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Impact of polymorphisms within genes involved in regulating DNA methylation in metastatic colorectal cancer patients enrolled in three independent, randomized, open-label clinical trials: a meta-analysis from TRIBE, MAVERICC, and FIRE-3.

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Abstract

Background—CpG island DNA hypermethylation and global DNA hypomethylation are hallmark characteristics of CRC. Therefore, we aim to explore the effect of genetic variations within the genes that regulate the DNA methylation and demethylation pathways on outcomes in

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Conflict of interests.

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metastatic CRC (mCRC) patients treated with first-line therapy and enrolled in three independent, randomized, open-label clinical trials.

Methods—A total of 884 mCRC patients enrolled in TRIBE, MAVERICC, and FIRE-3 trials were included. Single nucleotide Polymorphisms (SNPs) within genes involved in DNA methylation and demethylation pathways were analyzed. The prognostic value of each SNP across all treatment arms was quantified using the inverse-variance-weighted effect size, a meta-analysis approach implemented in the METASOFT software.

Results—In the meta-analysis, *DNMT3A* rs11681717 was significantly associated with OS (HR=1.26; 95%CI 1.08–1.46; P=0.002; FDR=0.016), accounting for seven tests in the DNA methylation pathway. In addition, there was suggestive evidence of association for *TET* genes variance with tumor response (*TET1* rs3814177, OR=0.76, 95%CI 0.59–0.97, P=0.025, FDR=0.087; *TET3* rs7560668, OR=1.44; 95%CI 1.10–1.89; P=0.009; FDR=0.062).

Conclusions—We showed that polymorphisms within the genes responsible for the DNA methylation and demethylation machineries are correlated with outcomes in mCRC patients enrolled in three independent, randomized, open label, phase II/III clinical trials. In addition, we demonstrated the feasibility of a meta-analysis approach to identify stronger and more convincing association between gene polymorphisms and outcome, potentially leading the way to a new method of analysis for similar dataset.

Keywords

colorectal cancer; DNA methylation; single nucleotide polymorphism; meta-analysis; clinical trials

1. INTRODUCTION

The body of knowledge describing epigenetic abnormalities in human cancers has been largely developed over the last few decades. It is now believed that alterations in the epigenetic landscape are a hallmark of cancer. Unlike genetic mutations, the potential reversibility of epigenetic modifications encouraged the advancement of epigenetic therapies, especially in hematologic malignancies, but also in solid tumors [1].

The epigenetic biomarker research in colorectal cancer (CRC) has led to the identification of methylation markers for early detection, prediction of prognosis, and treatment response. To date, some methylation markers for early detection of CRC (such as SEPT9, NDRG4, and BMP3) have been incorporated in the FDA-approved commercial tests, Epi proColon® and Cologuard®, respectively [2]. In advanced disease, the availability of blood-based epigenetic biomarkers would increase tumor detection at earlier and more treatable stage, as well as a better selection of patients who would benefit from specific treatments. However, to date, neither predictive nor prognostic biomarkers have made the translation to clinical practice.

Among epigenetic alterations, DNA methylation has been the most widely studied in CRC and considered as one of the main molecular pathways that lead to CRC development. Promoter CpG island DNA hypermethylation has been shown to promote CRC by silencing tumor suppressor gene expression. Moreover, global DNA hypomethylation is also

considered a common characteristic of CRC, promoting genomic instability and proto-oncogene activation [3].

The CpG island methylator phenotype (CIMP) represents about 15% of CRCs, characterized by a unique epigenome with a high frequency of specific CpG island DNA methylation [4]. CIMP tumors have been shown to be associated with both clinical (right-sided colon cancer, female gender and older age) and molecular (*BRAF*^{600E} mutation and microsatellite instability high - MSI-H) features [5]. However, the lack of a global consensus for the definition of CIMP status and the controversial results reported in several prior studies regarding to its predictive and prognostic values [6] have impeded the application of CIMP status in clinical practice so far.

DNA methyltransferases (DNMTs) are responsible for the transfer of a methyl group from *S*-adenosyl-L-methionine (*SAM*) to the C-5 position of cytosine residues in DNA. Previous reports have highlighted a significant association between *DNMT3A* polymorphisms and increased risk of colorectal cancer [7], as well as the association between *DNMT3B* overexpression with CIMP-high CRC [8].

