



de Gunzburg Myeloma Research Fund
Dana-Farber Cancer Institute
Interim Report on Milestones Achieved
June 2015

In 2012, the de Gunzburg Myeloma Research Foundation established the de Gunzburg Myeloma Research Fund to further myeloma research at Dana-Farber Cancer Institute's 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 December 2014, Constantine Mitsiades, MD, PhD, and Paul Richardson, MD, have continued their close and comprehensive collaboration with the goal to help enhance the overall outcome of multiple myeloma (MM) patients. The success of this collaboration is evidenced by the February 2015 approval by the US Food and Drug Administration (FDA) of panobinostat for the treatment of relapsed/refractory MM. Earlier preclinical studies conducted by Dr. Mitsiades laid much of the groundwork for this approach, and the clinical research efforts of Dr. Richardson, in conjunction with other members of Dana-Farber's Jerome Lipper Multiple Myeloma Center, provided the platform for panobinostat's approval.

Panobinostat, which falls within a category of drugs called HDAC inhibitors, is the first of its kind to receive FDA approval for clinical use in MM patients—providing a new therapeutic option for patients who no longer respond to standard treatment. Nearly a decade ago, Dr. Mitsiades' preclinical studies first demonstrated that broad-spectrum anti-HDAC drugs can augment the response of myeloma cells to proteasome inhibitors (such as bortezomib) or to immunomodulatory thalidomide derivatives. The pivotal randomized phase III clinical trial PANORAMA 1, combining the broad-spectrum HDAC inhibitor panobinostat with bortezomib, validated Dr. Mitsiades' work. Dr. Richardson was senior investigator on the panobinostat trial, which provided the basis for the drug's FDA approval.

The panobinostat approval represents an example of the successful bench-to-bedside translational collaboration between Drs. Mitsiades and Richardson. This most recent development further underscores the significance of the support of the de Gunzburg Foundation to the Mitsiades laboratory, as well as the clinical research project led by Dr. Richardson and his colleagues.

The following report outlines Drs. Mitsiades' and Richardson's 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 generous support of the de Gunzburg Myeloma Research Foundation, the Mitsiades laboratory continues its studies to determine which molecular defects allow myeloma cells to become refractory to currently available therapies or to those that are in advanced stages of preclinical evaluation and could soon become part of the standard therapeutic armamentarium. The Mitsiades laboratory also studies other potential therapeutics in order to identify those which can overcome the resistance of myeloma cells to these existing therapies.

During the last six months, the Mitsiades laboratory:

- Continued its studies of human myeloma cells growing within bone-like scaffolds implanted in immunocompromised mice. This system has allowed investigators to achieve engraftment and expansion of patient-derived myeloma cells in numbers sufficient for further comprehensive studies that were previously extremely difficult in the myeloma field.
- Further examined the molecular features of MM cells that grow in this novel model. For instance, investigators have performed extensive analyses of these cells with whole-exome sequencing to determine which subpopulations of MM cells from a given patient can grow more efficiently in this environment.
- Expanded the analyses of myeloma cells that develop treatment resistance in this research model, using whole-exome sequencing to examine the coding portions of genes and detect which mutations are enriched in myeloma cells after they have developed resistance to treatment(s). These studies have been performed with both human myeloma cell lines, which grow indefinitely in laboratory conditions or in mice, and with patient-derived myeloma cells.
- Adopted and further developed novel approaches to interrogate, in large scale, candidate genes associated with resistance to both established and investigational treatments. As part of these efforts, the Mitsiades laboratory has been actively studying and further validating which of these candidate-resistance genes protect MM cells against specific treatments, but not others.
- Expanded the capabilities of the Mitsiades laboratory to engineer “customized” myeloma cell lines, which harbor defined molecular defects, to match the lesions detected in myeloma cells from specific patients. Through this effort, scientists will generate panels of MM cell lines that match the genetic lesions present in the tumor cells of myeloma patients with resistance to conventional therapies. Researchers will then use these engineered cell lines as tools for evaluating candidate therapeutics that could be capable of circumventing the resistance of corresponding patients to existing therapies.

In a presentation at the December 2014 annual meeting of the American Society of Hematology (ASH), Dr. Mitsiades and his colleagues presented findings from a study that used a gene editing tool, called CRISPR-Cas9, to detect genes that play a role in bortezomib resistance. They identified candidate-resistance genes that are associated with pathways linked to proteasome inhibition or response. This ongoing work is expected to assist the Mitsiades laboratory in

creating a roadmap on how the future treatment of myeloma could be adjusted on the basis of the specific molecular features of patients' tumor cells.

