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Original article

Tumor mutation burden, DNA mismatch repair status and checkpoint immunotherapy markers in primary and relapsed malignant rhabdoid tumors

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ABSTRACT

Introduction: Malignant rhabdoid tumor (MRT) is a rare, aggressive pediatric tumor of nuclear lineage. It is mainly characterized by germline or somatic *SMARCB1 (INI1)* driver mutations. To characterize the potential for immunotherapy in untreated and treated MRT, current study investigated tumor mutational burden (TMB) and other biomarkers in MRT.

Material and methods: Normal-tumor paired whole exome sequencing (WES) and/or immunohistochemistry (IHC) of DNA mismatch repair (MMR) proteins, PD-L1, PD-1 and CD8 were performed in 16 cases, some with both primary and relapsed tumor.

Results: Five cases subjected to WES demonstrated germline *SMARCB1 (INI1)* mutations. TMB was 0.7–1.07/Mb in 4 of the 5 primary untreated tumors, and 33.81/Mb in one case with pathogenic MMR, POLD, and POLE mutations. Ten cases tested for MMR status by IHC showed retained nuclear expression of the proteins. Eight of the 16 cases (8/16, 50%) showed membranous expression of PD-L1 in 10–70% of tumor cells (tumor proportion score, TPS). Nine cases (9/16, 56.3%) showed high (> 2/HPF) tumor infiltrating lymphocytes with PD-1 staining ranging 10–60%, correlating with tumor PD-L1 staining ($p < 0.0001$). Between post-treatment metastatic tumors and the pre-treatment primary tumors, TMB was similar while PD-L1 TPS was similar or lower.

Conclusion: MRT has a low TMB. Nonetheless, because a subset of MRT cases have a PD-L1 TPS greater than the cutoff for checkpoint therapy in other malignancies, the utility of immune checkpoint inhibitors should be studied in this patient population.

1. Introduction

Malignant Rhabdoid Tumor (MRT), an aggressive malignancy first described in the kidney in 1978, predominantly affects infants and young children [1,2]. Subsequently, tumors with similar morphology and an aggressive clinical course arising from extrarenal sites were elucidated including atypical teratoid/rhabdoid tumor (ATRT), the central nervous system (CNS) MRT equivalent [3,4]. Regardless of the site of origin, MRT share a common genetic signature, namely mutations involving the *SMARCB1 (INI1)*, gene or rarely the *SMARCA4* gene [5,6]. These genes encode proteins that are components of the chromatin remodeling complex SWI/SNF. Aggressive treatment regimens including surgery, chemotherapy, and radiation are often used in combination [7], however, the patient outcomes are dismal with reported survival rates between 20 and 35% [8,9]. Additionally, with the current treatment regimens patients experience significant treatment related toxicities [10]. Thus, there is a need for better therapeutic options for these patients.

In the past few decades, studies have unraveled the relationship between tumor cells and tumor microenvironment and the potential for harnessing the native immune system to control cancer progression [11]. Tumor cells can evade immune surveillance mechanisms by utilization of checkpoint inhibition and this pathway can be targeted to prevent tumor progression [12,13]. Blockade of the programmed cell

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death 1/programmed cell death ligand 1 (PD-1/PD-L1) immune checkpoint pathway has proven to be one of the most promising approaches. Several clinical trials of PD1/PD-L1 inhibition for various adult cancers have shown durable clinical responses with milder side effect profiles when compared to conventional therapies, and FDA approved treatments are now available [12].

Nonetheless, currently there is no single biomarker that can reliably predict the response to immune checkpoint blockade. Expression of PD-L1 in tumor cells (tumor percentage score, TPS) has been the most widely studied, with data suggesting immunohistochemistry (IHC) based PD-L1 expression as an important, though yet not a definitive, biomarker [14]. Additional biomarkers that have shown predictive treatment response include tumor mutational burden (TMB), neoantigen levels, microsatellite instability (MSI) and DNA mismatch repair (MMR) functional status, and HLA subtypes [14–16].

