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Molecular Profiling Use in Patients with Resectable *versus* Unresectable Metastatic Colorectal Cancer

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Abstract

Background: The effect of molecular profiling on clinical treatment practices in metastatic colorectal cancer remains to be elucidated.

Materials and Methods: Retrospective cohort study using a tertiary referral cancer hospital clinical database (March 2006 to August 2016). Metastatic colorectal cancer patients who underwent molecular profiling were identified and divided into 2 groups: Primary Resectable *vs*. Primary Unresectable. Tumor molecular profile data, clinical treatments rendered and overall survival were analyzed and compared.

Results: Median time from diagnosis of metastatic disease to molecular profiling was 7.7 months in the Primary Resectable group *vs*. 2 months in the Primary Unresectable group. Two or three mutations were detected in 58% of patients. KRAS was the most common actionable mutation. Among the KRAS mutated patients with known treatment, 59% received bevacizumab. KRAS wild type was present in 52% of patients, and 19% of this group received cetuximab or panitumumab. BRAF was mutated in 5% of our cohort, and a targeted therapy was used in 5% of this group. Median time from initial cancer diagnosis to molecular profiling was 12.2 months overall, 29.9 months in the Primary Resectable Group, and 2 months in the Primary Unresectable Group ($p<0.001$). Median survival time was 4.7 years overall, 5.7 years in the Primary Resectable Group, and 2.7 years in the Primary Unresectable group ($p<0.001$).

Conclusion: There was use of a targeted therapy based on KRAS mutations identified by molecular profiling. Further research is necessary to correlate molecular profiling data, targeted therapy use and clinical trial accrual with survival outcomes in metastatic colorectal cancer patients.

Keywords: Molecular profiling; Colorectal cancer; Targeted therapy

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Introduction

Colorectal Cancer (CRC) is the third most commonly diagnosed cancer and the third leading cause of cancer death in the United States in both men and women [1]. According to the 2017 Surveillance, Epidemiology, and End Results (SEER) program data, at initial diagnosis, 36% of patients with CRC have locally advanced disease with spread to regional lymph nodes, and 23% of patients have synchronous distant metastatic disease [2]. Metastatic Colorectal Cancer (mCRC) carries a poor prognosis, with an overall 5-year survival of only 13% [2,3].

Molecular Profiling (MP) is the broad term used to describe technologies that measure the expression of multiple genes in a single tissue sample. Next Generation Sequencing (NGS) is a subset of MP that involves high-throughput technology that is able to rapidly detect single nucleotide variants and other mutations in DNA [4]. Gene mutations identified by MP are being used more commonly to guide therapy for CRC patients that have distant metastatic disease, with emphasis being placed on detecting alterations in KRAS and BRAF. Theoretically, the results of MP may also allow clinicians to assess tumors for the use of novel, approved and experimental therapies in clinical trials to personalize or target treatment.

The Epidermal Growth Factor Receptor (EGFR) is a therapeutic target in mCRC due to its role in activating signaling pathways involved in cellular proliferation [5,6]. KRAS encodes a protein that acts as a signal transducer for EGFR [5]. Oncogenic mutations in KRAS are found in 30% to 40% of mCRC patients and it has been shown to result in significant clinical ramifications [5]. Studies show that patients with certain mutations in KRAS are resistant to EGFR directed therapy

and have an overall poor prognosis [5-9]. Furthermore, studies have indicated that a mutation in BRAF is also associated with resistance to anti EGFR therapies, as well as an overall poorer prognosis in CRC [10,11]. Conversely, it has been demonstrated that KRAS Wild Type (WT) mCRC patients have a clinical benefit with the use of EGFR targeted therapies. Anti-EGFR agents such as panitumumab and cetuximab lead to disease stabilization and response rates of 30% and 10% respectively in these patients [5,7].

