P40: Tumour immunity signatures to expand current diagnostic approaches in mismatch repair deficient cancers in the context of Lynch syndrome through InSituPlex technology and Tissue Phenomics integration

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Background

The tumour microenvironment (TiME) is a complex system comprising of cells of the immune system, fibroblasts and endothelial cells. Mismatch repair deficient (dMMR) tumours are characterised by the expression of highly immunogenic peptides that stimulate lymphocytic infiltration as well as the upregulation of inflammatory cytokines. Although the increased density of T-cells is associated with an improved prognosis in many tumour types, the tumour has several ways in which it can escape the immune response. In response to chronic, endogenous inflammation and cytokine signaling; tumour cells and indeed immune cells can aberrantly express immune checkpoints. dMMR tumours represents a unique molecular subtype of solid tumours to decipher these inflammatory and immunosuppressive mechanisms. In this pilot study we comprehensively evaluated spatial immune infiltration patterns (topographic phenomics) across a series of dMMR and pMMR colorectal and endometrial cancers using integrative InSituPlex technology and Tissue Phenomics.

Methods

Colorectal and endometrial cancer samples from the ACCFR and ANGELS studies were selected that included dMMR (Lynch syndrome CRC and EC, MLH1 methylated and sporadic MMR loss) and proficient mismatch repair (young onset colorectal cancer [YOCRC] and BRCA1^{MT} endometrial cancer) tumours. 4µm tissue sections were stained with the entire UltiMapper IO portfolio (PD-L1, PD-1 CD3, CD8, CD11c, CD20, CD45RO, CD68, CD163, Granzyme B, Ki67, MHCII and pan-cytokeratin) capturing the co-localisation and differential expression of immune cell phenotypes (**Figure 1**). These slides were then digitally acquired at 20x magnification using a Zeiss fluorescent slide scanner. Images then imported into a proprietary Tissue Phenomics platform where customised rule sets were developed that quantitatively phenotyped the TiME (**Figure 1 and Table 1**). Individual 50µm intra- and peri-tumoural borders were automatically generated for each sample and all phenotypes quantified within these regions.

Results

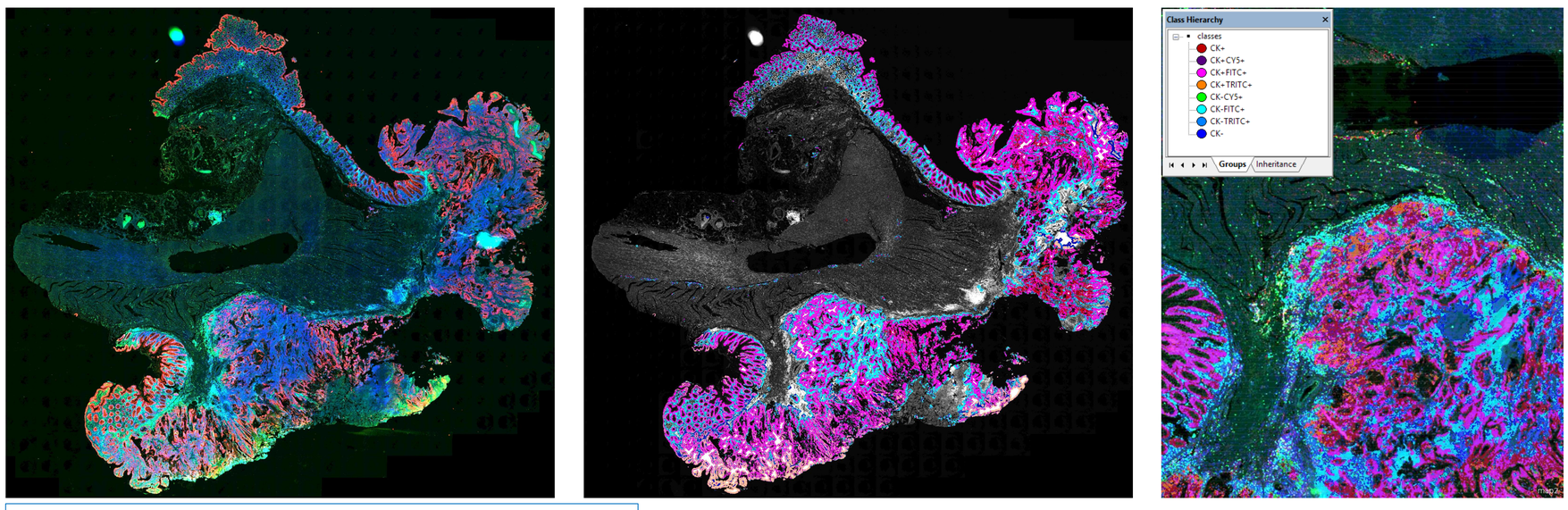
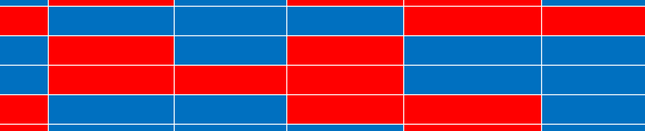


