RESEARCH HIGHLIGHTS

AACR-SU2C RESEARCH ALERTS

GLIOBLASTOMA: NEW FINDINGS

Title: "Neoadjuvant nivolumab modifies the tumor immune microenvironment in resectable glioblastoma"
DOI: http://dx.doi.org/10.1038/s41591-018-0339-5
SU2C Author: Kurt Schalper, MD, PhD
Title: "Immune and genomic correlates of response to anti-PD-1 immunotherapy in glioblastoma"
DOI: http://dx.doi.org/10.1038/s41591-019-0349-y
SU2C Author: Raul Rabadan, PhD
Grants: SU2C Dream Teams: SU2C-American Cancer Society Lung Cancer Dream Team, SU2C Colorectal Cancer Dream Team (Schalper); NSF/SU2C/V-Foundation Convergence Grant and Phillip A. Sharp Innovation in Collaboration Award (Rabadan)
Published: *Nature Medicine*, Feb. 11, 2019

In studying the impact of immunotherapy on glioblastoma, SU2C-funded researchers have identified promising results on the use of the immunotherapy agents called PD-1 inhibitors, as well as clues to the origin of resistance to this class of drugs.

Summary by the AACR:
Glioblastoma is the most common form of brain cancer. Its prognosis remains dismal. Unfortunately, the standard first-line treatment of surgery and chemoradiation has limited efficacy. Immune checkpoint blockade has been a promising therapeutic strategy in many cancers, including melanoma and lung cancer. In light of this, researchers have explored whether immune checkpoint blockade, specifically PD-1 blockade, can also be effective in treating glioblastoma.
SU2C-funded investigators have shared their contribution to this important effort in two research papers in the prestigious journal *Nature Medicine*.

Kurt Schalper, MD, PhD, and his colleagues studied whether PD-1 inhibitors can also be used to treat glioblastoma patients before they undergo surgery (a treatment regimen called neoadjuvant therapy). Although the number of patients treated was not big enough to make definitive conclusions about the survival benefit of the treatment, they observed that the PD-1 inhibitor induced a more diverse population of the immune cells called T cells at the tumor site.

A more diverse T cell population is a sign of an effective immune system. In light of the positive effects of a PD-1 inhibitor on the immune system even when given as a single-agent and given before surgery, Dr. Schalper's team anticipates that combining anti-PD-1 inhibitors with other immunotherapies can bring us one step closer to effectively treating glioblastoma patients.

Raul Rabadan, PhD, led a team that studied patient samples from 66 glioblastoma patients who were treated with PD-1 inhibitors. They found that patients who did not respond to the treatment had significantly more mutations in the *PTEN* gene. These mutations may be causing conditions in the tumor microenvironment that keep

immune cells from attacking the tumor. Patients who responded to PD-1 inhibitors, on the other hand, had mutations in genes involved in a signaling pathway in the cell known as MAPK. Dr. Rabadan's results, therefore, suggest that it may be beneficial to combine PD-1 inhibitors with targeted drugs against the MAPK pathway.

RESEARCH HIGHLIGHTS

AACR-SU2C RESEARCH ALERTS

NEW APPROACH TO PANCREATIC CANCER

Title: "Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers"
SU2C Author: Martin McMahon, PhD
Published: *Nature Medicine*, March 4, 2019
DOI: https://doi.org/10.1038/s41591-019-0367-9

Promising results from the laboratory of Martin McMahon, PhD, at Huntsman Cancer Institute, and in the clinic, demonstrate the therapeutic promise of combining RAF/MEK/ERK inhibitors plus autophagy blockers for the treatment of pancreatic cancer. These data pave the way for a clinical trial funded, in part, by the Pancreatic Cancer Collective.

