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Original Article

MDM2/MDM4 amplification and *CDKN2A* deletion in metastatic melanoma and glioblastoma multiforme may have implications for targeted therapeutics and immunotherapy

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Abstract: Metastatic melanoma has a five-year survival of ~10%, with a paucity of biomarkers predicting metastasis to specific anatomic sites or targeted therapies for metastases. We analyzed 1015 primary and 358 metastatic melanomas and found metastatic disease is enriched for *MDM2* and *MDM4* amplifications compared to primary disease, and amplifications are associated with lower overall survival. *MDM2/4* amplifications are associated with a higher rate of metastasis to the brain and liver. Two negative regulators of p53, *USP7* and *PPM1D*, are also altered in metastatic melanoma compared to primary disease. These findings suggest that patients with metastatic melanoma have a dysregulated TP53 pathway compared to primary disease. We propose that patients with metastatic melanoma and wild-type *TP53* may be more likely to benefit from MDM2, MDM4, USP7, and PPM1D inhibitors. Patients with *MDM2/4* amplification display deep deletions in *CDKN2A*, alterations also associated with a higher rate of metastasis to the brain. Patients with a *CDKN2A* deletion have a higher rate of alterations in *TTN*, *MUC16*, *LRP1B*, and *NF1*, alterations previously associated with favorable response to immune-checkpoint inhibitors in melanoma. We propose *CDKN2A* alteration as a potential biomarker to predict response to immunotherapy in melanoma. We found that GBM displays the highest rate of *MDM4* amplifications (9.63%) and *CDKN2A* deletions (54.39%) across all cancer types. In 592 GBM samples we found that 8.45% display *MDM2* amplification. We suggest that patients with melanoma or GBM and amplifications in *MDM2/4* and *CDKN2A* alterations may benefit from combinations of targeted inhibitors of MDM2/4 and CDK4/6, as well as immunotherapy.

Keywords: MDM2, MDM4, melanoma, GBM, CDKN2A, cancer therapy, immunotherapy

Introduction

In the United States in 2022, there will be an estimated 99,780 new cases of melanoma and 7,650 deaths [1]. The incidence of malignant melanoma has been increasing at a faster rate than that of any cancer except lung cancer in women, and it is currently the fifth most common cancer type in men and sixth most common in women [2]. Melanoma tends to affect individuals at a younger age than other solid tumors, with an average age at diagnosis of 57 years, and there may be association with reproductive factors [3]. While the five-year survival rate for patients with melanoma in situ is 97%, patients with stage IV disease have a five-year survival rate of only 10% [4]. By contrast to the curative nature of surgical resection for early-stage melanoma, there exists no therapy to date that can predictably improve the overall survival of patients with metastatic disease.

Melanoma arises from transformed melanocytes whose precursor cells are derived from

neural crest cells. Early markers of malignant transformation are mutations in the proto-oncogenes *BRAF*, *NRAS*, and *KIT*, as well as loss of the tumor suppressors *PTEN* and *CDKN2A*, while later stages are characterized by loss of E-Cadherin and upregulation of N-Cadherin [5]. The most frequently hyperactivated pathways are the MAPK and PI3K/AKT pathways [6]. Interestingly, despite being one of the most mutated malignancies, metastatic melanoma rarely has mutations in *TP53*, with the locus remaining intact in >95% of cases [7].

Cancers that retain wild-type *TP53* status often find other ways to disrupt its function, either through alteration of upstream regulators or inactivation of downstream targets. Two major negative regulators of p53, MDM2 and MDM4, are known to have non-redundant functions in regulating p53 activity and are promising targets to reactivate p53 function. Moreover, recent studies have shown that stabilization of the p53-MDM2-MDM4 complex is controlled by the ubiquitin-proteasome complex, with the deubiquitinating enzyme USP7 playing a key role. USP7 protects MDM2 and MDM4 from ubiquitination-mediated proteasomal degradation [8]. Inhibition of USP7 promotes MDM2 and MDM4 degradation, thereby activating the p53 signaling pathway. Moreover, PPM1D is a negative regulator of p53, known to accelerate tumorigenesis in several mouse tumor models [9], and its inhibition may also enhance an anti-tumor response.

Melanoma accounts for 10% of all patients who develop brain metastases, and an estimated 1/3 of patients newly diagnosed with melanoma also present with brain metastases [9]. Evidence indicates that because melanoma cells have evolved from a primary site in the brain, they have subsequent cerebral tropism. For cancer cells to migrate through the blood brain barrier, the barrier must be compromised, suggesting that mutations contributing to metastatic melanoma may also increase the permeability of the BBB [10]. Surgical resection and stereotactic radiosurgery are indicated for symptomatic patients with brain metastases, while whole-brain radiotherapy is reserved for patients with diffuse involvement. Standard chemotherapy for metastatic melanoma in the brain includes temozolamide, fotemustine, and thalidomides, but these therapies have very low response rates [11]. Recently, immune checkpoint inhibitors have become the cornerstone of treatment for brain metastases, namely ipilimumab and nivolumab [5, 12], but immunotherapies achieve long-term survival in only 50% of metastatic patients. Further understanding of the molecular mechanisms driving metastasis to the brain is necessary in order to uncover novel therapeutic targets to help improve patient survival.

Previous work has revealed potential genomic links between melanoma and glioma, demonstrating that the incidence rate of gliomas was greater among melanoma cases than in the general population [13], and that melanomas were over-represented among patients with glioblastoma multiforme (GBM) [14]. This predisposition, termed melanoma and neural system tumor syndrome, results from a common germline mutation in CDKN2A [15], but little else is known regarding additional genomic alterations that contribute. Patients with GBM have an extremely poor prognosis, showing resistance to a number of targeted therapies and immunotherapies, and displaying a three-year survival of a mere 10.5% [16]. Early identification of genomic markers that may predispose individuals to GBM is needed, and such alterations have the potential to serve as druggable targets.

Because additional efforts are needed to identify therapeutic targets in metastatic melanoma to improve clinical prognosis, we sought to compare genomic alterations in metastatic and primary disease to better understand the molecular mechanisms predisposing to metastasis. We identified an enrichment of alterations in four negative regulators of p53, namely MDM2, MDM4, USP7, and PPM1D, in metastatic disease compared to primary. Moreover, a subgroup of patients with MDM2/4 amplifications also displayed alterations in CDKN2A. We show that alteration in MDM2, MDM4, and CDKN2A in patients with melanoma are all associated with a higher rate of metastasis to the brain compared to patients lacking these alterations. Additionally, because previous studies have demonstrated a potential link between melanoma and gliomas, we sought to uncover additional genomic similarities between the two. We reveal that in addition to a high rate of deep deletions in CDKN2A, both melanoma and GBM display amplification in MDM2 and MDM4. Together, our results propose therapeutic targets that may be particularly beneficial for patients with metastatic

melanoma and GBM, and highlight potential genomic links between these two cancer types.

Methods

TCGA PanCancer Atlas Studies analyzed on cBioPortal included the following: Adrenocortical Carcinoma, Cholangiocarcinoma, Bladder Urothelial Carcinoma, Colorectal Adenocarcinoma, Breast Invasive Carcinoma, Brain Lower Grade Glioma, Glioblastoma Multiforme, Cervical Squamous Cell Carcinoma, Esophageal Adenocarcinoma, Stomach Adenocarcinoma, Uveal Melanoma, Head and Neck Squamous Cell Carcinoma, Kidney Renal Clear Cell Carcinoma, Kidney Chromophobe, Kidney Renal Papillary Cell Carcinoma, Liver Hepatocellular Carcinoma, Lung Adenocarcinoma, Lung Squamous Cell Carcinoma, Diffuse Large B-Cell Lymphoma, Acute Myeloid Leukemia, Ovarian Serous Cystadenocarcinoma, Pancreatic Adenocarcinoma, Mesothelioma, Prostate Adenocarcinoma, Skin Cutaneous Melanoma, Pheochromocytoma and Paraganglioma, Sarcoma, Testicular Germ Cell Tumors, Thymoma, Thyroid Carcinoma, Uterine Corpus Endometrial Carcinoma, Uterine Carcinosarcoma.

