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An EGFR Pathway-Oriented Pharmacogenetic Study in Metastatic Colorectal Cancer Patients Receiving Panitumumab and Irinotecan

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Abbreviations: AEs: Adverse Events; CRC: Colorectal Cancer; mCRC: Metastatic Colorectal Cancer; EGFR: Epidermal Growth Factor Receptor; NCICTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events; OR: Odds Ratio; OS: Overall Survival; PFS: Progression-Free Survival; RR: Relative Risk; SNP: Single Nucleotide Polymorphism; Wt: Wild-type.

ABSTRACT

We tested the predictive value of gene polymorphisms potentially linked to the pharmacodynamics of panitumumab and irinotecan.

Methods: 45 patients with metastatic CRC refractory to standard therapy were enrolled. Inclusion required wild-type KRAS tumor status (codons 12-13). Patients received panitumumab (6 mg/kg, day 1) associated with irinotecan (180 mg/m², day 1), Q2W until disease progression or unacceptable toxicity. Analyzed polymorphisms on blood DNA were EGFR (CA repeats in intron 1, -216G>T, -191C>A), EGF (61A>G), CCND1 (870A>G), UGT1A1 (*28).

Results: Cutaneous toxicity imputable to panitumumab was not linked to EGFR, EGF or CCND1 polymorphisms. Diarrhoea (no grade 4) was linked to CCND1 870G allele: 22% grade 2-3 in AA patients vs 63% in AG+GG patients (p=0.007). Neutropenia was more frequent in patients bearing the *28 allele of UGT1A1 gene (33% grade 2-3) vs 5% in *1/*1 patients (p=0.027). RAS mutation status was significantly related to the EGFR intron 1 CA-repeats polymorphism, with a higher RAS mutation rate in patients with both alleles ≥ 17 (63.6%).

Conclusions: The relationship between EGFR polymorphism in intron 1 and RAS mutation status merits further confirmation on a larger set of patients and suggests a new implication of EGFR intron 1 polymorphism.

INTRODUCTION

The epidermal growth factor receptor (EGFR) is a validated therapeutic target, and two monoclonal antibodies, cetuximab and panitumumab that target EGFR, have been approved in metastatic colorectal cancer (mCRC) [1-3]. In the pivotal BOND-1 study, the combination of cetuximab and irinotecan in patients with mCRC refractory to previous irinotecan-containing regimens gave

an objective response rate of 22.9% versus 10.8% with cetuximab alone, and a hazard ratio for progression-free survival (PFS) at 0.54 in favor of the combination therapy, suggesting that cetuximab could restore irinotecan sensitivity [2]. An exploration of response predictors revealed the dramatic role of KRAS mutation status as a negative predictive biomarker [4]. However, fewer than 20% of patients with KRAS codon 12-13 wild-type (wt) tumors will respond to cetuximab or panitumumab monotherapy, suggesting that other genetic or biologic alterations may also have a predictive role [5,6]. Among them, mutations in downstream effectors of the EGFR pathway have been shown to be associated with drug resistance [7]. A multicenter, phase II study recently evaluated the safety and efficacy of irinotecan-panitumumab combination in 65 heavily-pretreated mCRC patients with wt KRAS codon 12-13 tumors [8]. In complement to clinical data, translational research aimed at analyzing genetic alterations at both somatic and germinal levels was planned. A central re-assessment of full RAS status including rare KRAS and NRAS mutations, along with BRAF mutation V600E, was thus conducted on 60 available tumors [8]. In the 19 patients with either RAS-mutated, or BRAF-mutated tumors, no response was observed. In the remaining 41 patients with wt KRAS, NRAS, and BRAF tumors, objective response rate was 46.3%, PFS was 8.7 months and overall survival was 15.8 months. These data at the somatic level were published together with the clinical results [8]. In the last decade, pharmacogenetic studies have been performed in different areas including cancer [9]. The concrete applications of pharmacogenetic data to the clinical field have not met expectations. A complementary part of this translational research was thus dedicated to a pharmacogenetic study in order to evaluate the role of host genome variations in the pharmacodynamics of irinotecan-panitumumab therapy. The purpose of the present observational pilot ancillary study was to describe this pharmacogenetic analysis, with particular attention paid to EGFR pathway, mainly EGFR itself, its ligand EGF and the key EGFR-pathway regulated gene CCND1. Cyclin D1 is an important downstream effector of EGFR signaling that regulates cell cycle [10]. Of importance, long CA-repeats in intron 1 of EGFR gene have been shown to be linked with low EGFR expression [11].

MATERIALS AND METHODS

Patients

Forty-five patients with pathologically-confirmed mCRC refractory to standard therapy were enrolled in this ancillary pharmacogenetic study as part of a phase II, single-arm, multicenter trial that included 65 patients (PIMABI trial ID NCT 00655499, GERCOR) [8]. Inclusion criteria required wt KRAS codon12-13 status (analyses performed in each center, before extension to additional KRAS and NRAS rare mutations). Patients received panitumumab (6 mg/kg, 60-min IV infusion, day 1) associated with irinotecan (180 mg/m², 90-min IV infusion, day 1) until disease progression or unacceptable toxicity (day 1=day 14). The study protocol was approved by an ethics committee and all patients provided written informed consent for research participation. Adverse events (AEs) were collected during the treatment period and safety follow up phases and were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v3.0.

RAS Mutation Analysis

After inclusion, a central RAS re-assessment was performed in all eligible patients previously identified in each center as wt KRAS at codon 12-13. This screening included KRAS mutations at codons 12, 13, 59, 61, 117, 146 and NRAS mutations at codons 12, 13, 61. KRAS mutations at codon 12-13 were analyzed with TaqMan probes and additional KRAS and NRAS mutations were screened using direct sequencing of short amplicons [8]. Among these 45 patients, 12 harbored a RAS mutation: 5 patients exhibited a KRAS mutation at codon 12 (one G12C, 3 G12D, one G12V), and 7 patients displayed additional mutations: one KRAS A59T, 2 KRAS Q61H, one KRAS Q61K, one NRAS G13C, one NRAS Q61L and one NRAS Q61R.

Gene Polymorphism Analysis

Constitutional gene polymorphisms were analyzed on blood DNA. The CA-repeats polymorphism in intron 1 of EGFR gene (rs 11568315) was analyzed by fragment length analysis [12]. Due to the large number of genotypes (between 15 and 22 CA repeats), and according to our previous work, patients were split into 3 groups: patients with both alleles <17 vs patients with both alleles ≥ 17 vs others. EGFR -216G>T (rs 712829), EGFR -191C>A (rs 712830), EGF 61A>G (rs 4444903) and CCND1 870A>G (rs 9344) gene polymorphisms were analyzed by PCR-RFLP methods [12,13]. After PCR amplification, the TA-repeats in the UGT1A1 gene promoter were analyzed on Beckman-Coulter CEQ8000 sequencer [14]. The UGT1A1 *28 variant (rs 8175347) corresponds to the (TA)7 sequence while UGT1A1 *1 (wild-type allele) corresponds to the (TA)6 sequence. For UGT1A1 genotyping, DNA samples from 3 patients with known TA6/TA6, TA6/TA7 and TA7/TA7 were used as controls.

Statistics

With the exception of the EGFR intron 1 polymorphism considered as a ternary categorical variable (i.e. patients carrying both alleles with less than 17 CA-repeats vs patients carrying both alleles with ≥ 17 CA-repeats vs other patients), all other SNPs (single nucleotide polymorphism) were considered as binary variables (i.e. rare homozygous plus heterozygous genotypes vs wt/wt genotype). Due to the limited number of patients, Fisher's exact tests were applied to test associations between categorical variables, including linkage disequilibrium analyses. For estimation of odds ratio (OR) associated with toxicity, a logistic model was applied for modeling the occurrence of toxicity (0=no toxicity, 1=toxicity). The possible influence of genotypes on progression-free and overall survival (OS) was examined by means of Log Rank test, adjusted or not on RAS mutation status. Statistics were performed on SPSS software (v15.0).

RESULTS

Patient Characteristics

Characteristics of the 45 patients are detailed in **Table 1**. Median age was 60 years old (extremes 34-82). Median follow-up was 21 months. Complete response was observed in 3 patients, partial response in 13 patients, stable disease in 19 patients and progression in 9 patients, accounting for an objective response rate of 36.4% (one patient not assessable). Median progression-free survival (PFS) was 6.9 months (42 events) and median overall survival was 14.8 months (27 deaths). Re-assessed RAS status was a significant predictor of response rate (0% response in mut RAS vs 50% in wt RAS, $p=0.002$), progression-free survival (median 2.5 and 7.9 months in mut and wt RAS, respectively, $p<0.001$) and overall survival (median 4.5 and 18.2 months in mut and wt RAS, respectively, $p=0.048$). Treatment tolerance is described in **Table 2**, with no serious adverse event, no toxic death and no grade 4 toxicity. Cutaneous toxicity was not related to responsiveness. Toxicity was not related to patient gender.