In this study, we sought to determine tumor mutation burden (TMB) by tumor-normal paired whole exome sequencing; MMR mutation and function by both IHC and whole exome sequencing; and expression of multiple biomarkers relevant to immunotherapy approaches including PD-L1 expression in tumor cells and presence of CD8+ and/or PD-1+ tumor infiltrating lymphocytes (TILs).

2. Material and methods

2.1. Patient samples

With Institutional review board (IRB) approval (IRB # 201705056 and 201102311), a retrospective study with chart review and residual tissue study was conducted. Diagnosis of “malignant rhabdoid tumor” or “atypical teratoid/rhabdoid tumor” was searched for in departmental archives from 1990 to 2017. For cases identified, hematoxylin and eosin (H&E) and related immunohistochemical (IHC) staining slides were reviewed, and cases with sufficient tissue (formalin fixed paraffin embedded (FFPE) tissue from biopsy/resection specimens) were selected for inclusion.

2.2. DNA extraction and tumor mutation burden/load by normal-tumor paired whole exome sequencing

Whole exome sequencing (WES) was performed in cases with sufficient amount of both normal and tumor tissue. Genomic DNA was extracted from the normal tissue and neoplastic tissue following standard methods (see below).

2.2.1. DNA extraction

Following the manufacturer's recommended protocol, genomic DNA of both normal and neoplastic tissue was extracted in the Genome Technology Access Center (GTAC), Washington University, St Louis, MO using the AllPrep DNA/RNA FFPE Kit (Qiagen, cat#80234). Extracted DNA was qualified using a Tapestation 4200 (Agilent).

2.2.2. Whole exome sequencing

Whole exome sequencing was performed in the GTAC facility (as above). Genomic DNA was sonicated to an average size of 175 bp; fragments had Illumina's sequencing adapters ligated to their ends. The ligated fragments underwent amplification for 7 cycles. Fragments were hybridized to biotinylated RNA oligos specific to regions of interest (Clinical Research Exome (Agilent) and selected from remaining fragments using streptavidin beads. The enriched library was amplified for 14 cycles with primers that incorporate a unique indexing sequence tag. The resulting libraries were sequenced using the Illumina HiSeq-3000 as 150 bp paired end reads. Sequencing targeted 25–30 M reads for normal tissue and 45–50 M reads for neoplastic tissue.

2.2.3. Tumor mutation load/burden determination

VarScan2 was used to call somatic variants, and high-confidence somatic variant calls were extracted with the “processSomatic” command. The subset of high-confidence variants fulfilled several empirically-derived criteria, including tumor VAF > 15%, normal VAF < 5%, a somatic p-value of < 0.03, and indel length of 50 base pair (bp) or less. The high confidence SNVs and indels were totaled and divided by 54 Mb, the size of the exome target region, to obtain the tumor mutation burden (TMB) [17].

2.3. Immunohistochemistry (IHC)

Representative sections of tumor from each case were selected and IHC stains with appropriate controls were performed on FFPE tissue for the following markers: PD-L1 (1.61 μg/mL, rabbit monoclonal, clone SP263, Ventana, Tucson, AZ, USA); PD-1 (2.97 μg/mL, mouse monoclonal, clone NAT105, Ventana, Tucson, AZ, USA); CD8 (0.35 μg/mL, rabbit monoclonal, clone SP57, Ventana, Tucson, AZ, USA) and MMR [MLH1 (1.5 μg/mL, mouse monoclonal, clone M1, Ventana, Tucson, AZ, USA), MSH2 (3.62 μg/mL, mouse monoclonal, clone G219-1129, Cell Marque, Rocklin, CA, USA), MSH6 (0.101 μg/mL, mouse monoclonal, clone 44, Ventana, Tucson, AZ, USA), and PMS2 (14.32 μg/mL, rabbit monoclonal, clone EPR3947, Cell Marque, Rocklin, CA, USA)], following standard protocols on a Ventana automated stainer (Ventana Medical Systems, Tucson, AZ, USA) in the Anatomic and Molecular Pathology (AMP) Core Lab, Department of Pathology & Immunology, Washington University School of Medicine.

