Bladder cancer preps for its star turn

Karen Titus

February 2016—A streak of sibling rivalry emerges when experts ponder progress in the field of bladder cancer. Whether it's new markers or therapies, funding or advocacy, advances have come slowly, and the disease has long labored in the shadow of others.

"Urologic malignancies in general lag behind, compared to breast cancer and other tumor types, like colon and lung, where we've been envious for a while," says George Netto, MD, professor of pathology, urology, and oncology and director of surgical pathology molecular diagnostics, Johns Hopkins University School of Medicine.

Now, it appears bladder cancer is making a run at most-favored-child status. Or, as Dr. Netto puts it, "In urologic malignancy, we're catching up."

Progress has been made in the field of bladder cancer, particularly in the past year or two, says Dr. Jim Zhai (left), here with Dr. Richard Joseph, who says, "The closest to the clinic is PD-L1."

He pauses, then adds, "Finally."

"We've made a lot of progress, particularly in the last year or two," agrees Jim Zhai, MD, professor of pathology and laboratory medicine, and consultant pathologist, Mayo Clinic, Jacksonville, Fla. "The bad news is it's taken 30 years for a breakthrough."

The most spectacular leap concerns the use of anti-PD-1/PD-L1 immunotherapy to treat patients with metastatic urothelial bladder cancer. Physicians have known for some time that a subset of bladder cancer tumors express programmed death-ligand 1—anywhere from 25 to 50 percent, says Richard Joseph, MD, assistant professor of medicine, Mayo Clinic, Jacksonville. They've also known that patients are more likely to develop a recurrence if the primary bladder cancer tumor expresses PD-L1.

Programmed death 1 protein and PD-L1 play a role in one of the so-called checkpoints in the immune system.

When tumors learn to express the PD-L1 marker, they can evade immune surveillance, and PD-L1's "off" switch fails. Not surprisingly, PD-L1 appears to be influential in other cancers as well. A 2012 paper (Brahmer JR, et al. *N Engl J Med.* 366:2455–2465) reported that an antibody-mediated blockade of PD-L1 led to tumor regression and prolonged stabilization of disease in patients with advanced cancers, including non-small cell lung cancer, melanoma, renal-cell cancer, and ovarian cancer. Roche's MPDL3280A (anti-PD-L1) has received breakthrough designation from the FDA for both NSCLC and metastatic bladder cancer.

At ASCO's Genitourinary Cancers Symposium in January, Dr. Joseph and colleagues presented an abstract of the first phase two trial looking at anti-PD-L1 in metastatic bladder cancer. The trial, involving 316 patients, was a single-arm study of patients who had received chemotherapy and whose disease had progressed. Such patients "are really out of options," says Dr. Joseph, given the lack of proven second-line chemotherapies in metastatic bladder cancer.

The overall response rate was "significantly improved compared to a historical control of 10%," the authors wrote. Responses were durable and associated with higher PD-L1 expression, though poor prognostic factors did not preclude response. A randomized phase three study is ongoing.

FDA approval of anti-PD-L1 would be a major shift in treatment; it could also spur development of new markers. From a clinical viewpoint, it makes no sense to separate those two strands, says Dr. Joseph. "When there are no good treatments to target these markers, these markers are less important. For example, one of the most commonly mutated genes in all of cancer is *p53*, and while we know this gene contributes to the pathogenesis of the disease, it is not really a target we can act upon. So to me, as a medical oncologist, rare alterations that we can act upon are more important in the treatment of disease." An example of this type of marker in bladder cancer could be HER2 (more commonly seen in breast cancers, of course, though there's evidence to suggest it could also be a player in bladder cancers). The folate growth factor receptors might also predict benefit to anti-FGF drugs. "But the closest to the clinic is PD-L1. That's here, that's ready," says Dr. Joseph.

Adds Dr. Netto: "I believe in the near future—perhaps even this year—this will be something we are regularly asked to do on our patients with metastatic disease."

