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

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CMO, National Biomarker Development Alliance
CMO, ASU Complex Adaptive Systems Institute

Biomarkers and Diagnostics World Congress
Philadelphia, PA
May196, 2016

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

↑

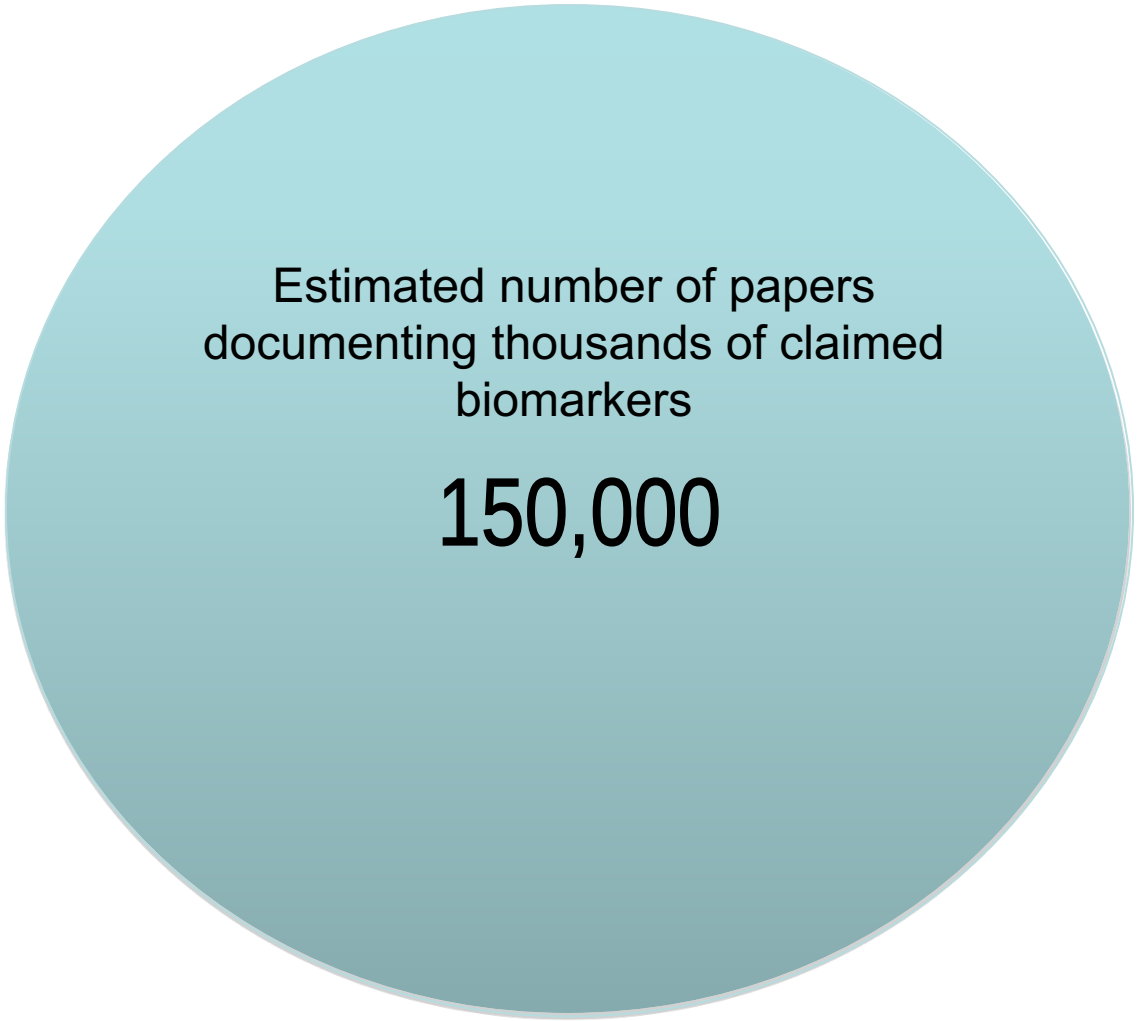
ALL OF THESE ARE BIOMARKER-DRIVEN

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



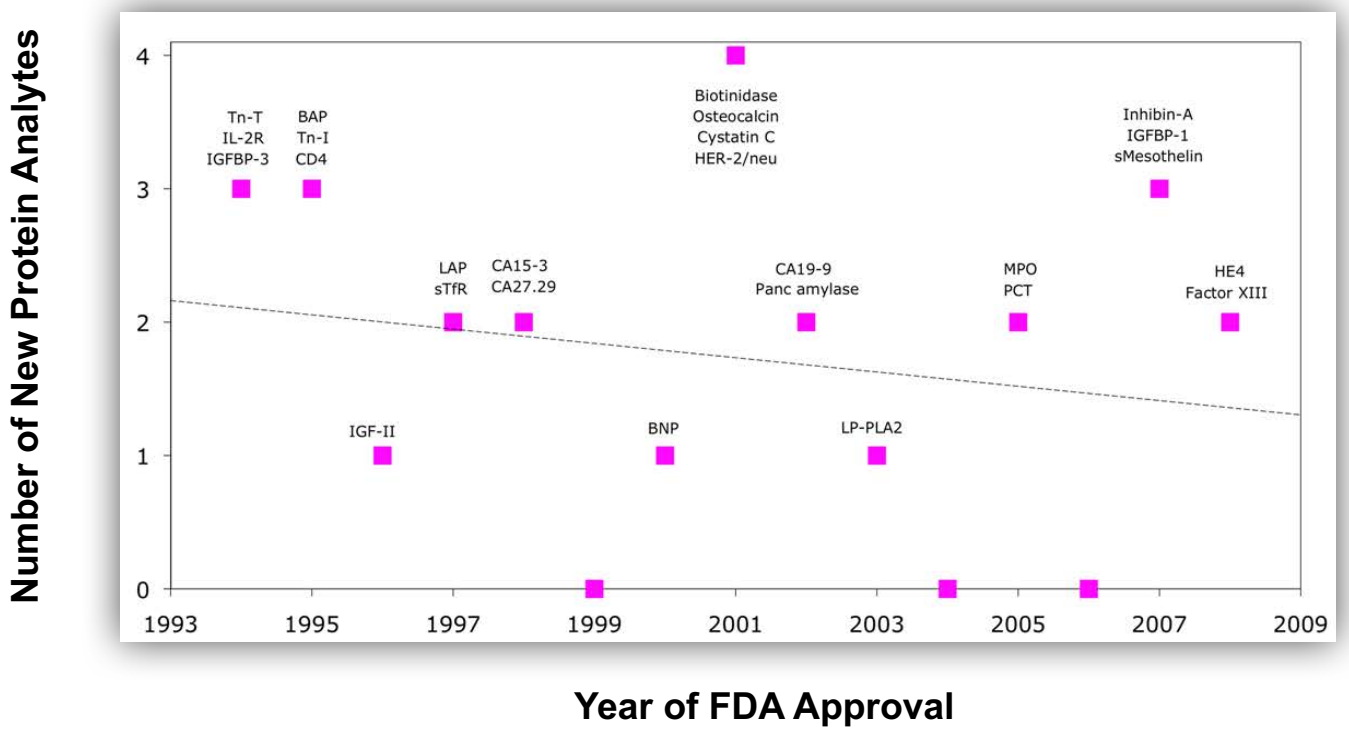
100

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**

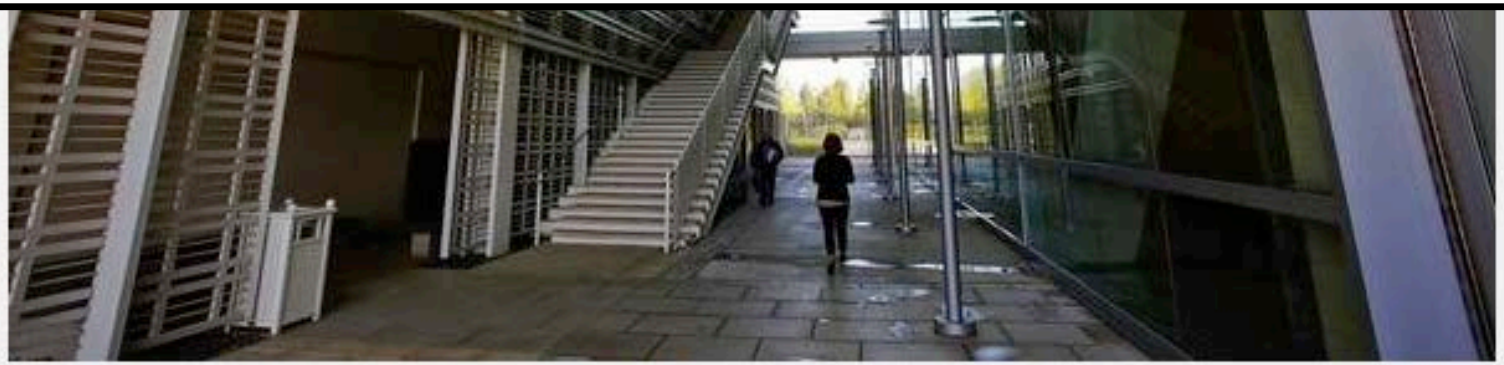
Time and attrition are both directly related to lack of validated biomarkers of efficacy and toxicity

Science has lost its way, at a big cost to humanity

Researchers are rewarded for splashy findings, not for double-checking accuracy. So many scientists looking for cures to diseases have been building on ideas that aren't even true.

Los Angeles Times, October 27, 2013

Amgen attempts to verify results of 53 landmark studies in oncology and hematology;
Only 6 (11%) could be reproduced.



A few years ago, scientists at Amgen set out to double-check the results of 53 landmark papers in cancer research and blood biology. Only six could be proved valid. Above is an Amgen building in Thousand Oaks. (Anne Cusack, Los Angeles Times / April 25, 2013)

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?

