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Review

Molecular and Clinical Insights into the Role and Significance of Mutated DNA Repair Genes in Bladder Cancer

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Abstract. DNA damage response and repair genes (DDR genes) are commonly mutated in bladder cancer. The biological impact of these mutations is an area of intense basic, translational, and clinical interest. As next generation sequencing increases its foothold in the treatment of localized and advanced bladder cancer, the role of DDR genes will continue to evolve. We review the inventory and biology of DDR genes in bladder cancer and describe known and candidate roles for loss of DDR genes to engender therapeutic susceptibilities to various anti-cancer agents.

Keywords: DNA damage response, DNA damage repair, cisplatin, immunotherapy, chemoresponse, bladder cancer, upper tract urothelial carcinoma

Cisplatin-based chemotherapy is the current standard of care for muscle-invasive bladder cancer (MIBC) in the neoadjuvant, adjuvant, and metastatic settings, with emergence of immunotherapy in metastatic patients who are platinum ineligible or refractory. Recent insights from multiplatform genomic analysis has revealed a significant proportion of MIBCs that harbor mutations in DNA damage and response/repair genes (DDR genes). MIBC is notable for a relatively high frequency of DDR gene mutations and the potential for these mutations to impact selection of therapy. Other diseases such as metastatic castrate resistant prostate cancer [1] and several familial cancer predisposition syndromes feature DDR alterations, but DDR loss of function through genetic alterations is otherwise rare in primary cancers. Mutations in MIBC driver genes identified by The Cancer Genome Atlas (TCGA) which have *bona fide* DDR roles include *ERCC2* and *ATM*. Additional DDR genes are recurrently mutated but with lesser frequency and may also impact tumor biology and therapeutic sensitivities, irrespective of their nomination as a common driver gene – the number of drivers will increase as more cancer genomes are sequenced. In this review we will compare and contrast DDR mutations in MIBC with DDR mutations in other cancers, discuss their potential impact on tumor biology, and highlight the emerging roles of DDR genes as biomarkers for therapy selection based on proposed therapeutic vulnerabilities.

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INVENTORY OF DDR GENES ALTERED IN MIBC

DDR genes *ERCC2* and *ATM* are TCGA-nominated bladder cancer driver genes [2]. Both

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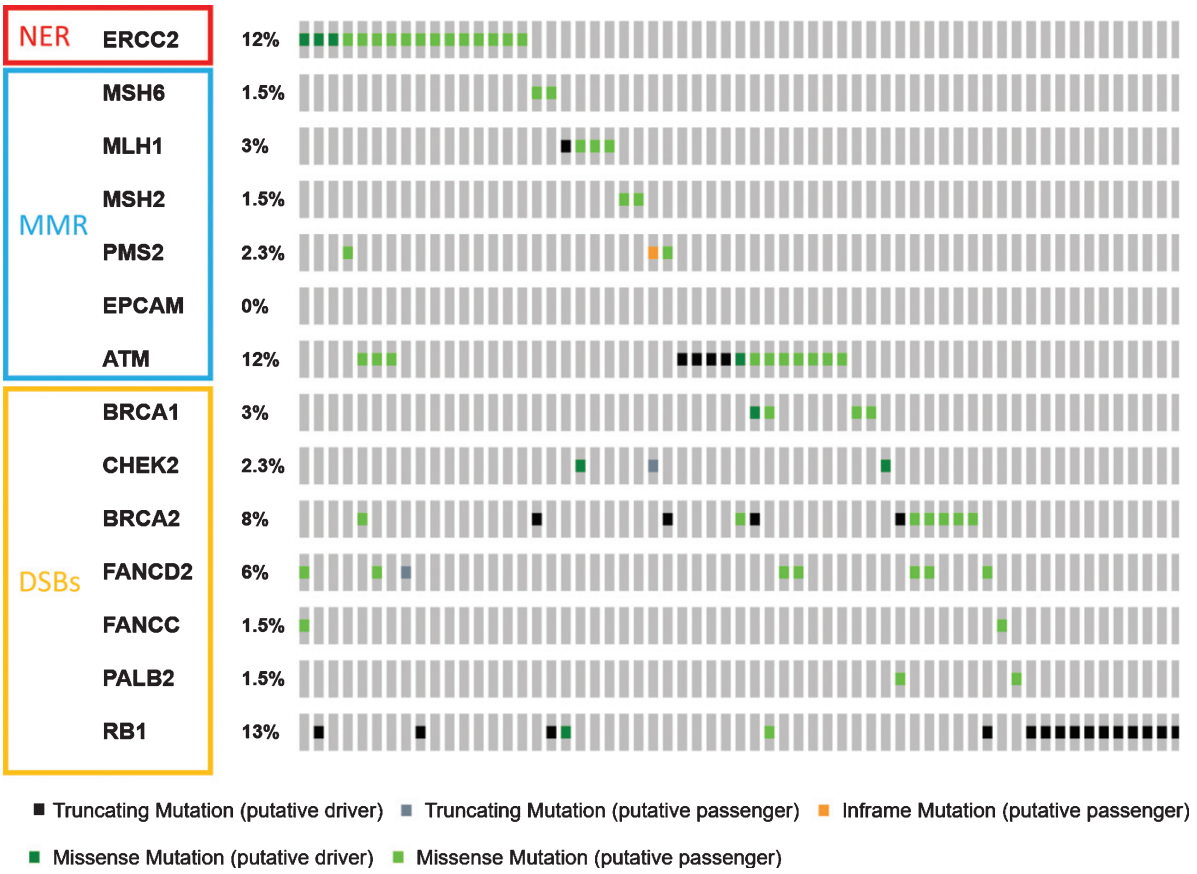

