Stratification of metastatic melanoma patients based on mutational signatures

Caterina Alfano
PhD program in Network Oncology and Precision Medicine
Sapienza University of Rome
Rome, Italy
caterina.alfano@uniroma1.it

Lorenzo Farina
Dept. of Computer, Control and Management Engineering
PhD program in Network Oncology and Precision Medicine
Sapienza University of Rome
Rome, Italy
lorenzo.farina@uniroma1.it

Manuela Petti
Dept. of Computer, Control and Management Engineering
Sapienza University of Rome
Rome, Italy
manuela.petti@uniroma1.it

***Abstract*—Genetic instability is one of the hallmarks of cancer, however mutations can occur for different causes and induce different effects. Mutational signatures are characteristic patterns of somatic mutations in cancer genomes, reflecting the underlying mutational processes. A mutational signature can be determined by studying the kind of mutations a patient has acquired during their life: patient stratification based on mutational signatures has become more and more useful in genomic studies, given its possible clinical implications. In this work we focused on Single Base Substitution (SBS) signatures to study a cohort of 115 metastatic melanoma patients. We inferred and identified two mutational signatures characterizing the patients. Based on these signatures we divided patients into two group: the bigger group was characterized by a signature associated with exposure to ultraviolet light, while the smaller group resulted to be mostly composed of patients which did not respond to immunotherapy (anti-PD1) and that presented a low mutational count. More importantly this second group showed a significantly worse survival outcome. The use of mutational signatures is clearly a powerful tool to identify disease sub-types that have a clinical relevance, however we believe that this topic needs further investigation focused on the characterization of patient subtypes with a multi-omics based approach.**

***Index Terms*—mutational signatures, metastatic melanoma, genomics, precision oncology, computational medicine, patient stratification**

I. Introduction

It is well established that genetic instability is one of the hallmarks of cancer [1], however, somatic mutations are present in all cells of the human body and occur throughout life [2]; they can result from errors in DNA replication during cell division, exposure to mutagens or a viral infection, or they can be inherited. When mutations accumulate in a cell genome they can lead to cancer cell survival and proliferation [12]. Different mutational processes generate unique combinations of mutation types, that are called "Mutational Signatures". In the last decade large-scale analyses have showed several mutational signatures across different human cancer types. Currently, four different variant classes of signatures have been defined [2]:

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- Single base substitutions (SBS) Signatures
- Doublet base substitutions (DBS) Signatures
- Small insertions and deletions (ID) Signatures
- Copy number signatures (CN) Signatures

Thanks to extensive previous studies, a curated resource of mutational signatures [2] was created. COSMIC Mutational Signatures is an online resource of signatures identified from analyses of the PCAWG (Pan-Cancer Analysis of Whole Genomes) dataset and through a curation of specific papers, and it provides a catalogue of signatures that serve as a reference set of high confidence signatures curated by experts in the field [2].

In the field of personalized genomics, applying comprehensive genomic approaches could prove to be most rewarding [3]. Previous studies have proven how "mutational signatures provide new insights into the causes of individual cancers, revealing both endogenous and exogenous factors that have influenced cancer development" [4]. By now conducting mutational signature analyses has become a standard practice while performing genomic studies. Indeed, this field is "heading towards being used in a clinically meaningful way" [4].

In this work we investigated SBS signatures (the most explored and consolidated throughout the literature) in a cohort of metastatic melanoma patients. The incidence of primary cutaneous melanoma has been increasing steadily for several decades; however, the overall survival rates have remained relatively constant. To this day melanoma remains the most lethal form of cutaneous neoplasm: almost 90% of patients die, accounting for 65% of all skin cancer-related deaths [5]. Melanoma is distinct from non-melanoma skin cancers in that it tends to spread locally, regionally, and distantly. An individual's risk of metastasis is directly related to the depth of invasion and ulceration of their primary lesion [6]. Approximately 4 percent of people are diagnosed with melanomas that have spread to distant parts of the body, according to the American Society of Clinical Oncology (ASCO). Common melanoma metastasis sites include the lymph nodes, lungs, liver, bones and brain. [7]. To this day immune checkpoint inhibitor-based therapy is the most effective treatment for metastatic

melanoma; there are three immune checkpoint inhibitors have been approved for the treatment of melanoma: two anti-PD-1 antibodies and one anti-CTLA-4 antibody. However, anti-PD-1 therapy does not effectively block tumour activity in all patients. As a result, PD-1 and PD-L1 inhibitors have been the major focus of research on the immunotherapy of melanoma [5].

