Clinical Assay Development, Validation & Training

Pharmacodynamic Assay Support of DCTD-Sponsored Early Clinical Trials

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Division of Cancer Treatment & Diagnosis

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SAIC-Frederick
Should Early Phase Trials Be Designed to Generate Evidence Supporting Proof-of-Mechanism?

• **Background:** Recent cancer small molecule development has been characterized by both high profile successes (crizotinib; vemurafenib) and failures (BSI-201). Successes were rapid and resulted from molecular stratification; failure associated with lack of P-of-M.

• **Feasibility:** Should we only develop agents that can be brought to the clinic under conditions that demonstrate P-of-M? Should resources be refocused around this paradigm with a consequent decrease in the number of trials performed and drugs evaluated?

• **Implications for success:** Fewer costly, late stage development failures; improved understanding of actual mechanism of action or resistance in the clinic; improved rationale for the selection of combination therapies for development.
Should Early Phase Trials Be Designed to Generate Evidence Supporting Proof-of-Mechanism?

- Demonstrate drug action on intended tumor target (proof of mechanism) in a human malignancy early in development
  - evaluate hypotheses surrounding mechanism of action *per se*
  - evidence of target modulation in the clinic assists decision to move agent forward, or not . . .
  - evaluate relationship of drug schedule and systemic exposure to target effects
  - examine relevance of marker chosen to represent target modulation
  - prior to expectation of efficacy

- **NOT** predictive of clinical benefit
  - only later stage (larger) trials can define relevance of target modulation to tumor growth inhibition
  - only consequent changes in cell biology (and perhaps biochemistry) would be expected to predict clinical benefit

Modern Drug Development Needs PoM/PoC-Based Trials

DCTD tasked FNLCR/SAIC-F to provide pharmacodynamic (PD) assay support
• PD assay development lab (PADIS) - develop, validate and prove assay fitness-for-purpose
• Clinical PD lab (NCTVL) – real time PD analysis of internal and CTEP trial specimens
• Long-term, open access to clinically proven assays, while maintaining assay quality

Portfolio of PD assays for high value molecular responses, based on expert input

Developmental Therapeutics Clinic to explore trial designs incorporating tumor PD

Mandatory target assessment during CTEP Phase 1 trials ("no assay, no trial")
Integrated PD Assay Support of the DCTD Program

**Frederick**

### Preclinical models and modeling
FNLCR/DTP/Biological Testing Branch
Melinda Hollingshead, DVM, PhD

### PD Assay Development, Validation and FF Purpose Demonstration, with Specimen Handling SOPs
FNLCR/SAIC-Frederick
PD Assay Development/Implementation Section (PADIS)
Robert Kinders, PhD

**Bethesda**

### Phase 0/1 Trial Designs for PoM
NCI-Bethesda/Devel Therapeutics Clinic
James Doroshow, MD, Shivaani Kummar, MD

### PD Analysis of Clinical Specimens (real-time)
FNL/SAIC-Frederick
National Clinical Target Validation Laboratory (NCTVL)
Jay Ji, PhD

**Development of “at-a-distance” assay QA/QC**
FNLCR/SAIC-Frederick
IQC Unit

**Incoming critical reagents**
Internally produced new reagents
Both Target Variability and Drug Drive PD Success

**Diagram A:**
- Target Activity vs. Increasing Dose
- Low sampling variability of target function
- Threshold for demonstration of target modulation
- Target modulation achieved at a nontoxic dose

**Diagram B:**
- Target Activity vs. Increasing Dose
- High sampling variability of target function
- Threshold for demonstration of target modulation
- Target modulation achieved at a nontoxic dose

**Diagram C:**
- Target Activity vs. Increasing Dose

**Diagram D:**
- Target Activity vs. Increasing Dose

Specimen Handling SOPs – a Key Fit-for-Purpose Issue

Develop with Clinically Relevant Samples

Stabilize the Analyte(s)

Cold (RT) Ischemia

Warm (37°C) Ischemia

Mean ± SEM (n=5 per group)

Relative Change from Mean of FF Biopsy (%)

