




Anti-PD-1 elicits regression of undifferentiated pleomorphic sarcomas with UV-mutation signatures

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ABSTRACT

Undifferentiated pleomorphic sarcoma (UPS), an aggressive soft-tissue sarcoma of adults, has been characterized by low tumor mutational burden (TMB) and high copy number alterations. Clinical trials of programmed death-1 (PD-1) blockade in UPS have reported widely varying efficacy. We describe two patients with recurrent scalp UPS that experienced clinical benefit from PD-1 blockade. These tumors had high TMB with a UV-induced mutational pattern. Analysis of additional head and neck UPS cases identified five out of seven tumors with high TMB and an ultraviolet (UV) mutational signature. Head and neck UPS tumors also had increased programmed death-ligand 1 (PD-L1) expression and CD8+ T cell infiltration as compared with UPS tumors arising from other sites. In summary, we found that UPS tumors of the head and neck, but not elsewhere, have a PD-L1+, T-cell-inflamed tumor microenvironment and high TMB, suggesting that these tumors represent a distinct genetic subgroup of UPS for which immune checkpoint inhibitor therapy might be effective.

BACKGROUND

Soft-tissue sarcomas (STS) comprise a heterogeneous group of cancers that arise from mesenchymal tissue and represent ~1% of adult malignancies. Undifferentiated pleomorphic sarcomas (UPS) are typically deep-seated lesions that enlarge rapidly and painlessly, frequently located in the limbs followed by the trunk, while superficial lesions are rare. The pathogenesis is unknown, although some tumors occur in a previously irradiated field.¹ Surgery plus radiotherapy remain the cornerstone of treatment of non-metastatic tumors. Relapse is common, with a 5-year overall survival rate of ~64%, and prognosis for patients with metastatic disease is poor, with a median survival of ~12 months.²

Treatment options for advanced UPS remain limited. Although PD-1 blockade has triggered tumor regressions in some patients, clinical trial results have been mixed. While Tawbi and colleagues reported a 40% (4/10) objective response rate to anti-PD-1, other trials have reported fewer responders or none at all, suggesting that a subset of UPS patients may respond to immune checkpoint blockade.^{3–5} Emerging evidence reveals an association between increased tumor-infiltrating immune cells and tumor regressions.⁶ Recent clinical trials have shown efficacy of PD-1 blockade in high tumor mutational burden (TMB) tumors, across many solid tumor types, leading to approval of the drug for treatment of high TMB tumors, regardless of tumor type.⁷ Additionally, >1% intratumoral PD-L1 expression in other tumor types is often associated with antitumor response to anti-PD-1, however, UPS generally have low TMB and the majority do not express high PD-L1.^{8–11} Further studies to determine how UPS patients who respond to immunotherapy differ from non-responders are necessary for prospectively identifying patients who may benefit from checkpoint blockade and to understand what aspects of the tumor and tumor immune microenvironment can promote response in UPS patients. Here, we report two patients with relapsed and metastatic UPS of the scalp who were found to have unusually high TMB, but were microsatellite stable. Both patients were treated with checkpoint blockade and experienced clinical improvement. We analyzed the mutational signatures and tumor microenvironment of these and other UPS tumors to determine if UPS of



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the head and neck have distinct characteristics relevant to response to checkpoint blockade.

METHODS

Patients and tumor samples

Both patients' primary UPS tumors were not associated with previous radiation therapy. Tumor tissues and blood were collected at Johns Hopkins Hospital (Baltimore, Maryland, USA) from patients with UPS. All samples were obtained in accordance with the Health Insurance

Portability and Accountability Act. Immunohistochemical analysis was performed on samples from the two patients detailed in the case report and 36 unique patients with UPS obtained from the Johns Hopkins Hospital surgical pathology archives from 2005 to 2017. All radiation-induced UPS tumors were excluded. Further details regarding patient treatment and demographics are provided in online supplemental table 1. Whole-exome sequencing (WES) data of head and neck UPS samples was obtained from the two case report patients, three