Previous studies have already focused on mutational signatures of melanoma patients: Shi et al. [8] analyzed 631 melanoma patients treated with immune checkpoint inhibitors (ICIs), found a total of four mutational signatures and noticed that male patients with signature SBS4 had an inferior ICI response rate and overall survival. They also found a immune subtype (based on mutational activities) that was associated with poor ICI survival in female patients. Kim et al. [9] considered splitting cutaneous melanomas (CMs) in two subgroups based on mutational signatures: UV-high and UV-low. They also showed that CMs belonging to the UV-low cluster showed significantly worse overall survival and landmark survival at 1-year than those in the UV-high cluster. Similar observations had previously been made by Trucco et al. [10] and Vicente et al. [11]. However these studies focused either on primary tumors or on samples of mixed nature (both primary and metastatic). Moreover, as Kim et al. state, patients belonging to the low-UV group have not been fully characterized yet. With this work we aim at further investigating this topic by leveraging the insights provided by mutational signatures as well as extending this study to metastatic melanoma patients.

II. Materials and Methods

A. Data

The data used in this study can be found in the "Metastatic Melanoma (DFCI, Nature Medicine 2019)" dataset [12], freely available for download on the cBioPortal for Cancer Genomics, an open-access and open-source resource for interactive exploration of multidimensional cancer genomics data sets [13]–[15]. The dataset consists of whole-exome and whole-transcriptome sequencing of pre-treatment tumors for a cohort of patients with metastatic melanoma treated with anti-PD1 immune-checkpoint blockade. For our purposes we only used mutational and clinical data. Mutational data was available for 118 patients.

B. Methods

Assessment of SNP mutational counts, estimation of SBS mutational signatures and patients' exposures were performed for all patients with the mutSignatures R package [16], a framework based on non-negative matrix factorization (NMF) to decompose a mutation matrix that contains the 96 base substitution classes with trinucleotide sequence pattern. These base substitution types are the permutation and combination of six main mutational categories (i.e., C > A, C > G, C >