The IHC staining was evaluated as follows; PD-L1: membranous staining, complete or incomplete, in tumor cells was identified and given a percentage (tumor proportion score, TPS) [18]. PD-1 and CD8: density of TILs and their expression of PD-1 was assessed. CD8+ lymphocytes were counted at 400× from 10 high power fields and > 20 cells in a tumor was scored as high [19]. PD-1 was assessed as the percentage of TILs showing positive cytoplasmic staining for PD-1. MMR: Nuclear staining in tumor cells was assessed for MLH1, MSH2, MSH6, and PMS2 and positive staining was assessed as retained expression.

2.4. Statistical methods

Survival time was defined as time from diagnosis to events including recurrence, metastasis, or death and was considered right-censored at the time of loss to follow-up. Kaplan–Meier estimates was obtained and compared between subjects with different levels of CD8 counts. A Cox proportional hazards model analysis was performed to examine the association of PD-L1, PD-1 and CD8 with survival time. All statistical analyses were two-sided at significance level 0.05. Data analysis was performed using SAS 9.4 (SAS, Cary, NC).

3. Results

The retrospective search from 1990 to 2017 identified 19 cases of MRT or ATRT, of which 16 had sufficient tissue for study by WES and/or IHC stains. The 16 patients were between the ages of 0–5 years. Six (6/16, 37.5%) patients were male and ten (10/16, 62.5%) female. Primary tumor sites included kidney, soft tissue, liver, and brain (Table 1). All cases had loss of INI1 expression demonstrated by immunohistochemistry.

3.1. Tumor mutation burden (TMB) by normal-tumor paired whole exome sequencing

Five cases were subjected to tumor-normal paired whole exome sequencing. All 5 cases demonstrated germline *SMARCB1/INI1* gene mutations, confirming the histologic diagnosis (Fig. 1). Among these 5 cases, 2 cases had paired primary (pre-treatment) and metastatic (post-treatment) tissue, 2 cases had primary, pre-treatment tissue only, and 1 case had pre-treatment and post-treatment tissue samples. At initial

Table 1
Patient demographics and primary tumor site.

Case number	Age at diagnosis	Gender	Primary location
1	Birth	F	Kidney
2	1 week	F	Soft tissue
3	9 weeks	F	Kidney
4	3 months	F	Kidney
5	6 months	M	Liver
6	9 months	M	Soft tissue
7	9 months	M	Brain, posterior fossa
8	10 months	M	Kidney
9	12 months	F	Brain, posterior fossa
10	14 months	F	Soft tissue
11	18 months	F	Brain, posterior fossa
12	19 months	M	Liver
13	19 months	F	Kidney
14	24 months	F	Brain, posterior fossa
15	24 months	M	Soft tissue
16	5.5 years	F	Soft tissue

Table 2
Tumor mutation burden.

Case number	Sequencing number	HC-som-SNPs	HC-som-indel	Total	Tumor mutation burden (/Mb)	PD-L1 IHC (Tumor cells) (%)	PD-1 IHC (TILs) (%)
Primary tumor, pre-treatment							
3	40310	31	14	45	0.83	< 1	< 1
6	40311	42	16	58	1.07	< 1	20
5	40312	28	10	38	0.70	< 1	30
8	40307	35	6	41	0.76	20	50
13	40309	1596	230	1826	33.81	10	30
Residual tumor, post-treatment							
6	40311	47	6	53	0.98	< 1	30
Metastatic tumor, post-treatment							
5	40312	19	1	32	0.59	< 1	< 1
8	40307	50	9	59	1.09	< 1	< 1

presentation, prior to therapy, 4 of these 5 cases (4/5, 80%) showed a low TMB ranging from 0.59 to 1.09 mutations/Mb; one case (1/5, 20%) revealed a TMB of 33.81/Mb (Table 2).

The difference between the TMB of primary pre-treatment and post-treatment tumors was minimal. For the two cases of (pre-treatment) primary versus (post-treatment) metastatic tumor, case 5 showed TMBs of 0.70 and 0.59 respectively, and case 8 showed TMBs of 0.76 and 1.09, respectively. For the case of pre-treatment primary and post-treatment recurrence, case 6 showed TMBs of 1.07 and 0.98.

