

Supplementary Materials

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

Authors

Laurene S. Cheung, Ph.D., Lingling Chen, M.D., Teniola Oke, B.S., Thomas B. Schaffer, Ph.D., Karim Boudadi, M.D., Jillian T. Ngo, John M. Gross M.D., Holly Kemberling, R.N., Luis A. Diaz, Jr., M.D., Evan J. Lipson, M.D., John-William Sidhom, M.S., Janis M. Taube, M.D., Robert A. Anders, M.D., Ph.D, Drew M. Pardoll M.D., Ph.D., Dung T. Le, M.D., Christian F. Meyer, M.D., Ph.D, Nicolas J. Llosa M.D.

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

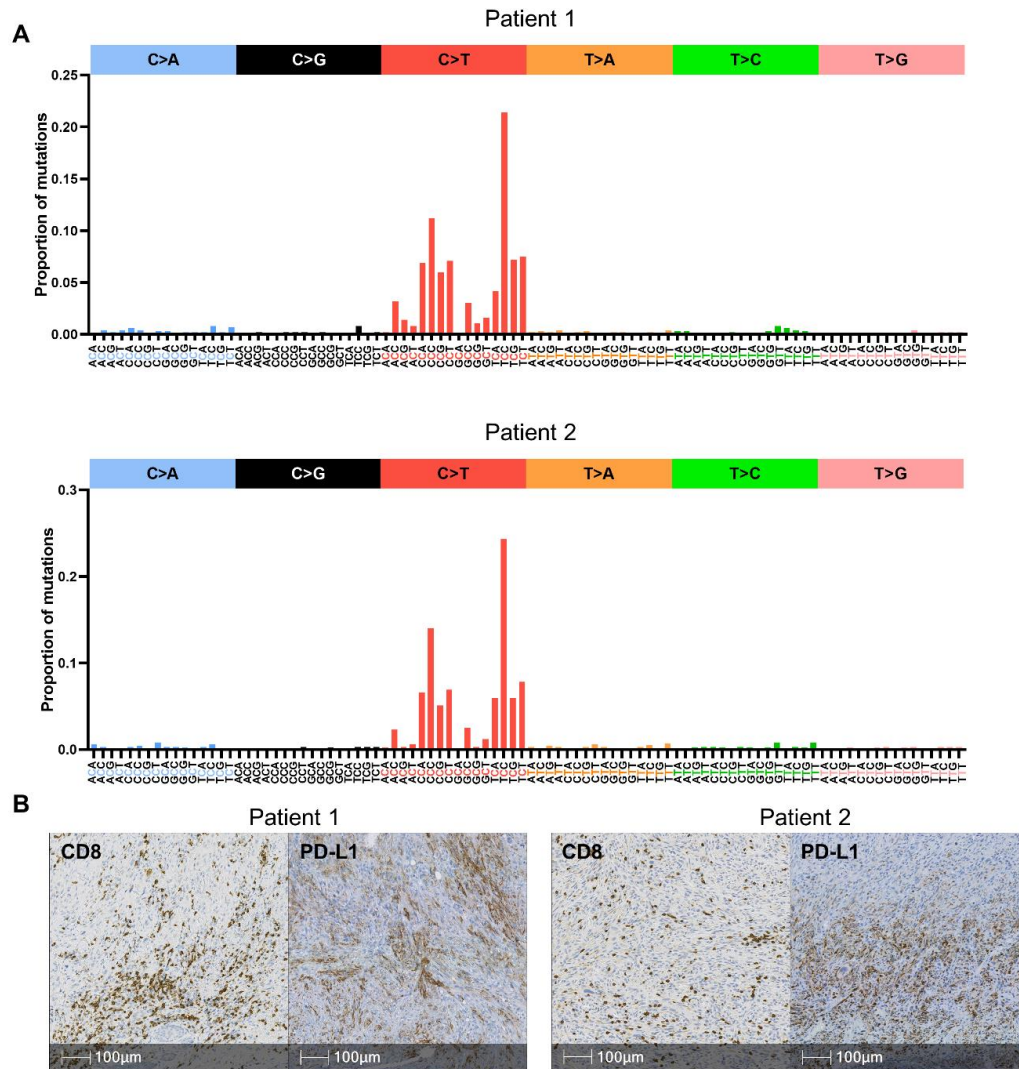
Pre-processed whole exome sequencing data from matched patient tumor and normal samples was aligned to the *hg19* human reference genome and subjected to a genomic copy number variation analysis pipeline using the Genome Analysis Toolkit (GATK version 4.1.4.0; the Broad Institute of MIT and Harvard, 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

