Cancer Genomics Webinar:
Clinical Research and Assays for Precision Medicine

Barbara A. Conley, M.D.
Oncologist, NCI Special Volunteer/Contractor

P. Mickey Williams, Ph.D.
Molecular Characterization Laboratory
Frederick National Laboratory for Cancer Research

Learning Objectives

- Review the updates on the NCI-MATCH clinical trial for adult cancers and the development of a gene panel specific to pediatric cancers
- Analyze the effects of next-generation sequencing on the speed of results from the clinical/pathology lab to oncologists to minimize the need for a painful and expensive rebiopsy
- Discuss a laboratory's implementation of next-generation sequencing for cancer clinical research

DISCLOSURES

- No conflicts of interest
- The opinions expressed in this presentation those of the presenters, and not those of the NCI, NIH, or DHHS or Thermo Fisher Scientific

Agenda of Discussion

- Introduction – What is Precision Medicine?
  - Clinical Research to Discover and Understand Biomarkers
  - Clinical Trials to Utilize Biomarkers
- NCI-MATCH as an example for discussion of Precision Medicine
- Precision Medicine requires robust clinical assays
- Discuss Selection and Development of Assays
  - Defining assay intended use
    - Retrospective research, clinical research or Dx
- Feasibility testing of the assay
- Analytical validation
- Summary

Precision Medicine

Dr. Barbara Conley MD, Oncologist
NCI Special Volunteer/Contractor

What Is an "actionable" biomarker?

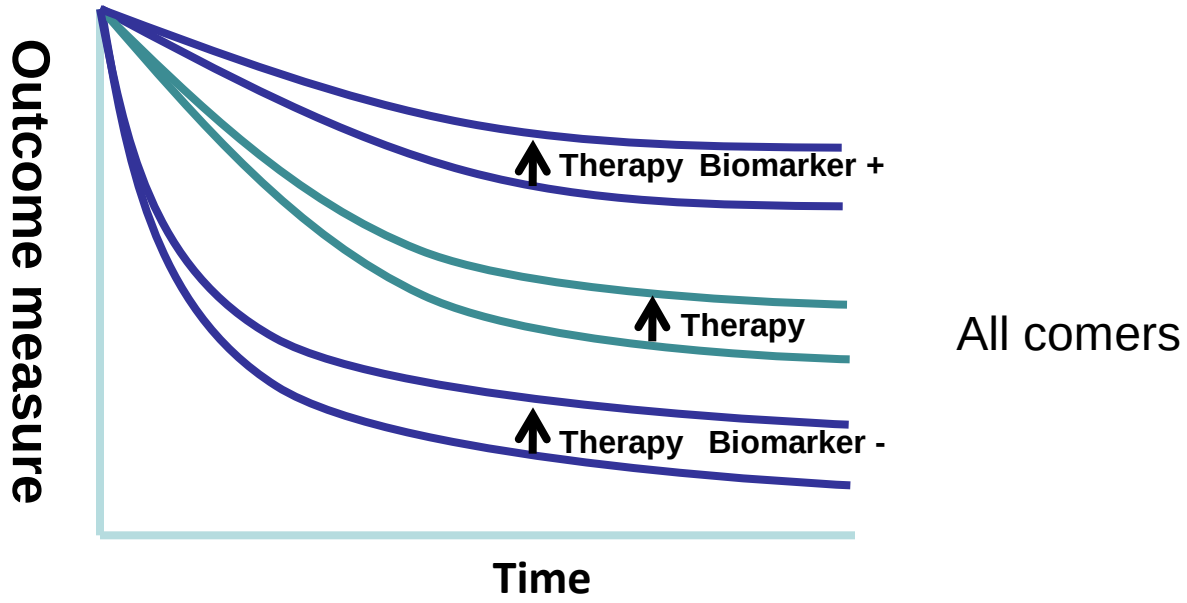
- **A biomarker that influences patient care**

- **Prognostic biomarker: Informs about disease course, irrespective of treatment**
  - Examples: OncotypeDx (colon); BRAFV600E (colon)
  - Useful when treatment can be omitted; e.g. such a good prognosis after surgery that adjuvant chemotherapy is not needed

- **Predictive biomarker: Informs about the likelihood of response or lack of response to a particular treatment**
  - Examples: KRAS mutation for treatment of colon cancer with cetuximab or panitumumab; HER2/neu amplification for treatment of breast cancer with trastuzumab (herceptin)
  - Very useful, but not many are available

