Genomic Assays Offered through the NCI National Clinical Laboratory Network

ETCTN Webinar

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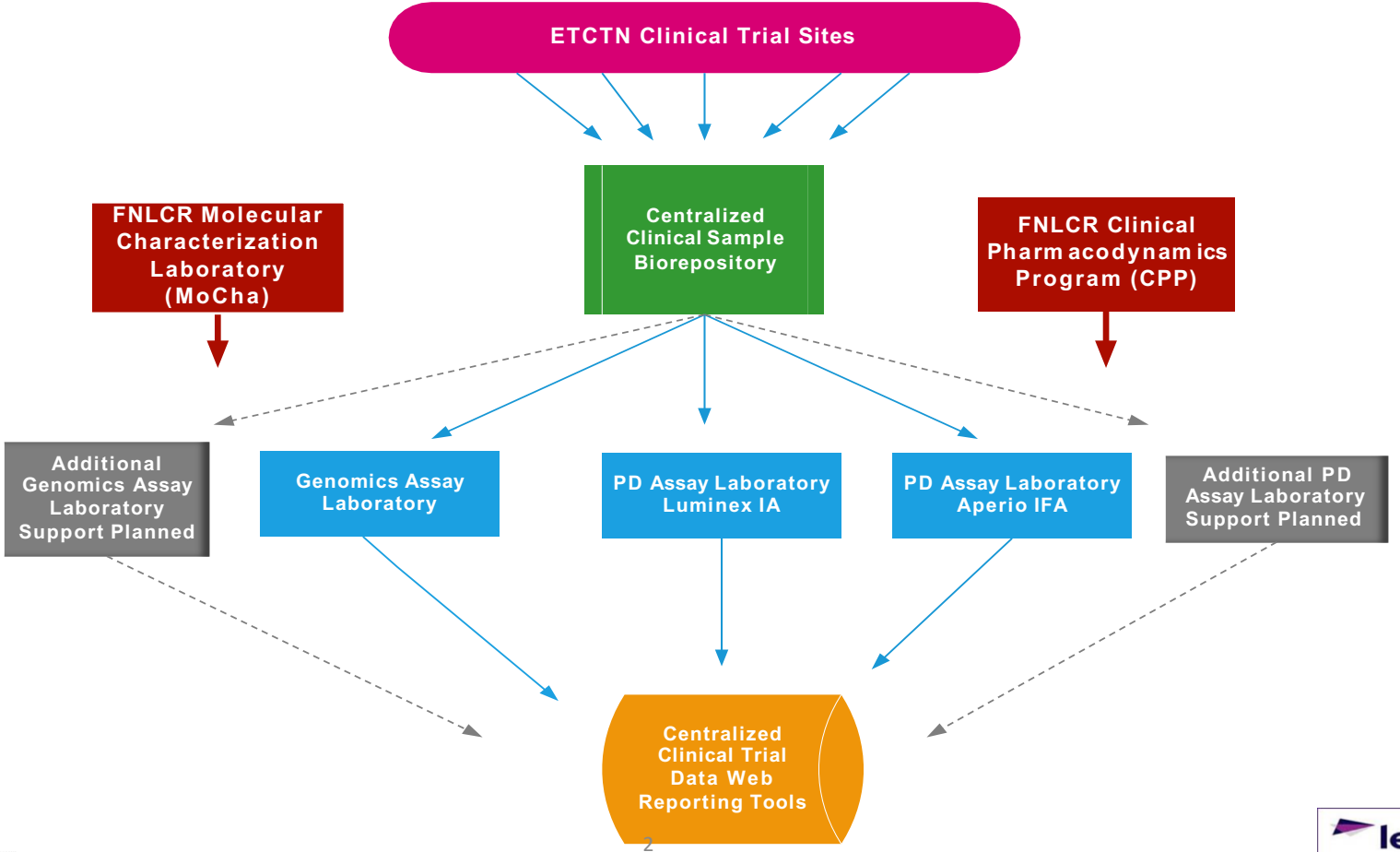
NIH NATIONAL CANCER INSTITUTE

National Clinical Laboratory Network for Precision Medicine



The NCLN is implementing validated assays that follow harmonized SOPs

- Uniform assay workflow, instrumentation & data analysis pipeline is being used for all assays across network labs
- NCI-MATCH central lab network achieved high inter-lab concordance (>96%) with this approach
- Pre-analytics for specimen collection and processing also follows strict SOPs