Vascular Epidermal Growth Factor (VEGF) is another therapeutic target in mCRC, as its overexpression is thought to catalyze the angiogenesis and subsequent proliferation of cancer cells [12]. The anti-VEGF agent bevacizumab is approved for use in mCRC, and studies have shown improvement in outcomes when used in combination with 5F-U or capecitabine regimens [9].

Currently, there are no standardized guidelines regarding the use of MP panels in CRC. Although MP is primarily utilized in the metastatic setting, specifics regarding patient selection, optimal timing of testing, interpretation of results, and use of the data to choose therapeutic options are left to the discretion of the ordering clinician. The aim was to explore the use of MP in a cohort of mCRC patients consisting of a Primary Resectable group (PR) and a Primary Unresectable group (PU).

Materials and Methods

Approval was obtained from an institutional review board. A retrospective review of the institutional clinical database was performed. The database identified 252 patients with mCRC that had MP between 2006 and 2016 as part of their care. Thirty-seven patients did not have clear MP data and were therefore excluded. The remaining 215 patients were divided into two groups. The Primary Resectable group (PR), consisted of patients whose disease was resectable at presentation, and the Primary Unresectable group (PU) consisted of patients whose disease was unresectable. Data collected included demographics, MP, systemic treatments rendered, and clinical trial accrual.

The Primary resectable group (PR)

Patients in the PR group presented with varying stages of disease, had R0 resection of all disease (with R0 metastasectomy if stage 4), but all later had a metastatic recurrence.

The Primary unresectable group (PU)

Patients in the PU group had synchronous metastatic disease at initial presentation, but were not surgical candidates based on multidisciplinary gastrointestinal tumor board discussions.

Molecular profiling panels and tissue analysis

Tissue samples were submitted for analysis using one or a combination of three panels. The Fox Chase Targeted Panel (FCTP) is an in-house MP panel which tests for 50 of the most commonly mutated genes in abdominal cancers. Specifically, the FCTP utilized The Ion AmpliSeq™ Hotspot Cancer Panel sequencing system, using Ion Torrent™ technology on the Ion Personal Genome Machine™. Formalin fixed paraffin embedded specimens were used. Twenty percent tumor content was required, and the analytical sensitivity equated to 10% variant allele frequency in variant detection. Metastatic lesions were used if available and appropriate for testing. Primary tumor biopsies or surgical resections were used for testing if metastatic lesions were not available. Pre-analytical assessment, analytical testing methods and post-analytical reporting at our institution did not change significantly throughout the study period.

Table 1: Patient Demographics & Clinical Characteristics.

Variable	Total	PR	PU	p
Patient #	215	93	122	-
Age at dx, y, median, (range)	60, (27-90)	57, (30-84)	62, (27-90)	0.065
Gender, n (%)				0.098
Male	125 (58)	60 (65)	65 (53)	
Female	90 (42)	33 (35)	57 (47)	
Ethnicity, n (%)				0.269
Caucasian	172 (80)	76 (82)	96 (79)	
Black / African American	26 (12)	8 (9)	18 (15)	
Hispanic / Latino	4 (2)	1 (1)	3 (2)	
Asian	5 (2)	3 (3)	2 (2)	
Another race	5 (2)	2 (2)	3 (2)	
BMI, median, (range)	26.7, (17-59)	27.3 (17.3-59)	26.5, (17-47.2)	0.614
Smoking history, n (%)				0.76
Current smoker	18 (8)	8 (9)	10 (8)	
Former smoker	82 (38)	33 (36)	49 (41)	
Non-smoker	112 (53)	51 (55)	61 (51)	
Site of primary tumor, n (%)				0.143
Colon	150 (70)	60 (65)	90 (74)	
Rectum	65 (30)	33 (35)	32 (26)	
***Stage at initial dx,** n (%)				
I	7 (3)	7 (8)	-	-
II	25 (12)	25 (27)	-	-
III	38 (18)	38 (42)	-	-
IV	143 (67)	21 (23)	122 (100)	-
Site of distant metastasis (initial dx or recurrence), n (%)				
Liver				
Lung	139 (65)	55 (59)	84 (69)	0.14
Peritoneum	81 (38)	47 (51)	34 (28)	0.001
Non-regional LN	45 (21)	18 (19)	27 (22)	0.62
Ovaries	11 (5)	0 (0)	11 (9)	0.003
Another distant site	15 (7)	4 (4)	11 (9)	0.003
	34 (16)	21 (23)	13 (11)	0.018

