de Gunzburg Myeloma Research Fund
Dana-Farber Cancer Institute
Interim Report on Milestones Achieved
December 2014

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 that myeloma cells develop when they resist existing therapies, as well as the development of novel therapeutics and combination regimens to overcome this resistance and help improve patient outcomes.

Since our last report in June 2014, Paul Richardson, MD, and Constantine Mitsiades, MD, PhD, continued their comprehensive and close collaboration with a singular goal: to improve the overall outcome of myeloma patients. The report below describes their progress during the past six months toward the Specific Aims and Anticipated Milestones/Timelines:

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

With the support of the de Gunzburg Foundation, the Mitsiades laboratory continues to advance its efforts to define which molecular defects allow myeloma cells to become resistant to existing therapies. In addition, the Mitsiades laboratory focuses on other potential therapeutics, many of which are in advanced stages of preclinical evaluation and could become part of the standard therapeutic armamentarium.

During the last six months of de Gunzburg Foundation funding, the Mitsiades laboratory:

- Studied the growth of human myeloma cells within bone-like scaffolds implanted in immunocompromised mice, which allowed researchers to achieve engraftment and expansion of patient-derived myeloma cells.
- Examined the molecular features that MM cells exhibit when they grow in this novel model.
- Analyzed myeloma cells using whole-exome sequencing to examine the coding portions of genes and detect mutations that are enriched in these cells once they develop resistance to certain therapies.
- Evaluated the molecular and functional characteristics of patient-derived myeloma cells and how these characteristics may change once myeloma cells develop resistance to established or investigational therapies.
- Examined which molecular features of MM cells may represent “progression events” and whether these defects confer to MM cells protection from specific treatments, but not others.

- Developed additional molecular tools required to engineer "customized" myeloma cells with defined molecular defects in order to generate panels of MM cell lines that match the genetic anomalies detected in patients who have developed resistance to conventional therapies.

The results of these studies may help investigators create a potential roadmap for targeting the treatment of myeloma patients based on the molecular features of their disease.

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 a broad range of conventional and investigational agents for their activity against myeloma cells. The support of the de Gunzburg Foundation has allowed Dr. Mitsiades and his team to further enhance the capability of the laboratory to conduct these studies for higher numbers of experimental conditions and with small numbers of cells per test.

During the last six months, the Mitsiades laboratory has:

- Conducted pilot screens of new libraries of compounds for their anti-myeloma activity.
- Optimized these assay systems for two- and three-dimensional cell cultures, as well as for the presence of nonmalignant cells in the bone marrow microenvironment, including bone marrow stromal cells.

These optimizations are designed to help the Mitsiades laboratory better mimic the context of natural tissues. In addition, they set the stage for upcoming applications in which these screening capabilities will test Food and Drug Administration-approved anti-cancer therapies, or their combinations with investigational agents, for their ability to kill not only cells lines, but also patient-derived myeloma cells.

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.

The generous funding of the de Gunzburg Foundation has allowed Dr. Mitsiades to enhance the capabilities of his laboratory to conduct wide-ranging bioinformatic studies in a timely manner.

During the last six months, Dr. Mitsiades and his team have:

- Increased their use of in-house storage servers and desktop systems to analyze large volumes of computational data related to activities outlined in this report.
- Applied this system to increase the volume of molecular profiling analyses and other computational studies that are necessary to advances the progress of the laboratory.

This infrastructure enables the analysis of large volumes of data, such as those derived from molecular profiling studies of cell samples.

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.

More than 20 clinical trials that focus on early- and advanced-stage myeloma are currently underway at Dana-Farber. Many of these clinical trials are based on laboratory studies conducted by Dr. Mitsiades:

- 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 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 I study has been presented at various meeting 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. FDA approval of panobinostat as the first histone deacetylase inhibitor, in combination with bortezomib and dexamethasone, for relapsed/refractory MM is hoped for in 2015.

- 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, with promising results to date.

- 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. This is now underway, with patients currently being enrolled.

- 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. They have submitted various papers to the *Journal of Clinical Oncology* and *Blood*, with a landmark meta-analysis previously presented at the American Society of Hematology (ASH) annual meeting in 2013 now provisionally accepted for publication in the *Journal of Clinical Oncology*.

- As a direct result of translational research conducted by the Mitsiades laboratory, Dr. Richardson and his clinical colleagues have completed 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. This study is being presented at the 2014 ASH meeting in San Francisco.

- 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 next 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 relationship and joint project with their colleagues 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, and are now informing future research and practice. With the support of the de Gunzburg Myeloma Research Foundation, a major study is being presented at the 2014 ASH meeting.

Thank You

The generous philanthropy of the de Gunzburg Myeloma Research Foundation drives the collaborative research described in this report. Drs. Richardson, Mitsiades, and their colleagues have made significant progress, both in the laboratory and in the clinic, in their efforts to offer patients safer, more effective therapies. We are grateful for your commitment to our lifesaving mission and for your partnership in making this vital work possible.