Bladder Cancer: A Disease Ripe for Major Advances

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Abstract: Despite high incidence rates and substantial financial burdens associated with the care of patients with bladder cancer, progress in bladder cancer management has been modest and no new therapeutic agents for bladder cancer have been approved over the past decades. Fortunately, a substantial improvement in our understanding of the biology of this disease has occurred owing to revolutionizing molecular analysis platforms and increased interest among physicians and scientists in bladder cancer. As a consequence, a number of promising novel therapeutic agents are in development and are expected to make their way into clinics in the near future. This review focuses on the unique aspects of current bladder cancer research that support the assertion that bladder cancer is a likely frontier for major advancements soon.

Keywords
Bladder cancer, immunotherapy, molecular diagnostics, novel opportunities, targeted therapy

Introduction

Bladder cancer is the most common malignancy of the urinary tract and the ninth most common malignancy in the world, with more than 350,000 new cases occurring each year worldwide.[1] It is the sixth most common cancer in the United States, and the American Cancer Society estimates that 74,690 new cases and 15,580 deaths from bladder cancer will occur in 2014.[2]

Furthermore, bladder cancer is the most expensive cancer per capita to treat in this country. Annual costs range from $96,000 to $187,000 per individual, which amounts to cumulative costs to the US health care system of $4 billion each year.[3] The high costs of bladder cancer management are due to relatively long-term survival and frequent recurrences in patients with early-stage disease, which necessitates extensive surveillance testing with imaging, cystoscopy, and cytology. According to recent estimates, 60% of the cost of bladder cancer care is associated with surveillance and treatment of recurrences, and 30% is attributable to management of complications.[4]

Despite high incidence rates and substantial financial burdens associated with care of patients with bladder cancer, progress in bladder cancer management has been modest, with no new therapeutic agent approvals over the past decades. As a result, clinical

outcomes of patients with bladder cancer have remained stagnant, with 5-year overall survival rates of less than 15% in patients with advanced-stage disease.[5] There is a clear unmet clinical need in the management of bladder cancer, specifically for alternative therapeutic approaches. In recent years, modern molecular diagnostic platforms and an increased number of researchers focusing on the bladder have produced substantial improvement in our understanding of the biology of this disease. The combination of rapidly emerging discoveries about bladder cancer biology, availability of tumor tissue for translational target identification and validation, and an increased cadre of bladder cancer–focused physician-scientists have poised bladder cancer as a disease ripe for major advancements in the near future.

Overview of Contemporary Management of Bladder Cancer

Urothelial carcinoma can develop anywhere from the bladder (90%), renal pelvis (8%), ureter, or proximal two-thirds of the urethra where transitional epithelium is present. Urothelial carcinoma is the predominant histologic type in the United States, accounting for 90% of all bladder cancers. In approximately 25% of cases, urothelial bladder cancer has a divergent differentiation that may include squamous, glandular, micropapillary, nested, lymphoepithelioma-like, plasmacytoid, neuroendocrine, and sarcomatoid variants.[6] Other rare histologies of bladder cancer include pure squamous cell carcinoma (3%), adenocarcinoma (1.4%), and small cell carcinoma of the bladder (1%).

Bladder cancers are commonly classified as non–muscle invasive, muscle invasive, or metastatic. Non–muscle-invasive bladder cancer (NMIBC) accounts for 75% of all bladder cancers at initial presentation. Although NMIBC is potentially a curable disease with tumor resection, which may be combined with intravesical immunotherapy such as BCG (bacillus Calmette-Guérin), most NMIBCs recur within 6 to 12 months. Between 10% and 25% of patients develop invasive or metastatic disease, which requires more extensive treatments such as radical cystectomy and systemic chemotherapy.[7]

Approximately 25% of patients with bladder cancer present with a tumor invading the muscle layer of the bladder wall.[8] Patients with muscle-invasive bladder cancer (MIBC) require aggressive local and often systemic therapies. There have been substantial advances in the management of MIBC such as development of new bladder-preserving multidisciplinary strategies, advances in surgical techniques, and the introduction of perioperative systemic chemotherapy. However, the 5-year mortality rate of patients with MIBC remains approximately 35%.[9,10] Bladder cancer deaths in this group are due to a high rate of systemic failure, underscoring the need for better systemic therapies in this patient population.

