Exposing the Heterogeneity of the Lipidome in the TME of Cutaneous Melanoma Following Treatment with NUC-7738 in Combination with anti-PD-1 Therapy

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Introduction

- Cancer cells adapt to hypoxic and nutrient deprived environments by reprogramming lipid biosynthesis to accelerate malignant behaviour[1]
- Lipids are not only structural components of cell membranes but also serve as signaling molecules within the tumor microenvironment (TME) to modulate immune cells[2]
- Cancer cells transitioning to a malignant phenotype demonstrate marked changes in lipid metabolism including:
  - Changes in the ratio of monounsaturated fatty acids (MUFAs) to polyunsaturated fatty acids (PUFAs)
  - Dysregulation of sphingolipids, crucial for tumor progression and survival
- Reprogramming lipid metabolism within the TME is a recognised strategy for boosting the effects of immunotherapy[3]

Lipid Metabolism in the TME

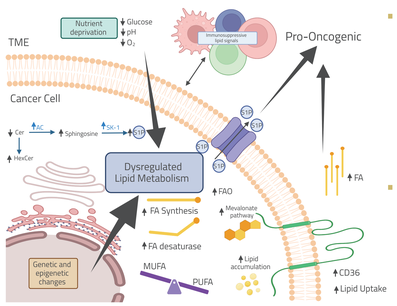


Figure 1: In nutrient deprived conditions cancer cells increase lipogenesis, fatty acid uptake via CD36 and fatty acid oxidation (FAO). Cancer cells rich in monounsaturated fatty acids (MUFA) prevent ferroptosis. Alterations in sphingolipid biosynthesis cause accumulation of the pro-tumorigenic and immunosuppressive sphingosine-1-phosphate (S1P) in the TME.

- NUC-7738 generates sustained intracellular levels of 3' deoxyadenosine triphosphate (3'-dATP), which profoundly alters RNA regulatory processes in tumor cells, resulting in changes in expression of proteins related to lipid metabolism
- Using paired biopsies from patients treated with treated with NUC-7738 ± pembrolizumab, we aim to investigate the potential role of NUC-7738 as a lipid reprogramming agent

Methods

- Snap frozen and FFPE biopsies were collected from 6 patients (1 x mucosal melanoma and 5x cutaneous melanoma) treated with NUC-7738 ± pembrolizumab. Biopsies were collected pre- and post- drug infusion (≤6h post infusion).
- TIC normalised data was exported into MetaboAnalyst 5.0 for statistical analysis (SAM, PCA, fold-change). LIPID MAPS® Structure Database was used to tentatively assign lipid species.

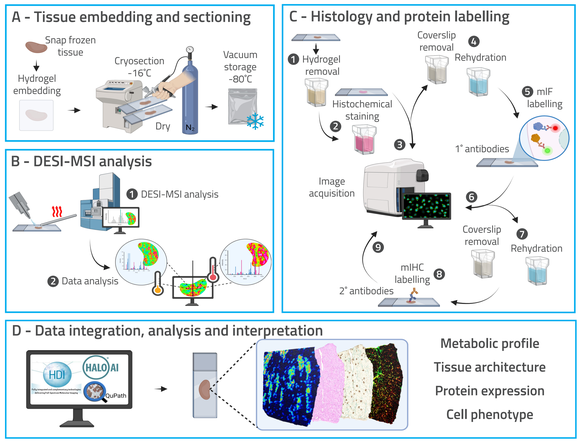


Figure 2: Multi-Modal Imaging Workflow for 10µm single-section tissue analysis

Lipid Profiles of Melanoma Cells Change Following Treatment

- The emergence of two distinct classes of lipids suggests NUC-7738 ± pembrolizumab changes the lipidome in the melanoma TME

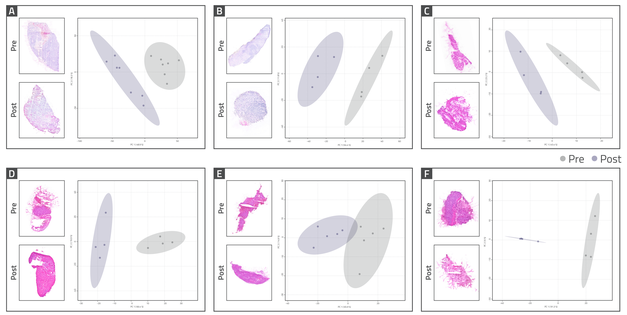


Figure 3: PCA 2D scores plots of lipids in tumor regions from biopsies pre- and post-treatment (A, mucosal and B-F, cutaneous melanoma). ROI were selected by high-magnification analysis of viable tumor regions of H&E-stained sections following DESI-MSI.

