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de Gunzburg Myeloma Research Fund
Dana-Farber Cancer Institute
Report on Milestones Achieved in Year One
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In 2012, The de Gunzburg Myeloma Research Foundation (DGRMF) 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.

Over the past year, Paul Richardson, MD and Constantine Mitsiades, MD, PhD engaged in a comprehensive and close collaborative effort with a singular goal - to improve the overall outcome of multiple myeloma (MM) patients. Below describes progress made 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 DGMRF, we advanced the cause of defining which molecular lesions allow myeloma cells to become clinically aggressive and resistant to existing therapies. For instance, we have utilized our recently developed model of myeloma cell growth in vivo in bone-like scaffolds implanted in immunocompromised mice (NSG strain). Using these systems, we have achieved engraftment and expansion of patient-derived tumor cells (with or without fractionation of tumor cells) from far-advanced cases of myeloma (including advanced cases of plasma cell leukemia or pleural effusion). We then proceeded to evaluate the molecular lesions present in these MM cells, before and after their proliferation in mice, using next-generation sequencing. Specifically, our samples have been analyzed by whole-exome sequencing (average read length of ~140-150 bp, more than 230,000 target regions in the genome of each sample, average coverage ~200x). The comprehensive analyses of these samples are ongoing and have already identified, candidate molecular lesions, which could serve to function as critical "progression events", because they are present in samples from more advanced stages of the disease (compared to earlier stages in the same patient) and therefore can be considered to contribute to the transition of myeloma from its earlier stages to its more advanced ones. To further complement this effort, we also initiated in our mouse models treatment of myeloma cells with established anti-myeloma therapeutics (e.g. proteasome inhibitors, thalidomide derivatives, alkylating agents), in order to identify molecular lesions which are selectively enriched for in myeloma cells once they develop in vivo resistance to these treatments. The first results from these comparative analyses of differential drug-specific candidate markers of in vivo resistance are expected in the coming weeks and will allow us to initiate in 2014 specific targeting of individual candidate lesions, with the intent to reverse resistance to existing anti-myeloma therapeutics.

We have also been establishing the molecular tools necessary for customized engineering of myeloma cells with molecular lesions present in patients with clinical resistance to established treatments for this disease. Some of these lesions are individually present in myeloma cell lines that our labs and others have previously worked with. However, up until now, there have been so far very few, if any, efforts in the myeloma field to stringently compare the behavior of myeloma cells which harbor one of these "progression lesions" compared to cells that do not harbor such "progression lesions" but are otherwise genetically identical. Furthermore, many myeloma patients treated in the era of novel agents (i.e. after the introduction of thalidomide and proteasome inhibitors in the therapeutic armamentarium) harbor more

than one of these lesions at the same time and there is very limited, if any, understanding in our field on how myeloma cells will behave differently when they harbor many of these lesions simultaneously vs. only one of them. The molecular tools that we have been developing represent a major step forward and a critical investment towards the ultimate success of this research program and we are greatly appreciative of the support of the DGMRF in this regard. Specifically, we have been conducting lentiviral transductions of myeloma cell lines which are wild-type for key mutational targets (e.g. p53) in order to introduce into the cell lines recurrent mutations of the respective genes and generate isogenic mutant variants of the cell lines. One such variant cell line (E285K TP53 in the ARD cell line background) is currently being evaluated in functional studies of our lab to document the role of this mutation in conferring resistance to established or investigational therapies, as well as altering the response of myeloma cells to immune effector therapies. Additional mutant variants will be generated during 2014. Moreover, specific efforts will be deployed towards generating in MM cell lines and functionally characterizing specific complex genotypes identified from our 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 long standing experience with high-throughput scalable testing of candidate therapeutics for their anti-myeloma activity. During this last year, we further improved our ability to perform these studies with small numbers of cells per experimental condition and, in preparation for studies using patient derived myeloma cells, conducted pilot screens in which myeloma cell lines were exposed to a library of FDA approved anticancer therapeutics, in order to survey the response of these tumor cells to these therapeutics or their combinations with other agents, established or investigational. We also optimized the deployment of these screens in the context of both two-dimensional and three-dimensional cultures, as well as in the presence versus absence of nonmalignant accessory cells of bone marrow microenvironment, such as bone marrow stromal cells. This progress, combined with the previously mentioned progress in expanding patient-derived tumor cells in our mouse models, will allow us in 2014 to test patient-derived myeloma with extended panels of investigational agents and their combinations with conventional therapeutics. The goal of these experiments will be to identify candidate therapeutics with selective activity against myeloma cells which harbor 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.