Prognostic biomarkers: Outcome, biology, change is agnostic to treatment

- Prognostic

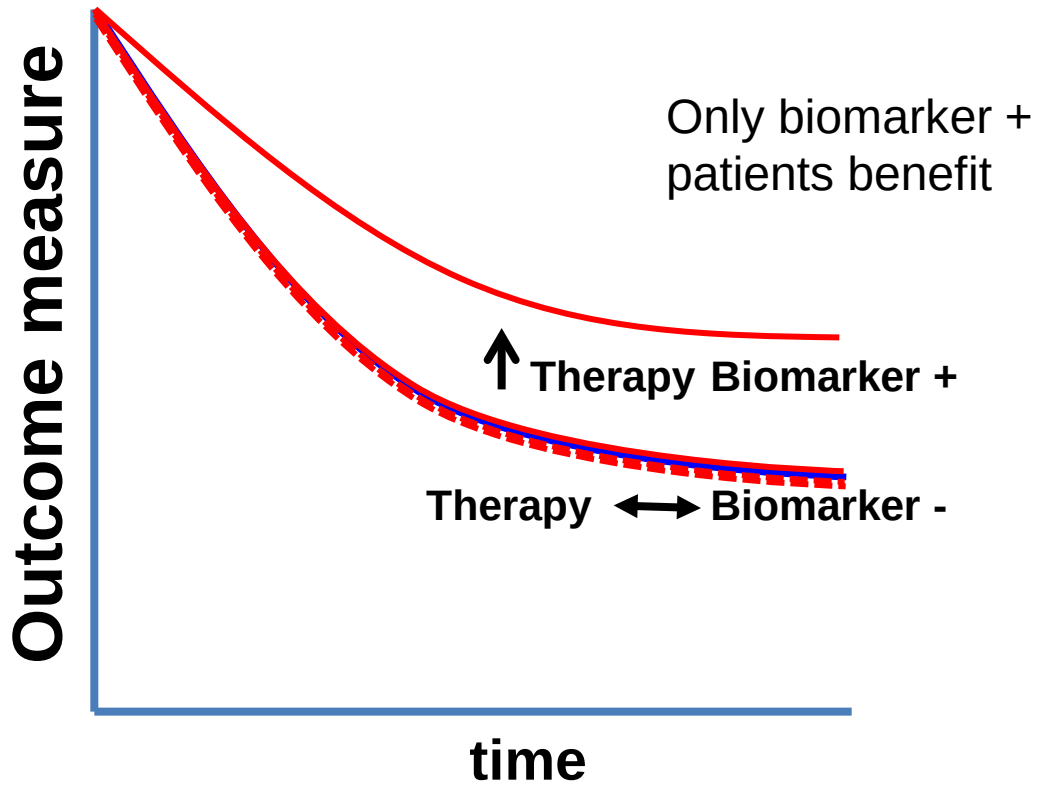


If the treatment effect is analyzed in both the biomarker positive and biomarker negative patients, we can see that the therapy benefits both groups approximately equally

Therefore this marker does not inform about which treatment to use, based on this biomarker

Predictive biomarker: Outcome is dependent on treatment

Predictive→



“Drivers” as predictive biomarkers: Clinically relevant questions

- Do “drivers” behave the same across tumors? Usually testing a particular predictive biomarker for a specific tumor (e.g. BRAFV600E predicts response to BRAF, MEK inhibitor in melanoma)
- What is the influence of other molecular alterations in the tumor, or in the host? Not all with the molecular abnormality will respond. Why not?
- Should trials concentrate on a single tumor histology, or group of tumors with same genomic abnormality

“Actionable” mutation

- An actionable mutation predicts clinical response to a specific treatment (predictive biomarker)
  - Activating mutations in oncogenes that up regulate signaling i.e. gain of function
  - Loss of function mutations in tumor suppressors and/or pathway inhibitors also lead to up regulated signaling
  - Loss of function in a pathway leading to synthetic lethality in combination with treatment derived pathway inhibition (e.g.DNA repair)
  - Mutation that predicts resistance
- What level of evidence is needed to assign a specific mutant locus as actionable?

