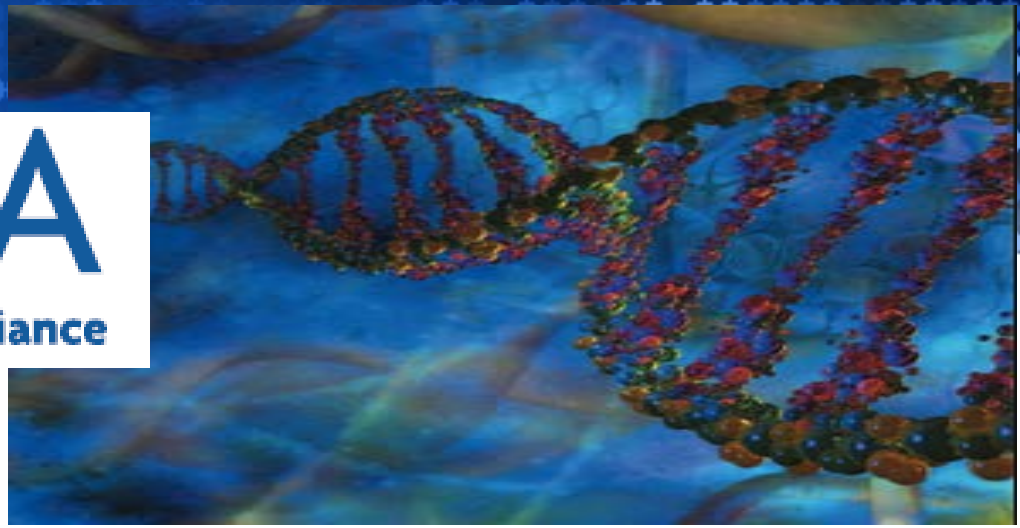

The "Garbage In" Problem in Cancer Research

Carolyn Compton, MD, PhD

Professor of Life Sciences, ASU

Professor of Laboratory Medicine and Pathology, Mayo Medical School

Adjunct Professor of Pathology, Johns Hopkins Medical Institutes

CMO, National Biomarker Development Alliance

CMO, Complex Adaptive Systems Institute

AACR Education Session
Philadelphia,PA
April 18, 2015

Disclosure Information

AACR Annual Meeting 2015

Carolyn Compton, MD, PhD

I have the following financial relationships to disclose:

Consultant for Indiuvmed

Board of Directors of HealthTell

- and -

I will not discuss off label use and/or investigational use in my presentation.

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Disappearing Line Of Demarcation Between Biomarker Discovery, Development And Clinical Use

Molecular Data

Clinical Care / Research

Diagnosis / Therapy

New Diagnostics
New Therapeutics

DETERMINES QUALITY HERE

PRECISION MEDICINE

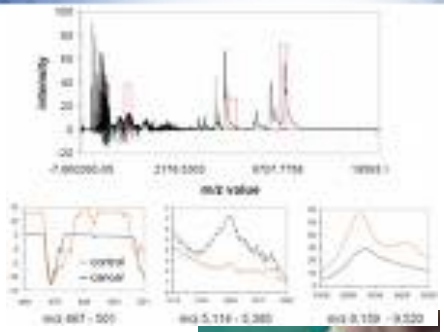
Biospecimen Analysis

Biospecimen Collection

QUALITY HERE

Biospecimen Processing and Stabilization

Biospecimen Quality Impacts Both Clinical And Research Outcomes

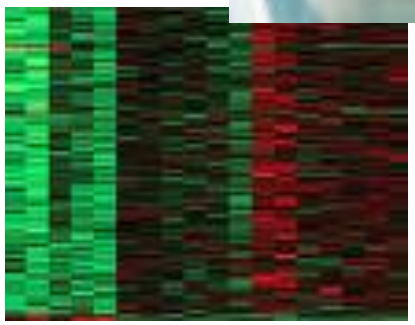


Effects on Clinical Outcomes

- 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
- Misinterpretation of artifacts as biomarkers



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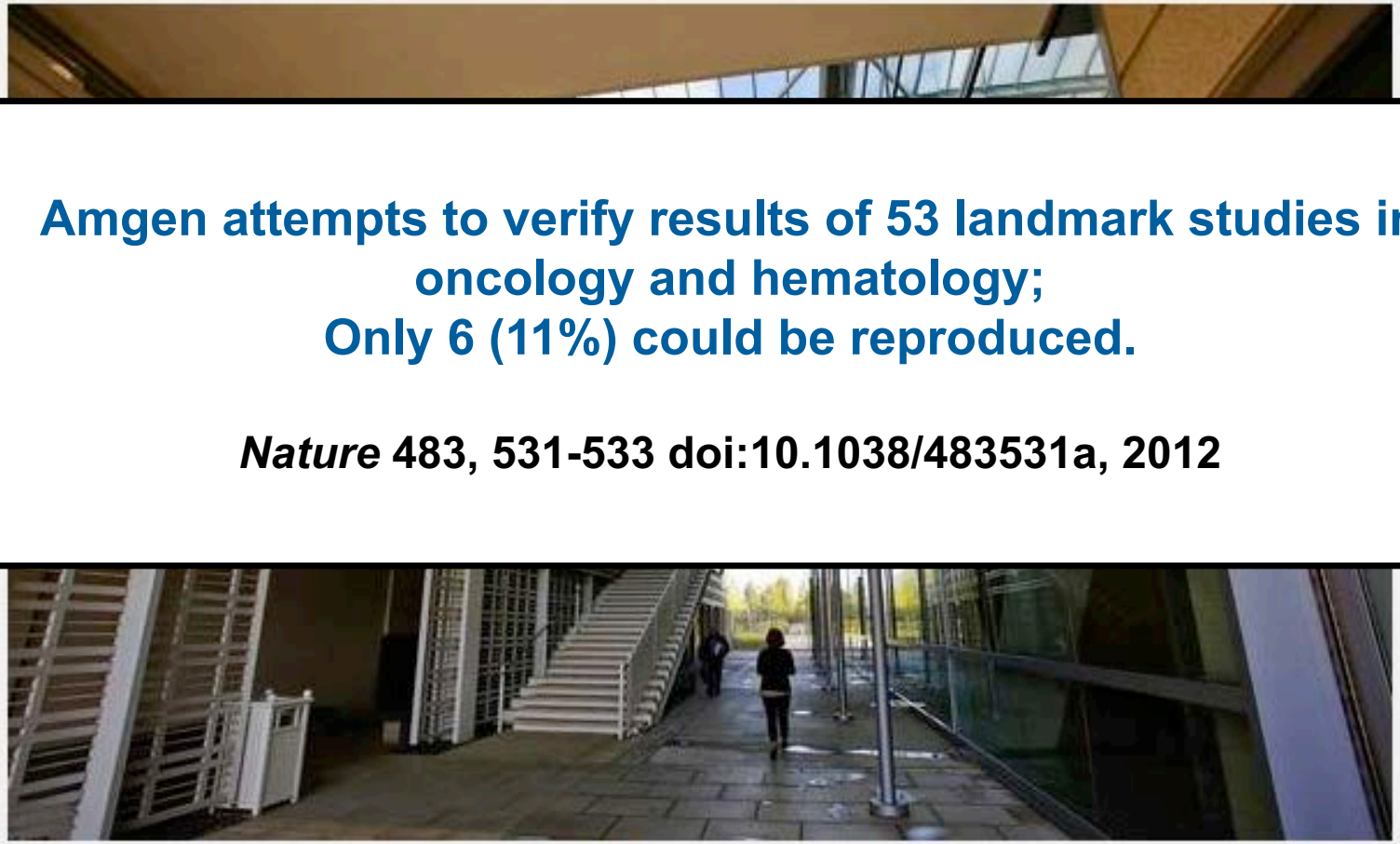
Science has lost its way, at a big cost to humanity