Fig. 1. A Mutations in DDR genes occurring the TCGA BLCA data set ($n = 130$). Note that only cases with variants present are depicted. Figures were generated using Cbioportal [62, 63].

have been linked to complete or near complete pathological response to cisplatin-based neoadjuvant chemotherapy as measured by pathological examination of surgical specimens at the time of radical cystectomy [3–5], highlighting the intense interest in these genes as biomarkers. We chose to focus on mutations in DDR genes because most of the high impact studies focus on mutations as the mechanism of loss of function or to correlate with clinical outcomes, as opposed to mRNA or protein expression, which has not been as well described in MIBC as it is in other malignancies [6]. Additionally, we focused on mutations rather than copy number alterations again because this type of alteration is what is reported in most studies and because deletion through copy number alteration does not occur in *ERCC2* or *ATM* in bladder cancer (Fig. 1). Indeed, because of *ERCC2*'s indispensable role in transcription, efforts to generate CRISPR knockout cell lines have not been successful, though point mutant variants can be viable (Mouw, Iyer, unpublished data, personal communication). Interestingly, the number of amplifications greatly outnumbers the number of deletions in this set of DDR genes in the TCGA, which is 'counter-culture' for tumor suppressor genes (Fig. 2). The clinical relevance of this observation is yet to be elucidated, but it is plausible to hypothesize that these tumors are hyper-proficient in repairing DNA damage and therefore may be intrinsically resistant to DNA-damaging chemotherapy regimens. We are not aware of convincing data to support this idea and will not speculate further though it may be a rationale for further study.

The exquisite tissue specificity of *ERCC2* mutations is notable. *ERCC2* has been found mutated in multiple bladder cancer cohorts at a rate of 10–18% [2–4, 7, 8] but not significantly in any other cancer cohort to our knowledge. Although the provisional TCGA cohort of adrenocortical carcinomas seems to have a high rate of D312N variants (unpublished), this variant is actually a common SNP with prevalence of 0.38 in the Exome Aggre-