Table 1. Patient and tumor characteristics.

		N	%
Sex	Male	26	57.8
	Female	19	42.2
WHO performance status	0	21	46.7
	1	19	42.2
	2	5	11.1
Primary tumor	Colon	28	62.2
	Rectum	15	33.3
	Both	2	4.4
Number of metastatic sites	1	33	73.3
	>1	12	26.7
Adjuvant chemotherapy	No	30	66.7
	Yes	15	33.3
1 st line chemotherapy	Oxaliplatin-based	29	64.0
	Irinotecan-based	14	31.1
	Oxaliplatin + Irinotecan	1	2.2
	Other	1	2.2
2 nd line chemotherapy	No second line	7	15.6
	Oxaliplatin-based	8	17.8
	Irinotecan-based	30	66.6
Prior treatment with bevacizumab	No	7	15.6
	Yes	38	84.4
RAS status at time of central re-assessment	Full RAS wt	33	73.3
	KRAS G12C	1	2.2
	KRAS G12D	3	6.7
	KRAS G12V	1	2.2
	KRAS A59T	1	2.2
	KRAS Q61H	2	4.4
	KRAS Q61K	1	2.2
	NRAS G13C	1	2.2
	NRAS Q61L	1	2.2
	NRAS Q61R	1	2.2

Table 2. Treatment Tolerance.

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Folliculitis	8	12	16	9	0
Paronychia	33	4	5	3	0
Diarrhea	12	12	13	8	0
Neutropenia	30	6	4	5	0
Mucositis	45	0	0	0	0

Note: Toxicities were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v3.0. (M aximum observed toxicity grade* at any cycle, N=45).

Gene Polymorphism Analysis and Relationships with RAS Mutation Status

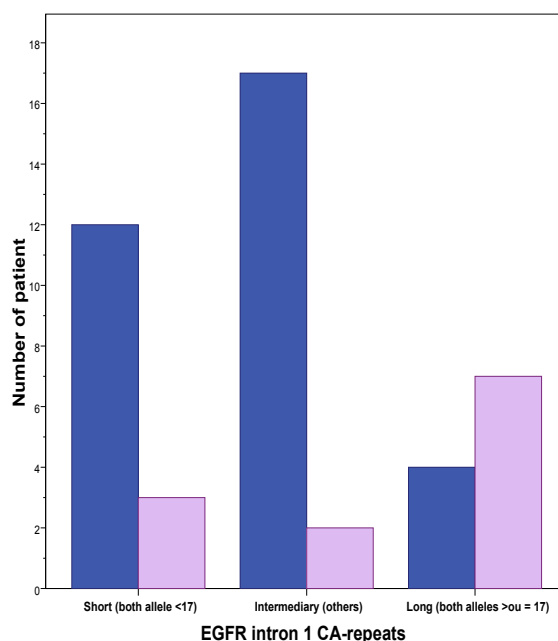
Table 3 shows the frequencies of analyzed gene polymorphisms. For -216G>T EGFR genotype, observed frequencies did not agree with those predicted by the Hardy-Weinberg equilibrium (Exact p value=0.007). As concerns EGFR gene, linkage disequilibriums were observed between -191C>A polymorphism and -216G>T (-216G associated with -191A, $p=0.03$) as well as between -191C>A and intron 1 polymorphism (short CA-repeats associated with -191C, $p<0.001$). No association was observed between other analyzed genotypes involved in the EGFR pathway (EGFR, EGF, CCND1).

Table 3. Gene polymorphism frequencies.

