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Case report

Molecular characterization of a bladder pleomorphic rhabdomyosarcoma in an adult patient

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ABSTRACT

Pleomorphic rhabdomyosarcoma (PRMS) is a rare but highly aggressive soft tissue tumor, accounting for 3% of soft tissue sarcomas. PRMS is the most frequent subtype of RMS in adulthood and it is mainly located in the large muscles of the extremities, particularly the lower limbs and the trunk, more rarely in other locations especially in the bladder. At our knowledge, only six cases of adult pleomorphic rhabdomyosarcoma of the bladder have been reported in the literature.

In this study, we report a case of PRMS of bladder with a very poor prognosis. In fact, the patient died a month after surgery. The tumor was characterized by poorly differentiated, medium-sized sometimes rhabdoid cells, mixed with large-sized and pleomorphic elements with evident anisonucleosis, and with large areas of necrosis. We used an extensive immunohistochemical panel to exclude other tumors much more frequently reported at this site. The positivity for myogenic markers such as actin, desmin, myogenin and MyoD1 allowed the correct diagnosis. Furthermore, since preliminary studies highlighted a series of specific molecular alterations in PMRS cell lines, we analyzed a panel of specific mutations and gene rearrangements by RT-PCR and FISH methods.

We showed a copy gains of *CCND1* and *MALT* genes in our samples, suggesting an accurate molecular characterization of PRMS to establish a better management of patients and new therapeutic opportunities.

1. Introduction

Rhabdomyosarcoma (RMS) is the most prevalent soft tissue tumor in children and adolescents, accounting for 5% of all pediatric tumors while it is rare in adults [1]. The World Health Organization (WHO) revised the classification of RMS subtypes as alveolar rhabdomyosarcoma (ARMS), embryonal rhabdomyosarcoma (ERMS), pleomorphic rhabdomyosarcoma (PRMS), and sclerosing/spindle cell rhabdomyosarcoma (SRMS) [2].

Despite the rarity, the most common subtype of rhabdomyosarcoma occurring in adults is the PRMS variant, accounting for up to 5% of all adult pleomorphic soft tissue sarcomas and frequently associated with a poor prognosis [3]. PRMS usually occurs in the extremities of adult men especially in the thigh of middle-aged adults and only rarely may occur at any other site. Particularly in the bladder, there are only few reported cases [4–6].

Due to the similarities in clinical manifestations and imaging features between PRMS and other soft tissue tumors, PRMS is often clinically misdiagnosed. Furthermore, its rare localization at visceral sites may suggest a differential diagnosis with other tumor types, mostly undifferentiated carcinoma and lymphoma. Immunohistochemical approach to identify PRMS is based on the positivity for myoglobin, myoD1, skeletal muscle myogenin, fast skeletal muscle myosin, other than desmin, MSA, SMA antibodies.

Abbreviations: RMS, Rhabdomyosarcoma; WHO, World Health Organization; ARMS, Alveolar rhabdomyosarcoma; ERMS, Embryonal rhabdomyosarcoma; PRMS, Pleomorphic rhabdomyosarcoma; SRMSSclerosing/spindle, cell rhabdomyosarcoma (); FISH, Fluorescence In Situ Hybridization; MyoD1Myogenic, Differentiation 1; MSAMitotic, spindle associated antigen; SMASmooth, muscle Actin; FOXO1Forkhead, box protein O1; CCND1Cyclin, D1; MDM2Mouse, double minute 2 homolog; MALT1Mucosa-associated, lymphoid tissue lymphoma translocation protein 1; BRAFv-raf, murine sarcoma viral oncogene homolog B1; NRASneuroblastoma, RAS viral oncogene homolog; KRASKi-ras2, Kirsten rat sarcoma viral oncogene homolog; HRASv-Ha-ras, Harvey rat sarcoma viral oncogene homolog; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PD-L1, Programmed Death-Ligand 1

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Recent molecular and genetic analysis of these tumors has produced substantial new insights into molecular cell biology, molecular cytogenetics, and tumorigenesis, leading to a better understanding of RMS development at the molecular level. However, there are limited studies on the biological pathways involved in PRMS, compared with the two more prevalent subtypes, ARMS and ERMS. Typical chromosomal aberration of alveolar rhabdomyosarcoma is *FOXO1* rearrangements, but fluorescence In Situ Hybridization (FISH) reveals sporadic amplification of *JUN, MYC, CCND1, INT2, MDM2*, and *MALT* in PRMS, suggesting their contribution in its pathogenesis [7].

