Characterization of tumor antigen expression and myeloid immune profiles to inform the development of immune stimulating antibody conjugates (ISACs)

Poster 2557

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Background & Rationale

Immuno-oncology (IO) has historically focused on T cell-driven effects, but a growing class of myeloid therapies are under investigation. This class includes immune-stimulating antibody conjugates (ISACs), which comprise a tumor-targeting antibody conjugated to an immune-stimulating payload. Novel therapeutics including ISACs, myeloid/tumor bispecifics, and certain investigational combinations may require both tumor-associated antigens and tumor-resident myeloid cells for activity.

While baseline T cell characteristics and their relationship with checkpoint inhibition prognosis are well understood, the myeloid immune landscape of solid tumor types has not been fully characterized. To address this need, we evaluated tumor microarray samples for target expression and immune cell infiltration by immunohistochemistry (IHC). As new IO strategies such as TLR-activating ISACs are developed, understanding the myeloid landscape is essential for cancer biology and drug development.

Methods

Tumor Samples Evaluated

Tumor Type	N	Stage I/II N (%)	Stage III N (%)	Stage IV N (%)	Stage NA N (%)	Primary Tumors N (%)
Colorectal Cancer (CRC)	232	59 (25%)	41 (18%)	116 (50%)	16 (7%)	216 (93%)
CRC: MSI-L/H	36	13 (36%)	6 (17%)	17 (47%)	0 (0%)	36 (100%)
CRC: MSS	152	32 (21%)	24 (16%)	86 (57%)	10 (7%)	142 (93.5%)
CRC: MSI data NA	44	14 (32%)	11 (25%)	13 (30%)	6 (14%)	38 (86%)
Breast Cancer (BC)	166	83 (50%)	68 (41%)	4 (2%)	11 (7%)	NA
BC: HER2+	37	9 (24%)	28 (76%)	0 (0%)	0 (0%)	37 (100%)
BC: Triple negative	61	25 (41%)	22 (36%)	4 (7%)	10 (16%)	NA
BC: HR positive	68	49 (72%)	18 (26%)	0 (0%)	1 (1%)	NA
NSCLC	48	0 (0%)	25 (52%)	21 (44%)	2 (4%)	NA
Gastric (GC)/GEJ	84	11 (13%)	68 (81%)	3 (4%)	2 (2%)	NA
HNSCC	66	0(0%)	0 (0%)	0 (0%)	66 (100%)	53 (80%)

NA = data not available

- Tumor microarrays with formalin-fixed paraffin-embedded (FFPE) samples were purchased from TriStar Technologies and used for an exploratory research study.
- There were no pre-specified statistical hypotheses. Comparisons were performed as post-hoc exploratory data analysis, without correction for multiple testing.

Staining and Scoring of Protein Expression

- ISACs targeting tumor antigens such as HER2, CEA, and PD-L1 are under investigation. Serial sections of FFPE samples from tissue microarrays were stained for the relevant tumor antigens (HER2, CEA, PD-L1), myeloid cell markers (CD68/CD163/CD11c/BDCA-2), and CD8+ T cells (duplex assay with PanCK).
- Staining was scored and cell populations enumerated across the samples.

Method	Antibodies	Clones	Scoring method	Biology measured
1) Single-plex IHC	CEA	CEA31	Pathologist	Tumor antigen
2) Single-plex IHC	HER2	4B5	Pathologist	Tumor antigen
3) Single-plex IHC	PD-L1	22C3	Pathologist	Tumor antigen and immune checkpoint
4) Dual-plex IHC	CD8 PanCK	SP239 AE1/AE3/PCK26	Automated	Adaptive immunity Tumor context
5) 4-plex IF	BDCA-2 CD11c CD68 CD163	992259 D3B1E SP251 MRQ-26	Automated	Myeloid markers

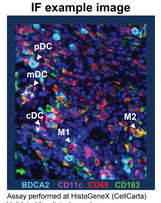
Immune Cell Subsets Were Defined Based on Combined Marker Expression

Myeloid cell definitions

	M1	M2	cDC	mDC	pDC
CD68	+	+/-	-	-	-
CD163	-	+	-	+	-
CD11c	+/-	-	+	+	+/-
BDCA-2	-	-	-	-	+

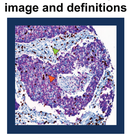
Total myeloid content
Macrophages (M1 + M2)
M1 macrophages *CD68+BDCA2-*
M2 macrophages *CD163+CD11c-BDCA2-*
DCs (cDCs + mDCs)
cDCs *CD11c+CD68-CD163-BDCA2-*
mDCs *CD11c+CD68+CD163-BDCA2-*
pDCs *BDCA2+CD68-CD163-*

Myeloid 4-plex IF example image



Assay performed at HistoGeneX (CellCarta)
Validated for clinical samples

CD8/PanCK example image and definitions



CD8+ (Total)
CD8+ (in tumor) *CD8 within PanCK+ tumor area*
CD8+ (out of tumor) *CD8 outside of PanCK+ tumor area*

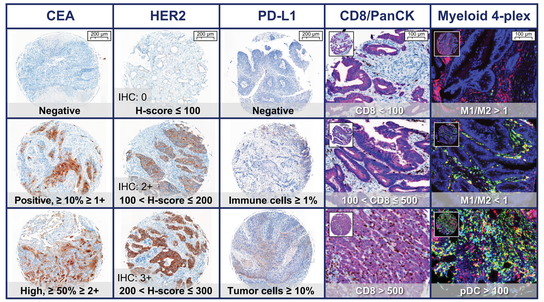
- Myeloid populations were quantified within the tissue core.
- CD8+ cells were quantified both within the core (total) and within the PanCK+ tumor area (in tumor).

References

1. Ackerman SE, et al. *Nature Cancer*. 2021;2:18–33.
2. Sharma M, et al. ESMO-IO 2021. Abstract 164P.

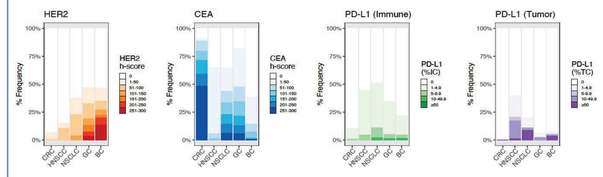
Results

Representative Tumor Antigen and Immune Marker Expression



IC = immune cells; TC = tumor cells

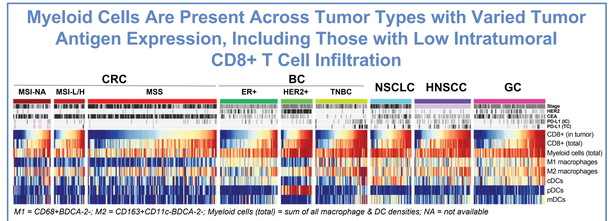
Prevalence of the Tumor-associated Antigens HER2, CEA, and PD-L1 Varies Across Tumor Types



	CRC	HNSCC	NSCLC	GC
CEA (≥10% 1+)	90.5%	33.3%	52.1%	69.0%
PD-L1 Immune Cells (≥1% 1+)	15.8%	49.2%	57.8%	44.2%
PD-L1 Tumor Cells (≥10% 1+)	0.5%	17.5%	11.1%	2.6%

- In line with prior expectations, HER2 was observed primarily in BC and GC tumors, CEA was observed primarily in CRC, GC, and NSCLC tumors, and PD-L1 was observed primarily in GC, NSCLC, and HNSCC. Percent prevalence data are not provided for HER2 or for BC because such numbers would mainly reflect the number of samples purchased for HER2+ BC versus TNBC.

Myeloid Cells Are Present Across Tumor Types with Varied Tumor Antigen Expression, Including Those with Low Intratumoral CD8+ T Cell Infiltration

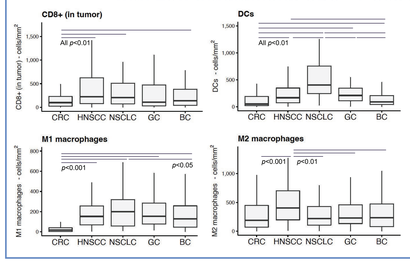


M1 = CD68+BDCA-2-; M2 = CD163+CD11c-BDCA-2-; Myeloid cells (total) = sum of all macrophage & DC densities; NA = not available

- Densities of myeloid cells and CD8+ cells are presented as a heatmap, together with the tumor types, cancer staging, and tumor antigen expression of the associated samples. PanCK was stained concurrently with CD8 enabling localization of CD8 infiltrates.
- Myeloid cells are present across tumor types and patient subsets, including cohorts with low intratumoral CD8+ cell infiltration such as MSS CRC.
- Each patient cohort has a distinctive profile of tumor antigen expression and immune cell densities, and statistical comparisons are presented in the subsequent data panels.

ND = not determined

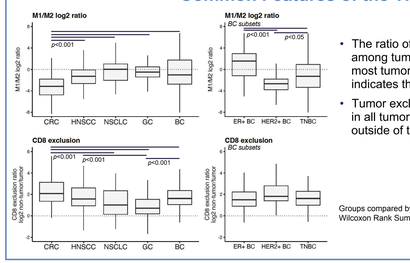
Myeloid Cells Are Present Across Tumor Types, Including Those with Low Intratumoral CD8+ T Cell Infiltration



- Densities of myeloid cells and CD8+ cells are presented as box plots for each tumor type.
- CD8+ and myeloid cell densities vary among tumor types.

Groups compared by Kruskal-Wallis test with post-hoc pairwise Wilcoxon Rank Sum tests, without correction for multiple testing.
Note that PanCK was stained concurrently with CD8 enabling localization of CD8 infiltrates.