Martin McMahon

"Autophagy is an evolutionarily ancient process by which pancreatic cancer cells cannibalize themselves to create building blocks for survival in the face of inhibition of KRAS signaling. We have a clinic-ready combination therapy which blocks KRAS signaling plus autophagy leading to tumor cell death. Support of the Pancreatic Cancer Collective is pivotal in allowing us to head into a clinical trial of this intervention." *Martin McMahon, PhD*

Summary by the AACR:

The *KRAS* gene is mutated in more than 80 percent of pancreatic cancers. A critical consequence of this mutation is the overactivation of the proteins RAF, MEK, and ERK. Although this overactivation has been shown to be pivotal in the development of pancreatic cancer, medicines that block these proteins have not been effective as single agents in treating the cancer.
In a paper published by the research group led by Martin McMahon, PhD, of the Huntsman Cancer Institute at the University of Utah, they demonstrate one mechanism by which pancreatic cancer cells evade the effects of RAF/MEK/ERK inhibitors. In the article in the highly rated journal *Nature Medicine*, the group reported that cells treated with RAF/MEK/ERK inhibitors undergo an increase in autophagy, or the process by which tumor cells recycle their internal contents. This key laboratory observation prompted them to explore whether pancreatic cells can be susceptible to combinations of RAF/MEK/ERK inhibitors and autophagy blockers. Given very promising results from their laboratory and animal experiments, Dr. McMahon's team initiated a clinical trial proposal to test the combination of the MEK inhibitor trametinib and the autophagy inhibitor hydroxychloroquine. A patient with metastatic pancreatic cancer was treated with this combination on a compassionate basis. This patient responded remarkably well.

Given the potential impact of this new therapeutic strategy, the newly formed Pancreatic Cancer Collective (PCC), the strategic partnership of the Lustgarten Foundation and Stand Up To Cancer, has decided to infuse at least up to $1 million in initial funding to Dr. McMahon's team, via the Collective's New Therapies Challenge grant program. McMahon's work is in line with one of the PCC's objectives: exploring the anticancer potential of medications that are already being used to

treat other illnesses. The two drugs that Dr. McMahon will use – trametinib and hydroxychloroquine – are already being used to treat melanoma and malaria, respectively.

Supported by this novel grant mechanism, Dr. McMahon will continue developing the MEK/autophagy inhibitor combination strategy through this year. If this therapeutic strategy continues to be promising, Dr. McMahon's team may be eligible for an additional $4 million for an expansion of the study into a larger clinical trial.

RESEARCH HIGHLIGHTS

AACR-SU2C RESEARCH ALERTS

NEW FINDINGS ON METASTASIS

Title: "Hepatocytes direct the formation of a pro-metastatic niche in the liver"
SU2C Author: Gregory L. Beatty, MD, PhD
Grant: 2017 Innovative Research Grant
Published: *Nature;* March 6, 2019
DOI: http://dx.doi.org/10.1038/s41586-019-1004-y

Promising results from the laboratory of SU2C Innovative Research Grant recipient Gregory L. Beatty, MD, PhD, point to possible therapeutic targets for preventing metastasis of pancreatic cancer to the liver.

Gregory L. Beatty

"The liver is the most common site of metastasis for pancreatic cancer. The liver becomes more favorable to metastasis through what we call the formation of a pro-metastatic niche. Our research indicates the importance of three specific proteins in the process. These findings give us the impetus to explore how we can prevent metastasis by blocking the activity of these proteins. "
Gregory L. Beatty, MD, PhD

Summary by the AACR:
The liver is the most common location of cancer metastasis. It is hypothesized that certain conditions develop in the liver that may promote cancer metastasis to this organ. In a paper published by the research group led by SU2C- funded early career investigator, Gregory L. Beatty, MD, PhD, of the University of Pennsylvania, they identified certain proteins that may promote metastasis of pancreatic cancer to the liver. They shared their findings in the journal *Nature*, the world's most highly cited journal of interdisciplinary science.

In their laboratory experiments, they found that pancreatic cancer cells, and certain normal cells in the tumor microenvironment, produce a protein called interleukin 6 (IL-6). IL-6 affects the liver, inducing the activation of a cellular pathway called STAT3 and consequently, production of a protein called serum amyloid A1 and A2 (SAA). They found that IL6, STAT3, and SAA were critical in promoting changes in the liver that favored metastasis.

Moving from their animal experiments, they looked at patient samples. They observed increased levels of SAA in the blood of pancreatic cancer patients. High levels of circulating SAA seemed to correlate with worse disease outcomes in these patients. Not only did they observe this in pancreatic cancer patients but they also observed increases in SAA in samples from lung cancer and colorectal cancer patients.

