Challenges in Analytical Validation of NGS Tests for Clinical Trials: NCI-MATCH Study

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Disclosure

- I have no financial relationships to disclose
- I will not discuss off-label use and/or investigational use in my presentation
- The views and opinions I express are my own and do not represent Leidos Biomedical Research nor NCI/NIH

Next Generation Sequencing

- Powerful multi-analyte (giga-analyte) assay method gaining popularity in all aspects of cancer research and patient clinical management
- NGS is a complex assay system:
  - Molecular biology techniques used to prepare and sequence DNA & RNA
  - Complex computer algorithms used to align the sequence to a reference and determine sequence variance
  - Every aspect of the assay system adds a unique bias to results
- 3 types of NGS of increasing complexity
  - Targeted sequencing (clinical)
  - Whole exome (research and clinical)
  - Whole genome (research and clinical?)
- Rapid improvements are occurring to these systems

Steps to Validating a NGS Assay System for Use in a Clinical Trial

1. Define intended use
2. Define assay system
3. Feasibility test the assay
4. Recommend consultation with FDA if specimens will be collected as part of the clinical trial specifically for this assay (Integrated or Integral)
5. Assess feasibility, mitigate assay weaknesses, define minimal analytical performance criteria and lock assay SOPs
6. Analytical validation of assay performance

Assay Intended Use

- Assay intended use as part of a clinical study:
  1. Pure clinical research (specimens not collected specifically for this use)
  2. Integrated assay (required for the clinical study but results not used for enrollment or treatment selection, e.g. specimens are collected for retrospective research)
  3. Integral assay (assay used for patient enrollment or treatment selection)

Assay System

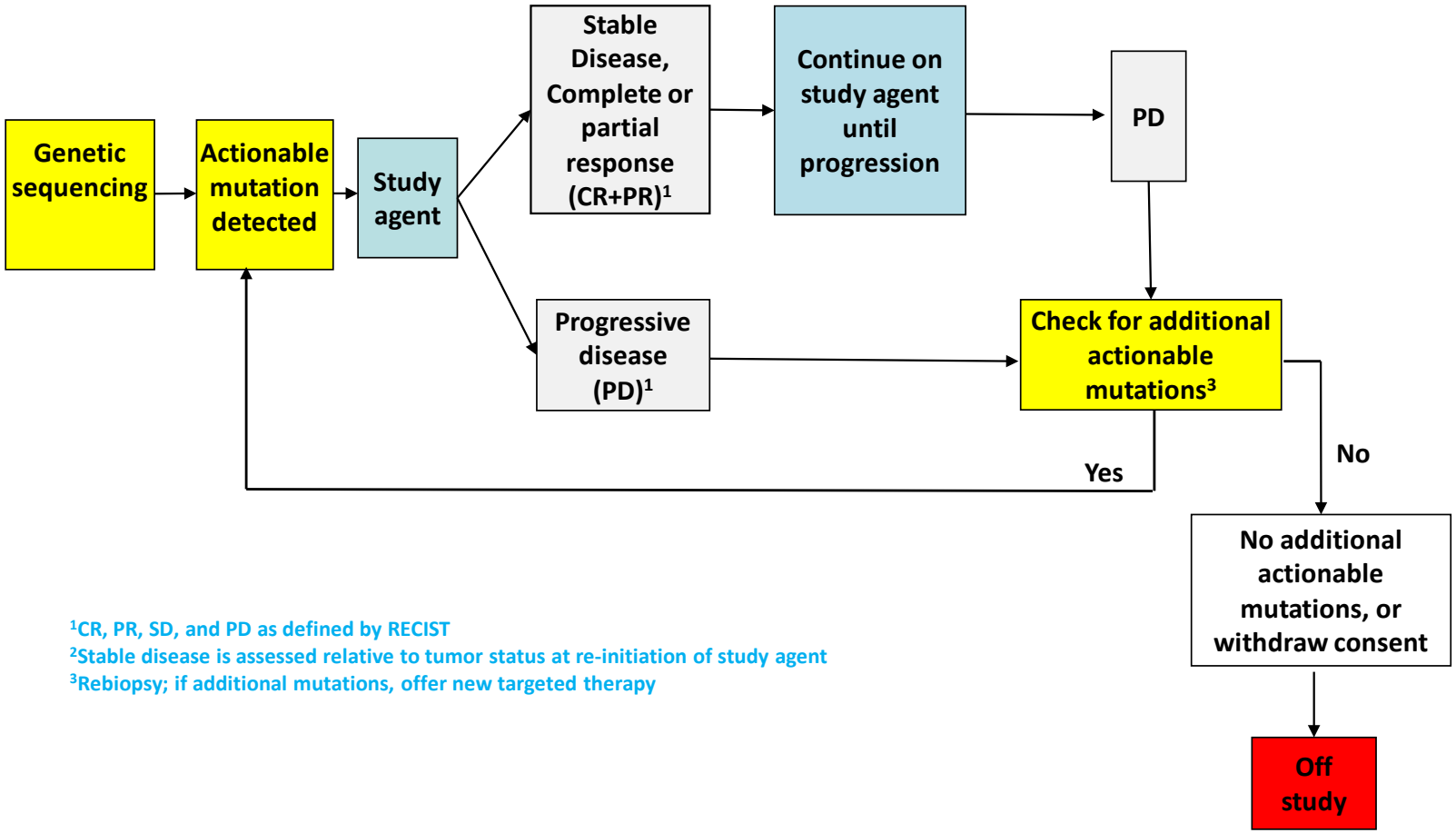
- Assays are systems that include all steps from biopsy through assay result reporting
- NGS assays are complex, any deviation from SOPs can confound data
- Document and lock down all aspects of the system in SOPs
- Assay systems each have a unique bias that at best is reproducible within the assay, but cannot be assumed to yield identical results to a different protocol
  - Changes in assay chemistry or data analysis will impact results