Current standard therapy for metastatic bladder cancer includes platinum-based combination chemotherapy. Two standard combination regimens, methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) and gemcitabine and cisplatin (GC), have demonstrated comparable efficacy.[11] Despite high response rates of 50% to 70% with cisplatin-based regimens, the duration of response is relatively short, and in the majority of patients the disease progresses within a year.[11,12] In addition, about half of patients with advanced bladder cancer are considered unfit for cisplatin therapy owing to advanced age and underlying comorbidities, such as renal insufficiency or poor performance status.[13] Furthermore, there is no established global standard second-line therapy for patients whose disease progresses after platinum-based chemotherapy.

The relatively short response duration of cisplatin-based first-line chemotherapy, the large portion of cisplatin-ineligible patients (who are faced with no well-established noncisplatin treatment options), and the lack of standard second-line regimens make the development of new therapies in bladder cancer all the more urgent. Unfortunately, there is no approved targeted therapy, and no new agent has been approved by the US Food and Drug Administration (FDA) in more than 2 decades for either localized or advanced bladder cancer. Intravesical BCG was introduced in 1976, and MVAC and GC systemic therapy regimens have been used since the early 1990s. Median survival for patients with recurrent or metastatic bladder cancer remains at only 14 to 15 months with cisplatin-based chemotherapy.[14]

Funding in Bladder Cancer Research

Challenges to the development of novel bladder cancer therapeutic agents have included lack of public awareness and disproportionately low research funding.[15] Bladder cancer is one of the most underrepresented malignancies compared with other cancers,[16] with relatively few organizations focused on bladder cancer.[17] Research funding for bladder cancer has been declining in the Unites States at a rate disproportionate to the increasing cost of bladder cancer care.[18] Research funding for bladder cancer is the lowest in terms of dollars per incident case according to data from the National Institutes of Health, and national research funding for bladder cancer is also the lowest relative to societal health costs incurred.[15,19] In 2013, National Cancer Institute (NCI) funding for bladder cancer research was $20.3 million out of a $4.79 billion dollar total budget, which is significantly lower than the $255.6 million allocated for prostate cancer research and the $45.5 million allocated for kidney cancer research (Figure).[20]