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.

***Nature* 483, 531-533 doi:10.1038/483531a, 2012**

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)

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Irreproducibility in Biomedical Research: A Crisis in Confidence (Public View)

The Economist | World politics | Business & finance | Economics | Science & technology | Cultur

Unreliable research

Trouble at the lab

Scientists like to think of science as self-correcting. To an alarming degree, it is not

Oct 19th 2013 | From the print edition

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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 research claim is true may depend on study power and bias, the number of other studies on the same question, and, importantly, the ratio of true to no relationships among the relationships probed in each scientific field. In this framework, a research finding is less likely to be true when the studies conducted in a field are smaller; when effect sizes are smaller; when there is a greater number and lesser preselection of tested relationships; where there is greater flexibility in designs, definitions, outcomes, and analytical modes; when there is greater financial and other interest and prejudice; and when more teams are involved in a scientific field in chase of statistical significance. Simulations show that for most study designs and settings, it is more likely for a research claim to be false than true. Moreover, for many current scientific fields, claimed research findings may often be simply accurate measures of the prevailing bias. In this essay, I discuss the implications of these problems for the conduct and interpretation of research.

THE NEW YORKER

ANNALS OF SCIENCE

THE TRUTH WEARS OFF

Is there something wrong with the scientific method?

BY JONAH LEHRER

DECEMBER 13, 2010

Many results that are rigorously proved and accepted start shrinking in later studies.

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 in the subjects' psychiatric symptoms. As a result, second-generation antipsychotics had become one of the fastest-growing and most profitable pharmaceutical classes. By 2001, Eli Lilly's Zyprexa was generating more revenue than Prozac. It remains the company's top-selling drug.

How Widespread Are Failures to Reproduce Published Reports?

- Mass spec diagnostic for ovarian cancer – results due to experimental artifact and bias – control and experimental specimens collected differently and 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)
- Assessment of 18 published microarray studies: 2 were reproducible (Science, 2011)
- Bayer Healthcare reported reproducibility rates of 25% in its attempt to reproduce discovery research (*Nature Reviews Drug Discovery* 10, 712 doi:10.1038/nrd3439-c1, 2011)

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Quality Analytical Data Begins with Quality Analytes

