National Cancer Institute

Molecular Profiling and Centralized Pharmacodynamic/Correlative Data Reporting in the ET-CTN

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

National Cancer Institute

Outline

- Reporting Goal
- Integral vs Integrated Markers
- Workflow
- Molecular Profiling Reports
- PD and other integrated reports
- Centralized Data vs Centralized Forms

Reporting Goal

- **The goal of the Theradex support of ET-CTN in the Medidata RAVE system is to facilitate clinical trial function by:**

  - centralized data collection

  - real time report generation

To facilitate trial accrual, completion and analysis

Markers

Integral Markers –

•Markers that are essential for performance of the trial

- used for medical-decision-making in specimen donor
- results given back to patient or physician
- Uses: eligibility criterion, treatment assignment, risk stratification, dose modification
- must be performed in a CLIA-approved laboratory

Integrated Markers –

•Markers that are research markers

- performed on all subjects but not for medical decision-making

OR

- performed on a predefined subset (e.g., QoL studies)

OR

- performed to test a hypothesis (e.g., PD markers)

Research (Correlative) Markers –

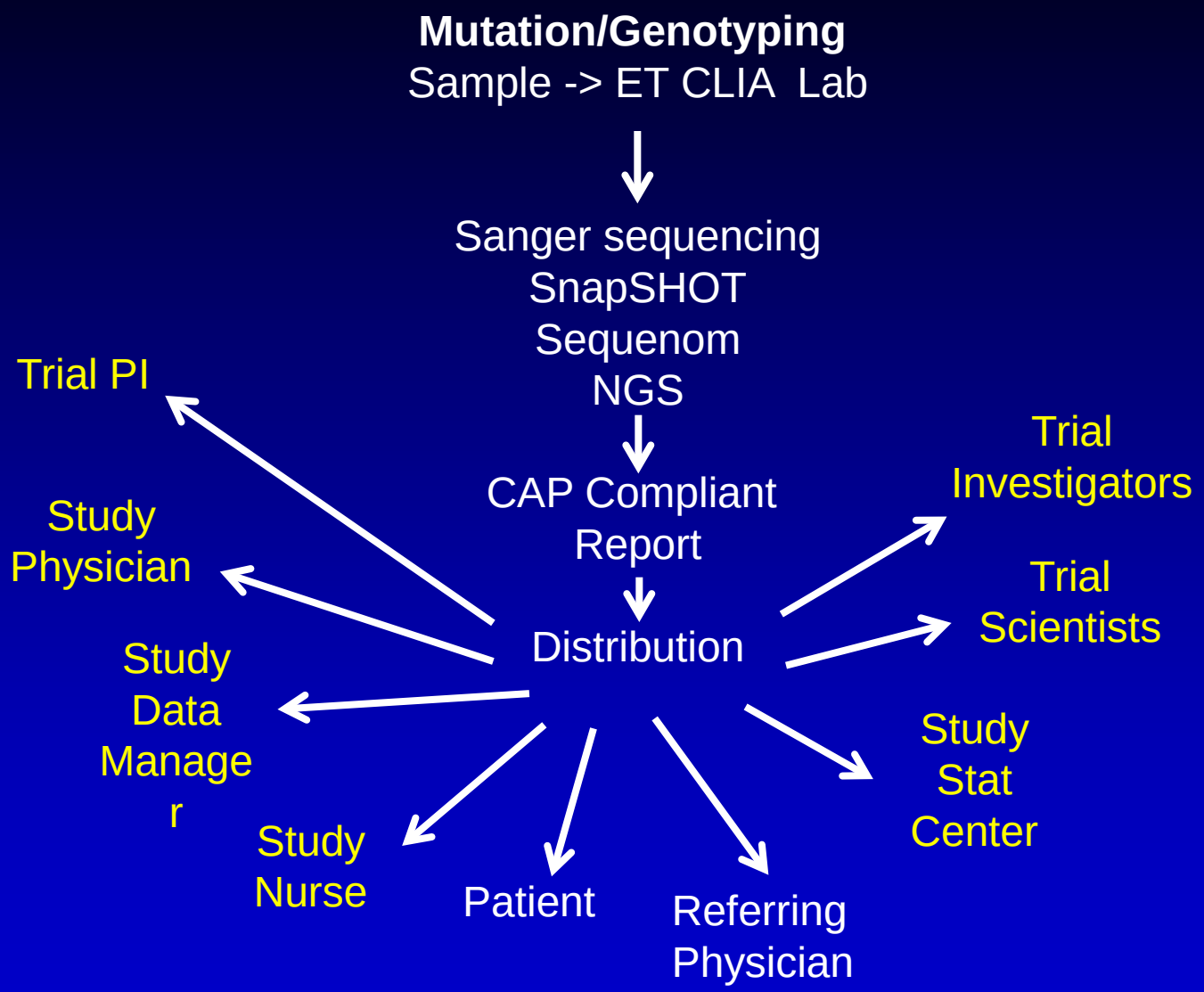
•Markers studied to generate hypotheses - exploratory

