Frederick National Laboratory for Cancer Research

The Molecular Characterization Laboratory (aka MoCha)

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MOCHA

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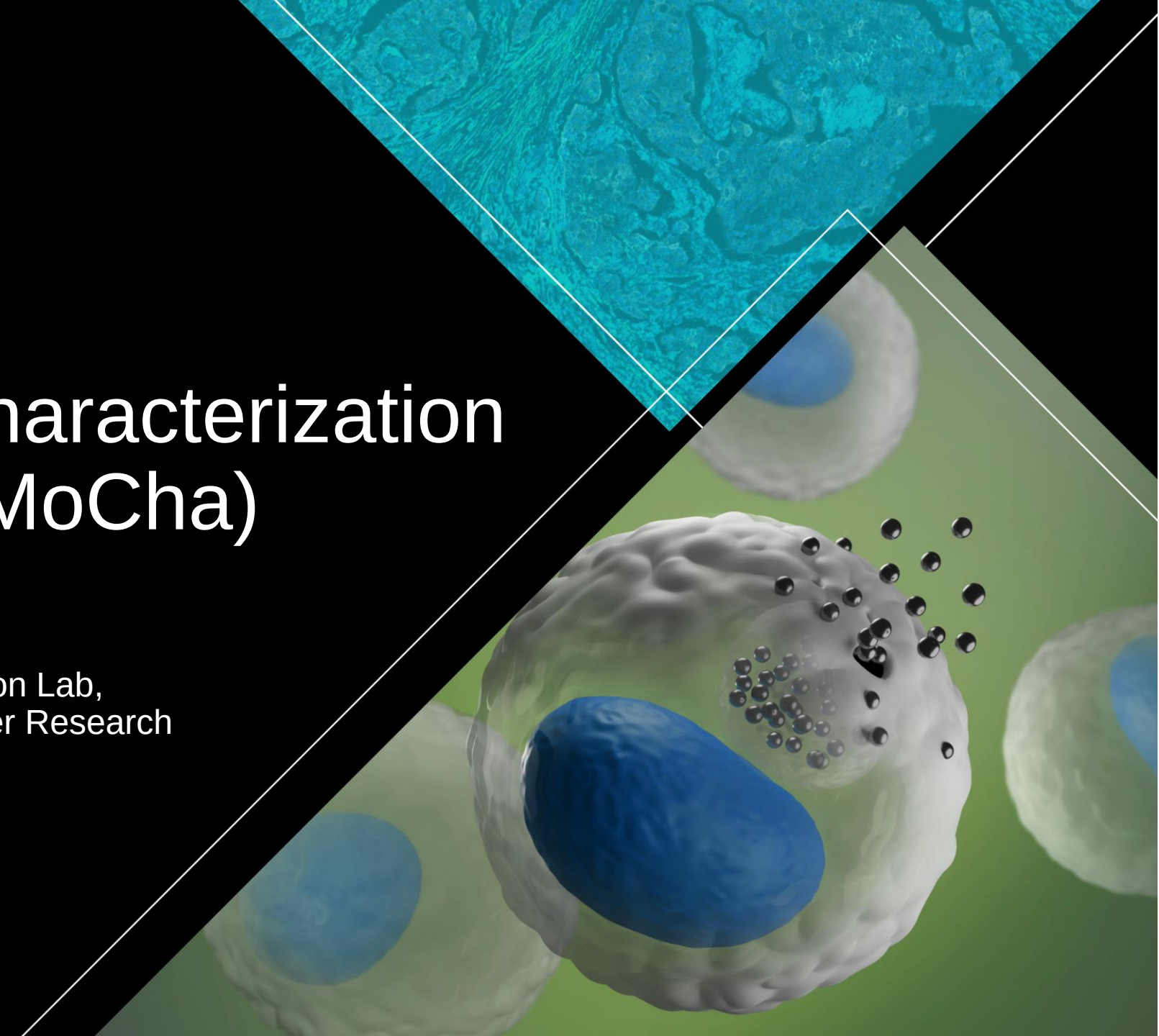

Our Goals

- **Provide cutting-edge genomic technologies and assays that are well characterized, accurate and reproducible in support of NCI pre-clinical research and clinical efforts (DCTD)**
- **Provide technical expertise in support of the development and functional oversight of Leidos sub-contracted laboratory activities**
- **Utilize NCI and Leidos CRADAs to provide novel technologies and assays to meet our goals**
  - Illumina (TSO500ctDNA liquid biopsy assay)
  - ThermoFisher (Genexus Myeloid assay)
- **Sharing all of our data publicly through NCI approved databases**

Laboratory Groups

- Moonshot Biobank
- Histology and Pathology (CLIA Accredited Complex Assay Lab)
- Research and Development
- CLIA Genomics Lab (CLIA Accredited Complex Assay Lab)
- Quality Assurance
- BioInformatics
- Administration

- MoCha began in April 2010

Cancer Moonshot Biobank Research Protocol
(NCI #10323)

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Cancer Moonshot Biobank: Primary Objective

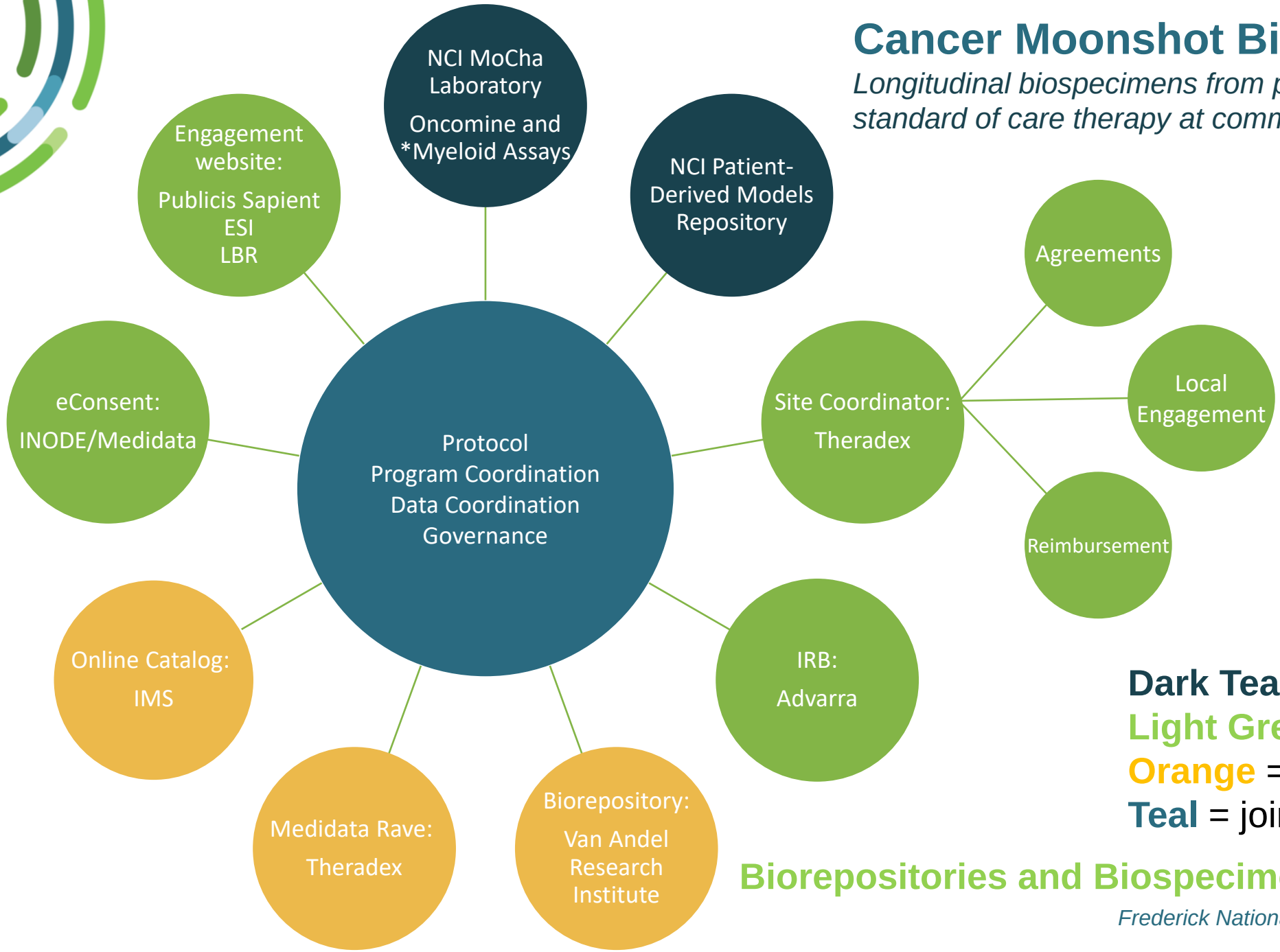
To support current and future investigations into drug resistance and sensitivity and other NCI-sponsored cancer research initiatives through the procurement and distribution of multiple longitudinal biospecimens and associated data from a diverse group of cancer patients who are undergoing standard of care treatment at NCI Community Oncology Research Program (NCORP) sites and other NCTN sites.