Considering the dismal progress in developing clinically useful bladder cancer markers, PD-L1 can be seen as a cause for celebration. One researcher even uses the word "frenzy" to describe the current atmosphere. Then again, experts in this field also sound eerily like Cubs fans—who, let us delicately point out, are counting 108 years since their team's last World Series championship: *This is the year!*

One advance, no matter how exciting, can't solve things overnight. The waters here are muddy, says Dr. Zhai. PD-L1 might be creating a splash—but it's a splash in a swamp.

For all his enthusiasm, even Dr. Joseph is careful neither to oversell nor undersell his study's findings. "The study demonstrated clinical benefit in about one-third of patients who received the drug, with very little toxicity," he says. Some had complete remissions, though they were the minority; most patients had either partial remissions or disease reduction that didn't quite qualify as a partial remission. The drug was most effective in those whose tumors were PD-L1 positive.

Should anti-PD-1/PD-L1 therapy prove to be worthwhile clinically, pathologists will still have high hurdles to clear.

"Defining PD-L1 expression has been a big-time challenge for pathologists," says Dr. Joseph. Many antibodies are currently in use, each with its own sensitivity and specificity. "And each can show different levels of expression even on the same tissues," he says. In the aforementioned phase two trial, the researchers used a proprietary antibody. (Roche/Genentech sponsored the study.) "I don't know if it's going to become a companion diagnostic test or not, but I hope that this antibody at least becomes publicly available."

Clinical trials for anti-PD-1/PD-L1 therapy have used different IHC antibodies, Dr. Zhai says. "So here the waters

become muddy again." Based on conversations he's had with a pharmaceutical company VP, Dr. Zhai says, with a laugh, "The FDA fully realizes this mess."

He also points to an editorial in *Archives of Pathology & Laboratory Medicine* (Cagle PT, et al. 2015;139:1329–1330). While the piece focuses on PD-L1 in the context of lung cancer, Dr. Zhai says it gives a framework for thinking about new markers in immune-oncology, chiefly immune checkpoint manipulation. "The news about pembrolizumab [the monoclonal antibody] is only the tip of the iceberg," the authors write. With several others also in clinical trials, "There are different proposed IHC companion diagnostics for each of these drugs."

This will need to be addressed, though Dr. Zhai says the current nodus is not paralyzing. "It's not the end of the world." In patients with positive tumors, 30 percent will respond to the treatment; among those with negative tumors, 10 percent will respond. "But we should have a guideline, based on data. I think we will be able to develop acceptable criteria eventually."

Another area of concern, says Dr. Joseph, is that some people believe that tumor expression is the most important aspect of PD-L1; others contend it's the tumor microenvironment—perhaps immune infiltrates, rather than the tumor itself, are expressing PD-L1. In his study, he says, the immune infiltrates appeared to be more important.

Right now PD-L1 "is the most exciting development in bladder cancer," says Dr. Joseph. But as in any cancer, bladder's potential markers are, like a to-do list, seemingly inexhaustible.

Case in point: Dr. Zhai is the coauthor (along with Jae Y. Ro, MD, PhD) of the recently published book *Advances in Surgical Pathology: Bladder Cancer* (Wolters Kluwer, 2016), which includes a chapter devoted to molecular pathology of urinary bladder neoplasms. The chapter (which Dr. Joseph helped write) contains an extensive overview of potential biomarkers, including p53, p21, p15, p16, p63, FGFR3, EGFR, VEGF, HER2, RB gene, PTEN, estrogen beta, EZH2, p27Kip1, and Cyclin D1. And those are just the tissue-based biomarkers.

As the Republican presidential campaign is demonstrating, a long list of candidates is no guarantee of, well, anything. Markers can be a disobliging bunch. "Up to this day, we don't do any markers on our bladder cancer biopsies in a routine fashion," says Dr. Netto.

"The field is unusually crowded right now," says David McConkey, PhD, professor of urology and cancer biology, and director of urological research, University of Texas MD Anderson Cancer Center. He attributes that, in part, to large genomics projects such as The Cancer Genome Atlas, which has generated not only excitement but also funding.