Garbage in…

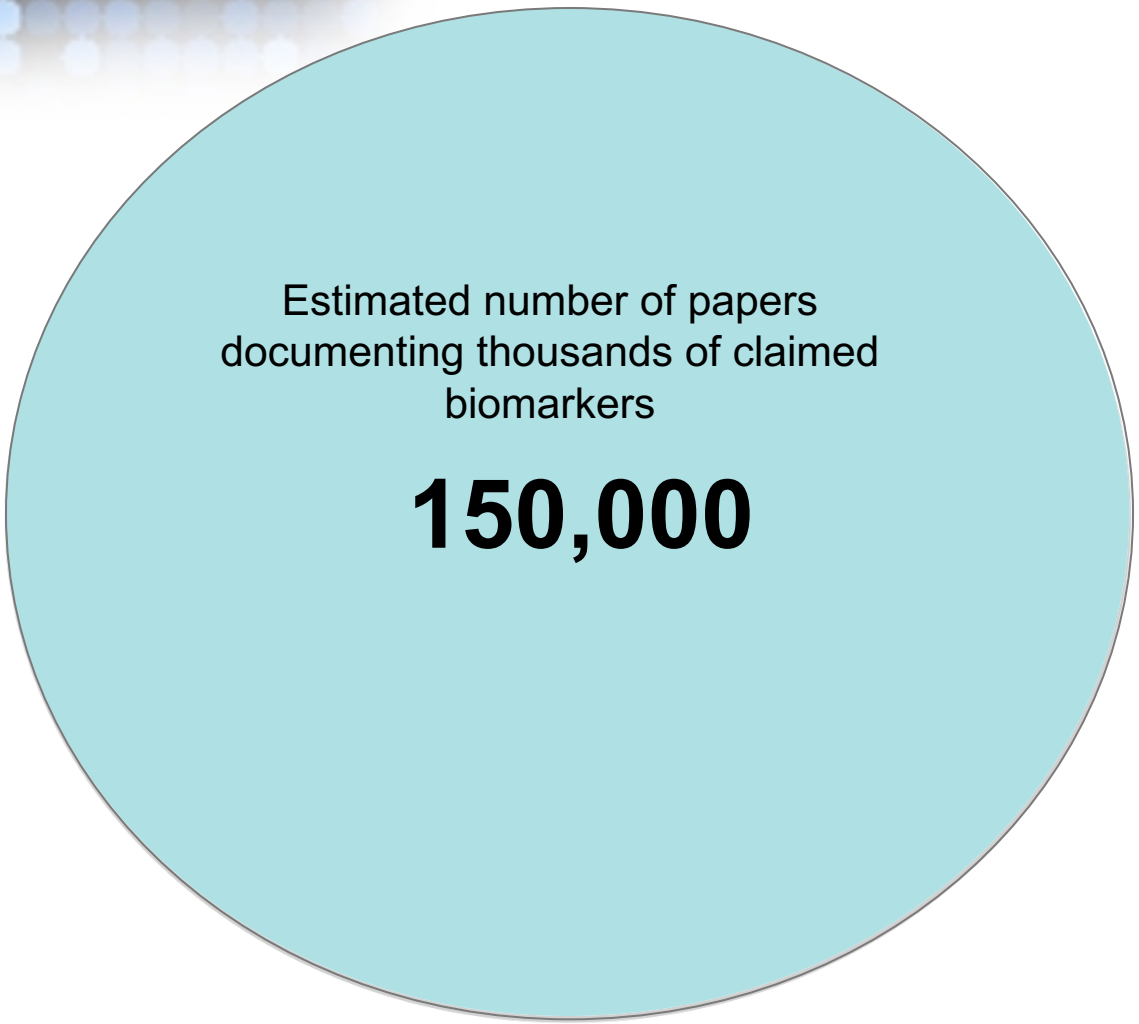
Purgamentum init,
exit purgamentum.

…Garbage out

Diamonds in…

Modified from Jerry Thomas

Biomarker Development: What's the Problem Here?



100

Estimated number of biomarkers routinely used in the clinic

Source: Poste G. Nature 469, 156-157 13 Jan 2011

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No Biomarkers, No Precision Oncology

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

- **Drug development – markers of efficacy, toxicity and surrogate endpoints for clinical trials**
- **Early detection (broad or specific detection/ corroboration of specific disease stage)**
- **Rational choice of treatments (patient stratification)**
- **Assessment of treatment effectiveness**
- **Prognosis, prediction**
- **Prevention, surveillance**
- **Treatment, disease monitoring**

Sources Of Bias In Molecular Marker Research In Cancer - Ransohoff and 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).

The Vision Of Precision Oncology Cannot Be Realized Without Biomarkers

Biomarker

- A measurable characteristic serving as an indicator of a biological state or condition
- Most often measured from biospecimens
- Required characteristics:

  - Quantifiable
  - Reproducible
  - Clinically relevant

All of these can be distorted by *pre-analytical* variation

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Pervasive Standards Deficits Contribute to the Lack of Progress in Biomarker Development

- Poor access to rigorously annotated, fit-for-purpose biospecimens from stringently phenotyped sources
- Insufficient control of pre-analytical parameters
- Low reproducibility of academic publications
- Variable analytical standards
- Idiosyncratic 'lab-specific' analytical methods
- Small studies lacking statistical power
- Chaotic data reporting formats and poor database interoperability
- Poor compliance with journal policies on reporting standards
- Non-existent quality management systems

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What Defines Biospecimen "Quality"?

- Requirements for biospecimen quality are related to:
  - The stringency of the analysis to be performed
  - The requirements of the specific platform used
  - The lability/stability of the molecular species to be analyzed

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Pre-analytical Factors Affect Both Molecular Quality And Molecular Composition

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

Factors (examples):
- Antibiotics
- [illegible]

Time 0

Factors (examples):
- Time at room temperature

Does it matter???

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

Pre-acquisition | **Post-acquisition**

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Pre-analytical Variables: Impact On *Biospecimen Quality* And Test Results

The facts:

- Between 32 and 75% of all laboratory test errors occur in the pre-analytical phase
- Insufficient specimen quality (or quantity) may account for over 60% of pre-analytical errors
- Genomic tests are not exempt from this issue

- Lippi et al. Clin Chem 2006; 52:1442.
- Stankovic et al. Clinics in Lab Med 2008; 28: 339-350.

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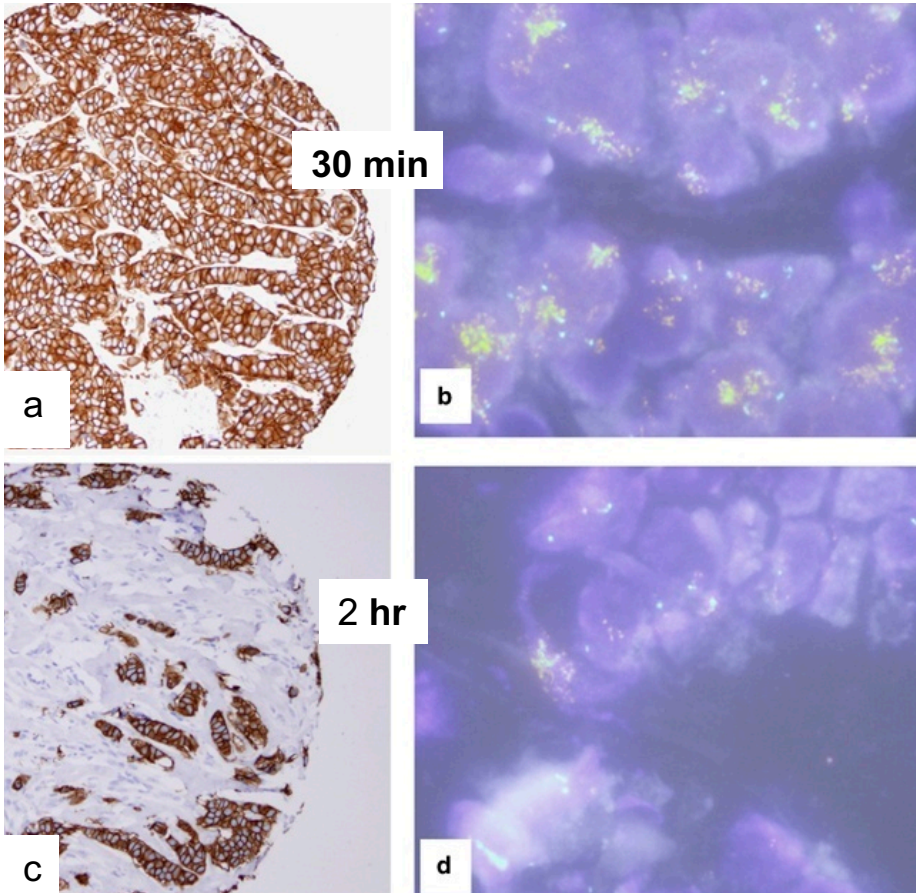
Pre-analytical Variables: Impact on Test Results

