

MULTIPLE  
MYELOMA  
RESEARCH  
FOUNDATION



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# Validating Compounds for Multiple Myeloma

An MMRF Roundtable

November 21-22, 2005  
New York, NY

Supported by grants from



Accelerating the Search for a Cure



## Table of Contents

Welcome	1
Agenda	2
Participants	4
Abstracts	8-23
<b>Session I</b> .....	8
In Vitro Modeling for Target Discovery	
<b>Session II</b> .....	14
Animal Models for Compound Validation in Myeloma	
<b>Session III</b> .....	19
How to Apply Model Systems for Validating Drug Combinations	
Notes	24



November 22, 2005

Dear Colleague:

Welcome to New York City and the **Validating Compounds for Multiple Myeloma** roundtable sponsored by the *Multiple Myeloma Research Foundation* (MMRF).

This exciting event will bring together leading experts in compound validation from academia as well as the pharmaceutical and biotech industries to reach consensus on a single algorithm to validate a compound (or combination of compounds) for myeloma. As part of this process, participants will also identify areas of weakness, as well as areas of need, in the algorithm. The MMRF will then issue requests for applications (RFAs) to the broader research community to address these areas of needs, to increase pre-clinical validation efforts in myeloma, and to promote the validation of novel and combination therapies for myeloma. We look forward to an open and stimulating discussion that will lead to the development of innovative targeted therapies for multiple myeloma patients.

This roundtable is made possible by the MMRF through an educational grant from McCarty Cancer Foundation, OncoGenex Inc., Infinity Pharmaceuticals, Inc., Keryx Biopharmaceuticals, Inc., and Nereus Pharmaceuticals, Inc.

Once again, thank you for joining us. We look forward to working together today and in future endeavors.

Kenneth C. Anderson, MD

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Multiple Myeloma Center  
Medical Director, Kraft Family Donor Center  
Professor of Medicine,  
Harvard Medical School  
Dana-Farber Cancer Institute

William S. Dalton, PhD, MD

Chief Executive Officer & Center Director  
H. Lee Moffitt Cancer Center & Research Institute  
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## Agenda

November 21-22, 2005

New York, NY

### CO-CHAIRS

Kenneth C. Anderson, MD – *Jerome Lipper Multiple Myeloma Center*

William S. Dalton, PhD, MD – *H. Lee Moffitt Cancer Center & Research Institute*

### MONDAY

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6:30 PM **Welcome Dinner**  
Joseph R. Bertino, MD – *The Cancer Institute of New Jersey*

### TUESDAY

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7:00 AM **Continental Breakfast**

8:00 **Introductions & Objectives**  
Kenneth C. Anderson, MD

8:10 **Compound Validation Overview – Proposed Algorithm**  
William S. Dalton, PhD, MD

8:30 **Session I – In Vitro Modeling for Target Discovery**  
Session Chair: William S. Dalton, PhD, MD

- **The Use of Human Myeloma Cell Lines to Identify Targets and Validate Compounds**  
Leif Bergsagel, MD
- **The Use of Cell Line Models to Identify Targets for Myeloma Treatment**  
Constantine S. Mitsiades, MD, PhD
- **Gene Expression Profiling of Drug Resistant Cell Lines to Identify Targets**  
William S. Dalton, PhD, MD
- **Cell Cycle as a Molecular Target in Myeloma**  
Selina Chen-Kiang, PhD

(All presentations – 20 minutes)

9:50 **Discussion – Strengths/Weaknesses of Current Data Set**

10:20 **Break**



## Agenda *(continued)*

- 10:40      **Session II – Animal Models for Compound Validation in Myeloma**  
                 Session Chair: Kenneth C. Anderson, MD
- **Validating Compounds for Multiple Myeloma: Utility of the SCID-hu Model**  
                 Joshua Epstein, DSc
  - **Animal Models for Compound Validation in Myeloma**  
                 Constantine S. Mitsiades, MD, PhD
  - **The Murine 5TMM Model in Myeloma Drug Discovery**  
                 Karin Vanderkerken, PRD
  - **New Targets and Strategies for Treating Multiple Myeloma**  
                 James R. Berenson, MD
- 12:00 PM    **Discussion – Strengths/Weaknesses of Current Data Set**
- 12:30      **Working Lunch**
- 1:00        **Session III – How to Apply Model Systems for Validating Drug Combinations**  
                 Session Chair: William S. Dalton, PhD, MD
- **High-Throughput Drug Screening**  
                 Said Sebti, PhD
  - **High-Throughput Drug Screening**  
                 James Bradner, MD
  - **Activity of IPI-504, a Water-Soluble Hsp90 Inhibitor, Combined with Bortezomib in a Human Multiple Myeloma Xenograft Model**  
                 Margaret A. Read, PhD
  - **The Pediatric Preclinical Testing Program (PPTP) – A Model for Drug Development in Rare Malignant Diseases**  
                 Peter J. Houghton, PhD
- 2:20        **Discussion – Strengths/Weaknesses of Current Data Set**
- 2:50        **Roundtable Summary/Next Steps**  
***Areas of Great Need to Expedite Compound Validation (Prioritization of Potential RFAs)***  
                 Session Chairs: William S. Dalton, PhD, MD, and Kenneth C. Anderson, MD
- 3:30        **Adjournment**



