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PHASE II STUDIES

Effect of simvastatin plus cetuximab/irinotecan for *KRAS* mutant colorectal cancer and predictive value of the *RAS* signature for treatment response to cetuximab

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Summary *Purpose* Preclinical data has demonstrated the potential of simvastatin to overcome cetuximab resistance in *KRAS* mutant CRC patients. Therefore, we designed a study using simvastatin/cetuximab/irinotecan for *KRAS* mutant CRC patients who are refractory to irinotecan and oxaliplatin-based chemotherapy. *Patients and methods* In this phase II study, patients received 500 mg/m^2 cetuximab, 150–180 mg/m^2 (day 1), and 80 mg simvastatin (once daily, days 1–14, every 2 weeks). The primary endpoint was the objective response rate (ORR). Secondary endpoints were progression-free survival (PFS), overall survival (OS), the disease control rate (DCR), and safety. We also analyzed the relationship between the *RAS* gene expression signature score and treatment response to simvastatin/cetuximab/irinotecan. *Results* Fifty-two *KRAS* mutant CRC patients were enrolled. The ORR (complete response [CR], 0; partial response [PR], 1) was 1.9 % (95 % confidence interval [CI], −1.8–5.6). The DCR (CR, 0; PR, 1; stable disease, 33) was 65.4 % (95 % CI, 52.5–78.3). The median PFS and OS from the time of study drug administration were 7·6 months (95 % CI, 4.4–10.8) and 12.8 months (95 % CI, 9.5–16.2), respectively. The most common grade 3/4 adverse events were anemia (28.8 %), neutropenia (13.5 %), and diarrhea (7.7 %). The *RAS* signature score was significantly correlated with the maximal change in target lesions from baseline (r=0.57, P=0.014). *Conclusion* The simvastatin/cetuximab/irinotecan regimen showed

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promising efficacy and safety in *KRAS* mutant CRC patients who failed irinotecan and oxaliplatin-based chemotherapy. The *RAS* signature may be a novel predictor of treatment response to cetuximab-combined chemotherapy in CRC patients.

Keywords Simvastatin · Cetuximab · KRAS · Colorectal cancer · RAS signature

Introduction

Cetuximab and panitumumab that target the epidermal growth factor receptor (EGFR) in combination with standard cytotoxic chemotherapy have proven to be efficacious for metastatic colorectal cancer (CRC) [1,2]. Recently, mutations in the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) have emerged as a negative predictive factor for treatment response in patients receiving cetuximab [3] and cetuximab or panitumumab failed to confer a survival benefit on CRC patients with *KRAS* mutations. Therefore, therapeutic options are limited for patients with metastatic CRCs that harbor *KRAS* mutations who have failed irinotecan-based or oxaliplatin-based regimens [3].

In our preclinical study and xenograft model, we showed that a cardiovascular dose of simvastatin overcomes cetuximab resistance in colon cancer cells with *KRAS* mutation by modulating BRAF protein and inducing the proapoptotic proteins BCL2L11 and BAD [4]. In a phase II study, we demonstrated that the addition of simvastatin to the standard irinotecan, 5-fluorouracil, and leucovorin (FOLFIRI) regimen did not result in significant toxicities [5]. So, we hypothesized that the addition of simvastatin to cetuximab and irinotecan may overcome cetuximab resistance in patients with irinotecan-refractory *KRAS* mutant CRCs. The *RAS* signature score, which is derived from *RAS* pathway-related genes across multiple datasets in lung cancer, CRC, and breast cancer, was shown to be superior to *KRAS* mutation status for the prediction of dependence on *RAS* signaling [6]. Herein, we designed a multi-center phase II study investigating simvastatin plus cetuximab and irinotecan in *KRAS* mutant CRC patients refractory to irinotecan. We also analyzed the correlation between the *RAS* signature score and treatment response to cetuximab and simvastatin in *KRAS* mutant CRCs.

Methods

Patients

Eligibility criteria included a histologically confirmed metastatic colorectal adenocarcinoma with *KRAS* mutations (codons 12, 13, or 61). Patients must have received one of the following irinotecan-based regimens for at least 6 weeks and must have had documented disease progression during treatment with FOLFIRI or XELIRI regimen or within 6 months thereafter. Patients treated with any prior statin therapy including simvastatin within 1 year from the date of study entry were excluded. This was an investigator-sponsored multicenter study, approved by the Institutional Review Board at Samsung Medical Center.