Gene	Polymorphism	Genotype	N	%
EGFR	CA-repeats in intron 1 (rs 11568315)	Both alleles <17	15	33.3
		Intermediary alleles	19	42.2
		Both alleles ≥ 17	11	24.4
	-216 G>T (rs 712829)	GG	17	37.8
		GT	28	62.2
		TT	0	0
-191 C>A (rs 712830)	CC	33	73.3	
	CA	11	24.4	
	AA	1	2.2	
EGF	61 A>G (rs 4444903)	AA	16	35.6
		AG	20	44.4
		GG	9	20.0
CCND1	870 A>G (rs 9344)	AA	18	40.0
		AG	19	42.2
		GG	8	17.8
UGT1A1	TA-repeats (rs 8175347)	*1/*1 (TA ₆ /TA ₆)	20	44.4
		*1/*28 (TA ₆ /TA ₇)	21	46.7
		*28/*28 (TA ₇ /TA ₇)	3	6.7

Interestingly, the frequency of RAS mutation was higher in patients with long CA repeats in EGFR gene intron 1. The RAS mutation rate was 63.6% (7/11) in patients with both alleles ≥ 17 vs 10.5% (2/19) in patients with intermediary alleles vs 20% (3/15) in patients with both alleles <17 (p=0.009, **Graph 1**). Limiting the analysis to KRAS mutations gave similar results, with mutation rates at 54.5% (6/11) in patients with both alleles ≥ 17 vs 10.5% (2/19) in patients with intermediary alleles vs 6.7% (1/15) in patients with both alleles <17 (p=0.010). Other genotypes involved in the EGFR pathway (EGFR, EGF, CCND1) were not linked to RAS mutation status.

Frequency distribution of RAS-mutated tumors (mut RAS, i.e. KRAS codons 12, 13, 59, 61, 117, 146 and NRAS codons 12, 13, 61) versus wild-type RAS (wt RAS) according to EGFR intron 1 polymorphism (number of CA-repeats). RAS mutation rate was 63.6% (7/11) in patients with both alleles ≥ 17 versus 20.0% (3/15) in patients with both alleles <17 versus 10.5% (2/19) in the remaining patients with intermediary alleles. Fisher's exact test gave a p value of 0.009. The nature of the identified mutation for each mutated tumor is noted in the "mut RAS" bar (**Graph 1**).



Graph 1. Frequency distribution of RAS-mutated tumors versus wild-type RAS according to EGFR intron 1 polymorphism.

Pharmacogenetic-Pharmacodynamic Relationships

Toxicity due to the absence of grade 4 toxicity and to the low frequencies of grade 3 relationships between genotypes and each toxicity pattern (folliculitis, paronychia, diarrhea, neutropenia, mucositis) was tested by considering both grade 3-4 and grade 2-3-4. So doing, cutaneous toxicity imputable to panitumumab, i.e. folliculitis and paronychia, was not linked to EGFR, EGF or CCND1 polymorphisms. Diarrhea was linked to CCND1 polymorphism, with 63.0% grade 2-3-4 in AG+GG patients vs 22.2%

in AA patients (OR=5.95, 95% CI 1.53-23.1, $p=0.014$). As expected, neutropenia was more frequent in patients bearing the *28 allele of UGT1A1 gene (33.3% grade 2-3-4 in patients bearing the *28 allele vs 5.0% in *1/*1 patients, OR=9.5, 95% CI 1.1-84.3, $p=0.027$). In contrast, diarrhea was not linked to UGT1A1 polymorphism.

Analyses restricted to the 33 patients with a wt RAS tumor did not reveal any significant relationship between analyzed gene polymorphisms and either responsiveness, PFS or OS. A tendency for a longer OS in patients bearing the *28 allele of UGT1A1 gene (median 25.0 months) relative to *1/*1 patients (median 14.8 months) was observed ($p=0.101$). Analyses performed on the entire group of 45 patients, with adjustment on RAS mutation status, did not reveal further relationships (UGT1A1, $p=0.093$).

DISCUSSION

Studies including the PRIME phase III trial comparing FOLFOX4 vs FOLFOX4-panitumumab as first-line therapy for mCRC have underscored the importance of RAS testing for selecting mCRC patients likely to benefit from anti-EGFR monoclonal antibody therapy [15,16]. Moreover, the PIMABI phase II trial has shown that the panitumumab-irinotecan combination appears to be an interesting treatment option in patients with mCRC resistant to standard chemotherapy, with 46% objective response rate and close to 16 months OS in patients with full wt RAS tumors, reinforcing the need for full RAS screening in these patients [8]. Treatment efficacy and toxicity may also be related to host characteristics. Pharmacogenetics complements pharmacogenomics by taking into account a degree of variability imputable to the host [17]. A second goal of PIMABI ancillary analyses was thus to explore DNA germinal polymorphisms. We herein analyzed polymorphisms of genes relevant for irinotecan (UGT1A1) and panitumumab (EGFR, EGF, CCND1), with a special focus on the possible links between the analyzed parameters. To our knowledge, such relationships have been seldom examined in the literature. However, since the number of patients presently studied was limited (N=45), this ancillary observational study must be considered as a pilot study. However, it should be mentioned that, in this sub-population of 45 patients, treatment outcome was comparable to that of the full PIMABI study group.

