Opposing CD68/CD163 tumor immune microenvironments revealed using a large multi-tumor tissue microarray (TMA) comprising cores from invasive margin (IM) and tumor center (TC)

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background

Macrophages contribute to cancer-associated inflammation, and those with M2-like phenotype have been reported to be involved in tumor progression, immunosuppression, metastasis and angiogenesis. CD163 is considered a marker of M2-like macrophages/monocytes and high expression has been associated with poor prognosis in various tumor indications.

methods

To investigate the relative contribution of macrophage/monocyte cell populations to the tumor immune microenvironment across multiple tumor types concurrently, we have utilised a formalin fixed paraffin embedded (FFPE) multi-tumor tissue microarray (TMA) comprising 30 different tumor indications. Each tumor type was represented by 10-13 cases (except thyroid n=9, sarcoma n=8, GIST n=5), with duplicate 1mm cores taken for each case (1 from invasive margin [IM] and 1 from tumour center [TC]). Serial sections of TMA slides (n=5) were stained by immunohistochemistry (IHC) for CD68 (clone PG-M1) and CD163 (clone 2G12) on the Ventana platform, and cells/mm²/core delivered by digital image analysis of scanned images (CellProfiler™).

results

As summarized in **Figure 1**, comparative analysis of all tumor types regardless of core location (IM and TC combined) revealed lung, renal and cervical cancers to be the most highly infiltrated with CD68+ macrophages, while the greatest CD163 staining was observed for sarcoma and glioblastoma (GBM).

Interestingly, a direct comparison of multiple tumor types simultaneously as performed here has revealed strongly opposing immune microenvironments in different tumor indications with respect to the balance of expression of CD68 and CD163 (**Figure 2**). Higher frequencies of CD68+ macrophages relative to low CD163 expression was observed for liver, pancreatic, prostate, small bowel, renal and cervical cancers for example. Conversely, a strong M2-like milieu exhibiting CD163+ cell frequencies exceeding CD68+ macrophages numbers was apparent for tumors such as sarcoma, GBM, mesothelioma, bladder, cutaneous squamous cell carcinoma. Tumor types such as triple negative breast, lung, gallbladder, colorectal, gastric, and esophageal cancers, all exhibited more equivalent levels of infiltrating CD68+ and CD163+ cells.

Representative staining for CD68 and CD163 is displayed in **Figure 3**.

Analyses of IM versus TC revealed further complexities of the tumor immune microenvironment. For some disease settings, divergent CD163:CD68 ratios were observed for IM versus TC, whereas for other indications the ratio remained unchanged (**Figure 4**).

conclusions

- Concurrent analysis of multiple tumor indications for CD68 and CD163 has revealed immunosuppressive M2-like macrophage/monocyte tumor immune microenvironments that vary markedly between tumor types and within some tumors.
- These data may help to identify those patients that may benefit from therapies that target this immune pathway.

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Figure 1

Comparative analysis of CD68 and CD163 across tumor indications

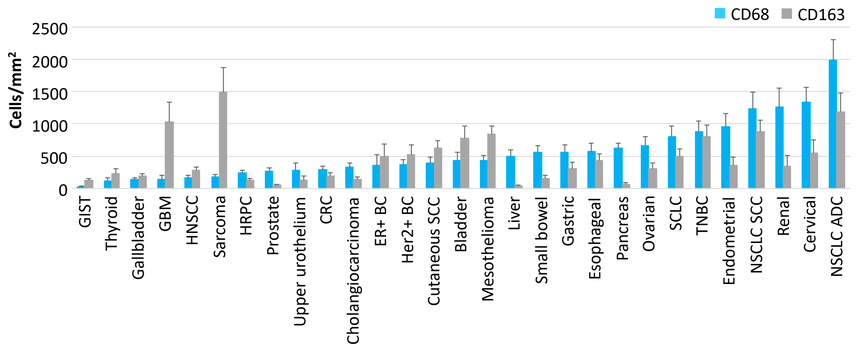

Figure 1. Comparative analysis of CD68 and CD163 across tumor types. Mean values for cells/mm² per tumor type for all cores regardless of location (IM and TC) are displayed (±SE). Data are plotted in increasing order with respect to CD68+ macrophage content.