Public Crisis in Confidence

The Economist

World politics | Business & finance | Economics

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

the Atlantic November 2010

Lies, Damned Lies, and Medical Science

MUCH OF WHAT MEDICAL RESEARCHERS CONCLUDE IN 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

PLOS | MEDICINE

Why Most Published Research Findings Are False

John P. A. Ioannidis

Published: August 30, 2005 • DOI: 10.1371/journal.pmed.0020124

Abstract

Summary

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

The Economist

Washington's lawyer surplus
How to do a nuclear deal with Iran
Investment tips from Nobel economists
Junk bonds are back
The meaning of Sachin Tendulkar

HOW SCIENCE GOES WRONG

99 Einsteinium

THE NEW YORKER

ANNALS OF SCIENCE

THE TRUTH WEARS OFF

Is there something wrong with the scientific method?

BY JONAH LEHRER

DECEMBER 13, 2010

On September 18, 2007, a few dozen neuroscientists, psychiatrists, and drug-company executives gathered in a hotel conference room in Brussels to hear some startling news. It had to do with a class of drugs known as atypical or second-generation antipsychotics, which came on the market in the early nineties. The drugs, sold under brand names such as Abilify, Seroquel, and Zyprexa, had been tested on schizophrenics in several large clinical trials, all of which had demonstrated a dramatic decrease

December 2011

THE WALL STREET JOURNAL.

WSJ.com

HEALTH INDUSTRY | DECEMBER 2, 2011

Scientists' Elusive Goal: Reproducing Study Results

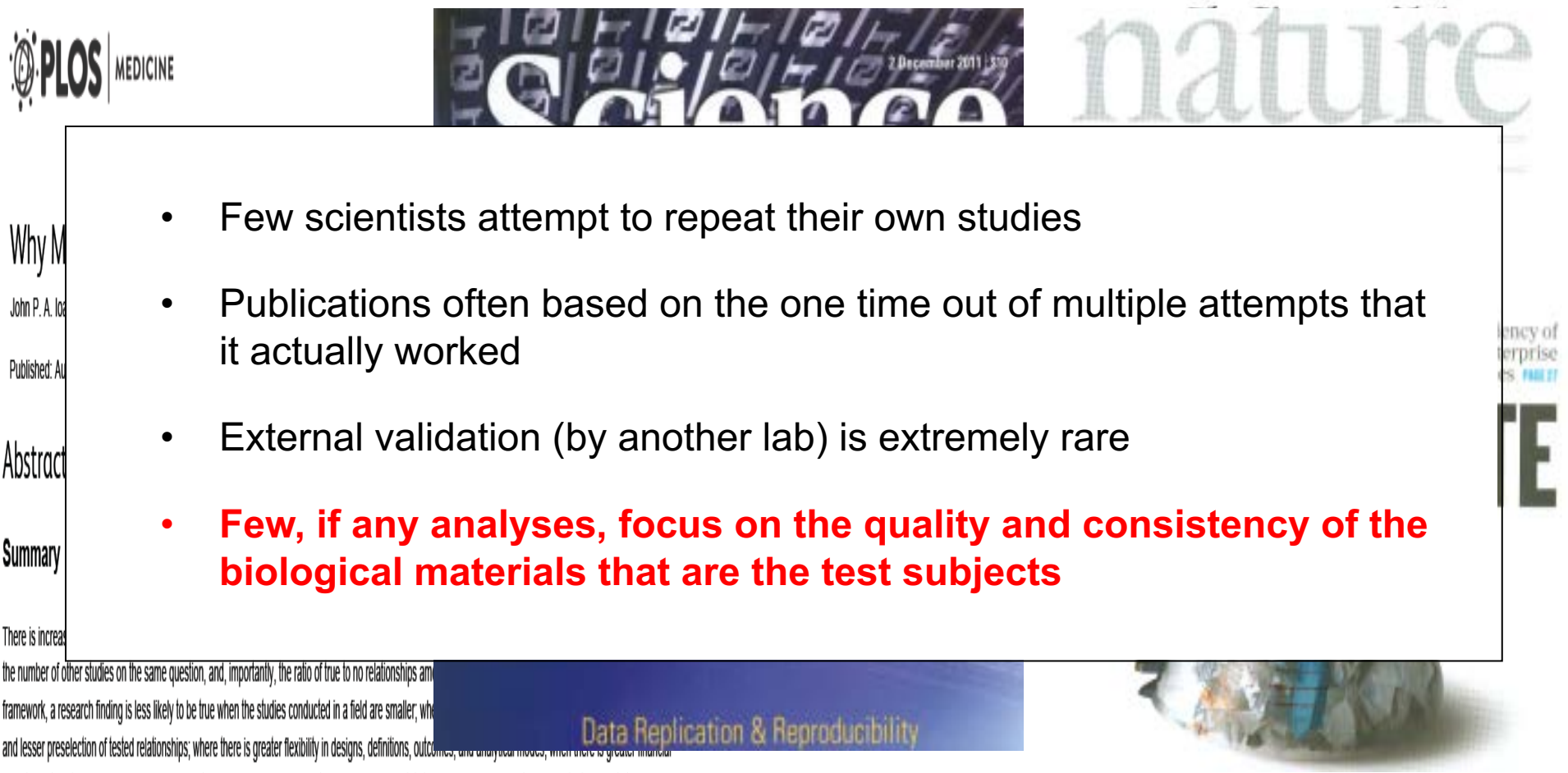
By GAUTAM NAIK

Two years ago, a group of Boston researchers published a study describing how they had destroy targeting a protein called STK33. Scientists at biotechnology firm Amgen Inc. quickly pounced dozen researchers to try to repeat the experiment with a goal of turning the findings into a drug.

