

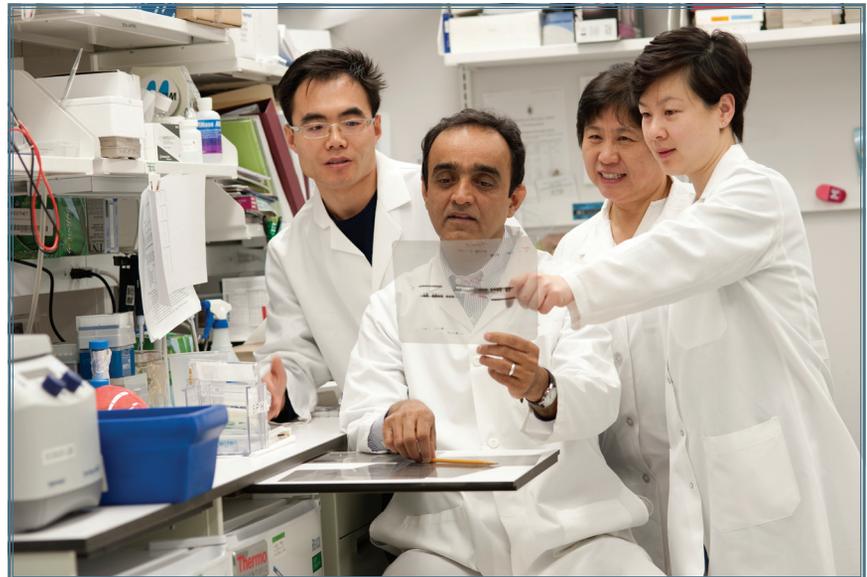
Among the Most Deadly

In 2012, Congress passed the Recalcitrant Cancer Research Act to draw attention to the deadliest cancers that afflict our society, those for which the odds of surviving for five years after diagnosis is less than 50 percent. Among the worst of the worst, pancreatic cancer—or, more specifically, pancreatic ductal adenocarcinoma (PDAC)—presents a five-year survival rate of less than five percent. With no early-detection markers, the disease is usually discovered at an advanced stage and once discovered, the response to chemotherapy is poor. Within CCR, diverse multidisciplinary efforts to understand and treat PDAC are gaining momentum.

When Pervez Hussain, Ph.D., started his group in CCR's Laboratory of Human Carcinogenesis in 2009, he decided to build a program in pancreatic cancer from scratch. "I said, let me take on this challenge. I want to understand the biology of this disease: what is the difference in this solid tumor as compared to others that makes it so aggressive? In my view, understanding the biology is the most important step because it will tell us where to strike," said Hussain.

PDAC tumors present at least two challenges: first, they are highly heterogeneous. For example, a paper published in *Science* in 2008 from Johns Hopkins researchers, found that on average, each tumor had multiple alterations affecting 12 core signaling pathways, but the particular mutations varied from tumor to tumor. In addition, unlike most solid tumors, PDACs have very few blood vessels and are surrounded by a dense tissue stroma, making therapeutic access a greater challenge.

By establishing collaborations around the world, Hussain's group



(Photo: R. Baer)

The Hussain team (left to right): Shouhui Yang, Ph.D., Pervez Hussain, Ph.D., Peijin He, B.S., and Jian Wang, Ph.D.

started collecting patient samples for analysis and validation in multiple independent cohorts. His laboratory took both a focused, hypothesis-driven approach to the mechanisms of pancreatic cancer progression, and a global approach to defining molecular distinctions through integrative analysis of the transcriptional and metabolic profiles with a focus on inflammatory mediators.

Inflammatory Targets

Although not well understood, many lines of evidence point to an important role for inflammation in pancreatic cancer. The most common precursors to PDAC, pancreatic intraepithelial neoplasms, are often found in association with areas of focal inflammation. Moreover, approximately 95 percent of pancreatic tumors have early mutations in the *KRAS* gene (see "RAS Takes

(Photo: B. Brantson)



Udo Rudloff, M.D., Ph.D., and Yaroslav Teper, Ph.D., in the lab

Center Stage,” *CCR connections* Vol. 7, No. 2). Among the many manifestations of these mutations is an increased inflammatory microenvironment.

