A New Molecular Taxonomy to Predict Immune Checkpoint Inhibitor Sensitivity in Prostate Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Immune checkpoint blockade therapy, especially using agents targeting the programmed death 1 (PD-1) and programmed death-ligand 1 (PD-L1) proteins, represents one of the greatest accomplishments of modern clinical oncology [1]. However, despite the fortunate fact that multiple PD-1 and PD-L1 inhibitors have been approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency to treat various types of solid tumors and hematological malignancies, none of these immune checkpoint agents have yet been approved for use in advanced prostate cancer [2]. This is largely because of the relatively modest response rates of approximately 10% (range, 5%–15%) when using PD-1 inhibitors in unselected patients with metastatic castration-resistant prostate cancer (mCRPC) [3–5] and the lack of readily available biomarkers of immunotherapy benefit. Moreover, sensitivity to PD-1 inhibitors in this disease does not appear to be related to expression of the PD-L1 ligand on tumor cells or immune cells [5, 6], despite the fact that PD-L1 expression is observed in at least one third of mCRPC tumor biopsies with further enrichment of PD-L1 expression in neuroendocrine and small cell prostate cancers [7].

A very significant advance in our understanding of PD-1 inhibitor sensitivity came with the recent discovery that cancers deficient in DNA mismatch-repair function (dMMR) or with microsatellite instability (MSI-high) demonstrate high rates of objective tumor responses with immune checkpoint therapies, partially owing to the much greater mutational load and neoantigen burden in these cancers [8], a finding that led to the FDA approval of pembrolizumab for the treatment of advanced dMMR/MSI-high cancers of any histologic type and independent of PD-L1 status. In this issue of *The Oncologist*, Manogue et al. [9] describe a patient with dMMR castration-resistant prostate cancer who achieved a dramatic response to single-agent pembrolizumab treatment. This patient was found to harbor a germline pathogenic rearrangement in the *MSH2* gene (the so-called Boland inversion [10], involving exons 1–7 of the *MSH2* locus), meaning that the patient had a form of Lynch syndrome. When treated with PD-1 blockade, despite having previously progressed on therapy with abiraterone and docetaxel, this patient

achieved a complete biochemical response (prostate-specific antigen [PSA] <0.01 ng/mL) to pembrolizumab, as well as a complete radiographic response in abdominal and pelvic lymph nodes. Further, using an investigational circulating tumor DNA (ctDNA) assay, the mutant allelic fraction (representing prostate cancer somatic alteration burden) also declined to the undetectable range following immunotherapy treatment. At the time of publication, the patient had an ongoing response lasting 12 months thus far, without evidence of PSA or radiographic progression of his disease.

Before embarking on discussing the specifics of this case, let us first consider the prevalence of MMR deficiency in prostate cancer. How common are germline and somatic mutations in the canonical MMR genes (*MSH2, MSH6, MLH1, PMS2*) in patients with mCRPC? Although exact estimates are difficult to discern, the overall prevalence of MMR gene mutations in the germline DNA of patients with advanced prostate cancer is around 1% (range, 0.5%–1.5%), mostly involving the *MSH2* and *MSH6* loci [11–14]. Conversely, the prevalence of somatic MMR gene mutations or MSI-high status in metastatic prostate cancers is in the order of 5% (range, 3%–8%), and all four genes can be affected [14–16]. In one study, the occurrence of somatic MMR gene mutations was reported at 12% [17], although this estimate was derived from an autopsy series that was enriched for lethal prostate cancer, and may not be representative of the overall mCRPC population. What is clear, however, is that the majority of MMR mutations in prostate cancer (at least 75% of them) occur at the somatic level and are not inherited. Therefore, both germline and somatic genetic testing are recommended to interrogate for MMR deficiency in men with prostate cancer. Further suspicion of MMR deficiency may be prompted by high-grade Gleason scores (especially primary pattern 5) [18] and variant histologies (including ductal and intraductal carcinoma, as well as small cell prostate cancer) [14, 18, 19].

