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Infection, Inflammation, and Prostate Cancer: "Smoking Gun" Evidence!

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***Partin**: "Even in a global pandemic, our mission remains the same: to defeat urologic disease."*

A Challenging Year

It's been quite a year for all of us. A year ago, I never imagined I would be writing to you after months of a global pandemic.

I can't begin to tell you how proud I am of The Brady's faculty and staff. When our labs were shut down, our scientists did their best to continue their momentum from home, through countless Zoom meetings and sharing data by email. Our surgeons, nurses and staff adapted like champions to new protocols. Many of our faculty and staff selflessly dedicated themselves to Covid-19 relief, creating test kits and PPE packs, and pursuing research to help lessen the toll of the virus. Although our hospital and clinic environments were very different, many things did not change: Unfortunately, prostate cancer didn't stop for Covid-19. Bladder cancer, kidney cancer, and testicular cancer didn't stop, either. Thus, our mission didn't change. We remained, and remain, dedicated to providing the very best care for our patients and defeating urologic disease.

Although this year's Patrick C. Walsh Prostate Cancer Research Fund Awards have been delayed by Covid-19 (which is why you won't see them in this issue, for the first time ever!), other very exciting research has come to fruition – and we're proud to present it here in *Discovery*.

As I write this, it looks like the sun is peeking out of the clouds, many children are back to school, businesses are opening back up, and our clinics and labs are buzzing with creative energy. However, 2020 has taught us that this can change very rapidly. If it does, I am confident that once again, The Brady and its people will rise to meet the challenge.

Best wishes,

Alan W. Partin, M.D., Ph.D.

The Jakurski Family Director and Professor
The James Buchanan Brady Urological Institute
Urologist-in-Chief, Johns Hopkins Medicine

COVER STORY:

Infection, Inflammation, and Prostate Cancer: "Smoking Gun" Evidence!

PART 1:

Because of the unique molecular tools used in this study, Sfanos, De Marzo and colleagues were able to catch the formation of these invasive cancers in real time.

What causes prostate cancer? A landmark study provides new evidence that one cause is bacterial infection. The study was led by Brady scientist Karen Sfanos, Ph.D., and her former graduate student, Eva Shrestha, Ph.D., in collaboration with Angelo De Marzo, M.D., Ph.D., Jonathan Coulter, Ph.D., and colleagues.

The bacterial culprit found in this study belongs to the family *Enterobacteriaceae*, which includes *E. coli*. Better known as a nasty gastrointestinal bug, *E. coli* causes inflammation in the urinary tract and is a known cause of bacterial prostatitis. As the scientists discovered, *colibactin*, a genotoxin produced by some strains of *E. coli*, can also instigate a series of unfortunate events in the prostate. Bacterial infection leads to acute and chronic inflammation, which can lead to the development of a lesion in the prostate called proliferative inflammatory atrophy (PIA), first described by De Marzo, William (Bill) Nelson, M.D., Ph.D., and other Brady scientists; it can also cause DNA damage. The presence of colibactin is even more ominous, because it can directly lead to double-stranded DNA breakage.

Sfanos suspects that this combination leads, in turn, to another development: fusion of two genes, *TMPRSS2* and *ERG*, that normally should remain separate, but in this case get abnormally spliced together. This *TMPRSS2/ERG* fusion – found in as many as half of all prostate cancers – is thought to be an early event leading to the development of prostate cancer.

"We found evidence in human tissues (from prostatectomy specimens) that bacterial infections are initiating the *TMPRSS2/ERG* fusion," says Sfanos. "We don't think this is the only way bacterial infections contribute to cause prostate cancer. But in this particular study, the way we looked at it was by tracking the presence of these *TMPRSS2/ERG* fusions."

It is entirely possible, notes De Marzo, "that other types of mutations or events could also be caused by bacterial infections or inflammation. But looking at these fusions gave us 'smoking gun' evidence that bacterial infection was the initiating event." Sfanos adds that "the colibactin-producing bacteria, *TMPRSS2/ERG* fusions, PIA, and tiny buds of cancer were all there, in the same place at the same time, a snapshot of prostate cancer being born." The team's early findings are published online in BioRxiv, a scientific data-sharing website, and a manuscript for publication is under review.

Bacterial infection is a known cause of other cancers. *H. pylori*, for example, is a well-established cause of stomach cancer. "We believe that many different types of microorganisms, certain types of sexually transmitted infections (STIs), and other infections in the prostate can certainly cause the same chain of events," says Sfanos.

How did the bacteria get into the prostate? They could have come from the urethra. "These bacteria are good crawlers," Sfanos says. De Marzo recalls what Don Coffey, Ph.D., the great and longtime director of The Brady's scientific labs, used to say: "The urethra is like the Holland Tunnel for bacteria."

Note: ***These tiny cancers are not the cancers that were biopsied and that led to the diagnosis of prostate cancer; they're too young even to achieve a Gleason grade.*** They're just new sites of cancer cropping up, in addition to the more mature cancer that was already there. Prostate cancer is multifocal: in most men with prostate cancer, several sites of cancer develop. But because of the unique molecular tools used in this study – looking for *TMPRSS2/ERG* fusions and "*ERG*-positive *PIA*"– Sfanos, De Marzo and colleagues were able to catch the formation of these invasive cancers in real time. "This might start to explain the multifocal nature of prostate cancer," says Sfanos. "There might be multiple infections or other inflammatory events that occur throughout a man's lifetime."

Sfanos suspects that the men whose tissue was used for this study "likely all had undiagnosed infections." These findings may lead to development of a new test, using urine or prostatic fluid, to look for *colibactin* or markers of inflammation in the prostate. Future studies may look at urine samples along with prostate tissue for such markers, and new imaging technology may one day be able to detect inflammation, as well.

For more than 20 years, De Marzo and Sfanos, with Brady scientists Bill Nelson, Srinivasan Yegnasubramanin, M.D. Ph.D., Elizabeth Platz, Sc.D., and William Isaacs, Ph.D., have studied inflammation as a risk factor for prostate cancer, particularly looking at *PIA*. Sfanos "has also been the major champion of infection" as a risk factor, De Marzo says. Now, these two paths of investigation have come together.

Could dietary changes make a difference? "Bill Nelson showed years ago that loss of expression of the *GSTP1* gene rendered prostate cells more susceptible to DNA damage caused by a chemical compound that is found in charred meat," says De Marzo. "Infection plus a bad diet might make this worse, and then combine that with the underlying genetics. There might be multiple culprits, a constellation of things over years." *Continued next page >*

ON THE COVER *From left: Angelo De Marzo, Karen Sfanos, Marikki Laiho, and Elizabeth Platz. The work of these four scientists and others has converged to show, for the first time, how infection leads to inflammation, which leads to prostate cancer -- and why fighting inflammation may be a powerful cancer-fighting strategy.*

COVER STORY, PART 2:

Why Anti-Inflammatory Drugs Might Protect Against Prostate Cancer

If inflammation can lead to prostate cancer (see Page 3), could anti-inflammatory agents help protect against it?

Epidemiologist Elizabeth Platz, Sc.D., has been intrigued by this possibility for many years. She is senior author of a new study on the use of aspirin and statins, published in Cancer Prevention Research. The study, of men in the placebo arm of the Prostate Cancer Prevention Trial, doesn't answer this question once and for all – but adds more weight to the idea that, for lowering the risk of developing potentially fatal prostate cancer, fighting inflammation is a good thing.

Evidence from observational studies has suggested that when taken regularly over time, aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs, including acetaminophen and ibuprofen) may lower the risk of prostate cancer. These drugs block enzymes that play a key role in the body's inflammatory response. Other studies have linked long-term use of statins, prescription drugs that are used to lower cholesterol but that also are anti-inflammatory, to a lower risk of advanced and metastatic prostate cancer.

In this most recent study, the investigators looked for inflammation markers in benign prostate tissue samples. "We compared the use of aspirin and statins with the presence and extent of inflammation in the prostate tissue," says Platz. They also looked at prostate biopsy slides for the presence of certain immune cells that are involved in inflammation.

"Of 357 men, 61 percent reported aspirin use, and 32 percent reported statin use," Platz continues. "Aspirin users were more likely to have low FoxP3, a T regulatory cell marker, and statin users were more likely to have a low CD68, a macrophage marker." "Our results suggest these medications may alter the immune environment of the prostate. A next step is to determine whether these immune alterations may underlie the epidemiologic observations that taking an aspirin or statin may protect against getting advanced prostate cancer, and dying from it."

COVER STORY, PART 3:

New Clue to Prostate Cancer Metastasis: Lipids

Prostate cancer gravitates to its own kind of junk food – the cellular version of deep-fried Oreos with a side of chili cheese fries. Scientist Marikki Laiho, M.D., Ph.D., director of the Division of Molecular Radiation Sciences, and colleagues have figured out how the body enables prostate cancer's terrible diet, in research funded by the Patrick C. Walsh Prostate Cancer Research Fund. Her basic science work may explain why anti-inflammatory drugs might help protect against prostate cancer.