"This is one of medicine's dirty secrets: Most results, including those that appear in top-flight peer-reviewed journals, can't be reproduced"

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

JOURNAL OF CLINICAL ONCOLOGY

Official Journal of the American Society of Clinical Oncology

Table 1. Sources and "Locations" of Bias in Marker Research

Source of Bias	Location of Bias: Before or After Specimens Are Received in the Laboratory: Before	After	Example
Features of subjects, determined in selection: Age Sex Comorbid conditions Medications	X		Cancer subjects are male, whereas control subjects are mainly female. Bias: Assay results may depend on sex.
Specimen collection	X		Cancer specimens come from one clinic, whereas controls come from a different clinic. Bias: Assay results may depend on conditions that differ between clinics.
Specimen storage and handling	X	X	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.
Specimen analysis		X	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.

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 (ie, what is being measured and how it might be influenced).

Quality Data Begins with Quality Analytes

Purgamentum init,
exit purgamentum.

Garbage in…

…Garbage out

Diamonds in……

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

DETERMINES QUALITY HERE

CYCLE OF PROGRESS IN BIOMEDINE

Biospecimen Analysis

Biospecimen Collection

QUALITY HERE

Biospecimen Processing and Banking

Pre-analytical Factors Affect Both Molecular Composition and Molecular Quality

Specimen is viable and biologically reactive → Molecular composition subject to further alteration/degradation

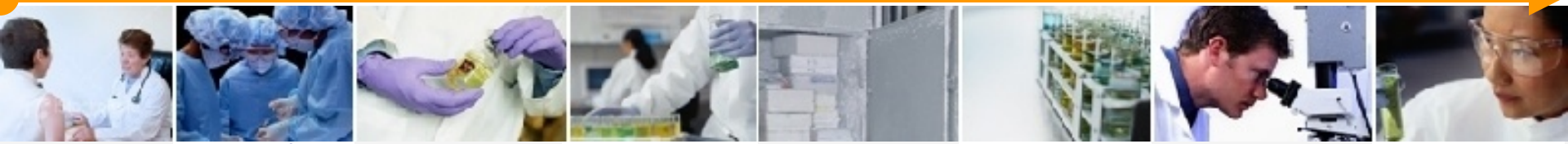
Factors (examples):

- Antibiotics
- Other drugs
- Type of anesthesia
- Duration of anesthesia
- Arterial clamp time

Time 0

Factors (examples):

- Time at room temperature
- Temperature of room
- Type of fixative
- Time in fixative
- Rate of freezing
- Size of aliquots



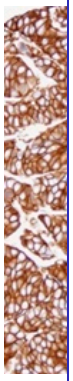
Patient | Medical/ Surgical Procedures | Acquisition | Handling/ Processing | Storage | Distribution | Scientific Analysis | Restocking Unused Sample

Pre-acquisition | **Post-acquisition**

Cold Ischemia and Molecular Assay Results

HER2 IHC and FISH in Breast Cancer:
Loss

pMAPK IHC of Colon Cancer :
to



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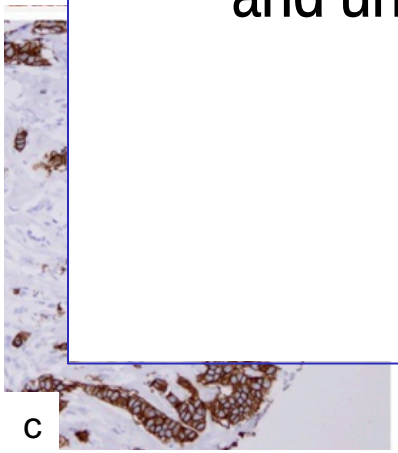

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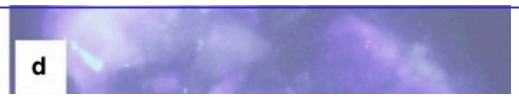

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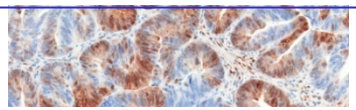


Without knowledge about tissue processing methods and assurance of rapid tissue fixation, protein expression data are unreliable, and understanding of pathway activity is impossible.