The current study reports an adult case of PRMS, with a very rare localization in urinary bladder, diagnosed by morphologic and immunohistochemical approach, molecularly characterized by qRT-PCR and FISH methods.

2. Case presentation

The present study was approved by the Ethics Review Committee of INT-IRCCS Pascale of Naples, and written informed consent was obtained from the patient. A 57 year-old-man was admitted to our hospital because of a pelvic mass apparently originating from the urinary bladder. Cystoscopy revealed a solid mass in the left lateral wall of the bladder and a diagnostic biopsy was taken from. Microscopic analysis revealed that the lesion infiltrated the suburothelial connective tissue and muscularis propria. The tumor was characterized by poorly differentiated, medium-sized cells, mixed with large-sized and pleomorphic elements with evident anisonucleosis (Fig. 1a,b), and with large areas of necrosis.

To rule out the possibility of a neuroendocrine carcinoma, an undifferentiated carcinoma, a sarcomatoid carcinoma or an high-grade lymphoma, an extensive immunohistochemical panel was performed on the sample. The initial immunohistochemical profile revealed a focal positivity for desmin (Fig. 2), a positivity for EMA in very few cells, and CD56, and a negativity for GATA3, CKAE1/AE3, CK7, CK20, chromogranin, synaptophysin, CD45, CD20, CD3, CD30, ALK(D5F3), S100 and SLUG1. For a definitive diagnosis, other markers have been added to the initial IHC panel and the subsequent immunohistochemical profile, with negativity for calponin, focal positivity for actin1A4 and MyoD1 and moderate for myogenin1, supported the diagnosis of pleomorphic rhabdomyosarcoma (Fig. 2 a,b).

Because of a severe bleeding, the patient shortly underwent to a radical bladder and prostate excision with removal of the left iliac lymph nodes. Gross examination revealed a well demarcated 8 × 6 cm mass. Microscopically, the tumor infiltrates the whole thickness of the bladder wall, the prostate stroma, and the ureteral wall with involvement also of the surgical margins. By the light microscopy, the mass consisted of a small round even rhabdoid cells similar to ones found in the original biopsy with more extensive pleomorphism and more abundant atypical mitotic figures. The immunohistochemical findings were the same confirming the initial diagnosis of pleomorphic rhabdomyosarcoma. A lymphovascular invasion and perineural infiltration were also present (Fig. 1 c,d).

The sample was subsequently molecularly characterized for the identification of some chromosomal aberrations known in rhabdomyosarcoma and for the search of a series of gene mutations, potentially predictive of target therapies. In addition, because therapies with checkpoint inhibitors begin to be investigated in sarcoma patients [8], we performed the immunohistochemical analysis of PD-L1, using two different antibody clones, SP263 and SP142. The results showed that only SP263 was moderately positive on the immune cells of the tumor microenvironment and not on tumor cells (Fig. 2c).

FISH analysis was performed on 4 μm-thick paraffin-embedded tissue according to the manufacturer's instructions kit using VP2000 machine (Abbott Molecular Inc, Des Plaines, IL). For detection of rearrangements involving the *FOXO1* gene located on chromosome 13q14 was used the Vysis *FOXO1* dual color Break Apart FISH Probe. The identification of the copy number alteration of *MDM2, MYC, CCND1* and *MALT1* genes were performed with different fluorescent-labeled probes: i) Vysis *MDM2*/CEP12 FISH Probe, a mix of two probes Spectrum Orange for detection of *MDM2* gene on 12q*15* and Spectrum Green probe for centromere chromosome 12 (12p11.1-q11), ii) Vysis LSI *IGH/MALT1* dual color dual fusion probes a mixture of Spectrum Orange probe spans which covers the *MALT1* gene region and Spectrum Green probe spans the IGH region; iii) Vysis LSI *MYC* dual color break-apart probe (8q24), a mixture of Spectrum Orange probe starts to centromeric *MYC* gene and Spectrum Green probe begins