ET-CTN Markers

- Usually either integral or integrated
- Integral markers most commonly markers used for eligibility
  - Somatic mutation, e.g, BRAF V600E, EML4-ALK
  - Pathway activation, e.g., phospho-FLT3 in AML
    - These need to be performed in a CLIA approved lab
    - Result to patient, their physician and the study team (PI, etc.)

- Integrated markers often used to assess biological response to therapy
  - Phosphorylation of DNA/proteins, e.g., γH2AX for DNA damage
  - DNA Methylation, e.g., Me-CpG LINE1
  - Protein Levels, e.g., Topoisomerase 1
  - mRNA Levels (RT-qPCR), e.g., HIF1 alpha, HSP70
  - Circulating protein or cells, e.g., IL-6, CTC
    - These need to be preformed with ISO QC but not CLIA
    - Result to study team only (PI, coordinators, etc.)

ET-CTN Report Work Flow – Integral Markers



Data returned directly to Patient and Referring Physician

Data available through RAVE to CTSU Portal in Real Time

PMH Molecular Profiling Report – Positive Result

MOLECULAR PROFILING CLINICAL RESEARCH STUDY REPORT

Summary of Findings:

[Specimen #-block]

Tumor cells were identified in the specimen: YES
Sufficient DNA for validated genomic analysis: YES
The following somatic mutation(s) have been identified and confirmed in the subject's tumor sample:

PIK3CA E545K (21%)

Molecular alterations involving the PI3K/AKT pathway occur frequently in solid tumors such as breast, ovarian and large intestine and have been less frequently reported in cervical cancer. Alterations resulting in hyperactivity of the PI3K pathway include gain-of-function mutations in PIK3CA; these may respond favourably to PI3K/AKT/mTOR pathway inhibitors. Both genetic and biochemical data suggest that activation of the PI3K/AKT survival pathway contributes to cancer development and tumorigenesis. www.mycancergenome.org

Gene/Mutations Analysis:

Analysis was successful for all mutations: YES
Analysis was unsuccessful for the following mutations (list): NONE

Methodology:

Known mutations were identified using Sequenom primer extension technology, and verified by Sanger Sequencing and/or another validated test available in the CAP/CLIA Molecular Diagnostics Laboratory.

Sequenom Primer Extension: Sequenom Solid Tumor Panel v1.0 consists of 24 multiplexed assays, and can detect 280 mutations in 23 oncogenes. DNA is amplified using a PCR primer mix, and a single base extension reaction is performed using extension primers that hybridize immediately adjacent to the mutation. Multiplexed reactions are spotted onto a Chip and peaks with different mass are resolved by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) on a MassArray Compact Analyzer. Mutation calling is determined by using data generated from TyperAnalyzer software as well as manual analysis. This technology will detect a mutation if it is present at an allelic frequency of 10% or greater in the tissue examined. Neoplastic cells must be present at a minimum of 30% for mutation detection.

Sanger Sequencing: DNA is amplified using primers designed to cover the region of interest. The PCR product is sequenced in both directions after purification by SAP and ExoI digestion. Fluorescence-based cycle sequencing reactions are performed using the BigDye terminator v3.1 cycle sequencing kit (ABI). With dye terminator chemistry, each of the four dideoxynucleotide terminators is tagged with a different fluorescent dye. Fluorescence-labeled DNA fragments are separated using capillary electrophoresis on an ABI platform (3100, 3130, 3500). Sequences obtained from the data analysis software are compared with NCBI reference sequence to determine mutation status. This technology will detect a mutation if it is present at an allelic frequency of 10-15% or greater in the tissue examined. Neoplastic cells must be present at a minimum of 30% for mutation detection.