Study design

This was a non-randomized, open-labeled, multi-center phase II study. The primary endpoint was the objective response rate (ORR) according to the Response Evaluation Criteria in Solid Tumors version 1.1. Secondary endpoints were PFS, overall survival (OS), the disease control rate (DCR), and safety. Correlative biomarker analyses were pre-planned and outlined in the protocol.

Study treatment and assessments

Treatment comprised cetuximab (500 mg/m^2, day 1), irinotecan (150–180 mg/m^2, according to the immediate prior irinotecan-based regimen), and simvastatin (80 mg daily) every 2 weeks.

Tumor responses were assessed every 6 weeks during study drug administration and every 8 weeks after its cessation until disease progression. An independent radiological review was performed. Toxicities were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0.

Biomarker analyses

KRAS mutation tests were performed at the designated central laboratory as described previously [7]. Mutations in codons 12 and 13 of the *KRAS* gene were detected by direct sequencing of polymerase chain reaction products amplified from DNA extracted from representative tumor tissue. *BRAF* V600E direct sequencing and *PIK3CA* hotspot mutations were also tested.

RAS signature score according to gene expression profiling

Total RNA was extracted from 2 to 4 sections of 4-μm-thick formalin-fixed, paraffin-embedded tissue sections from representative primary tumor blocks using the High Pure RNA Paraffin kit (Roche Diagnostic, Mannheim, Germany), after removing non-tumor elements by manual macrodissection, guided by hematoxylin-eosin-stained slides. For the nCounter assay, 200 ng of total RNA was hybridized with the custom designed code set of 147 genes (105 upregulated genes, 42 downregulated genes for the *RAS* signature score) for 18 h at

65 °C and processed according to the manufacturer instructions [8]. Data were normalized to average expression levels of the internal reference genes recommended by the manufacturer.

RAS signature score according to gene expression profiling: *KRAS* wild-type cohort

Between 2007 and 2010, 72 patients with *KRAS* wild-type CRC were treated with cetuximab and irinotecan chemotherapy after failing to irinotecan and oxaliplatin-based chemotherapy. The *RAS* signature score was evaluated and correlative analyses with treatment response were performed. At the final analysis, 62 patients were included.

Statistics

According to the Simon two-stage design, a sample size of 47 patients was needed to detect an increase in the response rate from 2 to 12 %, the aim for our study. According to this assumption, at least one PR was required among the first 14 patients (and three among 47 patients). Accounting for a 10 % dropout rate, we planned to enroll 52 patients. PFS and OS were estimated using the Kaplan-Meier method and compared using the log-rank test. PFS was estimated from the date of first administration of the study treatment to death, documented progression, or the date of the last follow-up visit. OS was defined as the time from the date of the first administration of study treatment to death or the date of the last follow-up visit. All clinical data were held centrally (Clinical Trial Center, Samsung Medical Center, Seoul, Korea) and analyzed using SPSS (v18.0). All *P* values are two-sided. Pre-planned subgroup analyses were based on treatment outcome according to *KRAS* mutations in codons 12 vs 13. The study was registered at clinicaltrials.gov: NCT# 01281761.

Role of the funding source

The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. Cetuximab was donated by Merck Serono. Irinotecan, palonosetron, and simvastatin were donated by CJ Korea.

Results

Patients and *KRAS, BRAF,* and *PIK3CA* mutation status

Between December 2010 and September 2011, 52 CRC patients with *KRAS* mutations were enrolled at four Korean tertiary hospitals. Patient characteristics and mutation status are shown in Table 1. Twenty-three patients (44.2 %) had a

Table 1 Baseline characteristics of the patients (*n*=52)

