CDMRP

Department of Defense

Lung Cancer Research Program





U.S. Army Medical Research and Development Command

APPLICATION REVIEW PROCESS

The CDMRP uses a two-tier review process for evaluating applications, with both tiers involving dynamic interaction between scientists and disease survivors (consumers). The first tier of evaluation is a scientific peer review of the applications, measured against established criteria for determining scientific merit. The second tier is a programmatic review conducted by the Programmatic Panel, which is composed of leading scientists, clinicians, and consumers. The Programmatic Panel compares applications to each other and makes recommendations for funding based on scientific merit, potential impact, adherence to the intent of the award mechanism, relevance to program goals, and portfolio composition.

Vision: Eradicate deaths and suffering from lung cancer to better the health and welfare of Service members, Veterans, and the American public

Mission: Support and integrate research from multiple disciplines for risk assessment, prevention, early detection, diagnosis, and treatment for the control and cure of lung cancer

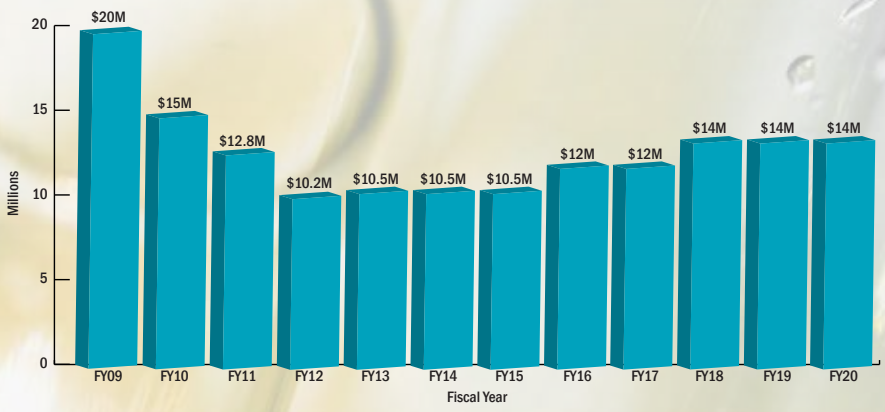
Congressionally Directed Medical Research Programs

The Office of the Congressionally Directed Medical Research Programs (CDMRP) was created in 1992 from a powerful grassroots effort led by the breast cancer advocacy community that resulted in a Congressional appropriation of funds for breast cancer research. This initiated a unique partnership among the public, Congress, and the military. Since then, the CDMRP has grown to encompass over 30 targeted programs and has been responsible for managing over $15 billion since its inception through fiscal year 2020 (FY20). Funds for the CDMRP are added to the Department of Defense budget, in which support for individual programs, such as the Lung Cancer Research Program (LCRP), is allocated via specific guidance from Congress.

Lung Cancer Research Program

Anyone can get lung cancer, whether you are a smoker or have never smoked. Lung cancer is the leading cause of cancer-related deaths in the United States and claims more lives each year than all other major cancers combined. The LCRP was established in FY09 with a Congressional appropriation of $20 million (M); since that time, it has received a total of $155.5M in Congressional appropriations through FY20. Over the past 11 years, the LCRP has played a critical role in helping accelerate high-impact translational research, encouraging innovation and stimulating creativity, bringing new investigators into the lung cancer field, and facilitating the creation of unique partnerships and resources. To address the critical needs of the lung cancer research and patient community, the LCRP adapts its investment strategy annually, focusing its support on underfunded and underrepresented areas.

FY09–FY20 LCRP Appropriations



Strategic Plan

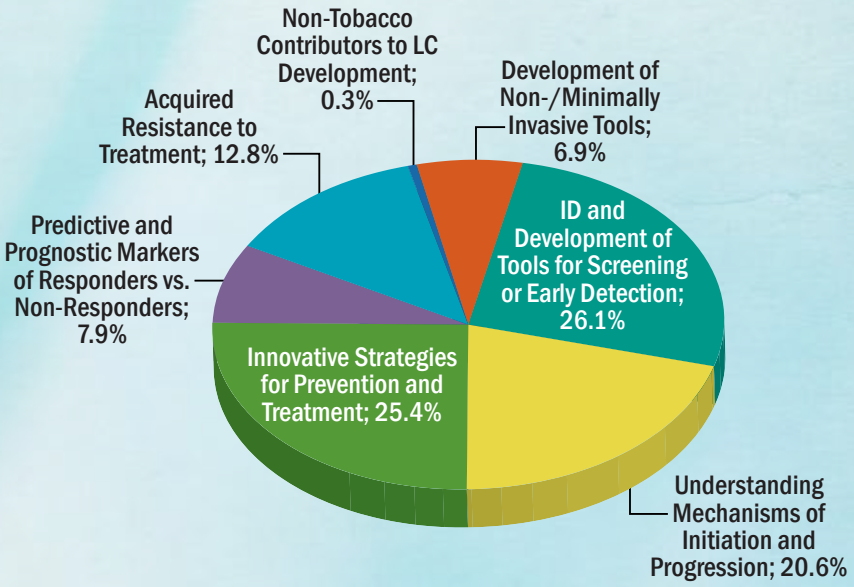
The LCRP recognizes that a broad range of unanswered research questions are potentially critical to advancing prevention, detection, treatments, and cures for lung cancer. Given the substantial need for further lung cancer research across the entire research spectrum (from prevention to biology/etiology, screening, detection, treatment, and cancer control/survivorship), the LCRP is not interested in limiting its focus to one or only a few of these research areas. Rather, the program's focus is better defined by the types of award mechanisms it typically offers, which include but are not limited to the following:

- Researcher Development Awards – Includes Career Development Awards, Clinical Fellow Awards, and New Investigator Awards
  - Designed to support promising scientists or research clinicians who are not yet established investigators or research clinicians and are focusing their research on lung cancer
- Early Idea Awards – Includes Concept Awards, Idea Development Awards, and Expansion Awards
  - Designed to encourage higher-risk/higher-return research and provide opportunities for continued investigation and further development of promising research
- Clinical/Translational and Team Science Awards
  - Designed to support projects with the potential to have a major impact on therapy by applying promising and well-founded preclinical research findings to the care of patients

The LCRP seeks to invest in its priorities based on its areas of emphasis; however, the program enables investigators to propose their best ideas and is interested in furthering high-impact, innovative lung cancer research. The program does not define which specific projects or products will be funded. The current overarching strategic priorities/areas of emphasis for the LCRP include the following:

- Identify innovative strategies for prevention of the occurrence of lung cancer
- Identify innovative strategies for the screening and early detection of lung cancer
- Understand the molecular mechanisms of initiation and progression to lung cancer
- Identify innovative strategies for the treatment of lung cancer
- Identify innovative strategies for the prevention of recurrence of or metastases from lung cancer
- Develop or optimize prognostic or predictive markers to assist with therapeutic decision-making
- Understand mechanisms of resistance to treatment (primary and secondary)
- Understand contributors to lung cancer development other than tobacco
- Identify innovative strategies for lung cancer care delivery (disparities/clinical management/surveillance/symptom management)

Dollars Invested per Area of Emphasis (FY09–FY18)



Research Outcomes

Clinical Trials

Multiple LCRP-funded projects have been successful, and their results have been translated to the clinic for testing in humans or have contributed resources for use by the scientific community. Some examples include the following:

Immunotherapy treatment with mesothelin-targeted chimeric antigen receptor T cells is being tested in phase I clinical trials in multiple cancers, including lung cancer (NCT02414269).