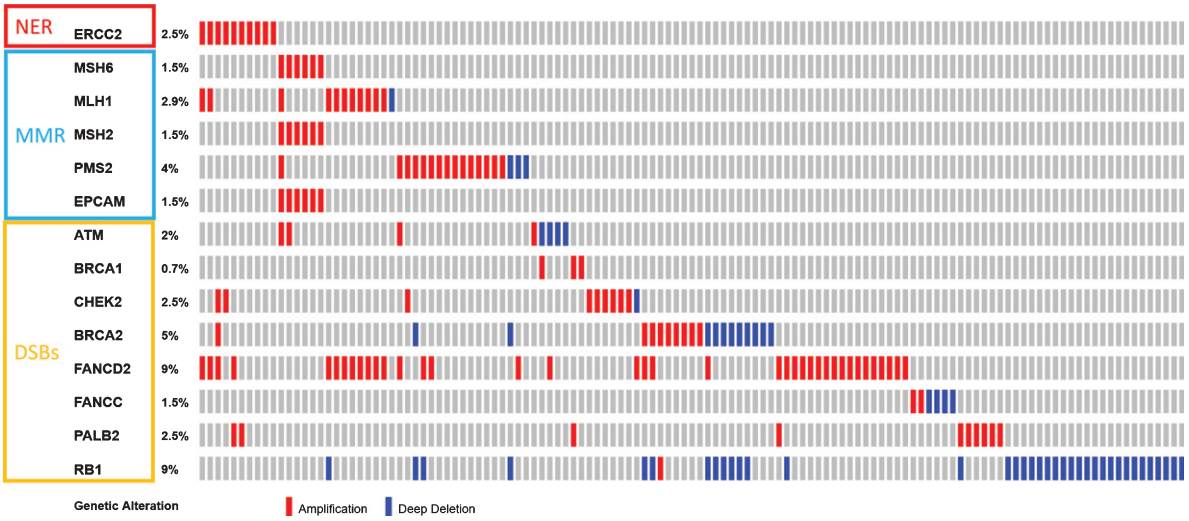

Fig. 2. Copy number alterations in DDR genes occurring in the TCGA BLCA data set ($n = 408$). Note that only cases with variants present are depicted. Figures were generated using Cbioportal [62, 63].

gation Consortium (ExAC), and is nondeleterious [9]. Germline *ERCC2* mutations are associated with xeroderma pigmentosum cross-complementation group D [10] (a hereditary skin cancer syndrome where patients develop normally), and trichothiodystrophy (an autosomal recessive condition where patients have abnormal development but no cancer predisposition [11, 12]). Interestingly, ERCC2 has two major functions relating to both transcriptional activation and nucleotide excision DNA repair (NER), but both xeroderma pigmentosum and trichothiodystrophy seem to be caused by a lack of DNA repair and not lack of transcriptional activity [13]. NER is both transcription-coupled and acts independent of transcription, and repairs a diverse set of DNA lesions such as cyclobutane pyrimidine dimers (from UV exposure), inter-strand and intra-strand cross links (such as by cisplatin), cyclopurines resulting from reactive oxygen, and bulky adducts [14]. It appears that the transcription-coupled NER pathway is most relevant to MIBC and that the global genome NER pathway may be less relevant. Liu and colleagues recently showed that *ERCC2*-mutant tumors have a specific trinucleotide mutation signature. This signature is enriched on the + strand of transcribed genes, strongly implicating transcription-coupled repair as the primary mechanism lost in *ERCC2*-mutated cancers [15]. Along these lines, *ERCC2*'s role in bladder cancer seems to stem from its role in transcription-coupled NER; no defect related to its role in transcription has been identified yet. Interestingly, 'ERCC2-ness' (i.e. the genomic mutation signature associated with ERCC2 loss of function n [16]) is not unique to *ERCC2*-mutated tumors, so it is possible that other alterations, whether genetic, epigenetic, or alterations expression or activity level, result in incapacitated transcription-coupled NER. In support of its critical role in transcription, all point mutations in the gene are missense and appear to be inactivating [4]. Often tumor suppressors have truncating or frame-shifting mutations, suggesting that complete inactivation of *ERCC2* through such a mechanism (or through deep deletion) results in reduced fitness and may be selected against.

In contrast to well-characterized hereditary cancer syndromes such as Von Hippel Lindau or familial adenomatous polyposis where the causative genes are also mutated in sporadic cancers, patients with deleterious germline *ERCC2* mutations do not develop bladder cancer, suggesting it may not have a role in initiation of the disease but possibly in progression. In support of this hypothesis, *ERCC2* is mutated more frequently in MIBC than nonmuscle-invasive bladder cancer (nMIBC [17]).