3.2. DNA mismatch repair and hypermutation gene analysis

Eleven (11) cases with sufficient tissue, including the five cases which had WES, were subjected to MMR immunohistochemistry and showed retained MMR staining. The germline and somatic sequences for the DNA mismatch repair genes (*MLH1, MSH2, MSH5, MSH6, and PMS2*), DNA polymerase epsilon (*POLE*) gene, and DNA polymerase delta (*POLD*) gene from the WES revealed several pathogenic variants (Table 3). Of note, Case 13, which had a TMB of 33.81, showed potentially pathogenic variants in genes *MSH6, POLE* and *POLD*.

3.3. Immunohistochemical analysis

Sixteen cases (including metastatic/recurrent tumor where available) were subjected to immunohistochemical studies, showing staining intra-tumoral heterogeneity as well as inter-tumoral heterogeneity. Eight out of 16 (8/16, 50%) cases showed PD-L1 membranous staining in tumor cells, with TPS ranging from 10 to 70%. The remaining 8 cases showed less than 1% PD-L1 membranous staining in the tumor cells (TPS < 1%).

CD8 stains were examined both within the tumor and at the tumor interface. Nine cases (9/16, 56.3%) showed a high percentage of TIL within the tumor. Twelve cases had a tumor interface with normal tissue available for evaluation. Among these 12 cases, 11 cases demonstrated high percentage of CD8 staining cells at interphase that was usually a few folds higher than CD8 density within tumor. PD-1 was variably positive in a subset (10/16, 62.5%) of TILs (Table 4). In addition, in 4 cases we evaluated paired primary and metastatic tumor (Table 5); the primary in all cases showed higher or similar expression of PD-L1/tumor, CD8 (within tumor) and PD1 in TILs when compared to the metastatic tumor (Fig. 2).

3.4. Correlation between PD-L1, PD-1, and clinical outcome

The level of expression of PD-L1 by tumor cells and the level of PD-1 expression in CD8+ TILs showed a positive Pearson correlation coefficient of 0.8652, which suggests a significant moderate to strong positive correlation of these markers (P-value < 0.0001). There was no correlation between the level of immunohistochemical staining and tumor location. Of the 14 cases for which follow-up data were available, 10 patients had events including metastasis, recurrence, and death within 5 years of initial diagnosis, and 4 patients showed no evidence of disease for follow-up ranging from 7–14 years (Table 4). Cox proportional hazards model analysis indicated that PD-L1, PD1, or CD8 expression are not a significant predictor of survival (p = 0.3579, 0.8451,

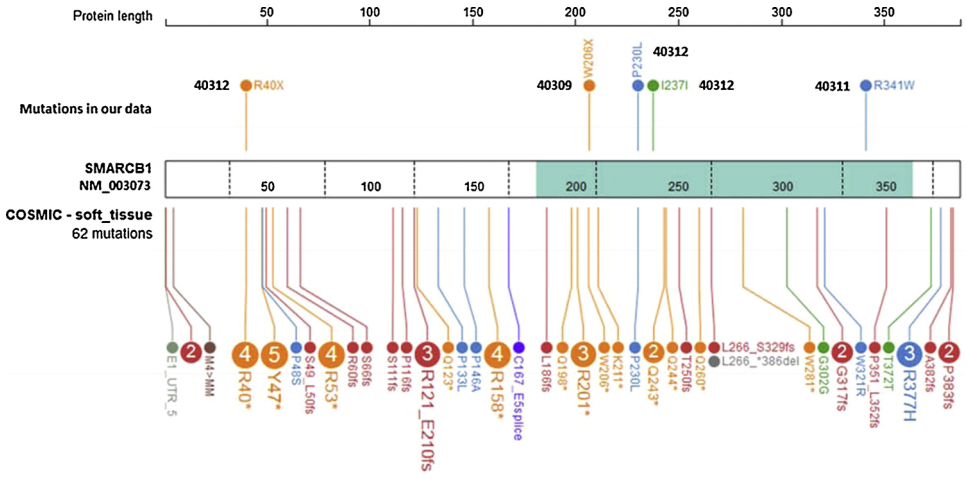

Fig. 1. *SMARCB1/INI1* germline mutation Lollipop plot of 5 patients.

Table 3
Potential significant somatic and germline mutations in DNA mismatch repair and hypermutation genes.

