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Interplay between TIM-3⁺ immune cells and other immune checkpoints in more than 40 different human carcinoma entities using 21 marker BLEACH&STAIN mfIHC

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Introduction & Objectives

An increasing number of therapy regimens using a combination of different immune checkpoint inhibitors have shown remarkable results in several different tumor entities. However, the likelihood of a positive response rate to combined immune checkpoint therapy is poor in most tumor entities and depends on several parameters including the tumor microenvironment. Particularly little is known about the spatial orchestration and spatial interplay between different immune checkpoint expressing cells.

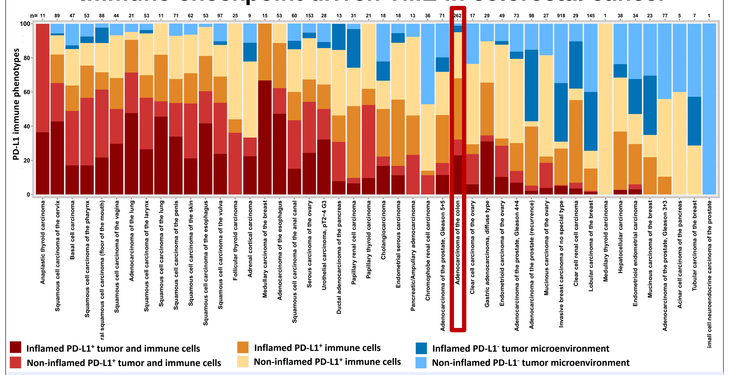
Given that the T-cell immunoglobulin and mucin domain-containing protein 3 (TIM3) is expressed on both immune cells as well as tumor cells and that several phase I/II studies are currently evaluating anti-TIM3 drugs, the interplay between these immune checkpoints in human cancers is of topical interest.

Materials & Methods

To study the spatial orchestration and interplay between TIM-3, CTLA-4, PD-1, and PD-L1 expression on T-cell subsets, macrophage subsets, CD11c⁺ dendritic cells, CD20⁺B-cells in relation to panCK⁺ malignant cells, CD31⁺ vessels and other structural tumor compartments, a multiplex fluorescence immunohistochemistry approach was used to stain 21 different antibodies on a set of tissue microarrays containing samples from more than 3000 carcinoma samples. In addition, a deep learning-based framework for cell type identification was developed and validated in this study.

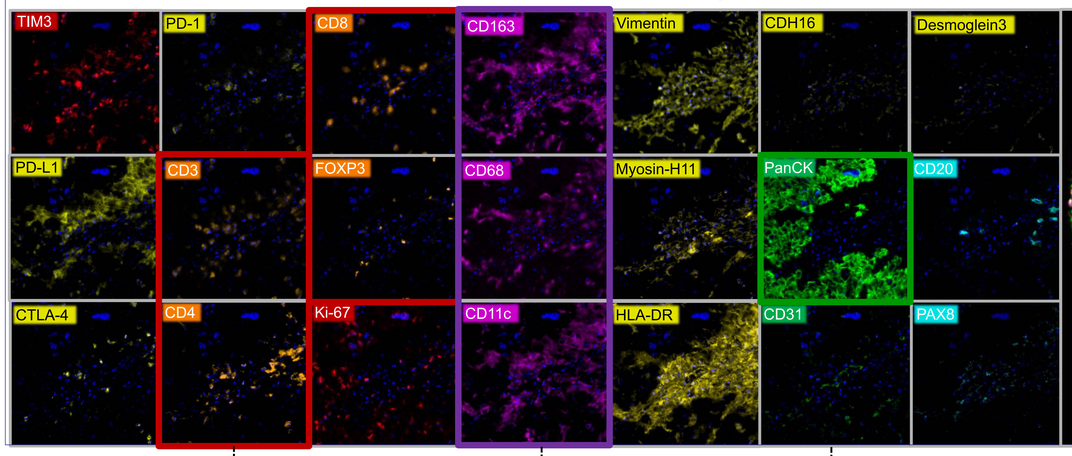
RESULTS

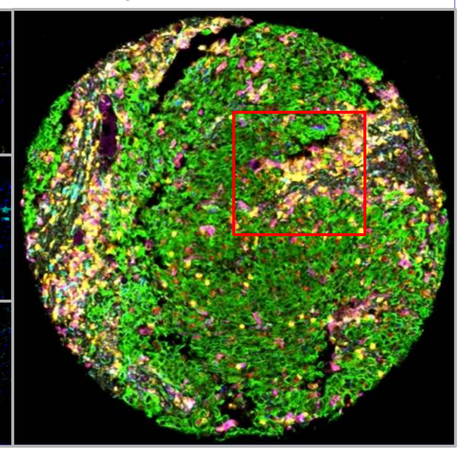
Immune checkpoint driven TME in colorectal cancer



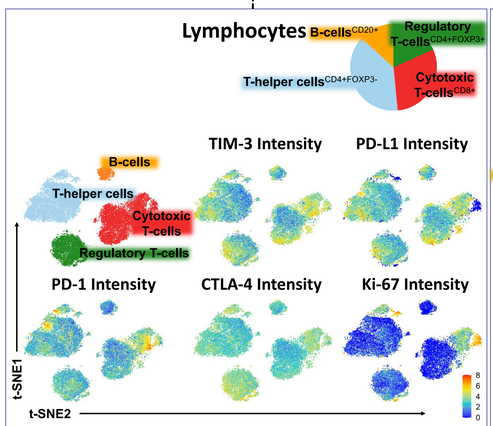
Previous analysis of the tumor microenvironment revealed a high proportion of patients with PD-L1 expression on Tumor- and immune cells in colorectal cancer. *unpublished data