Heterogeneity of Lipids and Immune Cells in the TME

- Dimension reduction analysis (UMAP) of data (>1000 lipids) reveals distinct regions within each biopsy

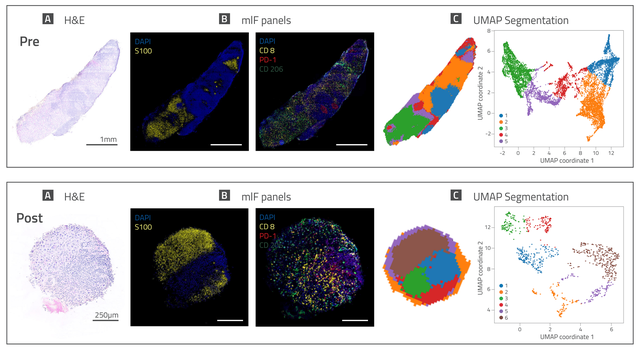


Figure 4: A) Post DESI-MSI H&E section, B) multiplex immunofluorescence (mIF) of DAPI co-localised with; s100 positive cells (indicative of malignant melanoma), CD 8 positive cells (T cells), CD 206 positive cells (macrophages) and PD-1 positive cells. C) UMAP segmentation of single 10µm section based on UMAP cluster data.

Spatial Co-registration for Quantitative Image Analysis

- Quantification of individual cell types within distinct metabolic regions of the tissue show which cell types may be driving changes in lipid signatures

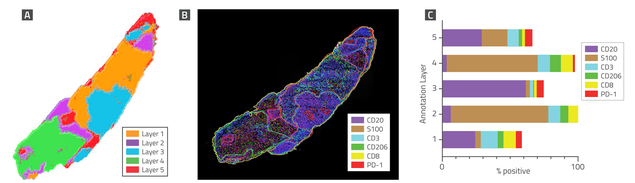
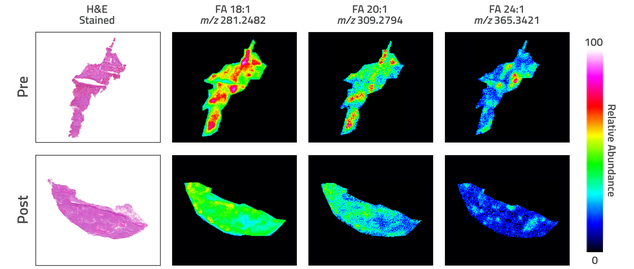


Figure 5: A) UMAP segmentation integrated into HALO AI analysis software as annotation layers. B) annotation layers applied to fused mIF and mIHC images. C) population of cells in each annotation layer determined by random forest classification.

Fatty Acid Reprogramming

- NUC-7738 + pembrolizumab changes the balance of fatty acids in favor of PUFAs, indicative of a shift towards a less aggressive oncogenic phenotype
- MUFAs have been linked to malignant behaviour and resistance to chemotherapy whilst PUFAs have been associated with susceptibility to reactive oxygen species and ferroptosis[4]

MUFAs



PUFAs

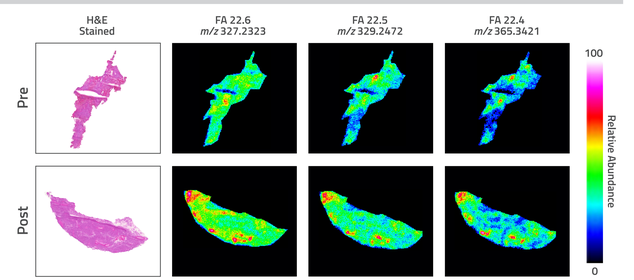


Figure 6: DESI-MSI ion heatmaps show spatial distribution and a decrease in the relative abundance of three MUFA species and increase in three PUFA species in viable tumor regions following NUC-7738 + pembrolizumab treatment

Changes in Apoptotic Related Lipids

- NUC-7738 + pembrolizumab causes a reduction in levels of HexCer species in tumor area
- HexCer, a subclass of sphingolipids are anti-apoptotic and associated with chemoresistance in melanoma patients[5]