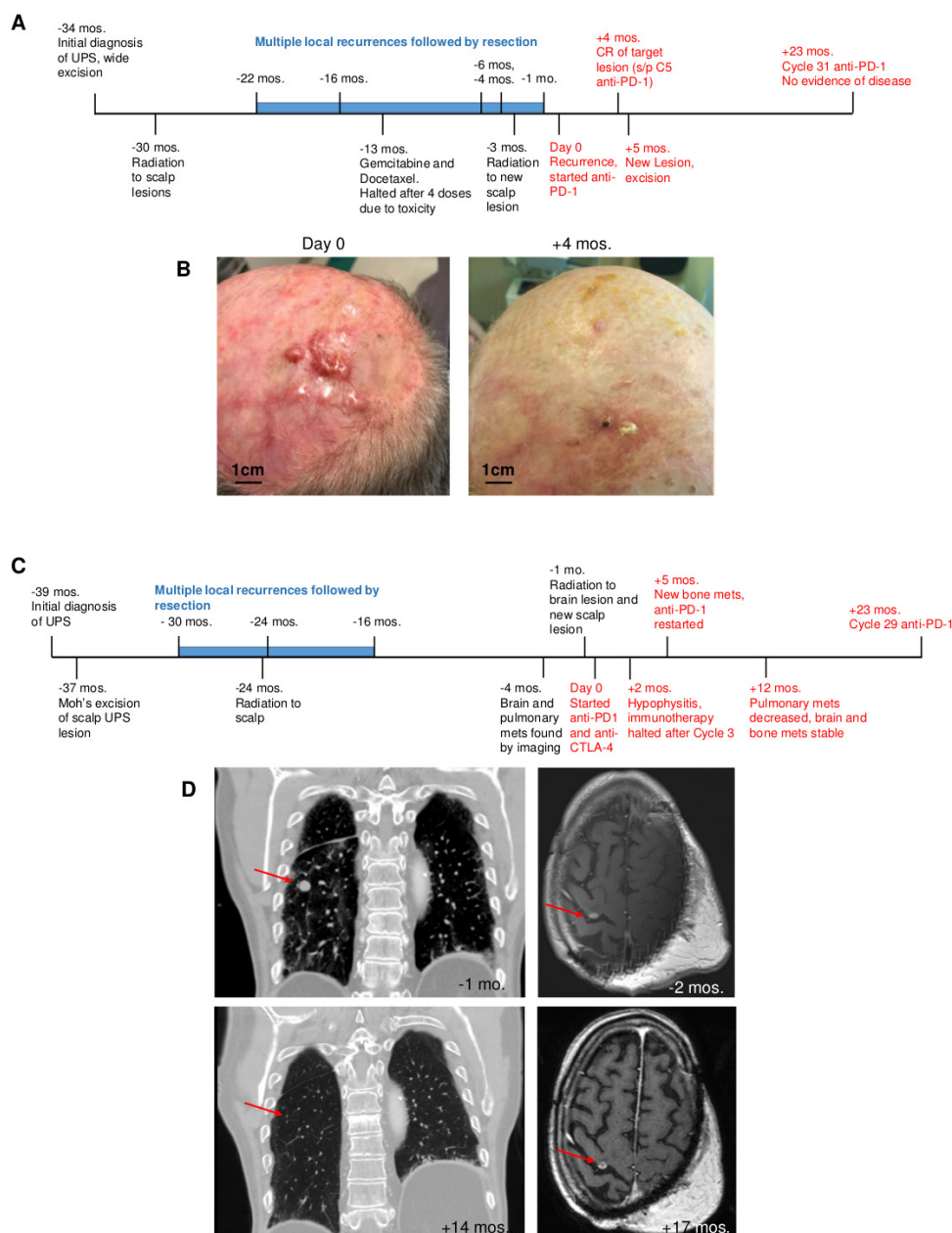


Figure 1 Clinical timeline and response to checkpoint blockade in two patients with ups of the scalp. Day 0 represents initiation of immunotherapy treatment. Events in red occurred while patients were treated with immunotherapy. (A) Clinical timeline of treatment for patient 1. (B) Images of patient 1 scalp lesions from before (left) and after achieving a CR of the target lesion 4 months after initiation of immunotherapy (right). (C) Clinical timeline of treatment for patient 2. (D) CT imaging of chest and MRI of brain from patient 2 before (top) and during (bottom) immunotherapy treatment exhibiting resolution of lung metastatic lesion and stabilization of brain metastatic mass. UPS, undifferentiated pleomorphic sarcoma.; CR, complete response.

patients from the Johns Hopkins Hospital surgical pathology archives, one patient from the Cancer Genome Atlas (TCGA) and one patient from MSK-IMPACT clinical sequencing cohort (online supplemental table 2).^{12 13} Genomic data for 48 non-head and neck UPS samples were from TCGA.

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks from tumor specimens were annotated by a pathologist, cut into 5 µm sections and mounted onto plus-charge glass slides. For each specimen, staining was performed according to standard protocol.

RESULTS

Case presentation number 1

A male in his late 60s with Fitzpatrick skin type II and a history of basal cell and cutaneous squamous cell carcinoma presented with a scalp lesion confirmed to be high grade UPS and was initially treated with surgical wide excision of the lesion, followed by radiation therapy. Over the next 18 months, the patient presented with multiple recurrences of scalp lesions, which were resected. He received gemcitabine and docetaxel, which was discontinued due to toxicities, and additional doses of radiation

to his scalp but continued to develop new scalp lesions. Targeted DNA sequencing of his lesions revealed a microsatellite stable high TMB tumor. From the time of diagnosis, the patient underwent six total surgical resections and two cycles of radiation, but due to progression of his disease and high TMB of his tumor, he was enrolled on a phase 2 clinical trial with anti-PD-1 therapy for high TMB neoplasms (ClinicalTrials.gov number, NCT01876511). The largest scalp lesion measured 20 mm in diameter with several additional satellite lesions. After 4 months of anti-PD-1 treatment, the patient had a complete response (defined by Response Evaluation Criteria in Solid Tumors, RECIST V.1.1) of his dominant scalp lesion and satellite scalp lesions.¹⁴ One month later, a new scalp lesion emerged. However, given that the dominant scalp lesion was still undetectable and there was no evidence of metastatic disease, the new lesion was resected and the patient continued on anti-PD-1 after surgical recovery. The patient has now been treated with anti-PD-1 for 22 months and continues to have no evidence of local or distant disease for the past 16 months (figure 1A,B).