The discovery of the importance of the role of these three proteins: IL-6, STAT3 and SAA, pave the way for designing therapeutics that can prevent pancreatic cancer metastasis to the liver. This is

especially critical given that the five-year survival rate for pancreatic cancer drops to a dismal 3 percent once it has metastasized.

RESEARCH HIGHLIGHTS

AACR-SU2C RESEARCH ALERTS

TWO-DRUG COMBINATION IN OVARIAN CANCER

Title: "Olaparib and α-specific PI3K inhibitor alpelisib for patients with epithelial ovarian cancer: a dose-escalation and dose-expansion phase 1b trial"
SU2C Authors: Alan D. D'Andrea, MD, team leader; Elizabeth M. Swisher, MD, co-leader; and others.
Grant: SU2C-Ovarian Cancer Research Alliance—National Ovarian Cancer Coalition Ovarian Cancer Dream Team
Published: *Lancet Oncology,* March 14, 2019
DOI: http://dx.doi.org/10.1016/S1470-2045(18)30905-7

Work by SU2C-supported researchers found encouraging indications for using drugs called PARP inhibitors to fight ovarian cancer in a set of patients in whom they would not usually be effective because their tumors feature a DNA repair pathway that is functioning normally. The team observed synergy with another drug that, among other things, inhibits the repair pathway and thereby makes cells vulnerable to PARP inhibitors, thus halting or slowing down the cancer.

Summary by the AACR:
The homologous recombination repair (HRR) pathway is one of the major pathways involved in repairing damaged DNA in cells. BRCA1 and BRCA2 proteins are involved in the repair of damaged DNA and play an important role in the HRR pathway. PARP inhibitors (PARPi) are drugs that are FDA-approved in patients with HRR deficiencies, such as those having BRCA1/2 mutations. Unfortunately, patients often have an intact HRR pathway or they acquire HRR proficiency, leading to resistance to PARPi. Hence, researchers have investigated the idea of combining PARPi with drugs that can inhibit the HRR pathway. One such class of drugs are PI3K inhibitors that not only inhibit the HRR pathway but have also shown synergy with PARPi in pre-clinical studies. Members of the SU2C-Ovarian Cancer Research Alliance—National Ovarian Cancer Coalition Ovarian Cancer Dream Team initiated a phase 1b clinical trial (NCT01623349) of olaparib, a PARPi, combined with either buparlisib (pan-PI3K inhibitor) or alpelisib (PI3Kα inhibitor) in patients with ovarian cancer and patients with breast cancer. Initial studies with buparlisib showed no advantage in combining it with olaparib and resulted in toxic side effects in the central nervous system. To overcome this, in this study the team evaluated the combination of alpelisib with olaparib, with the aim to assess the safety and find a recommended phase 2 dose of the drug combination. The team enrolled 34 patients (30 with epithelial ovarian cancer and four with breast cancer) on the trial with this combination.

The combination did not yield any unexpected toxic side effects. Data from the trial shows that 36 percent of patients had a partial response, 50 percent had stable disease, and 3 percent progressed. They did not observe any benefit of this combination in platinum-resistant patients with BRCA mutations. However, they observed an overall response rate of 33.3 percent with this combination in platinum-resistant patients with no BRCA mutations, compared to the reported 5 percent overall response with the use of PARPi only in this population. Prior studies have also not reported much

success in using PI3K inhibitors alone in epithelial ovarian cancer. Overall, these findings provide evidence of synergy in the combination of a PI3K inhibitor and PARPi in HRR-proficient ovarian cancers.

The team published its findings in the prestigious journal *Lancet Oncology.*

RESEARCH HIGHLIGHTS

AACR-SU2C RESEARCH ALERTS

NEW DRUG TARGET IN PANCREATIC CANCER

Title: "A multiscale map of the stem cell state in pancreatic adenocarcinoma"
SU2C Authors: Daniel D. Von Hoff, MD; Haiyong Han, PhD; Andrew M. Lowy, MD; Tannishtha Reya, PhD
Grants: Pancreatic Cancer Collective New Therapies Challenge; SU2C—Cancer Research UK—Lustgarten Foundation Pancreatic Cancer Dream Team
Published: *Cell,* April 4, 2019
DOI: https://doi.org/10.1016/j.cell.2019.03.010

Promising results from SU2C-funded researchers point to a new drug target in pancreatic cancer stem cells resistant to chemotherapy: the receptor protein ROR gamma. With ROR gamma pathway blockers already being tested in Phase II trials for autoimmune diseases, these results point to the possibility of repurposing these agents to treat pancreatic cancer.

