HIGHLIGHT ARTICLE

Pharmacogenetics in Pancreatic Cancer

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ABSTRACT

Pancreatic cancer is an aggressive malignancy with a poor overall survival rate. Given advances in pharmacogenomics, numerous gene mutations have been identified that could be potential targets for drug development. Therefore, future research strategies may identify prognostic and predictive markers aiming to improve outcome by maximizing efficacy whilst lowering toxicity.

In this commentary, we summarize several interesting results regarding pancreatic cancer pharmacogenetics that have been presented in the 2014 American Society of Clinical Oncology (ASCO) Annual Meeting. In particular, we focus on Abstract #4124, which investigated the potential predictive role of human equilibrative nucleoside transporter 1 (hENT1) in patients treated with adjuvant gemcitabine for pancreatic cancer, on Abstract #4125, which examined the tolerability of a modified FOLFORINOX study based on UGT1A1*28 genotype guided dosing of IRI in patients with advanced pancreatic cancer, and on Abstract #4130, which confirmed the predictive role of circulating tumor and invasive cells (CTICs) from patients with unresectable pancreatic cancer in second-line chemotherapy treatment setting.

Introduction

Pancreatic cancer is considered the fourth leading cause of cancer in the United States, as approximately 37000 patients die from this aggressive malignancy each year [1]. Despite developments in detection and management of the disease, survival remains extremely poor, with only about 5% of patients living 5 years after diagnosis. Hence, there is a need to better understand the biological mechanisms that contribute to pancreatic carcinogenesis and furthermore to identify pharmacogenetics that would help formulating more successful treatment of the disease through patient- oriented, personalized medicine.

What Did We Know Before 2014 ASCO Annual Meeting?

Pancreatic cancer remains an aggressive disease, with limited therapeutic options in terms of efficacy and tolerance. As gemcitabine is considered the mainstay for treating patients with pancreatic cancer, studies have focused on investigating the mechanisms of resistance to gemcitabine in terms of CDA deregulation, tumor-level impaired drug transport (e.g. hENT1 and hCNT3), polymorphisms affecting the ribonucleotide reductase target, activating and deactivating enzymes (e.g. dCK and dCTD) or hedgehog-related changes in tumor stroma [2].

Increased intratumoral hENT1 expression has been identified in randomized trials as a potential predictive marker for response to gemcitabine in the adjuvant setting [3]. Hence, hENT1 expression failed to be predictive of gemcitabine response in treatment-naive patients receiving gemcitabine in the metastatic setting [4]. Effective systemic treatment of pancreatic cancer is hampered by drug resistance and treatment related toxicities, as in the case of irinotecan, that has been associated with unpredictable toxicities, including myelosuppression and diarrhea, which could be severe and even fatal [5]. A major challenge in research is biological interpretation of complexity of pancreatic cancer somatic mutation profiles. Therefore, studies investigated the selection of cytotoxic treatment based on gene expression profiling and the use of circulating tumor cells (CTCs) as a surrogate tissue for monitoring response to therapy and tumor relapse [6, 7].

What Did We Learn from 2014 ASCO Annual Meeting?

We analyze the recent work presented at the 2014 ASCO Annual Meeting on pharmacogenetics in pancreatic cancer. Table 1 summarizes the related abstracts and their main findings.

Hent1 Expression in Patients with Pancreatic Cancer Treated With Gemcitabine after Curative Intended Resection: Results from the CONKO-001 Trial

The CONKO-001 study is a multicenter, phase 3 randomized trials that was designed to compare adjuvant

Key words gemcitabine; Pancreatic Neoplasms; Pharmacogenetics
Abbreviations CDA, cytidinedeaminase; hENT1, human equilibrative nucleoside transporter 1; hCNT3, human concentrative Na+-nucleoside cotransporter 3; dCK, deoxycytidine kinase; dCTD, deoxycytidylatedeaminase.
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intravenous gemcitabine with observation alone in the adjuvant setting. The study demonstrated that the use of adjuvant gemcitabine for 6 months compared with observation resulted in increased overall survival (OS) as well as disease-free survival (DFS) in patients undergoing complete resection of pancreatic cancer. In order to investigate hENT1 expression as a potential predictive marker for response to gemcitabine (Figure 1), Sinn et al., studied tumor samples of 156 patients (88 patients gemcitabine-treated and 68 patients observation alone) [8]. Tissues microarrays (TMA) on paraffin blocks were prepared and immunostaining for expression of hENT1 was performed by using the rabbit monoclonal anti-hENT1 SP120 antibody. With an unequivocal membrane staining in more than 50% of tumor cells, hENT1expression was assessed high, while with a less percentage of tumor cells stained, the hENT1 expression was considered as low.

The median overall survival (mOS) and the median disease free survival (mDFS) for the gemcitabine-treated patients was 22.7 months and 12.9 months, while for the observation alone patients the median value was 19.1 months and 6.2 months respectively, results comparable to the overall study population of the trial. High hENT1 expression was not associated with improved mDFS or mOS in either group of patients (Table 2).