## Co-Chairs

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# Session I: In Vitro Modeling for Target Discovery

Session Chair:  
William S. Dalton, PhD, MD

SESSION I



## Abstracts

### The Use of Human Myeloma Cell Lines to Identify Targets and Validate Compounds

*Leif Bergsagel, MD*

There are currently many available drugs that may be active in multiple myeloma. We need a simple screening algorithm to help to prioritize their further preclinical and clinical evaluation. There is no widely accepted standard to guide the choice for preclinical drug selection. Generally they will be evaluated for activity in two or three immortalized cell lines, and in the best cases evaluated further for activity in co-culture and *in vivo* xenograft models. Unfortunately, we have no simple assays that tell us that a given drug will be particularly active in multiple myeloma (MM), and perhaps more importantly, we have no assays that can identify drugs that induce long remissions (e.g., melphalan), as opposed to only short remissions (e.g., dex). As we enter the era of targeted therapies (particularly with signal transduction pathways) it is likely that any given pathway may be active in only a subset of myeloma (e.g., FGFR3). For this reason it becomes imperative to have a simple initial screen that encompasses as much of the heterogeneity in myeloma as possible. Finally, it is important in an initial screen to demonstrate that a given agent is not a non-specific toxin.

We have assembled a panel of 50 human myeloma cell lines (HMCL) and performed extensive characterization. They are all mycoplasma negative, EBV negative (excluding ARH77, IM9, MC/CAR), B cells/Plasma Cells (excluding KMS5). In particular they have been analyzed for metaphase karyotype by G-banding, Ig translocation, N and K ras mutation, FGFR3 mutation, p53 mutation, p18 deletion. Their gene expression has been profiled on the LymphoChip, Affymetrix Hu95A, Hu133A+B, HU133Plus2, Stewart's Myeloma Array. They have been analyzed by conventional and array CGH. A complete cytogenetic analysis by spectral karyotype has been performed. In addition to the cell lines that display genetic heterogeneity, there are four matched sublines of each of 8226 and U266 developed by Bill Dalton that have been selected for resistance to various chemotherapy (melphalan-LR5, doxorubicin-DOX6 and DOX40, mitoxantrone-MR20). In addition there are matched sublines of MM.1 with differential sensitivity to dexamethasone (MM.1S and MM.1R).

These cell lines represent a valuable resource for target identification and compound validation in MM. However there are several limitations to their use. We do not have any cell lines developed from patients with hyperdiploidy (TC D1), representing about 40% of MM patients. The cell lines are all highly proliferative and independent of the bone marrow (BM) microenvironment. To supplement their use we need a model of nonproliferative BM localized monoclonal plasma cells, and have developed a novel mouse model with these features.

*Submitted by Leif Bergsagel, MD, Mayo Clinic, Scottsdale, Arizona, USA*



## Abstracts

### **The Use of Cell Line Models to Identify Targets for Myeloma Treatment**

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*Constantine S. Mitsiades, MD, PhD*

Tumor cell lines have been a basic resource for the pre-clinical *in vitro* and *in vivo* evaluation of novel therapeutics for multiple myeloma (MM), as well as the vast majority of other human neoplasias. In this presentation, we will review our experience in the study of a large set of human MM cell lines to characterize the patterns of expression of putative therapeutic targets for MM; to assess the patterns of sensitivity or resistance of MM cells to various investigational anti-tumor agents; as well as to identify molecular markers correlating with the degree of responsiveness of MM cells to these agents. In addition, we will present our ongoing experience with cell line-based studies for the rational design of novel combination therapies for MM. Importantly, we will discuss the putative caveats of cell line models, as well as strategies to address them, within the context of our efforts to delineate the optimal strategies for bench-to-bedside translation of novel classes of anti-MM therapeutics, and hopefully, improvement of clinical outcome of MM patients.

