Case study

JAK2, *PD-L1*, and *PD-L2* (9p24.1) amplification in metastatic mucosal and cutaneous melanomas with durable response to immunotherapy[☆],[☆☆]

Sounak Gupta MBBS, PhD, Chad M. Vanderbilt MD, Paolo Cotzia MD, Javier A. Arias Stella III MD, Jason C. Chang MD, Yingbei Chen MD, PhD, Laura H. Tang MD, PhD, Deborah F. DeLair MD, Jinjuan Yao MD, PhD, Marc Ladanyi MD, Dara S. Ross MD*

Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

Summary As immune checkpoint inhibitors are rapidly developing into the standard of care for patients with advanced melanoma, the value of diagnostic metrics to predict response to immunotherapy is steadily increasing. Next-generation sequencing–based parameters include tumor mutation burden (TMB) and genomic amplification of *PD-L1/PD-L2/JAK2* at 9p24.1. At present, there are limited studies documenting response to checkpoint blockade in 9p24.1-amplified solid tumors. Herein, we have compared a cutaneous melanoma with a mucosal melanoma, both with 9p24.1 amplifications and durable response to immunotherapy. Although the cutaneous melanoma had a high TMB, the mucosal melanoma had a lower TMB compared with the mean TMB for all melanomas within the institutional clinical sequencing cohort. In summary, *PD-L1/PD-L2/JAK2* amplification was associated with durable response to therapy for both cases, and this genomic signature is a potential valuable metric in predicting response to immunotherapy.
© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Food and Drug Administration (FDA)–approved agents for the treatment of patients with advanced melanoma include BRAF/MEK-targeted therapeutics (BRAF: vemurafenib, dabrafenib; MEK: trametinib and cobimetinib) and immune checkpoint inhibitors [1,2]. Ligands such as programmed death ligand-1 (*PD-L1*; also known as *CD274/PDCD1LG1*) and programmed death ligand-2 (*PD-L2*; also known as *CD273/PDCD1LG2*) expressed on the tumor cell surface bind to specific immune cell receptors, such as programmed

death 1 (PD-1), to evade the host immune response [2]. Immunotherapeutic agents disrupt this interaction to restore the host immune response, and currently approved agents include ipilimumab (anticytotoxic T-lymphocyte–associated protein 4; CTLA4), and nivolumab and pembrolizumab (anti–PD-1), and response rates as high as 55% have been reported with anti–CTLA4/PD-1 therapy [2,3].

Most melanomas, including mucosal melanomas, elicit an adaptive immune response where host T cells up-regulate PD-1 expression in response to an increased load of tumor neoantigens, whereas cytokines such as interferon-γ released by these activated T cells promote the expression of PDL-1 on tumor cells, allowing them to escape the host immune response [2].

Biomarkers used to predict response to immunotherapy include metrics to assess the immune microenvironment (CD8+ cell density), tumor neoantigen load (tumor mutational burden), and the expression of PD-L1 itself [2,4]. Constitutive PD-L1/PD-L2 expression, often secondary to genomic amplification at the 9p24.1 locus, is a rare event seen in less than 1% of melanomas [5,6]. The presence of *JAK2* at the 9p24.1 locus is significant because prior studies have shown that *JAK2* signaling can further up-regulate PD-L1 expression [7]. Hematolymphoid neoplasias with *PD-L1/PD-L2/JAK2* coamplifications such as classical Hodgkin lymphoma, primary central nervous system lymphoma, and primary testicular lymphoma have shown good response to nivolumab; however, studies in solid tumors with a similar genomic signature are lacking [8-10].

A prior report documented an exceptional response to anti-PD1 therapy in a patient with a metastatic basal cell carcinoma with a 9p24.1 amplification, but this patient also had a high tumor mutation burden (TMB) and therefore exhibited multiple genomic signatures that would predict response to immunotherapy [11]. A more recent study involving various 9p24.1-amplified solid tumors with a low-to-intermediate TMB (based on sequencing of 1.2 Mb of the genome; low TMB defined as ≤5 mutations/megabase [mt/Mb], intermediate TMB defined as 6-19 mt/Mb, high TMB defined as ≥20 mt/Mb) documented objective responses to the administration of immune checkpoint blockade agents for 6 (66.7%) of 9 cases, emphasizing the importance of this genomic signature [6].

