2019 MRF Grant Awardees

The Melanoma Research Foundation (MRF) is committed to advancing research across the spectrum of melanoma- from prevention through diagnosis, staging and treatment. In 2019, the MRF awarded 21 research grants encompassing not only cutaneous melanoma, but also the rare melanoma types. Further, MRF-supported research spanned scientists early in their career (e.g. medical students and post-doctoral fellows) as well as senior investigators. Additional details of the 2019 grant awardees are noted below.

Uveal Melanoma
Team Award

Principal Investigator: **William Sellers, MD**
Broad Institute, Inc.

Co-PI: Keith Flaherty, MD
Massachusetts General Hospital

Proposal Title
The development and characterization of cellular models of uveal melanoma

Description

Uveal melanoma (UM) is an aggressive cancer arising in the eye and is the second most common melanoma after those that arise in the skin. In some patients, the initial tumor can be successfully removed by surgery or treated with radiation, but despite these treatments, nearly 50% of patients develop untreatable metastatic spread of the disease. The discovery of recurrent mutations in two critical genes named GNAQ and GNA11 has generated significant interest in new drugs called PKC inhibitors that can act against these mutant genes. However, in large part, there is a major deficit in the landscape of new therapeutic targets for this disease.

In other cancers, rapid progress has been made in discovering new therapeutics against mutated genes. However, such progress typically requires the ability to grow cancer cells in the laboratory where drug testing and other experiments can be carried out. In uveal melanoma, these types of experiments have been severely limited by the poor availability of uveal melanoma cancers growing in the laboratory setting (we call these cancer models "cell lines"). The currently available cell lines for uveal melanoma grow poorly and are not clearly good representations of real uveal melanoma cancers.

MRF MELANOMA RESEARCH FOUNDATION

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To enable the uveal melanoma research community to conduct more robust experiments and test new drugs in uveal melanoma, we aim to develop new cell line models of this cancer beginning with primary tumors after biopsies are obtained. In parallel, we are optimizing growth conditions of the 16 existing uveal melanoma cell lines to empower high-throughput experiments. To this end, we have assembled world-class research teams from Massachusetts General Hospital (MGH) and the Broad Institute who are expert in the care, treatment, and study of uveal melanoma patients and have robust expertise in the development of new cell line models, respectively. The creation of new cell line models should pave the way for new target discovery efforts by a broad range of investigators and will enable drug discovery screens to proceed.

In parallel, it is clear that immunotherapy is making remarkable in-roads in the treatment of many cancers. In uveal melanoma, however, we know very little about how the patient's immune response is reacting to the tumor. Drawing on the significant patient volume seen at MGH, and their well-established biopsy program we seek to enhance our understanding of the immune response to uveal melanoma by systematically characterizing the immune cells that are found in patient biopsies of metastatic uveal melanoma tumors. We believe that we can create a map of the immune cells in uveal melanoma and that this will help clarify what immuno-oncology clinical trials should be prioritized in this disease.

Established Investigator Award

Principal Investigator: J. William Harbour, MD
University of Miami

Proposal Title
Cellular and Genomic Landscape of Uveal Melanoma at Single Cell Resolution

Description

Uveal melanoma (UM) is a highly aggressive eye cancer that leads to metastatic death in up to half of patients, with no measurable improvement in survival over the past half century. As such, there is a critical need to develop new therapies for metastatic UM. In contrast to cutaneous melanoma, checkpoint inhibitor immunotherapy is largely ineffective in UM, which is likely due at least in part to the tumor creating an immunosuppressive microenvironment. We hypothesize that the suppressive immune microenvironment in UM is triggered by genomic aberrations that arise during tumor evolution. We propose to address this hypothesis using the latest advances in single-cell DNA and RNA sequencing to study the genomic and immune cell landscape of UM tumors obtained from patients (Aim 1). We will further explore these findings using a novel mouse model of UM developed in our laboratory that will allow us to study the interplay between genomic abnormalities and the host immune system (Aim 2). The overall objective of our research program is to provide the first comprehensive genomic and cellular atlas of UM at single cell resolution. The clinical relevance of the proposal is to stimulate new strategies for effective therapy. This proposal benefits from a unique collaboration between our lab and 10X Genomics to develop and optimize new methods for analyzing mutations in single-cell DNA sequencing data. We also utilize single-nucleus methodology, which can be performed on snap-frozen samples, thereby allowing our large biorepository of annotated samples

to be available for analysis. This technology may have clinical applications in the future and provides a critical resource to the scientific community.

