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A genetic variant in Rassf1a predicts outcome in mCRC patients treated with cetuximab plus chemotherapy: results from FIRE-3 and JACCRO 05 and 06 trials

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Abstract

The Hippo pathway is involved in colorectal cancer (CRC) development and progression. The Hippo regulator Rassf1a is also involved in the Ras signaling cascade. In this work, we tested single nucleotide polymorphisms within Hippo components and their association with outcome in CRC patients treated with cetuximab. Two cohorts treated with cetuximab plus chemotherapy were evaluated (198 RAS wild-type (wt) patients treated with first-line FOLFIRI plus Cetuximab within the FIRE-3 trial and 67 Ras wt patients treated either with first-line mFOLFOX6 or SOX plus Cetuximab). In these two populations, Rassf1a rs2236947 was associated with overall survival, as patients with a CC genotype had significantly longer OS compared to those with CA or AA genotypes. This association was stronger in patients with left-side CRC [HR: 1.79 (1.01–3.14); *P*=0.044 and HR: 2.83 (1.14–7.03); *P*=0.025, for Fire 3 and JACCRO cohorts, respectively]. Rassf1a rs2236947 is a promising biomarker for patients treated with cetuximab plus chemotherapy.

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Keywords

Rassf1a; cetuximab; SNP; colorectal cancer; biomarker

Introduction

Salvador-Warts-Hippo pathway controls organ size by regulating tissue growth. In recent times, several studies have highlighted the implication of deregulated Hippo signaling in cancer development and progression[1]. This novel pathway acts as a complex tumor suppressor network controlling cell growth, proliferation, stem-cell maintenance and epithelium mesenchymal transition[2]. Hippo's signaling core consists of a complex of kinases whose activation ultimately leads to the phosphorylation of the oncoproteins YAP and TAZ preventing their translocation to the nucleus. On the contrary, if YAP/TAZ are not phosphorylated they can translocate to the nucleus where they regulate the activity of several transcription factors that control the expression of the Hippo target genes. These target genes include amphiregulin, Sox2 or Birc5 among others. Additionally, Hippo pathway interacts with other pathways such as Wnt, TGFβ or Notch[3]. These pathways connections are of particular relevance for colorectal cancer (CRC) development and progression. Moreover, some of Hippo's upstream regulators like Rassf1a are also crucial players in CRC. Rassf1a is a tumor suppressor that interacts with Ras signaling through a Ras interaction domain and with the Hippo pathway, specifically with MST, through a SARAH interaction domain. Rassf1a is also involved in microtubule stability, cell-cycle regulation and apoptosis[4].

Rassf1a is methylated in a high percentage of CRC samples (12% to 81% depending on the series), representing an alternative mechanism of aberrant Ras signaling[5] and, interestingly, a mutually exclusive relationship with KRAS mutations has been reported[6, 7]. Rassf1a has also been found to regulate the EGFR ligand amphiregulin by Hippo activation[8].

The growing interest in the Hippo pathway in cancer is slowly translating into multiple translational research works that underscore the clinical relevance of this pathway in CRC tumors. The expression of Hippo's oncoproteins YAP and TAZ has been correlated with the prognosis of CRC patients. A potential explanation for this correlation could be that TAZ/YAP signaling contributes to chemoresistance conferring cancer stem cell-related traits[9, 10]. Recently, in colon cancer cell lines YAP was reported to contribute to 5-Fluorouracil (5-Fu) resistance by inducing cellular quiescence as well as contributing to a stem cell-like phenotype[11]. Not only the expression of YAP and TAZ appear to be useful in predicting the patients' prognosis in CRC. Single nucleotide variations within genes involved in the Hippo pathway have also been investigated as biomarkers in colorectal cancer patients. In stages II and III colorectal cancer polymorphisms located within TAZ and Rassf1a were found to be associated with the recurrence risk[12]. However, in the metastatic colorectal cancer (mCRC) setting to our knowledge genetic variants within genes involved in the Hippo pathway have not been evaluated. In mCRC, a combination of anti-EGFR therapies plus chemotherapy is considered a standard of care in Ras wild-type patients[13–16]. Despite of the presence of Ras mutations as strong biomarkers to select the patients that benefit the most from anti-EGFR, approximately 25–30% of the patients do not respond to

treatment and, moreover, survival among responders can vary significantly. The mechanisms for this lack of response and survival differences remain unknown. We hypothesized that the critical role of the Hippo pathway in CRC development and progression might play a role in these differences. In this work, we evaluated single nucleotide polymorphisms within the Hippo pathway as biomarkers in mCRC patients treated with cetuximab plus chemotherapy.