*Submitted by Constantine S. Mitsiades, MD, PhD, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA*



## Abstracts

### Gene Expression Profiling of Drug-Resistant Cell Lines to Identify New Drug Targets

*William S. Dalton, PhD, MD*

During the past two decades, our laboratory has developed myeloma drug-resistant cell lines by exposing cell lines to gradually increasing drug concentrations resulting in the selection of cell lines with acquired resistance to various drugs. The human myeloma cell lines used for drug selection include RPMI 8226, U266, and H929. Drugs used for selection of drug-resistant cell lines include doxorubicin, mitoxantrone, and melphalan. Over the years, these cell lines have been valuable for elucidating mechanisms of acquired drug resistance and ultimately examining patient specimens to determine the clinical relevance of these mechanisms. Presumably, either preventing or reversing these drug-resistant mechanisms could increase the efficacy of established drugs; and new drugs that are active in drug-resistant cell lines likely have novel targets for activity.

Two new drugs that demonstrate activity in classic drug-resistant cell lines include bortezomib and tipifarnib, among others. More recently, we compared gene expression profiles of cell lines with classical acquired drug resistance with cells that exhibit the CAM-DR phenotype associated with myeloma tumor cell: bone marrow microenvironment. The CAM-DR phenotype is a form of *de novo* drug resistance associated with either physical contact of myeloma cells with bone marrow stromal elements or exposure to soluble factors, such as cytokines, found in the bone marrow microenvironment.

A recent comparison examined the genotypic and phenotypic profiles for melphalan resistance in the isogenic human 8226 myeloma cell line when cells were either selected for acquired resistance or adhered to the bone marrow extracellular matrix, fibronectin (FN). We found that CAM-DR resistance was a transient phenotype that occurred only while cells were adherent and rapidly reversed when cells detached from FN. We have since observed similar phenotypes when other myeloma and leukemia cell lines are adhered to FN or other extracellular matrices that bind via  $\beta 1$  and/or  $\beta 7$  integrins. Comparing GEP from cell lines that are adherent vs. grown in suspension, we are able to gain insight into mechanisms related to *de novo* and acquired drug resistance. For example, the comparison of GEP profiles for 8226 myeloma cell lines with acquired melphalan resistance and 8226 cells adhered to FN has led us to focus on the signaling pathway involving the pro-apoptotic molecule Bim, and the cholesterol synthesis pathways, as possible important pathways for predicting drug response or resistance. In addition, we observed that the FA/BRCA pathway was critical for melphalan response and the increased expression of the FA/BRCA genes caused resistance to melphalan and radiation.



## Abstracts

### Gene Expression Profiling of Drug-Resistant Cell Lines to Identify New Drug Targets

(continued)

Our goal is to describe molecular/biochemical profiles that describe both de novo and acquired drug resistance in cell lines and then test these profiles using patient specimens. We currently are testing several myeloma cell lines with different genetic backgrounds, especially as they relate to Ras mutations, integrin expression, and mutations and overexpression of c-maf. We will be analyzing these cell lines for similarities and differences in GEP for de novo and acquired drug resistance, focusing on melphalan, topoisomerase II inhibitors, dexamethasone, and eventually new agents bortezomib and tipifarnib. At all times we will be comparing GEP with *ex vivo* drug sensitivity testing results (hence the term molecular/biochemical profiles). We will use this iterative process, first using different myeloma cell lines and then analyzing results from patient specimens, to develop molecular signatures for treatment response. Ultimately, we will analyze GEP (with and without adhesion) and *ex vivo* drug testing of patients enrolled in clinical trials to predict treatment response and time-to-treatment failure.

*Submitted by William S. Dalton, PhD, MD, H. Lee Moffitt Cancer Center & Research Institute, at the University of South Florida, Tampa, Florida, USA, on behalf of Lori Hazlehurst, PhD, and William S. Dalton, PhD, MD*



## Abstracts

### Cell Cycle as a Molecular Target for Multiple Myeloma

*Selina Chen-Kiang, PhD*

Multiple myeloma (MM), the second most common hematopoietic cancer, ultimately becomes refractory to treatment when self-renewing MM cells begin unrestrained proliferation by unknown mechanisms. In contrast to the extensive effort in understanding the loss of apoptotic control, the mechanism of cell cycle dysregulation remains to be defined. Here one, but not more than one, of the three early G1 D cyclins is elevated in each case of MM. Cyclin D1 or D3 expression does not vary in the clinical course, but that alone is insufficient to promote cell cycle progression unless Cdk4 is also elevated, in the absence of Cdk6, to phosphorylate the retinoblastoma protein Rb. By contrast, cyclin D2 and Cdk6 are coordinately increased, thereby overriding the inhibition by Cdk inhibitors, p18<sup>INK4c</sup> and p27<sup>Kip1</sup>, and phosphorylating Rb in conjunction with the existing Cdk4. Thus, cyclin D1 pairs exclusively with Cdk4 and Cdk6 pairs only with cyclin D2, although cyclin D2 can also pair with Cdk4 in MM cells. The basis for this novel and specific Cdk-D cyclin pairing lies in differential transcriptional activation. In addition, cyclin D1 or D3 expressing MM cells are uniformly distributed in the bone marrow, whereas Cdk6-specific phosphorylation of Rb occurs in discrete foci of bone marrow MM cells before proliferation early in the clinical course, then is heightened with proliferation and disease progression. Mutually exclusive Cdk4-cyclin D1 and Cdk6-cyclin D2 pairing, therefore, is likely to be a critical determinant for cell cycle reentry and progression and may play a pivotal role in the expansion of self-renewing MM cells.