Case presentation number 2

A man in his late 70s with Fitzpatrick skin type II and a history of cutaneous squamous cell carcinoma presented

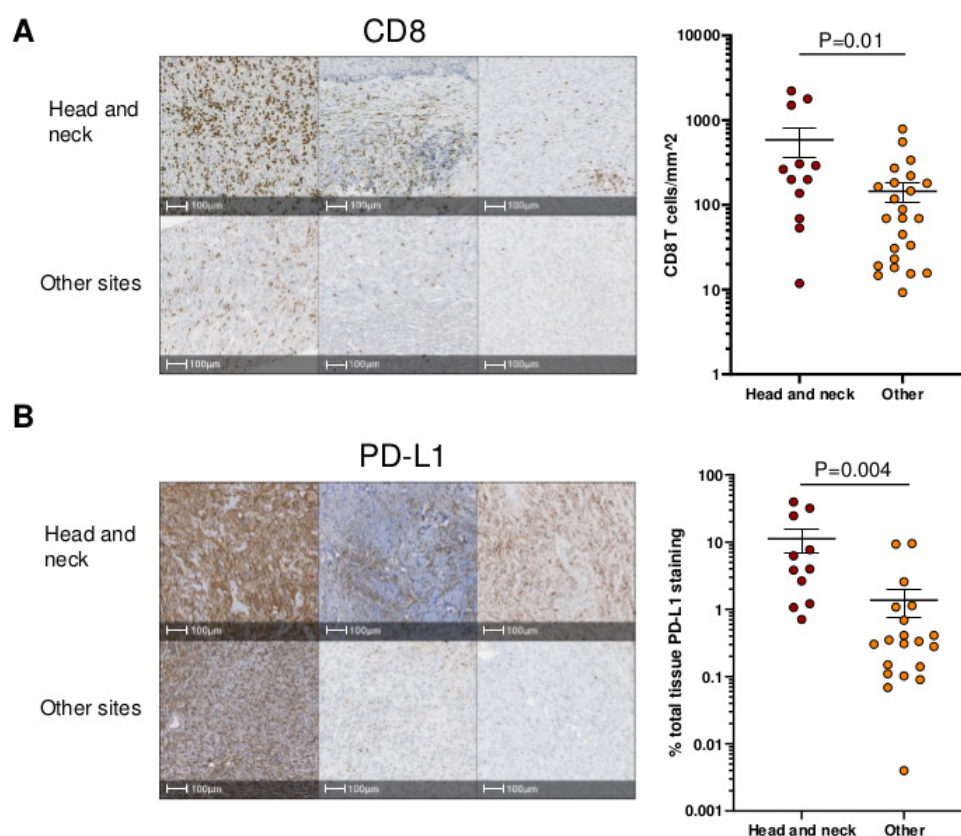


Figure 2 CD8+ T cell infiltration and PD-L1 expression in UPS tumors of head and neck compared with other sites. (A) Representative images and quantitation of IHC staining of UPS tumors for CD8. The number of CD8+ T cells mm² of tumor tissue is shown. (B) Representative images and quantitation of IHC staining of UPS tumors for PD-L1. Per cent PD-L1 expression of tumor tissue is shown. I bars show the SE. P values were calculated using a two-tailed t-test. UPS, undifferentiated pleomorphic sarcoma; IHC, immunohistochemistry.

with a high grade UPS of his occipital scalp. Targeted DNA sequencing of the tumor found it to be microsatellite stable with a high TMB. He underwent wide excision but experienced disease relapse 7 months later requiring a radical resection, followed by adjuvant radiation therapy. Despite undergoing several surgical resections, the patient developed brain, osseous, and lung metastases. The patient received radiation to the brain lesion and to a new scalp lesion. One month later on follow-up CT, several lung nodules were noted to be increasing in size. The lung mass measured 13mm and the brain lesion was 7mm in diameter pretreatment. Immunotherapy with combination anti-PD-1 and anti-CTLA-4 was started. After 1 month of anti-PD-1, the patient had regression of lung nodules with stabilization of the brain lesion. At month 2 of anti-PD-1 and anti-CTLA-4, the patient developed hypophysitis requiring administration of replacement hydrocortisone therapy and immunotherapy was halted. Imaging demonstrated new osseous metastases, but pulmonary nodules continued to shrink and the brain lesion remained stable and anti-PD-1 monotherapy was restarted 3 months later. The patient has had complete resolution of pulmonary nodules, with stable brain and bone lesions for the last 18 months after 23 months of anti-PD-1 therapy (figure 1C,D).