Although *DNMT3A* and *DNMT3B* are expressed at low levels in somatic tissues, both are overexpressed in human cancers, including CRC, and are thought to be involved in generating cancer-specific DNA methylation profiles [9]. DNMTs can also act as corepressors to silence gene expression, in part through their association with histone deacetylases (HDACs) that help maintain chromatin in a compact and silent state [10].

On the other hand, the DNA demethylation machinery and the major players involved in this pathway have been poorly understood [11]. The ten-eleven translocation (TET) family of enzymes was discovered in 2009 [12, 13] as the main regulators of DNA demethylation. The three mammalian TET proteins, namely TET1, TET2 and TET3, are Fe²⁺- and 2-oxoglutarate-dependent dioxygenases that successively oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), and then can further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [14]. In addition, TET activities are increased by vitamin C, which induces DNA demethylation [15]. *TET1* downregulation and 5hmC reduction are common features of CRC [16]. In *vitro* and in *vivo* experiments showed that *TET1* downregulation is not only linked to tumor progression and malignancy but is also necessary for tumor initiation and growth in CRC, leading to a downregulation of the inhibitors of the Wnt pathway, that eventually is constitutively activated [17]. Additionally, 5hmC, the main product of TET enzymes, was shown to regulate gene expression during colon cell differentiation and controls gene expression in human colon cancers [18].

The impact of the genes involved in the DNA methylation and demethylation machineries on prognosis in metastatic CRC (mCRC) has not been well established. Therefore, the significant role of epigenetic modifications in CRC development and progression, together with these promising pre-clinical data, led us to explore the effect on outcomes of genetic variations within these genes in mCRC patients treated with first-line therapy and enrolled in three independent, randomized, open-label clinical trials.

2. MATERIALS AND METHODS

2.1 Patient population and study design

A total of 884 mCRC patients enrolled in randomized, open-label TRIBE (NCT00719797) [19], MAVERICC (NCT01374425) [20] and FIRE-3 (NCT00433927) [21] trials were included in this study. Patients were randomly assigned to two different treatment arms in each trial. In the TRIBE trial, patients received either FOLFIRI plus bevacizumab or FOLFOXIRI plus bevacizumab; in MAVERICC, they received either FOLFIRI plus bevacizumab or mFOLFOX6 plus bevacizumab; in FIRE-3, they received either FOLFIRI plus bevacizumab or FOLFIRI plus cetuximab.

All patients provided informed consent for the molecular analysis and local ethics committees for each participating site approved this study.

2.2 Selected polymorphisms and genotyping

Single nucleotide polymorphisms (SNPs) within genes involved in DNA methylation and demethylation pathways were selected according to two major criteria: 1) minor allele frequency (MAF) in Caucasians > 10% (www.ensembl.org); and 2) potential role in changing gene function based on public databases (<https://snpinfo.niehs.nih.gov>; <https://www.ncbi.nlm.nih.gov>). Linkage disequilibrium among selected SNPs was identified through SNAP search service (<http://archive.broadinstitute.org/mpg/snap/>).

Genomic DNA from blood samples was extracted using the QIAmp DNA easy kit (Qiagen, Valencia) and then genotyped through the OncoArray, which is a custom array manufactured by Illumina (San Diego, CA, USA), including 530K SNP markers [22]. Fourteen functional SNPs within ten genes involved in DNA methylation (*DNMT1/3A/3B*, *MDM2*) and demethylation pathways (*TET1/2/3*, *IDH1/2*, *MBD4*) were analyzed. The characteristics of these SNPs are depicted in Supplementary Table S1.