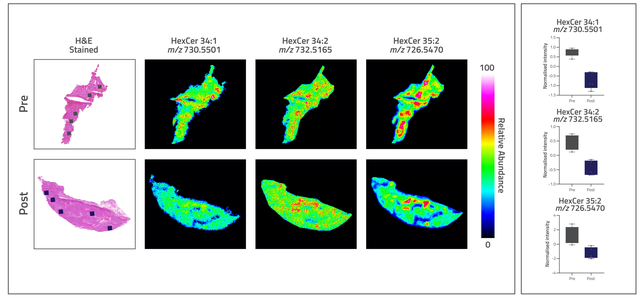


Figure 7: DESI-MSI ion heatmaps show spatial distribution and a significant decrease (|d| < 5) in the relative abundance of three HexCer species in viable tumor regions following NUC-7738 + pembrolizumab treatment

- Pro-apoptotic ceramide (Cer) species increase following treatment

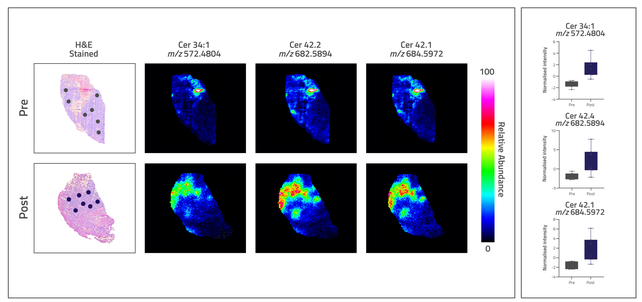


Figure 8: DESI-MSI ion heatmaps show spatial distribution and a significant increase (|d| > 3) in the relative abundance of three Cer species in viable tumor regions following NUC-7738 treatment

Multi-Modal Imaging Confirms Lipidomic Reprogramming

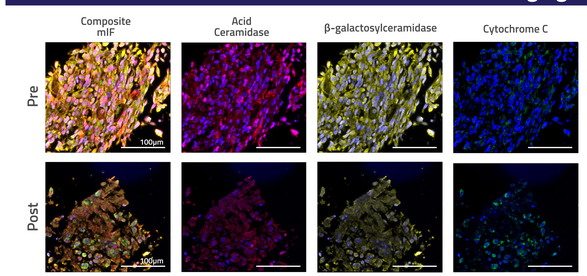


Figure 9: mIF of 3µm FFPE section show a decrease in the protein expression of key lipid metabolizing enzymes and an increase in the apoptotic marker cyt c following NUC-7738 treatment

- Pro-apoptotic ceramide (Cer) species increase following treatment with NUC-7738 corresponding with a decrease in protein expression of acid ceramidase (AC) which hydrolyses ceramides into sphingosine and is associated with tumor progression[6]
- β-galactosylceramidase, a key enzyme in sphingolipid metabolism often upregulated in melanoma progression and associated with poor prognosis[7] decreases following NUC-7738 treatment
- Protein expression of Cytochrome C, a marker of apoptosis increases following treatment

CONCLUSION

- Novel methodology developed to analyse the spatial relationship between the lipidome and TME of patient biopsies within a single-tissue section
- Preliminary results suggest that NUC-7738 ± pembrolizumab reprograms cancer cell lipid metabolism in the TME
- This platform provides a powerful investigative tool to simultaneously explore the myriad of factors in the TME that contribute to tumorigenesis and therapeutic response

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ABBREVIATIONS: 3'dATP: 3'deoxyadenosine Triphosphate AC: acid ceramidase β-gal: beta-galactosylceramidase CD36: cluster of differentiation 36 Cer: Ceramide Cyt c: cytochrome C DESI-MSI: desorption electrospray ionisation- mass spectrometry imaging FA: fatty acids FAO: fatty acid oxidation H&E: hematoxylin and eosin HexCer: hexosylceramide IHC: immunohistochemistry mIF: multiplex immunofluorescence MUFA: monosaturated fatty acids PCA: principal component analysis PUFA: polyunsaturated fatty acids ROI: region of interest S1P: sphingosine-1-phosphate SAM: significance of microarray SK-1: sphingosine kinase -1 TIC: total ion count TME: tumor microenvironment UMAP: uniform manifold approximation and projection