Genes/Mutations Analysed

AKT1: E17K
AKT2: E17K, S302G, R371H
AKT3: E17K
BRAF: G464R, G464V/E, G466R, F468C, G469S/E/A/V/R, D594V/G, F595L, G596R, L597S/R/Q/V/P, T599I, V600E/K/R/L/D/G/M, K601N, K601E
CDK4: R24C, R24H
CTNNB1: A13T, A21T, V22A, D32Y/N/H, D32G/A/V, S33C/F/Y, S33P/A, G34E/V, G34R, S37A/P, S37C/F/Y, T41A/P/S, T41I, S45C/F/Y, S45P/A
EGFR: R108K, T263P, A289V, G598V, E709K/Q, E709A/G/V, G719S/C/A, E746_A750del, E746_T751del, L747_T751del, L747_S752del, A750P, T751A/I, S768I, D770_N771insG, D770E, T790M, T854A, L858R, L861Q
ERBB2: L755S, L755P, D769H, G776S, G776LC, A775_G776insYVMA, G776VC, V777L, S779_P780insVGS, P780_Y781insGSP
FGFR1: G70R, S125L, T141R, P252T, V664L
FGFR2: S252W, Y375C, N549K
FGFR3: R248C, S249C, G370C, S371C, Y373C, A391E, K650Q/E, K650T/M, G697C
HRAS: G12R/S, G12V/A/D, G13C/R/S, Q61H, Q61L/R/P, Q61K
KIT: D52N, Y503_F504insAY, K550_K558del, W557R/G, K558_V560del, K558_E562del, V559I, V559D/A/G, V559del, V559_V560del, V560del, V560D/G, E561K, Y570_L576del, L576P, D579del, K642E, V654A, D816V, D816H/Y, D816E, D820Y/H, D820A/G, N822H/Y, N822I, N822K, Y823D, V825A, A829P, E839K
KRAS: G12V/A/D/C/S/R/F, G13V/D, A59T, Q61E/K, Q61L/R/P, Q61H
MEK1: Q56P, P124L
MET: H1112Y, H1112R/L, Y1248C, Y1248H, Y1253D, M1268T
NOTCH1: L1586P, F1593S, L1594P, R1599P, L1601P, I1617N, L1679P, Q2460*
NRAS: G12V/A/D, G12C/R/S, G13V/A/D, G13C/R/S, A18T, Q61L/R/P, Q61H, Q61E/K
PDGFRA: V561D, S566_E571>K, T674I, F808L, D842V, D842Y, D842_H845del, I843_S847>T, I843_D846del, D846Y, N870S, D1071N
PIK3CA: R38H, R88Q, N345K, C420R, P539R, E542K/Q, E545K/Q, E545A/V/G, Q546K/E, H701P, C901F, Y1021C, T1025A, M1043I, H1047R/L, H1047Y, G1049R/S
RET: C634R, C634Y, C634W, E632_L633del, A664D, M918T
SMO: T640A
STK11: Q37*, Q170*, D194Y, D194N, D194V, G196V, E199K/Q/*, P281L, W332*, F354L

References:

Thomas RK, et al. High-throughput oncogene mutation profiling in human cancer. Nature Genetics 2007; 39(3): 347-351
MacConaill LE, et al. Profiling Critical Cancer Gene Mutations in Clinical Tumor Samples. PLoS ONE 2009; 4(11): e7887

The mutations tested may not include all mutations present in the genes listed above. This report has been produced solely for research purposes as part of a clinical trial. Content is for information purposes only, and cannot be guaranteed to be complete; nor is it intended to substitute for professional medical advice.

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PMH Molecular Profiling Report – Negative Result

MOLECULAR PROFILING CLINICAL RESEARCH STUDY REPORT

Summary of Findings:

[Specimen #-block]

Tumor cells were identified in the specimen: YES
Sufficient DNA for validated genomic analysis: YES
The following somatic mutation(s) have been identified and confirmed in the subject's tumor sample:
NONE

Gene/Mutations Analysis:

Analysis was successful for all mutations: YES
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ERBB2: L755S, L755P, D769H, G776S, G776LC, A775_G776insYVMA, G776VC, V777L, S779_P780insVGS, P780_Y781insGSP
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PMH Molecular Profiling Report – Background Information