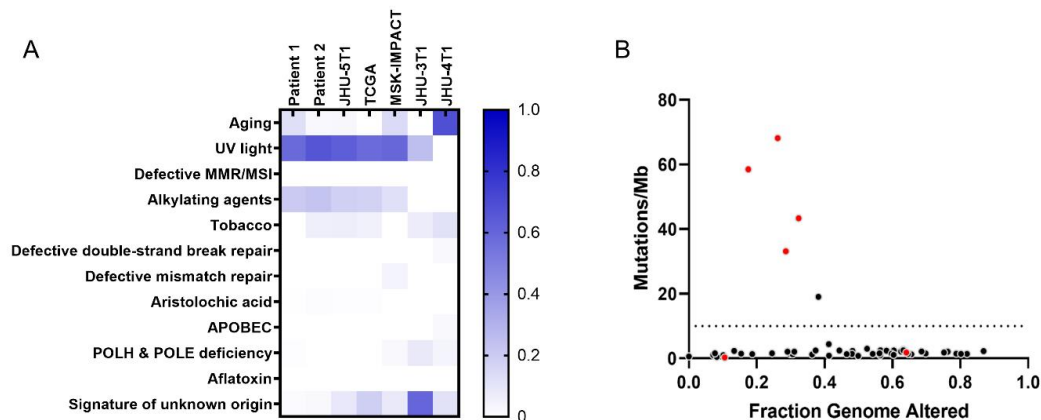
Histopathology, IHC, and image analysis of FFPE specimens were stained with hematoxylin and eosin combination, CD3 (clone PS1, Leica Biosystems), CD8 (clone C8144B, Cell Marque), IDO1 (clone SP260, Abcam/Spring Bio), PD-L1 (clone SP142), and CD163 (clone Novacastra10D6, Leica Biosystems) according to 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.

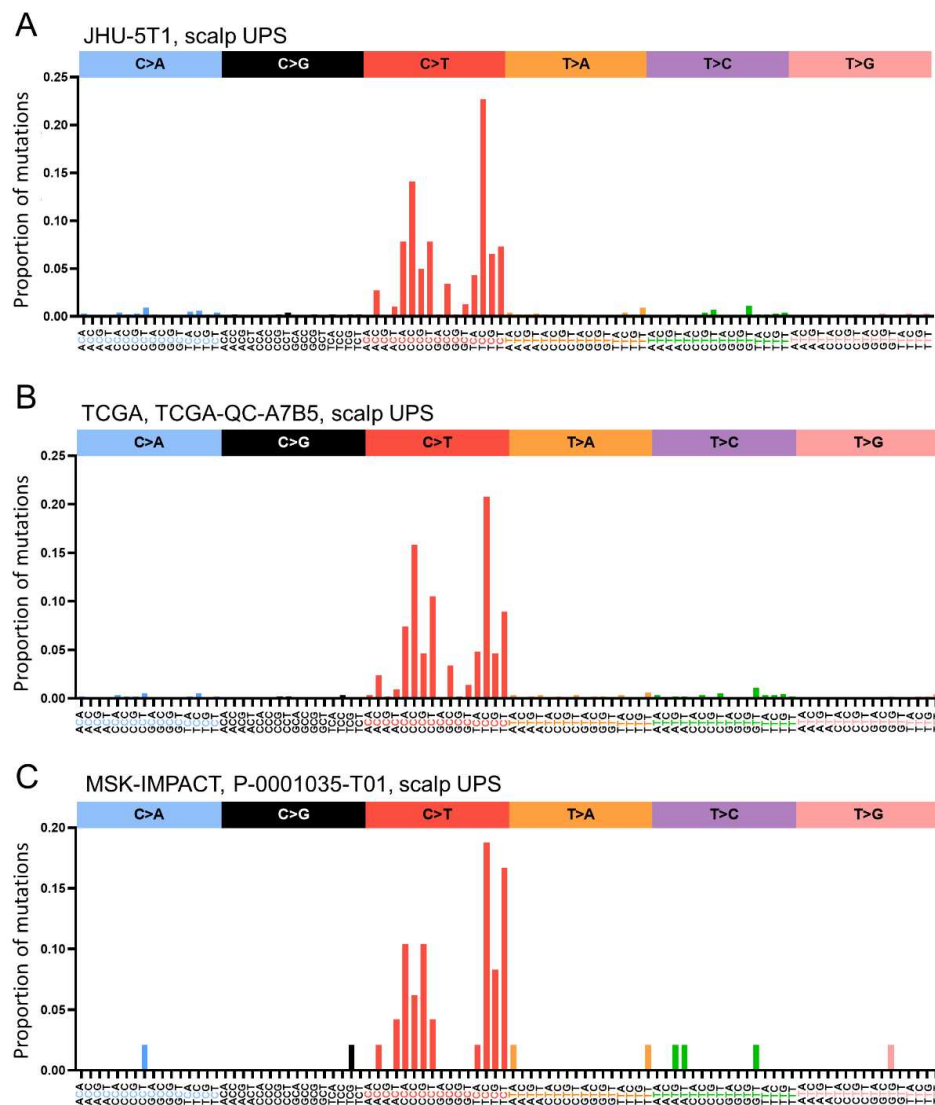
Supplementary Figure 2



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.
2. 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.
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.