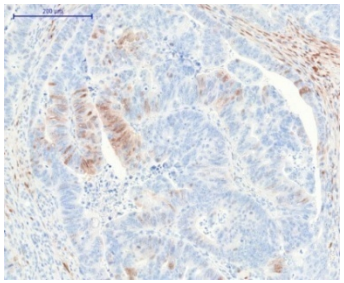
HER2 IHC and FISH in Breast Cancer: Loss of Signal with Time to Fixation



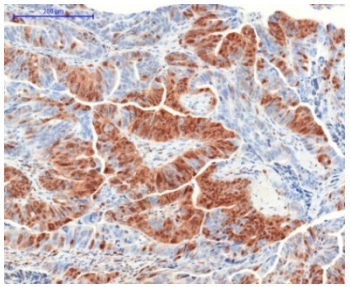
Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

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

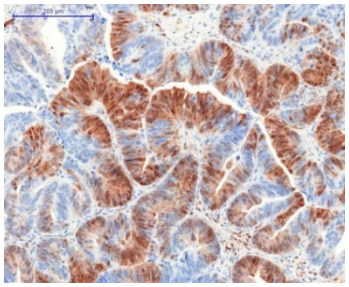
10 min



20 min



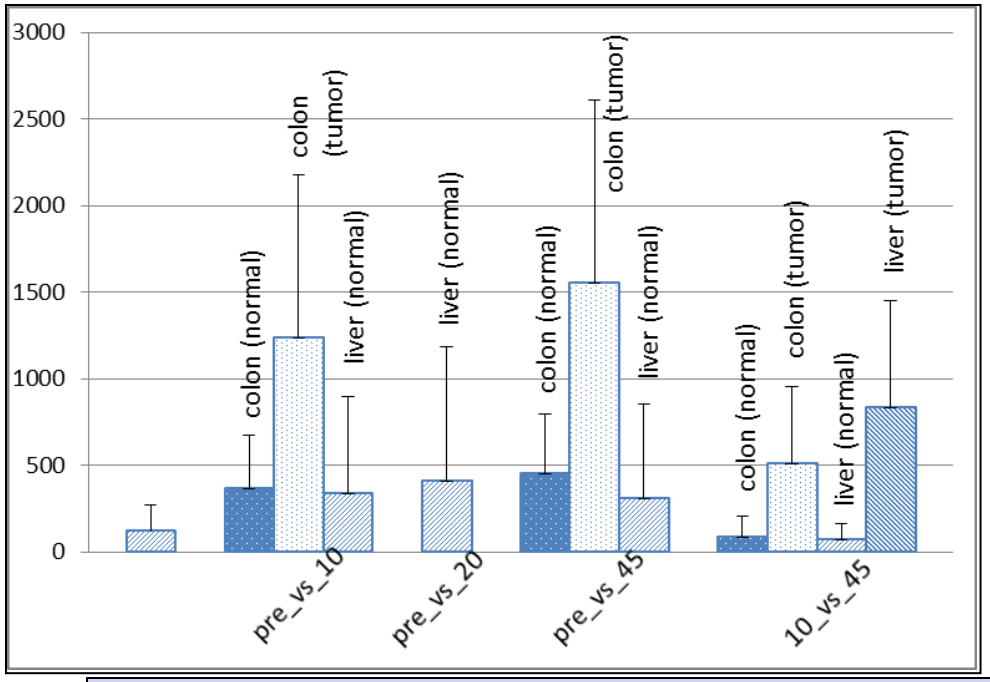
60 min



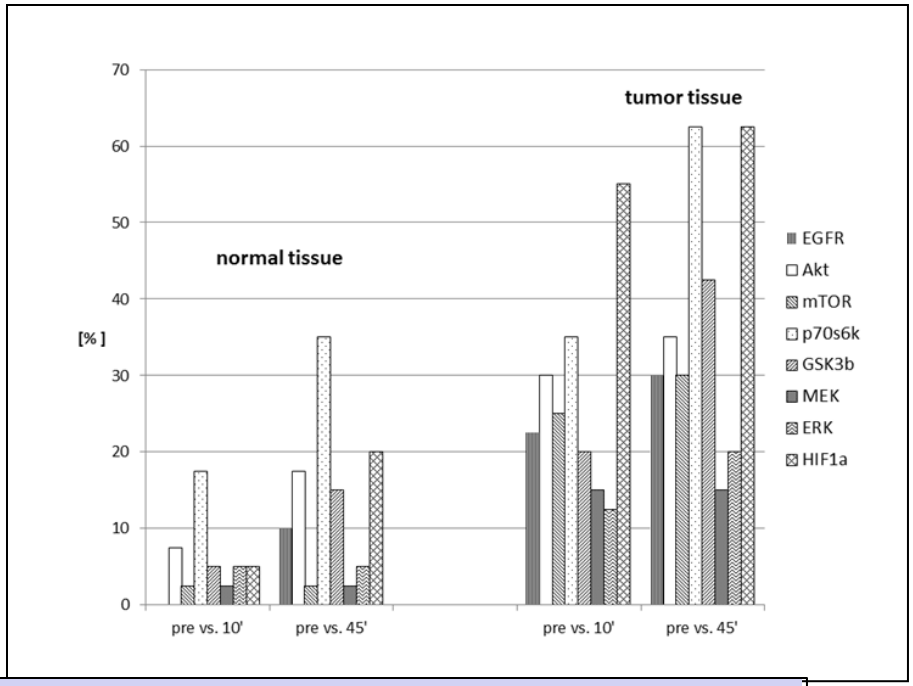
Hartmut Juhl, Indivumed GmbH, BRN

Pre-analytical Variables: Surgery and Pathology Contributions

Number of Genes Showing
>2-Fold Change in Expression Level
Pre vs. Post Surgery



Percentage of Patients with
>2-Fold Change in Selected Protein Expression Level
Pre vs. Post Surgery



Expression of >15% of genes and up 60% of selected proteins change >2-fold during surgery and postsurgical processing time

K David et al, Oncotarget November 2014

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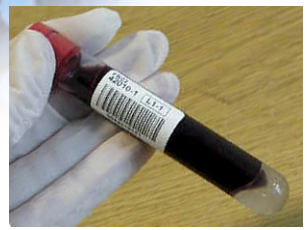
Blood Collection And Plasma Processing: Circulating Genomic Biomarkers And Tumor Cells



Collection Tubes and Order of draw