Don't assume that different assays or different laboratories will yield identical results!

NCI MATCH Study

- A multi-arm basket study
  - Multiple treatment arms each including multiple tissues
- Identify mutations/amplifications/gene fusions in patient tumor sample - eligibility determination
- Assign patient to relevant agent/regimen using a rules based approach
- Requires screening large numbers of tumors and have large numbers of targeted treatments

SCHEMA



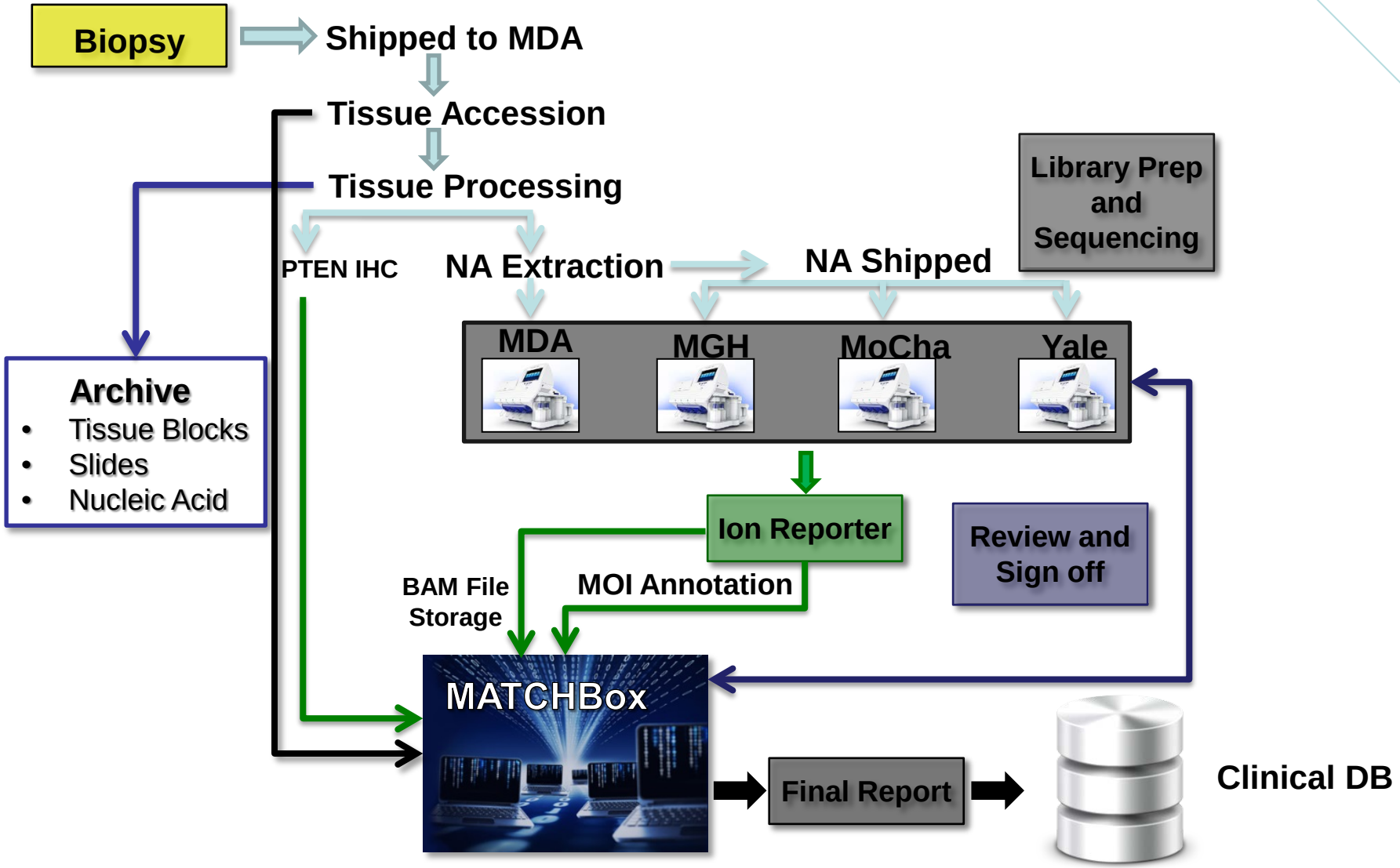
[1]CR, PR, SD, and PD as defined by RECIST
[2]Stable disease is assessed relative to tumor status at re-initiation of study agent
[3]Rebiopsy; if additional mutations, offer new targeted therapy

