Multidimensional spatial characterization of the tumor microenvironment in synchronous melanoma metastases yields insights into mixed responses to therapy

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Background

Background: Although both targeted and immune therapies have significantly improved outcomes for metastatic melanoma patients, only a minority of patients experience durable responses with many patients with multiple synchronous melanoma metastases demonstrating differential responses to therapy. We performed multidimensional spatial characterization of immune markers in synchronous melanoma metastases from melanoma patients treated with targeted and immune therapies to improve our understanding of correlations and determinants of response. **Methods:** NanoString's Digital Spatial Profiling research platform was used on 6 synchronous melanoma metastases from 3 patients (treatment-naïve; BRAF + MEK targeted therapy treated; anti-PD-1 immunotherapy treated) for 30 immune and signaling proteins. For analysis, we selected and compared immune-rich (CD45+) and tumor-rich (S100B+) regions. **Results:** Striking differences in patterns of expression were detected, including in Ki67, CD68 myeloid cells, and the potent immunosuppressor B7-H3. Synchronous metastases progressing after targeted therapy demonstrated higher pAKT and PD-L1 expression, consistent with described resistance mechanisms. Large differences in expression of PD-L1 were noted following anti-PD-1 therapy, which could contribute to heterogeneous responses. Differential expression patterns in the tumor microenvironment associated with response were also detected, including increases in CD4 and CD14 cells in progressing lesions. **Conclusions:** Striking intrapatient differences were observed, providing potential explanations for the heterogeneous clinical responses frequently observed in metastatic melanoma patients. Studies are ongoing to further characterize interactions and spatial distribution of cell types, as well as integrate these findings with previous molecular and immune profiling data (whole exome sequencing, gene expression profiling, flow cytometry, IHC, TCR sequencing) in these and additional patients to identify actionable strategies to homogenize responses across metastases in metastatic melanoma patients.

Rationale

Figure 1. Intrapatient differential responses to targeted therapy and immune checkpoint blockade are universal in patients with synchronous melanoma metastases. Radiographic responses by RECIST1.1 in n=60 patients with multiple synchronous metastases treated with combination BRAF/MEK inhibition **(A)** or PD-1 blockade **(B)**.

Prior Studies

Figure 2. Molecular and immune profiling of synchronous melanoma metastases. A) Metastases resected for therapeutic purposes were processed in parallel and molecular and immune profiling was performed. **B)** Representative CT scans from a patient with three synchronous metastases demonstrating heterogeneous responses to therapy 25 weeks following treatment initiation. Green = Treatment-naïve, Red = Targeted therapy, Blue = Immunotherapy.

Patients

	Age (Gender)	Prior Therapy	# of Metastases	Tumor Sites		RECIST Class	Change in Tumor Size
Intertumoral Heterogeneity							
Treatment-naive (n=4)							
Patient #1	54 (F)	None	2	M1	Left axillary lymph node	NA	NA
				M2	Left axillary lymph node	NA	NA
Patient #2	43 (M)	None	2	M1	Left axillary mass	NA	NA
				M2	Left axillary mass	NA	NA
Patient #3	55 (M)	None	2	M1	Left axillary mass	NA	NA
				M2	Right supraclavicular mass	NA	NA
Patient #4	59 (F)	None	2	M1	Left scapula	SD	-11%
				M2	Right hip	PD	+4%
Targeted therapy (n=3)							
Patient #5	43 (M)	BRAFi + MEKi	2	M1	Left abdomen	PD	30
				M2	Right abdomen	PD	153%
Patient #6	55 (M)	MEKi + AKTi	2	M1	Right flank	NA	NA
				M2	Right shoulder	NA	NA
Patient #7	48 (M)	BRAFi + MEKi	3	M1	Right axillary lymph node	PR	-50%
				M2	Right neck lymph node	SD	15%
				M3	Left neck mass	SD	-21%
Immunotherapy (n=8)							
Patient #8	60 (F)	PD-1 Blockade	2	M1	Left inguinal lymph node	SD	1.4%
				M2	Left popliteal fossa	PD	71%
Patient #9	35 (M)	PD-1 Blockade	2	M1	Left abdominal mass	NA	NA
				M2	Right abdominal mass	NA	NA
Patient #10	63 (M)	CTLA-4 + PD-1 Blockade	2	M1	Mid abdominal mass	NA	NA
				M2	Mid abdominal mass	NA	NA
Patient #11	43 (M)	PD-1 Blockade	4	M1	Right buttock	NA	NA
				M2	Left back	SD	-2
				M3	Left back	NA	NA
				M4	Left back	NA	NA
Patient #12	39 (F)	CTLA-4 Blockade	4	M1	Right breast	PD	69%
				M2	Left breast	PD	62%
				M3	Left breast	SD	-17%
				M4	Right breast	SD	-4%
Intratumoral Heterogeneity							
Targeted therapy (n=1)							
Patient #13	29 (M)	BRAFi	2	M1A-B	Left axilla	SD	5.8%
				M2	Right chest	PD	113%
Immunotherapy (n=2)							
Patient #14	62 (F)	PD-1 Blockade	1	M1A-D	Right thigh	NA	NA
Patient #15	76 (F)	CTLA-4 Blockade	2	M1A-F	Spleen	SD	7%
				M2A-B	Right abdominal wall	PD	24%