Mutation report for BRAF V600E

Status: complete — Last saved by: on 05/05/2011 02:30 PM

Frequency of V600E mutation in BRAF in the top tumour types

	Tumour	Frequency	Samples
1.	large intestine serrated polyp	62.646%	(1018/1625 samples)
2.	ns malignant melanoma	51.364%	(320/623 samples)
3.	eye benign melanocytic nevus	50.000%	(14/28 samples)
4.	thyroid carcinoma	45.753%	(8953/19568 samples)
5.	skin benign melanocytic nevus	42.216%	(865/2049 samples)
6.	skin malignant melanoma	38.108%	(3203/8405 samples)
7.	skin atypical spitzoid tumour	36.000%	(18/50 samples)
8.	ovary low malignant potential (borderline) tumour	33.268%	(169/508 samples)
9.	central nervous system ganglioglioma	32.143%	(9/28 samples)
10.	large intestine aberrant crypt foci	27.500%	(11/40 samples)

BRAF characteristics

Full name: v-raf murine sarcoma viral oncogene homolog B1

Raf kinases, a family of three serine/threonine kinases, are part of the ras-MAPK signaling cascade and phosphorylate MEK. Upon growth factor stimulation, Raf-1 (or c-Raf) is activated by GTP-bound Ras and recruited to the cell membrane. This activation process is tightly regulated by a number of factors including phosphatases (e.g. PP1, PP2A, PP5), kinases (e.g. Src, ERK, Akt, PKC) and proteins that bind directly to Raf-1 (e.g. RKIP, 14-3-3zeta, KSR, Hsp90). Raf-1 is also thought to be able to dimerize with wild type B-Raf in a Ras-dependent process. B-raf is commonly mutated and thereby activated in many human cancers, the most frequent mutation being the V600E mutation of the kinase domain. Whilst wt b-Raf and Raf-1 are strongly activated by growth factor signals via Ras and Src, a-Raf is only modestly activated and has low basal activity. All three isoforms of Raf are considered to be oncogenic.

BRAF V600E characteristics

The functional consequence of this mutation is: **activating**. Reference (PMID): pmid:18631381, pmid:12198537

Clinical and Preclinical Studies

1. NS malignant melanoma - 51.364%

In this tumour type, the clinical significance of this mutation has been examined by **prospective** clinical trials.

A phase I trial of BRAF inhibitor PLX4032 in BRAF V600E metastatic melanoma demonstrated improved response rates and progression free survival compared to historical data. Results from a phase III study are not yet published.

Reference: pmid:20818844 - evidence IA

2. thyroid carcinoma - 45.753%

In this tumour type, the clinical significance of this mutation has been examined by **retrospective** clinical trials.

Retrospective studies have demonstrated that BRAF V600E mutant papillary thyroid cancer is associated with poorer outcomes.

Mutation report for BRAF V600E

Reference: pmid:18682506 - evidence IVD

3. large intestine carcinoma - 10.072%

In this tumour type, the clinical significance of this mutation has been examined by **retrospective** clinical trials.

Retrospective studies have demonstrated that BRAF V600E mutant metastatic colorectal cancer is associated with inferior progression free and overall survival compared to BRAF wildtype, when treated with cetuximab and chemotherapy. There is debate as to whether BRAF V600E mutations are also associated with poorer response rates to EGFR targeted therapy.

Reference: pmid:19571295, pmid:20619739 - evidence IIB

4. breast carcinoma - 1.608%

In this tumour type, the clinical significance of this mutation has been examined by **preclinical** studies.

Molecular characterization of 41 breast cancer cell lines demonstrated that BRAF mutations occur in basal-like breast subtype

Reference: pmid:19593635 - evidence VD

Availability of Investigational Agents

The available investigational agents PLX4032 have documented efficacy: **effective**

