TriStar Technology Group

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Comparative multiplex digital phenotyping of the tumor microenvironment across multiple tumor types using a well characterized multi-tumor tissue microarray

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background

The tumor microenvironment (TME) represents a complex interaction of immune and host cells and can be described broadly as 'inflamed', 'immune excluded' and 'desert'; categories that may serve as predictive parameters for response to different immunotherapies. Multiplexed fluorescence and associated digital imaging platforms are important tools for defining the spatial components of the TME and can accelerate development of novel immunotherapy strategies.

methods

To investigate the TME across multiple tumor types simultaneously we have utilized a multi-tumor tissue microarray (TMA) covering 11 different tumour types comprising a total of 142 cases on 2 TMA slides, each case in duplicate with cores (1mm) taken from invasive margin (IM) and tumor center (TC). The TMA was stained using the UltiMapper I/O Immuno8 panel, which includes markers for CD3, CD4, CD8, FOXP3, CD68, PD-1, PD-L1, and a pan-CK/SOX10 cocktail as a tumor indicator. Stained TMAs were scanned on a fluorescence whole slide scanner (20X magnification) and marker images aligned using the UltiStacker software using the nuclear counterstain images as references from two rounds of imaging, to provide accurate marker colocalization data. Scanned images are displayed in **Figure 1A**. Digital image analysis was performed using Visiopharm software to generate cell phenotype data, including localization to tumor and stroma, for each of the 288 cores (**Figure 1B-D**).

results

Comparative analysis of all tumor types regardless of core location (IM and TC combined) revealed gastric cancer, CRC, TNBC and NSCLC to be most highly infiltrated with CD3+ T cells, and ER+ and Her2+ breast cancers least infiltrated (**Figure 2**).

The contribution of cytotoxic T cells (CD3+CD8+) to the TME was greater for gastric cancer (approx. 50%) compared with NSCLC (approx. 20%) and the proportion of cytotoxic T cells activated/exhausted as defined by expression of PD-1 ranged from approx. 25% (e.g. TNBC, CRC) to 1% (e.g. ER+ BC) (**Figure 2**).

Spatial localization analyses of cytotoxic T cells revealed pancreatic and hepatic cancers as the most 'excluded' as evidenced by low CD3+/CD8+ T cell numbers in tumor (CK+) compared with stromal regions (CK-) (**Figure 3**). Interestingly, cytotoxic T cells with an exhausted phenotype (CD3+CD8+PD-1+) were preferentially infiltrated within tumor (CK+) areas compared with stroma (CK-) for inflamed tumors such as CRC and TNBC.

Heterogeneity was observed with regard to spatial localization of T cell subpopulations (CTLs & exhausted CTLs) between tumor types (**Figure 4**). In general, the distribution of CTLs between tumor and stromal regions in cores taken from IM vs TC was similar, although more marked variations were observed for exhausted PD-1+ CTLs.

conclusions

- These data highlight the benefits of combining a fast, multiplexed staining protocol with a detailed digital analysis of immune phenotypes spatially within well characterized tumor samples to better understand the TME (e.g. inflamed, excluded) and identify tumor types more likely to benefit from different immunotherapeutic strategies.
- The study demonstrates further utility for specially designed TMAs in the simultaneous screening of multiple tumour types as part of immuno-oncology investigation and provides confidence in the use of this type of TMA to enhance high throughput immunohistochemical and digital image analysis technology.

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Figure 1

Multiplex digital phenotyping of the TME: 8-plex immunofluorescence images & digital image analysis strategy

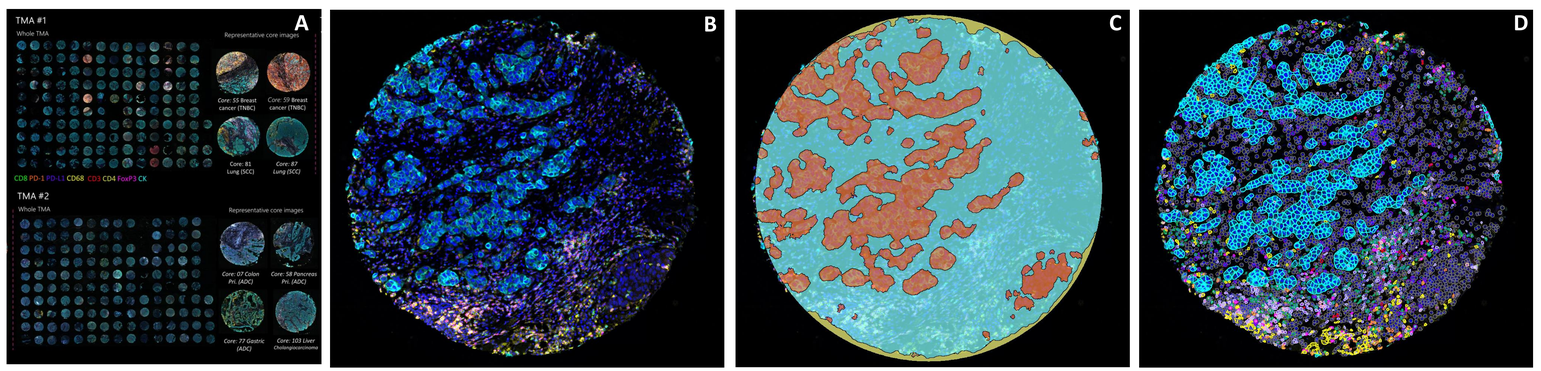

Figure 1. Scanned 8-plex fluorescent images for TMA 1 and TMA 2 with accompanying enlarged views of 4 selected cores for each TMA (**A**); 8-plex staining within 1 TMA core (**B**); segmentation of core showing Tumor (orange overlay), Stroma (blue overlay) and Glass/Artefact (yellow overlay) (**C**); cellular analysis of different immune cell phenotypes within the TME (**D**).

