The Increasing Role of Pharmacogenetics in the Treatment of Gastrointestinal Cancers

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ABSTRACT

Despite improvements in the treatment of gastrointestinal cancers, 5-year survival rates for advanced-stage patients remain disappointing. Therefore, the need exists to develop innovative new therapies while optimizing the current ones. Pharmacogenetics can be helpful in this context. Metabolism of cancer drugs varies according to age, gender, diet, concurrent use of other drugs, and existing comorbidities, including impaired liver and renal function. In addition, metabolizing enzymes, drug-transport proteins, metabolites, and drug receptors are genetically determined. It has also been demonstrated that genetic mutations within a tumor can be a determining factor with regard to response to treatment. The most common agents used in the treatment of digestive system tumors — 5-fluorouracil, oxaliplatin, cisplatin, irinotecan, gemcitabine, and newly developed biologic agents bevacizumab, cetuximab, panitumumab, and erlotinib — will be reviewed from a pharmacogenetic perspective. The US Food and Drug Administration has approved the UDP-glucuronosyltransferase 1A1 test in patients treated with irinotecan, and additional approval of newer tests is anticipated. Increasing availability of these sophisticated assays is expected to facilitate the delivery of more effective, less toxic chemotherapy regimens in the management of relatively resistant tumors of the gastrointestinal tract.

Gastrointest Cancer Res 3:197–203. ©2009 by International Society of Gastrointestinal Oncology

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Tumors of the digestive system are the leading group of cancers in terms of frequency and cause of cancer deaths.[1] These malignancies are generally diagnosed at an advanced stage, metastatic disease is rarely curable, and the rate of recurrence is quite high in earlier-stage resectable gastrointestinal cancers. The objective of adjuvant therapy is to decrease postoperative recurrence rate and prolong disease-free and overall survival. At certain stages of colon, rectum, gastric, and pancreas cancers, the benefit of adjuvant chemotherapy alone or in combination with radiotherapy has been demonstrated. As for cancers of the anal canal, a high rate of cure can be achieved with neoadjuvant chemoradiotherapy. On the other hand, the role of adjuvant therapy in esophagus, liver, and biliary tract cancers has not been established clearly.

Despite recent advances in medical treatment of gastrointestinal cancers, 5-year survival rates remain disappointing. Effective new treatments are urgently needed, and existing therapies need to be refined and improved.[2,3] Moreover, the ability to distinguish which patients are or are not likely to respond to a given therapy, as well as to identify patients at risk of developing severe toxicity, will enable clinicians to optimize cancer treatment, increase the chances of therapeutic success, and improve patient outcomes.[4] In this respect, pharmacogenetics represents, perhaps, the most promising method of offering patients such individualized therapy.[5]

Doses for anticancer agents are generally close to maximum tolerated levels, as determined in phase I trials; however, optimal therapeutic doses have not been established for many cancer drugs. Thus, a certain incidence of grade 3/4 side effects might be expected in some patient populations. Metabolism of chemotherapy agents varies depending on patient age, gender, diet, concomitant drug use, comorbidities, and hepatic and renal functions. However, enzymes that metabolize the drug, proteins that transport the drug and its metabolites, and drug receptors are determined based on a patient's genetic profile. Pharmacogenetics focuses on investigating the influence of genetic structure on cancer treatment. In this report, primary drugs used in the treatment of gastrointestinal cancers — 5-fluorouracil (5-FU), oxaliplatin, cisplatin, irinotecan, gemcitabine, and the novel biologic agents, bevacizumab, cetuximab, panitumumab, and erlotinib — will be reviewed from a pharmacogenomic perspective. Table 1 shows mechanisms of action, metabolisms, and excretions of these drugs.

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Table 1. Mechanisms of action, metabolism, and excretion of drugs used in patients with gastrointestinal cancers.