Cancer Moonshot Biobank: Secondary Objectives

- **To provide a service of value to study participants and their medical providers through the performance of molecular profiling assays on tumor samples in a CLIA-certified laboratory and reporting of results to physicians and patients that they may opt to use in clinical management.**
- **Enable the development of patient-derived models such as cell lines and xenografts for cancer researchers through the provision of biospecimens from 20% of study participants to the NCI's Patient Derived Models Repository (PDMR), a national resource available to investigators (https://pdmr.cancer.gov/).**
- **To develop increased capabilities in U.S. community hospitals and clinics for contribution to cancer research through biobanking activities.**

Cancer Moonshot Biobank

Longitudinal biospecimens from patients undergoing standard of care therapy at community hospitals



Dark Teal = NCI laboratory
Light Green = LBR subcontract
Orange = NCI contract
Teal = joint NCI/LBR activities

Biorepositories and Biospecimen Research Branch

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Cancer Moonshot Biobank: Status

- **Study launch date: September 16, 2020**
- **Full data set (including histological and radiological images) released in dbGaP in 2022; planning for release to CTDC**
- **Enrollment as of February 20, 2023: 188**
- **60 clinical reports returned to patients and treating physicians**
- **Focus areas:**
  - Engagement of patients and sites to increase enrollment
  - Expansion of sites
  - Continued assessment of specimen and data quality
  - Preparation for data release

Genomic Landscape of NCI's Patient-Derived Models Repository

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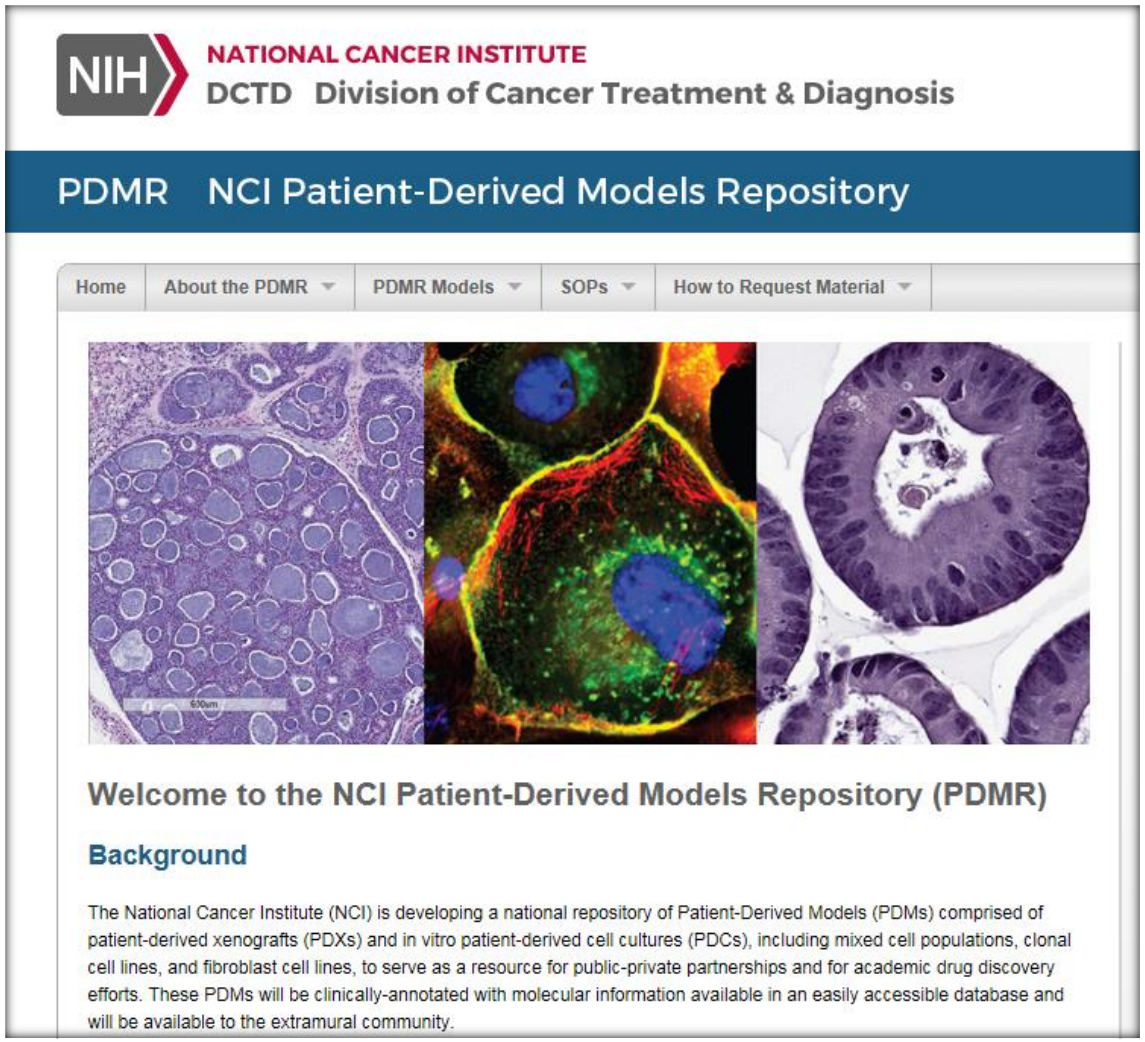
NCI Patient-Derived Models Repository (PDMR)

