Facilitating Collaborations to Develop Combination Investigational Cancer Therapies: NCI Perspectives on Preclinical Issues in Co-Development

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Challenges to Development of Combination Targeted Therapeutics

- Incomplete understanding of mechanisms of action for a growing number of targeted agents available for trial
  - Inability to assess target effect
    - Lack of assays, imaging tools
    - Lack of assay standardization
    - Lack of commercially-available agents formulated for in vitro use
    - Lack of available investigational agents for in vitro use
- Lack of preclinical models for combinations
  - To evaluate efficacy, schedule effects, biomarker utility, toxicity
- Clinical trials methodology
  - Need to screen large numbers of patients?
  - Need for tumor biopsies?
  - Is histologic homogeneity relevant?
  - Pharmacokinetic interactions? SD vs RR?
- Intellectual property & regulatory challenges to novel combinations
<table>
<thead>
<tr>
<th>Drug</th>
<th>Biomarker</th>
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</thead>
<tbody>
<tr>
<td>Anti-estrogens</td>
<td>ER, PR, genomic signature</td>
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<tr>
<td>Trastuzumab</td>
<td>Her2 FISH, IHC</td>
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<tr>
<td>EGFR small molecule inhibitors</td>
<td>Mutation status</td>
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<tr>
<td>B-Raf, ALK inhibitors</td>
<td>Mutation status</td>
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<tr>
<td>Anti-VEGF/VEGFR agents</td>
<td>??</td>
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<tr>
<td>IGF-I receptor antagonists</td>
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<tr>
<td>Src inhibitors</td>
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<tr>
<td>Cdk/Cyclin D1 inhibitors</td>
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<tr>
<td>HDAC/DNMT inhibitors</td>
<td>??</td>
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<tr>
<td>Anti CTLA-4 Antibody</td>
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</tbody>
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Role of Proof of Mechanism Studies in Early Phase Trials of Molecularly Targeted Combinations

• Demonstration of mechanism(s) of action (or resistance) of the combination in tumor early in development provides:
  — Evaluation of the actual versus presumed sites of target engagement
  — Evidence to support further development of the combination
  — Demonstration of the relationship of drug schedule and systemic exposure to target effects
  — Data to determine the relevance of the biomarker chosen to represent modulation of the target—downstream effects can be studied as well as direct target inhibition

• Ability to investigate molecular effects of the combination of agents in surrogate (non-malignant) tissues
  — Evaluate the relevance of the non-malignant tissue as a marker of target engagement
  — Opportunity to study molecular toxicology and other safety signals in a range of normal organs

• Not necessarily predictive of clinical benefit—requires larger, later stage trials
### Pharmacodynamic Assay Development

<table>
<thead>
<tr>
<th>Concept</th>
<th>Feasibility &amp; Development</th>
<th>Validation</th>
<th>Launch</th>
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<tbody>
<tr>
<td><strong>Target</strong></td>
<td><strong>Application</strong></td>
<td>Platform</td>
<td>Feasibility</td>
</tr>
<tr>
<td>γ-H2AX Protein (tumor)</td>
<td>DNA Damaging Agents</td>
<td>ELISA</td>
<td>✓</td>
</tr>
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</tr>
<tr>
<td>Top 1 Protein</td>
<td>TOPO Inhibitors</td>
<td>ELISA</td>
<td>✓</td>
</tr>
<tr>
<td>Top 1 Protein</td>
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<td>IFA Commercial Reagents</td>
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</tr>
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<td>MET TK domain and Grb2 Docking Site</td>
<td>Kinase Inhibitors</td>
<td>IFA Custom Reagents</td>
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<tr>
<td>PARG mRNA</td>
<td>PARP Inhibitors</td>
<td>RT-qPCR</td>
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<td>PARP 1 mRNA</td>
<td>PARP Inhibitors</td>
<td>RT-qPCR</td>
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<tr>
<td>PARP 1,2 Activity (PAR levels)</td>
<td>PARP Inhibitors</td>
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<td>PARP 2 mRNA</td>
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<tr>
<td>Stem Cell Proteins -ALDH 1A1 -OCT 3/4 -NANOG -CD44v6</td>
<td>Tumor Stem Cell Inhibitors</td>
<td>IFA</td>
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</tbody>
</table>

