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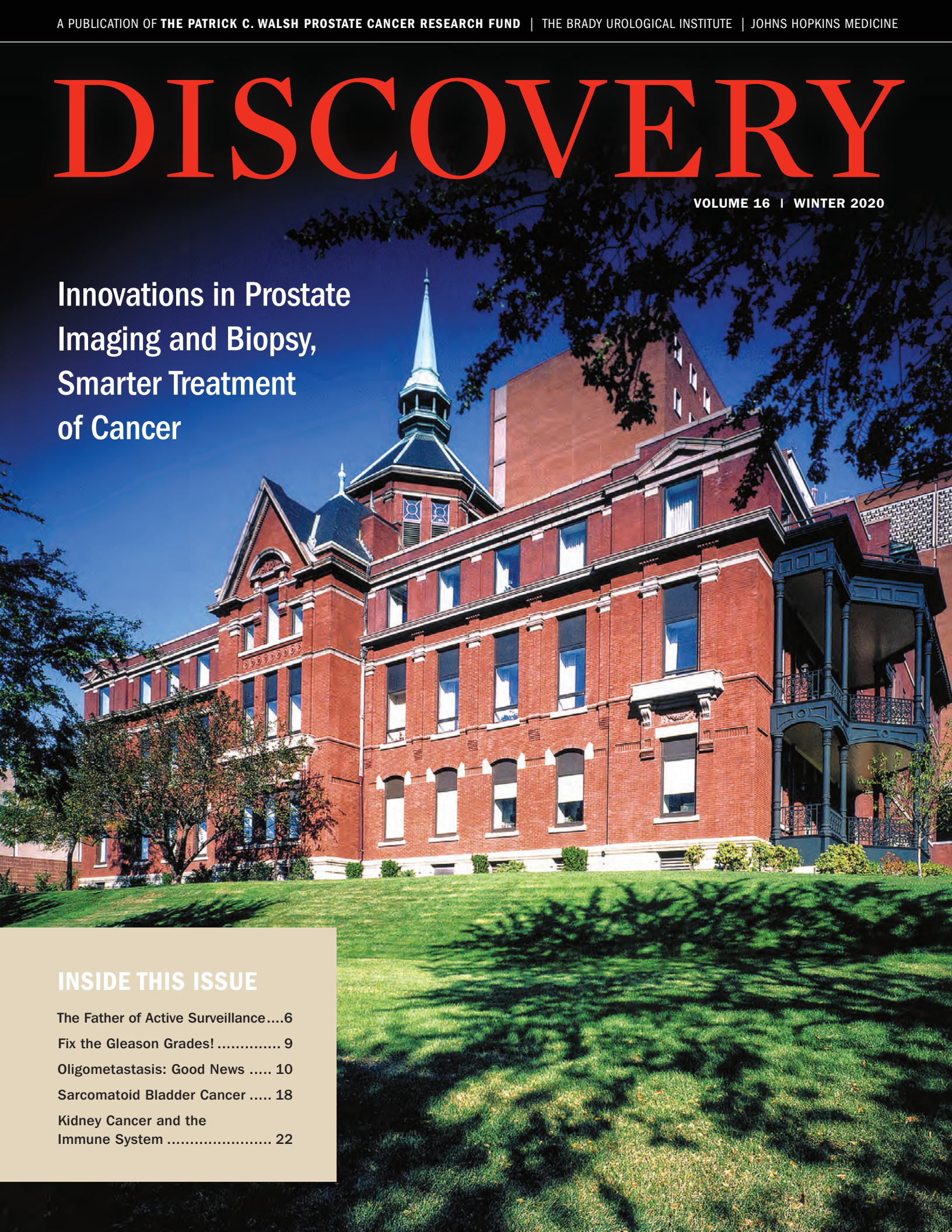
Innovations in Prostate Imaging and Biopsy, Smarter Treatment of Cancer



INSIDE THIS ISSUE

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***Partin**: Unprecedented precision in treatment.*

Individualized Treatment Through *Discovery*

We have always provided precision treatment for every man with prostate cancer who comes through our doors. Now, surgical innovation, dramatic improvements in imaging, better understanding of molecular biomarkers, more accurate biopsy techniques and more precise diagnosis have come together to allow us to provide truly individualized treatment. This means that we can tell a man with confidence that he can safely enter active surveillance; it also makes us far less likely to miss significant cancer that needs to be treated, and for men at higher risk, it gives us the opportunity to offer additional treatments aimed at curing their disease and maximizing their quality of life.

Milestones: I am proud to report that H. Ballentine Carter and Trinity Bivalacqua have received two of the highest awards in our field (see pages 6 and 20). Bal Carter has retired after a remarkable 32 years at the Brady, but his legacy will continue for many more years, thanks to a research fund (see page 8) established in his name.

We continue to lead the field in treatment and research in bladder, kidney, and testicular cancer (see pages 18, 22 and 23), and to treat an ever-broader range of patients who, not so many years ago, would have had incurable disease.

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:

Advanced Imaging, Better Biopsy, Smarter Treatment, Fewer Side Effects

Prostate cancer is a curable disease for many patients. In fact, for some men, it requires no treatment at all – but we at the Brady want to make sure we correctly and safely identify those men. For men who do need treatment, we want to address their cancer with few to no side effects.

ACTIVE SURVEILLANCE: BETTER IMAGING, SAFER BIOPSY

H. Ballentine Carter, M.D., as Director of the Brady's Prostate Cancer Program, showed that most men with low-grade prostate cancer do not require immediate treatment, and instead can be monitored safely with active surveillance. Based on that strong foundation, our next step is to minimize the number of blood tests and prostate biopsies that are required for men on surveillance. Most men on active surveillance who go on to require treatment likely had higher-grade cancer all along; it simply evaded detection on previous biopsies. We are now focusing on ways to decrease the intensity of active surveillance by doing a better job detecting significant cancers at the time of initial diagnosis.

The key to this goal begins with advanced imaging; particularly, multi-parametric MRI, or mpMRI (a form of MRI that looks at the prostate in several ways, including with a contrast dye injected in the blood). This sophisticated form of MRI shows suspicious areas of the prostate that can be targeted for biopsy. With the use of MRI-targeted biopsy, the likelihood of identifying clinically significant prostate cancer increases substantially. We are making it a priority to ensure that all men in the active surveillance program are routinely imaged with MRI and then, when appropriate, given a targeted biopsy.

And to make these biopsies safer, to perform the procedure by avoiding the rectum and the risk of infections – instead reaching the prostate through the perineum, an area of skin located between the scrotum and rectum.

Why change the way urologists perform prostate biopsies? With the traditional transrectal biopsy, potentially dangerous bacteria can be transmitted from the rectum to the blood stream. This is very concerning for men on active surveillance, who typically require multiple biopsies. With the transperineal approach, instead of passing through the rectum, the needles go through an area of skin, which can be thoroughly cleansed before the procedure. We used to routinely swab a man's rectum to see what bacteria he had, and we would give him antibiotics based on those bacteria. Despite our best intentions, sometimes those antibiotics would fail to prevent an infection. Additionally antibiotics can cause complications on their own. With the transperineal approach, we don't have to give any antibiotics.

In the Brady's Active Surveillance Program for Prostate Cancer, at least 90 percent of men are getting a transperineal biopsy. It is safer and is quickly becoming the new standard of care throughout the world. Mohamad Allaf, M.D., Vice Chairman at the Brady, and colleagues have developed a technique to perform MRI-guided prostate biopsy through the transperineal approach. It's not only cleaner, but there is reason to believe the transperineal approach is more accurate, better able to sample the prostate's anterior region – the area where cancer commonly develops in African American men.

With our novel biopsy techniques and imaging strategies, we can ensure the lowest possible risk of side effects to men on active surveillance. Prostate MRI and transperineal biopsy are not just for men on active surveillance: the vast majority of new patients presenting with an elevated PSA level nowundergo an MRI-guided trans-perineal prostate biopsy.

But there are other new innovations. In an ongoing clinical trial funded through The Patrick C. Walsh Prostate Cancer Research Fund, we are investigating whether PSMA-targeted PET imaging plus mpMRI can improve the diagnosis of clinically significant prostate cancer. PSMA (short for prostate-specific membrane antigen) is a molecule that sits on the surface of prostate cancer cells.

Hopkins radiologist Martin Pomper, M.D., Ph.D., has engineered a number of radioactive compounds that target PSMA and can be used for PET imaging. One of these agents, called 18F-DCFPyL, has been studied extensively by colleagues at the Brady and is poised for approval in the U.S.

Can prostate cancer treatment be minimized even further? Yes. The innovations in imaging are allowing something that has never been considered safe or feasible before: focal ablation of the prostate.

For men with a very small amount of intermediate risk prostate cancer, instead of treating the entire prostate, we can treat just the portion of the gland that has the cancer – greatly minimizing the potential side effects of incontinence and erectile dysfunction that are sometimes seen with radical prostatectomy and radiation. To make this possible requires sophisticated equipment that allows the user to record the exact location of cancerous areas found on an MRI- or PET- targeted biopsy, a concept known as prostate cartography. Because this makes it possible to know exactly where the biopsy needles went, we can now return to areas that were positive for cancer and deliver focal treatments.

Our cover story continues on the next page >

ON THE COVER

The Marburg Building was built in 1889 and is one of the three original hospital buildings remaining and still in use. In 1982, it was renovated to serve as the new home for the James Buchanan Brady Urologic Institute.

Cover story, continued >

Brady urologists have begun performing focal cryoablation (controlled freezing of the prostate) in highly selected men using a technique called "preoperative thermal dosimetry". Utilizing special software, cryoablation procedures can be planned with precision – similar to the way radiation oncologists determine where to deliver radiation, and how much the dose should be.

Because this is still a very new concept, the efficacy and safety of the procedure is being rigorously evaluated. One avenue for study is through a collaboration started at the FDA, known as the SPARED registry. SPARED is a multi-center effort to evaluate the outcomes of various forms of focal therapy that are being performed throughout the country. The FDA remains aware of the many unknowns of ablative therapy for prostate cancer, and has provided resources to allow urologists to study the safety and efficacy of this emerging form of prostate cancer treatment.

But what about the multi-focal nature of prostate cancer? Hopkins scientists previously have shown that the majority of men with prostate cancer will develop tumors in multiple, distinct areas of the gland. However, just because the cancer is multifocal doesn't necessarily mean you have to treat all the areas. Perhaps just treating certain areas, like those you can see on MRI or those that have a certain molecular signature, is sufficient, especially for older men who need a less durable solution for their prostate cancer. Also, there are some men who truly have only one or two small areas of cancer—something that can now be more readily determined using advanced imaging and biopsy techniques—and ablation may serve as the preferred treatment option for these men, regardless of age.

Because this is all so new in concept, we are proceeding very cautiously. All men who are undergoing focal treatment will be enrolled in the SPARED registry and required required to adhere to strict follow-up surveillance. We cannot be too cautious, but we also must push boundaries if we are to continue to improve the care of men with prostate cancer. ■

The Urinary Microbiome Begins in Childhood

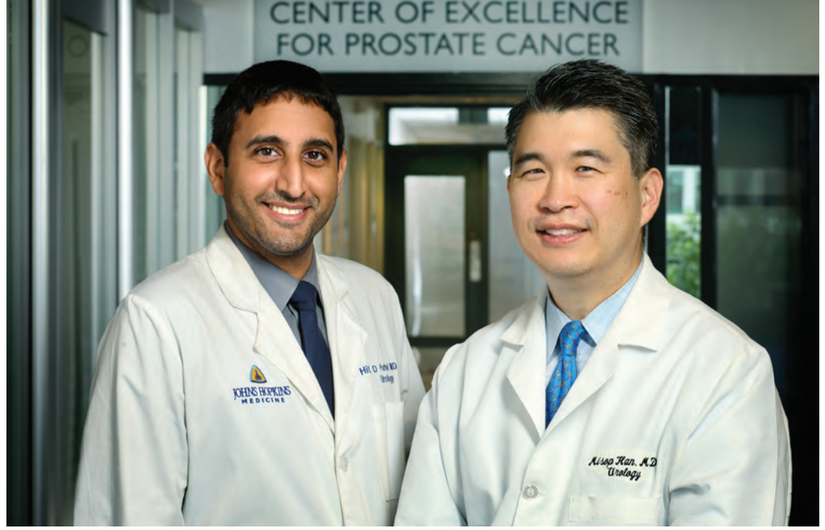
Previously in *Discovery*, we reported on a startling finding made by Brady molecular biologist Karen Sfanos, Ph.D.: Contrary to popular belief, urine is not sterile! Sfanos found not only that bacteria *do* exist in the urinary tract, but that this bacterial community, or microbiome, may contribute to the development of urologic diseases. Sfanos and colleagues also reported last year in the *Journal of Urology* that the bacteria in the urine of men with prostate cancer are different from the "urinary microbial communities" in men who don't have prostate cancer.