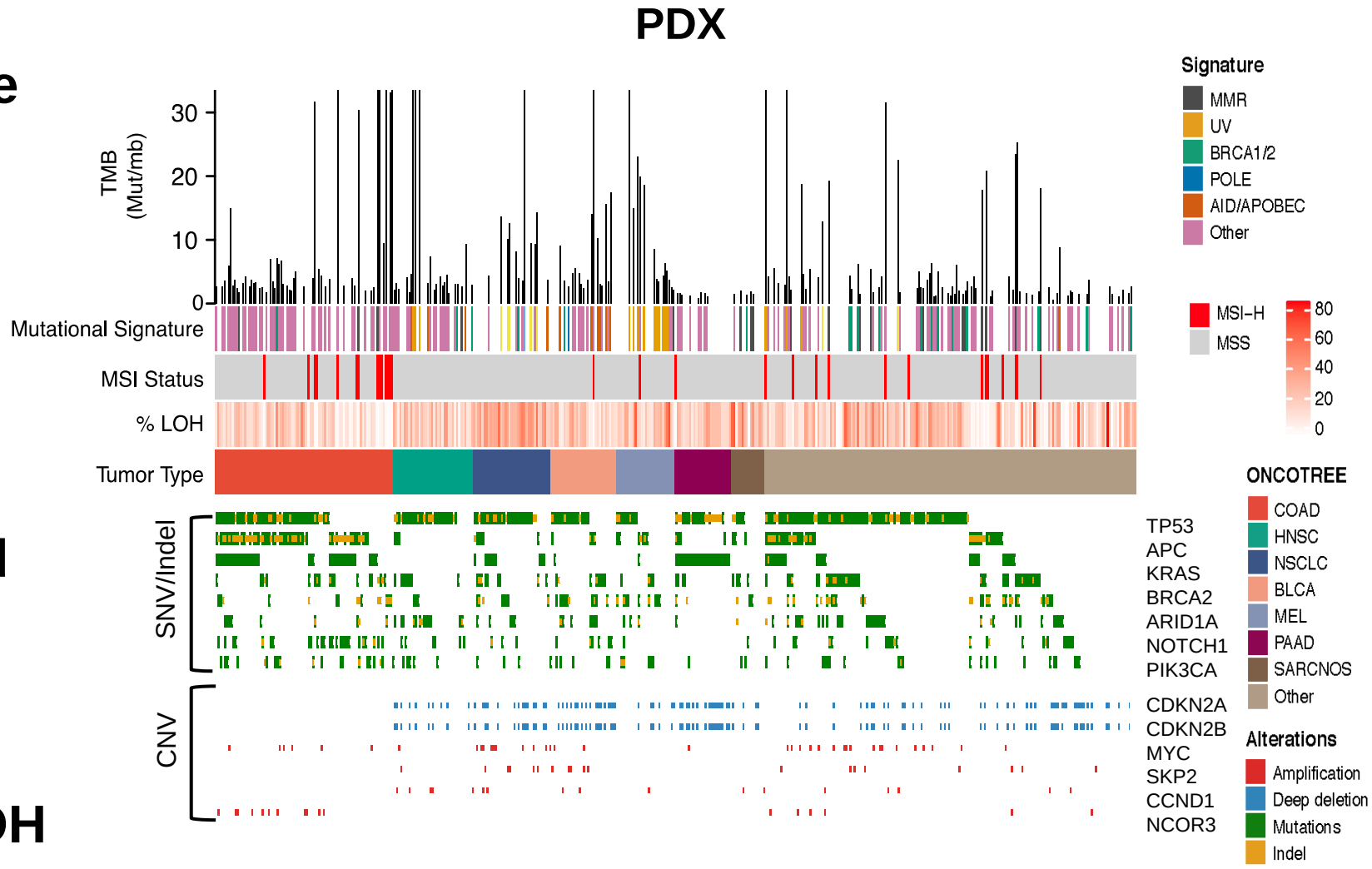


- **NCI has developed a national repository of Patient-Derived Models (PDM)**
  - Patient-derived xenografts (PDX)
  - Patient-derived cell lines (PDC)
  - Patient-derived organoid models (PDOrg)
  - Cancer associated fibroblast cell lines (CAF)
- **Models are available to the extramural research community**
- **All models have clinical and molecular data (WES and RNASeq) in an easily accessible database**

https://pdmr.cancer.gov

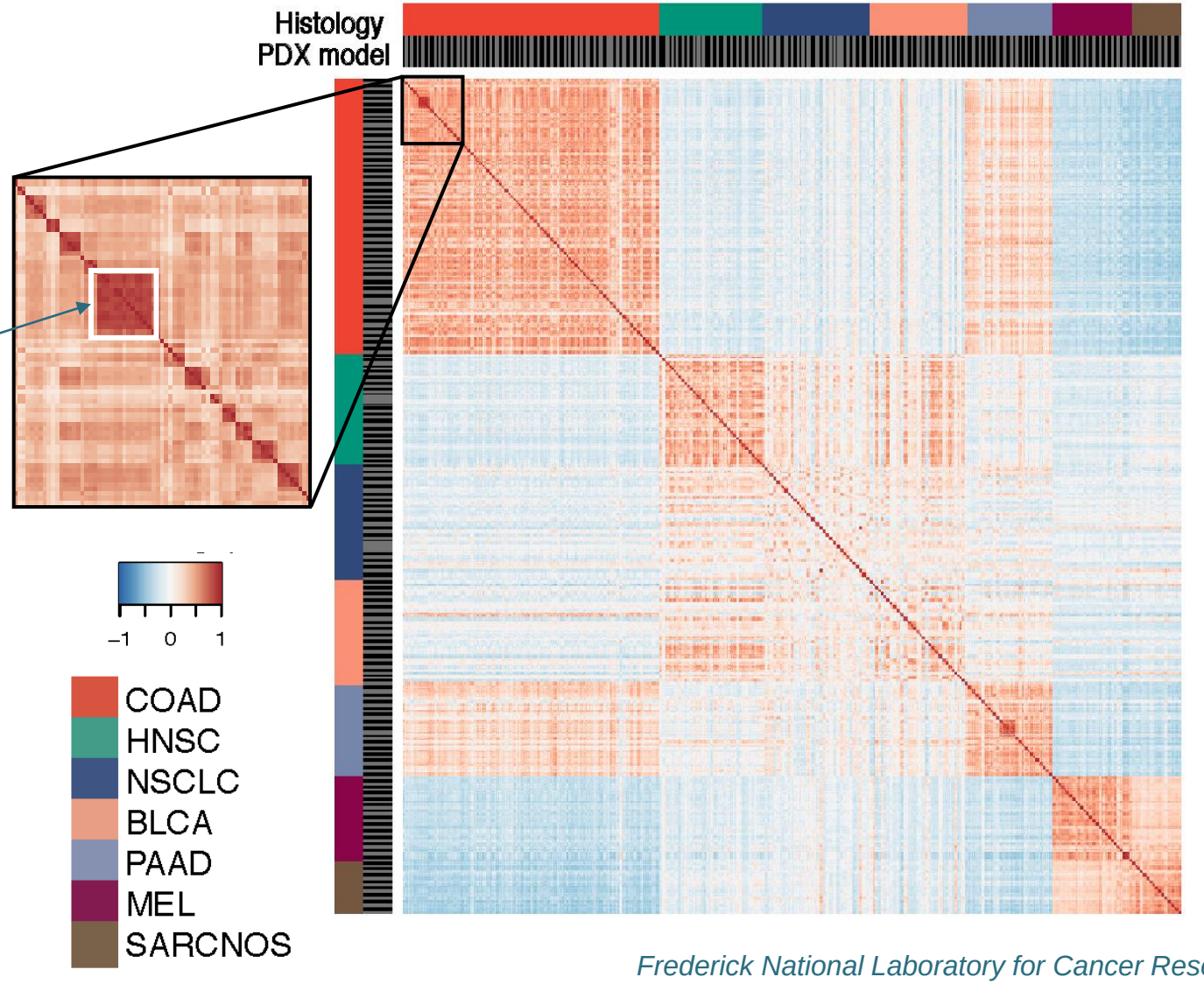
Genomic Landscape of Altered Genes and Clinically Relevant Biomarkers in NCI PDMR