Our findings suggest that myeloma may be sub-typed according to the cyclin D1-Cdk4 and cyclin D2-Cdk6 pairing. The prognostic value of our findings as well as the development of small molecule Cdk inhibitors for myeloma will be discussed.

*Submitted by Selina Chen-Kiang, PhD, Weill-Cornell Medical College, New York, New York, USA*



# Session II: Animal Models for Compound Validation in Myeloma

Session Chair:  
Kenneth C. Anderson, MD

SESSION II



## Abstracts

### Validating Compounds for Multiple Myeloma: Utility of the SCID-hu Model

Joshua Epstein, DSc

Our appreciation of the effects of tumor cell interactions with their microenvironment on their survival and their susceptibility to therapeutic agents has come to the forefront of tumor biology and therapy. Intuitively, primary human tumor cells that grow in their natural microenvironment and produce the typical disease manifestations would constitute the most relevant system for validating potential therapeutics. The SCID-hu model, in which primary human myeloma cells grow in a human bone marrow microenvironment and produce typical myeloma manifestations, encompasses all these characteristics. This model helped clarify the intimate, reciprocal relationships between myeloma cells and bone active cells and demonstrated a clear biological difference between classical, medullary myeloma and advanced myeloma with extramedullary growth, a disease state from which most myeloma cell lines have been derived. These biological differences translated to differential responses to inhibitors of bone resorption. The SCID-hu model also helped demonstrate unequivocally that in order to exert its antimyeloma activity, thalidomide must be metabolized, a finding not popular at the time but widely accepted now. Yet, even such a seemingly 'ideal' model is fraught with limitations, inherent and adopted. Among the inherent limitations, common to all murine models, is the difference in metabolism and metabolic rates between mice and humans. In all xenograft models, the interactions between the human tumor cells and the microenvironment are different than in the patients, potentially leading to different responses to agents. Even in the SCID-hu model where the tumor cells grow in a human microenvironment, the systemic contribution of the murine host differs from that of the patient, potentially altering the effectiveness of various agents.

Other issues, relevant only to *in vivo* validation models, need to be addressed: Should one start treatment with a tested drug once tumor has been established, reflecting the clinical situation where diagnosis preceded treatment, or, since introduction of millions of tumor cells amounts to instantaneous establishment of a tumor, should treatment start with administration of tumor cells? Such an approach will more closely emulate the metastatic process, associated with tumor progression and reduced life expectancy. How should response be evaluated? Unlike patients, in model systems treatment is compared with a control condition. Therefore, response can be seen as cure, or more often as less aggressive tumor progression, i.e., slower increase in tumor burden. Such observations cannot be made in individual patients where only net reduction in tumor burden or manifestation is considered. Therefore, extrapolation from the model to the clinic certainly is not a straight line, as an agent's efficacy or lack of it in the model does not necessarily predict similar results in the clinic. While *in vitro* and *in vivo* model systems provide a platform for studying the biology of tumors and for developing and testing agents that interfere with processes critical for the survival of tumor cells, the clinical efficacy of the successful agents may have very little to do with their intended mechanism or with their effects in model systems, as has been experienced by all. From the drug development point of view, if an agent shows activity in a tumor model, the result may encourage further development; but, if it does not work in a 'validation' model system, should it no longer be considered for clinical use?

Submitted by Joshua Epstein, DSc, University of Arkansas for Medical Sciences, College of Medicine, Little Rock, Arkansas, USA



## Abstracts

### **Animal Models for Compound Validation in Myeloma**

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*Constantine S. Mitsiades, MD, PhD*

The evaluation of safety and efficacy of novel candidate drugs in tumor-bearing animal models is a critical component of pre-clinical development of new therapeutics for multiple myeloma (MM), and human neoplasias, more generally. In this presentation, we will review our experience on the technical features of various *in vivo* models that we have applied in our Center towards the goal of evaluating the anti-MM properties of novel therapeutics. In particular, we will discuss the properties and examples of application of models of diffuse GFP+ and/or Luc+ MM bone lesions in SCID/NOD mice, as well as the SCID-hu model. The imaging modalities and biochemical assays that are useful for monitoring the disease burden in these models will be discussed. Particular emphasis will also be placed on the relative merits, as well as limitations, of these *in vivo* models, as well as on approaches to address the latter, through parallel use of models with mutually complementing features. Finally, we will discuss the relative role of these models in providing insight into the pathophysiology of MM, particularly the interaction of its tumor cells with the bone marrow microenvironment, and how such studies can inform the process of target identification and compound validation for MM.

