The overarching vision of the PRCRP remains the same as it has been since its inaugural year of FY09: to improve quality of life by decreasing the impact of cancer on Service members, their families, and the American public. This singular theme emphasizes the PRCRP’s strategy of funding research into cancers that may develop due to exposures relevant to unique military situations/settings and addressing knowledge gaps in cancer care that may have a profound effect on mission readiness and the health and well-being of all military beneficiaries. Through innovative mechanisms, militarily relevant focus areas, and targeted investment strategies to develop the next generation of cancer researchers, the PRCRP is answering the need to successfully promote high-impact research for cancer prevention, detection, treatment, and survivorship for Service members, their families, and the American public.
The Program Cycle

The PRCRP holds an annual Vision Setting meeting to identify research and patient care knowledge gaps and to define an investment strategy for the program’s upcoming FY. During Vision Setting, the Programmatic Panel (an expert group of scientists, clinicians, and consumers [patients or caregivers affected by cancer]) identifies the critical needs of the research and patient communities in order to advance the impact of cancer research funded by the PRCRP.

Upon receipt of grant applications, the PRCRP utilizes the CDMRP two-tier review process, which is based on the recommendations set forth in 1993 by the National Academy of Medicine, formerly called the Institute of Medicine. Peer Review and Programmatic Review have different goals, but they are complementary. Peer Review, the first tier of application evaluation, is a scientific review of individual applications measured against established criteria for determining scientific merit. Programmatic Review then compares the applications against one another, assessing the portfolio and the original intent of the program announcement to make recommendations for funding based on program goals.
Steven Silverstein serves as a consumer reviewer on the PRCRP Programmatic Panel and is a stage 4 melanoma survivor. His journey through his initial diagnosis and the ensuing treatment makes for a “long story,” as he says. At first, he was misdiagnosed, and by the time doctors realized that he had melanoma, the disease had progressed to stage 4. Steven went through months of surgeries and treatments that had no effect. Finally, he began receiving Interleukin 2 treatment at Columbia University, and after 4 months of treatment, there was no evidence of disease.

Steven’s life is quite different now. When he was undergoing the months of grueling treatment and facing a grim prognosis, he sold his company. Now he has repurposed his life, volunteering with the Melanoma Research Foundation (MRF) and serving on the board of a local hospital, in addition to other activities. In his view, “The mission as the patient voice in the melanoma community is a compelling mission.” Steven has been the MRF Chair for the last 3½ years and is striving to fulfill the organization’s mission to connect with patients and their families in meaningful and impactful ways. He is encouraged by his participation on the PRCRP Programmatic Panel. Steven has been able to meet and interact with “wonderful people from the science and government communities who work tirelessly to see the process work as perfectly as possible.” He is hopeful about the impact that PRCRP-funded studies will have on the melanoma field.
Lisa Peabody  
Pediatric Brain Tumor Consumer, Peer Review Panel

The National Brain Tumor Society (NBTS) nominated Lisa Peabody to serve as a PRCRP Consumer Reviewer for the FY16 Pediatric Brain Tumor Peer Review Panel. She became familiar with the work of the NBTS after her daughter, Caroline, was diagnosed in 2004 with a pilocytic astrocytoma in her brain stem. Caroline was 13 months old and was referred to a neurologist when she did not meet her 9-month crawling milestone. An MRI (magnetic resonance image) showed the inoperable mass. Despite starting chemotherapy within a week, the tumor grew. She became too sick to travel and was admitted into an adult radiation trial at the National Institutes of Health. Lisa knew going into the trial that her daughter was dying and there were no data for this radiation protocol on a 13-month-old with a brain tumor; it was all unknown and a gamble. Within a few weeks, Caroline’s symptoms worsened and new symptoms appeared. Lisa had hoped that her daughter would make radiation history and be a survivor. Instead, Caroline died 63 days after diagnosis. She was 15 months old. In 2004, Lisa led her first race team in the NBTS’s Race for Hope in Washington, DC, a tradition that she continues today. Lisa and her husband also began an annual golf tournament, which ran for 10 years, to benefit brain tumor research. Together, they have raised $1M for the NBTS. She was hired by the NBTS in 2016 to be their Public Policy Associate, where she helps forge relationships with members of Congress and educates people about the challenges of a brain tumor diagnosis. When Lisa joined the PRCRP Peer Review Panel, she was impressed with the program and felt that the scientific panel was detailed and particular. The reviewers’ approach to each application was thorough and thoughtful, and she “felt well-respected and acknowledged throughout the process.”

James Randolph Hillard  
Stomach Cancer Survivor and Consumer, Peer Review Panel

As a 7-year stomach cancer survivor, James Randolph Hillard, M.D., Professor of Psychiatry at Central Michigan University College of Medicine, is a committed patient advocate promoting awareness and acceptance for the stomach cancer community. He is the founder of the Michigan Chapter of Debbie’s Dream Foundation: Curing Stomach Cancer, as well as the Chair of the Medical Advisory Board for Hope for Stomach Cancer. His advocacy work has also influenced and begun to blend in with his recreational hobbies, such as his organization of “International Swim with Your Chemoport Day!”