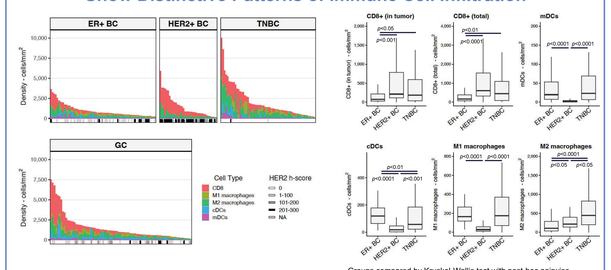
Higher M2, Relative to M1, and Exclusion of CD8+ Cells Are Common Features of the TME



- The ratio of M1/M2 macrophages differs among tumor types, with M2 predominant in most tumors. An M1/M2 log2 ratio below 0 indicates that M2 are more frequent than M1.
- Tumor exclusion of CD8+ cells was observed in all tumor types, with higher CD8+ densities outside of the tumor.

Groups compared by Kruskal-Wallis test with post-hoc pairwise Wilcoxon Rank Sum tests, without correction for multiple testing.

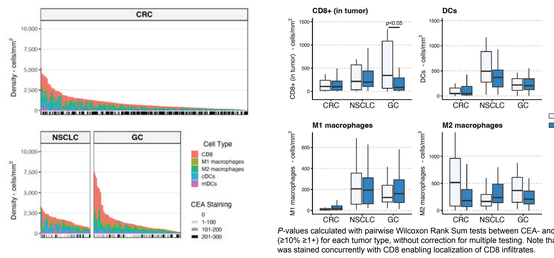
BC and GC, Which Have HER2 Expression in a Subset of Patients, Show Distinctive Patterns of Immune Cell Infiltration



Groups compared by Kruskal-Wallis test with post-hoc pairwise Wilcoxon Rank Sum tests.

- HER2+ BC samples showed higher CD8+ T cell infiltration relative to the ER+ BC cohort, and lower DC and M1 macrophage densities relative to both ER+ BC and TNBC.

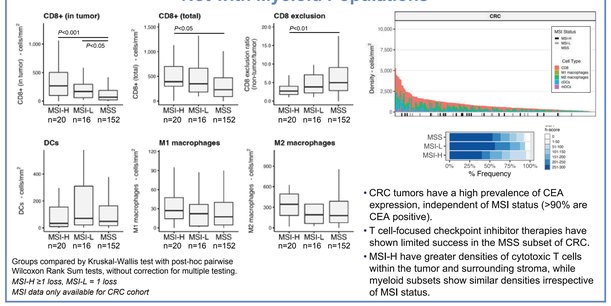
CEA+ and CEA- Tumor Samples Have Similar Immune Infiltrates



P-values calculated with pairwise Wilcoxon Rank Sum tests between CEA- and CEA+ (≥10% 1+) for each tumor type, without correction for multiple testing. Note that PanCK was stained concurrently with CD8 enabling localization of CD8 infiltrates.

- No associations between CEA status and immune cell densities were observed in CRC or NSCLC, with slightly higher intratumoral CD8+ cell densities in CEA- GC as compared to CEA+ GC.

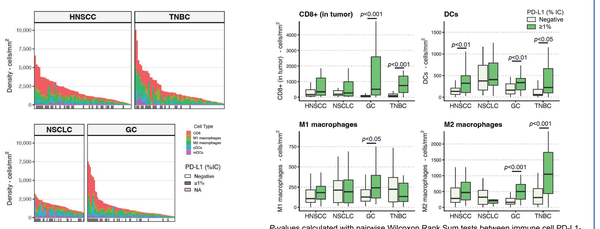
MSI Status in CRC Correlates with Intratumoral CD8+ Cells, but Not with Myeloid Populations



Groups compared by Kruskal-Wallis test with post-hoc pairwise Wilcoxon Rank Sum tests, without correction for multiple testing.
MSI-H ≥1 loss, MSI-L = 1 loss
MSI data only available for CRC cohort

- CRC tumors have a high prevalence of CEA expression, independent of MSI status (>90% are CEA positive).
- T cell-focused checkpoint inhibitor therapies have shown limited success in the MSS subset of CRC.
- MSI-H have greater densities of cytotoxic T cells within the tumor and surrounding stroma, while myeloid subsets show similar densities irrespective of MSI status.

PD-L1 on Immune Cells Is Associated with Higher CD8+ and Myeloid Cell Densities



P-values calculated with pairwise Wilcoxon Rank Sum tests between immune cell PD-L1- and PD-L1+ samples (≥1%) for each tumor type, without correction for multiple testing.

- In several relevant tumor types, tumors with PD-L1 staining on immune cells also showed higher infiltration of CD8+ and/or myeloid cells.

Conclusions

- Myeloid cells are abundant in many solid tumor types, independent of CD8+ T cell infiltration.
- HER2+ BC samples have higher CD8+ T cell infiltration relative to the ER+ BC cohort, and lower DC and M1 macrophage densities relative to both ER+ BC and TNBC. Immune infiltration is independent of CEA expression in CRC and NSCLC.
- In contrast to CD8+ cells, myeloid cells do not differ by MSI status in CRC.
- PD-L1 on immune cells is associated with higher CD8+ and myeloid cell densities in GC and TNBC and higher DC densities in HNSCC.
- The presence of myeloid cells in multiple tumor types offers broad therapeutic targets for ISACs and other myeloid-directed therapies that can activate the innate immune system as a bridge to adaptive immunity.