Career Development Award

Principal Investigator: Stefan Kurtenbach, PhD
University of Miami

Mentor: J. William Harbour, MD

Proposal Title
Role of PRAME in Epigenetic Reprogramming and Chromosomal Instability

Description

Uveal melanoma (UM) is a highly aggressive eye cancer that leads to metastatic death in up to half of patients. UM has a propensity to undergo early micrometastasis prior to treatment of the primary tumor, with later emergence of overt metastatic disease. Unfortunately, there has been no dramatic improvement in survival over the past half century. PRAME (Preferentially Expressed Antigen In Melanoma) is a gene that is usually only found in testis, is also found highly expressed in a variety of tumors. We have recently reported that PRAME does correlate with metastatic risk in UM, which is also true for many other cancer types including cutaneous melanoma. Besides the broad interest in PRAME, how PRAME promotes tumor progression and metastasis is not yet understood. In this proposal, we present preliminary data showing that PRAME is not only a biomarker for poor outcome, but plays a role in the formation of metastasis itself. We further show that PRAME expression may cause a defect in proper genomic DNA maintenance, which could be exploited for treatment with PARP inhibitors. Indeed, we present preliminary data for PARP inhibitors that is very promising, where UM cells expressing PRAME are more susceptible to PARP treatment. This proposal will cover two aims, where we intend to (1) generate a comprehensive map of where PRAME binds in the genome to regulate expression of genes involved in genomic instability and tumor progression, and (2) test PARP inhibitors for their suitability as a treatment option in our mouse metastasis model. For Aim 1, we will make use of a unique set of cell lines we have generated allowing for the inducible expression and knockdown of PRAME, including normal human uveal melanocytes, presenting a unique resource. Further, will use a variety of state-of-the-art next generation sequencing techniques to decipher the mechanism by which PRAME modulates gene expression. For Aim 2, we will utilize a mouse metastasis model we have established that allows for imaging of tumor growth and metastatic spread in live animals.

Together, the results of this proposal will draw a detailed picture of how PRAME re-shapes the epigenetic landscape around genes important for tumor development, as well as exploit PRAME's function in

chromosomal maintenance for treatment using a mouse model, which could have a strong translational impact in the clinical setting.

Melanoma Brain Metastases
Career Development Award

Principal Investigator: Venkata Saketh Sriram Dinavahi, PhD
The Pennsylvania State University College of Medicine

Mentors: Gavin Robertson, PhD; Sheri Holmen, PhD; & Arthur Berg, PhD

Proposal Title
Modulating p53 Transcriptional Activity to Reduce Melanoma Brain Metastasis

Description
Successful management of melanoma will require eliminating both the primary cancer as well as its spread. The survival of melanoma patients reduces significantly if the cancer spreads to the brain. Five-year patient survival after melanoma spreads to brain is only 5%. The key contributor for growth of melanoma is changes in a protein called BRAF. Similarly, the major factor that influences melanoma to spread to the brain is a protein called AKT. To reduce cancer spread, a number of novel treatments are currently being evaluated. Our hypothesis is that targeting the AKT and BRAF pathways will reduce both cancer as well as its spread to the brain, by increasing an important protein, called p53. One such treatment strategy to achieve this is inhibition of AKT and WEE1, WEE1 being a downstream protein in the BRAF pathway. We have previously shown that targeting AKT and WEE1 is superior to targeting either of the proteins alone in reducing melanoma development. Therefore, the central hypothesis of this project is to test the effect of p53-modulation on melanoma metastasis to the brain. The project will be accomplished by first identifying the best strategy to target the p53 pathway using genetic modifications and drugs. The best identified strategy will be tested for its effect on the p53 pathway and regulation of growth of melanoma cells. Finally, this strategy will be evaluated to decrease melanoma growth and its spread to brains in a mouse model. This discovery would identify unique approaches to overcome melanoma brain metastasis thereby improving the survival of patients.