As a consumer reviewer for the PRCRP, he was at first apprehensive about his involvement on the Peer Review Panel. “I was initially concerned that the scientists on the review panel would consider me the team mascot or something.” Thankfully, that was not the case. Dr. Hillard was an integral member of the peer review process and provided a perspective that the other panel members were eager to hear: “...they were all extremely interested in what I had to say. They felt that I had something important to add.”

Overall, Dr. Hillard’s experience as a consumer reviewer for the PRCRP was positive. “I did find that my contributions really did make a positive impact on the process.” His tireless work as an advocate for stomach cancer patients has given voice to people affected by this deadly worldwide disease, and he has been rewarded for his efforts by seeing a visible shift in public awareness of stomach cancer. “I feel that our advocacy has led to a much needed increased focus on this disease.”
Military Focus
Throughout the history of the PRCRP, congressional language has directed the amount to be appropriated, the different topic areas for research funding, and the requirement that research should be relevant to Service members and their families. As a research funding program, the most significant method the PRCRP has to influence the quality of life of Service members and their families is through creative and impactful research funding solutions that emphasize the health and well-being of this community. In order to continue to address the critical needs of Service members and their families, the PRCRP includes a requirement that all research must address at least one militarily relevant focus area (see text box).

In the first of the two focus areas, health risks associated with unique military environments may be investigated. The Department of Veterans Affairs (VA) has acknowledged that certain exposures increase the cancer risk of Service members and their families. Areas of deployment across the world present multiple hazards. Service members perform their duties in both developed and developing nations. Exposure related to cancer risks include, but are not limited to, chemical weapons, including storage; ionizing radiation; herbicides; electromagnetic fields; jet fuel; organic materials; biological agents; ultraviolet (UV) radiation; etc. Depending on the deployment environment, exposures and risk will vary. For example, in the developing world, the use of exposed asbestos as a building material puts Service members at risk of such cancers as mesothelioma and lung cancer, as well as cancer of the larynx, pharynx (throat), stomach, colon, and rectum. The environment in which Service members serve may lead to increased incidence of carcinogenesis years or decades later and should be researched and the risks understood and mitigated.

The second potential military focus area of investigation is within the cancer care spectrum (see Cancer Care Spectrum [CCS], page 9). A cancer diagnosis of a Service member affects not only the individual Soldier, Airman, Marine, or Sailor, but every part of the unit and mission as well. Each Service member plays a critical role in mission readiness. A Service member at risk or under treatment decreases the mission-ready state of the unit. This also extends to the Service member’s family. When a Soldier’s support system is threatened because a family member is facing a diagnosis of cancer, the Soldier is affected too. The illness may lead to a request for transfer, exceptional status, or even separation, all of which impact mission readiness. Therefore, the second focus area strives to answer this call by targeting knowledge gaps and funding areas of research that represent dire needs so that Service members can be ready when called to duty. A healthy family unit, free of serious illness, allows the Service member to focus on his or her role as a Warfighter and facilitates the overarching military mission.

The PRCRP Militarily Relevant Focus Areas

- Militarily relevant risk factors associated with cancer (e.g., ionizing radiation, chemicals, infectious agents, and environmental carcinogens)
- Gaps in cancer prevention, screening, early detection, diagnosis, treatment, and/or survivorship that may affect the general population, but have a particularly profound impact on the health and well-being of military members, Veterans, and their beneficiaries

With these different militarily relevant focus areas, the PRCRP is poised to answer the call of Congress and advocates to ensure a healthy and cancer-free military.
The PRCRP funds a variety of cancer topic areas, as designated by Congress (see table below). Four topic areas were offered in FY09, the inaugural year of the PRCRP. The number of topic areas funded by the PRCRP has steadily increased since then; there are 14 designated topic areas in FY17. It is important to note that the topic areas provided in a single year are not guaranteed to be present in subsequent years, and the congressional language used to describe a topic area may be modified from year to year.

<table>
<thead>
<tr>
<th>Topic Area (FY appropriation):</th>
<th>FY09 ($16M)</th>
<th>FY10 ($15M)</th>
<th>FY11 ($16M)</th>
<th>FY12 ($12.8M)</th>
<th>FY13 ($15M)</th>
<th>FY14 ($25M)</th>
<th>FY15 ($50M)</th>
<th>FY16 ($50M)</th>
<th>FY17 ($60M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Cancers</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer in Children, Adolescents, and Young Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancers Related to Radiation Exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal Cancer</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Genetic Cancer¹</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney Cancer²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Listeria Vaccine for Cancer³</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma/Skin Cancer⁴</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloproliferative Disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Invasive Ablation⁵</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatic Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pediatric Brain Tumors</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pediatric Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiation Protection Utilizing Nanotechnology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Topic area includes FY09 congressional language: genetic cancer and its relation to exposures to the various environments that are unique to the military lifestyle, as well as FY10 congressional language: genetic cancer research and genomic medicine.
² Kidney cancer research will be funded under the Kidney Cancer Research Program, which is new for FY17.
³ In FY17, the congressional language for the Listeria topic area was changed from “Listeria Vaccine for Cancer” to “Listeria-based Regimens for Cancer.”
⁴ Topic area includes FY09 congressional language: melanoma and other skin cancers as related to deployments of Service members to areas of high exposure, as well as FY10-FY17 congressional language: melanoma and other skin cancers.
⁵ Non-invasive cancer ablation treatment, including selective targeting with nanoparticles.
PRCRP Investment

Topic Area Investment

The PRCRP strives to fund the best research across each year’s designated topic areas and aims to fund projects across all topic areas.