The next most commonly mutated DDR gene in MIBC is *ATM*. Like *ERCC2*, germline mutations of *ATM* are causative of a heritable cancer syndrome, ataxia telangiectasia. Germline mutations of this gene are also increasingly being recognized in nonsyndromic patients with advanced cancer, especially those with metastatic prostate cancer [1, 18, 19]. This kinase works upstream of p53 and acts as a part of DNA damage sensor pathway, sensing DNA double-

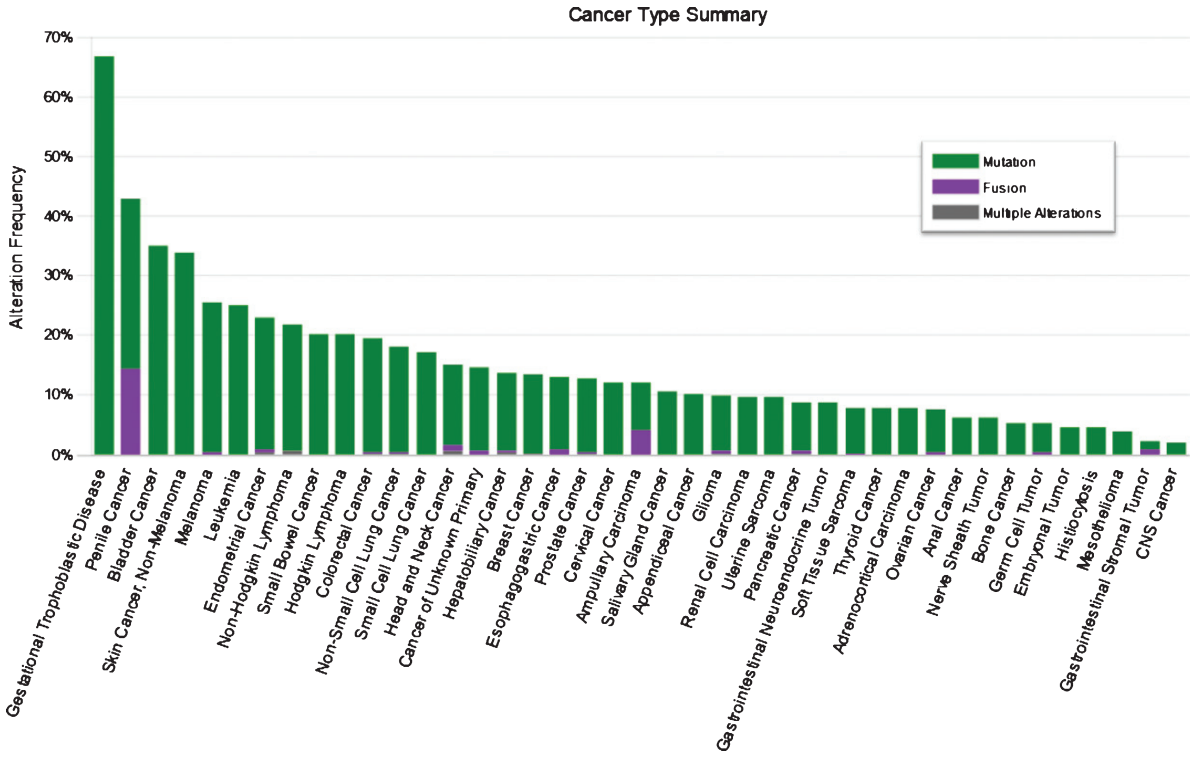

Fig. 3. Pan-cancer analysis of mutations in DDR genes listed in Fig. 1 as described in >10,000 tumors using MSK-IMPACT. Figures were generated using Cbioportal [62, 63].

stranded breaks (DSBs) [20–22]. Similar to *ERCC2* carriers, *ATM* carriers also are not known to have increased risk of bladder cancer, again suggesting a role in progression but not initiation. *ATM* mutations in TCGA [2, 23] and those reported by our group [5] are also missense substitutions in the majority of cases. These alterations often affect key residues that, when mapped onto the three-dimensional structure of the protein, are found in the core of the protein that likely result in the inability to fold properly [5].

