

**U54 NCI Drug Resistance and Sensitivity Center (DRSC):
Mayo Clinic Arizona/Rochester and U. Minnesota**

Information for Potential Administrative Supplement Collaborators

Supplement Funding Announcement released April 11, 2018: Administrative Supplements to NCI Grant and Cooperative Agreement Awards to Support Collaborations with the Drug Resistance and Sensitivity Network (DRSN) (PAR-18-752): <https://grants.nih.gov/grants/guide/pa-files/PAR-18-752.html>.

Section 1: Mayo Clinic Arizona/Rochester and U. Minnesota: Current DRSN-related research projects

Overarching DRSC Study Title: Overcoming Drug Resistance in Multiple Myeloma	
Primary Contact for Collaborative Supplement Inquiries	
Name/Title:	A. Keith Stewart, M.B., Ch.B., Program Director
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Research Project 1 Title:	
High throughput drug screening and correlations with mutational status in myeloma cell lines and patient samples	
Project 1 Summary:	
<p>Despite recent advances in therapy the majority of multiple myeloma (MM) patients are not cured but rather suffer from a chronic relapsing, yet ultimately fatal disease. Two challenges immediately become evident. Most urgent is the need to find alternative therapies for patients who fail existing potent drug classes. Second, is to understand why patients may be resistant in the first place and to seek methodologies, dosing strategies and new drug combinations which can prevent or overcome drug-resistant relapse.</p> <p>We propose an innovative strategy of "direct to drug" screening of 500 primary patient samples with chemogenomic interrogation which addresses both of these two big questions. Our hypothesis is that a direct to drug analysis of individual patients will improve response rates, lower unnecessary toxicity and reduce drug costs through identification of the most effective combinations of FDA approved drugs for each patient. This strategy will also, for the purposes of this proposal, provide a database of samples and clinical data sets from which to explore genomic or clinical correlates of drug sensitivity and resistance.</p> <p>Our goal will be attained through the successful pursuit of three specific aims, building upon extensive cell line and primary patient sample data. First, we will measure the in vitro sensitivity of 79 MM therapeutics including CTEP compounds in 500 primary myeloma patient samples. Second, we will conduct combination screens to seek synergistic combinations of IMiDs and proteasome inhibitors as base compounds with other active MM therapeutics such as bromodomain inhibitors which can be tested in animal models such as the human CRBN mouse in project 2. Third, using our M3P mutation panels, we will conduct a chemogenomic interrogation to examine specific correlates of drug sensitivity and resistance utilizing pre-treatment and surviving cells from primary patient robotic screens. These studies will determine the frequency of specific genetic mutation or cellular subsets resistant to the most active drugs or drug combinations used in MM therapy and will be shared throughout the program for further epigenetic and transcriptional analysis and bi-directional feedback derived from both Projects 2 and 3.</p> <p>Critical to the current U54 application this strategy will provide a database of 500 multiple myeloma samples and linked clinical data sets which together build a mosaic of drug sensitivity and clinical phenotype from which all elements of this program grant can exploit to explore genomic or clinical correlates of drug sensitivity and resistance.</p>	

Project 1 scientific assays and models used:

1. Drug sensitivity assay using cytotoxicity endpoint for MM 70 drug panel in Human Myeloma Cell Lines (HMCLs) and primary myeloma patient cells
2. High throughput drug screening as single agents and combinations
3. MM Mutation panels containing markers of drug resistance to IMiDs and a proteasome inhibitor resistance gene panel
4. Database of samples and clinical data sets from which to explore genomic, proteomic, or clinical correlates of drug response

Project 1 Lead

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Research Project 2 Title:

IMiD Resistance in Patients and Humanized Mice with Multiple Myeloma

Project 2 Summary:

Thalidomide, Lenalidomide and Pomalidomide (IMiDs) mediate their anti-myeloma effect by binding to human (but not mouse) CRBN, leading to degradation of IKZF1/3. We propose to study IMiD resistance by analyzing serial primary patient samples from patients before, and after the development of IMiD resistance, using a large panel of genetically characterized human myeloma cell lines with differential sensitivity to IMiDs, and using a humanized mouse model of multiple myeloma, hCRBN Vk*MYC, in which hCRBN, but not mCRBN, is expressed.