Herein, we report 2 patients with metastatic melanoma that had a 9p24.1 amplification that showed a durable response to immunotherapy.

2. Materials and methods

2.1. Methods

Immunohistochemistry for PD-L1 (Cell Signaling Technology, Danvers, MA; clone E1L3N, 1:400 dilution) was performed on representative whole tissue sections.

The Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay was used for molecular profiling, and details of this assay have been previously reported [12]. In brief, the MSK-IMPACT assay involves hybridization capture-based library preparation, followed by deep sequencing of up to 6614 protein-coding exons of 468 genes in the current panel. Approximately 1.5 Mb
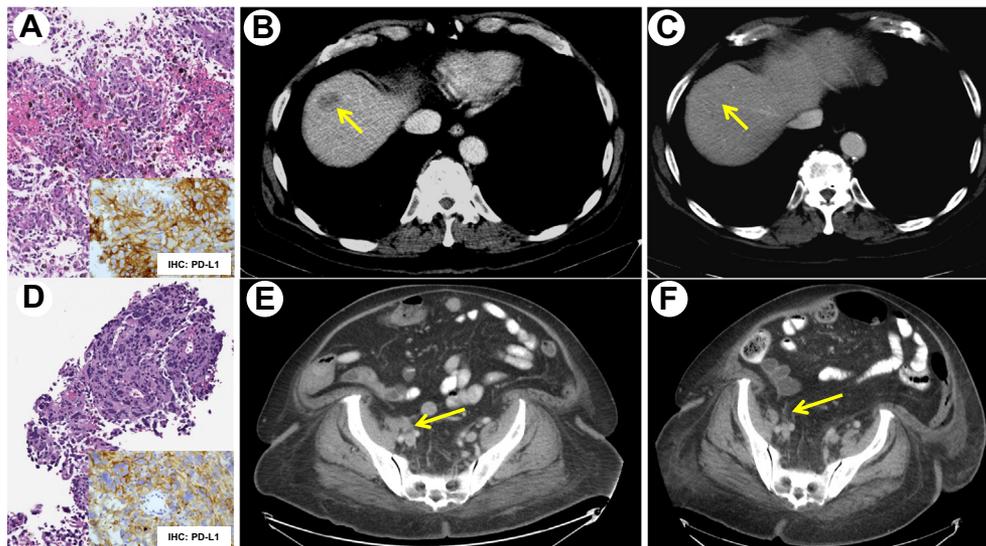
Fig. 1   Histopathology and imaging. Representative histology of a liver biopsy showing metastatic cutaneous melanoma involving the liver (A, case 1) and computed tomographic imaging showing response to therapy with pembrolizumab (B, 2.5 × 1.6 cm on day 1; C, 0.7 × 0.6 cm on day 313). A, Representative immunohistochemistry for PD-L1 is depicted as well (inset). D, Histology of an inguinal mass biopsy showing involvement by metastatic mucosal melanoma (case 2) and corresponding immunohistochemistry for PD-L1 (inset). Computed tomographic imaging showing resolution of the lesion after therapy with pembrolizumab (E, 2.4 × 1.9 cm, day 256; F, no appreciable adenopathy, day 1050) is depicted. Arrows demonstrate the lesion of interest.