Determination that defective apoptosis plays a large role in the emergence of resistance in lung cancer, leading to phase I clinical trial (NCT02520778) testing of a combination therapy of an apoptotic stimulator with a targeted therapy in lung cancer.

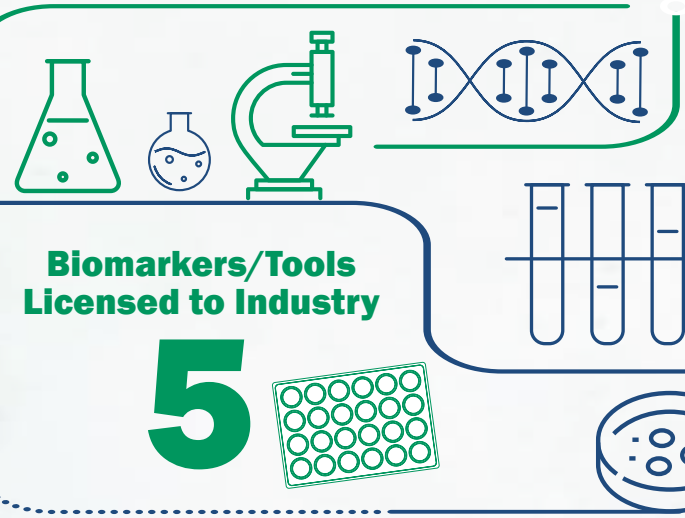
In vivo demonstration that inhibition of focal adhesion kinase (FAK) specifically inhibits high-grade lung cancer, leading to clinical testing of the FAK inhibitor defactinib in non-small cell lung cancer (NSCLC) patients harboring KRAS mutations (NCT01951690).

Very promising results from an LCRP-funded phase I trial of an immune checkpoint inhibitor plus stereotactic ablative radiotherapy in patients with inoperable stage I NSCLC (NCT02599454) have spurred the National Cancer Institute to sponsor a randomized phase III trial of stereotactic body radiation therapy with and without atezolizumab in early-stage inoperable NSCLC patients.

Study inducing metabolic crisis using combination therapy of a glutaminase inhibitor CB-839 plus an epidermal growth factor receptor (EGFR) inhibitor, erlotinib, provided rationale for a phase I clinical trial (NCT02071862).

Resources

The Lung Cancer Biorepository Network contributed hundreds of samples to the lung cancer-focused APOLLO 1 project of the Cancer Moonshot Program, which is exploring whether specific gene mutations or specific gene expression signatures are associated with disease recurrence, to enable further testing of these molecular changes as prognostic markers that can be used in clinical decision-making.



Biomarkers/Tools Licensed to Industry

5

369

Publications

Patents & Patent Applications

4

Patents

10

Patent Applications

Concept Award
(New, Innovative Ideas)

- 19 Principal Investigators funded for $2,463,120:
  - 27 Publications
  - 29 Follow-On Awards of $15,099,070
  - Return on Investment = $6.1 for every $1 invested

Strategic Partnerships

Working hand-in-hand, lung cancer researchers, physicians, and consumers ensure that the LCRP funds the most relevant, groundbreaking, and high-risk/high-reward projects. Each participant in the review process, whether consumer, physician, or scientist, has an equal voice; their thoughts are welcomed and valued by the panels on which they serve.

"The LCRP has had a lasting impact on my survival. When I was able to participate on the panel, the researchers and scientific community were incredibly supportive, knowledgeable, and, most importantly, passionate about lung cancer. I believe that the people who participated with me are leading the way in continuing to find a cure for my cancer while also keeping the patients' needs at the forefront of their research. Words cannot express my appreciation for the effort and time these people put in for us as survivors, as well as the meaning they place behind their work. I am extremely grateful for the scientists who choose to work on lung cancer because, without them, lung cancer will continue to be the deadliest cancer in men and women."

Rachael Malmberg, Consumer Reviewer

"Lung cancer claims more lives than any other cancer in the United States and affects military Veterans at higher rates than the general population. As Chief of the Hematology/Oncology Service at Walter Reed National Military Medical Center, I have directed the care of lung cancer patients who have served in every major war since WWII. And as a doctor deployed to Operation Iraqi Freedom, I have shared the same exposures that led our newest Veterans to fear for their future health. The work of the LCRP is essential for all those touched by this disease. The collaborative reviews by academic, Federal Government, military, and VA [Department of Veterans Affairs] experts ensure that funding is directed to the best science with the highest potential impact. Only through such steadfast advancements in medical science will we be able to prevent future deaths and improve the lives of those living with lung cancer right now."

COL David Van Echo, Programmatic Panel Chair

"Serving as a scientific reviewer for the CDMRP LCRP peer review panels is a highlight of my year. The panels are incredibly well balanced between scientists, translational researchers, and patient advocates. Each panel allows me to gain valuable insight into state-of-the-art techniques for lung cancer research, but also connects me to key thought leaders in the field. I have grown immensely as a scientist and clinician through these interactions. More importantly, work funded by the CDMRP has led to groundbreaking advances in lung cancer that has directly benefited all types of patients with lung cancer, particularly military Veterans. I could not be more proud to serve as a scientific reviewer for such an important cause."

