

# Correlation of tumor mutational burden and treatment outcomes in patients with colorectal cancer

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**Background:** The Cancer Genome Atlas (TCGA) showed that 16% of colorectal cancers (CRCs) display DNA repair mechanisms and high tumor mutational burden (TMB). Although, there is accumulating evidence of greater benefit of immunotherapy in tumors with high-TMB, its impact on response to chemotherapy is unknown.

**Methods:** In this retrospective cohort study, we investigated the impact of TMB on progression-free survival (PFS) of CRC patients treated at tertiary care oncology clinics who had their tumors profiled by next-generation sequencing (NGS). Low TMB (TMB-L) and intermediate/high TMB (TMB-I/H) were defined as  $\leq 5$  mutations per base (MB) or  $\geq 6$  MB, respectively.

**Results:** Seventy-four CRC patients (61 colon and 13 rectal cancers) were identified from the database. In the TMB-L cohort, irinotecan-based chemotherapy treated patients had improved PFS compared to oxaliplatin-based chemotherapy treated patients (11.9 vs. 6.5 months,  $P < 0.001$ ). No difference in PFS was observed between the two treatment cohorts in TMB-I/H group. There was also no difference in time to recurrence in the TMB-L and TMB-I/H arms in patients treated with oxaliplatin-based therapy in perioperative setting.

**Conclusions:** TMB-L may be a predictive biomarker in a subset of CRC patients treated with chemotherapy but these results need to be confirmed in larger studies.

**Keywords:** Chemotherapy; oxaliplatin; colorectal cancer (CRC); next-generation sequencing (NGS); irinotecan; tumor mutational burden (TMB)

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## Introduction

Colorectal cancer (CRC) is the third most common cancer diagnosed in both men and women in the United States and approximately 134,490 new cases will be diagnosed in 2016

alone (1). The Cancer Genome Atlas (TCGA) identified 16% of CRCs to have defects in mechanisms that repair spontaneous DNA damage, and consequently these tumors have increased mutational burden (2). While three quarters are due to mismatch repair defect (MMR-D) phenotype,

otherwise known as high-frequency microsatellite instability (MSI-H) CRC, the remaining 25% consist of somatic mutations in one or more DNA repair genes such as *POLE*, *BRAF*, *PIK3CA* and *PTEN* (2,3).

Recent evidence showing improved responses to anti-PD-1 antibodies in MSI-H CRC has renewed the interest in its detection (4). Traditionally detected using immunohistochemistry or polymerase chain reaction (PCR), it is now possible to predict MSI-H CRC phenotype using mutational load extracted from next generation sequencing (NGS) (5,6). NGS can detect MSI-H CRC with concordance rates as high as 97% compared to standard testing (7).

Studies using MSI-H as a biomarker for chemotherapy response have yielded inconsistent results. A pivotal clinical trial using MSI-H as a biomarker showed that 5-fluorouracil (5-FU) adjuvant chemotherapy was not beneficial in decreasing colon cancer recurrence in MSI-H CRC (8). However, MSI-H tumors were shown to be sensitive to irinotecan chemotherapy with response in 57.1% (4 of 7) compared to only 10.8% (7 of 65) with microsatellite stable (MSS) CRC ( $P=0.009$ ) (9). A trend toward longer DFS was also observed with the combination of irinotecan, 5-FU and leucovorin (LV) on MSI-H tumors as compared with those receiving 5-FU/LV (HR =0.57; 95% CI, 0.42–0.71 *vs.* HR =0.76; 95% CI, 0.64–0.88;  $P=0.07$ ) (10). Another retrospective study of CRC patients treated with irinotecan based regimen in first line metastatic setting showed median progression-free survival (PFS) difference of 8.85 *vs.* 6.82 months in MSI-H and MSS respectively, but this difference did not reach statistical significance ( $P=0.089$ ) (11).