Table   Next-generation sequencing by MSK-IMPACT

	Case 1. Metastatic cutaneous melanoma (to liver)	Case 2. Mucosal (urethral) melanoma
Tumor purity (facets [a]) and coverage	36% purity; 810× coverage	36% purity; 661× coverage
TMB (mt/Mb)	52.7 (468-gene panel) [b]	3.9 (410-gene panel) [b]
Mutations include	*TERT* (g.1295228C>T) and *TP63* (p.E609K)	*TP53* (p.R175L and p.R333fs) and *ATRX* (p.C1122fs)
Copy number gain	3.3 FC at 9p24.1 (*JAK2/PD-L1/PD-L2*)	3.6 FC at 9p24.1 and 2.1 FC at 4q12 (*PDGFRA/KIT*)
Copy number loss	NA	NA
Structural variants	Not detected	Not detected
UV signature	Present	Absent

Abbreviations: FC, fold change; NA, not applicable.
 [a] Shen and Seshan 2016 [15].
  [b] Average TMB for melanoma in MSKCC-IMPACT cohort: 7.9 mt/Mb.

of the human genome is targeted by this assay, with homogenous distribution of single-nucleotide polymorphism tiling probes across the genome, which allows for accurate genome-wide copy number assessment. This assay is currently FDA authorized as a class II in vitro diagnostic test.

TMB is defined as the total number of nonsynonymous mutations that occur as a fraction of the total genomic target region (mutations per megabase; mt/Mb) identified using MSK-IMPACT. In this report, the metastatic cutaneous melanoma involving the liver was sequenced using a 468-gene panel, and the mucosal melanomas were sequenced using a prior version of the assay with a 410-gene panel.

2.2. Case report: case 1

An 88-year-old man with a remote history of prostatic adenocarcinoma and cutaneous melanoma presented with widely metastatic disease involving the liver, lung, and bone. Biopsy of the 2.5-cm hepatic lesion revealed metastatic cutaneous melanoma (Fig. 1A and B). Immunohistochemistry for PD-L1 revealed strong, diffuse, and membranous expression of PD-L1 in most neoplastic cells (Fig. 1A, inset). Molecular profiling by MSK-IMPACT revealed a high TMB of 52.7 mt/Mb (average TMB for melanomas in the MSK-IMPACT clinical sequencing cohort, 7.9 mt/Mb); an abundance of G>A
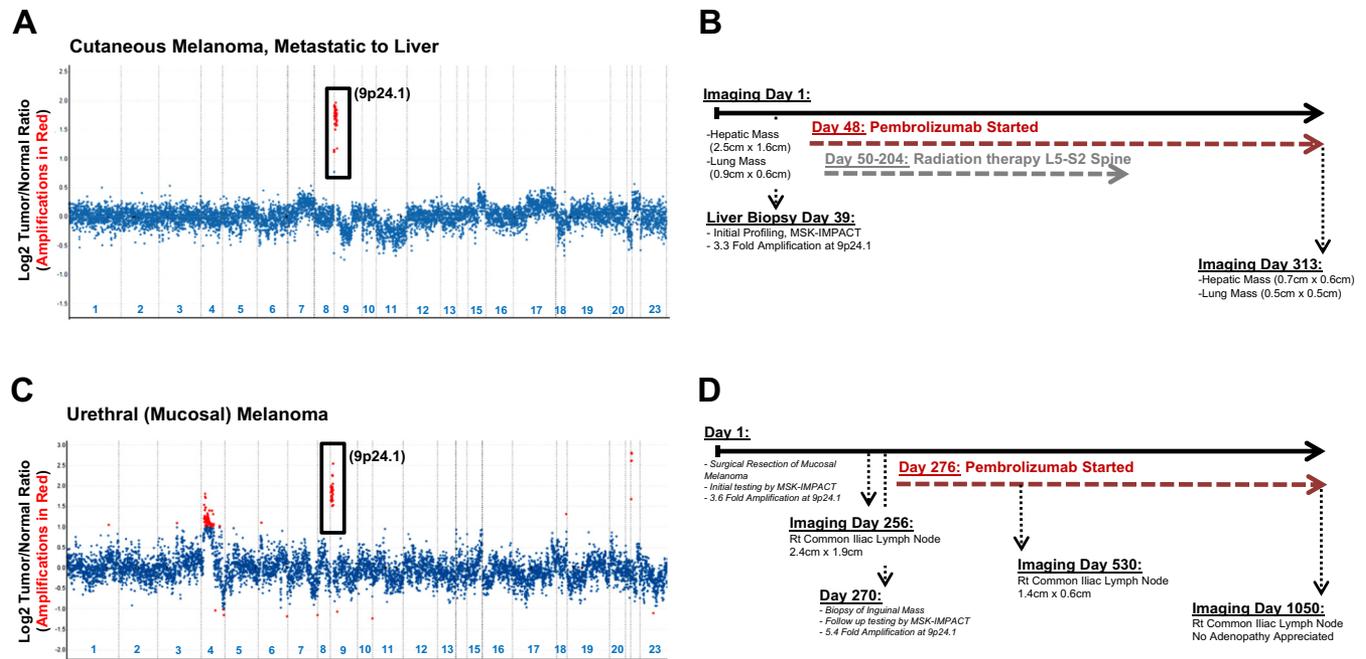
Fig. 2   Timeline and copy number assessment in melanomas with 9p24.1 amplification. Copy number plots have been shown for both patients (A: case 1, cutaneous melanoma metastatic to liver; C: case 2, urethral melanoma). Relative (log$_2$) tumor/normal ratios (*y*-axis) and corresponding chromosomes (*x*-axis) are displayed, with each blue dot representing an individual probe region. Amplified regions are shown in red. Corresponding timelines detailing key dates pertaining to imaging, molecular testing, and immunotherapy have been shown for the cutaneous metastatic melanoma involving the liver (B, case 1) and the mucosal melanoma (D, case 2).

