Meeting Report

Preclinical models for defining efficacy of drug combinations: mapping the road to the clinic

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A 1-day symposium on *Preclinical models for defining efficacy of drug combinations: mapping the road to the clinic* was sponsored by the Developmental Therapeutics Program (DTP) of the National Cancer Institute at National Cancer Institute-Frederick on April 11, 2003. About 100 researchers attended with invited speakers coming from Canada, Germany, and various institutions in the United States. The symposium, coordinated by Dr. Edward Sausville, Associate Director of National Cancer Institute's Division of Cancer Treatment and Diagnosis for the DTP, and Dr. Anne Monks, Science Applications International Corporation-Frederick, was organized with the purpose of reaching consensus recommendations for the use of preclinical models in the development of combination therapies to predict clinical outcomes. The meeting was formatted as a presentation of reports focusing on different preclinical approaches to studying drug combinations, as well as tools and models for analyzing and interpreting drug combinations. Each presentation was followed by a brief discussion period. The symposium ended with a roundtable discussion seeking to reach consensus on a recommendation for an optimal set of data to advance combination therapies into the clinical setting for evaluation. This report summarizes the meeting and provides, for consideration by the cancer research community, a preliminary set of criteria for selecting and prioritizing drug combinations for clinical evaluation.

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The scientific program began with an introduction by Dr. Sausville highlighting the importance of preclinical models for combination drug therapy. He noted that combination therapy has been the basis for most success stories in cancer treatment, which is understandable when components of a combination have a favorable pharmacological interaction (same target, but different large-organ toxicities). A more recent foundation for the success of some cancer treatments involves a mechanistic basis, under which a combination of drugs is used to affect different molecular targets to circumvent resistance. A barrier to major advancements in combination therapy, however, has been a lack of understanding about the intersection of critical signaling pathways. Synergy might be induced through the effect of drugs on the same as well as parallel pathways. Because the number of drug combinations is limitless, a strategy for determining the most promising combinations and prioritizing their evaluation is crucial. Dr. Sausville posed a number of questions (see ''Roundtable Discussion'') to both speakers and participants with the main objective of coming to a consensus resolution on *what data should act as the ''roadmap'' to the clinic for a ''rational'' combination therapy*.

Participants were also invited to provide their input on a proposal by DTP to develop a plated set of compounds of known mechanism of action to facilitate combination drug studies for users with no access to robotics.[1] This set would include most approved anticancer drugs, as well as compounds of novel mechanisms that have not been tested in humans. The feedback obtained overwhelmingly indicated that researchers would like to have such a resource available.

The talk by Dr. Ting-Chao Chou, Memorial Sloan-Kettering Cancer Center, concerned mathematical modeling and computer simulations to calculate the expected effect of different drug combinations in an effort to better understand how chemotherapeutic combinations might be used clinically. *In vivo*, favorable synergistic drug combinations may result in increased efficacy, decreased dosage, reduced toxicity, and minimized development of resistance. Dr. Chou presented the history and derivation of his *Median Effect Equation* (Chou) and *Combination Index Equation* (Chou-Talalay). The isobologram curves generated represent additive, synergistic, or antagonistic interactions caused by different drug combinations. These

[1] For more information on the proposed ''Plated compound set for combination studies,'' contact Dr. Susan Holbeck, DTP, National Cancer Institute, at holbecks@mail.nih.gov.

methods have been extensively applied by many researchers, including several of the speakers, to determine optimal drug combination ratios for use in the clinical setting.

Dr. William Greco, Roswell Park Cancer Institute, vividly described the concept behind and applications of surface response models, including his own. He explained that the joint effect of two active agents could be viewed as a three-dimensional response surface. While these models for assessing joint action are the most comprehensive, he cautioned that they are also highly complex and difficult to create. Moreover, Dr. Greco noted that the true link between empirical synergy and the underlying biochemical, molecular, and physiological mechanisms is very difficult to discover.

To close the session on the theoretical and computational aspects of drug combination analysis, Dr. John Weinstein of the Laboratory of Molecular Pharmacology, National Cancer Institute, shared information on some bioinformatics resources developed in his laboratory for the analysis of drug combination data (*e.g.*, *COMBO*, a computer program package), as well as for the interpretation of such data (*e.g.*, *MedMiner*, *LeadMiner*). He agreed with previous speakers in that there is no single best model for the analysis of drug combination data. He suggested a matrix format in which good dose-response curves for each individual drug, at a restricted concentration range near the IC_{20}, are obtained on a single plate, and drug combinations screened on the same plate. Those combinations that show apparent synergy could then be tested further.