CRC with unusually high mutational load (>150) may be attributed to a hypermutating phenotype such as the P286R hotspot *POLE* mutation (12). While such isolated mutations occur rarely, other DNA repair defects may cause tumors to have a higher mutation burden. Our recent study exploring the TCGA database showed that CRC cases carrying mutations in one of the 13 most frequently mutated DNA repair genes (*ATM*, *MRCA2*, *MSH6*, *MLH1*, *LIG1*, *POLE*, *BRCA1*, *MSH2*, *SLX4*, *FANCM* and *FANCD2*) exhibited higher mutational burden (13). While there is accumulating evidence suggesting that tumors with high mutational burden may respond better to checkpoint inhibition (4,14), the relationship between tumor mutational burden (TMB) status and response to chemotherapy is unknown. In this retrospective study, we investigated if TMB was associated with treatment response to chemotherapy administered to

CRC patients. Specifically, the research project aimed to address the following questions: (I) Is there a difference in PFS between low TMB (TMB-L) and intermediate/high TMB (TMB-I/H) patients treated with chemotherapy in first line metastatic setting? (II) Do TMB-L and TMB-I/H patients respond differently to oxaliplatin and irinotecan? (III) Is there an association between time to recurrence and TMB status in stage 2 and 3 CRC patients who received perioperative oxaliplatin?

## Methods

### *Patient identification*

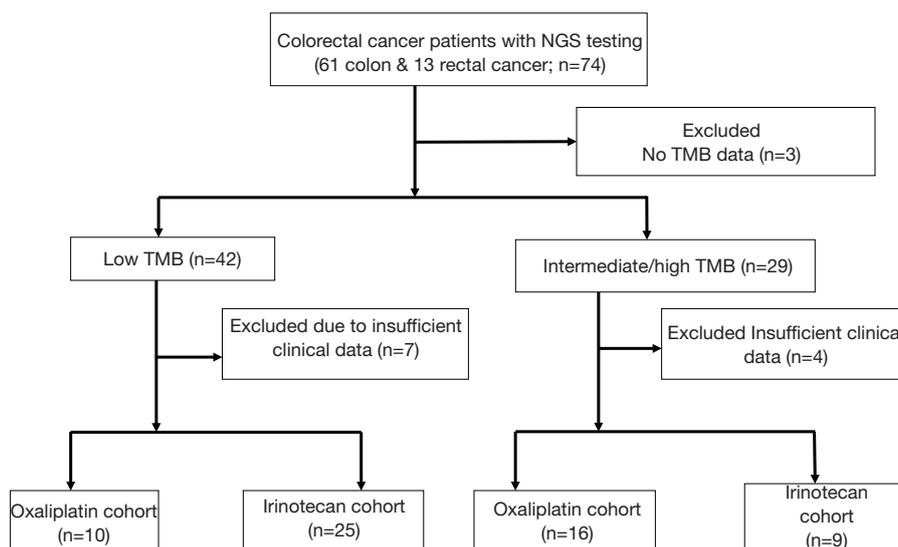
Following IRB approval, CRC patients treated at Northwestern University oncology clinics between June 1, 2013 through May 31, 2016 who had their tumors submitted for NGS with FoundationOne® (Foundation Medicine Inc., Cambridge, MA, USA) were identified. The most recent tumor samples available in the repository were sent for NGS during routine clinical care of the patients.

### *Procedures*

Demographic (date of birth, sex, race, date of diagnosis) and clinical information (including imaging results and assessment of response to treatment, chemotherapy treatment history) were obtained by chart review. Treatment in the first-line metastatic setting was captured as ‘oxaliplatin-based’ if the chemotherapy regimen contained oxaliplatin (such as FOLFOX or XELOX) or ‘irinotecan-based’ such as FOLFIRI. No patients received oxaliplatin and irinotecan concomitantly. Dose modifications for oxaliplatin or irinotecan, or omission of the drug due to toxicity were not captured. Details of adjuvant chemotherapy received were noted. Patients were followed longitudinally until the progression of the cancer by periodic imaging (CT or MRI) as per the treating physician.