Cutaneous Melanoma
Team Award

MELANOMA RESEARCH FOUNDATION BREAKTHROUGH CONSORTIUM Accelerating Research for a Cure

MRF Breakthrough Consortium Young Investigator Team Award to Advance the Field of Translational Immuno-Oncology *(generously funded through a grant by BMS)*

2019 MRF Grant Awardees

Principal Investigator: Genevieve Boland, MD, PhD
Massachusetts General Hospital

Co-PIs: David Liu, MD; Dana Farber Cancer Institute

Srinivas Saladi, PhD; Massachusetts Eye and Ear Infirmary

Proposal Title
Characterizing the Role of the Hippo Pathway During Melanoma Immunotherapy

Description

The Hippo pathway in cancer, mediated by YAP1, has been implicated in therapy resistance and aggressive tumor behavior in melanomas treated with targeted therapies. However, the role of this pathway in response and resistance to immunotherapy has not yet been characterized. There is existing data suggesting that activation of this pathway may lead to immune evasion by tumors. Therefore, we hypothesize that concurrent inhibition of the Hippo pathway in combination with immunotherapy may be complementary. We will use a unique resource of longitudinal tumors from patients treated with immunotherapy in whom analysis of molecular data is already underway to assess if there is regulation of the Hippo pathway during treatment with immunotherapy in melanoma and if this correlates with response or resistance to therapy (Aim 1). We will then use cell lines to establish the mechanism of Hippo pathway activity in melanoma (Aim 2), and finally will examine the impact of activating/inactivating the Hippo pathway in combination with immunotherapy in a mouse model of melanoma. This proposal draws from our respective strengths in translational medicine (Dr. Boland), computational biology (Dr. Liu) and mechanisms of gene regulation (Dr. Saladi).

Established Investigator Award

Principal Investigator: Donald McDonnell, PhD
Duke University Medical Center

Proposal Title
Pharmacological targeting of estrogen receptor to enhance melanoma immunity

Description

The development of new classes of drugs which target pathways required for melanoma growth and approaches to increase tumor immunogenicity (Immune Checkpoint Blockade (ICB)), have had a significant impact on outcome in this disease. However, notwithstanding the fact that “cures” are seen in ~20% of melanoma patients treated with ICBs, there remains a clear need to increase response rate. Our group has had a long-standing interest in targeting sex steroid receptors in cancer and have developed several first-in-class drugs that are now in clinical development. Not surprisingly, we were intrigued by studies which

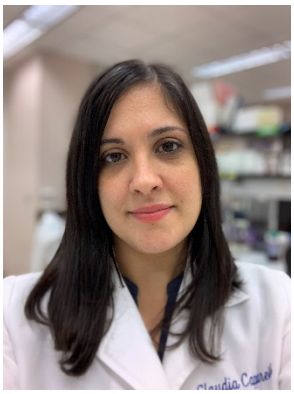
revealed that the magnitude of benefit from ICB is greater in males than females. Among the possible explanations for these findings are sex hormone dependent baseline differences in the functionality of the immune system. In support of this idea we have generated compelling data in animal models of melanoma showing that estrogens influence tumor pathobiology through actions in intratumoral immune cells. With a view to improve delivery of existing drugs, and to inform the development of the next generation of drugs for this disease, we will explore the specific mechanisms by which estrogens affect melanoma. The utility of measuring the expression of estrogen regulated genes in intratumoral immune cells as biomarkers of response to ICB therapies will also be explored. Further, preclinical studies will be performed to assess the utility of using approved anti-estrogens to increase the efficacy (patient response rates) and reduce the toxicities associated with ICB and attenuate/reverse ICB resistance. This project specifically addresses the special topic area of identification of mechanisms and biomarkers for predicting/monitoring therapeutic response.