Dr. Patrick Reynolds, Children's Hospital Los Angeles, made an interesting contribution by describing a fluorescence-based, high-throughput system, called digital image microscopy-based cytotoxicity assay (DIMSCAN), for *in vitro* testing of cytostatic or cytotoxic drug combinations. He elaborated on five principles for this type of testing: (*a*) the assay system should have a wide dynamic range, ideally 3 to 4 logs of cell kill; (*b*) the cell line panel should employ multiple cell lines, including drug-resistant lines; (*c*) major mechanisms of resistance should be identified and used to structure the cell line panel; (*d*) exposure to drugs should be at clinically achievable levels and schedules; and (*e*) because hypoxia may antagonize drug action, it is essential to test under hypoxic conditions. Dr. Reynolds listed a number of completed, ongoing, and planned clinical trials based on DIMSCAN and concluded that the best approach for selecting drug combinations for evaluation is the one based on molecular targets; studies should first be performed *in vitro* and then confirmed with *in vivo* models before clinically testing the drug combination. He also stressed that drug antagonism is frequently used to exclude drug combinations.

The next series of presentations covered a broad spectrum of examples of combination modalities for cancer treatment at the preclinical and clinical levels. Dr. Patricia Burke, University of California, Davis, elegantly described how radioimmunotherapy in combination with antiangiogenic agents might enhance efficacy. This combined modality can increase both the delivery of the radiopharmaceutical dose to the tumor by increasing penetration and trapping of large monoclonal antibodies and the effect of the dose that is taken up by the tumor by sensitizing tumor cells, altering the effect of radioimmunotherapy-induced growth factors, and targeting endothelial cells supporting the tumor. Dr. Burke remarked that the treatment schedule, particularly the timing, for the antiangiogenic agent is as important as the agent and the radiopharmaceutical themselves. She also touched on how gene profiling of endothelial cells treated with different antiangiogenic agents can serve to predict synergy of these agents before *in vivo* assessment. By combining this information with the gene expression profile of patients' tumors, a rational design of the best combination therapy might be feasible. Efficacy can then be assessed using serum tumor markers or noninvasive imaging methods.

A stimulating presentation by Dr. Steven Grant, Medical College of Virginia, addressed the synergistic interactions between novel signal transduction modulators and established cytotoxic agents, cell cycle inhibitors, and differentiation inducers. While the first type of drug combination involves toxic concentrations of the cytotoxic agent to produce modest potentiation *in vitro*, the second and third types induce a high degree of synergism at concentrations of both agents that are nontoxic (ineffective) when given individually. Drug combinations between cell cycle inhibitors or these agents and differentiation inducers have also been shown to produce significant synergistic activity *in vitro*. The number of signal transduction modulator combinations associated with synergistic interactions of cell death is virtually limitless. Whether these combinations are therapeutically superior to those between conventional cytotoxic agents or those between conventional and novel agents and whether they represent a new paradigm for combination chemotherapy remains to be determined.

Dr. Judith Sebolt-Leopold, Pfizer Global Research and Development, contributed to the understanding of the critical issues impacting the design of preclinical combination chemotherapy studies, particularly for signaling antagonists. She illustrated how *in vitro* studies have demonstrated sequence dependence when cytotoxic agents are combined with signaling antagonists targeting ErbB or MEK. For inhibitors of the Ras-mitogen-activated protein (MAP) kinase pathway, apoptosis can be significantly enhanced by administration of the cytotoxic agent first (*e.g.*, Taxol, gemcitabine), followed by the signaling antagonist (*e.g.*, CI-1033, PD 098059). Animal studies have also shown that toxicity patterns and efficacy are greatly impacted by dosing sequence. Dr. Sebolt-Leopold believes that *in vitro* combination studies should always be corroborated with *in vivo* models, because the former cannot predict for toxicity and may not adequately predict for the degree of efficacy observed in cell cultures.

An additional illustration of the potential synergism between a cytotoxic agent and a signaling antagonist was provided by Dr. Hayley McDaid, Albert Einstein College

of Medicine. All combinations of the MEK inhibitor CI-1040 and Taxol tested in xenograft-bearing nude mice show synergism compared with the administration of single agents. The importance of drug sequencing, especially for molecular targeted therapies was again highlighted. Dr. McDaid also provided evidence for the selective enhancement of Taxol's antitumor activity *in vivo* by discodermolide, a microtubule-stabilizing agent. In both models, the *in vivo* data have corroborated the *in vitro* findings. Heterotransplanted models are currently being evaluated to determine whether these preclinical models can predict clinical response.