Sensitivity and Resistance Conferred by Mutation

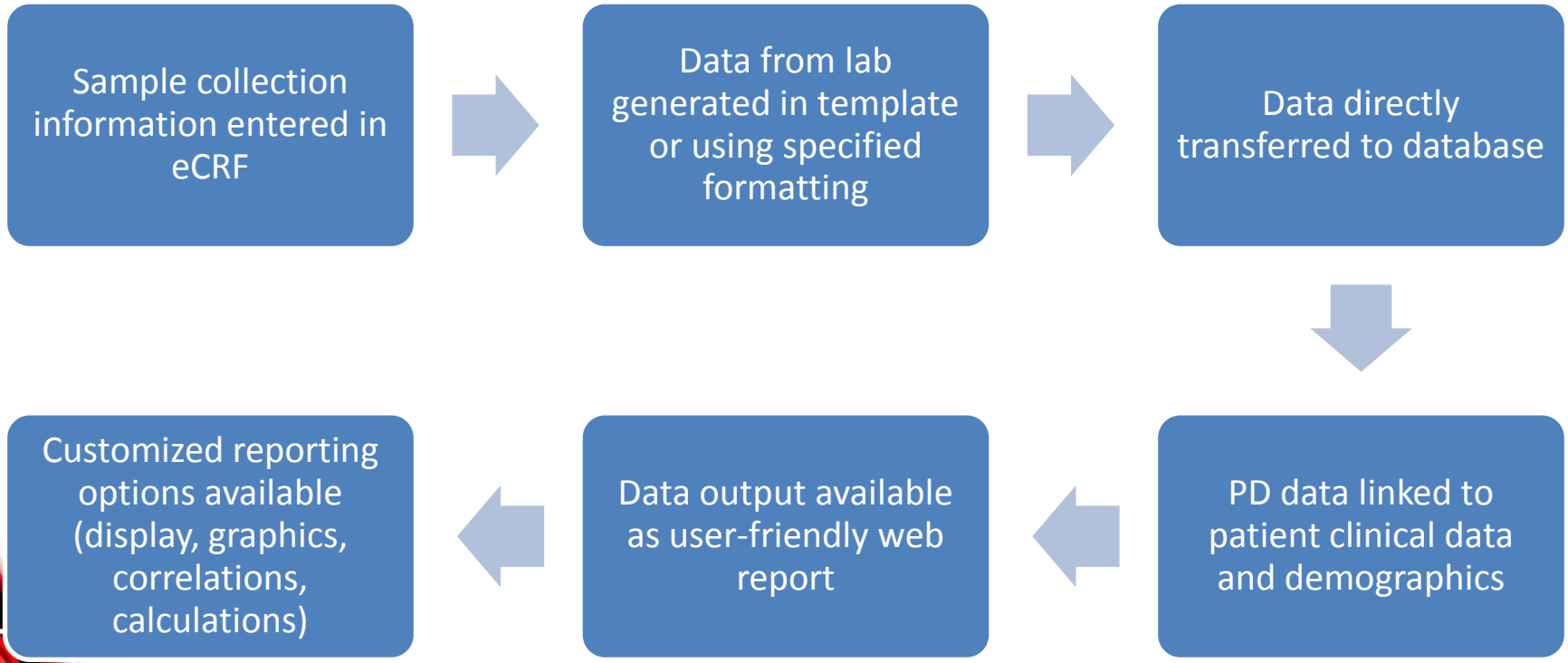
This mutation **may** confer **sensitivity** to: BRAF and MEK inhibitors

