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Improved Pathologic response to chemoradiation in MGMT methylated locally advanced rectal cancer

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ABSTRACT

Background and Purpose: With the growing interest in total neoadjuvant treatment for locally advanced rectal adenocarcinoma (LARC) there is an urgent unmet need to identify predictive markers of response to long-course neoadjuvant concurrent chemoradiotherapy (LCRT). O6-Methylguanine (O6-MG)-DNA-methyltransferase (MGMT) gene methylation has been associated in some malignancies with response to concurrent chemoradiotherapy. We attempted to find if pathologic response to LCRT was associated with MGMT promoter hypermethylation (MGMTh).

Materials and Methods: Patients were identified with LARC, available pre-treatment biopsy specimens, and at least 1 year of follow-up who received LCRT followed by surgical resection within 6 months. Biopsies were tested for MGMTh using a Qiagen pyrosequencing kit (Catalog number 970061). The primary outcome of LCRT responsiveness was based on tumor regression grade (TRG), with grades of 0–1 considered to have excellent response and grades of 2–3 considered to be non-responders. Secondary outcomes included overall survival (OS) and recurrence free survival (RFS).

Results: Of 96 patients who met inclusion criteria, 76 had samples which produced reliable assay results. MGMTh corresponded with higher grade and age of the biopsy specimen. The percentage of responders to LCRT was higher amongst the MGMTh patients than the MGMTn patients (60.0% vs 27.5%, p value = 0.0061). MGMTh was not significantly associated with improved OS (2-year OS of 96.0% vs 98.0%, p = 0.8102) but there was a trend for improved RFS (2-year RFS of 87.6% vs 74.2%, p = 0.0903).

Conclusion: Significantly greater tumor regression following LCRT was seen in MGMTh LARC. Methylation status may help identify good candidates for close observation without surgery following LCRT.

Introduction

Pathological complete response (pCR) rates after long-course neoadjuvant chemoradiotherapy (LCRT) in locally advanced rectal cancer (LARC) has ranged from approximately 8–20%. To avoid the morbidity of surgery, a watch-and-wait (W&W) strategy is sometimes used for patients with an adequate clinical response. Complete clinical response rates of around 40% have been achieved using total neoadjuvant therapy (TNT) with pCR rates of up to 25% [1–5]. The results of the OPRA trial appeared to show organ preservation rates with W&W substantially higher than pCR rates, demonstrating the potential feasibility of W&W with TNT for select patients with a complete or near complete clinical

Abbreviations: pCR, Pathological complete response; LARC, locally advanced rectal cancer; LCRT, long-course neoadjuvant concurrent chemoradiotherapy; W&W, watch-and-wait; TNT, total neoadjuvant therapy; MGMT, O6-Methylguanine-DNA-methyltransferase; O6-AG, O6-alkylguanine; AGT, DNA alkyltransferase; RFS, recurrence free survival; OS, overall survival; 5-FU, Fluorouracil; TRG, tumor regression grade; UA, unsuccessful assay; SA, successful assay.

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Table 1
Patient and Treatment Characteristics by Success of Assay.