ERCC2 and *ATM* are mutated frequently and specifically in sporadic MIBC. Mutation of caretaker genes probably confers a selective advantage to cancer cells through generation of a diverse tumor ecology with intratumor heterogeneity, but DDR mutations are simply not as common in other sporadic cancers when compared to MIBC. Although sporadic DDR mutations have been described in several cancers, (*e.g. BRCA1* and *BRCA2* in sporadic breast/ovarian cancers) their frequency is <5% in aggregate in each cancer. For instance, in our query of the recent MSK-IMPACT study of >10,000 tumors with targeted sequencing for the genes listed in Fig. 1, bladder cancer ($n = 422$) tops the list of >40 tumors types with mutations in these genes in >30% of cases, excluding rare cases gestational trophoblastic disease ($n = 11$) and penile cancer ($n = 7$) [24] (Fig. 3). In terms of individual gene mutations, Hodgkins lymphoma had a 20% rate of *MLH1* mutation, but very few cancers had a mutation rate >5% in the other genes (on a per-gene basis), underscoring the uniqueness of bladder cancer as a disease of DDR.

One notable DDR mutation in sporadic cancers is the *POLE* hotspot mutation which was initially identified in uterine cancer [25]. These hotspots result in DNA Polymerase ε loss of exonuclease function. This polymerase mediates DNA backfill at sites of DNA excision due to damage/repair, and *POLE* mutations are associated with accumulation of thousands of mutations per genome – a hypermutated phenotype – resulting from loss of DDR capacity. This mutation occurs in 7% of uterine cancers and at lower frequencies in other cancers.

Mismatch repair genes (MMR genes), germline mutation of which cause Lynch or Muir-Torre syndromes, are associated with upper tract urothelial cell carcinomas (UTUCCs [26, 27]) and are prob-

ably under-recognized in metastatic UTUCC [28]. MMR is a highly evolutionarily conserved process which was first described in *Streptococcus pneumoniae* [29] and *Escherichia coli* [30] among other prokaryotes and yeast. Its most well-studied function is to repair mismatches and indels which occur during mostly DNA replication (as opposed to ATM and ERCC2 proteins which function in repair of DNA damage). In whole exome sequencing studies, colorectal tumors which arise in Lynch Syndrome patients acquire mutations at a rate at least one order of magnitude higher than sporadic tumors as a result of the loss of MMR [31, 32]. Although UTUCCs of Lynch syndrome patients have not been studied using whole exome sequencing to our knowledge, it seems likely that they behave in a similar manner. For instance, Lynch syndrome patients with endometrial tumors (another Lynch-associated cancer) form tumors with much higher mutational burden than non-Lynch endometrial cancers [33]. Given the evolutionarily conserved role of these proteins in DDR, a plausible explanation for UTUCC carcinogenesis is loss of caretaker function in Lynch cases with accumulation of mutations leading to carcinoma.

MMR is clinically measured by quantitating microsatellite instability (MSI). Microsatellites are short repeats, most often CA repeats, which occur at thousands of sites throughout the genome and often at the 3' end of genes. The lengths of these repeats becomes unstable in tumors with MMR deficiency, and differences between the length of the repeat in germline (which is constant) and tumor (which becomes variable) can be measured to assess if MSI (and MMR deficiency by proxy) is present. Although likely relevant in UTUCCs, it should be noted, that MMR deficiency is extremely uncommon in bladder cancer. A recent study showed that 1/253 cases of bladder cancer in the TCGA cohort had microsatellite instability [34], in agreement with prior studies [35, 36].

Mutations in *ERCC2*, *ATM*, and MMR genes are the most well established and studied DDR alterations occurring in bladder cancer, but additional DDR mutations occur in bladder cancer that are not TCGA-nominated bladder cancer genes or well-known familial cancer genes. Genes involved in homologous repair such as *BRCA1* and *BRCA2* are recurrently but infrequently mutated. Similarly, multiple genes in the Fanconi Anemia pathway such as *PALB2*, *FANCD2*, and *FANCC* are recurrently but infrequently mutated in bladder cancer.