Material and Methods

Selected polymorphisms

A total of 4 single nucleotide polymorphisms (SNPs) were selected based on previously reported results and based on their potential relevance in cetuximab treated patients[12]. The selected polymorphisms were: rs2073498 and rs2236947 located in the Rassf1 gene, rs558614 located in the LATS2 gene and rs3811715 located in the TAZ gene (also known as WWTR1). Rassf1 rs2073498 polymorphism is a missense change (Ala133Ser) located in exon 3. LATS2 rs558614 polymorphism is also a missense change (Ala324Val) located in exon 4. The rest of the analyzed polymorphisms are located intronically.

DNA was extracted from FFPE tissue samples and genotypes were obtained using PCR-based direct sequencing. 5% of the samples were re-sequence to ensure the accuracy of the results revealing a concordance higher than 99%. The author that performed the genotyping was blinded to the clinical data set.

Patients' clinical characteristics

These 4 SNPs were tested first in cohort 1 that comprised of all Ras wild-type patients enrolled in the arm A of Fire 3 trial. Those SNPs significantly associated with survival were subsequently evaluated in an independent cohort 2 that included all Ras wild-type patients enrolled in JACCRO 05 and JACCRO 06 trials.

Cohort 1 consisted of a total of 199 Ras wild-type patients enrolled in the arm A of Fire 3 trial (NCT00433927) treated with FOLIRI plus cetuximab. Cohort 2 consisted of a total of 67 patients enrolled in JACCRO 05 (UMIN000004197) or 06 (UMIN000007022) who received oxaliplatin based chemotherapy (FOLFOX or SOX) plus cetuximab. The clinical characteristics of these two cohorts have been described in detail somewhere else[13, 17, 18]. Table 1 describes the baseline clinical characteristics of the patients included in the study.

This study was performed following the REMARK recommendations for the reporting of biomarkers[19]. The study was approved by the ethics committees and all patients signed an informed consent.

Statistical analysis

The endpoints of the current study included overall survival (OS), progression-free survival (PFS), and tumor response per RECIST 1.0. Overall survival was measured as the time period from randomization or registration to death from any cause. PFS was defined as the time from the date of randomization in FIRE 3 and registration in JACCRO 05 or 06 to disease progression or death from any cause. PFS and OS were censored at the last follow-up if progression and death were not observed.

Deviations from distribution of the Hardy-Weinberg equilibrium were examined using χ^2 test. The true inheritance mode of the candidate polymorphisms had not been known yet, therefore a codominant, dominant or recessive model was assumed whenever appropriate. The associations of the SNPs and PFS or OS were analyzed using Kaplan Meier curves and log-rank tests. In the multivariable Cox regression analysis, the model was adjusted by baseline prognostic factors. The associations between the SNPs and tumor responses were examined using χ^2 tests.

All analyses were conducted using SAS statistical package version 9.4 (SAS Institute, Cary, NC, USA). All tests were 2-sided at a significance level of 0.05. *P* values were adjusted for multiple testing using the false discovery rate (FDR). The FDR-adjusted *P* values <15% were considered as statistically significant.

Results

The median follow up for cohort 1 was 34.1 months (range 0.03–70.8) and the median overall survival reached 33.1 months. For the JACCRO 05 and 06 cohort, the median follow up was 31.6 months (range 5.5–42.9) and the median survival was 33.9 months.

Of all the analyzed samples, genotypes were achieved in at least 90% of the cases for each polymorphism. In those failed cases, genotypes were not obtained due to a limited DNA quantity or poor DNA quality.

The four analyzed polymorphisms were within the probabilities limits of the Hardy-Weinberg equilibrium (p>0.05). For the Fire 3 cohort, the minor allele frequency was 47% and for the Japanese cohort 27% (expected 46% and 21% respectively, according to www.Ensembl.org).

In cohort 1, the rs2236947 polymorphism was associated with overall survival. In the dominant model, patients with a CC genotype had a median overall survival (OS) of 46.3 months (95% CI; 21.8–70.8), whereas patients with a CA or AA genotypes had a median OS of 30.6 (95% CI, 23.9–38.3); *P*= 0.023. In the multivariable Cox regression model adjusting for sex, ECOG performance status (0 vs 1–2) and primary tumor site (right, left vs NA) and number of metastatic sites (1–2 vs 3 or more) the hazard ratio (HR) was 1.50 (95% CI, 0.94–2.38); *P*=0.088. This SNP did not associate with the response rate (RR) or the progression-free survival (PFS) in this population.

The rest of the analyzed polymorphisms did not yield any association regarding RR, PFS or OS. Table 2 shows in detail all the analyzed associations.

The rs2236947 located in the Rassf1a gene was analyzed in the second cohort of patients. In this population, the rs2236947 was also associated with OS: patients harboring a CC genotype had a median OS of 42.8 months (95% CI, 27.1–42.8) compared with the patients with a CA or AA genotypes whose median OS was 19.0 months (95% CI, 13.4–42.9); *P*=0.057. In the multivariable Cox regression model adjusting for ECOG performance status the HR was 2.72 (95% CI, 1.23–6.04); *P*=0.014. In this cohort, an association was found also regarding PFS. Table 3 shows in detail these results.

These polymorphisms were also evaluated in an exploratory cohort of 190 patients enrolled in the arm B of the FIRE 3 arm and treated with FOLFIRI plus Bevacizumab. In this population no associations were found regarding response, PFS or OS based on the rs2236947 genotype (Online only Supplementary Table 1).

Subgroup analysis

The association of Rassf1a rs2236947 with OS was stronger in patients bearing left-side tumors. In cohort 1, patients with a CC genotype had a median OS of 59.0 months (95% CI, 23.8–70.8) compared to 38.3 (95% CI, 29.8–41.2) months for the patients with a CA or AA genotypes, *P*=0.013. In multivariable analysis this association remained statistically significant with a HR of 1.79 (1.01–3.14); *P*=0.044 (Figure 1, Table 4). No association was found regarding Rassf1a rs2236947 genotype in patients harboring right-side colon tumors.

In cohort 2, patients harboring a CC genotype had a median OS of 42.8 months (95% CI, 30.5–42.8) whereas patients with a CA or AA genotypes had a median OS of 23.2 (13.4–42.9), *P*=0.056. In the multivariable analysis the HR was 2.83 (1.14–7.03); *P*=0.025 (Figure 2, Table 3).

In this cohort, the rs2236947 SNP was also associated with PFS in patients harboring left-side tumors. Patients with a CC genotype had a median PFS of 15.2 months (95% CI, 8.8–18.0) compared to 10.0 months (95% CI, 8.5–11.7) for the patients with a CA or AA genotype, P=0.059. In multivariable analysis the HR was 1.98 (95% CI, 1.02–3.84); *P*=0.045.

Discussion

The polymorphism rs2236947 located in the Rassf1 gene was found to be associated with overall survival in two independent cohorts of patients treated with chemotherapy plus the anti-EGFR monoclonal antibody cetuximab. Moreover, this association appears to be stronger in patients bearing left-sided tumors. Additionally, in the JACCRO population this SNP was also associated with progression-free survival.

Rassf1a is a tumor suppressor frequently methylated in colorectal cancer. Rassf1a is involved not only in Ras signaling, but also it is a recognized upstream regulator of Hippo signaling interacting with MST through its SARAH domain[5]. The critical importance of Ras signaling in mCRC is widely known[20]. Regarding Hippo signaling, several recent works are highlighting influence of Hippo not only in the prognosis of CRC patients[21, 22], but also in the lack of response to chemotherapy by favoring stemness and quiescence status of tumor cells[23, 24]. Moreover, recent works have associated Hippo's oncogene YAP1 activation with resistance to cetuximab treatment[25].

The signaling of Rassf1a through Ras and Hippo pathways make this protein an attractive drug candidate, particularly, to influence outcome in those patients treated with anti-EGFR therapies. Until now, polymorphisms within the Rassf1 gene had never been evaluated as predictive or prognostic marker in patients treated with anti-EGFR therapies. In a previous work, our group studied several SNPs within the Hippo pathway as recurrence predictors for

patients with high-risk stage II and stage III colon cancer. Interestingly, in this work the rs2236947 polymorphism correlated with recurrence-free probability at 3 years after surgery. Patients with an AA genotype had significantly higher recurrence rate[12]. This result is in keeping with the present work in which, patients with at least an A allele for the rs2236947 SNP had significantly shorter OS compared to those patients with a CC genotype.

The association of rs2236947 with OS was significantly stronger in patients harboring left-sided colorectal tumors. Over the past few years, mounting evidence is appearing regarding the differences between left and right colon cancer[26–28]. Particularly, in mCRC tumor location appears to have a strong implication in the patients' prognosis as well as in the benefit derived from targeted therapies. It has been suggested that left side colon cancer location might be a predictor of cetuximab efficacy[29, 30]. In our study, the fact that value to predict survival for rs2236947 polymorphism was stronger in patients with left-sided colorectal tumors could be associated to these molecular differences. Rassf1a is implicated in Ras signaling, and Ras signaling is of high relevance for cetuximab efficacy. Therefore we hypothesize this is the reason for an association of rs2236947 with outcome only in left-side colorectal cancer patients. Nonetheless, due to the low number of patients with right-sided tumors we cannot firmly conclude that this SNP has no value in this population.

Overall, this study reveals a promising new biomarker for patients treated with chemotherapy plus cetuximab regardless of the chemotherapy backbone. Additionally, the value of rs2236947 as a biomarker could be confirmed in two different populations, Caucasian and Japanese, despite of the different minor allele frequencies. However, this work also has some limitations. First, the biological mechanism behind the association of Rassf1a rs2236947 with OS is not understood. This SNP is located intronically and its functionality is not known. This SNP is in high linkage disequilibrium with a missense polymorphism (rs13100173) located in the HYAL3 gene. However, whether this SNP can explain the association found is unknown. Nonetheless, *in silico* analysis using data from the ENCODE project[31] has revealed a potential functionality for rs2236947 by affecting transcriptional regulation and the expression of target genes (www.Regulomedb.org)[32]. Second, although the SNP did not associate with response, in the Japanese cohort was also associated with PFS whereas no association was found in the FIRE 3 population. In the FIRE 3 trial no association with PFS was found when comparing the cetuximab and the bevacizumab arms[13].