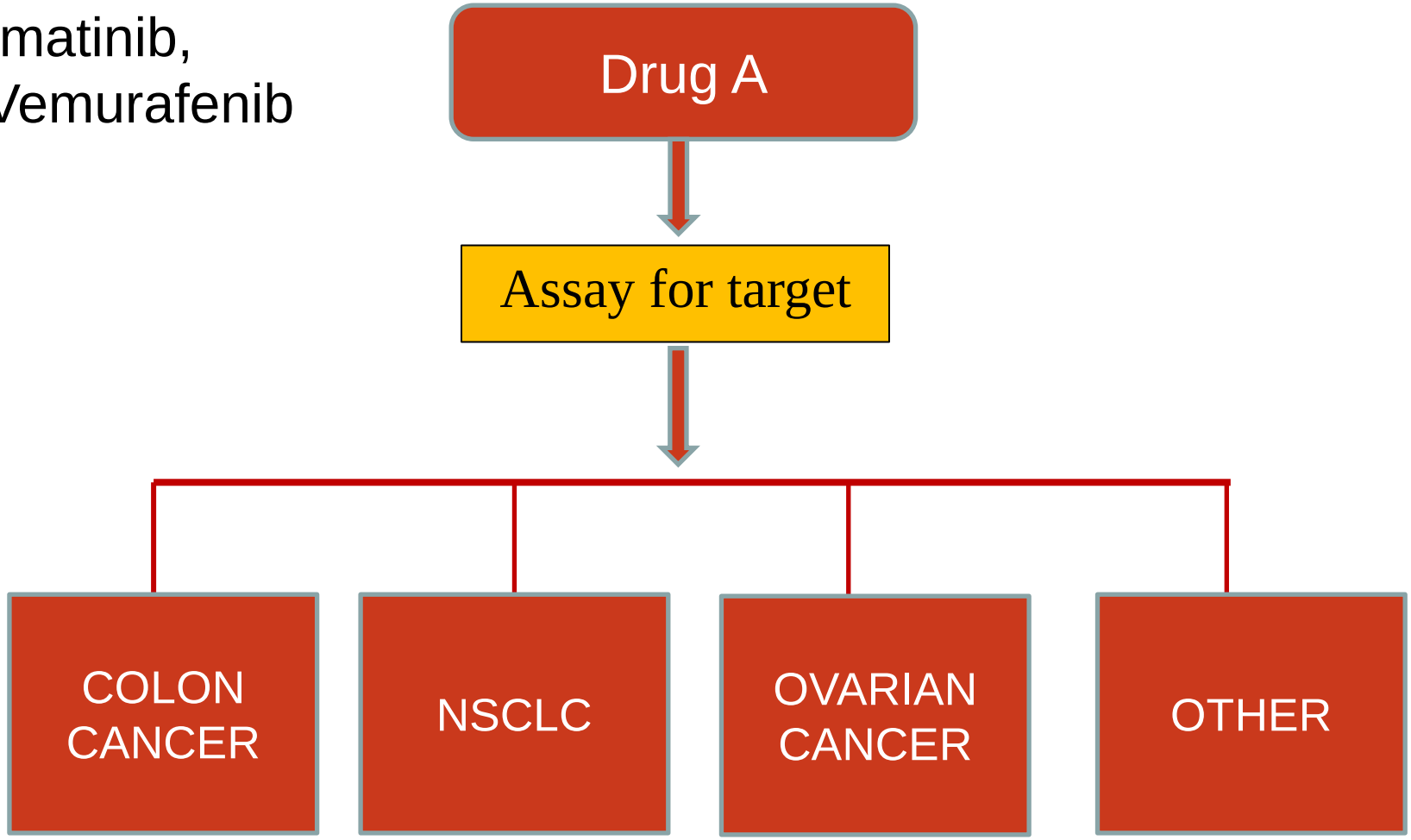
Precision Oncology: Challenges

- Right drug: Targeting the Driver
- Assay: move from research lab to clinic
- Assignment rules and evidence
- Multidisciplinary medical teams: medical, radiation and surgical oncologists, pathologists, laboratorians, statisticians
- Patient and clinician acceptance, education
- Frequency of biomarker in population tested
- Secondary findings (germline)
- Trial design – is marker also prognostic?