Sfanos and her team found bacteria in urine and fecal samples of boys as young as three months.

Building on this work, Sfanos' team, in collaboration with pediatric urologist Ming-Hsien Wang, M.D., aimed to determine exactly when the microbial populations in the urinary tract begin to show up, "and if the development of the urinary microbiome could be influenced by antibiotics in childhood." The team, which included Johns Hopkins medical student Borna Kassiri, who worked in the Sfanos laboratory as part of a Persky summer fellowship, examined both urine and fecal samples collected from young boys, ranging in age from three months to eight years.

"Surprisingly, we found that *all* of the samples contained microbial populations," says Sfanos. "Furthermore, we discovered significant differences in both the urinary and fecal microbiomes in children with prior antibiotic exposure." The study, recently accepted for publication in the journal, *Urology*, provides one of the first characterizations of the urinary microbiome in prepubertal males. ■

Sfanos and Kassiri: *"We discovered significant differences in both the urinary and fecal microbiomes (communities of bacteria) in children with prior antibiotic exposure."*



Patel and Han: *Reassuring news: The vast majority of our prostatectomy patients don't need all the pain medication they are prescribed after they leave the hospital.*

Reducing Opioids after Radical Prostatectomy

"We saw a reduction in opioid prescribing by nearly 47 percent, a reduction in opioid use by nearly 27 percent, and increased disposal of leftover opioids by nearly 14 percent."

A first-of-its-kind Brady intervention study points the way to smarter prescription and use of opioids after radical prostatectomy. Among other findings, the Brady's Opioid Reduction Intervention for Open, Laparoscopic, and Endoscopic Surgery (ORIOLES) initiative determined that the vast majority of our patients don't need all the pain medication they are prescribed after they leave the hospital.

The study involved two groups of patients: 214 men in the pre-intervention group, and 229 men in the post-intervention group. "Before intervention, on average, patients were prescribed 30 pills of 5 mg oxycodone, but used only three pills," says Brady Chief Resident Hiten Patel, M.D., M.P.H., who launched and led the study, along with urologist Amin Herati, M.D., and urologist Misop Han, M.D. "Overall, *77 percent of the opioid medication that was prescribed was never used*," and just over 9 percent of patients properly disposed of their leftover pain pills – which can be dangerous to leave lying around. These findings were true for both the open procedure and robotic prostatectomy; the investigators found no difference in prescribing or use between these groups of patients.

The team's intervention had notable success: "We saw a reduction in opioid prescribing by nearly 47 percent, a reduction in opioid use by nearly 27 percent, and increased disposal of leftover opioids by nearly 14 percent," says Patel, who presented the study's results at the 2019 meeting of the American Urological Association. "Only five patients required additional opioid medication."

Patel and colleagues also identified risk factors for higher use of opioids after surgery. One of these, for some men, was simply having a higher prescription; fewer pills prescribed led to fewer pills taken. Also more likely to take more opioids were men with a higher body mass index (BMI); and men who had used opioids for other conditions before surgery. "Notably, just having had a history of pain – without opioid use – before surgery was not a significant predictor of greater use." After the intervention, only 2.2 percent of patients reported needing additional opioid medication, fewer than 1 percent needed to obtain a prescription, and only 1.3 percent had long-term opioid use. "This is the first pre-post opioid intervention study in urology to report an improvement in prescribing practices after radical prostatectomy and to demonstrate factors associated with greater use," says Patel. ■

Preventing Blood Clots After Radical Prostatectomy

They don't happen often, but Brady urologists would like to prevent them altogether: blood clots in the legs or lungs after radical prostatectomy.

A new study, led by Brady Chief Resident Hiten Patel, M.D., M.P.H., and directed by Mohamad Allaf, M.D., Vice Chairman and Professor of Urology, has shown that a blood-thinning drug, heparin, given subcutaneously (under the skin), can help – and, importantly, that it doesn't cause extra problems.

For half a century, Patel notes, subcutaneous heparin has gotten a bad rap: "Many urologists in the U.S. have been wary of subcutaneous heparin, because of early reports that it might increase the rate of lymphoceles (buildup of lymphatic fluid in the pelvis) after radical prostatectomy. But Patel and Allaf thought heparin deserved another look, and Patel has just completed the largest-ever prospective randomized trial, assessing the impact of subcutaneous heparin in 500 men who underwent radical prostatectomy at Johns Hopkins. The trial is called PREVENTER (PREvention of VENous ThromboEmbolism Following Radical Prostatectomy).

"We found that the rate of symptomatic blood clots (those felt by the patient, usually due to pain or swelling in the legs) after radical prostatectomy is already very low, at about 2 percent, without using subcutaneous heparin," says Patel. "While not statistically significant, subcutaneous heparin further reduced the rate of blood clots by about 1 percent." The scientists also screened for asymptomatic blood clots – clots too small for the patient to feel – using Duplex ultrasound in about one-third of patients.

"Most importantly, we did not observe any increase in adverse events or bleeding," Patel continues. "The occurrence of symptomatic lymphoceles was the same, regardless of whether patients received subcutaneous heparin or not. Our findings suggest it is safe to give subcutaneous heparin around the time of radical prostatectomy," and this may be most beneficial for men at higher risk, including those with blood disorders or a history of blood clots. ■

***Bal Carter** is the 2019 winner of the prestigious Eugene Fuller Triennial Prostate Award, given once every three years by the American Urological Association to an individual who has made an outstanding contribution to the study of the prostate and its associated diseases. This award is named after the famous 19th-century New York City urologist, Eugene Fuller, who pioneered the open suprapubic surgical approach for treatment of benign prostatic enlargement, and supported by a trust fund established by his family. Since 1977, the Award has been awarded to 13 urologists, nine of them former Brady residents or faculty: Clarence Hodges, William W, Scott, Patrick C. Walsh, John T. Grayhack, Donald S. Coffey, William J. Catalona, Thomas A. Stamey, and Peter C. Albertsen. The award was presented by AUA President **Robert Flanigan**.*

The Father of Active Surveillance

Carter, retiring as the Bernard L. Schwartz Distinguished Professor of Urologic Oncology, reflects on his 32 years at the Brady.

The history of Johns Hopkins medicine is rich in fortuitous coincidence: the right people who happen to be at the right place at the right time. We are fortunate that one of these is urologist H. Ballentine Carter, M.D., who came to the Brady in 1987, after graduating second in his class at the Medical University of South Carolina and completing an internship and residency at New York Hospital-Cornell Medical Center.

"I was awarded an American Urological Association (AUA) Scholarship to work with Don Coffey (the Brady's longtime Director of Research) for two years," Carter recalls. "His lab was an amazing place to be, not just for the opportunity to work with Don, but with so many people who shaped my career, including: Evelyn Barrack, Tom Chang, Bill Nelson, John Isaacs, William Isaacs, Ken Pienta, Alan Partin, and Jonathan Simons," to name a few. The galvanizing research environment inspired Carter to stay in academic medicine as a surgeon-scientist, and in 1989, then-Director Patrick Walsh hired Carter as a member of the Brady faculty.

"To say that Patrick Walsh had an impact on my professional life would be an understatement. His generosity in mentorship truly launched my career in urology." Carter also credits his "amazing colleagues" at the Brady, who "generously gave advice to a junior faculty member," including Fray Marshall, Chaz Brendler, Jacek Mostwin, Ray Stutzman, Bob Jeffs, and John Gearhart.

Entering the PSA Era

So here we have Carter at the right place to begin a fruitful career. But what about the right time? In the late 1980s, discoveries by Walsh and others had dramatically shaken up the field of prostate cancer treatment. Walsh's transformation of the radical prostatectomy into a safer operation, and his discovery of the neurovascular bundles (allowing preservation of potency) suddenly made surgery into the gold standard for curing localized disease. At the same time, the discovery of PSA made it possible, for the first time, to learn about prostate cancer from a blood test. The problem was that nobody knew what to do with PSA, or how to use it along with the digital rectal exam and the new use of transrectal ultrasound-guided biopsy in diagnosing prostate cancer early. Widespread screening for prostate cancer did not exist.

"Until several papers were published, by William Catalona, William Cooner, and others, demonstrating that PSA was the most effective way to go about identifying prostate cancer early as a primary screening tool, there was controversy about even using PSA," Carter recalls. "There were voices saying, 'Beware, we're going to uncover a lot of cancers that never should have come to light.'"

Next came controversy over the PSA threshold: what was the magic number that would indicate the need for a prostate biopsy? "In retrospect, that was probably the wrong thing to be asking," Carter says. "There was just not a good understanding then of PSA as a continuum."

Carter pioneered the concept of *PSA velocity* – the rate at which PSA rises over time. "But that's never been tested as a screening tool. I honestly believe if we had not focused on a single, absolute threshold, and instead had focused on changes in PSA to alert us that someone has an aggressive cancer, in the long run we may have identified more individuals with the cancers that need to be treated, and eliminated more who don't need to be treated. But that will require a carefully done, prospective trial."

Once PSA screening started, as Carter, Walsh and others had predicted, there was a bubble – tens of thousands of men were diagnosed with prostate cancer – and then, over time, this leveled out. But in the early 1990s, Carter notes, "nobody knew that PSA levels would change. Until they started looking at it."

"Have You Ever Heard of the BLSA?"

Carter started looking at it, in studies that would lay the foundation for PSA screening and also for safe, vigilant active surveillance as a mainstream treatment for low-risk prostate cancer. How that came about, he says, "was really the brilliance of Pat Walsh. When I first joined the faculty, he came to me and said, 'What do you think would happen if we looked at changes in PSA?' I said, 'I think they'll probably rise faster in people who have prostate cancer. How can we study that?' And he said, 'Have you ever heard of the Baltimore Longitudinal Study of Aging (BLSA)?'" The BLSA was conceived in 1958, when gerontologists at the Baltimore City Hospitals were trying to find a better way to study aging. At that time, and even today, scientists would compare men and women who were in their twenties to people who were decades older. But these scientists had a better idea: to revisit the same person every two years, with a history and physical and blood samples that were stored. This made it possible to look at men who had no prostate disease, benign enlargement, or localized or metastatic prostate cancer –and then work backward, describing the changes in PSA over the previous 20 to 30 years.

"I had never heard of it," Carter continues. "He said, 'Why don't you go and shake the trees and see what falls out? I know they have a large frozen serum bank, and we might be able to use some of that to look at the question: do PSA levels rise faster in men who have aggressive disease vs. men who don't have prostate cancer?' Sure enough, that's the way it turned out. I did the work, but believe me, virtually everything that came out of the Brady in that era, Pat was the instigator, the mind behind the hypothesis. He's just an amazingly brilliant man. I've never worked with anyone who could think more clearly. He has an amazing way of getting to the bottom of things."

Patrick Walsh, in turn, places the credit squarely on Carter. "Simply stated and without exaggeration," Walsh says, "Bal has changed the way prostate cancer is treated today around the world." Although Carter is "a great surgeon," he has done his best not to operate on men who don't need it. "He was a voice of reason at a time when the diagnosis and treatment of the disease underwent revolutionary changes. With the introduction of widespread PSA testing in 1990, the diagnosis of prostate cancer reached epidemic acceleration and led to abuses fed by the greed of many fellow urologists. Those are tough words, but there is no other way to explain it. Bal emphasized the harm of overdiagnosis and overtreatment, proposed solutions based on improved screening practices, and developed guidelines for identifying men who should not be treated. He began by learning about PSA."

Using blood samples that had been collected for decades by the BLSA, Carter described how age and prostate disease influenced PSA. "Based on his unique observations, he proposed new ways to interpret PSA levels, and specified intervals for testing that were the most informative," says Walsh. As Chairman of the AUA's Guidelines Panel, Carter developed recommendations for how all urologists should screen for prostate cancer.

Active Surveillance in Prostate Cancer

He also changed the way prostate cancer is treated for many men with localized disease. "In 1994, (Brady pathologist) Jonathan Epstein published his landmark work on the use of prostate biopsy criteria and PSA density to predict the presence of small-volume, low-grade prostate cancer at radical prostatectomy," says Carter. "Recognizing that PSA testing was uncovering a substantial number of prostate cancers consistent with what was found at autopsy in most men who died of other causes, and that these men were undergoing radical prostatectomy for cure, we began to use the Epstein criteria to enroll men in a longitudinal study of expectant management, or active surveillance." In 1995, Carter began the Brady's active surveillance program for men with low-risk prostate cancer (about 40 percent of all men who are diagnosed with the disease). Since then, and with the help of Patricia Landis, who has coordinated the program for more than 20 years, the program has enrolled more than 2,000 men.

"Because of Bal Carter, active surveillance has become accepted as a standard-of-care approach to the management of favorable-risk disease around the world," says Brady Director Alan Partin, M.D., Ph.D., The Jakurski Family Director and Professor, *"and because of his work, many men have been able to avoid the side effects of unnecessary treatment."*

In recognition of his preeminence as an educator, Carter was elected President of the American Board of Urology. And in recognition of his outstanding contributions in the field of prostate cancer, he was awarded the highest honor bestowed by the Society of Urologic Oncology, the Charles Huggins Medal, and the American Urological Association's Eugene Fuller Triennial Medal and its Distinguished Contribution Award.

Because of Carter's work, urologists now recommend that men start getting their PSA tested in their forties. But when should they stop? Carter recalls asking that question at a meeting with other BLSA scientists. "No one really knew what would happen if you measured PSA very early in life, in midlife, and then looked at outcomes later on. I had gotten really interested in screening for cancer, so I combed the literature. It just dawned on me, mainly from cervical cancer screening literature: What if very young men have PSA levels that are extremely low, and have cumulative numbers of negative tests – would that suggest later in life that they are very low-risk? As it turns out, if you're in your 50s or early 60s and you have very low PSA, it's unlikely that you're going to be diagnosed with prostate cancer later in life. Is there an age and a PSA level where you could tell an older man, 'Congratulations, you made it, and you don't ever need to have a PSA test again?' Sure enough, you reach an age around 70, 75, and your PSA is less than 3, it is extremely unlikely that you will be diagnosed with lethal prostate cancer."

What about intervals for screening? In another study, published in *JAMA*, Carter and colleagues found that "PSA levels really don't change rapidly in most people, even those who are going to develop prostate cancer. We found that intervals of two to four years were perfectly reasonable for detecting the type of cancers we wanted to detect."

Looking back over his 32 years at the Brady, Carter says, "I would not change anything. I landed in the perfect spot, with unbelievable mentors and colleagues. It could not have been for me a better place, or better situation. I got to do everything I wanted to do." With his wife, Lillian Carter, Ph.D., a professor of health education at Towson University, Carter has moved to "a house we built in 1993 on Bald Head Island," in North Carolina. He is learning Spanish, which he intends to use while hiking in Latin America, and very soon will be hiking across England with a Brady alumnus, urologist Joel Nelson. "I'm also going to be looking for volunteer work," he says, "to help people who are not as fortunate as I have been throughout my life." ■

Ballentine Carter by Peter Egeli: *Oil on canvas, 2019, Courtesy of The Alan Mason Chesney Medical Archives of The Johns Hopkins Medical Institutions.*

The H. Ballentine Carter, M.D., Prostate Research and Innovation Fund

"We know a lot, but certainly there is more to learn, about the markers, the disease itself, and how to deal with it when something should be done."

Urologist H. Ballentine Carter, M.D., has dedicated his career to filling the "gaps in knowledge" in the use of prostate-specific antigen (PSA) to detect prostate cancer. Some of his major contributions (see Page 6) include:

PSA velocity (PSA's rate of change over time) is associated with the presence of prostate cancer, and directly associated with lethal prostate cancer.

Frequency of testing: Testing every other year is sufficient to find the cancers that most need to be diagnosed.

Median PSA levels based on age: PSA levels above the median for age can predict the development of prostate cancer 20 or 30 years from now.

When it's safe to stop PSA testing: A man over 75 who has a PSA below 3 ng/ml (this "represents two out of three men in the population," Carter says) is highly unlikely to develop lethal prostate cancer.

Active surveillance can be done safely in men with favorable-risk prostate cancer. In 1995, when Carter began the active surveillance program for prostate cancer at the Brady, "there was substantial resistance to monitoring men with a diagnosis of prostate cancer, regardless of grade," he recalls. "Our goal was to demonstrate the safety of this approach for carefully selected men. We now know that for these men, the risk of death from prostate cancer, or development of metastatic disease, is 26-fold lower than the risk of death from other causes over 15 years."

To honor Carter's research legacy and ensure the continuation and furtherance of his work at the Brady Urological Institute, patients, their families, and Carter's colleagues established the H. Ballentine Carter, M.D., Prostate Research and Innovation Fund. Already, the fund's donors have committed nearly $1.5 million. Many of those who have contributed are new donors to Johns Hopkins, who were moved to give by Carter's standards for care. Others are surgical patients whose cancer was successfully treated by Carter as long as 25 years ago, and still others are men who have been in active surveillance for years, or even decades.

One of these generous donors is Bill Clarke, who met Carter nearly 20 years ago, at age 49, when Carter performed his radical prostatectomy. Clarke was not the first in his family to have prostate cancer; his father had also had a radical prostatectomy done in Boston, by a surgeon using the then-new nerve-sparing procedure developed by Patrick Walsh. After his cancer was cured, Clarke says, "I stayed in touch with him, and I also was in a position to help him with his research."

Clarke, a philanthropist who has been very involved with the Bloomberg School of Public Health, says, "Giving back to Bal Carter's work made a lot of sense to me, and I have been a partner in that sense since 2003. Indeed, I really gravitated toward the research, because it was using some of the same techniques that I had been using in my career as a commodity futures trader." Clarke developed computer models to make sense of vast amounts of data, and "to understand where the trends were." For example, "by analyzing price data and comparing it to what has happened in the past, you can know with some form of certainty what will happen in the future. That basically is the same technique that Bal was using – assembling data so he could know more about where men were on the spectrum of prostate cancer.

"I'm really proud of what Bal has done," distilling findings from decades' worth of data, to help patients with localized prostate cancer and their doctors make informed decisions about treatment, and also to avoid needless treatment. "We can back up that decision with an incredible database that has been the core of Bal's research over the years."

With this Fund, "we will continue research in refining the data, and continue the path that Bal has been on," continues Clarke. "We know a lot, but certainly there is more to learn, about the markers, the disease itself, and how to deal with it when something should be done. It is my intention to honor Bal's career with my contribution. Bal became a friend, and shared his research with me. I could see how much he appreciated, not only being able to do the operations and cure patients, but also to do the research so that he and the people who came after him could treat patients in a better fashion. That's really what it's all about to me." ■

If you are interested in contributing to the H. Ballentine Carter, M.D., Prostate Research and Innovation Fund as a means of supporting the Prostate Cancer Program at the Brady and honoring Dr. Carter's legacy at Johns Hopkins, please contact MaryAnn Jones at mjone263@jhmi.edu or 443-287-6048.

GLEASON GRADE GROUPS

GRADE GROUP	GLEASON SCORE
1	3 + 3
2	3 +4
3	4 + 3
4	4 + 4
5	9 or 10

Fix the Gleason Grades!

At many hospitals worldwide, Gleason scoring is still less accurate.

Several years ago, world-renowned Brady pathologist Jonathan Epstein, M.D., *the Rose-Lee and Keith Reinhard Professor of Urologic Pathology*, came up with a much-needed solution to confusion in grading prostate cancer: he developed a new grading system called Grade Groups. Epstein and colleagues validated this overhauled system in a large, multi-institutional study. Worldwide, pathologists found it to be such an improvement that it was endorsed by the 2014 International Society of Urological Pathology Consensus Conference.

In the Grade Group system, Grade Group 1 (GG1) is a Gleason score of 6 or lower, GG2 is Gleason score 3+4=7, GG3 is Gleason score 4+3=7, GG4 is Gleason score 8, and GG5 is Gleason score 9-10. "In part, we proposed the new grading system because of anecdotal evidence that Gleason scores were incorrectly combined in the literature," says Epstein. A big problem: cancer that is Gleason 3 + 4 = 7 is different from cancer that is Gleason 4 + 3 = 7, yet both used to be lumped together as Gleason 7 disease. Gleason 8 cancer is different from Gleason 9 and 10, yet Gleason 8-10 were crammed into the same category in the old system.

There are important differences in these cancers; they have different prognoses and respond differently to treatment. They deserve their own spots in the scoring system. Thus, "the old system was less accurate," Epstein says.

Guess what? At many hospitals worldwide, Gleason scoring is *still* less accurate.

In a new study, published in *European Urology*, Epstein and colleagues looked at how Gleason scores were grouped worldwide. "We found that the most common method in current use was still the D'Amico risk stratification groups (categorized as Gleason ≤ 6, 7, 8–10)," Epstein says, "which is incorrect as it combines Gleason scores, despite very different prognoses." Things are getting slightly better; in 2016, the authors found, only 10 percent of published articles used Grade Groups or Gleason Score equivalents, and in 2017, this nearly doubled to 19.4 percent.

Unfortunately, says Epstein, "today only a minority of published articles on prostate cancer group Gleason scores accurately. This could lead to inaccurate results and affect patient care with different prostate cancer grades. Our study calls for more widespread adoption of the five Grade Groups." ■

High-Grade Prostate Cancer and Inherited Gene Mutations

"We found that three genes, ATM, BRCA2, and MSH2, were mutated at a significantly higher rate in high-grade cancers."

Previously in *Discovery*, we reported on the work of William Isaacs, Ph.D., *the William Thomas Gerrard, Mario Anthony Duhon and Jennifer and John Chalsty Professor of Urology*, and colleagues showing that inherited mutations in a few genes involved in repairing damaged DNA are significantly more common in men who die from metastatic prostate cancer, compared to men who have less aggressive, more slow-growing cancer.

Since then, Isaacs' research group has worked to understand more about mutations in these genes, including *BRCA2* and *ATM*. "We wanted to find out whether there was a correlation between mutations in genes associated with lethal disease and the *grade* of prostate tumors," says Isaacs.

In a collaborative study with Ambry Genetics, the scientists studied 1,694 men who underwent radical prostatectomy at Johns Hopkins, including 708 patients with the two highest tumor grades (grade groups 4 and 5) and 988 patients with low-grade (grade group 1) disease. They looked for mutations in 14 DNA repair genes – the genes Isaacs and his group previously had shown to be most commonly mutated in metastatic prostate cancer.

"Overall," Isaacs says, "we found that the number of men carrying inherited (germline) mutations in the 14 genes was significantly higher in patients with high-grade disease," compared to men with the lowest-grade cancer. "We also found that three genes, *ATM*, *BRCA2*, and *MSH2*, were mutated at a much higher rate in high-grade cancers."

Isaacs and colleagues were surprised to find that men in grade group 5 had about 20 times the number of mutations as men in grade group 1, and three to six times more mutations than men in grade group 4. "While much more work is necessary, the strong enrichment of mutations in these three critical DNA repair genes and tumors among men in grade group 5 is greater than expected," says Isaacs. "This suggests a fundamental association between loss of normal tissue architecture, acquisition of aggressive tumor behavior, and inherited inactivation of specific, key components of critical DNA repair pathways." Better understanding of this association "could provide a basis for novel, gene-targeted treatment." ■

Oligometastasis: Good News from the ORIOLE Study

"The men in the SABR group did considerably better. This is a definite signal that we can perhaps modify metastatic disease."

The boundary used to be very clear: prostate cancer was either confined to the prostate or prostate bed, or it wasn't. Like a light switch with no dimmer, there was no in-between: a man with only one metastasis was believed to face the same fate, eventually, as a man with widespread metastases. It was just a matter of time.

Thank goodness, that's not the case today!

Previously in *Discovery*, we reported on the Baltimore ORIOLE study, led by radiation oncologist Phuoc Tran, M.D., Ph.D. Tran was enrolling patients in this small study to see if men with *oligometastasis* – up to three small bits of cancer that have broken away from the main prostate tumor and started to grow elsewhere – would benefit from stereotactic ablative radiotherapy (SABR, highly focused, intense doses of radiation), in addition to treatment of their primary tumor.