WES and mutational signature

As targeted sequencing uses a panel of commonly mutated genes and can only provide an estimated TMB, WES was performed to determine the exact TMB for each tumor. High TMB is generally defined as ≥ 10 mutations/MB.¹⁵ TMB was 33 and 43 mutations/MB for the patients, respectively, while the average TMB of UPS tumors in the TCGA is 2.7 mutations/MB, indicating these scalp tumors were likely subjected to a different mutational process. To assess mutational signatures of these tumors, we compared the frequencies of single nucleotide variants to reference signatures in the COSMIC database. We found that for both tumors, COSMIC signature 7 was the dominant signature, with a prevalence of C>T mutations in a dipyrimidine context, which is characteristic of UV-induced DNA damage and repair and is frequently found in melanoma (online supplemental figures 1A, 2A).¹⁶ This finding is interesting as a previous study characterizing genomics of STS identified only one out of 44 UPS tumors with high TMB due to a mutation in DNA mismatch repair machinery, rather than being due to UV exposure.⁸

To determine whether UV damage is common in head and neck UPS tumors, we analyzed mutational signatures of UPS tumors in publically available databases as well as tumors from three patients who underwent surgical resection at Johns Hopkins Hospital. Of three head and neck UPS tumors resected from Johns Hopkins patients that we sequenced, one patient also had high TMB at 68 mutations/MB with a UV mutational signature, while the other two patients had less than 10 mutations/MB. Additionally, we found two patients with primary UPS tumors

arising from the head and neck in the TCGA and MSK-IMPACT databases and both tumors had high TMB (53 and 62 mutations/MB) with a UV mutational signature (online supplemental figures 2, 3, online supplemental table 2).^{12,13} In the TCGA dataset, one non head and neck UPS tumor had high TMB, located at the thigh/knee with a UV signature (data not shown), however, 26 other UPS tumors located on the leg were low TMB. In summary, of seven head and neck UPS tumors for which sequencing data was available, five tumors had high TMB due to UV exposure.

T cell infiltration and PD-L1 expression in the tumor microenvironment

CD8 +T cell infiltration and PD-L1 expression have been found to correlate with anti-PD-1 response, independently of TMB.¹⁷ While UPS tumors generally have a paucity of T cells and low expression of PD-L1, tumors from patient 1 and 2 had high levels of infiltrating CD8 +T cells and expression of PD-L1 (online supplemental figure 1B).¹¹ We analyzed 36 additional UPS samples from the Johns Hopkins Hospital archive, comparing the levels of CD8 +T cells and PD-L1 expression in head and neck UPS tumors to those arising from other sites in the body (online supplemental table 1). Head and neck UPS tumors had increased CD8 +T cell infiltration and expression of PD-L1 versus UPS tumors from other sites of the body (figure 2A,B). High TMB did not predict the level of CD8+ T cells or PD-L1 in the tumor.

DISCUSSION

We report two patients with recurrent scalp UPS tumors with high TMB and UV mutational signatures who experienced clinical benefit with anti-PD-1 therapy. Together with analysis of additional head and neck UPS samples, our findings suggest that UPS tumors arising in this location may represent a distinct genetic subgroup of UPS with predominantly UV-induced mutations that confer susceptibility to checkpoint blockade. Both patients had developed frequent locally recurrent tumors, and in one case, metastatic lesions before starting immunotherapy. Additionally, patients were unable to continue with standard chemotherapy due to treatment failure or intolerable toxicities. On treatment with anti-PD-1, both patients derived clinical benefit from checkpoint blockade. Further analysis of head and neck UPS tumors revealed additional cases with a UV mutational signature and a T-cell-inflamed immune signature as compared with UPS tumors arising from other sites of the body.

UPS tumors have been previously reported to respond to immunotherapy, however, the majority are not sensitive to anti-PD-1 treatment.³⁻⁵ In these trials, the location of the primary tumors (ie, head and neck vs elsewhere) were not reported. UPS generally has low TMB, and therefore, generates fewer tumor neoantigens, decreasing the likelihood of T cell recognition of the tumor.¹⁸ High TMB can occur due to environmental factors such as exposure to carcinogens or mutations in DNA repair pathways that allow for the accrual

of errors during DNA replication.^{19,20} UV rays only penetrate the epidermis and dermis, causing DNA damage in tumors such as melanoma and squamous cell carcinoma. While most UPS tumors originate in the deep tissues, a subset of tumors arise in the dermis, making these tumors more likely to have UV-induced high TMB.²¹ Our data suggest that UV damage can cause high TMB in UPS tumors of the head and neck, due to increased sun exposure of these sites, making these tumors more likely to be sensitive to checkpoint blockade than tumors arising from other sites. Of seven head and neck UPS tumors for which we obtained WES data, five tumors had high TMB with a UV signature, suggesting this may be a frequent phenomenon. Additionally, 1 of 26 leg UPS tumors also had high TMB, indicating that although non-head and neck tumors are less frequently high TMB, some UPS tumors arising in other sun-exposed sites may also be amenable to checkpoint blockade. Steele *et al* have also identified a subset of high TMB UPS tumors due to DNA repair pathway defects associated with IFN gamma response pathway enrichment, suggestive of increased immune infiltration, which mirrors our observations in UPS tumors with UV mutational signatures.²⁰