- **TP53, APC, and KRAS are the most mutated genes**
- **Histology-specific enrichment of mutational signatures were observed**
- **MSI-high and POLE-mutated PDX models had higher TMB values**
- **Models with BRCA1/2 signatures had high %LOH**



Transcriptome Profiles Are Related by Histology and PDX Model Origin

- Pairwise Spearman correlation was conducted on gene expression profiles of PDX samples using normalized count values
- Samples in several common disease types are shown
- White box in the figure indicates samples in the same model
- PDX samples were ordered by their disease types



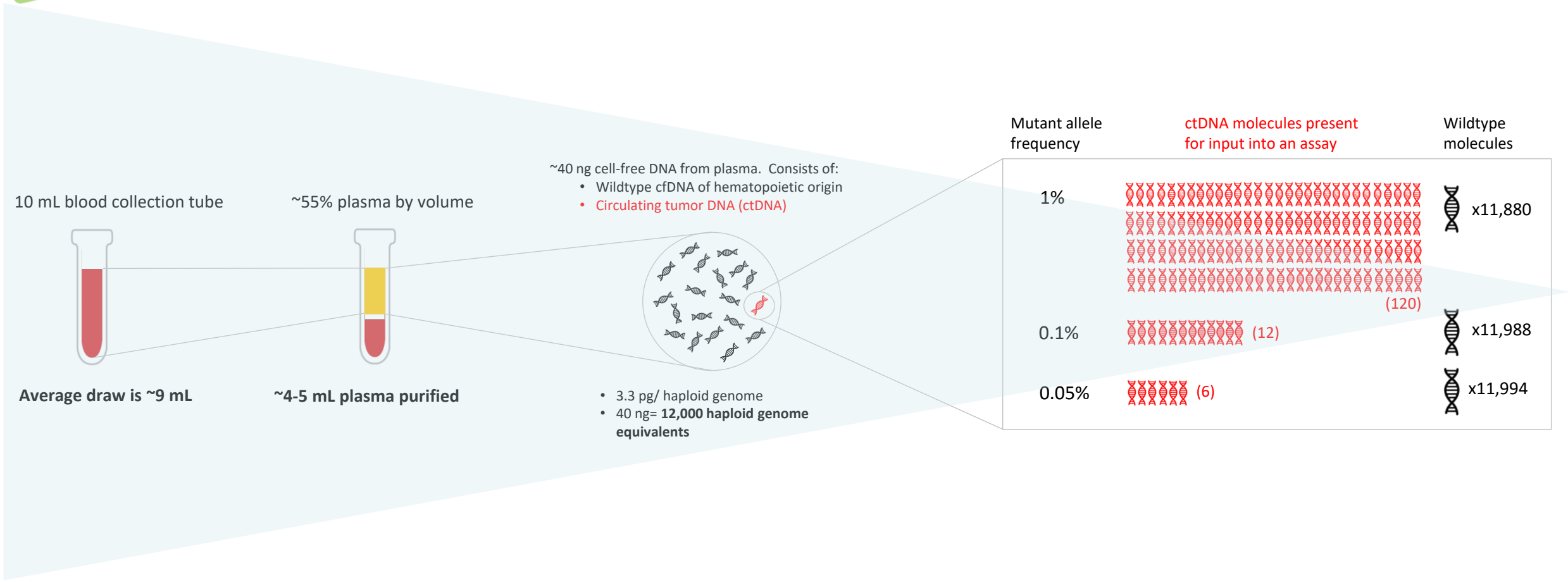
PDMR General Observations to Date

- **822 preclinical models from 775 patients have both WES and RNASeq data**
- **Multiple levels of evidence indicate genomic aberrations in patient tumors are maintained and propagated during early passages (P0 through P2) of the PDX models**
  - Driver mutations, CNA profiles, transcriptomic profiles, and the associated clinically relevant biomarkers LOH and MSI
- **The majority of somatic SNV/indels observed in the patient originator specimen detected in the individual specimens for each model**
- **The NCI PDMR has established a large repository of preclinical models from diverse solid tumor histologies, including rare cancers, with accompanying clinical, histological, and molecular datasets providing a robust resource for pre-clinical drug development**

Blood-Based Comprehensive Genomic Profiling: TSO500 ctDNA Assay

- **After assessment of 4 different assay technologies, it was decided to move forward with an Illumina pre-commercial assay**
  - TSO500ctDNA provided:
    - Largest gene panel, 523 cancer relevant genes and all exons sequenced including the large tumor suppressor genes, e.g.BRCA1 & 2 and ATM
    - Gene copy amplification, MSI, and TMB are reported
    - Tiling of relevant introns in clinically relevant gene translocations
- **Work performed under Leidos and NCI CRADA's**
- **Close collaboration with Illumina assay development and bioinformatic teams**