Tran believed that the lines of prostate cancer were not so clear-cut as scientists had assumed; that instead of two circles – localized and metastatic cancer – that didn't connect, we might be dealing with a Venn diagram, with oligometastasis as the critical area where the two circles overlap. "It may be that the window of curability is wider than we thought," he said last year.

Now, we are pleased to report, the results of this multicenter trial are even better than Tran hoped. In the ORIOLE trial, 54 men with oligometastasis were randomly assigned either to treatment with SABR or to observation. To find and keep track of the oligometastases, the study used PSMA-PET scanning, which uses a small molecule linked to PSMA (prostate-specific membrane antigen, found on the surface of prostate cancer cells) as a radioactive tracer. Developed by Martin Pomper at Johns Hopkins, this PSMA-targeting tracer can highlight areas of cancer as small as a BB – much smaller than can be seen on regular PET or CT imaging. "PSMA-PET allows us to treat lesions we otherwise couldn't see," Tran explains "A CT or bone scan would miss those lesions, and patients would presumably not do as well."

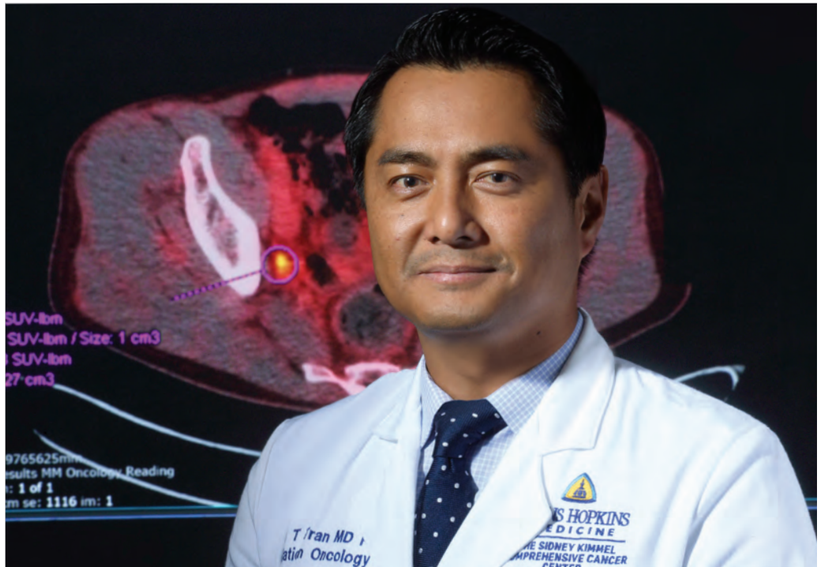
At six months, 61 percent of the men in the observation group progressed – compared to only 19 percent of the men who received SABR. "We also saw a significantly decreased risk of new metastatic lesions using PSMA PET-CT," says Tran. "The men in the SABR group did considerably better. This is a definite signal that we can perhaps modify metastatic disease."

This was a Phase 2 study, and "we need larger Phase 3 trials," he says. "But this is very positive, and we hope that in the future, we will be able to change the course of metastatic disease in some men."

What's happening here? The spread of cancer is one of colonization, Tran explains. A few pioneers set forth on a journey to a new land. At first, it's touch-and-go; their survival is tenuous. Just think of the early colonists in the U.S., from England, France, or Spain. Until they took root in the new land, these nascent colonies were frail: they needed reinforcements from their mother countries – medicine, weapons, tools, food – and "eventually they did survive." So it is with the seeds of cancer; either the cancer cells themselves, or their messages (in the form of genetic and chemical telegrams) are dispatched to the primary tumor, the mother country. If the mother country is no more – if it has been eradicated by surgery or radiation – then small cancer outposts might get similar support from visiting each other. But if those outposts are destroyed by SABR, even if there are a few cancer cells remaining in the tissue or bloodstream, it doesn't matter: the environment is too hostile, and the numbers are too few for new colonies to survive – "or, if they did, it would take much longer."

Insights: In the ORIOLE trial, Tran and colleagues looked for circulating tumor DNA (ctDNA), and identified certain gene signatures that can tell if a man is more likely to respond to SABR. "Patients who don't have these mutations responded very well," he says. They also have learned from this and other research that men with oligometastasis fall into three basic groups. "Some men do really well after one course of SABR," with no recurrence of cancer. A second group of men have a small recurrence. "Another site pops up; a microscopic metastasis that we couldn't see before establishes itself into a macroscopic metastasis. It's a limited return of cancer and it responds to another round of SABR." Then some men, after a few months, have multiple new areas of cancer. "For these men, the SABR doesn't control the disease at all."

***Tran**: "This has the potential to be practice-changing. We are very excited by our results."*



Imagine a green lawn, with one or two dandelions, Tran tells his patients: "You can pluck those two or three weeds, and wait and see. Sometimes you get lucky; sometimes another weed or two pops up, and you pluck them. It's like Whack a Mole. You can do that for a while," with repeated SABR treatments. "But unfortunately, sometimes there will be a whole bunch of seeds all at once, and at that point, you need weed killer all over the lawn," or systemic therapy.

Looking ahead: In a follow-up trial, called RAVENS, men with oligomestatic prostate cancer are randomly given either SABR alone, or SABR plus radium-223 (Xofigo). "What we have seen in the men in that second group – the ones who have more isolated spots of cancer popping up – is, they're not failing where they received the SABR, but in areas that were microscopic, and commonly in the bone." Radium-223 targets cancer in bone. "It releases a radioactive particle that is very toxic but is so focused that it only kills in a radius of two-three cell depths. It's ideal for microscopic disease."

In the future, Tran envisions, men with oligometastasis will require more vigilant monitoring, and ideally, regular follow-up PSMA-PET scanning. "This has the potential to be practice-changing. We are very excited by our results, and by the potential to modulate the course of metastatic prostate cancer." ■

Discovered: A "Gate-keeper" Gene for CRPC

We've known this for decades: some prostate cancer cells respond to androgens (male hormones), and some don't. This is why androgen deprivation therapy (ADT), although "an extremely targeted and highly effective treatment for prostate cancer," is not a cure, says Shawn Lupold, Ph.D.: because it doesn't stop the cancer cells that aren't affected by androgens. Even when the androgen-dependent cells are under control, these other cells keep right on growing and dividing. Eventually, the balance tips, and ADT is no longer enough to keep the cancer in check; this is called castration-resistant prostate cancer (CRPC).

Lupold, looking at different targets for treatment, has been studying genes activated by the androgen receptor (AR). An androgen such as testosterone binds to an AR like a key in a lock. This, in turn, "activates" the AR, "allowing it to enter the nucleus of the cell, bind DNA, and activate a large number of genes," Lupold explains. "This pathway is exceedingly critical for prostate cancer cells." In CRPC, androgen-resistant cancer cells can make use of the AR pathways – which are supposed to be shut down – through tricky genetic maneuvers: imagine a crook jimmying a lock or making a skeleton key. These genetic alterations "allow cancer cells to use androgen-like molecules, or low levels of androgens, to re-activate the pathway. In fact, some tumors even evolve mechanisms to produce their own androgens." Pretty sneaky!

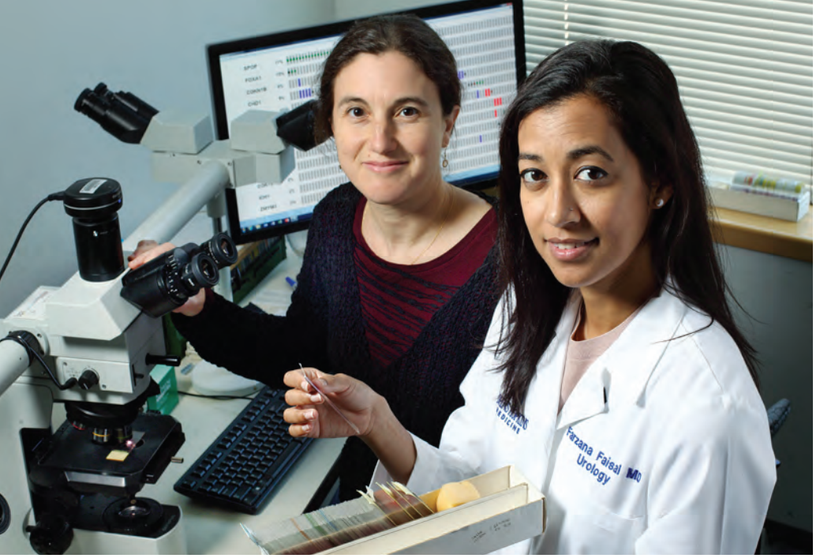
In sophisticated, painstaking research that took more than a decade, Lupold and colleagues set out to find the particular AR genes that promote prostate cancer cell survival and castration resistance. They began by looking at newly discovered genes called microRNAs (MiRNAs). Unlike most genes, microRNAs do not make proteins; instead, they are troublemakers that prevent a gene's protein from being made. Imagine you go into a diner and order a grilled cheese sandwich; the waitress writes your ticket and sends it to the short-order cook – but before the ticket reaches the kitchen, somebody tears it up! That's what MiRNAs do on a genetic level: they tear up tickets. Lupold and colleagues zeroed in on one particular microRNA called miR-21. "We discovered that elevated levels of miR-21 could stimulate prostate cancers to develop castration resistance." This discovery, led by Judit Ribas and published in *Cancer Research* in 2009, "set us on a new journey: to determine how miR-21 drives castration resistance." Specifically: which gene did miR-21 use to accomplish its mischief?

A postdoctoral fellow in Lupold's lab, Fatema Rafiqi, using complex computer algorithms, sifted through thousands of genes and analyzed likely candidates with Ross Liao, now a Johns Hopkins medical student. Postdoctoral fellow Koji Hatano identified a likely suspect: *PDCD4* (Programmed Cell Death 4), a tumor suppressor gene. Postdoctoral fellow Kenji Zennami began studying this gene in prostate cancer cell and tumor models, in work he and Lupold's lab recently published in *Molecular Cancer Research.*

"We found that androgens significantly reduced *PDCD4* production in prostate cancer cells, and that ADT or AR inhibition (androgen receptor-blocking drugs such as enzalutamide) triggered *PDCD4* expression. This activity was reduced when we blocked miR-21, providing a direct link between the AR, miR-21, and *PDCD4*." When they shut down *PDCD4*, prostate cancer cells multiplied – and cancer cell death slowed. "Like miR-21 over-expression, *PDCD4* inhibition caused prostate cancer cell growth and hormone resistance." These results were so striking that Lupold believes *PDCD4* may be a gate-keeper for prostate cancer's response to ADT. He sees *PDCD4* as a key "thermostat" for prostate cancer, whose presence or absence helps determine whether the cancer cells will die, or go on to become independent of hormonal control.

Lupold sees PDCD4 *levels as a key "thermostat" for prostate cancer, whose presence or absence helps determine whether the cancer cells will die, or go on to become independent of hormonal control.*

"We don't yet understand how *PDCD4* regulates prostate cancer cell proliferation or androgen sensitivity," Lupold adds. "Further studies are required to solve this puzzle." In the meantime, *PDCD4* has the potential to be a new biomarker for higher-risk patients. "Preliminary studies from human prostate tissues indicate that *PDCD4* levels are lower in more-aggressive cancer (high Gleason grade)." The next steps are to determine whether *PDCD4* also has the potential to become an entirely new mode of treatment for high-risk or advanced prostate cancer. ■



***Lotan** and **Faisal**: "The poor prognosis of patients with deletions in the* CDKN1B *gene has important clinical implications for African American men."*

Racial Differences in Prostate Cancer: New Genetic Targets

"Some of these alterations are directly associated with poor prognosis."

Why are African American men 1.5 times more likely to get prostate cancer, and *more than twice as likely to die* of it as men of European descent? Brady investigators have discovered critical, inherited genetic mutations in men of African descent. Their work gives scientists new genes to look for – and new targets for precision diagnosis and treatment.

"We have identified several molecular and genomic alterations of prostate cancer that are *unique to African American men*," says Brady resident Farzana Faisal, M.D., who began this research several years ago as a Johns Hopkins medical student, working with Brady scientists Tamara Lotan, M.D., and Edward Schaeffer , M.D., Ph.D. (now Director of Urology at Northwestern University). "Importantly, some of these alterations are directly associated with poor prognosis."

Many studies of prostate cancer patients worldwide have been done on groups of entirely or primarily Caucasian men; but the Hopkins scientists have learned that the genetic markers that can signal aggressive prostate cancer in men of one race don't always apply to men of other races, and vice versa. Thus, Faisal, with the direction of Lotan, performed targeted genome sequencing in a group of African American patients – the largest cohort to date.