The afternoon session opened with a lively presentation by Dr. Andrew Janoff of Celator Technologies, Inc., in Canada, on commercial solutions for bringing effective drug combinations to the clinic. Celator is working on the identification of critical ratios at which drug combinations act synergistically to kill tumor cells, with the objective of fixing those synergistic ratios in a liposome-based delivery vehicle (CombiPlex) designed to target tumors following i.v. injection. Two *leitmotifs* appeared throughout the presentation: ''The *in vitro* synergistic activity depends on specific drug ratios'' and ''the *in vivo* activity depends on maintaining the synergistic ratios.''

Dr. Heinez Fiebig, University of Freiburg in Germany, provided some insight on the combination of epithelial growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) inhibitors with cytotoxic agents as measured by a panel of human tumor xenografts developed by his group. He stressed the importance of characterizing the xenograft panel for the expression of specific molecular targets, thus allowing the selection of tumors overexpressing the target of interest for both *in vitro* and *in vivo* studies. The tumor xenografts have been characterized for VEGF expression as well as for vascular permeability and vessel density. Using sensitive xenograft models, Dr. Fiebig has demonstrated synergistic activity between gemcitabine and a human antibody against VEGF. Similarly, he has shown synergistic responses between BIBX (an EGFR tyrosine kinase inhibitor) and Taxol.

Dr. Vassilios Avramis, Children's Hospital Los Angeles, shared information on his *in vitro* and *in vivo* models for the determination of drug synergism between cytotoxic and cytostatic drugs. Adaphostin, a potent tyrphostin inhibitor of $p210^{bcr/abl}$ and other tyrosine kinase activities that also inhibits VEGF secretion and tumor cell growth, is highly synergistic with idarubicin (IDA), IDA + 1-β-D-arabinofuranosylcytosine (ara-C), and IDA + fludarabine + ara-C over the respective cytotoxic drug regimen in drug-resistant human leukemia cell lines. Adaphostin also shows synergistic activity both *in vitro* and *in vivo* against human glioblastoma cells when given in combination with Flt-1/Fc chimera, an antibody against VEGF. Using Dr. Chou's methods for analyzing drug combination efficacy, several drug combinations have been translated from Dr. Avramis' laboratory to the clinic, including fludarabine + ara-C + IDA and STI-571 (Gleevec) + mitoxantrone + ara-C, among others.

To complete the afternoon session, participants were treated to an exciting exposition on clinical combinations by Dr. David Schrump of the Thoracic Oncology Section, National Cancer Institute. The importance of targeting molecular end points rather than cytotoxicity was highlighted. Whether plasma drug concentrations in patients correlate with *in vitro* exposure conditions and induce target gene expression and whether the target gene modulation correlates with clinical response are relevant factors in translational research on molecular targeted cancer therapy. On the basis of the knowledge that synergistic induction of gene expression can be mediated by combination treatment with DNA demethylating agents and histone deacetylase inhibitors, *in vitro* studies in lung cancer cell lines were conducted with decitabine plus depsipeptide under clinically relevant exposure conditions. Combination treatment resulted in a synergistic induction of the tumor antigen NY-ESO-1, with concomitant enhancement of cancer cell growth inhibition and apoptosis induction. Initial molecular analysis in single-agent clinical trials with decitabine and depsipeptide has corroborated the *in vitro* findings. A combination Phase I trial of the two agents is currently ongoing in patients with pulmonary or pleural malignancies.

Roundtable Discussion

The panel, consisting of the speakers and Drs. Janet Dancey of the Cancer Therapy Evaluation Program, National Cancer Institute, and Peter Lassota of Novartis, was reminded of the questions posed by Dr. Sausville in his introductory presentation: (*a*) What are the parameters that define synergy or antagonism and the best model for assessing these *in vitro*? (*b*) Do combination data generated *in vitro* correspond to the response of *in vivo* models? (*c*) Do combination data generated *in vitro* or *in vivo* predict for clinical response? (*d*) What additional information could help improve the predictability of these models (sequence of drug administration; duration of experimental conditions; number of models required to define a credible interaction)? (*e*) How important is it to evaluate combinations based on previously determined pharmacology of the drugs? and (*f*) What classes of drugs should be combined for potential therapeutic advantage?

Because of the financial and logistical limitations to clinically evaluating all possible drug combinations tested—one way or another—in preclinical models, a set of criteria is needed that would assist researchers in selecting and prioritizing those combinations that would be most valuable for testing in the clinic. The following section recapitulates the main points addressed at the roundtable discussion and ends with a preliminary set of criteria proposed for assisting with the selection and prioritization of drug combinations for clinical testing.