### *Extraction of DNA and NGS*

At least 50 ng DNA was extracted from archival formalin-fixed, paraffin embedded tumor tissue and mutational data obtained by using hybridization capture of 3,769 exons of 315 cancer-related genes plus introns from 28 genes commonly rearranged in cancer (15,16). All classes of genomic alterations (base substitutions, small insertions and deletions (INDELS), rearrangements, copy number



**Figure 1** Consort diagram showing different cohorts. TMB, tumor mutation burden; NGS, next generation sequencing.

alterations) were determined. TMB was calculated by counting all synonymous and nonsynonymous variants as well as indels across a 1.25 megabase coding region spanning 315 genes. Germline polymorphisms were filtered using a proprietary somatic/germline algorithm (SGZ) and by comparing dbSNP, ExAC as well as internal FMI databases. Due to the high mutation rate associated with oncogenes and tumor suppressors, these genes were removed to limit biasing in the calculation of TMB (17). TMB-L and TMB-I/H were defined as  $\leq 5$  mutations per base (MB) or  $\geq 6$ /MB respectively. MSI-status was detected using a validated novel computational method developed by Foundation Medicine Inc. (7).

### Statistical analysis

Continuous variables were reported as medians and inter-quartile ranges and the two cohorts TMB-L ( $\leq 5$ /mbp) or TMB-I/H ( $\geq 6$ /mbp) compared between groups via the Wilcoxon rank-sum test. Categorical variables were reported as frequencies and percentages and compared between groups via Fisher's exact test. PFS was defined as time from date of start of first line chemotherapy to the date of progression noted on imaging. Survival estimates were compared between TMB-L and TMB-I/H groups via the log-rank test. Hazard ratios were obtained via Cox regression. All analyses were conducted using SAS (SAS Institute Inc., Cary, NC, USA, Version 9.4).

### Results

Seventy-four patients with CRC whose tumors were submitted to NGS testing were identified; 61 patients had colon cancer and 13 rectal cancer. Fourteen patients were excluded from analysis due to incomplete data (3 with no TMB data; 11 with insufficient clinical data) (Figure 1).

#### Impact of TMB status on PFS in metastatic CRC

When outcomes of TMB-L ( $n=39$ ) and TMB-I/H ( $n=26$ ) were compared, without considering the nature of chemotherapy received in first line metastatic setting, a trend towards improved PFS was observed in the TMB-I/H compared to TMB-L (9.9 vs. 5.8 months,  $P=0.18$ ) (Figure 2).

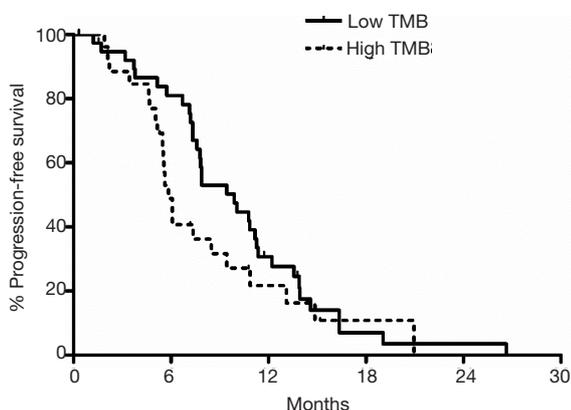
On subset analysis of TMB-L, patients in irinotecan cohort had improved PFS (11.7 months,  $n=25$ ) compared to oxaliplatin (6.4 months,  $n=10$ ), which was statistically significant ( $P=0.0002$ ; HR =0.22; 95% CI, 0.09–0.52) (Figure 3A). However, oxaliplatin cohort had more stage 4 patients (60% vs. 44%) and a higher median age (57.1 vs. 46.4 years at initial diagnosis,  $P=0.34$ ; 61.3 vs. 50.4 years at metastatic disease,  $P=0.24$ ) compared to irinotecan cohort (44%) (Table 1). Majority of the patients in irinotecan cohort had received prior oxaliplatin-based therapy in adjuvant setting (95%). There was no significant difference in the molecular characteristics in terms of MSI-H status, median number of mutations, KRAS, NRAS and BRAF

status between the two cohorts.

