

Consent NA.

REFERENCE

1. Kluger H, Tawbi H, Ascierto M, *et al.* Defining tumor resistance to PD-1 pathway blockade: recommendations from the first meeting of the SITC Immunotherapy Resistance Taskforce. *J Immunother Cancer*. 2020; **8**:3000398.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0228>

229

DISCOVERY OF GANGLIOSIDE GM2 ACTIVATOR AS A NOVEL PROTEOMIC BIOMARKER ASSOCIATED WITH RESPONSE TO TREATMENT IN FIRST-LINE MELANOMA SUBJECTS TREATED WITH PD-1 IMMUNOTHERAPY

¹Kristina Beeler*, ¹Jakob Vowinkel, ¹Martin Soste, ²Domenico Mallardo, ²Mariaelena Capone, ²Madonna Gabriele, ²Antonio Sorrentino, ²Vito Vanella, ¹Kamil Sklodowski, ¹Daniel Heinzmann, ²Paolo Ascierto. ¹*Biognosys, Schlieren, Switzerland*; ²*Istituto Nazionale Tumori IRCCS, Napoli, Italy*

Background Immune checkpoint inhibitors (ICI) have greatly improved the treatment options for patients with metastatic melanoma. Yet, a large percentage of melanoma patients do not respond to ICIs, there is a need for biomarkers that can predict patients' clinical benefit thereby identifying the patient population most likely to respond. Here, we apply unbiased discovery proteomics to deeply characterize global tumor proteomes and associate proteins and pathways at baseline with clinical response to anti-PD-1 immunotherapy.

Methods Unbiased, data-independent acquisition (DIA) mass spectrometry was used to analyze Formalin Fixed Paraffin Embedded (FFPE) tumor tissue from subjects with stage IIIc-IV melanoma which were resected prior to initiation of first-line anti-PD-1 therapy. The selected samples represent two distinct clinical subgroups; those who received clinical benefit (CR or PR by RECIST criteria or OS >1 year with SD by RECIST criteria, n = 13), and those with no clinical benefit (PD by RECIST criteria or OS <1 year with SD by RECIST criteria n = 9). Previously, the sample cohort had been analyzed by a 2-hour LC-MS/MS gradient setup operated in DIA mode. In this study, all samples were analysed with a longer gradient of 4-hours which enabled the quantification of 1'000 more proteins and enabled an updated analysis with a deeper level of characterization.

Results 8'548 proteins were quantified across all samples, with 7'416 quantified on average per sample. Univariate statistical testing between groups identified 285 proteins that were significantly regulated in subjects who received clinical benefit. Through partial least squares discriminant analysis (PLS-DA) a set of 25 proteins was identified that describe the variance between the two sample groups. Ganglioside GM2 activator (GM2A) and other members of its interaction network such as HEXB, HRNR and CPPED1 were identified to be upregulated in the non-responder group.

Conclusions Global profiling of the baseline tumor proteome provides a unique characterization of melanoma tumor biology. A signature of 25 protein markers was identified as a driver of separation between responder and non-responder patients to PD-1 blockade. Among the protein markers, GM2A and its interactors, were previously shown to perturb T cell function, which might explain their enrichment in the non-responder group and provide an attractive target for improving patient response to immunotherapy.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0229>

230

SINGLE CELL PI3K GENE EXPRESSION PATTERNS SUPPORT DUVELISIB (PI3K-DELTA, GAMMA INHIBITOR) TREATMENT OF MELANOMA AND OTHER TUMORS AFTER CHECKPOINT INHIBITOR THERAPY

Samantha Hidy*, David Weaver. *Verastem Oncology, Needham, MA, USA*

Background Duvelisib, an FDA-approved oral phosphoinositide 3-kinase (PI3K)- δ,γ inhibitor, targets tumor cells of B/T cell malignancies, but may modulate non-malignant immune cells in the tumor microenvironment (TME) of many cancers. PI3K- δ and PI3K- γ downmodulate immunosuppressive Tregs and myeloid cells in solid tumors.¹⁻³ We used single-cell RNA analysis of PIK3CD and PIK3CG to explore resistance mechanisms to checkpoint inhibitors (CPI).

