Characterization of TIM3 and its Ligands in Colorectal Cancer

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Abstract ID:341

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Background

- TIM-3 is an inhibitory checkpoint glycoprotein found on innate and adaptive immune cells and is highly expressed on tumor infiltrating lymphocytes.
- TIM-3 and its ligands, Galectin 9 (Gal9), HMGB1 and CEACAM1 play a critical role in immune regulation
- Preclinical data suggest a role in the pathogenesis of colorectal cancer (CRC).
- We aimed to characterize the molecular features and prognostic value of TIM3 and its ligands in CRC.

Methods

- Tumor molecular profiling was performed from 15,026 FFPE samples by NextGen Sequencing on DNA (592 genes or WES) and RNA (WTS) and immunohistochemistry (IHC) at Caris Life Sciences (Phoenix, AZ).
- Top quartile transcripts per million (TPMs) for *TIM-3, Gal9, HMGB1 and CEACAM1* were considered high (Q4) while bottom quartile low (Q1) expression (exp).
- X^2/Fisher-exact tests were used for comparison and significance was determined as *P*-value adjusted for multiple comparison and this was found for the results reported here ($Q < 0.05$).
- Cell infiltration in the tumor microenvironment (TME) was estimated by quanTIseq.
- Real-world overall survival (OS) information was obtained from insurance claims data and Kaplan-Meier estimates were calculated for molecularly defined pts.

Results

Tables 1. (A-D) show patient demographic information for *TIM3* and its ligands.

A.

	TIM3 Q1	TIM3 Q4
Count (N)	3697	3697
Median Age (range)	62.0 (20 - >89)	63.0 (15 - >89)
Male	55.7% (2060/3697)	53.0% (1961/3697)
Female	44.3% (1637/3697)	47.0% (1736/3697)

B.

	GAL9 Q1	GAL9 Q4
Count (N)	3697	3697
Median Age (range)	61.0 (15 - >89)	63.0 (17 - >89)
Male	55.4% (2047/3697)	54.7% (2024/3697)
Female	44.6% (1650/3697)	45.3% (1673/3697)

C.

	HMGB1 Q1	HMGB1 Q4
Count (N)	3697	3697
Median Age (range)	62.0 (14 - >89)	63.0 (17 - >89)
Male	54.9% (2029/3697)	55.3% (2046/3697)
Female	45.1% (1668/3697)	44.7% (1651/3697)

D.

	CEACAM1 Q1	CEACAM1 Q4
Count (N)	3697	3697
Median Age (range)	62.0 (15 - >89)	63.0 (17 - >89)
Male	53.4% (1976/3697)	56.0% (2071/3697)
Female	46.6% (1721/3697)	44.0% (1626/3697)

Figure 1: (A-D)IO marker prevalence (%) for *TIM3* and its ligands.

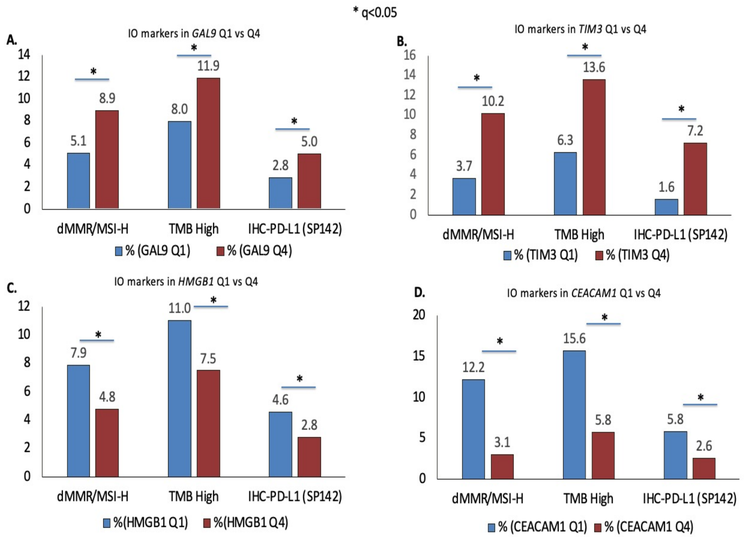
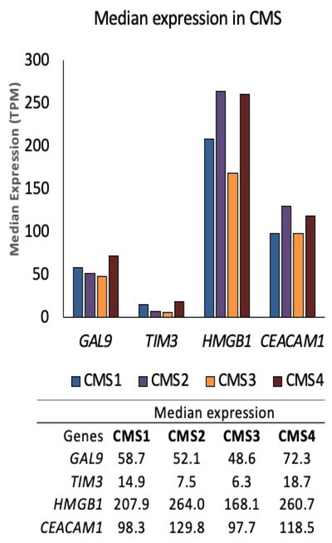


Figure 2: Median expression for CMS



Median expression

Genes	CMS1	CMS2	CMS3	CMS4
GAL9	58.7	52.1	48.6	72.3
TIM3	14.9	7.5	6.3	18.7
HMGB1	207.9	264.0	168.1	260.7
CEACAM1	98.3	129.8	97.7	118.5

Figure 4: (A-D) Real world overall survival (rwOS) for *TIM3* and its ligands in CRC.