		All, n (%) or median (range)	Successful, n (%) or median (range)	Unsuccessful, n (%) or median (range)	P-value
Sex	Male	62 (64.6%)	49 (64.5%)	13 (65.0%)	0.9651
	Female	34 (35.4%)	27 (35.5%)	7 (35.0%)	
Race	White	72 (75.0%)	57 (75.0%)	15 (75.0%)	1.0000
	Hispanic	14 (14.6%)	11 (14.5%)	3 (15.0%)	
	Black	5 (5.2%)	4 (5.3%)	1 (5.0%)	
	Other	5 (5.2%)	4 (5.3%)	1 (5.0%)	
Clinical T-stage	T2	7 (7.3%)	5 (6.6%)	2 (10.0%)	0.6288
	T3	78 (81.3%)	63 (82.9%)	15 (75.0%)	
	T4	11 (11.5%)	8 (10.5%)	3 (15.0%)	
Clinical N-stage	N0	43 (44.8%)	34 (44.7%)	9 (45.0%)	0.9242
	N1	41 (42.7%)	32 (42.1%)	9 (45.0%)	
	N2	12 (12.5%)	10 (13.2%)	2 (10.0%)	
Clinical Group Stage	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.9330
	2	44 (45.8%)	35 (46.1%)	9 (45.0%)	
	3	52 (54.2%)	41 (54.0%)	11 (55.0%)	
	4	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Grade	1	5 (5.2%)	4 (5.3%)	1 (5.0%)	1.0000
	2	79 (82.3%)	62 (81.6%)	17 (85.0%)	
	3	12 (12.5%)	10 (13.2%)	2 (10.0%)	
Surgery	TAE	6 (6.3%)	6 (7.9%)	0 (0.0%)	0.5267
	LAR	46 (47.9%)	35 (46.1%)	11 (55.0%)	
	APR	44 (45.8%)	35 (46.1%)	9 (45.0%)	
Concurrent Chemotherapy	5-FU	77 (80.2%)	62 (81.6%)	15 (75.0%)	0.5350
	Capecitabine	19 (19.8%)	14 (18.4%)	5 (25.0%)	
Tumor Regression Grade	0	19 (19.8%)	16 (21.1%)	3 (15.0%)	0.8772
	1	16 (16.7%)	13 (17.1%)	3 (15.0%)	
	2/3	61 (63.5%)	47 (61.8%)	14 (70.0%)	
Age (years)		60 (29, 87)	59.5 (32, 87)	65 (29, 82)	0.1591
Follow-up Time (years)		4 (0, 15)	4 (1, 15)	2 (0, 14)	0.0144
Time from CRT to Surgery (days)		57.5 (16, 192)	57.5 (25, 192)	55 (16, 140)	0.7590
Age of Biopsy		5 (1, 15)	6 (1, 15)	3 (1, 13)	0.0508
Number of LNs examined		13 (0, 59)	13 (0, 59)	17.5 (5, 32)	0.0075
First distant met to lung		11 (11.5%)	10 (13.2%)	1 (5.0%)	0.4493
First distant met to liver		10 (10.4%)	7 (9.8%)	3 (15.0%)	0.4298
Pelvic failure		2 (2.1%)	2 (2.3%)	0 (0.0%)	1.0000

Abbreviations: MGMT, O6-Methylguanine-DNA-methyltransferase; TAE, transanal excision; LAR, low anterior resection; APR (abdominal perineal resection); 5-FU, fluorouracil; CRT, chemoradiation.

response [6]. However, up to 40% of patients have minimal response or even progression with up-front LCRT or TNT [2–6]. With increased clinical response incorporating LCRT as part of TNT, there is continued interest in finding predictive biomarkers to guide treatment and W&W selection [7].

The DNA-repair protein O6-alkylguanine (O6-AG) DNA alkyltransferase (AGT) is encoded by the O6-Methylguanine (O6-MG)-DNA-methyltransferase (MGMT) gene. DNA damage from sources like ionizing radiation can increase MGMT protein expression. Protein expression can be turned off with methylation of specific CpG islands of the promoter region of the gene. Enhanced killing can then take place due to decreased capacity for DNA damage repair [8]. Similar to rates of 30–40% in glioma, MGMT gene hyper methylation is found in around 27–40% of colorectal cancers (CRCs) [9–15].

Clinically, MGMT methylation testing is most used to help guide glioma and glioblastoma management. MGMT hypermethylated (MGMTh) tumors have increased response to temozolomide and radiation, whether given together or independently, as well as other alkylating agents [9,16–20]. Other malignancies have shown varying degrees of response by MGMT methylation with both alkylating and non-alkylating agents [21–23]. Fluorouracil (5-FU) or its oral pre-cursor capecitabine are the standard chemotherapy agents given concurrently with RT in LCRT. 5-FU acts principally by inhibiting thymidylate synthase (TS) and subsequent DNA replication. Oxaliplatin, an alkylating-like agent, is added as part of TNT [6,24,25].

We hypothesized that there would be better pathologic response in MGMTh tumors following LCRT in LARC. Secondarily, we sought to find if there were associations with recurrence free (RFS) and overall survival (OS).

