Flow cytometric characterization of the tumor-infiltrating immune compartment: Significant differences in the tumor immune repertoire versus peripheral blood

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Abstract:

Characterization of the tumor microenvironment (TME) is expected to be critical in informing prognosis and guiding response prediction[1,2]. Recent studies have classified the TME based on tumor infiltrating leukocytes (TILs), programmed death-ligand 1 (PDL1) expression, and the nature of inflammatory responses[1,3]. Tumor studies involving transcript profiling and IHC have begun to inform the diversity and complexity of the tumor-infiltrated immune compartment (TIIC) by defining aspects of cellular composition, spatial distribution, and function. The use of flow cytometry allows the elucidation of an array of immune cell subtypes across entire samples. This study characterizes immune cell population subsets and tumor cells in four cancer types (breast, head and neck, colorectal, and gall bladder) from patients using multi-color flow cytometry. A good correlation was observed (r= 0.89, p=0.003) between tumor content, as evaluated by a pathologist (Hematoxylin and Eosin staining), and tumor content (EpCAM/panCK[7/8]+ cells) as estimated by flow cytometry (for all cancers excepting head and neck). Using pathology scoring we divided the samples into high-content (>10% tumor) and low-content (<10% tumor) samples. Samples with high tumor content were further examined by comparing the immune population in the tissue with their matched peripheral blood mononuclear cells (PBMC). We observed significantly higher proportions of T-cells, T-regulatory cells (Tregs) and exhausted T-cells (PD-1+ T-cells) in TIIC in comparison to its matched PBMC, while a lower proportion of B-cells and NK cells was noted. Also, samples with high tumor content had a significantly increased proportion of CD8 T-cells, Tregs and NK cells and a lower proportion of effector CD4 (CD4+Foxp3-) T-cells in comparison to the samples with low tumor content. The results from this study demonstrate the differences in the peripheral immune repertoire from the TIIC, reaffirming the need to robustly characterize the local TME. Additionally, the similarity in Treg and exhausted T-cell profiles across the indications examined may support a broad utility for therapeutics that can successfully target immune exhaustion or suppression.

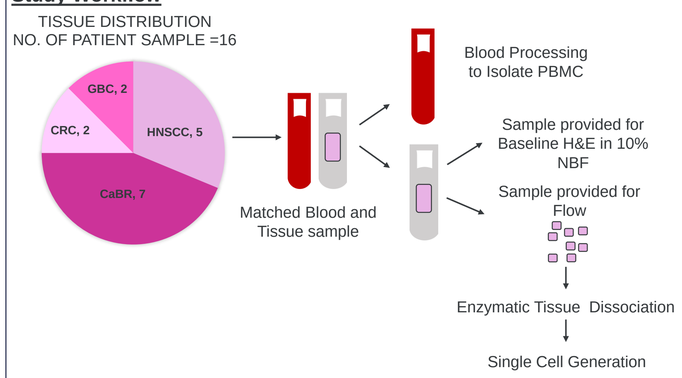
Methodology:

PBMC isolation: Blood sample was collected in ACD tubes (#02-684-26, BD) prior to surgery and transferred at 4^{0}C. PBMC was isolated using Histopaque 1077 (#10771, Sigma) by layering 4ml of Blood over 4ml of Histopaque 1077 at room temperature. It was centrifuged at 700g for 20 minutes with break setting at acc 9/ dec 5. The layer in the interface was pipetted out into a separate tube and washed with PBS.

Tissue dissociation: The tissue samples (small fragments on ~2x2x2mm) were dissociated into single cells using Tumor Dissociation kit (#130-095-929, Miltenyi Biotec). For each sample enzyme mix was prepared in 5ml of RPMI (without FBS) containing 40µL of Enzyme H, 20µL of Enzyme R, and 5µL of Enzyme A in a C Tube. Tissue fragments were added and dissociated using gentleMACS Octo Dissociator (130-095-937, Miltenyi Biotec) using in-built programs. Post dissociation Wash buffer containing FBS was added and filtered using 70µm filter. The cells were washed in PBS.

Flow Staining: The cells were first stained with Live/Dead dye in PBS, followed by Fc blocking in FACS buffer (2% FBS in PBS). Then the cells were stained with the surface marker cocktail. Then the cells were fixed and permeabilized and stained with intracellular marker cocktail.

Study Workflow

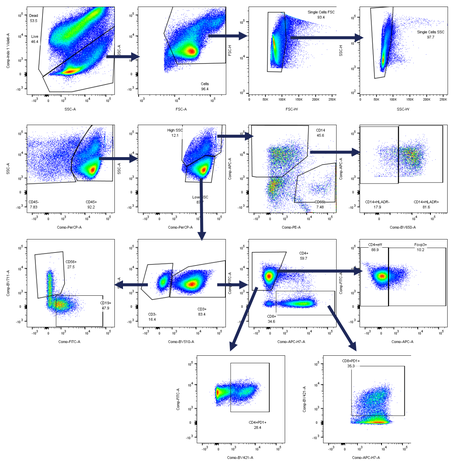


Flow Cytometry Based Assay Design

Panel: 11color 13 marker

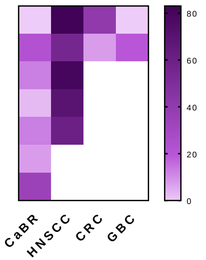
ANTIBODY	FLUROCHROME	CLONE
CD45	PERCP	2D1
CD3	BV510	OKT3
CD4	FITC	SK3
CD8	APC H7	SK1
CD14	PE CY7	M5E2
CD56	BV711	NCAM.16
CD66b	PE	G10F5
PD-1	BV421	EH12.2H7
CD19	FITC	HIB19
HLA-DR	BV650	L243
FOX P3	APC	236A/E7
EpCAM	PE	EBA1
CYTOKERATIN (7/8, CK)	PE	CAM5.2
LIVE/DEAD™ Fixable Blue	indo-V	NA