Figure 2

Contribution of T cell phenotypes to the TME

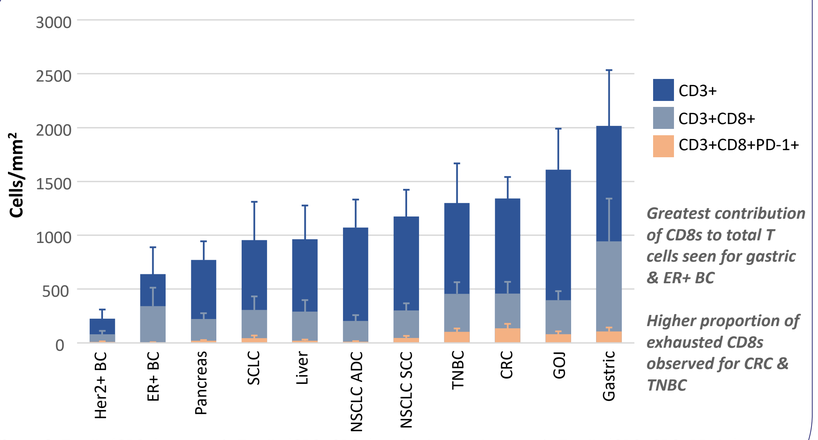

Figure 2. T cells (CD3+), cytotoxic T cells (CD3+CD8+) and exhausted cytotoxic T cells (CD3+CD8+PD-1+) were enumerated per core and data displayed as mean (±SE) values for all cores regardless of location (IM/TC) for each tumor type. Data are plotted in increasing order with respect to total CD3+ T cell content.

Figure 3

Spatial localization of CTLs in tumor vs stroma

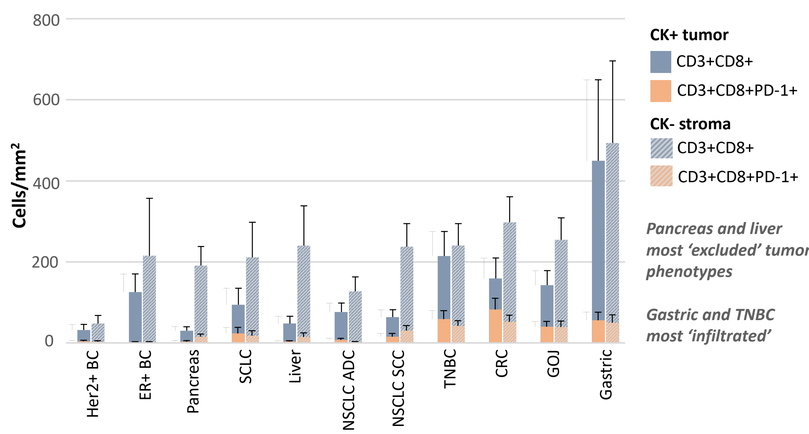

Figure 3. Cores were segmented into tumor and stroma on the basis of CK expression and CTLs (CD3+/CD8+)/ exhausted CTLs (CD3+CD8+PD-1+) evaluated for each tumor type. Data are plotted in order of increasing CD3+ T cell infiltration.

Figure 4

Spatial distribution of CTLs & exhausted CTLs in tumor vs stroma within cores taken from different tumor regions (invasive margin vs tumor center)

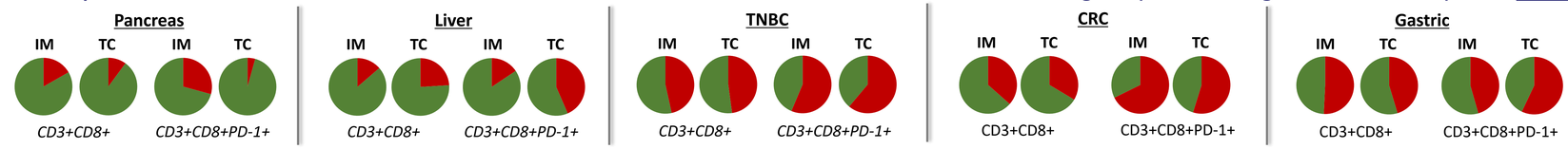

Figure 4. Each pie chart represents the ratio for either CTLs (CD3+CD8+) or exhausted CTLs (CD3+CD8+PD-1+) in tumour (■) vs stroma (■) for cores taken from either invasive margin (IM) and tumor center (TC)