**KEY:**
- In Progress
- Completed
- Delayed
- CA Commercially Available
- NA/UIN Not Applicable or Uninformative
- Technical Difficulty
- H On Hold
- R Ready
γH2AX: Pharmacodynamic Marker of DNA Double Strand Breaks

Nucleosome with H2AX Tail

- H2AX: Histone protein phosphorylated by ATM, ATR, or DNA-PK on serine c-4 following DNA DSBs (many sources) in geographic proximity to the DSB, forming foci
- DNA repair proteins accumulate around the phosphorylated γH2AX focus—platform for DNA damage response
- Detect by counting immuno-fluorescent foci: each focus contains hundreds of γH2AX molecules
- Decay of foci reflects DNA rejoining
- Downstream indicator of the effects of the formation of DNA-topoisomerase I covalent complex

Quantitative Immunofluorescence Assay: γH2AX Measured in A375 Xenografts after Top1 Inhibitor Therapy (18G Biopsy)

Dose Response of γH2Ax to 724998 at +2 Hours A375 Xenograft

Vehicle

25 mg/kg iv NSC 724998

γ-H2AX: A Non-Invasive Marker of DNA Double Strand Breaks in CTCs

Critical Pathways for Development of Multiplexed Pharmacodynamic Assays

- RAS/RAF/MEK/ERK
- PI3Kinase/AKT/mTOR
- Glycolytic and mitochondrial energy metabolism
- DNA repair
- Circulating tumor cells
- NOTCH
- Apoptosis
- Autophagy
- EMT
Challenges to Development of Combination Targeted Therapeutics

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COMBO set 1
- 87 compounds of diverse mechanism
- Includes many older FDA-approved anticancer agents

FDA-approved COMBO set:

**Approved Oncology Drugs Set Information: A set of FDA-approved anticancer drugs to enable cancer research.**

This plated set (2 microtiter plates/set) contains most current FDA-approved anticancer drugs. The set consists of 88 agents and is intended to enable cancer research, drug discovery and combination drug studies. Details on the drugs included in this plated set can be found by clicking on Approved Oncology Drugs Plated Set (Plate 1, Plate 2.) Clickable links within the excel files will dynamically query the DTP databases to retrieve up to date DTP information, including NCI60 data, for each drug. Compounds in this set are provided as 20 microliters at 10mM in 100% DMSO; plates are shipped frozen, with dry ice. All proprietary agents in this set were obtained through commercial sources.
Combination Plate: Dilution Series

Dilution series of plated set - mini dose-response curves
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Using the NCI 60 Panel to Develop Combinations of Cancer Therapeutic Agents: Drug Concentrations Chosen Based on Cell Line Activity

6-MP: 1/3 concentrations are < Human $C_{max}$

Dasatinib: 4/5 concentrations are < Human $C_{max}$
Combination of Dasatinib (NSC 732517) and 6-MP (NSC 755) More Than Additive Across a Wide Range of Dasatinib and 6-MP Concentrations in LOX IMVI Melanoma Cells In Vitro
For any 2-drug combination:

Bars to left indicate loss of benefit relative to best single-agent results.

Bars to right indicate overall benefit to using combo relative to best single-agent results.
Statistics of Pilot Phase

Initial Studies

• 31 pairwise drug combinations x 60 cell lines
  = 1,759 evaluable experiments to date

• 25,045 total dose combinations (5 concentrations of
  one drug; 3 of the second)
  ✓ 11,287 (45%) better than or equal to expected additive value
  ✓ 3,042 (12%) are better than both single agents at the same concentration
  ✓ 1,129 (4.5%) are antagonistic

Goal: 100 commercially available drugs with 5000 unique combinations
Growth Inhibition by Combination Is Not Predictable from Single Agent Activity

Dasatinib activity

6-MP activity

Combo benefit

6-MP sensitive

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Dasatinib/6-MP Combination: LOX IMVI Melanoma Xenografts

10 mice per group

Cell Line: LOX IMVI

- Untreated
- 6-Mercaptopurine: 25 mg/kg PO QDx15, d3
- Dasatinib: 50 mg/kg PO QDx15, d3
- Combo, 6-MP then Dasatinib
- Combo, Dasatinib then 6-MP 4 h later

Median Tumor Weight (mg)

Mean Net Body Weight (g)

Days Postimplant
Why Is the Dasatinib/6-MP Combination of Interest?