Figure 2

Opposing immune (CD68:CD163) microenvironments across tumor types

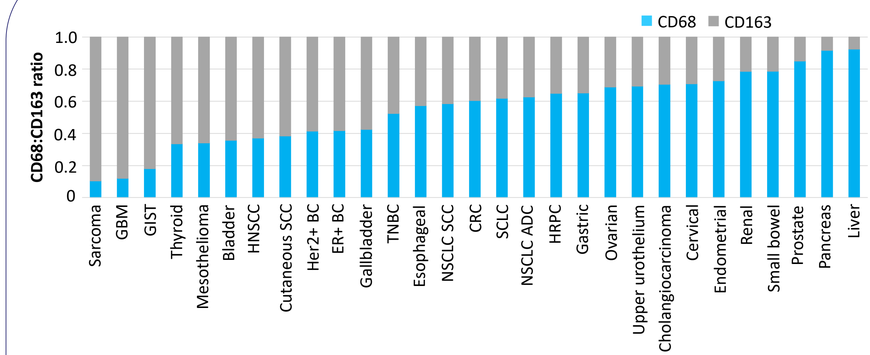

Figure 2. Balance of CD68:CD163 across tumor types. The ratio of CD68:CD163 was determined for all tumor indications using the mean cells/mm2 for each marker as shown in Figure 1. Results are plotted in order of increasing CD68 composition.

Figure 3

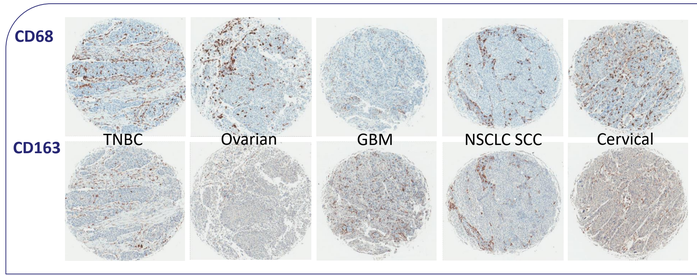

Figure 3. Representative images for CD68 and CD163

Figure 4

Tumor localization examples for CD68 and CD163

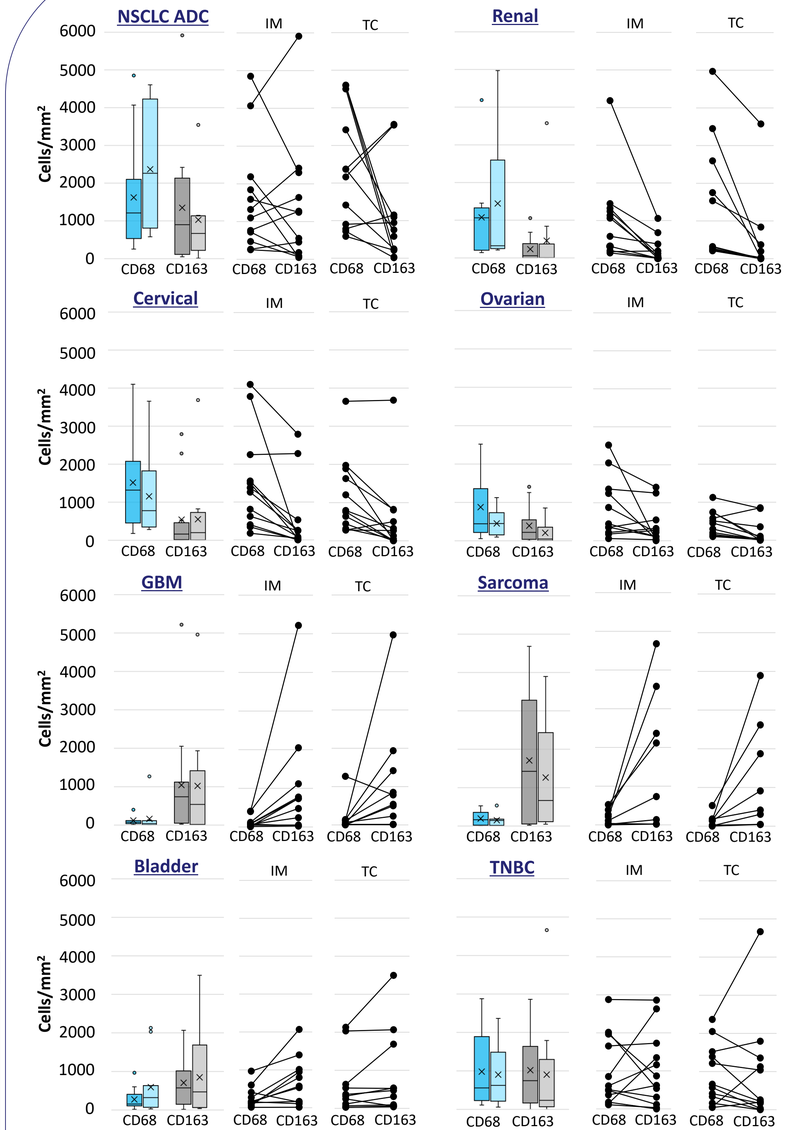

Figure 4. Distribution of CD68 and CD163 in invasive margin versus tumor center. For each tumor indication depicted, results are displayed as box-and-whisker plots showing the distribution of CD68 and CD163 in IM vs TC (■ CD68 IM; ■ CD68 TC; ■ CD163 IM; ■ CD163 TC), followed by line graphs showing individual case data.