BIOLOGY OF DDR GENES ALTERED IN MIBC

An intuitive and plausible mechanism of chemosensitivity manifests in the idea that loss of the ability to sense or repair DNA damage brought on by cytotoxic chemotherapy results in persistent DNA lesions which ultimately cause the death of the cell through intrinsic apoptosis pathways. In lower eukaryotic organisms, this paradigm is known as synthetic sickness or lethality [37, 38], where mutation or loss of either of two pathways or genes permits the cell to persist but loss of both genes or pathways results in loss of viability [39]. This paradigm has led to the successful application of PARP inhibitors to patients with breast or ovarian cancer who are *BRCA1*/*BRCA2* carriers [40–42]. BRCA1 and BRCA2 proteins are intimately involved in homologous repair (HR) of DSBs, so inhibition of the base-excision repair pathway by PARP inhibitors will convert single strand breaks (SSB) to DSBs which cannot be repaired in tumor cells descendent from *BRCA1* or *BRCA2* carriers [39, 43]. And recently, PARP inhibitors were shown to have significant activity in patients with metastatic prostate cancer but activity was limited to patients carrying germline or somatic mutation in DDR genes, most often *BRCA1*, *BRCA2*, and *ATM* [1]. Similar application of PARP inhibitors to *BRCA2*-mutant tumors might be feasible, given the rate of *BRCA2* deleterious mutations in urothelial cancer, mostly via truncating mutations (Fig. 1).

A similar therapeutic relationship is emerging between cisplatin and *ERCC2*, *ATM*, and other DDR mutations in MIBC with respect to cisplatin sensitivity. When *ERCC2* mutations are present in MIBCs, the overall mutation rate in each tumor is tripled [4], reminiscent of a hypermutator phenotype, and suggesting these mutations do primarily result in the inability of the tumor to repair DNA damage. Indeed, tumor-associated somatic *ERCC2* variants lack the ability to rescue viability in fibroblasts derived from patients with germline *ERCC2* loss when treated with cisplatin [4]. A similar increase in somatic mutations per tumor occur when *ATM*, *RB1*, or *FANCC* are mutated in bladder cancers: chemotherapy responders had 43% more alterations in a panel of 216 clinically-relevant genes than nonresponders [5]. DDR mutations also strongly associate with complete or near complete response in multiple bladder cancer cohorts from independent institutions [3, 5].

Table 1
Functional annotation (ClinVar) of germline DDR SNPs reveals that most of these SNPs are either benign variants or a VUS

	Pathogenic	Likely pathogenic	VUS	Other	Total	% not known to be delterious
ATM	464	290	1772	753	3279	77.0%
BRCA1	2445	210	1980	1199	5834	54.5%
BRCA2	2738	222	3265	1557	7782	62.0%
ERCC2	21	4	41	46	112	77.7%

This therapeutic susceptibility may be relevant more generally. For instance, when a subset of DDR genes is mutated at a lower frequency as detected by panel targeted sequencing, there is associated increase in survival in patients with advanced urothelial cancers who were treated with cisplatin [44]. DDR gene mutations identified in panel targeted sequencing also associated with prolonged survival in MIBC patients treated with chemoradiation [45], suggesting DDR gene mutations may be prognostic markers in this setting. In another study, patients with somatic DDR gene mutations or carriers of germline DDR mutations had prolonged recurrence free survival, again pointing to their potential utility as prognostic biomarkers. However, this latter study does not stratify germline variants of unknown significance from deleterious or likely deleterious variants. Many of these germline or somatic variants may be nondeleterious because the majority of SNPs (which are common in *ATM*, *BRCA1*, *BRCA2*, *ERCC2*) are either benign or variants of unknown significance. We calculated the percentage of variants found in the ClinVar database [46] not known to be deleterious for each of these genes (Table 1). In each case, half to three-quarters of the variants have known benign or unknown effects. This underscores the importance of functional annotation against known databases or experimental validation of variants when identified in such studies.

It is not clear whether a different set of mutations engenders therapeutic susceptibility to radiation compared to chemotherapy. The damage from chemotherapy, particularly cisplatin, causes intra- and interstrand crosslinks, as compared to radiation which causes DSBs. Other DNA damaging chemotherapeutic agents used in bladder cancer such as doxorubicin cause different types of damage as well. This distinction is critical because each type of genetic insult requires a specific pathway for repair, and therefore the mutation causing pathway loss of function would be expected to dictate the therapeutic susceptibility. For instance, *BRCA2* loss-of-function mutations would wipeout the cell's ability to repair DSBs, potentially resulting in radiotherapy or PARP inhibitor susceptibility.