The culprit is a lipid-regulating protein called *CAVIN1*, the scientists reported in the journal, *Molecular Cancer Research*. In lab studies, when *CAVIN1* was removed from cells in and around the prostate tumor, the fatty acid that was in those cells spilled into the tumor's microenvironment. The effect on prostate cancer cells was dramatic: the cancer cells soaked up the lipids, which then acted as turbo fuel to make the cancer spread more aggressively.

"In every prostate cancer cell line we tested," says research fellow Jin-Yih Low, Ph.D., the study's first author, "tumor cells universally had an appetite for the lipids, using them to strengthen the protective membrane around the cell, synthesize proteins and make testosterone to support and fuel the cancer's growth. The tumor cells then behaved more aggressively, exhibiting invasive and metastatic behavior. Just having access to the lipids gave the tumor cells more power; the tumor's behavior changed."

But wait! There's more: nearby cells changed, too. Deprived of their lipids, normal stromal cells started to churn out inflammatory molecules, adding fuel of their own to the fire.

Laiho's team then confirmed their findings in mouse models, comparing tumors with and without *CAVIN1* in the stromal cells. In the mice, Laiho says, "although the presence or absence of *CAVIN1* did not affect the speed of tumor growth, lack of *CAVIN1* definitely caused the cancer to spread. All of the mice with tumors that lacked *CAVIN1* had a twofold to fivefold increase in metastasis. The tumors also had a fortyfold to hundredfold increase in lipids and inflammatory cells."

The investigators were surprised at these results, Laiho adds. "We suspected *CAVIN1* was important, but we didn't realize how important. The tumor's microenvironment matters, and the amount of lipids matters a lot." Just changing the level of lipids "created a situation of rampant metastasis."

What could come from this research? One possibility is development of a new biomarker: a loss of *CAVIN1* in local or locally advanced cancer, for example, could signal a higher risk of metastasis. The next step is to understand more about the inflammatory process in the tumor's microenvironment. "We want to understand why the inflammation brings in macrophages, immune cells that further exacerbate the inflammatory process, instead of T cells, which should attack the cancer." ■

Slowing Down Prostate Cancer

This novel therapy slowed the growth in 93 percent of mice with prostate cancer, and 53 percent of mice with pancreatic cancer.

Promising results in mice may one day lead to a new way to treat prostate cancer.

Scientist Alan Friedman, M.D., in collaboration with Kenneth Pienta, M.D., the Donald S. Coffey Professor of Urology, Lei Zheng, M.D., Ph.D., and Elizabeth Jaffee, M.D., of the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, has developed a novel cell-based therapy that slowed the growth in 93 percent of mice with prostate cancer, and 53 percent of mice with pancreatic cancer.

This work, published in the *Journal for Immunotherapy of Cancer*, "builds on the finding that several cancers grow slower in mice that lack a protein called p50 in myeloid cells, a type of white blood cell," says Friedman. When mice with cancer were given engineered white blood cells that lack p50, "this activated cells in the immune system to attack the cancer," which curtailed cancer's ability to grow and spread.

Friedman's laboratory is now working to develop human immature myeloid cells lacking p50 – looking at prostate cancer patients' blood or bone marrow cells, and also at patient-derived adult stem cells – as a new form of immunotherapy. ■

A New Urine Test for Aggressive Prostate Cancer?

The test looks for proteins in the urine that are made by clinically significant prostate cancer.

"The majority of men with low-risk prostate cancer can be managed by active surveillance," says Alan W. Partin, M.D., Ph.D., the Jakurski Family Director and Professor, "but there is a pressing need for differentiating low-risk and high-risk patients."

In a recent study, Partin and colleagues have come up with a potential way to do this: a simple urine test that looks for proteins that are made by aggressive cancer. The study was led by Hui Zhang, M.S., Ph.D., Director of the Mass Spectrometry Core Facility at the Center for Biomarker Discovery and Translation.

The test uses quantitative glycoproteomics – a way to look at many different proteins all at once – to analyze proteins shed by prostate cancer that find their way into the urine. "Glycoproteins play essential roles in cancer development, and we found several glycoproteins made by aggressive prostate cancer," says Partin. "They can be used either individually or in combination to detect patients with clinically significant cancer that needs to be treated." Of these, one protein, urinary ACPP, outperformed the others.

"Urinary ACPP, used with a blood PSA test, can further improve our ability to discriminate aggressive cancer from cancer that can safely be monitored in active surveillance."

In the study, the team analyzed quantitative mass spectrometry data of glycopeptides from urine samples of 74 men with aggressive (Gleason score 8 or higher) and 68 men with non-aggressive (Gleason score 6) prostate cancer. The next step is to validate these results with a larger, multi-center study. These results have been accepted for publication. ■

SelectMDx: Could This Be a "Liquid Biopsy" for Active Surveillance?

Nobody likes prostate biopsy. In fact, says epidemiologist Bruce J. Trock, M.P.H., Ph.D., the Frank Hinman, Jr. Professor in Urology, this is a major reason why men with low-risk prostate cancer, who otherwise could stay in active surveillance, choose to get treatment with surgery or radiation. "Surveillance requires that a man undergo regular repeat biopsies, usually every one to two years," he says, "and many men will elect to undergo treatment – despite absence of evidence that their tumor has worsened – rather than continue repeated biopsies."

For years, scientists have been searching for a liquid biopsy, a noninvasive test using blood or urine that could provide information about the aggressiveness of a man's prostate cancer. One of the most promising of these tests is SelectMDx, a urine test developed by MDxHealth, Inc. SelectMDx measures two genes, *HOXC6* and *DLX1*, and combines those measurements with a man's age, PSA level, prostate volume and digital rectal exam result to create a score from one to 100 indicating potential tumor aggressiveness.

Recently, scientists at MDxHealth approached Trock and Alan Partin, M.D., Ph.D., to develop a study to evaluate SelectMDx's ability to identify men on active surveillance who are likely to experience upgrading. Because men in the Johns Hopkins Prostate Cancer Active Surveillance Program provide a urine test when they enter the program, and provide further samples every six to 12 months afterward, "it gave us the perfect opportunity for a rigorous test of SelectMDx."

Trock designed a study comparing three groups of men: men who upgraded on their first surveillance biopsy; men who upgraded during years two to six on active surveillance; and men who went six or more years without upgrading on repeat biopsies. The urine samples taken when the men entered active surveillance were sent to MDxHealth for analysis, blinded to each man's identity and group status. Once MDxHealth had analyzed the two genes in the urine, Trock sent them the clinical information (without personal identifiers) to allow them to calculate the SelectMDx score.

"The results were very promising," says Trock. "A 10-unit increase in the SelectMDx score was associated with a more than five-fold increase in the probability of being upgraded at the first surveillance biopsy, and a two-fold increase in the probability of being upgraded during years two through six. If the study population was restricted to men at very low risk, the results were even stronger: a 10 unit increase in the SelectMDx score was associated with a more than seven-fold increase in the risk of being upgraded at the first surveillance biopsy, and a nearly three-fold increase in risk of being upgraded in years two through six."

These exciting results of the performance of SelectMDx as a liquid biopsy are being prepared for publication. ■

Zhang and Partin: *"There is a pressing need for differentiating low-risk and high-risk patients with prostate cancer."*

Pavlovich: *For men in AS who go on to need treatment, "we continue to push the envelope and try to make it even better, with even fewer side effects."*

"Robust, Innovative, Forward-Thinking"

Personalized Active Surveillance, Safer Surgery.

What's the best course of treatment for men with localized prostate cancer? That's actually a trick question: it depends on the individual patient, there are several good choices – and urologist Christian Pavlovich, M.D., the Bernard L. Schwartz Distinguished Professor in Urologic Oncology, is working to expand the range of options even further.

Pavlovich is the new Director of the Prostate Cancer Active Surveillance (AS) Program, a job originated and held for many years by renowned urologist H. Ballentine Carter, M.D., who retired last year. "My goal is to keep our AS program robust, innovative, and forward-thinking," says Pavlovich. Some exciting projects on the horizon include:

A personalized medicine approach to AS: "The longer we follow men on AS, the more we learn about some of the finer points of monitoring their prostate cancer," says Pavlovich. In addition to a yearly rectal exam and repeat biopsies every two to five years, men in The Brady's AS program – more than 1,500 since the program launched – get their PSA checked every six months. What do Pavlovich and colleagues do with all that information? They look at the bigger picture: *PSA kinetics* is the study of the complex variability of PSA over time. "PSA itself is not a good trigger; just because it hits 8 or 10 doesn't mean cancer has progressed. We don't have a hard and fast PSA threshold, or PSA velocity (rate of change over time) threshold, to trigger recommendations for biopsy or treatment. What has been most predictive in AS is PSA density (PSA divided by prostate volume) and the complex assessment of PSA kinetics."

Pavlovich and colleagues are working on an "Active Care Tool", a machine learning algorithm generated from the data of many men in AS who had radical prostatectomy at Hopkins. The Active Care software was developed several years ago, "but we are in the process of refining it. We hope to make it even more helpful and accurate in its predictions for men on AS, and then ideally to roll it out for other AS programs as well." How does it work? Let's say you are in AS, and have been for several years. All of the data you have generated – from multiple biopsies, results of MRI, PSA tests – can be used to predict what's happening with your cancer. Do you need another biopsy? The program compares your results with those of other men who have been in AS and ultimately had a prostatectomy. "It's not just, 'Your PSA is now 4, it used to be 3,'" says Pavlovich, "but, 'there's an approximately 17-percent chance that you are actually harboring more aggressive cancer.' If we can confirm the accuracy, I would like to design a clinical trial using these predictive analytics to guide safer AS."

Improving surgery: Many men who enroll in AS go on to have their cancer treated, and for these men, Pavlovich is working to make surgery safer and even less invasive. "My expertise is in extra-peritoneal robotic prostatectomy," he explains. "I do not operate in the peritoneal cavity at all," but about a centimeter or two below that, "in the retropubic space where Dr. Walsh initially described the operation and where there's no risk of abdominal complications." Many of his surgical patients are at increased risk for abdominal surgery, including men with Crohn's disease, previous inguinal hernia repair other abdominal surgery, with possible abdominal scar tissue.

For these surgeries, Pavlovich makes the five dime-sized incisions within a very small area for safe removal of the prostate. However, with urologist Mohamad Allaf, M.D., Pavlovich is also performing a "single port" method of robotic prostatectomy, using a new Da Vinci robot that requires only one small incision. "We put the port right on top of the prostate and take it out with minimal side effects to patients; we do not have to manipulate the bowel and often never even see it."

"It's not just, 'Your PSA is now 4, it used to be 3,' but, 'there's an approximately 17-percent chance that you are harboring more aggressive cancer."

These refinements to the operation invented by Patrick Walsh 40 years ago (see story on page 7), are exactly in the tradition established by Walsh himself of "tweaking" the operation," says Pavlovich. "We continue to push the envelope and try to make it even better, with even fewer side effects."

Biomarkers: Pavlovich's research also includes work with Brady scientist Jun Luo, Ph.D., and others, to find better biomarkers for prostate cancer. Most recently, Pavlovich and Luo performed molecular urinalysis to detect prostate cancer cells in the urine of men with an elevated PSA. This work was supported with a grant from the Patrick C. Walsh Prostate Cancer Research Fund, Pavlovich points out. Through that endowment, Pavlovich was "the Bernard Schwartz Scholar, and now here I am again with Mr. Schwartz's philanthropy supporting me as Distinguished Professor." ■

40th Anniversary of a Lifesaving Discovery

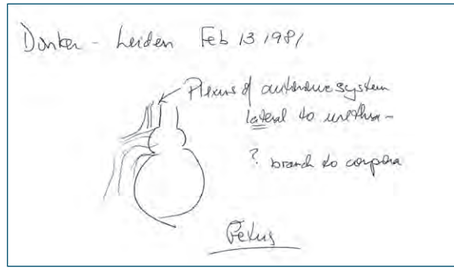
In 1982, only 7 percent of men with prostate cancer underwent surgery. By 1992, 70 percent of men in their 50s and half of men in their 60s underwent surgery. That year, 100,000 radical prostatectomies were performed. By 2002, deaths from prostate cancer had declined by 30 percent – more than for any other cancer in men or women during the same decade.

In 1980, few men with prostate cancer were treated with curative intent. Radiation therapy in that era was underpowered and unable to control the disease, and although surgery had the potential to cure, it was rough: life-threatening bleeding during the operation, and severe incontinence plus lifetime impotence afterward. "Most men felt that the cure was worse than the disease," says Patrick Walsh, M.D., University Distinguished Service Professor of Urology Emeritus, "and what they feared the most was the loss of sexual function."

Surgeons knew why this happened – injury to the nerves that controlled erections – but they were mistaken about where it happened. They thought these nerves lived inside the prostate, and were an unavoidable casualty of removing the cancer. They didn't realize that the nerves were outside the prostate – and as they were removing the prostate, they were cutting them and leaving them in place.

All of this changed on February 13, 1981, when a major discovery was made by Walsh and Professor Pieter Donker, the retired Chair of Urology at the University of Leiden, the Netherlands. Walsh was in Leiden for five days as a Visiting Professor at the Boerhaave Surgical Symposium, operating, lecturing, and visiting laboratories. On his last day there, he went to the anatomy laboratory where Donker was working. The precise location of the nerves to the bladder had never been found because of difficulties using the adult cadaver. So Donker, with the help of a dissecting microscope, was painstakingly identifying them in a stillborn infant, where they were easier to locate. Walsh asked if Donker could show him the location of the branches to the nerves that control erections. "I've never looked," Donker replied. Together, they found them three hours later – outside the prostate! Over the next year, Donker and Walsh worked long-distance to confirm the findings, and Walsh developed the technique to use the neurovascular bundle to identify them during surgery. On April 26, 1982, Walsh performed the first purposeful nerve-sparing operation. This man recovered his potency quickly, and he remains cancer-free today.

It is no exaggeration to say that this discovery revolutionized the field of prostate cancer treatment. In 1982, only 7 percent of men with prostate cancer underwent surgery. However – with the timely development of PSA testing to identify men with curable disease – by 1992, 70 percent of men in their fifties and 50 percent of men in their sixties underwent surgery. That year, 100,000 radical prostatectomies were performed. By 2002, deaths from prostate cancer had declined by 30 percent – more than for any other cancer in men or women during the same time interval. ■



A Fortunate Discovery*: Walsh's illustration from the day he and Pieter Donker discovered the location of the branches to the corpora cavernosa.*

How it All Started

New podcast on prostate cancer from *Boston Globe Magazine* features Dr. Patrick Walsh

Writer Mark Shanahan chronicled his prostate cancer journey in an article for *Boston Globe Magazine*, and also in a six-part podcast. In episode 3: "Hands of God," Shanahan makes his fateful decision: He will have his prostate removed. So he hunts for the best surgeon he can find. The trail begins with Dr. Patrick Walsh, "a guru in the field of urology who revolutionized prostate cancer treatment by preserving men's sexual function."

For more information, visit:
https://podcasts.apple.com/us/podcast/mr-80-percent/id1529316279

Just Invented: A Robotic Ultrasound Probe for the Prostate

The Brady's own Dan Stoianovici, Ph.D., Director of the Urology Robotics Program and inventor of numerous robotic devices, has done it again: With the help of surgeon Misop Han, M.D., the David Hall McConnell Professor in Urology, Stoianovici has developed a novel robotic ultrasound probe especially for the prostate. The work was supported by the Patrick C. Walsh Prostate Cancer Research Fund.

"The probe integrates ultrasound and robotic components within the same structure, and it is the first construction of this kind," Stoianovici says. Its anticipated uses are not only for better imaging of the prostate, but also for "image-guided interventions," says Han: "Currently, ultrasound probes are mostly operated manually. Robot assistance will allow hands-free probe operation, 3-Dimensional ultrasound scanning, and automated needle targeting for ultrasound-guided procedures such as biopsy and ablation."

Stoianovici's lab has built a prototype of the new probe, and used it in preclinical tests – that were so promising, the FDA has approved the device for clinical trials.

Today's targeted biopsy fuses the results of prostate multiparametric (mp)MRI with ultrasound. Although this combined imaging approach is much more accurate than the traditional transrectal ultrasound biopsy, there is still room for improvement. "Targeted biopsy misses a significant number of clinically significant prostate cancers," says Stoianovici, "because of errors in fusion between the MRI and ultrasound, and in manual targeting. In addition, not all clinically significant prostate cancer is detected in MRI." Another drawback, notes Han, is that "the lesions detected by mpMRI that we sample in targeted biopsy are not necessarily those that are biologically dangerous. Further advances in technology are needed, and the new probe has the potential to make our targeted biopsy even more accurate."

Stoianovici's lab has built a prototype of the new probe, and used it in preclinical tests – that were so promising, the FDA has approved the device for clinical trials. "Based on this approval, the Johns Hopkins University IRB has recently approved the study protocol, and we plan to start a safety and feasibility trial soon." Ultimately, Stoianovici believes, the robotic probe has the potential to "reduce the dependency of clinical results on physician's skill and training, and also reduce variability among physicians." ■

Making Robotic Prostatectomy Even More Minimally Invasive

A new da Vinci robotic system involves only one small incision, near the patient's navel. How does it compare to the standard robotic prostatectomy?

If you have had a robotic prostatectomy, you've got a handful of dime-sized scars around your abdomen – one for each incision, or "port" the surgeon made. This has been the standard for minimally invasive removal of the prostate, but that may be about to change. The company that makes the surgical robot, da Vinci, has introduced a single-port system. As its name suggests, this new system involves only one small incision, near the patient's navel. How does it compare to the standard robotic approach?

To answer this question, a team led by Mohamad Allaf, M.D., the Mohamad E. Allaf Director of Minimally Invasive Urology, recently compared outcomes between 376 men who underwent the standard robotic procedure at Hopkins and 208 men who had surgery using the single-port system at Hopkins and elsewhere. Their findings were encouraging: "Overall, we found that blood loss, complication rates, and pain were similar between the two approaches," says Mitchell Huang, a Johns Hopkins medical student and the study's lead author. "The only significant differences were a slightly longer operating time and fewer lymph nodes removed in the single-port group, which might be due to a small learning curve in adopting the new technology."

Of note, there was no difference in post-surgery opioid medication use between the two approaches. "A current point of emphasis in the treatment of prostate cancer is reducing opioid use by patients after surgery," notes Hiten Patel, M.D., M.P.H., a recent graduate of the Brady residency program and current urologic oncology fellow at Loyola University. "Because minimally invasive approaches to surgery generally reduce pain, we might expect that patients who receive the single-port approach to prostate surgery would have less pain. While we didn't see this in our study, this might be something that we will observe as surgeons gain more experience with the technology."

The results of this study, presented virtually at this year's American Urological Association and European Association of Urology meetings, indicate that the single-port approach is safe. "We anticipate that the single-port approach should continue to expand its usage as surgeons become familiar with the surgical system," says Allaf. "The bigger question now is whether this new approach offers enough relative benefit over the standard robotic platform to justify the additional cost to patients with prostate cancer." ■

Virtual Urologic Robotic Surgery

Fighter pilots do it, and so do professional athletes: they spend hours learning and honing their muscle memory on computer simulators. Why not Urology residents?

Thanks to a generous philanthropic gift, "Johns Hopkins Urology is at the forefront of a revolution in medical education," says Marisa Clifton, M.D., Director of the Urology Residency Program. Simulation and deliberate practice are helping the next generation of urologists tackle increasingly diverse and complex surgeries.

Clifton, in collaboration with Andrew Cohen, M.D., Director of Trauma and Reconstructive Urologic Surgery, is developing a six-year educational program that is poised to become a national standard for urologic robotic training.

Burnett: *"We showed that the pelvic nerve network for penile erection can be distinguished from surrounding tissues of the prostate."*

New Imaging Techniques Highlight Specific Erection Nerves

Four decades ago, after his discovery of the bundles of nerves that control erection, Patrick Walsh, M.D., performed the first purposeful nerve-sparing radical prostatectomy (see page 7). Building on that work, urologist Arthur Burnett, M.D., the Patrick C. Walsh Professor of Urology, has been working for many years to figure out how to protect these nerves.

Most recently, using new imaging techniques, he and colleagues in the Johns Hopkins Departments of Radiology and Radiological Science and Electrical and Computer Engineering have been working to identify the specific nerves out of these bundles that are involved in erection.

"Erectile dysfunction remains a considerable potential risk of radical prostatectomy today, despite modern technical improvements of this surgery, because the nerves coursing around the prostate are susceptible to traumatic injury," says Burnett. Even if these nerves aren't cut, they can be stretched, nicked, or bruised during the procedure.

During prostatectomy, the surgeon uses visual landmarks to spot the general location of the nerves, so they can be protected. However, "although a wide variety of nerve-localization techniques have been brought to this surgery, prior studies have not yet established the exact nerves that produce penile erection – distinct from nerves that do not have any role in erection or cause erection decline."

In this collaborative investigation, using a rodent model that mimics the prostate and pelvic anatomy of humans, Burnett and colleagues used voltage-sensitive dye imaging while they induced erections, "which caused the relevant erection nerves to selectively light up. We showed that the pelvic nerve network for penile erection can be distinguished from surrounding tissues of the prostate." Burnett plans to bring this advance to radical prostatectomy surgery soon, "with hopes for improving erection recovery after this surgery." Their work was published in *Scientific Reports* ■

Prostate Cancer Immunotherapy: A New Target

"Despite enrolling patients who had a high risk of prostate cancer recurrence, the majority of these men receiving Enoblituzumab remain cancer-free for 12 months or more after prostatectomy."

Immunotherapy – "checkpoint inhibitor" drugs that help the immune system recognize and fight off cancer – works much better in kidney cancer and melanoma than in prostate cancer. Why?

Scientist Emmanuel S. Antonarakis, M.D., professor of oncology and urology, believes these drugs may be targeting the wrong immune mechanisms in prostate cancer. "Checkpoint inhibitors unlock specific molecules, such as PD-1 and CTLA-4, that act as handcuffs on the body's powerful T cells, which normally fight off enemy invaders." With the restraints lifted, T cells seem to wake up, notice that something is very wrong, and attack cancer cells.

Building on the work of former Brady urologist Ashley Ross, M.D., Ph.D., Antonarakis and cancer immunologist Eugene Shenderov, M.D., D.Phil., are investigating a promising new target: a molecule called B7-H3. "B7-H3 is not only highly expressed on prostate cancer cells," says Antonarakis, "but it appears to be associated with more rapid progression of prostate cancer following local treatment with surgery or radiation."

The team designed a clinical trial to study the anti-tumor, immunological and clinical effects of targeting B7-H3 in high-risk men with localized prostate cancer who are about to undergo prostatectomy. Prostatectomy patients present an ideal opportunity for pathologists to study "before and after" tumor tissue – the biopsy samples taken at diagnosis, and then the removed prostate specimen. "In our trial, 32 patients were treated with Enoblituzumab, a humanized monoclonal antibody against B7-H3, before surgery." Then, after surgery, expert prostate cancer pathologist, Angelo De Marzo, M.D., Ph.D., examined the tissue, looking for evidence of an antitumor immune response.

Prelimary results are promising: "Enoblituzumab was well tolerated," Shenderov says, "and did not seem to produce as many side effects as other immunotherapy drugs." Also, "prostatectomy samples from men who received Enoblituzumab showed an altered tumor microenvironment in a fashion that indicates enhanced immune infiltration – a hallmark of responsiveness to immunotherapy."

Even more exciting: "A significant proportion of patients had a drop in their PSA level as well as a decrease in their Gleason scores after receiving Enoblituzumab," adds Antonarakis. "Despite enrolling patients who had a high risk of prostate cancer recurrence, the majority of these men remain cancer-free for 12 months or more after prostatectomy."

These results are so promising that Antonarakis and Shenderov are designing new studies to target B7-H3 in men with metastatic prostate cancer – the first studies of their kind. ■

Genes, Race, and Prostate Cancer

Understanding Why African American Men Are at Higher Risk of Aggressive Cancer

"In Baltimore City, five out of six men who die from prostate cancer are men of African descent," says molecular geneticist William Isaacs, Ph.D., the William Thomas Gerrard, Mario Anthony Duhon, and Jennifer and John Chalsty Professor of Urology. "It's like an epidemic." This high death rate is not limited to the U.S. All over the world, men of African descent are diagnosed at a younger age with aggressive prostate cancer.

And yet, these men who are at highest risk of dying of prostate cancer are least represented in major studies, Isaacs says. "At Johns Hopkins, we have blood and tissue samples from 20,000 men who have undergone radical prostatectomy. But fewer than 10 percent are African American (AA). If you just looked at those results, you'd think this was a disease of white men." Isaacs recently did a search for papers on clinical trials for prostate cancer, "and the average participation of African Americans was 3 percent. These men should be overrepresented, not underrepresented! They're more likely to get prostate cancer, but are rarely included in trials that might result in better treatment and a better outcome." Similarly, in genetic studies, "AA men are grossly underrepresented. Our lack of knowledge about the molecular drivers of lethal prostate cancer among AA men remains a major barrier to precision medicine in this population."

Good news: "That is changing." Isaacs and colleagues at the Brady including molecular pathologist Tamara Lotan, M.D., and immunologist Jelani Zarif, Ph.D., in partnership with Tamaro Hudson, Ph.D., and Angel Byrd, M.D., Ph.D., at Howard University, have proposed a groundbreaking project to identify factors in the genome and immune system in AA men with aggressive, locally advanced or metastatic prostate cancer. "This project is first-in-field and long overdue," says Isaacs.

Isaacs: *"In African American men, the team has found mutations in some genes that are much less well-studied."*

Supported by the Patrick C. Walsh Hereditary Prostate Cancer Program, Isaacs' research group is also conducting the largest-ever, whole exome sequencing (WES, looking for variations in every protein-coding region in the entire genome) study of germline (inherited) DNA from AA men with prostate cancer.

So far, in preliminary analyses of the germline data, Isaacs and colleagues have found no significant differences among races in the frequency of mutations of genes such as *BRCA2* and *ATM* – well-known genes linked to lethal prostate cancer in Caucasian men. However, in AA men, the team has found mutations in some genes that are much less well-studied – genes including *LZTR1*.