Previously, studies of UPS immune infiltrates have shown low levels of CD8+ T cells and PD-L1 expression, despite being more inflamed when compared with other STS.^{10,22} We found that head and neck UPS tumors had increased CD8+ T cell infiltration and intratumoral PD-L1 expression as compared with UPS tumors located in other sites. This T-cell-inflamed phenotype could indicate a pre-existing anti-tumor immune response that has subsequently become exhausted, making these tumors more likely to respond to anti-PD-1 therapy.²³

In conclusion, we describe two patients with high TMB scalp UPS tumors who experienced an anti-tumor immune response to checkpoint blockade. Head and neck UPS showed a higher frequency of UV-induced high TMB and a T-cell inflamed phenotype compared with UPS arising from other sites. Taken together, these findings support the screening of head and neck UPS tumors for high TMB and consideration for treatment with PD-1 blockade.

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Contributors LSC and NL conceptualized and designed the study. LSC, LC, and TBS analyzed data. LSC, LC, TFO, JT and HK collected clinical data. LSC, LC and NL interpreted data. LAD, EL, J-WS, JT, RA, DMP, DTL and CFM provided insight and assisted in interpretation of data. DTL, KB and CFM treated the cases. LSC and NL wrote the manuscript. NL supervised the study. All authors reviewed and edited manuscript.

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Competing interests LC is employed by Janssen Research and Development at the time of this submission. LAD is a member of the board of directors of Personal Genome Diagnostics (PGDx) and Jounce Therapeutics. He is a paid consultant to PGDx, 4Paws (PetDx), Innovatus CP and Neophore. He is an uncompensated consultant for Merck but has received research support for clinical trials from Merck. LAD is an inventor of multiple licensed patents related to technology for circulating tumor DNA analyses and mismatch repair deficiency for diagnosis and therapy from Johns Hopkins University. Some of these licenses and relationships are associated with equity or royalty payments directly to Johns Hopkins and LAD. He holds equity in PGDx, Jounce Therapeutics, Thrive Earlier Detection and Neophore. His spouse holds equity in Amgen. The terms of all these arrangements are being managed by Johns Hopkins and Memorial Sloan Kettering in accordance with their conflict of interest policies. EJJ has received institutional research grant funding from Bristol-Myers Squibb, Merck, and Regeneron. EJJ is a consultant for Array BioPharma, Bristol-Myers Squibb, EMD Serono, MacroGenics, Novartis, Merck, Regeneron, Sanofi Genzyme. JMT serves on advisory boards for BMS, Merck, Akoya Biosciences, Astra Zeneca and has received research funding from BMS, Akoya Biosciences. RAA serves on advisory boards for Merck, Bristol Myers Squibb and FLX bio and has received research funding from Merck, Bristol Myers Squibb, and FLX bio. DMP serves on advisory boards for Merck, Bristol Myers Squibb and Compugen and has received research funding from Bristol Myers Squibb and Compugen. DTL serves on advisory boards for Merck and Bristol Myers Squibb and has received research funding from Merck, Bristol Myers Squibb, Aduro Biotech, Curegenix, Medivir, and Nouscom. She has received speaking honoraria from Merck and is an inventor of licensed intellectual property related to technology for mismatch repair deficiency for diagnosis and therapy (W02016077553A1) from Johns Hopkins University. The terms of these arrangements are being managed by Johns Hopkins. CFM was a paid consultant for Bayer and has received speakers bureau honoraria from Novartis. NJL has received funding support from Bristol Myers Squibb.

Patient consent for publication Obtained.

Ethics approval This study was conducted in accordance with the ethical principles stated in the Belmont Report and the US Common Rule. It was approved by the Johns Hopkins Institutional Review Board and all samples were obtained in accordance with the Health Insurance and Accountability Act. This study was conducted under an Institutional Review Board approved protocol with a waiver of consent for archived tissues and patients gave written informed consent for prospective tumor collection.

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Supplementary Materials

Anti-PD-1 elicits regression of undifferentiated pleomorphic sarcomas with UV-mutation signatures

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SUPPLEMENTARY METHODS

Human subjects and tissues

Patient 1 was enrolled in a phase II study of pembrolizumab treatment for patients with Microsatellite Unstable (MSI) and High Tumor Mutation Burden Tumors (ClinicalTrials.gov number, NCT01876511). Patient 2 was identified in the setting of clinical testing and consented to our IRB approved protocol for studying the Immunobiology of Blood and Tissues. Patient 2 was initially treated with nivolumab and ipilimumab until the development of hypophysitis after 2 months of treatment. Immunotherapy was halted and the patient was restarted on nivolumab alone three months later. Due to insurance issues, Patient 2 was switched to pembrolizumab after one year of nivolumab treatment. Comparison cohort of patients examined under this

study was approved by the Johns Hopkins University Institutional Review Board and conducted in accordance with the ethical standards of the Declaration of Helsinki. All samples were obtained in accordance with the Health Insurance Privacy and Accountability Act. Specimens from 38 unique patients with UPS were obtained between 2005 and 2017 from the Johns Hopkins Hospital surgical pathology archives.