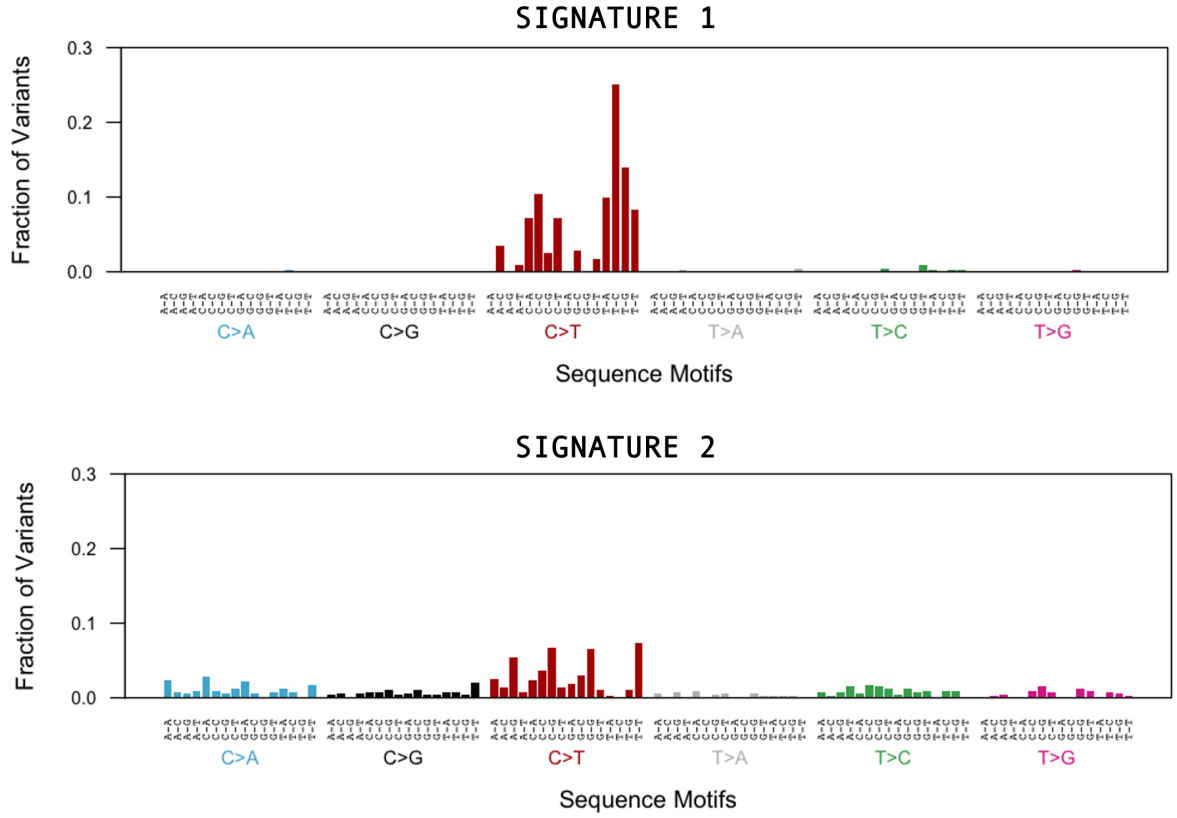

Fig. 1. SBS Mutational signatures extracted from the patients' SNP (Small nucleotide polymoprhism) mutations

T, T > A, T > C, and T > G) and their surrounding adjacent bases. The estimated signatures were compared with the ones validated by COSMIC.

All statistical tests and data analyses were performed using R. Patient survival analyses were performed using the survival R package [17]. To asses patient enrichment in the clinical variable ”Best Response” (BR), we divided patients into ”Responders” if they were classified as ”Complete Response”, ”Partial Response” or ”Mixed Response” and ”Non responders” if the case of ”Stable disease” or ”Progressive Disease”. Enrichment analysis was performed using hypergeometric test with a significance threshold of 0.05.

III. Results

A. Mutational signatures

The considered dataset contains a total of 14703 with SNP mutations related to 118 patients. After removing mismatch mutations and aggregating count mutation types, we obtained a cohort of 115 patients and we were able to estimate two signatures (Fig. 1). The first one is dominated by the presence of C > T mutations, while the second one presents a wider range of SNPs. By checking the patients' exposure to the signatures we noticed that, while most patients were mostly exposed to the first signature, there was a small group mostly exposed to the second one (Fig. 2). This was found among the patients with smaller mutational count (Fig. 3).

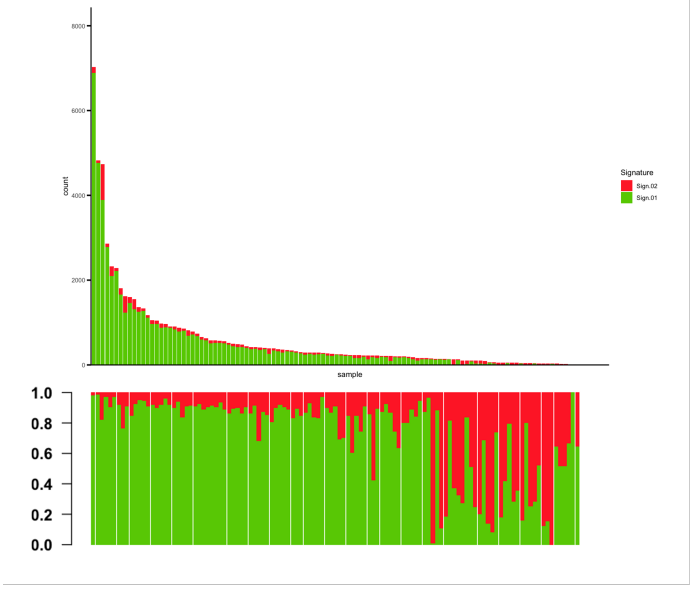

Fig. 2. Histogram showing the patients' exposure to the extracted signature. Every bar represents a patient (ordered by amount of mutations) and the color shows how many mutations of that patient can be attributed to a signature. In the bottom histogram the same information is represented as percentage over the total mutations of that patient. Signature 1 is shown in green and signature 2 in red.