*Submitted by Constantine S. Mitsiades, MD, PhD, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA*



## Abstracts

### The Murine 5TMM Model in Myeloma Drug Discovery

Karin Vanderkerken, PRD

The murine 5TMM models were initially developed by J. Radl. Multiple myeloma (MM) developed spontaneously in 0.5% of mice (of the inbred strain C57BL/KaLwRij) older than 2 years. The MM cells were localized in the bone marrow (BM) of the mice and the serum paraprotein concentration correlated well with the development of the disease. The latter was associated with a decreased concentration of normal polyclonal immunoglobulins. The primary diseased BM was intravenously transplanted into young syngeneic animals and by doing so several *in vivo* growing 5TMM lines were developed, each with its own characteristics. The 5TMM model hereby belongs to the *de novo* myelomas and its clinical characteristics resemble the human disease closely: the tumor cells are located in the bone marrow, the serum paraprotein concentration is a measure of disease development, neovascularization is increased (this was determined for 5T2MM and 5T33MM), and in certain lines a clear osteolytic bone disease develops. All the original 5TMM models are maintained and propagated *in vivo*.

The 5T2MM model best represents human MM, with a moderate growth and the development of osteolytic bone lesions. These osteolytic lesions are associated with a decrease in cancellous bone volume, decreased bone mineral density and increased numbers of osteoclasts. The 5T33MM model has a more rapid tumor take.

The 5T2 and 5T33MM models have been extensively characterized. Specific monoclonal antibodies have been raised against the idiotype of both 5T2 and 5T33MM allowing the detection, with great sensitivity, of the serum paraprotein by ELISA and the specific staining of the tumor cells both by FACS analysis and immunostaining of histological sections. The sequence analysis of the V<sub>H</sub> gene enables the detection of cells by RT-PCR and Northern blot analysis.

The 5TMM models can be used for both *in vitro* and *in vivo* experiments. The specific antibodies allow the separation of MM cells by flow cytometry or with magnetic beads, generating pure MM cell populations for further *in vitro* investigation. The 5TMM models generate a typical MM disease and different methods are available to assess tumor load in the bone marrow, serum paraprotein concentrations, bone marrow angiogenesis (by measuring the microvessel density) and osteolytic bone lesions (by a combination of radiography, densitometry, and histomorphometry). The investigation of these latter parameters allows the use of the 5TMM models in the study of the biology of myeloma in a complete syngeneic microenvironment and allows the use of the model in preclinical testing. Mice can be treated either from injection with the tumor cells on or after onset of the disease (as measured by the presence of detectable serum paraprotein). Compounds such as osteoprotegerin, zoledronic acid, broad spectrum matrix metalloproteinase inhibitors, picropodophyllin (= IGF-1R inhibitor) and VEGFR inhibitors have been tested successfully in the 5TMM models.

Submitted by Karin Vanderkerken, PRD, Vrije Universiteit Brussel, Brussels, Belgium