Studies analyzed on cBioPortal included the following: Melanoma (MSKCC, Clin Cancer Res 2021; https://www.cbioportal.org/study/summary?id=mel_mskimpact_2020), Melanomas (TCGA, Cell 2015; https://www.cbioportal.org/study/summary?id=skcm_tcga_pub_2015), Metastatic Melanoma (DFCI, Science 2015; https://www.cbioportal.org/study/summary?id=skcm_dfci_2015) [17], Metastatic Melanoma (MSKCC, JCO Precis Oncol 2017; https://www.cbioportal.org/study/summary?id=skcm_vanderbilt_mskcc_2015) [18], Metastatic Melanoma (DFCI, Nature Medicine 2019; https://www.cbioportal.org/study/summary?id=mel_dfci_2019) [19], Metastatic Melanoma (UCLA, Cell 2016; https://www.cbioportal.org/study/summary?id=mel_ucla_2016) [20], Non-Small Cell Lung Cancer [21, 22].

Results

Metastatic melanoma displays a higher rate of amplification in MDM2 and MDM4 compared to primary disease, and alteration predicts a worse prognosis

Because wild-type p53 is expressed in melanoma without functioning as a tumor suppressor, we sought to determine the roles that *MDM2/4* may play in this dysregulation. We analyzed 1055 primary melanoma samples and 358 metastatic melanoma samples and found that *MDM2/4* have higher rates of alteration in metastatic disease compared to primary. Among all metastatic melanoma studies included in cBioPortal, the highest frequency of alterations in *MDM2* was 15.79% and in *MDM4* was 13.16% (UCLA, Cell 2016; https://www.cbioportal.org/study/summary?id=mel_ucla_2016) [20]. Among these MDM2 alterations, 10.53% were amplifications and 5.26% were mutations (**Figure 1A**). Similarly, among these MDM4 alterations, 10.53% were amplifications and 2.63% were deep deletions (**Figure 1B**). Among primary melanoma studies, the highest frequency of amplifications in *MDM2* was 2.08% and in *MDM4* was 0.29% (MSKCC, Clin Cancer Res 2021; https://www.cbioportal.org/study/summary?id=mel_mskimpact_2020). When pooling the two genes together and looking at the percentage of patients who had either an *MDM2* or an *MDM4* alteration, we found that the highest frequency in any metastatic study was 21.05% (UCLA, Cell 2016; https://www.cbioportal.org/study/summary?id=mel_ucla_2016) [20] compared to 1.44% in any primary study (MSKCC, Clin Cancer Res 2021; https://www.cbioportal.org/study/summary?id=mel_mskimpact_2020) (**Figure 1C**). In primary melanoma, *MDM2* and *MDM4* alterations display a tendency toward co-occurrence (P=0.372) (**Table 1A**), while they display a tendency toward mutual exclusivity (P=0.570) in metastatic disease (**Table 1B**). Together, these findings demonstrate that metastatic melanoma displays a higher frequency of MDM2/4 dysregulation compared to primary disease.

We explored whether alteration in *MDM2/4* could be used as a prognostic biomarker to predict patient overall survival. We found that among patients with an *MDM2/4* alteration, the median months of overall survival was 64.44 compared to 94.61 in patients without an alteration (P=0.0167) (**Figure 1D**). Moreover, when comparing the response grade among patients with an *MDM2/4* alteration to those without, we found that 71.43% of patients with alterations in *MDM2/4* displayed a poor response in comparison to 28.81% of patients without an alteration. Moreover, 37.29% of patients without an alteration displayed an

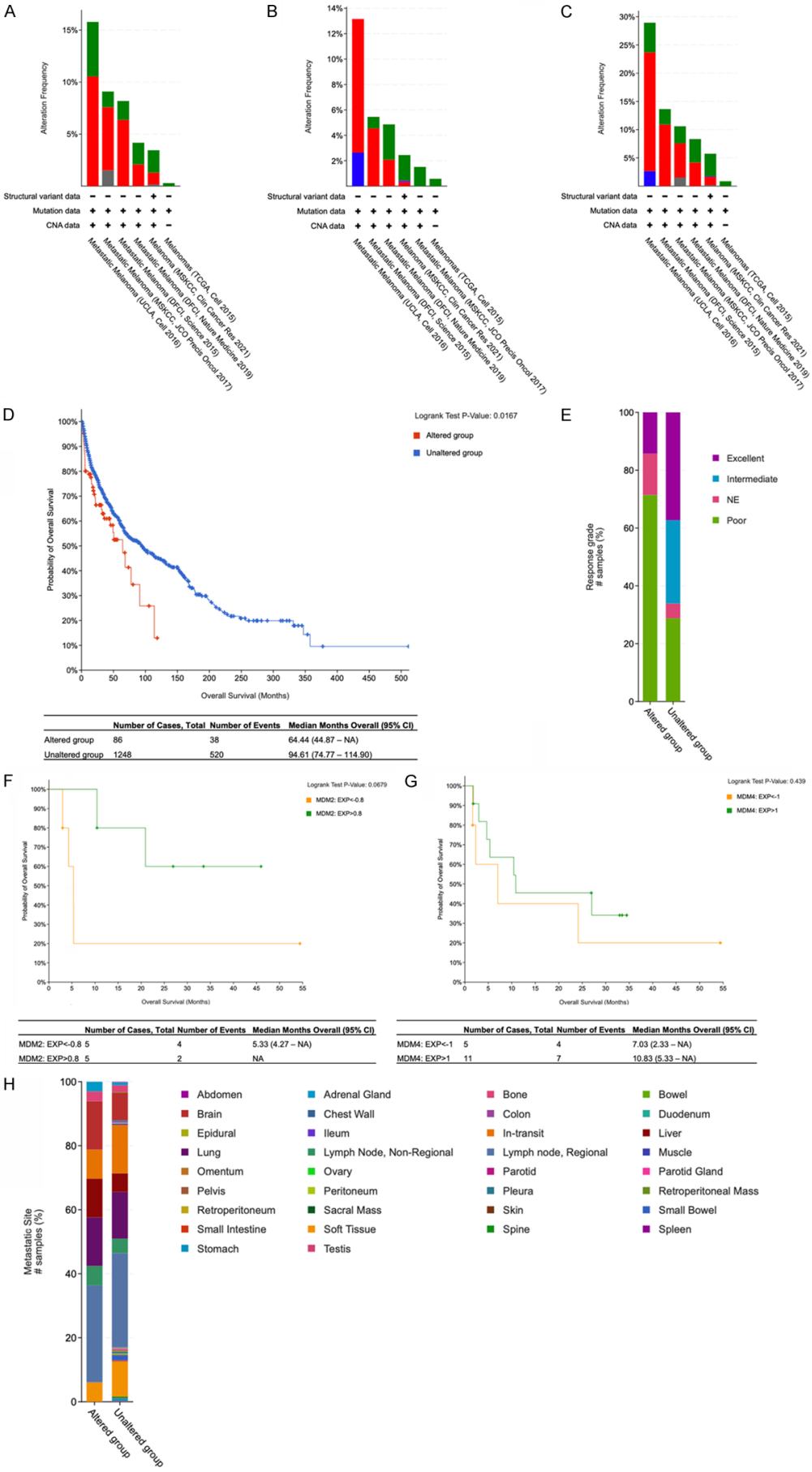

A
B
C
Alteration Frequency
Structural variant data
Mutation data
CNA data
Melanomas (TCGA, Cell 2015)
Metastatic Melanoma (MSKCC, Clin Cancer Res 2021)
Metastatic Melanoma (DFCI, Nature Medicine 2019)
Metastatic Melanoma (MSKCC, JCO Precis Oncol 2017)
Metastatic Melanoma (DFCI, Science 2015)
Metastatic Melanoma (UCLA, Cell 2016)
D
Logrank Test P-Value: 0.0167
Altered group
Unaltered group
Probability of Overall Survival
Overall Survival (Months)
Number of Cases, Total
Number of Events
Median Months Overall (95% CI)
Altered group 86 38 64.44 (44.87 – NA)
Unaltered group 1248 520 94.61 (74.77 – 114.90)
E
Excellent
Intermediate
NE
Poor
Response grade
samples (%)
F
Logrank Test P-Value: 0.0679
MDM2: EXP<-0.8
MDM2: EXP>0.8
MDM2: EXP<-0.8 5 4 5.33 (4.27 – NA)
MDM2: EXP>0.8 5 2 NA
G
Logrank Test P-Value: 0.439
MDM4: EXP<-1
MDM4: EXP>1
MDM4: EXP<-1 5 4 7.03 (2.33 – NA)
MDM4: EXP>1 11 7 10.83 (5.33 – NA)
H
Metastatic Site
samples (%)
Abdomen
Adrenal Gland
Bone
Bowel
Brain
Chest Wall
Colon
Duodenum
Epidural
Ileum
In-transit
Liver
Lung
Lymph Node, Non-Regional
Lymph node, Regional
Muscle
Omentum
Ovary
Parotid
Parotid Gland
Pelvis
Peritoneum
Pleura
Retroperitoneal Mass
Retroperitoneum
Sacral Mass
Skin
Small Bowel
Small Intestine
Soft Tissue
Spine
Spleen
Stomach
Testis