Minutes

0 1 2 15 30 45 60

0 50 100 150 200

pY1235 MET

Total MET

pY1235 MET

Total MET
Minimize the Influence of Specimen Processing - Key for Immunoassay of Hif-1α (Hif1α-IA)

Stabilize the Analyte(s)

Mean ± SD

P=0.01 (unpaired t-test, 2-tailed)

LLQ = 2.6
Development of Indenoisoquinolines with Clinical PD Assays

Unique, non-camptothecin Topo I inhibitors
- chemically stable
- low cross-resistance with camptothecin analogs (irinotecan; topotecan)
- not substrates for ABCG2 efflux pump
- prolonged stability of complex
- unique patterns of DNA cleavage
- produce dose- and time-dependent DNA double strand breaks → γH2Ax

Discovery/Development Path
- discovered by Yves Pommier (NCI intramural program)
- developed by DCTD
- FIH Randomized NCI Phase I trial of NSC 724998 vs 725776

Develop comprehensive PD package for proof of mechanism evaluation PRIOR to FIH studies:

1° level PD: TOP1-immunoassay (new)
2° level PD: γH2Ax-qIFA
2° level PD: γH2Ax-CTC
Effect of Topotecan on TOP1 Levels in Xenograft Bx Specimens

4h Topotecan (15 mg/kg) vs Vehicle Control

Effect of NSC 724998 on TOP1 Levels in A375 Xenografts

Vehicle Range:
Solid red line = Avg
Dashed red line = Avg ± 1 and 2 SD
Black line = Dose Response of indeno NSC 724998

PlosOne, submitted
Immunoassay for total Topoisomerase 1 (TOP1-IA) - Clinical

Unpublished, ASCO 2012
Quantitative Immunofluorescence Assay for γH2Ax (γH2Ax-qIFA)

Topotecan-Treated Mice; A375 Xenografts

Topotecan-Treated Non-Tumor Bearing Mice

Quantitative Immunofluorescence Assay for γH2Ax (γH2Ax-qIFA)

in vitro tolerance of TOP1 inhibitors by human and murine hematopoietic stem cells (CFU-GM)

<table>
<thead>
<tr>
<th>Investigational Agent</th>
<th>Mouse IC90 (nM)</th>
<th>Human IC90 (nM)</th>
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</thead>
<tbody>
<tr>
<td>Topotecan Hydrochloride (Hycamtin)</td>
<td>120 ± 50 (64 - 160)</td>
<td>5.9 ± 5.1 (1.7 - 15)</td>
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<tr>
<td>NSC 724998</td>
<td>29 ± 12 (18 - 41)</td>
<td>27 ± 14 (7.1 - 45)</td>
</tr>
<tr>
<td>NSC 725776</td>
<td>26 ± 12 (12 - 35)</td>
<td>6.6 ± 2.6 (4.1 - 10)</td>
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Direct comparison study conducted on 3 mouse, 6 human marrow specimens
Adapting the γH2Ax Assay to Circulating Tumor Cells (γH2Ax-CTC)
CTC γH2Ax Response to Indenoisoquinolines

\[ R = \]

- CH\(_3\) \quad \text{NSC 314622}
- (CH\(_2\))\(_3\)-NH-(CH\(_2\))\(_2\)OH \quad \text{NSC 706744 (MJ-III-65)}
- (CH\(_2\))\(_3\)-N=\quad \text{NSC 725776}
- (CH\(_2\))\(_3\)-N=\quad \text{NSC 724998}

![Chemical structures and graphs showing total CTCs (7.5 mL) and percent γH2AX-positive CTCs for various samples before (D1) and after (D3) treatment with different compounds.](image-url)
Pushing CTC Technology toward Universal Analysis

**DESIGN FEATURES from SAIC-F to Meet Expected Needs in CTC Analysis**

- Cell surface antigen-independent separation of CTCs from blood (EpCAM-neg CTCs)
- Capable of evaluating carcinomas, sarcomas and lymphomas
- Clinically validated with small volume samples (0.1 – 1.0 cc)
- Interfaces directly with down-stream molecular analyses – both PD and Dx
- Capable of evaluating non-clinical cancer models