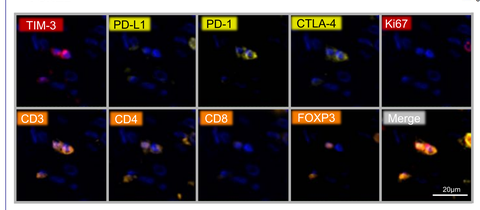
21+1 marker BLEACH&STAIN multiplex fluorescence immunohistochemistry





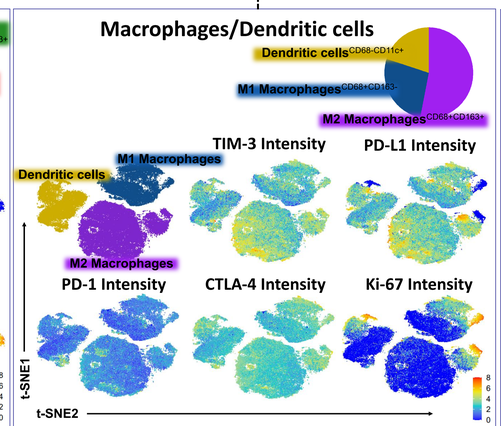
Lymphocytes

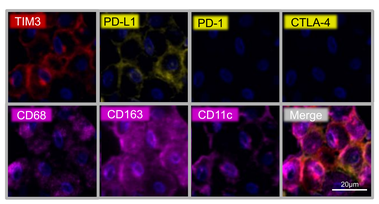




Most T-cell subsets (CD4⁺ helper T-cells, CD8⁺ cytotoxic T-cells and FOXP3 regulatory T-cells) showed a co-expression of TIM-3, PD-1 and/ or CTLA-4.

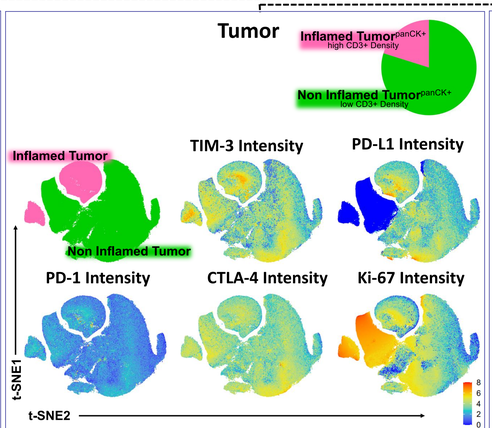
Macrophages/Dendritic cells

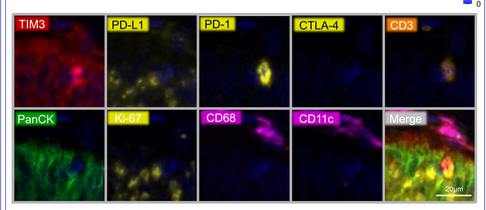




Most CD68⁺CD163± macrophages as well as CD11c⁺ dendritic cells showed co-expression of TIM-3 and PD-L1.

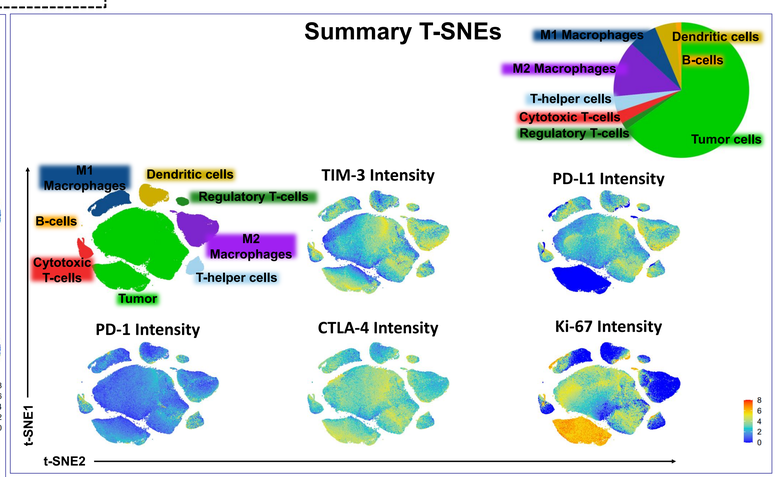
Tumor





A high TIM-3 expression on tumor cells was linked to an elevated Ki-67 labeling index.

Summary T-SNEs



Conclusions

- Deep profiling of 21 biomarkers in more than **40 different carcinoma entities** – especially in colorectal cancer – showed a **complex interplay of TIM-3 with PD-L1, PD-1 and CTLA-4.**

CD68 TIM-3 PD-L1 CTLA-4 PD-1 CD163 CD20 CD3 CD11c FOXP3 CD4 CD8 panCK Ki-67 CD31 Vimentin HLA-DR Myosin-H11 Desmoglein3 PAX8 CDH16

Conflicts of interest: The TIM3, PD-L1, CTLA-4, CD20, CD4, panCK, Ki-67, CD31, HLA-DR, Myosin H11, Desmoglein3 and CDH16 antibody clones were provided by MS Validated Antibodies GmbH (owned by a family member of GS)

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