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Variation in FOLFOX, FOLFIRI, and FOLFOXIRI effects on CD8+ T cell and PDL1 levels in MSS CRC patients

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Abstract

Background: Colorectal cancer (CRC) is a heterogenous disease treated with FOLFOX, FOLFIRI, and FOLFOXIRI chemotherapy regimens. About 85% of CRC patients have microsatellite stable (MSS) tumors that resist immune checkpoint blockade (ICB). Some studies suggest that chemotherapy modulates the MSS CRC tumor microenvironment (TME) to enhance CD8+ T cell infiltration, but ICB remains ineffective. We evaluated the TME of MSS CRC patients to investigate chemotherapy impact on immune markers.

Methods: CRC patient samples (n = 16,827) underwent DNA (592-gene or whole exome)/RNA (whole transcriptome) sequencing at Caris Life Sciences. Immune cell fractions within TMEs were estimated from RNA deconvolution (quanTIseq; Finotello, 2019; n = 11,109). PDL1 expression was assessed by IHC (SP142; ≥2+/5%). Patients who received FOLFOX (n = 425), FOLFIRI (n = 88), or FOLFOXIRI (n = 19) < 1 year prior to tumor collection or who didn't receive these treatments > 4000 days before tumor collection (untreated, n = 6,608) were analyzed. Statistical significance was determined using chi-square, Fisher's exact, and Mann-Whitney U tests, where appropriate.

Results: FOLFOX-treated CRC (n = 213) had a higher CD8+ T cell fraction vs untreated (n = 3,449, FC = 1.9, p < 0.01) CRC and there was no difference among FOLFIRI-treated (n = 37, FC = 1.1, p = 0.13) or FOLFOXIRI-treated (n = 9, FC = 2.2, p = 0.49) CRC. Survival outcomes were similar in FOLFOX-treated CD8+ T cell-high (n = 82) vs low (n = 161, HR = 1.3, p = 0.12) and in FOLFOX-treated PDL1+ (n = 13) vs PDL1- (n = 338) CRC (HR = 1.6, p = 0.14). The CD8+ T cell fraction was similar in untreated KRAS wild-type (WT, n = 1714) vs untreated KRAS-mutated (mut, n = 1718) CRC (FC = 1.3, p < 0.01). FOLFOX-treated KRAS-mut CRC had a higher CD8+ T cell fraction (n = 1718 untreated, 96 treated, FC = 2.3, p < 0.01) and CD69 expression (n = 1724 untreated, 97 treated, FC = 1.7, p < 0.01) vs untreated KRAS-mut CRC. This FOLFOX-dependent effect on CD8+ T cells was lower in WT KRAS CRC (n = 114 FOLFOX-treated, n = 1714 untreated, FC = 1.6, p < 0.01) but survival outcomes were similar in FOLFOX-treated WT (n = 210) vs KRAS-mut (n = 210) CRC (HR = 1.0, p = 0.82). The CD8+ T cell fraction was similar in untreated right- (R; n = 803) vs left-sided CRC (L; n = 927, FC = 1.3, p < 0.01) but PDL1+ IHC rates were significantly higher in R-CRC (n = 1340 L, 1124 R, FC = 2.2, p < 0.01). The CD8+ T cell fraction was similar in untreated WT (n = 3144) vs BRAF-mut CRC (n = 248, FC = 0.8, p < 0.01), but BRAF-mut were more frequently PDL1+ (n = 4622 WT, 340 mut, FC = 4.2, p < 0.01). PDL1+ rates were similar in untreated (n = 1124) vs FOLFIRI-treated R-CRC (n = 12, FC = 2.0, p = 0.46) but were higher in FOLFIRI-treated (n = 9) vs untreated (n = 1340) L-CRC (n = 9, FC = 6.0, p = 0.04).

Conclusions: We describe an association between FOLFOX and subsequent infiltration by CD8+ T-cells and high PDL1+ rates in R-CRC, BRAF-mut CRC, and FOLFIRI-treated L-CRC. The findings provide insights for future therapeutic strategies.

Results

CRC patient cohort design

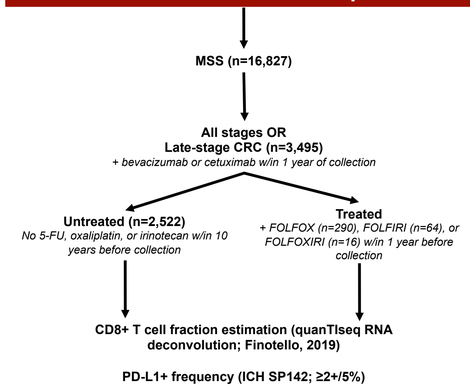
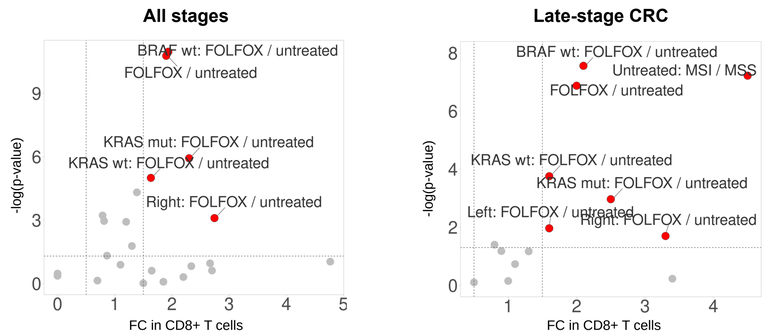


Figure 1. MSS tumors were selected from a cohort of patients with colorectal cancer with samples in the Caris Life Sciences CODEai database. A cohort of patients with late-stage disease was estimated by selecting for treatment with bevacizumab or cetuximab within 1 year of collection. Patients were further divided into untreated or treated cohorts. Levels of tumor-infiltrating CD8+ T cells and PD-L1+ frequency were analyzed in each cohort.

FOLFOX is associated with CD8+ T cell infiltration in MSS CRC
Stronger association in KRAS-mutant, BRAF-wt, and right-sided tumors

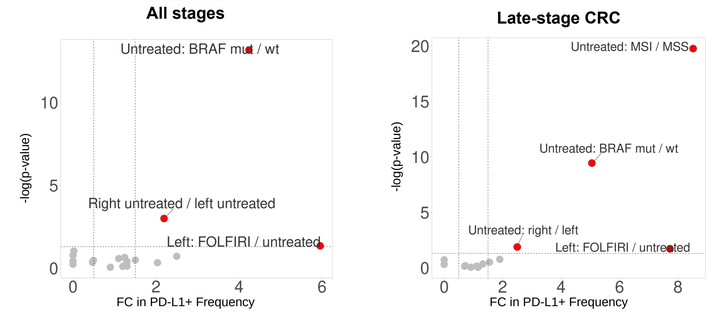


Treatment	N	%CD8+ T cells
Right: FOLFOX / untreated	33 / 803	1% / 0.5%
KRAS mut: FOLFOX / untreated	96 / 1718	0.9 % / 0.4%
BRAF wt: FOLFOX / untreated	190 / 3144	0.9% / 0.4%
FOLFOX / untreated	213 / 3449	0.8% / 0.4%
KRAS wt: FOLFOX / untreated	114 / 1714	0.8% / 0.5%
CMS4: FOLFOX / untreated	106 / 1223	0.8% / 0.6%
Left: FOLFOX / untreated	44 / 927	0.5% / 0.4%
Untreated: KRAS wt / mut	1714 / 1718	0.5% / 0.4%
KRAS wt: FOLFIRI / untreated	20 / 1714	0.4% / 0.5%
Untreated: BRAF mut / wt	248 / 3144	0.5% / 0.4%
Left untreated / right untreated	927 / 803	0.4% / 0.5%

Treatment	N	%CD8+ T cells
Untreated: MSI / MSS	33 / 1365	2% / 0.4%
Right: FOLFOX / untreated	26 / 347	1% / 0.4%
KRAS mut: FOLFOX / untreated	66 / 716	1% / 0.4%
BRAF wt: FOLFOX / untreated	129 / 1222	0.9% / 0.4%
FOLFOX / untreated	146 / 1365	0.9% / 0.4%
Left: FOLFOX / untreated	29 / 361	0.6% / 0.3%
KRAS wt: FOLFOX / untreated	77 / 643	0.8% / 0.5%

Figure 2. Levels of tumor-infiltrating CD8+ T cells were compared across various patient cohorts. Comparisons with significant differences (0.5>FC>1.5; p-val<0.05) are shown in red. The tables below each graph list the number of patients in each cohort comparison and absolute differences in CD8+T cell percentage in the tumor. FC, fold change.

Chemotherapy is not associated with changes in PD-L1+ rate
High PD-L1+ rates in BRAF-mutant, R-CRC, and FOLFIRI-treated L-CRC



Treatment	N	PD-L1+ Frequency
Left: FOLFIRI / untreated	9 / 1340	11% / 2%
Untreated: BRAF mut / wt	340 / 4622	9% / 2%
Right untreated / left untreated	1340 / 1124	4% / 2%

Treatment	N	PD-L1+ Frequency
Untreated: MSI / MSS	63 / 1912	25% / 3%
Left: FOLFIRI / untreated	6 / 510	17% / 0.2%
Untreated: BRAF mut / wt	153 / 1728	11% / 2%
Untreated: right / left	471 / 510	5% / 2%

Figure 3. Levels of PD-L1+ tumors (assessed by IHC SP142; ≥2+/5%) were compared across various patient cohorts. Comparisons with significant differences (0.5>FC>1.5; p-val<0.05) are shown in red. The tables below each graph list the number of patients in each cohort comparison and absolute differences in PD-L1+ frequency. L-CRC, left-sided colorectal cancer.

High CD8+ T cell infiltration, PD-L1 expression do not predict survival in FOLFOX-treated MSS CRC

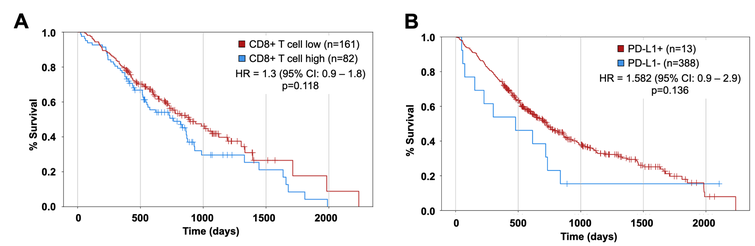


Figure 4. A) FOLFOX-treated patients with MSS CRC were divided into CD8+ T cell low (25th percentile) or high (75th percentile) cohorts based on quanTIseq RNA deconvolution. B) FOLFOX-treated patients were divided into PD-L1+ or PD-L1- cohorts based on IHC SP142; ≥2+/5%.

Conclusions

- **Immunotherapy remains ineffective for patients with MSS CRC.**
- There is an association between FOLFOX and high tumor infiltrating CD8+ T cell levels.
- CD8+ T cell levels and PD-L1 expression do not predict survival in FOLFOX-treated patients.
- There is an association between high PDL1+ rates and R-CRC, BRAF-mut CRC, and FOLFIRI-treated L-CRC.

Future Directions

We are currently investigating:

- Various chemotherapy + immunotherapy treatment schedules *in* vivo to identify optimal strategies for patients with MSS CRC.
- Immune cell activation state along the course of chemotherapy treatment in patients with MSS CRC.