Characteristic	Number (%)	
Age (years)		
Median	57	
Range	33–78	
Sex		
Male	25	(48.1)
Female	27	(51.9)
ECOG performance status		
0	3	(5.8)
1	49	(94.2)
Prior treatment		
Adjuvant	7	(13.5)
Curative surgery	13	(25.0)
Palliative surgery	30	(57.7)
None	4	(7.7)
Primary site		
Colon	38	(73.1)
Rectum	13	(73.1)
Colon and rectum	1	(1.9)
No. of metastatic sites		
One	23	(44.2)
Two	20	(38.5)
Three	7	(13.5)
>Three	2	(3.8)
Metastatic sites		
Liver	36	(69.2)
Lung	31	(59.6)
Peritoneal seeding	2	(3.8)
Bone	1	(1.9)
Others	14	(26.9)
Number of previous systemic anticancer therapies		
2	44	(84.6)
3	5	(9.6)
≥4	3	(5.8)
K-ras mutation(amino acid substitution)		
Codon 12		
G12D (Aspartate)	23	(44.2)
G12V (Valine)	13	(25.0)
G12C (Cysteine)	6	(11.5)
G12A (Alanine)	2	(3.8)
G12S (Serine)	2	(3.8)
G12R (Arginine)	0	(0.0)
Codon 13		
G13D (Aspartate)	4	(7.7)
G13S (Serine)	1	(1.9)
G13A (Alanine)	0	(0.0)
Codon 61		
G61L	1	(1.9)
PIK3CA mutation (*n*=34)		
E542K	1	(2.9)

Table 1 (continued)

Characteristic	Number (%)	
D1056N	1	(2.9)
Wild type	31	(91.2)
BRAF mutation (*n*=41)		
Positive	0	(0.0)
Wild type	41	(100.0)
Concomitant K-ras and PIK3CA mutations		
KRAS G12D and PIK3CA E542K	1	(2.9)
KRAS G12C and PIK3CA D1056N	1	(2.9)

KRAS codon 12 G12D mutation, 13 had G12V (25.0 %), 6 had G12C (11.5 %), and 5 had a G13D codon 13 mutation (9.6 %). None of the 41 patients tested for BRAF mutation harbored the BRAF V600E mutation. Two patients had concomitant *KRAS* and *PIK3CA* mutations: *KRAS* G12D and *PIK3CA* E542K and *KRAS* G12C and *PIK3CA* D1056N.

Safety

Patients completed a median of 6 cycles of treatment (range, 1–21 cycles). The dose intensity was 98 % for cetuximab, 89.5 % for irinotecan, and 95 % for simvastatin. Table 2 lists the toxicities observed in at least one cycle. The most frequent grade 3/4 adverse events were anemia (28.8 %), neutropenia (13.5 %), and diarrhea (7.7 %). Creatine kinase elevations were considered to be related to simvastatin, and 13 patients experienced transient elevations, which were reversible by temporary study drug cessation. No drug-related mortality was observed in this study.

Treatment outcome

Five patients were not evaluable for treatment response. In intent-to-treat analysis including all patients, the ORR (CR, 0; PR, 1) was 1.9 % (95 % confidence interval [CI], −1.8–5.6). The DCR (CR, 0; PR, 1; stable disease [SD], 33) was 65.4 % (95 % CI, 52.5–78.3; Table 3). As shown in the waterfall plot (Fig. 1), tumor shrinkage compared to baseline tumor measurement was observed in 14 patients (26.9 %). An expert radiologist blinded to the treatment response and treatment outcome (Fig. 1b) performed a separate independent review. The median PFS and OS were 7.6 months (95 % CI, 4.4–10.8) and 12.8 months (95 % CI, 9.5–16.2), respectively. The DCR (61 % vs 80 %; *P*=0.637) and median PFS (9.1 vs 5.3 months; *P*=0.152) did not differ significantly according to the *KRAS* mutation type (codons 12 vs 13; 46 vs 5 patients) (Fig. 2). The patient with concomitant *KRAS* G12C and *PIK3CA* D1056N mutations demonstrated SD for 6 months. Another patient with concomitant KRAS G12D and PIK3CA E542K mutations showed progressive disease after 3 cycles of cetuximab/irinotecan/simvastatin.

Table 2 Overview of adverse events

Toxicity profile	No. of patients (%) with G3/4 adverse events	
Hematologic		
Anemia	15	(28.8)
Neutropenia	7	(13.5)
Thrombocytopenia	0	(0)
Febrile neutropenia	0	(0)
Non-hematologic		
Nausea	1	(1.9)
Mucositis	3	(5.8)
Diarrhea	4	(7.7)
Neuropathy	0	(0)
Hand-foot syndrome	0	(0)
Muscle enzyme elevation[a]	34	(65.4)
CK elevation[a]	13	(25.0)
AST elevation[a]	24	(46.2)
ALT elevation[a]	23	(44.2)

[a] include all grades adverse events

RAS signature score and cetuximab response in *KRAS* mutant and *KRAS* wild-type CRCs