MASTER PROTOCOLS: BASKET TRIALS

- Examples

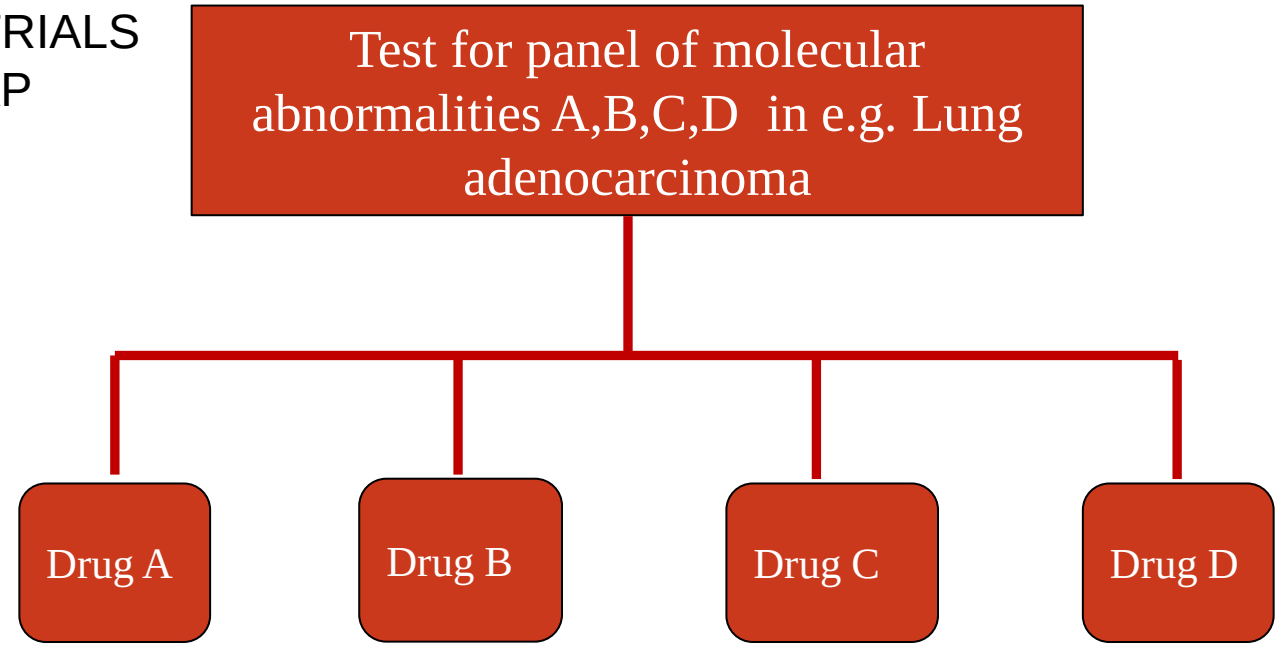
  Imatinib,
  Vemurafenib



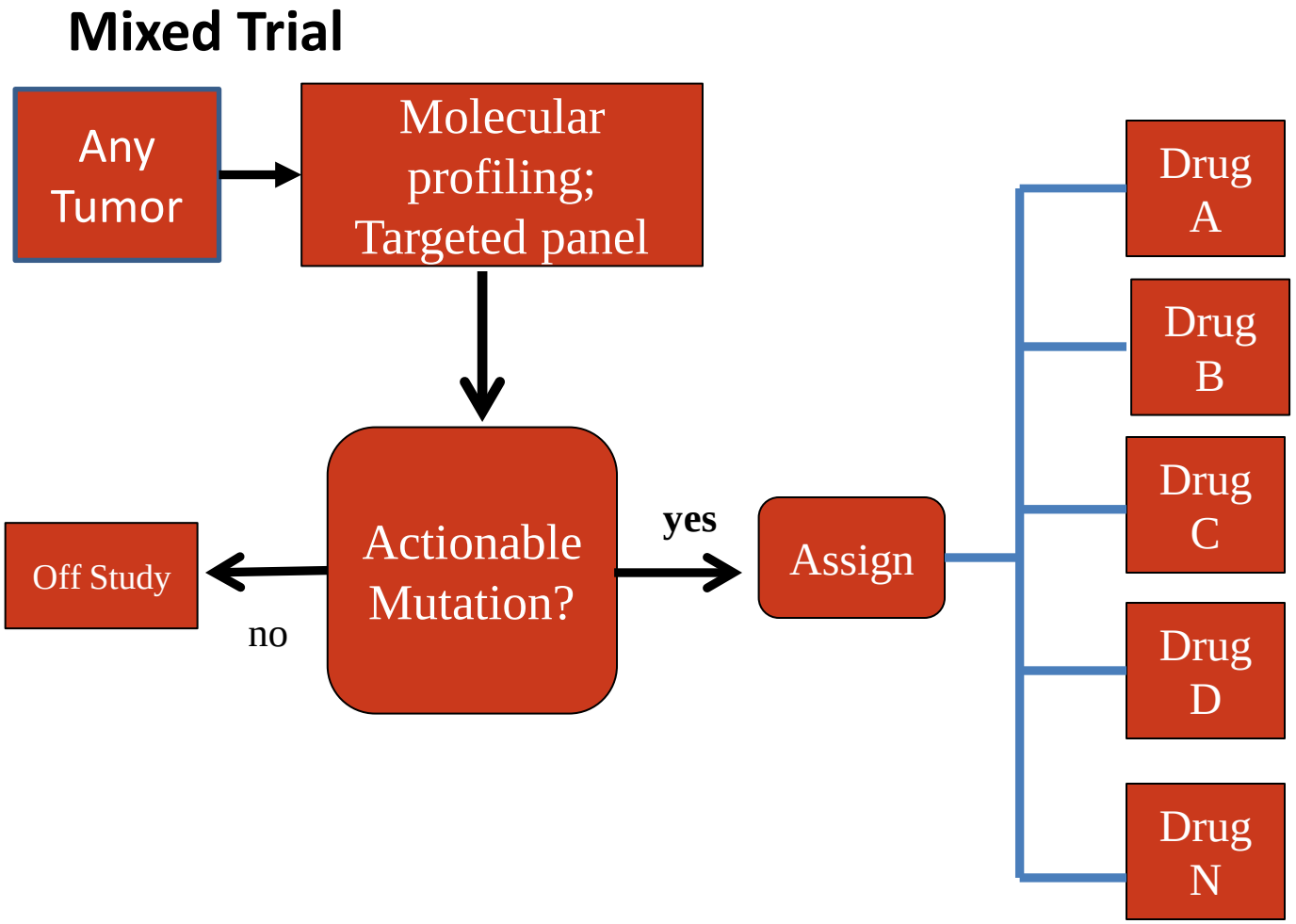
Umbrella “Genotyping Trial” Single Malignancy

- Examples

BATTLE TRIALS
LUNG MAP



“Genotyping Trial” all solid tumors: NCI-MATCH (Molecular Analysis for Therapy Choice)



Precision Medicine

- *BRAFV600E* melanoma responds to BRAF inhibitors or BRAF inhibitors combined with MEK inhibitors; but colorectal cancers with the same mutations appear not to respond
- Various tumors with *NTRK* fusions appear to respond to NTRK inhibitors (ASCO 2017)
- We need to know more about which tumors will respond to agents targeted to “driver” mutations
- Most driver mutations are relatively rare

What is NCI-MATCH?

THIS PRECISION MEDICINE TRIAL EXPLORES TREATING PATIENTS BASED ON THE MOLECULAR PROFILES OF THEIR TUMORS

NCI-MATCH IS FOR ADULTS WITH:

- solid tumors (including rare tumors), lymphomas, and myeloma
- tumors that no longer respond to standard treatment

ECOG-ACRIN cancer research group | NIH NATIONAL CANCER INSTITUTE

NCI-MATCH Objective

- To determine whether matching certain drugs or drug combinations in adults whose tumors have specific gene abnormalities will effectively treat their cancer, regardless of the cancer type
- This is a signal-finding trial—treatments that show promise can advance to larger, more definitive trials

Selection and Development of Assays for Precision Medicine

P. Mickey Williams, PhD, Director
