NCI Experimental Therapeutics Program (NExT):
Molecular Pharmacodynamics In Drug Discovery And Development

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OUTLINE

• Pharmacodynamics (PD) for proof of mechanism (POM) and proof of concept (POC) in anticancer drug development: Example of cMET TKIs
• Lability and preservation of biomarkers at the point of collection and point of processing
• DNA damage response biomarkers and NCI early phase clinical trials
• Apoptosis biomarkers in support of CBC drug discovery
• FNLCR PD efforts in support of the extramural community
• What is ahead on the PD biomarker horizon?
Pharmacodynamic Biomarkers

- Pharmacodynamics (PD) is broadly defined as “what a drug does to the body”
- At the molecular level, it’s the intended biochemical response of the target and its downstream pathways to drug action

Pharmacological Principles in PD:

- Proof of Mechanism of Action (POM): confirming that a drug acts as designed upon its intended target in a living tumor
- Proof of Concept (POC): confirming that a drug’s MOA leads to a therapeutic effect
Pharmacodynamic Biomarkers – POC and POM of MET-TKIs

**Single Dose POM Studies**

- **Cabozantinib**: XL-184 (NSC 761068)
- **Foretinib**: XL880 (NSC 755775)
- **tepotinib**: EMD-1214063 (NSC 758244)

**POC using PD-Guided Dose Scheduling**

- **Vehicle XL880**: 17, qd
- **XL880**: 44, bid
- **Vehicle XL184**: 44, bid
- **XL184**: 13, bid
- **Vehicle EMD**: 13, bid
- **EMD1214063**: 13, bid

Srivastava et al. Mol Cancer Ther 2018;17:698-709

Frederick National Laboratory for Cancer Research
PD-guided Clinical Development of MET-TKIs?? Made Possible by Generation of a Specific mAb

ANTI-PY1235-MET IMMUNOLOGICAL BINDING REAGENT AS CANCER DIAGNOSTIC

SUMMARY

This technology consists of highly specific rabbit monoclonal antibodies reactive with phosphorylated tyrosine located at amino acid 1235 in the human MET sequence. Binding to this pY1235 residue is independent of the phosphorylation of other tyrosines in the vicinity (1230 and 1234), does not cross-react with these nearby phosphotyrosine residues, and does not occur when Y1235 is unphosphorylated. Researchers at the NCI seek licensing and/or co-development research collaborations to commercialize and develop a companion diagnostic for selective MET inhibitors.

PRODUCT TYPE

Diagnostics

KEYWORDS

Antibody, MET, Diagnostic, Companion Diagnostic, Rabbit Monoclonal Antibody, Tyrosine Kinase Inhibitor

COLLABORATION OPPORTUNITY

This invention is available for licensing and co-development.

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301-402-6655

PCT Application

No. PCT/US17/22783
filed March 16, 2017
Have Clinical Trials Properly Assessed c-Met Inhibitors?

Veronica S. Hughes¹,* and Dietmar W. Siemann¹

The c-Met/HGF pathway is implicated in cancer progression and dissemination. Many inhibitors have been developed to target this pathway. Unfortunately, most trials have failed to demonstrate efficacy. However, clinical trials have not

Pathway activity should be verified in patients using an appropriate biomarker, yet biomarkers are rarely validated. Although a validated phospho-Met immunoassay has been developed, it is not currently used in clinical trials [12].

Assays such as this must be utilized if we are to advance therapeutics. Enrolling patients whose tumors do not express phospho-Met in a clinical trial of c-Met inhibition is unlikely to have a positive outcome, and is also unjust to the patients. Ultimately, potentially beneficial drugs may be discarded.
Highly Labile Post-translational Phosphorylation of Tyrosines: METpY1235 and pY1356 Are Examples

Stability vs Ischemia Time in core needle biopsies full-length MET and pMET (SNU5 xenografts)

Demonstrating Fitness-for-Purpose 18-gauge core needle biopsy of xenograft tumor models

SOP: 340507

Title: “Tumor Frozen Needle Biopsy Specimen Collection and Handling”

Purpose: point-of-care specimen handling for PD biomarker preservation

Method: snap-freeze within 2 minutes of collection

research community access via the

DCTD Website for Validated Biomarker Assays


Dr Hollingshead, from Kinders et al. Clin Cancer Res 2008;14:6877-6885
PD Biomarkers are Dynamic and Highly Labile but are Evaluable if Pre-analytical Variables Are Controlled

phospho-Ser\textsuperscript{473}-AKT in human GE tumors and HT-29 colon cancer xenografts