Based on the literature, the response rate of KRAS mutant CRCs to cetuximab is extremely low with

Table 3 Response according to RECIST (version 1.1) and survival outcome

Response	Number of patients (%, 95 % CI)
Complete response	0 (0)
Confirmed partial response	1 (1.9, −1.8–-5.6)
Confirmed stable disease	33 (63.5, 50.4–76.6)
Progressive disease	13 (25.0, 13.2–36.8)
Not evaluable	5 (9.6, 1.6–17.6)
Disease control rate (CR + PR + SD)	3 (65.4, 52.5–78.3)
Survival outcome	Months (95 % CI)
Progression-free survival	7.6 (4.4–10.8)
3-month progression-free survival rate	82 %
6-month progression-free survival rate	57 %
1-year progression-free survival rate	14 %
Overall survival – months	12.8 (9.5–16.2)
6-month overall survival rate	81 %
1-year overall survival rate	65 %

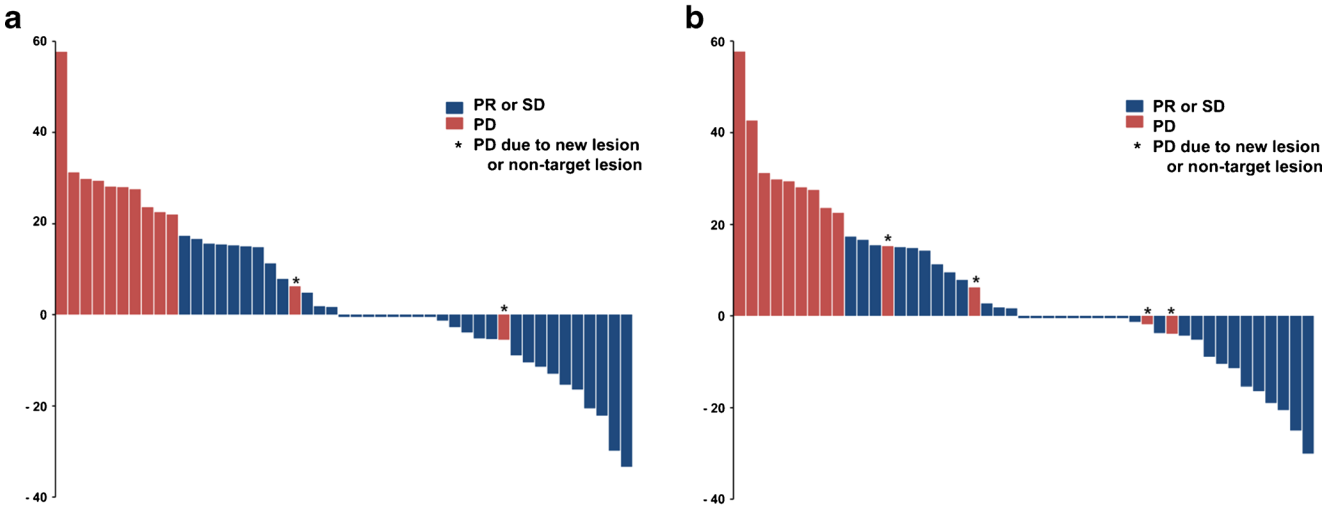

Fig. 1 **a** Waterfall Plot by Investigators **b** Waterfall Plot by Independent Radiologic Review

<10 % in RR. Of the 30 *KRAS* mutant CRCs, 18 patients had low ras signature score. Of these 18 CRC mutant patients with low ras signature, 8 patients demonstrated some degree of tumor shrinkage. One patient who achieved PR for >6 months had low ras signature (Fig. 3). In the *KRAS* wild-type cohort, 38 (64.4 %) of 59 patients had low ras signature and 24 (63.1 %) had some tumor shrinkage after cetuximab/irinotecan treatment. In contrast, approximately 70 % of the KRAS wild-type CRC patients with high ras signature had disease progression ranging from 30 to 80 % increase in tumor burden by RECIST 1.1.