2.3 Statistical analysis

SNP variation within DNA methylation and demethylation pathways was evaluated for association with mCRC patient outcomes, overall survival (OS), progression-free survival (PFS), and tumor response (TR). OS was defined as time from randomization until death from any cause. Patients still alive were censored at the last date of follow-up. PFS was defined as time from randomization until disease progression, death or until last follow-up in patients who were alive and remained free of disease progression. TR was defined as the percentage of patients who achieved either a complete (CR) or a partial (PR) remission according to the Response Evaluation Criteria in Solid Tumors (RECIST) classification. Associations between SNPs and clinical outcomes were estimated separately for each treatment arm. Survival outcomes were modeled using Cox proportional hazards regression, and TR was modeled using logistic regression. Regression models included the following study-specific adjustment covariates; TRIBE included gender, age, Eastern Cooperative Oncology Group (ECOG) performance status, primary tumor site, liver limited disease, adjuvant chemotherapy, BRAF status and RAS status; MAVERICC included age, ECOG performance status, number of metastases, primary tumor resected; and FIRE-3 included

gender, ECOG performance status, liver limited disease and BRAF status. Models also included the first three principle components of the European ancestry SNPs, computed separately for each cohort. SNPs were coded using an additive genetic model for the number of variant alleles, i.e., the common homozygote is represented by 0, the heterozygote by 1, and the variant homozygote by 2.

All treatment arms differed in either population or treatment, leaving no natural division of the data into discovery vs validation sets. Therefore, we took the alternative approach of jointly evaluating cohort-specific results in a meta-analysis. This approach enabled us to evaluate, 1) evidence of a common effect across cohorts, which would imply a prognostic effect, and 2) evidence of differences in an effect between cohorts, which would imply either predictive effects or population-specific effects. The prognostic value of each SNP across all treatment arms was quantified using the inverse-variance-weighted effect size estimate [23] which is most powerful when the magnitude of the effect size is the same across studies, implemented in the METASOFT software [24]. We also tested for heterogeneity of effects across arms using Cochran's Q statistic. In addition, we jointly evaluated sets of SNPs corresponding to DNA methylation/demethylation pathways to identify multiple weak effects across multiple SNPs, studies, and treatment arms. Pathway tests were conducted using a statistically powerful approach called Pegasus [25] applied to results from the meta-analyses. The Pegasus approach combines p-values for SNP-outcome association tests, modeling dependencies from independent Linkage Disequilibrium (LD) estimates. Here, pathway tests were conducted using meta-analysis results, and LD among SNPs was estimated from 1000 Genomes data [26].

To investigate possible SNP effect modification by gender, primary tumor location, RAS or KRAS status, interaction terms were included in the regression models described above and evaluated using Wald tests. For each pathway, multiple testing was accounted for using Benjamini-Hochberg (BH) false discovery rate (FDR) control at the 0.05 level. All analyses were performed using the SAS statistical package, version 9.4 (SAS Institute, Cary) and R, version 3.4.0.

3. RESULTS

Patient characteristics

A total of 884 mCRC patients from six treatment arms of three independent phase III trials, were included in this study. Some component studies differed in treatment and other patient characteristics, such as age, performance status, primary tumor site, number of metastases, primary tumor resected, and RAS status (Table 1). Median follow-up and median survival times are summarized in Table 2.

Clinical outcomes

Nominally significant P-values ($P < 0.05$) from association tests did not appear to cluster by SNP, outcome, or treatment arm (Figure 1), however SNPs in *DNMT* and *TET* genes (*DNMT1*, *DNMT3A*, and *TET1*) did meet the significance level for association with at least one of the three outcomes after FDR adjustment.

In the TRIBE FOLFIRI/bevacizumab (bev) arm, *DNMT3A* rs11681717, and *TET1* rs3814177 had significant associations with OS ($P=0.001$, FDR=0.010), and TR ($P=0.008$, FDR=0.056), respectively. In the TRIBE FOLFOXIRI/bev arm, *DNMT3A* rs2276598 was suggestively significantly associated with TR ($P=0.012$, FDR=0.087). In the MAVERICC mFOLFOX6/bev arm, *DNMT1* rs2228611 was significantly associated with TR ($P=0.001$, FDR=0.007). (Figure 1, Supplementary Table S2 and S3).

The meta-analysis of effects of gene polymorphisms on outcomes across treatment cohorts yielded a single SNP within *DNMT3A*, rs11681717, that achieved the 0.05 FDR level and was therefore considered significant (Table 3). rs11681717 was associated with OS across the six treatment cohorts (meta-analysis HR=1.26, 95% confidence interval [CI] 1.08–1.46, $P=0.002$, FDR=0.016), accounting for seven tests in the DNA methylation pathway. This effect appears to be driven by results from the TRIBE study (Figure 2A), where variant alleles were associated with shorter survival. In addition, there was suggestive evidence of association for *TET* gene variance with TR (*TET1* rs3814177, OR=0.76, 95% CI 0.59–0.97, $P=0.025$, FDR=0.087 [Figure 2B]; *TET3* rs7560668, OR=1.44, 95% CI 1.10–1.89, $P=0.009$, FDR=0.062 [Figure 2C]).