Molecular Characterization Laboratory
National Cancer Institute

Defining the Assay's Intended Use

Define the intended use of the assay, early in process

- *Is assay used for pure research or clinical applications: integrated or integral in a trial OR Dx*
- *Categories of Clinical Use: 1) Dx to determine is disease is present, 2) prognostic or 3) predictive (Rx)?*
- *Sample characteristics?*
- *Desired turn around time?*
- *What results are reported? What will the physician need to know in order to take clinical action?*
- *How will assay performance characteristics impact intended use? Is sensitivity more important then specificity? What risk will result from an incorrect assay report?*
- *Does the use of this assay require regulatory discussions (FDA?) If unclear, always best to discuss directly via pre-submission!*
- *Determine components of the assay system*
- *Plan analytical validation to demonstrate required assay performance and identify and mitigate assay weaknesses*

Some Challenges of Assay Selection

- Single analyte versus multi-analyte:
  - Many Companion Dx IVD assays are single or few analytes
    - Long and costly development
    - Developed for 1 drug at a time
    - If many different targeted drugs exist for disease (e.g. NSCLC), is sufficient tissue available for all of the different Co-Dx assays
- NGS has proven valuable, multi-analyte targeted panels
  - Is tissue specific or pan-cancer panel best?
  - Dx assay reimbursement issues with larger panels (do you need all of the genes?)

