

Hyperprogression after anti-programmed cell death ligand-1 therapy in a patient with recurrent metastatic urothelial bladder carcinoma following first-line cisplatin-based chemotherapy: a case report

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Background: Immune checkpoint blockade targeting programmed cell death ligand-1 (PD-L1)/programmed death-1 (PD-1) signaling was approved recently for locally advanced and metastatic urothelial bladder carcinoma (UBC). Some patients experience a very rapid tumor progression, rather than clinical benefit, from anti-PD-L1/PD-1 therapy.

Case presentation: A 58-year-old male diagnosed with non-muscle-invasive bladder cancer 3 years ago received transurethral resection of bladder tumor (TURBT) and intravesical chemotherapy. TURBT was repeated a year later for recurrent and progressive UBC. Following further disease progression, he received a radical cystectomy (RC), pathologically staged as T2bN2M0, and adjuvant cisplatin-containing combination chemotherapy. When his disease progressed to metastatic UBC, he was started on anti-PD-L1 monotherapy and experienced ultrarapid disease progression within 2 months; imaging scans ruled out pseudoprogression. We observed a fourfold increase in tumor growth rate, defined as the ratio of post- to pretreatment rates. Next-generation sequencing of formalin-fixed paraffin-embedded RC tissues showed *MDM2* amplification without *MDM4* amplification, *EGFR* aberrations, or *DNMT3A* alterations. Immunohistochemistry showed grade 2+ PD-L1 labeling intensity of the RC tissues, with 15%–25% and 5%–10% PD-L1 immunopositive tumor cells and tumor-infiltrating immune cells, respectively.

Conclusion: Even in cases with PD-L1-positive tumors, *MDM2* gene amplification may result in failure of anti-PD-L1 immunotherapy and rapid tumor growth. Therefore, genomic profiling may identify patients at risk for hyperprogression before immunotherapy.

Keywords: urothelial bladder carcinoma, programmed cell death ligand-1, immune checkpoint blockade, hyperprogression, *MDM2*

Introduction

Although platinum-based combination chemotherapy often prolongs the survival of patients with locally advanced or metastatic urothelial bladder carcinoma (UBC), progression remains almost inevitable with a median overall survival of only 14 months in 2014.¹ The recent US FDA approval of immune checkpoint inhibitors that target the programmed cell death ligand-1 (PD-L1)/programmed death-1 (PD-1) receptor axis has changed how advanced or metastatic UBC is managed.² Monoclonal PD-L1 antibodies can revitalize and enhance anticancer immunity by preventing PD-L1 from binding to PD-1 receptors.³

PD-L1 antibody was confirmed to produce durable objective responses and to have good tolerability in patients with inoperable advanced or metastatic UBC,⁴⁻⁷ leading to

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its approval for use in patients whose disease progressed during or within 12 months following neoadjuvant or adjuvant platinum-based chemotherapy.⁸ However, immunomodulatory therapies, such as PD-L1 immunotherapy, can produce opposing effects in a subset of patients. Indeed, there have been several recent reports of patients who experienced rapid tumor progression while on immune checkpoint blockade (ICB), consistent with ICB-promoted hyperprogression.^{9–14} Thus, there is a critical and urgent need to identify the predictors and mechanisms of such hyperprogression to prevent tragic adverse outcomes of ICB. A recent study showed an association between tumor hyperprogression and specific genomic alterations, including *MDM2* family amplification and *EGFR* aberrations.¹⁴

Here, we report the case of an adult male patient with recurrent metastatic UBC whose disease progressed following platinum-based chemotherapy and then hyperprogressed shortly after initiation of ICB. UBCs have been reported to have relatively high PD-L1 expression among all cancers, and elevated PD-L1 expression intensity has been related to a higher probability of clinical response.^{6,7,15–17} Thus, we investigated the genomic profile and PD-L1 protein expression of the patients' primary tumor following radical cystectomy (RC).

Case presentation

Patient characteristics and history

A 55-year-old man presented with left hip pain in October 2014. An initial workup revealed a left posterior mass in his bladder. Transurethral resection of bladder tumor (TURBT) pathology indicated stage-TaG3 UBC. After the TURBT procedure, he began a 12-month course of intravesical instillation of epirubicin chemotherapy. However, 14 months after the resection surgery, a cystoscope examination revealed bladder tumor recurrence. TURBT pathology indicated that the recurrent tumor was stage T1G3. The patient then received an additional 12-month course of adjuvant intravesical epirubicin chemotherapy instillations.

