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UGT1A1*28 Polymorphism in Advanced Colorectal Cancer: The Story Is Not Yet Ended

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ABSTRACT

Background: UGT1A1*28 polymorphism is associated with neutropenia and diarrhea in previous reports, while this study tried to investigate correlation with other toxicities like vomiting. Patients and Methods: This is a prospective case control study including all eligible cases of advanced colorectal cancer. The genotypes of UGT1A1*28 was assessed in the peripheral blood and/or in tissues by PCR. Patients were divided into two groups, Group 1: patients with no mutation, Group 2: patients with homo or hetero mutation. All patients received standard IFL regimen. Primary objectives were: 1) comparison between the 2 groups as regard vomiting, 2) assessment of the incidence of UGT1A1*28 polymorphism. Secondary objectives were: comparison between the 2 groups as regard: neutropenia, diarrhea, treatment delay, progressive diseases (PD), progression free survival (PFS) and overall survival (OS). Results: 46 cases of advanced colorectal cancer present to National Cancer Institute, Cairo University, aged between 19 and 71 years with a median age of 45 years were included and followed up during the period from September 2010 to January 2013 with a median follow-up of 9 months. UGT1A1*28 polymorphism was present in 20 patients (43%), of whom 15% are homozygous. Grade (II-IV) vomiting was found in 8.3% of Group 1 versus 52.5% of Group 2 (P = 0.01). Grade (II-IV) neutropenia was found in 20.8% of Group 1 versus 64.7% of Group 2 (P = 0.03). Grade (II-IV) diarrhea was found in 37.5% of patients of Group 1 and 27.5% of patients with Group 2. (P = 0.75). Treatment delay occurred in 29.16% of Group 1 versus 72.4% of Group 2 (P = 0.02). 25% of Group 1 showed PD versus 25% of Group 2 (P = 0.8). 1-year PFS was 19% in Group 1 versus 23% in Group 2 (P = 0.8) while there was a trend towards better OS in Group 1 (47% versus 35%) (P = 0.07). Conclusions: UGT1A1*28 polymorphism is present frequently (43%) in a Caucasian population and is associated with more vomiting, neutropenia and treatment delay.

1. Introduction

Colorectal cancer is the fourth most frequently diagnosed cancer and the second leading cause of cancer-related death in the United States [1].

Because Irinotecan-based chemotherapy is a standard treatment in the management of metastatic CRC, interpatient variation of the enzymes involving its metabolism has been investigated extensively. Irinotecan (CPT-11) is

a water-soluble analogue of 20(S)-camptothecin (CPT) and is an inactive prodrug. Its major metabolite, SN-38, is a potent active topoisomerase I-inhibitor and is known to be toxic [2]. One limitation of CPT-11 is the unpredictable and occasionally fatal gastrointestinal and hematologic toxicity, which varies greatly among individuals [3]. Predictive markers of CPT-11 toxicity, may thus be deduced from the CPT-11 metabolic pathway.

SN-38 undergoes glucuronide conjugation to the pharmacologically inactive SN-38 glucuronide by the hepatic

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uridine diphosphate glucuronosyltransferase 1A1 (UGT 1A1) enzyme. The glucuronidation of SN-38 may be protective against irinotecan-induced gastrointestinal toxicity [4]. This protection may be genetically determined, as UGT1A1 enzyme is known to exist in polymorphic states.

The specific polymorphism (UGT1A1*28) is the presence of a (TA)7TAA sequence in the promoter region, instead of (TA)6TAA. Studies with human liver microsomes, have demonstrated an association between this polymorphism and SN-38 glucuronidation, with significantly lower SN-38 glucuronidation rates in liver samples heterozygous or homozygous for the (TA)7TAA polymorphism [5]. Thus, knowledge of the UGT1A1 polymorphism status could help guide the selection of appropriate starting dosages, reducing the risk of severe toxicity and improving the chances that therapy could be maintained.

Roughly 10% of the US population is homozygous for UGT1A1*28. The frequency of the UGT1A1*28 allele varies among ethnicities, being highest in those of African (43%) or European (39%) descent and lowest in those of Asian (16%) descent [6].

Among diplotypes of UGT1A genes, patients with the haplotypes harboring UGT1A1*6 or *28 had significantly reduced area under concentration curve ratios, with the effects of UGT1A1*6 or *28 being of a similar scale. In multivariate analysis, the homozygotes and double heterozygotes of *6 and *28 (*6/*6, *28/*28 and *6/*28) were significantly associated with severe neutropenia in patients who received irinotecan monotherapy [7].

2. Aim of Work

Primary objectives were:

1) Comparison between patients with wild and mutant UGT1A1*28 as regard vomiting.

2) Assessment of the incidence of UGT1A1*28 polymorphism.

Secondary objectives were:

Comparison between the 2 groups as regard: neutropenia, diarrhea, treatment delay, progressive diseases (PD), progression free survival (PFS) and overall survival (OS).

3. Patients and Methods

This is a prospective case control study including all eligible cases of advanced colorectal cancer. The genotypes of UGT1A1*28 was assessed in the peripheral blood and/or in tissues by PCR. Patients were divided into two groups, Group 1: patients with no mutation, Group 2: patients with homo or hetero mutation.

The study was conducted according to the Declaration of Helsinki and the guidelines for Good Clinical Practice. The local ethics committees approved the protocol, and informed consent was obtained from all patients before study entry.

3.1. Inclusion Criteria

Patient must have a histologically confirmed diagnosis of colon cancer. Stage III & Stage IV colon cancer. Patient is at least 18 years of age. Patient has a performance status of (ECOG Scale) $\leq$ 2. Patient has adequate bone marrow function, (WBC count $\geq 3.0 \times 10^9$ /L, ANC $\geq 1.5 \times 10^9$ /L platelet count $\geq 100 \times 10^9$ /L, hemoglobin level ≥ 9 g/L). Patient has adequate liver function; serum bilirubin $< 1.5 \times$ ULN, ALT and AST levels $<$ three times normal values; ALT and AST levels $<$ five times normal limits allowed in patients with known liver metastases. Patient has adequate Kidney function; plasma creatinine level < 1.5 times normal value. Patients should have compliance, mental state and geographic proximity that allow adequate follow up and they have to provide written informed consent before any study-specific procedure.

3.2. Exclusion Criteria

Patient is pregnant or breastfeeding. Patients with a "currently active" second malignancy. Patient who is currently involved in another clinical trial.

3.3. Treatment Plan

All patients received standard IFL (Irinotecan, 5FU, Leucovorin) regimen: Irinotecan 125 mg/m^2 weekly, Leucovorin 20 mg/m^2 weekly and 5FU 425 mg/m^2 weekly 4 weeks on 2 weeks off up to 6 months in responding patients.

3.3.1. Study Assessment

Pretreatment assessment included complete medical history and physical examination.

Further assessment conducted within 7 days before treatment included vital signs, performance status (ECOG), complete blood count with differential and full biochemical panel, including liver and renal function tests were performed and repeated before each treatment course. CEA and CA19.9 were done at base line and then every 6 weeks. Radiological evaluation including computerized tomography (CT) scan of the chest, Abdomen & pelvis. Additional radiological imaging such as bone scan… were done if indicated. Imaging was repeated every 6 weeks.

Evaluation was done according to RECIST [8].

Post treatment evaluation included:

Medical history and physical examination every 3 weeks. CBC and chemistry every 3 weeks. CT chest, abdomen and pelvis every 6 weeks. CEA and CA19.9 every 6 weeks. Other investigations were done if indicated.

3.3.2. Toxicity

Toxicity evaluation was done according to the NCI Common Terminology Criteria for Adverse Events v4.0 (CT-CAE) [9].

3.3.3. Statistical Methods

SPSS package (version 17.0) was used for data analysis. Mean and standard deviation were reported to describe quantitative data. The Chi-square and Fischer exact tests were used to evaluate the differences in the distribution of the variables. The Kaplan-Meier method was used to estimate the overall and progression free survival and the Log rank test to evaluate differences in survival among groups. P value of ≤0.05 was considered statistically significant.

3.3.4. UGT1A1 Assessment

UGT1A1*28 polymorphism is characterized by the presence of an additional TA repeat in the TATA sequence of the UGT1A1 promoter ((TA)7TAA, instead of (TA)6TAA). In the current study, UGT1A1*28 polymorphism was assessed in the blood and/or tissues by PCR as previously described by Iyer *et al*., 2002 [10] and Akiyama *et al*., 2008 [11].

1) Extraction of DNA from blood Mononuclear cells (MNCs)

Blood samples (7 ml) were collected from each patient on day 1 of the cycle (before starting chemotherapy). Density gradient separation of the MNCs from the collected blood was done using FicollHypaque Solution. DNA was extracted from the MNCs by phenol: chloroform: isoamyl alcohol after proteinase K digestion according to standard protocols. The extracted DNA was used to detect UGT1A1*28 polymorphism by PCR.

2) DNA Extraction from formalin fixed paraffin embedded tissues (FFPET)

For each tumor sample included in the study, five micron thick sections (5 sections) were obtained in a sterile, eppindorff, plastic tube. DNA was extracted from the homogenized FFPET sections by phenol: chloroform: isoamyl alcohol after proteinase K digestion according to standard protocols. Assessment of the concentration and purity of the extracted DNA was done using the spectrophotometer followed by visualization of an ethidium bromide-stained gel.

3) PCR amplification and genotyping of UGT1-A1*28

The isolated DNA was amplified by polymerase chain reaction (PCR) using the primer sequences and conditions of Iyer *et al*., 1999 [5].

Primers:
5'-GTC ACG TGA CAC AGT CAA AC-3'
5'-TTT GCT CCT GCC AGA GGT T-3'
PCR conditions

Initial denaturation step at 95˚C for 5 minutes, 30 cycles of: 95˚C for 30 seconds, 58˚C for 40 seconds, 72˚C for 40 seconds and final extension at 72˚C for 5 minutes.

Genotypes were assigned as follows:
6/6: homozygous for (TA)6TAA.
6/7: heterozygous for each (TA)6TAA/(TA)7TAA.
7/7: homozygous for (TA)7TAA.
5/8: heterozygous for each (TA)5TAA/(TA)8TAA.

4. Results

46 of advanced colorectal cancer cases presenting to National Cancer Institute, Cairo University, aged between 19 and 71 years with a median age of 45 years were included and followed up during the period from September 2010 to January 2013 with a median follow up of 9 months.

4.1. Patients' Characteristics

Table 1 summarizes patients' characteristics with regard to age, sex, stage, pathology, side, and smoking history.

4.2. Incidence of UGT1A1 Polymorphism

UGT1A1*28 polymorphism were present in 20 patients (43%), homozygous in 7 patients (15%) and heterozygous in 13 patients (28%) (**Figure 1**).

Genotypes were:
6/6 in 26 patients (57%).
6/7 in 9 patients (20%).
7/7 in 7 patients (15%).

Table 1. Patients' characteristics.

Characteristic		No. of patients (%)		P-value
		Wild	**Mutant**	
All patients		26 (100)	20 (100)	
Mean age		45 years	44 years	
Sex:	Male	14 (54)	12 (60)	0.4
	Female	12 (46)	8 (40)	0.4
Stage:	III	10 (38)	6 (30)	0.39
	IV	16 (62)	14 (70)	0.39
Pathology:	Adenocarcinoma GII	17 (66)	12 (60)	0.4
	Adenocarcinoma GIII	4 (17)	2 (10)	0.4
	Mucinous Adenocarcinoma GII	4 (17)	5 (25)	0.3
	Undifferentiated Carcinoma	-	1 (5)	0.4
Side:	Left	7 (27)	6 (30)	0.5
	Right	19 (73)	14 (70)	0.5
Smoking history		4 (15)	3 (15)	0.4

5/8 in 4 patients (8%).

4.3. Toxicity

Vomiting: There was a statistically significant lower incidence of vomiting in patients with wild type UGT-1A1*28 compared to patients with mutant type: Grade (II-IV) vomiting was found in 8.3% of Group 1 versus 52.5% of Group 2 (P = 0.01) (**Table 2**).

Neutropenia: There was a statistically significant lower incidence of neutropenia in patients with wild type UGT1A1*28 compared to patients with mutant type:

Grade (II-IV) neutropenia were found in 20.8% of Group 1 versus 64.7% of Group 2 (P = 0.03) (**Table 2**).

Diarrhea: Grade (II-IV) diarrhea was found in 37.5% of patients of Group 1 and 27.5% of patients with Group 2 (P = 0.75) (**Table 2**).

4.4. Treatment Delay

There was a statistically significant lower incidence of treatment delay in patients with wild type UGT1A1*28 compared to patients with mutant type:

Treatment delay occurred in 29.16% of Group 1 versus 72.4% of Group 2 (P = 0.02) (**Table 2**).

Other toxicities included 1 case of thrombocytopenia grade III (in Group 1), 1 case of oral mucositis grade II

(in Group 1), 1 case of nausea grade II (in Group 2), 2 case of urinary tract infection (in Group 1), 2 cases of gastritis grade II (1 in each group) and 2 cases of abdominal pain grade II (in Group 2).

4.5. Clinical Response

There was no significant difference between the 2 groups as regard clinical response. 25% of Group 1 showed PD versus 25% of Group 2 (P = 0.8) (**Table 3**).

4.6. Survival

Progression free survival (PFS): There was no significant difference between the two groups as regard PFS. 1 year PFS was 19% in Group 1 versus 23% in Group 2 (P = 0.8) (**Figure 2**).

Overall survival (OS): There was a trend towards better OS in patients with wild type UGT1A1*28 compared to patients with mutant type: One year OS in Group 1 was 47% versus 35% in Group 2 (P = 0.07) (**Figure 3**).

5. Discussion

The main problem with Irinotecan, which is an important drug in the treatment of colon cancer, is its toxicity. Patients receiving Irinotecan may experience some kind of severe toxicity, mainly diarrhea and neutropenia, resulting either in dose reduction, treatment withdrawal or dy-
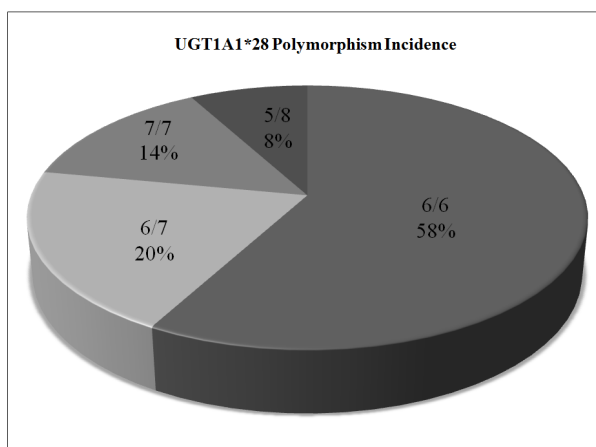

Figure 1. UGT1A1*28 polymorphism incidence.

Table 2. Correlation between UGT1A1*28 polymorphism and toxicities (Grades II, III, IV).

Toxicity	Wild	Homo + Hetero	P value
Diarrhea	37.5%	27.5%	0.7523
Neutropenia	20.8%	64.7%	0.0325[*]
Vomiting	8.3%	52.5%	0.0109[*]
Treatment delay	29.16%	72.4%	0.0241[*]
Dose reduction	12.5%	32%	0.2502

Table 3. Clinical response.

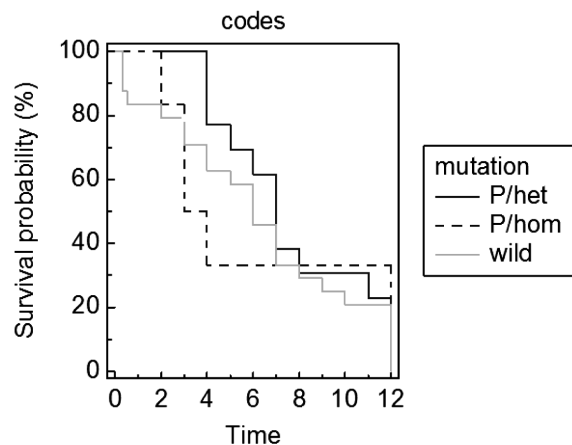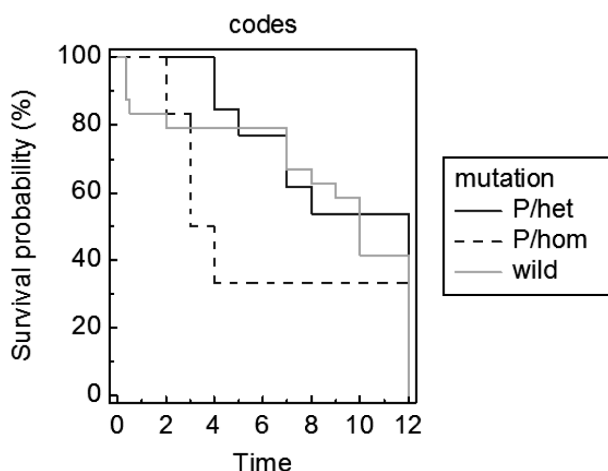| Response	Wild	Hetero + Homo
CR	**10%**	**15%**
PD	**25%**	**25%**
PR	**30%**	**35%**
SD	**35%**	**25%**

P = 0.8927.

Figure 2. One year progression free survival.

Figure 3. One year overall survival.

ing. UGT1A1*28 polymorphism is associated with neutropenia and diarrhea in previous reports, our study tried to investigate correlation with other toxicities like vomiting.

This is a prospective case control study including all eligible patients presenting to National Cancer Institute (NCI), Cairo University during the period from September 2010 to January 2013. Patients were divided into 2 groups according to the condition of UGT1A1*28 gene polymorphism to wild (6/6) and mutant genotype (6/7 and 7/7).

UGT1A1*28 polymorphism were present in 43% of patients, 15% homozygous (7/7) and 28% heterozygous (6/7). In Christoph Schulz et al. (2009) study, 9.5% of patients were homozygous and 49.5% were heterozygous for UGT1A1*28 [19]. In other studies, UGT1A1*28 polymorphism was present in a lower percentage such as Y. Akiyama et al. (2008) study, in which there was 16.4% of Japanese patients having this polymorphism, and 10.5% in Xiaoqing Zhang et al. (2012) study (Han population), 15.3% in Zhang A et al. (2007) study (Dong population), 28.6% in Tang et al. (2005) study (Taiwanese population), 18.7% in Zhou et al. (2009) study (Singaporean population), 13% in Saeki et al. (2006) study (Japanese population), 14% in Yea et al. (2008) study (Korean population) and 28.6% in Thomas et al. (2006) study (Caucasian population) [11-19]. It is noteworthy that ethnic differences do exist in the UGT1A1*28 polymorphism, that's why there is differences between different studies of different ethnicities.

To no surprise, the current study showed that, UGT1A1*28 polymorphism plays a significant role in the occurrence of hematological toxicity in patients treated with Irinotecan, especially neutropenia (20.8% vs. 64.7%, P = 0.03), this finding is in concordance with previous studies which showed similar results such as Chun-Yu et al. (2007) (53.8% vs 4.9%; P < 0.01), Innocenti et al.

(2004) which stated that patients with the 7/7 genotype had a 9.3-fold higher risk (95% confidence interval, 2.4 - 36.4) of developing grade 4 neutropenia than patients with the 6/6 and 6/7 genotypes, L Iyer et al. (2001) in which there was a correlation between absolute neutrophil count (ANC) and UGT1A1 promoter genotypes in patients after irinotecan treatment with Significant trend in ANC nadirs (6/6 > 6/7 > 7/7, z = −2.05, P = 0.04, non-parametric trend analysis), Kristine et al. (2006) with increased risk of grade 3 or 4 neutropenia (P = 0.001) and Rouits et al. (2004) that confirmed statistically significant risk of neutropenia (P = 0.001) [10,19-23].

The current study showed no significant difference between the 2 study groups regarding risk of diarrhea (incidence 37.5% vs. 27.5%, P = 0.75) which may be attributed to small sample size of the present study. Some previous studies shows no difference like Christoph Schulz et al. (2009) in which result [(6/7, 7/7) vs (6/6) plymorphismwas 13.0% vs 6.2%, P = 0.08] and Rouits et al. (2004) that found no significant statistical difference (P = 0.559). (9, 14) Other studies showed a statistically significant difference like: Chun-Yu et al. (2007) study which showed a statistically significant difference regarding diarrhea (26.9% vs 5.9%; P < 0.01). Kristine et al. (2006) showed that there was an increased risk of grade 3 or 4 diarrhea (P = 0.005). L. Iyer et al. (2001) stated that patients with the (TA) 7TAA repeat in the promoter region of UGT1A1 may be expected to exhibit a higher incidence of diarrhea and neutropenia than those without the polymorphism [10,20,21].

The interesting finding in this study is that, although all patients received premedication antiemetic, patients with UGT1A1*28 polymorphism had significantly more risk for vomiting than patients with wild type (52.5% in mutant type group versus 8.3% in the wild type group with P value of 0.01.). To our knowledge this is the first study to primarily address correlation between UGT1A1*28 polymorphism and vomiting.

The current study showed no significant difference between the two groups regarding toxicity related dose adjustments (12.5% versus 32%, P = 0.25) whereas there was a statistically significant more dose delay in the mutant group (29% versus 72%, P = 0.02). These results are to a large extent similar to those of previous studies: In Christoph Schulz et al. (2009) study, treatment delays incidence was; 25.1% vs. 19.3%, [P = 0.24] in [(6/7, 7/7)] group vs. (6/6) group respectively and dose reductions incidence was 21.5% vs 27.2%, [P = 0.07] in [(6/7, 7/7) group vs. (6/6) group respectively. Toffoli et al. (2006) showed that dose reduction occurred in 17.5%, 23.2%, and 18.2% of TA6/TA6, TA7/TA6, and TA7/TA7 patients, respectively, with no significant association with genotypes. Chun-Yu et al. (2007) study states that requirement for irinotecan dose reduction was significantly

greater in patients who had the genetic variant (42.3% vs 12.7%; P < 0.01) [19,20,26].

Although the treatment delay was significantly greater in patients with the TA6/TA7 or TA7/TA7 genotypes when compared with wild type (72.4% versus 29.16% respectively), (P = 0.02) there was no significant difference between the two groups as regard clinical response, P value = 0.89. This goes with most of previous published studies like Christoph Schulz *et al*. (2009) study, which stated that the overall response rate (OR) was similar between patients carrying the (6/7, 7/7) or the wild genotype (6/6) (44.3% vs 43.2%, P = 0.75) and Chun-Yu *et al*. (2007) study which showed that the response rate to irinotecan-based chemotherapy were not affected with dose reduction that was required for the UGT1A1*28 polymorphism group (42.3% vs 45.1%; P = 0.80). (9, 10) But some studies such as Glenn *et al*. (2009) reported that, the *28 heterozygotes had a nonsignificantly higher response rate (RR, 1.09; 95% CI 0.83 - 1.43), and *28 homozygotes had a significantly higher response rate (RR, 1.70; 95% CI 1.24 - 2.33; P = < 0.001), and Toffoli *et al*. (2006) stated that, the UGT1A1 TA7/TA7 genotype seemed to be associated with increased clinical benefit and tumor response, as homozygous TA7/TA7 patients had a significantly reduced risk of PD or SD compared with the wild-type genotype (OR, 0.32; 95% CI, 0.12 to 0.86) and considering clinical benefit, the homozygous TA7/TA7 patients had a significantly lower risk of experiencing progression (OR, 0.19; 95% CI, 0.04 to 0.89) [24].

In our study, we did not find a statistically significant difference between the two groups as regard PFS and OS, (PFS: 19% in wild group vs. 23% in mutant group, P = 0.8), although there was a trend toward a statistical significant difference for OS at one year (wild: 47% vs. mutant: 35%, P = 0.07). It confirms the previous studies results. In Christoph Schulz *et al*. (2009) study neither time to progression [(TTP) 8.1 vs. 8.2 months, P = 0.97] nor overall survival [(OS) 21.2 vs. 18.9 months, P = 0.73] differed significantly in patients who carried the (6/6) or (6/7, 7/7) genotypes respectively. Chun-Yu *et al*. (2007) study stated that although the requirement for irinotecan dose reduction was significantly greater in patients who had this genetic variant, it did not significantly affect progression-free survival (10 months vs 11 months; P = 0.94) or overall survival (19 months vs 18 months; P = 0.84). The data from Toffoli *et al*. (2006) and Marcuello *et al*. (2004) studies reported that none of the differences were statistically significant. Findings from Toffoli *et al*. (2006) study were in the direction of improved survival for *28 homozygotes versus non wild genotype patients, whereas the Marcuello *et al*. (2004) study reported a survival advantage for the wild genotype individuals [19,20, 25,26].

6. Conclusion

The current study showed that UGT1A1*28 polymorphism is present frequently (43%) in a Caucasian population and is a key determinant for predicting irinotecan-induced toxicities as vomiting, neutropenia and treatment delay for patients with advanced colorectal cancer with a trend toward improvement of survival. Further prospective studies including larger sample size are warranted for using this polymorphism to optimize irinotecan-based chemotherapy with special emphasis on other toxicities which may be related to this polymorphism.

Acknowledgements

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