Parameters & Complexities of Clinical NGS Assay Systems

1. Specimen acquisition (Dx block or a recent core needle biopsy) *How does genetic landscape change with treatment?*
2. Specimen storage and shipment conditions (Formalin or fresh frozen)
3. Pre-analytics processes (tumor enrichment, etc.) *How is allele fraction or CNV impacted by tumor enrichment protocols? How will allele fraction or CNV for treatment decision?*
4. Nucleic acid purification methods and quantitation and quality checking
5. Choice of NGS assay methods *How do different platforms compare, how does probe capture versus PCR targeting compare, how does target gene selection compare?*
6. Data analysis (mapping and variant calling) *How do mapping and variant calling algorithms and the parameters selected impact assay results?*
7. Assay verification and laboratory director sign-off
8. Tumor Board or rules engine selects treatment options *Who attends tumor board or designs rules engine? What is the knowledge base and how is it applied with levels of evidence?*

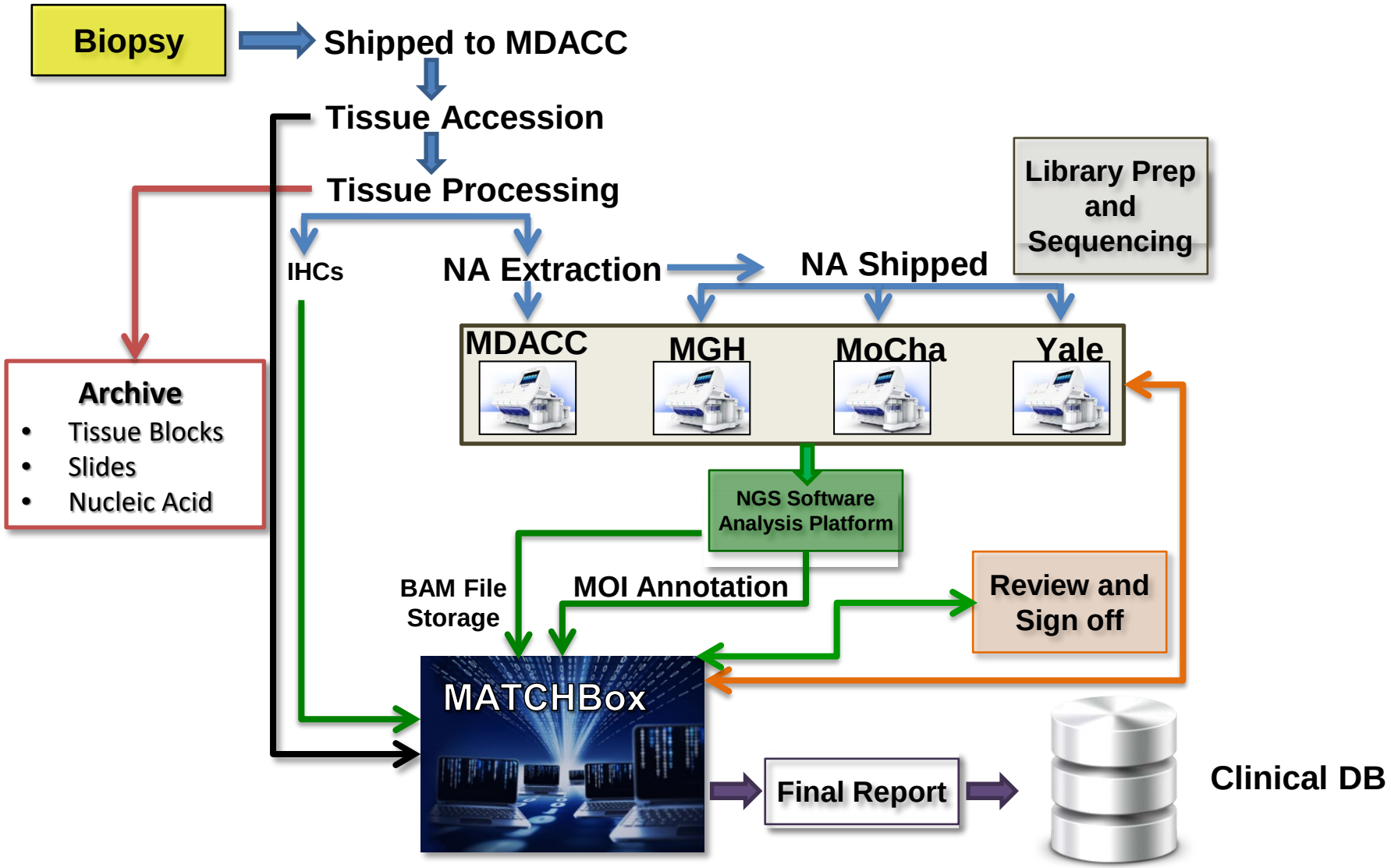
How to Determine if an Assay is "Good Enough" & How to Compare "2 Good Enough Assays"