Disease progression was detected 11 months later, and TURBT pathology indicated that the advancing lesion was a stage T2G3 N0 UBC. He then received a RC, and the removed tumor was pathologically staged as T2bN2M0. Subsequently, he was treated with adjuvant cisplatin-containing combination chemotherapy for 3 months.

Twelve months after the RC, follow-up chest radiography and computer tomography (CT) revealed metastases in the right lumbar muscles, left adrenal gland, and lungs (Figure 1). In addition to bladder cancer, patient had no other history of cancer. The patient's right lumbar mass biopsy puncture

results indicated urothelial carcinoma. The patient was started on PD-L1 blockade monotherapy on December 19, 2017. Chest radiography and a full-body CT on January 15, 2018 showed pronounced enlargement of a left lung metastasis (1,004% increase from preimmunotherapy size) and progression of the right lumbar muscle and left adrenal gland metastases, as well as new multiple lymph node metastases involving a mediastinal, a left supraclavicular, and two hilar lymph nodes (Figure 1). He had developed a progressively enlarging right back mass with localized swelling and persistent severe pain, and was therefore admitted to our hospital.

In the hospital, while still receiving PD-L1 blockade monotherapy, the patient experienced unusually rapid disease progression demonstrated in repeated CT scans to rule out pseudoprogession. The patient terminated the immunotherapy after receiving two cycles of PD-L1 blockade treatment due to his rapid disease progression. A full-body CT, upper abdomen MRI, and positron emission tomography-CT on January 29, 2018 showed rapid progression of the metastatic lung lesions (1,078% increase from pre-immunotherapy cumulative size) and continued growth of the right lumbar muscle and left adrenal metastases, as well as the emergence of three liver metastases and at least seven bone metastases. Upon discovery of these changes, the patient's treatment plan was changed to cisplatin/gemcitabine chemotherapy. One month after the patient began cisplatin/gemcitabine chemotherapy, we observed drastic reductions in lesion size (Figure 1).

To evaluate the patient's treatment responses, we calculated tumor growth rate (TGR) vis-à-vis comparisons of tumor volume over time. TGR ratio was defined as the ratio of tumor volume growth change after, relative to that observed prior to, the treatment of interest. Comparing the TGR for the 8-week period following ICB to that for the 1-week period prior to ICB, we determined that the patient had a TGR ratio of 4.0, reflecting a fourfold increase in growth rate in association with ICB onset, meeting the criteria for hyperprogression (Figure 2). We employed Kato et al's definition of hyperprogression criteria as follows: time-to-treatment failure (TTF) <2 months; increase in tumor burden >50%; and a >2-fold increase in TGR.¹⁴ All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of Shanghai Tenth People's Hospital (SHSY-IEC-4.0/17-16/01) and with the 1964 Helsinki declaration and its amendments or comparable ethical standards. Written informed consent was obtained from the patient to have the case details and any accompanying images published. The publication of the case details was approved by ethics committee of Shanghai Tenth People's Hospital.