Significance: The results of our preliminary studies strongly suggest that estrogens negatively impact anti-tumor immune response which may explain the decreased efficacy of ICB in females compared to males. With the goal of developing approaches to exploit these findings in near-term clinical trials, we will assess the extent to which anti-estrogens improve the response to ICBs and evaluate the utility of using estrogen regulated genes/signatures as biomarkers to predict patient responses to ICB.

Innovation: Gender differences in response to ICB have been attributed to differences in neoantigen burdens related to differences in lifestyle. We propose the additional/alternate hypothesis that estrogens, in and of themselves, impact tumor immunity in a manner that is clinically meaningful. We believe that the information gained from dissecting estrogen action in immune cells can be used to improve the delivery of existing drugs/modalities, reveal new therapeutic targets and provide new biomarkers of ICB response.

Impact: Clinical trials of an ICB/ER modulator combination, the design of which will be informed by an understanding of ER action in tumor immunity, is a likely outcome of our studies.

Career Development Award



Principal Investigator: **Claudia Capparelli, PhD**
Thomas Jefferson University

Mentor: Andrew Aplin, PhD

Proposal Title
SOX10 role in WT BRAF melanoma

Description

Metastatic melanoma is the most lethal skin cancer. In the past decade the number of new melanoma cases diagnosed annually has increased by 53%. This increase is due to many different factors, such as elevated usage of tanning salons in large cities and inadequate UV protection with sun-blocking products. Because of these issues, it is crucial to study melanoma in order to better understand its molecular causes and to uncover new potential therapies to help the ever-growing population of melanoma patients across the world.

2019 MRF Grant Awardees

Despite some progress in melanoma research, a large portion of patients still do not respond to treatments. Therapeutic options are even more limited for a subgroup classified as wild-type BRAF melanoma.

In our previous publications we showed that the combination of two different drugs targeting proteins named MEK and ErbB3 can reduce the growth of wild-type BRAF melanoma in animal models. These data led to the approval of a new clinical trial to investigate the effect of targeting MEK and ErbB3 in wild-type BRAF melanoma patients. Melanoma is a highly dynamic and heterogenous tumor at the cellular level. The presence of cellular subpopulations with different characteristics and the ability to adapt to different types of insults are two of the main reasons leading to the onset of resistance to therapy. In the present proposal we aim to investigate how the differential cellular expression of a protein, SOX10, affects the tumor behavior and the response to the combinatorial therapy of MEK inhibitor plus anti-ErbB3 drug.

Principal Investigator: Matthew Griffin, PhD
The Rockefeller University

Mentor: Howard Hang, PhD

Proposal Title
Augmenting Melanoma Response to Immunotherapy via Commensal Microbiota

Description

Cancer immunotherapy (CI) drugs activate our own immune system to directly kill cancer cells. These drugs have revolutionized how we treat many cancers including melanoma. However, not all patients respond equally to CI treatment, and we still do not fully understand why. Surprisingly, microorganisms in our gastrointestinal tract known collectively as the gut microbiota may be necessary for effective CI treatment. Melanoma, lung, and kidney cancer patients who responded to CI drugs contained a higher abundance of specific bacteria including Enterococci species compared to non-responding patients. These results suggest that individual bacterial species in the gut may improve CI therapy. Nevertheless, how this occurs is still unclear.

In parallel, our laboratory has found that one Enterococcus species, Enterococcus faecium (Efm), can protect against infection by activating the immune system. Efm produces SagA, an enzyme that breaks down the bacterial cell wall to produce small molecules that stimulate innate immunity via the sensor Nod2. Based on these results, I hypothesize that Enterococcus species can also improve CI drug effectiveness through the direct activation of host immunity via a similar mechanism. To test this, I will first examine how colonization of the GI tract with Enterococci can directly change the growth of melanoma in mice and alter immune cell activation within the tumor during CI treatment. To decipher how Enterococci can alter drug response, I will then test whether the SagA enzyme and the resulting small molecule it produces are sufficient to improve CI drug effectiveness against melanoma. Finally, I will determine whether Enterococci-mediated melanoma suppression also requires the host sensor Nod2.

