Specimens and Standards: Banking on Gold for Biomarker Development in Neurofibromatosis

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Biomarkers and Diagnostics World Congress
Philadelphia, PA
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Disclosures

- Nothing to disclose
The Vision for NF Research

- 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
Molecular Biomarkers

**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
Biomarkers: Many Are Reported, Few Are Qualified

150,000 Estimated number of papers documenting thousands of claimed biomarkers

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
Consequence: The Product Development Pipeline - 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
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)
Biomedical Science Reproducibility Rate of 10-30%

- Flipping a coin would be superior to reading *Science* or *Nature* in making business decisions for Pharma.

- 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

  - Additional waste in privately funded science

- 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?
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 probability that a research claim is false may range from about 15% (for those finding in the top most journals) to 90% (for most research in some fields). Such research outcomes are even more likely to include false results. What is the cause of these problems? One explanation is that published research results are often much more highly biased than is generally appreciated. Authors, journal editors, and reviewers may often assume that their research is true and therefore fail to defend it in a vigilant way. In this essay, I discuss the implications of these problems and suggest several ways to improve the reliability of research results.
A Cultural Norm in Biomedical Science

• 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
# Sources of Bias in Molecular Marker Research in Cancer

- David F. Ransohoff and Margaret L. Gourlay, 2010

**Table 1. Sources and “Locations” of Bias in Marker Research**

<table>
<thead>
<tr>
<th>Source of Bias</th>
<th>Before</th>
<th>After</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Features of subjects, determined in selection:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>Cancer subjects are male, whereas control subjects are mainly female. Bias: Assay results may depend on sex.</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comorbid conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen collection</td>
<td>X</td>
<td></td>
<td>Cancer specimens come from one clinic, whereas controls come from a different clinic. Bias: Assay results may depend on conditions that differ between clinics.</td>
</tr>
<tr>
<td>Specimen storage and handling</td>
<td>X</td>
<td>X</td>
<td>Cancer specimens are stored for 10 years because it takes longer to collect them, whereas control specimens are collected and stored over 1 year. Bias: Assay results may vary with duration of storage, or with different numbers of thaw-freeze cycles.</td>
</tr>
<tr>
<td>Specimen analysis</td>
<td></td>
<td>X</td>
<td>Cancer specimens are run on one day, whereas control specimens are run on a different day. Bias: Assay results may depend on day of analysis in a machine that “wanders” over time.</td>
</tr>
</tbody>
</table>

NOTE. The table shows examples of different sources of bias and the location of the bias before or after specimens are received in the laboratory. The list is not exhaustive; other biases may be important, and the biases listed may or may not be important in any given research study, depending on details of biology and technology (i.e., what is being measured and how it might be influenced).
Quality Data Begins with Quality Analytes

Garbage in... Purgamentum init, exit purgamentum.

Diamonds in......

...Garbage out

Modified from Jerry Thomas
The US Takes Action on Irreproducibility

- 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 any reference to human biological materials

- Private Sector: The Reproducibility Project in Cancer Biology, 2013
  - 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 any reference to human biological materials
Rigor and Reproducibility for Biomarker Measurement in the Clinical Lab: How Is It Assured?

- **Place** where test is done
  - CLIA/CAP laboratory accreditation
- **People** doing the test

> More is known about the quality of beef in the supermarket than is known about the quality of human biospecimens used in research

- SOPs
- Quality management
- **Patient samples** to be tested
  - WILD WEST
Biospecimens Driving Progress for Patients

Molecular Data → Diagnosis / Therapy

Biospecimen Analysis → DETERMINES QUALITY HERE

CYCLE OF PROGRESS IN BIOMEDINE

Biospecimen Collection → QUALITY HERE

Biospecimen Processing and Banking
Pre-analytical Factors Affect Both Molecular Composition and Molecular Quality

Specimen is viable and biologically reactive

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

Molecular composition subject to further alteration/degradation

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Molecular composition subject to further alteration/degradation
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
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 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
- Molecular Analysis
- Patient Consent and Preparation

Processing Procedure:

1. Blood Draw Procedure
2. Collection Tubes and Order of draw
3. Processing Procedure, Temperature and Time
4. Distribution & Storage
5. Molecular Analysis
6. Patient Consent and Preparation
And It’s Getting Far More Challenging

It all starts with the “Right Stuff”.

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

Courtesy of G. Poste
Powerful Tools: Powerful Risks

• Technology development is exponential, not linear

• Analysis technologies become ever faster, better, cheaper

• 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

• No technology can spin straw into gold – you must begin with gold!
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
Biospecimens Flank
End-To-End Biomarker Development

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

The National Biomarker Development Alliance (NBDA)* Workshop

The National Biomarker Development Alliance (NBDA) Workshop

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Convergence Conference on Biospecimen Challenges for Biomarker Development

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

March 26-28, 2021

The Royal Palms Resort & Spa
5200 East Camelback Road, Phoenix, AZ 85018

Phone: 1-602-904-3510 Fax: 1-602-904-3520

*Mission of the NBDA: To enable the design, development, and implementation of a standards-based “end-to-end” system for biomarker discovery and validation in the context of patient care.

February 2021

Healthcare conference for biomarker development

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NBDA Convergence Conference: The Top 10 List

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*)
Think: Pareto Principle (20/80 rule)

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

## Tissue

<p>| | |</p>
<table>
<thead>
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| 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

<p>| | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Time to processing</td>
</tr>
</tbody>
</table>
| 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) |
• Pre-analytics for Precision Medicine: 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
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

• A “bar” is established that may be electively raised as needed to meet requirements of specific analysis types/platforms

• This will confer a baseline degree of quality and consistency for NF patients treated anywhere

• A networked biobank of NF institutions can implement now and can raise the bar as new ideas and new analysis technologies require
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
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Realizing an End-To-End, Standards-Based Approach to Biomarker Development

Standards are needed at every step and across the continuum