Srivastava et al, 2018 (unpublished)
### Preservation of phosphoProtein Biomarkers at Point of Collection and at Point of Processing (Lab)

DCTD Website for Validated Biomarker Assays

#### SOP 340507
**Title:**
“Tumor Frozen Needle Biopsy Specimen Collection and Handling”

**Purpose:**
Point-of-care core biopsy handling for PD biomarker preservation

**Method:**
Snap-freeze within 2 min of collection

#### SOP 341401
**Title:**
“Tumor Biopsy Lysate Preparation and Fractionation for IFA”

**Purpose:**
Lab core biopsy processing for analysis of PD phosphobiomarkers

**Method:**
Thaw specimen under 4°C extraction buffer containing Roche PhosphoStop and Roche cOmplete™ Mini Protease Inhibitor Cocktail

#### SOP 340522
**Title:**
“Tumor Frozen Needle Biopsy Preparation for IFA”

**Purpose:**
Lab core biopsy processing for analysis of PD phosphobiomarkers

**Method:**
Thaw specimen under 10% neutral buffered formalin
# ETCTN Portfolio: DNA Damage Response Modifiers

## ETCTN Early Clinical Development of DDR modulators

<table>
<thead>
<tr>
<th>Pathway Target</th>
<th>Molecular Target</th>
<th>Agents</th>
<th>DDR PD Biomarker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single/Double Strand Break Induction</td>
<td>multiple TOP1i</td>
<td>any chemoRx agents irinotecan, topotecan, indenoisoquinolines</td>
<td>pNBS1, γH2Ax, RAD51, ERCC1 γH2Ax w/cCasp3 (apoptosis)</td>
</tr>
<tr>
<td>Single Strand Break Response: BER</td>
<td>PARP1/2i</td>
<td>veliparib, olaparib, talazoparib</td>
<td>PAR polymer</td>
</tr>
<tr>
<td>Single Strand Break Response: TMB, MSI</td>
<td>APE blockade w/TOP2 sensitivity PD1/L1 blockade</td>
<td>pembrolizumab, nivolumab, durvalumab, atezolizumab</td>
<td>Late DNA damage (pNBS1, γH2Ax, RAD51) MLH1, MSH2, MSH6, PMS2</td>
</tr>
<tr>
<td>DDR Sensors</td>
<td>ATRi</td>
<td>M6620 (formerly VX-970)</td>
<td>pS1989 autophosphorylation</td>
</tr>
<tr>
<td>DNA-PKi</td>
<td>M3814 M9831 (aka VX-984)</td>
<td>γH2Ax, pKAP1 (recent project plan)</td>
<td></td>
</tr>
</tbody>
</table>
Multiplex Evaluation of DDR Biomarkers to Support ETCTN Portfolio of DNA Damage Response Modifiers

Baseline Biomarker levels
DT Clinic – CRC Series

Marker expression (% NAP or % cells ≥ 5 Rad51 foci)

Patient number

Vehicle-treated
Topotecan-treated
(4.7 mg/kg; 4 hours post-treatment)

**DDR Biomarkers:** Rad51, pS343-Nbs1, γH2AX, DAPI

Wilsker, Dull and Kinders, submitted 2018
What Does a γH2AX Response Mean?

**Figure 1: Tumor Volume and γH2AX Response**

**MDA-MB-231**
- Vehicle
- 4 mg/kg birinapant
- 12 mg/kg birinapant

**OVCAR-3**
- Vehicle
- 4 mg/kg birinapant
- 12 mg/kg birinapant

**Figure 2: Biomarker Analysis**

**MDA-MB-231**
- γH2AX⁺
- γH2AX⁺ / CC3(bleb)⁺

**OVCAR-3**
- 12 mg/kg birinapant

Dull et al. Oncotarget 2018; 9:17104-17116
What Does a γH2AX Response Mean?

Dull et al. Oncotarget 2018; 9:17104-17116
DNA Damage Response in Spontaneous Cancer Model (Canine Lymphoma Pts Treated with Topo 1 Inhibitors)

Patient A (indimitecan (LMP776), >60% tumor reduction)

Pre-dose 2 h 6 h

Patient B (indotecan (LMP400), >60% tumor reduction)

Pre-dose 2 h 6 h

reported assay values*

max % cells in apoptosis

Responders

Patient A: 9%
Patient B: 5%

Non-Responders

7 pts: <1.5%

max % of apoptotic cells in the γH2Ax population

Responders

Patient A: 82%
Patient B: 38%

*15,000 cells evaluated per Bx

Dull et al. Oncotarget 2018; 9:17104-17116

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Intrinsic Apoptosis Sandwich Immunoassay v1.0