Whole exome sequencing and mutational signature analysis

Whole exome sequencing was performed on DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissues. Library preparation and sequencing were performed by Personal Genome Diagnostics (Baltimore, MD).[1] Mutation data from UPS patients in public datasets were obtained from the TCGA and MSK-IMPACT cohort.[2] Tumor-specific mutations were identified through comparison between tumor and matched normal tissue. Mutational signature analysis of somatic single nucleotide variants was performed using the online tool MuSiCA.[3] Mutational signatures were based on classification of single nucleotide substitution in every possible trinucleotide context and shown as the relative proportion of each mutation type across all single nucleotide substitutions. Resemblance between sample mutational signatures and known mutational signatures is quantified along a 0-1 scale using cosine similarity, where the closer the value is to 1, the more similar the signatures are to each other.

Fraction genome altered analysis

Pre-processed whole exome sequencing data from matched patient tumor and normal samples was aligned to the *hg19* human reference genome and subjected to a genomic copy number variation analysis pipeline using the Genome Analysis Toolkit (GATK version 4.1.4.0; the Broad Institute of MIT and Harvard, Cambridge, MA). In brief, read coverage counts were collected for pre-filtered reads overlapping each genomic interval

targeted by the sequence capture probes. Read coverage counts were then standardized to a \log_2 scale and denoised of systematic artifacts against a panel of sequencing data from three similarly-sequenced normal tissues of patients uninvolved in this study.

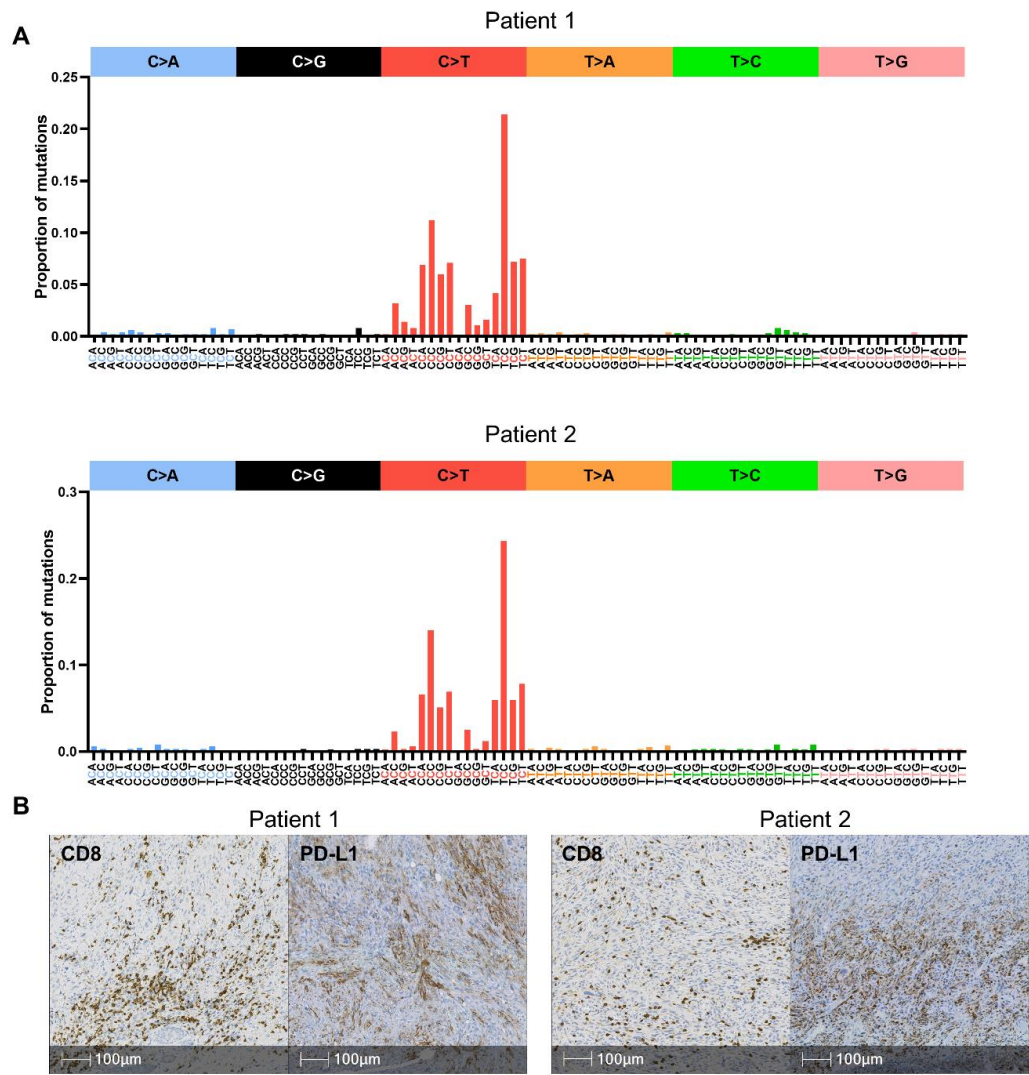
By modeling haplotypes in the sequence data, allelic fractions for each sequenced region in normal and tumor study samples were calculated and used to model amplification or loss of contiguous genomic loci in standardized, denoised tumor read coverage counts data. The total fraction genome altered for each patient tumor sample was calculated by dividing the sum of all genomic segments exhibiting a \log_2 fold change greater than 0.2 or less than -0.2 by the total length of all modeled segments in the sequencing data.