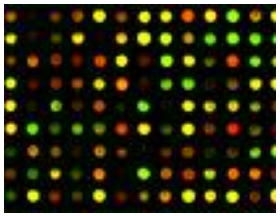
Processing Procedure, Temperatur and Time

Blood Draw Procedure

Distribution & Storage

Patient Consent and Preparation

Molecular Analysis



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Plasma Biomarkers: Pre-analytical Variations With Known Effects On Analyte Assays

Procedure	Variations
Venipuncture	Needle gauge Priming volumes
Phlebotomy	Patient position (seated /reclining) Tourniquet time Tube orders Venipuncture sites
Collection device	Tube types
Blood derivatives and processing	Anticoagulant types Temperatures Centrifugation speeds Processing time
Time between collection and storage	Variable or unknown times
Storage and shipping	Temperature Duration

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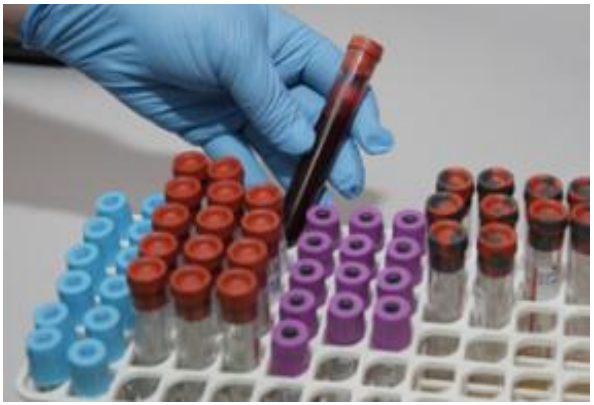
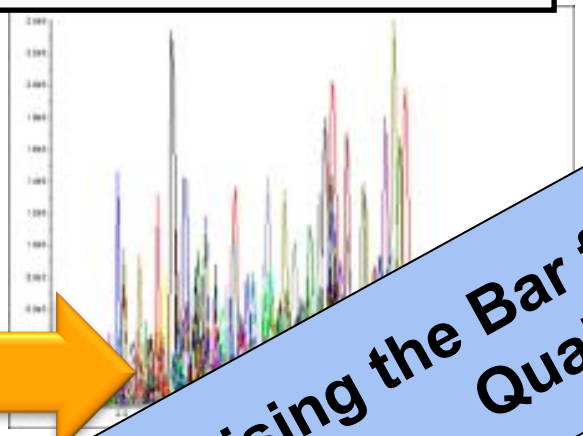
Evolution Of Biomarker Testing In The “Omics Era”

Unianalyte Tests



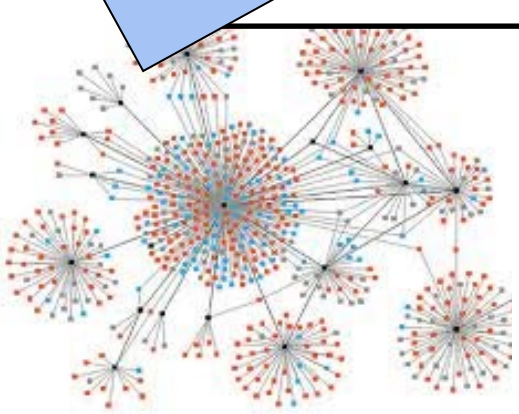
Multianalyte Tests

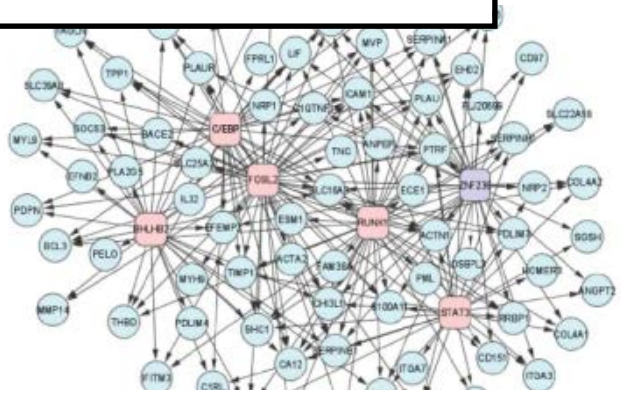


Whole Genome Sequencing

Raising the Bar for Specimen Quality!