More than 30 clinical studies have analyzed the relationships between irinotecan-related toxicity and UGT1A1*28 polymorphism, some studies reporting significant links whereas others do not. Moreover, two meta-analyses gathering 821 and 1998 patients, respectively [18, 19], have demonstrated that the frequency of hematotoxicity grade 3-4 is significantly higher in homozygous *28/*28 patients relative to others, with an OR at 3.2 and at 4.8 for intermediary irinotecan dose (150-250 mg/m²) such as that administered in the present study [18,19]. Accordingly, in the present study, 33.3% of patients bearing the *28 allele developed grade 2-3-4 neutropenia as compared to 5% in patients homozygous for the common allele (*1/*1), with an OR at 9.5 (95% CI 1.1-84.3). In contrast, diarrhea was not linked to UGT1A1 *28 polymorphism but to cyclin D1 (CCND1) polymorphism. In fact, we presently observed 63% grade 2-3-4 diarrhea in CCND1 870AG/GG patients as compared with 22% in 870AA patients (OR=5.95, 95% CI 1.5-23.1). Experimental studies have reported that the CCND1 870A>G polymorphism impacts the cyclin D1 half-life [20]. Numerous case-control studies conducted on large cohorts of patients have reported that CCND1 870A allele is a low-penetrant risk factor of CRC (colorectal cancer), especially for rectal cancer in Caucasian populations [21,22]. Reports in the literature for a role of this gene polymorphism in the pharmacodynamics of anticancer agents are scarce. A recent study conducted on 498 stage II-III colon cancer patients reported that CCND1 870A allele was significantly associated with shorter time to recurrence in patients treated with 5FU-based therapy, whereas no influence was demonstrated in patients treated with curative surgery alone [23]. Additionally, more basic investigations are encouraged so as to clarify the possible interactions between irinotecan-panitumumab and CCND1 polymorphisms.

As RAS is a key molecular step in the EGFR signaling pathway, we felt it relevant to examine the relationship between RAS mutation status and EGFR gene polymorphism. In our opinion, the original result from the present pharmacogenetic pilot study is the link between intron 1 EGFR gene polymorphism and full RAS mutation status. Interestingly, the RAS mutation rate including rare KRAS and NRAS mutations was more than 4-fold higher ($p=0.009$) in patients bearing two EGFR alleles with long CA-repeats in intron 1 (63.6%) as compared to others (14.7%). An explanation for this observation may lie in tumorigenesis phenomena which are mutually exclusive when considering cancer causality. Given the fact that long CA-repeats in intron 1 have been shown to be associated with low EGFR tumor expression, it can be hypothesized that tumors with long EGFR CA-repeats, and thus low EGFR expression, could be prone to be RAS-mutated [11,24]. Such a hypothesis is consistent with the carcinogenicity of the EGFR pathway and the fact that oncogenic factors often exclude each other. For instance, EGFR mutations and RAS mutations are mutually exclusive in lung cancer, as are RAS and BRAF mutations in CRC [25,26]. Interestingly, EGFR CA-repeats gene polymorphism on intron 1 has recently been associated with a risk of lung cancer [27]. The present original observation merits further confirmation and may suggest a new role for EGFR intron 1 polymorphism, with a possible relationship with CRC risk.

CONCLUSIONS

In total, the present pharmacogenetic pilot study provides new information relative to the combination of irinotecan and panitumumab in mCRC. The importance of UGT1A1 *28 polymorphism regarding the risk of neutropenia related to irinotecan is confirmed and a role of CCND1 870A>G polymorphism in the occurrence of diarrhea is strongly suggested. The new and original observation of a relationship between EGFR germinal polymorphism at intron 1 and full RAS mutation rate at the tumor level points to the need for a more thorough evaluation of this germinal trait as a possible risk factor of CRC. The present findings need to be confirmed on a larger set of patients and we hope they will stimulate further research into this topic.

Competing interests

G rard Milano has received honorarium from AMGEN.