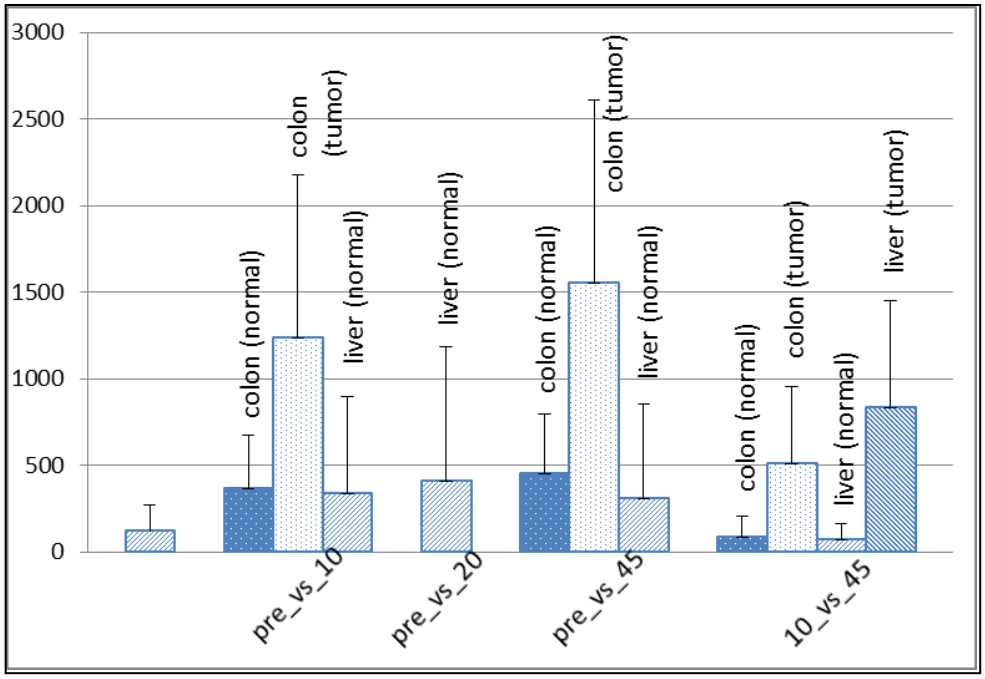
- Hartmut Juhl, CEO Indivumed

Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

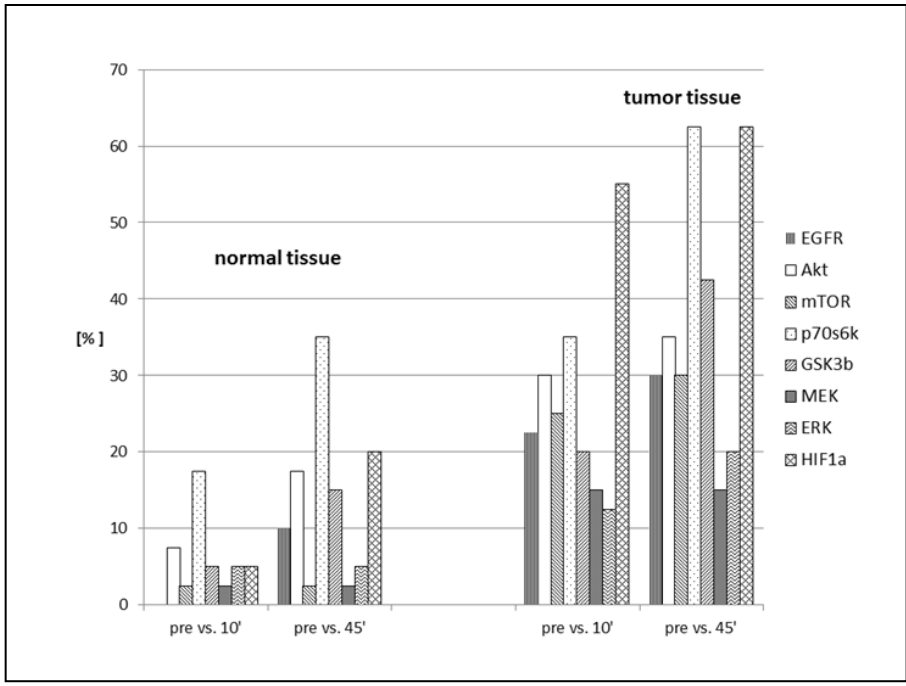
Hartmut Juhl, Indivumed GmbH, BRN

Expression of >15% of Genes and Up to 60% of Selected Proteins Change >2-fold during Surgery and Postsurgical Processing Time