- Assays used for clinical decisions, must undergo analytical validation and be performed in a clinically accredited laboratory
- If used as part of a clinical study and the assay will be considered integral, should include assay details as part of IND or IDE-pre submission
- There is a great need for Oncology NGS sequencing standards and performance panels to compare assays
  - NIST's Genome in a Bottle
  - Cancer cell line panels
  - Plasmids
  - Require some basic consensus of what on genes , variants and limits of detection should be tested

Selection of NGS Platform and Development of Laboratory Network

- NGS platform chosen after evaluation of RFI:
  - Low input of nucleic acid (core needle biopsy)
  - Accurate sequence from FFPET
  - Rapid turn-around time *(accommodate small batch size)*
  - Targeted assay capable of detection of many variant types: SNVs, indels, CNVs and gene fusions
  - Version 1: 143 genes & 4066 annotated variants
  - SNV, indel, CNV, targeted translocations
- Network of clinical laboratories selected by RFP (MDACC, MGH, Yale and MoCha)

Adult MATCH Assay System & Work Flow



Development of the NGS Assay System for Adult MATCH

1. **Defined intended use:**
   a) Assay will be used for enrollment and selection of treatment
   b) Assay is integral for trial enrollment and treatment selection
   c) Assay system and performance requirements were established in keeping with defined use:
      i. Pre-analytics (FFPET CNB, minimal NA),
      ii. TAT (~14 days)
      iii. Analytical performance (requires high specificity, high reproducibility within and across laboratories)
2. **Assess risk of use of assay in the clinical study in discussions with FDA**
   - Significant risk, requires IDE application
   - *Non significant risk, requires "Abbreviated IDE" **

* Up to date clinical trial binders/documents, versioned SOPs etc. Sponsor site audits, prepared for regulatory audits