"We found that mutations in the *TP53* gene, deletions in the *CDKN1B* gene, and overall burden of genome alteration (percent of the genome with variations) were associated with increased risks of metastasis in African American men," notes Faisal. "Our study was the first ever to correlate tumor genomic sequencing data with risk of metastasis in African American prostate cancer." The team's findings were presented at the American Urological Association, at the American Society of Clinical Oncology's Genitourinary Symposium, and published in the *Journal of Clinical Oncology* and the *Journal of Urology*.

Faisal and colleagues are most excited about bringing the *CDKN1B* gene to light: "The poor prognosis of patients with deletions in the *CDKN1B* gene is a novel discovery that has important clinical implications for African American men," Faisal says. One foreseeable implication might be in the type of treatment recommended: for example, an African American man diagnosed with localized prostate cancer who tests positive for a deleted *CDKN1B* gene might be encouraged to seek surgery or radiation, rather than undergo active surveillance. "These deletions were more common in younger men with higher-grade and -stage disease." For this work, Faisal received the Physician Scientist Award at the 2019 Young Investigators' Day at Johns Hopkins. ■

Aggressive Cells in Primary Tumors

Under the microscope, pathologists can see it: differences in prostate cancer cells, some clearly more ragged-looking and aggressive than other, more well-defined and orderly cells. "But we don't know why some prostate tumors behave more aggressively," says Brady pathologist Tamara Lotan, M.D.

Looking for answers, Lotan worked with Hopkins medical oncologists Mario Eisenberger, M.D., and Emmanuel Antonarakis, M.D., and with two physician-scientists at the University of Washington, Colin Pritchard and Michael Schweizer, to genetically profile aggressive cells found in primary prostate tumors.

"We found that these tumors frequently have mutations in the genes involved in DNA repair activity in the cell," Lotan says.

Sometimes these mutations seem to have happened spontaneously – just in the tumor, but not in other cells. But in many cases, they're in every cell, which means the patient was born with a genetic defect, called a germline mutation, that led to cancer.

"These patients *and their family members* may be carrying these germline mutations, passing them down from generation to generation," Lotan explains.

In a study of 49 patients with Gleason score 9 and 10 carcinoma (the most aggressive Gleason grade), Lotan and colleagues found that 34 percent of patients had at least one mutation in a DNA damage repair gene, and more than half of these patients had germline mutations. This study was recently accepted for publication in the *Journal of Clinical Oncology-Precision Oncology*. In a separate study in the same journal, the group found almost half of patients with a rare prostate cancer variant, called ductal adenocarcinoma, had similar mutations in DNA repair genes. And in collaboration with Brady pathologist Jonathan Epstein, M.D., Lotan's group found that half of tumors with giant cells (formed by several distinct cells joined together) carried these mutations; this work was published in *Histopathology*.

"Our findings all suggest that men with aggressive prostate cancer should be routinely screened for *underlying germline mutations in DNA repair genes*, and these patients may also benefit from PARP inhibitors currently in clinical trials," says Lotan. (For more on PARP inhibitors, see story below). ■

For Some Men with Certain Genes: PARP Inhibitors Instead of ADT

"We are using two PARP inhibitors, olaparib and rucaparib, in two clinical trials to maximize clinical benefit while avoiding the long-term toxicity of ADT."

Nobody likes androgen deprivation therapy (ADT). Not doctors, and certainly not patients. ADT is a mainstay of treatment for advanced prostate cancer because it prolongs life for years, and sometimes even decades – but at a cost. Just a few of the many side effects of ADT (beyond the loss of testosterone itself) are weight gain, depression, and a higher risk of diabetes, heart attack, stroke, and dementia.

So established, in fact, is ADT that although there are other forms of treatment for advanced and metastatic prostate cancer – including androgen receptor blockers, chemotherapy, new checkpoint-blocking immunotherapy drugs, and platinum chemotherapy drugs – ADT is still the gateway through which these drugs must pass. At some point, all men with advanced prostate cancer will start ADT, and they often never get off of it. If the PSA starts to rise and cancer starts to advance, they add another drug on top of it – but don't stop taking the ADT.

We've been stuck with ADT because there hasn't been a way around it. But now, we have entered the era of "precision oncology," says medical oncologist Emmanuel Antonarakis, M.D. "Our goal is to use genetic information to tailor therapies for the right patients at the right time – something we couldn't do before." Precision treatment for prostate cancer requires knowing which faulty genes are involved in an individual man's cancer, and knowing what to do with that information.

Some of the most common defective genes in prostate cancer are the DNA repair genes, whose job is to fix errors made as cells grow and divide. Genetic mistakes happen all the time in everyone, and it's the job of these genes – including *BRCA1*, *BRCA2*, and *ATM* – to make sure that these mistakes are fixed before anything goes wrong. They do their jobs using a repair method called homologous recombination (HR). If one of these HR, or quality-control, genes stops working, harmful mistakes can eventually lead to cancer.

"In recent years, we have seen the advent of drugs called poly-ADP ribose polymerase (PARP) inhibitors," says medical oncologist Mark Markowski, M.D., Ph.D. PARP inhibitors zero in on these faulty HR genes, and in someone whose cancer has one of these mutated genes, "inhibiting PARP can result in profound anti-cancer effects."

Together, Markowski and Antonarakis are working – as Antonarakis puts it – "to exploit these HR mutations to develop non-hormonal therapies for men with recurrent and metastatic prostate cancer." In other words, instead of shutting down the hormones that help drive the cancer, and then waiting for ADT to fail in these men, they are cutting right to the chase: going after the bad genes that caused the cancer in the first place. *Without ADT.*

"We are using two PARP inhibitors, olaparib and rucaparib, in two clinical trials being conducted to maximize clinical benefit while avoiding the long-term toxicity of ADT," Antonarakis continues.

One trial, NCT03047135, is testing olaparib (a PARP inhibitor that has shown success in treating breast and ovarian cancer in women who have mutated *BRCA1* and *BRCA2* genes) without ADT in men with high-risk prostate cancer who have experienced a rise in PSA after prostatectomy. "In early results, we have observed that olaparib is well-tolerated and has very promising activity in some men," says Markowski, "particularly – but not exclusively – in men who have mutations in *BRCA2* and *ATM*, both of which are HR genes."

A second trial, NCT03413995, also called the TRIUMPH study, is treating metastatic prostate cancer patients with the PARP inhibitor, rucaparib, "also in the absence of ADT," Antonarakis says. "All men in the TRIUMPH study must have an inherited (or "germline") HR gene mutation, and must feel comfortable postponing ADT." This study has just begun enrolling patients, but already, "preliminary observations suggest potential benefit of PARP inhibition in this patient population, especially in men with germline *BRCA2* mutations."

These trials are being supported by Hopkins scientist Tamara Lotan, M.D., who is the lead pathologist on both studies. Lotan's lab is conducting in-depth molecular analyses on tissue specimens to help determine which men will benefit the most from these PARP inhibitors.

To find out more about these studies, please call Rana Sullivan (410-614-6337) or Serina King (410-614-6139). ■

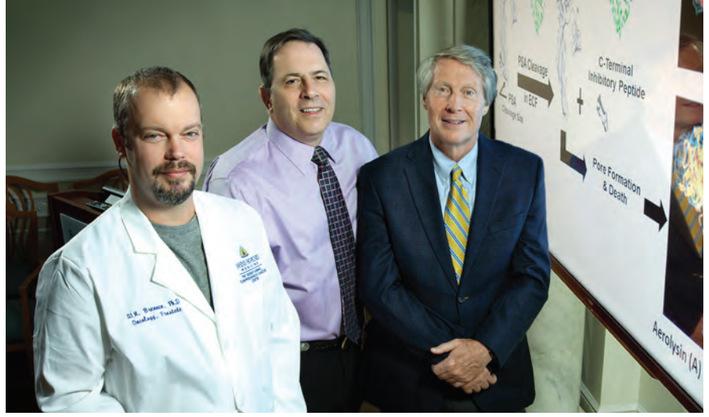
Uneven Accuracy in IHC Testing

Immunohistochemical staining (IHC), a technique using antibodies to detect specific molecules on cells, is an essential tool that pathologists use in diagnosing cancer. But as Brady scientist Angelo M. De Marzo, M.D., Ph.D., and colleagues recently discovered, there's a huge variation in the quality of IHC tests available – and this could affect accuracy.

"IHC is used in thousands of research and clinical laboratories," De Marzo says, "but there is widespread misunderstanding about the two classes of antibodies used in IHC staining: clinical grade and research grade. Clinical grade antibodies are validated for accuracy prior to their use in hospital pathology labs, but are limited in number," with about 500 antibodies in use. "On the other hand, the majority of commercially available research-grade antibodies are not held to the same standards of validation, and there are now more than 3.8 million of these. Overall, we estimate that at least half of published studies using research IHC assays have potentially incorrect staining results due to lack of antibody validation."

"A cornerstone of scientific research is the ability to reproduce findings of colleagues' studies, to either affirm or reject them," says Brady scientist Karen Sfanos, M.S., Ph.D., co-author of this study. "The problem with having many incorrect IHC results in the literature is that it makes it difficult to rely on prior results, which can significantly slow down research."

This work was published in a special issue of the *Asian Journal of Urology* that was dedicated to the late Donald Coffey, Ph.D., longtime Director of the Brady Research Labs. In the publication, the Brady scientists provide numerous examples of validated assays, literature and other resources to help pathologists and scientists find the right IHC antibodies and assays. Other authors and contributors included Srinivasan Yegnasubramanian, William Nelson, Tamara Lotan, Ibrahim Kulac, Jessica Hicks, Qizhi Zheng, Charles Bieberich and Michael Haffner. ■



***Brennen, Denmeade, and Isaacs**: Protoxin mimics the way immune cells kill cancer.*

Special Delivery to Metastatic Prostate Cancer: PSMA-Targeted Poison

This protoxin is a copycat killer: like immune cells, it pokes lethal holes in cancer cells.

When all goes well, the body's immune cells should recognize prostate cancer as something bad that shouldn't be there, and then attack the cancer. In effect, they should stab it to death – poking holes in the cancer cells' surface. Unfortunately, for a variety of reasons, so far prostate cancer has proven to be resistant to immunotherapy.

So how can we get to metastatic prostate cancer cells to kill them? Scientist John Isaacs, Ph.D., working with colleagues Nathaniel Brennen, Ph.D., Emmanuel Akinboye, Ph.D., and Samuel Denmeade, M.D., has come up with a solution: special delivery. The "package" to be delivered is chimeric protoxin, a deadly (to cancer!) chemical weapon that's *the next best thing to having an army of cancer-killing immune cells*. "It short-circuits the lack of immune cells by mimicking the way these cells kill cancer," says Isaacs. This protoxin, in other words, is a copycat killer: like immune cells, it pokes lethal holes in cancer cells. What really matters, Isaacs adds, are the holes – not the hole-punchers. "It comes down to whether enough holes are produced to kill the cancer cells. Our approach doesn't require the immune cells themselves."

To put the "address" on the package – to make sure it goes only to the right cells, and doesn't harm normal cells – Isaacs and colleagues made it prostate-specific. Even though metastatic cancer cells have spread far from the prostate, they still have PSMA (prostate-specific membrane antigen), a molecule that sits on their surface. The PSMA is the address. Metastatic prostate cancer cells also still make PSA, and here, the PSA acts as the detonator for the protoxin.

"Both PSMA and PSA function as molecular scissors, capable of cutting proteins at specific sites," explains Isaacs. Using protein engineering, the scientists fused the cancer-killing poison to serum albumin (a protein made by the liver and carried in the blood), "to keep this chimeric protoxin from binding to normal cells throughout the body, and to increase delivery to the tumor," when the drug is administered systemically via the bloodstream. This protein is engineered to *require cutting by PSA* to activate the cell-killing pore forming (hole-poking) ability of the liberated bacterial toxin."

In elegant work, Isaacs and colleagues modified the protoxin so that when it is out for delivery – when it's circulating in the blood – it is inactive, and harmless. But when it reaches cells that have PSMA, watch out! "It selectively binds to the surface of metastatic prostate cancer cells that express PSMA, and this binding enables PSA to activate the protoxin by acting as a molecular scissor," Isaacs says. In other words, the mail bomb only goes off when PSA snips open the package. "We are accumulating the preclinical validation needed for clinical development of this PSMA-targeted chimeric protoxin activated by PSA."