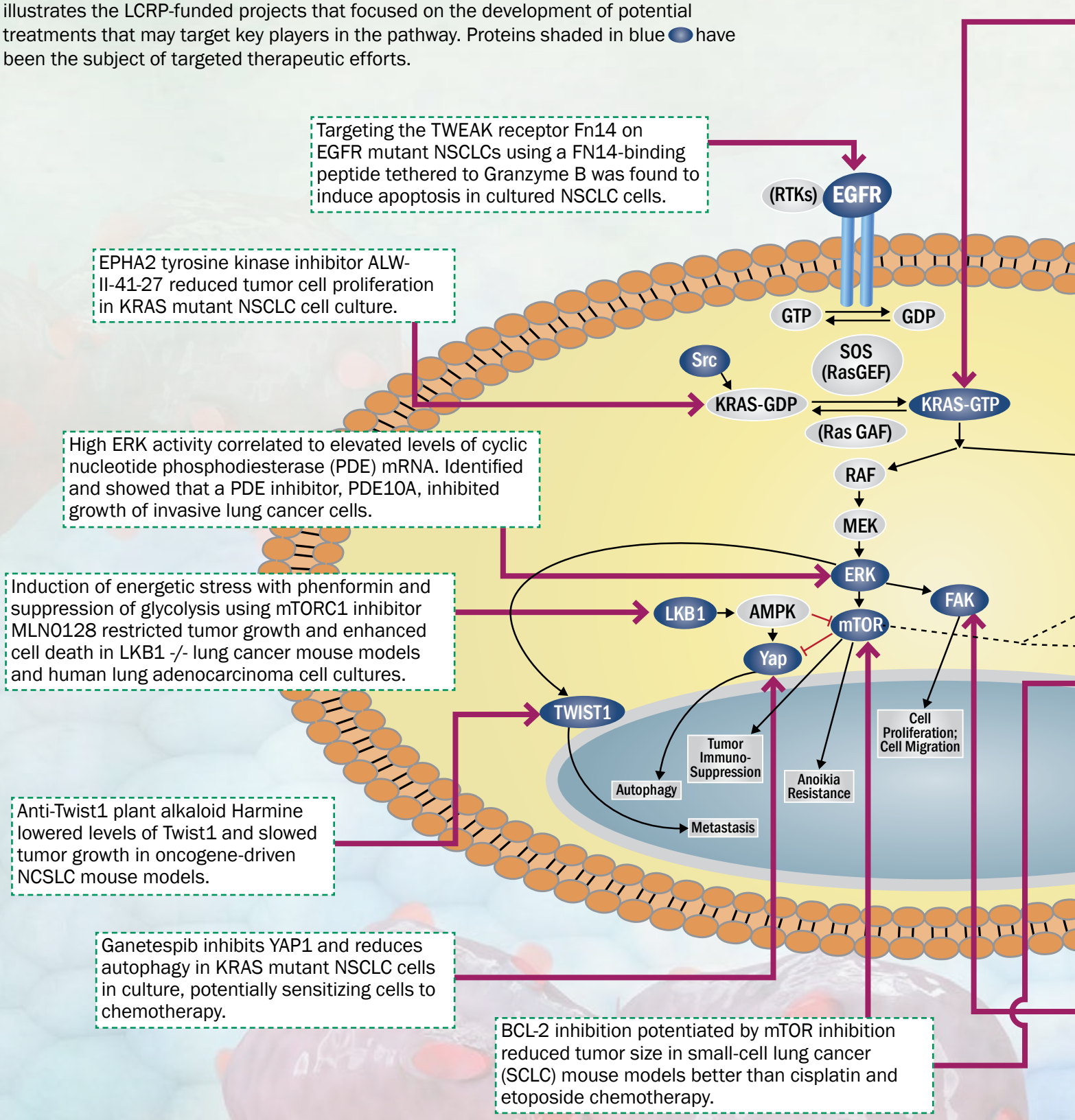
Brenden Stiles, MD, Peer Reviewer

"Why do I participate in programs like the LCRP? I do so to make my journey make sense. I do it to give my experience meaning and purpose. I involve myself in advocacy, in outreach, and in research because I want to be part of the solution, and I am so grateful that the LCRP gives me an opportunity, in an important way, to help influence the direction of lung cancer research."

Laura Greco, Programmatic Panel Consumer Reviewer

Portfolio Highlights - Novel Treatment Approaches

Since its inception, the LCRP has funded a wide range of projects focused on the oncogenic KRAS pathway. This pathway, which relays signals from the cell surface to the nucleus and is capable of prompting uncontrolled cell growth, is a key focus for the development of therapeutics in the lung cancer field. A simplified version of the KRAS pathway below illustrates the LCRP-funded projects that focused on the development of potential treatments that may target key players in the pathway. Proteins shaded in blue have been the subject of targeted therapeutic efforts.



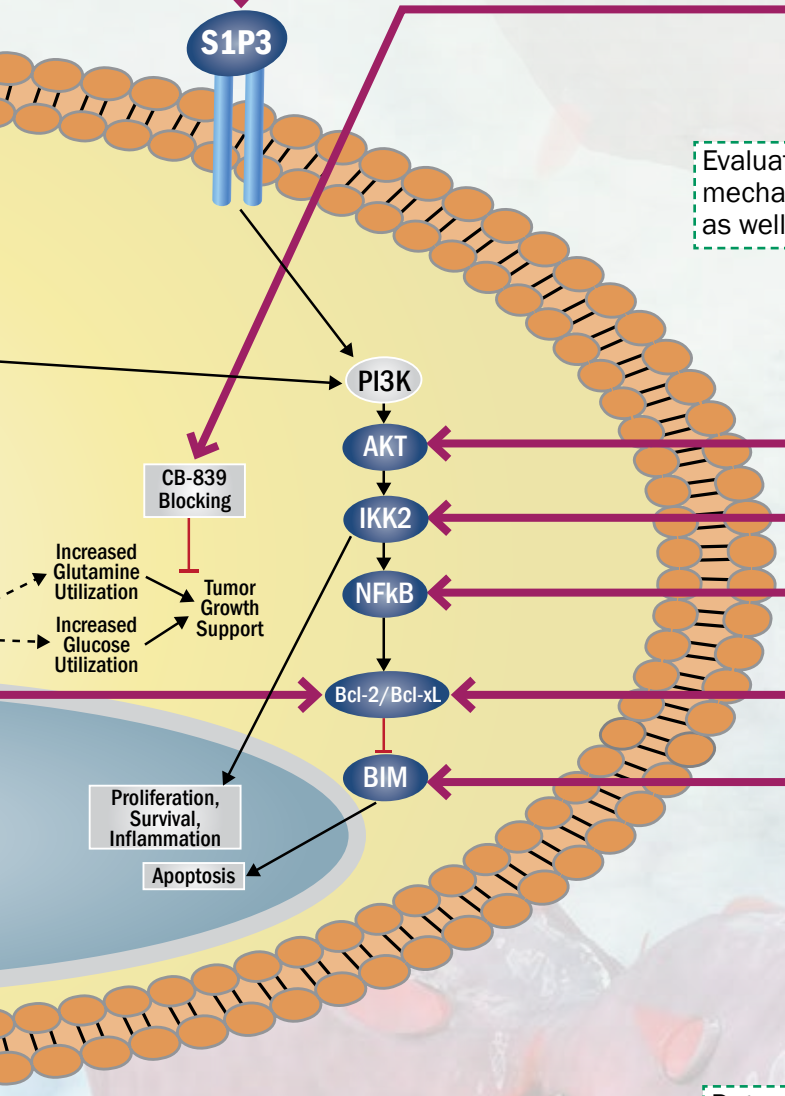
to Oncogene-Driven Lung Cancers

Refined pharmacological properties of previously developed small molecule inhibitor of KRAS G12C.

Designed rigosertib, a small molecule inhibitor that disrupts KRAS binding and downregulates KRAS signal pathway in patient-derived mouse xenografts (PDX) and a KRAS mouse model.

Developed an SIPR3 inhibitor that abrogated lung cancer tumorigenesis in vitro and in a mouse model.

Co-suppression of glycolysis and glutaminolysis with erlotinib and CB-839 induced energetic stress in EGFR-mutant tumors in vivo, activation of the AMPK pathway, and autophagy, ultimately leading to tumor cell death. Results supported the rationale for clinical trial NCT02071862.



