed GmbH, BRN
Expression of >15% of Genes and Up to 60% of Selected Proteins Change >2-fold during Surgery and Postsurgical Processing Time
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
Biospecimen Quality Impacts Both Clinical And Research Outcomes

Effects on Clinical Outcomes

• Potential for incorrect diagnosis

• Potential for incorrect treatment
  – Therapy linked to diagnostic test on a biospecimen

Effects on Research Outcomes

• Irreproducible results
  – Variation in mutation data
  – Variation in gene expression data
  – 11-25% reproducibility of published biomedical data

• Misinterpretation of artifacts as biomarkers
Evolution of Biomarker Testing

Unianalyte Tests

Multianalyte Tests

Omics Analyses

Networks and Systems

Raising the Bar for Specimen Quality Even Further!
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
Powerful Tools: Powerful Risks

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• The technological capacity exists to produce low-quality data from low-quality analytes with unprecedented efficiency

• We now have the ability to get the wrong answers with unprecedented speed
Molecular Analysis for Therapy Choice:
The NCI MATCH Trial To Link Targeted Cancer Drugs to Gene Abnormalities

IN THE LAB

Shoddy biopsies deny cancer patients a shot at personalized treatment

By ELIE DOLGIN  @eliedolgin
JANUARY 22, 2016
The Right Answers Depend on the Right Stuff

Tumor cells are typically mixed with normal tissue. Tumor content may be enriched by macro-dissection.

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

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

Natural and introduced inhibitors may interfere with amplification.

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

Are pathologists prepared for what’s coming?

From Ken Bloom, MD, GE Healthcare, June 2014
Biomarker Development Is a Team Sport
NBDA: Understanding the Issues in Biomarker Development and Building Solutions

The National Biomarker Development Alliance (NBDA)* Workshop

"Biomarker Discovery or Uncharted Territory"

March 26-28, 2014
The Royal Palms Resort
5200 East Camelback Road, Phoenix, AZ
Phone: 1-800-840-2010

"Mission of the NBDA: To Enable the design of a standards-based "end-to-end" system of a star of a star..."

February

"Challenges in Creating a New Generation Biomarker-Driven..."

July 14-15, 2014
The Phoenician
A Luxury Collection Resort
6000 East Camelback Road
Scottsdale, AZ 85251
www.thephoenician.com
The Process of Biomarker Development Is Siloed and Fragmented

- **Early Discovery** (Biology Verified Patient Samples)
- **Translatable Discovery** (Clinical Measure Established)
- **Assay Development** (Analyte - Reagents - Technology - Robust)
- **Assay Performance** (Analytical Validation)
- **Biomarker Qualification** ("Fit for Clinical Purpose)
- **Biomarker Validation** (Clinical Validation)

**Academia**

**Physicians**

**Regulators**

**Industry**

**Patients**

**Funding Agencies**

**Professional Bodies**
Realizing an End-To-End, Standards-Based Approach to Biomarker Development

Standards are needed at every step and across the continuum
The National Biomarker Development Alliance (NBDA) Workshop

“Biomarker Discovery or Uncharted Territory?”

THE NATIONAL BIOMARKER DEVELOPMENT ALLIANCE (NBDA) WORKSHOP

“The Biomarker(s) 510(k), PMA or Uncovering the New Pathways”

March 26-27, 2017
The Royal Palms Resort & Spa
5200 East Camelback Road, Phoenix, AZ

“The mission of the NBDA: to enable the design and implementation of a standards-based “end-to-end” system for biomarker discovery and development”

Convergence Conference on Biospecimen Challenges for Biomarker Development

“Creating a New Pathway”

NBDA WORKSHOP V

“Rethinking and Redesigning (and/or Realigning) Biomarker Discovery”

February 15, 2017
The Westin Kierland Resort & Spa
5000 E.ovable Dr., Scottsdale, AZ

“The mission of the NBDA: to enable the design and implementation of a standards-based “end-to-end” system for biomarker discovery and development”

NBDA: Understanding The Issues - Building Towards Solutions
55 Attendees – Representing All Stakeholder Groups and Points of View

- Academic genomics experts (scientists: basic and translational)
- Academic proteomics experts (scientists: basic and translational)
- Expert molecular pathologists
- CAP leadership:
  - President
  - President Elect
  - Immediate Past President
- Surgeons
- Patient advocacy group leaders: JDRF
- Funders: NCI
- Regulators: FDA
- Leadership of professional societies: ASCO, AACR
- Payers: CMS, Palmetto, Aetna, BC/BS
- Industry (Pharma, Platform manufacturers, Tissue providers): Illumina, Genetech, Caprion, Indivumed, Becton-Dickerson, Novartis, Abbott)
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

- Identify where the greatest value can be delivered in the control of pre-analytical variation (biggest quality bang for the buck)
NBDA Genomics Convergence Conference: Defining a Benchmark for Patient Biospecimens

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)
Action

- Pre-analytics for Precision Medicine Project Team: College of American Pathologists
- Verification of the Top 5 lists for Tissue and Blood Specimens from NBDA Convergence: literature review, CLIA, ISBER, NCI
- Develop a Top 5 for cytology specimens
- Establish performance metrics around the Top 5’s
  - DATA-DRIVEN
  - PRACTICAL
- 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
CAP Validated Practicable Benchmarks: Tissue

1. Time to stabilization: 60 minutes or less
2. Method of processing
   - **Section thickness:** $\leq 5 \text{ mm}$
   - **Mass/volume formalin ratio:** $\geq 4:1$, optimal $\geq 10:1$
   - Transport temperature: ambient
3. Method of stabilization
   - **Type of fixative:** 10% neutral phosphate-buffered formalin
   - **Time in fixative:** 6-24 hours (includes time in formalin in processor)
4. Tissue processor variables
   - Maintenance schedule: Manufacturer’s recommendation or a validated deviation
   - **Paraffin type:** low melt $<$60°C
   - **Total time in processor:** 7.5-8 hours (forbid non-standard practices: e.g., “topping off with non-standard solutions”)
5. Storage conditions: Ambient (e.g., 20-25°C)
6. [Metadata to be collected]: Any deviation from the above recommendations
NEW YORK (GenomeWeb) – A group of pathologists at the annual College of American Pathologists meeting last week in Las Vegas, Nevada, said the work that they are doing to raise recognition of the need for higher quality patient samples could provide significant benefits for patient care, and lead to more reliable molecular test outcomes.

The pathologists stressed the importance to the accuracy and reliability of molecular testing of developing and following standard practice guidelines and recommendations related to the provision of higher quality preanalytical patient samples.

Preanalytics are the key to the molecular quality of specimens, which in turn determines the data quality of molecular analysis, said Carolyn Compton, chief medical officer of the National Biomarker Development Alliance in Scottsdale, Arizona, during a presentation at CAP16. Little attention is paid to controlling factors that affect patient sample quality before molecular testing is done, she added.
**Envisioned Result**

Historic transformation of practice with far-reaching impact:

- Variably variable and unknown quality ➔ uniform, known quality that is consistent with molecular analysis

- Simultaneous impact on both clinical and research results

- “Convenience samples” 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
Our Challenge

Garbage in...

...Garbage out