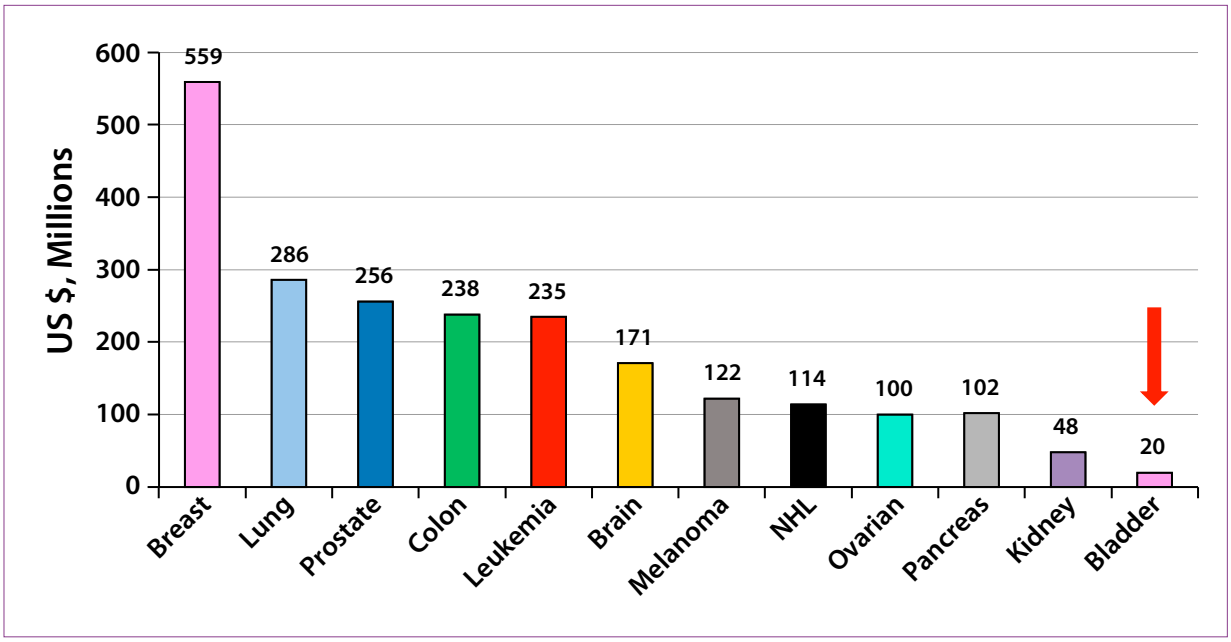

Figure. 2013 Annual National Cancer Institute funding according to cancer type.[20]

NHL, non-Hodgkin lymphoma.

In recent years, nonprofit organizations, the government, and philanthropic groups have employed novel approaches to promote research in bladder cancer. Since its establishment in 2005, the Bladder Cancer Advocacy Network has provided more than $1 million in bladder cancer research grants. Although only one NCI Specialized Program of Research Excellence award, totaling $13.9 million, has been awarded specifically for bladder cancer research, the award mechanism remains open for additional consideration of applicant institutions. Lastly, in a unique strategy, the Johns Hopkins Greenberg Bladder Cancer Institute was established in 2014 through a joint private and academic coinvestment with the intention of accelerating highly impactful bladder cancer research throughout the world. Although more support is needed, these additional avenues for funding encourage talented investigators to pursue bladder-focused research careers.

New Era in Bladder Cancer Research

A better understanding of the underlying molecular biology of the disease is essential for the development of new therapeutics. It has been well recognized that bladder cancer is a heterogeneous disease harboring more genetic and epigenetic alterations than any other human carcinomas except for lung cancer and melanoma.[21] Also, these molecular alterations undergo dynamic evolution at different disease stages and after exposure to different therapies, requiring repeated tissue analyses for an accurate, thorough understanding. Urothelial carcinoma of the bladder presents unique anatomical advantages in that direct tissue biopsy and urine cytology are relatively easily obtained, and tissue acquisition before and after intravesical and systemic chemotherapy is often the standard of care.

The Cancer Genome Atlas Project

With rapid advances in molecular techniques, especially next-generation sequencing technologies, which permit more rapid and extensive analyses, there has been an explosion of molecular data in recent years. A significant milestone of recent advances in molecular research of bladder cancer is The Cancer Genome Atlas (TCGA) project.[22] TCGA is a multi-institutional, comprehensive, coordinated effort to understand the molecular basis of cancer through various high-throughput genome analysis techniques. The bladder TCGA project started in 2010. An integrated analysis of data from 131 muscle-invasive urothelial carcinoma patient samples was recently published. TCGA bladder cancer data include data on DNA copy number, somatic mutations, messenger RNA (mRNA) and microRNA (miRNA) expression, protein and phosphorylated protein expression, DNA methylation, transcript splice variations, gene fusion, viral integration, pathway perturbation, clinical correlates, and histopathology. Various types of new-generation platforms and algorithms have been used, including next-generation HiSeq platform, DNAseq for whole-genome/exome sequencing and RNAseq for mRNA and miRNA expression, Infinium human methylation 450 (HM450) microarray for methylation analysis, reverse-phase

proteomic array for protein expression, SNP Array 6.0 microarray and BIC-seq for somatic copy number alteration, MutSig algorithm for mutation significance analysis, BreakDancer and Meerkat for translocations discovery, SpliceSeq for splice variation, VirusSeq for virus integration, and bootstrapped ensemble clustering algorithms.

The Bladder Cancer Analysis Working Group performed the integrated analysis of TCGA mRNA, miRNA, protein expression, and pathologic histology and identified 4 distinct bladder cancer subgroups (clusters). Cluster I is enriched in the papillary phenotype and *FGFR3* gene alterations. Cluster II is characterized by high *ERBB2* and *ESR2* expression and *GATA3* and *FOXA1* gene alterations resembling luminal A type breast cancer. Cluster III has squamous differentiation and expresses stem cell markers sharing characteristics with the basal-like breast cancer subtype. The most encouraging of the TCGA data is the identification of somatic mutations, copy number variations, or epigenetic alterations in genes with targets currently in drug development for 69% of patients.[22] Commonly altered pathways include the p53/Rb pathway, the receptor tyrosine kinase (RTK)/Ras/phosphatidylinositol 3-kinase (PI3K) pathway, switch/sucrose nonfermentable (SWI/SNF) pathway, and alterations of genes related to chromatin modification.

Circulating Tumor Cells and Circulating Free Tumor DNA

Complementary to analysis of patient tumor tissues, various techniques to detect bladder cancer circulating tumor cells (CTCs) and circulating free tumor DNA (cf-DNA) have been introduced. Detection of CTCs and cf-DNA offers the advantage of a so-called liquid biopsy approach rather than invasive core or fine-needle aspiration techniques. Utilizing the epithelial marker–, flow cytometry–based CellSearch platform, investigators have reported CTC detection rates of 44% to 58% in patients with metastatic bladder cancer.[23-25] In addition, recent CTC investigations have found an association between presence of CTCs and recurrence rates in high-risk NMIBC as well as evidence of CTCs as a potential surrogate marker of clinical staging.[26-28] The validation of CTCs as a prognostic factor for patient outcomes is ongoing in prospective clinical trials with metastatic bladder cancer patients.

Although promising, the majority of current CTC platforms present limitations, including (1) the requirement of specimen processing and analysis within 72 hours of collection; (2) the inability to perform whole-genome analysis of CTC DNA; and (3) the dependence on epithelial cell surface markers for CTC detection, thus eliminating detection of epithelial to mesenchymal transformed cells. Therefore, investigators have examined more readily available means to identify, quantify, and characterize tumor genomic content circulating within patients. The study of cf-DNA from peripheral blood and urine has demonstrated feasibility in early studies.[29] Specifically, high rates of cf-DNA detection have recently been reported across a wide range of metastatic malignancies including bladder cancer.[30] A particular advantage of cf-DNA platforms is the ability to analyze banked frozen patient samples, provided the original samples were collected in ethylenediaminetetraacetic acid or heparinized tubes.

Unique Bladder Cancer Tumor Models

Novel in vivo research models in bladder cancer have also been introduced in the past few years. Historically, several rodent-based bladder cancer models including orthotopic, genetically engineered, gene knock-in/knock-out, and carcinogen-induced models have been used. However, the data from these models may not accurately mimic human bladder cancer biology. Patient-derived xenograft (PDx) models present a novel platform to account for the tumor heterogeneity frequently encountered in human bladder cancer. In PDx models, primary tumors from individual patients are directly transferred into immunodeficient mice. This model maintains stromal and tumor biologic characteristics similar to the original cancers. Consequently, the PDx model may permit more accurate and personalized prediction of therapeutic efficacy.[31] Use of naturally occurring canine invasive bladder carcinoma as a novel model for translational efforts is another area of active research. Unlike experimentally carcinogen-induced tumors in murine models, the naturally occurring canine model allows for study of tumor invasion, metastases, and progression mechanisms while accounting for tumor heterogeneity in an immunocompetent, biologically relevant model. As such, it permits the identification of diagnostic markers and treatment responses that can be translated to humans with bladder cancer throughout all cancer stages.[32]