Likewise, he says, exuberance over PD-1/PD-L1 checkpoint blockade/immunotherapy appears to have spread to other areas of bladder cancer research. It's not news that activating mutations in *FGFR3* are common in both nonmuscle-invasive and muscle-invasive bladder cancers; moreover, he says, plenty of companies have developed small molecules that inhibit FGFR3. "But industry hasn't been all that interested in doing clinical trials in bladder cancer. They've been more focused on breast and multiple myeloma and other disease types. But now all of a sudden we're flooded with companies that want to work with us on FGFR3," says Dr. McConkey.

Here again, years of neglect have taken their toll. Even PD-L1, which was cloned nearly 20 years ago, had a hard time generating interest, says Dr. Zhai. "At the time, people didn't pay attention to it," given limited treatment options. The trio of surgery, chemotherapy, and radiation barely budged in decades. And in looking for therapeutic targets, researchers tended to focus on tumor cells alone, he says. "We found a lot of molecular markers, a lot of changes on tumor, but the environment was kind of ignored." That meant potential immune therapy was overlooked, too.

Lack of research funds has also delayed progress, says Dr. Netto. "Bladder cancer is, in that regard, an orphan disease. And bladder cancer patients for the longest time did not have huge advocacy groups," he adds, though

that's changing with the establishment of the Bladder Cancer Advocacy Network, or BCAN, which is lobbying not only for philanthropic support but looking to secure funds from government agencies to sponsor clinical trials.

The tide began to turn with the 2012 *NEJM* publication. With anti-PD-L1 therapy "melting" tumor cells in the metastatic setting, says Dr. Zhai, "people got very excited." Suddenly, bladder cancer became a hoverboard. Pharmaceutical companies saw the future, Dr. Zhai says, "and they started jumping on this."

The need for markers in bladder cancer is strong. Among solid tumors, it's the most expensive cancer per patient, says Dr. Netto. The majority of patients present with superficial disease that typically recurs, thus incurring costs related to follow-up, including repeat cystoscopies and biopsies. "Finding markers that could change the follow-up approach from invasive procedures for surveillance to utilizing molecular urine markers, be it cell-free DNA or DNA from tumor cells that are shed in the urine, for detecting cancer recurrence, or lack of recurrence, will be very valuable," he says.

Better markers of surveillance is only one need. Clinicians would also like to see better prognostic markers, which might include small IHC panels that have their roots in gene expression signatures. Current research is hinting at signatures of aggressive and nonaggressive disease. "I think this is one of the hottest areas in bladder cancer research," says Dr. Netto, "where people are talking about bladder cancer types similar to what has been shown in breast cancer: a luminal type, a basal type, and a p53 wild-type." At this point, says Dr. Netto, the signatures are based on hundreds of genes, but he's reasonably confident they can be whittled down to a more manageable size using IHC-based signatures or surrogates.

"I think these signatures will finally translate into routine testing," Dr. Netto continues. Markers could include different cytokeratins, such as cytokeratin 20 and high-molecular-weight cytokeratins 5 and 6, along with CD44, HER2, and *FGFR3*. "We could build this small panel that would classify tumors into one of these three, or four, intrinsic genetic signatures, based on such methodology."

Signatures might also be used theranostically, Dr. Netto says, particularly related to use of neoadjuvant chemotherapy in metastatic disease. Those who respond to such therapy enjoy good survival rates, he says. But those who don't certainly won't benefit from a three-month delay in cystectomy. Assuming signatures of this type are reimbursed, says Dr. Netto, there could be a financial advantage. He says he's more optimistic about an IHC panel being reimbursed rather than a gene panel assay. "If we can identify a neoadjuvant signature, you can make sure you're putting your chemotherapy cost [toward] the right patients."

Delving more deeply into current research efforts shows just how complex this work is.
Dr. Netto's own area of interest lies in early detection markers. He and his colleagues have been sequencing tumors for several markers that are altered and detectable in tumor cells that are shed in urine.

TERT promoter mutations look promising, he says. Activating mutations in this gene occur in 66 percent of muscle-invasive urothelial carcinomas. In a pilot study, he and his colleagues in the Bert Vogelstein Laboratory at the Ludwig Cancer Research Center at Johns Hopkins looked at *TERT* promoter mutations in urine samples in patients with prior bladder cancer and noted that every patient who had a mutation detected in his or her urine sample had a recurrence, and those without the mutation did not (Kinde I, et al. *Cancer Res.* 2013;73:7162–7167). In subsequent studies they looked at more patients and expanded their panel to include 11 additional genes. Based on findings from The Cancer Genome Atlas and other studies, "We know how we can design an assay of around 10 or 12 genes. We're beginning to prove that genetic alterations in urine samples tightly mirror those in associated bladder tumors, and we're finding some encouraging results in terms of developing a very specific assay for a bladder cancer detection screen," says Dr. Netto.

Their panel of urine genetic markers is also applicable to the surveillance setting, where the need is huge, Dr. Netto says. And he'd love to see automated next-generation-sequencing–based panels replace costly and labor-intensive FISH-based assays that have been in use.

“We also need markers of BCG response.” With Bacillus Calmette-Guérin immunotherapy as the mainstay treatment in patients with superficial disease, he says, “We need to identify patients whose tumor will resist BCG and progress to muscle-invasive disease.”

Dr. McConkey takes it a step further. “BCG is very effective, but in some sense this is an albatross around our necks—it’s effective but nondurable.” As a result, patients need to be followed their whole lives, and ultimately many eventually require cystectomies. “We’re not reducing the cost burden with BCG. So what we really need is a replacement for it. Again, the immune checkpoint blockade frenzy has spilled over into the nonmuscle-invasive cancers.” At Johns Hopkins, he says, Noah Hahn, MD, is leading a multi-institutional effort to develop novel immunotherapy approaches for this type of disease.

Nonetheless, BCG is not departing anytime soon. With that reality in mind, Dr. McConkey notes that colleagues at MD Anderson have developed a cytokine biomarker panel to measure patient response post-BCG but before recurrence.

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He also highlights the work of Ellen Zwarthoff, PhD, in the Netherlands, who is developing DNA-based biomarkers to detect early recurrence. The hope is to create a noninvasive, more accurate alternative to cystoscopy. Most nonmuscle-invasive cancers have activating *FGFR3* mutations and telomerase promoter mutations, he explains, and DNA methylation biomarkers are very common in nonmuscle-invasive cancers; Dr. Zwarthoff is developing methods to measure them in voided urine, with an eye toward using them in a surveillance strategy.

Apart from these efforts, researchers are looking at proteomics, microRNAs, and exosomes. “It’s a crowded space,” says Dr. McConkey, with understatement.
He and his colleagues are focusing on markers related to muscle-invasive disease. The reason is simple, says Dr. McConkey: “It’s the lethal form of bladder cancer. And frontline therapies—cystectomy, with or without perioperative cisplatin-based combination chemotherapy—have not changed in decades.”

One of the immediate clinical needs is to determine who needs cystectomy. While bladder preservation is a high priority, “Most of my urology friends, at least here at MD Anderson, would be very concerned about waiting on

that," says Dr. McConkey. "They would rather err on the side of caution. There's definitely a culture here of cystectomy."

While there's level one evidence to suggest benefit of using neoadjuvant cisplatin-based combination chemotherapy in all patients with muscle-invasive disease, the impact on disease-specific survival is only five to 10 percent, he continues. "So our No. 1 priority has been to develop methods to distinguish patients who will benefit from those who won't."

Their early attempts involved generating genomic data sets to compare outstanding responders to those who didn't respond well, and looking for messenger RNAs that were differently expressed by the two groups. They ultimately rejected this approach and next opted "to be more unbiased," Dr. McConkey says, by generating whole-genome mRNA expression profiling data sets from large cohorts of patients. When they looked at clusters that were generated by subsequent analyses, "Lo and behold, we found that muscle-invasive bladder cancers group into what we now call intrinsic subtypes that share common features with human breast cancers."