During 2014, we hope to advance, with the help of the DGMRF, the goal of developing a comprehensive system of bioinformatics and computational support for the Mitsiades laboratory. These systems would facilitate the analysis of the higher volumes of data that we expect to get from the molecular analyses of samples evaluated from our in vitro and in vivo studies. In preparation for these efforts, members of our team have obtained access to institutional servers, in which R or Matlab scripts for computational analyses can be run. We are specifically planning for expansion of our capabilities to perform pathway-based analyses, gene set enrichment analyses, processing of next-generation sequencing data (e.g. RNA sequencing), as well as integrative analyses of transcriptional, mutational and DNA copy number data.

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.

Two thousand and thirteen (2013) has been a busy and productive year in the MM clinical research program. Over 20 trials are currently in process in both the early and advanced stage setting (Phase I, II

and III). Specifically, studies have been initiated or completed in smoldering myeloma, newly diagnosed myeloma, early relapse and relapsed/refractory disease (RR MM). Moreover, studies continue in the context of maintenance post-transplant. Further, transplant-based strategies including tandem transplantation, consolidation, and maintenance as well as maintenance-only approaches have been prospectively evaluated. In 2012, the program enrolled approximately 360 patients. In 2013, the annualized rate of enrollment is expected to exceed last year's total.

Numerous presentations at the 2013 American Society of Hematology (ASH) meeting in New Orleans reflected this remarkable productivity. Our oral presentation of the first-in-class orally –bioavailable boronate peptide ixazomib combined with lenalidomide and dexamethasone reported on results of the combination of lenalidomide and dexamethasone with ixazomib in newly diagnosed patients and generated overall response rates of 95%, with 75% very good partial response (VGPR) or better, with favorable tolerability (Richardson et al, ASH 2013, abstract 535). This all oral, three-drug regimen is the first to achieve such high quality overall and complete responses in this setting. The high levels of minimal residual disease (MRD) negativity determined in this study were especially promising (Richardson et al, ASH 2013, abstract 535). Other clinical trials presented at the meeting included pomalidomide, bortezomib and dexamethasone (PVD) combined in treatment of patients with RR MM in which an overall response rate of 70% was reported with excellent tolerability in patients who were both heavily pretreated and had received multiple lines of prior therapy (Richardson et al, ASH 2013 abstract 1969). Importantly, updated results of a single-arm phase II multicenter trial of the novel combination of bortezomib and dexamethasone combined with panobinostat (PANAROMA 2) reported a 35% PR or better with a clinical benefit rate approaching 50% in a study of 55 pts with RR MM. Tolerability was favorable and toxicity proved manageable (Richardson et al, ASH 2013, abstract 1970). This study was particularly noteworthy because at the same meeting, positive results were announced for the PANORAMA 1 trial, a large, international phase III evaluation of panobinostat, bortezomib, and dexamethasone compared to bortezomib and dexamethasone placebo. This large study, enrolling over 600 patients, achieved its primary endpoint with a highly statistically significant result in favor of the 3-drug regimen. Additional data from this trial will be presented next year at various meetings and it are anticipated will lead to the FDA approval of panobinostat as the first histone deacetylase inhibitor in combination with bortezomib and dexamethasone to receive regulatory authorization in RR MM.