Novel Prognostic and Predictive Molecular Diagnostics

Several molecular prognostic and predictive markers have been studied in both local and advanced bladder cancer to predict response to therapy. In MIBC patients, Theodorescu and colleagues have developed the novel coexpression extrapolation (COXEN) methodology to predict response to neoadjuvant chemotherapy.[33] The COXEN approach profiles whole-genome gene expression from an individual's bladder tumor and compares it with the gene expression profiles of the NCI-60 human cancer cell lines to find the best match in tumor biology.[33] It then uses the 50% inhibitory concentration data on more than 45,000 compounds for the NCI-60 cell lines to rank optimal therapies for the individual

Table. Selected Ongoing Clinical Trials of Targeted Therapies

Target	Drug	Population	Regimen	Phase	Identifier
VEGFR	Sunitinib	High-risk NMIBC	BCG→sunitinib	2	NCT00794950
FGFR	Dovitinib	BCG-refractory NMIBC	Dovitinib	2	NCT01732107
VEGFR	Bevacizumab	First-line mUC	GC ± bevacizumab	3	NCT00942331
EGFR/ VEGFR	Vandetanib	First-line mUC	GCarbo ± vandetanib	2	NCT01191892
mTOR	Everolimus	First-line mUC	Everolimus ± taxol	2	NCT01215136
VEGFR	Pazopanib	Postplatinum mUC	Taxol + pazopanib	2	NCT01108055
PIK3CA	Buparlisib	Postplatinum mUC	Buparlisib	2	NCT01551030
EGFR/HER2	Afatinib	Postplatinum mUC	Afatinib	2	NCT02122172
c-Met	Cabozantinib	Postplatinum mUC	Cabozantinib	2	NCT01688999
mTOR	Temsirolimus	Postplatinum mUC	Temsirolimus	2	NCT01827943
HSP27	Apatorsen	Postplatinum mUC	Docetaxel ± apatorsen	2	NCT01780545

BCG, bacillus Calmette-Guérin; EGFR, epidermal growth factor receptor 1; FGFR, fibroblast growth factor receptor; GC, gemcitabine and cisplatin; GCarbo, gemcitabine and carboplatin; HER2, human epidermal growth factor receptor 2; HSP27, heat shock protein 27; mTOR, mammalian target of rapamycin; mUC, metastatic urothelial carcinoma; NMIBC, non–muscle-invasive bladder cancer; PIK3CA, phosphatidylinositol 3-kinase, catalytic subunit alpha; VEGFR, vascular endothelial growth factor receptor.

patient. The ability to test drug sensitivity a priori rather than retrospectively at the conclusion of large clinical trials represents a significant advantage of the COXEN approach. This strategy is being tested in the prospective SWOG 1314 (Southwest Oncology Group study 1314) clinical trial in patients with MIBC who are undergoing neoadjuvant cisplatin-based chemotherapy.

In addition to the COXEN approach, there has been a growing interest in identification of genomic alterations with predictive value using next-generation sequencing platforms. Using computational methods on whole-exome sequencing data of more than 300 common cancer-related genes obtained from pretreatment tumor and germline DNA, Van Allen and colleagues recently demonstrated a strong association between *ERCC2* gene mutations and cisplatin sensitivity in patients with MIBC treated with cisplatin-based neoadjuvant chemotherapy.[34] In addition, Plimack and colleagues have also reported genomic predictors of response to cisplatin-based chemotherapy using a similar, but different, next-generation sequencing assay of more than 300 cancer-related genes.[35] In their analysis, all patients with alteration in 1 or more of 3 genes, *ATM*, *RB1*, and/or *FANCC*, achieved a complete response (positive predictive value, 100%) to neoadjuvant dose-dense MVAC, whereas only 1 patient without a variant in any of these 3 genes achieved a complete response (negative predictive value, 96%).[35] If validated, these predictive biomarkers will be particularly useful in bladder cancer management given that bladder cancer is a disease of the elderly, with a median patient age of 73 years, and the majority of patients have multiple comorbid conditions.