Case No	Disease State	Sample	Chromosome	Start	End	Ref	Alt	Function	Gene	Comments	Type
Significant somatic variation in DNA mismatch repair and hypermutation genes											
13	Primary	40309	Chr12	10684816	106848416	A	-	exonic	*POLR3B*		
Significant germline variation in DNA mismatch repair and hypermutation genes											
8	Primary	40307	chr6	31727989	31727989	A	G	Exonic	*MSH5*	Deleterious (in Silico tools)	Nonsynonymous SNV
			chr19	50906795	50906795	G	A	Exonic	*POLD1*	Deleterious (in Silico tools)	Nonsynonymous SNV
13	Primary	40309	chr2	48023170	48023170	C	T	Exonic	*MSH6*	Hot-spot region with 14 pathogenic variants, deleterious	Nonsynonymous SNV
			chr12	133257750	133257750	T	C	Exonic	*POLE*		Nonsynonymous SNV
			chr7	44157262	44157262	C	T	Exonic	*POLD2*	Deleterious (in Silico tools)	Nonsynonymous SNV
3	Primary	40310	chr2	48025841	48025841	G	A	Exonic	*MSH6*	Hot-spot region with 18 pathogenic variants out of 20 classified variants	Nonsynonymous SNV
5	Metastatic	40312	chr7	6042169	6042169	C	T	Exonic	*PMS2*	Hot-spot region with 11 pathogenic variants out of 15 classified variants	Nonsynonymous SNV

and 0.6328, respectively).

4. Discussion

MRT is a rare malignancy in children in which germline or somatic *SMARCB1 (INI1)*, or rarely *SMARCB4*, mutations are considered to be the driving oncogenetic event. This study is the first to investigate tumor mutation burden (TMB) in both primary, pretreatment, and posttreatment metastatic or recurrent tumors by normal-tumor whole exome sequencing; MMR and hypermutation genes variants by both WES and IHC; and PD-L1, PD1, and CD8 in the tumor microenvironment. Our data indicate that most cases of MRT have a low TMB. For the first time, we identify a case of MRT with high TMB and pathogenic variants of MMR and hypermutation genes. Our data further demonstrate that a subset of cases have a PD-L1 TPS greater than the cutoff typically used to identify patients who will benefit from checkpoint therapy in other malignancies. Our results suggest that the utility of immune checkpoint inhibitors should be studied in this patient population.

4.1. Tumor mutation burden

Somatic non-synonymous mutations in cancer cells can generate neoantigens which are recognized by the immune system as non-self and thus help initiate an immune response [20,21]. TMB are traditionally divided into three groups: low (1–5 mutations/Mb), intermediate (6–19 mutations/Mb), and high (≥20 mutations/Mb) [22]. Because, theoretically, more mutations will lead to more antigenic neoepitopes, TMB is often used as a surrogate to predict the likelihood that a particular patient will show a clinical response to immune checkpoint inhibitor therapy, an approach that is supported by results that show a higher mutation load is correlated with a better response to CTLA-4 inhibitors and better survival, as seen in melanoma, non-small cell lung cancer, colon cancer, and so on [23–26]. There is a wide range of TMB in tumors [27–29]. At the low end, tumors such as pilocytic astrocytoma and acute lymphoblastic leukemia usually have a TMB < 1 mutations/Mb (for comparison, adult malignancies such as melanoma and squamous lung cancer have TMBs between 100–1,000 mutations/Mb). Our demonstration that 80% of ATRT/MRT have a mutational load of 0.70–1.76 mutations/Mb is consistent with prior results demonstrating a TMB usually below 2 [29]. Our finding of a case with a TMB of 33/Mb suggests more heterogeneity in the oncobiology of ATRT/MRT than has previously been recognized.

MRT/ATRT is genetically defined by germline or somatic *SMARCB1*, or rarely *SMARCB4*, mutations as the key oncogenic drivers. Other pediatric malignancies are also characterized by a key, powerful driver mutation such as the Ewing sarcoma (EWS) which characteristically harbors a balanced chromosomal translocation between *EWS* and a member of the *ETS* gene family; of note, the TMB in EWS is almost always < 2 mutations/Mb [29,30]. However, in EWS, there is apparently a 2- to 3-fold increase in the TMB in relapsed tumors [30], which contrasts with the lack of a significant increase in the TMB in metastatic

Table 4
Immunohistochemistry results of PD-L1, CD8, PD-1 and MMR expression.