"This is pretty much, hands down, the most important genetic risk marker I've seen for African American men."

Genetic Variants: Risk SNPs

In this project, and also as part of an international team of scientists, Isaacs is looking at variant stretches of DNA called *single-nucleotide polymorphisms*, or SNPs (pronounced "snips"). SNPs are almost-but-not-quite-right bits of DNA. Isaacs, a pioneer in discovering faulty genes involved in prostate cancer, has identified and studied many SNPs.

What's the difference between bad genes and SNPs? "Mutated genes like *BRCA2* are 'high penetrance' genes," Isaacs explains. "Although the mutations are rare, they have a big impact." The opposite is true for these common genetic variants, "which typically are not even in a gene; they're close to genes and affect the expression and the regulation of the gene. Instead of a six-fold higher risk of getting cancer, which you might have with *BRCA2*, you'll have maybe a 1.1- or 10-percent increase in risk. However, there are many of these risk SNPs," 170 discovered by the international team at last count. "You don't look at just one SNP or two, but many of them, and then you just count," with the cancer risk going up or down depending on how many out of the bunch of SNPs a man has. "That's why you really need to look at them as a group."

In 2008, Isaacs, Wake Forest scientist Jianfeng Xu, Ph.D. (now at NorthShore University Health System), and Henrik Gronberg, M.D., from Sweden, were the first to publish their discovery of five SNPs that can raise a man's risk of prostate cancer. The risk, they found, depends on how many variants he has, and also on whether he has inherited a "risk allele," one of a pair of DNA sequences at the same place on a chromosome that control the same characteristic (like hair color, or color blindness). "Each one of these SNPs has a risk and a non-risk allele." In that study, Isaacs' team showed that "if you inherit the risk allele for each one of these five SNPs, your risk is five times higher." The risk is cumulative: the more risk SNPS and alleles, the higher your odds of getting prostate cancer. The opposite is also true: if you hit the genetic jackpot and have very few bad stretches of prostate cancer-related DNA, your lifetime risk is reassuringly low.

Isaacs was part of a 2020 study directed by Chris Haiman, Ph.D., at USC, published in *European Urology*, showing that men of African – but not European – descent have a particular SNP that raises the odds of getting prostate cancer more than most SNPs do. "It's not in individuals of European ancestry, only in men of African descent," but men who have this variant have a twofold increase in their risk of prostate cancer. "If you have a family history and you have it, your risk goes up about 28 percent, and if you have a family history of early onset cancer, diagnosed under age 60, your risk goes up as high as 40 percent. There's no other variant or DNA mutation that can dramatically increase your risk of familial prostate cancer, and this one is quite common in men of African descent. This is pretty much, hands down, the most important genetic risk marker I've seen for AA men. It's a major finding that will have implications for screening men of African descent." ■

Targeting "Non-Coding" Genes for Prostate Cancer Radiation Therapy

"By inhibiting miR-21 in localized prostate cancers, we could potentially reduce the amount of radiation needed for primary or salvage radiation therapy – or achieve a greater cancer-killing effect with standard radiation doses."

In the search for better treatments for prostate cancer, protein-coding genes – genes that make proteins that cancer needs to grow and spread – have been a major focus of scientific attention. "Proteins are often good targets for cancer drugs," says scientist Shawn Lupold, Ph.D., the Catherine Iola and J. Smith Michael Distinguished Professor in Urology, "because they contain unique features that can be specifically targeted with tiny molecules or antibody-based therapies. However, there is an entirely different class of genes that contribute to prostate cancer growth but do not encode a single protein."

These non-coding genes "are proving to be novel biomarkers and therapeutic targets for cancer," explains Theodore DeWeese, M.D., a pioneer professor of radiation oncology and molecular radiation sciences, who brought the first gene therapy for prostate cancer to clinical trial at Hopkins. Instead of proteins, these genes make RNA molecules, "which are more difficult to target with small molecules and antibodies." Lupold and DeWeese are developing strategies for prostate cancer radiation therapy aimed at specific RNA molecules, called microRNAs.

For more than 20 years, Lupold's laboratory has been studying microRNAs in prostate cancer – particularly, one promising microRNA gene: *miR-21* "This microRNA is highly active in multiple types of cancers, and is believed to drive cancer development, growth, metastasis, and resistance to treatments," Lupold says. In previous studies, Lupold and colleagues showed that *miR-21* is upregulated in prostate cancer, associated with high Gleason grade, and capable of driving resistance to hormonal therapy. Lupold and DeWeese recently made another discovery about *miR-21*: it plays an important role in how prostate cancer cells respond to radiation therapy. "We were looking to identify microRNAs that inhibited DNA repair and enhanced the cell-killing effects of radiation therapy," says DeWeese, "but we found that *miR-21* caused the opposite effect. Cells that received *miR-21* appeared resistant to radiation therapy, with almost three times more cancer cells surviving radiation treatment!" Shireen Chikara, a postdoctoral fellow working with Lupold and DeWeese, has validated these findings in several different prostate cancer cell models. Importantly, her work found that inhibiting *miR-21* made radiation therapy even more effective.

Chikara has used two different strategies to block *miR-21* in prostate cancer: natural chemicals that prevent cells from making *miR-21*, and RNA-based *miR-21* inhibitors. "By directly injecting *miR-21* inhibitors, I have been able to significantly reduce the levels and activity of *miR-21* in human prostate tumors grown in mice," she says. Chikara's work has exciting implications for radiation therapy, notes DeWeese: "Radiation resistance is a significant factor contributing to the progression of localized prostate cancers following unsuccessful primary or salvage radiation therapy. By inhibiting *miR-21* in localized prostate cancers, we could potentially reduce the amount of radiation needed for primary or salvage radiation therapy – or achieve a greater cancer-killing effect with standard radiation doses." ■

DeWeese and Lupold*: "*miR-21 *is believed to drive cancer development, growth, metastasis, and resistance to treatments."*

***Pomper**: PyL successfully localized sites of disease in men with biochemical recurrence – even men with low PSA levels.*

PSMA PET/CT with PyL Reaches a Key Milestone toward Approval

"PyL PET/CT detected localized disease in most men with biochemical recurrence presenting with negative or equivocal conventional imaging, and led to changes in management in the majority of patients."

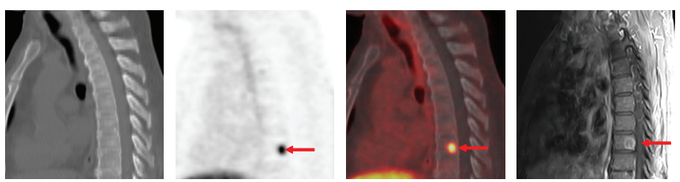
It is no exaggeration to say that the work of Martin Pomper, M.D., Ph.D., Director of Nuclear Medicine and Medical Imaging, has revolutionized our ability to see tiny bits of prostate cancer anywhere in the body, and that his discoveries are actively expanding our ability to treat recurrent and metastatic prostate cancer. Pomper was the first to figure out how to engineer a small-molecule, harmless radioactive tracer to target prostate-specific membrane antigen (PSMA), a protein that lives in high concentrations on the surface of prostate cancer cells. Those tagged cells then "light up" on a PET scan to show very small areas of cancer.

The original support to develop this technique came from the Patrick C. Walsh Prostate Cancer Research Fund in 2012. Several years ago, Pomper tested the first PSMA-targeted PET agent in a clinical trial. Then he refined this into a more sensitive and specific, second-generation agent that provides sharper images, called [18F]DCFPyL (PyL).

PyL is now another step closer to FDA approval. "Current imaging techniques are inadequate for localizing and characterizing disease in men with biochemically recurrent prostate cancer, particularly in men with a low PSA (less than 2 ng/ml)," says Pomper. "But although PyL, and analogs of it, have been used in tens of thousands of patients worldwide to detect and help manage prostate cancer – to detect primary disease, metastases, and to guide focal therapy – no PSMA-targeted agent has yet garnered FDA approval in the U.S."

Recently, PyL finished the second of two Phase 3 prospective trials, the CONDOR trial, with results presented at the 2020 meeting of The American Society of Clinical Oncology. "CONDOR met its primary endpoint," Pomper says. PyL successfully localized sites of disease in men with biochemical recurrence – even men with low PSA levels. "PyL PET/CT detected localized disease in most men with biochemical recurrence presenting with negative or equivocal conventional (bone scan plus CT) imaging, and led to changes in management in the majority of patients. Clinicians find PSMA PET imaging useful in men with recurrent or suspected metastatic prostate cancer."

CONDOR and an earlier trial, OSPREY, which focused on men with high-risk prostate cancer, have established the performance characteristics of PyL. A new drug application (NDA) for PyL is nearing submission as another step toward FDA approval. ■



These are images of a 56-year-old man with a rising PSA after prostatectomy. Although a CT scan was negative, PyL found oligometastataic bone disease, which was confirmed with MRI. The man was treated with stereotactic radiation therapy, after which his PSA level dropped.