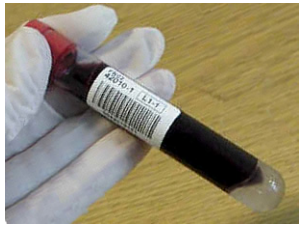
Gene Expression
Pre vs. Post Surgery



Protein Expression
Pre vs. Post Surgery



Blood Collection and Plasma Processing: Biomarkers and Circulating Tumor Cells



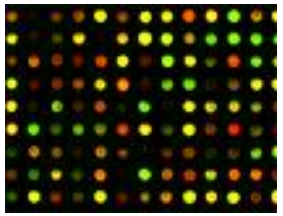
Collection Tubes and Order of draw

Processing Procedure, Temperature and Time

Distribution & Storage

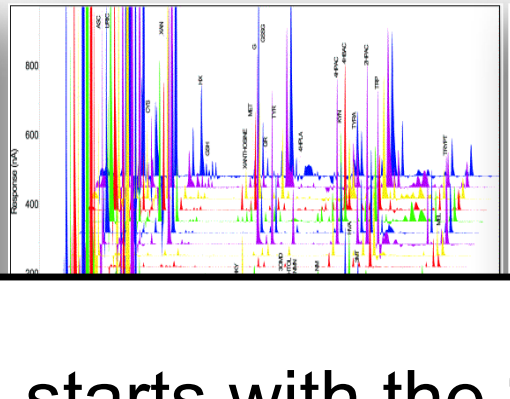
Blood Draw Procedure

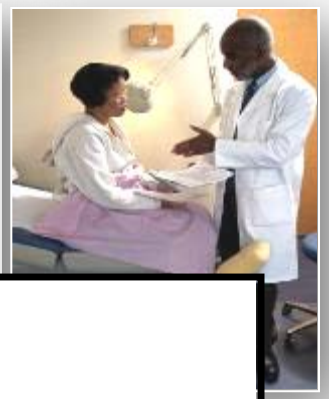
Patient Consent and Preparation



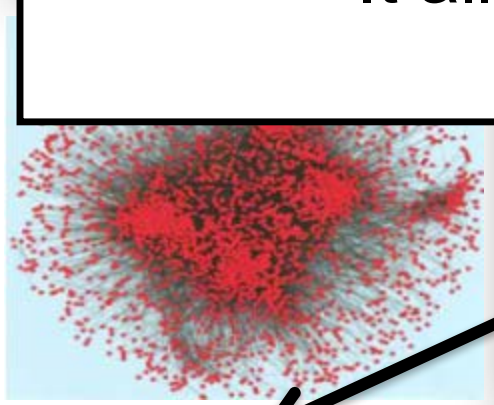
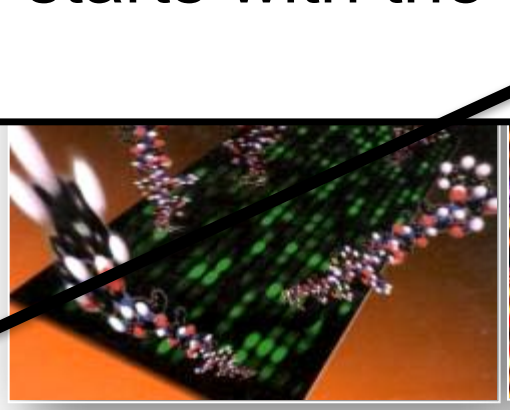
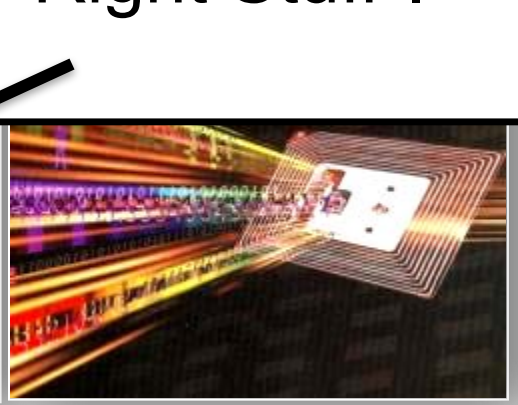
Molecular Analysis

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)

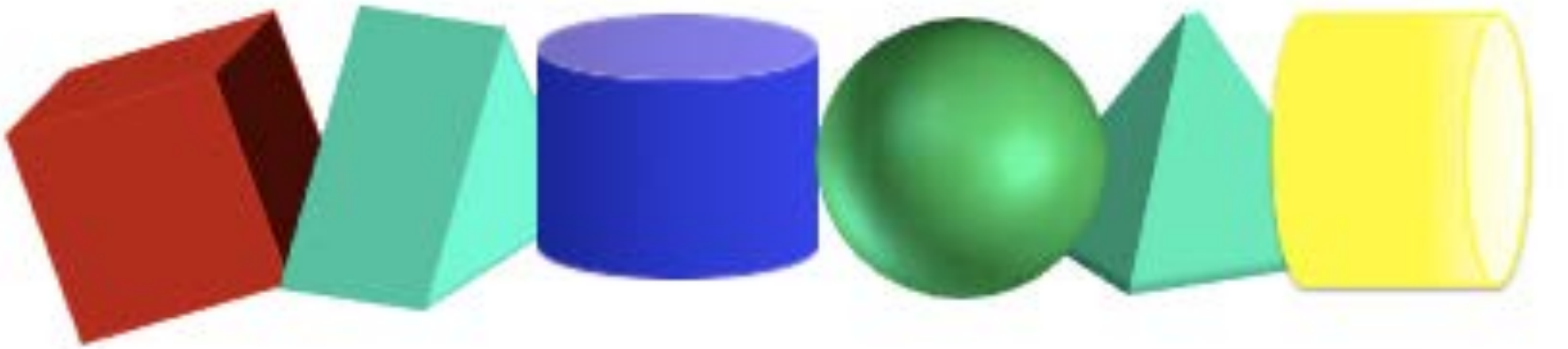
Translatable Discovery
(Clinical Measure Established)

Assay Development
(Analyte - Reagents- Technology – Robust)

Assay Performance
(Analytical Validation)

Biomarker Qualification
("Fit for Clinical Purpose)

Biomarker Validation
(Clinical Validation)