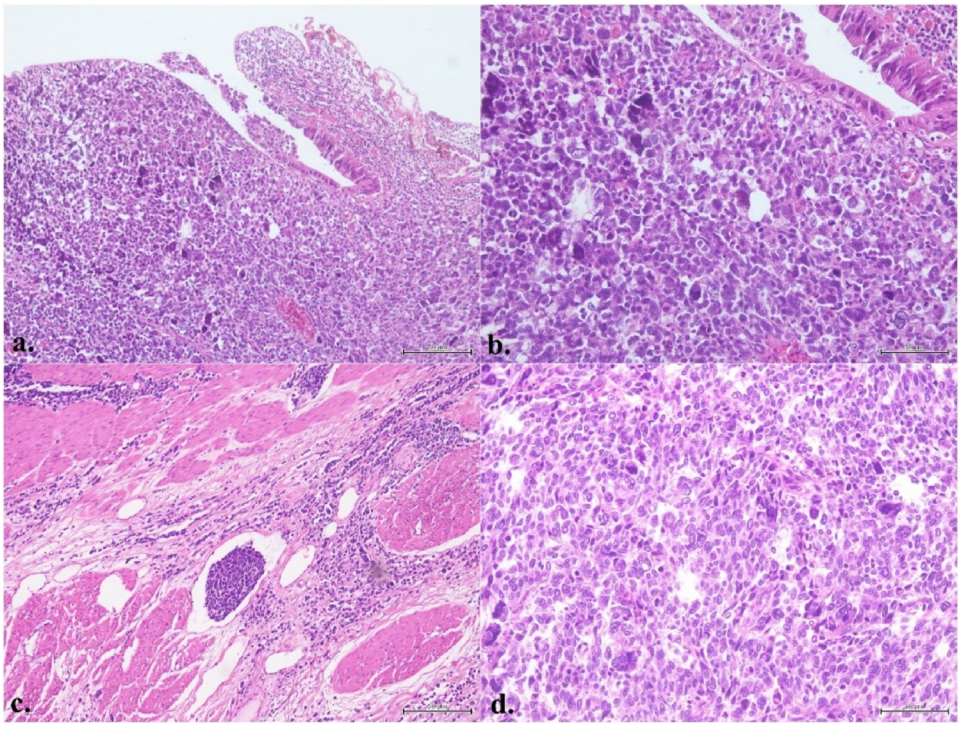

Fig. 1. Bladder PRMS microscopic features. (a) Hematoxylin–eosin of a biopsy fragment (10X); (b) Hematoxylin–eosin with details of atypical, roundish, elongated and pleomorphic cells in the biopsy (20X) (c) Hematoxylin–eosin of surgical specimen showing infiltration of the bladder wall with a neoplastic intravascular aggregate (10X); (d) Hematoxylin–eosin of the surgical specimen with details of atypical, roundish, elongated and pleomorphic cells (20X).

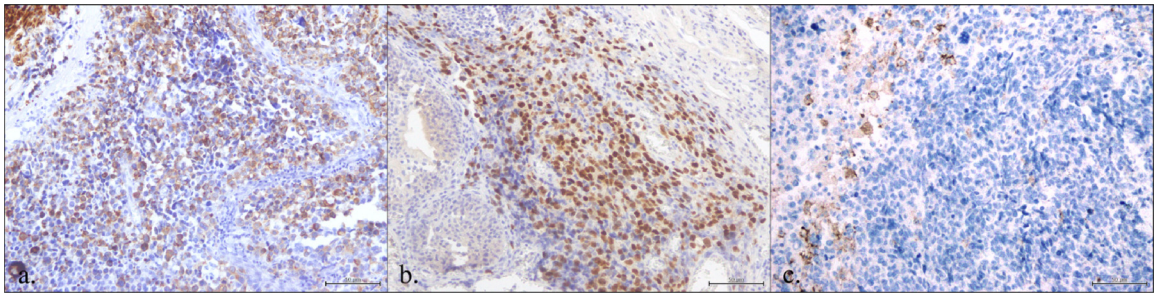

Fig. 2. Immunohistochemical profile of PRMS. (a) Focal positivity for Desmin (20X); (b) Diffuse and strong positivity for myogenin (20X); (c) Moderate positivity for PD-L1 in immune cells of the microenvironment. PDL1 was completely absent in neoplastic cells (20X).