Tannishtha Reya

"We must find a better way to treat pancreatic cancer. Our work in mapping molecular dependencies in chemotherapy-resistant pancreatic cancer stem cells points to a possible new path: drugs already being evaluated in autoimmune disease. They may also be effective against pancreatic cancer."
Tannishtha Reya, PhD

Summary by the AACR:
Chemotherapy remains the standard of care for pancreatic cancer. Unfortunately, only 30 percent of patients respond, and the disease soon progresses in most of them. The rapid disease recurrence after chemotherapy indicates that there are drug-resistant cells that survive chemotherapy and grow back rapidly; these are known as stem cells. In a paper published by the research group jointly funded by SU2C-Cancer Research UK and Lustgarten Foundation, scientists identified a therapeutic target in these drug-resistant cells. They shared their findings in the journal *Cell*.

In an effort to develop new treatment strategies to target these drug-resistant cells, Tannishtha Reya, PhD, and the team identified the proteins that impart unique characteristics to these pancreatic cancer stem cells. They saw that one of these proteins, ROR gamma, is already being targeted in the treatment of autoimmune diseases in phase II clinical trials. Thus, work already being done with ROR gamma inhibitors in autoimmune diseases may help to hasten their use in the clinic if they are found to be effective against pancreatic cancer also.

The group also confirmed that combining a ROR gamma pathway blocker with the chemotherapeutic drug gemcitabine was more effective in stopping pancreatic growth in the laboratory than gemcitabine alone. They looked at samples from pancreatic cancer patients and observed that the levels of ROR gamma correlated with aggressiveness of the cancer. These results

strengthen the case that ROR gamma plays an important role in pancreatic cancer progression, and that blocking ROR gamma is a promising treatment strategy for this deadly disease.

This research falls within the portfolio of the Pancreatic Cancer Collective, the strategic partnership of Lustgarten Foundation and SU2C.

RESEARCH HIGHLIGHTS

AACR-SU2C RESEARCH ALERTS

FINDING NEW TARGETS IN CANCER

Title: "Prioritisation of cancer therapeutic targets using CRISPR-Cas9 screens"
SU2C Author: Mathew J. Garnett, PhD
Grants: SU2C-Dutch Cancer Society Tumor Organoids Dream Team
Published: *Nature,* April 10, 2019
DOI: https://doi.org/10.1038/s41586-019-1103-9

SU2C-supported scientists have used the powerful new genome editing technology CRISPR-Cas9 to identify genes that are important to the growth and function of cells in several different tumor types. These genes can be used as targets in the development of drugs, hopefully identifying new targeted therapies for different types of cancer.

Mathew J. Garnett

"Patients will benefit from greater availability of therapies that are targeted to their specific cancers. Our research is dedicated to finding the vulnerabilities in tumor genomes that can be targeted with new drugs. We hope our research, by identifying the most promising targets, will help bring forth new drugs to fight cancer."
Mathew J. Garnett, PhD

Summary by the AACR:
Targeted cancer therapies are continuously being developed to more effectively treat patients based on patients' genetic characteristics. However, there still remains a lot to learn on the characteristics of tumors that can be targeted by drugs. In a paper published by the research group jointly funded by SU2C and the Dutch Cancer Society, scientists used an emerging genetic technique, called CRISPR-Cas9, to identify new target proteins in cancer cells. They shared their findings in *Nature,* the world's most-cited journal of interdisciplinary science.

CRISPR-Cas9 can be used to effectively shut down the function of a specific protein to identify which proteins are most needed by cells. The research group knocked down 18,009 different genes in more than 300 cancer cell lines, representing 30 different types of cancers. The cancer types that they tested included the most common (lung, colon, and breast), and those that have caused the highest number of deaths (lung and pancreatic).

The team developed a statistical method to distinguish which genes affected most of the cancer cell lines. They surmised that targeting these genes, although may seem advantageous, may also affect normal cells and therefore, cause toxicity in patients. Instead of focusing on these genes, they focused on prioritizing those which were important to the growth of specific subsets of cancer cell lines that share similar genetic characteristics or tissues-of-origin. They identified 628 priority targets, 120 of which are promising to pursue for drug development.