Targeted Therapies Development

Based on an improved understanding of underlying bladder cancer molecular alterations, significant increases in clinical trial options for patients with bladder cancer have taken place in recent years. Multiple targeted agents are being evaluated in various stages in bladder cancer studies either as a monotherapy or in combination with cytotoxic chemotherapeutic agents. Relevant pathways under investigation as bladder cancer therapeutic targets include those mediated by vascular endothelial growth factor receptor, c-Met (hepatic growth factor receptor), epidermal growth factor receptor 1 (EGFR/ErbB1), human epidermal growth factor receptor 2 (HER2/ErbB2), PI3K, protein kinase B (PKB/Akt), mammalian target of rapamycin (mTOR/TOR1/TOR2), tuberous sclerosis 1 and 2, neurofibromatosis 1, fibroblast growth factor receptor, chromatin remodeling (CREBBP/EP300), and protein chaperones (heat shock protein 27). A summary depicting the large number of agents in various stages of clinical trial development is shown in the Table.

Immunotherapy

In addition to an increasing knowledge of bladder cancer molecular biology, the opportunities for significant breakthroughs in bladder cancer outcomes have been further enhanced by an improved understanding of cancer immunology with early, but extremely promising, clinical data of several immunotherapeutic agents in bladder cancer. Local intravesical immunotherapy with BCG has been a standard of care since its introduction in the 1970s

for high-grade NMIBC. Intravesical BCG elicits a nonspecific cytotoxic immune reaction against bladder cancer cells through both innate and adoptive immune mechanisms.[36,37] The established clinical benefits of intravesical BCG immunotherapy suggest the potential for benefit from systemic immune-based therapies in bladder cancer. The finding that tumor-infiltrating lymphocytes in patients with bladder cancer are associated with favorable clinical outcomes provides rationale for further development of immunotherapy for bladder cancer.[38,39]

Among various systemic immunotherapeutic approaches, immune checkpoint inhibition has provided the most encouraging early results. It is recognized that one of the important mechanisms of tumor immune system evasion is adaptive upregulation of negative regulatory molecules in immune cells, tumors, and the microenvironment. Blockade of the inhibitory receptors cytotoxic T-lymphocyte–associated antigen 4 (anti-CTLA-4) and programmed death 1 (PD-1) and its ligand, PD-L1, has been extensively studied in various cancer types. Ipilimumab (Yervoy, Bristol-Myers Squibb), a monoclonal antibody targeting CTLA-4, was approved by the FDA for treatment of melanoma and also has been shown to provide durable disease control in subsets of patients with renal cell carcinoma[40] and lung cancer.[41] A multicenter phase 2 trial of ipilimumab in combination with GC in treatment-naive bladder cancer patients and several phase 1 studies with bladder cancer expansion cohorts are ongoing.[42]

Powles and colleagues recently presented the most compelling evidence to date demonstrating the validity of the PD-1/PD-L1 pathway as a viable bladder cancer target. In a phase 1 expanded cohort study of 67 postplatinum therapy, metastatic urothelial carcinoma patients treated with MPDL3280A, a human anti-PD-L1 monoclonal antibody, 52% of patients whose tumors were PD-L1 positive by immunohistochemistry had objective responses, including 2 complete responses after 12 weeks of follow-up.[43] Furthermore, the agent demonstrated an excellent safety profile, with no grade 4 or grade 5 adverse events reported. Based on this finding, the FDA recently granted MPDL3280A a breakthrough therapy designation for expedited development. In addition to MPDL3280A, other PD-1/PD-L1 immune checkpoint inhibitors including pembrolizumab (Keytruda, Merck), nivolumab, and MEDI-4736 are all being studied in phase 1 clinical trials with bladder cancer patients enrolled.

