K-Ras Mutations and Treatment Outcome in Colorectal Cancer Patients Receiving Exclusive Fluoropyrimidine Therapy

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Abstract

Purpose: K-Ras mutations predict resistance to anti – epidermal growth factor receptor (EGFR) monoclonal antibodies. Because combinations of anti-EGFR with 5-fluorouracil (5-FU)-based chemotherapy are promising treatments, we analyzed the effect of K-Ras mutations in patients having received exclusive 5-FU therapy.

Experimental Design: This study was conducted on 93 stage IV colorectal cancer patients with unresectable measurable liver metastasis receiving 5-FU-leucovorin (56 men and 37 women; 77 cancer deaths). Liver metastases ($n = 93$) along with primary tumors ($n = 48$) were analyzed for K-Ras mutations (codons 12 and 13), p53 mutations (exons 4-9), p53 polymorphism (codon 72), thymidylate synthase (TS) polymorphism (28-bp repeats including G>C mutation), methylenetetrahydrofolate reductase polymorphism (677C>T, 1298A>C), thymidylate synthase (TS) activity, dihydropyrimidine dehydrogenase activity, folylpolyglutamate synthase activity, and p53 protein expression.

Results: Thirty-six of 93 (38.7%) metastases were K-Ras mutated (30 at codon 12 and 6 at codon 13). Mutated primary tumors (16 of 48) matched perfectly with mutated metastases. The additional analyzed tumor markers were not different between K-Ras mutated and wild-type tumors. The objective response rate was 37%: 44.4% in K-Ras mutated versus 32.1% in wild-type K-Ras metastasis ($P = 0.27$). Low TS activity in metastasis was the only significant predictor of tumor response ($P = 0.047$). K-Ras status did not influence specific survival.

Conclusions: The present data indicate a perfect concordance of K-Ras mutations between primary and liver metastasis and suggest that any predictive and/or prognostic value of K-Ras mutations in treatments combining anti-EGFR monoclonal antibodies with 5-FU should be exclusively linked to the anti-EGFR agent.

Ras proteins are membrane-localized G proteins that function as signal switches that link receptor tyrosine kinase activation to downstream effectors (1). Ras proteins are thus involved in signaling pathways controlling cell proliferation and differentiation. Ras mutations usually lead to constitutive activation of the signaling pathways and are involved in the development of colorectal cancer. Activating K-Ras mutations are found in ~30% of human cancers, mainly in pancreatic, colorectal, endometrial, biliary tract, lung, and cervical cancers (1). The clinical effect of K-Ras mutations has been particularly studied in colorectal cancer. A large multicentric study conducted on 3,439 colorectal cancer patients showed that the presence of a glycine to valine mutation at codon 12 of K-Ras significantly decreased the failure-free and overall survival (2). Importantly, Ras occupies a key position in the epidermal growth factor receptor (EGFR) signaling pathway (3). Several clinical studies have shown that the presence of a K-Ras mutation is a significant predictor of resistance to anti-EGFR therapy in colorectal cancer patients (4 – 7). These studies were conducted on patients receiving cetuximab (4 – 7) or panitumumab (5) given alone (4, 5, 7) or in combination with chemotherapy (4 – 6) consisting in irinotecan for the majority of patients.

5-Fluorouracil (5-FU) still remains a major drug in the treatment of colorectal cancer. Recent results of the Crystal study have shown on 1,200 patients that the 5-FU/leucovorin/irinotecan (FOLFIRI) regimen combined with cetuximab significantly increased the response rate and progression-free survival compared with FOLFIRI alone in advanced colorectal cancer patients (8). From this result, the combination of anti-EGFR therapy with fluoropyrimidine-based chemotherapy represents a promising treatment in colorectal cancer. Interestingly, connections have been suggested between fluoropyrimidine

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Received 11/15/07; revised 1/17/08; accepted 2/10/08.

Grant support: French Research Ministry (Programme Hospitalier de Recherche Clinique).