Feasibility Testing, Validation Plan and Analytical Validation

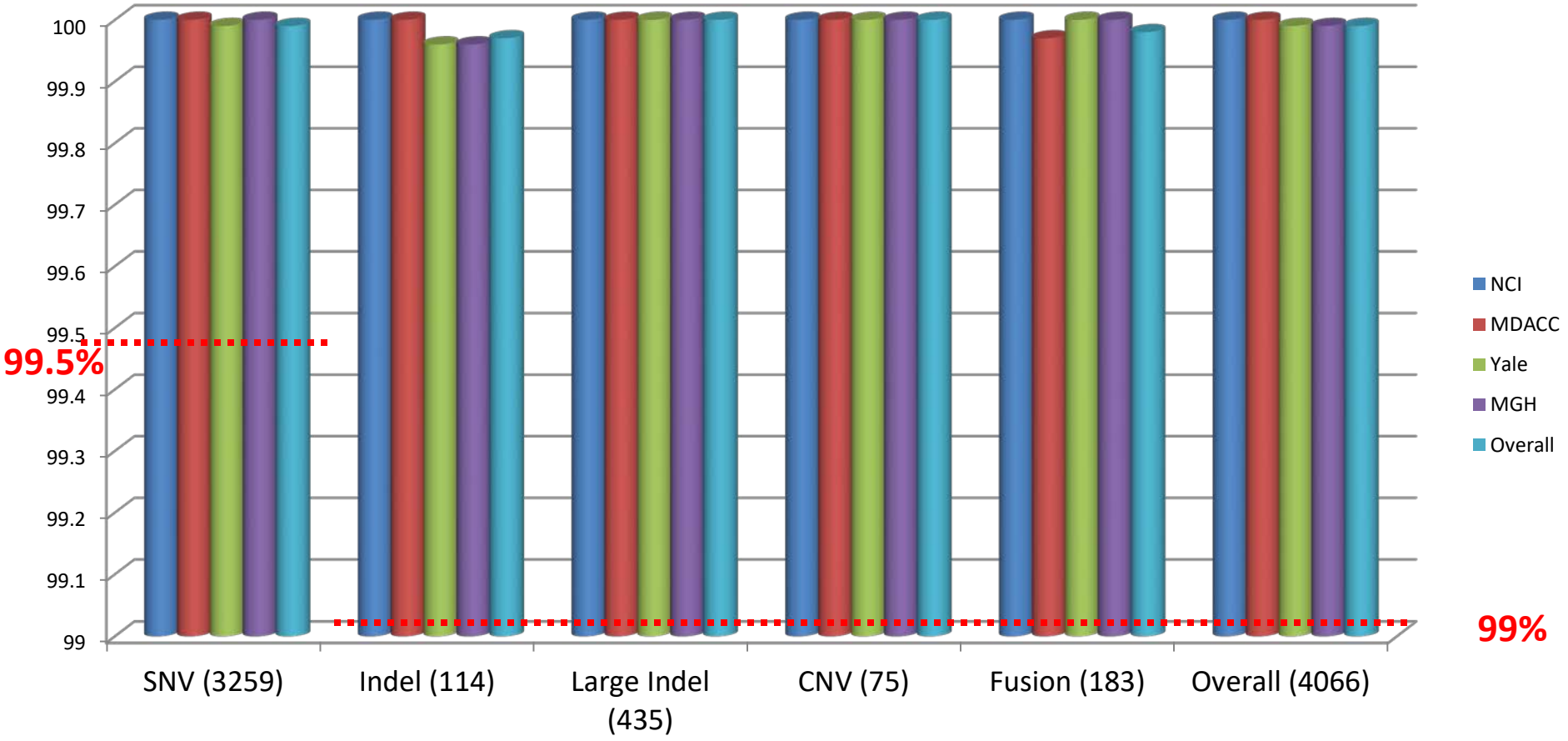
- Feasibility:
  - Test overall performance of assay platform
  - Help guide analytical performance testing and Validation Plan
- Lock SOPs and Complete Validation Plan
  - Key lab personnel met in Frederick to complete SOPs
  - Pre-submission with FDA for Validation Plan
- Perform Analytical Validation
  - Validation report submitted with IND

Specificity

Critical Parameter for Intended Use

True Negative / (True Negative + False Positive) over 4066 MOI loci

Acceptance criteria met by all laboratories



Reproducibility in Detected Variants

Reproducibility across 16 specimens	Nucleic Acid	Type	Mean Concordance
Positive Concordance	DNA	Intra-Operator	96.20
	RNA	Intra-Operator	100
	DNA	Within lab Inter-Operator	96.20
	RNA	Within lab Inter-Operator	100
	DNA	Cross lab Inter-Operator	96.29
	RNA	Cross lab Inter-Operator	100
Overall Concordance	DNA	Intra-Operator	99.99
	RNA	Intra-Operator	100
	DNA	Within lab Inter-Operator	99.99
	RNA	Within lab Inter-Operator	100
	DNA	Cross lab Inter-Operator	99.99
	RNA	Cross lab Inter-Operator	100

NCI-MATCH Success

- In June, 2017, the trial successfully reached its goal to sequence the tumors of 6K patients, nearly two years early
- Its availability through more than 1100 participating sites reflects the broad interest in the promise of genomics, and the ability of such a study to deliver that promise to the community

NCI-MATCH Overall Testing Rates

- For patients sequenced to date within the trial
  - Labs complete tumor testing for ~**94%** of patient cases
    - Lack of acceptable tumor samples is the major reason why the labs are unable to complete tumor testing for some patients
    - The lab network routinely out performs the industry, which averages ~80% for completion of tumor gene testing

Summary

- Time spent up front determining assay intended use and performance characteristics will pay off later
- Precision Medicine is a complex field and moving quickly
- Regulatory environment is complex and requires laboratory engagement to insure safety and utility of results
- A bad assay is just as harmful as a bad drug (D. Hayes)

Thanks!