When PSA Comes Back: GC Test Helps Predict Risk of Metastasis

"As men are approaching low-volume metastasis, there's a perfectly reasonable period in which you can ask the question: Does local therapy change the metastatic process?"

Phuoc Tran, M.D., Ph.D., professor of radiation oncology and molecular radiation sciences, oncology, and urology, is working to push the boundaries of curable cancer.

Last year in Discovery, we reported on the encouraging results of Tran's ORIOLE trial (Observation vs Stereotactic Ablative RadiatIon for OLIgometastatic Prostate CancEr). In that trial, men with oligometastasis – a few tiny bits of cancer outside the prostate area – were treated with stereotactic radiation therapy (SABR). Building on those results and promising early results of ORIOLE's successor, the RAVENS trial (RAdium-223 and SABR Versus SABR for oligomEtastatic Prostate caNcerS) at treating the early spread of prostate cancer, Tran is now looking for a better early warning test to detect it.

"Gleason score and PSA alone are not enough to accurately identify men with more biologically aggressive disease," he says. Better biomarkers are urgently needed, and Tran and his NRG Oncology colleagues may have found one: the Decipher genomic classifier (GC), developed from tissue samples of prostatectomy patients. Tran's group recently became the first to validate the GC in a national study, the NRG/RTOG 9601 Phase 3 placebo-controlled, randomized trial, in which 760 men with a returning PSA after prostatectomy received salvage radiotherapy (sRT) plus around two years of hormonal therapy with bicalutamide (Casodex).

Being able to predict the risk of future metastasis is especially important in men receiving early sRT who have a very low but rising PSA – below 0.7 ng/mL. "Our study demonstrates that among men receiving earlier sRT, those with a higher GC had more than an 11-percent improvement in 12-year distant metastasis, and nearly a 5-percent improvement in overall survival from the addition of hormonal therapy. The GC was able to predict which men were more likely to develop distant metastasis, and to die of prostate cancer – and thus, which men could really benefit from the addition of bicalutamide to sRT."

Soon, better imaging – particularly, PSMA-PET – "will undoubtedly help determine the true state of tumor burden, particularly in newly diagnosed men when the PSA is rising but is less than 10," he continues. "Conventional imaging really is not useful when the PSA is 5 or 10."

Tran believes the number of men with oligometastasis in the U.S. is huge – "much higher than the number of men diagnosed with upfront metastatic prostate cancer each year." For now, "systemic therapy is the standard of care for patients with metastatic disease. But in that gray area of biochemical recurrence (PSA creeping back up after prostatectomy or radiation of the primary tumor), as men are approaching low-volume metastasis, there's a perfectly reasonable period in which you can ask the question: does local therapy change the metastatic process?" That was the question behind the ORIOLE trial.

"If this were not a spectrum, and if local therapy could not alter that natural history of metastasis, then we shouldn't be able to affect progression with local therapy alone. Patients should progress no matter what. We did not see that" in the ORIOLE trial, nor in early results so far of the RAVENS trial. "Obviously, stronger evidence is needed," but Tran believes that all evidence points to this conclusion: "the course of early metastasis is not set in stone." ■

Coulter Joins Research Faculty

Could timing make a difference with drugs that target the androgen receptor, combined with radiation and other treatments?

Meet the newest research scientist to join our faculty: Jonathan Coulter, Ph.D., M.H.S., who joins The Brady research faculty after completing his postdoctoral fellowship in Radiation Oncology and Molecular Radiation Sciences at Johns Hopkins, and serving as Chief Research Fellow of the Sidney Kimmel Comprehensive Cancer Center.

Coulter earned his Ph.D. from the Johns Hopkins Bloomberg School of Public Health prior to starting his research fellowship, which was supported in part by an award from the Department of Defense. He credits his interest in prostate cancer to "the work of pioneers like Donald Coffey, who helped us understand the organization of massive amounts of DNA inside the tiny nucleus of a cell."

Could timing make a difference with drugs that target the androgen receptor (AR), combined with radiation and other treatments? Coulter and colleagues including Ted DeWeese, M.D., Srinivasan Yegnasubramanian, M.D., Ph.D., Angelo De Marzo, M.D., Ph.D., and Daniel Song, M.D., believe that it might. "It might even lead to higher cure rates and less toxicity in patients with early or late-stage prostate cancer," says Coulter. The team published a report showing that AR activity damages DNA in prostate cancer cells as it attempts to navigate the complex 3D-structure of DNA, and that this activity could be exploited. ■

***Jonathan Coulter** credits his interest in prostate cancer to "the work of pioneers like Donald Coffey, who helped us understand the organization of massive amounts of DNA inside the tiny nucleus of a cell."*

***Choi and McConkey**: Choi, McConkey and colleagues are investigating "whether tumors that display low-oxygen gene expression are uniquely sensitive to therapies that target tumor blood vessels,"*

DISCOVERY IN BLADDER CANCER

Targeting Hypoxia in Bladder Cancer

The Brady team is investigating whether these cancers are uniquely sensitive to therapies that target tumor blood vessels.

A novel avenue of treatment for subtypes of bladder cancer builds on Nobel Prize-winning work by a Johns Hopkins scientist, Gregg Semenza, M.D., Ph.D., who described how tissues respond to low oxygen concentrations (hypoxia).

It turns out that hypoxia happens in some aggressive forms of bladder cancer, as well. As part of their discovery of the basal and luminal molecular subtypes of bladder cancer, Brady scientists Woonyoung Choi, M.S., Ph.D., and David McConkey, Ph.D., Director of the Johns Hopkins Greenberg Bladder Cancer Institute and the Erwin and Stephanie Greenberg Professor of Urology, recognized that "basal bladder cancers express genes associated with the low-oxygen pathway discovered by Dr. Semenza and his colleagues," says McConkey.

Working with UK scientist Catharine West, Ph.D., and her colleagues in Manchester, Choi and McConkey confirmed that low-oxygen gene expression is associated with more aggressive and fatal cancers, and that a class of biomarkers known as microRNAs can be used to identify these tumors. In an exciting collaborative project with Johns Hopkins scientist Daniele Gilkes, who trained with Semenza, along with investigators at Memorial Sloan-Kettering Cancer Center, Dana-Farber Cancer Institute, and Duke University, the Brady team is investigating "whether tumors that display low-oxygen gene expression are uniquely sensitive to therapies that target tumor blood vessels," says Choi. Two recently-completed Phase 3 clinical trials, based on the pivotal results of a Phase 2 clinical trial – led by oncologist Noah Hahn, M.D. – of gemcitabine/cisplatin plus bevacizumab (a therapy that targets tumor blood vessels), confirmed that "these agents extend some patients' lives," says McConkey. The team's research also suggests that oxygen-related biomarkers can determine which patients are likely to benefit from blood vessel-targeted therapies. ■

New Immunotherapy Targets: B Cells

B cells play a vital role in the immune response against cancer.

B cells are immune system cells that help fight cancer. They're not as famous as other immune cells – T cells, for instance – and, in fact, have been overlooked as a focus of immunotherapy. New research at The Brady is changing this.

Burles (Rusty) Johnson III, M.D., Ph.D., a clinical fellow in oncology, is investigating the functional role of B cells in bladder cancer.

"Although immunotherapy has improved responses in some patients with advanced bladder cancer, unfortunately these therapies do not significantly shrink tumors in most patients," Johnson says. "Thus, we need to identify the mechanisms that are preventing immunotherapy from being as effective as it could be." While most immune-related research in bladder cancer has focused on stimulating cancer attack by T cells, Johnson continues, "little research has focused on B cells." B cells play a vital role in the immune response against cancer. "They can stimulate or inhibit anti-tumor immunity, depending on how the patient's immune system responds to cancer growth." Specifically, Johnson is looking to identify B cell subsets that block the immune response against cancer and, instead, help bladder cancer to grow. ■

Tackling a Rare, Aggressive Form of Cancer

Small cell neuroendocrine bladder carcinoma (SCBC) is an aggressive, lethal variant of urothelial carcinoma, and much about it, until recently, has been a mystery. Because it's so rare – affecting less than 1 percent of all people with bladder cancer – "its molecular characteristics remain elusive, and no standard treatment options are available," says scientist Woonyoung Choi, Ph.D.

It turns out that small cell neuroendocrine bladder carcinoma has a lot in common with small cell neuroendocrine lung cancer, and this may lead to new strategies for treatment.

Choi, in collaboration with medical oncologist Jean Hoffman-Censits, M.D., is working to shed some light on SCBC. Recent genomics studies have shown the overall similarity of small cell neuroendocrine tumors in the lung and other sites, Choi says. Based on these observations, she discovered subtypes of SCBC that are driven by three key transcriptional regulators – which are similar to those found in small cell lung cancer. "The next step is to comprehensively characterize the biological characteristics of each subtype, in order to identify novel therapeutic targets for patients with deadly SCBC."