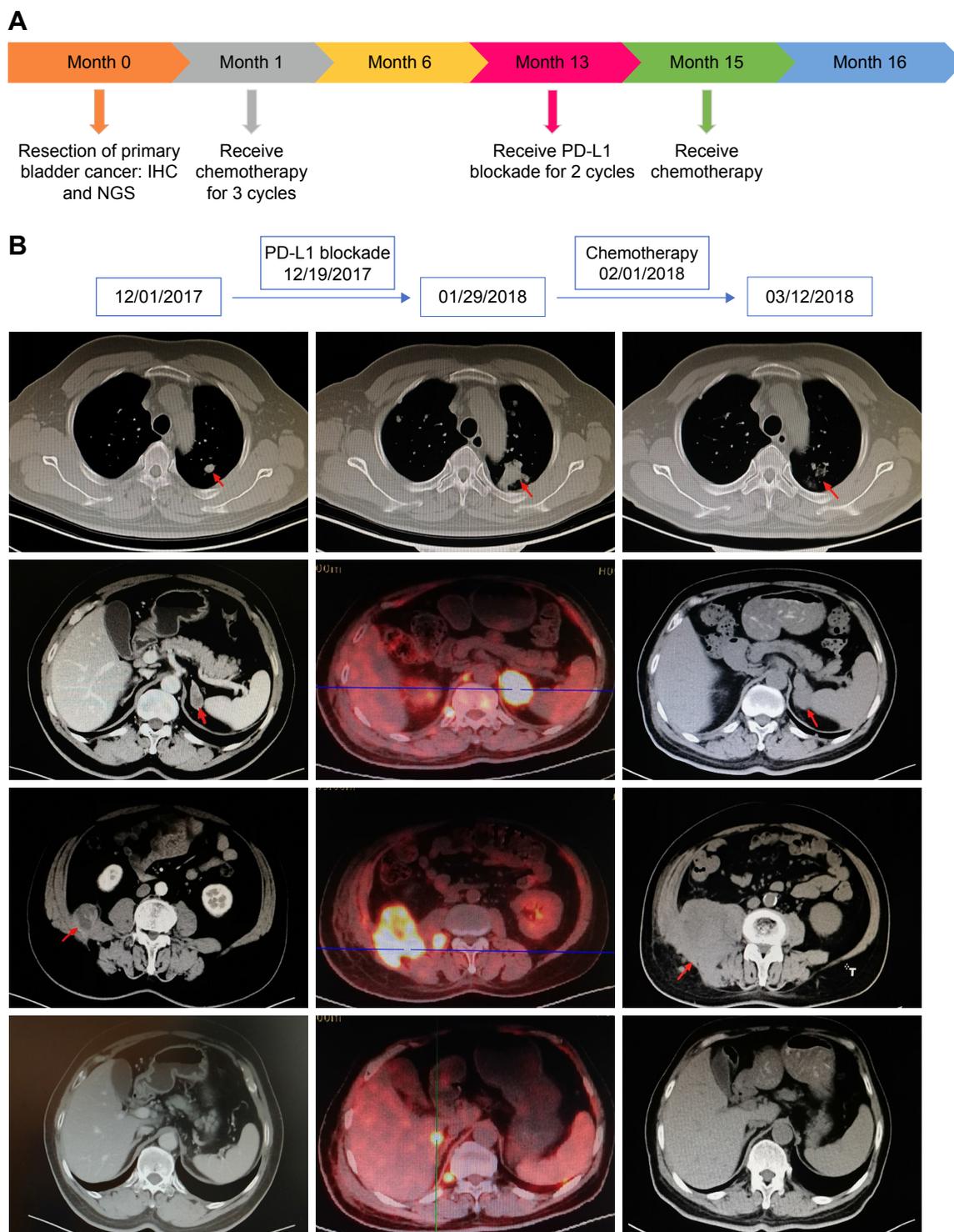


Figure 1 Treatment intervention process and imaging of disease progress after PD-L1 blockade.

Notes: (A) Summary of interventions received by the present patient. Arrowheads indicate time points for each intervention. (B) PET/CT or CT images for metastatic lesions before and after PD-L1 blockade.

Abbreviations: PET, positron emission tomography; CT, computer tomography; PD-L1, programmed cell death ligand-1; IHC, immunohistochemistry; NGS, next-generation sequencing.

Assessments

Formalin-fixed paraffin embedded RC tissue samples were obtained from the Department of Pathology, Shanghai Tenth People's Hospital. The samples were subjected to

next-generation sequencing (NGS) and immunohistochemistry (IHC) with the aim of identifying possible predictive factors for immunotherapy-triggered hyperprogression. NGS was performed with a 499-gene panel assay (Table S1).