Project 2 scientific assays and models used:

1. CD138-selected BM cells from patients before and after development of resistance to IMiDs will be analyzed by RNAseq, and NGS for identification of acquired mutations (SNV, CNA, and SV)
2. A large panel of human MM cell lines will be characterized for IMiD sensitivity, IMiD-induced modulation of key regulatory factors (E.g., IKZF1/3, MYC, IRF4) and IMiD-induced changes of enhancer structure and function
3. hCRBN Vk*MYC MM transplantable cell lines will be engrafted into various C57Bl/6 genetic backgrounds to study the contribution of various host elements to IMiD-induced anti-MM effects.

Project 2 Lead

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Research Project 3 Title:

Modeling Proteasome Inhibitor Response and Resistance in Cell Lines and Patient Samples with Single Cell Analysis of Subpopulations

Project 3 Summary:

Proteasome inhibitors form the backbone of current treatment approaches in multiple myeloma (MM). Wide inter-individual variation in response to proteasome inhibitors (PIs) is a major limitation in achieving consistent therapeutic effect in MM. Such heterogeneity in the depth and duration of response to treatment is governed in large part by the underlying molecular characteristics of the tumor, including differences in the expression of genes involved in mechanisms of chemo-resistance; although germline variations in patients may also impact response as well as toxicities due to effects on drug pharmacodynamics and pharmacokinetics. Deciphering variations (sequence, expression, proteome) leading to the variation in sensitivity to chemotherapy is therefore essential to predict effective response or resistance to one of the major front line therapeutic approaches in myeloma. There is certainly tumor heterogeneity among patients that impacts response, but as we and others have observed, resistance may emerge by selective expansion of resistant sub-clones.

The central hypotheses we will address are: 1) Genetic and phenotypic signatures can be identified within myeloma tumors that will predict the depth and duration of response to PIs; 2) Tumor and germline sequence variations can be identified that are associated with toxicities precluding adequate therapy; 3) Myeloma tumors show selective clonal evolution that can lead to emerging resistance that will be predicted through single cell analyses of individual patient tumors; and 4) Identification of the responsible genes will allow therapeutic targeting of the resistance factors and thus recapturing or enhancing the efficacy of PIs.

Project 3 scientific assays and models used:

Assays: Cytotoxic profiles to therapeutic drugs; gene expression profiling; single cell transcriptomics; targeted analysis of genetic variation (SNPS); CyTOF; CRISPR gene editing.

Models: Myeloma cell lines; myeloma primary patient samples; bone marrow biopsies

Project 3 Lead

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Section 2: DRSC information for a potential collaborative supplement study**Types of assays, technologies, or model systems that our DRSC would be willing to utilize and/or share with other researchers in cancer drug resistance, who might be a recipient of a DRSN supplement award:**

Project 1: Myeloma gene mutation panel profiling; profiling of new experimental drugs in HMCLs and primary MM patient cells; profiling of primary cells in our drug panel, IMiD and Proteasome inhibitor sensitive and resistant cell lines, database of drug sensitivity and resistance across MM and lymphoma cell lines

Project 2: IMiD sensitive and resistant MM cell lines, hCRBN Vk*MYC MM mice and transplantable cell lines (when complete, currently not fully generated)

Project 3: CyTOF analyses; gene panels for variant detection; single cell profiling

Our DRSC limits to collaborative interactions or assistance to supplement awardees:

Primary patient samples are not available retrospectively but can in certain circumstances be obtained or accepted prospectively for collaborative studies. DNA and RNA from primary patient materials may be available but not always. Currently the hCRBN Vk*MYC mice are not complete.

Optimal year(s) for a collaborative supplement study with our DRSC (i.e., 2018, 2019, 2020, 2021, 2022):

2019 onwards

Otherwise, any year would be acceptable with our DRSC, which is preferred by NCI to allow more flexibility for supplement studies:

Any

Suggestions to potential supplement applicants:

Studies which involve novel investigational agents of promise in Myeloma, studies involving primary human materials are of highest interest