Material and methods

Institutional review board approval was obtained for this study with funding provided through an internal research award. Patients were included with adenocarcinoma of the rectum, retrievable pre-treatment biopsies, and at least 1 year of follow-up who started LCRT from June 2006 to July 2020, followed by surgical resection within 6 months of LCRT completion. Unstained sections were cut from the original biopsy tissue blocks. Areas of tumor were identified using Hematoxylin and Eosin stained slides corresponding to each pathology sample, and tumor cells were retrieved using laser capture microdissection technique. This was followed by genomic tumor DNA extraction with subsequent bisulfite conversion and PCR amplification. The samples then underwent a MGMT gene hyper-methylation assay using a commercial Qiagen pyrosequencing kit (Catalog number 970061). Characteristics of patients whose samples failed tumor DNA extraction, subsequent bisulfite conversion, or final MGMT status determination were allocated to the unsuccessful assay (UA) group, while completed MGMT assays were allocated to the successful assay (SA) group. SA tumors were subsequently grouped by the presence or absence of MGMT hypermethylation (MGMTh and MGMTn, respectively). Patients were required to have 5% methylation of at least one CpG site in exon 1 of the MGMT gene to be considered MGMTh.

Radiation treatments were acceptable if given using external beam photons to doses of 4500 cGy to the pelvis, with or without a sequential boost tumor bed boost of 540–900 cGy at 180 cGy per fraction. Treatment with prone or supine patient positioning was allowed and daily imaging for setup verification was required. All patients received concurrent 5-FU or capecitabine.

Surgical pathologic specimens were originally assigned tumor

Table 2
Patient and Treatment Characteristics by MGMT Status.

		All, n (%) or median (range)	Methylated, n (%) or median (range)	Unmethylated, n (%) or median (range)	p-value
Sex	Male	49 (64.5%)	13 (52.0%)	36 (70.6%)	0.1117
	Female	27 (35.5%)	12 (48.0%)	15 (29.4%)	
Race	White	57 (75.0%)	20 (80.0%)	37 (72.6%)	0.4860
	Hispanic	11 (14.5%)	3 (12.0%)	8 (15.7%)	
	Black	4 (5.3%)	2 (8.0%)	2 (3.9%)	
	Other	4 (5.3%)	0 (0.0%)	4 (7.8%)	
Clinical T-stage	T2	5 (6.6%)	0 (0.0%)	5 (9.8%)	0.1156
	T3	63 (82.9%)	24 (96.0%)	39 (76.5%)	
	T4	8 (10.5%)	1 (4.0%)	7 (13.7%)	
Clinical N-stage	N0	34 (44.7%)	12 (48.0%)	22 (43.1%)	0.7235
	N1	32 (42.1%)	9 (36.0%)	23 (45.1%)	
	N2	10 (13.2%)	4 (16.0%)	6 (11.8%)	
Clinical Group Stage	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.4664
	2	35 (46.1%)	13 (52.0%)	22 (43.1%)	
	3	41 (54.0%)	12 (48.0%)	29 (56.9%)	
	4	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Pathologic T-stage	0	16 (21.1%)	7 (28%)	9 (17.65%)	0.8446
	1	5 (6.6%)	1 (4%)	4 (7.84%)	
	2	21 (27.6%)	7 (28%)	14 (27.45%)	
	3	29 (38.2%)	9 (36%)	20 (39.22%)	
	4	5 (6.6%)	1 (4%)	4 (7.84%)	
Pathologic N-stage	0	48 (69.6%)	16 (69.57%)	32 (69.57%)	1.0000
	1	19 (27.5%)	6 (26.09%)	13 (28.26%)	
	2	2 (2.9%)	1 (4.35%)	1 (2.17%)	
Pathologic Group xStage	0	16 (23.2%)	7 (30.43%)	9 (19.57%)	0.5702
	1	15 (21.7%)	3 (13.04%)	12 (26.09%)	
	2	17 (24.6%)	6 (26.09%)	11 (23.91%)	
Age (years)	3	21 (30.4%)	7 (30.43%)	14 (30.43%)	0.6986
Grade	1	4 (5.3%)	2 (8.0%)	2 (3.9%)	<0.0001
	2	62 (81.6%)	14 (56.0%)	48 (94.1%)	
	3	10 (13.2%)	9 (36.0%)	1 (2.0%)	
Surgery	TAE	6 (7.9%)	1 (4.0%)	5 (9.8%)	0.4927
	LAR	35 (46.1%)	14 (56.0%)	21 (41.2%)	
	APR	35 (46.1%)	10 (40.0%)	25 (49.0%)	
Concurrent Chemotherapy	5-FU	62 (81.6%)	20 (80.0%)	42 (82.4%)	1.0000
	Capecitabine	14 (18.4%)	5 (20.0%)	9 (17.7%)	
Tumor Regression Grade	0	16 (21.1%)	7 (28.0%)	9 (17.7%)	0.0138*
	1	13 (17.1%)	8 (32.0%)	5 (9.8%)	
	2/3	47 (61.8%)	10 (40.0%)	37 (72.6%)	
Age (years)		59.5 (32, 87)	59 (32, 87)	60 (35, 80)	0.6986
Time from CRT to Surgery (days)		57.5 (25, 192)	64 (33, 192)	55 (25, 142)	0.2639
Follow-up Time (years)		4 (1, 15)	5 (1, 15)	4 (1, 15)	0.8102
Age of Biopsy (years)		6 (1, 15)	7 (2, 14)	5 (1, 15)	0.0046
Number of lymph nodes examined		13 (0, 59)	12, (0, 21)	13 (0, 59)	0.2000
First distant met to lung		10 (13.2%)	2 (8.0%)	8 (15.7%)	0.4824
First distant met to liver		7 (9.2%)	1 (4.0%)	6 (11.8%)	0.4147
Pelvic failure		2 (2.6%)	0 (0.0%)	2 (3.9%)	1.0000