Academia

Industry

Physicians

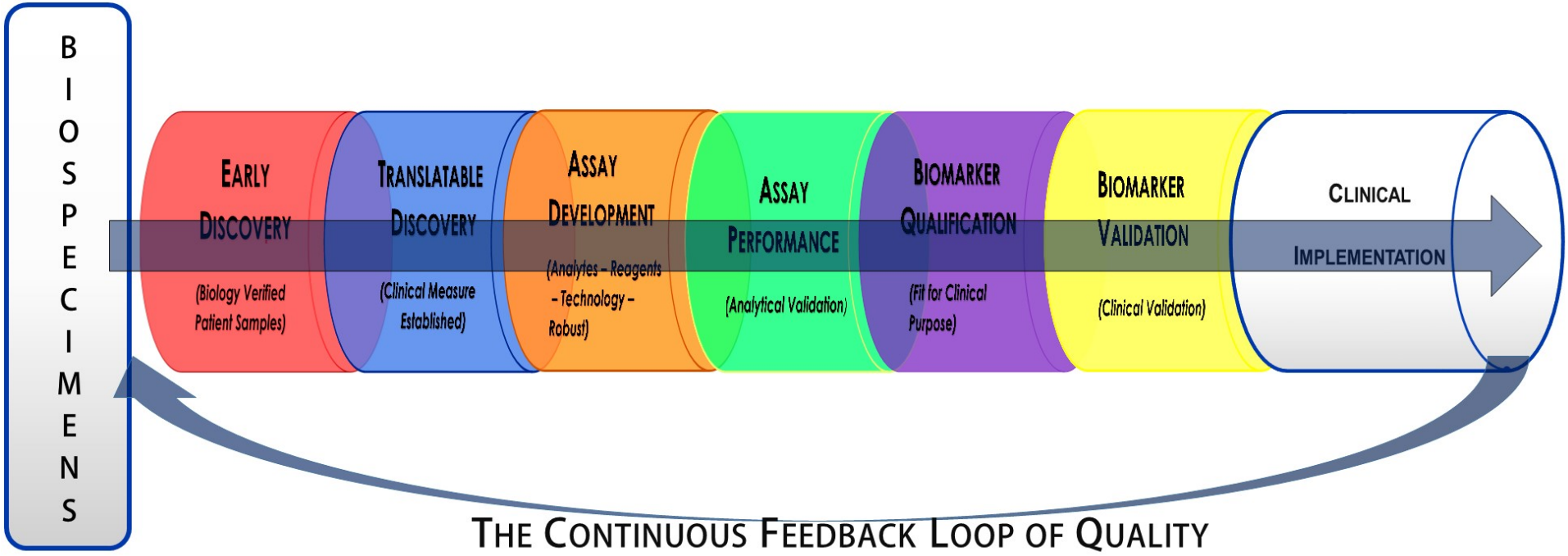
Patients

Regulators

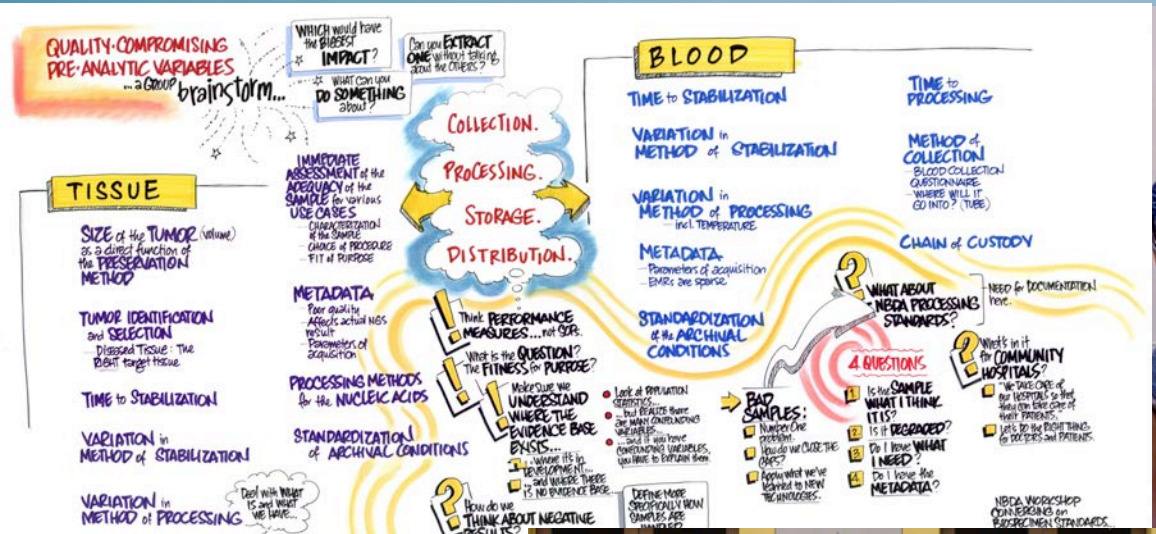
Funding Agencies

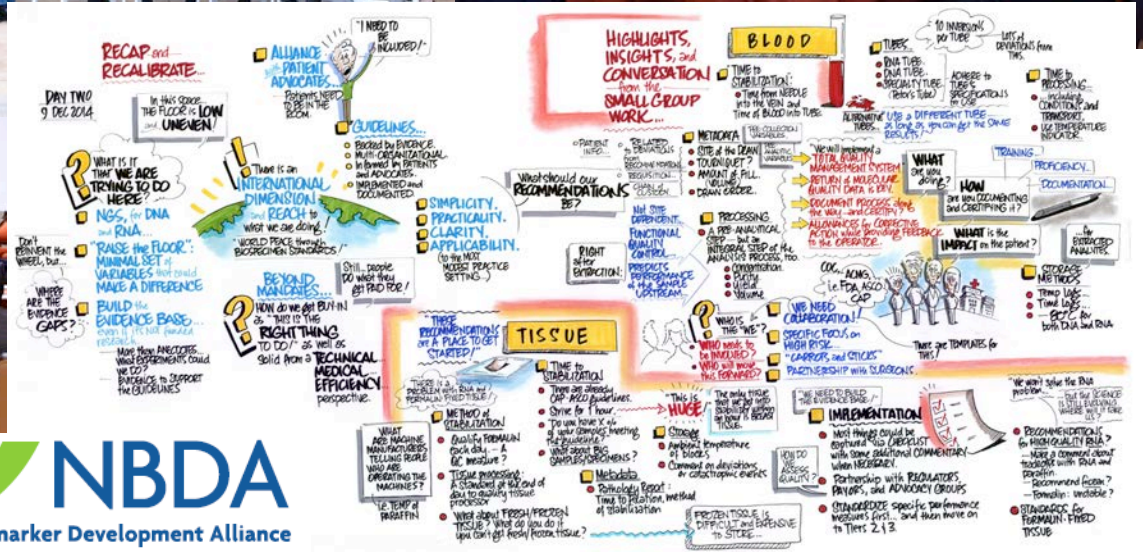
Professional Bodies

Biospecimens Flank
End-To- End Biomarker Development



NBDA: Understanding The Issues - Building Towards Solutions





NBDA
National Biomarker Development Alliance

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*)

NBDA Genomics Convergence Conference: Defining a Benchmark for Patient Biospecimens

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: 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?"