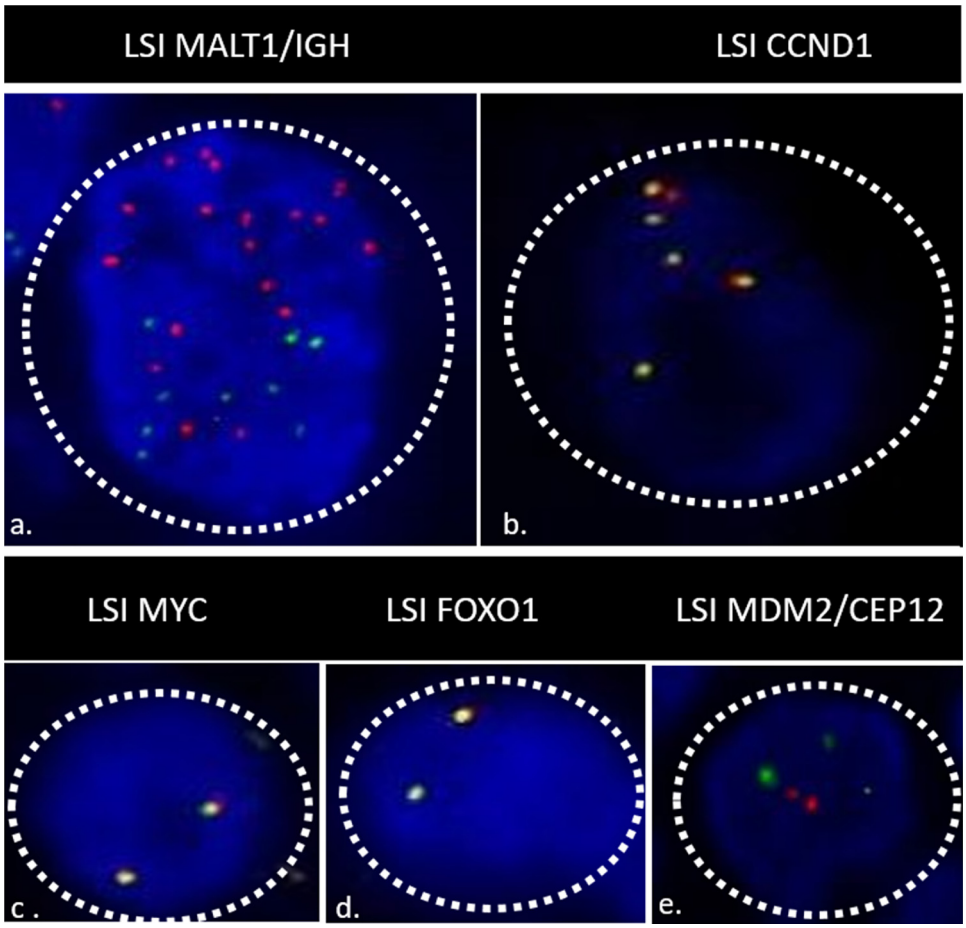

Fig. 3. Representative result of FISH analysis on PRMS sample. Abnormal signals pattern for (a) *MALT1* (6 Orange signals) (b) *CCND1* (6 fusion signals) (c) *MYC* no rearranged 2 fusion signals (d) *FOXO1* no rearranged 2 fusion signals and (e) Normal FISH results for the *MDM2* gene (2 orange signals) and CEP12 (3 Green signals).