A 10 mL Tube of Blood Contains Very Little ctDNA

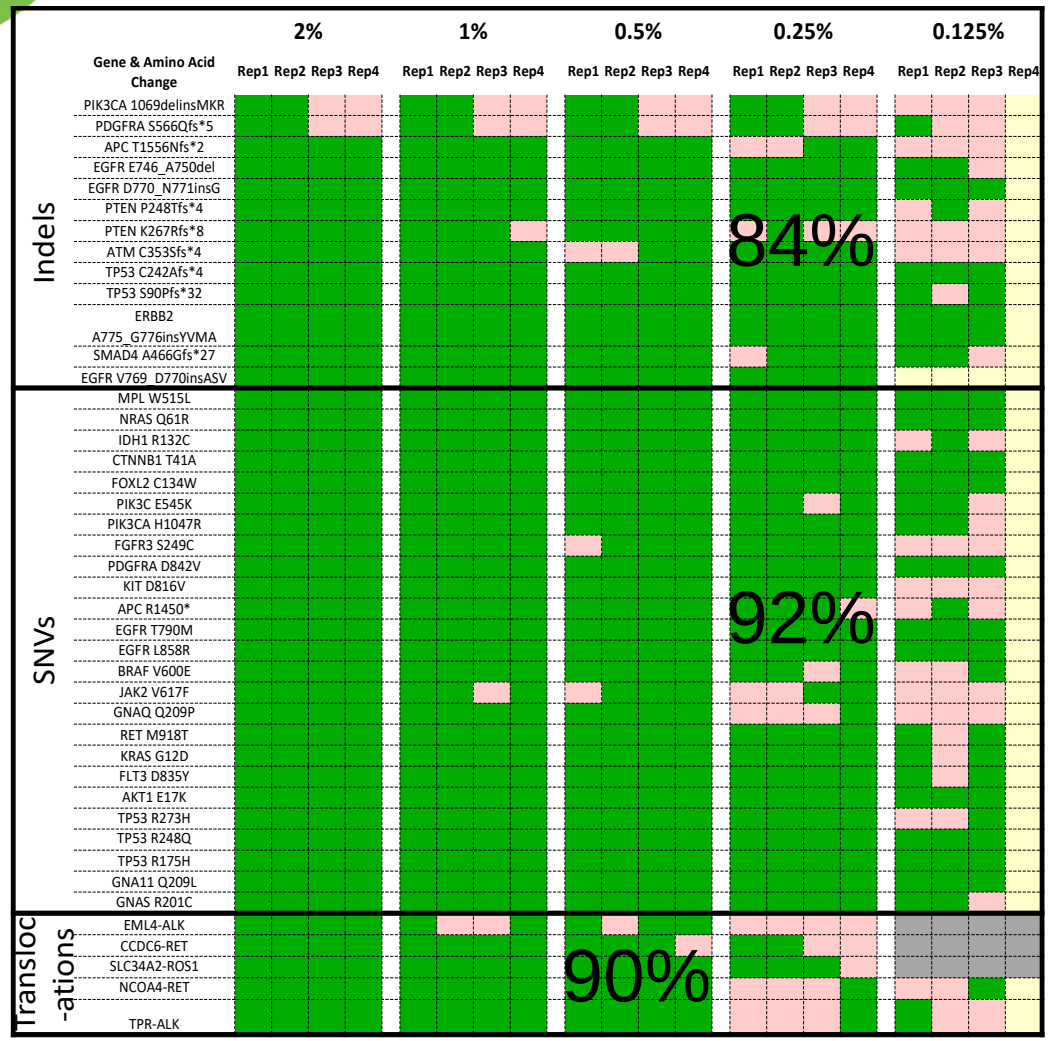


Intended Use of a ctDNA Assay

- **Intended use: Initial use as an Integrated clinical research assay,**
- **If needs arise move into an Integral predictive or prognostic biomarker assay for trial support**
  - Integrated assays have specimens collected during a clinical trial for use in research

    No results are returned to physician or patient
  - Integral assays are used to enroll, stratify or manage treatment of patients in a clinical trial

LoD80 Established for 3 Variant Types



	Titration Point	Rep1	Rep2	Rep3	Rep4
EGFR	A	2.644	2.666	2.671	2.625
	B	1.832	1.833	1.832	1.828
	C	1.327	1.325	1.316	1.307
	D	ND	ND	ND	ND
	E	ND	ND	ND	ND

	Titration Point	Rep1	Rep2	Rep3	Rep4
ERBB2	A	2.169	2.193	2.169	2.172
	B	1.389	1.38	1.39	1.374
	C	1.191	1.182	1.181	1.171
	D	ND	ND	ND	ND
	E	ND	ND	ND	ND

	Titration Point	Rep1	Rep2	Rep3	Rep4
MET	A	2.093	2.094	2.074	2.122
	B	1.459	1.469	1.483	1.487
	C	1.226	1.215	1.233	1.245
	D	ND	ND	ND	ND
	E	ND	ND	ND	ND

	Titration Point	Rep1	Rep2	Rep3	Rep4
MYCN	A	1.99	2.006	1.994	1.941
	B	1.354	1.353	1.362	1.341
	C	ND	ND	ND	ND
	D	ND	ND	ND	ND
	E	ND	ND	ND	ND

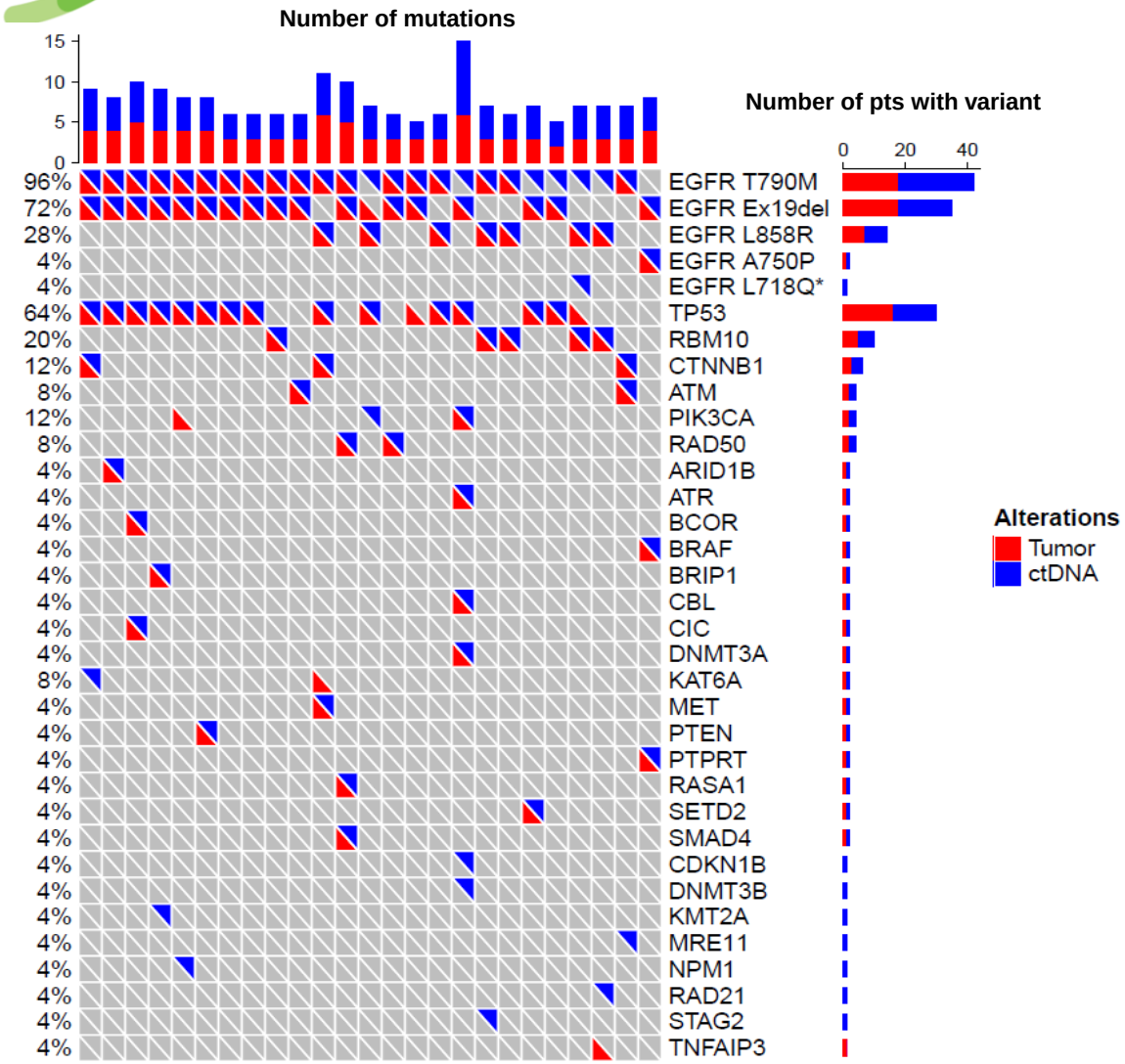
LOD_{80}: the lowest VAF at which at least 80% of replicates can be detected

Limit of Reporting Thresholds

- SNVs ≥ 0.5% VAF
- Indels ≥ 0.5% VAF
- Translocations ≥1.0% VAF
- ≥ 3 supporting reads
- CNVs ≥1.3-fold change

Harrington et al. AMP Annual Meeting 2020 poster TT21

SNV and Indel Molecular Landscape: NSCLC Tissue vs. Blood



- **Oncogenic/Likely oncogenic OncoKB SNVs and Indels were identified in 25 patients with matched plasma and tissue**
- **All pts had somatic alterations in tumor and plasma**
- **One pt had a sub-clonal EGFR L718Q mutation (VAF = 0.49%) in plasma only**
- **TP53 mutations were identified in 64% of pts**
- **Discordant variants (i.e. tumor+/ plasma- or tumor-/plasma+) were mostly sub-clonal as inferred from the variant allele frequency (VAF) (not shown).**