We believe that further evaluations of the rs2236947 polymorphism in independent cohorts as well as functionality studies are needed to confirm the prognostic/predictive value of Rassf1a rs2236947.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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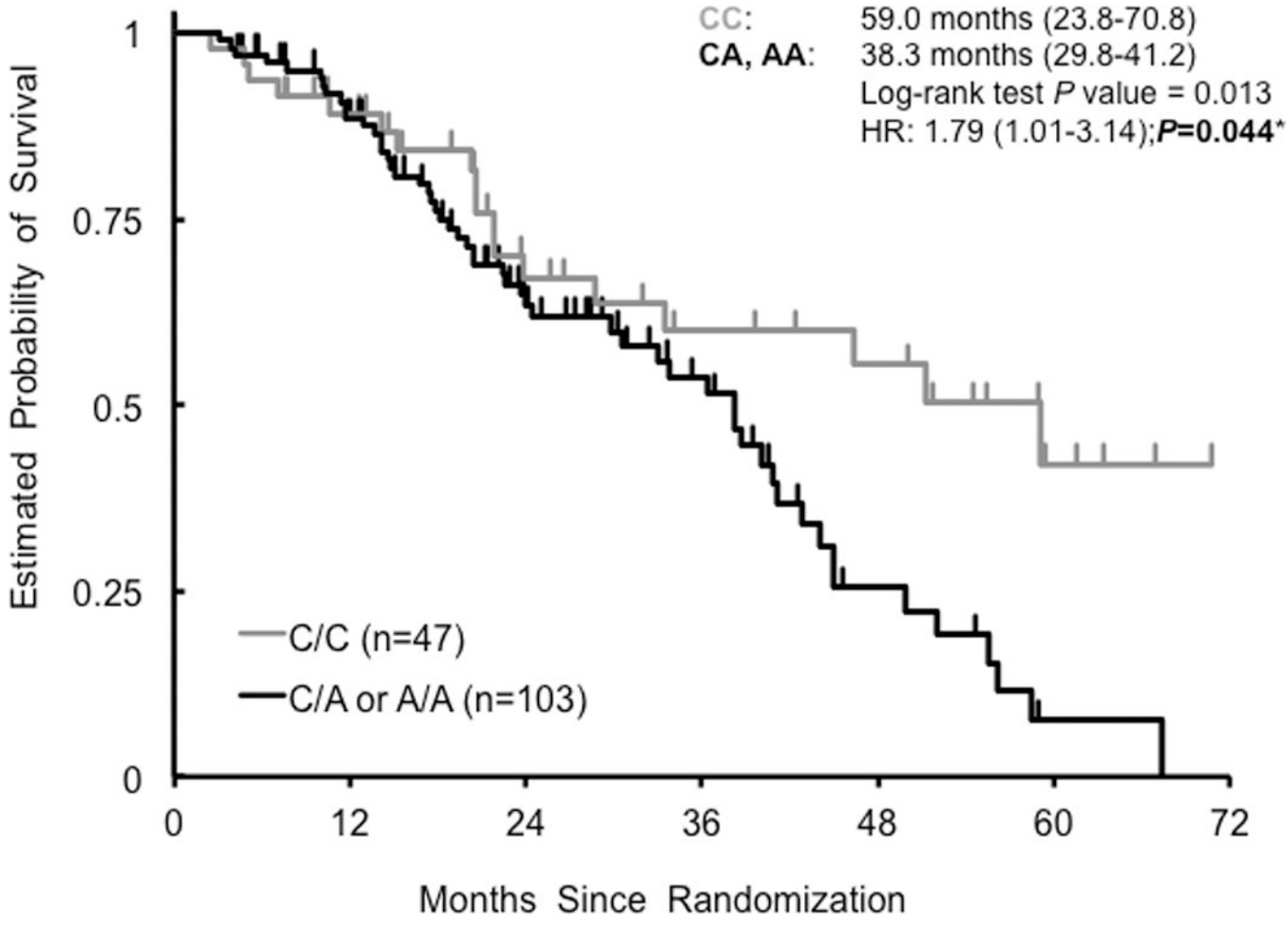

figure 1.

Rassf1a rs2236947 is associated with OS in Ras wt left-sided mCRC patients treated with FOLFIRI plus cetuximab in Fire 3.

*Wald test in the multivariable Cox Regression model adjusting for sex, ECOG, and number of metastatic sites

