de Gunzburg Myeloma Research Fund
Dana-Farber Cancer Institute
Interim Report on Milestones Achieved
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In 2012, the de Gunzburg Myeloma Research Foundation established the de Gunzburg Myeloma Research Fund at Dana-Farber Cancer Institute to further myeloma research at the Jerome Lipper Center for Multiple Myeloma. The Fund provides essential resources for research into drug resistance, including the characterization of the features of myeloma cells that develop resistance, the development of novel therapeutics to overcome resistance, and the use of combination therapies.

Since our last report in December 2013, Paul Richardson, MD, and Constantine Mitsiades, MD, PhD, continued to engage in a comprehensive and close collaborative effort with a singular goal—to improve the overall outcome of myeloma patients. Below describes progress made over the past six months toward the identified Specific Aims and Anticipated Milestones/Timelines:

Specific Aim 1: To define in detail the molecular lesions that allow MM cells to become clinically aggressive and resistant to existing therapies.

With the support of the de Gunzburg Foundation, the Mitsiades laboratory advances efforts to define which molecular lesions allow myeloma cells to become clinically aggressive and resistant to existing therapies, and potentially others that are being tested clinically and could eventually become part of the standard therapeutic armamentarium.

During the first year of de Gunzburg Foundation funding, the Mitsiades laboratory applied in its studies a model of myeloma cell growth within bone-like scaffolds implanted in immunocompromised mice. This system allowed researchers to achieve engraftment and expansion of patient-derived myeloma cells from advanced cases of the disease, including plasma cell leukemia and myelomatous pleural effusion. In addition, Dr. Mitsiades' team continues to discern the molecular features of MM cells, before and after their proliferation in mice, using next-generation sequencing. Specifically, they analyze such samples using whole-exome sequencing, which examines the coding portions of genes to detect rare mutations, to target more than 230,000 regions of the genome in each sample. This model provides a powerful platform for scientists to evaluate the molecular and functional characteristics of patient-derived myeloma cells.

Over the past six months, Dr. Mitsiades used the results of these studies to examine molecular lesions that could represent "progression events" that drive MM growth. He placed particular emphasis on molecular pathways that are enriched for genes with mutations, deletions, or amplifications that are present more frequently in samples derived from MM patients after they become resistant to a given treatment compared to earlier disease stages.

Dr. Mitsiades has now begun examining whether these progression events are treatment-specific or if they are influenced by the underlying genetic makeup of the MM cells at the time of exposure to a particular treatment. For example, he treated myeloma cells from a mouse model with established anti-MM drugs, including pomalidomide (a thalidomide derivative) and alkylating agents such melphalan, to determine which molecular defects are selectively enriched in myeloma cells after they

become resistant to each of these treatments. Researchers are now identifying lesions for functional studies, which will determine specific and actionable therapeutic directions that may help reverse resistance.

Building on the progress achieved during the first year of de Gunzburg Foundation funding, Dr. Mitsiades' laboratory continues to develop additional molecular tools required for the customized engineering of myeloma cells with defined molecular characteristics. This approach generates panels of MM cell lines that match the genetic features in MM cell lines from patients who have developed resistance to conventional therapies.

This effort addresses several longstanding issues in the field of myeloma research. Due to the complexity of the genome of MM tumor cells, for example, several molecular defects associated with high-risk disease have not been comprehensively characterized in terms of their specific contribution to drug resistance. Moreover, molecular lesions associated with high-risk disease often co-occur in patients, but it has not been clearly established whether simultaneous targeting of these lesions is necessary to achieve improved clinical responses. Notably, many of these patterns of co-occurring molecular lesions are not represented in myeloma cells available to the Mitsiades laboratory.

Because many of the cell lines available for myeloma research were generated from patients before the introduction of thalidomide and proteasome inhibitors, there is concern that existing panels of cell lines do not harbor the molecular features conducive to understanding drug-resistance mechanisms. With the support of the de Gunzburg Foundation, Dr. Mitsiades has generated, over the past six months, MM cell lines in which critical molecular lesions have been introduced. The goal is to compare the functional properties of these cells with the parental cell lines in which these lesions are absent.

For instance, the Mitsiades laboratory introduces in myeloma cells mutated versions of specific tumor suppressor genes that are frequently altered in myeloma and other cancers. In addition, they developed "inducible" constructs, which allow scientists to gradually increase or decrease the levels of molecules frequently targeted by dysregulated gene expression. Dr. Mitsiades and his colleagues currently are evaluating one such variant cell line to document its role in conferring resistance to established or investigational therapies. They also are altering its response to immune effector therapies, which mount an immune response against the myeloma cells. Later this year, Dr. Mitsiades will generate additional mutant variants, and efforts will be geared toward functionally characterizing specific complex phenotypes identified from the laboratory's molecular profiling studies.

Specific Aim 2: To serially test in the laboratory the response of primary MM cells isolated from patients to extended panels of investigational agents and combinations with conventional ones.

The Mitsiades laboratory has extensive experience with high-throughput testing of the anti-myeloma activity of investigational drugs. Funding from the de Gunzburg Foundation enabled Dr. Mitsiades to enhance the laboratory's capability to perform these studies with small numbers of cells. These studies are conducted in preparation for pilot screens, which allow scientists to test both myeloma cell lines and patient-derived myeloma cells for their response to a library of Food and Drug Administration-approved anti-cancer therapies, as well as their combinations with other agents, including investigational drugs.