## Abstracts

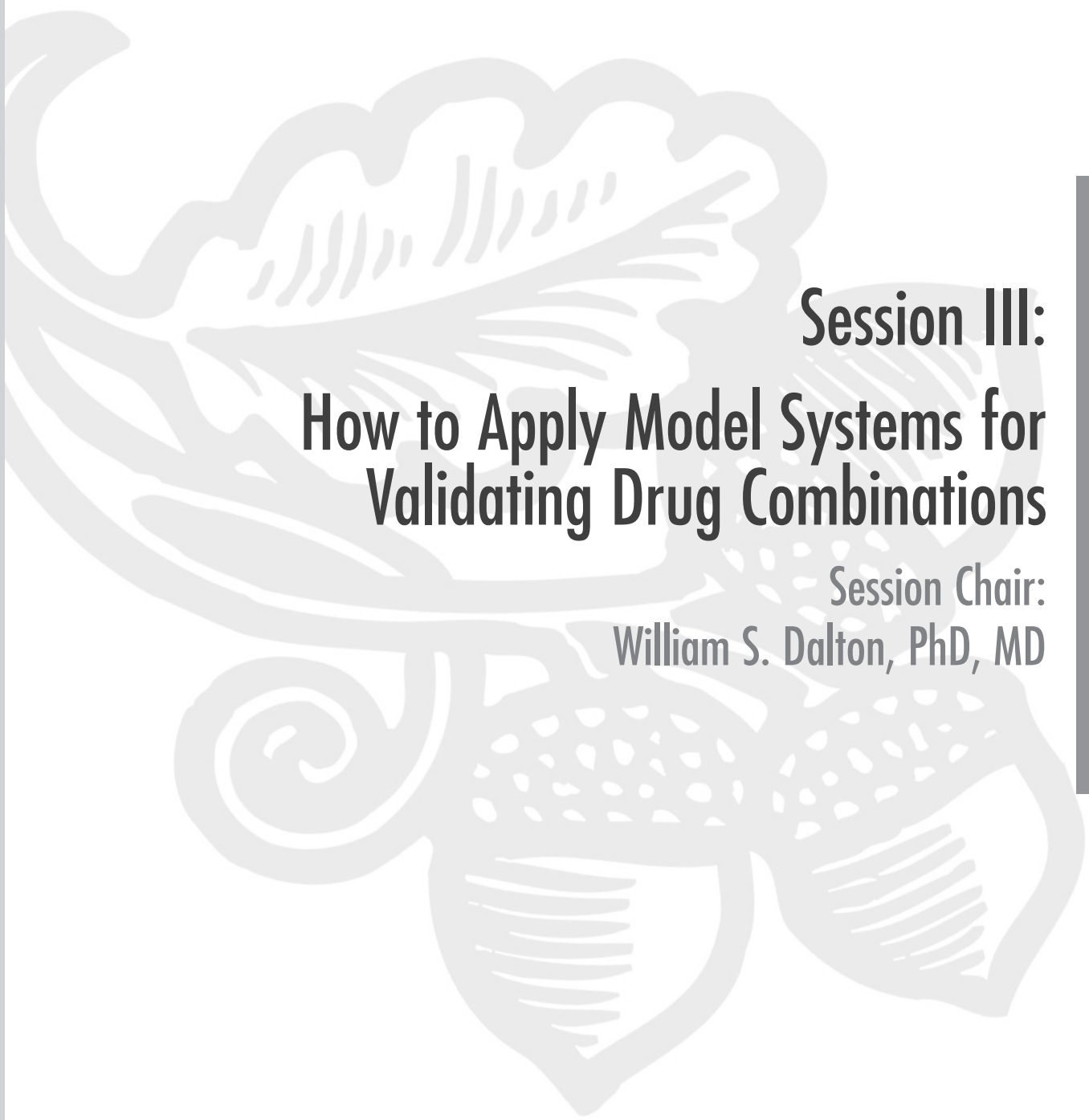
### New Targets and Strategies for Treating Multiple Myeloma

*James R. Berenson, MD*

We have generated new murine models of human multiple myeloma (MM) tumors for pathophysiological studies and rapid evaluation of new anti-myeloma therapies. Fresh whole core bone marrow (BM) biopsies taken from MM patients, or peripheral blood from plasma cell leukemia (PCL) patients, were engrafted into the hind limb muscle of SCID mice. The resulting tumors produced human monoclonal IgG (hIgG) and displayed the features of human MM, including morphology, immunophenotype, and BM plasmacytosis. Following intramuscular passage, we generated large numbers of mice with predictable increases in tumor growth and human paraprotein levels. We have used these models to determine the anti-myeloma effects of traditional and novel anti-myeloma agents, alone and in combination. In our first model, LAG $\lambda$ -1, we found that melphalan did not significantly alter tumor growth, except minimally at high doses, reflecting the resistance of this patient's tumor to this drug. In contrast, paraprotein secretion was reduced in LAG $\lambda$ -1-bearing mice receiving clinically relevant doses of arsenic trioxide (ATO) or bortezomib. Importantly, combinations of ATO and bortezomib, or ATO, vitamin C, and melphalan, significantly inhibited tumor growth and hIgG secretion at doses that were ineffective when used singly. We also used this model to optimize the dosing schedule of liposomal doxorubicin. Low doses administered once daily three days per week decreased tumor growth and hIgG levels, whereas much higher doses given once weekly had no antimyeloma effects. We are also using these SCID-hu models to evaluate novel agents with potential anti-MM activity. We have found that a newly developed histone deacetylase inhibitor reduced MM tumor growth and hIgG production at both doses tested and that an inhibitor of superoxide dismutase 1 re-sensitized a bortezomib-resistant SCID-hu model, LAG $\kappa$ -1B, to bortezomib treatment.