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 long-standing experience with high-throughput, scalable systems to evaluate diverse sets of conventional and investigational agents for their activity against myeloma cells, both in conventional monoculture conditions and in the presence of nonmalignant accessory cells of the bone marrow microenvironment, where myeloma cells primarily reside. The support of the de Gunzburg Foundation enabled Dr. Mitsiades and his team to enhance their capabilities for conducting these studies in high numbers of experimental conditions and small numbers of cells per test.

During the last six months, the Mitsiades laboratory has:

- Further optimized its assays for two- and three-dimensional cultures, and has examined new approaches to study how nonmalignant cells from the bone marrow microenvironment, including bone marrow stromal cells, can influence the response of myeloma cells to treatments applied in these types of cultures.
- Based on these resources and capabilities, completed a first-level functional mapping of the myeloma "kinome" using a large library of small molecule kinase inhibitors as chemical probes. Kinases are important regulators of tumor growth, and kinase inhibitors have revolutionized the treatment of many cancers. These proteins, however, have not yet become a major component in the treatment of myeloma. Through this functional mapping of the myeloma kinome, the Mitsiades laboratory has identified certain kinase inhibitors that can be highly active against specific molecularly-defined subgroups of myeloma cells, thus creating new opportunities for the therapeutic targeting of this disease. This work was presented at the December 2014 ASH meeting.

These advances have helped the Mitsiades laboratory to test candidate therapeutics under conditions that better mimic the context of the patients' tissues in which myeloma cells grow.

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 support of the de Gunzburg Foundation has enabled Dr. Mitsiades to enhance the capabilities of his laboratory to perform wide-ranging bioinformatic studies. During the last six months, Dr. Mitsiades and his team have:

- Further improved the laboratory's in-house storage server capacity and desktop systems in order to analyze large volumes of computational data for the many studies highlighted in this report.

- Applied these systems to increase the volume of molecular profiling analyses and other computational studies that are necessary to advance the overall progress of the Mitsiades laboratory.
- Improved the capabilities of the laboratory for complex statistical analyses correlating clinical data with molecular data from matched patient-derived samples.

This infrastructure enables the analysis of large volumes of data, such as those derived from molecular profiling studies of cell samples, as well as correlation of these molecular profiling results with functional data derived from experimental studies. In addition, these enhanced capabilities supported a recent joint publication by Drs. Mitsiades and Richardson on the clinical outcome and molecular features of myeloma patients with extramedullary disease, which is now in press with the *British Journal of Haematology*.

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 and are led by Dr. Richardson:

- Data from the PANORAMA 2 study, a phase II multi-center trial examining the novel combination of bortezomib, dexamethasone, and panobinostat led by Dr. Richardson as principal investigator, builds on the laboratory work by Dr. Mitsiades in the area of HDAC 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. Moreover, this bench-to-bedside collaboration of Drs. Mitsiades and Richardson on broad-spectrum HDAC inhibitors provided a framework from which other colleagues—Teru Hideshima, MD, PhD, James Bradner, MD, and Kenneth Anderson, MD—developed selective HDAC6 inhibitors.
- Additional data from the PANORAMA program and specifically the pivotal phase III PANORAMA 1 study was presented at the 2014 ASH meeting 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 both principal investigator and the European leader. They found that a combination of panobinostat, bortezomib, and dexamethasone led to an increase in progression-free survival in patients with relapsed/refractory MM, precipitating FDA approval of panobinostat as the first HDAC inhibitor to treat the disease (see page 1).
- 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 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 also led by Drs. Laubach and Richardson in the United States. This trial 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. A landmark meta-analysis presented by Drs. Laubach and Richardson at the 2013 ASH meeting showed that proteasome inhibitors, such as bortezomib, were associated only a low grade of heart failure in newly diagnosed patients, as well as those with relapsed or refractory MM. A paper has been accepted and is now in press at 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 shows promise in targeting pathways subserved by N-Ras, a key genetic mutation that becomes more common as myeloma progresses in each patient. Presented at the 2014 ASH meeting, the trial demonstrated that PRLX 93936, as a single agent, has activity against the activated Ras pathway in patients with relapsed or refractory MM. The findings warrant studies involving PRLX 93936 as part of combination therapy with other anti-MM drugs.
- 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 in 2015 for the treatment of relapsed and refractory myeloma under the leadership of Drs. Laubach and Richardson.
- Finally, Drs. Richardson, Laubach, and Mitsiades have developed a joint project with their colleagues to examine the nature and biology of extramedullary disease (EMD) in 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, the Dana-Farber researchers presented their findings at the 2014 ASH meeting and the paper describing these results is in press with the *British Journal of Haematology*. They found no evidence, as was previously thought, that a front-line combination of bortezomib and lenalidomide for MM precipitated a more rapid development of EMD.

Thank You

The generous philanthropy of the de Gunzburg Myeloma Research Foundation drives the collaborative research described in this report. Drs. Mitsiades, Richardson, 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.