As a consequence, we divided patients into two groups based on their exposure to the found signatures; each patient was assigned to the signature if their exposure to said signature (and

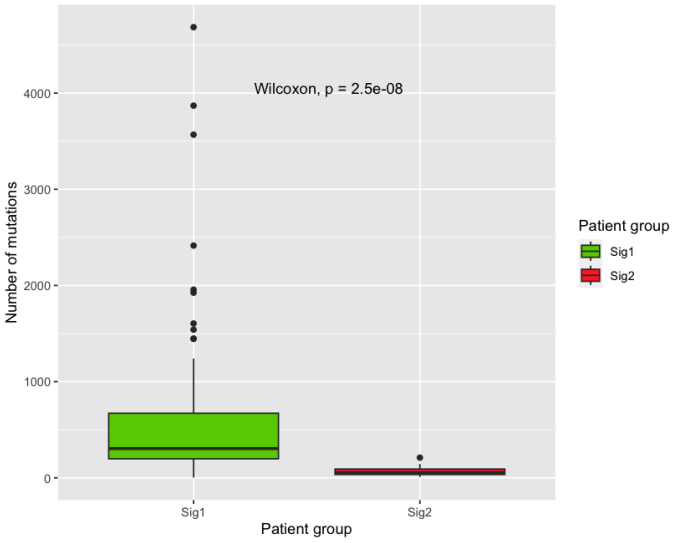

Fig. 3. Boxplot showing the difference in terms of amount of mutations between the two identified groups

therefore the contribution of the signature to their mutations) was grater or equal than 50%. We then focused on the 21 patients which belong to the smaller group associated to the second signature. We noticed that 12 of them are women and 9 men (even thought the dataset contains more men than women) and that they do not respond to treatment (p-val = 0.021). Additionally, they have worse mortality rate and tendency to lower LDH values. Finally, the majority of them had also already undergone a CTLA4 treatment (Fig. 4).

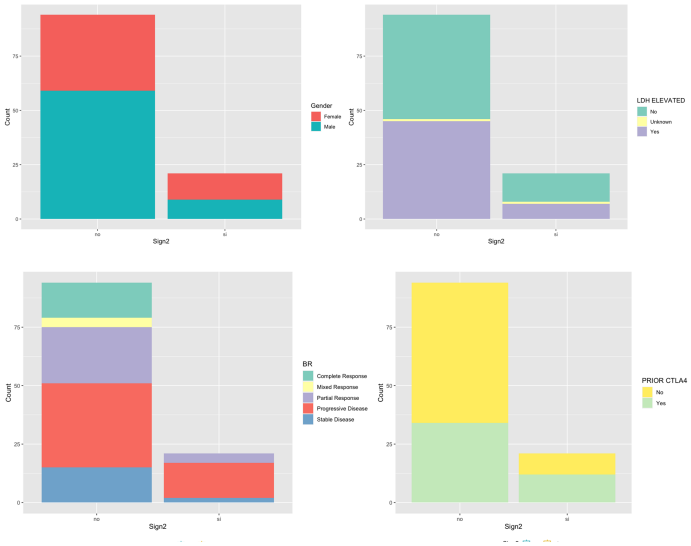

Fig. 4. Stackbar plots comparing the 21 identified patients to the rest of the samples in terms of clinical information (sex, high ldh, therapy response and prior CTLA4 therapy)

To better understand the mutational signatures we had found, we compared them to COSMIC signatures (Fig. 5). By setting a threshold of 0.7 on the cosine similarity (cs), we can