Case Number	PD-L1 (%)	CD-8 + TILs (within tumor,/HPF)	CD-8+ TILs (at interface,/HPF)	PD-1 + TILs (interface, %)	MMR	Last FU* (time from initial dx)	Disease status
1	< 1	Low	11	< 1	Retained	12 days	Expired
2	30	High	120	80	Not done	NA	NK
3	< 1	Low	< 1	< 1	Retained	7 weeks	Disease Progression
4	20	High	180	80	Retained	10 years	Disease Free
5	20	High	80	30	Retained	NA	NK
6	< 1	Low	7	30	Retained	13 years	Disease Free
7	10	High	70	30	Retained	2 years	Disease Progression
8	20	High	220	80	Retained	5 months	Disease Progression
9	< 1	Low	5	50	Not done	1 year	Recurrence, Disease Progression
10	30	High	100	50	Retained	3 months	Expired
11	< 1	Low	NA	< 1	Retained	10 years	Disease Free
12	< 1	High	NA	< 1	Not done	7 years	Disease Free
13	10	High	80	30	Retained	14 years	Disease Free
14	< 1	Low	NA	< 1	Not done	3 years	Recurrence, Disease Progression
15	< 1	Low	NA	< 1	Not done	1 year	Disease Progression
16	70	High	> 250	30%	Retained	5 years	Expired

FU: Follow up. NA: Not applicable. NK: Not known.

Table 5
Comparison between PD-L1 expression, CD8/PD-1 + TILs in primary versus metastatic tumor.

	CASE 5		CASE 8		CASE 10		CASE 16	
	Primary	Metastatic	Primary	Metastatic	Primary	Metastatic	Primary	Metastatic
Location	Liver	Lungs	Kidney	Lung	Soft tissue	Lungs	Soft tissue	Lungs
PD-L1 (%)	20	< 1	20	5	30	10	70	5
CD8 + TILs within tumor	High	Low	High	High	High	Low	High	Low
PD-1 + TILs (%)	30	< 1	80	< 1	50	20	30	30

or relapsed MRT/ATRT. While the number of cases of MRT/ATRT and EWS for which the TMB has been reported is admittedly quite small, the results to date suggest that for pediatric tumors types characterized by powerful driver mutations (whether SNVs or translocations), the TMB is generally < 2 mutations/Mb, similar to the TMB in leukemias and lymphomas [27,28].

4.2. Tumor mutation burden and DNA mismatch repair

An increase in mutational burden often associated with a deficiency of the DNA mismatch repair (MMR) pathway via either an inherited or acquired mutation in the DNA mismatch repair enzymes, or via somatic epigenetic modifications that result in decreased expression of key DNA MMR enzymes [20] or mutations in genes encoding DNA polymerase proofreading domains, such as *POLE* and *POLD* [15,31]. It is not surprising therefore that the one case in our study (case 13) that showed a high TMB of 33.81 mutations/Mb harbored pathogenic variants of the *MSH6, POLE*, and *POLD* genes (Table 3) it is well established that loss of function mutations in MMR genes may encode proteins that are still detected by routine immunohistochemical analysis, which likely explains the retained expression of *MSH6* we observed in case 13 [31]. Given that prior studies have demonstrated the expected correlation between deficient DNA MMR, increased TMB, a subsequent increase in the number of tumor neoantigens, and a better response to checkpoint blockade therapy [32,33], our results from case 13 emphasize that not only the oncobiology of some cases of MRT/ATRT is more complicated than previously recognized but also some cases of MRT/ATRT may be responsive to checkpoint blockade therapy.