This work was supported by The Patrick C. Walsh Prostate Cancer Research Fund and the Allegheny Fund at Johns Hopkins. ■

The Keystone to Resistance after Chemotherapy for Prostate Cancer

When cancer cells are treated with chemotherapy, the Keystone cancer cells stop dividing, and seem to hibernate. Because chemotherapy kills cells that are dividing, "the Keystone cancer cells survive."

Consider, if you will, the Monterey Pine tree. Its cones only open after a fire reaches a certain temperature. Its whole survival has adapted so that after a wildfire, it will release many seeds at once to repopulate the burnt forest.

Brady scientists Sarah Amend, Ph.D., and Ken Pienta, M.D., *the Donald S. Coffey Professor of Urology*, have found that something similar happens in prostate cancer cells in response to the stress of chemotherapy. "Using an imaging system that allows us to track cells over time, we observed prostate cancer cells responding to chemotherapy stress," says Amend. "We found, existing within the larger cancer cell population, a distinctive rare cell subtype," which they have named the Keystone cancer cell. These Keystone cancer cells are giant and have extra DNA as compared to the other cancer cells. "These cells have been observed by scientists for a century, but have long been dismissed as insignificant, thought to be artifacts or dying cells," adds Pienta.

They seem to be just the opposite: Amend and Pienta have discovered that these cells – like the Monterey pine cone seeds after fire – "emerge under therapeutic stress," says Amend. "Based on our recent observations, where we observed them as live, functioning cells, we now believe Keystone cells are critical operators of resistance." They found that when cancer cells are treated with chemotherapy, the Keystone cancer cells stop dividing and seem to hibernate. Because chemotherapy kills cells that are dividing, "the Keystone cancer cells survive."

After the stress of treatment subsides, the Keystone cancer cells wake up, start dividing again, "and give rise to a generation of now-resistant daughter cells," Amend continues. "We believe that these Keystone cancer cells are critical to therapy resistance and disease recurrence, and unless they are eliminated, cancer is going to come back after chemotherapy." Amend and Pienta are now working to learn more about how these Keystone cancer cells are formed, "so we can learn how to target them, to make treatment for advanced prostate cancer much more effective." Amend and her colleagues have received an award from The Patrick C. Walsh Prostate Cancer Research Fund to build on this work. For the next steps of their project, please see page 17. ■

Coming Soon: A New Urine Test for Prostate Cancer

Prostate-specific RNA in the urine may lead to a new test for clinically significant prostate cancer, and may show which men have more aggressive disease.

Why do we rely so heavily on blood samples to tell us what's happening in the prostate? Why not urine? Indeed, says scientist Jun Luo, Ph.D., "given that exfoliated cells and secretions from the prostate can be found in the urine, it represents an ideal source of biomarkers for localized prostate cancer."

Luo, leading a team of investigators to explore novel possibilities of urine testing in prostate cancer, has developed an innovative procedure to microscopically identify prostate cells recovered from urine. Using multiplex *in situ* hybridization (a technique that allows scientists to find precise sequences of DNA or RNA), the team looked for prostate-specific RNA targets. They fluorescently labeled prostate cells and evaluated RNA sequences at the single-cell level, using a method developed by Brady research fellow Jillian Eskra, Ph.D. The results were as exciting as they had hoped: "Using this technique, it is possible to visualize malignant and benign prostate cells in urine specimens," says Luo.

Once they had a test, Luo's team worked with urologist Christian Pavlovich, M.D., to evaluate how well it worked, in urine collected from 98 patients. The test performed like a champ: "When we compared the results of the urine test with clinical and pathological findings," Luo says, "we found that the majority of patients who tested positive for cancer had clinically significant disease." Even better: "Positive detection also correlated with high-risk cancer features" detected in needle biopsy. "These preliminary results indicate the urine test is highly specific for detecting clinically significant prostate cancer, and holds promise as a tool for distinguishing men who harbor aggressive prostate cancer from those with indolent disease."

This work was presented at the annual AUA meeting in May 2019. Results were published in the *Journal of Urology*, and a second journal publication is being prepared. ■

Read About the Research You Have Helped Make Possible.

THE PATRICK C. WALSH PROSTATE CANCER RESEARCH FUND

We are looking for innovative ways to stop lethal prostate cancer and to make life better for men with localized prostate cancer: these are the exciting research projects you helped us fund this year. Since its inception in 2005, The Patrick C. Walsh Prostate Cancer Research Fund has awarded millions of dollars to Johns Hopkins scientists in every discipline with good ideas worth pursuing that can help us understand more about prostate cancer — to help us save lives, to find better ways to treat it at every stage, and even to help prevent it. These awards wouldn't have been possible without the tremendous and amazing generosity of our patients and friends.

For 2019, we asked scientists to submit one of two types of research proposals: for pilot projects, as in previous years, and for team science awards. These team projects are aimed at developing the necessary preliminary data and team structure needed to apply for NIH program project grants (such as P01 or U01), or similar funding from other sponsors. Applications were reviewed by nine separate investigators with appropriate expertise related to each project. Projects were scored on a scale of 1 to 10 (1 being the best possible score) and then ranked, based on their adjusted average (the highest and lowest scores were eliminated before taking the average of the remaining seven scores).

2019 AWARDEES

Jun Luo, Ph.D.,
The Virginia and Warren Schwerin Scholar
Department of Urology

Bruce J. Trock, M.P.H., Ph.D
The Ambrose Monell Foundation Scholar, *Department of Urology*

Sarah R. Amend, Ph.D.,
The Carolyn and Bill Stutt Scholar,
Department of Urology

Hui Zhang, M.S., Ph.D.,
The Beth W. and A. Ross Myers Scholar,
Department of Pathology

Precision Medicine in Prostate Cancer: Finding Subsets of Patients

We've known for many years that prostate cancer fine-tunes itself from patient to patient. This is why, although two men may appear to have identical prostate cancer, with the same Gleason grade and same stage, one man may respond to a drug, and the other will not. Thanks to advances in prostate cancer genetics – many of them made right here at the Brady – we now know that men with prostate cancer can be divided into subsets. One subset, for example, is men with mutated *BRCA1/2* genes, who may do better on PARP inhibitors than other men.

"The treatment landscape for men with advanced prostate cancer is rapidly evolving," says Brady scientist Jun Luo, Ph.D., who adds that "disruptive changes are being introduced by multiple treatment modalities. Although progress has been made in prostate cancer genetics and genomics, there remains a critical knowledge gap that limits the impact of these advances on the overall goal of prostate cancer control."

"We will use our team science award to develop genetic marker tests and characterize subsets of patients with distinct treatment responses. Such tests could help choose treatment at critical disease states along the prostate cancer spectrum."

Luo, with Brady scientists William Isaacs, Ph.D., and Shawn Lupold, M.D., Ph.D., will use a team science award to develop genetic marker tests and characterize subsets of patients with distinct treatment responses. Such tests could help choose treatment "at critical disease states along the prostate cancer spectrum." These pilot efforts will lead to a NCI PO1 project, "with the overall goal of realizing precision medicine in prostate cancer." ■

Could Metabolomics Explain Some Racial Differences in Men with Prostate Cancer?

Many metabolic patterns can be modified – by changing diet, exercise, other behaviors, or medications. "Identifying key metabolic differences could provide clues about changes that may help lower the risk in African American men."

Metabolomics is the study of metabolites: small molecules found inside cells, body fluids, or tissues. Could it help explain racial differences in prostate cancer? Bruce Trock, M.P.H., Ph.D., and co-investigator Angelo De Marzo, M.D., Ph.D., believe it might.

As we discuss elsewhere in *Discovery* (see page 12), African American men are more likely to develop prostate cancer, and to die of it, than men of European descent. There's no simple explanation: in addition to genetic differences, many factors contribute to this discrepancy: certainly, issues such as "differences in income, education, diet, and access to medical care," are important pieces of this puzzle, Trock notes. "But there also appear to be unexplained differences between African American and white men in the biology of prostate tumors. Although some differences in gene expression or mutations have been identified, they don't provide a clear indication of how biological functions in the prostate may contribute to the excess risk in African American men."

Metabolites may provide a new window for discovery. "Unlike genes and proteins, metabolites reflect the actual workings of cells and organs," says Trock; in other words, what makes the prostate the prostate. Another advantage of studying metabolites is that many metabolic patterns can be modified – by changing diet, exercise, other behaviors, or medications. "Comparing patterns of metabolism between African American and white men with prostate cancer may reveal differences in the underlying biology. Identifying key metabolic differences could, in turn, provide clues about changes that may help to lower the risk in African American men. This type of approach is an example of precision urology."

With their grant from The Patrick C. Walsh Prostate Cancer Research Fund, Trock and De Marzo will be comparing metabolic patterns in the prostatic fluid taken from men who underwent radical prostatectomy at Johns Hopkins. "This fluid is secreted by glands within the prostate – and these glands are the structures where prostate cancer originates." The scientists believe these metabolites "should closely reflect the biological activity giving rise to prostate cancer." They will compare prostatic fluid metabolites between closely matched African American and white prostate cancer patients whose tumors did, or did not, recur after surgery. "This may identify whether the biology leading to aggressive prostate cancer differs between African American and white men," says Trock, "which will help us form new hypotheses for reducing risk and tailoring treatment to the biological aggressiveness of the tumor." ■

Keystone Cells and Resistance and Lethality of Prostate Cancer

"We will test the ability of α-particle irradiation to eradicate these Keystone cancer cells."

On page 15, we talked about "Keystone" cells – so named by scientists Sarah Amend, Ph.D., and Ken Pienta, M.D., because they seem to play a pivotal role in advanced prostate cancer that defies treatment. These Keystone cells – distinctive, large cells that emerge after chemotherapy – "have been recognized for more than 100 years, but are often dismissed as unimportant, thought to be artifacts or dying cells," says Amend. "However, our preliminary data suggest that, in fact, Keystone cells are the critical mediators of therapy resistance. Therefore, unless they are eradicated, cancer will recur in treated patients."

In a team project with Brady scientist Angelo De Marzo, M.D., Ph.D., and Stavroula Sofou, Ph.D., a biomolecular engineer at the Johns Hopkins Whiting School of Engineering, Amend will build on this work. "We will determine how and when Keystone cells are formed during prostate cancer progression," Amend says. "We will also explore how they survive anti-cancer therapy by exiting the cell cycle and how they repopulate a therapy-resistant population. Then we will test the ability of α-particle irradiation to eradicate them." Amend and colleagues believe the results of this project "will fundamentally change our understanding of how and when therapeutic resistance arises, and will introduce a candidate treatment to eliminate Keystone cells and increase the chances for long-term survival in patients with aggressive disease." ■

What Happens at the Beginning of Metastasis?

Many, but not all, men diagnosed with low-risk prostate cancer can be safely monitored without treatment. There's still some uncertainty, because we don't have a way to tell who is truly low-risk, and whose risk might go up over time. Much needed, says Hui Zhang, Ph.D., is "a new test that could distinguish patients with truly low-risk disease from patients with high-risk features." Such a test, she adds, would provide peace of mind to men who choose active surveillance, and allow doctors to recommend this strategy with greater confidence.

"A urine test that could distinguish patients with truly low-risk disease from patients with high-risk features would provide peace of mind to men who choose active surveillance."

With co-investigator Alan W. Partin, M.D., Ph.D., Director of the Brady Urological Institute, Zhang will work on developing a urinary test to determine cancer risk using urinary glycoproteins, which have proven to be useful biomarkers in detecting prostate cancer from blood. Zhang and Partin have identified four likely candidate glycoprotein biomarkers. Says Zhang: "We will determine the clinical performance of detecting patients with different levels of high-risk tumors, using mass spectrometry as well as immunoassays for these four candidates from urine." ■

***Hahn**: "Neoadjuvant multi-agent chemotherapy"– using a triple punch of cisplatin, gemcitabine, and docetaxel – before cystectomy is helping sarcomatoid bladder cancer patients. "Our initial results are very encouraging."*

DISCOVERY IN BLADDER CANCER

Sarcomatoid Bladder Cancer: Encouraging Responses to New Regimen

"Four out of six patients demonstrated a complete response, with no identifiable residual tumor seen at the time of surgery."

New hope for patients with a rare, extremely aggressive form of bladder cancer: "very encouraging responses" to an intense, triple-drug, neoadjuvant chemotherapy approach, followed by cystectomy (surgical removal of the bladder).