Abbreviations: MGMT, O6-Methylguanine-DNA-methyltransferase; TAE, transanal excision; LAR, low anterior resection; APR (abdominal perineal resection); 5-FU, fluorouracil; CRT, chemoradiation.
*P-value of methylated vs. unmethylated responder rate (60.0% vs 27.5% with tumor residual grades of 0 and 1) is 0.0061.

regression grade (TRG) scores using the 4-tiered scheme recommended by the College of American Pathologists [26]. Patients with TRGs of 0 (no viable cancer cells) or 1 (single cells or rare groups of cancer cells) were considered to have excellent response to treatment (the responders). Patients with TRGs of 2 (evident tumor regression but more than single cells or rare groups of cancer cells) or 3 (no evident tumor regression) were considered to have poor response to treatment (the non-responders).

After surgery, patients were followed every 3–6 months for 2 years followed by every 6 months up to 5 years or more. Clinical examination and imaging were used to identify recurrences. Recorded survival and follow-up times were based on the interval from the start of LCRT to the date of last follow-up or death.

Sample characteristics were described using frequencies and percentages for categorical variables and median and ranges for continuous ones. Bivariate analysis assessed patient and tumor characteristics, as well as outcomes, by MGMT methylation status in SA patients. Similar analysis was performed to compare characteristics and outcomes of SA and UA patients. Chi-square tests were used to assess associations between categorical variables while Wilcoxon rank-sum tests were used to assess differences in continuous variables between groups. Kaplan Meier methods were used to generate survival curves and log-rank tests were used to assess differences in OS time and RFS time between groups. RFS included both distant and local recurrences. Statistical significance was determined by p-values less than 0.05. Statistical analysis was done using SAS version 9.4 (SAS Institute Inc., Cary, NC).

Results

Ninety-six patients were identified who met study inclusion criteria. Of these, 76 (79.2%) were able to have MGMT status reliably determined after successful completion of each assay step. SA samples were associated with increased follow-up time and fewer examined lymph nodes compared to UA samples. Otherwise, there was no significant difference in patient characteristics or treatment. (See Table 1).

In SA samples, MGMTh was associated with increased biopsy age and grade. There was no significant difference by MGMT status of patient race, gender, and age or by stage, surgery type, time to surgery,