Flow Gating Strategy

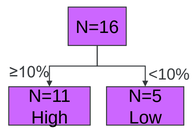


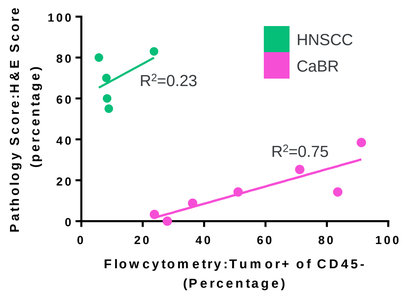
Tumor Content in tissues :
Pathology scoring vs Flow cytometry determination using EpCAM and CK

Tumor Content (H&E Score by Pathologist)

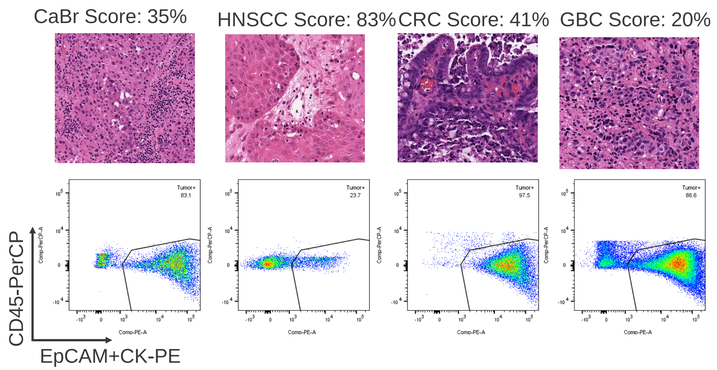


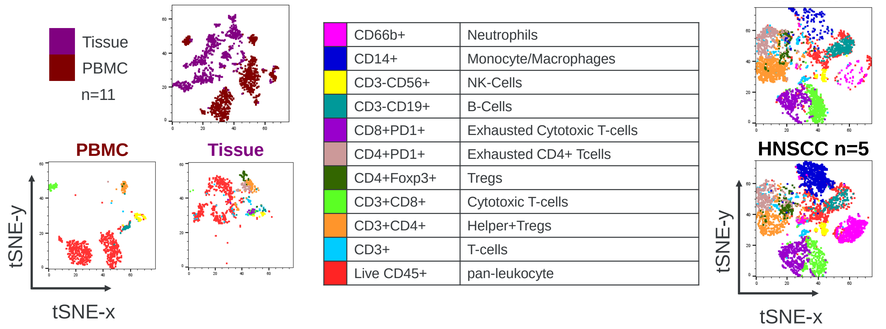
Cohorts based on Tumor Content



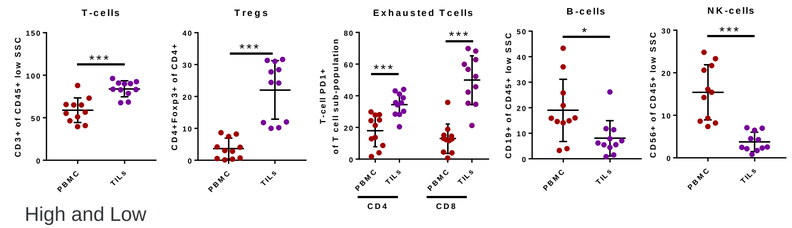


High tumor containing sample cohort:

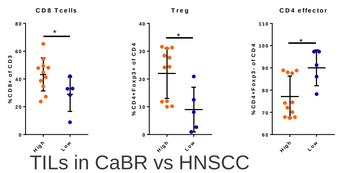




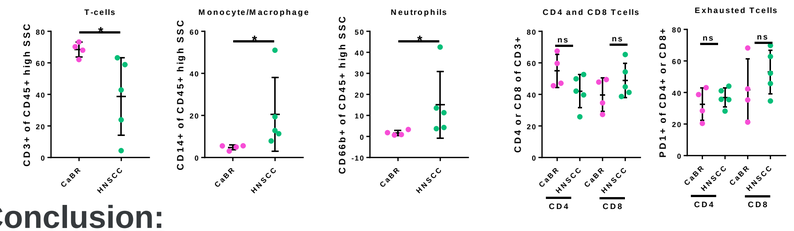
Peripheral Blood PBMC immune cells vs TILs:



High and Low



TILs in CaBR vs HNSCC



Conclusion:

References:

1.Marshall, Henry, et al. "Immuno-oncology: emerging targets and combination therapies." Frontiers in oncology 8 (2018): 315.
2.Li, Xiaotong, et al. "Immune profiling of pre-and post-treatment breast cancer tissues from the SWOG S0800 neoadjuvant trial." Journal for immunotherapy of cancer 7.1 (2019): 88.
3.Binnewies, Mikhail, et al. "Understanding the tumor immune microenvironment (TIME) for effective therapy." Nature medicine 24.5 (2018): 541-550.

Acknowledgement

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