Medical oncologist Noah Hahn, M.D., presented results of the Johns Hopkins experience in treating sarcomatoid bladder cancer at the International Bladder Cancer Network's Annual Meeting in Aarhus, Denmark, in October. Based on a complete response to neoadjuvant chemotherapy in a patient who initially was considered to have unresectable cancer, Hahn and urologist Trinity Bivalacqua, M.D., Ph.D., began offering "neoadjuvant multi-agent chemotherapy" – using a triple punch of cisplatin, gemcitabine, and docetaxel – before cystectomy to sarcomatoid bladder cancer patients seen at Johns Hopkins who were eligible for cisplatin-based chemotherapy. "Thus far, six patients have been treated with this more intense approach, followed by cystectomy," says Hahn. "Our initial results are very encouraging, with four out of the six patients (67 percent) demonstrating a complete response, with no identifiable residual tumor seen at the time of surgery." The news was good for the other two patients, as well: "Neither of the patients who had residual tumor in the surgery specimen, had growth of their cancer on treatment, and neither had any severe, unexpected surgical complications."

Now, the scientists are examining the pre-treatment tumor samples from these patients "to examine the genetic basis of these encouraging responses," says Hahn, "to help guide physicians in deciding whether all or only a portion of patients with sarcomatoid bladder cancer should be treated with this new, multimodality approach."

Grant from National Cancer Institute: Previously in *Discovery*, we reported on Hahn's leadership of a clinical trial of an immune checkpoint-inhibitor drug, durvalumab, in patients with metastatic bladder cancer. "This study led to the FDA approval of durvalumab and provided bladder cancer patients with another much-needed therapy option," says Bivalacqua, Director of Urologic Oncology. Recently, a team of Hopkins investigators led by Hahn, including Bivalacqua, Luigi Marchionni, Kellie Smith, Woonyoung Choi, Alex Baras, Marianna Zahurak, Gary Rosner, David McConkey, and Drew Pardoll, received a $3.2 million R01 award from the National Cancer Institute for their novel clinical and translational work proposed within the ADAPT-BLADDER trial.

"Within this study, bladder cancer patients with BCG-relapsing and BCG-unresponsive, non-muscle invasive bladder cancer will be treated with a number of novel immunotherapy combination approaches," says Hahn, "including durvalumab plus BCG, and durvalumab plus radiation therapy." The award will also fund novel genetic and functional immunology investigations, using patient samples collected within the study. "The ADAPT-BLADDER trial is one of the first studies to bring urology, medical oncology, radiation oncology, pathology, and translational medicine together to create a new multidisciplinary care model for non-muscle invasive bladder cancer," notes Hahn. "In this respect, it stands to serve as a transformative study for the bladder cancer field." ■

Upper Tract Urothelial Cancers: Who Will Benefit from Chemotherapy?

When it comes to chemotherapy for urothelial cancer, does location matter? Hopkins scientists are working to find out.

"Recent studies have led to the identification of genetic biomarkers, including molecular subtypes, that can be used to predict benefit from presurgical (neoadjuvant) chemotherapy in urothelial cancers located in the bladder," says medical oncologist Jean Hoffman-Censits, M.D. "However, we don't know if these same biomarkers can predict chemotherapy benefit" if these tumors are located elsewhere – namely, in the ureter or renal pelvis; these are upper tract urothelial cancers (UTUCs).

Urologist Philip Pierorazio, M.D., and Hoffman-Censits are collaborating with other investigators to establish a research-based Center of Excellence at the Brady and the Greenberg Bladder Cancer Institute. In the recently completed Eastern Cooperative Oncology Group's Phase II clinical trial, they and other Hopkins investigators demonstrated that neoadjuvant chemotherapy has clinical benefit in patients with UTUCs. Now, in a project supported by philanthropic donation from Jim and Pam Harris, they are performing molecular genetic studies to identify biomarkers that can help predict which patients with UTUCs are most likely to benefit. These studies are being performed in close collaboration with urologist Surena Matin, of MD Anderson. (To read more about our work on UTUCs, please visit https://www.hopkinsmedicine.org/greenberg-bladder-cancer-institute/utuc.) ■

Bladder Immune Cells May Determine Response to Chemotherapy

Scientist Woonyoung Choi, Ph.D., who led genomic studies that identified the basal and luminal molecular subtypes of bladder cancer, has discovered that the immune system plays a powerful role in how patients respond to chemotherapy.

"Differences in the numbers and characteristics of the immune cells – in particular, the T lymphocytes – that are present in bladder cancers strongly influence their sensitivity to neoadjuvant chemotherapy," she says. With a $1.5 million grant from the Department of Defense, she is collaborating with a co-investigator in Texas to develop a new molecular subtyping test, and to perform mechanistic studies to determine whether T lymphocytes are required for bladder cancers to respond to chemotherapy. ■

Evaluating alternatives to BCG: Is combination chemotherapy the next frontier?

Maintenance dosing of intravesical combination chemotherapy "leads to more durable responses than induction treatment alone."

If you are diagnosed with early-stage bladder cancer, you will probably be given a locally delivered immunotherapy called BCG. Soon, there may be other options, as well. Previously, Brady scientists Max Kates, M.D., and Trinity Bivalacqua, M.D., Ph.D., showed that BCG's success is largely due to an influx of T cells into bladder tumors. "A single supplier in the United States is currently unable to meet demand for the treatment" notes Kates. "However, BCG is not the only effective treatment for this disease."

Over the past five years, and with the help of collaborators from the Center for Nanomedicine at Johns Hopkins, Kates and his colleagues have designed novel chemotherapy approaches to enhance drug absorption into the bladder wall. Following the lead of colleagues from the University of Iowa, the team has begun to offer intravesical combination chemotherapy, including gemcitabine/docetaxel, for patients who do not have access to or are not candidates for BCG.

Recent work by Kates and colleagues has shown that "maintenance dosing of this combination leads to more durable responses than induction treatment alone," he says. "We are in the midst of a new wave of interest in combination intravesical chemotherapy for bladder cancer. Our current efforts are focused on sequencing these tumors to identify predictors of response to these new and exciting therapies." For these discoveries, the American Cancer Society has given Kates's lab a five-year Clinical Scientist Development Award. This work was published in *Clinical Cancer Research* and *Cancer Immunological Research*. ■

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., *the Erwin and Stephanie Greenberg Professor of Urology* and Director of the Johns Hopkins Greenberg Bladder Cancer Institute; he 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." ■

***Bivalacqua** has made numerous contributions in two areas of urology: sexual dysfunction and urologic oncology. The coveted Gold Cystoscope Award was presented by AUA President **Robert Flanigan**.*

Bivalacqua Wins Gold Cystoscope Award

"Trinity Bivalacqua defines a translational surgeon-scientist."

Brady urologist Trinity Bivalacqua, M.D., Ph.D., *the R. Christian B. Evensen Professor*, has received this year's Gold Cystoscope Award, one of the highest honors awarded by the American Urological Association. The award recognizes the young urologist who has made the most contributions during the first 10 years after completing residency.

Bivalacqua is in very good company: recipients of this award have a distinguished history of going on to become leaders in the field. And, over the years, many Gold Cystoscope awardees have been Brady faculty and former residents, including: Patrick Walsh, William Catalona, Mani Menon, Herbert Lepor, Louis Kavoussi, Alan Partin, Jeffrey Cadeddu, and William Roberts.

"Trinity Bivalacqua defines a translational surgeon-scientist," says Partin, Director of the Brady, "and he has made numerous contributions in two fields – sexual dysfunction and urologic oncology."

After earning a bachelor's degree in Cell and Molecular Biology from Tulane University, he entered the M.D./Ph.D. program at Tulane's School of Medicine. "My Ph.D. thesis described the influence of systemic disease states on dysfunctional genes responsible for aberrant vascular control of the heart, pulmonary and penile circulation," he recalls. Bivalacqua was the first to show that both gene and stem cell-based therapies improved penile vascular function in preclinical models of erectile dysfunction.

"I knew I wanted a career that would allow me to combine basic science research with clinical responsibilities," Bivalacqua says. Urology was a perfect fit, and he "purposefully sought out a residency at Johns Hopkins, due to the Brady's rich tradition of training surgeon-scientists in urologic oncology." He applied for a postdoctoral fellowship during his research year in residency, and obtained an M.D./Ph.D. Fellowship from the AUA Foundation – the first resident to do so. In part because of Bivalacqua's groundbreaking success, the AUA Care Foundation now encourages residents to apply for postdoctoral fellowships.

After his fellowship, Bivalacqua joined the Brady faculty. With a K08 Career Development Award from the NIH/NIDDK, he studied next-generation therapies for nerve regeneration following radical prostatectomy – research that led to discoveries in the field of peripheral nerve biology and highlighted the detrimental effects of neuroinflammation in autonomic nerve degeneration. For this work, he was awarded the esteemed AUA Rising Star Award.

Over time, his clinical and research practice shifted to focus on bladder cancer. As the Brady's Director of Urologic Oncology, Bivalacqua has built a program around multi-disciplinary care of urologic oncology patients. He heads a translational research program in genitourinary cancers and tissue engineering, using preclinical animal models of disease – in particular, transgenic mouse models of bladder cancer and of erectile dysfunction. "My research uses cell culture models of urothelial carcinoma and genetically engineered rodent models of bladder cancer to study the inflammatory and immune responses that mediate development and ultimate progression of urothelial carcinoma."

The carcinogen model developed in Bivalacqua's lab has led to the first preclinical model to study non-muscle invasive bladder cancer. Using this model, which has been characterized at the genetic and molecular level, Bivalacqua's team has developed novel intravesical therapies. Recently, they developed a genetically engineered, recombinant BCG that over-expresses a STING (stimulator of interferon genes) agonist, "resulting in enhanced interferon signaling and greater therapeutic response than BCG alone." His work has resulted in a patent and licensing of rBCG-STING, "which is soon to be tested in a phase 1 clinical trial in patients who have been unresponsive to BCG."

Bivalacqua believes his greatest accomplishment – so far – has been the successful development and completion of a tissue-engineered conduit that may one day lead to development of a new artificial bladder. The trial, "Phase I Open Label Single Center Exploratory Study of Autologous Neo-Urinary Conduits in Subjects Requiring Incontinent Urinary Diversion following Cystectomy," uses autologous cell-based, engineered genitourinary tissue – instead of a surgically removed section of intestine – to replace the lower urinary tract, to reduce complications following surgery. "This trial was conceived and designed from research performed in the lab, and was brought to the first in-human clinical trial in this field," Bivalacqua says.

In addition to his clinical and scientific work, Bivalacqua has served on numerous national committees for the AUA, the Society of Urologic Oncology and Sexual Medicine Committee of North America. ■

Bladder Immune Molecular Subtypes Can Help Determine Who May Benefit from Chemotherapy for Urothelial Cancer

Urothelial cancer, also known as transitional cell cancer, is the most common type of bladder cancer; it's also more complicated, and has more subtle differences from person to person, than scientists used to think. It encompasses several molecular subtypes, "each with its own distinct clinical and biological characteristics," says urologist Trinity Bivalacqua, M.D., Ph.D., Director of Urologic Oncology.

These subtypes can be classified as either basal or luminal. "Basal tumors, while more aggressive, show the greatest improvements in survival outcome, with platinum-based neoadjuvant chemotherapy (NAC)," Bivalacqua continues. Luminal tumors, in contrast, "tend to be less aggressive, but may also receive less benefit from NAC" – and here is where understanding molecular subtypes may provide valuable insight.

"In the field of bladder cancer, we are desperately trying to find biomarkers for the selection of patients for NAC before surgery," Bivalacqua says.

"If accurate, they could be used on tumor tissues obtained at transurethral resection of bladder tumor (TURBT) to identify high-risk patients who would benefit from chemotherapy, while low-risk patients might be spared the side effects of chemotherapy."

Bivalacqua and Brady urologist Max Kates, M.D., have teamed up with other urologists worldwide "to determine whether we can use the molecular subtypes to help us select patients with high-risk, non-muscle-invasive bladder cancer who should be offered chemotherapy" at the time of radical cystectomy. "This is necessary," Bivalacqua explains, "because some patients with T1 disease are actually understaged," and more or higher-grade cancer is found after the removed bladder is examined by a pathologist. "This means these patients have more aggressive cancer, and thus may benefit from chemotherapy prior to radical cystectomy (bladder removal)."