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doi:10.1158/1078-0432.CCR-07-4906

efficacy and EGFR signaling pathway. A recent study reported that adjuvant treatment with the 5-FU prodrug tegafur, combined with uracil, significantly prolonged survival in lung cancer patients with EGFR wild-type (wt) tumors but not in patients with EGFR mutant tumors (9). In addition, an experimental study by Houghton and colleagues (10) on a thymidylate synthase (TS)-deficient colon cell line showed that mutated K-Ras transfection significantly decreased the ability of the cell to undergo apoptosis in response to thymidine deprivation. Because TS inhibition and subsequent thymidine deprivation is one of the main mechanisms of 5-FU cytotoxicity, we wondered whether K-Ras mutation may influence 5-FU efficacy. These experimental and clinical backgrounds motivated the present study aimed at examining the link between K-Ras mutations and treatment outcome in stage IV colorectal cancer patients receiving exclusive 5-FU–based therapy. We previously reported on a limited group of 56 advanced colorectal cancer patients that K-Ras and p53 mutations were not linked to 5-FU responsiveness (11). We have here expanded this study on a larger group of 93 patients. Moreover, the K-Ras investigation has been coupled with a multivariate biological analysis, including various potential prognostic and/or predictive tumoral markers, so as to compare these markers in mutated and nonmutated K-Ras tumors.

Materials and Methods

Patients. Patient characteristics are presented in Table 1. All exhibited stage IV colorectal cancer with unresectable liver metastases assessable by computed tomography scan. Inclusion criteria excluded patients who had received previous 5-FU–based chemotherapy. A liver metastatic biopsy (mean, 200 mg) was taken at time of laparotomy done either for primary tumor resection or for an unsuccessful attempted removal of the metastasis. In addition, for 48 patients, a biopsy of the primary (mean, 600 mg) was obtained. Tissue samples were immediately frozen and stored in liquid nitrogen until processed. After laparotomy, all patients received chemotherapy combining 5-FU and folinic acid (racemic form) given i.v. Clinical response was assessed (computed tomography scan) 3 mo after the start of chemotherapy according to WHO criteria. The same chemotherapy was maintained until disease progression (liver metastases were never resected).

K-Ras mutation analyses at codons 12 and 13. Mutations at K-Ras codons 12 and 13 (exon 1) were detected by a sensitive enrichment double PCR-RFLP assay, as previously described (12). A first PCR-RFLP was done for introducing restriction sites for *Bst*XI (codon 12 wt specific) or *Xcm*I (codon 13 wt specific; enzymes were from New England Biolabs). Digestion of the generated amplicon was then done with each specific restriction enzyme, which cut only wt amplicons and allowed mutant amplicons to be further amplified. Each digestion product was then submitted to a second PCR-RFLP, which favors amplification of the mutated amplicons.

A second specific digestion by *Bst*XI (codon 12 wt) and *Xcm*I (codon 13 wt) allowed identification of mutated codons 12 and 13. DNA from tumor cell lines, SW620 (mutated at codon 12), LOVO (mutated at codon 13), and WIDR (wt at codons 12 and 13), were used as controls.

p53 polymorphism at codon 72. *p53* codon 72 genotype (Arg>Pro) was analyzed by means of PCR-RFLP, as previously described (13). PCR generates a 279-bp amplicon and the digestion by the restriction endonuclease *Bst*UI (New England Biolabs) results in a single 279-bp band for Pro/Pro variants and in 160- and 119-bp bands for Arg/Arg variants.

Additional tumoral variables. TS activity (tritium release assay), folylpolyglutamate synthetase (FPGS) activity (radioenzymatic assay), dihydropyrimidine dehydrogenase (DPD) activity (radioenzymatic assay), p53 protein (immunoluminometric assay), exons 4 to 9 p53 mutations (denaturing gradient gel electrophoresis), TS genotype (28-bp repeats, PCR analysis), and methylenetetrahydrofolate reductase (MTHFR) genotypes (677C>T and 1298A>C, melting curve analyses) had been analyzed as previously reported (14, 15). The presence of a G→C mutation in the E-box of the second repeat of the 3R allele was investigated (PCR-RFLP; ref. 16). This G→C mutation allows samples to be classified as a function of the number of functional E-box binding sites likely to bind USF proteins (class 2 = 2R2R or 2R3RC or 3RC3RC; class 3 = 2R3RG or 3RC3RG; class 4 = 3RG3RG).