Dr. McConkey now says the approach they chose was a fortunate choice. "It turns out the factors that generate aggressive disease are complicated and probably differ according to a tumor's intrinsic subtype." Basal cancers tended to be associated with advanced stage and metastatic disease at clinical presentation. Particularly in the absence of perioperative chemotherapy, they were also the most aggressive, and patients with this subtype had the worst clinical outcome. "In addition, we noticed that these cancers were enriched with squamous and sarcomatoid features, and that they tended to be somewhat more enriched in women." When they looked at other major variants of bladder cancer, they saw enrichment, in both basal and luminal subtypes, in many of them. "So I think the story here might be that these variants tend to be exaggerated biological versions of the major intrinsic subtypes," Dr. McConkey says.

Further investigation revealed that a large fraction of the basal subtypes responded to perioperative chemotherapy. "If there's one patient population we think should definitely get chemotherapy, it's the population with these basal cancers," Dr. McConkey says. (That's also true with breast cancer, he adds—the efficacy of neoadjuvant therapy is highest in the basal and HER2-enriched subtypes. And with a new basal variant having been identified in pancreatic cancer, which might also be more responsive, "We think that being basal might be a sign of being chemosensitive across disease types.")

The luminal cancers were separated into two distinct subtypes. One was enriched with papillary features and activating mutations in FGFR3; the other was infiltrated with fibroblasts and some lymphocytes, Dr. McConkey says. The latter subtype—referred to by some researchers as "infiltrated" or p53-like (because they had gene expression signatures consistent with active, wild-type p53)—appears to be resistant to neoadjuvant chemotherapy. Looking at additional cohorts, the researchers have validated these observations, Dr. McConkey says. "We found the same patterns to be true every time we looked. They're not completely resistant in every cohort, but we're a little concerned that some of the downstaging that's attributed to neoadjuvant chemotherapy might actually be due to surgery," he says. It might also be due to a combination of the two.

It's possible fibroblasts have something to do with chemoresistance, Dr. McConkey says, noting that they've been implicated in pancreatic and other cancers and have been associated with tumor quiescence. Further complicating the picture, he says, he and his colleagues have found that a few basal cancers slip into this category, although TCGA analyses found this cluster to be 100 percent luminal. "We don't think it's a particularly stable subtype," he says. Some of the papillary luminal cancers that aren't downstaged by neoadjuvant therapy "switch over" and become p53-like after chemotherapy. "We worry that this phenotype, if it persists, could undermine attempts to re-treat these patients with other cytotoxic agents." Again, he draws a possible parallel to luminal A breast cancers, which don't benefit from neoadjuvant chemotherapy.

While Dr. McConkey's work is looking through the mRNA lens, others are looking through the DNA perspective at mutations such as *ERCC2, ERBB2, ATM, FANCC,* and *RB1*. "There's good evidence that some of these mutations may also be able to inform the use of neoadjuvant therapy," he says.

For all the genuine excitement, the current clinical realities are still sobering. “Today, what do we order on every patient routinely?” asks Dr. Netto. “The answer is: nothing.”

Nevertheless, he remains positively chipper about the future. “At no time have I been more optimistic than I have in the last year or two,” Dr. Netto says. “And I will be very surprised in the upcoming year if we don’t start doing a panel of CK20, CK5/CK6, HER2, CD44 expression, and *FGFR3* mutation.”

“And,” he adds, “we’re starting to get clinical requests for PD-L1 markers.”

Naturally, new markers will place new demands on pathologists. But Dr. Zhai also suggests that old demands are worth revisiting, too.

That would include the basic diagnostic categories. “We routinely classify bladder cancer into three major groups,” says Dr. Zhai. Superficial disease is either noninvasive or superficially invasive; this is considered to be a low-risk group. “You follow up, you use topical BCG, curettage, or local resectioning—all the things that are less dramatic interventions,” he says. On the other end of the spectrum lies metastatic cancer.

“We’re flooded with companies that want to work with us on FGFR3.”
David McConkey, PhD

In between is muscle-invasive disease. “I wrestle with this,” Dr. Zhai concedes. Muscle involvement can entail either the muscularis mucosae, which is essentially superficial involvement, or muscularis propria. “When oncologists and urologists talk about muscle involvement, they mean muscularis propria.”