In a study published in European Urology, Bivalacqua, Kates, and Hopkins colleagues looked at how various molecular subtypes fared when pathologists examined the removed bladder specimen – particularly, at the cancers that turned out to have spread beyond the bladder – in a multi-institutional cohort of patients with clinical T1-T2 bladder cancer who were treated with radical cystectomy. "This study provides valuable guidance," says Bivalacqua. "We found that luminal tumors were less likely to have spread outside the bladder compared with basal tumors." ■

Blue Light Shows Urothelial Cancer

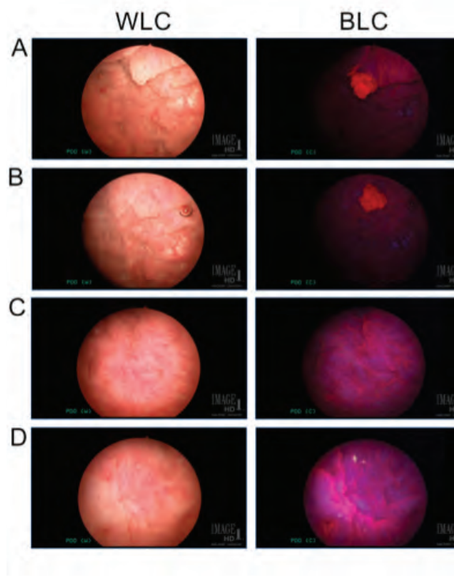
Have you ever gone to an event and had your hand stamped? The stamp may not show up at all unless someone shines a black light on it, and then there it is, plain as day! Pathologist Andres Matoso, M.D., and colleagues have discovered something similar by changing the color of light – from white to blue – used during cystoscopy. "Carcinoma in situ (CIS; abnormal cells that may become cancerous and spread; also called stage 0 disease) is difficult to visualize with white light cystoscopy," says Matoso, "but blue light cystoscopy, using photosensitizing agents, improves detection rates" of urothelial cancer, also known as transitional cell carcinoma, the most common type of bladder cancer.

In a recent study, Matoso and colleagues assessed the sensitivity of blue light cystoscopy, and compared the results with the final pathology diagnoses (see photo). "We also focused on cells that looked abnormal in blue light cystoscopy and had a pathology diagnosis that was suspicious, but not diagnostic of CIS. We found that blue light cystoscopy allows us to detect CIS that would have been underdiagnosed with the conventional white light cystoscopy." Their results were published in *Human Pathology*.

Bladder cancer that invades the muscle wall: Matoso and colleagues recently completed another study to evaluate the clinical significance of invasive urothelial carcinoma that *might* be invading the muscle wall of the bladder – but again, it might not. "Urothelial carcinoma that invades the muscle wall has a much worse prognosis than cancer that is non-invasive, or that just invades superficially," says Matoso. "While most patients can confidently be diagnosed as having either superficial or muscle-invasive bladder cancer, there's a small subset of cases that are difficult to classify."

In this study, Matoso and colleagues looked at invasive urothelial carcinoma that appeared ambiguous for muscle wall invasion on initial transurethral resection of bladder tumor (TURBT). They compared clinical and pathologic information from patients whose urothelial carcinoma was considered ambiguous to samples from patients with muscle-invasive disease and from patients diagnosed with superficial bladder cancer who underwent radical cystectomy (surgery to remove the bladder). "We found that the great majority of patients who have ambiguous invasion on initial TURBT turn out to have advanced disease" when the removed bladder specimen is examined. "This emphasizes the need for early repeat TURBT – or even consideration of early cystectomy to lower the risk of worse pathological findings, and to prolong survival."

This study was published in the *World Journal of Urology*.



Look at the difference a change in light makes! The white light used with conventional cystoscopy is on the left; the same views using blue light are on the right. A and B show a small papillary lesion – which really shows up well under blue light cystoscopy, highlighted by red fluorescence. C and D show areas (in red under blue light cyscoscopy) that need to be biopsied.

***Allaf and Pierorazio**: "Understanding the emotional and psychological stresses involved with having a small renal mass are just as important as an assessment of a patient's physical health."*

DISCOVERY IN KIDNEY CANCER

Physical, Mental Health and Active Surveillance for Kidney Cancer

"Half of the patients who chose delayed intervention did so because their cancer grew or changed; the other half decided to have surgery because of anxiety or uncertainty about their cancer. "The good news is that none of these patients had a recurrence of cancer."

Who's the ideal patient for active surveillance for a small kidney cancer?

"Although we have made significant strides in our understanding of active surveillance for these patients, there are no universally accepted criteria for selecting patients," says urologist Phillip Pierorazio, M.D., who with urologist Mohamad Allaf, M.D., Ph.D., began the Delayed Intervention and Surveillance for Small Renal Masses (DISSRM) Registry at the Brady 10 years ago. "However, recent studies led by Brady trainees have highlighted the importance of physical and mental health as determining factors."

In one study, published in the *Journal of Urology*, Brady Chief Resident Hiten Patel, M.D., and colleagues compared 410 patients who chose active surveillance with 341 patients who chose surgery to treat their small renal mass. They generated a DISSRM Score, incorporating age, tumor size, and other medical issues.

"Importantly," notes Allaf, who holds *the Mohamad E. Allaf Directorship in Minimally Invasive Surgery*, "the DISSRM Score also incorporated how patients felt about their own *physical health* – their ability to perform moderately strenuous activities like pushing a vacuum cleaner or playing golf." Asking patients about their physical health "may be an important step in assessing suitability for surveillance," says Patel. "A patient's perception of physical health takes into account age and other medical issues, and it's easy for patients and physicians to understand."

Physical health is just one part of the assessment of patients with small renal masses. In another study, published in *Urologic Oncology*, Mohit Gupta, M.D., urologic oncology fellow, and colleagues evaluated patients who enrolled in active surveillance but later went on to have surgery. "About 15 percent of patients in active surveillance later choose surgery," says Gupta. "We found that half of these patients who elected delayed intervention did so because their small renal mass grew or changed in a manner suspicious for cancer. But the other half elected to have surgery because of anxiety or uncertainty about their cancer." The good news is that none of the patients who underwent delayed surgery had a recurrence of cancer.

In addition to active surveillance, treatment options for small renal masses include partial nephrectomy, radical nephrectomy, and thermal ablation of the tumor. "Few studies have compared these management options simultaneously," notes urology resident Ridwan Alam, M.D., who led a third recent study, published in the *British Journal of Urology International*. The study found that all four management options had excellent oncological outcomes. "Partial nephrectomy and ablation were generally preferred over radical nephrectomy, as they spare kidney function," he says. Active surveillance was confirmed to be a reasonable option for well-selected patients with comparable mental health and oncologic outcomes. "We also found that mental and emotional health improved over time for patients undergoing immediate surgery and active surveillance."

"Understanding the emotional and psychological stresses involved with having a small renal mass are just as important as an assessment of a patient's physical health," says Pierorazio, "we want patients to be confident in their choices. If they choose surveillance, we want them to safely and assuredly remain in the program." ■

Potential Biomarker for Kidney Cancer: The Immune System?

Can what's happening to the immune system in kidney cancer be tracked? Are there biological footprints that might lend themselves to a test? Quite possibly!

There's no PSA equivalent for detecting or monitoring kidney cancer. Brady investigators, supported by funding from Brady Advisory Board members Patricia and Kevin Kiernan, are hoping to change that.

They have been building an understanding of kidney cancer biomarkers and, in the process, learning much more about the disease. "When we started looking for kidney cancer biomarkers, we thought we would find sugars and proteins in the urine or blood that were released by the cancerous cells," says urologist Phillip Pierorazio, M.D. "It turns out, we are more likely to detect sugars and proteins that describe the immune system's response to these kidney tumors. This is because kidney cancers suppress the body's immune system as they grow and spread."

Can what's happening to the immune system in kidney cancer be tracked? Are there biological footprints that might lend themselves to a test?

Quite possibly! The Kidney Cancer Biomarker Group, headed by Pierorazio, examined the urine of 100 patients with either benign tumors, benign-behaving kidney cancer, or aggressive kidney cancer, and found a number of lactose-containing molecules that could differentiate these groups. "Importantly, these lactose-containing molecules were cell-surface antigens – tiny sugar molecules used by the immune system to identify good from bad cells," says Pierorazio.

Scientist Jelani Zarif, Ph.D., and colleagues evaluated the role of macrophages in the development of early to aggressive forms of kidney cancer. "Macrophages are immune cells that engulf and digest cellular debris, bacteria – and, in some circumstances, cancer cells," Zarif explains. But certain types of macrophages, called M2, can actually promote cancers by suppressing the immune system around a malignant cell. Zarif's study, reported in *European Urology Oncology*, found more macrophages and more immunosuppression in kidney cancer cells than in the normal surrounding kidney tissue.

Scientist Richard Zieran, M.D., Ph.D., and colleagues are evaluating nano-vesicles in the blood and urine of patients with kidney cancer. "Traditionally, researchers thought these were the 'trash cans' of cells," says Zieran. "But we are increasingly finding evidence that these nano-vesicles actually contain valuable information in the form of proteins and nucleic acids. They are a form of communication between cells." Zieran's early work, presented at the American Association for Cancer Research's meeting in Atlanta, suggests that cancer cells may produce more vesicles than healthy cells, and thus influence the tumor environment.

"As we learn more about the immune system, we believe we are getting closer to finding the elusive biomarker that will help us diagnose and guide treatment for many patients with kidney tumors," says Pierorazio. ■

Conditional Survival After Kidney Cancer

"How long am I going to live?" There's a new way to answer this question, and it's called conditional survival. "The term refers to the improving probability of surviving long-term after the diagnosis of cancer or another chronic condition.," says Joseph Cheaib, M.D., postdoctoral researcher in the Brady.

Cheaib recently evaluated the conditional survival of patients with kidney cancer, using the national SEER database and the records of about 3,000 patients in the Johns Hopkins database. "We found a stage-specific conditional survival," he explains. "In particular, patients with advanced kidney cancers had a greater probability for long-term survival for every year they survived after the diagnosis of kidney cancer."

Cheaib's findings, recently published in *Seminars in Urologic Oncology*, indicate that patients with stage I and II kidney cancer did not experience a conditional survival – because they consistently survive at about 98 percent and 90 percent, respectively, no matter how long they are followed. Patients with metastatic kidney cancer showed the greatest increase in survival, increasing from 23 percent to 59 percent, and 31 percent to 76 percent over a five-year period in the SEER and Johns Hopkins datasets, respectively.

"These data have two important implications for our kidney cancer survivors," says Phillip Pierorazio, M.D., the study's senior author. "First, for patients with early-stage disease: once they undergo curative surgery, we can limit the extent of follow-up imaging after a relatively short period of time. And for patients with advanced cancer, it is very encouraging to see that our treatments are getting more and more effective. This is an incredibly hopeful time for patients with metastatic kidney cancer!" ■

DISCOVERY IN TESTICULAR CANCER

Much-Needed Guidelines for Treating Testicular Cancer

"Many urologists may go for years without treating a single patient with testicular cancer."

The long-term survival for a man diagnosed with testicular cancer is excellent – about 95 percent – thanks to good treatment, including chemotherapy and retroperitoneal lymph node dissections.

"However, the disease is relatively rare," says Phillip Pierorazio, M.D., Director of the Brady's Division of Testicular Cancer, "with only about 9,000 men diagnosed with it in the U.S. every year. Many urologists may go for years without treating a single patient with testicular cancer, and there has been little guidance on treating testicular cancer for the average urologist."

That's no longer the case. In May, the American Urological Association (AUA) released its first-ever guidelines for the management of early-stage testicular cancer. The AUA Guidelines include 45 statements to direct the diagnosis and management of men with early-stage testicular cancer – and each of those statements is supported by evidence from the Evidence-based Practice Center (EPC) at Johns Hopkins, led by Eric Bass, M.D., M.P.H. The EPC team – made up of Bass, Pierorazio, and Brady residents and fellows – evaluated hundreds of medical manuscripts about testicular cancer and generated five reports, on topics including the proper diagnostic and staging tests for testicular cancer patients, the comparative effectiveness of surveillance, chemotherapy, radiation and surgery, surveillance after therapy, and survivorship for men with early forms of the disease.

"This was a much-needed guideline for the standardization of testicular cancer care in the U.S. and around the world," says Pierorazio. "We are confident this will translate into improved care and outcomes for testicular cancer patients everywhere." ■

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