A Spatial Proteomics Approach Identifies Novel Immuno-Oncology Markers for Merkel Cell Carcinoma

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Background

- New Zealand has one of the highest incidences of Merkel Cell Carcinoma in the world (0.88 per 100,000).
- In NZ the majority are Merkel Cell Polyomavirus (MCPyV) negative and triggered by the accumulation of ultra-violet light DNA damage.
- MCC patients relapse quickly after chemotherapy so alternative treatments are needed.
- Clinical trials have shown great promise for immunotherapies that target the PD-1/PD-L1 axis such as Pembrolizumab and Avelumab.
- Further understanding of the immune status of MCPyV negative tumours could benefit patients by offering alternative checkpoints for therapeutic targetting.

Aims

- To compare heterogeneity between and within metastatic MCPyV negative MCC skin tumours
- To identify the immune cell types present within primary lesions
- To analyse the expression of immune checkpoint proteins in MCCs

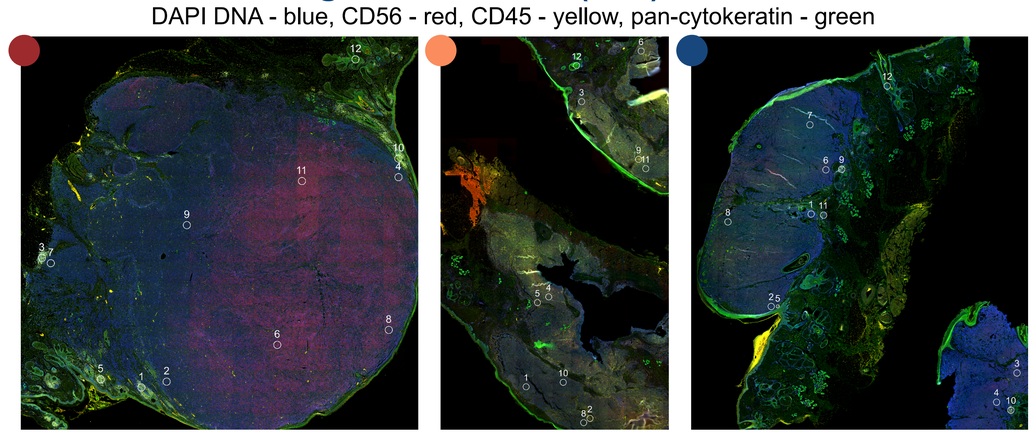
Methods

Nanostring GeoMx Digital Spatial Profiling (DSP) Technology hybridises a cocktail of antibodies with photocleavable oligonucleotide tags to quantitate selected proteins within a defined region of formalin fixed paraffin embedded material. DSP was used to quantitate 38 proteins in selected 100-200µm regions of interest (ROI) enriched for tumour (CD56+) or immune cell (CD45+) clusters selected using multicolour immunofluorescent staining. 12 ROI were chosen on each 5µm tumour slide and 6 primary tumours were analysed (ethical approval 13/NTB/173).

GeoMX Digital Spatial Profiling unravels the immune response within Merkel Cell Carcinomas

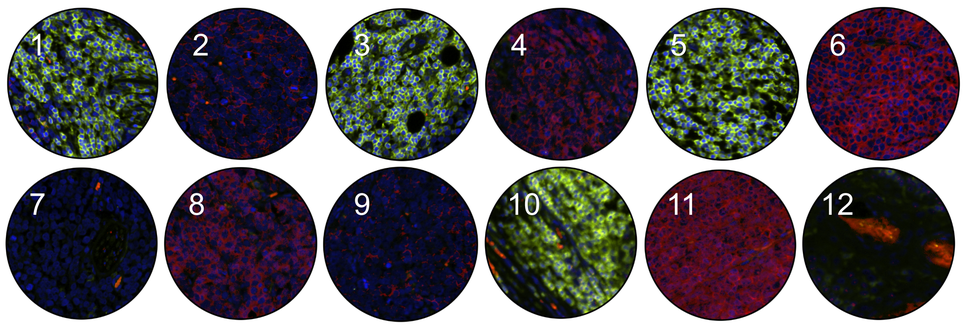
DSP region of interest (ROI) selection

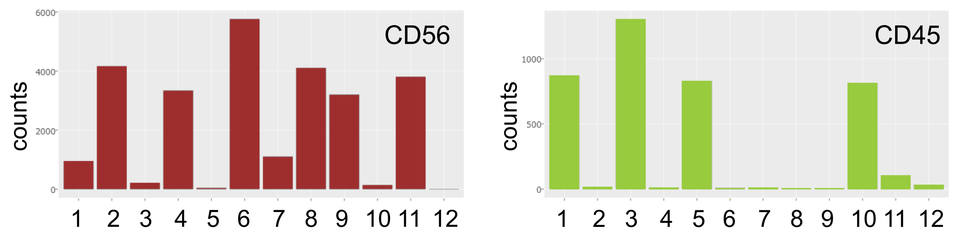
DAPI DNA - blue, CD56 - red, CD45 - yellow, pan-cytokeratin - green