Karlovich et al. AMP Annual Meeting 2020

Past Clinical Trials

- **NCI-MPACT: a pilot precision medicine study, MoCha provided a targeted NGS clinical assay**
- **NCI-MATCH:**
  - Implemented a 4 clinical laboratory network
  - Harmonized and analytically validated a central laboratory assay in all 4 labs
  - Supported screening of the first 6,000 patients
  - Implemented 29 laboratory designated laboratory network
    - Vetted analytical validation and required concordance testing
- **Pediatric MATCH**
  - Utilized existing central laboratory network and NCI-MATCH clinical assay

MDNet: Laboratory Support for 3 New Precision Medicine Trials

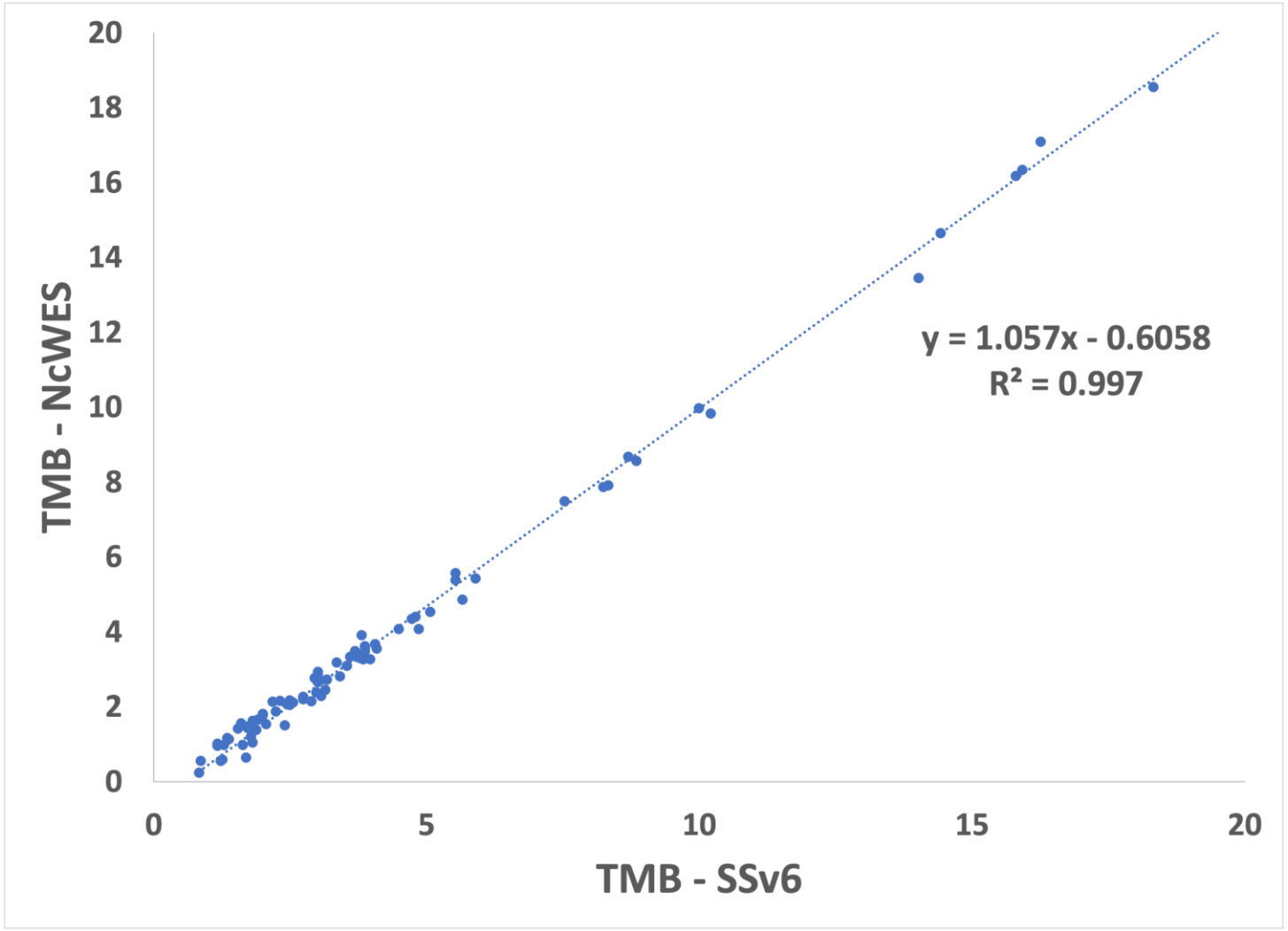
- **iMATCH**
  - Pilot trial will use 2 biomarkers:
    - TMB via harmonized cWES in MoCha and 1 sub-contracted lab
    - Tumor inflammation score (TIS), 2 sub-contracted laboratories working with NanoString
- **MyeloMATCH; (IDE)**
  - 3 assays requiring 72-hour turn-around-time
    - Cytogenetics (reflux FISH); sub-contracted
    - Targeted NGS; MoCha and 1 sub-contracted
    - FLOW; 1 sub-contracted lab
- **COMBO-MATCH**
  - NCI-MATCH Designated Lab Network (increased to ~40 labs)

Clinical Whole Exome Sequencing (cWES) Assay

- **Analytically validated WES assay for iMATCH (TMB Integral assay), Integrated assay for ComboMATCH and ETCTN clinical trials**
- **~44 Mb target region**
- **Higher coverage in the exons of 671 genes for increased sensitivity for SNV/Indels (genes annotated in OncoKB database as oncogenic or likely oncogenic)**
- **Integrated and Exploratory Biomarkers:**
  - Coverage in intronic regions of actionable fusion genes to identify translocations
  - Additional probes (tiled across genome at 1MB intervals) for identifying LOH regions, focal amplifications
  - WES data analysis pipeline will also report MSI and HLA Class I typing
  - Detection of 7 oncogenic virus family
- **Fast turnaround time needed for prospective reporting (<2 weeks)**