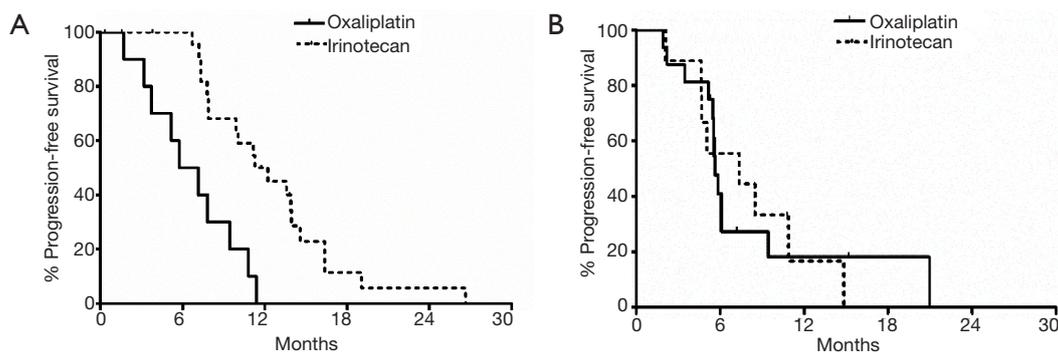
There was no difference in PFS between the irinotecan and oxaliplatin cohorts in TMB-I/H group, where the clinical and molecular characteristics were comparable between the two cohorts (Table 2) (Figure 3B).

### TMB status and CRC recurrence after perioperative oxaliplatin based regimen

Twenty-nine patients had been diagnosed with stage 2



**Figure 2** Progression free survival of patients treated with chemotherapy in first line metastatic setting comparing low TMB (solid line) vs. intermediate/high TMB (dashed line) cohort: there is a trend towards improved progression free survival in TMB-L (n=39) compared to TMB-I/H (n=26) (9.9 vs. 5.8 months), however did not reach statistical significance (P=0.18). TMB-L, low TMB; TMB-I/H, intermediate/high TMB.



**Figure 3** Progression free survival (PFS) comparing oxaliplatin (solid line) vs. irinotecan (dashed line) based regimen in 1st line metastatic setting. (A) Low TMB cohort: patients on irinotecan-based regimen have improved progression free survival in low TMB cohort: 6.4 months (oxaliplatin, n=10) versus 11.7 months (irinotecan, n=25), P=0.0002; (B) there was no difference in PFS in intermediate/high TMB cohort: 5.6 months (oxaliplatin, n=16) versus 7.4 months (irinotecan, n=9), P=0.99.

and 3 CRC and received perioperative chemotherapy. All patients were treated with oxaliplatin-based chemotherapy in perioperative setting. There was no difference in time to recurrence in the TMB-L and TMB-I/H groups in these patients (Figure 4).

### Discussion

Our results suggest that a TMB obtained from NGS tumor profiling panel based on the numeric quantitation of the mutation load can serve as a predictive biomarker for tumor response to chemotherapy. Patients with TMB-L seemed to have improved PFS when treated with irinotecan-based chemotherapy compared to oxaliplatin.

Population level genetics show better prognosis in MSI-H phenotype, a tumor traditionally known to have high TMB (18,19). Patients with TMB-I/H in our study tended to have worse outcomes, with decreased PFS, although statistically not significant. This may partly be explained by sampling bias as NGS was obtained retrospectively in only those MSI-H tumor patients who progressed to metastatic CRC. This may inadvertently have selected the aggressive phenotype of otherwise less aggressive MSI-H phenotype.