DSP vs immunofluorescence

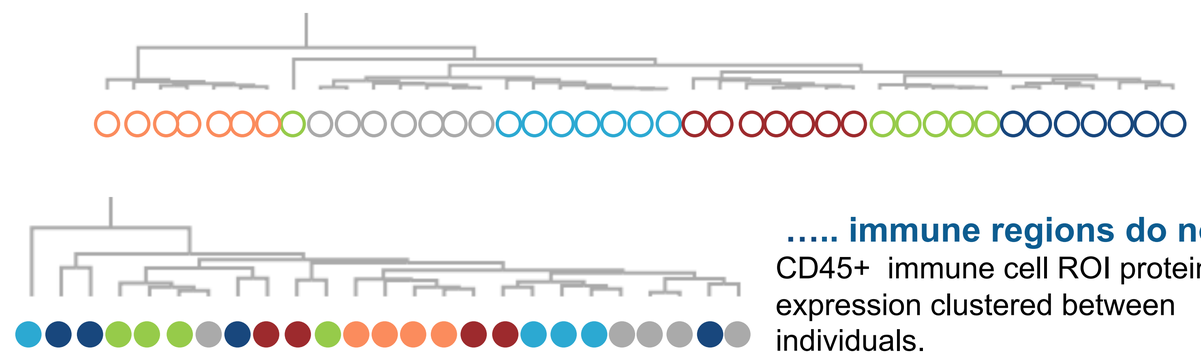
Immunofluorescent staining of CD56 (NET tumour: red) and CD45 (leukocytes: green-yellow) correlated well with the DSP data, validating this novel method - a first in the world use for NET research





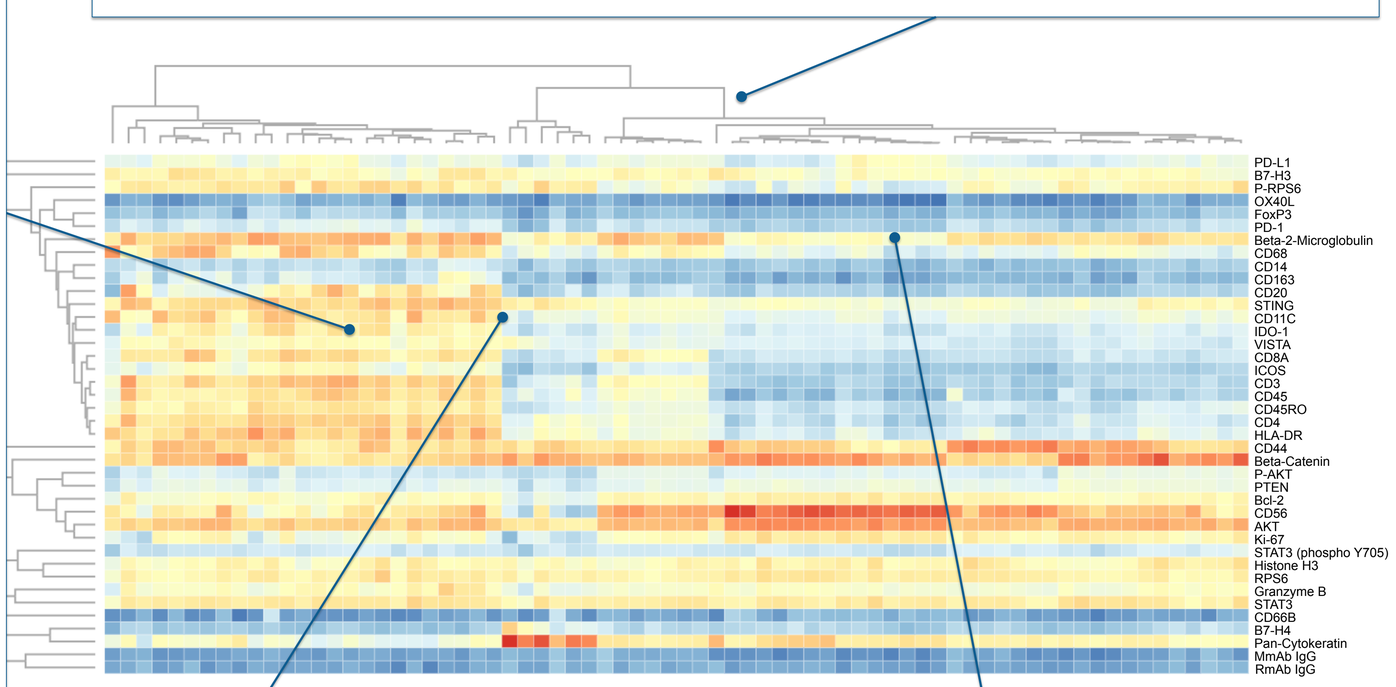
Tumour regions *within* a lesion have similar protein expression