Together, these experiments will provide direct evidence for how individual species of commensal bacteria can activate the host immune system during immunotherapy treatment of melanoma. Results from this

proposal may allow us to predict how well patients will respond to CI drugs by detecting the presence of SagA-expressing Enterococci bacteria in their GI tract. Moreover, the microbial and host components I identify may provide new ways to improve clinically approved CI drugs via new probiotic or small molecule combination therapies.

Principal Investigator: Vito Rebecca, PhD
The Wistar Institute

Mentor: Meenhard Herlyn, DVM, DSc

Proposal Title
Targeting Melanoma Developmental Programs to Overcome Therapy Resistance

Description

Melanoma is the most aggressive form of skin cancer. Although progress has been made for advanced melanoma patients with 13 new FDA-approved therapies since 2011, resistance arises in most cases. Our focus is on melanomas that harbor activating BRAF mutations (~50% of patients). Most of these patients respond dramatically to combination therapy with a BRAF and MEK inhibitor (BRAFi/MEKi). However, four out of every five patients relapse within two years due to the persistence of therapy-resistant subpopulations of melanoma cells. This expanding BRAFi/MEKi-resistant patient cohort is the greatest challenge of the field; few experience durable benefit from immune therapy and no alternative effective therapies exist. Therefore, there is an unmet need to develop more effective strategies.

We have characterized therapy-resistant subpopulations and identified common features; 1) existence prior to therapy, 2) a slow-growing state, 3) high metastatic potential and 4) stem cell-like molecular and biological properties that allow for high adaptability in stressful conditions including therapy. Shared gene signatures by stem cells and melanoma cells are poorly understood. In our initial studies, we identified a developmental receptor, LPAR1, as key for the survival of melanoma and stem cells. LPAR1 increases the proliferation of neuronal stem cells and aggressiveness of breast and lung cancer. We show LPAR1 expression increases with progression in melanoma patient tumor tissue relative to benign nevi. Further, a) LPAR1 expression is higher in BRAFi/MEKi resistant melanoma cells, b) hyperactivation of a down-stream LPAR1 effector, YAP1, increases the presence of resistant stem cell-like melanoma cells, and c) genetic or pharmacological targeting of LPAR1 kills BRAFi/MEKi resistant melanoma cells. This provides strong scientific rationale for investigating LPAR1 as a novel target to overcome BRAFi/MEKi resistance.

We propose to validate LPAR1 as a clinically relevant target by using models that closely mimic the in vivo biology of melanoma. This includes 3D human skin-, spheroid-, and a collection of >500 patient-derived xenograft (PDX)-models where patient tumor material is inoculated directly into mice, including >200 patients that relapsed on BRAFi/MEKi. Towards this goal, we will define the molecular consequences of inhibiting LPAR1 on the survival and growth of stem cell-like melanoma cells and in BRAFi/MEKi resistance. We will identify the most potent LPAR1 inhibitor that can synergize with BRAFi/MEKi to eliminate all tumor cells without causing toxicity.

2019 MRF Grant Awardees

As LPAR1 inhibitors are currently being clinically investigated, we expect our proposed studies will provide the scientific rationale to clinically test new therapeutic strategies that will increase the curative potential of BRAFi/MEKi and facilitate the development of future clinical trials.

Principal Investigator: Maria Sosa, PhD
Icahn School of Medicine at Mount Sinai

Mentor: Marisol Soengas, PhD

Proposal Title
Therapeutic strategies to induce dormancy in disseminated melanoma cells

Description

Metastasis is the main cause of melanoma death. Currents treatments mostly fail to cure metastasis. It is of common knowledge that disseminated cancer cells (DCCs) are the seeds of future metastasis. Once these DCCs arrive to secondary organs they undergo a dormancy program that involves cell cycle arrest or quiescence. DCCs could remain in this state for months to almost decades and they are able to survive current treatments. The reason is because most current therapies target anti-proliferative cells, thus dormant DCCs remain unaffected. Eventually these DCCs reactivate and they start forming metastases.