These Design Features were incorporated into a SAIC-F RFP to develop instrumentation that moves past limitations of the Veridex Cell Search and other marker-based systems.
Selection of ApoCell, Inc to Deliver a Universal CTC Device

Dielectrophoretic Field-Flow Fractionation (DEP-FFF)

- DEP-FFF utilizes balance of physical forces in a laminar flow chamber to isolate CTCs from blood cells
- Throughput is high compared to other systems; 1 ml of blood can be processed in <30 minutes
ApoStream prototype isolated viable EpCAM negative tumor cells: spiked human ovarian carcinoma and canine osteosarcoma cell lines

<table>
<thead>
<tr>
<th>preoptimization</th>
<th>Recovery</th>
<th>Viability</th>
<th>Enrichment</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>Fold</td>
</tr>
<tr>
<td>preoptimization</td>
<td>33+/−1.3</td>
<td>&gt;95</td>
<td>1326</td>
</tr>
<tr>
<td>IGROV-1 (EpCam Neg)</td>
<td>83+/−6.0</td>
<td>&gt;95</td>
<td>1446</td>
</tr>
<tr>
<td>BW.KOSA (Canine)</td>
<td>78.5+/−0.5</td>
<td>&gt;95</td>
<td>4440</td>
</tr>
</tbody>
</table>

IGROV-1 cells (EpCAM neg) spiked into human PBMCs
BW.KOSA cells spiked into dog blood

PD response (γH2AX) of a canine OS cell line to indenoisoquinoline ex vivo:

Fibronectin- γH2A EGFR
Alpha Prototype Delivery and Use at the FNLCR

- Fit-for-Purpose demonstration using blood specimens from a canine clinical trial of the indenoisoquinolines
  - Ongoing
  - Uses Breadboard Prototype
  - Dog Lymphoma phenotyping
  - Use $\gamma$H2Ax as the PD marker

- Alpha prototype delivery:
  - ApoCell in August 2012
  - FNLCR/PADIS in October 2012

- SOP-based Methods Transfer
  - December 2012

- Initiate clinical trial support
  - March-May 2013
Creating User Groups for Validated, Proven PD Assay

PD assay development (PADIS)

Reagent Supply Chain
Incoming critical reagents
Internally produced new reagents

“at-a-distance” Assay QA/QC (ICQ unit)

Real-time clinical specimen analysis (NCTVL)

Training courses at FNLCR
Assay transfers
Reagent QC
Centralized SOPs (web-based)
Trouble shooting
Shared lessons learned

Developmental Therapeutics Clinic
DCTD/CTEP

External Phase 1 Clinical Trial Sites

Frederick
SAIC-F
NCI

Bethesda
Drug Development Community
A) Onsite, laboratory-based training classes at the FNLCR

<table>
<thead>
<tr>
<th>Assay</th>
<th># of classes</th>
<th># of attendees</th>
<th>universities &amp; research institutions</th>
<th>NIH programs</th>
<th>pharma/ Biotech/CRO</th>
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</thead>
<tbody>
<tr>
<td>PAR-IA</td>
<td>9</td>
<td>29</td>
<td>16</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>γH2Ax-qIFA</td>
<td>5</td>
<td>18</td>
<td>9</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>γH2Ax-CTC</td>
<td>3</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>2</td>
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<tr>
<td>TOP1-IA</td>
<td>preparation/scheduling</td>
<td></td>
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as of May 2012

B) Quality controlled supply chain for key reagents
- Assays faced with using R&D grade, rather than Dx grade, reagents and suppliers
- Custom orders of reagents/subcontracts to specifications (Epitomics, Argonne Natl Lab)
- Acceptance criteria applied to incoming batches before distribution to clinical labs
  - batches both of PcAb and MoAb have been rejected (fate of these in the community is unknown)

C) Web accessible current SOPs, training dates, and forms to request key reagents

D) Assay “User Groups”
- Centralized change control of SOPs
- Assay troubleshooting results shared with all assay sites
- Recalls of key reagent batches are possible via a distribution tracking system
### Selection of Molecular Targets in Early Assay Development