Total Amount Invested in Each Topic Area FY09-FY16 (% of Portfolio)

- Melanoma/Skin Cancer: 20%
- Pediatric Cancer: 6%
- Stomach Cancer: 8%
- Pancreatic Cancer: 8%
- Liver Cancer: 8%
- Colorectal Cancer: 9%
- Bladder Cancer: 3%
- Blood Cancers: 7%
- Cancers-Radiation Exposure: 1%
- Genetic Cancer: 8%
- Immunotherapy: 5%
- Kidney Cancer: 6%
- Listeria Vaccine for Cancer: 1%

No full applications were submitted for Radiation Protection Utilizing Nanotechnology.

Funding Mechanism Investment

The PRCRP uses a variety of different funding mechanisms to address the research priorities identified during the annual Vision Setting meeting. This includes investing in early career investigators to establish the next generation of researchers; innovative, potentially high-impact projects that address research gaps in each topic area; and team projects that foster collaboration within and/or between institutions. In recent years, the PRCRP renewed efforts to push forward the translation of bench research to the clinic by offering translational awards. Additionally, all mechanisms offered in FY17 required PIs to discuss the military relevance of the submitted applications.

Total Amount Invested in Each Type of Funding Mechanism FY09-FY16 (% of Portfolio)

- Innovative Exploratory: 40%
- Collaboration: 19%
- Translational: 19%
- Career Development: 28%
The Cancer Care Spectrum (CCS) provides a framework to succinctly categorize the types of research funded by the PRCRP: biology/etiology, prevention, diagnosis/detection, prognosis, treatment, and survivorship. From the basic research of biology and etiology through the more applied research of prevention to survivorship, the impact of research in each category affects progress in all the others. The CCS is a useful tool to assess in which areas the PRCRP portfolio has most strongly invested, identify gaps in research, and determine areas that may need more resources. It can also be used to visualize the state of the science across the different topic areas in which the PRCRP invests.

For example, the field of melanoma is relatively advanced. There are effective screening methods available that lead to melanomas being diagnosed at an early stage of disease. Additionally, recent advances in immunotherapies have led to prolonged survivorship, even for patients diagnosed at late stages of disease. The state of the science is more immature for other cancers. Pancreatic cancer is often not diagnosed until the late stages of disease, and the current standard-of-care therapies are rarely effective. With pediatric brain tumors, a tumor may be diagnosed at an early disease stage, but the therapies are either ineffective or, if effective, leave survivors with devastating developmental and psychological side effects. Using the CCS, the program can classify the knowledge gaps and strategically plan the investment for PRCRP funds for each topic area and each part of the spectrum.
PRCRP Topic Areas Along the Cancer Care Spectrum

Examples of research gaps along the CCS

Risks factors for many cancers are well understood. For example: exposure to asbestos in mesothelioma; UV damage in melanoma; *H. pylori* infection in stomach cancer; hepatitis infection in liver cancer; etc. Yet, the known risk factors and exposures do not explain the etiology of all subtypes of each cancer, leaving gaps in this area, including the possible identification of other risk factors and exposures.

Biology and etiology studies provide important information that guides research further along the entire spectrum. For example, the etiology of many pediatric cancers is not well understood, complicating research into other areas along the CCS, like prevention and detection.

Pancreatic, liver, mesothelioma, and stomach cancers are frequently not diagnosed until the advanced stages of disease or advanced age. This severely limits a patient’s therapeutic options and leads to poor survivorship.

Five-year survival rates*:
- Pancreatic Cancer – 8.7%
- Mesothelioma – 9.1%
- Liver Cancer – 17.6%
- Stomach Cancer – 31.1%

* Based on SEER (Surveillance, Epidemiology, and End Results) data from patients diagnosed between 2007-2013
Cancers with moderate survival rates (bladder, colorectal, and lymphoma) have treatment options that work in the majority of patients. However, additional research is needed to understand and differentiate between patients who do and do not respond to particular therapeutic strategies in all cancers.

Five-year survival rates:
- Bladder Cancer – 78.0%;
- CRC – 65.6%;
- Lymphoma – 90.2% (diagnosed between ages 0-19), 59.8% (diagnosed over the age of 65)

The field of immunotherapy, which includes the use of Listeria vaccines, is advancing rapidly. The Food and Drug Administration (FDA) approved the first checkpoint inhibitor for use in melanoma patients in 2011. Then in June 2017, for the first time, the FDA approved the use of the checkpoint inhibitor, pembrolizumab, in patients with specific genetic features, rather than basing its approval on a specific cancer type. However, research is still needed to extend these exciting advances to more patients and a wider subset of cancers.