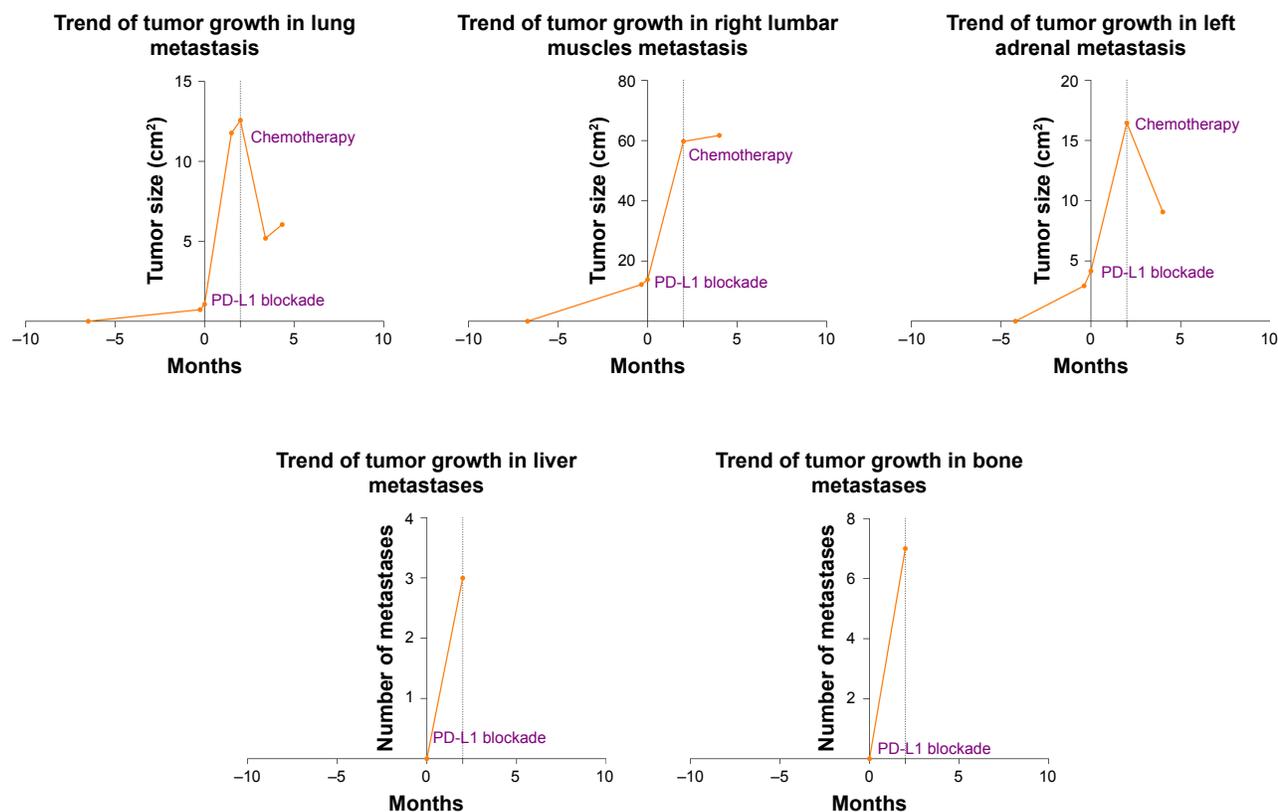


Figure 2 Tumor metastasis changes over time. The 0-month time point represents the start of PD-L1 blockade treatment. **Abbreviation:** PD-L1, programmed cell death ligand-1.

The panel included sequences for multiple gene variants previously suggested to be associated with hyperprogression including *MDM2* family amplification, *EGFR* aberration, and *DNMT3A* alteration sequences. The mean sequencing coverage depth exceeded 15,000 \times . The NGS method employed revealed copy number alterations, gene rearrangements, and somatic mutations with 95% specificity and >90% sensitivity. The presence of ≥ 3 gene copies was considered gene amplification. IHC carried out with monoclonal rabbit anti-PD-L1 antibody (clone MXR003, working solution for 15 hours; Fujian Maixin, Fujian, PR China), goat anti-rabbit and -mouse secondary antibody (PV-6000, working solution for 1 hour; ZSGB-BIO, Beijing, PR China), and horse-radish peroxidase to enhance visualization (ZLI-9017; ZSGB-BIO). IHC-AP cell membrane staining intensity was graded as follows: 0, none; 1+, weak or incomplete; 2+, weak to medium; 3+, medium to strong and complete.

Predictors of hyperprogression

NGS showed that the RC specimen from the present case had several malignancy-related alterations, including *MDM2* amplification, a *KRAS* mutation, and a *KMT2D* mutation. It was not harboring an *MDM4* amplification, *EGFR* aberrations, or *DNMT3A* alterations. The genomic alterations found are reported

in Table 1 with descriptive information, including abundance, location, base and amino acid changes, and type of mutation.

To calibrate PD-L1 expression relative to the proportion of tumor cells present in the RC specimen, alternate sections were subjected to H&E staining and anti-PD-L1 IHC prior to evaluating PD-L1 expression. In the H&E-stained sections (Figure 3A), we observed a 40% tumor cell ratio; >100 PD-L1 immunopositive tumor cells were examined under a light microscope. PD-L1 staining was localized primarily to cell membranes, with some non-specific cytoplasm staining. Tumor-associated immune cells had PD-L1 immunopositive cytoplasm and membranes. Both tumor cells and tumor-infiltrating immune cells had grade 2+ PD-L1 staining intensity. We found that 15%–25% and 5%–10% of tumor cells and tumor-infiltrating immune cells, respectively, showed PD-L1 immunopositivity (Figure 3B).