After the detection of a melanoma lesion and treatment, patients enter a phase of remission in where no symptoms of disease are detectable and one can say that patients are “cured”. However, the majority of these patients will develop metastasis that will arise from DCCs. Clinical analyses suggest that dissemination of DCCs happens very early during melanoma progression. Therefore, understanding how DCCs enter dormancy and what makes them reactivate to form metastasis is of extreme significance to design novel treatments.

We designed a protocol to reprogram tumor cells into long-term dormancy by combining an inhibitor of methylation plus retinoic acid. These reprogrammed dormant cells upregulated a transcription factor named NR2F1, which was responsible for the dormancy phase. We validated that NR2F1 could be used as a biomarker to determine dormancy status of DCCs in breast and prostate cancer patients. Moreover, the above-mentioned protocol is now part of a clinical trial to treat advanced cancer patients. In addition, previous results showed that patients with melanoma positive for a secreted factor named Midkine relapsed earlier than those patients that were negative for Midkine. Interestingly, when we blocked Midkine the levels of NR2F1 factor were upregulated reinforcing dormancy of DCCs and reducing metastasis formation.

Thus, we propose a therapeutic strategy to keep those DCCs in a dormancy phase by blocking the reactivation signal (midkine) and inducing dormancy signals (NR2F1). We believe this treatment could stop DCCs from forming life-threatening metastases.

Medical Student Awards

Principal Investigator: Rebecca Chen (Weill Cornell Medical College)
Mentor: Eleni Linos, MD, DrPh

2019 MRF Grant Awardees

Proposal Title: Geographic Analysis of indoor tanning salon clustering around high risk communities

Principal Investigator: **Michelle Ferreira** (Yale School of Medicine)
Mentor: Marcus Bosenberg, MD, PhD
Proposal Title: Evaluating the Role of the COX2/PGE2 Pathway in Anti-Melanoma Immunity

Principal Investigator: **Hannah Knochelmann** (Medical University of South Carolina)
Mentor: Chrystal Paulos, PhD
Proposal Title: Determining Mechanisms of Enhanced Anti-Tumor Efficacy of Briefly Expanded Th17 Cells for Melanoma

Principal Investigator: **Cory Kosche** (Rush Medical College)
Mentor: Caroline LePoole, PhD; Jennifer Choi, MD
Proposal Title: Understanding Skin Rash Secondary to Checkpoint Inhibitor Immunotherapy

Principal Investigator: **Michael Lee** (Eastern Virginia Medical School)
Mentor: Jeremy Etzkorn, MD
Proposal Title: Characterizing Trends in Utilization of Mohs Micrographic Surgery for Melanoma in the United States

Principal Investigator: **Victor Lin** (UNT Health Science Center)
Mentor: Yu-chieh Wang, PhD
Proposal Title: Enhancing Immune Checkpoint Therapy by Targeting Protein Deglycosylation in Melanoma

Principal Investigator: **Dianne Lumaquin** (Weill Cornell Medical College)
Mentor: Richard White, MD, PhD
Proposal Title: Adipocyte-derived Lipids as Drivers of Endoplasmic Reticulum (ER) Stress and Invasion in Melanoma

Principal Investigator: **Alicia Mizes** (University of Pittsburgh School of Medicine)
Mentor: Louis Falo, MD, PhD
Proposal Title: A Novel Chemoimmunotherapy for Cutaenous Melanoma Using Dissolvable Microneedle Arrays

Principal Investigator: **Kristina Navrazhina** (Weill Cornell Medical College)
Mentor: Elena Piskounova, PhD
Proposal Title: Elucidating the Role of AMPK Signaling in Melanoma Metastasis

Principal Investigator: **Kyle Tegtmeyer** (Northwestern Feinberg School of Medicine)
Mentor: Jeffrey Sosman, MD
Proposal Title: Understanding Mechanisms of Immune Checkpoint Inhibitor Induced Colitis in Melanoma

2019 MRF Grant Awardees

Principal Investigator: **Eliot Zhu** (University of Iowa)
Mentor: Adam Dupuy, PhD
Proposal Title: Identification of Src Family Kinases that Drive Resistance to Mutant BRAF Inhibition