It is not uncommon for survivors to face post-cancer issues; yet these issues, and how to help patients deal with them, are often underfunded areas. Colorectal Cancer (CRC) survivors may have to cope with the stigma associated with having temporary or permanent ostomies. Pediatric, adolescent, and young adult cancer patients undergo chemotherapy and radiation treatments that may lead to long-term side effects on health (cardiovascular, neurocognitive, fertility, etc.).
Investigating Novel Treatment Strategies for Virus-Associated Lymphomas

Zhiqiang Qin, M.D., Ph.D., Louisiana State University Health Sciences Center–New Orleans

Kaposi sarcoma-associated herpes virus (KSHV) can cause several types of cancers, including primary effusion lymphoma (PEL). The prevalence of KSHV in the population is relatively low in the United States, but can be found at very high rates in certain countries of sub-Saharan Africa, according to the Department of Health and Human Services. With limited therapeutic strategies for high-mortality lymphomas, there is a significant need to understand the mechanism of KSHV carcinogenesis. In FY14, Dr. Qin received a Career Development Award under the Blood Cancer topic area to investigate the role of hepatocyte growth factor (HGF) and its receptor (c-Met) in PEL pathogenesis. Using an immune-deficient xenograft mouse model, Dr. Qin is testing whether a selective small-molecule inhibitor of c-MET, PF-2341066, can slow PEL progression and reduce established tumors. PF-2341066 reduced HGF production, and treatment with PF-2341066 effectively prevented PEL expansion in a xenograft model. Notably, there have been eight peer-reviewed publications based on outcomes from this research, including results published in Blood, demonstrating that targeting the HGF/c-MET pathway induced KSHV and PEL cell apoptosis through cell cycle arrest and DNA damage. Dr. Qin hopes to use these results to support future clinical trials that will evaluate strategies to target HGF/c-MET for the treatment of deadly, virus-associated lymphomas.

Skin cancer remains a serious disease burden worldwide. New estimates from the World Health Organization state that one in three cancers diagnosed are a type of skin cancer. Within the United States, one in five Americans is expected to develop some form of skin cancer. Military personnel are also particularly vulnerable to these cancers due to occupational exposure at duty locations with high UV radiation.

Dr. Wu is an early career investigator developing tools to identify new druggable targets for melanoma therapies, focusing specifically on proteins that interact with oncogenic proteins in melanoma tumors. With an FY12 Career Development Award, Dr. Wu used chemical probes that target palmitoyl acyltransferases (PATs). PATs are proteins that are responsible for adding a 16-carbon fatty acid palmitate to proteins, which is a required step for activation and translocation to the cellular membrane for some proteins, including NRAS. Overactivation of NRAS may contribute to the genesis of cancer. With the chemical probes Dr. Wu developed, he identified PATs that had increased activity in melanoma cells with activated NRAS. Interestingly, only one of the top four PATs with elevated enzymatic activity in these cells also showed elevated mRNA expression.

To test whether targeting highly active PATs rather than highly expressed PATs could result in more effective inhibition of NRAS signaling, Dr. Wu developed NRAS mutant cell lines with reduced expression of five candidate PATs with varying activity and expression levels. From these experiments, only DHHC5, a PAT with elevated enzymatic activity but no significant overexpression, was able to efficiently block NRAS palmitoylation and membrane localization. However, knockdown of the highest-expressing PATs with low activity in melanoma cells had little to no effect on NRAS modification. Furthermore, melanoma cell lines with reduced expression of DHHC5 also showed reduced downstream NRAS signaling. This research project has uncovered a critical component for NRAS activity within melanoma cells and a potential target for future drug development. Importantly, the results from this project also present a strong case for profiling proteins based on their activity within tumor cells to identify new cancer drug targets.

1 http://www.who.int/uv/faq/skincancer/en/index1.html
A Novel Therapeutic Strategy to Target Colorectal Cancer

Jae-Il Park, Ph.D., University of Texas MD Anderson Cancer Center

CRC is the third most frequently diagnosed cancer among VA patients, according to the VA Central Cancer Registry. Development of novel colorectal interventions is thus highly relevant to military Service members, Veterans, and their beneficiaries. Better treatment options and outcomes are possible with early detection, but survival of late-stage disease remains poor.

With support from an FY14 Career Development Award, Dr. Jae-Il Park and his research team demonstrated that transmembrane protein TMEM9 is up-regulated in CRC cells and amplifies Wnt signaling. Overactivation of Wnt signaling may lead to CRC; however, due to the essential role of Wnt in normal cellular function, it is not a viable target. Using genetically engineered mouse models, Dr. Park’s team showed that increased expression of TMEM9 initiated intestinal tumorigenesis. Furthermore, complete loss of TMEM9 expression could protect mice from developing genetically induced colon cancer. The researchers hypothesize that inhibition of TMEM9 might block aberrant Wnt signaling, thus suppressing oncogenesis. Exploiting the novel target of TMEM9 could lead to improved treatment options for patients with CRC.