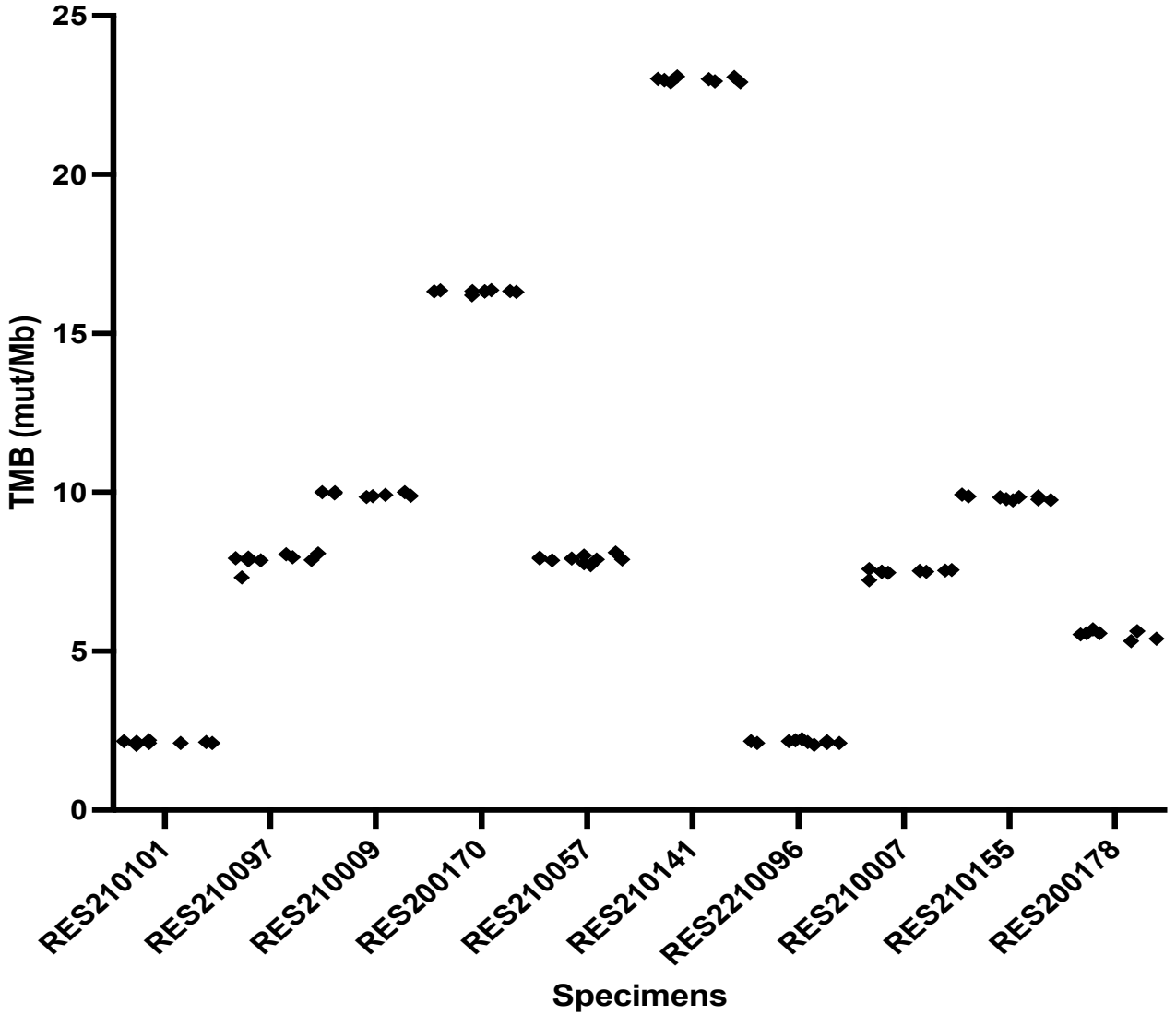
Correlation of TMB with an Orthogonal Assay

- Correlation study for TMB was performed using Research WES assay as the orthogonal assay
- 91 specimens tested
- TMB is ranging from 0-140 mut/Mb



Precision of cWES TMB

- **Precision of TMB values across the reportable range of 5-20 mut/Mb is high (% CV <2.87)**
- **It is within the acceptance criteria (%CV <20%)**



NCI-Myeloid Assay

- **Developed under NCI CRADA with Thermo-Fisher**
- **Sequencing chemistry uses isothermal amplification of targeted DNA/RNA followed by synthesis-based sequencing**
- **Minimal sample input 30 ng DNA and RNA**
- **Fully automated workflow:**
  - Load a plate of DNA and RNA from patient samples
  - Receive all data for review and a clinical report
  - **Faster TAT (1-2 days)**

NCI-Myeloid Assay – Version 2 (NMAv2)

DNA hotspots				
ABL1	*ANKRD26*	*BRAF*	*CBL*	*CSF3R*
DDX41	*DNMT3A*	*FLT3*	*GATA2*	*HRAS*
IDH1	*IDH2*	*JAK2*	*KIT*	*KRAS*
MPL	*MYD88*	*NPM1*	*NRAS*	*PPM1D*
PTPN11	*SETBP1*	*SF3B1*	*SMC1A*	*SMC3*
SRSF2	*U2AF1*	*WT1*		
DNA Full Gene				
ASXL1	*BCOR*	*CALR*	*CEBPA*	*ETV6*
EZH2	*IKZF1*	*NF1*	*PHF6*	*PRPF8*
RB1	*RUNX1*	*SH2B3*	*STAG2*	*TET2*
TP53	*ZRSR2*			
RNA Fusion Driver Genes				
ABL1	*ALK*	*BCL2*	*BRAF*	*CCND1*
CREBBP	*EGFR*	*ETV6*	*FGFR1*	*FGFR2*
FUS	*HMGA2*	*JAK2*	*KMT2A (MLL) +PTDs*	*MECOM*
MET	*MLLT10*	*MLLT3*	*MYBL1*	*MYH11*
NTRK3	*NUP214*	*NUP98*	*PDGFRA*	*PDGFRB*
RARA	*RBM15*	*RUNX1*	*TCF3*	*TFE3*
BAALC	*MECOM*	*MYC*	*SMC1A*	*WT1*

- **NMAv2 covers**
  - 45 DNA genes and 35 fusion driver genes
  - Includes 28/30 (93.3%) genes mutated with >=3% frequency in AML.
  - Includes 36/50 (72%) genes mutated with >1% frequency in AML.
  - Includes 779 unique fusions reported in AML
- **Can detect all genetic alterations needed for**
  - WHO classification of AML, except inv 3
  - NCCN/ELN risk stratification, except inv 3
- **NMAv2 can detect**
  - FLT3-ITD up to 120bp
  - Alterations in CEBPA

Validation Summary

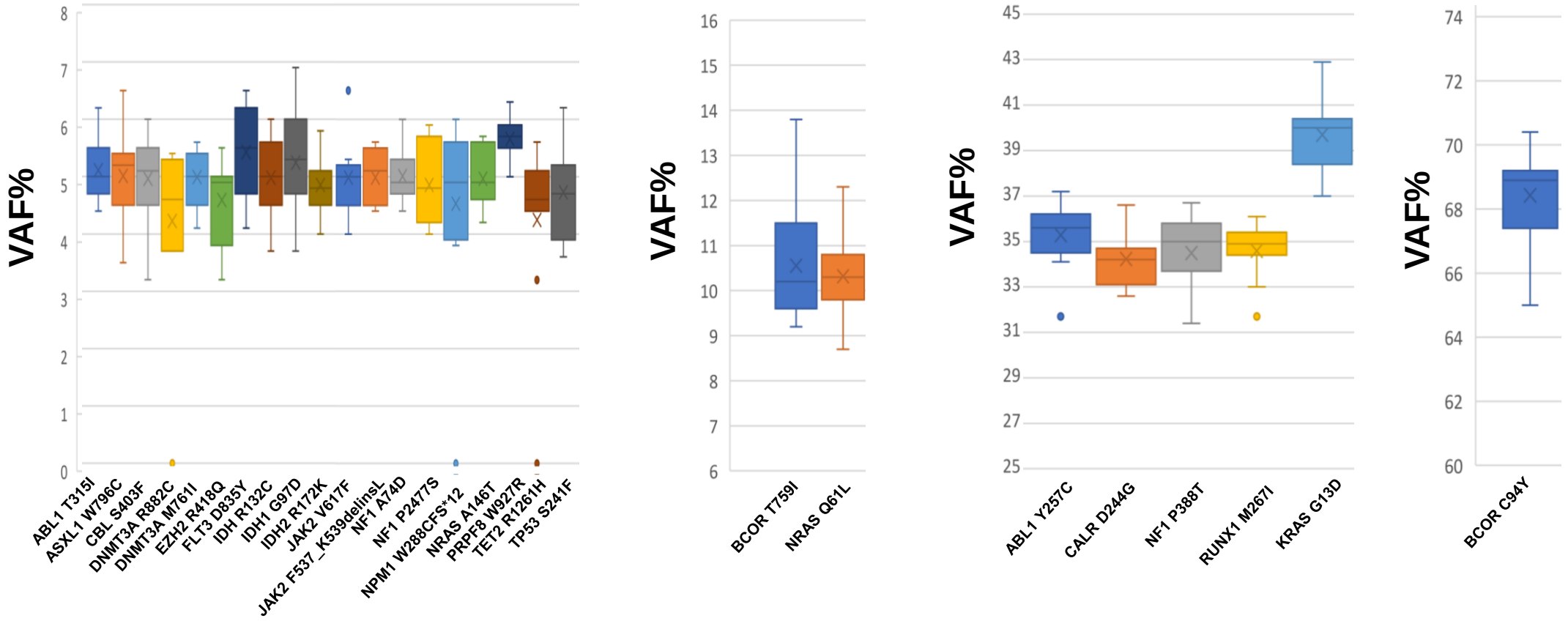
NMAv2 Performance:

- **Specificity: 100%**
- **Overall sensitivity: 98.9%**
  - SNVs: 97.78%
  - Indels: 100%
  - Fusions: 100%
- **Reproducibility**
  - Mean PPA: 98.33%
  - Mean NPA: 100%
- **LOD**
  - SNVs: HS <0.06%, Non-HS <2%
  - Indels: HS <2%, Non-HS <3%
  - FLT3-ITDs: 0.3% for 40bp
  - Fusion: 40 read counts, 0.1% tumor content

LOR: Will report out SNV/Indels >5% VAF, but assay is validated to report

1. ≥2.5% for all SNVs
2. ≥3% for all indels
3. 1% for FLT3-ITD
4. ≥100 read counts or two reproducible calls for fusions detected at <100 reads, with exception of the KMT2A-PTD fusion which requires reporting if detected at ≥2000 read counts.

Precision of NMAv2 Assay



Contrived material and leukemia cell lines, sequenced multiple times (≥35)

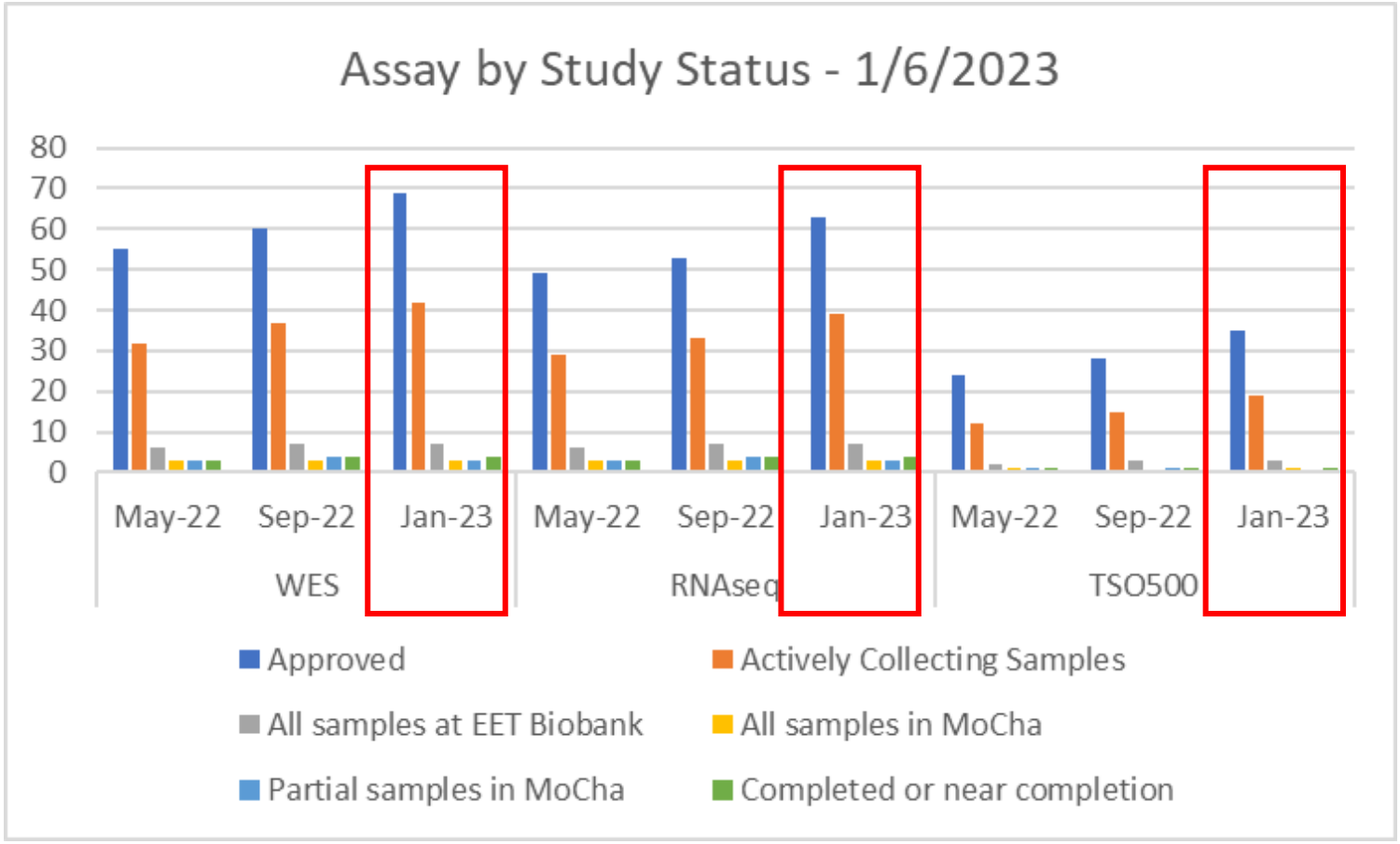
Reference Materials, Quality Control Materials and Diagnostic Harmonization Efforts

- **Precision Medicine efforts require Dx assays for cancer patient management**
- **Difficult to judge accuracy and comparability of these complex assays**
- **MoCha has engaged in:**
  - Genome in a Bottle; human genome RM (NIST)
  - Developed and Implemented Oncology RM (SeraCare CRADA)
  - Co-Developed Copy Number RM (NIST)
  - Developing ctDNA QCM (FNIH, NIST)
  - FOCR TMB Comparability Study
  - FOCR HRD Comparability Study
  - Contributed to CIMAC genomic assay harmonization effort
  - SRS Somatic Reference Samples (based on Genome in a Bottle RM)

National Clinical Laboratory Network (NCLN)

- **National Clinical Laboratory Network supports Early Treatment Clinical Trial Network**
- **Serve as expert evaluators of study proposals**
- **Provide robust analytically validated assays for Pharmacodynamic and Genomic Analysis (PADIS and MoCha)**
  - MoCha is providing genomic assay support for ETCTN
    - WES
    - RNASeq
    - ctDNA predictive and longitudinal

NCLN Genomic Assay Status



Acknowledgements to MANY!!

- **NCI**
- **Our many subcontractors and collaborators**
- **Many non-MoCha collaborators within Leidos: FNLCR**
- **All of the staff at MoCha**
  - Chris Karlovich PhD, Associate Director
  - Lily Chen PhD, BioInformatic/Computational Biology
  - Bishu Das PhD, R&D
  - DJ Jiwani MD PhD, CLIA Lab Director/Histology
  - Sean McDermott PhD, Moonshot Biobank

- **OPEN TO COMMENTS AND QUESTIONS?**

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