Importantly, this data builds on promising translational work derived from our laboratories and including seminal work by Dr. Mitsiades. Dana-Farber has a leadership role in PANORAMA 1 (specifically Dr. Richardson is the Senior Investigator of the trial), and more broadly in the field of histone deacetylase inhibitors both with vorinostat and panobinostat as well more recent efforts with the HDAC6 selective compound ACY-1215. In the context of the PANORAMA program, Dana-Farber also provided leadership for the phase I study clinically, as well as leadership for the phase II and most recently senior leadership for the phase III trial as detailed above.

In addition, with Dr. Richardson's senior leadership and the Principal Investigatorship of Dr. Jacob Laubach, in collaboration with Dr. Mitsiades' laboratory, a comprehensive review of proteasome inhibitor –related cardiac toxicity was presented at the ASH meeting. Dr. Mitsiades laboratory is integrally involved in this project in the context of molecular markers and correlative science (Laubach et al, ASH 2013, abstract 3187). This is a very important area as proteasome inhibitor toxicity in the treatment of multiple myeloma remains a top priority with the goal of improving therapeutic index and enhancing clinical efficacy of proteasome inhibitors more broadly.

In the context of early-phase clinical trials developed in the last year pursuant of direct translational work previously conducted by Dr. Mitsiades laboratory, the phase I study of PRLX 93936 with or without dexamethasone infused three times a week, two weeks on and one week off, has been pursued in RR MM

(DFCI # 12-220). Approximately 10 patients have been enrolled with favorable tolerability seen so far. Modest single-agent activity has been demonstrated and further studies are planned in combination given the synergy seen in experimental models from Dr. Mitsiades' lab. Importantly, PRLX 93936 was developed as an agent that is potently active against tumor cells with Ras mutations and is considered a potentially important combinatorial agent going forward for the treatment of RR MM, especially as the frequency of Ras mutations is increased as the natural history of myeloma evolves.

Other translational studies initiated in 2013 in which a direct laboratory role has been derived from Dr. Mitsiades include the combination of panobinostat, lenalidomide, bortezomib, and dexamethasone (PAN RVD) building upon the experience derived above (DFCI # 13-262). This has been led by Dr. Laubach, the Principal Investigator, with Dr. Richardson acting as Senior Investigator. This phase I/II multicenter trial is exploring a dose-escalation schedule of panobinostat, lenalidomide, bortezomib, and dexamethasone and has begun enrollment with 3 patients enrolled in the first cohort and several patients in screening. Tolerability has been favorable to date and whilst it remains early to report on overall activity, clinical benefit has been seen. Further, a prospective phase II study of lenalidomide, bortezomib, and dexamethasone (RVD) administered subcutaneously has been in development and is now ready for submission, with approval and activation planned for 2014. Dr. Mitsiades' laboratory has provided key support for correlative science and molecular markers for this trial which will be part of an international collaborative effort with Ireland, in which a parallel study from the Myeloma Ireland Consortium (MIC) will be pursued. Another novel translational effort is the development of a bromodomain inhibitor (TH101) derived from the JQ1 molecule developed by the Mitsiades' laboratory and Dr. Jay Bradner's group, in combination with immunomodulatory-based therapy (specifically pomalidomide and dexamethasone). This study is anticipated to be further developed, approved, open and activated in 2014 with Dr. Laubach acting as Principal Investigator and Dr. Richardson as Senior Investigator.

Thank you

The support kindly provided to Dana-Farber by the de Gunzburg Myeloma Research Foundation has allowed us to achieve significant progress towards the goals that we initially set out to reach. Major progress has been achieved in all aspects of our studies in the laboratory and in animal models, as well as in the clinic. We are highly optimistic that this progress will translate into exponential acceleration of productivity of our studies in the next few years; we are especially excited about a highly productive and innovative 2014.