Immunohistochemistry staining and quantification

Histopathology, IHC, and image analysis of FFPE specimens were stained with hematoxylin and eosin combination, CD3 (clone PS1, Leica Biosystems), CD8 (clone C8144B, Cell Marque), IDO1 (clone SP260, Abcam/Spring Bio), PD-L1 (clone SP142), and CD163 (clone Novacastra10D6, Leica Biosystems) according to the standard protocols. Analysis was performed using HALO image analysis software. CD8+ T cells were quantified as cells per unit area. PD-L1 was quantified as percent positive staining per unit area.

SUPPLEMENTARY FIGURES

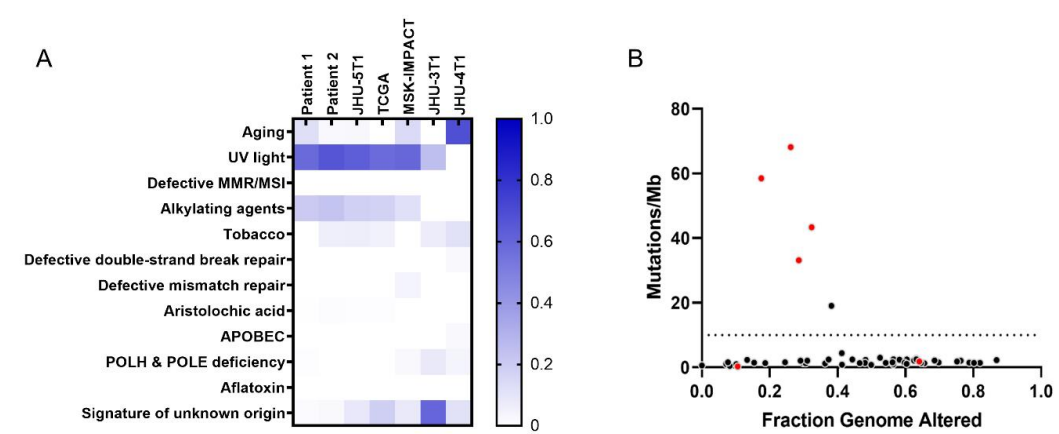
Supplementary Figure 1



Supplementary Figure 1. Tumor mutational signatures and immunophenotyping of tumor microenvironment in two scalp UPS patients.

(A) Distribution of single nucleotide substitutions in a trinucleotide context for the tumors of each patient as compared to matched normal tissue. Mutations are displayed based on the type of substitution (C>A, C>G, C>T, T>A, T>C, T>G). The vertical axis is the proportion of mutations that are of the designated type. **(B)** Photomicrographs of immunohistochemical stains for CD8 and PD-L1 of tumors from Patient 1 (left) and Patient 2 (right) demonstrating a robust immune infiltration of specimens. Both samples were obtained prior to immune checkpoint blockade.