Drugs	Indication	Mechanism of action	Metabolism	Renal excretion	Biliary excretion	Dose reduction
5-Fluorouracil	Colorectal cancer, gastric cancer	Antimetabolite, thymidylate synthase blocking	Thymidylate synthase, thymidylate phosphorylase, dihydropyrimidine dehydrogenase	Inactive metabolites in urine	Inactive metabolites in bile	Enzymatic catabolism by DPD in the liver (>90%); Biluribin > 5 mg/dL: omit use
Oxaliplatin	Colorectal cancer	Alkylator of DNA	–	50%	–	Reduce dose for renal dysfunction
Cisplatin	Anal cancer	Alkylator of DNA	–	90%	<10%	Creatinine clearance 10–50 mL/min: 25% reduction
Irinotecan	Colorectal cancer, gastric cancer	Topoisomerase I inhibition	Carboxylesterase, CYP3A4, UGT1A1, topoisomerase I	>25%	>50% hepatic metabolism and biliary excretion	Metabolized in the liver. Consider in the presence of hepatic dysfunction
Gemcitabine	Pancreatic carcinoma	Metabolized intracellularly to the active diphosphate and triphosphate	Cytidine deaminase, deoxycytidylate deaminase	Nearly entirely excreted in urine	–	Metabolized in the liver. Consider in the presence of hepatic dysfunction
Bevacizumab	Colorectal cancer	Monoclonal antibody for VEGF-A		–	–	–
Cetuximab	Colorectal cancer	Monoclonal antibody to EGFR		–	–	No adjustment needed for renal or hepatic impairment
Panitumumab	Colorectal cancer	Monoclonal antibody to EGFR				
Erlotinib	Pancreas cancer	Inhibits intracellular phosphorylation of EGFR tyrosine kinase		8% renal elimination	Hepatic metabolism through CYP 3A4; 83% hepatic elimination	Consider in severe liver impairment

Abbreviations: VEGF = vascular endothelial growth factor; EGFR = epidermal growth factor receptor

5-FLUOROURACIL

5-FU has been used in the treatment of gastrointestinal cancers for over 50 years.[6] 5-FU itself is a prodrug that is converted to 5-fluoro-2′-deoxyuridine-5′-monophosphate (5-FdUMP) within the cell to inhibit thymidylate synthase (TS). Thus, synthesis of pyrimidine and subsequently DNA is suppressed. Eighty-five percent of 5-FU is catabolized to its inactive metabolites via dihydropyrimidine dehydrogenase (DPD) (Figure 1).[7]

Dihydropyrimidine Dehydrogenase

In cases of DPD enzyme deficiency, blood levels of 5-FU and its active metabolites increase. DPD enzyme deficiency can result in fatal myelotoxicity, mucositis, and cerebellar toxicity.[7–15] Determination of DPD enzyme activity in mononuclear cells may be of benefit; however, it is technically very difficult.[12] The gene encoding DPD enzyme is located at 1p22 and consists of 23-
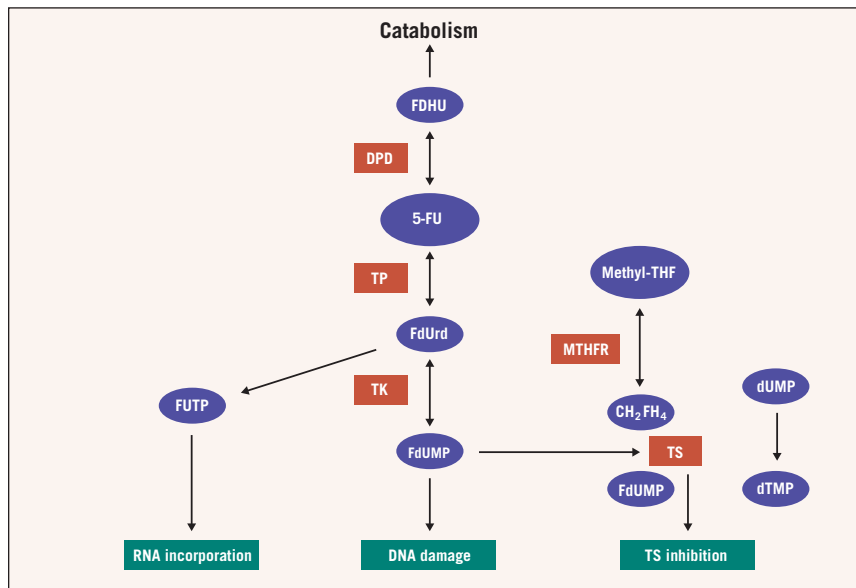

Figure 1. 5-Fluorouracil metabolism

Abbreviations: 5-FU = 5-fluorouracil; DPD = dihydropyrimidine dehydrogenase; dTMP = thymidine monophosphate; dUMP = deoxyuridine monophosphate; FDHU = 5-fluorodihydrouracil; FdU = fluorodeoxyuridine; FdUMP = 5-fluoro-2-deoxyuridine monophosphate; FdUrd = 5-fluorodeoxyuridine; FUTP = 5-fluorouridine triphosphate; MTHFR = methylenetetrahydrofolate reductase; THF = tetrahydrofolate; TK = thymidine kinase; TP = thymidilate phosphorylase; TS = thymidylate synthase

exons.[13] Conditions resulting in DPD deficiency include base substitutions, splicing deficits, and frameshift mutations. More than 40 different polymorphisms related to this enzyme have been reported.[15] Severe 5-FU toxicity is associated with 17 of these mutations. In the general population, homozygote and heterozygote DPD dysfunction is estimated to be 0.1% and 3% to 5%, respectively. DPYD*2A is the most common DPD polymorphism associated with 5-FU toxicity. In this case, exon 14 is skipped as a result of the G→A translocation at intron 14, and inactive enzyme is formed. With this polymorphism, the heterozygote form is characterized by severe toxicity; the homozygote form is characterized by mental deficiency.

Dihydropyrimidine dehydrogenase enzyme deficiency was demonstrated in 61% of patients exhibiting severe 5-FU toxicity. DPYD*2A polymorphism was identified in 50% of patients with grade 4 neutropenia. However, DPD enzyme activity is normal in most patients with severe 5-FU toxicity. Therefore, multiple factors and genes are thought to be involved in 5-FU toxicity.[14,15] Genetic variations related to other enzymes involved in 5-FU metabolism are also important, particularly TS and thymidylate phosphorylase (TP).

Thymidylate Synthase

Thymidylate synthase is a target of 5-FU. It plays a significant role in folate metabolism. TS enables conversion of deoxyuridylate to deoxythymidylate.[16,17] In tumors, increased TS enzyme expression is associated with resistance to 5-FU and capecitabine.[18] In particular, intratumoral TS levels in metastatic lesions are indicative of 5-FU resistance.[18,19] This is a result of differences between TS expressions of primary and metastatic lesions.[20]

In adjuvant treatment of stage III colon cancer, response of patients with TSER3 polymorphism is poor. TS 5′-untranslated region (5′-UTR) polymorphism is useful for determining response to 5-FU. While the response rate is 50% in those with 2R/2R, it is 8% in those with 3R/3R.[20] Apart from response, TS polymorphism also affects survival. Median survival in cases of 2R/2R is 16 months vs. 12 months in cases of 3R/3R.[21] TS polymorphism is also relevant in predicting response to neoadjuvant 5-FU treatment in rectal cancer. Cases of 3R/ 3R are associated with a poor response.[22]

Thymidylate Phosphorylase

Thymidylate phosphorylase mRNA levels in patients not responding to 5-FU were 2.6-fold higher than in responding patients in pretreatment biopsies of patients with colorectal cancer.[23] Survival was significantly increased in patients with both TS and TP under nonresponse cutoff values, and low intratumoral expression of TS and TP was associated with a response to 5-FU and improved survival.[21,23,24]

GEMCITABINE

Gemcitabine is a nucleoside analog of deoxycytidine used in treatment of localized and advanced-stage pancreatic cancer.[25] Gemcitabine undergoes metabolic activation by kinases to form a cytotoxic trinucleotide in the cell.[26] Metabolic inactivation of gemcitabine by deamination is catalyzed by cytidine deaminase (CDA) or — after phosphorylation — by deoxycytidylate deaminase (DCTD).[27] In a study of patients with pancreatic carcinoma treated with gemcitabine, intratumoral SLC29A1 expression (as measured by immunohistochemistry) was related to prolonged survival.[28] This is because functional nucleoside transporters are necessary for gemcitabine cytoxicity, and nucleoside-transporter–deficient cells are highly resistant to gemcitabine.[29] SLC29A1 is the most abundant of the nucleoside transporters.[30] Analysis of SLC29A1 mRNA expression also showed a significant correlation between increased mRNA expression and longer survival in patients with pancreatic cancer treated with gemcitabine.[31]

Deoxycytidine kinase (DCK) deficiency was previously the most common form of acquired resistance to gemcitabine in vitro.[32] Studies have shown a correlation between higher levels of DCK activity and increased gemcitabine sensitivity in patients with advanced pancreatic cancer treated with gemcitabine, whereas low tumoral DCK immunohistochemical expression was associated with a worse overall survival and progression-free survival.[30]

Ribonucleotide reductase (RR) is a target enzyme for gemcitabine.[33] The pharmacology and pharmacogenetics of ribonucleotide reductase subunit M1 (RRM1) is of particular interest due to its potential role in gemcitabine chemosensitivity and synergy with other chemotherapeutic agents, particularly cisplatin.[33–35] In a study of genetically modified lung cancer cell lines, RRM1 expression correlated inversely with gemcitabine sensitivity.[36]

Deactivating enzymes of gemcitabine include 5′, 3′-nucleotidase, cytosolic (NT5C), deoxycytidylate deaminase (DCTD), and cytidine deaminase (CDA).[26,33] Upregulation of CDA may play a role in gemcitabine resistance, while impaired activity may result in increased toxicities.[33,34] Germline genotyping revealed homozygosity for CDA 208A in a recent report of a Japanese patient with pancreatic cancer treated with gemcitabine and cisplatinum who developed severe hematologic and nonhematologic toxicity.[35]

IRINOTECAN (CPT-11)

Irinotecan is a topoisomerase I inhibitor.[37] Among gastrointestinal malignancies, it is used in the treatment of colorectal cancer and gastric cancer. Activation, transportation, and deactivation of irinotecan is complex, and many enzymes, including carboxylesterase (CE), CYP3A4, uridine diphosphate glucuronosyltransferase (UGT1A1), and topoisomerase I are involved in the metabolism of this drug.[37–39] Irinotecan is converted to its active metabolite, SN38, by CE present in the gastrointestinal tract (Figure 2).[38,39] This enzyme has many allelic variations and genotypes and is involved in irinotecan toxicity.[38]

UGT1A1, present in the liver, is the enzyme that inactivates SN38 via glucuronidation.[40,41] The association of irinotecan toxicity and UGT1A1 polymorphism is currently under investigation. UGT1A1 inactivates SN38 via phase II reaction.[40,41] The UGT1A1*28 polymorphism is associated with reduced UGT1A1 expression and, as a result, decreased glucuronidation of SN38. This, in turn, increases blood levels of active metabolites resulting in increased toxicity.[39–42] The pharmacokinetics of irinotecan is poorly associated with body surface area.

Since SN38 undergoes glucuronidation to a lesser extent in patients with Gilbert and Crigler Najjar syndromes, irinotecan toxicity increases in these patients, because of reduced or deficient expression
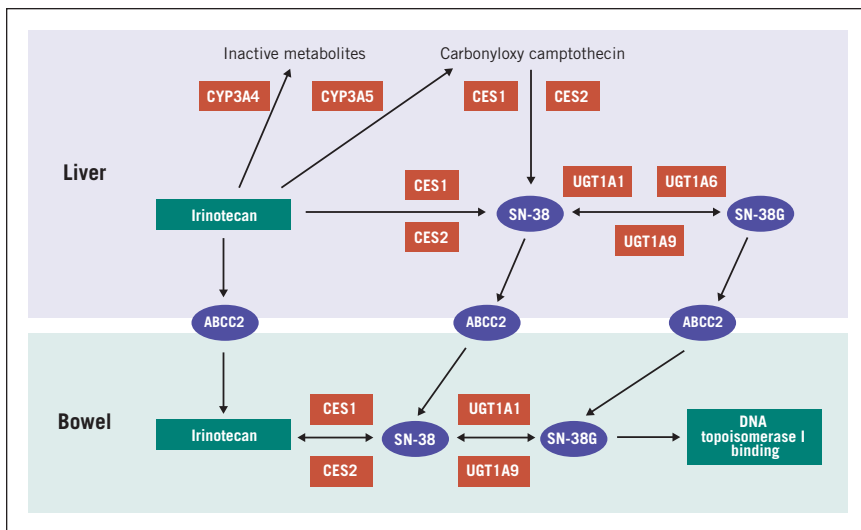
Figure 2. Irinotecan metabolism

levels of UGT1A1. Gilbert syndrome results from the UGT1A1*28 homozygote transition of promoter polymorphism caused by seven TA repetitions. In UGT1A1*28 polymorphism, transcription is decreased by 70% and toxicity is increased. Patients with the 7/7 genotype (homozygous for seven TA repetitions) exhibit a 9.3-fold increase in risk of grade 4 neutropenia, and irinotecan is associated with severe side effects in this population.[40–43]

In a meta-analysis of 10 studies assessing the correlation between irinotecan-induced hematologic toxicities in UGT1A1*28 patients, irinotecan dose, and overall toxicity, risk of experiencing irinotecan-induced hematologic toxicity for homozygous UGT1A1*28 patients was found to be a function of the dose of irinotecan administered, and genotyping was recommended at high doses (> 200 mg/m^2) of irinotecan.[42] Genotyping was of modest benefit at intermediate doses, such as 180 mg/m^2 used in the FOLFIRI (folinic acid/5-FU/irinotecan) regimen. Unless administered concomitantly with another myelotoxic agent, UGT1A1*28 testing was not recommended at doses < 150 mg/m^2.

PLATINUM COMPOUNDS (Cisplatin and Oxaliplatin)

Cisplatin and oxaliplatin are commonly used in gastrointestinal cancers.[44,45] Platinum analogs block DNA replication by forming different DNA adducts. DNA repair enzymes ERCC1 and ERCC2 — also known as XPD and glutathione S transfer-

ase (GSTP) enzymes — are involved in the activity of these agents.[46] High expression of the genes that code for these enzymes is inversely correlated with therapeutic response in colorectal and gastric cancer.[47]

In single nucleotide polymorphisms involving transformation of lysine forming on ERCC2 gene, codon 751, to glutamine, the response rate is 24% in lysine, the inactive form of this enzyme, and 10% in other forms.[48] This trial was performed in patients who had previously received chemotherapy. These results have not been confirmed in patients receiving first-line therapy.

COMBINATION CHEMOTHERAPY

The N9741 trial is a phase III, randomized trial designed to compare the efficacy of FOLFOX (folinic acid/5-FU/oxaliplatin), IROX (irinotecan/oxaliplatin), and IFL (irinotecan/bolus 5-FU/folinic acid) in patients with metastatic colorectal cancer.[49] The pharmacogenetic evaluation performed in this trial revealed that both the objective response rate and incidence of grade 3/4 side effects, particularly diarrhea, were lower in black patients. The low rate of response was particularly marked in the FOLFOX arm. Overall, the rate of response was 41% and 30% in white and black patients, respectively (P = .015). The rate of severe toxicity was 48% in whites and 34% in black patients in the FOLFOX arm (P = .047). Although no significant median survival difference was observed between

these two patient groups in the FOLFOX arm, median survival was lower in black patients in both the IFL and IROX groups.

In all arms, black patients experienced less toxicity, particularly diarrhea, than white patients did. In this trial, UGT1A1 7/7 involved in irinotecan metabolism was identified at a rate of 21% and 9% in black and white patients, respectively. However, the determinant role of UGT1A1 gene polymorphism with respect to response and toxicity could not be demonstrated. Significant differences were also detected between white and black patients in prevalence of other pharmacogenetic variances such as CYP3A, MDR (multidrug resistance), ERCC1, ERCC2, and GSTP. These genes are important in the metabolism and detoxification of irinotecan and oxaliplatin.[50] In the same trial (Intergroup N9741), GSTP1 polymorphism was demonstrated to be associated with early development of oxaliplatin neuropathy in patients receiving FOLFOX.

A small study showed that ERCC codon 118 polymorphism predicted response to oxaliplatin/5-FU chemotherapy in patients with advanced colorectal cancer. In this study, response rate was 61.9%, 42.3%, and 21.4% in T/T, C/T, and C/C groups, respectively (P: 0.018).[51]

BIOLOGIC AGENTS

Biologic agents used in gastrointestinal cancers include bevacizumab, cetuximab, panitumumab, and erlotinib. Increased VEGF expression is involved in tumoral angiogenesis and associated with poor prognosis. The therapeutic benefit of bevacizumab has been shown in the treatment of patients with advanced-stage colorectal cancer. Thus far, however, adequate pharmacogenetic data have not been produced to predict toxicity, response, or resistance.

In the BOND-2 trial, metastatic colorectal cancer patients progressing after irinotecan-based chemotherapy were randomized to receive irinotecan plus bevacizumab plus cetuximab (CBI) or bevacizumab and cetuximab (CB).[52] In this trial, germline polymorphisms involved in angiogenesis (vascular endothelial growth factor [VEGF], interleukin-8 [IL-8], transforming growth factor [TGF]-β), the epidermal growth factor receptor (EGFR)

pathway (EGFR, cyclooxygenase-2, E-cadherin), DNA repair (ERCC1, ERCC2, XRCC1, xeroderma pigmentosum group D [XPD]), and drug metabolism pathway (GSTP1, UGT1A1) were investigated.[52,53] Results showed a correlation between TGF-β polymorphism and response; UGT1A1, cyclin D1, and time to disease progression (TTP); and TGF-β polymorphism and tumor response in the CBI arm. In addition, a correlation between EGFR 497 and overall survival was found. As for the CB arm, the investigators found a trend in association between polymorphisms of Fc fragment of IgG–3A and tumor response; a significant correlation between ERCC2, TGF-β and TTP; and ERCC2 and overall survival were correlated.[52–54]

A correlation between RAS mutation and resistance to the EGFR antibodies cetuximab and panitumumab has been demonstrated. KRAS mutations account for approximately 30% to 40% of patients who are not responsive to treatment with these agents.[55–57] Patients with RAS mutation in tumor tissue have lower rates of response to cetuximab and panitumumab and shorter progression-free survival time. Recently, a BRAF V600E mutation was detected in 11 of 79 patients who had wild-type KRAS.[58] This BRAF mutation is associated with no response to cetuximab and panitumumab with significantly shorter progression-free and overall survival compared to wild-type patients.[58,59]

Erlotinib is a small-molecule tyrosine kinase inhibitor that targets HER1/EGFR.[60,61] In addition, it is a potent inhibitor of CYP1A1, and a moderate inhibitor of CYP3A4 and CYP2C8, as well as a strong inhibitor of glucuronidation by UGT1A1 in vitro.[62] Potent inducers of CYP3A4 may reduce the efficacy of erlotinib, whereas potent inhibitors of CYP3A4 may lead to increased toxicity.[60–62] Concomitant treatment with these types of agents should be avoided. The inhibition of glucuronidation may cause interactions with substrates of UGT1A1 exclusively cleared by this pathway. Patients with low expression of UGT1A1 or genetic glucuronidation disorders (eg, Gilbert's disease) may exhibit increased serum concentrations of bilirubin and must be treated with caution.[62]

In a recent study including 569 patients with advanced pancreatic cancer, erlotinib in combination with gemcitabine showed statistically superior overall survival compared with gemcitabine alone (6.4 months vs. 5.9 months, respectively).[63] In this study, patients responded equally well to treatment with erlotinib regardless of whether their tumors expressed abnormal levels of EGFR. Survival was longer in patients with wild-type KRAS in comparison to KRAS-mutated patients (7.5 months vs. 3.7 months, respectively) in erlotinib-treated patients. In the wild-type group, median survival was 3.4 months in the erlotinib arm and 7 months in the placebo arm. Survival was also longer in EGFR wild-type patients and in patients with high EGFR copy numbers as demonstrated by fluorescence in situ hybridization.[64]

In the AViTA study, patients with advanced-stage pancreatic cancer were treated with gemcitabine plus erlotinib with or without bevacizumab. In this study, survival was positively correlated with severity of rash, but no statistically significant difference was detected in terms of overall survival with the addition of bevacizumab.[65] Therefore, reassessment of erlotinib treatment is recommended in patients who do not develop rash within the first 4 to 8 weeks of treatment.

DISCUSSION

Based on the results of pharmacogenetic studies, the US Food and Drug Administration has approved the UGT1A1 test for patients receiving irinotecan. Additionally, RAS mutation assay is required to determine whether patients are candidates for treatment with cetuximab and panitumumab. New tests are on the horizon and, based on data from many of the trials reviewed herein, are expected to be approved. Increasing availability of these sophisticated assays is expected to facilitate the delivery of more effective, less toxic chemotherapy regimens by individualizing treatments for patients with relatively resistant tumors of the gastrointestinal tract.

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Disclosures of Potential Conflicts of Interest

Dr. Yalçin indicated no potential conflicts of interest.