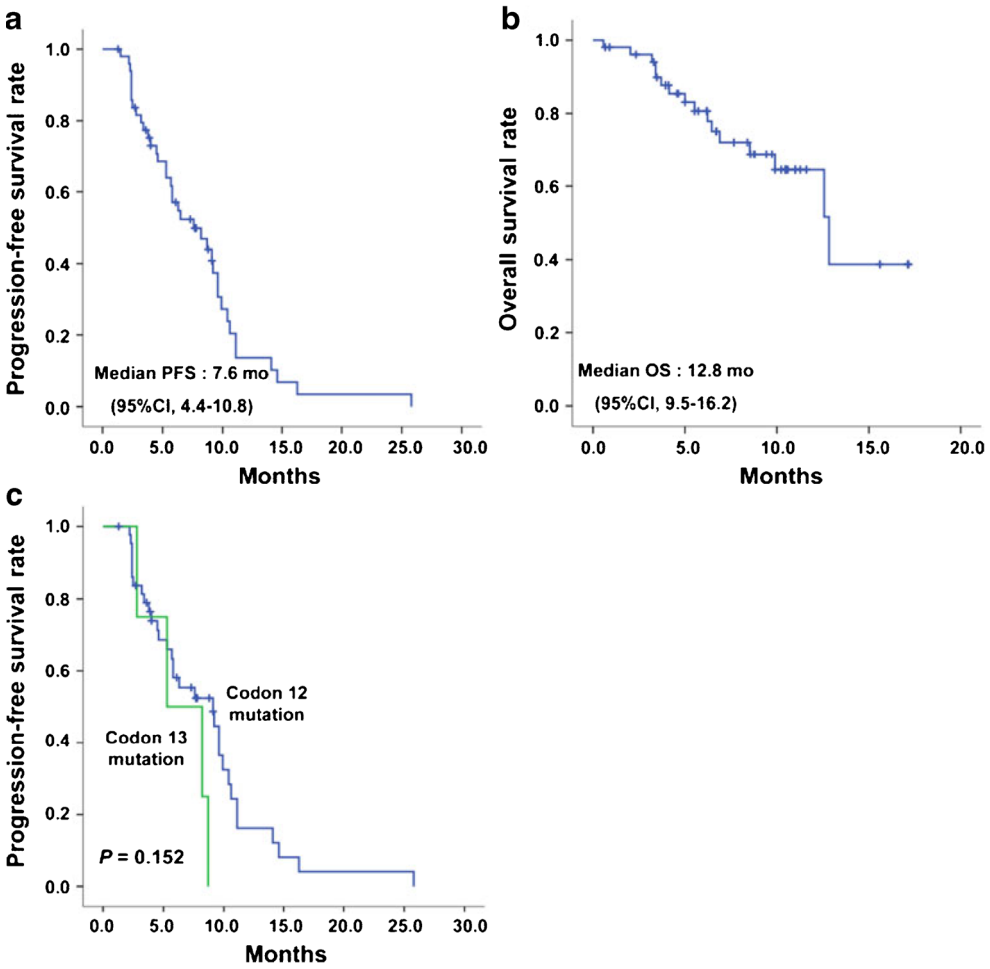

Fig. 2 **a** Progression-free survival **b** Overall survival **c** Overall survival according to codon 12 and codon 13 KRAS mutations