Figure 1. *MDM2* and *MDM4* alterations are enriched in metastatic melanoma compared to primary disease and are associated with metastasis to the brain and a worse survival. A. Frequencies of alteration events in *MDM2* in primary melanoma and metastatic melanoma. B. Frequencies of alteration events in *MDM4* in primary melanoma and metastatic melanoma. C. Frequencies of alteration events in either *MDM2* or *MDM4* in primary melanoma and metastatic melanoma. Alterations include mutations (green), amplifications (red), deep deletions (blue), and multiple alterations (gray). D. Kaplan-Meier curve comparing survival between patients with melanoma with either an *MDM2* or *MDM4* alteration (red) and those lacking an alteration (blue). E. Classifying response grades by either excellent (purple), intermediate (blue), NE (pink), or poor (green), and comparing responses in patients with an *MDM2/4* alteration to those without. F. Kaplan-Meier curve comparing survival between patients with metastatic melanoma according to *MDM2* mRNA expression (z-score= ±2 relative to diploid samples, RNA Seq FPKM, EXP< -0.8 or EXP>0.8). G. Kaplan-Meier curve comparing survival between patients with metastatic melanoma according to *MDM4* mRNA expression (z-score= ±2 relative to diploid samples, RNA Seq FPKM, EXP<-1 or EXP>1). H. Comparing anatomic sites of metastases in patients with an MDM2/4 alteration to those without.

Table 1. A. Alterations in *MDM2*, *MDM4*, *USP7*, and *PPM1D*, and tendencies toward mutual exclusivity or co-occurrence in primary melanoma. B. Alterations in *MDM2*, *MDM4*, *USP7*, and *PPM1D*, and tendencies toward mutual exclusivity or co-occurrence in metastatic melanoma

A.									
A	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	*p*-Value	q-Value	Tendency
USP7	PPM10	331	12	2	1	>3	0.109	0.653	Co-occurrence
MDM4	MDM2	999	18	24	1	1.209	0.372	0.943	Co-occurrence
MDM2	PPM1D	956	24	24	0	<-3	0.556	0.943	Mutual exclusivity
MDM4	PPM1D	961	19	24	0	<-3	0.629	0.943	Mutual exclusivity
MDM4	USP7	331	2	13	0	<-3	0.926	-0.962	Mutual exclusivity
MDM2	USP7	332	1	13	0	<-3	0.962	0.962	Mutual exclusivity
B.									
A	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	*p*-Value	q-Value	Tendency
USP7	PPM1D	267	11	8	5	>3	<0.001	0.002	Co-occurrence
MDM4	USP7	260	15	13	3	2.000	0.067	0.201	Co-occurrence
MDM2	PPM1D	259	19	11	2	1.309	0.239	0.478	Co-occurrence
MDM2	USP7	256	19	14	2	0.945	0.324	0.486	Co-occurrence
MDM4	MDM2	312	18	26	1	-0.585	0.570	0.572	Mutual exclusivity
MDM4	PPM1D	261	17	12	1	0.355	0.572	0.572	Co-occurrence

excellent response in comparison to 14.29% of patients with an alteration (P=0.0643) (**Figure 1E**). We also sought to determine whether mRNA expression of *MDM2/4* predicted a difference in clinical prognosis. Contrary to genomic alterations, we found that, though not statistically significant, higher mRNA expression of either *MDM2* (P=0.0679) (**Figure 1F**) or *MDM4* (P=0.439) predicted a trend toward better overall survival (DFCI, Science 2015; https://www.cbioportal.org/study/summary?id=skcm_dfci_2015) [17] (**Figure 1G**). Together, these results display the potential for alteration of MDM2/4 to predict both response grade and clinical prognosis.

Patients with MDM2/4 amplifications display a higher rate of metastasis to the brain and liver

Given that *MDM2/4* amplifications were associated with a poorer clinical prognosis, we asked whether alterations in these genes were correlated with metastases to specific anatomic sites. We found that patients with an *MDM2/4* alteration displayed a notably higher rate of metastasis to the brain (15.15% vs. 8.56%) and liver (12.12% vs. 5.83%). Alteration in *MDM2/4* did not seem to strongly influence metastasis to the regional lymph nodes (30.3% vs. 29.33%), lungs (15.15% vs. 14.57%), non-regional lymph nodes (6.06% vs. 4.55%), bone (3.03% vs. 2.19%), or adrenal glands (3.03% vs. 0.91%) when compared to patients lacking an alteration (**Figure 1H**). Patients lacking an *MDM2/4* alteration displayed a higher rate of in-transit metastases (15.12% vs. 9.09%) compared to patients with an alteration. These results suggest that genomic profiling of patients with melanoma to detect alteration in *MDM2/4* could be useful in predicting subsequent metastasis to particular anatomic sites.

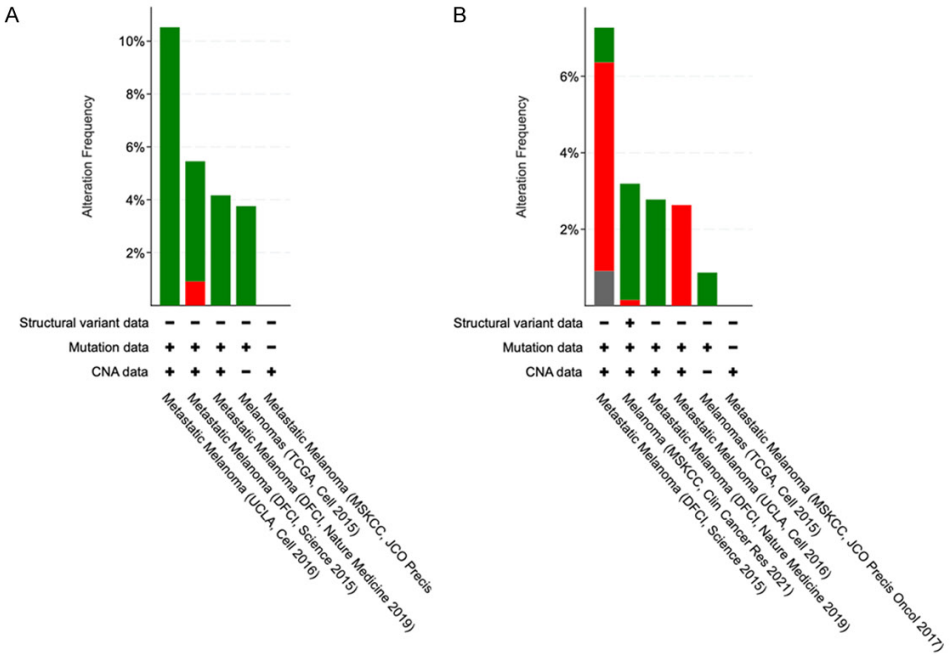
Metastatic melanoma has a higher frequency of alteration in USP7 and PPM1D compared to primary disease