Pathway analysis suggested that genetic variation in the DNA demethylation pathway was associated with both TR and PFS (TR, $P=0.016$; PFS, $P=0.038$; OS, $P=0.22$). These results are partially explained by associations involving the *TET1* SNP rs3814177, which is significant at the $P<0.05$ level in the meta-analysis for both of these outcomes. Of note, *TET1* rs3814177 has a negative coefficient for TR and positive coefficients for PFS and OS.

Pathway tests using the Pegasus approach for effects across multiple SNPs were not significant for the DNA methylation pathway (TR, $P=0.90$; PFS, $P=0.16$; OS, $P=0.13$). None of the SNPs analyzed here exhibited evidence of heterogeneity of effects across the 6 treatment arms after accounting for multiple tests (Table 3).

4. DISCUSSION

Here we demonstrate that SNPs in DNA methylation and demethylation pathways are significantly correlated with clinical outcomes in mCRC patients.

Genome-wide association studies (GWAS) have spread our knowledge in terms of novel pathways with specific roles in carcinogenesis, and the application of GWAS has provided opportunities for drug discovery as well as for cancer prevention [27]. However, as single GWAS have normally low sample size and low statistical power, the validation in independent cohorts or the conduction of a meta-analysis increase power and reduce false-positive findings [28]. Although this approach is a current method of choice to pinpoint the genetic variations predisposition to complex disorders [29], including CRC [30], we are the first to explore its application to investigate the impact of genetic variants on outcomes in patients affected with mCRC. The originality of this study lies in the fact that we applied a meta-analysis approach to identify the association between functional candidate SNPs and outcomes in a large cohort of mCRC patients. In addition, a common approach for identifying genomic elements associated with complex traits is to evaluate combinations of

variants in known pathways; therefore, we applied a novel statistically powerful method (called Pegasus), which has been recently shown to outperform the other existing methods of gene scores [25], to analyze the effect of methylation and demethylation pathways on outcomes in this cohort of patients.

To perform our meta-analysis, we applied the additive model that assumes that there is a uniform, linear increase in risk for each copy of a specific allele. A common practice for GWAS is to examine additive models only, as the additive model has reasonable power to detect both additive and dominant effects [31]. The importance and the clinical impact of these types of analysis have been recently highlighted by Nelson *et al* [32], who estimated that drug mechanisms with genetic support would succeed twice as often as those without it, leading to lower rates of failure due to lack of efficacy in clinical development.

Herein, we showed that *DNMT3A* rs11681717 is significantly associated with worse OS in mCRC and might serve as a prognostic factor for these patients (Table 3 and Figure 2). The expression of *DNMT3A* has been shown to be an independent poor prognostic indicator both in gastric [33] and in lung cancer [34]. Accordingly, *DNMT3A* mutations are highly recurrent in acute myeloid leukemia (AML) and are independently associated with a poor outcome [35]. Recently, Lin *et al.* showed a higher *DNMT3A* expression in CRC compared to normal tissue and demonstrated that *DNMT3A* mediates the function of HIF1A-AS2 (a long non-coding RNA), which affects cell proliferation, invasion and epithelial–mesenchymal transition (EMT) in CRC [36]. Additionally, *DNMT3A* has been shown to be a direct target of miR-143 which is frequently downregulated in CRC, resulting in an upregulation of *DNMT3A* [37]. The restoration of the miR-143 expression in colon cell lines decreased tumor cell growth and soft-agar colony formation and downregulated the *DNMT3A* expression in both mRNA and protein levels, highlighting tumor-suppressive role of miR-143 in CRC development [37].