Figure 1: A) InSituPlex image with B+C) Tissue Phenomic classification

Phenotype	dMMR MLH1 CRC	dMMR MSH2 EC	dMMR Sporadic CRC	pMMR BRCA1 EC	pMMR BRAF^{MT} YOCRC	MLH1 methylated CRC 33.84 Mt/MB	MLH1 methylated CRC 82.20 Mt/MB
TiME Classification (Figure)	Type II	Type I	Type IV	Type IV	Type III	Type I	Type IV
Tumour Signature I							
Tumour Signature 2							
Proliferating Tumour cell							
Infiltrating T cell							
Infiltrating Cytolytic cell (iCD8)							
Infiltrating, Proliferating cytolytic T cell							
Infiltrating Memory Cell							
Infiltrating Exhausted T Cell							
Infiltrating Exhausted Memory Cell							
PDL1^{+} Tumour cell (tPD-L1)							
Infiltrating cytotoxic T cell							
Infiltrating M_1 Φ							
PD-L1^{+} immune cells (margin)							
M1 Φ (margin)							
Cytotoxic T cell margin							
Φ mediated inflammation (Tumour)							
Φ mediated inflammation (Margin)							
Infiltrating Dendritic cell							
Infiltrating M_2 Φ							
Infiltrating B cell							
Infiltrating M_2 Φ and B cell (Tumour)							
Infiltrating M_2 Φ and B cell (Margin)							
Dendritic cell (margin)							
M_2 Φ margin							
B cell margin							
Epithelial: Non-epithelial CD8 ratio							
CD8: CD20 ratio (Tumour)							

Table 1: Phenotype distribution by tumour type and MMR category.
Type I; high iCD8 and high tPD-L1
Type II; high iCD8 and low tPD-L1
Type III; low iCD8 and high tPD-L1
Type IV; low iCD8 and low tPD-L1



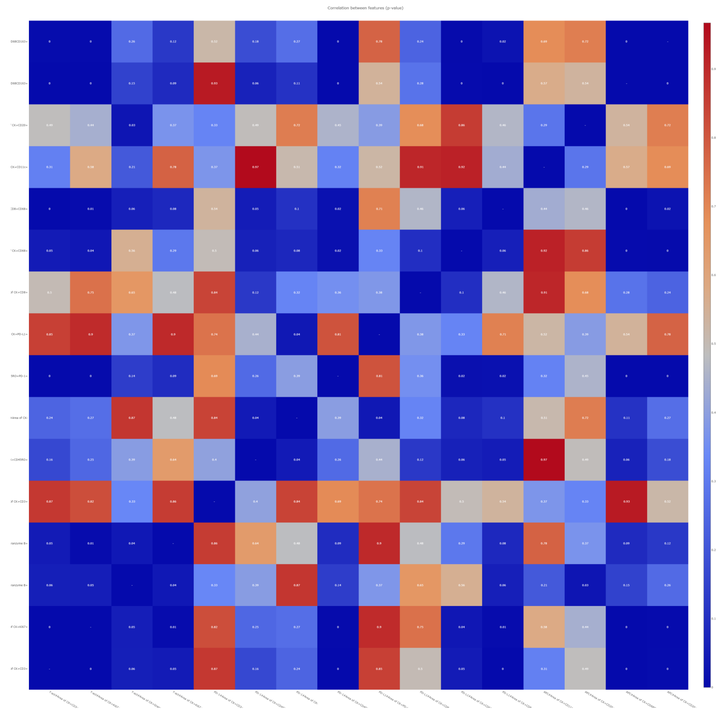
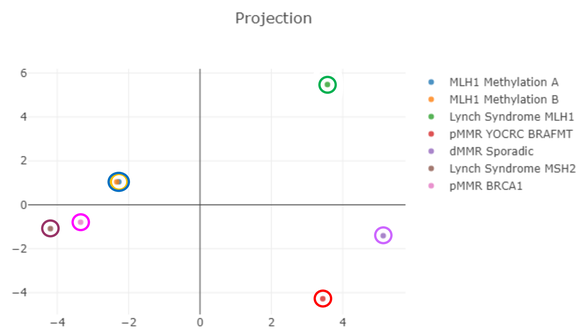