Networks and Systems





Powerful Tools: Powerful Risks

- 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
- Starting materials of known, consistent quality are required to assure analysis data of known, consistent quality

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NGS Is One Of Those Powerful Tools Moving Rapidly Into Clinical Application

- Rational choice of treatments (patient stratification)
- Assessment of treatment effectiveness / disease evolution
- Treatment/disease monitoring
- Risk assessment
- Prognosis (outcome)
- Early detection

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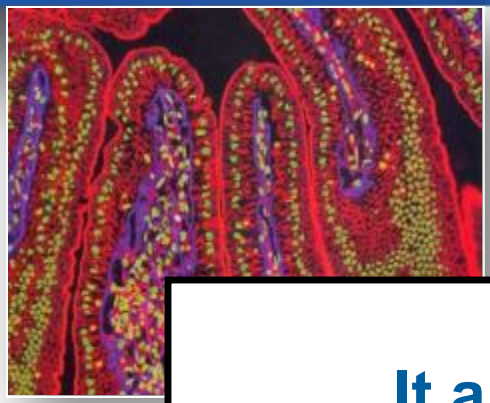
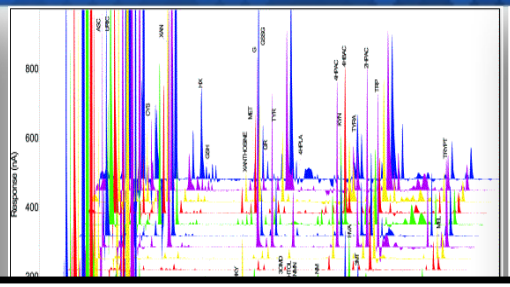
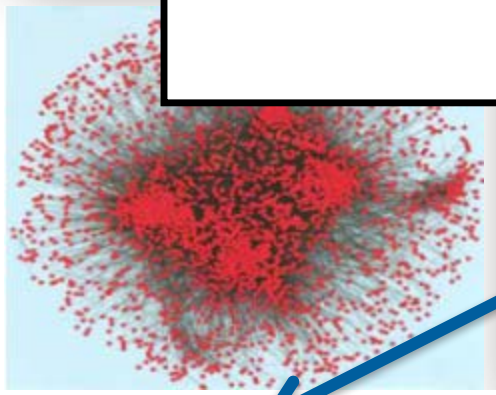
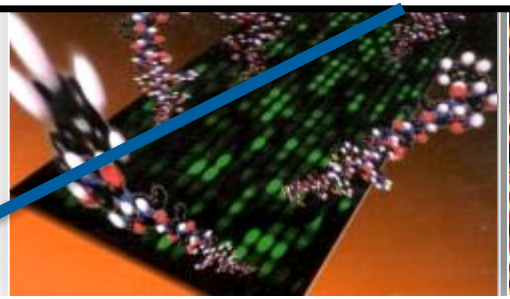
Genomics and Proteomics Are Only Part of the Equation - Complexity Is Increasing


It all starts with the "Right Stuff".

Biospecimens and Analysis of Molecular Pathway/ Network Perturbations

Multiplex Assays and Complex Signal Deconvolution Algorithms

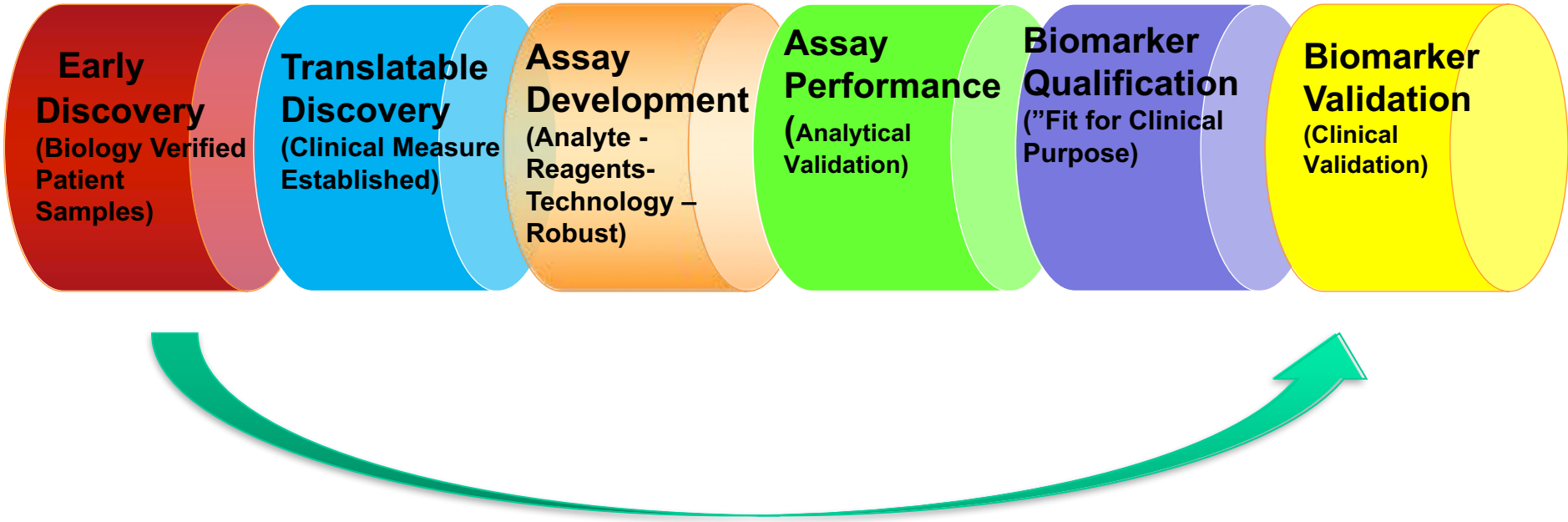
Novel Instrumentation, Automation and Large Scale Informatics

