Guanylate cyclase 2C (*GUCY2C*) expression and the tumor immune microenvironment (TIME) in gastrointestinal (GI) cancers

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Background

- An immunosuppressive TIME can reduce the efficacy of immune checkpoint inhibitors (ICI) in GI cancers.
- *GUCY2C* is preferentially expressed in colorectal (CRC), gastroesophageal junction (EJC) and gastric adenocarcinoma (GA).
- There is currently an ongoing trial of GUCY2C-directed bispecific T cell engager in advanced GI cancers.
- Better characterization of the TIME of *GUCY2C*-high GI cancers will be important as additional directed immunotherapies are developed.

Methods

- CRC (N = 15,285), EJC (N = 3,276) and GA (N = 2,420) tumors were tested at Caris Life Sciences (Phoenix, AZ) with NextGen Sequencing on DNA (592 genes or whole exome) and RNA (whole transcriptome).
- PD-L1+ expression was assessed by IHC (22C3: TPS ≥ 1% [CRC] or 28-8: ≥2+, ≥80% [EJC, GA]).
- A combination of IHC and NGS was used to assess deficient mismatch repair/microsatellite instability high (-MSI, stable: -MSS).
- *GUCY2C*-High (H) and -Low (L) (transcripts per million, TPM) was defined for each molecularly defined subtype as top and bottom quartile, respectively.
- Cell infiltration was estimated by QuantiSEQ. Mann-Whitney U and χ^2/Fisher's exact tests were applied as appropriate ($p < .05$, adjusted for multiple comparisons).
- Real-world overall survival (OS) and survival since start of ICI was obtained from insurance claims and Kaplan-Meier estimates were calculated for molecularly defined patients.

Results

1. *GUCY2C* expression across GI cancers

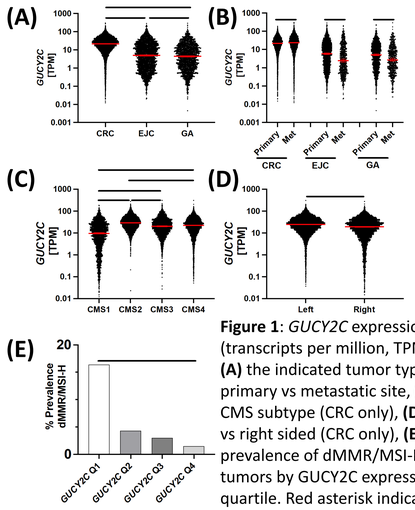


Figure 1: *GUCY2C* expression (transcripts per million, TPM) in **(A)** the indicated tumor type, **(B)** primary vs metastatic site, **(C)** by CMS subtype (CRC only), **(D)** left vs right sided (CRC only), **(E)** prevalence of dMMR/MSI-H CRC tumors by GUCY2C expression quartile. Red asterisk indicates statistical significance ($p < 0.05$).

2. Cohort characteristics

EJC	GUCY2C Q1	GUCY2C Q2	GUCY2C Q3	GUCY2C Q4	*q*-value
Count (N)	819	819	819	819	
Median Age	66	67	67	67	0.460
Male	82.9% (679/819)	84.7% (694/819)	84.5% (692/819)	86.1% (705/819)	0.454
GA					
Count (N)	605	605	605	605	
Median Age	67	66	65	65	0.159
Male	60.8% (368/605)	58.8% (356/605)	51.6% (312/605)	58.2% (352/605)	0.019
CRC MSI					
Count (N)	629	163	115	59	
Median Age	71	65	70	66	0.002
Male	36.7% (231/629)	52.1% (85/163)	42.6% (49/115)	59.3% (35/59)	<0.001
CRC MSS					
Count (N)	3197	3652	3708	3762	
Median Age	62	61	62	63	<0.001
Male	53.3% (1704/3197)	56.3% (2057/3652)	55.2% (2046/3708)	55.9% (2104/3762)	0.062

3. Genomic landscape by *GUCY2C* expression

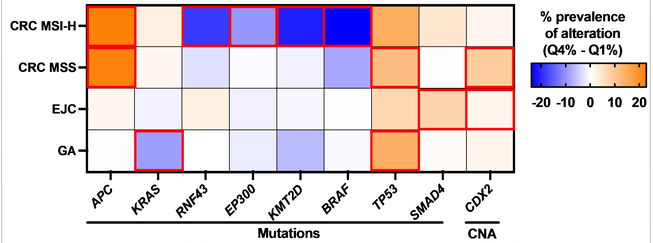


Figure 3: % prevalence of alteration (GUCY2C Q4-Q1) for the indicated tumor types and genes (mutations: SNV/indels and CNA: copy number amplification). Red box indicates statistical significance ($p < 0.05$). Genes that had p < 0.05 and Q4-Q1% greater than 7% in at least one tumor type are shown.