It is widely established that Wnt signaling is a hallmark of CRC, and *APC* mutations represent the main mechanism by which the Wnt pathway is constitutively activated. However, epigenetic silencing of Wnt inhibitors by DNA hypermethylation has also been observed as another potential mechanism of Wnt pathway disruption [38]. Accordingly, an elevated *DNMT3A* expression coincides with repressed *SFRP5*, a Wnt antagonist, leading to an upregulation of the Wnt pathway, eventually responsible for CRC development and progression [39]. Noteworthy, the up-regulation of *DNMT3A* and *DNMT3B* has been reported as a feature of the colorectal adenoma–carcinoma sequence [40], suggesting that epigenetic modifications are an early event in CRC development. Taken together, our findings add another proof of the importance and the critical role of *DNMT3A*, and more widely by the aberrant DNA methylation, in CRC development, progression and prognosis in mCRC patients.

We showed that *TET1* rs3814177 and *TET3* rs7560668 were significantly associated with outcomes, highlighting their potential role on prognosis in mCRC patients, thus further studies are warranted to investigate whether they may be used as potential targets for drugs development. As mentioned above, α -ketoglutarate (α -KG) is required for TET protein function. α -KG is provided by isocitrate dehydrogenase (IDH) enzymes through oxidation

of isocitrate. Therefore, the TET and IDH functions are strongly correlated. Although *IDH* and *TET* mutations do not seem to be common in CRC, downregulated *TET1* expression is an early event in cell transformation and has been related to colon cancer growth by leading to a constitutive activation of the Wnt pathway [17]. Interestingly, vitamin C regulates both DNA and histone demethylation, as an essential cofactor for TET dioxygenases and JMJC domain-containing histone demethylases [41, 42]. Vitamin C has gained widespread attention in the last few years due to its impact in CRC. It selectively kills KRAS and BRAF mutated CRC cells, leading the cell towards metabolic stress and eventual apoptosis [43]. Furthermore, vitamin C acts as an effector of 5-Aza-CdR (decitabine)-based DNA demethylation. Combining vitamin C with 5-Aza-CdR treatment of cancer cells results in a synergistic boost in DNA demethylation, as both active and passive mechanisms of DNA demethylation are activated [44].

Finally, the pathways tests using Pegasus approach for effects across multiple SNPs demonstrated the association of the DNA demethylation pathway with outcome in terms of TR ($P = 0.016$) and PFS ($P = 0.038$), which is most likely due to the effects of *TET1* variant. However, no significance was shown for the DNA methylation pathway. These results warrant some caution in the interpretation, and further studies will be needed to confirm our findings.

Immunotherapy have shown striking results in several cancer types, although only small subsets of patients benefit from these drugs. Recent studies have demonstrated the mutual relation between DNA methylation and immune system: DNA demethylating drugs, i.e. 5-azacitidine, can enhance CTLA-4 blockade-mediated T cell responses [45] and promote a significant enrichment of immunomodulatory pathways [46]. Recently, Kato *et al.* [47] showed that *DNMT3A* alterations were associated with poorer clinical outcomes (time-to-treatment failure <2 months) in patients treated with immune checkpoint inhibitors, highlighting, once again, the strong and intertwined relationship between DNA methylation and immunotherapy.

Given the extraordinary potential of DNA methylation inhibitors as therapeutic agents, several phase I/II clinical trials in mCRC patients are ongoing to investigate the efficacy azacitidine, decitabine or SGI-110 in combination with standard chemotherapy, immune checkpoint inhibitors or anti-EGFR antibodies, suggesting that epigenetic therapies may become an exciting new option for CRC patients in the near future.

5. CONCLUSION

Epigenetic aberrations, primarily DNA methylation and demethylation, are considered to be a crucial driver of CRC development and progression. Here, we showed that polymorphisms within the genes responsible for the DNA methylation and demethylation machineries, especially *DNMT3A* rs11681717, are correlated with outcomes in mCRC patients enrolled in three independent, randomized, open label, phase II/III clinical trials. Our results highlight the strong impact that these pathways have on prognosis for mCRC patients. In addition, we demonstrated the feasibility of a meta-analysis approach to identify stronger

and more convincing association between gene polymorphisms and outcome, potentially leading the way to a new method of analysis for similar datasets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

1. Epigenetic alterations are hallmark characteristics of colorectal cancer.
2. The impact of SNPs within methylation genes on mCRC outcome has not been studied yet.
3. 884 mCRC patients enrolled in TRIBE, MAVERICC, and FIRE-3 trials were included.
4. Here we observed that *DNMT3A* rs11681717 was significantly associated with worse OS.
5. We also showed the feasibility of a meta-analysis approach for this kind of dataset.

Figure 1. P-value plot of associations between SNPs and outcomes (TR, PFS and OS) in six treatment arms.

P-values for associations between SNPs and outcomes (TR, PFS and OS) in six treatment arms. P-values were generated from likelihood ratio tests of association for each SNP, coded additively for the minor allele, and each outcome. OS and PFS were modeled using Cox proportional hazards regression and TR was modeled using logistic regression. Adjustment covariates varied across studies (see Methods).

Figure 2. Forest Plots of meta-analysis results for the four significant genes.

Forest Plots of meta-analysis results for the four significant genes. Log odds ratios (OR) or log hazard ratios (HR) are shown with 95% confidence intervals (CI). The summary row shows the inverse-variance-weighted effect size with 95% CIs, combining the six estimates for the individual arms into a single summary measure. A positive log OR or HR implies a negative influence on TR or survival, respectively. SNPs with statistically significant or suggestive summary effects are plotted here. **A.** *DNMT3A* rs11681717 was significantly associated with OS (HR = 1.26, 95% CI 1.08–1.46, $P = 0.002$, FDR adjusted $P = 0.016$). **B.** *TET1* rs3814177 was suggestively associated with TR (OR = 0.76, 95% CI 0.59 – 0.96, $P = 0.025$, FDR adjusted $P = 0.087$) and PFS (HR = 1.14, 95% CI 1.01 – 1.30, $P = 0.041$, FDR adjusted $P = 0.14$). **C.** *TET3* rs7560688 was associated with TR (OR = 1.44, 95% CI 1.10 – 1.89, $P = 0.009$, FDR adjusted $P = 0.062$). **D.** *IDH1* rs1437410 was suggestively associated with PFS (HR = 1.15, 95% CI 1.01 – 1.31, $P = 0.034$, FDR adjusted $P = 0.14$).

Table 1.

Baseline characteristics of patients enrolled in the six treatment arms within the three clinical trials analyzed.

Characteristics	Total N=884	TRIBE N=324		MAVERICC N=324		FIRE3 N=236		P value*
		FOLFIRI BEV N=215	FOLFOXIRI BEV N=109	FOLFIRI BEV N=163	mFOLFOX6 BEV N=161	FOLFIRI BEV N=107	FOLFIRI CET N=129	
Sex								0.063
Male	571	132(61%)	66(61%)	103(63%)	101(63%)	70(65%)	99(77%)	
Female	313	83(39%)	43(39%)	60(37%)	60(37%)	37(35%)	30(23%)	
Age								0.005
65	589	156(73%)	78(72%)	101(62%)	117(73%)	62(58%)	75(58%)	
>65	295	59(27%)	31(28%)	62(38%)	44(27%)	45(42%)	54(42%)	
Performance status								<0.001
ECOG 0	586	177(82%)	95(87%)	97(60%)	81(50%)	56(52%)	80(62%)	
ECOG 1	296	37(17%)	14(13%)	66(40%)	79(49%)	51(48%)	49(38%)	
Unknown	2	1(1%)	0(0%)	0(0%)	1(1%)	0(0%)	0(0%)	
Primary tumor site								<0.001
Right-sided	261	53(25%)	30(28%)	67(41%)	64(40%)	25(23%)	22(17%)	
Left-sided	599	147(68%)	73(67%)	96(59%)	97(60%)	81(76%)	105(81%)	
Unknown	24	15(7%)	6(6%)	0(0%)	0(0%)	1(1%)	2(2%)	
Number of metastases								<0.001
2	657	178(83%)	89(82%)	106(65%)	101(63%)	83(78%)	100(78%)	
>2	219	37(17%)	20(18%)	57(35%)	60(37%)	20(19%)	25(19%)	
Unknown	8	0(0%)	0(0%)	0(0%)	0(0%)	4(4%)	4(3%)	
Liver limited disease								0.64
No	379	150(70%)	70(64%)	NA	NA	75(70%)	84(65%)	
Yes	181	65(30%)	39(36%)	NA	NA	32(30%)	45(35%)	
Primary tumor resected								<0.001
No	447	80(37%)	31(28%)	153(94%)	148(92%)	12(11%)	23(18%)	
Yes	437	135(63%)	78(72%)	10(6%)	13(8%)	95(89%)	106(82%)	
Adjuvant chemotherapy								0.081
No	760	188(87%)	94(86%)	143(88%)	146(91%)	86(80%)	103(80%)	
Yes	123	27(13%)	15(14%)	20(12%)	15(9%)	21(20%)	25(19%)	
Unknown	1	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(1%)	
RAS status								<0.001
Wildtype	233	50(23%)	34(31%)	NA	NA	66(62%)	83(64%)	
Mutant	203	110(51%)	57(52%)	NA	NA	17(16%)	19(15%)	
Unknown	124	55(26%)	18(17%)	NA	NA	24(22%)	27(21%)	
BRAF status								0.59
Wildtype	432	168(78%)	88(81%)	NA	NA	81(76%)	95(74%)	
Mutant	30	10(5%)	9(8%)	NA	NA	4(4%)	7(5%)	
Unknown	98	37(17%)	12(11%)	NA	NA	22(21%)	27(21%)	

* P-value is based on the Chi-square test.

Unknown group is not included in the analysis.

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Table 2.

Follow-up and survival time summary.

	TRIBE N=324		MAVERICC N=324		FIRE3 N=236	
	FOLFIRI BEV N=215	FOLFOXIRI BEV N=109	FOLFIRI BEV N=163	mFOLFOX6 BEV N=215	FOLFIRI BEV N=107	FOLFIRI CET N=129
Follow-up						
Median (months)	48.9	54.5	23.3	26.8	26.7	29.1
PFS						
Median (months)	9.7	10.8	12.5	10.1	11.5	12.8
OS						
Median (months)	26.2	26.0	27.4	24.7	31.4	49.8

Abbreviations: BEV, bevacizumab; CET, cetuximab;

Table 3.

Meta-analysis results for tumor response, PFS and OS for methylation and demethylation pathways SNPs.

SNPs	Tumor Response			Progression-free Survival			Overall Survival		
	P value for FE	Q Statistics	P value for Q	P value for FE	Q Statistics	P value for Q	P value for FE	Q Statistics	P value for Q
Methylation pathway									
DNMT1 rs2228611	0.460	13.841	0.017	0.927	6.434	0.266	0.784	8.600	0.126
DNMT3Ars1 1681717	0.920	8.994	0.109	0.200	4.974	0.419	0.002 (0.016)	7.626	0.178
DNMT3A rs2276598	0.760	9.876	0.079	0.185	5.699	0.337	0.577	3.866	0.569
DNMT3B rs2424932	0.426	6.870	0.230	0.901	5.006	0.415	0.221	3.450	0.631
MDM2rs 1690916	0.324	6.571	0.255	0.119	3.513	0.621	0.695	2.886	0.718
MDM2 rs2279744	0.741	5.626	0.344	0.271	3.380	0.642	0.815	1.357	0.929
MDM2 rs2870820	0.730	5.526	0.355	0.052	4.149	0.528	0.540	4.762	0.446
Demethylation pathway									
MBD4rs1 40696	0.340	3.612	0.607	0.427	7.786	0.168	0.949	14.065	0.015
TET1 rs12241767	0.367	4.311	0.505	0.091	6.111	0.296	0.129	4.720	0.451
TET1 rs3814177	0.025 (0.087)	8.411	0.135	0.041	6.776	0.238	0.120	2.607	0.760
TET2 rs7670522	0.058	2.223	0.817	0.705	6.584	0.253	0.528	2.243	0.815
TET3 rs7560668	0.009 (0.062)	3.003	0.699	0.207	3.901	0.564	0.178	5.733	0.333
IDH1 rs1437410	0.807	5.772	0.329	0.034	1.550	0.907	0.432	1.669	0.893
IDH2rs10459702	0.945	2.503	0.776	0.316	2.867	0.720	0.163	2.348	0.799

FE indicates fixed effects inverse-variance-weighted estimates for allelic effects that are common across studies. Q denotes Cochran's Q statistic for assessing heterogeneity, differences in effect estimates across treatment cohorts. In bold, P-values that achieved a nominal 0.05 significance level.

Adjusted P values after FDR achieved 0.1 level are shown in the parentheses.