Utilizing a different strategy, studies with multiple vaccine approaches to treat bladder cancer are underway. DN24-02 is an investigational HER2-targeted autologous cellular immunotherapy consisting of antigen-presenting cells cultured with BA7072, a recombinant HER2-derived antigen linked to granulocyte-macrophage colony-stimulating factor. DN24-02 is currently under investigation in a phase 2 adjuvant trial in patients with surgically resected HER2-positive urothelial cancer at high risk for recurrence.[44] Other experimental immunologic therapeutics in early stages of trials include antitumor vaccines targeting human chorionic gonadotropin beta and NY-ESO-1, the survivin peptide vaccine, personalized peptide vaccines, and infusion of autologous T-helper cells.[45-48]

Novel Bladder Cancer Clinical Trial Designs

With the rapid rate of new bladder cancer molecular discoveries, it seems unlikely that the traditional randomized trials with unselected treatment groups can successfully evaluate the bevy of new targeted therapeutic agents, especially those targeting molecular subsets that are relatively rare. With the promise for discovery of predictive biomarkers, innovative clinical trial designs incorporating individual molecular characteristics will be required. This need for critical change in trial design has recently been recognized and addressed by bladder cancer leaders, and novel clinical designs have been proposed.[49]

An initial solution for bladder cancer patients with advanced disease is a mutation-enriched, umbrella trial design. In this strategy, all patients undergo large-scale molecular profiling and are assigned to a specific targeted therapeutic based on the presence of "druggable" genomic aberrations. This design typically aims to test the efficacy of several experimental agents targeting different molecular aberrations in an accelerated fashion. This can be further accelerated by coordinating multiple trials through the established multicenter networks, facilitating molecularly eligible patient enrollment. Several pharmaceutical companies are planning umbrella trials in bladder cancer.

A more advanced approach uses a staged adaptive design, which combines an umbrella screening trial and subsequent parallel, targeted therapy trials. A small number of patients are randomly assigned to treatment arms based on biomarker profiles in an initial phase. Response rates are analyzed using a Bayesian model, with more patients adaptively assigned to each treatment arm according to efficacy results.[50] For each biomarker profile, better-performing arms will have higher randomization rates. Low-performing arms may be suspended for new patient entry based on early stopping rules for avoiding exposure to toxicities from agents that are unlikely to provide benefit. This concept has already been adopted in other cancer studies such as the BATTLE-1 (Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination) trial in lung cancer and the I-SPY 2 TRIAL (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis 2) in breast cancer.[51,52]

Of note, it is essential to obtain a tumor biopsy before treatment, and biopsies are also desirable at the time of

progression in future clinical trials of molecularly targeted agents. This will allow for validation of predictive biomarkers for candidate agents and analysis of mechanisms of resistance, which potentially can be used for identification of the next candidate therapeutic agent.[53]

Conclusion

Although few major changes in the management of patients with bladder cancer have occurred in decades, bladder cancer patients and investigators have real reason for optimism about the future. At no other time previously have we seen such a flurry of simultaneous scientific discoveries emerging from the laboratory bench, rapidly making their way into bladder cancer–specific clinical trial opportunities for our patients. In the absence of government-supported research funding, the bladder cancer community has, by necessity, had to shoulder the burden of pushing onward to identify new models to support and accelerate bladder cancer research. Although the model for bladder cancer may be somewhat nontraditional, in recent years it has been very successful. We now observe significant momentum in both the bladder cancer research laboratories and clinics. Taken together, sufficient evidence exists for us to remain optimistic that we will witness profound advances in bladder cancer treatments in the near future.

Disclosures

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