and C>T transitions, consistent with UV exposure; and no clear driver alteration (Table). A greater than 3-fold amplification at the 9p24.1 genomic locus including *JAK2/PD-L1/PD-L2* was identified (Table, Fig. 2A), and immunotherapy with an anti-PD1 agent (pembrolizumab) was initiated. The patient was also treated with radiation therapy to the sacrum. At last follow-up (day 313), the patient had stable disease, including radiologic resolution of the hepatic mass (Figs. 1C and 2B).

2.3. Case report: case 2

A 73-year-old woman presented with a urethral mass, which was biopsied and revealed a mucosal melanoma. MSK-IMPACT testing revealed a low TMB of 3.9 mt/Mb (Table), *KIT/PDGFRA* amplification at 4q12 likely serving as a driver alteration, and 3.6-fold amplification at 9p24.1 (Table, Fig. 2C). On follow-up, the patient developed a metastasis to the right common iliac lymph node (Fig. 1D and E), and MSK-IMPACT testing revealed a similar genotype, TMB, and copy number alterations, including a 5.4-fold amplification at 9p24.1. This 9p24.1 amplification was concordant with immunohistochemistry for PD-L1, which revealed strong, diffuse, and membranous expression of PD-L1 in most neoplastic cells (Fig. 1D, inset). This patient was treated with pembrolizumab as well, starting on day 276, and showed radiologic improvement by day 530 and resolution of the abdominopelvic lymphadenopathy at last follow-up (day 1050; Figs. 1F and 2D).

3. Discussion

Immunotherapeutic options are steadily being incorporated into the standard of care for patients with advanced melanoma. The expression of ligands such as *PD-L1*, *PD-L2*, and *CD80* (also known as *B7.1/CD28LG*) on the tumor cell surface allows them to bind receptors on T cells within the tumor microenvironment, such as PD-1 and CTLA4, to down-regulate the host immune response [2,13]. FDA-approved immune checkpoint inhibitors such as ipilimumab (anti-CTLA4), and pembrolizumab and nivolumab (humanized and fully human anti–PD-1 monoclonal antibodies, respectively) counteract this immune evasion and have shown promising responses [2,13]. Response rates as high as 50% and durable response to therapy in many cases, with concurrent or sequential therapy with ipilimumab and nivolumab in one study, highlight the success of these agents [2,13].

The primary biomarker used to predict response to immunotherapeutic agents is PD-L1 protein expression assessed by immunohistochemistry, whereas others include CD8-positive T-cell density and specific transcriptional signatures [2,13]. Recently, TMB, a surrogate metric for assessing tumor immunogenicity by determining the load of neoepitopes, has emerged as a promising biomarker for predicting response to immunotherapy [4,14]. Although cutaneous melanomas often

have a high TMB secondary to UV exposure, characterized by an abundance of G > A and C > T transitions, noncutaneous melanomas, such as mucosal melanomas, lack a similar mutational signature and tend to exhibit a lower TMB. A recent study of 9p24.1 amplifications, as determined by next-generation sequencing–based methodology, in more than 100,000 tumors showed that most of these tumors in fact have a low (defined as ≤5 mt/Mb) to intermediate TMB (defined as 6-19 mt/Mb) [6].

Comprehensive molecular profiling of tumors using panels such as MSK-IMPACT therefore provides metrics such as TMB and copy number status of genes such as *PD-L1/PD-L2/JAK2* at 9p24.1 to help guide immunotherapeutic strategies [11]. Although most melanomas, including mucosal melanomas, exhibit an induced pattern of PD-L1 expression secondary to signaling events within the tumor microenvironment, a small percentage of cases exhibit constitutive expression secondary to 9p24.1 amplifications [2,5,6]. Although the literature is limited in regard to treatment outcomes for solid tumors with 9p24.1 amplifications, a few case series and reports suggest that such a signature is likely to predict favorable response to immunotherapy [6,11].

Herein, we compared a case of metastatic cutaneous melanoma with a case of a metastatic mucosal melanoma. Both cases showed 9p24.1 amplifications and exhibited a durable response to immunotherapy. A high TMB, as defined by at least 20 mt/Mb by Goodman et al [4,6], was associated with favorable response to immunotherapy for the metastatic cutaneous melanoma. However, in the case of the mucosal melanoma, it was lower than the mean TMB for all melanomas in the MSKCC clinical sequencing cohort. In contrast, *PD-L1/PD-L2/JAK2* amplification events were correlated with durable response to therapy for both cases and suggest that such genomic signatures should be carefully documented and correlated with response to checkpoint blockade administration to guide future immunotherapeutic decision making.

References

[1] Coit DG, Thompson JA, Algazi A, et al. NCCN guidelines insights: melanoma, version 3.2016. J Natl Compr Cancer Netw 2016;14:945-58.

[2] Taube JM, Galon J, Sholl LM, et al. Implications of the tumor immune microenvironment for staging and therapeutics. Mod Pathol 2018;31:214-34.

[3] Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 2015;373:23-34.

[4] Goodman AM, Kato S, Bazhenova L, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther 2017;16:2598-608.

[5] Kaunitz GJ, Cottrell TR, Lilo M, et al. Melanoma subtypes demonstrate distinct PD-L1 expression profiles. Lab Investig 2017;97:1063-71.

[6] Goodman AM, Piccioni D, Kato S, et al. Prevalence of PDL1 amplification and preliminary response to immune checkpoint blockade in solid tumors. JAMA Oncol 2018;4(9):1237-44.

[7] Green MR, Monti S, Rodig SJ, et al. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. Blood 2010;116:3268-77.

[8] Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 2015; 372:311-9.

[9] Nayak L, Iwamoto FM, LaCasce A, et al. PD-1 blockade with nivolumab in relapsed/refractory primary central nervous system and testicular lymphoma. Blood 2017;129:3071-3.

[10] Younes A, Santoro A, Shipp M, et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. Lancet Oncol 2016;17: 1283-94.

[11] Ikeda S, Goodman AM, Cohen PR, et al. Metastatic basal cell carcinoma with amplification of PD-L1: exceptional response to anti-PD1 therapy. NPJ Genom Med 2016;1:16037.

[12] Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017;23:703-13.

[13] Achkar T, Ali SM, Welsh A, Dhir R, Gollin SM, Parikh RA. A prolonged response to platinum-based therapy in a patient with metastatic urothelial carcinoma harboring a single rearranged and truncated NF2 gene. Genes Chromosomes Cancer 2018;57(8):430-3.

[14] Hellmann MD, Callahan MK, Awad MM, et al. Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer. Cancer Cell 2018;33:853-861.e854.

[15] Shen RL, Seshan VE. FACETS: algorithm involving allele-specific copy number analysis from next-generation sequencing data. Nucleic Acids Res 2016;44(16):e131.