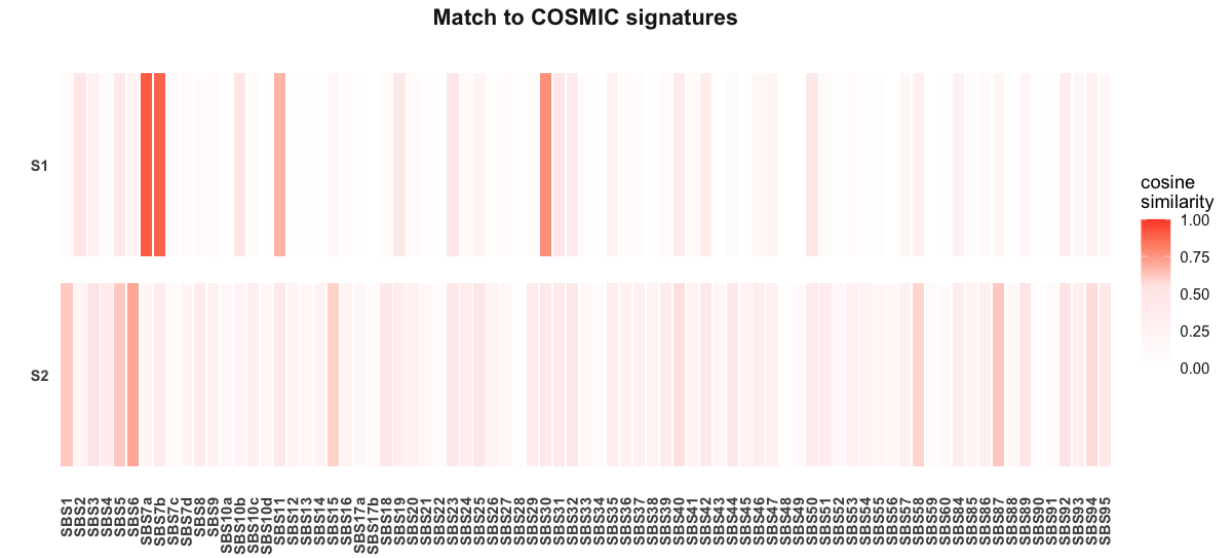

Fig. 5. Heatmap showing the cosine similarity score between the identified signatures and the 87 COSMIC signatures

highlight that signature 1 is highly similar to both SBS7a (cs = 0.91) and SBS7b (cs = 0.88), as well as presenting a strong affinity with SBS30 (cs = 0.76). The first two signatures are likely to be due to exposure to ultraviolet light, while SBS30 is due to deficiency in base excision repair due to inactivating mutations in NTHL1. On the other hand, for signature 2 we identified only one relevant similarity with SBS6 (cs = 0.71) which is associated with defective DNA mismatch repair and is often found in microsatellite unstable tumours.

B. *Survival analysis*

As a second step of our analysis we compared the survival of the 21 patients characterized by signature 2 with the survival of the other patients. As we can see from Fig. 6, the smaller subgroup has a significantly worse survival outcome (pval =