Statistical analysis. Statistics were done on logarithm 10–transformed data for TS, FPGS, and p53. Mean comparisons were done using Student's *t* test or ANOVA. Relationships between categorical variables were assessed by means of Fisher's exact test. K-Ras mutations at codons 12 and 13 were always analyzed both separately and together (i.e., mutation at codon 12 or 13). Relationships between K-Ras mutations or p53 polymorphism with additional tumoral variables were only tested in metastasis to limit the number of statistical tests and thus avoid significant *P* value that may arise by chance due to multiple testing. The two-sided significance level was fixed at 0.05. Specific survival (cancer-related death, excluding chemotherapy-related death), computed from the start of chemotherapy, was analyzed according to the Kaplan-Meier method. The median follow-up for living patients was 47 mo. Survival comparisons between factor levels were done by log-rank tests. In addition, potential survival predictors were analyzed according to the Cox proportional hazard regression model. For stepwise multivariate Cox analysis, the probability for entry and removal was 0.05 and 0.10, respectively. Statistics were done on Statistical Package for the Social Sciences (SPSS) software version 15.0.

Results

K-Ras mutations and tumoral variable description. Among the 93 analyzed metastatic biopsies, 30 exhibited a mutation at K-Ras codon 12 and 6 additional samples were mutated at codon 13, thus accounting for a total of 38.7% mutated samples (36 of 93). Analysis of K-Ras mutations in 48 paired biopsies of primary tumors (16 of 48 mutated samples) showed

Table 1. Patient characteristics

Gender (men/women)	56/37
Age	
Mean	65
Extremes	40-82
Liver metastasis status	
Synchronous/metachronous	82/11
Single/multifocal	9/84
Primary tumor location	
Right colon	14
Transverse colon	1
Left colon	32
Sigmoid	10
Rectum	36
Treatment description	
5-d continuous infusion of 5-FU (350 mg/m^2/d) + FA (200 mg/m^2/d), day 1 = day 28.	66
Weekly 24-h infusion of 5-FU (1,300 mg/m^2) + FA (200 mg/m^2)	8
2-h FA infusion (200 mg/m^2/d) followed by 5-FU bolus (400 mg/m^2/d) and 22-h 5-FU infusion (600 mg/m^2/d) for 2 d, day 1 = day 14	19

Abbreviation: FA, folinic acid.

Table 2. Relationships between the K-Ras mutation status and tumoral variables measured in liver metastases

	wt K-*Ras* at codons 12 and 13	Mutated K-*Ras* at codon 12 or 13	Statistics*
TS activity (fmol/min/mg protein)			
Geometric mean †	129	160	$P = 0.56$
1st-3rd quartile	24-564	34-757	
n	57	36	
DPD activity (pmol/min/mg protein)			
Mean	169	167	$P = 0.90$
1st-3rd quartile	105-230	106-233	
n	55	35	
FPGS activity (fmol/min/mg protein)			
Geometric mean †	1,127	1,062	$P = 0.64$
1st-3rd quartile	725-1,592	736-1,667	
n	57	36	
p53 concentration (ng/mg protein)			
Geometric mean †	0.87	0.53	$P = 0.31$
1st-3rd quartile	0.10-8.51	0.12-2.43	
n	53	34	
p53 mutations			
wt	21	18	$P = 0.59$
Stop mutation	4	2	
Nonstop mutation	30	16	
p53 polymorphism			
Arg/Arg	27	18	$P = 0.96$
Arg/Pro	22	14	
Pro/Pro	8	4	
TS polymorphism (28-bp repeats)			
2R2R	15	5	$P = 0.13$
2R3R	24	14	
3R3R	14	16	
TS polymorphism (taking into account the G>C mutation in the second repeat of the 3R allele)			
Class 2	34	15	$P = 0.056$
Class 3	12	16	
Class 4	3	1	
MTHFR polymorphism (677C>T)			
CC	32	13	$P = 0.18$
CT	16	15	
TT	9	8	
MTHFR polymorphism (1298A>C)			
AA	28	21	$P = 0.76$
AC	21	11	
CC	8	4	

*P value of Student's t test for continuous variable or Fisher's exact test for categorical variables.
† Geometric means are given for variables analyzed as logarithm 10.

the perfect concordance of K-Ras mutations between primary tumor and liver metastasis.