*AJCC 6th edition
*2 pts in PR group had unknown stage at initial dx

Expanded MP panels used in this study were foundation one and caris molecular intelligence, which test for single nucleotide variants in a larger panel of genes. All panels in this study utilized Next Generation Sequencing (NGS) technology. The panel chosen for each patient was at the discretion of the ordering physician.

Statistical analysis

Statistics were calculated using chi-squared tests, fisher's exact tests, and wilcoxon tests. Missing data points were excluded from final calculations. Survival curves were generated using the Kaplan-Meier method, and survival trends were compared using the log-rank test.

Results

Clinical characteristics

Clinical characteristics are listed in Table 1. A total of 215 patients with mCRC were identified and retrospectively reviewed. There were 93 patients in the PR group and 122 patients in the PU group. The median age at diagnosis was 60 years (range 27-90). The majority of our cohort consisted of Caucasian (n=172) males (n=125) with colon adenocarcinoma (n=150). The median BMI was 26.7 and most patients were non-smokers (n=112).

In the PR group, the stages at initial diagnosis are as follows: Stage 1 (n=7), Stage 2 (n=25), Stage 3 (n=38), stage 4 (n=21). Data for the initial stage at diagnosis was missing for 2 patients in the PR group. Among the 21 patients in the PR group that presented with stage 4 diseases, 100% underwent R0 metastasectomy in addition to R0 resection of the primary tumor; seventeen patients had hepatic resections and 4 patients had lung resections. The most common surgical resections performed in the PR group are as follows: Segmental colon resection (n=55), low anterior resection (n=28), abdominoperineal resection (n=7), transanal excision (n=4), and total colectomy (n=2).

In the PU group, 33% patients had palliative surgical resection for an acute issue, most commonly for perforation or obstruction (n=40).

The median time from initial resection to metastatic recurrence in the PR group was 1.8 years (range =6 months to 14.4 years). The most common sites of metastatic recurrence in the PR group were liver (n=55), lung (n=47), peritoneum (n=18), bone (n=7), ovaries (n=4), brain (n=3), and adrenal (n=3). In the PU group, the most common sites of synchronous metastasis at initial presentation were liver (n=84), lung (n=34) and peritoneum (n=27).

Molecular profiling data

Molecular profiling panels are listed in Table 2. The majority of the cohort (n=177, 82%) was tested *via* FCTP (Table 2). Thirteen patients had selective testing at our institution using FCTP to assess only for alterations in KRAS, BRAF, or MSI. Six of those thirteen patients went on to receive an outside expanded MP panel, while the remaining seven patients had no further testing. Thirty-two patients were tested exclusively *via* caris molecular intelligence or foundation one. One patient was tested by both FCTP and an expanded MP panel.

The tissue source for molecular profiling data was captured for the PR and PU groups. In the PR group, patients had MP after R0 resection; 45 patients had the primary tumor profiled and 48 patients had a metastatic lesion profiled. In the PU group, 74 patients had the primary tumor profiled following endoscopic biopsy, 48 patients had a metastatic lesion profiled.

Table 2: Molecular Profiling Panels.

MP Panel	Cohort Total	PR	PU	p
FCTP	177 (82)	76 (82)	101 (83)	0.86
Selective MP at FCCC	13 (6)	6 (6)	7 (6)	0.99
Caris Molecular Intelligence	22 (10)	11 (12)	11 (9)	0.51
Foundation One	10 (5)	6 (6)	4 (3)	0.34

Table 3: Number of Mutations.