4.3. Immunohistochemical staining of checkpoint therapy markers

At present, the immunohistochemical markers usually considered as predictive of a response to checkpoint blockade therapy include PD-L1 expression on tumor cells, and PD1 expression in TILs. In our set of cases, the tumor cells demonstrated variable, heterogeneous but significant expression of PD-L1 within and among tumors, with TPS ranging from < 1% in 50% of cases, to 10–70% in 50% of cases. Two previous studies have reported IHC staining results of PD-L1 only in ATRT cases. Our results are consistent with those from a recent study showing positive PD-L1 expression (using a semiquantitative scoring method) in 8 out of 20 (40%) ATRT cases [34]. Another study reported negative PD-L1 expression in 100% of cases of ATRT, but only two cases were evaluated; thus, the different result may be due to different antibodies used, analytic methods, or tumor heterogeneity, or simply a result of the small sample size [35].

The current study is the first to investigate the expression of IHC markers used to predict the response to immunotherapy between primary, untreated tumor and post-treatment metastatic or relapsed tumor. In all 4 cases, the primary tumor showed similar or higher expression of PD-L1 by the tumor cells, similar or higher number of CD8 + TIL within the tumor, and a higher number of immune cells expressing PD1 when compared with the metastatic or recurrent disease. These results have implications for the efficacy of checkpoint blockade therapy in post-treatment disease.

While our results show that 50% of MRT have a TPS higher than 10%, a finding that suggests checkpoint blockade may be of clinical benefit in some patients, it is worth noting that although PD-L1 IHC has been used as a companion or complimentary test for checkpoint immunotherapy, the IHC result is not always predictive of the clinical response to anti PD-1/PD-L1 therapy [36]. Complicating matters, several IHC scoring systems have been proposed, but there is as yet no consensus on either PD-L1 scoring itself or the level of staining required to drive clinical therapy. In addition to the tumor percentage score (TPS) popularly used in lung cancer (with different cutoff in different situations), the combined percentage score (CPS, which combines positive staining of tumor and immune cells), has been used in many other malignancies with cutoffs for a positive result ranging from 1% in gastric carcinoma, to 10% in urothelial carcinoma, to 20% in head and neck squamous cell carcinoma (HNSCC) [37–40]. In current study, TPS and CPS showed similar category of results, with the differences lying in percentage of positive staining. The FDA has approved various anti-PDL1 therapies with recommended companion/complimentary diagnostic tests. For example, the FDA recommends use of IHC-22C3 as a companion diagnostic test for treatment selection of previously untreated metastatic non-squamous non-small cell lung cancer (NSCLC) patients whose tumors express PD-L1 (TPS) at a level of ≥50%, second line for NSCLC patients with ≥1% expression and patients with advanced gastric or gastroesophageal junction adenocarcinoma who have CPS of ≥1 [41].

In our study there was no correlation between TMB and IHC PD-L1 staining results, which may be due to the small number of cases we evaluated. Thus, in an individual patient, a tumor with a low TMB should still be evaluated for PD-L1 expression, since a low TMB (even coupled with retained DNA MMR function) does not exclude the possibility of a level of PD-L1 expression that would indicate that immunotherapy would be of clinical benefit.

Finally, all 5 cases on which genetic analysis was performed showed germline *SMARCB1* mutations, consistent with the recognized rhabdoid tumor predisposing syndrome. Given that there are other, admittedly rare, tumors that show complete loss of *SMARCB1* including cribriform neuroepithelial tumor, renal medullary carcinoma, and epithelioid sarcoma it will be of interest to determine whether these tumors show a similar pattern of TMB; DNA MMR abnormalities; and PD-L1, PD-1, and CD8 staining as MRT/ATRT [42].

In summary, the current study demonstrates that MRT/ATRT, a pediatric malignancy defined by germline or somatic *SMARCB1*, or rarely *SMARCB4*, mutations as the key oncogenic driver, usually harbors a very low TMB, with no significant increase in TMB between primary and metastatic or recurrent tumors. However, 50% of cases show a PD-L1 TPS ranging from 10–70%, and PD1 expression in TIL ranging from 10% to more than 50%, with no correlation of TMB with level of IHC staining for PD-L1. Taken together, the findings suggest that even in patients whose tumor shows a low TMB, PD-L1 expression should be evaluated to identify those cases which could putatively benefit from checkpoint blockade immunotherapy.

Conflict of interest

The authors have no financial disclosures and no conflicts of interest.