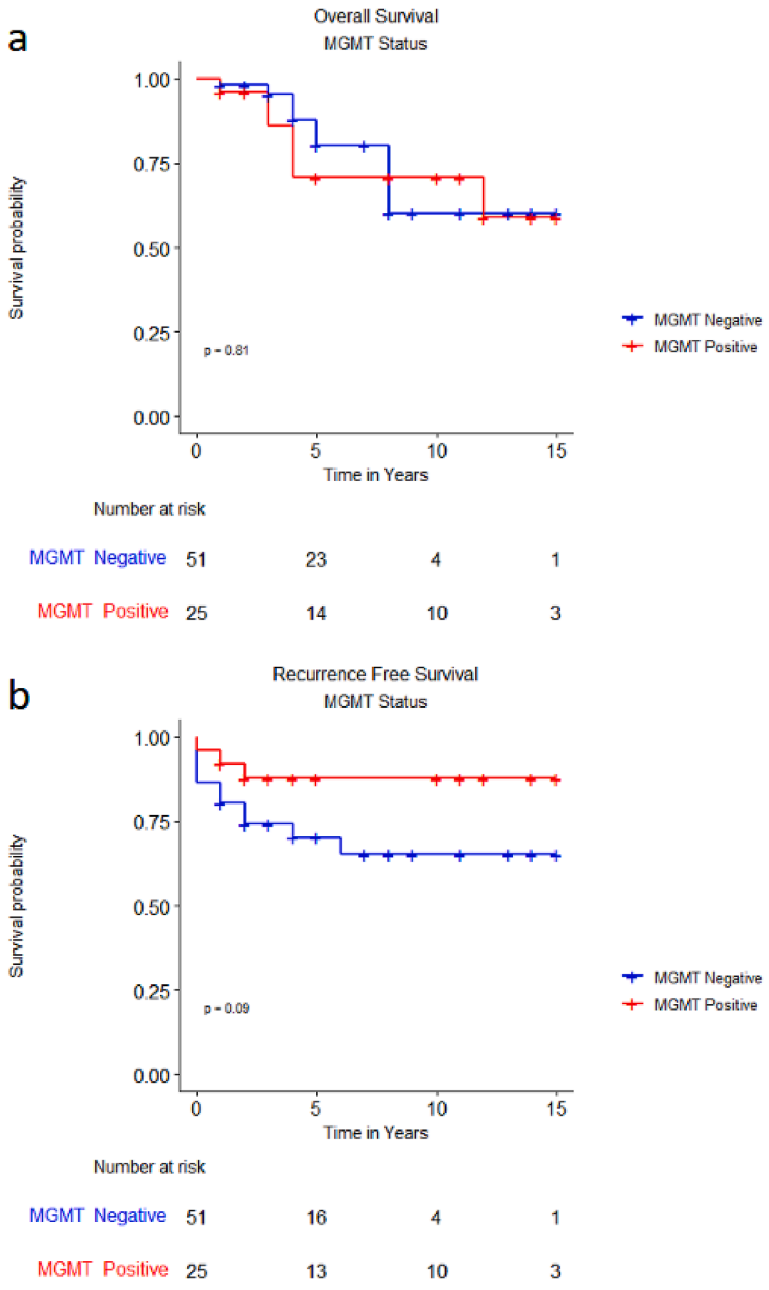

Fig. 1. A) recurrence free survival and b) overall survival by mgmt status in patients with locally advanced rectal cancer.

chemotherapy type, number of nodes examined, and biopsy age. Follow-up time was similar, as was frequency of pelvic failure and the frequency of lung vs liver deposits as the first metastatic site (See Table 2).

The LCRT responder frequency was significantly higher amongst MGMTh vs MGMTn patients (60.0% vs 27.5%, p-value = 0.0061). The rate of pCR alone (TRG of 0) was not significantly higher in MGMTh vs MGMTn patients (29.2% vs 17.7%, p-value = 0.2560). Pathologic nodal staging following chemoradiation was not significantly lower in MGMTh vs MGMTn patients (p-value = 1.0000). MGMTh was not significantly associated with improved OS (2-year OS of 96.0% vs 98.0%, log-rank p = 0.8102, hazard ratio [HR] = 1.126, 95% confidence interval [CI] 0.414 to 3.066), but there was a trend towards improved RFS (2-year OS of 87.6% vs 74.2%, log-rank p = 0.0903, HR = 0.369, CI 0.107 to 1.276). See Fig. 1.

Discussion

This is the largest report in the literature exploring the association of MGMT hypermethylation on pathologic tumor response to LCRT in rectal cancer. It is also the first to suggest a significantly increased rate of treatment response in MGMTh patients.