- Panel 1: Bak, Bax, total Casp-3, total Lamin-B, SMAC
- Panel 2: Bad, **Bax::Bcl2**, Bcl-XL, Bim, Mcl1
- Panel 3: cCasp-3, **Bcl-XL::Bak**, Mcl1::Bak, pS99Bad, survivin

Apoptosis Biomarkers (Multiplex Immunoassay Elisa)

Responsive Model
MDA-MB-231
- vehicle
- 4 mg/kg birinapant
- 12 mg/kg birinapant

PD Biomarker-Informed Drug Discovery by CBC Project Teams: MCL1 Inhibitors

Srivastava AK et al, AACR 2018, ASCO 2018
**Apoptosis Biomarker Immunoassay – Community Access**

**Intrinsic Apoptosis Panel v1.0**
- Panel 1: Bak, Bax, total Casp-3, total Lamin-B, SMAC
- Panel 2: Bad, Bax::Bcl2, Bcl-XL, Bim, Mcl1
- Panel 3: cCasp-3, Bcl-XL::Bak, Mcl1::Bak, pS^{99}Bad, survivin

**Commercialization - Bio-Rad Inc w/Myriad RBM**
- Panel 1: Bak, Bax, total Casp-3, total Lamin-B, SMAC
- Panel 2: Bad, Bax::Bcl2, Bcl-XL, Bim, Mcl1
- Panel 3: cCasp-3, Bcl-XL::Bak, Mcl1::Bak, pS^{99}Bad, survivin


**Community Access**

**User Model** –
Direct purchase of assay kits from BioRad, coupled with NCI-Frederick assay training program
(see [https://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm](https://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm))

**Fee-for-Service Model** –
Ampersand Biosciences Inc (NY), GLP but not CLIA uses

**Contract Model** –
RBM Myriad Lab (TX), CLIA-certified lab for diagnostic uses

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Contributions to the Extramural Community

Assay Support of NExT/CBC Discovery Teams and NExT/ETCTN Development Teams

• Generating new Mabs to measure precise MOAs (MET-pY^{1235}, MET-pY^{1356}, ATR-pT^{1989})
• Qualifying commercial Mabs for intended use, and QC of supply chain
• Creating recombinant protein calibrators (e.g., heterodimeric proteins, such as Mcl1::Bak for the apoptosis panel)
• Distributing assay kits
• Transferring Validated, Fit-for-Purpose Assays to End-User Laboratories
  – lab-based training of certified assay operators at the NCI-F campus
  – enable formal, SOP-based assay transfer by providing qualified key reagents and proficiency panels
  – monitor assay performance as a lab network
  • root cause analysis in case of an assay failure
  • assessment of the proposed solution
  • amend the SOP to the next version, if necessary
  • issue a recommended hold on clinical specimen analysis during the correction process
• Providing centralized lab assay support to NCI-sponsored clinical trials in the ETCTN
Contributions to the Extramural Community (2)

Initiative to improve core biopsy quality and suitability for pharmacodynamic and other biomarker analyses

- Recognized biopsy quality as a major issue to solve for the success of POM and POC studies
- Created tumor “biopsy board” at NCI – regular communication between clinical trial oncologists, pathologists, radiologists, and PD biomarker laboratory to connect clinical and lab findings
- May 2017 NCI conference on re-thinking biopsy collection for non-diagnostic purposes
- Developed or changed laboratory methods to increase biopsy evaluableity (tumor cell segmentation, lab practices/procedures)
What’s Ahead on the PD Biomarker Horizon?

- Signaling Pathways (MEK/ERK/RP6, PI3K/AKT) → PI3Kβ/δi
- Cell Death mechanisms → multiple agents
  - apoptosis-intrinsic v2.0, apoptosis-extrinsic, necroptosis, ferroptosis, anaphase catastrophe, pyroptosis, oxeiptosis, perforin/granzymes, autophagy
- DNA Damage Response and “Cell Cycle Catastrophe”
  - enhance chemotherapy-induced DNA damage → PARPi, BERi, ATRi, DNA-PKi
  - exploit intrinsic tumor defects in DNA repair (HRD, mutATM, MMR status) - PARPi, ATRi
  - exploit intrinsic tumor defects in cell cycle control during DNA repair (CDKi) → Wee1i
- Protein Homeostasis, Oxidative ER Stress (ubiquitination, proteasome inhibitors) → p97i
- Methylation of protein and DNA (p16/p21 induction, methylome patterns) → WDR5i, KDM5i, DNMT1,3i
- Immune Checkpoint Inhibitors and CTL function (CTLA4i, integration of PD1 and TCR signaling) → SHP2i
- Mapping targets to EMT and CSC subpopulations to treat biological tolerance of therapy → multiple
- Adaptation of biopsy-based assays for hematologic malignancies and for CTCs → apoptosis
DCTD Pharmacodynamic Biomarkers Program