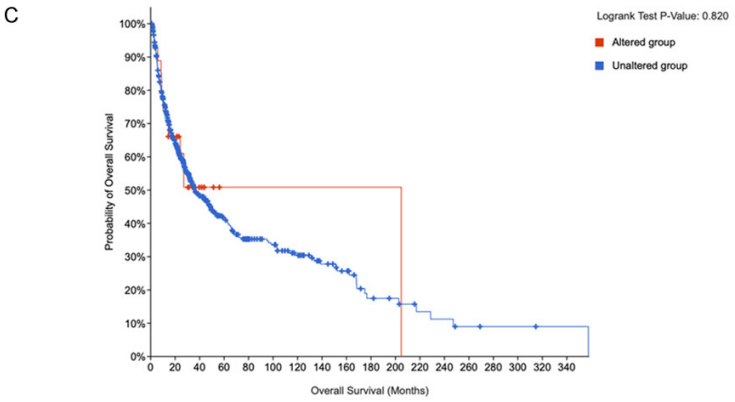
Given the enhanced dysregulation of *MDM2/4* that we found in metastatic melanoma compared to primary disease, we next asked whether other negative regulators of p53 displayed a similar pattern. The highest frequency of alteration in *USP7* across any metastatic melanoma study in cBioPortal was 10.53%, with 100% of these alterations being mutations (UCLA, Cell 2016; https://www.cbioportal.org/study/summary?id=mel_ucla_2016) [20] (**Figure 2A**). In comparison, the highest frequency of alteration in *USP7* in primary melanoma was 4.17%, with 100% of these alterations also being mutations (TCGA, Cell 2015; https://www.cbioportal.org/study/summary?id=skcm_tcga_pub_2015). Similarly, the highest frequency of *PPM1D* alteration among metastatic studies was 7.27%, with 5.45% of these events being amplifications and 0.91% being mutations (DFCI, Science 2015; https://www.cbioportal.org/study/summary?id=skcm_dfci_2015) [17] (**Figure 2B**). On the contrary, the highest frequency of alteration in *PPM1D* in primary disease was 3.19%, with 3.04% of these events being amplifications and 0.15% being amplifications (MSKCC, Clin Cancer Res 2021; https://www.cbioportal.org/study/summary?id=mel_mskimpact_2020). Interestingly, though not statistically significant, the median months of overall survival was 204.74 for patients with a *USP7* alteration in comparison to 35.95 months in those without (P=0.820) (**Figure 2C**), suggesting that *USP7* alteration may be a favorable prognostic factor. We found that 89.2% of *USP7* mutations were missense (**Figure 2D**). Like the pattern seen with *MDM2/4* alteration, alteration in *PPM1D* predicted a worse clinical prognosis, though not statistically significant, with 51.32 median months of overall survival displayed in those with an alteration compared to 92.14 months in those without (P=0.796) (**Figure 2E**). Together, these findings demonstrate that metastatic melanoma also displays an enhanced dysregulation in *USP7* and *PPM1D* compared to primary disease.

We explored whether patients with an *MDM2/4* alteration were more likely to also have an alteration in *USP7* or *PPM1D*. Interestingly, we found that 17.39% of patients with an *MDM2/4* alteration also had an alteration in *USP7*, compared to 4.19% of patients without an *MDM2/4* alteration (P=0.0184). Similarly, 4.55% of patients with an *MDM2/4* alteration also had an alteration in *PPM1D*, compared to 2.80% of patients without an *MDM2/4* alteration (P=0.296) (**Figure 2F**). In primary disease, *USP7* and *PPM1D* displayed a tendency toward co-occurrence, while *MDM2* and *PPM1D*, *MDM4* and *PPM1D*, *MDM2* and *USP7*, and *MDM2* and *USP7* all displayed a tendency toward mutual exclusivity (**Table 1A**). In comparison, dysregulation among these gene pairs were more likely to co-occur in metastatic disease; *USP7* and *PPM1D*, *MDM4* and *USP7*, *MDM2* and *PPM1D*, *MDM2* and *USP7*, and *MDM4* and *PPM1D* all displayed a tendency toward co-occurrence in metastatic melanoma (**Table 1B**). Together, these results suggest that patients with metastatic melanoma show an enhanced broad dysregulation among multiple negative regulators of p53 compared to primary disease, and that the tendency for compounded alterations among these genes is more strongly seen in metastatic disease.

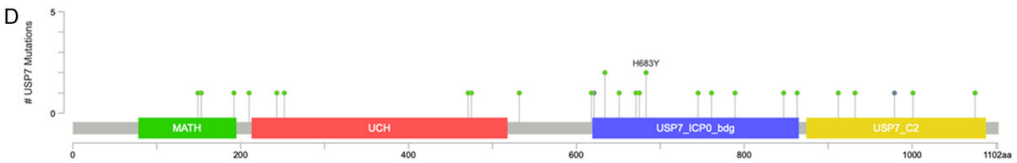
Patients with an MDM2/4 amplification also display CDKN2A alterations

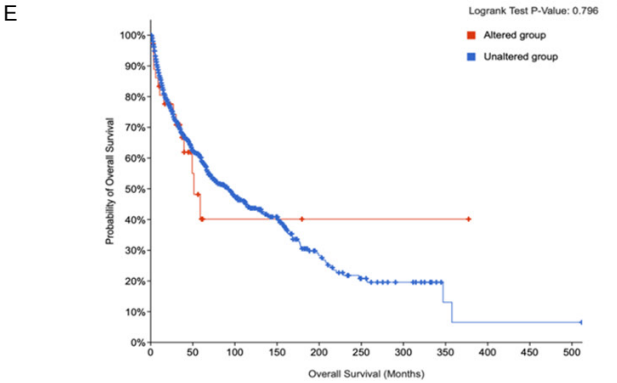
Given that germline alteration in *CDKN2A* is the highest-risk predisposition gene for melanoma, we asked whether patients with an *MDM2/4* alteration also showed dysregulation in *CDKN2A*. We found that 34.78% of patients with an *MDM2* alteration and 29.73% of patients with an *MDM4* alteration also had alterations in *CDKN2A* (**Figure 3A**). Among the studies we analyzed in cBioPortal, the highest rate of alteration in *CDKN2A* was 45.11% in primary melanoma (MSKCC, Clin Cancer Res 2021; https://www.cbioportal.org/study/summary?id=mel_mskimpact_2020), followed by 36.36% in metastatic melanoma (MSKCC, JCO Precis Oncol 2017; https://www.cbioportal.org/study/summary?id=skcm_vanderbilt_mskcc_2015) [18] (**Figure 3B**). In primary disease, the most frequent alteration event was deep deletions, representing 25% of alterations, followed by mutations, representing 19.25% of alterations. On the contrary, in metastatic disease, the most frequent alteration event was mutations, representing 19.7% of alterations, followed by deep deletions, repre-





	Number of Cases, Total	Number of Events	Median Months Overall (95% CI)
Altered group	28	13	204.74 (24.13 – NA)
Unaltered group	610	330	35.95 (31.41 – 48.88)





	Number of Cases, Total	Number of Events	Median Months Overall (95% CI)
Altered group	36	15	51.32 (39.61 – NA)
Unaltered group	1260	516	92.14 (72.76 – 112.57)

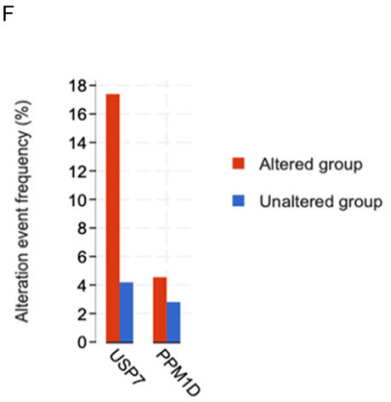


Figure 2. Alterations in *USP7* and *PPM1D* are enriched in metastatic melanoma compared to primary disease. A. Frequencies of alteration events in *USP7* in primary melanoma and metastatic melanoma. B. Frequencies of alteration events in *PPM1D* in primary melanoma and metastatic melanoma. Alterations include mutations (green), amplifications (red), and multiple alterations (gray). C. Kaplan-Meier curve comparing survival between patients with melanoma with a *USP7* alteration (red) and those lacking an alteration (blue). D. Schematic of *USP7* mutations. Depicted are VUS missense mutations (light green, 25 total) and VUS truncating mutations (gray, 3 total). E. Kaplan-Meier curve comparing survival between patients with a *PPM1D* alteration (red) and those lacking an alteration (blue). F. Comparison of alteration events in *USP7* and *PPM1D* in patients with either an *MDM2/4* alteration (red) and those without (blue).

senting 16.67% of alterations. Among patients with a *CDKN2A* mutation, we found that 49.49% of these mutations were truncating (**Figure 3C**). Patients with a *CDKN2A* alteration also displayed a higher rate of metastasis to the brain; 10.18% of patients with a *CDKN2A* alteration had a brain metastasis, in comparison to 7.82% of patients without an alteration (**Figure 3D**). These findings demonstrate a co-occurrence of alterations in *MDM2/4* in addition to *CDKN2A* and suggest a common preferential metastasis to the brain.