Methods Single-cell melanoma (SKCM) RNAseq datasets: GSE120575;⁴ CD45+ cells from 48 CPI responders and non-responder tumors, and GSE115978;⁵ 33 treatment-naïve and CPI-progressing (resistant) tumors. Cancer cells and CD45+ TME subpopulations, specified by gene expression signatures and tSNE plots, had PI3K gene expressions profiled. Differential gene expression (DE) was gated in MAST/Seurat. Fishers test Odds Ratio (OR) was calculated for 'high' expression.

Results PIK3CD expression is higher in SKCM than most cancers (10.8 median RSEM log 2).⁷ By single-cell analysis, PIK3CD (> 0.3 log₂ TPM) occurs in 68.2% of cancer cells, with PIK3CB, PIK3CA, and PIK3CG expressed in 32.3%, 12.0%, and 7.2% respectively. PIK3CD-high cancer cells (>4 log₂ TPM) have a 711-gene DE gene signature mostly related to immune processes. A higher proportion of cancer cells in CPI resistant tumors express PIK3CD, than untreated tumors (OR 2.02, 95% CI 1.65–2.48, p=3.04 × 10⁻¹²), as do PIK3CD+PIK3CG-expressing cancer cells (OR 2.14, 95% CI 1.47–3.13, p=4.2 × 10⁻⁵). Additionally, in PI3K- δ or PI3K- γ high melanoma cell lines duvelisib inhibited proliferation, p-AKT and c-myc.⁷ PIK3CD and PIK3CG are prominently expressed in many SKCM CD45+ TME cells (84.5% and 31.7% respectively). PIK3CD (>0.3 log₂ TPM) occurs in a high fraction of T (85.7%), CD8+ T (86.3%), CD4+ T (86.9%), B (78.5%), macrophages (88%), and NK (85%). PIK3CG is highest in B, dendritic, cycling lymphocytes and plasma cells. Strikingly, a significantly higher proportion of PIK3CD+ cells occur in resistant tumors compared to untreated for all CD45+ cells, (OR 1.64, 95% CI 1.40–1.94, p=4.79 × 10⁻¹⁰), CD8+ T (OR 2.15, 95% CI 1.61–2.86, p=6.5 × 10⁻⁸), and an exhausted C8+ T subpopulation (OR 3.17, 95% CI 1.89–5.37, p=2.95 × 10⁻⁶). PIK3CD +PIK3CG-expressing CD45+ cells are significantly increased in CPI-resistant tumors (OR 1.22, 95% CI 1.07–1.39, p=0.002).

Conclusions These findings support a mechanism where CPI therapies may contribute to modulation of PI3K δ expression in cancer cells and the immune TME. The PI3K- δ,γ inhibitor duvelisib is being investigated in combination with CPI and evaluated in the context of CPI resistance in clinical trials: pembrolizumab (HNSC, NCT04193293), and nivolumab (Richter's Syndrome, NCT03892044).

REFERENCES

1. Ali K, Soond DR, Pineiro R, Hagemann T, Pearce W, Lim EL, Bouabe H, Scudamore CL, Hancox T, Maecker H, Friedmann L, Turner M, Okkenhaug K, Vanhaesebroeck B. Inactivation of PI(3)K p110 δ breaks regulatory T-cell-mediated immune tolerance to cancer. *Nature* 2014; **510**(7505):407–411.
2. Kaneda MM, Messer KS, Ralainirina N, Li H, Leem CJ, Gorjestani S, Woo G, Nguyen AV, Figueiredo CC, Foubert P, Schmid MC, Pink M, Winkler DG, Rausch M, Palombella VJ, Kutok J, McGovern K, Frazer KA, Wu X, Karin M, Sasik R,