This mutation **may** confer **resistance** to: EGFR targeted therapies

Report History

Date	Confirmed by	Comment
11/21/2012 07:48 PM		

Proposed PD data transfer process

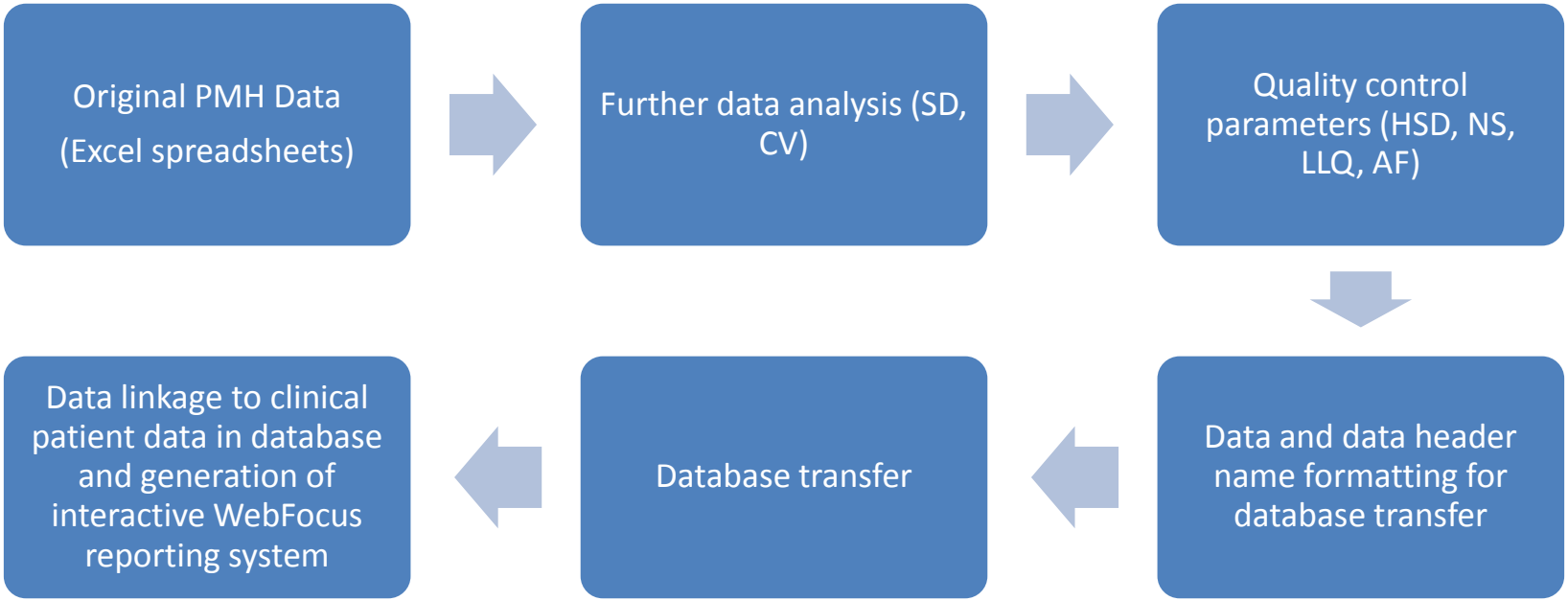


Theradex® ONCOLOGY | EXPERTS

PMH PD Data Test Case

- Princess Margaret Hospital (PMH) Data Types:
  - ELISA data for 7 different growth factors/cytokines
    - Standard curve data was included for each ELISA type
    - Opted to display the standard curve elements (slope, intercept, R2, and blank value) with data
  - Flow cytometry thymidine phosphorylase assay data

Steps from raw PMH PD data to Web Reports



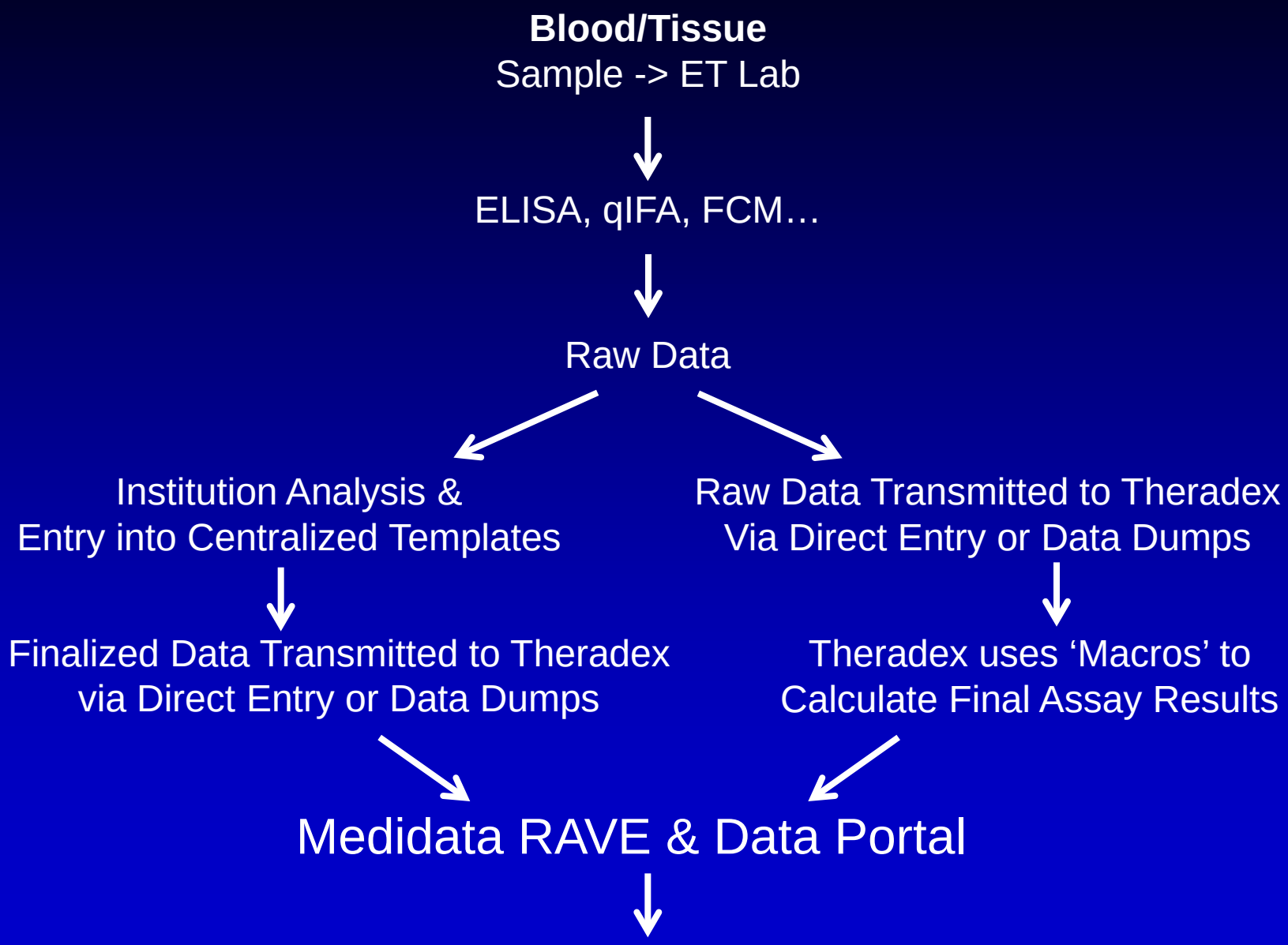
PMH data was formatted by Theradex as a special case in order to facilitate database transfer and Web data reporting.