CDKN2A altered tumors display a higher rate of alterations in genes previously associated with a favorable response to immunotherapy in melanoma

Given the high frequency of alterations in *CDKN2A* in melanoma, we asked whether *CDKN2A* has potential to serve as a biomarker to predict response to immunotherapy. Because alterations in *TTN* [23], *MUC16* [24], *LRP1B* [25], and *NF1* have all been previously demonstrated to predict a positive response to immune checkpoint inhibitors in melanoma, we asked whether patients that had a *CDKN2A* alteration were more likely to have alterations in these genes. Interestingly, we found that patients with a *CDKN2A* alteration had a statistically significant higher frequency of alterations in each of these genes compared to patients lacking a *CDKN2A* alteration (**Figure 3E**). This pattern was also seen in non-small cell lung cancer (**Figure 3F**), but not seen in GBM (**Figure 3G**). These findings suggest that alteration in *CDKN2A* may serve as a prognostic biomarker to predict outcomes to treatment with immune checkpoint inhibitors in patients with melanoma.

Similarities between genomic alterations associated with melanoma and glioblastoma multiforme

Given prior work that has demonstrated that patients with melanoma display a higher incidence of gliomas when compared to the general population, we sought to find potential genomic links between the two. Because alteration of *MDM2/4* in melanoma was associated with a higher rate of metastasis to the brain, we asked whether GBM also displayed dysregulation of *MDM2/4*. We found that, across all cancer types screened in TCGA, GBM displayed the highest frequency of *MDM4* alterations (**Figure 4A**) and the fourth highest frequency of *MDM2* alterations (**Figure 4B**). *MDM4* was altered in 11.64% of GBM (**Figure 4C**) and *MDM2* was altered in 8.73% of GBM (**Figure 4D**) (TCGA, PanCancer Atlas). Skin cutaneous melanomas displayed the eighth highest frequency of alterations in *MDM4* and the ninth highest frequency of alterations in *MDM2* in TCGA. In GBM, 10.85% of *MDM4* alterations were amplifications, while 7.41% of *MDM2* alterations were amplifications, the most highly represented alteration seen in both genes. When combining amplifications in *MDM2* or *MDM4*, we found that 19.58% of GBM patients displayed an alteration in either of these genes (**Figure 4E**). Unlike in melanoma, alteration in *MDM2/4* does not appear to significantly influence clinical prognosis in GBM, as patients with an alteration display a median overall survival of 12.76 months in comparison to 14.73 months in those without (P=0.382) (**Figure 4F**).

We also aimed to determine whether *USP7* and *PPM1D* showed a similar pattern of dysregulation in GBM. We found that, in contrast to melanoma, *USP7* is only altered in 1.3% of GBM (**Figure 5A**), and alteration does not seem to impact overall survival, as patients with an alteration displayed 15.12 median months of overall survival compared to 14.40 months in patients without an alteration (P=0.476) (**Figure 5B**). Similarly, *PPM1D* is altered in only 1.06% of GBM (**Figure 5C**), and alteration predicts 10.75 median months of overall survival in comparison to 14.40 months in patients without an alteration (P=0.967) (**Figure 5D**). Together, these results suggest

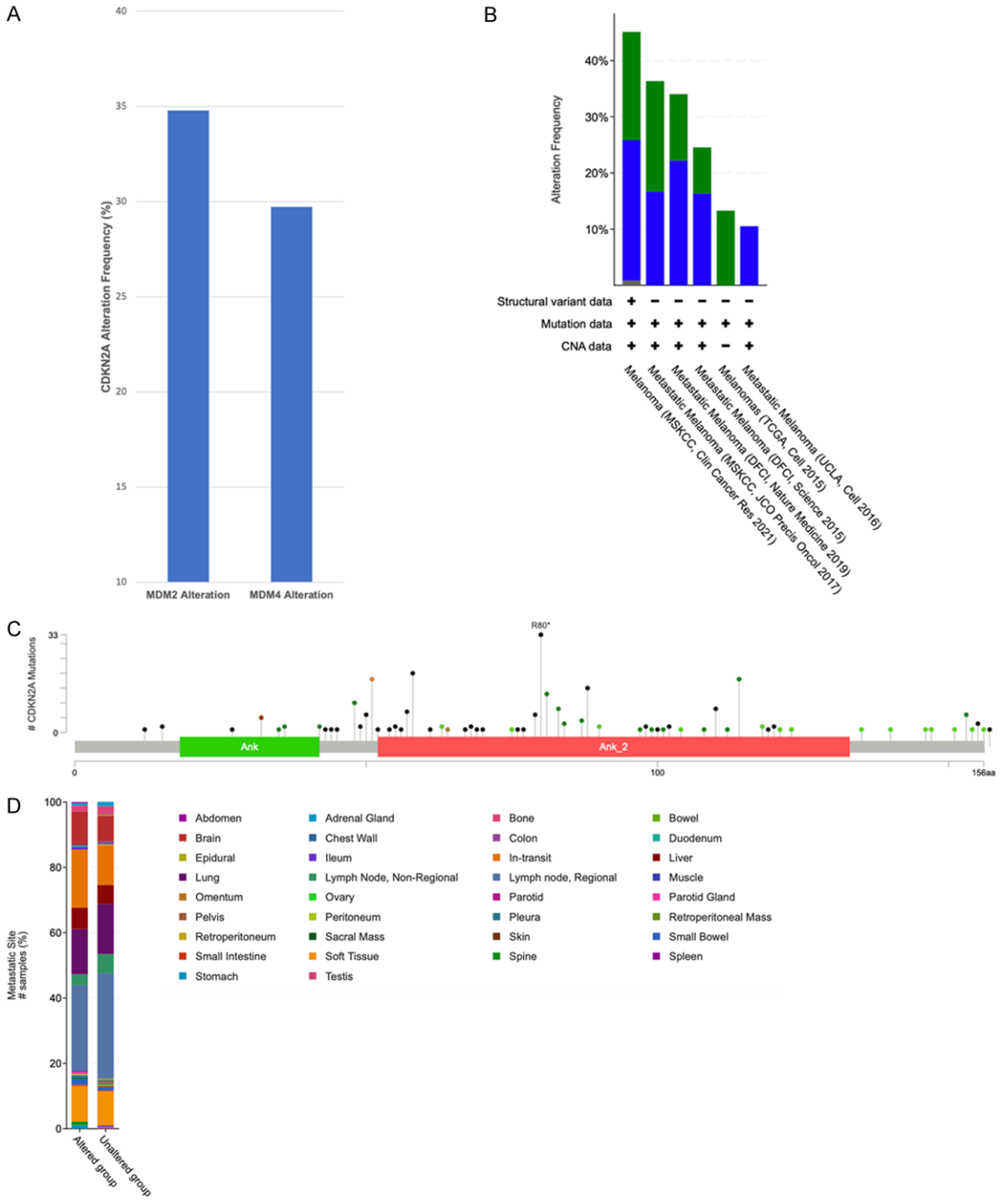
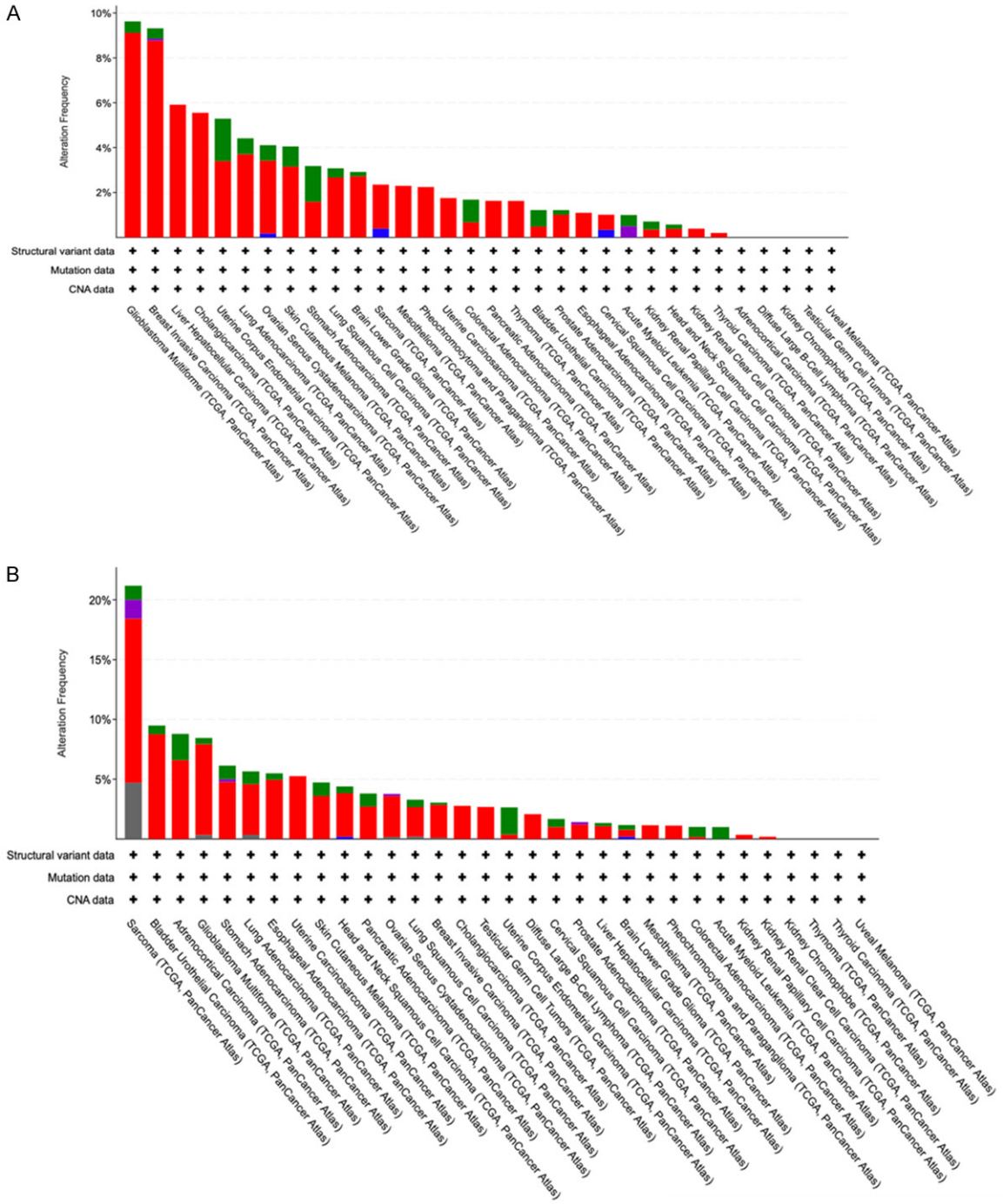