Core genomic technologies supported by the NCLN

- Oncomine pan-cancer targeted gene panel (OCAv3)
  - screened first 6000 pts for NCI-MATCH
- Whole Exome Sequencing (WES)
  - Somatic and germline variant-calling using matched germline WES
- RNA Sequencing (RNA-Seq)
  - Gene expression signatures; fusion-calling will be implemented in the coming weeks
- TSO500 targeted gene panel for ctDNA
  - 523-gene targeted panel

The Oncomine Comprehensive Assay (OCAv3)

Hotspot Genes (87)			
AKT1	FGFR4	MED12	SRC
AKT2	FLT3	MET	STAT3
AKT3	FOXL2	MTOR	TERT
ALK	GATA2	MYC	TOP1
AR	GNA11	MYCN	U2AF1
ARAF	GNAQ	MYD88	XPO1
AXL	GNAS	NFE2L2	
BRAF	H3F3A	NRAS	
BTK	HIST1H3B	NTRK1	
CBL	HNF1A	NTRK2	
CCND1	HRAS	NTRK3	
CDK4	IDH1	PDGFRA	
CDK6	IDH2	PDGFRB	
CHEK2	JAK1	PIK3CA	
CSF1R	JAK2	PIK3CB	
CTNNB1	JAK3	PPP2R1A	
DDR2	KDR	PTPN11	
EGFR	KIT	RAC1	
ERBB2	KNSTRN	RAF1	
ERBB3	KRAS	RET	
ERBB4	MAGOH	RHEB	
ERCC2	MAP2K1	RHOA	
ESR1	MAP2K2	ROS1	
EZH2	MAP2K4	SF3B1	
FGFR1	MAPK1	SMAD4	
FGFR2	MAX	SMO	
FGFR3	MDM4	SPOP	

Full-Length Genes (48)	
ARID1A	PALB2
ATM	PIK3R1
ATR	PMS2
ATRX	POLE
BAP1	PTCH1
BRCA1	PTEN
BRCA2	RAD50
CDK12	RAD51
CDKN1B	RAD51B
CDKN2A	RAD51C
CDKN2B	RAD51D
CHEK1	RB1
CREBBP	RNF43
FANCA	SETD2
FANCD2	SLX4
FANCI	SMARCA4
FBXW7	SMARCB1
MLH1	STK11
MRE11A	TP53
MSH2	TSC1
MSH6	TSC2
NBN	
NF1	
NF2	
NOTCH1	
NOTCH2	
NOTCH3	

Copy Number Genes (43)	
AKT1	MDM2
AKT2	MDM4
AKT3	MET
ALK	MYC
AR	MYCL
AXL	MYCN
BRAF	NTRK1
CCND1	NTRK2
CCND2	NTRK3
CCND3	PDGFRA
CCNE1	PDGFRB
CDK2	PIK3CA
CDK4	PIK3CB
CDK6	PPARG
EGFR	RICTOR
ERBB2	TERT
ESR1	
FGF19	
FGF3	
FGFR1	
FGFR2	
FGFR3	
FGFR4	
FLT3	
IGF1R	
KIT	
KRAS	

Gene Fusions (Inter- and Intra-genic)	
AKT2	NF1
ALK	NOTCH1
AR	NOTCH4
AXL	NRG1
BRAF	NTRK1
BRCA1	NTRK2
BRCA2	NTRK3
CDKN2A	NUTM1
EGFR	PDGFRA
ERBB2	PDGFRB
ERBB4	PIK3CA
ERG	PPARG
ESR1	PRKACA
ETV1	PRKACB
ETV4	PTEN
ETV5	RAD51B
FGFR1	RAF1
FGFR2	RB1
FGFR3	RELA
FGR	RET
FLT3	ROS1
JAK2	RSPO2
KRAS	RSPO3
MDM4	TERT
MET	
MYB	
MYBL1	

- OCAv3 is a harmonized, highly reproducible CLIA assay used by the central NCI-MATCH lab network
  - Used for screening in NCI-MATCH and Pediatric MATCH Trials
- detects over 8000 variants in 161 cancer-related genes, relevant across all major cancer types
- SNV, Indels, CNV and RNA fusions
- Sample Input: 10ng per pool (20ng DNA, 20ng RNA)

Assay	Total Gene #	DNA	RNA
OCAv3	161	146	51

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Whole Exome Sequencing (WES)

WES has been developed as a research assay for exploratory, retrospective testing and comprehensive genomic analysis