Analyses done in metastatic biopsies showed that neither p53 concentration, TS, FPGS, nor DPD activity was significantly linked to the presence of K-Ras mutations (Table 2). In addition, the presence of K-Ras mutations was not associated with the presence of p53 mutations, p53 polymorphism, MTHFR polymorphisms at positions 677C>T or 1298A>C, nor 28-bp repeat TS polymorphism (Table 2). The presence of a G>C mutation in the E-box of the second repeat of the 3R allele of the *TS* gene was analyzed and no significant relationship was observed between TS class genotype and K-Ras mutations (Table 2).

Relationship with clinical outcome. Clinical response on liver metastasis was assessable for 92 patients. Complete response was observed in 3 patients, partial response in 31 patients, stable disease in 20 patients, and progressive disease in 38 patients, giving an objective response rate of 37% (34 of 92). Tumor responsiveness was not linked to clinical characteristics of metastases (synchronous versus metachronous, single versus multifocal). Response rate was 44.4% in mutated K-Ras metastases versus 32.1% in wt K-Ras metastases ($P = 0.27$; Table 3). p53 polymorphism at codon 72 and 28-bp repeat TS polymorphism including the G>C mutation or not did not influence clinical response (Table 3). The only tumor marker that significantly influenced tumor responsiveness was TS activity measured in metastasis, with lower TS activity in responding patients compared with nonresponding patients ($P = 0.047$; Table 3).

At the time of analysis, 77 patients had died from their disease, 4 had died from other causes, and 12 were alive. Median specific survival was 16.4 months. Patient age and multifocal status of metastasis were not significant survival predictors. Specific survival of patients with synchronous metastases was significantly shorter than that of patients with metachronous metastases (relative risk, 2.33; $P = 0.037$). K-Ras

mutation status did not influence specific survival (Fig. 1). Among tumoral variables measured in metastasis, the only significant variables were TS activity (relative risk for patients with TS activity > median value = 1.60; $P = 0.041$), p53 mutation status (relative risk for patients with stop mutations versus wt = 3.17; global $P = 0.030$), and 1298A>C MTHFR polymorphism (relative risk for CC versus AA = 2.37; global $P = 0.006$). A multivariate Cox analysis showed that independent significant prognostic factors were MTHFR polymorphism ($P = 0.004$), p53 mutation status ($P = 0.013$), and TS activity ($P = 0.014$).

Discussion

Current chemotherapy protocols in advanced colorectal cancer include 5-FU-leucovorin, oxaliplatin, irinotecan, cetuximab, or bevacizumab. As first-line therapy, combinations of 5-FU/leucovorin/oxaliplatin (FOLFOX) or FOLFIRI are considered as standards (17). Cetuximab has shown activity in combination with chemotherapy in both first- and second-line therapy (8, 18). In addition, it has been recently shown that cetuximab combined with 5-FU–based therapy (FOLFIRI) was superior to FOLFIRI alone in metastatic colorectal cancer patients (8). Although treatment of colorectal cancer is becoming increasingly complex with this panel of drugs, fluoropyrimidines still occupy a central place. As recently stressed by Mayer (19), adding oxaliplatin or irinotecan plus targeted therapies to infusional 5-FU-leucovorin has prolonged the median survival from 12 to >20 months. Thus, the part of survival benefit brought by 5-FU-leucovorin remains significant among the current combination schedules.

Recent data on patients receiving anti-EGFR treatment have shown that the presence of a K-Ras mutation allows unresponsive patients to be identified (4–7). Of note, the predictive value of K-Ras mutations was observed in patients receiving cetuximab associated with chemotherapy (4–6), whereas the intrinsic activity of cetuximab alone is rather low in colorectal cancer, with ~10% objective response rate (20). Although combinations between anti-EGFR treatment and chemotherapeutic agents have proven to be supra-additive based on preclinical data (21, 22), it is hard to understand that K-Ras mutation may have such an influence on treatment outcome combining cetuximab and chemotherapy. It could be hypothesized that K-Ras mutation may also influence chemotherapy outcome, irrespective of the administered drug. The influence of K-Ras mutation in chemotherapy efficacy has not been widely studied. Experimental data have shown that mutation of K-Ras in TS-deficient colon carcinoma cells significantly decreases the ability of these cells to commit apoptotic death (10). This raised the possibility that K-Ras mutations may reduce the capacity of cells to respond to chemotherapeutic agents targeting TS protein, such as 5-FU. The present study was thus exclusively centered on 5-FU-leucovorin therapy administered on a homogeneous group of 93 advanced colorectal cancer patients with measurable liver metastases. The major result is that the presence of a K-Ras mutation at codon 12 or 13