A
CDKN2A Alteration Frequency (%)
MDM2 Alteration
MDM4 Alteration
B
Alteration Frequency
Structural variant data
Mutation data
CNA data
Metastatic Melanoma (UCLA, Cell 2016)
Melanomas (TCGA, Cell 2015)
Metastatic Melanoma (DFCI, Science 2015)
Metastatic Melanoma (DFCI, Nature Medicine 2019)
Metastatic Melanoma (MSKCC, JCO Precis Oncol 2017)
Melanoma (MSKCC, Clin Cancer Res 2021)
C
CDKN2A Mutations
R80*
Ank
Ank_2
156aa
D
Metastatic Site
samples (%)
Abdomen
Adrenal Gland
Bone
Bowel
Brain
Chest Wall
Colon
Duodenum
Epidural
Ileum
In-transit
Liver
Lung
Lymph Node, Non-Regional
Lymph node, Regional
Muscle
Omentum
Ovary
Parotid
Parotid Gland
Pelvis
Peritoneum
Pleura
Retroperitoneal Mass
Retroperitoneum
Sacral Mass
Skin
Small Bowel
Small Intestine
Soft Tissue
Spine
Spleen
Stomach
Testis
Altered group
Unaltered group
E
Alteration event frequency (%)
TTN*
MUC16*
LRP1B*
NF1*
F
Mutation frequency (%)
G
Alteration event frequency (%)
TTN
MUC16
LRP1B
NF1

Figure 3. *CDKN2A* deletions coincide with *MDM2/4* alterations and are also associated with metastasis to the brain. A. Percentage of patients with an *MDM2* alteration or an *MDM4* alteration who also have a *CDKN2A* alteration. B. Frequencies of alteration events in *CDKN2A* in primary melanoma and metastatic melanoma. Alterations include mutations (green) and deep deletions (blue). C. Schematic of *CDKN2A* mutations. Depicted are driver missense mutations (dark green, 69 total), driver truncating mutations (black, 121 total), driver in-frame mutations (maroon, 4 total), driver splice mutations (orange, 23 total), VUS missense mutations (light green, 25 total), and VUS in-frame mutations (gold, 1 total). D. Comparing anatomic sites of metastases in patients with a *CDKN2A* alteration to those without. E. Frequencies of alteration events in melanoma among genes whose alterations have previously been implicated in predicting a favorable response to immune therapies in patients with a *CDKN2A* alteration (red) or without (blue). F. Frequencies of alteration events in non-small cell lung cancer among genes whose alterations have previously been implicated in predicting a favorable response to immune therapies in patients with a *CDKN2A* alteration (red) or without (blue). G. Frequencies of alteration events in glioblastoma multiforme among genes whose alterations have previously been implicated in predicting a favorable response to immune therapies in patients with a *CDKN2A* alteration (red) or without (blue).