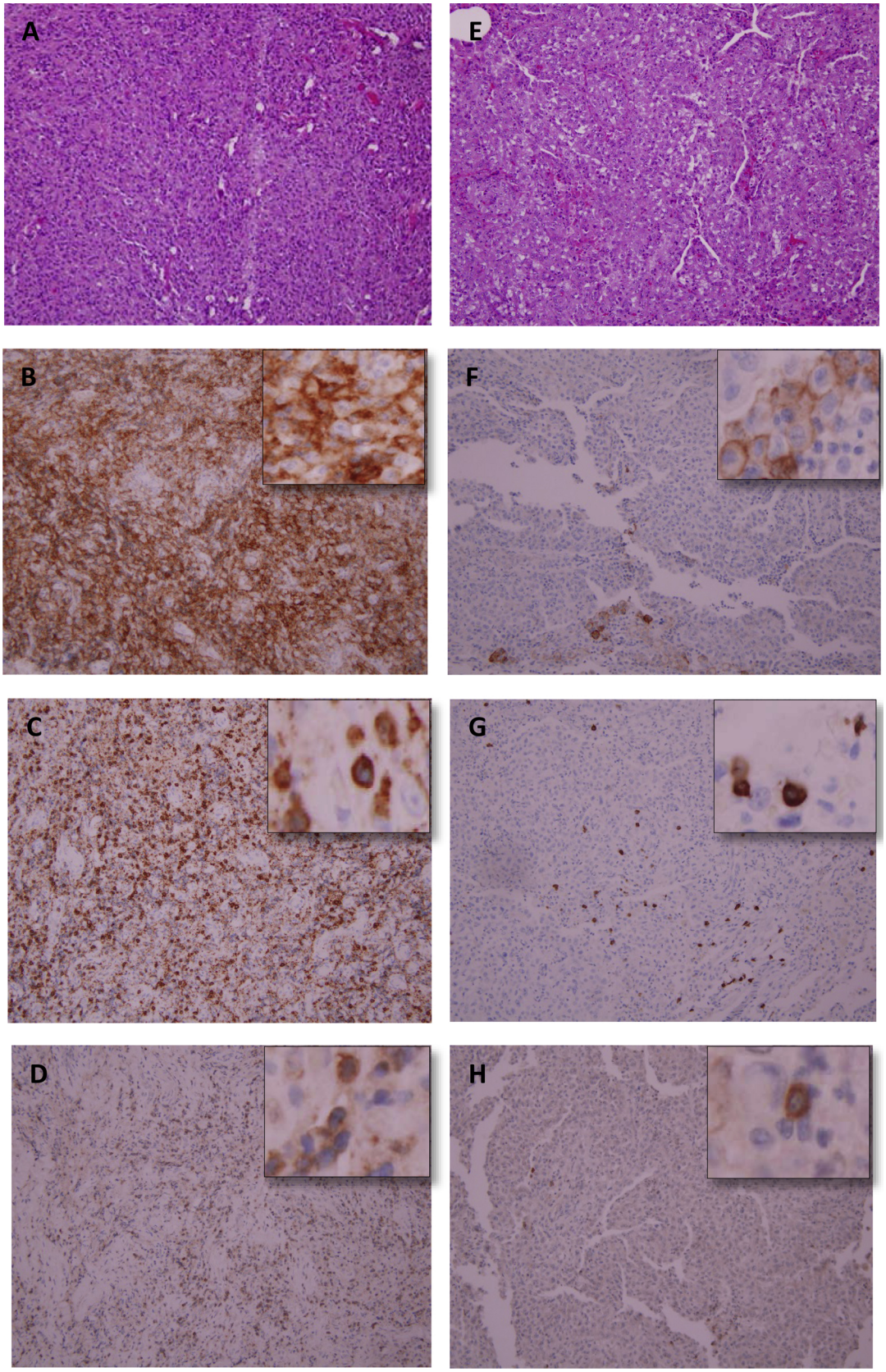

Fig. 2. Representative images from primary and metastatic tumor, case 16; (A–D) Primary tumor- H&E, PD-L1, CD-8, PD-1; (E, F) Metastatic tumor- H&E, PD-L1, CD-8, PD-1. Images captured at 20× magnification (insets captured at 40× magnifications).

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