Theradex® ONCOLOGY | EXPERTS

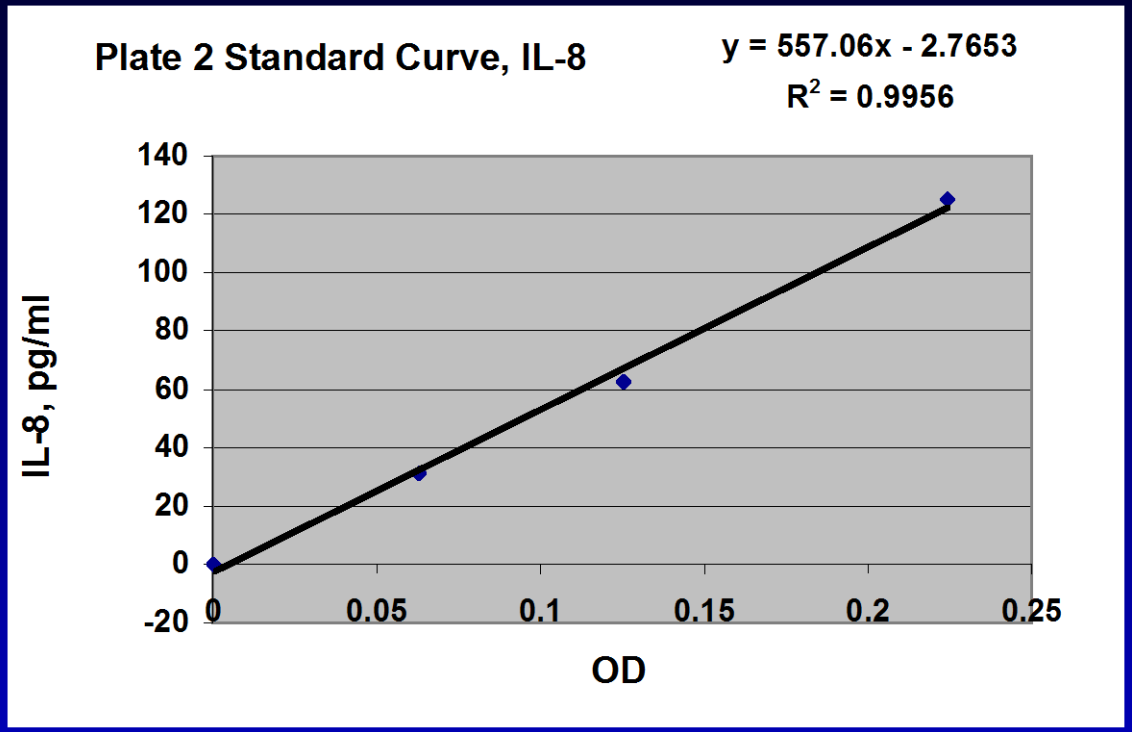
Data templates

- Based on PMH data, sample templates have been developed so that future generated data will be formatted for transfer and reporting.
- Templates specify what data placeholders are designed for textual and/or numeric entries.
- Data formatting is specified.