Let us now delve a little deeper into the molecular characteristics of this patient's tumor, which highlights many of the challenges and pitfalls of interpreting and understanding MMR deficiency in prostate cancer. First, although this patient had a known germline structural rearrangement in

the *MSH2* gene, his somatic tumor DNA testing from his prostate cancer biopsy did not reveal an apparent *MSH2* mutation, despite demonstrating microsatellite instability and a large number of somatic mutations (many of which were frameshift alterations occurring in genes with known microsatellite repeat sequences). Immunohistochemical (IHC) staining helped to confirm the diagnosis of MMR deficiency by revealing absent expression of both the MSH2 and MSH6 proteins, implying an underlying genomic lesion in *MSH2* (conversely, loss of MSH6 protein with intact MSH2 staining would imply an underlying genomic lesion in *MSH6*). Second, although the microsatellite status was classified as MSI-high, this was based on a shift in only two of the five NIH-defined microsatellite markers by polymerase chain reaction (PCR) analysis (i.e., three of the classic microsatellite markers were stable), potentially confusing the interpretation of MSI status. Third, as observed here, most clinical-grade somatic genomic assays do not report loss of heterozygosity (LOH) of tumor suppressor genes including MMR genes, so biallelic inactivation cannot be determined. This is important because only biallelic MMR gene inactivation would be expected to lead to functional mismatch repair deficiency. In the current case, LOH of the wild-type *MSH2* allele was only suggested by the investigational research-grade ctDNA assay, and was missed or not reported on the biopsy-based genomic assay. Finally, even if true MMR deficiency is confirmed at the DNA and protein level, and even if this results in microsatellite instability and hypermutation, only a proportion of such patients with prostate cancer will respond favorably (or durably) to PD-1 inhibition [14, 20, 21]. Each of these pitfalls will be dissected in more detail below.

The first challenge exemplified by this case was the apparent lack of an MMR gene mutation on somatic DNA sequencing from this patient's tumor biopsy. Although the cancer appeared to be MSI-high and hypermutated, no mutation was reported in any of the canonical MMR genes. Given the known Boland inversion in *MSH2* detected on germline DNA sequencing, this was a false-negative result that occurred because standard exon-only sequencing will miss the vast majority of genomic structural rearrangements, especially those occurring within intronic (i.e., noncoding) DNA regions. A recent study suggested that complex rearrangements of the *MSH2* and *MSH6* genes may account for a significant fraction of somatic MMR mutations [17], meaning that we might perhaps be underestimating the true prevalence of dMMR prostate cancers. Whole-genome sequencing (covering both coding and noncoding regions) may solve this problem in the future, but is not readily available in current clinical practice. An additional caveat observed here was that this patient did have a pathogenic protein-truncating mutation in the *MLH3* gene, but this is probably a passenger rather than a driver mutation that was caused by the primary MSH2-deficient state. Importantly, inactivating mutations in the noncanonical MMR genes (such as *MLH3*), unless accompanied by a true MMR gene mutation, do not predict sensitivity to immunotherapy agents.

A second pitfall is that most clinical-grade genomic assays do not report biallelic mutations (e.g., hemizygous deletion or LOH) in tumor suppressor genes including MMR genes. This limitation may also affect other tumor suppressor genes, such as *BRCA2* inactivation, in the context of potential poly ADP-ribose polymerase (PARP) inhibitor use. In the case of the

MMR genes, biallelic genomic inactivation can almost always be inferred by performing IHC analyses for the four MMR proteins, which has become a standard practice in many pathology laboratories. To this end, loss of both MSH2 and MSH6 implies underlying genomic *MSH2* inactivation, whereas MSH6 loss alone implies underlying *MSH6* mutation. Similarly, loss of both MLH1 and PMS2 implies genomic *MLH1* inactivation, whereas PMS2 loss alone implies underlying *PMS2* mutation.

A third caveat has to do with the definition of MSI status and the method used to determine this. It must be remembered that the classic five-marker NIH panel of mononucleotide repeat sequences (BAT-25, BAT-26, NR-21, NR-24, MONO-27) assessed by PCR was validated primarily in dMMR colorectal cancer and not prostate cancer. It is possible, for example, that these five microsatellite sequences might be less relevant in dMMR prostate cancers than in dMMR colorectal cancers. In a recent case series of 13 MMR-mutated advanced prostate cancers, 27% of these patients had no MSI marker shifted, 36% had one to two markers shifted, 36% had three to four markers shifted, and none had all five markers shifted [14]. A more appropriate way to determine MSI status in prostate cancer might be to use an expanded panel of prostate cancer-relevant microsatellites interrogated by next-generation sequencing, which has been shown to increase the sensitivity for detecting MSI-high status in prostate cancer without compromising specificity [22]. To this end, in a series of 29 known dMMR prostate cancers, the five-marker PCR assay had a sensitivity of 72% whereas a 60-marker next-generation sequencing method had a sensitivity of 93% (detecting six cases of MSI that were missed by the conventional PCR method) [22].