Discussion

Blockade of the PD-1/PD-L1 pathway has produced durable clinical responses for some solid tumors and anti-PD-L1 agents have demonstrated a manageable safety profile and favorable clinical activity in patients with advanced, previously treated UBC.^{2,5-7} Currently, it is still a challenge to select the patients most likely to respond to

Table 1 Summary of NGS-revealed gene mutations

Gene	Location	Base mutation	Amino acid change	Abundance	Mutation type
KRAS	chr12:25398284	c.35G>A	p.Gly12Asp	22.82%	Missense
KMT2D	chr12:49426895	c.11593C>T	p.Gln3865Ter	21.78%	Nonsense
MDM2	–	–	–	11.92 copies	Amplification
SPEN	chr1:16264490	c.10693C>T	p.Arg3565Ter	1.26%	Nonsense
NOTCH2	chr1:120462059	c.5657G>A	p.Arg1886His	1.09%	Missense
AR	chrX:66765516	c.528C>A	p.Ser176Arg	95.11%	Missense
MUTYH	chr1:45798136	c.715G>A	p.Val239Ile	1.52%	Missense
DDR2	chr1:162748503	c.2417G>A	p.Arg806Gln	1.43%	Missense
TCF7L2	chr10:114910785	c.904C>T	p.His302Tyr	13.55%	Missense
PTPN11	chr12:112926915	c.1535G>A	p.Arg512Gln	1.09%	Missense
IDH2	chr15:90630711	c.775G>A	p.Asp259Asn	1.19%	Missense
IGF1R	chr15:99465453	c.2278G>A	p.Ala760Thr	1.32%	Missense
PLCG2	chr16:81902844	c.505A>G	p.Ile169Val	46.35%	Missense
AXIN2	chr17:63554353	c.386G>A	p.Arg129Gln	1.08%	Missense
SMARCA4	chr19:11100064	c.1190G>A	p.Arg397Gln	1.16%	Missense
LRP1B	chr2:141283458	c.7981G>A	p.Gly2661Arg	1.55%	Missense
CASP8	chr2:202136289	c.533C>A	p.Ser178Tyr	53.30%	Missense
BAP1	chr3:52442077	c.272G>T	p.Cys91Phe	15.58%	Missense
EPHA5	chr4:66231683	c.2017T>A	p.Ser673Thr	46.75%	Missense
TET2	chr4:106155794	c.695A>G	p.Gln232Arg	46.42%	Missense
INPP4B	chr4:143043366	c.2050G>A	p.Val684Ile	27.15%	Missense
FAT1	chr4:187524812	c.10868C>T	p.Thr3623Met	44.02%	Missense
FAT1	chr4:187541475	c.6265G>A	p.Val2089Ile	41.88%	Missense
PDGFRB	chr5:149501461	c.2326G>A	p.Asp776Asn	50.00%	Missense
ARID1B	chr6:157405827	c.2069C>T	p.Thr690Met	37.91%	Missense
ETV1	chr7:14027789	c.55G>A	p.Gly19Arg	45.92%	Missense
MAGI2	chr7:78150951	c.550G>A	p.Gly184Ser	1.45%	Missense
KMT2C	chr7:151860428	c.10234C>T	p.Arg3412Trp	1.07%	Missense
KAT6A	chr8:41906155	c.341G>C	p.Gly114Ala	4.56%	Missense
PREX2	chr8:69033224	c.3664C>A	p.Pro1222Thr	50.00%	Missense
GID4	chr17:17942909	c.131G>C	p.Arg44Pro	12.48%	Missense
SOX10	chr22:38370185	c.718A>C	p.Thr240Pro	10.47%	Missense

Abbreviation: NGS, next-generation sequencing.

treatment with immunotherapeutic agents. Robertson et al reported that clustering by mRNA, lncRNA, and miRNA expression converged to identify subsets with differential epithelial–mesenchymal transition status, carcinoma-in-situ scores, histologic features, and survival in bladder cancer.

Their analyses identified five expression subtypes that may stratify response to different treatments. Among these, mRNA luminal-papillary subtype and basal-squamous subtypes show increased expression of CD270 (PD-L1) and PD-1 immune markers, which correspond to lncRNA 1

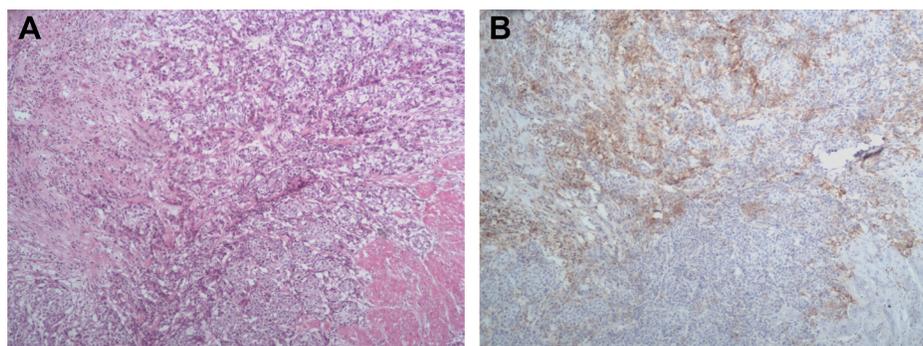


Figure 3 Anti-PD-L1 immunohistochemistry of bladder cancer tissues.