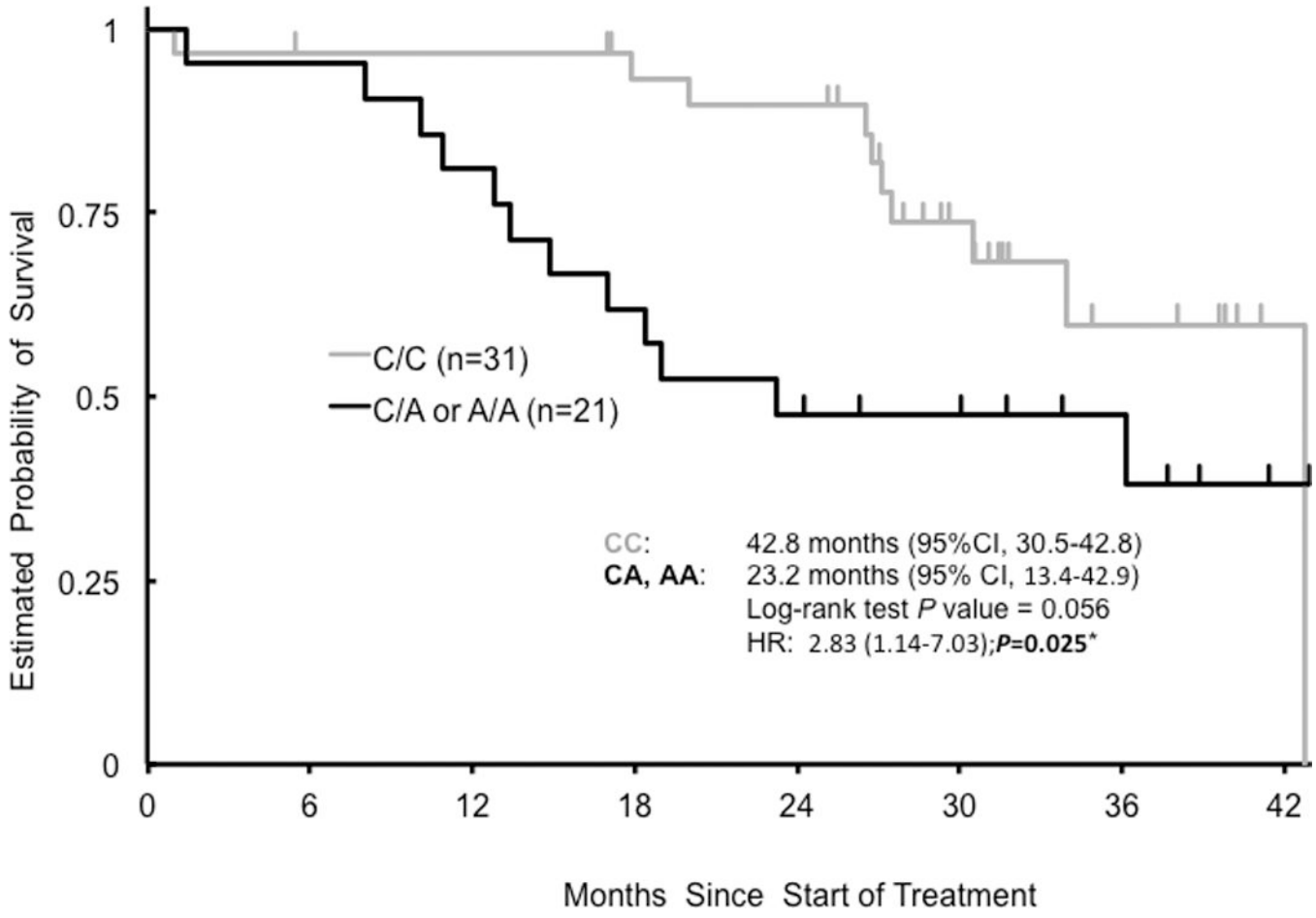

figure 2.

Rassf1a rs2236947 is associated with OS in Ras wt left-sided mCRC patients treated with oxaliplatin-based chemotherapy plus cetuximab.

*Wald test in the multivariable Cox Regression model adjusting for ECOG and regime (FOLFOX vs SOX).

Table 1

Baseline characteristics of the two cohorts

	Cohort 1: Fire-3 Arm A		Cohort 2: JACCRO 05 and 06	
	N=297	%	*N*=77	%
Age, years				
Median (range)	64 (38–79)		63 (39–79)	
≤65	158	53.2	45	58.4
> 65	139	46.8	32	41.6
Sex				
M	213	71.7	44	57.1
F	84	28.3	33	42.9
ECOGPS				
0	154	51.8	69	89.6
1–2	143	48.2	8	10.4
Primary tumor site				
Right	54	18.2	11	14.3
Left	236	79.5	64	83.1
Unknown	7	2.4	2	2.6
Metastatic sites, n				
1	123	41.4	33	42.9
>1	174	58.6	44	57.1
Time to mets				
Synchronous	217	74.3	59	76.6
Metachronous	75	25.7	18	23.4
Unknown	5			
Adjuvant therapy				
No	226	77.4	71	92.2
Yes	66	22.6	6	7.8
Unknown	5			
Mutation Status				
All RAS wildtype	199	83.6	67	87.0
Mutant	39	16.4	10	13.0
Unknown	59			

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Table 2

Hippo pathway SNPs and clinical outcomes in patients with all RAS wild-type mCRC treated with first-line FOLFIRI+Cetuximab in Fire-3

		Tumor response, RECIST		Progression-Free survival (PFS)			Overall survival (OS)		
SNP	**N**	**CR+PR**	**SD+PD**	**Median, ms (95%CI)**	**HR (95%CI)†**	**HR (95%CI)‡**	**Median, ms (95%CI)**	**HR (95%CI)†**	**HR (95%CI)‡**
RASSF1a rs2073498									
C/C	155	98 (74%)	34 (26%)	10.0 (8.0, 11.5)	1 (reference)	1 (reference)	29.8 (23.7, 38.3)	1 (reference)	1 (reference)
C/A§	31	26 (84%)	5 (16%)	11.1 (9.5, 14.3)	0.74 (0.50, 1.10)	0.84 (0.56, 1.28)	56.2 (20.5, 67.4)	0.72 (0.43, 1.21)	0.86 (0.50, 1.48)
A/A§	6								
P value*			0.35		0.13	0.42		0.20	0.58
RASSF1a rs2236947									
C/C	57	37 (76%)	12 (24%)	10.1 (7.8, 11.1)	1 (reference)	1 (reference)	46.3 (21.8, 70.8)	1 (reference)	1 (reference)
C/A, A/A§	132	88 (78%)	25 (22%)	10.5 (9.3, 13.0)	0.95 (0.67, 1.34)	0.91 (0.64, 1.29)	30.6 (23.9, 38.3)	1.65 (1.05, 2.59)	1.50 (0.94, 2.38)
P value*			0.84		0.76	0.58		0.023 (0.14)[a]	0.088 (0.20)[a]
LATS rs558614									
A/A	120	80 (78%)	22 (22%)	10.4 (9.2, 13.0)	1 (reference)	1 (reference)	38.7 (27.1, 49.8)	1 (reference)	1 (reference)
A/G	55	35 (73%)	13 (27%)	10.0 (7.8, 11.8)	1.15 (0.81, 1.63)	1.10 (0.77, 1.57)	23.8 (18.1, 37.1)	1.49 (0.98, 2.27)	1.17 (0.76, 1.80)
G/G	11	5 (63%)	3 (38%)	13.0 (6.1, 70.8)	0.53 (0.23, 1.20)	0.58 (0.25, 1.36)	45.0 (7.1, 70.8)	0.78 (0.31, 1.96)	0.89 (0.34, 2.31)
P value*			0.46		0.17	0.36		0.11	0.73
TAZ rs3811715									
C/C	124	77 (75%)	26 (25%)	10.4 (9.0, 12.2)	1 (reference)	1 (reference)	33.4 (24.4, 45.0)	1 (reference)	1 (reference)
C/T§	57	42 (78%)	12 (22%)	10.6 (8.0, 13.3)	0.98 (0.70, 1.36)	1.03 (0.73, 1.43)	30.6 (19.3, 40.9)	1.15 (0.77, 1.72)	1.13 (0.75, 1.70)
T/T§	4								
P value*			0.84		0.89	0.88		0.50	0.57

**P* value was based on Fisher's exact test for response, log-rank test for PFS and OS in the univariable analysis (†) and Wald test for PFS and OS in the multivariable Cox regression model (‡) adjusting for sex (male vs female), ECOG performance status (0 vs 1–2), primary tumor site (right, left, vs NA), and number of metastatic disease (1, 2 vs 3+).

[a] *P* value adjusted by FDR (false discovery rate).

§ A dominant model was used.

Table 3

Rassfa1 rs2236947 and clinical outcomes in Japanese patients with all RAS wildtype mCRC treated with first-line oxaliplatin+cetuximab in JACCRO 05 and 06

		Tumor response, RECIST		Progression-Free survival (PFS)			Overall survival (OS)		
SNP	**N**	**CR+PR**	**SD+PD**	**Median, ms (95%CI)**	**HR (95%CI)†**	**HR (95%CI)‡**	**Median, ms (95%CI)**	**HR (95%CI)†**	**HR (95%CI)‡**
All patients									
C/C	35	26 (81%)	6 (19%)	13.8 (6.6, 17.4)	1 (reference)	1 (reference)	42.8 (27.1, 42.8)	1 (reference)	1 (reference)
C/A, A/A§	27	20 (77%)	6 (23%)	9.4 (5.8, 11.3)	1.44 (0.81, 2.54)	1.69 (0.93, 3.07)	19.0 (13.4, 42.9)	1.96 (0.96, 3.99)	2.72 (1.23, 6.04)
P value*			0.75		0.18	0.088		0.057	0.014
Left-sided CRC									
C/C	31	24 (86%)	4 (14%)	15.2 (8.8, 18.0)	1 (reference)	1 (reference)	42.8 (30.5, 42.8)	1 (reference)	1 (reference)
C/A, A/A§	21	17 (81%)	4 (19%)	10.0 (8.5, 11.7)	1.75 (0.91, 3.34)	1.98 (1.02, 3.84)	23.2 (13.4, 42.9)	2.21 (0.95, 5.14)	2.83 (1.14, 7.03)
P value*			0.71		0.059	0.045		0.056	0.025