Table 3. Analysis of ras mutation, p53 polymorphism, TS polymorphism, and TS activity measured in metastasis as a function of clinical response

	Responders (CR + PR), *n* (% response rate)	Nonresponders (SD + PD), *n* (% nonresponse)	Statistics*
Ras mutation at codon 12			
wt	20 (32.2)	42 (67.8)	$P = 0.25$
Mutated	14 (46.7)	16 (53.3)	
Ras mutation at codon 13			
wt	32 (37.2)	54 (62.8)	$P = 1.00$
Mutated	2 (33.3)	4 (66.7)	
Ras mutation at codon 12 or 13			
wt	18 (32.1)	38 (67.9)	$P = 0.27$
Mutated	16 (44.4)	20 (55.6)	
p53 polymorphism			
Arg/Arg	13 (29.5)	31 (70.5)	$P = 0.36$
Arg/Pro	15 (41.7)	21 (58.3)	
Pro/Pro	6 (50)	6 (50)	
TS polymorphism (28-bp repeats)			
2R2R	10 (50)	10 (50)	$P = 0.36$
2R3R	14 (37.8)	23 (62.2)	
3R3R	9 (30)	21 (70)	
TS polymorphism (including G>C)			
Class 2 (2R2R or 2R3RC or 3RC3RC)	17 (35.4)	31 (64.6)	$P = 0.73$
Class 3 (2R3RG or 3RC3RG)	12 (42.9)	16 (57.1)	
Class 4 (3RG3RG)	1 (25)	3 (75)	
TS activity (fmol/min/mg protein)			
Mean	268	664	$P = 0.047$
Median	91	242	
Extremes	0-2,864	0-9,796	
n	34	58	

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
*Fisher's exact test for categorical variable or Student's *t* test for quantitative variable (on logarithm 10–transformed data for TS activity).

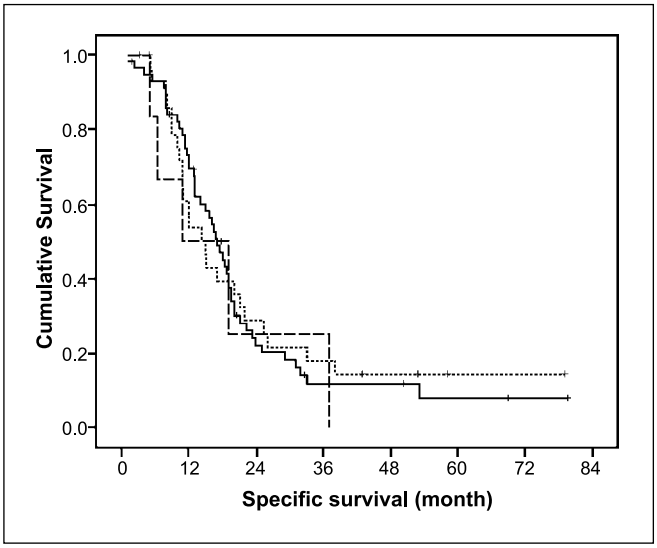

Fig. 1. Plot of cumulative specific survival according to K-Ras mutations measured in metastasis. ——, wt tumor (57 patients; 48 events; median survival, 16.9 mo); - - - - -, mutation at codon 12 (30 patients; 24 events; median survival, 14.3 mo); – – – – –, mutation at codon 13 (6 patients; 5 events; median survival, 10.8 mo). Log-rank test comparison of wt versus mutation at codon 12 versus mutation at codon 13 gave a *P* value at 0.89; wt versus mutation at codon 12 or 13 gave a *P* value at 0.95.