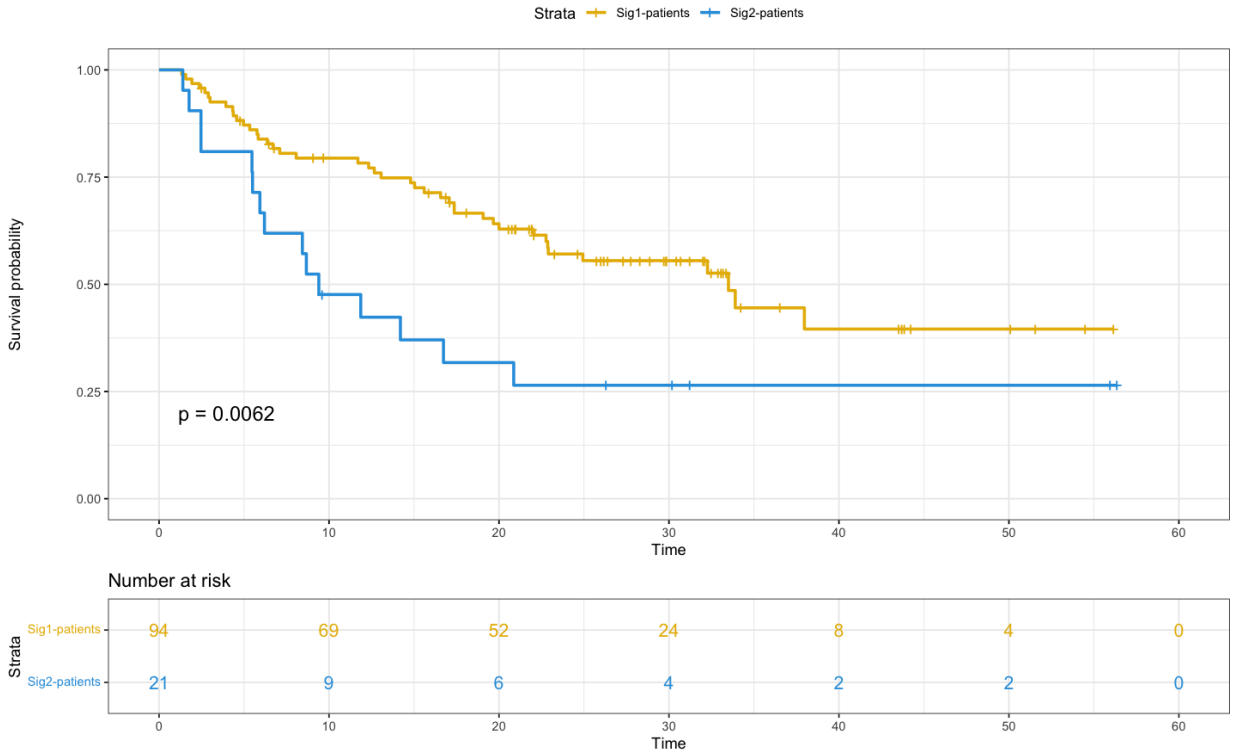

Fig. 6. Survival curve of the 21 identified patients compared with the others. The p-value of this significant survival difference is also reported in the image

0.0062). By inspecting the survival curve we can notice how the survival of these patients is worse from the first months.

IV. Discussion

Melanoma is currently the most lethal form of skin cancer, with a 90% mortality rate. Previous studies have shown that melanoma patients could be clustered based on their mutational information; however these studies focused either on primary melanoma or on mixed dataset, with samples related both to primary and metastatic melanoma. In this work, we extended these analysis on SBS mutational signatures and patients' survival by focusing only on metastatic melanoma patients. Similarly to Kim et al. [9], Turcco et al. [10] and Vicente et al. [11], we highlighted two different groups of patients based on two mutational signatures that we identified. A first group is composed of 94 patients: they are characterized by high number of mutations and strongly associated to a signature extremely similar to COSMIC signatures 7a and 7b, known to be caused by ultraviolet light exposure and with a predominance of C-to-T nucleotide transition. The second group (21 patients), on the other hand, was characterized by a mutational profile not associated with UV-light exposure and presented a quite low number of mutations. This proves that this signature-based classification can also be applied to metastatic melanoma. Moreover, the difference in survival outcome is also confirmed. The smaller group (the one not associated with ultraviolet light exposure) showed, in fact, a worse overall survival. To better characterize these patients, we saw that they are enriched in being non responders to anti-PD1 therapy. Moreover, by comparing their mutational signature to known COSMIC signatures we found an affinity with SBS6 (whose proposed aetiology is "defective DNA mismatch repair"), while the already mentioned study by Kim et al. [9], who found similar results but focused mostly on primary tumors, had instead found a similarity with SBS1 (spontaneous or enzymatic deamination of 5-methylcytosine) and SBS5 (unknown). We can conclude that as in primary melanoma, it is possible to distinguish different sub-types with different prognosis. These sub-types also show different responses to anti-PD1 immunotherapy. We believe that this topic, and specifically the characterization of the sub-type with worse prognosis, needs further investigation: their genomic profile might be the result a mixture of joint causes therefore a multi-omics approach might lead to insightful results. Therefore, further steps of this investigation will focus on integrating also transcriptomics data.

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References

[1] Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. Cell 144, 646–674. https://doi.org/10.1016/j.cell.2011.02.013 (2011).

[2] https://cancer.sanger.ac.uk/signatures/

[3] Nik-Zainal S, Morganella S. Mutational Signatures in Breast Cancer: The Problem at the DNA Level. Clin Cancer Res. 2017 Jun 1;23(11):2617-2629. doi: 10.1158/1078-0432.CCR-16-2810. PMID: 28572256; PMCID: PMC5458139.