“When you look at pancreatic tumors, you see a lot of markers of inflammation,” said Hussain. “Even the tumor cells produce inflammatory mediators: chemokines, cytokines, and growth factors.” Hussain’s laboratory has pursued two of these mediators: macrophage migration inhibitory factor (MIF) and nitric oxide (NO).

Hussain and his colleagues found that the tumor cells in their patient samples were expressing high levels of MIF, a factor that is hypothesized to be a connecting link between inflammation and cancer. Moreover, they found that increased levels of MIF were associated with a more aggressive phenotype and a poorer prognosis in patients with PDAC.

Pursuing this observation in cell signaling studies, the Hussain

group has found that altering MIF expression (either through overexpression or knockdown in cell lines and in animal models), resulted in changes to a signaling pathway that enhances the epithelial-to-mesenchymal transition, a key process in the development of metastases and disease progression to distant organs.

They now have taken this work into models. In a well-validated model of pancreatic cancer derived from pancreas specific mutations in *KRAS* and *P53* (the KPC mouse), Hussain’s laboratory has found that further genetic modification to delete MIF significantly increases the survival of these mice by several months. Instead of a genetic deletion, the next step is to use small molecule inhibitors and monoclonal antibodies to target MIF in these mice. “If this ongoing preclinical study shows us that it regresses the tumor and enhances the survival of these mice, we will have very strong

evidence to pursue a clinical trial,” said Hussain.

A similar pattern is emerging in Hussain’s studies of NO. Nitric oxide synthase 2 (NOS2) is expressed under conditions associated with inflammation, including cancer. Once expressed, it produces high levels of NO for prolonged periods. Hussain has found that increased expression of NOS2 in patient samples is also associated with poorer prognosis. Moreover, a genetic deletion of NOS2 increases survival in the KPC mouse model. His laboratory is currently working to define the cellular pathways that account for these observations.

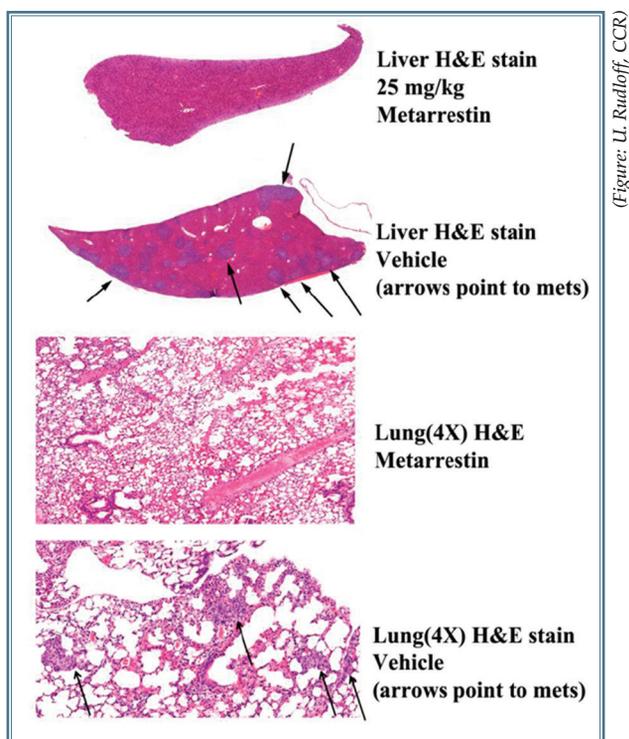
“In the KPC model, mice start dying at about three to four months of age with fully metastasized tumors. But when we deleted either MIF or NOS2, median survival significantly improved,” said Hussain. “That’s a pretty good start.”

Increasing the Odds

“The big theme in my lab is drug development in pancreatic cancer,” said Udo Rudloff, M.D., Ph.D., Investigator in CCR’s Thoracic and Gastrointestinal Oncology Branch. Like Hussain, Rudloff joined CCR in 2009, after training in surgical oncology at Memorial Sloan Kettering Cancer Center. “Surgery is still associated with the best outcomes for early stages of the disease, but in nearly all patients, the cancer comes back eventually. Once metastases set in, the answer is not surgery any more. The answer to the deadliest cancers is to develop new and better drugs.”