clearly does not influence the objective response rate (44.4% versus 32.1% in mutated and wt K-Ras, respectively) in patients receiving exclusive 5-FU-leucovorin protracted infusion schedules. This observation concords well with the fact that distribution of tumoral markers of 5-FU activity, such as TS activity (23), TS gene polymorphism (24), DPD activity (25), FPGS activity (26), MTHFR gene polymorphism (15), and p53 status (27), was similar in mutated K-Ras and nonmutated K-Ras metastases (Table 2). In line with numerous clinical data having shown a predictive role of TS in 5-FU–based therapy (28), the only significant predictor of 5-FU responsiveness in the present study was TS activity (Table 3).

The previous RASCAL study including >3,000 colorectal cancer patients showed that K-Ras mutation was significantly associated with decreased failure-free and overall survival, irrespective of the administered treatment (2). Among other survival predictors, we analyzed whether the presence of a K-Ras mutation was a prognostic marker. K-Ras mutations at codon 12 or 13 did not influence specific survival on the present population. Given the experimental data from Houghton and colleagues (10) reporting the effect of K-Ras mutation on apoptosis induced by thymineless stress, we further analyzed the influence of K-Ras mutation after adjustment on TS activity measured in metastasis (TS > or ≤ median value) and observed no significant influence of K-Ras mutation on survival (unshown data). The significant independent survival predictors arising from the multivariate analysis were the TS activity, MTHFR polymorphism, and p53 stop mutation status. These results concur well with previous clinical data (29–31).

The treatment target of advanced colorectal cancer being in most cases distant metastases, it was relevant to examine whether the K-Ras status provided by the primary tumor, usually available, concurs well with that of the metastasis on which treatment is applied and for which biopsy is not systematically available. Literature data are controversial about the concordance of K-Ras mutations between primary and matched metastases, some authors reporting the absence of concordance (32) whereas others reporting perfect similarity (33). The present study clearly indicates an absolute concordance for K-Ras mutation status between the primary lesion and the liver metastasis.

In total, the present data are of clinical significance in the context of EGFR targeted therapy in advanced colorectal cancer. They suggest that any predictive and/or prognostic value of K-Ras mutations in treatments combining anti-EGFR monoclonal antibodies with 5-FU–based therapy should be exclusively linked to the presence of the anti-EGFR agent. This statement is particularly relevant in the context of the current protocols administered in colorectal cancer that combine FOLFIRI regimen with either cetuximab (8) or bevacizumab (34). The fact that the presence of a K-Ras mutation does not impair 5-FU efficacy, while predicting resistance to anti-EGFR therapies, could indicate that patients with a K-Ras mutated tumor are more likely to benefit from the bevacizumab-FOLFIRI association. The K-Ras mutation status may thus represent a promising marker for guiding the choice of the targeted therapy associated with 5-FU–based chemotherapy in advanced colorectal cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer 2007;7:295–308.
2. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. Br J Cancer 2001;85:692–6.
3. Bianco R, Melisi D, Ciardiello F, Tortora G. Key cancer cell signal transduction pathways as therapeutic targets. Eur J Cancer 2006;42:290–4.
4. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66:3992–5.
5. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 2007;67:2643–8.
6. Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. Br J Cancer 2007;96:1166–9.
7. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007;25:3230–7.
8. Van Cutsem E, Nowacki M, Lang I, et al. Randomized phase III study of irinotecan and 5FU/FA with or without cetuximab in the first-line treatment of patients with metastatic colorectal cancer: The CRYSTAL study [abstract 4000]. Proc Am Soc Clin Oncol 2007;25:4000.
9. Suehisa H, Toyooka S, Hotta K, et al. Epidermal growth factor receptor mutation status and adjuvant chemotherapy with uracil-tegafur for adenocarcinoma of the lung. J Clin Oncol 2007;25:3952–7.
10. Houghton JA, Ebanks R, Harwood FG, Tillman DM. Inhibition of apoptosis after thymineless stress is conferred by oncogenic K-Ras in colon carcinoma cells. Clin Cancer Res 1998;4:2841–8.
11. Rosty C, Chazal M, Etienne MC, et al. Determination of microsatellite instability, *P53* and K-Ras mutations in hepatic metastases from patients with colorectal cancer: relationship with response to 5-fluorouracil and survival. Int J Cancer 2001;95:162–7.
12. Schimanski CC, Linnemann U, Berger MR. Sensitive detection of K-ras mutations augments diagnosis of colorectal cancer metastases in the liver. Cancer Res 1999;59:5169–75.