Differentiating between the two types can be difficult, says Dr. Zhai. “We can use morphology, we can use immunohistochemistry, and occasionally we can’t tell.

“As a surgical pathologist,” he continues, “I think it is OK to communicate with the clinician: ‘I’m not sure what kind of muscle—we should probably rebiopsy the patient before anything dramatic is done.’ So surgical pathologists don’t write casually, ‘muscle-involving urothelial carcinoma.’”

Pathologists also need to determine if the bladder cancer is indeed urothelial carcinoma. Most cases are, although a minority fall into other categories: stromal tumors, squamous cell carcinoma, and adenocarcinoma, for example. “In the stromal tumor you have sarcoma, but there is also pseudosarcoma,” Dr. Zhai warns. Mistaking the latter for the former can lead to unnecessary cystectomy. Micropapillary variants can also pose a diagnostic problem. Much

of the time, such variants are seen when the tumor has already deeply invaded the muscle. If pathologists are unaware of this feature and call the tumor superficially invasive, clinicians will mistakenly treat these patients as if they had low-risk disease.

Micropapillary variants tend to be more aggressive, Dr. Netto says; interestingly, using HER2 as a marker and targeting these variants with Herceptin shows some promise. “It opens clinicians’ eyes to a potential target of therapy,” he says.

Carcinoma in situ has its own challenges. These flat, high-grade lesions by their nature produce very limited amounts of material. “It’s been difficult to do good genomics on them,” says Dr. McConkey. He’s hopeful that technological breakthroughs will push researchers over that barrier soon, however. “I think within the next year we’ll have a better idea of what CIS looks like, whether it looks like muscle-invasive disease, whether it consists of both basal and luminal tumors, whether it’s got unique patterns of DNA alterations, etc.”

Dr. McConkey sees an expanded role for pathologists as a fresh grammar of bladder cancer is established. Variants that are seen under the microscope will likely also be revealed through RNA expression profiling or RNA sequencing, for example. Citing the work of MD Anderson colleague Bogdan Czerniak, MD, PhD, he reports that tumors with squamous cell features, for example, tend to have molecular characteristics consistent with the squamous focus throughout the tumor. Dr. Czerniak has also seen this with sarcomatoid tumors and micropapillary tumors, Dr. McConkey says. The upshot: “From a diagnostic perspective, I think our pathology calls will be a lot more accurate. And I think ultimately our therapeutic decision-making is going to change, based on whether we know the tumor looks squamous, or sarcomatoid, or micropapillary, or small cell, etc.”

Beyond that, of course, identifying the basal/luminal origins of the cancers will likely be critical, Dr. McConkey says. Researchers are already developing tools that will enable pathologists to make those calls without having to perform deep molecular profiling, he says. At a conference in Madrid in March 2015, participants discussed development of an immunohistochemical classifier. The subsequent consortium tasked with doing this hopes to use RNA sequencing to assign large cohorts of tumors from two independent clinical trials; at the same time, they’ll try to make subtype assignments using IHC biomarkers already approved for use in other cancer types, including breast. “So we would hypothesize that using just two antibodies might be sufficient for us to make these calls. If we could assign the tumors to the subtypes using routine immunohistochemistry, that would make this type of assignment accessible everywhere.”

Dr. McConkey, in short, leaves pathologists with a clear message: They have every reason to be excited about new developments in bladder cancer. In fact, they may be the ones leading the way out of the swamp.

Embracing genomics as part of pathologic diagnosis is probably the wave of the future, he says. For some, that spells tension. “I can sense there are sometimes these struggles, kind of an us-versus-them mentality,” Dr. McConkey says. “That’s not helpful.” What pathologists see under the microscope can be understood with genomics, but genomics is not ready to replace pathology, he says. IHC-based testing for anti-PD-1/PD-L1 makes that point loud and clear.

“If anything,” says Dr. McConkey, “this is the time when we need pathologists, more than ever, to be actively involved in this work.” Perhaps bladder cancer is, at long last, emerging from its twin curses of being invisible and being complex. *Is this the year?*
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