There are several publications investigating the significance of MGMT hypermethylation in CRCs as a broad group. Findings have been mixed regarding MGMT methylation and polymorphisms as a risk factor for CRC [27–34]. The evidence of MGMT methylation as a prognostic marker in CRC is mixed. MGMT hypermethylation was associated with decreased rates of recurrence in the study by Nagasaka et al [35]. However, the patient population in this study underwent surgery followed by adjuvant chemotherapy. None of the patients in this study received neoadjuvant therapy. Another study showed no association of MGMT methylation with CRC outcomes [36]. However, the MGMT methylation in this study was examined with immunohistochemistry

and details on treatment were not reported. Notably, our study was different from these studies in that we examined the association of MGMT methylation status with response to LCRT specifically in LARC.

Data specific to the significance of MGMT hypermethylation in LARC is sparse. In 131 rectal cancer patients, Kim et al. found that decreased recurrence after curative surgery correlated with MGMT hypermethylation (methylated vs. unmethylated, 4% vs. 21.7%, $P = 0.026$) although it did not significantly affect survival outcomes. Unfortunately, preoperative chemoradiation was an exclusion criterion and the rate of neoadjuvant chemotherapy alone was not reported [34]. A smaller study from China on 34 LARC patients required LCRT but rather than testing DNA from tumor tissue as in the present study, they used plasma cell-free DNA. This study found a higher baseline MGMT promoter methylation status in the good response group (Dworak TRG 3,4) than the poor response group (88.9 vs. 50%, $p = 0.04$) [37].

In a phase 1 study, Jeong et al. combined temozolomide with capecitabine-based LCRT in 22 patients with LARC. Pathologic complete response was observed in 37.5% and 16.7% of the hypermethylated and unmethylated MGMT groups, respectively ($P = 0.616$). To this author's knowledge, there have not been subsequent published studies building off these results [38].

The use of temozolomide up-front in Jeong's study is notable, as temozolomide has failed to find much of a foothold in this setting outside of gliomas [18]. In metastatic CRC (mCRC) with MGMT hypermethylation, dacarbazine and its oral analog temozolomide have yielded only modest activity, with an overall response rate of 10–16% [10–15]. These studies have shown that MGMT silencing at the genetic and protein level, while necessary for patient response to temozolomide, has little predictive value [39–42]. Response seems to be further restricted to microsatellite stable (MSS) patients. The more recently published MAYA trial showed some promise with temozolomide priming in MSS, MGMT silenced (by pyrosequencing and lack of expression with immunohistochemistry) mCRC. However, this priming was done to take advantage of mutations in mismatch repair genes and hypermutation linked to acquired TMZ resistance [43]. This induction of hypermutation may be a feature of nearly all temozolomide-sensitive tumors [44]. Secondary hypermutation and MGMT expression expansion have been shown to be mutually exclusive mechanisms of acquired temozolomide resistance in GBM patients [45–48]. None of the chemotherapy agents typically used as part of LCRT or TNT are true directly alkylating agents. It is possible that any substantially increased benefit for MGMTh patients from the addition of Temozolomide or other direct alkylating agents may be in the definitive setting combined with newer therapies [49,50].

Surgery after LCRT in LARC leads to increased acute and chronic morbidity. MGMT hypermethylation and other epigenetic markers may help identify the patients who can be sufficiently treated solely with definitive combinations of chemotherapy and radiation. These same markers may identify patients with an increased propensity for chemotherapy and radiation resistance. This population may be best suited for atypical neoadjuvant therapies or even upfront resection. Other methylation markers also have the potential application for treatment response prediction and the development of novel treatment strategies [34,51].

Our findings have the inherent limitations of a retrospective study. The patient cohort was modestly sized and notably heterogeneous in stage and surgery type. Reassuringly, assay success was not inversely correlated with specimen age. However, MGMTh was significantly correlated with age of the biopsy specimen. Consistent methodology was used regardless of age, so it is unclear why this relationship would exist. Results should be viewed as hypothesis generating. More work needs to be done in larger cohorts for confirmation, preferably with testing of other epigenetic markers and mutations for detection of genetic confounders.

Conclusions

MGMT hypermethylation was significantly associated with LCRT response. Continued research into epigenetic markers related to DNA repair is needed to improve treatment response prediction and selection of patients with LARC who are potential candidates for a W&W strategy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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