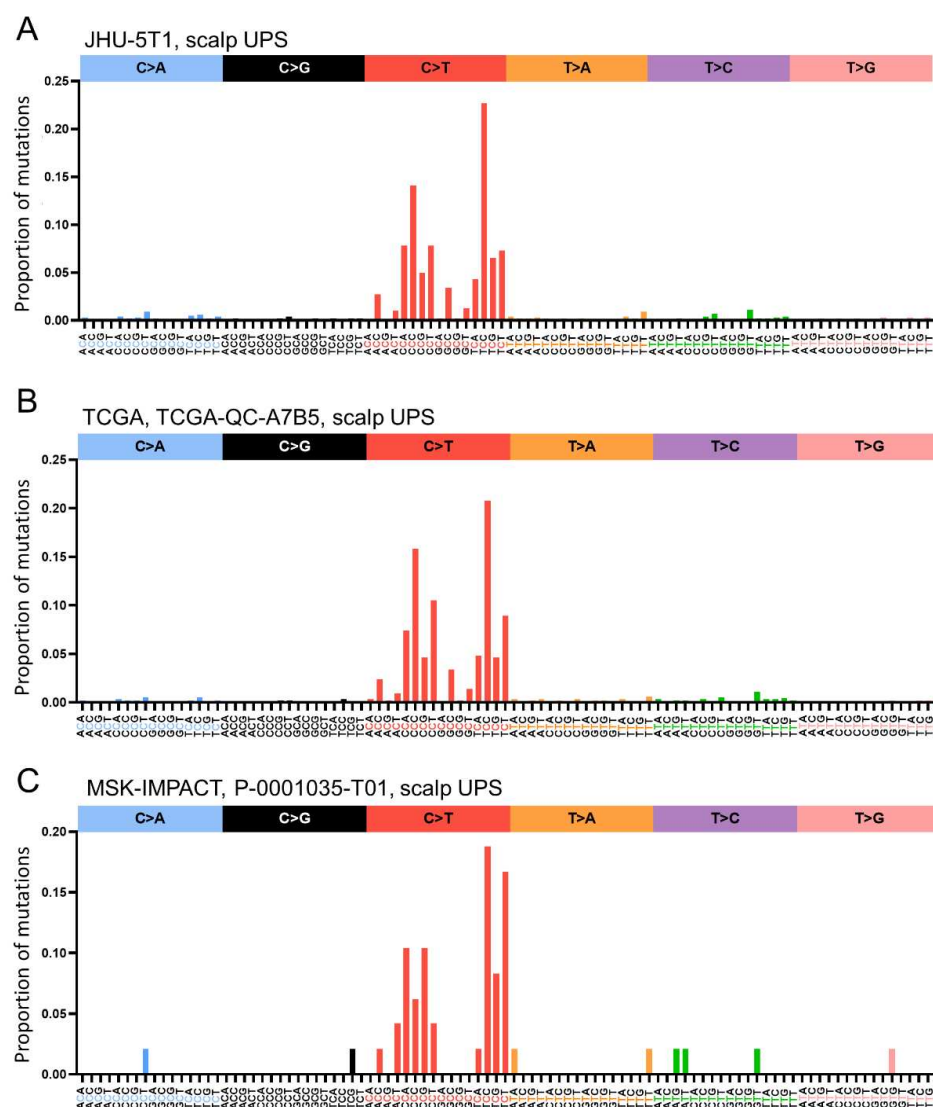
Supplementary Figure 2



Supplementary Figure 2. Mutational signature analysis and genomic alterations of head and neck UPS sarcomas.

(A) Heat map depicting correspondence of sample mutational signature to mutational signature of specified etiology. Level of similarity shown on scale of 0 to 1, a value of 1 indicates the signatures are identical. **(B)** Tumor mutational burden and fraction genome altered of UPS tumors from the TCGA and 5 Johns Hopkins patients are shown. ● represent head and neck UPS cases; ● represent UPS cases originating from other sites of the body. The dotted line at 10 mutations/Mb shows the lower cutoff for high TMB tumors.

Supplementary Figure 3



Supplementary Figure 3. Mutational signatures of head and neck UPS tumors with high TMB.

The horizontal axis shows the type of nucleotide substitution in a trinucleotide context in tumor tissue as compared to matched normal tissue. The vertical axis is the proportion of mutations that are of a specific type. **(A)** Scalp UPS from a patient seen at Johns Hopkins who was not treated with immunotherapy. **(B)** Scalp UPS from the TCGA database with the patient ID listed. **(C)** Scalp UPS from the MSK-IMPACT cohort with

the patient ID listed. All the tumors showed a predominance of C>T mutations at dipyrimidine sites.

Supplementary Table 1. Demographics of Johns Hopkins UPS patients for IHC analysis

Characteristic	Head and neck	Other sites
	(N=12)	(N=26)
Male sex--no.(%)	9 (75)	14 (54)
Age, years	71 ± 10.1	61 ± 14.4
Previous treatment--no.(%)		
None	10 (83)	12 (46)
Chemotherapy	0	2 (8)
Radiation	2 (17)	5 (19)
Chemotherapy and radiation	0	7 (27)
Sample type--no.(%)		
Primary tumor	8 (67)	22 (85)
Recurrence	4 (33)	3 (12)
Metastasis	0	1 (4)

*Plus-minus values are means ± SD

Supplementary Table 2. Whole exome sequencing sample summary

Patient	Source	Tumor location	Tumor mutation burden (mutations/MB)
Patient 1	JHU	scalp	33
Patient 2	JHU	scalp	43
JHU-5T1	JHU	scalp	68
TCGA-QC-A7B5	TCGA	scalp	53
P-001035-T01	MSK-IMPACT	scalp	62
JHU-3T1	JHU	scalp	0.27
JHU-4T1	JHU	cheek	1.8

SUPPLEMENTARY REFERENCES

1. Jones S, Anagnostou V, Lytle K, et al. Personalized genomic analyses for cancer mutation discovery and interpretation. *Sci Transl Med*. 2015;7.
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3. Díaz-Gay M, Vila-Casadesús M, Franch-Expósito S, et al. Mutational Signatures in Cancer (MuSiCa): A web application to implement mutational signatures analysis in cancer samples. *BMC Bioinformatics*. *BMC Bioinformatics*; 2018;19:1–6.