REFERENCES

1. Chibaudel B, et al. Therapeutic strategy in unresectable metastatic colorectal cancer. *Ther Adv Med Oncol*. 2012;4:75-89.
2. Cunningham D, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med*. 2004;351:337-345.
3. Jonker DJ, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med*. 2007;357:2040-2048.
4. Li vre A, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol*. 2008;26:374-379.
5. Amado RG, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008;26:1626-1634.
6. Karapetis CS, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*. 2008;359:1757-1765.
7. De Roock W, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer:a retrospective consortium analysis. *Lancet Oncol*. 2010;11:753-762.
8. Andr  T, et al. Panitumumab combined with irinotecan for patients with KRAS wild-type metastatic colorectal cancer refractory to standard chemotherapy:a GERCOR efficacy, tolerance, and translational molecular study. *Ann Oncol*. 2013;24:412-419.
9. Garcia-Gonzalez X, et al. Clinical implementation of pharmacogenetics. *Drug Metabol Personal Ther*. 2016.
10. Brandt B, et al. Mechanisms of egfr gene transcription modulation:relationship to cancer risk and therapy response *Clin Cancer Res*. 2006;12:7252-7260.
11. Gebhardt F, et al. Modulation of epidermal growth factor receptor gene transcription by a polymorphic dinucleotide repeat in intron 1. *J Biol Chem*. 1999;274:13176-13180.
12. Etienne-Grimaldi MC, et al. Analysis of the dinucleotide repeat polymorphism in the epidermal growth factor receptor (EGFR) gene in head and neck cancer patients. *Ann Oncol*. 2005;16:934-941.
13. Dahan L, et al. Pharmacogenetic profiling and cetuximab outcome in patients with advanced colorectal cancer. *BMC Cancer*. 2011;11:496.
14. Inoue Y, et al. Irinotecan cytotoxicity does not necessarily depend on the UGT1A1 polymorphism but on fluoropyrimidine: a molecular case report. *Oncol Rep* 2006; 16:971-974.
15. Douillard JY, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med*. 2013;369:1023-1034.
16. Wang J, et al. Q-TWiST analysis of panitumumab plus FOLFOX4 versus FOLFOX4 alone in patients with previously untreated wild-type RAS metastatic colorectal cancer. *Curr Med Res Opin*. 2016;1:1-7.
17. Ivanov M, et al. Epigenomics and interindividual differences in drug response. *Clin Pharmacol Ther*. 2012;92:727-736.
18. Hoskins JM, et al. UGT1A1*28 genotype and irinotecan-induced neutropenia: dose matters. *J Natl Cancer Inst*. 2007;99:1290-1295.
19. Liu X, et al. Association of UGT1A1*28 polymorphisms with irinotecan-induced toxicities in colorectal cancer:a meta-analysis in Caucasians. *Pharmacogenomics J*. 2014;14:120-129.
20. Betticher DC, et al. Alternate splicing produces a novel cyclin D1 transcript. *Oncogene* 1995;11:1005-1011.
21. Yang J, et al. CCND1 G870A polymorphism is associated with increased risk of colorectal cancer, especially for sporadic colorectal cancer and in Caucasians: a meta-analysis. *Clin Res Hepatol Gastroenterol*. 2012;36:169-177.
22. Chen B, et al. Cyclin D1 (CCND1) G870A gene polymorphism is an ethnicity-dependent risk factor for digestive tract cancers:a meta-analysis comprising 20,271 subjects. *Cancer Epidemiol*. 2012;36:106-115.
23. Absenger G, et al. The cyclin D1 (CCND1) rs9344 G>A polymorphism predicts clinical outcome in colon cancer patients treated with adjuvant 5-FU-based chemotherapy. *Pharmacogenomics J*. 2014;14:130-134.
24. Zhang W, et al. Exploring the relationship between polymorphic (TG/CA)_n repeats in intron 1 regions and gene expression. *Hum Genomics*. 2009;3:236-245.
25. Karachaliou N, et al. KRAS mutations in lung cancer. *Clin Lung Cancer*. 2013;14:20-205.

26. Berg M and Soreide K. EGFR and downstream genetic alterations in KRAS/BRAF and PI3K/AKT pathways in colorectal cancer: implications for targeted therapy. *Discov Med.* 2012;14:207-214.
27. Feng X, et al. Association of epidermal growth factor receptor (EGFR) gene polymorphism with lung cancer risk: a systematic review. *J Recept Signal Transduct Res.* 2014;345:333-334.