Other limitations include, biopsies for NGS were obtained as warranted for the clinical care of the patients and the most recent biopsy available were sent, hence we cannot ensure uniformity in the timing of biopsy. The dose intensity received and concurrent administration of biologics was not captured. This is a small data set of patients from a single institution in USA and findings

**Table 1** Characteristics of colorectal cancer patients with low TMB ( $\leq 5$ /mbp)

Characteristics	Oxaliplatin cohort, n=10	Irinotecan Cohort, n=25	P value
Age (median, IQR), years			
At diagnosis	57.1 (38.8, 61.2)	46.4 (38.7, 57.2)	0.34
At metastasis	61.3 (38.8, 64.9)	50.4 (40.1, 58.6)	0.24
Difference	1.0 (0.1, 2.3)	1.3 (0.9, 2.0)	0.41
Sex			0.46
Male	7 (70.0%)	13 (52.0%)	
Female	3 (30.0%)	12 (48.0%)	
Race			0.16
White	2 (20.0%)	13 (52.0%)	
Black	0 (0.0%)	1 (4.0%)	
Others	8 (80.0%)	11 (44.0%)	
Site			0.64
Colon cancer	9 (90.0%)	19 (76.0%)	
Rectal cancer	1 (10.0%)	6 (24.0%)	
Stage at diagnosis			0.03
Stage 2	2 (20.0%)	0 (0.0%)	
Stage 3	2 (20.0%)	14 (56.0%)	
Stage 4	6 (60.0%)	11 (44.0%)	
Treatment characteristics			
Prior oxaliplatin based chemotherapy	3 (50.0%)	21 (95.5%)	0.02
Duration of chemotherapy, months	7.9 (6.1, 10.4)	5.3 (5.0, 6.1)	0.04
Time to recurrence from last chemo, months	8.8 (2.8, 14.9)	5.9 (3.9, 16.0)	0.88
Adjunct therapy			0.99
Bevacizumab	7 (100.0%)	18 (90.0%)	
Cetuximab	0 (0.0%)	2 (10.0%)	
Biopsy characteristics			
Site of biopsy			0.99
Colon	5 (50.0%)	12 (48.0%)	
Liver	3 (30.0%)	7 (28.0%)	
Lung	1 (10.0%)	4 (16.0%)	
Lymph node	0 (0.0%)	0 (0.0%)	
Other	1 (10.0%)	2 (8.0%)	
Timing of biopsy			0.88
At Initial diagnosis	3 (30.0%)	9 (36.0%)	
Post neo-adjuvant therapy	1 (10.0%)	4 (16.0%)	
At recurrence	6 (60.0%)	12 (48.0%)	

**Table 1** (continued)

**Table 1** (continued)

Characteristics	Oxaliplatin cohort, n=10	Irinotecan Cohort, n=25	P value
MSI-status			0.26
Not performed	5 (50.0%)	7 (28.0%)	
MS stable	5 (50.0%)	18 (72.0%)	
Total number of mutations reported	1 (0, 1)	1 (0, 1)	0.95
<i>KRAS</i> mutation status			0.44
Wild	5 (50.0%)	8 (32.0%)	
Mutant	5 (50.0%)	17 (68.0%)	
<i>BRAF</i> mutation status			0.26
Wild	8 (88.9%)	25 (100%)	
Mutant	1 (11.1%)	0 (0.0%)	
<i>NRAS</i> mutation status			0.24
Wild	6 (85.7%)	22 (100%)	
Mutant	1 (14.3%)	0 (0.0%)	

TMB, tumor mutation burden; mbp, mega base pairs; IQR, interquartile range; MSI, microsatellite instability.

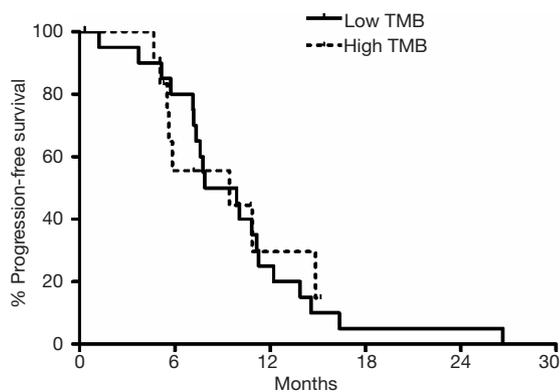
**Table 2** Characteristics of colorectal cancer patients with intermediate and high TMB. Patients with intermediate/high TMB ( $\geq 6$ /mbp)