Blockade of TMEM9 suppresses CRC.
A. TMEM9 knockout inhibits lethal phenotype of CRC mouse models.
B. Low expression of beta-catenin (indicated by reduction of yellow stain), a key molecule of Wnt signaling in TMEM9 knockout tumors. Areas inside the green boxes on the upper and lower left panels are magnified in the upper and lower right panels, respectively.
Integrated Microfluidic Magnetic CTC Sorter and Enumerator for Early Diagnosis and Management of Pancreatic Cancer

Sunitha Nagrath, Ph.D., University of Michigan

Exposure to certain chemicals and radiation puts our Service members and Veterans at an increased risk for developing pancreatic cancer. However, due to the lack of effective screening tools, the majority of pancreatic cancer cases are not diagnosed until the cancer has already metastasized, resulting in a 5-year survival rate of only 8.2%, based on SEER data from 2007-2013. The most prevalent form of pancreatic cancer, pancreatic ductal adenocarcinoma, is particularly aggressive and rarely responds to conventional or targeted therapies. Additional challenges in obtaining pancreatic tissue at time of diagnosis and/or during treatments hamper efforts to better understand pancreatic cancer biology and disease progression and to develop better therapeutics.

It is well established that primary tumors shed circulating tumor cells (CTC) into the bloodstream. These cells exhibit properties of the primary tumor and eventually lead to metastasis by seeding tumors at secondary organ sites. CTC can be isolated from patient blood samples, allowing clinicians to gather important information about tumor pathology, genetics, and treatment response without invasive biopsy procedures. The leading challenge in developing CTC tools is the rarity of the cells, which have a frequency as low as 1 CTC per 1 billion blood cells. Traditional methods for isolating CTC from patient blood samples often involve extensive processing that prevents downstream analysis of the CTC once isolated. Dr. Nagrath received an FY12 Career Development Award to develop a cell-sorting device that (a) rapidly processes blood samples, (b) yields highly purified CTC, and (c) maintains the viability of the CTC for downstream cell culture and analysis.

The cell-sorting device combines two cell separation techniques into one platform—the first stage separates the typically larger CTC from smaller blood cells by size, and the second stage uses antibody-bound magnetic beads that recognize a tumor cell-specific protein marker (EpCAM) to further purify the CTC from the blood cells. This cell-sorter has the advantage, compared to previous methods and devices, that the enriched population of CTC is still viable for a variety of downstream analyses, such as gene expression analyses and cell culture. To validate the dual-phase device, normal human blood samples were spiked with known amounts of pancreatic cancer cells and run through the cell-sorter. These control experiments demonstrated that the final population of cells collected from the device were in fact highly purified pancreatic CTC (an average of 83% of the final sample was composed of CTC); over 90% of the CTC present in the initial sample were captured; and this version of the cell-sorter had an approximate limit of detection of 3 CTC per milliliter (1 cm³) of whole sample. These results were published in 2016. With the proof of concept established, more sophisticated studies ensued to develop the utility of the device for clinical assessments of pancreatic cancer.

Dr. Nagrath conducted a small-scale study using blood samples from eight pancreatic cancer patients to compare gene expression across CTC isolated from patients who either had locally advanced cancer or metastatic disease. The results demonstrated clear differences in the levels of gene expression between the two groups (local versus metastatic). In a second study, refinement of the magnetic sorting module of the microfluidic device allowed separation of pancreatic cancer cells into subpopulations, based on the level of EpCAM expressed on the cell surface (low, moderate, or high). When the isolated subpopulations of cancer cells were grown in tissue culture, they demonstrated a range of growth and migration properties. Cells expressing low levels of EpCAM tended to grow more slowly, while cells expressing high levels of EpCAM grew more rapidly. Analysis of six pancreatic cancer patient samples, using this refined cell separation approach, showed that each patient displayed a unique distribution of CTC across the different subpopulations of EpCAM expression.
The full potential and implications for this device have not been completely elucidated. However, these early studies provide the foundation necessary to translate this device into a clinically meaningful tool. Method validation could lead to the device being a non-invasive tool for regular screening of high-risk individuals, potentially catching the disease at an earlier, more treatable stage. Characterization of CTC gene expression may provide clinicians an important tool for matching a pancreatic cancer patient to a treatment regimen that is best suited for their specific tumor. Developing a more complete understanding of how EpCAM expression relates to disease status could give clinicians better prognostic information or allow them to see how/whether a patient is responding to treatment. These are just a few gaps along the cancer capability spectrum that this device could address in the care of pancreatic cancer patients. Dr. Nagrath is continuing her research to refine the capabilities of the cell-sorter, better define the characteristics of pancreatic cancer CTC, and determine more explicitly the biological and clinical significance of CTC.

Plasma Metabolomic Fingerprint of Early Gastric Cancer

Ying Bao, M.D., Sc.D., Brigham and Women’s Hospital, Inc.

The majority of patients diagnosed with gastric cancer present with incurable, late-stage disease because early-stage gastric cancer is often asymptomatic, and there are currently no established screening tests. A major risk factor for developing gastric cancer is exposure to the bacterium Helicobacter pylori. According to the Centers for Disease Control and Prevention, H. pylori infection rates are greater in developing countries compared to developed nations, meaning that US Service members deployed outside of the United States are at greater risk of H. pylori exposure and subsequently at risk of developing gastric cancer.¹ With an FY15 Career Development Award, Dr. Bao is using patient plasma samples to measure systemic metabolic changes associated with gastric cancer growth. If successful, she would identify the first-ever metabolomic fingerprint that could be used to detect gastric cancer at its earliest stages, when a cure is still attainable. Moreover, identification of gastric cancer-associated metabolites and their pathways could provide new targets for preventive and therapeutic interventions, as well as tailored therapies based on patient-specific metabolic fingerprints.