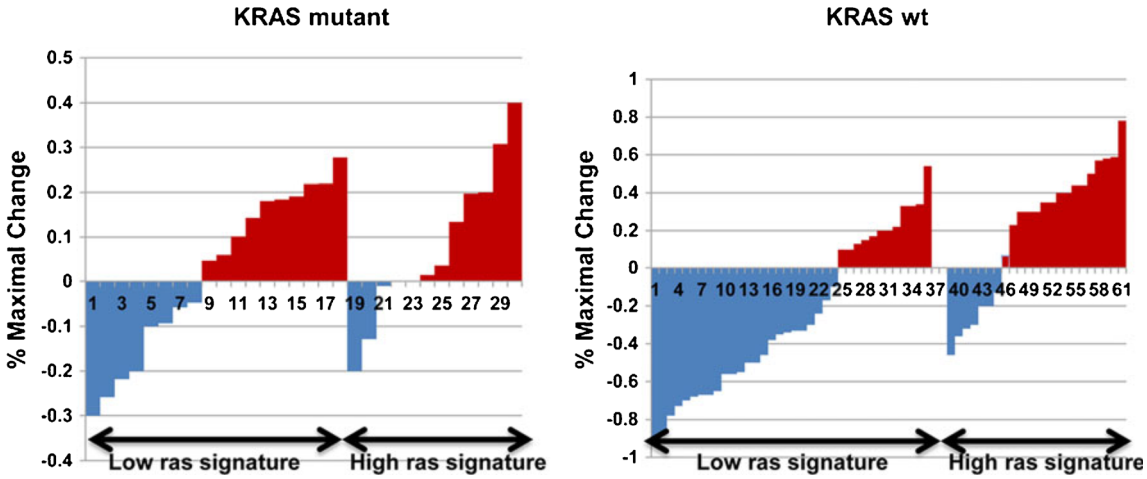

Fig. 3 Ras signature and Cetuximab Treatment Response

Discussion

Therapeutic options for *KRAS* mutant CRC patients after failing oxaliplatin and irinotecan treatment are limited. The CORRECT trial randomized 760 CRC patients to receive regorafenib or placebo in a 2:1 ratio and demonstrated, for the first time, a survival benefit with regorafenib (median OS, 6.4 months vs 5.0 months; P=0.0052) [9]. The response rate, the primary endpoint, in our study for cetuximab/irinotecan/simvastatin was 1.9 %, similar to that observed in the regorafenib arm of the CORRECT trial (1.0 %). Further, the DCR was higher for cetuximab/irinotecan/simvastatin treatment (63.5 %) than for regorafenib (41 %) [9]. However, any definitive conclusions should be reserved until these results are confirmed in a randomized trial, since this was a phase II trial with a selected patient population.

Moreover, the DCR and PFS achieved in our study with cetuximab/irinotecan/simvastatin was more favorable than that reported for *KRAS* mutant tumors treated with cetuximab plus chemotherapy in a large retrospective analysis (DCR, 49.1 % [124/253]; PFS, 12 weeks) [10]. In that study, approximately 15 % of the KRAS mutant CRCs were reported to harbor concomitant *PIK3CA* mutations [10]. There is increasing evidence that, aside from *KRAS* mutations, *PIK3CA* mutations are potentially associated with a low response rate to cetuximab [11,12]. Over 90 % of our patients had *PIK3CA* wild-type CRC. In one patient with concomitant *KRAS* G12C and *PIK3CA* exon 20 D1056N mutations, the addition of simvastatin to cetuximab and irinotecan stabilized the disease for 6 months. Notably, this patient had low ras signature.

KRAS mutation status is the first predictor to be applied in the clinic for CRC. However, the search for new biomarkers for anti-EGFR antibody therapy is still ongoing, since up to 50–65 % of patients with *KRAS* wild-type tumors are resistant to EGFR monoclonal antibodies [13,14]. As part of a preplanned biomarker analysis, we analyzed the *RAS* signature score in terms of response to cetuximab/irinotecan and simvastatin, calculated as described previously [6,15]. This score was a significant predictor of sensitivity to MEK inhibition and resistance to AKT inhibition in lung cancer and predicted resistance to cetuximab in CRC [6]. Hence, the *RAS* signature may be a transcriptional readout of *RAS* pathway dependence, reflecting not only *KRAS* mutations but also other potential alternative aberrations such as *BRAF* and/or *PIK3CA* mutations. Although limited by a small sample size, our data demonstrated that *KRAS* mutant CRCs with low ras signature were likely to benefit from cetuximab/irinotecan/simvastatin. In *KRAS* wild-type CRCs, most patients with a high *RAS* signature score were unlikely to respond to cetuximab. However, approximately 70 % of KRAS wild-type CRCs with low signature responded to cetuximab/irinotecan. Since our results are preliminary, we plan to prospectively validate the predictability of ras signature for cetuximab treatment responsiveness in a larger cohort of patients.

In conclusion, the addition of 80 mg simvastatin to cetuximab and irinotecan after failed treatment with oxaliplatin and irinotecan-containing regimens provided tumor stabilization in 63 % of patients with *KRAS* mutant CRCs. The treatment outcome did not differ between the *KRAS* codon 12 and 13 subgroups. The addition of simvastatin did not seem to reverse cetuximab resistance in *KRAS* mutant CRCs with a high *RAS* signature score. and patients with *KRAS* wild-type CRCs with a high *RAS* signature score did not respond well to cetuximab/irinotecan treatment. We plan to validate *RAS* signature scores in a larger patient population and analyze the activated *RAS* pathways in relation to *KRAS*, *PIK3CA*, and *BRAF* mutations and PTEN loss. A randomized, placebo-controlled trial with cetuximab/irinotecan with and without simvastatin should be conducted in patients with *KRAS* mutant CRC, especially those with a low *RAS* signature score.

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