Evaluating new chemopreventive, p-XS-Asp, to determine its mechanism of action and efficacy correlated to stage-specificity, as well as biomarkers to evaluate efficacy.

Discovered that blocking IKK2 activates inflammatory responses and reduces cell proliferation, which slowed tumor growth in KRAS G12D mice and increased their life span.

Parthenolide analogs that bind NF-κB interferes with drug resistance in cultured cancer stem cells.

Two-pronged attack by Rova-T and CBL0137, targeting DLL3 and FACT (leading to downregulation of NFkB), reduces SCLC PDX tumor volume in mice and increases survival.

Created for inhalation delivery targeted nanoparticles using a peptide that binds cancer-expressed Luteinizing Hormone Releasing Hormone Receptor and slowly releases chemotherapy agents and siRNAs designed to knock out MDR1 and BCL2.

Inhibiting or silencing FAK sensitizes KRAS mutant cancers in mice to radiation and chemotherapy. Results supported clinical trial NCT01951690.

Determined that acquired resistance to EGFR inhibitors treatment develops from loss of or resistance to apoptotic response due to loss or downregulation of BIM. Results supported rationale for clinical trial NCT02520778.


Amer A. Beg, Ph.D.


Mengyu Xie, Ph.D.

MEK Inhibition Modulates TNFα Response in Lung Cancer to Enhance Therapeutic Activity

Amer A. Beg, Ph.D., H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL

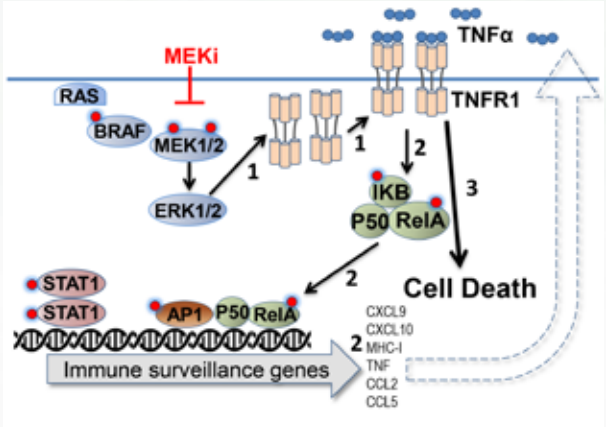
The increase in Food and Drug Administration (FDA)-approved immunotherapies for lung cancer over the past 5 years has opened a promising new avenue for treatment in many patients; in select subsets of lung cancer patients, these treatments have been very successful in improving outcomes. In general, however, immunotherapies only work in very specific variants of the disease, so a primary focus of immunotherapy bench research is to increase the response to treatment.

Dr. Amer Beg's research at the Moffit Cancer Center is focused on methods that can increase immune surveillance within tumors and therefore increase the likelihood of response to an immune-stimulating treatment. Immune surveillance refers to the ability of an individual's immune system to access a tumor, identify it as "foreign," and destroy tumor cells. This function is commonly repressed in lung cancer tumors; however, increased immunotherapy effectiveness is often associated with patients who have a more active immune surveillance response. With funding from an FY14 Idea Development Award (Established Investigator), Dr. Beg investigated the roles of specific oncogenic mutations in the suppression of the immune surveillance response and tested methods to overcome this suppression, leading to increased response to immunotherapy.

Dr. Beg's study builds off the prior discovery that MEK inhibitors (MEKi), which have been used to limit the uncontrolled growth of cancer cells, increase the number of T cells that are able to infiltrate the tumor, improving immune surveillance. While investigating the molecular pathways involved in MEK inhibition, Dr. Beg and his team found that there is cross-talk between MEKi and cytokine signaling pathways that plays a key role in triggering immune response and inflammation. Treatment with MEKi and TNFα + IFNγ significantly increased cell growth arrest and induction of cell death via apoptosis. A key underlying mechanism discovered in this study was the ability of MEKi to increase cell surface expression of TNFα receptor 1 (TNFR1).

Dr. Beg's team then compared the effect of MEKi in combination with cytokines TNFα and IFNγ or immune checkpoint inhibitors (e.g., PD-1 antibodies). The findings of these experiments demonstrated that the combined treatment (in both cases) is the most effective at reducing tumor size. However, the combination of MEKi and PD-1 antibody had the most dramatic impact on tumor growth. These results suggest that combining MEKi with agents that promote TNFα and IFNγ expression, such as checkpoint blockade or T cell adoptive cell therapy, may help achieve greater benefit in lung cancer patients than MEKi alone or in combination with direct systemic administration of TNFα and /or IFNγ, which is expected to be highly toxic.

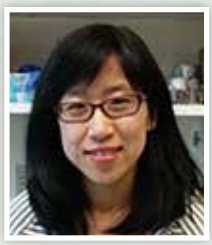
These results show significant promise for those patients hoping to have access to immunotherapies, but who may have not responded well to immunotherapy alone. By combining two FDA-approved therapies, it may be possible to improve patient outcomes and translate these results to the clinic in a much shorter timeline than completely new therapies. Dr. Beg is extending upon this idea of combination therapies to improve response to immunotherapy and has moved on to clinical trials to test the safety and efficacy of similar combination treatments.



MEKi lead to inhibition of downstream ERK kinases to increase cell surface expression of TNFR1 through a presently unclear mechanism (step 1 in figure). This in turn activates the NF-kB transcription factor (p50+RelA complex) and potentially other TNFR1 regulated transcription factors such as AP1 (step 2 in figure). The combined action of NF-kB, AP1, and IFNg induced STAT1 transcription factor leads to increased expression of immune surveillance genes. The increase in TNF expression establishes a positive feedback signaling loop with TNFR1 to augment signaling. Enhanced signaling from TNFR1 significantly increases cell death via apoptosis (step 3 in figure).

Publication:

Xie M, Zheng H, Madan-Lala R, et al. 2019. MEK Inhibition Modulates Cytokine Response to Mediate Therapeutic Efficacy in Lung Cancer. *Cancer Res* 79(22):5812-5825.



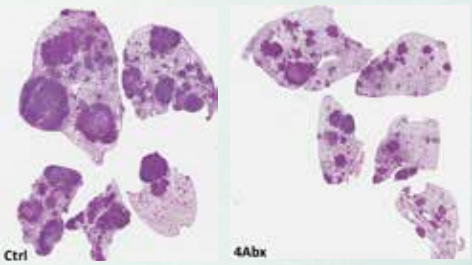
Investigating the Role of the Lung Microbiome in Non-Small Cell Lung Cancer Tumor Initiation and Progression

Chengcheng Jin, Ph.D., Massachusetts Institute of Technology

NSCLC is associated with low survival rates and accounts for about 85% of lung cancer cases. The genetic underpinnings that trigger this type of cancer have been widely explored, yet only recently are scientists aiming to understand the role of the lung microbiome in tumorigenesis. Bacterial infections occur in up to 70% of lung cancer patients and can drastically affect clinical outcomes. Although the lungs were once thought to be a sterile environment, microbial communities were recently confirmed to exist in the lower respiratory tract and can even lead to pulmonary disorders such as chronic obstructive pulmonary disease and asthma. The mucosal lining of the lungs is exposed to a wide array of foreign bacteria through inhalation, and scientists are now investigating how these diverse groups of bacteria, localized to the mucosal lining of the lung and known as the commensal microbiota, can play a role in tumor initiation and progression.

With support from an FY14 LCRP Concept Award, Dr. Chengcheng Jin from the Massachusetts Institute of Technology investigated the role of the commensal microbiota of the lung in promoting NSCLC development. To accomplish this, Dr. Jin and her team utilized a mouse model that replicates the two main drivers of human lung adenocarcinoma – point mutations in KRAS and loss of p53 function – to characterize the bacterial composition and abundance of lung microbiota associated with NSCLC. Dr. Jin's team optimized methods for collecting fluid and tumor tissue from the lungs and then extracted total DNA from the bacterial cells found within the samples. Sequencing analysis was performed to compare the bacterial DNA found in the lungs to that of bacterial DNA found in stool samples, as the gut microbiome is well-defined. The results revealed that distinct bacterial communities existed within the lung compared to the gut. Dr. Jin also discovered that development of lung adenocarcinoma was associated with increased total bacterial burden in the lung and airway tissue, as well as an alteration of the microbiota composition, compared to cancer-free mice.

The results supported by the LCRP award enabled Dr. Jin to further investigate the role of commensal microbiota in regulating tumor initiation and progression in the lung by comparing germ-free mice (mice bred and raised under aseptic conditions, lacking all microorganisms) with conventional mice with normal microbiome. In the germ-free, aseptically raised animals, both tumor burden and number were substantially decreased. Moreover, combined antibiotic treatment with ampicillin, neomycin, metronidazole, and vancomycin resulted in significantly reduced tumor burden in the conventional mice, but failed to inhibit tumor cell growth in vitro, highlighting the critical role that intact commensal microbiota in the lung play in promoting tumorigenesis. This finding also provides strong evidence that manipulation of the microbiota in the lung could lead to new treatment strategies. Currently, Dr. Jin is investigating host immune pathways involved in mediating the effect of the lung microbiome on tumor development. Using flow cytometry, various immune cells associated with lung adenocarcinoma have been characterized, and lung-resident γδ T lymphocytes were found to be particularly important in driving local inflammation and tumor promotion. Thus far, results confirmed that tumor progression is associated with increased bacterial burden and immune activation in the lung.



Treatment of specific pathogen-free KP mice (GEM model of human LUAD using conditional alleles of Kras LSL-G12D; p53 flox/flox) with combined antibiotics (4Abx) in drinking water starting at 6.5 weeks post-tumor initiation. Tumor burden and grade were analyzed at 15 weeks p.i.

The results of Dr. Jin's work, published in *Cell* (Jin, 2019), indicate that there is a clear link between the local microbiota and lung tumor development, specifically in NSCLC. The FY14 LCRP Concept Award funded work that ultimately catapulted further research, providing insights into the role of commensal microbiota in tumor pathogenesis, and could lead to new treatment strategies for lung cancer patients through targeting the microbiota of the lung.

Publication:

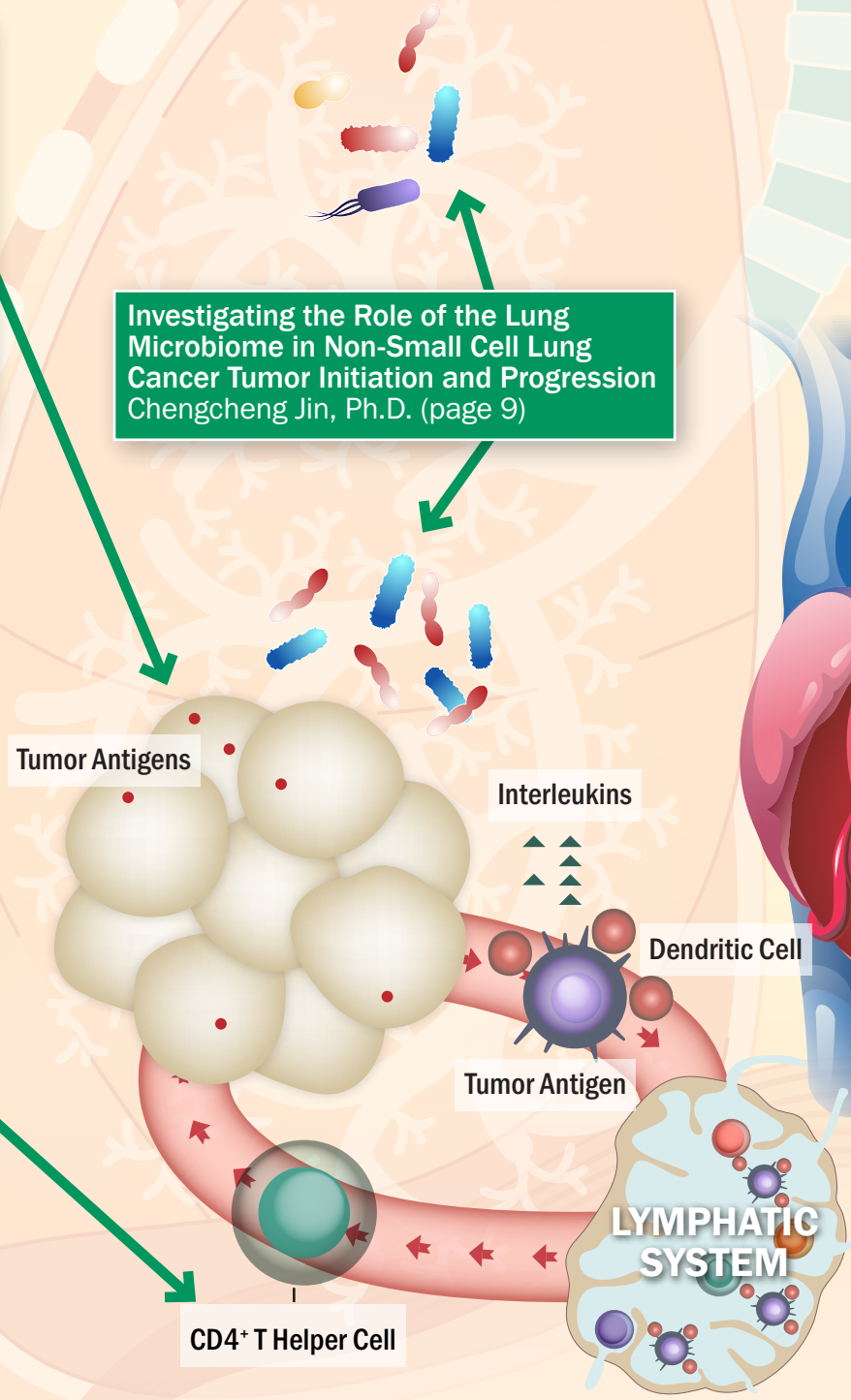
Jin C, Lagoudas GK, Zhao C, et al. 2019. Commensal Microbiota Promote Lung Cancer Development via γδ T Cells. *Cell* 176(5):998-1013.

Portfolio Highlights-Immunotherapy

Lung Cancer Vaccines
Aimed to develop a preventative vaccine that could be used to inhibit carcinogenesis in subjects at high risk for lung cancer. The Principal Investigator successfully identified 14 possible targets that are overexpressed in lung cancer and are necessary for tumor cell survival. Possible vaccine epitopes were confirmed for five of these targets and are currently being evaluated for efficacy in combination with chemotherapy in animal models of lung cancer.

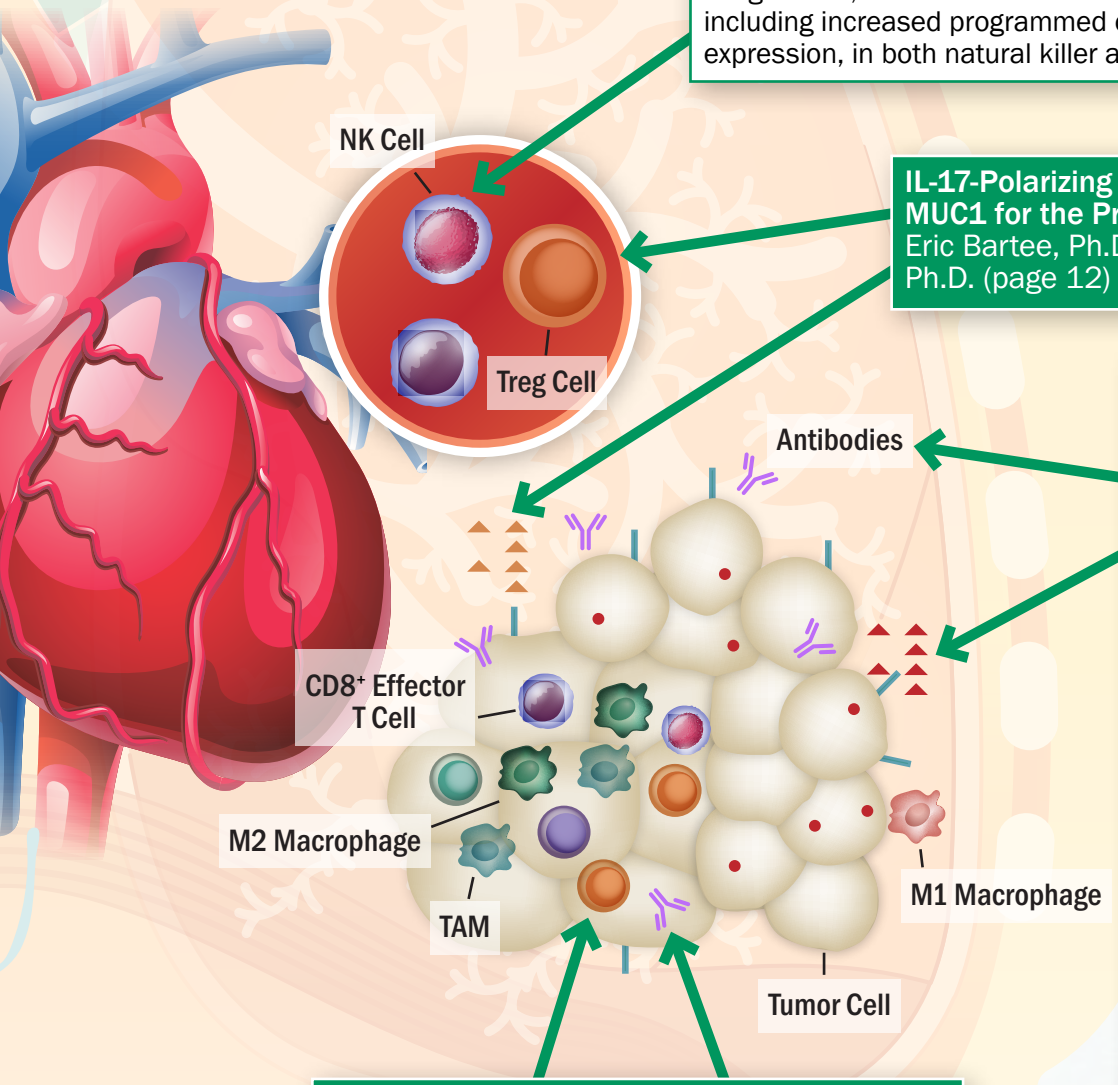
Investigating the Role of the Lung Microbiome in Non-Small Cell Lung Cancer Tumor Initiation and Progression
Chengcheng Jin, Ph.D. (page 9)

Role of Pathogenic CD4⁺ T Cells in Lung Cancer
The hypothesis for this study stemmed from the discovery that the inflammatory immune response plays an important role in lung adenocarcinoma tumor development. Results demonstrated that, by targeting a specialized subset of helper CD4⁺ T cells (Th17 cells) that is primarily found in lung tumors, it is possible to reduce tumor burden. Meanwhile, regulatory T cells found in KRAS mutant lung tumors are capable of inhibiting immunosurveillance. Finally, research funded under this award led to discovery of a novel atypical antitumor CD8 T cell that appears to prevent the infiltration of functional antitumor T cells in KRAS mutant lung cancers.



- *CD4⁺ T Helper Cell* – Key players in immunosurveillance that release interleukins in response to the expression of specific antigens.
  - Treg Cell (Regulatory T Cell) – A type of CD4+ T cell that downregulates immune response (commonly found in tumor microenvironment).
- *CD8⁺ Effector T Cell* – A cytotoxic immune cell that triggers cell death when foreign antigens are detected.
- *Dendritic Cell* – Identifing and expressing antigens and presenting them to T cells to prompt an immune response.
- *Interleukin (Cytokine)* – Signaling molecules that trigger and facilitate immune responses.
- *NK Cell (Natural Killer Cell)* – Capable of identifying and "killing" tumor cells regardless of whether these cells are expressing antigens that have been recognized by the immune system as foreign.
- *TAM (Tumor-Associated Macrophage)* – Commonly found in tumor microenvironments and believed to promote tumor proliferation and suppress the immune response to tumor invasion.
  - *M1 Macrophage* – Promotes inflammation and immune response (not typically found in tumor microenvironment).
  - *M2 Macrophage* – Contributes to suppression of antitumor immune response and tumor angiogenesis by suppressing inflammation (a common phenotype assumed by TAMs).
- *Tumor Antigen* – Unique tumor signature that can be identified by the immune system and used to selectively target tumor cells.

Nicotine Effects on the Immune System
Focusing on determining the influence that nicotine might have on the immune response to lung cancer, this project found that exposure to cigarettes, even e-cigarettes, results in increased immune suppression, including increased programmed death protein 1 (PD-1) expression, in both natural killer and T cells.



IL-17-Polarizing Vaccination Targeting MUC1 for the Prevention of Lung Cancer
Eric Bartee, Ph.D. and Adam C. Soloff, Ph.D. (page 12)

Predictive Markers for Immunotherapy Treatment
Investigated the immune microenvironment in the hope of identifying predictors of response to PD-1 targeting immunotherapies. Efforts led to a method for direct measurement of cytokine levels using mRNA probes or protein assays in fixed tissue samples, thereby overcoming difficulties associated with reliably measuring such targets. Identified two new markers, LAG-3 and IL-8, which not only may predict response to anti-PD-1 treatment, but also may serve as candidate immunotherapy targets beyond PD-1/PD-Ligand 1 (PD-L1) in NSCLC. Clinical trials targeting these molecules are currently being conducted.

Functional Characterization and Modeling of Acquired Resistance to Immune Modulation in Lung Cancer
Katerina Politi, Ph.D. (page 13)


Adam C. Soloff, Ph.D.


Eric Bartee, Ph.D.

IL-17-Polarizing Vaccination Targeting MUC1 for the Prevention of Lung Cancer

Adam C. Soloff, Ph.D., University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

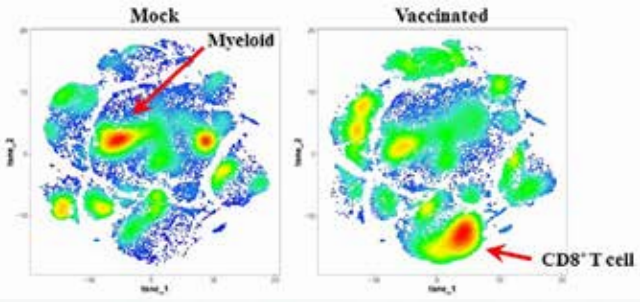
Eric Bartee, Ph.D., University of New Mexico, Albuquerque, New Mexico

Vaccines help prevent disease by exposing individuals to a safe, disease-related substance that induces the body's killer T cells to mature and antibodies to be prepared without causing infection or damage. Scientists hypothesize that vaccines targeting abnormal proteins uniquely expressed by cancer cells can heighten the immune system's ability to recognize these cells as foreign, potentially eliminating rogue cancer cells before they become tumors. One such protein is mucin 1 (MUC1), a protein that is normally expressed on cells that line the gut and respiratory system as a first line of defense from infection. As cells become cancerous, MUC1 becomes uniquely altered; this abnormal tumor-associated MUC1 is overexpressed on the surface of cancer cells (lung cancer in particular). Some MUC1-targeting vaccines paired with other lung cancer treatments are currently being studied in clinical trials and show promise in safety and efficacy, but yield no more than 6 months of progression-free survival. Dr. Adam Soloff hypothesized that the key to achieving a longer-lasting effect from lung cancer vaccines is to enhance T cell activity while targeting the abnormal tumor-associated MUC1 on cancer cells.

Supported by an FY16 LCRP Concept Award, collaborators Dr. Soloff and Dr. Eric Bartee have been making progress toward developing a vaccine that enhances the body's own immune system to attack cancer. Pro-inflammatory T helper cells that produce a protein called IL-17 (TH17 cells) are particularly long-lived, and the presence of a protein called inducible T cell costimulatory ligand could increase the antitumor function of these cells (the TH17 response) (Carrell, 2018). This project is based on the hypothesis that a vaccine capable of triggering the immune system's ability to target abnormal MUC1 would produce a persistent anti-cancer effect while also heightening the TH17 response by elevating ICOS-L levels. To test this, the researchers first created a vaccination using a genetically modified virus carrying the gene for tumor-associated MUC1 found on cancer cells. Using this "Trojan horse" means of vaccination, the injected adenovirus vaccine will cause a strong immune response as the body recognizes the virus as foreign, while simultaneously producing a large amount of the tumor-associated MUC1 target. In this manner, the body's immune system learns to mount a potent attack against the tumor-associated MUC1 target that normally generates only low-level immune reactions. When mice that are tolerant to MUC1 as a "self" protein were vaccinated, they developed strong immune responses against tumor-associated MUC1, including specialized TH17 T cell-responses and antibodies, confirming that the vaccination can generate antitumor immunity (Carrell, 2018).

Dr. Soloff and his team then created a second adenoviral vaccine that would produce ICOS-L in order to drive greater TH17 T cell maturation. When mice were vaccinated with adenoviruses producing MUC1 and ICOS-L, the immune response was enriched in TH17 cells and persisted 10 months after vaccination (Carrell, 2018). Dr. Soloff reported that the TH17 enhancement extended only to helper T cells, not to killer T cells, and also increased production of anti-MUC1 antibodies. Moreover, regulatory T cells were not activated by vaccination, so one would not expect any quelling of the immune response by their action.

As Drs. Bartee and Soloff continue their vaccine development for lung cancer, these results already provide hope that the immune system can be primed to recognize and attack cancer cells before disease occurs. Their accomplishments could represent a landmark achievement in the struggle to harness the immune system to help fight lung cancer, particularly in the development of a vaccine with a long-lasting effect.



Prophylactic Ad.M/I vaccination induced robust accumulation of CD8+ T cells within lung tumors and disease inhibition, whereas mock vaccination resulted in increased myeloid cell subsets and progressive disease.

Publication:

Carrell RK, Stanton RA, Ethier SP, et al. 2018. ICOSL-augmented adenoviral-based vaccination induces a bipolar Th17/Th1 T cell response against unglycosylated MUC1 antigen. *Vaccine* 36(42):6262-6269.

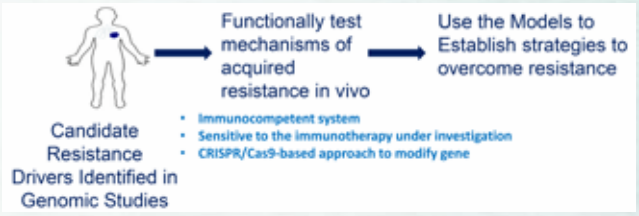
Functional Characterization and Modeling of Acquired Resistance to Immune Modulation in Lung Cancer

Katerina Politi, Ph.D., Yale University

The FDA has approved four immune checkpoint inhibitors (ICIs) for lung cancer treatment to date, including an antibody that targets anti-PD-1. PD-1 is a negative immune regulator protein expressed on the surface of T cells. When PD-1 binds to PD-L1, the T cell activity to recruit other immune cells to attack a foreign object is suppressed. Tumor cells can express PD-L1 in order to block T cell activation. Anti-PD-1 blocks tumor cells from suppressing T cell activity by binding to PD-1 first (without structurally triggering T cell deactivation), thereby preventing PD-L1 from accessing and binding to PD-1. In patients who qualify, ICIs often impede cancer growth longer than traditional treatments due to the more active immune response; however, some patients still develop acquired resistance. The mechanisms by which acquired resistance to ICIs develops in lung cancer patients are largely unknown. Once the likely mechanisms of resistance have been identified, it will be possible to make improvements to ICIs and develop ICI combination treatments that will allow the successful treatment of lung tumors with immunotherapy.

Dr. Katerina Politi from Yale University, with support from an FY16 Idea Development Award from the LCRP, is studying the cellular and molecular mechanisms that lead to acquired resistance to ICIs. She has been studying mechanisms of resistance to ICIs, including anti-PD(L)1 and anti-CTLA4 agents. Dr. Politi's team profiled tumor biopsies to assess the genomics and transcriptomic profiles of tumors before and after receiving ICI treatment. Genetic profiling (whole exome and RNA sequencing) of these samples did not demonstrate consistent gene mutations in treated tumors that were resistant to treatment as opposed to those that remained susceptible. However, acquired alterations in components of the antigen presentation and processing machinery were observed, some of which could affect human leukocyte antigen (HLA) class I antigen presentation. The HLA Class I presents antigens on the cell surface, signaling to T cells to target the cell as a foreign object, thereby activating an immune response. In particular, the gene-encoding Beta-2 microglobulin (B2M), a component of the HLA Class I antigen molecule, was either genomically lost or downregulated in a subset of tumors resistant to ICIs. From select ICI-resistant patient tumor samples, her team developed PDXs to further study B2M in vivo. Western blot and flow cytometry analysis of tumor cells from PDXs confirmed weak or lack of B2M expression, and in some, also a lack in HLA class I expression entirely, allowing the cancer cells to effectively hide from T cell recognition.

Her team then developed mouse B2M knockout lung cells via CRISPR technology and injected mice with tumor cells. These were then treated with anti-PD-1. In contrast to wild-type mice with similar tumor growth, the B2M knockout tumors continued to grow when treated with anti-PD1. This confirmed that, without B2M activity in tumor cells, cancer cells can effectively evade T cell recognition and develop resistance to anti-PD-1 treatment. These results were published in *Cancer Discovery*'s December 2017 issue (Gettinger, 2017). Dr. Politi's laboratory has been using this model of B2M-deficient lung cancer to study approaches to therapeutically target tumors that are resistant due to MHC I loss or downregulation. In addition to these studies, the genomic studies of ICI-resistant tumors have uncovered additional potential genes that contribute to resistance to ICIs. Using similar approaches used to study the role of B2M, Dr. Politi's laboratory has been functionally validating additional mechanisms of resistance to ICIs. Taken together, this research is shedding light on mechanisms of resistance to ICIs, which are likely to become an increasingly important clinical problem given the widespread use of these agents to treat patients with lung cancer.



Approach to functionally validate candidate resistance drivers in vivo and develop new models of resistance to ICIs.

Publication:

Gettinger S, Choi J, Hastings K, et al. 2017. Impaired HLA class I antigen processing and presentation as a mechanism of acquired resistance to immune checkpoint inhibitors in lung cancer. *Cancer Discov* 7(12):14201435.

Research on the Horizon:

Augmenting Antitumor T-Cell Recruitment and Activation in Localized NSCLC Tumors to Improve Checkpoint Inhibitor Therapy

Dr. Edwin Manuel, City of Hope Beckman Research Institute

Dr. Manuel aims to develop and characterize biological beacons ("bio-beacons") that can secrete chemokines and recruit cytokines to activate T cells within NSCLC tumors. This project will study the impact bio-beacons could have on T cells' and tumor cells' response rates to checkpoint inhibitor therapy.

Identifying Mutational Signatures of Military Carcinogens in Lung Adenocarcinomas of Service Members and Veterans

Dr. Zeynep Gumus, Icahn School of Medicine at Mount Sinai

This project aims to identify the entire spectrum of genomic alterations in tumors of individuals with lung adenocarcinoma who were previously exposed to jet fuel during their military service.

Exosomes in Immunotherapy Resistance

Dr. Rajagopal Ramesh, University of Oklahoma, Health Sciences Center

Dr. Ramesh's team aims to determine tumor-derived exosomes' role in therapy resistance. Specifically, the team plans to investigate whether molecular targets, such as PD-L1 and PD-1, are expressed on the surfaces of these exosomes and whether these exosomes participate in competitive binding of anti-PD-L1 or anti-PD-1 therapeutics, thereby helping tumor cells evade T cell recognition.

Identifying Metabolic Hallmarks of Cancer Initiation in Lung Tumor-Adjacent Normal Tissue

Dr. Narasimhan Rajaram, University of Arkansas, Fayetteville

This project will assess the metabolomics profile of NSCLC tumors and nearby tissue. The goal is to identify a unique signature that is indicative of cancer versus benign tissue.

Targeting ART1, a Novel Immune Checkpoint, for the Treatment of Lung Cancer

Dr. Brendon Stiles, Joan & Sanford I Weill Medical College of Cornell University

Preliminary results from Dr. Stiles' group showed that knockdown of ribosyltransferase 1 (ART1) in mouse models of lung cancer yielded an increase of CD8+ T cell infiltration and decrease of tumor burden. With this award, the research team will develop an immunotherapy that inhibits ART1 via a monoclonal antibody (mAb) and will evaluate its effect on NSCLC tumors when combined with radiation therapy.

Targeted Cell-Based Therapies for Metastatic Lung Tumors

Dr. Khalid Shah, Brigham and Women's Hospital

This project aims to improve treatment for brain metastasis of NSCLC by engineering bimodal nanobodies to target EGFR and death receptor 4 and 5 to block cell growth and inhibit apoptosis evasion while simultaneously delivering a potent variant of tumor necrosis factor apoptosis-inducing ligand to induce apoptosis of tumors.

Advancing Lineage-Directed Therapy as Personalized Medicine for Small Cell Lung Cancer

Dr. Christopher Vakoc, Cold Spring Harbor Laboratory

In preliminary work, Dr. Vakoc and his team found that SCLC tumor tissue included expression of a unique tuft cell epigenome. His team aims to characterize the unique tuft cell variant of SCLC and strategize possible targeted therapeutic approaches.

Hybrid Breath Analysis/Computer-Assisted Image Processing System for Early Assessment of Lung Nodule Malignancy

Dr. Ayman El-Baz, University of Louisville

This project aims to develop a novel clinical diagnostic system that is faster and more accurate, sensitive, and specific than current diagnostic methods by integrating data from a single low-dose CT scan and a single breath test.

A Novel Immune-Modulating Antibody for the Treatment of Lung Cancer

Dr. Edward Patz, Duke University
Dr. You-Wen He, Duke University

Together, Drs. He and Patz developed a mAb therapeutic that targets complement factor H (anti-CFH), mimicking the naturally occurring autoantibody found in high incidence in early-stage NSCLC patients who never develop recurrence or metastasis (low incidence of anti-CFH autoantibody found in late-stage cancer patients). With this award, these researchers will evaluate the effectiveness of their anti-CFH antibody to mimic its natural counterpart, induce complementary dependent toxicity, and kill tumor cells.

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