Notes: (A) H&E stained tumor section with 40% tumor cell proportion. (B) Image of IHC PD-L1 labeled section subjected to PD-L1 percentage scoring. The percentages of tumor cells and tumor-infiltrating immune cells are 15%–25% and 5%–10%, respectively.

Abbreviations: PD-L1, programmed cell death ligand-1; IHC, immunohistochemistry.

and miRNA 2 subtypes, lncRNA 4 and miRNA 4 subtypes, respectively. These two subtypes may serve as predictive markers for response to immune checkpoint therapy.¹⁸ However, the occurrence of immunotherapy-induced hyperprogression in some patients with various cancer types has drawn attention to a critical potential risk of immunotherapy.^{13,14} Reports of UBC hyperprogression with anti-PD-1 antibody treatment specifically are rare. To the best of our knowledge, the presently reported circumstance of dramatic growth and metastatic spreading of neoplastic lesions following anti-PD-L1 antibody initiation in an *MDM2*-amplified patient with UBC is quite rare. The rapid shrinking of multiple metastatic lesions, especially in the lungs, observed during the subsequent cisplatin-gemcitabine treatment indicated that the ICB-associated progression observed in this patient was not pseudoprogression but rather true hyperprogression.

Predictors of and mechanisms underlying ICB-triggered hyperprogression remain to be elucidated. The limited information available to date has implicated two clinical variables, namely older age and regional recurrence in an irradiated field,¹³ and a handful of genomic alterations, namely *MDM2/4* amplification, *EGFR* aberrations, and *DNMT3A* alterations, in hyperprogression.¹⁴ In a study of 131 patients, encompassing 21 tumor types, treated with PD-1/PD-L1 pathway blockade, without genomic profiling, Champiat et al observed rapid progression in 12 patients (9%), including 2/8 patients (25%) with bladder cancer.¹³ In a study of 155 patients with diverse cancers, Kato et al reported that 49 patients (31.6%) had poor clinical outcomes of immunotherapy, defined as a TTF <2 months. Molecular profiling of Kato et al's patient group showed that those with a poor clinical outcome harbored *MDM2/4*, *EGFR*, and/or *DNMT3A* alterations, each of which emerged as an independent predictor of a poor outcome. Six patients had *MDM2* or *-4* amplification, and all of them experienced hyperprogression, including one patient with bladder cancer harboring an *MDM2* amplification.¹⁴

In the presently reported case, this patient was only 58 years old and had not received radiation therapy (RT). Upon starting anti-PD-L1 antibody treatment, the patient experienced rapid clinical deterioration with a marked acceleration in tumor growth (fourfold increase in progression rate and TTF of 1.4 months) accompanied by the emergence of new liver and bone metastases. IHC revealed PD-L1 expression in up to a quarter of RC tumor cells and up to a tenth of tumor-infiltrating immune cells, which suggests that PD-L1 immunopositivity is not a reliable indicator of immunotherapy sensitivity. Retrospective genomic profiling by NGS aimed at identifying hyperprogression predictors and clues regarding its mechanism showed *MDM2* ampli-

fication without accompanying *MDM4* or *ERGR* alterations. Similarly, Kriegmair et al found that patients with low *MDM4* and high *MDM2* expression tended to have poor muscle-invasive bladder cancer outcomes.¹⁹ These data point to *MDM2* amplification as a predictive biomarker candidate for rapid ICB-triggered cancer progression.

Normally, PD-1/PD-L1 pathway activation is associated with anti-tumor immunity evasion that enables immunogenic tolerance. However, unfortunately, in some patients with UBC, the PD-1/PD-L1 pathway appears to have been linked with oncogenic signaling that triggers tumor proliferation and progression. Melanoma cell-intrinsic functions of PD-1/PD-L1 signaling might modulate several alternative signaling networks, including some that favor tumor growth.²⁰ Such an effect may be secondary to an accumulation of oncogenes in tumor cells. Because our patient's tumor had *MDM2* amplification, in the absence of a p53 mutation, it may be that amplification of *MDM2* inhibited the wild-type p53 tumor suppressor.²¹ Indeed, antigen-specific CD4⁺ T-cell responses have been reported to down-modulate tumor suppressor p53 through T-cell receptor signaling by decreasing expression of p53 while escalating expression of *MDM2*, the protein product of which mediates posttranscriptional inactivation of p53.²² In addition to T-cell receptor signaling increasing interferon- γ suppression of the PD-1 pathway – which activates JAK-STAT signaling thereby increasing interferon regulatory factor-8 expression – it may also induce *MDM2* expression.^{23–26}

Immune checkpoints occupy crucial regulatory pathways for the maintenance of immune homeostasis. Numerous immune cell subsets express PD-1 in tumor microenvironments, including macrophages, T cells, B cells, natural killer cells, and dendritic cells.²⁷ Thus, ICB could trigger compensatory mechanisms and adaptive immune resistance, enabling an acceleration of tumor growth.