**P* value was based on Fisher's exact test for response, log-rank test for PFS and OS in the univariable analysis (†) and Wald test for PFS and OS in the multivariable Cox regression model (‡) adjusting for ECOG performance status (0 vs 1), and regimen (FOLFOX vs SOX)

§A dominant model was used

Table 4

Hippo pathway SNPs and clinical outcomes in patients with all RAS wild-type left-sided mCRC treated with first-line FOLFIRI+Cetuximab in Fire-3

		Tumor response, RECIST		Progression-Free survival (PFS)			Overall survival (OS)		
SNP	**N**	**CR+PR**	**SD+PD**	**Median, ms (95%CI)**	**HR (95%CI)†**	**HR (95%CI)‡**	**Median, ms (95%CI)**	**HR (95%CI)†**	**HR (95%CI)‡**
RASSF1a rs2073498									
C/C	120	83(79%)	22(21%)	10.4(9.3,12.9)	1(reference)	1(reference)	38.7(30.6,45.0)	1(reference)	1(reference)
C/A§	33	23(82%)	5(18%)	12.2(9.6,14.3)	0.81(0.53,1.24)	0.82(0.53,1.28)	56.2(23.9,67.4)	0.82(0.46,1.45)	0.82(0.46,1.46)
A/A§									
P value *			0.80		0.32	0.39		0.49	0.50
RASSF1a rs2236947									
C/C	47	32(80%)	8(20%)	10.4(9.2,12.2)	1(reference)	1(reference)	59.0(23.8,70.8)	1(reference)	1(reference)
C/A, A/A	103	74(81%)	17(19%)	11.5(9.6,14.1)	0.98(0.66,1.45)	0.92(0.62,1.38)	38.3(29.8,41.2)	1.91(1.11,3.29)	1.79(1.01,3.14)
P value *			1.00		0.92	0.69		0.013 (0.092)[a]	0.044 (01.8)[a]
LATS rs558614									
A/A	95	68(83%)	14(17%)	12.2(9.7,14.1)	1(reference)	1(reference)	44.1(33.8,55.5)	1(reference)	1(reference)
A/G	42	29(76%)	9(24%)	9.9(7.8,11.8)	1.23(0.82,1.83)	1.23(0.80,1.89)	23.8(19.3,42.8)	1.66(1.00,2.76)	1.31(0.76,2.28)
G/G	10	5(63%)	3(38%)	13.0(6.1,70.8)	0.49(0.20,1.22)	0.51(0.20,1.28)	45.0(7.1,70.8)	0.79(0.28,2.21)	0.73(0.25,2.10)
P value *			0.27		0.12	0.19		0.091	0.47
TAZ rs3811715									
C/C	98	65(76%)	20(24%)	10.4(8.1,12.2)	1(reference)	1(reference)	38.7(29.8,49.8)	1(reference)	1(reference)
C/T§	47	35(85%)	6(15%)	12.9(10.3,14.1)	0.87(0.60,1.28)	0.90(0.61,1.32)	40.0(28.7,52.0)	1.04(0.64,1.71)	1.05(0.64,1.73)
T/T§									
P value *			0.35		0.48	0.59		0.86	0.85

* *P* value was based on Fisher's exact test for response, log-rank test for PFS and OS in the univariable analysis (†) and Wald test for PFS and OS in the multivariable Cox regression model (‡) adjusting for sex (male vs female), ECOG performance status (0 vs 1–2), and number of metastatic disease (1, 2 vs 3+).

[a] *P* Value adjusted by false discovery rate (FDR).