Defining Hepatocellular Carcinoma Subtypes and Treatment Responses in Patient-Derived Tumorgrafts

(Shown from left to right)
Akbar Waljee, M.D., Veterans Education and Research Association of Michigan; Daniel Siegwart, Ph.D., Amit Singal, M.D., Adam Yopp, M.D., Hao Zhu, M.D., University of Texas Southwestern Medical Center

Hepatocellular carcinoma (HCC) is the fastest growing cause of cancer-related death in the United States. Furthermore, according to the VA, the incidence of HCC has more than tripled among US Veterans over the past 10 years. Fewer than 15% of patients with HCC respond to curative therapies, and the vast majority are treated with systemic and/or locoregional therapies that extend survival by months, not years. HCC is poorly understood on the molecular level, partly because tissue biopsies have not been evaluated for over 80% of the HCC population. To increase understanding of this disease, Drs. Zhu, Siegwart, Singal, Yopp, and Waljee plan to analyze a large collection of patient-derived xenograft (PDX) models of HCC tumors, in which human liver cancers are implanted, grown, and studied in mice. The researchers, with an FY15 Translational Team Science Award, are investigating the human-mouse PDX models’ susceptibility to experimental treatments using small RNA inhibitors or microRNAs that target essential pathways in HCC pathogenesis. Together, the research team has designed this project to differentiate between early versus advanced HCCs and determine whether different stages of HCC have distinct signatures, which could contribute to a specific therapeutic response. The researchers hope their data will lead to the establishment of a platform for studying experimental therapeutics and, ultimately, to the development of patient-specific therapeutic regimes that attack the molecular vulnerabilities of HCC to improve patient outcomes.
**Targeting Neuroblastoma with Expanded Autologous NK Cells**

(Shown from left to right)
Dean A. Lee, M.D., Ph.D., Nationwide Children's Hospital
Mitchell S. Cairo, M.D., New York Medical College
Robert C. Seeger, M.D., M.S., Children’s Hospital Los Angeles

Neuroblastoma (NB) is the most common extracranial solid tumor in children, affecting civilian populations as well as children of active duty personnel. Current treatments include chemotherapy, surgery, and radiation, which can be followed by immunotherapy. Children with high-risk NB have less than a 50% chance of survival, as this cancer frequently gains resistance to current treatments. With the support of an FY16 Translational Team Science Award, Drs. Lee, Cairo, and Seeger will assess the utility of targeting NB, particularly chemotherapy-resistant NB, with autologous expanded peripheral blood activated natural killer (aNK) cells. Natural killer (NK) cells are a component of the innate immune system capable of killing a variety of cancer cells. This research group developed a system for removing NK cells from NB patients, activating and growing the NK cells to increase their number and improve function, and then returning the aNK cells to the patient. By leveraging samples collected through a multi-institutional clinical trial, the researchers will assess how these aNK cells behave in conjunction with anti-GD2 antibody treatment alone or in combination with lenalidomide, an NK cell activator. They will then assess whether the aNK can be engineered to improve NB targeting and overcome resistance. Results from this study could also be applied to other cancers of civilian and military importance that are sensitive to NK cells or express GD2, including sarcomas and melanoma.
Epha2-/- NK Cell Therapy Against Malignant Pleural Mesothelioma

(Shown from left to right)
Nasreen Najmunnisa, Ph.D., University of Florida; Co-Investigators, Frederic Kaye, M.D., and Kamal Mohammed, Ph.D., University of Florida

Malignant pleural mesothelioma (MPM) is a rare and aggressively fatal cancer with few treatment options and a short life expectancy for patients. The VA acknowledges that Service members and their families are at risk for developing mesothelioma due to previous exposure to asbestos. The development of new treatment options for mesothelioma patients is therefore of critical importance to the military health community. One emerging target for novel therapeutic development is innate immune surveillance, an essential element used to defend the body from invaders such as microbes and tumor cells. NK cells are one branch of these “first-responders” that are able to seek and destroy their targets on site without additional sensitization from other immune cells. NK cells’ cytotoxic function is triggered when the presence of activating stimuli overwhelm the inhibitory stimuli present at the site of action. This cytotoxicity makes NK cells an attractive immunotherapeutic tool; however, their utility in the clinic has stalled due to limited long-term antitumor benefits. The need to develop methods for boosting NK cell function and to train them to more effectively target cancer cells is essential.