If the presently observed hyperprogression phenomenon is specific to anti-PD-1/PD-L1 monotherapy, it might be solved with mechanistically sound combination therapies. In metastatic castration-resistant prostate cancer mouse models, intratumoral myeloid-derived suppressor cells inhibited CD4⁺ and CD8⁺ T-cell proliferation, and PD-1/PD-L1 blockade combined with myeloid-derived suppressor cell-targeted therapies yielded excellent synergistic efficacy against ICB resistance.²⁸ Indeed, RT has been reported to enhance T-cell recognition of malignant cells through induction of *MHCI* expression and neoantigen generation.²⁹ Meanwhile, PD-L1 has been found to be upregulated after RT,^{12,30} and combining RT with PD-L1 blockade has been found to enhance anti-tumor treatment effects.^{30,31} Likewise, chemotherapy has been reported to augment intra-tumor

CD8⁺ T-cell infiltration, consistent with the notion that immunogenic chemotherapies could increase the anticancer efficacy of ICB.^{32–34} These studies support the strategy of developing innovative combination therapies to overcome undesirable tumor responsiveness to PD-1/PD-L1 blockade.

In summary, genomic testing of malignant tumors prior to treatment, preferably in an early stage, may reveal which patients harbor genetic alterations associated with hyperprogression. The present case indicates that patients with *MDM2* amplification in particular should not receive anti-PD-L1 monotherapy, even in cases where tumor cells or tumor-associated immune cells are found to express PD-L1. Large-cohort studies are needed to confirm this link. ICB-triggered hyperprogression may be avoided with a combined treatment.

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Author contributions

YY and XY designed and guided the present study. SM, JZ, and YG collected the study data. SM, YW, ZZ, and WZ analyzed and interpreted the data. LW, JZ, and YG made figures and tables. SM was a major contributor in writing the manuscript. JG, YY, and XY revised the manuscript. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Gene detection list