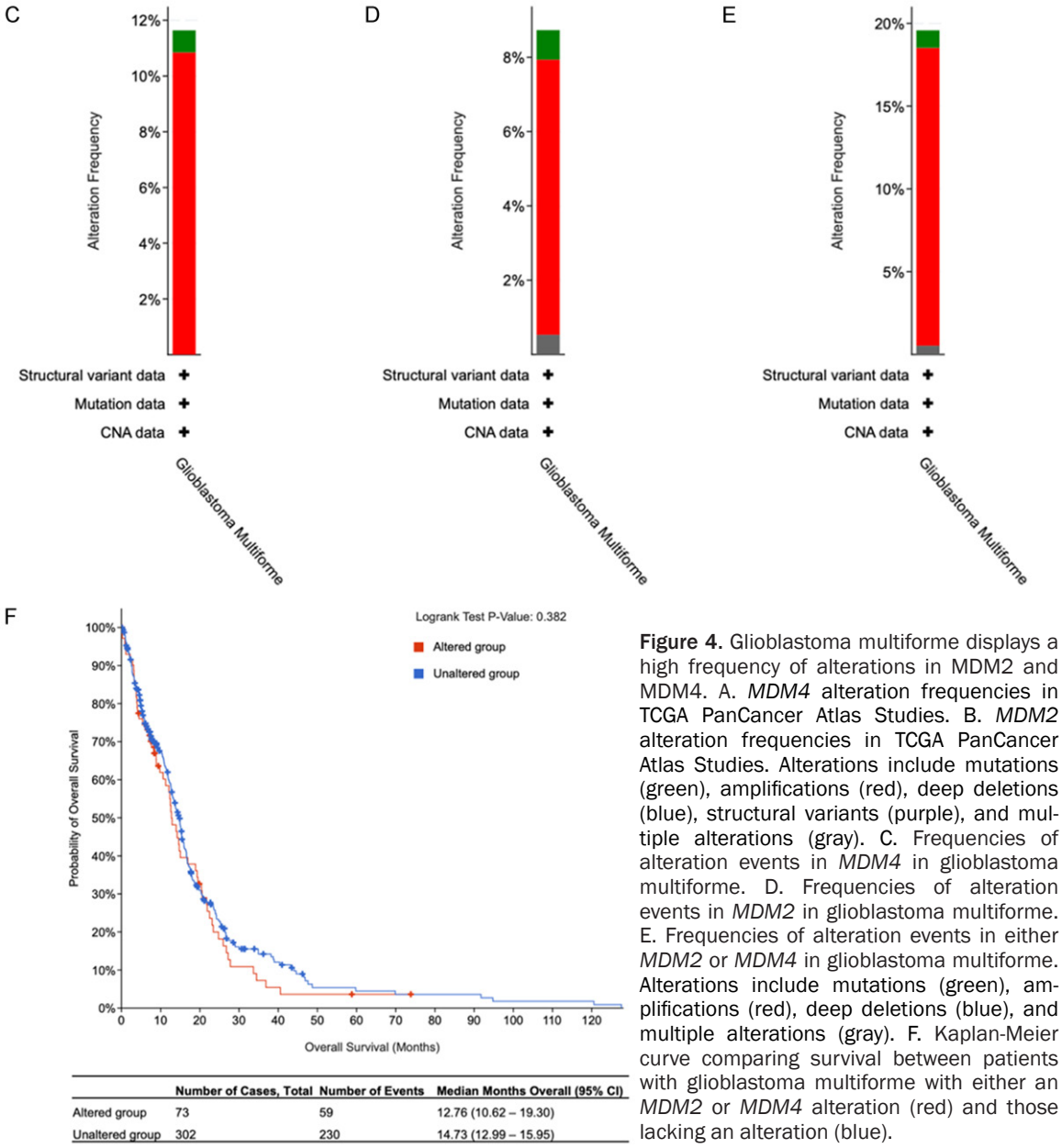


Figure 4. Glioblastoma multiforme displays a high frequency of alterations in MDM2 and MDM4. A. *MDM4* alteration frequencies in TCGA PanCancer Atlas Studies. B. *MDM2* alteration frequencies in TCGA PanCancer Atlas Studies. Alterations include mutations (green), amplifications (red), deep deletions (blue), structural variants (purple), and multiple alterations (gray). C. Frequencies of alteration events in *MDM4* in glioblastoma multiforme. D. Frequencies of alteration events in *MDM2* in glioblastoma multiforme. E. Frequencies of alteration events in either *MDM2* or *MDM4* in glioblastoma multiforme. Alterations include mutations (green), amplifications (red), deep deletions (blue), and multiple alterations (gray). F. Kaplan-Meier curve comparing survival between patients with glioblastoma multiforme with either an *MDM2* or *MDM4* alteration (red) and those lacking an alteration (blue).

similar genomic dysregulation in *MDM2* and *MDM4*, but not in *USP7* or *PPM1D*, in metastatic melanoma and GBM.

Because a germline mutation in *CDKN2A* is the strongest risk factor for the development of melanoma, we sought to determine the extent of alteration in *CDKN2A* seen in GBM. We found that GBM displayed the highest frequency of *CDKN2A* alterations, 55.41%, across all cancer types screened in TCGA, while skin cutaneous melanoma had the eighth highest frequency (**Figure 6A**). *CDKN2A* alterations in GBM patients were largely deep deletions, seen in 54.39% of patients, followed by mutations in 0.68% of patients and structural variants in 0.34% of patients (**Figure 6B**). Like the mutational pattern we found in melanoma, the most frequent type of *CDKN2A* mutation in GBM was truncating, representing 50% of all mutations (**Figure 6C**). Alterations in *CDKN2A* predicted a statistically significant poorer overall survival, with a median of 16.70 months of overall survival seen in patients with an alteration com-

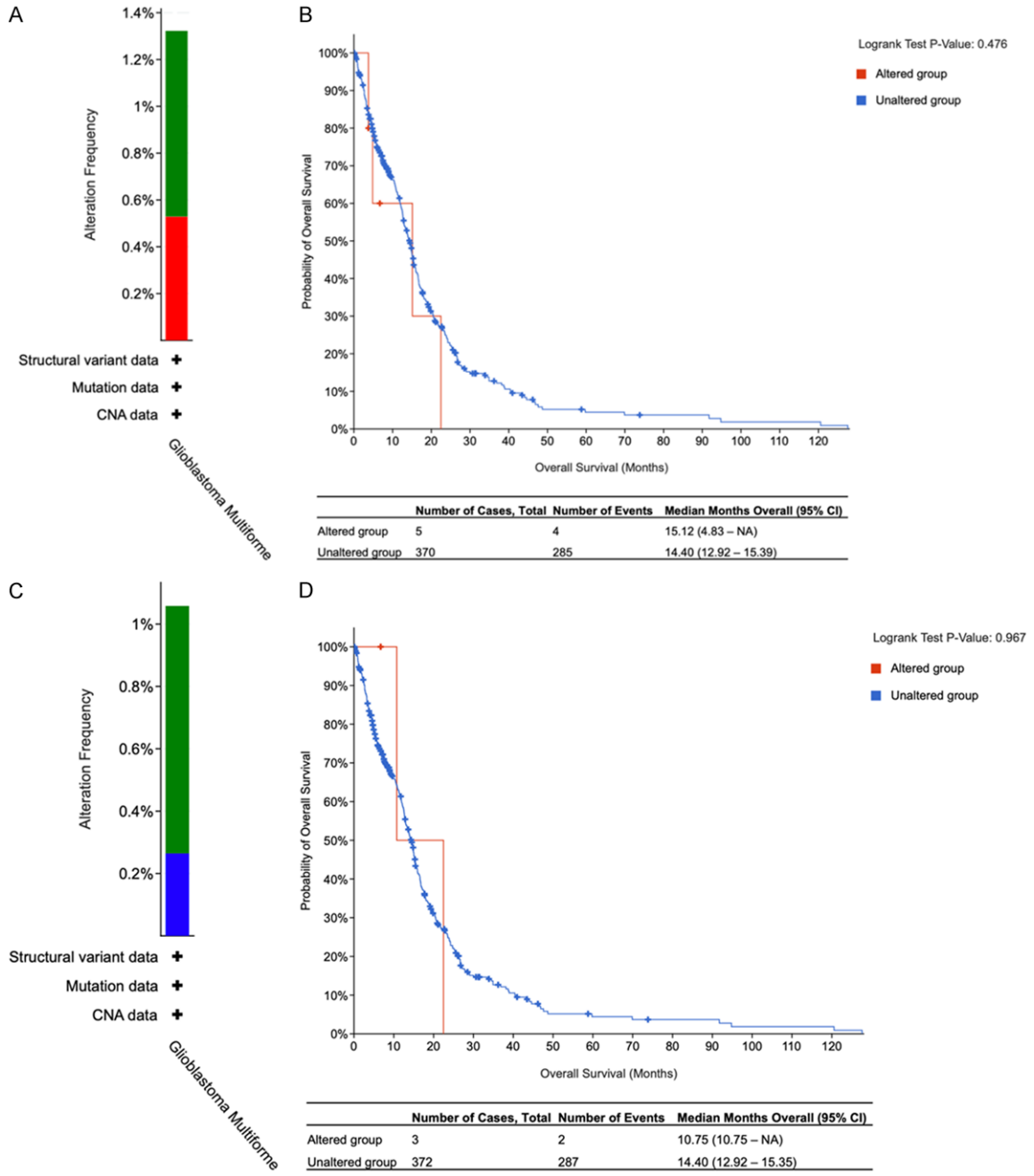


Figure 5. USP7 and PPM1D are not significantly altered in glioblastoma multiforme. A. Frequencies of alteration events in *USP7* in glioblastoma multiforme. B. Kaplan-Meier curve comparing survival between patients with glioblastoma multiforme with a *USP7* alteration (red) and those lacking an alteration (blue). C. Frequencies of alteration events in *PPM1D* in glioblastoma multiforme. Alterations include mutations (green), amplifications (red), and multiple alterations (gray). D. Kaplan-Meier curve comparing survival between patients with glioblastoma multiforme with a *PPM1D* alteration (red) and those lacking an alteration (blue).

pared to 43.23 months in those without (P=0.00) (**Figure 6D**). These findings support the common predisposition to both melanoma and GBM through alteration of *CDKN2A* and highlight that *CDKN2A* may serve to predict clinical prognosis in patients with GBM.