4. Immune landscape

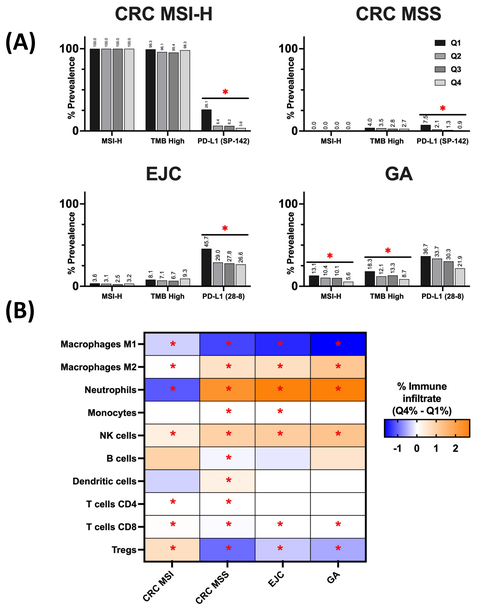
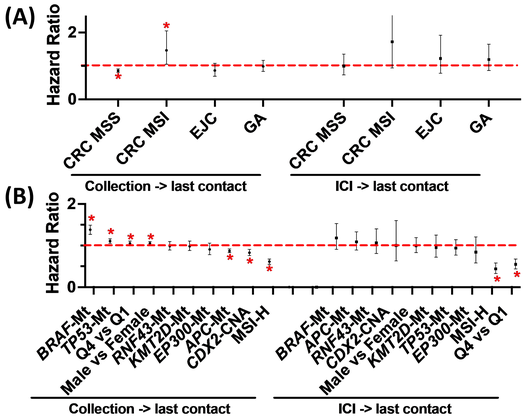


Figure 4: **(A)** The prevalence of MSI-H, TMB High or PD-L1 positive tumors (by IHC) for the indicated tumor types across GUCY2C expression quartiles. **(B)** Heat map of the difference in % immune infiltrate between quartile 4 and 1 of GUCY2C expression (TPM). Red asterisk indicate statistical significance ($p < 0.05$).

5. GUCY2C CRC tumors have increased survival from start of ICI



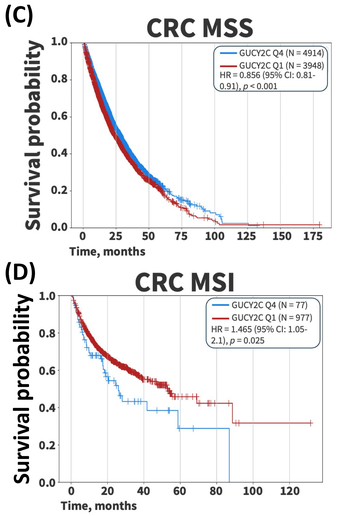


Figure 5: **(A)** Forest plot of either overall survival (OS, collection to last contact) or OS since start of ICI. Red asterisk indicates statistical significance. **(B)** Forest plot (multivariate analysis) of either overall survival (OS, collection to last contact) or OS since start of ICI in CRC for biomarkers with an imbalance between Q4 and Q1 of GUCY2C expression. Red asterisk indicate statistical significance ($p < 0.05$)**(C, D)** Kaplan Meier curves for OS for the indicated tumor type (univariate analysis).

Study Highlights

- *GUCY2C* expression is higher in CRC vs EJC or GA
- *GUCY2C*-H tumors are characterized by an "Immune cold" TIME (low M1, high M2 macrophage infiltrate.
- In MSS CRC, high expression of GUCY2C was associated with improved OS.

Conclusions

- These results show that *GUCY2C* expression is associated with an immune cold microenvironment and could be an attractive target for immune activating therapeutics.

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