Patient Profiling, Rational Rx and Health Monitoring

Courtesy of G. Poste

NBDA: Understanding The Issues - Building Towards Solutions

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



Standards are needed at every step and across the continuum

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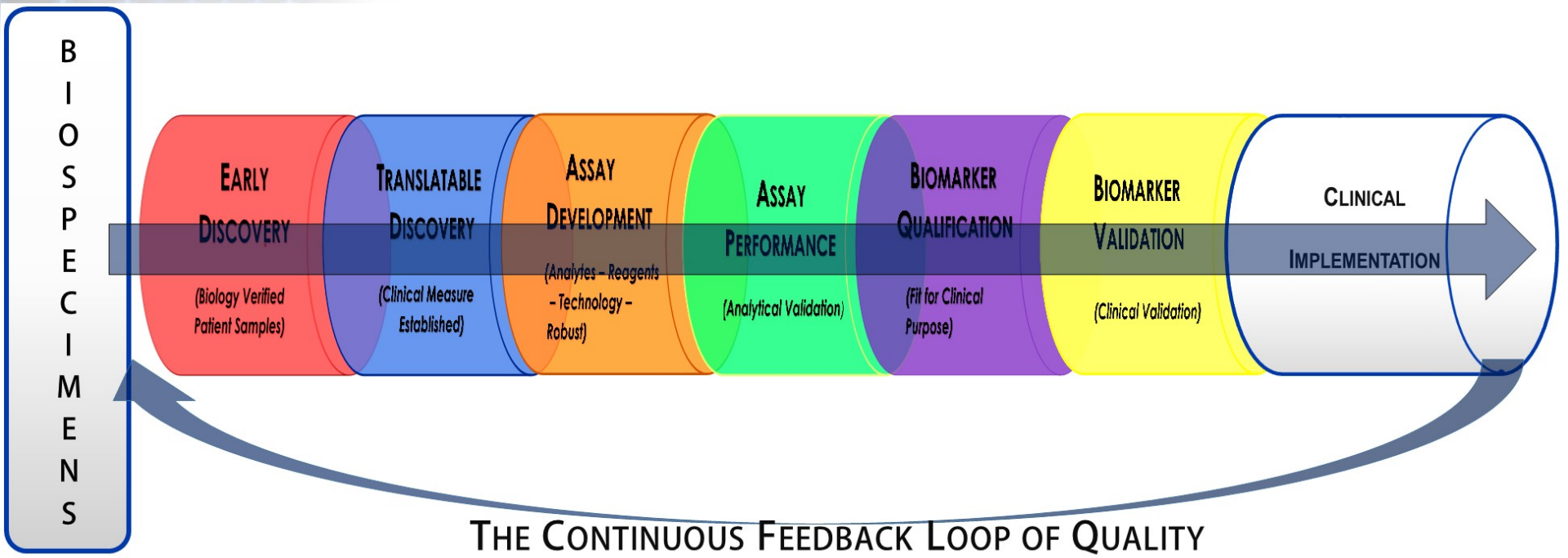
Biospecimens Flank End-To- End Biomarker Development



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Stakeholders Are Both Part of the Solution and Beneficiaries of the Solution

- Academic discovery scientists
- Clinical investigators
- Inductsry (pharma, biotech, diagnostics)
- CROs
- Clinicians
- Regulators
- Accreditation organizations
- Payors
- Patients

- Better science, greater efficiency, cost savings, better medicine – because there are patients waiting

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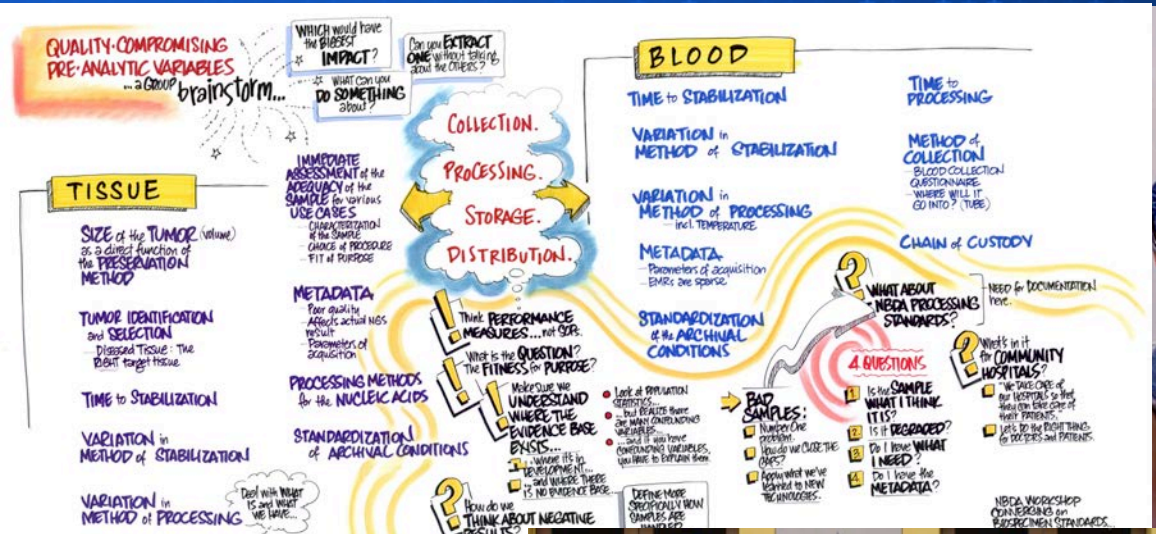