A fourth complication is that even in prostate cancers with confirmed genomic and proteomic MMR deficiency as well as MSI-high status, hypermutation does not always occur, and even when it does the tumor mutational burden is usually lower than in other dMMR cancers. In that same prior study of 13 dMMR prostate cancers [14], only 63% of patients with MSI-high status using the five-marker PCR assay had tumor mutational loads of ≥20 mutations/Mb and the median mutational burden was only 18 mutations/Mb (range, 3–165 mutations/Mb). This implies that dMMR prostate cancers may generate fewer mutation-associated neoantigens than other dMMR cancers such as colorectal or endometrial cancers. In another study of 14 MSH2-deficient prostate cancers [18], only 61% demonstrated microsatellite instability whereas 83% showed hypermutation with a median tumor mutational burden of 26 mutations/Mb (range, 3–104 mutations/Mb). Interestingly, in this second study, only 54% of the MSH2-deficient cases demonstrated a high CD8+ tumor-infiltrating lymphocyte density (>200 CD8+ T cells/mm^2) in primary prostate tumors [18], suggesting either that dMMR/MSI-high status is not sufficient to induce a CD8+ T-cell response or that other immunosuppressive factors are preventing T-cell infiltration.

Finally, a sobering reality is that only a proportion of prostate cancer patients with MMR-deficiency and hypermutation will respond favorably to PD-1 inhibitor therapy. In the largest case series to date of 10 patients with dMMR mCRPC [20], only half achieved a >50% PSA response to PD-1/PD-L1 inhibitor treatment and only 2 men had objective tumor responses. In a second study of six patients with dMMR prostate cancer [21], the PSA response rate to anti-PD-1 therapy

was only 17% and the median progression-free survival was 7.8 months. In a third study of four dMMR prostate cancer cases [14], half of patients achieved a PSA response (both also had an objective tumor response), and the median progression-free survival was 9.0 months. These relatively modest PSA and objective response rates to PD-1/PD-L1 blockade therapy in dMMR prostate cancers is somewhat disappointing, although there are clearly patients (such as the one presented in the accompanying article) [9] who do much better than this. However, combination immunotherapies (e.g., immune checkpoint blockade plus vaccination) or other strategies are clearly needed to augment the response rates and durability of responses further in these patients.

This leads to the conclusion that the field is in need of a new molecular taxonomy to more accurately define immunotherapy response in prostate cancer. To this end, in addition to MMR deficiency, there may be other genomic markers of immune checkpoint inhibitor sensitivity. Some of these may include inactivating mutations in the *CDK12* gene that lead to increased gene fusion-associated neoantigens [23, 24], exonuclease domain mutations in the DNA polymerase genes *POLE* and *POLD1* that lead to ultramutation without underlying microsatellite instability [25, 26], deletion of the 3' untranslated region of the *CD274*

(PD-L1) locus resulting in overexpression and stabilization of PD-L1 transcripts [27], and perhaps inactivation of homologous recombination DNA repair genes (e.g., *BRCA2*, *ATM*) [5, 6]. Further refinement of this genomic taxonomy of advanced prostate cancer, and a better understanding of the suppressive mechanisms inhibiting antitumor immune responses even in the presence of these favorable molecular subtypes, remain our challenges for the future.

Disclosures

Emmanuel S. Antonarakis: Janssen, Astellas, Sanofi, Dendreon, Medivation, ESSA, AstraZeneca, Amgen, Clovis, Merck (C/A), Janssen, Johnson & Johnson, Sanofi, Dendreon, Genentech, Novartis, Tokai, Bristol-Myers Squibb, AstraZeneca, Celgene, Clovis, Merck (RF), Qiagen (IP).
(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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