With an FY14 Idea Award with Special Focus, Dr. Najmunnisa and her team, Drs. Kaye and Mohammed, have isolated and modified NK cells from EphA2-deficient mice to make them more effective at destroying tumor cells. EphA2 is an oncogenic member of the receptor tyrosine-kinase family of proteins that is overexpressed in many types of cancers. In preliminary studies, Dr. Najmunnisa observed that tumors transplanted into mice lacking the EphA2 gene grew more slowly than those transplanted into wild-type mice. She hypothesized that this slowdown could be due, in part, to a more effective clearance of the tumor by the immune system (Figure 1). Examining the cytotoxic potential of NK cells from these mice, she observed that EphA2 knockout NK cells (EphA2-/- NK) have increased expression of activating receptors and decreased inhibitory receptors when compared to wild-type cells. This difference in receptor expression likely makes the EphA2-/- NK cells more sensitive to activating stimuli and likely more cytotoxic. Furthermore,
in co-culture experiments with cancer cells, the EphA2-/‐NK cells showed greater lysis of cancer cells, with up to a 40% increase of target cell death. These NK cells also had a significant increase in pro-inflammatory cytokine expression and production of cytotoxic granules, supporting the idea that EphA2-/‐NK cells could produce a more robust response to – and a superior cytotoxic effect against their target than – unmodified NK cells.

Additionally, Dr. Najmunnisa engineered these highly cytotoxic NK cells with the ability to target mesothelioma. Mesothelioma cells express high amounts of mesothelin, which may be recognized by chimeric antigen receptors (CAR) when expressed by immune cells. By engineering NK cells that express mesothelin-recognizing CAR, Dr. Najmunnisa developed NK cells with enhanced cytotoxicity that preferentially target mesothelin-expressing cells. When cytotoxicity for these NK cells was tested against MPM cell lines in culture, the amount of MPM cell death tripled in comparison to NK cells lacking the anti-mesothelin CAR. Now, in the final year of her award, Dr. Najmunnisa plans to test these enhanced NK cells in clinically relevant mouse models of mesothelioma. If successful, her findings have the potential to improve the cytotoxicity of NK cell-based immunotherapies and provide a pathway toward developing a therapeutic for mesothelioma, for which no FDA-approved targeted therapies currently exist.

**Intraventricular Delivery of Engineered Oncolytic Herpes Simplex Virotherapy to Treat Localized and Metastatic Pediatric Brain Tumors**

Gregory K. Friedman, M.D., University of Alabama at Birmingham, Children’s of Alabama

Medulloblastoma (MB) is a high-grade aggressive tumor that is diagnosed in 250-500 children in the United States each year. The survival rates vary depending on the age of the child at time of diagnosis and whether the tumor has metastasized to the spinal cord. If the disease has remained localized, then the 5-year survival rate is 70%-80%; if it has metastasized, the rate goes down to about 60%.

Further complications of this disease are that the current standard of care—a combination of surgery, radiation, and chemotherapy—can have long-term consequences for a survivor's physical and mental well-being. Thus, the development of effective, less toxic therapies for MB and other high-grade tumors represents an unmet need in pediatric brain tumors.

The development of oncolytic herpes simplex virotherapy (oHSV) to destroy tumors offers a promising avenue as a targeted therapeutic option. oHSV employs a DNA virus that has been engineered to prevent infection of normal cells, but will infect tumor cells. The virus replicates in the tumor cells and causes the cells to rupture; new virus particles are then released that can infect other tumor cells. Additionally, it is possible to engineer the oHSV to produce inflammatory products. The inflammatory products are released into the tumor microenvironment when the tumor cells rupture and can elicit the host’s immune system, further promoting the tumor-fighting properties of the virus. Multiple Phase I trials in adult brain tumors have proven that oHSV can be used safely in humans, and a pediatric trial is ongoing (clinicaltrials.gov NCT02457845). The current major limitation of oHSV is that it must be delivered directly into the tumor, necessitating an invasive brain surgery and preventing multiple doses from being administered.

Delivery of oHSV into the spinal fluid (via intraventricular [IVT] delivery) offers several advantages. The need for invasive surgery is reduced, and multiple doses can be given to combat both the primary tumor and spinal metastases. Additionally, IVT delivery eliminates the problem of the virus crossing the blood–brain barrier. However, there are toxicity concerns involved with IVT delivery of oHSV. Dr. Friedman received a Career Development Award in FY14 to determine the underlying cause of toxicity using mouse
models. He is testing the hypothesis that the toxicity is due to an inflammatory immune response to the HSV and that it can be prevented by administering an immune regulator that reduces this response.

To characterize the nature of the toxicity that arises due to IVT delivery of oHSV, Dr. Friedman injected normal mice (i.e., the mice had a functioning immune system and did not have MB) with one of two different strains of live oHSV (G207 or M002), inactivated M002 virus, or inert components of the injection (e.g., saline solution or glycerol). Only the mice injected with live virus developed toxic reactions, indicating that toxicity is due to the live virus and not viral antigens (markers recognized by a host that stimulate an immune response) or other components of the injection. Interestingly, M002, which produces an immune stimulatory molecule not produced by G207, induced a stronger toxic reaction. Dr. Friedman is currently investigating the mechanisms driving these different reactions.

For a second set of experiments, Dr. Friedman injected normal mice with a lower dose of either G207 or M002, and neither virus elicited a toxic response. Based on these promising observations, Dr. Friedman tested the lower dose of G207 in a mouse model of MB. The mice treated with the lower dose of G207 exhibited prolonged survival and reduced spinal metastases compared to untreated mice (see figure). Ongoing studies seek to confirm and expand upon these observations in additional models of MB.