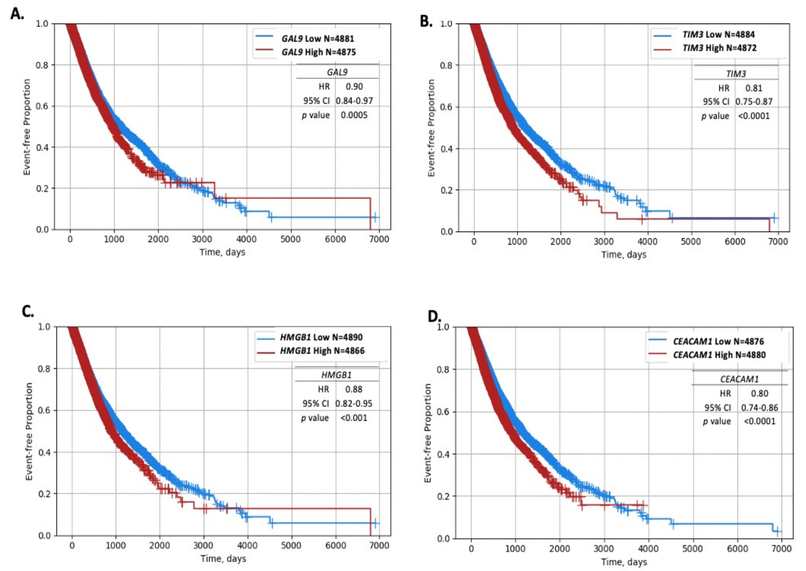


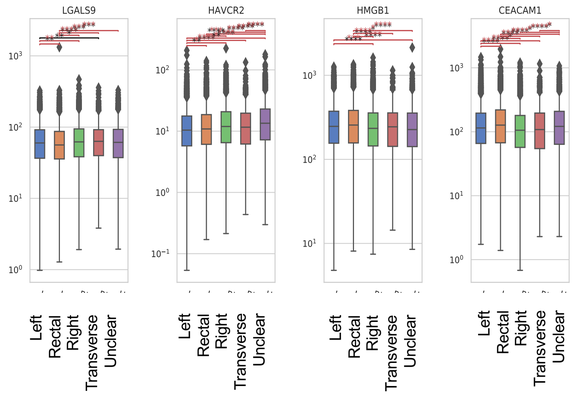
Table 2: A. Immune infiltration summary showing significant infiltration with gene expression within MSS tumors.

Immune Cell	GAL9	TIM3	HMGB1	CEACAM1
B cell	increase	increase	increase	decrease
Macrophage M1	increase	increase	decrease	decrease
Macrophage M2	increase	increase	increase	decrease
Neutrophil	increase	N.S.	increase	increase
NK cell	increase	increase	increase	increase
T cell CD4+ (non-regulatory)	increase	increase	increase	increase
T cell CD8+	increase	increase	decrease	decrease
T cell regulatory (Tregs)	increase	increase	decrease	decrease
Myeloid dendritic cell	increase	increase	increase	increase
Monocyte	decrease	N.S.	decrease	decrease

B. Significantly different alteration summary within MSS tumors (Q1 vs Q4).

Alteration	*GAL9*	*TIM3*	*HMGB1*	*CEACAM1*
TP53 mut	lower (80% vs 75%)	lower (78% vs 73%)	higher (68% vs 86%)	higher (73% vs 78%)
FLT1 amp	lower (3% vs 2%)	lower (3% vs 2%	higher (1% vs 4%)	higher (73% vs 78%)
FLT3 amp	lower (4% vs 2%)	lower (4% vs 2%)	higher (2% vs 5%)	higher (2% vs 4%)
CDX2 amp	lower (13% vs 7%)	lower (12% vs 6%)	higher (7% vs15%)	higher (7% vs 12%)
CDK8 amp	lower (3% vs 1%)	lower (3% vs 1%)	higher (1% vs 4%)	higher (1% vs 3%)
APC mut	N.S.	lower (82% vs 69%)	higher (69% vs 85%)	higher (67% vs 83%)
NRAS mut	lower (5 vs 3%)	N.S.	higher (3% vs 6%)	higher (3% vs 5%)
GNAS mut	N.S.	higher (2% vs 4%)	lower (5% vs 1%)	lower (4% vs 2%)
FBXW7 mut	lower (10% vs 8%)	N.S.	lower (10% vs 8%)	lower (10% vs 8%)
RNF43 mut	N.S.	higher (2% vs 3%)	lower (3% vs 2%)	lower (3% vs 2%)

Figure 3: Tumor sidedness and differences in expression for *TIM3* and its ligands



Gene	Left (median TPM)	Rectal (Median TPM)	Right (Median TPM)	Transverse (Median TPM)	Unclear (Median TPM)
GAL9	59.9	56.2	62.0	63.1	61.1
TIM3	10.4	10.9	11.9	11.6	13.5
HMGB1	246.9	256.1	233.8	244.0	226.2
CEACAM1	114.6	127.0	105.6	108.1	121.2

Conclusions

- Gal 9/TIM3-high tumors had higher prevalence (prev) of high tumor mutational burden ((12% vs. 8%; 14% vs. 6%), deficiency in mismatch repair (dMMR) (9% vs. 5%; 10% vs. 4%), PD-L1 (5% vs. 3%; 7% vs. 2%).
- Expression of Gal9/TIM3 was highest in CMS4, and in right sided and transverse tumors compared to left sided tumors.
- In contrast, HMGB1 and CEACAM1-high tumors had lower prev of dMMR (5% vs. 8%, 3% vs. 12%), PD-L1 exp (3% vs. 5%, 3% vs. 6%) and TMB-H (8% vs. 11%, 6% vs. 16%), and was highest in CMS2.
- In MSS tumors, Gal9/TIM3-high tumors were associated with lower frequency of TP53, amplifications of FLT1/3, CDX2, FOXO1, and CDK8 whereas CEACAM1 and HMGB1 were associated contrasting alteration rates.
- High expression in TIM3 and its ligands were associated with worse overall survival (all p<0.01).
- These findings provide rationale for further evaluation of TIM3 and its ligands in CRC as prognostic and predictive biomarkers and potential therapeutic targets modifying the tumor microenvironment.

ASCO Annual 2022