approximately telomeric to the *MYC* gene; iv) LSI *CCND1* dual color break-apart probe (11q13) a mixture of Spectrum Green probe hybridizes centromeric to *CCND1* and Spectrum Orange probe spans and covers *CCND*1 (Abbott Molecular Inc, Des Plaines, IL). FISH analysis showed the presence of the *CCND1* (average of 4.01 signals/cell) and *MALT* (average of 5.2 signals/cell) copy gains. No chromosomal aberrations in *MDM2, FOXO1* and *MYC* genes were found (Fig. 3). Regarding mutation analysis, one slide stained with hematoxylin and eosin (H&E), was evaluated by the pathologist for the quantitative presence of tumor cells and DNA isolation was performed after the macrodissection from FFPE whole sections. Genomic DNA was derived from FFPE tissue using Qiagen's QIAamp FFPE Mini Kit (QIAGEN, Dusseldorf, Germany), according to the manufacturer's instructions. The case was evaluated for V600E *BRAF, NRAS, KRAS, HRAS* and *PIK3CA* mutations using three real-time PCR mutation assays based on mutation-specific PCR (Entrogen, Woodland Hills, CA). The first kit detects V600E exon 15 *BRAF*, codons 12 – 13 exon 2 *KRAS*, codon Q61 exon 3 *NRAS* and G12 V, G13R and Q61R *HRAS* mutations. The second kit detects the 13 most common mutations in codons 12, 13, 59, 61, 117 and 146 of *NRAS* gene (EntroGen). The third kit detects codons 542 – 545 exon 9 and codon 1047 exon 20 *PIK3CA* mutations. The samples showed no mutations in hot spot regions of genes analyzed.

All details of molecular and immunohistochemical panels are summarized in Table 1.

The patient died about a month after surgery before starting any chemotherapy regimen.

3. Discussion

PRMS is a rare tumor, accounting for up to 5% of all adult pleomorphic soft tissue sarcomas. PRMS is usually detected in the extremities especially in the thigh of middle-aged adults while urinary bladder involvement is very rare with only 6 cases reported in the literature [4–6]. Historically, Stout first introduced PRMS into the

Table 1
Immunohistochemical antibodies, FISH probes, and mutation assays panels.

Immunohistochemical analysis	Antibodies	Clones	Manufacturing Company
	GATA3	L50 − 823	Ventana Medical Systems, Arizona, USA
	CKAE1/AE3	AE1/AE3	DAKO, Agilent, Santa Clara, CA 95051, USA
	CK7	RN7	Leica Biosystems, Buffalo Grove, IL 60089, USA
	CK20	SP33	Ventana Medical Systems, Arizona, USA
	Chromogranin	5H7	Leica Biosystems, Buffalo Grove, IL 60089, USA
	Synaptophysin	27G12	Leica Biosystems, Buffalo Grove, IL 60089, USA
	CD45	SP19	Ventana Medical Systems, Arizona, USA
	CD20	L26	Ventana Medical Systems, Arizona, USA
	CD3	2GV6	Ventana Medical Systems, Arizona, USA
	CD30	BER-H2	Ventana Medical Systems, Arizona, USA
	ALK(D5F3)	D5F3	Ventana Medical Systems, Arizona, USA
	S100	GA50A	DAKO, Agilent, Santa Clara, CA 95051, USA
	SLUG1	sc-15,391	Santa Cruz Biotechnology, Inc, CA, USA
	Calponin	EP789Y	Ventana Medical Systems, Arizona, USA
	Actin1A4	1A4	DAKO, Agilent, Santa Clara, CA 95051, USA
	MyoD1	5.8A	DAKO, Agilent, Santa Clara, CA 95051, USA
	Myogenin	L026	Leica Biosystems, Buffalo Grove, IL 60089, USA
	PD-L1	SP263, SP142	Ventana Medical Systems, Arizona, USA
FISH analysis	Probes	Manufacturing Company	
	LSI FOXO1 Dual Color, Break Apart Rearrangement Probe	Vysis, Abbott Molecular, Abbott Park, Illinois, U.S.A.	
	MDM2/CEP 12 FISH probe	Vysis, Abbott Molecular, Abbott Park, Illinois, U.S.A.	
	LSI IGH/*MALT1* dual color dual fusion	Vysis, Abbott Molecular, Abbott Park, Illinois, U.S.A.	
	LSI *MYC* dual color break-apart	Vysis, Abbott Molecular, Abbott Park, Illinois, U.S.A.	
	LSI *CCND1* dual color break-apart	Vysis, Abbott Molecular, Abbott Park, Illinois, U.S.A.	
Mutational analysis	Real-time PCR mutation assays	Manufacturing Company	
	BRAF (codon 600)	Entrogen, Woodland Hills, CA, USA	
	KRAS (codons 12 − 13)	Entrogen, Woodland Hills, CA, USA	
	NRAS (codons 12, 13, 59, 61, 117 and 146)	Entrogen, Woodland Hills, CA, USA	
	HRAS (codons 12, 13, 61)	Entrogen, Woodland Hills, CA, USA	
	PIK3CA (codons 542 − 545, 1047)	Entrogen, Woodland Hills, CA, USA	

literature in 1946 as “classical” rhabdomyosarcoma [9]. It is currently defined as a high-grade sarcoma composed of atypical spindle or pleomorphic cells and/or undifferentiated roundish cells that show skeletal-muscle differentiation without embryonal or alveolar components.