Other DDR mutations also associate with chemoresponse. Whereas studies focusing on *ERCC2* mutations used whole exome sequencing, other studies have used panel next generation sequencing (NGS). Using a clinical NGS panel, it was shown that mutation of *ATM*, *RB1*, or *FANCC* associated with partial response and progression-free and overall survival [5]. Although *in vitro* functional validation was not undertaken to characterize these mutations, molecular modeling of the 3-D structures of the variant ATM and RB1 proteins revealed that most of the mutations fell within residues which were critical to protein folding or kinase function (for ATM) or interaction with DP1/E2F transcription factors (for RB1).

MMR deficiency may require an approach different from synthetic lethality though. MMR primarily repairs DNA replication errors and not DNA damage, therefore application of DNA damaging therapeutics might not be expected to elicit a strong apoptotic response. Some studies in other tumor types have suggested that loss of expression of MMR proteins associates with cisplatin resistance [47–50] but the relationship between MMR status and chemosensitivity status is yet to be borne out in urothelial cancer.

However, another candidate therapeutic vulnerability has been identified in tumors with high mutation burdens: PD1/PDL1-directed immunotherapy with monoclonal antibodies [31]. The rationale for such an approach rests in the finding that patients who respond to immune checkpoint blockade tend to have a higher number of mutations per tumor than non-responders [51–54]. Each mutation can give rise to one or more than one neoantigen, defined as the mutant peptide that arises from a nonsynonymous or frameshifting variant. These can be recognized as non-self by the immune system. Neoantigen

burden is directly correlated to mutation load: about half of nonsynonymous variants give rise to a neoantigen, with almost 40% of those being in an expressed gene [55]. In this sense, neoantigens are like lottery tickets – the more tickets one purchases, the more likely one is to hit the jackpot. Alternatively, many smaller jackpots may lead to a big payday as well. To this end, several well-done studies have shown that indeed anti-neoantigen CD8 T cell responses can be detected in patients who respond to immune checkpoint blockade [52–54] and other immune therapies [56] and that the number of anti-neoantigen T cells increases as patients respond. MMR deficiency, and possibly DDR deficiency more broadly, therefore may enable successful use of immune checkpoint blockade in patients with MIBC. In light of the high response rate to these drugs in clinical trials [57–61] and the high burden of DDR mutations in patients with advanced or metastatic urothelial cancers, it seems likely that there is significant overlap. DDR mutations could plausibly be seen as a predictive biomarker of response to these drugs, but this hasn't been explored or reported yet. Again, it is important to note, this approach may be even more relevant in UTUCCs given the higher incidence of presumed MMR deficiency, the expected high number of neoantigens arising from frameshifts, and the potential for increased immunogenicity of such neoantigens [31, 32].

CONCLUSION

The finding that DDR mutations are associated with susceptibility to chemotherapies and immunotherapies is a transformative avenue of research for clinic trialists and basic and translational researchers. More effort in this area is likely to yield numerous benefits to patients with localized or metastatic bladder cancer. It is foreseeable that the benefit immune checkpoint blockade may extend outside of MMR deficient tumors to tumors with any DDR mutation or even a high neoantigen burden with WT DDR genes. However, the bladder cancer research community must be cognizant of the potential to advance the care of the same subset of patients repetitively without developing improved therapies and outcomes for patients with WT DDR pathways. It is possible that the DNA damaging agents and immunotherapies benefit the same subset of patients, in which case the argument can be made that the improvement is more incremental than transformative. In any case, these findings have added significant understanding to the fundamental mechanisms of how anticancer therapies work as well as paradigm-shifting rationales for treatment selection in urothelial cancer. In time, these findings will translate to personalized medicine algorithms for a subset of patients with DDR mutations, with the hope of extending these benefits to all patients using the same or likely yet untested urothelial cancer agents.

CONFLICT OF INTEREST

(ERP) Methods for Screening Muscle Invasive Bladder Cancer Patients for Neoadjuvant Chemotherapy Responsiveness. U.S. Patent Application No.: 14/588,503, Filed 1/2/2015.

(PHA) No conflicts of interest.

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