Total # of Mutations	Cohort Total	PR	PU	p
0 mutations	12 (6)	4 (4)	8 (7)	0.12
1 mutation	42 (20)	18 (19)	24 (20)	
2 mutations	66 (31)	26 (28)	40 (33)	
3 mutations	57 (27)	22 (24)	35 (29)	
4 mutations	26 (12)	15 (16)	11 (9)	
≥ 5 mutations	12 (6)	8 (9)	4 (3)	

Table 4: Most Common Mutations.

Most Common Mutations	Cohort Total	PR	PU	p
P53	129 (60)	60 (65)	69 (56)	0.2
APC	107 (50)	44 (48)	63 (52)	0.58
KRAS	102 (47)	45 (48)	57 (47)	0.8
SMAD4	26 (12)	14 (15)	12 (10)	0.23
PIK3CA	25 (12)	15 (16)	10 (8)	0.06
FBXW7	13 (6)	7 (8)	6 (5)	0.57
PTEN	12 (6)	3 (3)	9 (7)	0.24
BRAF	11 (5)	6 (7)	5 (4)	0.54
STK11	3 (1)	0 (0)	3 (2)	0.26
EGFR	3 (1)	1 (1)	2 (2)	0.99

**12 pts had no mutations; 4 PR and 8 PU

Table 5: Overall Systemic Chemotherapy Data.

Variable	Cohort Total	PR	PU	p
Systemic chemotherapy	191 (93)	79 (93)	112 (91)	0.91
Chemo + any biologic	132 (67)	56 (67)	76 (68)	0.86
Bevacizumab	113 (55)	44 (52)	69 (62)	0.17
Cetuximab or panitumumab	24 (12)	16 (19)	8 (7)	0.013

**Total patients with known systemic chemo data N=205
**Total patients with known biologic chemo data N=196
**Total patients with known bevacizumab data N=197

The total numbers of mutations for each group are listed in Table 3. Twelve patients (6%) did not have any detectable mutations (n=12.4% PR *vs.* 7% PU). 89% of patients had between 1 and 4 mutations (n=191, 87% PR *vs.* 90% PU). Within this group, 64% had 2 or 3 mutations (n=123, 52% PR *vs.* 61% PU). 6% of our cohort had 5 or more mutations (n=12, 9% PR *vs.* 3% PU). The overall number of mutations was similar between PR and PU patients (p=0.12).

The most common mutations for each group are listed in Table 4. The most common mutations overall were p53 (n=129), APC (n=107), and KRAS (n=102). The most common actionable mutations were: KRAS (48% PR *vs.* 47% PU, p=0.81), SMAD4 (5% PR *vs.* 10% PU, p=0.23), and PIK3CA (16% PR *vs.* 8% PU, p=0.07). BRAF was mutated in 11 patients (7% PR *vs.* 4% PU, p=0.54). EGFR was mutated in 3 patients (1% PR *vs.* 2% PU, p=0.99).

Clinical treatments rendered and correlation with mutation status

Systemic chemotherapy data is listed in Table 5. Systemic chemotherapy data was known for 205 patients, of which 93% received chemotherapy during their treatment (n=192, 93% PR *vs.* 91% PU, p=0.91). Data regarding the use of biologic agents was known for 196 patients, of which 69% received a biologic agent as part of their treatment (n=132, 67% PR *vs.* 68% PU, p=0.86). Among these patients, 55% received bevacizumab (n=113, 52% PR *vs.* 62% PU, p=0.17), and 12% received cetuximab or panitumumab (n=24, 19% PR *vs.* 7% PU, p=0.01).

Correlation between KRAS/BRAF status and use of targeted therapy is summarized in Table 6. Among the 93 KRAS mutant patients with known chemotherapy data, 4% of patients with mutated

Table 6: Correlation Between KRAS/BRAF Status and Targeted Chemotherapy.

Variable	Cohort Total	PR	PU	p
KRAS mutant on bevacizumab	55 (59)	22 (52)	33 (65)	0.23
KRAS mutant on EGFR-I	4 (4)	4 (10)	0 (0)	0.02
KRAS WT on bevacizumab	58 (56)	22 (51)	36 (59)	0.43
KRAS WT on EGFR-I	20 (19)	12 (28)	8 (13)	0.06
BRAF mutant on bevacizumab	4 (36)	3 (50)	1 (20)	0.55
BRAF mutant on EGFR-I	1 (9)	0 (0)	1 (20)	0.46

**KRAS mutant w/ known tx N=93
**KRAS WT w/ known tx N=104
**BRAF mutant w/ known tx N=11
**EGFR-I = cetuximab or panitumumab

Table 7: Correlation Between KRAS/BRAF Status and Clinical Trial Enrollment.

Variable	Cohort Total	PR	PU	p
Clinical Trial Enrollment	18 (9)	10 (11)	8 (7)	0.33
KRAS mutant on CT	12 (12)	7 (16)	5 (9)	0.36
KRAS WT on CT	6 (5)	3 (6)	3 (5)	0.99
BRAF mutant on CT	0 (0)	0 (0)	0 (0)	-

**KRAS mutant w/ known CT data N=101
**KRAS WT w/ known CT data N=110

KRAS received cetuximab or panitumumab. Chemotherapy data was known for 104 patients with KRAS Wild Type (WT). Among KRAS WT patients, 56% received bevacizumab (n=58, 51% PR *vs.* 59% PU, p=0.43) and 19% of patients received cetuximab or panitumumab (n=20, 28% PR *vs.* 13% PU, p=0.06). Among the 11 patients with mutated BRAF, 4 patients received bevacizumab as part of their systemic chemotherapy, 1 patient received cetuximab, and 6 patients did not receive any biologic agent.

Clinical trial enrollment is summarized in Table 7 and occurred for 9% of patients in our cohort (n=18, 11% PR *vs.* 7% PU, p=0.33). KRAS was mutated in 67% of patients enrolled in a trial (n=12). The clinical trials included a phase 1 study of mogamulizumab (an anti CC4 antibody), a phase 1 trial of an anti CEA antibody, a trial using panitumumab plus an investigational agent, a trial using a c-Met inhibitor, and two trials investigating different chemoradiation regimens (FOLFOX + bevacizumab *vs.* FOLFIRI + bevacizumab).

Clinical time intervals

The various clinical time intervals are summarized in Table 8. The overall median length of follow up was 1.8 years. There was a statistically significant difference in time from initial diagnosis to MP between the PR group and the PU group (29.9 mo *vs.* 2.1 mo, $p \leq 0.001$). There was also a statistically significant difference in time from diagnosis of metastatic disease to MP between the PR and PU groups (15.7 mo *vs.* 2 mo, p=0.001). Lastly, there was a statistically significant difference in time from initial diagnosis to death between the PR and PU groups (5.7 years *vs.* 2.7 years, $p \leq 0.001$).

Table 8: Clinical Time Intervals.

Median Time Interval	Overall	PR	PU	p
Initial diagnosis to MP, mo (yrs)	12.2 (1.01)	29.9 (2.49)	2.0 (0.17)	<0.001
R0 resection to metastatic recurrence, mo (yrs)	-	21.5 (1.79)	-	-
Metastatic recurrence to MP, mo	-	7.7	-	-
Metastatic recurrence to death, mo (yrs)	-	34.2 (2.9)	-	-
Diagnosis of metastatic disease to MP, mo	2.8	15.7	2	0.001
MP to death, mo	17.3	17.2	17.9	0.298
Initial diagnosis to death, mo (yrs)	56.0 (4.7)	68.7 (5.7)	32.8 (2.7)	<0.001