- **Low DNA input** requirement (100 ng) conserves nucleic acid
  - DNA and RNA are extracted from the same specimen at NCH biorepository
- Hybridization capture-based target enrichment (Agilent SureSelect V6+COSMIC)
- Sequencing on Illumina NovaSeq 6000 with dual indexed libraries
- Somatic and germline variant-calling using matched germline WES
- Currently **400x median target coverage** (MTC)
- Data analysis pipeline calls TMB, MSI, mutational signatures & %LOH
- Performance characteristics:
  - **Sensitivity at 400x MTC is >93% for SNVs at 10% allele fraction**
  - **Specificity is >99.999%**

RNA Sequencing (RNA-Seq)

RNA-Seq has been developed as a research assay for exploratory, retrospective testing and transcriptomic analysis

- **40-100 ng** total RNA from good-quality FFPE (250 ng preferred)
- Illumina TruSeq RNA Exome (transcript-based probe capture)
- Sequencing on Illumina NovaSeq 6000 with dual index libraries
- ~50 million reads; sufficient for broad dynamic range of expression profiling
- Can identify **gene fusions**, but use of a targeted gene fusion panel at MoCha is preferred if fusions are important for your study

ctDNA Assay

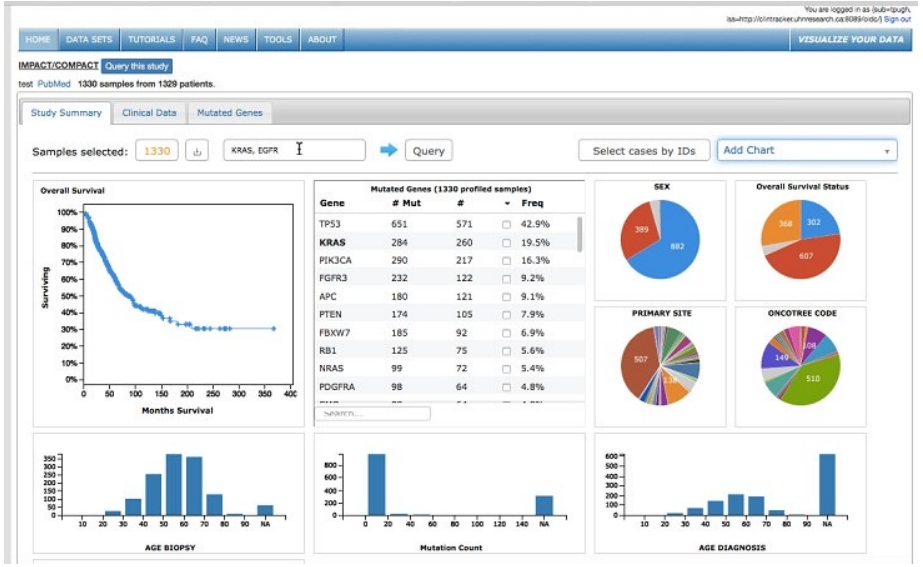
ctDNA assay: 523-gene targeted panel (TSO500 ctDNA)

- Reports SNVs, Indels, clinically relevant fusions, CNVs and TMB
- Upcoming version will include MSI status
- ***Technology***: Hybrid capture; incorporates unique molecular indices (UMIs) and error correction algorithms
- ***Status***:
  - Comprehensive validation complete
  - 350 libraries tested to establish performance characteristics
  - LOD80 is 0.25% for SNVs and Indels
- ***Blood collection requirements***: 2 Streck tubes (preferred)

➢ TSO500 ctDNA will be a CLIA laboratory test at MoCha and could potentially be used as an integral or integrated assay in ETCTN trials

ETCTN investigators will visualize NCLN genomics data through cBioPortal

- NCLN labs will upload raw genomics data through a common data analysis pipeline
- Investigators can visualize their processed genomics data in cBioPortal
  - It will be possible to integrate clinical & genomics
  - Investigators will have restricted access to view only their study data
- Final data sets will ultimately be uploaded to the Genomic Data Commons



Access to the NCLN

- The first step will be for the investigator to request collaboration with the NCLN in their LOI. As part of the review of an LOI, CTEP might also recommend use of the NCLN

- Use of the NCLN is voluntary. The alternative option of an entirely investigator-driven biomarker plan, through BRC review if necessary, is still completely open

- The specific NCLN assays to be performed for a trial will be agreed upon by a consensus process between the trial investigators, the NCLN laboratory(ies) and CTEP. This process will take place in parallel with drug commitment

Advantages of using the NCLN

- World-class laboratory collaborators with state-of-the-art platforms
- Assays performed at NCI's expense—no need for identified source of funding
- Validated SOPs and workflows, including biorepository support, already in place
- Standardized assays will yield data that is comparable across supported studies
- No BRC review