ET-CTN Report Work Flow – Integrated Markers



Example of An ELISA Form



- Standard curve for plate 2 of an ELISA run that will be presented on next slide.
- These are taken from an Excel Spreadsheet that is designed to accept transferred data that is either formatted or 'fits' into a template with macros to create defined sets of data.

Example of An ELISA Form

Analyte	PLATE	Patient ID	Specimen ID	Time Point	Temp-coding	Date Collection	OD 1	OD 2	Concentration average (pg/ml)	S.D. (pg/mL)	RSD
IL-8	2	004-001	004-001ANG1	ANG1 = Cycle 1 Day 1	49	6/1/14	0.1138	0.1069	**58.7**	**2.7**	**4.63%**
IL-8	2	004-001	004-001ANG2	ANG2 = Cycle 1 Day 22	50	6/22/14	0.1193	0.1301	**66.7**	**4.3**	**6.38%**
IL-8	2	004-001	004-001ANG3	ANG3 = Cycle 2 Day 1	51	7/13/14	0.0987	0.1015	**53.0**	**1.1**	**2.08%**
IL-8	2	004-002	004-002ANG1	ANG1 = Cycle 1 Day 1	52	6/8/14	0.1325	0.1199	**67.5**	**5.0**	**7.35%**
IL-8	2	004-002	004-002ANG2	ANG2 = Cycle 1 Day 22	53	6/29/14	0.1537	0.1482	**81.3**	**2.2**	**2.66%**
IL-8	2	004-002	004-002ANG3	ANG3 = Cycle 2 Day 1	54	7/20/14	0.0591	0.0664			**NS**
IL-8	2	004-003	004-003ANG1	ANG1 = Cycle 1 Day 1	55	6/8/14	0.0578	0.0602			**NS**
IL-8	2	004-004	004-004ANG1	ANG1 = Cycle 1 Day 1	56	8/26/14	0.2264	0.2062	**117.7**	**8.0**	**6.76%**
IL-8	2	004-004	004-004ANG2	ANG2 = Cycle 1 Day 22	57	9/16/14	0.2324	0.2275	**125.3**	**1.9**	**1.54%**
IL-8	2	004-004	004-004ANG3	ANG3 = Cycle 2 Day 1	58	10/7/14	0.1425	0.1533	**79.6**	**4.3**	**5.34%**

RSD is % CV (QC term) – Flags are attached if %CV>10% or value is outside measurable range; NS is No Sample or Not Sufficient Sample; SUMMARY Reports will be available for PI.

National Cancer Institute

Centralized Reports – Two Ways to Get There Need Input from ET-CTN Investigators

- Assay data analyzed at lab gives local investigators quick access to data
  - Centralized forms can standardize output and allow for easy uploading into a CDMS
  - Raw data kept at lab but not centralized in a CDMS
  - But …. amazing how many ways there are to fill out a form
  - Transfer to Theradex may entail transcription errors to and from form

- Assay data analyzed at Theradex
  - Use centralized, common ‘macros’ or ways to analyze data
  - Raw data could be read off output from lab machines, reduce transcription and possible errors
  - Raw data at lab and Theradex
  - Output available to trial team when analysis finished

WOULD LIKE INPUT FROM INVESTIGATORS ON WHICH IS FAVORED FOR INTEGRATED MARKERS

National Cancer Institute

SUMMARY

- Integral marker data will be forwarded to Theradex to put into Rave and the Data Portal using CAP compliant data forms

- Integrated marker data will be generated at the sites and

EITHER

- forwarded to Theradex for data reduction using standardized processes and templates

OR

- assay data will be put into standardized report templates at each site and forwarded to Theradex for distribution through Rave and the Data Portal

- These approaches will allow faster presentation of data to the study team to enable assessment of
  - **accrual**
  - **status of specimen collection**
  - **status of assay results**
  - **assessment of hypothesis testing**
  - **correlation to various other clinical parameters (e.g., response and/or toxicity)**

ACKNOWLEDGMENT

PMH
Siu
Degendorfer
Kamel-Reid
Oza
Cheiken

NCI/SAIC
Ivy
Smith
Montello
Cropp
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Gray
Ferry-Galow
Parchment
Williams

Theradex
Boran
Anderson
Valnoski
Rinker
Davey