• Unexpected result based on “standard” understanding of the mechanism of action of either agent:
  ➢ Thiopurines (6-MP, 6-TG):
    - Inhibition of de novo purine synthesis
    - Incorporation into DNA
    - ALSO: Trigger mismatch repair-induced apoptosis that is dependent on homologous recombination apparatus and, thus, selectively kill BRCA2 defective cells (Cancer Res. 70: 6268, 2010; Molec. Cancer Res. 9: 206, 2011)
  ➢ Dasatinib:
    - Inhibits BCR-ABL tyrosine kinase as will as c-KIT, EPHA, SRC, and PDGFR-β RTKs
    - ALSO: Inhibition of physiological c-ABL, absent translocation, strongly impairs DNA DSB repair (Oncogene 27: 4380, 2008)

• Suggests that “systematic” screening will provide novel, hypothesis-generating data that can be used to develop potential therapeutic combinations broadly
Accelerating Cancer Diagnosis and Drug Development

**Developmental Therapeutics**
- Jerry Collins
- Joe Tomaszewski
- Melinda Hollingshead
- Ralph Parchment
- Robert Kinders
- Tom Pfister
- Jay Ji

**DCTD**
- Jason Cristofaro
- Barbara Mrochowsk
- Michael Difilippantonio

**CTEP**
- Jamie Zweibel
- Jeff Abrams

**Center for Cancer Research**
- Yves Pommier
- Lee Helman
- Bob Wiltrout
- Shivaani Kummar
- William Bonner

**Cancer Imaging**
- Paula Jacobs

**Cancer Diagnosis**
- Barbara Conley
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<tr>
<th>Targets</th>
<th>Regimen</th>
<th>Full doses of individual agents</th>
<th>MTD of the combination (% of full dose)</th>
<th>Main DLTs within 1-2 cycles or after prolonged therapy</th>
</tr>
</thead>
</table>
| VEGF + VEGFR| Bevacizumab + Sorafenib | • BV: 10 mg/kg q2w  
• Sorafenib: 400 mg BID                                                                 | • BV 5 mg/kg q2w (50%) + Sorafenib 200 mg BID, 5 days on/2 days off (35%)  
• In patients with RCC: BV 5 mg/kg q2w + sorafenib 200 mg QD (25%) | Hand and foot syndrome; Hypertension; Proteinuria; Thrombocytopenia; |
|             | Bevacizumab + Sunitinib  | • BV: 10 mg/kg q2w  
• Sunitinib: 50 mg/D, 4wks on, 2 wks off                                                                 | • Full doses based on cycle toxicities  
• Intolerable with prolonged therapy in RCC patients | HTN, headache. Thrombotic microangiopathy after prolonged therapy in RCC patients |
| VEGF + mTOR | Bevacizumab + Temsirolimus| • BV: 10 mg/kg q2w  
• Tem: 25 mg qw                                                                 | Full doses                                                                                         | Mucositis, hyperlipidemia |
|             | Bevacizumab + Everolimus | • BV: 10 mg/kg q2w  
• Eve: 10 mg/d                                                                                                        | Full doses                                                                                         | |
|             | Temsirolimus + Sorafenib| • Tem: 25 mg qw  
• Sorafenib: 400 mg BID                                                                                           | Tem: 25 mg qw (100%) + Sorafenib 400 mg BID                                                        | Hand-foot syndrome; thrombocytopenia |
|             | Temsirolimus + Sunitinib| • Tem 25 mg qw  
• Sunitinib: 50 mg/D, 4wks on, 2 wks off                                                                                 | Intolerable despite 40-50% dose reduction of both agents (MTD exceeded)                             | Rash, thrombocytopenia, asthenia, diarrhea, stomatitis, |
| VEGF + EGFR | Erlotinib + Bevacizumab  | • Erlotinib: 150 mg/d  
• BV: 10 mg/kg q2w                                                                                              | Full doses                                                                                         | No DLT |
|             | Erlotinib + Sorafenib   | • Erlotinib: 150 mg/d  
• Sorafenib 400 mg BID                                                                                          | Erlotinib: 100 mg/d (67%) + Sorafenib 400 mg BID (100%)                                           | LFT abnl, HFS, diarrhea, lipase abnl |
|             | Tipifarnib + Sorafenib  | • Tipifarnib 300 mg BID  
• Sorafenib: 400 mg BID                                                                 | Tipifarnib 100 mg BID (33%) + Sorafenib 400mg am/200mg pm BID (75%)                               | Rash, Fever, Diarrhea |