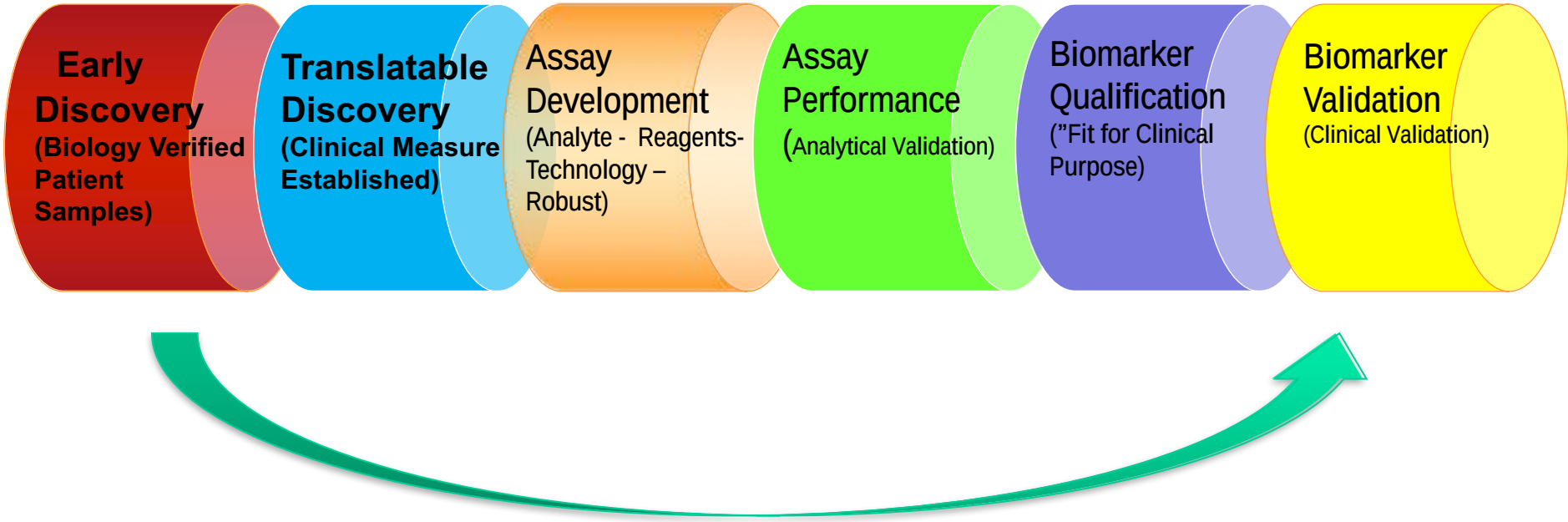
- John Wooden, Coach UCLA

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

Carolyn Compton, MD, PhD
Chair, Scientific Advisory Committee, Indivumed GmbH
Professor of Life Sciences, ASU
Professor of Laboratory Medicine and Pathology, Mayo Clinic
Adjunct Professor of Pathology, Johns Hopkins
CMO, National Biomarker Development Alliance
CMO, ASU Complex Adaptive Systems Institute

Biomarkers and Diagnostics World Congress
Philadelphia, PA
May196, 2016

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



Standards are needed at every step and across the continuum