In this study we described a 57 year-old-man with a diagnosis of PRMS with a very rare presentation in the bladder which required an extensive immunophenotypic characterization to exclude other entities, especially sarcomatoid carcinoma which is much more frequent in this particular site. The IHC panel allowed us to support the suspected morphological diagnosis.

Because of the high propensity for metastasis, the unfavorable clinical behavior and poor to chemotherapy responsiveness in PRMS, we performed an IHC evaluation for PD-L1 and specific molecular analyses. PD-L1 was moderately expressed only on lymphocytes of the tumor microenvironment, but at the moment the implication of this finding for the response to treatment with checkpoint inhibitor is not clear yet in sarcomas [8]. The molecular characterization has instead highlighted a biological and genomic complexity more similar to adult high-grade soft-tissue sarcomas than to paediatric RMS. PRMS is often characterized by a complex karyotype unlike that of alveolar RMS and of embryonal RMS [10]. Our case was molecularly characterized for the known mutations in *BRAF, NRAS, KRAS, HRAS* and *PIK3CA* genes, sporadically described in RMS, for *FOXO1* translocation typical of ARMS, and for *MDM2, MYC, CCND1* and *MALT1* genes rearrangements described in several PRMS cell lines [7].

No gene mutations, no rearrangement of *FOXO1, MDM2* and *MYC* was highlighted in our sample. Indeed, we found copy gains of *CCND1*and *MALT* genes by FISH analysis.

Gene amplification is an important cytogenetic manifestation of genetic instability. *CCND1* is a proto-oncogene that encodes cyclinD1, which is a key regulator of the G1 phase of the cell cycle. CyclinD1 binds and activates *CDK4* and *CDK6*, and this complex catalyzes Rb protein phosphorylation resulting in the release of transcriptional regulators E2F from Rb, which promotes cell cycle progression. Deregulated expression via mutations, gene rearrangements, or amplification of CCND1 has been reported in various cancer types showing a critical role in tumor initiation and progression by proto-oncogene activation [11]. In sarcoma cells *CCND1* amplification has been reported in a synovial sarcoma cell line and *CCND1* rearrangement in a small percentage of cells in a subset of patient samples [12]. A copy gain (3–5 copies) of *CCND1* was found in sporadic cases of well-differentiated/dedifferentiated liposarcoma [13], and in HS-RMS-2 cells, a cell line established from a pleomorphic type of rhabdomyosarcoma. In 2015, Palbociclib, a small molecule inhibitor of *CCND1/CDK4* kinase activity and *CDK6*, was approved for advanced breast cancer and several other studies suggested that *CCDN1* amplification can be predictive of drug response in different tumors [12].

MALT1 has been shown to be a key mediator in NF-κB activation in T and B lymphocyte, and its genetic alterations are considered critical events in the pathogenesis of certain types of lymphoma. *MALT1* contributes to cell survival by regulating growth factor-induced NF-κB pathway also in solid tumors [14–16]. *MALT1* amplification, other than found in some B-cell non-Hodgkin lymphomas [17], was described in HS-RMS-2 cells from PRMS [7].

The therapeutic value of *MALT1* is associated with the protein’s caspase-like domain, which contains an arginine-specific protease activity [18]. Although *MALT1* inhibitors currently represent potential therapeutic targets only for some subtypes of lymphomas, more recent studies also suggest their use in several solid tumors [19]. Moreover, *MALT-1* expression appeared as a predictive biomarkers in other cancers. For example, intrahepatic cholangiocarcinoma *MALT1*-positive patients are more sensitive to the use of drugs such as Regorafenib, a potent oral inhibitor of multiple kinases [20].

Due to the aggressive nature of PRMS and its high tendency to metastasize to various sites, a molecular characterization of this tumor

could offer a better management of patients at risk of progression and could suggest new therapeutic opportunities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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