There was an animated discussion as to what constitutes a significant degree of synergism and whether it can be graded on a scale; one participant explained that in his studies of fludarabine plus ara-C, a 5-fold synergy *in vitro* is considered the minimum value acceptable for

validating a combination regimen in animal models and, possibly, in the clinic. Other participants argued that synergism is a continuum and that grading is unnecessary or not possible.

There was collective agreement that understanding of molecular targets is essential for predicting the patient population most likely to benefit from a particular combination regimen. Knowledge of both the molecular targets *in vitro* and the pharmacokinetics of the agents can guide the rational selection of promising combinations for further development. Moreover, lack of these data could actually result in an ineffective or even an antagonistic clinical outcome.

From the clinical investigator's perspective, a drug combination would more likely be approved for clinical trials if clinical single-agent data (*e.g.*, pharmacokinetics, favorable toxicity profile, and consistent evidence of antitumor activity in a refractory patient population) were available. In this case, *in vitro* studies of the drug combination would be recommended only for determination of potential antagonism; animal models would be recommended only if toxicity concerns were pressing. The case for a combination trial would be even stronger if mechanistic data on single agents as well as preclinical pharmacokinetic data on the combination were available. However, the predictive value as well as the required diversity of the preclinical data, especially *in vivo* models, were questioned. In general, the more preclinical data available on the single agents and drug combination, in addition to single-agent clinical activity, the higher the priority for clinically testing the combination.

Countering this view, a panel member questioned the value of conducting Phase II studies with particular single agents when preclinical data suggest that optimum efficacy will be obtained with combination regimens. Preclinical models of drug combinations should suffice for moving the combination forward into clinical testing, especially if single-agent pharmacology data are available to adjust the *in vitro* models to the clinical situation. The general sentiment among panel members was that it is dangerous to go directly from *in vitro* studies to clinical trials with combination regimens; at a minimum, limited *in vivo* safety studies are needed to exclude toxicity concerns. Determination of optimum sequencing schedules would be desirable. The importance of pharmacokinetic and biodistribution determinations, as well as the use of orthotopic tumor models with drug combinations, was also addressed.

The following characteristics were recommended as a preliminary set of criteria for selecting and prioritizing drug combinations for clinical evaluation: (*a*) a significant degree of synergism—and no antagonism—*in vitro* at pharmacologically achievable or relevant concentrations; (*b*) evidence of synergistic activity across a spectrum of tumor types or biologically delimitable subset of tumors; (*c*) activity of the drug combination against primary human tumor cells at pharmacologically achievable or relevant concentrations; (*d*) *in vitro* selectivity against tumor cells—that is, the synergistic combination is nontoxic to normal tissues; (*e*) synergism in the presence of human plasma; (*f*) clear, compelling, or at least reasonable mechanism of action of the agents, with the drug combination based on a mechanistic approach that can be validated; (*g*) some rationale for the clinical activity of one, if not both, agents in existing clinical trials; and (*h*) *in vivo* data showing no evidence of significant toxicity and definite evidence of efficacy (or pharmacodynamic activity) of the drug combination. The latter criterion could be weighed based on how compelling the other seven criteria are.

DTP would like the cancer research community to react to these criteria and have investigators provide the National Cancer Institute with feedback.

Appendix

Speakers

Dr. Edward A. Sausville, Developmental Therapeutics Program, National Cancer Institute, NIH, Rockville, MD, USA

Dr. Ting-Chao Chou, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Dr. William R. Greco, Roswell Park Cancer Institute, Buffalo, NY, USA

Dr. John Weinstein, Laboratory of Molecular Pharmacology, National Cancer Institute, NIH, Rockville, MD, USA

Dr. C. Patrick Reynolds, Childrens Hospital Los Angeles, Los Angeles, CA, USA

Dr. Patricia A. Burke, Sacramento Medical Center, University of California, Davis, Sacramento, CA, USA

Dr. Steven Grant, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, USA

Dr. Judith Sebolt-Leopold, Pfizer Global Research and Development, Ann Arbor, MI, USA

Dr. Hayley McDaid, Albert Einstein College of Medicine, Bronx, NY, USA

Dr. Andrew Janoff, Celator Technologies, Inc., Vancouver, BC, Canada

Dr. Heinz H. Fiebig, University of Freiburg, Freiburg, Germany

Dr. Vassilios I. Avramis, Childrens Hospital Los Angeles, Los Angeles, CA, USA

Dr. David S. Schrump, Thoracic Oncology Section, National Cancer Institute, NIH, Rockville, MD, USA

Additional Members of the Roundtable Discussion

Dr. Janet Dancey, Cancer Therapy Evaluation Program, National Cancer Institute, NIH, Rockville, MD, USA

Dr. Peter Lassota, Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA