Prognostic and Therapeutic Implications of DNA Repair Gene Mutations in Advanced Prostate Cancer

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Abstract: Recent work directed toward understanding the molecular features of advanced prostate cancers has revealed a relatively high incidence of both germline and somatic alterations in genes involved in DNA damage repair (DDR). Many of these alterations likely play a critical role in the pathogenesis of more aggressive prostate cancers—leading to genomic instability and an increased probability of the development of lethal disease. However, because the ability to repair DNA damage with a high degree of fidelity is critical to an individual cell's survival, tumor cells harboring alterations in DDR pathway genes are also more susceptible to drugs that induce DNA damage or impair alternative DNA repair pathways. In addition, because the genomic instability that results from these alterations can lead to an inherently higher number of mutations than occur in cells with intact DDR pathways, patients with genomic instability may be more likely to respond to immune checkpoint inhibitors, presumably owing to a correspondingly high neoantigen burden. In this review, we discuss the emerging molecular taxonomy that is providing a framework for precision oncology initiatives aimed at developing targeted approaches for treating prostate cancer.

Introduction

Failure to repair DNA damage and replication errors accurately can lead to the accumulation of mutations and an increased risk for cancer. It is therefore not surprising that mutations in DNA repair genes have been associated with several cancer predisposition syndromes.[1-5] Studies across a variety of malignancies have also shown that when DNA damage repair (DDR) deficiency occurs—often as a result of homozygous loss-of-function mutations in *BRCA1/2*, *ATM*, and other genes involved in homologous recombination (HR)—intrinsic genomic instability is present, which can render cells vulnerable to agents that induce DNA damage or inhibit alternative DNA repair pathways. Poly(ADP-ribose) polymerase (PARP) has been shown to be a key mediator in this respect, and strategies to inhibit PARP activity have been shown to be effective in a number of cancers with impaired HR.[6-10] In addition, more

recent data have shown that targeting PARP activity may be an effective strategy to augment the antitumor effects of other DNA-damaging agents (eg, alkylating agents and platinum chemotherapeutic agents) in cancers with intact DDR pathways.[10,11] Tumors with homologous recombination deficiency (HRD) also appear to be exquisitely sensitive to DNA-damaging chemotherapeutic agents.[12,13]

In addition, because alterations in mismatch repair (MMR) pathway genes can lead to the accumulation of vastly more mutations than occur in tumors with an intact MMR pathway (ie, hypermutation), it has been hypothesized that such tumors will have a higher neoantigen burden, which renders them more susceptible to immune checkpoint inhibitors. A recent study testing this hypothesis has led to the first US Food and Drug Administration (FDA) tumor-agnostic approval for pembrolizumab (Keytruda, Merck) in patients with MMR gene mutations or microsatellite instability (MSI), a marker of genomic fragility.[14-16]

Alterations in the DDR pathway are present in upward of 20% of men with metastatic castration-resistant prostate cancer (mCRPC) and in up to 12% of men with metastatic prostate cancer harboring a germline alteration in one of these genes.[5,17] Given how prevalent these mutations are, it is not surprising that a number of precision oncology approaches are being developed to treat patients who have advanced prostate cancer with impaired DDR. This review outlines the clinically relevant DDR pathways as they pertain to prostate cancer and discusses efforts to develop drugs targeting these pathways.

DNA Damage Repair: Overview

A multitude of events occur daily that lead to DNA damage that requires subsequent repair. The ability to repair DNA damage with a high degree of fidelity is both critical to an individual cell's survival and necessary to prevent malignant transformation. As such, germline alterations in DDR genes can increase replicative DNA stress, the accumulation of mutations, and the risk for cancer.[18,19] Because of the critical role that DDR pathways play in maintaining cellular viability, a complex network of cellular pathways has evolved to deal with DNA damage by detecting and repairing it as it arises—herein referred to as DDR pathways.[20-23]

The DDR pathways are signal transduction pathways consisting of sensors, transducers, and effectors.[24,25] The ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and RAD3-related (ATR) proteins are key kinases involved in sensing and regulating the response to DNA damage and are intimately involved in several DDR pathways.[26] If DNA damage is detected, cell cycle arrest occurs, providing an opportunity either for damaged

DNA to be repaired via a number of DDR pathways or for apoptosis to occur if catastrophic genomic instability has occurred.[19,22,27] Some key proteins involved in regulating the cell cycle include the following: ATM (G1/S checkpoint), ATR (S-phase checkpoint), CHK1 (G2/M and S-phase checkpoints), CHK2 (G1/S checkpoint), DNA-PK (S-phase checkpoint), WEE1 (S-phase and G2/M checkpoints), and TP53 (G1/S checkpoint).[22]

Following the detection of DNA damage, overlapping downstream DDR pathways are activated to resolve double-strand DNA (dsDNA) damage or single-strand DNA (ssDNA) damage.[27] The key pathways involved in ssDNA repair are MMR, base excision repair (BER), and nucleotide excision repair (NER). The main pathways involved in dsDNA damage repair are HR and nonhomologous end joining (NHEJ).[28-38] A third pathway responsible for rescuing damaged dsDNA is called translesion DNA synthesis. Redundancies in these pathways ensure that even with loss-of-function mutations in one of these pathways, an individual cell may still be able to survive. Key proteins involved in these overlapping pathways are outlined in Table 1.[1,25,26]

Given the complexity of the DDR pathways, an exhaustive review of the topic is beyond the scope of this article. Instead, we focus on the pathways that appear most clinically relevant to the prognosis and treatment of prostate cancer.

Targeting Homologous Recombination Deficiency

Mutations in the genes involved in HR are frequently observed in men with metastatic prostate cancer.[5,17] Nearly 12% of unselected patients with metastatic prostate cancer have been found to have germline alterations in HR genes, and approximately 20% to 25% of patients with mCRPC harbor alterations in HR genes (somatic and/or germline), with *BRCA2*, *ATM*, and *BRCA1* the most commonly affected genes.[5,17] Studies examining the effect of germline *BRCA1/2* mutations on prostate cancer risk have reported that *BRCA2* confers an 8.6-fold increased risk for prostate cancer and that *BRCA1* confers a 3.4-fold increased risk.[39-43] *BRCA1/2* germline alterations have also been shown to be associated with a higher Gleason score, a higher T stage, nodal involvement, and metastases at diagnosis.[43] Rates of cause-specific overall survival and metastasis-free survival are also significantly lower for patients with localized prostate cancer and a germline alteration in *BRCA1* (hazard ratio, 2.6; *P*=.01) or *BRCA2* (hazard ratio, 2.7; *P*=.009).

The most genotoxic form of DNA damage is dsDNA damage because both strands of DNA are affected.[22,44] The 2 key pathways involved in resolving dsDNA damage

Table 1. Key DNA Damage Repair Pathway Sensors, Transducers, and Effectors

	dsDNA Repair Pathways			ssDNA Repair Pathways		
	HR	NHEJ	alt-NHEJ	BER	MMR	NER
Causes of damage	Radiation, topoisomerase I inhibitors, nucleoside analogues	Radiation, topoisomerase II inhibitors	Radiation, topoisomerase II inhibitors	Radiation, alkylating agents, oxidation, deamination	Replication/recombination errors, alkylating agents	Ultraviolet light, polycyclic aromatic hydrocarbons, platinum chemotherapy
Sensors	MRN complex	Ku70-Ku80	PARP	APE1, 9-1-1 complex, PARP, RPA complex	MLH1, MSH2, MSH3, MSH6, PMS2	XPC, DDB2, CSA
Transducers	ATM, ATR, BRCA1, BRCA2, PALB2	ATM, DNAPK				RPA complex
Effectors	BLM, FANCJ, PARI, POLQ, RAD51, RECQL5	LIG4, PAXX, XLF, XRCC4	LIG1, LIG3, POLQ, XRCC1	LIG1, LIG3A, POLB, XRCC	EXO1, LIG1, POLD	ERCC1, POLE, POLD1, LIG 1, LIG 3, XPG

alt-NHEJ, alternative NHEJ; BER, base excision repair; dsDNA, double-strand DNA; HR, homologous recombination; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; ssDNA, single-strand DNA.

are NHEJ and HR. It is important to note that although HR results in error-free repair of dsDNA damage and uses the undamaged sister chromatid as a template, NHEJ is an error-prone repair mechanism that can lead to a large number of chromatid breaks and aberrations, which can result in loss of cell viability.[44,45] As mentioned earlier, HR is the major pathway for high-fidelity DNA repair following an insult that results in dsDNA damage. Cancers in which the tumor cells have biallelic loss-of-function mutations in genes involved in HR are sensitive to agents that induce DNA damage.

PARP Inhibitors in Prostate Cancer

PARPs (especially PARP1, PARP2, and PARP3) are key enzymes involved in BER and are required to repair ssDNA damage efficiently. Without PARP1 function, single-strand gaps in DNA persist, and degeneration to double-strand breaks can occur if a replication fork encounters these genomic defects.[45-48] Under normal conditions, such dsDNA damage can be repaired via the HR pathway; however, in the case of HRD, replication forks collapse and chromatid breaks persist, leading a cell down a pathway toward apoptosis.[48-50] In addition, PARP1 is involved in repairing dsDNA breaks through the alternative NHEJ pathway and can therefore further impair the ability to repair dsDNA breaks in HR-deficient tumors.[51-53] Preclinical studies have supported this model, demonstrating that *BRCA1/2*-deficient cell lines

are sensitive to pharmacologic PARP1 inhibition.[45,48]

Proof of concept for this approach is derived from TOPARP (A Phase II Trial of Olaparib in Patients With Advanced Castration Resistant Prostate Cancer).[6] This was a phase 2 study testing olaparib (Lynparza, AstraZeneca) at an oral dose of 400 mg twice daily in men with mCRPC. The primary endpoint was the response rate, which was defined as the presence of any of the following: an objective radiographic response per the Response Evaluation Criteria in Solid Tumors (RECIST) criteria v1.1, a reduction in the prostate-specific antigen (PSA) level of at least 50% from baseline (ie, a PSA_{50} response), or a confirmed reduction in the number of circulating tumor cells (CTCs) from at least 5/7.5 mL of blood to fewer than 5/7.5 mL of blood. Of the 50 patients with mCRPC who were enrolled, all had received prior docetaxel, and 49 had received prior abiraterone acetate (Zytiga, Janssen Biotech) or enzalutamide (Xtandi, Astellas/Medivation). There were 16 patients (33%) who met the primary endpoint, achieving a response according to the composite definition. Most notably, responses to olaparib were enriched in the subset of patients with loss-of-function alterations (homozygous deletions, deleterious mutations, or both) in HR genes (eg, *BRCA1/2*, *ATM*, Fanconi anemia genes, *CHK2*); the observed response rate was 88% in this biomarker-positive cohort. Interestingly, genomic reversions of germline and/or somatic DNA repair mutations that restore the open reading frame (ORF) were

described as driving secondary resistance in this trial.[54] Several subsequent studies have since been launched to evaluate PARP inhibitors further in men with recurrent or advanced prostate cancer (Table 2).

DNA-Damaging Agents

The induction of DNA damage is one of the most common mechanisms by which chemotherapeutic agents exert their cytotoxic effects. Given the importance of HR in repairing dsDNA damage, it is intuitive that cells with impaired HR activity will be sensitive to any number of DNA-damaging agents. Indeed, preclinical models have shown that *BRCA1* and *BRCA2* are important mediators of platinum-induced DNA damage, and loss of function of these genes can enhance platinum sensitivity.[45,55] Consistent with this finding is the observation that ovarian cancers with mutations in *BRCA1* or *BRCA2* are more susceptible to platinum chemotherapy.[56]

Several older trials that did not include next-generation sequencing of tumor samples tested platinum-based chemotherapy regimens in men with advanced prostate cancer.[57-61] Because most of these studies tested combination regimens, it is difficult to estimate the contribution of the platinum agent to the observed response rate. Many studies have reported PSA_{50} response rates of 15% to 30%—approximating the incidence of HRD in patients with CRPC.[17] A phase 2 study reported by Ross and colleagues is particularly informative. In that trial, the authors reported that of 34 men with CRPC that had progressed during or within 45 days of completion of docetaxel-based chemotherapy, 18% had a decline in PSA of at least 50% following treatment with docetaxel (60 mg/m²) plus carboplatin (area under the curve [AUC], 4).[57] One can surmise that because this study enrolled only men with previously progression on docetaxel, the observed clinical effects were most likely the result of carboplatin activity.

Emerging data support HRD as a predictive biomarker for prostate cancer response to DNA-damaging agents. In a small case series, Cheng and colleagues reported on 3 heavily pretreated patients with mCRPC who had extreme responses to platinum-based chemotherapy; all of the men had deleterious alterations in HR genes.[12] Similarly, a recent retrospective analysis of patients with mCRPC who were receiving platinum-based chemotherapy revealed that PSA_{50} response rates were higher in men with known pathogenic germline *BRCA2* alterations. In this study, by Pomerantz and colleagues, 6 of 8 carriers (75%) of a pathogenic *BRCA2* variant had a PSA_{50} response following carboplatin plus docetaxel vs 23 of 133 men (17%) without a pathogenic *BRCA2* variant (*P*<.001).[62] On the basis of these data, a precision oncology trial testing docetaxel plus carboplatin

in patients with mCRPC who have HRD was recently launched (NCT02598895).

Combination PARP Inhibitors and DNA-Damaging Agents

Because DDR inhibitors impair a cell's ability to resolve DNA damage, combining a PARP inhibitor with a conventional cytotoxic therapy could in theory potentiate the effects of the cytotoxic therapy. Consistent with this idea, PARP inhibitors have been shown across multiple preclinical tumor models to potentiate the antitumor effects of DNA-damaging cytotoxic agents (eg, alkylating agents, platinum chemotherapy) as well as of radiation.[63-67] Importantly, many of these studies have shown that the observed antitumor effects are not restricted to cell lines with a biallelic loss of HR pathway genes.

On the basis of preclinical work demonstrating synergy between PARP inhibitors and temozolomide, a number of trials testing PARP inhibitors in combination with temozolomide have been launched.[22] A pilot study testing low-dose veliparib with temozolomide in patients with mCRPC after docetaxel was previously reported by Hussain and colleagues.[68] Of the 26 patients eligible for this study, 25 were evaluable for PSA_{30} response (the primary endpoint). Overall, 2 of 25 patients (8%) had a confirmed PSA_{30} response, and there were no objective radiographic responses in the 16 patients with RECIST-evaluable disease. The authors questioned whether the low dose of veliparib (40 mg twice daily) tested in this trial could have affected the overall efficacy of the combination. In addition, temozolomide is not particularly active in prostate cancer and may not have yielded sufficient DNA damage in this tumor type. Somatic tumor sequencing was unfortunately not performed in this study, and the underlying HRD status of the enrolled subjects is not known.

The more recent I-SPY 2 trial (Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer) tested veliparib in combination with carboplatin as a neoadjuvant therapy in patients with breast cancer.[8] This study was a multicenter, randomized, phase 2 "platform" trial testing the addition of multiple experimental regimens to a control "backbone" regimen. Patients with high-risk primary breast cancer planning to undergo surgery were eligible. The control arm received 12 weekly cycles of paclitaxel followed by 4 cycles, every 2 to 3 weeks, of doxorubicin/cyclophosphamide. One of the experimental arms received a combination of 50 mg of veliparib by mouth twice daily and carboplatin (AUC, 6) concurrently with the weekly paclitaxel. The primary endpoint was the pathologic complete response (pCR) rate as assessed at the time of surgery. Among the patients with triple-negative breast cancer (ie, negative for human epidermal growth factor 2 [HER2], estrogen receptor [ER],

Table 2. Selected Ongoing Clinical Trials Testing PARP Inhibitors in Men With Prostate Cancer

Agents Being Tested	Trial Phase	Disease State	Key Eligibility Criteria	Sample Size	Primary Endpoint	Identifier
Olaparib +/- degarelix (Firmagon, Ferring Pharmaceuticals)	Phase 1	Localized	Intermediate- to high-risk disease Planning to undergo prostatectomy	20	Determination of PARP inhibition	NCT02324998
Olaparib +/- cediranib	Phase 2	mCRPC	Two or more prior lines of therapy for mCRPC	84	Radiographic PFS	NCT02893917
Rucaparib (Rubraca, Clovis Oncology)	Phase 2	mCRPC	HRD After taxane and 1-2 next-generation AR signaling inhibitors	160	Objective response rate PSA response rate	NCT02952534
Rucaparib vs abiraterone, enzalutamide, or docetaxel	Phase 3	mCRPC	HRD After next-generation AR signaling inhibitor	400	Radiographic PFS	NCT02975934
Niraparib (Zejula, Tesaro)	Phase 2	mCRPC	Progression on ≥1 taxane-based chemotherapy regimen and ≥1 AR signaling inhibitor	160	Objective response rate	NCT02854436
Niraparib + enzalutamide	Phase 1	mCRPC	—	—	MTD	NCT02500901
Olaparib	Phase 2	Biochemical recurrence	After prostatectomy Nonmetastatic disease	50	PSA response rate	NCT03047135
Olaparib + abiraterone	Phase 2	mCRPC	After docetaxel	159	Safety Radiographic PFS	NCT01972217
Abiraterone vs olaparib vs olaparib + abiraterone	Phase 2	mCRPC	HRD Before docetaxel	70	PFS	NCT03012321
Olaparib vs enzalutamide or abiraterone	Phase 3	mCRPC	HRD After abiraterone and/or enzalutamide	340	Radiographic PFS	NCT02987543
Olaparib + pembrolizumab*	Phase 1	mCRPC	After docetaxel	210	PSA response rate Safety	NCT02861573
Niraparib + radium-223	Phase 1	mCRPC	—	6	MTD	NCT03076203
Niraparib + apalutamide or abiraterone	Phase 1	mCRPC	After docetaxel	60	MTD Safety	NCT02924766

AR, androgen receptor; HRD, homologous recombination deficiency; mCRPC, metastatic castration-resistant prostate cancer; MTD, maximum tolerated dose; PARP, poly(ADP-ribose) polymerase; PFS, progression-free survival; PSA, prostate-specific antigen.

* This is a multiple-arm study testing pembrolizumab in combination with several prostate cancer therapies, including olaparib.

and progesterone receptor [PR]), the estimated pCR rates were 51% (95% Bayesian probability interval [PI], 36%-66%) in the veliparib/carboplatin arm and 26% (95% PI, 9%-43%) in the control group. It is notable that this study was not restricted to patients with DDR deficiency, although the percentage of patients in the veliparib/carboplatin arm with deleterious mutations in *BRCA1* or *BRCA2* (12/72, 17%) was higher than the percentage in the control arm (2/44, 5%). Given that platinum-based chemotherapy has shown promise in mCRPC, it would be reasonable to test platinum/PARP inhibitor combination strategies in men with advanced prostate cancer.

Although mounting evidence suggests synergistic efficacy when PARP inhibitors are combined with DNA-damaging agents, this likely comes at the expense of increased toxicity. For instance, in the aforementioned I-SPY 2 trial, grade 3 or higher neutropenia occurred in 71% of patients receiving paclitaxel in combination with veliparib and carboplatin compared with 2% in patients receiving only paclitaxel.[8] Although some of the increased bone marrow toxicity observed in the experimental arm of I-SPY 2 was likely due to the addition of carboplatin, the stark difference in the rates of neutropenia cannot be completely explained solely by the addition of carboplatin, and it seems probable that veliparib compounded this risk. Similarly, increased toxicity was observed in a randomized phase 2 study, reported by Oza and colleagues, comparing olaparib, paclitaxel, and carboplatin followed by maintenance olaparib vs paclitaxel and carboplatin alone in women with recurrent platinum-sensitive ovarian cancer.[69] This study reported grade 3 or higher neutropenia in 43% of patients receiving PARP inhibitor combination therapy and in 35% of patients receiving chemotherapy only. Larger studies are needed to better define the clinical benefit, as well as overlapping toxicity, of PARP inhibitor/chemotherapy combinations.

Homologous Recombination Deficiency and Inhibition of Androgen Receptor Signaling

Hussain and colleagues recently reported on the activity of abiraterone, a cytochrome P_{450} (CYP) 17 inhibitor able to decrease the production of androgens in extragonadal (eg, intratumoral and adrenal) sources with or without veliparib.[70] Their rationale for combining an inhibitor of androgen receptor (AR) signaling with a PARP inhibitor was based on preclinical data demonstrating that PARP is involved in the AR transcriptional machinery, and that inhibiting PARP can downregulate AR activity.[71] Randomization to this study was stratified by expression of the ETS protein as determined by immunohistochemistry (IHC) on the basis of the hypothesis that the presence of AR-regulated *ETS* oncogene fusions would predict a response to PARP inhibition. The primary endpoint was

the PSA_{50} response rate (ie, the proportion of patients with decreases in PSA of ≥50% from baseline). This trial accrued 148 subjects, with 72 randomly assigned to abiraterone alone and 76 to the combination arm. The study ultimately failed to meet its primary endpoint, with similar PSA_{50} response rates in the 2 arms (63.9% with abiraterone vs 72.4% with the combination; *P*=.27), and ETS IHC status did not predict response to therapy. A secondary analysis involved next-generation sequencing of tumor samples (N=80) to evaluate for other genomic biomarkers that might predict response. This analysis revealed that 20 patients (25%) had alterations in HR genes (ie, *BRCA1, BRCA2, ATM, FANCA, PALB2, RAD51B,* and *RAD51C*), and interestingly, a post hoc analysis revealed that alterations in these genes predicted improved response rates irrespective of the treatment arm (PSA_{50} response rates, 58% vs 39%; *P*=.013).

A contemporary phase 2 study reported by Chi and colleagues tested abiraterone vs the next-generation AR antagonist enzalutamide in patients with newly diagnosed mCRPC, with crossover following PSA progression.[72] The coprimary endpoints were response and time to PSA progression following crossover. The study accrued 202 patients and randomized them equally between the groups. The PSA_{50} response rates at 12 weeks were 53% for abiraterone and 73% for enzalutamide (*P*=.004). Circulating cell-free tumor DNA (ctDNA) was sequenced as part of this study, and in contrast to the results reported by Hussain and colleagues, the presence of deleterious *BRCA2* or *ATM* mutations (n=14) did not predict improved outcomes. Chi and colleagues instead found an association between HRD and shorter time to progression (hazard ratio, 5.34; *P*<.001).

We now have 2 studies with conflicting results regarding the use of HRD to predict response to AR-signaling inhibitors. To a certain extent, the study of Chi and colleagues confirms our biases derived from natural history studies that have revealed more aggressive biology in patients with DDR alterations.[43] Caution should be exercised, however, in relying too heavily on these results. Both analyses used exploratory secondary endpoints, with relatively small subsets of patients who had HRD in each trial. The assays used in these studies were also different; Hussain and colleagues relied on tissue sequencing, whereas Chi and colleagues used newer methods to sequence selected target genes from ctDNA samples. Finally, the definitions of a DNA repair lesion in the 2 studies may have been different, in terms of both the spectrum of genes included in the biomarker panel and the designation of pathogenicity (monoallelic vs biallelic).[73] Confirmatory studies to assess the efficacy of HRD as a predictive biomarker of response/resistance to AR-signaling inhibition are therefore needed.

Targeting Mismatch Repair Deficiency and Somatic Hypermutation

The MMR pathway is responsible for correcting base-base mismatch and insertion-deletion loops, which occur during DNA replication and recombination. In tumors with MMR deficiency, long tracks of repetitive DNA sequences, known as microsatellites, are prone to strand slippage, which can result in persistent insertion-deletion loops and the rapid accumulation of mutations.[18] As such, MMR-deficient tumors have been described as exhibiting a "mutator" phenotype, which is characterized by MSI (defined as differences in microsatellite tracks between normal germline DNA and somatic tumor DNA) and somatic hypermutation (≥10 mutations per megabase of coding DNA).[74]

Lynch syndrome is a cancer predisposition syndrome characterized by germline loss of function of MMR genes and is a well-established risk factor for colorectal, endometrial, ovarian, and upper tract urothelial cancer in addition to other malignancies, including prostate cancer.[18,75] This syndrome has most commonly been associated with alterations in genes involved in the MMR pathway, including *MLH1, MSH2, MSH6,* and *PMS2*, which occur in 32%, 39%, 15%, and 14% of cases of colorectal Lynch syndrome, respectively.[76] Clinically, this syndrome can be defined with the Amsterdam criteria, in which a germline alteration in an MMR pathway gene is assumed if a family meets the following criteria: (1) 3 or more family members with a Lynch syndrome–associated cancer; (2) 2 or more successive generations affected; and (3) 1 or more family members with cancer developing before the age of 50 years.[77,78] The pathogenic role of MMR gene alterations in prostate cancer risk is not well defined, however. Pritchard and colleagues found deleterious germline MMR gene alterations in 4 of their cohort of 692 men (0.6%) with metastatic prostate cancer.[5] Estimates of MMR mutations in metastatic prostate cancer (combined somatic and germline) are likely higher, however, with series reporting mutations in anywhere from 3% to 12% of cases. Rates of MMR deficiency may be higher in more aggressive histologic subtypes.[17,79,80]

Defining the true incidence of MMR-deficient prostate cancer has been further challenged by the limitations of the assays commonly used to determine MSI status. Most MSI assays involve multiplex polymerase chain reaction (PCR) testing on a handful of genomic loci (the National Institutes of Health panel includes 5 microsatellite loci) and rely on comparisons of microsatellite loci amplified from tumors and matched normal controls.[3,75,81] The loci tested in these assays and the threshold for declaring MSI have, for the most part, been validated and optimized to detect MSI only in colorectal cancer.

Because the performance of these PCR-based MSI assays for prostate cancer is unknown, clinicians should not rely too heavily on their results. Less-biased approaches for determining MSI status from next-generation sequencing data are available, and these tests may be more appropriate for noncolorectal histologies.[82]

The determination of whether an MMR gene is altered in a prostate cancer is also challenged by the fact that hypermutated prostate cancers often occur as a consequence of complex structural genomic rearrangements in MMR genes.[79,80,83] This contrasts with the inactivating mutations, loss of heterozygosity, and epigenetic silencing typical of colorectal cancers in patients with Lynch syndrome. Next-generation sequencing assays that sequence only the exons of target genes (which are the most common type of DNA-sequencing assays in clinical use) will therefore miss MMR gene alterations that arise as a result of rearrangements involving intronic regions. Assays that provide complete target gene coverage are more appropriate in this instance because they can accurately identify complex genomic rearrangements that may lead to MMR-deficient prostate cancer.[79] However, such assays are not in wide clinical use. A simpler screening approach could be to use standard IHC for MMR protein loss. For example, a recent paper used a validated IHC assay to screen 1176 primary prostate cancers for loss of MSH2, the most commonly inactivated MMR protein in prostate cancer. Although MSH2 deficiency was rare in the entire cohort (1%), MSH2 loss was enriched in patients with primary Gleason pattern 5 cancers (8%) and small cell prostate cancers (5%).[83] If these data can be replicated, screening for MSH2 inactivation in patients with primary Gleason 5 cancers and small cell prostate cancers might facilitate the identification of patients with MMR deficiency.

Because the loss of MMR gene function is often associated with a high mutational load, it has been hypothesized that individuals with this loss will have a higher tumor neoantigen burden, possibly predisposing them to respond to immune checkpoint inhibitors.[14,84-86] Proof of concept that MMR-deficient tumors may respond well to checkpoint inhibition comes from a phase 2 study that tested the anti–programmed death 1 (anti–PD-1) agent pembrolizumab in patients who had metastatic carcinomas with and without MMR deficiency (ie, MSI-high and MSI-low carcinomas, respectively).[14] In this study, 40% of the patients with MSI-high colorectal cancer had an immune-related objective response (irOR), compared with 0% of the patients with MSI-low colorectal cancer. Similarly, pembrolizumab was associated with a 50% response rate in patients with hypermutated noncolorectal gastrointestinal malignancies—supporting the hypothesis that mutational load may predict response to immune

checkpoint blockade in a range of malignancies. This study paved the way for the recent FDA approval of pembrolizumab in the treatment of patients with unresectable or metastatic MSI-high or MMR-deficient solid tumors that have progressed following prior treatment and who have no satisfactory alternative options. Of note, the approval of pembrolizumab for this indication is the FDA's first tissue-agnostic approval for a cancer therapy, which includes therapy for MMR-deficient advanced prostate cancer.[15]

Overall, immune checkpoint inhibitors have demonstrated only modest activity in unselected advanced prostate cancer, which may be a consequence of the relatively low mutational load observed in cohorts with unselected prostate cancer.[87] To date, the results of 2 phase 3 studies testing the anti–cytotoxic T-lymphocyte–associated antigen 4 (anti–CTLA-4) agent ipilimumab (Yervoy, Bristol-Meyers Squibb) in mCRPC have been negative.[88,89] Similarly, rates of response to anti–PD-1 therapy in patients with unselected prostate cancer have been low, with no responses identified in the phase 1 study of nivolumab (Opdivo, Bristol-Meyers Squibb) and an objective response to single-agent pembrolizumab in only 13% of patients.[90,91] It is worth noting, however, that a small trial testing combination enzalutamide plus pembrolizumab documented dramatic PSA declines in 3 of 10 patients.[92] In that study, 2 responders had adequate tumor material for sequencing, and one of them was found to have underlying MSI—providing a partial explanation for the high response rate observed in that study. Cases of other patients with MSI-high prostate cancer responding to PD-1 pathway inhibitors have also been reported, and studies designed to determine the rate of response to immune checkpoint inhibitors in MSI-high mCRPC are planned (Durvalumab in Treating Patients With Metastatic Hormone-Resistant Prostate Cancer; NCT02966587).[80] In another recent study, 2 of 8 patients who had mCRPC and measurable disease achieved an objective response to a combination of ipilimumab and nivolumab; neither of the 2 responding patients had MSI or hypermutation.[93]

Given that PARP inhibitors may be able to induce genomic instability, leading to neoepitope formation and enhanced sensitivity to checkpoint blockade, trials testing PARP inhibitors combined with PD-1 pathway inhibitors in advanced prostate cancer have also been launched. In an ongoing study testing the anti–programmed death ligand 1 (anti–PD-L1) agent durvalumab (Imfinzi, AstraZeneca) in combination with olaparib, 7 of 16 patients enrolled for longer than 2 months have had documented PSA$_{50}$ responses.[94] It should be noted that although most of the patients with a PSA$_{50}$ response had evidence of HRD, some patients with an intact HR pathway responded favorably to combination therapy. Therefore, the presence of an HRD mutation or an MMR mutation may be neither necessary nor sufficient for a response to immune checkpoint inhibitors in prostate cancer.

Conclusion

During the past few years, our understanding of the recurrent molecular alterations defining advanced prostate cancer has increased dramatically. Somewhat unexpectedly, we have learned that a significant subset of men with this disease harbor alterations in DDR pathway genes, and precision oncology strategies designed to exploit these cellular vulnerabilities are being pursued actively, including in multiple large-scale efforts aimed at developing PARP inhibitors for patients who have prostate cancer with HRD. Several retrospective reports have also shown that platinum-based chemotherapy can be highly effective in patients with HRD, which is encouraging given that these drugs are readily available.[12,62] In a similar vein, pembrolizumab has recently been approved for MSI-high or MMR-deficient advanced solid tumors, including prostate cancers, in patients who lack a reasonable alternative therapy. With this rapidly evolving treatment landscape, it is becoming increasingly important to define the genomic features of each patient's tumor so that all potentially beneficial therapies can be explored. However, as we strive toward a precision oncology framework for treating prostate cancer, critical issues surrounding the acquisition of tumor material for next-generation sequencing and the development of assays able that can accurately identify relevant somatic alterations are becoming apparent.

Currently, metastatic biopsy is the gold standard for obtaining tumor DNA for sequencing. Germline DNA assessments are insufficient because they do not capture all the relevant DDR pathway alterations used to guide therapeutic decision making. In addition, selective pressure during treatment can lead to clonal evolution, so that freshly obtained tumor DNA is preferred because it provides a snapshot of the current spectrum of mutations. Obtaining fresh tumor material is not a trivial matter, however. Prostate cancer is an osteotropic disease, and extracting DNA from osseous metastases for next-generation sequencing can be challenging.[95,96] Metastatic biopsies are also painful, potentially morbid, and expensive. Fortunately, sequencing ctDNA is quickly becoming a viable alternative.[97] These so-called liquid tumor biopsies have the advantage of allowing genomic material to be sampled easily and repeatedly as needed.

Several commercial ctDNA sequencing assays are currently available; however, caution should be exercised before blood-based assays not optimized for use on prostate cancer samples are undertaken. For example, most com-

mercially available assays are not designed to identify accurately genomic copy number changes, which are some of the most frequent alterations found in mCRPC tumors.[17] A number of groups are actively developing strategies to detect copy changes in ctDNA, and these approaches may provide a more accurate means for detecting the spectrum of mutational events that can lead to DDR pathway inactivation.[98-100] Until these technologies are widely disseminated, however, metastatic biopsy should still be considered the standard for evaluating DDR pathway alterations.

Recurrent genomic rearrangements are another hallmark of prostate cancer, and many commercial sequencing assays—based on both ctDNA and tumor tissue—do not provide sufficient gene coverage to identify such changes accurately.[101] This problem has specific relevance to MMR pathway genes because complex genomic rearrangements involving these genes have been described as a frequent cause of hypermutation in prostate cancers.[79] In addition, most PCR-based MSI assays rely on the testing of a limited number of microsatellite loci, which have been selected on the basis of data from colorectal cancer cohorts. Less-biased MSI assays that cover a larger number of microsatellite loci are currently available, however, and may be more appropriate for testing prostate cancers.[102] With the recent approval of pembrolizumab for treating MSI-high and MMR-deficient tumors, it is increasingly important to choose tests that can accurately identify these alterations across a spectrum of tumor types.

Mutations affecting DDR pathway genes are both a liability—increasing the likelihood of cancer development—and potentially a therapeutic opportunity. Bridges first described the concept of synthetic lethality in the 1920s after observing that 2 mutations were necessary to induce lethality in a fruit fly, whereas either mutation in isolation had no effect on the insect's health.[103] Only recently have we applied these principles to treating prostate cancer, developing precision oncology strategies to select patients whose tumors have lost critical DDR pathway functionality. These tailored approaches for treating patients with advanced prostate cancer have tremendous potential and should provide hope that a wave of highly effective therapies are around the corner.

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