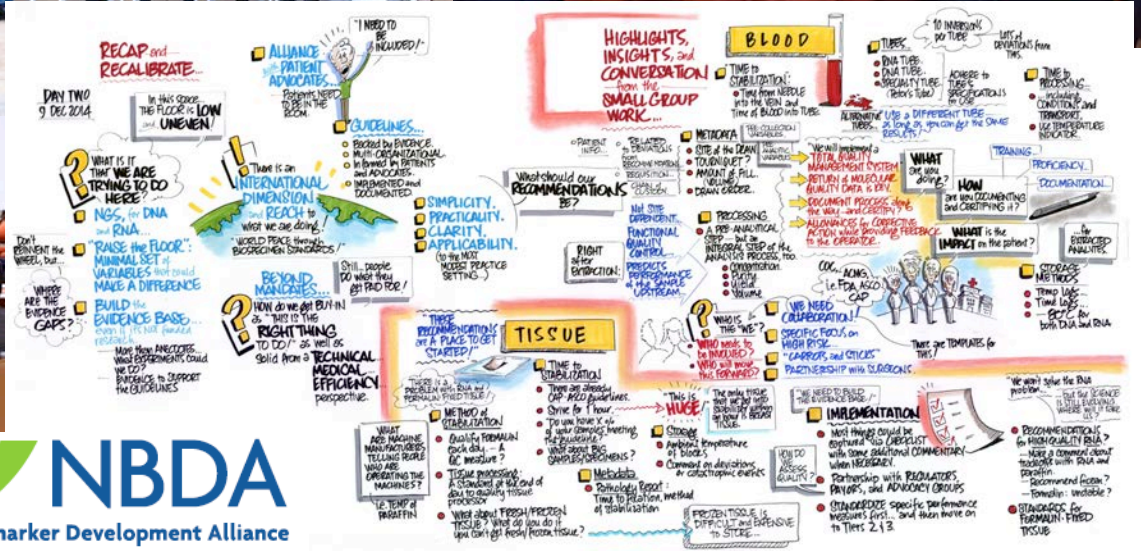

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NBDA Convergence Conferences Focused On Biospecimens For Molecular Analysis

The goal:

- Converge on the pre-analytical steps of the biospecimen lifecycle that most compromise the quality of tissue and blood for NGS and mass spec
  - "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 3 List"
- Define the performance metrics required to achieve control of the highest-value variables
- Define a cost-effective strategy for implementation and compliance with those metrics

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NBDA Genomics Convergence Conference Focused On Biospecimens For NGS

Think: Pareto Principle (20/80 rule)

For many events 80% of the effects come from 20% of the cause

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Top 5 List

1. Time to stabilization

Tissue: Fixation within 1 hour

Blood: N/A to blood extraction

2. Method of processing

Tissue: Time in formalin 6-24 hours

- Section thickness <3 mm

Blood: Room temp (15-25° C)

- Maintained in transport

3. Method of stabilization

Tissue: Standardize formalin and tissue - fixative volume ratio

Blood: 3 tubes: RNA, DNA optional specialty tube

- Minimum 10 inversions

4. Metadata collected

Tissue: Time to fixation

Deviations

Fixative QC

Blood: Site (vein or line)

Tourniquet

Draw order

Volume of tube fill

5. Storage conditions

Tissue blocks: room temp (15-25° C)

Blood analytes -80° C

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The CAP Isoving Ahead

Goal:

- Implementation of the Top 5 through the College of American Pathologists (CAP) Laboratory Accreditation Program checklists
- New reimbursements codes sought, if needed
- Reinforcement through FDA guidance, funder requirements, etc.

Next steps:

- MOU between the NBDA and the CAP in process
- Personalized Healthcare Committee (PHC) of CAP begins education and implementation through theCAP Laboratory Accreditation Program
- PHC further develops, refines and updates key pre-analytics

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Envisioned Result

Historic transformation of practice with far-reaching impact:

- **Variably variable and unknown quality to uniform, known quality that is consistent with molecular analysis**
- **Simultaneous impact on both clinical and research results**
- **"Convenience samples" will be fit for purpose!**

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If Your Research Involves Human Biospecimens, Think Sample Quality

"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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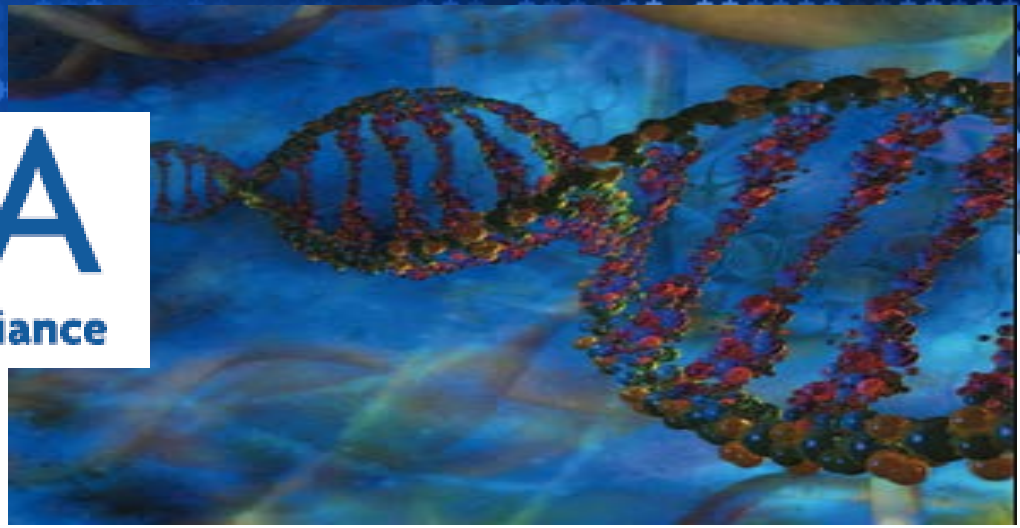

The "Garbage In" Problem in Cancer Research

Carolyn Compton, MD, PhD

Professor of Life Sciences, ASU

Professor of Laboratory Medicine and Pathology, Mayo Medical School

Adjunct Professor of Pathology, Johns Hopkins Medical Institutes

CMO, National Biomarker Development Alliance

AACR Education Session
Philadelphia,PA
April 18, 2015