We identified a new molecular target for antimyeloma therapy, pleiotrophin (PTN), an 18 kD heparin-binding protein, known to promote cellular proliferation and angiogenesis. PTN levels have been found to be elevated in some solid tumors, where constitutive expression promotes tumor growth, vascularization and metastasis, but it has not been previously evaluated in hematologic malignancies. We have found that serum PTN levels were higher in MM patients than in normal age-matched controls, and expressed at intermediate levels in monoclonal gammopathy of undetermined significance (MGUS) patients. Serum PTN levels increased at the time of disease progression; conversely, patients who responded to anti-MM therapy exhibited significant decreases of PTN as compared with their pre-treatment levels. In the BM of myeloma patients, PTN is expressed by the malignant cells. Using a polyclonal anti-PTN antibody in our LAG $\lambda$ -1 myeloma model, we found marked decreases in tumor growth. We have also shown that PTN promotes tumor-directed angiogenesis by a novel mechanism: transdifferentiation of monocytes into vascular endothelial cells. Co-culture of the monocytic cell line THP-1 with MM cells or the serum from MM patients induces the expression of the vascular endothelial genes Tie-2, Flk-1 and von Willebrand's factor (vWF) in these cells; the addition of anti-PTN antibody abrogates this effect. We confirmed these results by co-culturing CD14+ peripheral monocytes from normal donors on collagen I with MCSF, VEGF, and PTN. In the presence of PTN these cells express endothelial genes and form tube-like arrays *in vitro*. When GFP-marked THP-1 cells were co-transplanted with MM cells into SCID mice, GFP+ tubes carrying red blood cells were found within the MM tumors. We have identified the PTN receptors on monocytes and MM cells and are currently defining the signaling pathways leading to transdifferentiation and MM tumor growth. Our findings identify PTN as a potential new molecular target to inhibit tumor growth and angiogenesis in MM patients and should have profound clinical implications.

*Submitted by James R. Berenson, MD, Institute for Myeloma and Bone Cancer Research, West Hollywood, California, USA*



# Session III: How to Apply Model Systems for Validating Drug Combinations

Session Chair:  
William S. Dalton, PhD, MD

SESSION III



## Abstracts

### High-Throughput Drug Screening

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*Said M. Sebti, PhD*

Completion of the human genome project is truly a major scientific milestone of the century that has far-reaching implications for the treatment of human disease. However, in order to translate this genome-based knowledge into health benefits, one needs to understand gene product function (proteomics) and to identify chemical probes/small molecules to manipulate protein function in accurate and predictable ways. One important way to identify chemical probes and, ultimately, drug candidates is to use high throughput screening (HTS) to evaluate chemical libraries against molecular targets. HTS results in hits that through medicinal chemistry and lead optimization result in drug candidates for clinical developments. Thus, HTS meets the scientific need to identify novel anticancer drugs. HTS efforts at the Moffitt Cancer Center resulted in the successful identification of chemical probes for many molecular targets such as Akt, PDGF, VEGF, GGTase I, mdm2/p53, BclXL/Bax, Rb/Raf, STAT3, SHP2, and Aurora. Some of these are in advanced preclinical trials and one, an Akt inhibitor, is undergoing hypothesis-driven clinical trials.

*Submitted by Said M. Sebti, PhD, H. Lee Moffitt Cancer Center & Research Institute at the University of South Florida, Tampa, Florida, USA*



## Abstracts

### High-Throughput Drug Screening

*James Bradner, MD*

Advances in ligand discovery have improved the efficiency of drug development. Among the many remarkable, recent improvements in discovery technologies, high-throughput screening (HTS) has most favorably affected the process of small molecule discovery and target validation. Though primarily the focus of the pharmaceutical industry, approaches to HTS are an emerging feature of academic research and a critical component of academic and governmental scientific infrastructure. These technologies rely on automation, curated libraries, robust biological assays, and advanced biostatistics to test the activity of millions of compounds in a screening run. HTS methodologies are increasingly diverse, comprising multiple platform capabilities and accommodating a myriad of scientific questions. Our Institute has developed an efficient, academic HTS platform designed for interfacing with the academic research expert in a relevant area of cell biology. Through these collaborations, tool compounds are identified that catalyze our fundamental understanding of cell and disease biology and serve as leads for pharmaceutical discovery.