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    the entire BTB support program
Protein Expression Laboratory - Dominic Esposito
Lab Animal Sciences Program
    Pathology/Histopathology Lab
    Animal Husbandry

Frederick National Laboratory for Cancer Research
Supplemental slides
PD Biomarkers in Clinical Translation of Novel Drug Combinations: EMT-based Survival of Nilotinib + Paclitaxel

Vehicle
Nilotinib + Paclitaxel

E-cadherin & Vimentin
H&E
E-cadherin & Vimentin
H&E

Day 1
Day 8
Day 15
Day 19
Day 58

Vehicle
Paclitaxel
Nilotinib + Paclitaxel

E-cadherin & Vimentin
H&E
E-cadherin & Vimentin
H&E
E-cadherin & Vimentin
H&E

Kinders, Wilsker, Navas, 2018 unpublished

Frederick National Laboratory for Cancer Research
PD Biomarkers in Clinical Translation of Novel Drug Combinations: EMT in the Clinic

Prostate 1  Colorectal 1  NSCLC  Esophageal  Parotid  Breast  Fibrosarcoma 2

H&E  IF Image  IF Image  IF Image  IF Image

Kinders, Navas, 2018 unpublished
Epithelial-Mesenchymal Transition (EMT)::
Image Analysis Pipeline to Quantify EMT in Tumor Bx

Kinders, Navas unpublished
PD Biomarkers in Clinical Translation of Novel Drug Combinations: Nilotinib + Paclitaxel

Holbeck S et al., Cancer Research, 2017

Intrinsic apoptosis pathway markers suggest cells remaining after two cycles of treatment appears to be resistance to apoptosis

Unpublished data show modulation of a necroptosis regulator
Supplementary Figure S1. CC3 puncta are associated with plasma membrane blebbing in tumor tissue from drug-treated xenograft models

MDA-MB-231

vehicle birinapant

OVCAR-3

vehicle birinapant

Dull et al. Oncotarget 2018; 9:17104-17116

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Disambiguating the Meaning of γH2Ax Response

Comprehensive PD Biomarker Portfolio with Frequent Use in NExT

- PD biomarker assays that visualize drug responses (multiplex fluorescence microscopy)
  - chemotherapy damage → nuclear γH2AxD(3,4,5,7,8,9,15)
    - add nuclear pNBS1, key in BRCA deficiency(15)
    - add nuclear RAD51 and pATR foci(16)
    - add cytoplasmic cCasp3+ “blebs”—key to distinguishing DDR from apoptosis(14)
      - add induction of LC-3 cytoplasmic “puncta” for autophagy(16)
  - cell cycle alterations → CDK1/2-pY15, pHH3(9)
  - immunoPD → CD8, CD3-pY142zeta, ZAP70-pY483, βCATN(11)
  - autophagy → LC3(16)
  - plasma membrane pY1235-MET (clone 7334), GLUT1 and NaKATPase-alpha(12)

- PD biomarker assays that survey drug responses in tissue extracts (sandwich immunoassays)
  - PARP1/2 signaling → PARylated protein (bid schedule used in >50 clinical trials)(1,2,5,7,11,15)
  - enzymatic MET signaling → full-length MET- pY1235, pY1234/1235, pY1356 (clones 7334, 23111)(12,13)
  - intrinsic apoptosis with recombinant heterodimer standards(10)
  - isoform-specific signaling → AKT1/2/3-pT308/pS473, rpS6-pS235/236/240/244 (16)

**Pharmacodynamic Biomarkers**

- Pharmacodynamics broadly defined as “what a drug does to the body”
- At the molecular level, a biochemical response of the intended target to drug action and its planned downstream biochemical and cellular consequences
- The sequence of events provides a framework for PD biomarker study design:

  - **1^e drug effect**
    - “Target Engagement”
    - Post-translational modification
    - Inhibition of activity
    - Conformational change
    - Change in stability

  - **2^e drug effect**
    - “Pathway Changes”
    - Signaling alterations
    - Stressor signals
    - DNA damage
    - Cell death signals
    - Gene de-silencing

  - **3^e drug effect**
    - “Cellular Changes”
    - Tumor cell death
    - Reduced proliferation
    - CTL lysis of tumor

Clinical surrogates:
- Medical
- Imaging
- Physical findings
- Survival surrogates

Therapeutic effect:
- Increased OS
- Increased PFS