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

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Outline

• Reporting Goal
• Integral vs Integrated Markers
• Workflow
• Molecular Profiling Reports
• PD and other integrated reports
• Centralized Data vs Centralized Forms
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 performed with ISO QC but not CLIA
    - Result to study team only (PI, coordinators, etc.)
ET-CTN Report Work Flow – Integral Markers

Mutation/Genotyping
Sample -> ET CLIA Lab

↓

Sanger sequencing
SnapSHOT
Sequenom
NGS

↓

CAP Compliant Report

↓

Distribution

Study Physician
Study Data Manager
Study Nurse

Trial PI

→

Patient
Referring Physician

→

Trial Investigators
Trial Scientists
Study Stat Center

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 hyperactivation 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 Analyzed:

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%-15% 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 Exel 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 deoxynucleotide 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.

References:


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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Tel: 416-940-4800 x 5739
Fax: 416-940-5596
PMH Molecular Profiling Report – Negative Result

MOLECULAR PROFILING CLINICAL RESEARCH STUDY REPORT

Summary of Findings:
[Specimen # selected]

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

Methodology:

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Genes/Mutations Analysed

AKT1: E17K
AKT2: E17K, S302G, R371H
AKT3: E37K
CDK4: R24C, R24H
CTNNB1: A13T, A21T, V22A, D32Y/N/H, D32Q/G/A, S33C/F/Y, S33P/A, G34E/V, G34R, S37A/P, S37C/F/Y, T41A/P/S, T41I, S45C/F/Y, S45P/A

References:


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 – 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 tumor types

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Frequency</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>large intestine serrated polyp</td>
<td>62.64%</td>
<td>(1018/1625 samples)</td>
</tr>
<tr>
<td>ns malignant melanoma</td>
<td>51.36%</td>
<td>(3206/6232 samples)</td>
</tr>
<tr>
<td>eye benign melanocytic nevus</td>
<td>50.00%</td>
<td>(1428 samples)</td>
</tr>
<tr>
<td>thyroid carcinoma</td>
<td>45.75%</td>
<td>(995/19368 samples)</td>
</tr>
<tr>
<td>skin benign melanocytic nevus</td>
<td>42.21%</td>
<td>(865/2049 samples)</td>
</tr>
<tr>
<td>skin malignant melanoma</td>
<td>38.10%</td>
<td>(3203/8405 samples)</td>
</tr>
<tr>
<td>skin atypical spitzoid tumour</td>
<td>36.00%</td>
<td>(16/50 samples)</td>
</tr>
<tr>
<td>ovary low malignant potential (borderline) tumor</td>
<td>33.26%</td>
<td>(169/506 samples)</td>
</tr>
<tr>
<td>central nervous system ganglioglioma</td>
<td>32.14%</td>
<td>(928 samples)</td>
</tr>
<tr>
<td>large intestine aberrant cryptic</td>
<td>27.50%</td>
<td>(1140 samples)</td>
</tr>
</tbody>
</table>

BRAF characteristics

Full name: v-rat sarcoma viral oncogene homolog B1

BRAF, a family of three serine/threonine kinases, are part of the ras-MAPK signaling cascade and phosphorylate MEK. Upon growth factor stimulation, Raf-1 (also 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. PTP1B, PP2A, PP5), kinases (e.g. Src, ERK, Akt, PKC) and proteins that bind directly to Raf-1 (e.g. FKBP, 14-3-3zetl, 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 isofoms of Raf are considered to be oncogenic.

BRAF V600E characteristics

The functional consequence of this mutation is activating. Reference (PMID): pmid:18682506 - evidence IVD

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 III 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:20818644 - evidence IA

2. Thyroid carcinoma - 45.75%

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.

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: 11/21/2012 07:48 PM
Proposed PD data transfer process

1. Sample collection information entered in eCRF
2. Data from lab generated in template or using specified formatting
3. Data directly transferred to database
4. Data output available as user-friendly web report
5. PD data linked to patient clinical data and demographics
6. Customized reporting options available (display, graphics, correlations, calculations)
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

Original PMH Data (Excel spreadsheets) → Further data analysis (SD, CV) → Quality control parameters (HSD, NS, LLQ, AF) → Data linkage to clinical patient data in database and generation of interactive WebFocus reporting system → Database transfer → Data and data header name formatting for database transfer

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

Blood/Tissue Sample -> ET Lab

ELISA, qIIF, FCM...

Institution Analysis & Entry into Centralized Templates

Raw Data Transmitted to Theradex
Via Direct Entry or Data Dumps

Raw Data Transmitted to Theradex
Via Direct Entry or Data Dumps

Theradex uses ‘Macros’ to Calculate Final Assay Results

Medidata RAVE & Data Portal

All Study Team Members access data in various report formats

Administrative Office
National Cancer Institute

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

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

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.
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

WOULDN'T LIKE INPUT FROM INVESTIGATORS ON WHICH IS FAVORED FOR INTEGRATED MARKERS
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
  - 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

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Williams

**PMH**  
Siu  
Degendorfer  
Kamel-Reid  
Oza  
Cheiken

**Theradex**  
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Anderson  
Valnoski  
Rinker  
Davey