With Hoffman-Censits, Choi is investigating how well these novel subtypes respond to therapy in a Phase 2 clinical trial of neoadjuvant chemotherapy plus atezolizumab in patients with SCBC. ■

Can Biomarkers Predict Who Will Benefit from Chemotherapy in Bladder Cancer?

New tests could identify who will benefit from chemotherapy, and spare those who will not.

Imagine a room full of patients with muscle-invasive bladder cancer. Which of them should get presurgical (neoadjuvant) chemotherapy? The answer right now is, "all of them" – but that's not the best answer.

"Although neoadjuvant chemotherapy is recommended for everyone with muscle-invasive bladder cancer, it only benefits a subset of those patients," says David McConkey, Ph.D., who is also Chair for Translational Medicine in the genitourinary division of the Southwest Oncology Group (SWOG). Together with Woonyoung Choi, M.S., Ph.D., McConkey is leading a nationwide effort to validate several panels of biomarkers. The biomarkers test for basal and luminal molecular subtypes of bladder cancer, and also for mutations in DNA damage repair genes, in tumors that were collected from patients enrolled in the SWOG's Phase 2 clinical trial comparing gemcitabine/cisplatin and MVAC chemotherapy.

"If the tests are validated, they will enable clinicians to use pretreatment biopsies to identify the subset of patients who will receive benefit, sparing the ones who will not," McConkey notes. "This would dramatically change clinical practice." ■

If Bladder Cancer Responds Well to Chemotherapy, Is It Safe to Skip the Cystectomy?

Who needs surgical removal of the bladder (radical cystectomy)? And who doesn't need it? Unfortunately, that question is still very hard to answer. Some research groups have proposed that for certain patients with bladder cancer – people whose tumors contain specific mutations – a very favorable response to chemotherapy may be enough to justify leaving the bladder in place, and then following the patient closely.

Not so fast, say Brady scientists. "That's a milestone we have not yet achieved," says Trinity Bivalacqua, M.D., Ph.D., the R. Christian B. Evensen Professor in Urology and Director of Urologic Oncology. "The standard of care for localized, muscle-invasive bladder cancer is neoadjuvant chemotherapy, followed by radical cystectomy and urinary diversion. We don't yet have reliable and accurate methods to identify who can safely avoid surgery."

In a recent study, Bivalacqua and Alex Baras, M.D., Ph.D., Director of Pathology Informatics, studied data from more than 300 bladder cancer patients who received chemotherapy and radical cystectomy at Johns Hopkins, evaluating doctors' accuracy in determining a patient's response to chemotherapy before cystectomy. Their findings, published in *European Urology*, showed that current strategies to identify patients who can safely avoid cystectomy, including tumor sequencing for DNA mismatch repair mutations, were not useful in identifying patients who had responded well to chemotherapy. "Even repeated visual (cystoscopic) examinations of the bladder with repeat biopsies failed to identify residual high-stage cancer in nearly a third of patients," says Baras.

"The idea that we could accurately pick out which patients have been cured by chemotherapy alone, and avoid surgery in those cases, is very intriguing," says urology resident Russell Becker, M.D., Ph.D., one of two lead authors on the study, along with Brady resident Alexa Meyer, M.D. "But we just aren't there yet. We have some work to do, to refine these techniques before they can safely be applied to guide management decisions – especially when the stakes are so high." Several large, ongoing clinical trials are attempting to use tumor sequencing and post-chemotherapy clinical restaging to select patients to forego cystectomy. "Our work strongly suggests that those trials are misclassifying quite a few patients – who may, in fact, have residual muscle-invasive disease – and are shunting them into an experimental, conservative management strategy that may ultimately be more hazardous than the surgery they are trying to avoid." ■

Non-Muscle Invasive Bladder Cancer: A New Approach, and Molecular Insights

Of the estimated 80,470 new cases of bladder cancer diagnosed in the U.S. in 2019, the vast majority – about 70 percent – are caught at an early stage: non-muscle invasive disease, with cancer limited to the epithelium, the tissue lining the bladder. The standard first-line treatment is transurethral resection of the bladder tumor, followed by immunotherapy: bathing the bladder with intravesical bacillus Calmette-Guerin (BCG), a modified form of tuberculosis bacteria.

"Initially, it's effective," says medical oncologist Noah Hahn, M.D. However, adds urologist Max Kates, M.D., "while up to 35 percent of patients have long-term, sustained remissions with intravesical BCG, as many as 60 percent of patients will have a recurrence of cancer within two years. Ultimately, 40 percent of these high-risk patients will progress to muscle-invasive stages and require radical cystectomy, surgical removal of the bladder." That many patients do not have a long-term response is one issue with BCG; another is a basic problem of supply. "We have had continuing supply issues and shortages in getting BCG," says Kates, "due to the fact that there is currently only one manufacturer."

"Clinical outcomes of the GEMDOCE combination were promising, approaching 50 percent recurrence-free survival at two years when used with monthly maintenance."

A better approach? Kates and colleagues have begun investigating a combination of two chemotherapy drugs, gemcitabine and docetaxel (GEMDOCE), delivered directly in the bladder – in the same way that BCG is instilled – for newly diagnosed bladder cancer patients. In previously published work at the Brady, as part of a multi-institutional study, Kates and Trinity Bivalacqua, M.D., Ph.D., evaluated patients who were given GEMDOCE when bladder tumors recurred after BCG. "Clinical outcomes of the GEMDOCE combination were promising," says Kates, "approaching 50 percent recurrence-free survival at two years when used with monthly maintenance." Based on these promising results, Kates has opened a Phase 2 clinical trial to evaluate this combination for newly diagnosed patients who have not had previous BCG. "We will also be looking for a biomarker that can predict response to GEMDOCE, which would help us guide newly diagnosed patients either to intravesical chemotherapy or BCG immunotherapy, based on their tumor biology." More information on this clinical trial can be found here: https://clinicaltrials.gov/ct2/show/NCT04386746.

Molecular characterization of CIS: Hahn recently presented insights on the key drivers of tumor and immune cell biology in patients with a specific type of non-muscle invasive bladder cancer, called carcinoma in-situ (CIS), at the International Bladder Cancer Network's Annual Meeting. Significant work by a Brady-led team of investigators on urothelial CIS and adaptive immune resistance to intravesical BCG in non-muscle invasive bladder cancer was published recently in Applied Immunohistochemistry Molecular Morphology. The team included Kara Lombardo, Max Kates, Woonyoung Choi, Trinity Bivalacqua, Andres Matoso, and others.

"The scant size of these tumors has presented challenges to unraveling tumor and immune cell biology unique to CIS patients," Hahn says. But with the help of sophisticated technology, he and colleagues have made unprecedented inroads in understanding gene expression in these patients. In a study with HTG Molecular Diagnostics, using that company's 1,392-gene Precision Immuno-Oncology RNA-based platform, the team was able to perform comprehensive profiling of gene expression in tissue from 43 out of 50 CIS patients. Even more exciting: "We identified unique immune gene expression signatures found only in patients with CIS," says Hahn. Next, the team plans to investigate whether these new CIS signatures are associated with response to BCG and other forms of bladder cancer immune therapy. ■

DISCOVERY IN KIDNEY CANCER

Our Multidisciplinary Kidney Cancer Group

Meet our two new faculty – who, with Phillip Pierorazio, M.D., newly named Director of the Kidney Cancer Program – form a multidisciplinary and translational kidney cancer group.

Nirmish Singla, M.D., M.S.C.S. has joined The Brady as an assistant professor in Urology and Oncology. Singla earned his undergraduate degree in Biomedical Engineering from the University of Michigan, and his M.D. from the University of Michigan Medical School. He completed his residency in Urology at the University of Texas Southwestern Medical Center (UTSW), and spent an additional year there as a postdoctoral research fellow through the NIH/NCI Physician Scientist Training Program. Singla earned a Master of Science in Clinical Science (M.S.C.S.) degree and pioneered a Physician Administrative Fellowship Program at UTSW, and then completed an advanced SUO-accredited clinical fellowship in Urologic Oncology at Memorial Sloan Kettering Cancer Center. Singla, who is Director of Translational Research in GU Oncology at Johns Hopkins, also treats patients with testicular cancer and urothelial malignancies.

Yasser Ged, M.B.B.S., finished medical oncology higher specialist training with Royal College of Physicians in Ireland, and then completed an Advanced Medical Oncology Fellowship at Memorial Sloan Kettering Cancer Center. Ged's clinical and research focuses on the management of advanced renal cell carcinoma; specifically,

he is studying rare kidney cancers, novel therapeutic agents, and developing immunotherapy biomarkers of clinical benefit." ■

Shedding Light on the "Trickle-Down" Effect of Mutated Genes in Kidney Cancer

"We have identified candidate drug targets that have been explored in other cancer types, but have yet to be evaluated in ccRCC."