Because they grow in a very hypoxic environment, with poor vascularization, pancreatic tumors contain an unusually high number of tumor initiating (stem) cells. These cells are able to survive without a lot of oxygen and in the presence of reactive oxygen species. Rudloff and his colleagues use cellular biomarkers

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(Figure: U. Rudloff, CCR)

Metarrestin effectively suppresses pancreatic cancer progression in the liver and lungs. H&E staining of untreated and treated representative liver (top) and lung (bottom) sections.

of these stem cells that correlate well with tumor progression.

“We have isolated pancreatic stem cells from patient tissue samples and put them into immunocompromised mice; they form tumors about one hundred times more frequently than nonstem cells,” said Rudloff. “These cancer stem cells appear to drive tumorigenesis, progression, and metastasis.”

Often cancer cell lines, growing

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in monolayers at the bottom of a petri dish *in vitro*, are a first port of call for drug screening. Rudloff’s team has been growing pancreatic cancer cells under conditions that promote the proliferation of cancer stem cells. The cells form spherical structures, creating their own internal microenvironments, which are more reflective of cancers *in vivo*.

“No one has used these spheres for drug screening. But our collaborators at the NIH’s National Center for Advancing Translational Sciences (NCATS) optimized growth conditions for high-throughput screening and found a compound with really strong activity in the spheres containing cancer stem cell fractions,” said Rudloff.

The compound, termed 10N, is a multikinase inhibitor, previously known for inhibiting the IL-2 T-cell kinase. In fact, Rudloff has conducted extensive proteomics analysis to show that the compound inhibits at least 16 kinases, four of which are important for cancer

stem cell function. His team has shown that the rate of apoptosis more than doubles when you knock out two of the compound’s kinase targets, suggesting the drug could create a disproportionately strong downstream effect.

“The cool thing is that this compound has intrinsic synergy; its targets cooperate, so disrupting them has an additive effect,” said Rudloff. “Cancer stem cells are hugely resistant to chemotherapy, which makes an inhibitor specific for the stem cell fraction very exciting.”

“Developing this drug would be a great opportunity,” said Rudloff. “It would be the first pancreatic stem cell inhibitor, with a completely novel target profile.”

In a parallel effort, Rudloff is working with scientists at NCATS who conducted a novel small molecule screen to identify inhibitors of metastasis. They took advantage of a poorly understood but prominent cell biological feature of metastatic cancer cells: the perinucleolar compartment (PNC). The PNC is found at the edge of the nucleolus, where it is enriched with RNA and RNA-binding proteins. It is especially prevalent in metastatic tumors, and, when found in tumor biopsies, indicative of a poor prognosis. A small molecule was discovered—metarrestin—which dramatically reduced the prevalence of this marker in metastatic cancer cell lines.

Rudloff tested metarrestin in an animal model of metastasis, which Research Fellow Yaroslav Teper, Ph.D., developed in his laboratory. Fluorescently tagged pancreatic cancer stem cell lines are injected into the pancreas of mice; the bioluminescence can be tracked not only to the pancreas, but to metastases of the liver and lungs. Metarrestin had little impact on the primary pancreatic tumor, but dramatically decreased the metastatic burden.

(Photo: R. Baer)



Tim Greten, M.D., in the clinic

Encouragingly, the toxicology profile of metarrestin in rodents indicates that it is very safe, and Rudloff is currently in discussions with the Food and Drug Administration (FDA) about further testing before a first-in-human clinical trial could be approved.

“The PNC compartment disassembles after administration of the drug, but we don’t know what the molecular target is,” said Rudloff. “We only know that it is very well correlated with metastatic progression. It’s a really good biomarker, which has been under development for several years. That’s why we are so excited about it.”

Requesting Immunity

Meanwhile, Tim Greten, M.D., Investigator, and Austin Duffy, M.D., Staff Clinician in CCR’s Thoracic and Gastrointestinal Oncology Branch, are taking a more immediate approach to potential clinical impact, by taking existing anticancer tools into pancreatic cancer treatment.

“Tim and I built up the GI cancer program from scratch since around 2009,” said Duffy, “It takes a while to set these things up, but we’re

now at a stage to capitalize on the investment we’ve made.”