More than 20 cancer types have a highly unstable genome because of an impairment in their DNA repair capacity. These are referred to as cancers with microsatellite instability (or MSI cancers). This characteristic can be seen in a subset of colon, ovarian, endometrial and gastric cancers. The research group identified a protein called WRN helicase as a promising target in MSI cancers. WRN helicase is what is referred to as a synthetic lethal target because blocking WRN function exacerbates the DNA repair deficiency of MSI cancers. The group suggests exploring the use of WRN antagonists in combination with approved immune checkpoint antibodies for MSI cancers. Dr. Garnett's work demonstrates how using the more novel genetic tool called CRISPR-Cas9, along with statistical and mathematical methods, can yield to new drug targets and therapeutic strategies.

RESEARCH HIGHLIGHTS

AACR-SU2C RESEARCH ALERTS

KEY GENES IN GLIOBLASTOMA IDENTIFIED

TITLE: "Genome-wide CRISPR-Cas9 screens expose genetic vulnerabilities and mechanisms of temozolomide sensitivity in glioblastoma stem cells"
SU2C AUTHORS: Graham MacLeod, PhD; Fiona J. Coutinho, PhD; Samuel Weiss, PhD; Peter B. Dirks, MD, PhD; Stephane Angers, PhD
GRANTS: SU2C Canada Cancer Stem Cell Dream Team
Published: *Cell Reports,* April 16, 2019
DOI: https://doi.org/10.1016/j.celrep.2019.03.047

SU2C Canada-supported scientists used the CRISPR-Cas9 tool to determine which genes in glioblastoma stem cells were required for the cells to proliferate and promote resistance to therapy.

Peter B. Dirks

"We think that, in one big experiment, we have uncovered many new targets for glioblastoma, some of which were surprising. These glioblastoma stem cells are also resistant to treatment, which is one reason that these tumors are so hard to cure. We need new ways to disrupt these cells specifically if we are going to give people a better chance of survival."
Peter B. Dirks, MD, PhD

Summary by the AACR:

Glioblastoma (GBM), a type of brain cancer remains largely incurable and very little progress has been made in identifying effective therapies for this disease. These tumours contain subpopulations of cells called GBM stem cells (GSCs), that drives growth and promotes resistance to therapy.

Members of the SU2C Canada Cancer Stem Cell Dream have used the gene editing tool called CRISPR-Cas9 in 10 patient-derived GSC cultures to perform a "cell fitness screen " to determine which genes in the GSCs were essential for growth and tumor progression. Multiple genes were identified as essential for tumor persistence in seven of the 10 GSC cultures tested, providing a list of potential drug targets for future therapeutic interventions.

One of the actionable drug targets is *DOT1L.* The team demonstrated the effectiveness of EPZ5676, a drug currently used to treat leukemia, to inhibit the DOT1L gene product in GSCs and animal models. The researchers also performed additional screens using a chemotherapeutic drug called temozolomide — a standard-of-care agent used to treat certain brain cancers — to identify the genes' underlying drug sensitivity and resistance. They found that most of the genes they identified are involved in numerous pathways used to repair damaged DNA. Some of these genes are potential drug targets, and this paves the way for developing strategies for combinatorial therapy.

RESEARCH HIGHLIGHTS

AACR-SU2C RESEARCH ALERTS

NEW APPROACH IN LUNG CANCER TREATMENT

Title: "Pulsatile MEK inhibition improves anti-tumor immunity and T cell function in murine *KRAS* mutant lung cancer"
Published*: Cell Reports,* April 16, 2019
SU2C Authors: Taha Merghoub, PhD; Kwok-Kin Wong, PhD; Jedd D. Wolchok, MD, PhD
Grant: SU2C-American Cancer Society Lung Cancer Dream Team
DOI: https://doi.org/10.1016/j.celrep.2019.03.

In mouse models of lung cancer bearing the *KRAS* mutation, phased administration of drugs called MEK inhibitors, plus immunotherapy, was more effective than continuous administration and may open a new path of combination treatment in humans.