Figure 2 Correlation matrix between quantitative phenotypes from the UltiMapper kits. Significant correlations are shown in deep blue (p <0.05), whereas non-significant correlations (P>0.05) are depicted in a gradient from light blue to red.



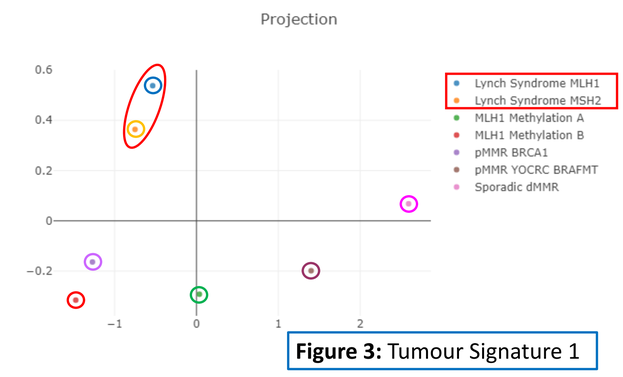


Figure 3: Tumour Signature 1

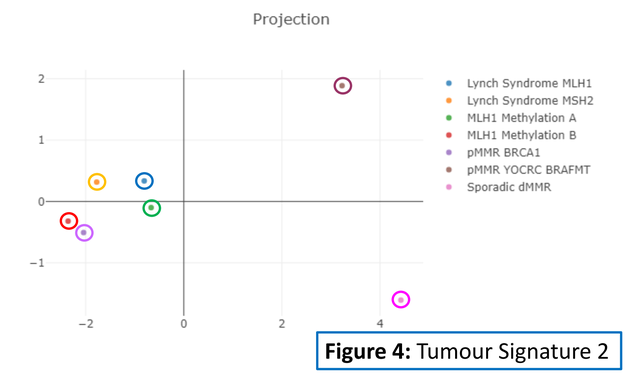


Figure 4: Tumour Signature 2

Results; The correlation between immune cell phenotypes is shown in **Figure 2**. Lynch-related colorectal and endometrial tumours have a higher ratio of intra-tumoural to peri-tumoural CD8^{+} T cells compared to other categories (p 0.02) and a lower area of proliferating tumour cells, whereas the pMMR YOCRC had the highest area of proliferating tumour cells. *Tumour signature 1* was higher in Lynch syndrome associated cancers compared to non-Lynch syndrome associated cancers (p 0.0004). There was a statistically significant difference between the means of the *tumour signature 2*, with a higher value being associated with high cell proliferation (p 0.03). We found that stromal PD-L1 expression was correlated with macrophage mediated inflammation at the invasion margin (p 0.004).

Conclusions

- This pilot study highlights the utility of integrating InSituPlex technology and Tissue Phenomics as a quantitative, next-generation pathology technique that enables spatially resolved, multiplexed phenotype measurements on a single FFPE slide. We used this data to define and generate tumour microenvironment signatures that may serve as predictive and prognostic biomarkers in solid tumours.
- Using dimension reduction modelling we show the predictive ability of our defined *in situ* tumour signatures to identify Lynch syndrome associated cancers (**Figure 3)** and the ability to elucidate underlying dMMR-associated biology (**Figure 4)**.
- We demonstrate that multiparametric analysis and generation of in situ tumour signature of dMMR tumours in the context of their dMMR category has the potential to guide which patients may benefit from immune checkpoint inhibitor monotherapy or combination immunotherapy.

***Acknowledgment's**: Sue Preston, Sharelle Joseland from the COG laboratory, the investigators, patients and families of the ACCFR and ANGELS studies and the R&D teams at Definiens and Ultivue.*