Discussion

The notion of targeted therapy and personalized medicine for CRC treatment has made MP increasingly popular. The existing literature on MP in CRC appears to emphasize the comparison between the various MP technologies, as well as, the turnaround time for results [13]. However, there are few studies detailing the clinical application of MP in CRC. This study explored the practice of MP among patients with CRC at a tertiary referral cancer center. There are no standardized guidelines regarding the use of MP in CRC, therefore we sought to analyze which patients were being selected for profiling, when profiling was being performed, which mutations were being identified, and how clinicians were using this data to guide therapy.

The cohort consisted exclusively of patients with mCRC, which is consistent with most of the MP literature that reports the use of MP in the metastatic setting [3,5-7,10,11,14,15]. Studies evaluating the timing of MP in the treatment course of mCRC patients are missing from the literature. This is the first study to our knowledge exploring this variable. Patients who were deemed resectable up front received MP significantly later in the treatment course when compared to those that were deemed unresectable (median time of 29.9 months *vs.* 2 months). This may reflect a focus on localized therapies and treatment options based on clinical guidelines in PR patients that may not necessitate early MP testing. Conversely, PU patients have may have received MP at an earlier point in their clinical course in hopes of identifying second or third line systemic chemotherapy options, or to accrue patients for clinical trials.

There has been no consensus to date regarding timing to incorporate EGFR-directed therapies for mCRC patients with wildtype KRAS and BRAF [15]. Furthermore, data detailing the clinical use of MP data regards to initiating targeted therapy is also missing from the literature. This may reflect the difficulty of capturing this data, as it requires reliance on precise and consistent documentation from clinicians during patient encounters.

Regarding the types of MP panels used, most of our cohort was tested via an in house 50-gene targeted panel, and conversely, expanded MP panel use was uncommon in our cohort. This was possibly secondary to ease of ordering an in-house test *vs.* a send out test. The current MP literature supports a turnover time of <7 working days for RAS testing [13]. The turnover time for receiving results of expanded MP panels such as foundation one and caris molecular intelligence was variable in our study. However, expanded panels failed to identify any novel targets for experimental therapies or clinical trials, as KRAS and BRAF status provided the most clinically useful information to ordering clinicians. As such, targeted panels may prove more useful and cost-effective in this patient population.

Clinical trials are typically employed late in a patient's clinical course after progression of disease despite trying several chemotherapy regimens. Overall, clinical trial enrollment was low for this cohort and seemed to be based primarily on the presence of KRAS or BRAF mutations. Based on the types of trials that patients in this cohort were enrolled in, there did not appear to be any trial based on any novel target discovered on a targeted MP panel or either of the expanded MP panels. Clinical trial accrual was based on patients who were refractory to second- and third-line therapies, but there were

no trials in our cohort that were conducted that gave any novel drugs based on MP data.

This study is the first to our knowledge to explore the practical clinical application of MP in CRC. However, this study has some limitations. The retrospective nature of data collection restricted the ability to determine rationales for certain clinical practices; including the administration of EGFR-inhibitor drugs to a small subset of KRAS mutated patients. Furthermore, it is unclear from review of the data why NRAS and MSI status were not routinely reported for all patients in the study. There is also an inherent selection bias, as only patients with mCRC either presenting initially with synchronous metastatic disease or with recurrent metachronous disease were selected for profiling.

Conclusion

KRAS status had the most influence on the choice of targeted chemotherapy and clinical trial accrual in mCRC. In the future, larger studies are needed to determine if MP data leads to the use of novel targeted therapies, as well as to correlate MP data to clinical trial enrollment. It remains to be seen whether earlier institution of MP in the metastatic setting could lead to prolonged survival. Furthermore, research is needed to determine whether there is a benefit in obtaining MP in all patients upfront regardless of stage as part of the standard care for CRC.

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