1. MATCH NGS Assay Intended Use

- MATCH NGS assay: intended use for patient entrance and treatment assignment (*Integral Assay)*
- Identical NGS assay system run 4 clinical laboratories (increased complexity)
  - MD Anderson Cancer Center (S. Hamilton)
  - Yale (J. Sklar)
  - MGH (J. Iafrate)
  - FNLCR (M. Williams)
- Levels of evidence established *a priori* matching of variants with treatment arms
- MATCHbox will select treatment based on established rules
- Consulted FDA CDRH (Devices) early via pre-submission meetings to determine best path for bringing assay into study

2. NCI-MATCH Assay System & Work Flow

11-14 Day Turnaround Time



3. MATCH Assay Feasibility Testing

- Gain familiarity with the assay prior to validation
- Identify strengths, weaknesses, mitigate assay deficiencies
- Develop quality check metrics and failure thresholds
- Develop and document data analysis and reporting process

4. Pre-Submission Discussion with FDA

- Meet with CDRH to discuss MATCH Study and Assay Intended Use and Validation Plan and expected assay minimal performance criteria
- The assay reports minimally 4048 known and annotated variants
- How can we validate such a large number of analytes?
- Demonstrate analytical performance with a subset of these variants

5. Assess Feasibility

- Assay performance was overall acceptable
- Identified several areas of weakness:
  - Difficult to sequence regions identified
  - Site to site variability in CNV normalization
  - In process of mitigation
    - Mark difficult sequence and increase confidence threshold for call prevent false positives, but increase false negatives
    - Test site specific normalization standard
- Met to harmonize laboratory procedures
- Finalize quality check metrics
- Finalize SOPs for full assay system
- Define minimal acceptable analytical performance criteria

6. Validation Plan

- Each laboratory will test analytical performance:
  - SENSITIVITY of 5 variant classes (FFPET clinical specimens with verified mutations):
    - SNV
    - Indel (small)
    - Indel (large)
    - CNV
    - Gene fusions
  - SPECIFICITY of all reportable variants (5 FFPET HAPMAP cell lines)
  - Accuracy (positive and negative accuracy)
  - Repeatability/Reproducibility (2 operator in each lab repeat 5 clinical specimens 2X each on 2 different instruments)
  - Full assay system ‘fit for purpose testing”

Some Final Comments

- NGS Assay systems are complex, *everybody is doing it,* but do different laboratories and assays get the same result?
  - Urgent need for reference materials (i.e. NIST Genome in a Bottle)
  - Need for minimal information guidelines for publication of NGS data (similar to MIAME)
- Plan for success in clinical studies, which will demonstrate clinical validity and prepare for demonstration of clinical utility
- There is a need for guidelines for clinically actionable variants, what level of evidence is needed before clinical action is taken?
- The community needs to work together collaboratively to insure successful integration of NGS into cancer patient management

Some Basic Guideline Documents

- Basic guidelines for development and clinical use of NGS assays, e.g.
  - CAP NGS Checklist
  - CLSI MM09-A2
  - "ACMG clinical laboratory standards for NGS", Genetics in Medicine; pg. 733-755 vol. 15 (9) 2013
  - CDC "Assuring the quality of NGS in clinical laboratory practice" Nat. Biotech. Pg. 1033-1036 vol. 30 2012