Characteristics	Oxaliplatin cohort, n=16	Irinotecan cohort, n=9	P value
Age (median, IQR), years			
At diagnosis	58.7 (49.1, 63.8)	54.7 (51.9, 67.9)	0.73
At metastasis	63.0 (52.5, 65.4)	57.0 (56.0, 69.6)	0.87
Difference	1.1 (0.2, 3.8)	1.5 (0.9, 1.7)	0.87
Sex			0.19
Male	9 (60.0%)	8 (88.9%)	
Female	6 (40.0%)	1 (11.1%)	
Race			0.99
White	8 (53.3%)	5 (55.6%)	
Black	2 (13.3%)	1 (11.1%)	
Others	5 (33.3%)	3 (33.3%)	
Site			0.99
Colon cancer	15 (93.8%)	8 (88.9%)	
Rectal cancer	1 (6.2%)	1 (11.1%)	
Stage at diagnosis			0.44
Stage II	2 (13.3%)	0 (0.0%)	
Stage III	5 (33.3%)	5 (62.5%)	
Stage IV	8 (53.3%)	3 (37.5%)	

**Table 2** (continued)

Table 2 (continued)

Characteristics	Oxaliplatin cohort, n=16	Irinotecan cohort, n=9	P value
Treatment characteristics			
Prior oxaliplatin based chemotherapy	6 (75.0%)	8 (100%)	0.47
Duration of chemotherapy, months	5.5 (5.0, 6.1)	5.6 (5.4, 6.0)	0.56
Time to recurrence from last chemotherapy, months	10.4 (3.5, 21.1)	10.0 (8.0, 11.0)	0.49
Adjuvant therapy			0.06
Bevacizumab	12 (100.0%)	6 (66.7%)	
Cetuximab	0 (0.0%)	3 (33.3%)	
Biopsy characteristics			
Site of biopsy			0.99
Colon	8 (50.0%)	4 (50.0%)	
Liver	4 (25.0%)	3 (37.5%)	
Lung	1 (6.25%)	0 (0.0%)	
Lymph node	1 (6.25%)	0 (0.0%)	
Other	2 (12.5%)	1 (12.5%)	
Timing of biopsy			0.99
At Initial diagnosis	8 (50.0%)	3 (37.5%)	
Post neo-adjuvant therapy	1 (6.25%)	1 (12.5%)	
At recurrence	7 (43.75%)	4 (50.0%)	
MSI-status			0.28
Not performed	5 (31.2%)	4 (44.4%)	
MS stable	11 (68.8%)	4 (44.4%)	
MSI-high	0 (0.0%)	1 (11.11%)	
Median TMB	8 (7, 9)	10 (8, 11)	0.18
KRAS mutation status			0.99
Wild	8 (50.0%)	4 (50.0%)	
Mutant	8 (50.0%)	4 (50.0%)	
BRAF mutation status			0.56
Wild	14 (87.5%)	5 (71.4%)	
Mutant	2 (12.5%)	2 (28.6%)	
NRAS mutation status			NA
Wild	12 (100%)	6 (100%)	
Mutant	0 (0.0%)	0 (0.0%)	



**Figure 4** Time to recurrence of colorectal cancer patients with stage 2 and 3 who underwent adjuvant chemotherapy with oxaliplatin based regimen comparing TMB-L (n=20, solid line) versus TMB-I/H (n=9, dashed line). There was no difference in PFS (9.9 vs. 10.9 months, P=0.64).

may not be generalizable. Our study did not analyze the origin of the cancer, which could have been of prognostic importance based on recent meta-analysis suggesting that left colon tumors were associated with reduced risk of death (HR =0.82; 95% CI, 0.79–0.84; P<0.001) (20). Further studies are needed corroborate these findings in a larger cohort, and we recommend investigating TMB on a tissue repository from clinical trials comparing irinotecan-based versus oxaliplatin-based regimens.

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### Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

*Ethical statement:* This study was approved by the Northwestern University Institutional review board of STU00200761. This approval exempted us from obtaining informed consent as this was a retrospective tissue study.

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