[4] Koh G, Degasperi A, Zou X, Momen S, Nik-Zainal S. Mutational signatures: emerging concepts, caveats and clinical applications. Nat Rev Cancer. 2021;21(10):619-637. doi:10.1038/s41568-021-00377-7

[5] Du F, Yang LH, Liu J, Wang J, Fan L, Duangmano S, Liu H, Liu M, Wang J, Zhong X, Zhang Z, Wang F. The role of mitochondria in the resistance of melanoma to PD-1 inhibitors. J Transl Med. 2023 May 23;21(1):345. doi: 10.1186/s12967-023-04200-9. PMID: 37221594; PMCID: PMC10207731.

[6] Sundararajan S, Thida AM, Yadlapati S, et al. Metastatic Melanoma. [Updated 2022 Dec 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470358/

[7] https://www.cancercenter.com/cancer-types/melanoma/types/metastatic-melanoma

[8] Shi F, Zhang W, Yang Y, et al. Sex Disparities of Genomic Determinants in Response to Immune Checkpoint Inhibitors in Melanoma. Front Immunol. 2021;12:721409. Published 2021 Nov 2. doi:10.3389/fimmu.2021.721409

[9] Kim YS, Lee M, Chung YJ. Two subtypes of cutaneous melanoma with distinct mutational signatures and clinico-genomic characteristics. Front Genet. 2022 Sep 29;13:987205. doi: 10.3389/fgene.2022.987205. PMID: 36246650; PMCID: PMC9557124.

[10] Trucco, L.D., Mundra, P.A., Hogan, K. et al. Ultraviolet radiation–induced DNA damage is prognostic for outcome in melanoma. Nat Med 25, 221–224 (2019). https://doi.org/10.1038/s41591-018-0265-6

[11] Vicente, A.L.S.A., Novoloaca, A., Cahais, V. et al. Cutaneous and acral melanoma cross-OMICs reveals prognostic cancer drivers associated with pathobiology and ultraviolet exposure. Nat Commun 13, 4115 (2022). https://doi.org/10.1038/s41467-022-31488-w

[12] Liu D, Schilling B, Liu D, et al. Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma [published correction appears in Nat Med. 2020 Jul;26(7):1147]. Nat Med. 2019;25(12):1916-1927. doi:10.1038/s41591-019-0654-5

[13] Cerami et al. The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. Cancer Discovery. May 2012 2; 401

[14] Gao et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. 6, pl1 (2013)

[15] de Bruijn et al. Analysis and Visualization of Longitudinal Genomic and Clinical Data from the AACR Project GENIE Biopharma Collaborative in cBioPortal. Cancer Res (2023).

[16] Fantini, D., Vidimar, V., Yu, Y. et al. MutSignatures: an R package for extraction and analysis of cancer mutational signatures. Sci Rep 10, 18217 (2020). https://doi.org/10.1038/s41598-020-75062-0

[17] https://CRAN.R-project.org/package=survival

[18] Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. Bioinformatics. 2011;27(12):1739-1740. doi:10.1093/bioinformatics/btr260

[19] de Matos Simoes R, Emmert-Streib F. Bagging statistical network inference from large-scale gene expression data. PLoS One. 2012;7(3):e33624. doi: 10.1371/journal.pone.0033624. Epub 2012 Mar 30. PMID: 22479422; PMCID: PMC3316596.

[20] Zhang, H.; Chen, Z.; Zhang, A.; Gupte, A.A.; Hamilton, D.J. The Role of Calcium Signaling in Melanoma. Int. J. Mol. Sci. 2022, 23, 1010. https://doi.org/10.3390/ijms23031010

[21] Alam MR, Rahman MM, Li Z. The link between intracellular calcium signaling and exosomal PD-L1 in cancer progression and immunotherapy. Genes Dis. 2023;11(1):321-334. Published 2023 Mar 24. doi:10.1016/j.gendis.2023.01.026

[22] Chen, G., Huang, A.C., Zhang, W. et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature 560, 382–386 (2018). https://doi.org/10.1038/s41586-018-0392-8