All protein expression clustered the CD56+ tumour ROIs within a lesion, showing that there is minimal intratumour heterogeneity relative to the differences found between individuals.



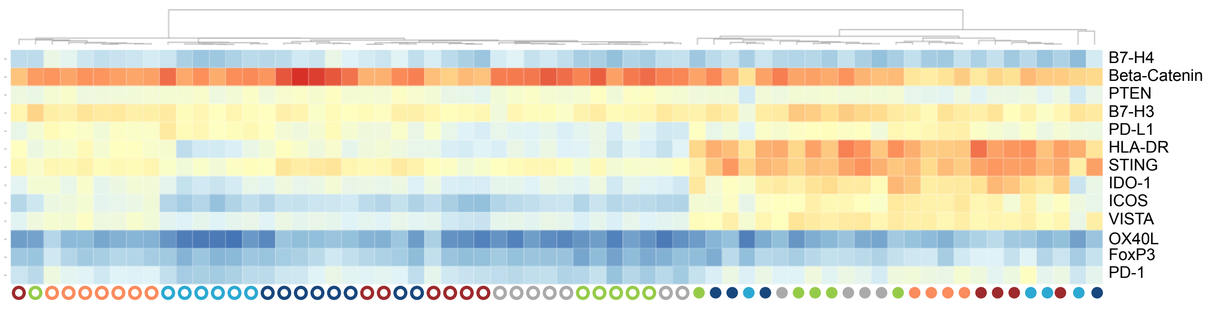
….. immune regions do not

CD45+ immune cell ROI protein expression clustered between individuals.



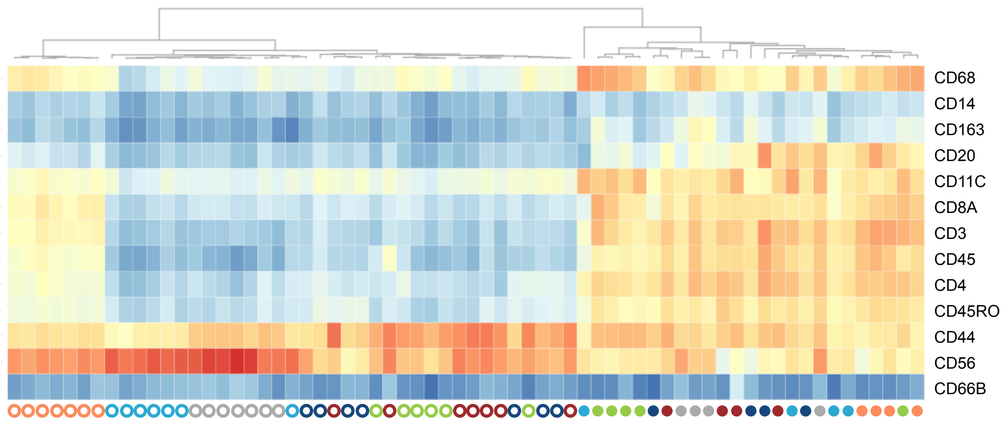
Immune checkpoint proteins are present in tumour and immune cell ROIs

PD-1/PD-L1 were expressed in individual tumours and this was associated with immune infiltration (brisk/non-brisk AJCC melanoma classification). Other immune regulatory markers such as B7-H3, STING and IDO-1 were also seen in tumour **O** and immune cell • ROIs.



MCPyV negative MCCs contain a mixture of immune cells

CD3+, CD4+ and CD8A+ (T cells) expression seen in both tumour and immune ROIs. CD68 (macrophages) and CD11C (dendritic cells) were also abundant in selected tumour **O** and immune cell • ROIs.



Conclusions

- Tumour regions show minimal heterogeneity in expression of the proteins assessed across an individual lesion. However, immune cell regions are spatially diverse.
- Alternative immune markers identified in MCPyV negative MCCs could offer new strategies for immunotherapy in PD-1/PD-L1 non-responders for the design of new trials.

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