ABL1	ABL2	ACVR1	ACVR1B	AGO2	AKT1	AKT2	AKT3	ALK	ALOX12B
AMER1	AR	APC	ANKRD11	ARAF	ARFRP1	ARID1A	ARID1B	ARID2	ARID5B
ASXL1	ASXL2	ATM	ATR	ATRX	AURKA	AURKB	AXIN1	AXIN2	AXL
B2M	BAP1	BARD1	BCL10	BCL2	BCL2L1	BCL2L11	BCL2L2	BCL6	BCOR
BCORL1	BIRC3	BLM	BMPRIA	BRAF	BRCA1	BRCA2	BRD3	BRD4	BRIP1
BTG1	BTK	C11orf30	CARD11	CALR	CARM1	CASP8	CBFB	CBL	CCND1
CCND2	CCND3	CCNE1	CD274	CD276	CD74	CD79A	CD79B	CDC42	CDC73
CDH1	CDK12	CDK4	CDKN1A	CDK6	CDKN2A	CDKN1B	CDK8	CDKN2B	CIC
CEBPA	CHD2	CHD4	CHEK1	CHEK2	CDKN2C	CREBBP	CRKL	CRLF2	CSDE1
CSF1R	CSF3R	CTCF	CTLA4	CUL3	CTNNB1	CTNNA1	CXCR4	CYLD	CYSLTR2
DAXX	DDR2	DICER1	DNMT3B	DNAJB1	DNMT1	DNMT3A	DIS3	DOT1L	DROSHA
DUSP4	E2F3	EED	EGF	EGFR	EIF1AX	EIF4A2	ELF3	EML4	EP300
EPAS1	EPCAM	EPHA3	EPHA5	EPHA7	EPHB1	ERBB2	ERBB3	ERBB4	ERCC1
ERCC2	ERCC3	ERCC4	ERCC5	ERF	ERG	ERRF1	ESR1	ETV1	ETV6
EZH2	FAM46C	FAM58A	FAM175A	FANCA	FANCD2	FANCE	FANCC	FANCF	FANCG
FANCL	FAS	FAT1	FBXW7	FGF10	FGF14	FGF19	FGF23	FGF3	FLT3
FGF4	FGF6	FGFR1	FGFR2	FGFR3	FGFR4	FLCN	FH	FLT1	FLT4
FOLR3	FOXA1	FOXL2	FOXO1	FOXP1	FRS2	FUBP1	FYN	GABRA6	GATA1
GATA2	GATA3	GATA4	GATA6	GID4	GLI1	GNA11	GNA13	GNAQ	GNAS
GOPC	GPR124	GREM1	GRIN2A	GRM3	GSK3B	GSTA1	H3F3A	H3F3B	HDAC1
HDAC4	HIST1H1C	HGF	HIST1H3B	HLA-A	HLA-B	HIST1H2BD	HNF1A	HIST1H3G	HOXB13
HRAS	HSP90AA1	HSD3B1	ID3	IDH1	IDH2	IFNGR1	IGF1	IGF1R	IGF2
IKBKE	IKZF1	IL10	IL7R	INHBA	INPP4A	INPP4B	INPPL1	INSR	IRF2
IRF4	IRS1	IRS2	JAK1	JAK2	JAK3	JUN	KAT6A	KDM5A	KDM5C
KDM6A	KDR	KEAP1	KEL	KIT	KLF4	KLHL6	KMT2A	KMT2B	KMT2C
KMT2D	KNSTRN	KRAS	LATS1	LATS2	LMO1	LRP1B	LRRK2	LYN	LZTR1
MAGI2	MALT1	MAP2K1	MAP2K2	MAP2K4	MAP3K1	MAP3K13	MAPK1	MAP3K14	MAPK3
MAX	MCL1	MDC1	MDM2	MDM4	MED12	MEF2B	MEN1	MET	MGA
MITF	MLH1	MPL	MRE11A	MSH2	MSH3	MSH6	MSI2	MST1R	MTOR
MUTYH	MYC	MYCL	MYCN	MYD88	MYO1	NAT2	NBN	NCOA3	NCOR1
NEGR1	NF1	NF2	NFE2L2	NFKBIA	NKX2-1	NOTCH1	NOTCH2	NOTCH3	NOTCH4
NPM1	NRAS	NSD1	NTRK1	NTRK2	NTRK3	NUF2	NUP93	OPRM1	PAK1
PAK3	PAK7	PALB2	PARK2	PARP1	PARP2	PAX5	PBRM1	PDCD1	PKI
PDGFRA	PDGFRB	PDPK1	PGR	PIK3CA	PHOX2B	PDCD1LG2	PIK3C3	PIK3C2B	PIK3C2G
PIK3CB	PIK3CD	PIK3CG	PIK3R1	PIK3R2	PIK3R3	PPP2R1A	PIM1	PLCG2	PMS1
PMS2	PNRC1	POLD1	PTPN11	POLE	PPARG	PPM1D	PPP6C	PRDM1	PRDM14
PREX2	PRKAR1A	PRKCI	PRKDI	PRKDC	PRSS8	PTCH1	PTCH2	PTEN	PTPRD
PTPRS	PTPRT	QKI	RAB35	RAC1	RAC2	RAD21	RAD50	RAD51	RAD51B
RAD51C	RAD51D	RAD52	RAD54L	RAF1	RANBP2	RARA	RASA1	RB1	RBM10
RECQL	RECQL4	REL	RET	RFWD2	RHEB	RHOA	RICTOR	RIT1	RNF43
ROCK1	ROSI	RPTOR	RUNX1T1	RRAGC	RPS6KB1	RPS6KA4	RRAS2	RRM1	RTEL1
RUNX1	RXRA	RYBP	SDHAF2	SDHA	SDHB	SLC19A1	SDHC	SDHD	SETD2
SF3B1	SMARCA4	SH2B3	SMARCB1	SHOC2	SHQ1	SMARCD1	SLIT2	SLX4	SMAD2
SMAD3	SNCAIP	SMAD4	SOCS1	SMO	SOS1	STAT5A	SOX10	SOX17	SOX2
SOX9	STAT5B	SPEN	STK11	SPOP	SPTA1	SUFU	SRC	SRSF2	STAG2
STAT3	SUZ12	STAT4	SYK	TAF1	TAP1	TAP2	TBX3	TCEB1	TCF3
TEK	TCF7L2	TERT	TGFBR1	TET1	TET2	TGFBR2	TOP1	TMEM127	TMPPRSS2
TNFAIP3	TNFRSF14	TOP2A	TP53	TP63	TP53BP1	TRAF7	TRAF2	TSC1	TSC2
TSHR	TYMS	U2AF1	VEGFA	VHL	WHSC1	WHSC1L1	WISP3	WT1	WWTR1
XIAP	XPO1	XRCC2	YAP1	YES1	ZBTB2	ZFHX3	ZNF217	ZNF703	

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