During the first year of this award, Dr. Friedman made the key observation that toxicity observed with IVT administration of oHSV is dose-dependent (i.e., toxicity is not observed at lower doses of virus). He was then able to build on this observation and show that this lower dose of oHSV is efficacious in a mouse model of MB. This information is critical and provides strong support for the continued development of IVT delivery of oHSV as a viable approach to treating MB.

1 https://www.stjude.org/disease/medulloblastoma.html
2 http://www.danafarberbostonchildrens.org/conditions/brain-tumor/medulloblastoma.aspx
Visible Light-Controlled Combination Strategy for Treating Nonmuscle Invasive Bladder Cancers

Youngjae You, Ph.D., University of Oklahoma, College of Pharmacy

Bladder cancer is among the 10 most common cancers diagnosed, based on the latest SEER (Surveillance, Epidemiology, and End Results) data.¹ Military Service members and Veterans who are exposed to contaminated drinking water and/or have a history of cigarette smoking have a higher prevalence of bladder cancer. One of the most prominent bladder cancers, nonmuscle invasive bladder cancer (NMIBC), can be detected and treated early; however, treatments are limited, patients experience side effects, and they are still at high risk of recurrence.

Dr. You and his team were recently awarded an FY16 Idea Award with Special Focus to support the development of a novel prodrug system to target and deliver chemotherapy to cancer cells. The idea is to use the FDA-approved technology of the formation of a photosensitizer (protoporphyrin IX) within a cancer cell to target light-activatable prodrugs to those cancer cells. Photodynamic therapy using visible light will then activate the prodrug to its cytotoxic form, ultimately resulting in the ablation of the cancer cells with minimal systemic side effects. If successful, this site-specific chemotherapy technology could improve therapeutic efficacy, alleviate side effects, and reduce the recurrence rate of NMIBC. Moreover, this technology can be applied to various types of cancers.


Schematic representation of the combined action of double mitochondriotropic prodrugs (HAL and Rh-L-Drug) for enhanced formation of PpIX in cancer cells and a light-activatable prodrug of anti-cancer drug.
PRCRP Impact

From FY09-FY16, the PRCRP has invested $199.8M in cancer research with the vision of improving the quality of life of Service members, their families, and the American public by decreasing the impact of cancer. During this period, the PRCRP has applied this vision to 20 topic areas. Peer and Programmatic Panels have offered their expert recommendations for research projects that would most likely advance the respective topic areas along the CCS. Across the CCS, career development; innovative, potentially impactful projects; and translational awards have been funded. Below are additional examples of high-impact projects in a variety of fields. Moving forward, the PRCRP aims to build on these early successes, continue identifying gaps in cancer research, and invest in research across the CCS to address identified priority areas.

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>PI Name(s)</th>
<th>Award Mechanism</th>
<th>Overview</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>Douglas Brash, Ph.D.</td>
<td>Collaboration/Translational</td>
<td>Dr. Brash and his collaborators at Yale University described the mechanism of chemiexcitation: DNA damage can occur long after the UV source has been removed. This is a paradigm shift in the understanding of UV damage and melanoma development. Dr. Brash is now working with L’Oreal to develop a new sunscreen to prevent post-UV exposure DNA damage (http:/cdmrp.army.mil/prcrp/research_highlights/15brash_highlight).</td>
</tr>
<tr>
<td>2011</td>
<td>Yue Wei, Ph.D.</td>
<td>Innovative Exploratory</td>
<td>Dr. Wei identified a mutation in the protein toll-like receptor 2 (TLR2) that deregulates innate immune signaling in bone marrow stem cells. The mutation is found in 10% of myelodysplastic syndromes (MDS) patients. This led to Dr. Wei’s collaboration with Opsona Therapeutics to test an antibody targeting TLR2 in patient cells. The humanized antibody to TLR2 is now undergoing Phase I/II trial evaluation in MDS patients; this is the first study to target innate immunity to treat MDS.</td>
</tr>
<tr>
<td>2012; 2015</td>
<td>Haining Yang, M.D./Ph.D.</td>
<td>Career Development; Translational</td>
<td>In her FY12 award, Dr. Yang discovered how mesothelioma patients with different mutations in BRCA1 associated protein-1 exhibit altered exposure outcomes. These results were leveraged to obtain an additional $1M in FY15 PRCRP funding.</td>
</tr>
<tr>
<td>2009</td>
<td>Deeann Wallis, Ph.D.</td>
<td>Innovative Exploratory</td>
<td>Dr. Wallis developed a novel screening platform to study the mechanisms responsible for driving radiation sensitivity and resistance. She identified seven genes that are involved in radiation resistance; of these, four have never before been implicated in radiation response. The results of this study could be used to develop novel strategies for increasing radiation therapy efficacy in cancer patients and enhancing radiation resistance for military personnel who are at risk for radiation exposure in the battlefield.</td>
</tr>
</tbody>
</table>
For more information, visit
http://cdmrp.army.mil
or contact us at:
usarmy.detrick.medcom-cdmrp.mbx.cdmrp-public-affairs@mail.mil
(301) 619-7071