“There’s such a huge unmet need to treat pancreatic cancer, and chemotherapy is only minimally effective,” said Greten. “Immunotherapy might help, but the drugs used so far haven’t shown the kind of efficacy seen with, for example, melanoma or lung cancer.”

The examples Greten highlighted come from the use of so-called immune checkpoint inhibitors, inhibitors of CTLA-4 and PD-L1 signaling, which normally operate as brakes on the immune response. Lifting those brakes has resulted in remarkable, durable responses in certain intractable cancers, but has thus far not had much impact on gastrointestinal solid tumors, including pancreatic cancer, despite the fact that immune cells are found in abundance in pancreatic tumors.

Greten’s laboratory has been studying tumor cell death and the effects on the immune response in preclinical models over the last decade. “Depending on how tumors die, you can have dramatic differences in the resulting antitumor activity.”

Based on this line of investigation, he believes that a combination of immune checkpoint inhibition with radiation therapy might deliver a strong one-two punch to pancreatic cancer. The idea would be that initial destruction of tumor cells would cause the tissue to release antigens, which would elicit a T-cell response. Checkpoint inhibitors would then strengthen that response.

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“Two years ago, we enrolled a similar cohort of patients with pancreatic cancer as part of a small multicenter study of two different vaccines; that study suggested that immune approaches in pancreatic cancer can be of benefit,” said Duffy. “It showed me that if you use stringent inclusion criteria, you may be able to get a signal and learn from these patients.”

Greten and Duffy have recently opened a study, in which they are combining the use of tremelimumab (an antibody inhibitor of CTLA-4) or MEDI4736 (an antibody inhibitor of PD-L1) with radiation treatment to the pancreas. The plan is to enroll 60 patients, who have previously had some form of standard treatment and

either progressed or did not tolerate the chemotherapy.

In designing the exact treatment schedule, Greten and Duffy have worked with Deborah Citrin, M.D., Senior Investigator in CCR’s Radiation Oncology Branch and her colleagues in the Branch, along with Jennifer Jones, M.D., Ph.D., Assistant Clinical Investigator in CCR’s Vaccine Branch, but there is very little systematic, comprehensive evidence to guide them to the optimal dosing. “We use whatever data is out there, but it’s very difficult to extrapolate from animal data to humans,” said Duffy.

By taking needle biopsies before and after the treatment, Greten and Duffy will be able to study the tumor immune response. Some patients will receive one dose of focused radiation, others will receive five; and computed tomography (CT) scans will be used to monitor the size of the tumor.

Looking Forward

“Eight to ten years ago, very few people were working on PDAC. It wasn’t a priority and it was an understudied cancer. That has changed,” said Hussain. “Every

year, we hold a symposium on pancreatic cancer in September, bringing together experts from the U.S. and around the world. Our goal is to exchange ideas between the extramural and intramural communities working on pancreatic cancer and foster collaborations. We’ve been doing this for the last three years and participation is growing.”

“Scientifically there is a lot of opportunity here at CCR for translational research. The proximity between lab and clinic doesn’t exist to the same extent in other places,” said Duffy. “Our pancreatic cancer study would be challenging to do in the community hospital setting, because of the nonstandard application of the radiation treatment. There’s more freedom to break new ground.”

Everyone acknowledges that there is still a lot to learn about pancreatic cancer, from its molecular subtypes and evolution, to the clinical implications of its physically constrained, hypoxic, stromal compartment. “We need completely new approaches,” said Rudloff. “The vast majority are going to fail, but we need to take the risks if we’re going to succeed.”

(Photo: B. Branson)



Austin Duffy, M.D., in the clinic

To learn more about Dr. Duffy’s clinical research, please visit his CCR website at <https://ccr.cancer.gov/austin-g-duffy>.

To learn more about Dr. Greten’s research, please visit his CCR website at <https://ccr.cancer.gov/tim-f-greten>.

To learn more about Dr. Hussain’s research, please visit his CCR website at <https://ccr.cancer.gov/s-perwez-hussain>.

To learn more about Dr. Rudloff’s research, please visit his CCR website at <https://ccr.cancer.gov/udo-rudloff>.