In addition, Dr. Mitsiades and his team optimized these screens in the context of two- and three-dimensional cell cultures that mimic natural tissues, as well as in the presence of nonmalignant cells

in the bone marrow microenvironment, including bone marrow stromal cells. Scientists in the laboratory will build on this progress and advance these studies in the latter part of this year and beyond, with the goal of identifying drugs that are highly potent against myeloma cells harboring specific combinations of molecular lesions associated with advanced disease.

Specific Aim 3: To develop a comprehensive system of bioinformatic and computational support for the Mitsiades laboratory that will facilitate the analysis of the volumes of data that result.

Generous funding from the de Gunzburg Foundation enabled Dr. Mitsiades to advance his laboratory's computational abilities toward building a comprehensive bioinformatics and computational support system. This infrastructure facilitates the analysis of high volumes of data, such as those derived from molecular analyses of cell samples. The Mitsiades laboratory has obtained access to institutional servers on which computational analyses can be conducted. Also, members of the Mitsiades laboratory have, in the past six months, developed additional in-house storage servers and implemented a new desktop system for analyzing large volumes of computational data. Dr. Mitsiades and his colleagues will use this system to increase the laboratory's volume of molecular profiling analyses.

Specific Aim 4: To devote a clinical research team to design and conduct trials of the most promising therapies being preclinically tested in the laboratory to develop new drugs or combinations of drugs specifically designed to overcome resistance.

Currently, more than 20 clinical trials at Dana-Farber focus on early- and advanced-stage myeloma, including studies on smoldering myeloma, newly diagnosed myeloma, early relapse, and relapsed/refractory disease, as well as stem cell transplant studies. Dr. Mitsiades' preclinical research has laid the groundwork for a number of these clinical trials, which, with Dr. Richardson's leadership in clinical research, have been translated rapidly into important advances, including regulatory approval.

Data from the PANORAMA 2 study, a phase II multi-center trial examining the novel combination of bortezomib, dexamethasone, and panobinostat, builds on seminal laboratory work by Dr. Mitsiades, Teru Hideshima, MD, PhD, and others (including chemical biologist James Bradner, MD, and Kenneth Anderson, MD, who as the overall laboratory program leader also provides advice and guidance at multiple different levels) in the area of histone deacetylase inhibition. This novel approach shows remarkable promise for panobinostat in advanced and highly resistant MM, both through the inhibition of aggresomal function as an escape pathway for proteasome inhibition and epigenetic stabilization.

Additional data from the PANORAMA program and specifically the pivotal phase III PANORAMA 1 study will be presented at various meetings this year by Dr. Richardson, who is the US clinical lead and overall senior investigator, and Jesus San Miguel, MD, of the University of Salamanca, who is principal investigator and the European leader. Scientists and clinical investigators alike anticipate that findings from this trial will lead to FDA approval of panobinostat as the first histone deacetylase inhibitor, in combination with bortezomib and dexamethasone, to receive regulatory authorization for relapsed/refractory MM.

Preclinical studies in Dr. Mitsiades' laboratory have also informed clinical trials that combine panobinostat, lenalidomide, bortezomib, and dexamethasone (so-called PAN-RVD). Jacob Laubach,

MD, who is Dr. Richardson's mentee and lead clinical colleague, and Dr. Richardson are conducting a phase I/II trial to determine the appropriate dosage of PAN- RVD and assess its clinical benefit.

In another correlative science effort, Dr. Mitsiades and his team have provided key molecular markers for a prospective phase II trial of RVD with subcutaneous bortezomib administration utilized in newly diagnosed myeloma, which is part of a large collaborative effort with the Myeloma Ireland Consortium and led by Drs. Laubach and Richardson in the United States.

In collaboration with Dr. Mitsiades' laboratory, Drs. Richardson and Laubach are lead investigators of a clinical trial reviewing proteasome inhibitor-related cardiac toxicity in MM, with the hope of developing future studies with molecular correlates. Specifically, Dr. Mitsiades and his colleagues have identified molecular markers used to assess adverse cardiac events caused by this treatment. Proteasome inhibitor toxicity is an important area of myeloma research, with the goal of more broadly enhancing the efficacy of these drugs both in this area and in the setting of neurotoxicity.

As a direct result of translational research conducted by the Mitsiades laboratory, Dr. Richardson and his clinical colleagues are conducting a multi-center phase I clinical trial of the investigational drug PRLX 93936, with or without dexamethasone, for patients with relapsed/refractory MM. In experimental models of MM, Dr. Mitsiades has shown that PRLX 93936 has modest single-agent activity, but in combination has great promise in targeting pathways subserved by N-Ras, a key genetic mutation that becomes more common as myeloma progresses in each patient.

In conjunction with Dr. Bradner, Dr. Mitsiades' laboratory played a critical role in the development of JQ1. This drug, known as a bromodomain inhibitor, will be combined with immunomodulatory-based therapies (specifically, lenalidomide and dexamethasone) in a phase I/II trial that is expected to open later this year for the treatment of relapsed and refractory myeloma under the leadership of Drs. Laubach and Richardson.

Finally, Drs. Richardson, Laubach, and Mitsiades have built a strong partnership and joint project with their colleagues in examining the nature and biology of extramedullary myeloma, a known barrier to cure and a major source of increased mortality in MM. Tissue collection and correlative studies have been a strong part of this work, which hopefully will inform future research and practice.

Thank You

The generous philanthropy of the de Gunzburg Myeloma Research Foundation powers the collaborative research described in this interim report, with the ultimate goal of improving patient outcomes. Thanks to your steadfast support, Dr. Richardson, Dr. Mitsiades, and their outstanding colleagues have achieved significant progress both in the laboratory and the clinic. They are optimistic that this will translate into the exponential acceleration of further productivity in myeloma research over the next few years. We are deeply grateful for your invaluable partnership in making this critically important work possible.