<table>
<thead>
<tr>
<th>Selection of Targets</th>
<th>Scientific Foundation</th>
<th>Feasibility</th>
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</table>
| **NCI** NExT Special Emphasis Panel prioritized drug discovery and development projects  
  • Includes early clinical trials | PD FWG | PD assay development lab |
| **PD Functional Working Group of the Therapeutic Subcomm, NCAB** | | |
| **SAIC-F** | ad hoc experts and consults | |
| | weekly PD program/assay meetings | |
## PD Assay Development Portfolio – Emphasis on Multiplex

<table>
<thead>
<tr>
<th>Concept (scientific foundation)</th>
<th>PD POM (MOA)</th>
<th>Pathway Consequences</th>
<th>Cell Stasis/Loss (POC)</th>
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<tbody>
<tr>
<td>pGSK3α/β-IA‡‡ Mer Kinase-IA‡‡</td>
<td>Energy Control-IA‡‡ AXIN, β-CATNN, PKA, LKB1, AMPK, PKCβ, AKT2, ULK1, GYS1, PDH-A1, PDP-1, BIM1</td>
<td>Cell Cycle Status Necrosis-qIFA Hydropic Degeneration-qIFA Caspase-independent Death-qIFA Oncosis-qIFA</td>
<td></td>
</tr>
<tr>
<td>JAK/pSTAT3-qIFAP‡‡ pATR-qIFAP</td>
<td>Rx2-qIFAP4 Rx1-qIFAP3 + vim/ker (DAPI)</td>
<td>Autophagy-IA LC3-II-qIFAP</td>
<td></td>
</tr>
<tr>
<td>ccTOP1-IA pMET-IA ver 2.0 (denaturing) pY1235/ pY1356MET-IA cIAP-qIFAP‡‡ HSP70 RT-qPCR‡‡</td>
<td>Glycolysis-IA‡‡ HK2, pPDHE1α, PKM2, LDH-A DDR1-qIFAP4 HR/BER/NHEJ/NER/MMR pNBS1, RAD51/--/--/ERCC1/γH2Ax (DAPI)</td>
<td>EMT1-qIFAP4 β-CATN, E/N-Cad, Vim or Ker (DAPI) Apoptosis (intrinsic)-IA Dimerized BAX-Bcl-2, BAX-BAX, BAK-BAX, BAK-BAK, Bak-Bcl-2, SMAC-SMAC Total pS39BAX, cleaved-Lamin-B, BAD, BAX, BAK, BIM, 17/19 Kd neoantigen cCasp-3, Mcl-1, Bcl-xl, survivin</td>
<td></td>
</tr>
<tr>
<td>pMET-IA pY1235/ pY1356MET-IA</td>
<td>Rx1-qIFAP3 γH2A/cCasp-3/Ki67 (DAPI) HIF1α-IA</td>
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### Development and Validation (PADIS)
- ccTOP1-IA
- pMET-IA ver 2.0 (denaturing)
- cIAP-qIFAP‡‡
- HSP70 RT-qPCR‡‡

### SOP-based Transfer (PADIS→IQC, NCTVL)
- pMET-IA pY1235/ pY1356MET-IA
- Rx1-qIFAP3 γH2A/cCasp-3/Ki67 (DAPI) HIF1α-IA
<table>
<thead>
<tr>
<th>Developmental Therapeutics</th>
<th>DCTD/OD</th>
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<tbody>
<tr>
<td>Jerry Collins</td>
<td>Jim Doroshow</td>
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<td>Melinda Hollingshead</td>
<td>Joe Tomaszewski</td>
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<td>Myrtle Davis</td>
<td>Shivaani Kummar</td>
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<td>Bev Teicher</td>
<td>Jason Cristofaro</td>
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<td></td>
<td>Barbara Mroczkowski</td>
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<td></td>
<td>Michael Difilippantonio</td>
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<td><strong>Center for Cancer Research</strong></td>
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<td>Yves Pommier</td>
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<td>Lee Helman</td>
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<td>Bob Wiltrout</td>
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<td>William Bonner</td>
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<td><strong>CTEP</strong></td>
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<td>Jamie Zwiebel</td>
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<td>Jeff Abrams</td>
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