The most prominent form of kidney cancer is clear cell renal cell carcinoma (ccRCC), and Hopkins investigators are part of a team that has delineated the molecular profiles of ccRCC. The work, done as part of the National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium initiative, was published in Cell. The paper's first author was David Clark, Ph.D., a postdoctoral fellow in the lab of Hui Zhang, M.S., Ph.D., Director of the Mass Spectrometry Core Facility in the Center for Biomarker Discovery and Translation.

Not only did the team identify several novel aspects of ccRCC biology, but "this represents the first major, large-scale proteogenomic characterization of ccRCC," explains urologist and clinical collaborator Phillip Pierorazio, M.D. "Many of the genetic alterations of kidney cancer are known, but this research demonstrates how those genetic alterations trickle down to proteins and active functions of the cells that define a kidney cancer." The implications of this research are exciting, he adds: "It tells us much more about potential targets for diagnosis and therapy than we previously knew."

Although many patients have the same type of cancer, there are many variations at the genomic level – tiny, subtle differences that can affect how well someone responds to treatment. In this study, Clark and colleagues showed that "protein features could be leveraged to stratify patients into specific, targeted treatment regimens that could be more effective," says medical oncologist Yasser Ged, M.B.B.S. "Some of these tumors progress down a pathway of vascular growth factors, some progress by manipulating the immune system – and some take advantage of a combination of vascular growth factors and the immune system. Fortunately, we have medications to target vascular growth factors and the immune system. Data from this study may help us understand how to personalize treatment – to couple or uncouple those therapies, as needed."

In addition, the team discovered "cellular switches," called kinases, that contribute to kidney cancer cell growth and the corresponding protein regulators. Even more exciting: "There are known, FDA-approved inhibitors" for these kinases," says Clark, "and we have identified candidate drug targets that have been explored in other cancer types, but have yet to be evaluated in ccRCC. We hope this work will expand the repertoire of treatments for our patients." ■

The Hunt for Urinary Biomarkers to Diagnose Kidney Cancer

Extracting these vesicles from the blood and urine could make it possible to diagnose kidney cancer noninvasively.

Kidney cancer is the perfect disease for urinary biomarkers," says scientist Sarah Amend, Ph.D. "These tumors arise

***Amend**: Looking for proteins and molecules released by kidney tumors into the blood and urine.*

from the filtering apparatus of the kidney, and should release proteins and molecules into both the blood and urine of patients with tumors. However, no biomarkers exist."

Amend and Brady scientists Richard Zieran, M.D., Ph.D., and Liang Dong, Ph.D., are actively working to change this. They have focused on extracellular vesicles, "tiny bubbles carrying RNA and proteins, which contain information about the cells that secreted them: cancer cells," says Zieran. These vesicles, adds Dong, "may transfer nucleic acids and proteins between cells, modulate a variety of cellular functions, and may be a marker of disease or disease state."

Extracting these vesicles from the blood and urine – and decoding them to diagnose kidney cancer – could make it possible to diagnose kidney cancer noninvasively. But right now, making sense of these bubbles is a bit like not seeing the forest for the trees: These vesicles are not the only 'bubbles' found in blood and urine," notes Amend. "Healthy cells also make vesicles that contain normal RNA and proteins." Thus, the team is studying vesicles that have been taken directly from kidney cancer tissues, "creating the pathway to identify the right biomarkers in the same patients' blood and urine." This work was published in *Medical Oncology*. ■

Newly Discovered Cells Shed Light on Immunotherapy in Kidney Cancer

Finding new potential biomarkers and targets for treating kidney cancer.

Immunotherapy – harnessing the body's powerful immune cells to fight cancer – has the potential to revolutionize how cancer is treated. But it's still pretty new: "We are still only beginning to learn about this approach," says urologist Phillip Pierorazio, M.D. He has teamed up with nephrologist Hamid Rabb, M.D., to study a newly discovered kidney T cell, the "double negative" T cell.

In work recently published in the *Journal of the American Society of Nephrology* and *Journal of Immunology*, in surgical specimens removed by Pierorazio, Rabb found double negative T cells in cancerous kidney tissue – and also in the normal tissue surrounding the cancer. Using sophisticated molecular tools including RNA sequencing of separated kidney white cells, he identified novel molecules on these cells that can be targets for future therapeutics.

"We identified another new white cell in the kidney, called 'intermediate macrophage'" says Rabb, "and we found that kidney-infiltrating immune cells express a molecule called NGAL that modifies kidney function and is an early biomarker." The team hopes their findings will lead to new strategies for early detection and treatment of kidney cancer. ■

DISCOVERY IN TESTICULAR CANCER

Early-Stage Testicular Cancer: Treating with Surgery Alone?

"Sometimes the best course is to do nothing," says urologist Phillip Pierorazio, M.D., Director of the Testicular Cancer Program. "Because most men with testicular cancer are young and can expect to be cured with surgery alone, there has been an increasing push to put men on surveillance after surgical treatment of testicular cancer."

A new Brady study, published in *European Urology Focus*, finds that in addition to preventing unnecessary side effects from chemotherapy and radiation, this approach might also be the most cost-effective option for men with a form of early stage testicular cancer called seminoma.

"For the vast majority of men with this type of low-risk testicular cancer, surgery alone is enough. If there is a recurrence later on, we can deal with it and the outcomes will still be excellent."

"We created a model based on well-established data from previous studies on testicular cancer," says Mitchell Huang, a Johns Hopkins medical student and the study's lead author. "We simulated outcomes for men with early stage seminoma and found that over a 10-year period, active surveillance resulted in a greater quality of life at a lower cost compared to other treatment options." In the study, the Brady team also reported that men had a very low rate of death from testicular cancer – regardless of what treatment option was selected.

"We made adjustments to the model to account for possible differences in clinical outcomes and costs," notes Brady urology resident Tony Su, M.D. "Our findings held up across a wide range of scenarios, which gives us confidence that active surveillance after surgery should be the preferred option for men with very low-risk testicular cancer."

The authors found very little benefit to starting men on radiotherapy or chemotherapy after surgery. "These findings really underscore what we've seen previously in the literature," says Pierorazio, leader of the research team. "For the vast majority of men with this type of low-risk testicular cancer, surgery alone is enough. If there is a recurrence later on, we can deal with it and the outcomes will still be excellent." ■

JAMA Study: Immunotherapy May Affect Male Fertility

This study is the first to link immunotherapy for cancer with reduced sperm-producing cells.

In a first-of-its-kind study, Brady scientists have shown that immunotherapy for cancer can have a major impact on sperm production. These findings were published in *Journal of the American Medical Association (JAMA) Oncology* (*JAMA Oncol.* 2020 Aug 1;6(8):1297-1299).

"Many forms of cancer therapy can be detrimental to fertility, if the therapy disrupts the reproductive tract, damages sperm-producing cells in the testis, or diminishes the testosterone-producing activity of the testes," says Brady urologist Amin Herati, M.D., Director of Male Infertility and Men's Health and senior author of the study.

Because immunotherapy is still so new, its effects on reproductive potential were not known. In this study, Herati and his team studied testicular tissue from seven men who received immunotherapy for metastatic melanoma, compared to tissue from six men with metastatic melanoma who did not receive immunotherapy or other potentially toxic treatment. The results were profound: "We found that 86 percent of the men who underwent immunotherapy had reduced sperm-producing cells, compared to only 33 percent of men in the control group," says Herati. This study is the first to link immunotherapy with reduced sperm-producing cells.

"Our findings have potential major implications for the use of immunotherapy among men who are planning on having children," Herati adds. Additional research is ongoing to confirm these findings. ■

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Johns Hopkins Medicine
600 North Wolfe Street, CMSC 130
Baltimore, Maryland 21287-2101
410.955.8434
www.hopkinsmedicine.org/brady-urology-institute

Patrick Walsh, M.D.
University Distinguished Service Professor of Urology Emeritus

Janet Farrar Worthington *Writer/Editor*
Laura LeBrun Hatcher *Design & Art Direction*
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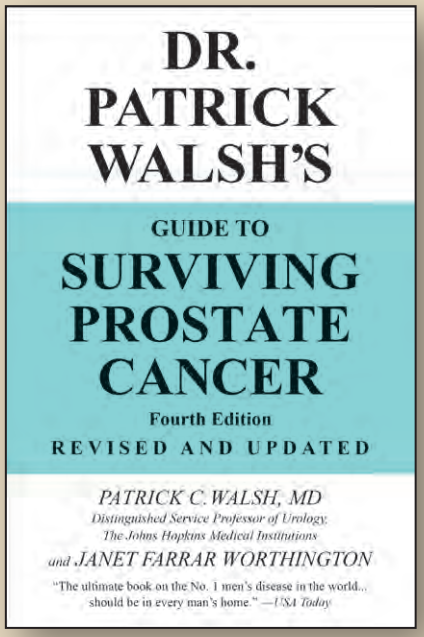

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