13. Xu Y, Yao L, Ouyang T, et al. p53 codon 72 polymorphism predicts the pathologic response to neoadjuvant chemotherapy in patients with breast cancer. Clin Cancer Res 2005;11:7328–33.

14. Etienne MC, Chazal M, Laurent-Puig P, et al. Prognostic value of tumoral thymidylate synthase and p53 in metastatic colorectal cancer patients receiving fluorouracil-based chemotherapy: phenotypic and genotypic analyses. J Clin Oncol 2002;20:2832–43.

15. Etienne MC, Formento JL, Chazal M, et al. Methylenetetrahydrofolate reductase gene polymorphisms and response to fluorouracil-based treatment in advanced colorectal cancer patients. Pharmacogenetics 2004;14:785–92.

16. Largillier R, Etienne-Grimaldi MC, Formento JL, et al. Pharmacogenetics of capecitabine is related to clinical outcome in advanced breast cancer patients. Clin Cancer Res 2006;12:5496–502.

17. Grothey A. Is there a third-line therapy for metastatic colorectal cancer? Semin Oncol 2006;33:S36–38.

18. Board RE, Valle JW. Metastatic colorectal cancer: current systemic treatment options. Drugs 2007;67: 1851–67.

19. Mayer RJ. Should capecitabine replace infusional fluorouracil and leucovorin when combined with oxaliplatin in metastatic colorectal cancer? J Clin Oncol 2007;25:4165–7.

20. Saltz LB, Meropol NJ, Loehrer PJ, Sr., Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 2004;22:1201–8.

21. Tortora G, Gelardi T, Ciardiello F, Bianco R. The rationale for the combination of selective EGFR inhibitors with cytotoxic drugs and radiotherapy. Int J Biol Markers 2007;22:S47–52.

22. Magné N, Fischel JL, Dubreuil A, et al. Sequence-dependent effects of ZD1839 ('Iressa') in combination with cytotoxic treatment in human head and neck cancer. Br J Cancer 2002;86:819–27.

23. Berg RW, Ferguso PJ, DeMoor JM, et al. The means to an end of tumor cell resistance to chemotherapeutic drugs targeting thymidylate synthase: shoot the messenger. Curr Drug Targets 2002;3:297–309.

24. Lenz HJ. Pharmacogenomics and colorectal cancer. Adv Exp Med Biol 2006;587:211–31.

25. van Kuilenburg AB. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. Eur J Cancer 2004;40:939–50.

26. Chazal M, Chéradame S, Formento JL. Decreased folylpolyglutamate synthetase activity in tumors resistant to fluorouracil-folinic acid treatment: clinical data. Clin Cancer Res 1997;3:553–7.

27. Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003;3:330–8.

28. Rustum YM. Thymidylate synthase: a critical target in cancer therapy? Front Biosci 2004;9:2467–73.

29. Russo A, Bazan V, Iacopetta B, et al. The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of p53 mutation: influence of tumor site, type of mutation, and adjuvant treatment. J Clin Oncol 2005;23:7518–28.

30. Grem JL. Intratumoral molecular or genetic markers as predictors of clinical outcome with chemotherapy in colorectal cancer. Semin Oncol 2005;32:120–7.

31. Duffy MJ, van Dalen A, Haglund C, et al. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. Eur J Cancer 2007;43:1348–60.

32. Albanese I, Scibetta AG, Migliavacca M, et al. Heterogeneity within and between primary colorectal carcinomas and matched metastases as revealed by analysis of Ki-ras and p53 mutations. Biochem Biophys Res Commun 2004;325:784–91.

33. Zauber P, Sabbath-Solitare M, Marotta SP, Bishop DT. Molecular changes in the Ki-ras and APC genes in primary colorectal carcinoma and synchronous metastases compared with the findings in accompanying adenomas. Mol Pathol 2003;56:137–40.

34. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350:2335–42.