Jedd D. Wolchok

"New treatments for lung cancer are urgently needed. We believe we have found a way of opening a window in which immunotherapy can be more effective in certain types of lung cancer. "
Jedd D. Wolchok, MD, PhD

Summary by the AACR:

Twenty to 30 percent of patients with lung cancer have mutations in their *KRAS* gene. However, no therapies have been approved by the US FDA to specifically treat patients with this mutation. Drugs called MEK inhibitors are being studied for this purpose, but resistance often develops. The SU2C-American Cancer Society Lung Cancer Dream Team aims to develop therapies for these patients. They have published promising results in the journal *Cell Reports* on a treatment strategy that may be effective.

The team found that MEK inhibitors, when given to mice with *KRAS* mutant lung cancer on a phased, or "pulsatile, " schedule (e.g., one week on and one week off), and used in combination with immunotherapy drugs, resulted in prolonged survival of the mice.

"In this study, we show that the pulsatile schedule alters the tumor microenvironment favorably by activating T cells and providing advantageous anti-tumor effect in KRAS driven lung cancer models, " the team wrote.

The team's work shows that treatments can be improved not only by discovering new drugs but also by optimizing the timing that the drugs are administered to patients. The team also demonstrated how a deeper understanding of the effects of targeted drugs can lead to rational drug combination approaches. The research is being carried forward by a phase I clinical trial where the team is comparing two different treatment regimen sequences of the MEK inhibitor trametinib and the immune checkpoint antibody anti-PD-1 (NCT03299088).

RESEARCH HIGHLIGHTS

AACR-SU2C RESEARCH ALERTS

NEW FACTOR IN PANCREATIC CANCER

TITLE: "Targeting LIF-mediated paracrine interaction for pancreatic cancer therapy and monitoring"
SU2C AUTHORS: Daniel Von Hoff, MD; Ronald M. Evans, PhD; Geoffrey M. Wahl, PhD; Andrew M. Lowy, MD, PhD; Tannishtha Reya, PhD; Michael Downes, PhD; Tony Hunter, PhD
GRANTS: SU2C Pancreatic Cancer Dream Team; SU2C-Cancer Research UK-Lustgarten Foundation Pancreatic Cancer Dream Team
Published: *Nature,* April 17, 2019
DOI: http://dx.doi.org/10.1038/s41586-019-1130-6

SU2C-supported researchers studying pancreatic cancer have discovered that a protein called leukemia inhibitory factor (LIF) plays a key role in the initiation and growth of pancreatic tumors. The finding could lead to therapies targeted at LIF which, if successful, could stop or slow down growth of the cancer.

Daniel D. Von Hoff

"Pancreatic cancer is a very tough problem, partly because of the dense tissue surrounding the tumor. By uncovering the previously under-appreciated role of LIF in tumor growth, we've contributed significantly to the search for new therapies for this terrible disease."
Daniel D. Von Hoff, MD

Summary by the AACR:

Pancreatic cancer has been particularly resistant to drugs, partly because the tumor is surrounded by a dense wound-like tissue called stroma. This stroma is produced by activated pancreatic stellate cells (PSCs). The activity of PSCs and pancreatic cancer cells (PCCs), and the communication between these cell types, influence pancreatic cancer progression and metastasis. The research group led by Tony Hunter, PhD, sought to understand the interaction between PSCs and PCCs, and consequently, identify new therapeutic strategies for this aggressive cancer. They shared their findings in the premier journal *Nature*.

Cells communicate with each other by secreting proteins that are recognized by other cells. The research group analyzed the proteins secreted by PSCs as well as the changes that resulted within the PCCs. They identified a protein called leukemia inhibitory factor (LIF), which has been previously been implicated in cancer development, due in part to its role in this intercellular communication. The researchers measured LIF levels in human pancreatic cancer tissues and observed that levels were elevated in all 77 PDAC cases that they tested. Furthermore, they observed that changes in LIF levels in the blood were correlated with how well patients responded to treatment.

Using a mouse model of pancreatic cancer, the team tested the effectiveness of an antibody that can block LIF activity. Combining the antibody with the chemotherapeutic drug gemcitabine significantly prolonged the survival of the mice. In addition, the combination was more effective in reducing tumor growth than gemcitabine alone.

The team's work indicates that LIF may not only be a biomarker of response of patients to other treatments, but also a therapeutic target by itself. The research is being carried forward by a Phase I clinical trial of an anti-LIF antibody (NCT03490669).