I. Molecular & Immune Heterogeneity

Figure 3. Genomic profiling reveals limited overlap in mutational burden between synchronous melanoma metastases. A) Number of non-synonymous exonic mutations (NSEM) in each metastasis within a given patient, as well a proportion of NSEM shared and unique within representative patients and treatment backgrounds. **B)** Aggregate genomic data showing number and proportion of shared and unique NSEM, as well as proportion of mutational signatures in all patients. Green = Treatment-naïve, Red = Targeted therapy, Blue = Immunotherapy.

Figure 4. Neoantigen prediction reveals high numbers of tumor-restricted neoantigens presenting high HLA-binding affinity. A) IC50 as predicted by NetMHC3.4 algorithm. Blue = Unique to Metastasis 1, Red = Unique to metastasis 2, Grey = Shared. **B)** Aggregate neoantigen numbers and shared and unique proportions in patients with synchronous melanoma metastases. Green = Treatment-naïve, Red = Targeted therapy, Blue = Immunotherapy.

Figure 5. Immune profiling shows heterogeneous immune infiltrates across synchronous melanoma metastases. A) Stacked bars illustrating the relative proportion of immune cell subsets detected in synchronous metastases as a percentage of CD45+ immune cells, as evaluated by flow cytometry. **B)** Aggregate data showing relative contribution of immune cell subsets in synchronous metastases in all patients. Green = Treatment-naïve, Red = Targeted therapy, Blue = Immunotherapy.

Figure 6. T cell receptor sequencing reveals significant heterogeneity in infiltrating T cell clones. A) Dot plot showing all clones detected based on the frequency at which they were detected. **B)** Dot plot showing the percentage of T cell clones detected across all metastases within any given patient. Green = Treatment-naïve, Red = Targeted therapy, Blue = Immunotherapy.

II. Digital Spatial Profiling Technology

Figure 7. Multiplexed digital spatial profiling of single FFPE tissue slides. Workflow showing the digital spatial profiling methodology performed on synchronous melanoma metastases from 3 patients (treatment-naïve, targeted therapy, immune checkpoint blockade). FFPE slides are first stained with oligo-conjugated and fluorescent antibodies, and regions of interest (ROIs) are selected based on morphology, expression of S100B (tumor cells) or of CD45 (immune cells). Oligos are then UV-cleaved from antibodies in ROIs, and aspirated for hybridization and quantification. Steps 3 to 5 are repeated for each ROI.

III. Region of Interest Selection

Figure 8. Selection of ROIs from immunofluorescently-labelled tumor FFPE section. Tumor section showing CD45 (green), S100B (purple) and DNA (gray) fluorescent stain. ROIs were selected based on intensity of CD45 and S100B for downstream analysis.

Figure 9. Quantification of CD45 expression in each ROI. Images of selected ROIs showing CD45 (green), S100B (purple), and DNA (gray) in each ROI.

IV. Immune & Tumor Analysis

Figure 10. Digital spatial profiling of synchronous melanoma metastases reveals engagement of distinct immune cell populations and signaling pathways. A) Quantification of tumor, signaling, survival, immune, and checkpoint molecules across immune- and tumor-enriched ROIs in patients with synchronous melanoma metastases. Black boxes highlight key intrapatient differences which could contribute to differential responses to therapy. Green = Treatment-naïve, Red = Targeted therapy, Blue = Immunotherapy. **B)** Selected immune and tumor ROIs in each patient.

Conclusions

- Synchronous metastases exhibit differential responses to therapy associated with genomic and immune heterogeneity;
- Digital spatial profiling allows highly multiplexed protein analysis of up to 30 proteins in a single FFPE tissue slide;
- Digital spatial profiling of synchronous metastases reveals differences in the immune microenvironment, as well as differences in tumor and immune cell signaling pathways;
- Digital spatial profiling of the tumor microenvironment may help identify the divergent resistance mechanisms at play in synchronous melanoma metastases.

Future Directions

- Digital spatial profiling of 20 patients with synchronous melanoma metastases progressing on PD-1 blockade;
- Integrated analysis of the tumor microenvironment through digital spatial profiling and correlation with lesion-specific responses;
- Correlation of spatial protein signatures with other biomarkers of therapeutic response;
- Identification and validation of targets involved in tumor resistance and mixed responses to therapy.

Acknowledgements

Together, we will end cancer.

2017 Merit Award Recipient

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