*Submitted by James Bradner, MD, Broad Chemical Biology Program, Broad Institute of Harvard and MIT, Dana-Farber Cancer Institute, Boston, Massachusetts, USA*



## Abstracts

### **Activity of IPI-504, a Water-Soluble Hsp90 Inhibitor, Combined with Bortezomib in a Human Multiple Myeloma Xenograft Model**

*Margaret A. Read, PhD*

IPI-504 is a novel chemical reduction product of 17-AAG, a well-characterized Hsp90 inhibitor. Unlike 17-AAG, which suffers from poor aqueous solubility, IPI-504 exists as a hydrochloride salt which is highly water soluble. *In vivo*, IPI-504 inter-converts with 17-AAG and exists in a pH and enzyme-mediated dynamic redox equilibrium. Thus, IPI-504 recapitulates the Hsp90 inhibitory properties and biological activity of 17-AAG without its formulation liabilities. IPI-504 has demonstrated *in vitro* and *in vivo* anti-tumor effects in a variety of cancers including xenograft and orthotopic models of multiple myeloma (MM), and is currently in a phase 1 dose-escalation trial in patients with relapsed/refractory MM. A preclinical study was conducted to evaluate activity of IPI-504 in combination with agents that are active in MM, including the proteasome inhibitor bortezomib. Both IPI-504 and bortezomib are known to impact cellular protein homeostasis, and they showed robust synergistic cytotoxic activity *in vitro* against MM cell lines. These data provided rationale for the combination of the two agents in an *in vivo* study to evaluate efficacy and pharmacodynamic markers of Hsp90 and proteasome inhibition.

RPMI-8226 human MM cells were implanted as subcutaneous xenografts in NOD/SCID mice and animals were randomized into the following treatment groups once tumors reached approximately 100 mm<sup>3</sup>: vehicle, 75mg/kg IPI-504, 0.25mg/kg bortezomib, combination of IPI-504 with bortezomib. All mice were dosed by intravenous injection 2x/week for up to 4 weeks. No significant body weight loss was seen in any groups. Administration of IPI-504 and bortezomib at these sub-effective concentrations resulted in tumor growth inhibition of 54%-56% and 10%-56%, respectively. The combination of IPI-504 and bortezomib resulted in tumor growth inhibition that was significantly greater than with either agent alone, ranging from 77% to 100%. Hsp70 levels in tumors increased in the IPI-504 treated groups, indicating inhibition of Hsp90, and levels of 20S proteasome inhibition were consistent with the bortezomib doses used. Plasma M protein levels decreased relative to decreases in tumor volume. Thus, destabilization of Hsp90 client proteins with IPI-504 while blocking their degradation with the proteasome inhibitor bortezomib can result in inhibition of tumor growth significantly more than either agent alone. These data provide rationale for clinical evaluation of these agents in combination.

*Submitted by Margaret A. Read, PhD, Infinity Pharmaceuticals, Inc., Cambridge, Massachusetts, USA, on behalf of MM Pink, CS Pien, VT Travaglione, E Normant, J Patterson, JK Tong, C Fritz, V Palombella, J Adams, and M Read*



## Abstracts

### **The Pediatric Preclinical Testing Program (PPTP): A Model for Drug Development in Rare Malignant Diseases**

*Peter J. Houghton, PhD*

In the U.S., approximately 12,500 new cases of cancer are reported annually in patients under the age of 21. Of these, it is estimated that 70% will be cured by current multimodality therapies. In those patients who ultimately succumb to their disease, the initial response to therapy is often very good, thus precluding most patients from experimental treatments at diagnosis. Consequently, the pool of patients eligible for experimental phase I/II therapies is small, restricting the number of new drugs that can be evaluated; further, this population may be highly resistant to chemotherapeutic agents and hence fail to identify agents that may be highly active at diagnosis. Thus, there are similarities between childhood cancer and multiple myeloma in terms of the challenges presented in identifying new effective therapies, or prioritizing the more than 400 anticancer entities currently in development.

The PPTP is an attempt to establish a preclinical program that will identify new agents that have significant activity in appropriate models of childhood solid tumors and acute lymphocytic leukemia. The program is based on extensive data generated in several laboratories that show that xenograft models of particular childhood cancers accurately identify those agents known to be active in the clinical disease, and prospectively identify agents that have significant clinical activity as single agents, as well as useful drug combinations.

A total of 58 *in vivo* models have been established that include panels of neuroblastomas, soft tissue and bony sarcomas, glioblastoma, non-GBM brain tumors, kidney tumors, and ALL. Tumor models were selected, in part, because their global expression profiles were similar to those determined in clinical specimens of the same histology, or where direct comparison with the patient tumor of origin showed very few changes in expression when tissue was heterografted into SCID mice. These tumor models are being characterized by expression profiling (Affymetrix U133), by CGH and SNP analysis, and proteomics approaches to create databases that will be linked to chemosensitivity profiles. In addition, 24 cell lines have been established for *in vitro* testing of single agents and drug combinations.

*Submitted by Peter J. Houghton, PhD, Dept. Molecular Pharmacology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA*