Discussion

Given the lack of known actionable mutations in metastatic melanoma beyond BRAF and poor patient prognoses, the identification of genes driving metastasis and the subsequent

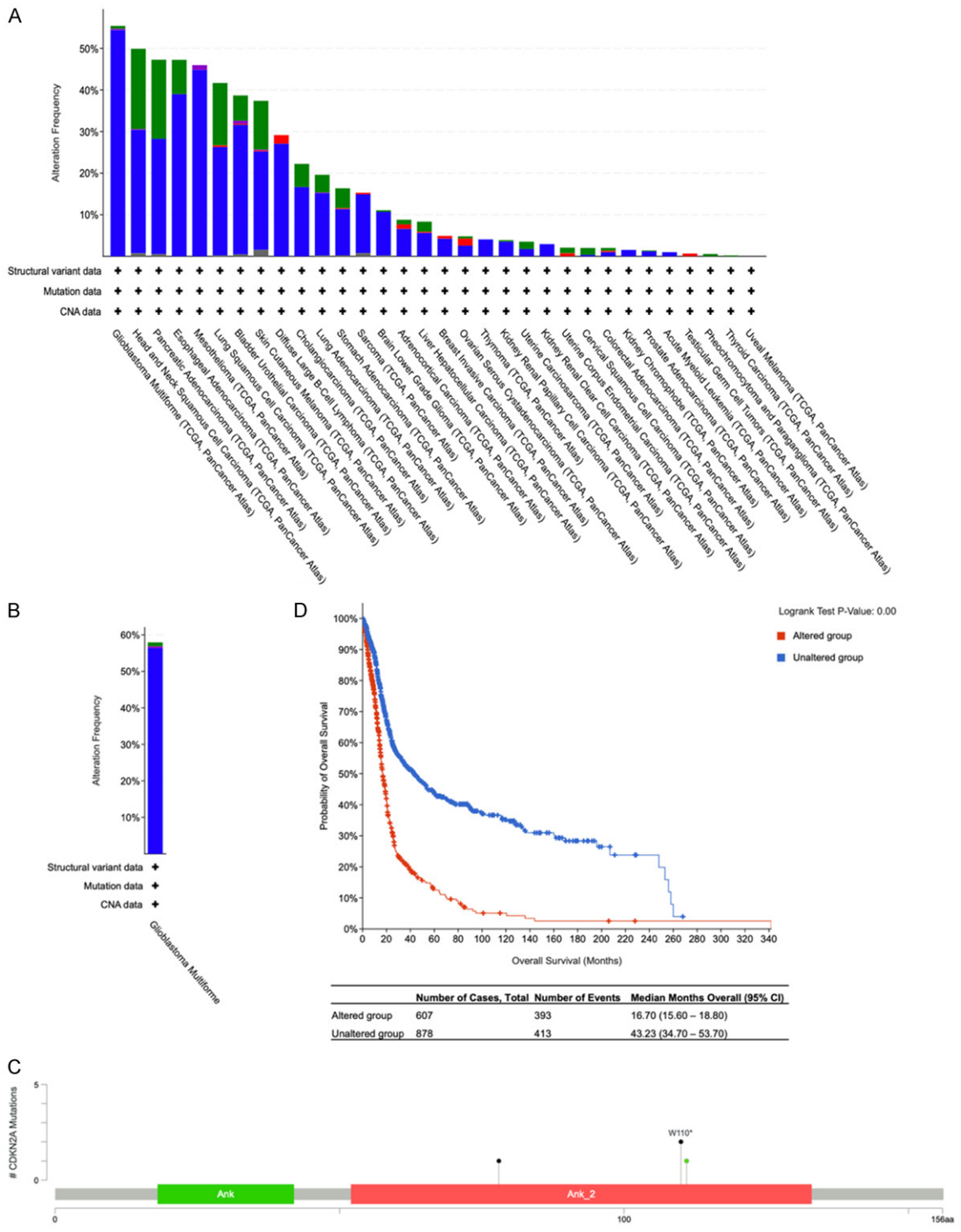


Figure 6. Glioblastoma multiforme displays the highest frequency of alterations in CDKN2A, and alteration predicts a worse survival. A. *CDKN2A* alteration frequencies in TCGA PanCancer Atlas Studies. B. Frequencies of alteration events in *CDKN2A* in glioblastoma multiforme. Alterations include mutations (green), amplifications (red), deep deletions (blue), structural variants (purple), and multiple alterations (gray). C. Schematic of *CDKN2A* mutations. Depicted are VUS missense mutations (green, 1 total) and driver truncating mutations (black, 3 total). D. Kaplan-Meier curve comparing survival between patients with glioblastoma multiforme with a *CDKN2A* alteration (red) and those lacking an alteration (blue).

development of therapies targeting these alterations are needed to help improve patient outcomes. In this study, we compared patients with primary melanoma to those with metastatic disease and propose *MDM2/4*, *USP7*, and *PPM1D* as druggable targets due to their enhanced dysregulation and tendency for alterations to co-occur in metastatic disease.

Because we found a predominance of missense mutations among patients with a *USP7* alteration, we suggest that these mutations are likely loss-of-function. Because loss-of-function of *USP7* would make MDM2 and MDM4 more prone to ubiquitin-dependent proteasomal degradation, there may be a compensatory amplification in these genes to overcome any predisposition for degradation. We therefore propose that restoration of wild-type TP53, through inhibition of MDM2/4, PPM1D, and USP7, both alone and in combination, may be a promising strategy for patients with melanoma, particularly those with metastatic disease.

We find that alterations in *MDM2/4* as well as deletion of *CDKN2A* are predictive of a higher rate of melanoma metastases to the brain. These findings offer evidence to support the idea that patients with melanoma should be stratified into distinct subgroups at the time of diagnosis according to genomic alterations such as *MDM2/4* and *CDKN2A*, as these alterations may help predict subsequent metastasis to particular anatomic locations such as the brain. We therefore propose that patients with melanoma brain metastases may show an enhanced response to *MDM2/4* inhibitors as well as *CDK4/6* inhibitors.

Moreover, patients with an *MDM2/4* alteration also predict a higher rate of metastasis to the liver, but such alterations do not appear to influence metastasis to the lungs. Previous work has demonstrated that patients with melanoma liver metastases responded worse to anti-PD-1 monotherapy and were more likely to progress than those with lung metastases [26]. Patients with melanoma liver metastases are therefore in need of improved therapies, and our findings suggest that they may benefit from MDM2/4 inhibitors, both alone and in combination.

We also propose that alteration in *CDKN2A* may have potential to serve as a predictor of response to immunotherapy. We find that patients with a *CDKN2A* alteration in melanoma are also statistically more likely to have alterations in several other genes that have previously been shown to predict a more favorable response to immune checkpoint inhibitors. We demonstrate that this pattern is seen in non-small cell lung cancer, which is known to respond favorably to immune checkpoint inhibitors [27], but not in glioblastoma, which is largely resistant to immunotherapies [28]. Previous work has demonstrated that evasion of the adaptive immune response is driven by inactivation of many tumor suppressor genes [29], so additional efforts are needed to better understand the role that *CDKN2A* may be playing in contributing to a possible favorable response to immunotherapies in melanoma.

Importantly, we report novel genomic links between metastatic melanoma and glioblastoma multiforme. *CDKN2A* is the major high-penetrance susceptibility gene for melanoma, with germline mutations identified in 20-40% of melanoma families, and germline mutations in *CDKN2A* have also been implicated in the development of familial astrocytoma. Here, we demonstrate potential roles of *MDM2/4* in contributing to this familial tumor predisposition syndrome, as significant amplification of both genes is seen in melanoma and GBM. We propose that patients with GBM may similarly benefit from treatment with MDM2/4 inhibitors, both alone and in combination.

Future work will aim to expand on the number of metastatic melanoma patient samples for analysis. Of note, this current study examined 1015 primary melanoma samples and 358 metastatic melanoma samples. Upon the inclusion of more samples from patients with metastatic melanoma, additional investigations can explore the indicated genomic alterations and compare dysregulation in patients with primary and metastatic disease when the sample sizes are better matched.

Additional pre-clinical investigation may help to determine which combinations of inhibitors targeting MDM2/4, USP7, and PPM1D might be most efficacious in patients with metastatic melanoma and GBM. Moreover, efforts could focus on determining whether combinations of inhibitors targeting the negative regulators of p53 show an enhanced response when used in

combination with CDK4/6 inhibitors, both in metastatic melanoma and GBM. It may be worthwhile to further examine whether patients with brain and liver metastases show a better response to MDM2/4 targeted therapies than those with lung metastases.

The findings reported here offer several therapeutic strategies that warrant further basic and clinical experimentation to expand therapeutic options for patients with metastatic melanoma and GBM. Because patients with these diseases continue to have limited treatment options and face devastating prognoses, novel targeted therapies are desperately needed. As the genomic landscape linking melanoma and glioblastoma becomes better defined, it will be important to understand both the mechanisms driving melanoma metastasis as well as to identify genes that predispose individuals to both diseases to help develop therapeutic options for these patients and improve clinical outcomes.

Disclosure of conflict of interest

None.

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