Thus, the researchers concluded that the predictive role of tumor hENT1 expression in treatment with gemcitabine was not confirmed in this randomized study. Reproducible standard procedures and more clinical trials are required in order to produce more definite results regarding validation or exclusion of hENT1 as predictive biomarker in pancreatic cancer.

A UGT1A1 Genotype-Guided Dosing Study of Modified FOLFIRINOX (mFOLFIRINOX)

While FOLFIRINOX improves survival compared with gemcitabine in advanced pancreatic cancer [10], there is a risk of severe, potentially life-threatening toxicity, mainly due to irinotecan. The active form of irinotecan SN-38, is subsequently inactivated through glucuronidation

Table 1. Summary of abstracts with pharmacogenetic interest presented at the 2014 ASCO Annual Meeting.

Abstract #	Abstract Title	Main findings
# 4124 Sinn, et al.	Hent1 expression in patients with pancreatic cancer treated with gemcitabine after curative intended resection: Results from the CONKO-001 trial.	Hent1 expression not confirmed to predict disease-free (DFS) and overall survival (OS).
# 4125 Sharma, et al .	A UGT1A1 genotype-guided dosing study of modified FOLFIRINOX (mFOLFIRINOX) in previously untreated patients with advanced gastrointestinal malignancies.	mFOLFIRINOX tolerable in UGT1A1*1/*1 pts at standard IRI dose, in *1/*28 pts at reduced dose. *28/*28 pts cannot tolerate reduced dose.
# 4130 Yu, et al.	Pharmacogenomic modeling of pancreatic cancer for prediction of chemotherapy response and resistance in second-line treatment setting.	CTICs' pharmacogenomic profiling predicts chemotherapy efficacy in patients with unresectable pancreatic Ca.

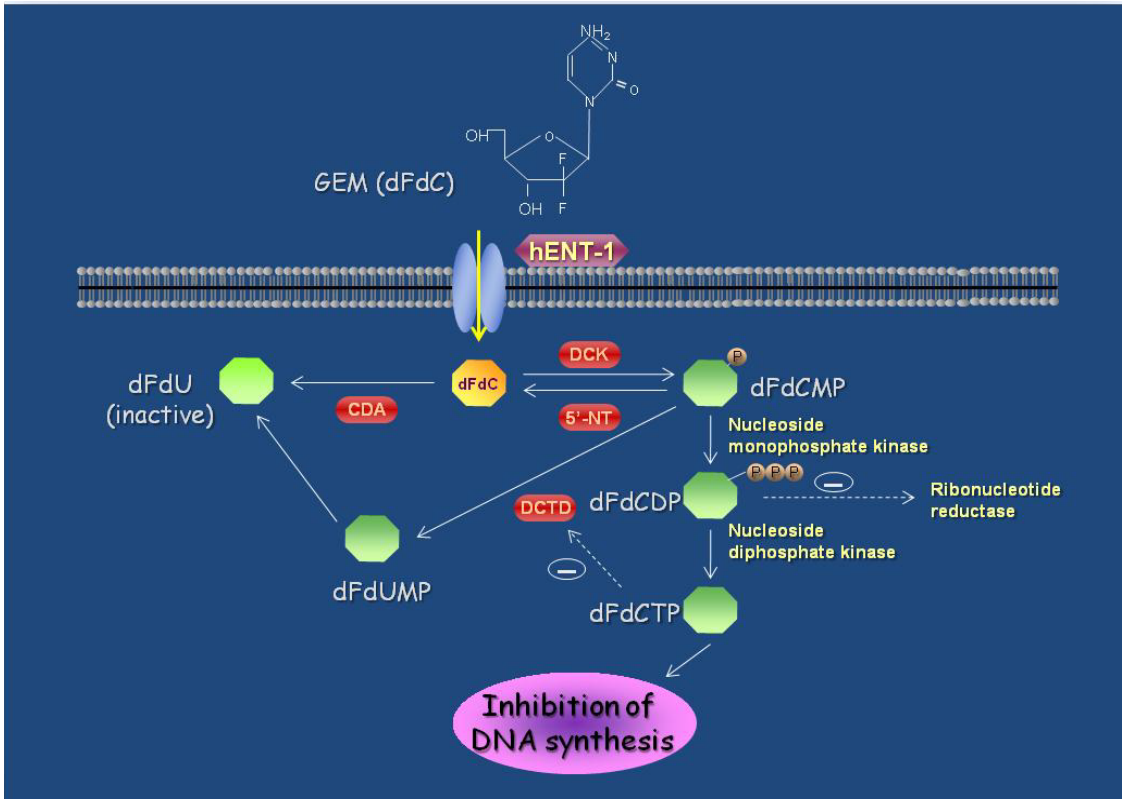

Figure 1. Cellular metabolism of gemcitabine.

dFdC: gemcitabine; dFdU: 2'-deoxy-2',2'-difluorouridine; DCK: deoxycytidine kinase; dFdCMP: gemcitabine monophosphate;dFdCDP: gemcitabine diphosphate; dFdCTP: gemcitabine triphosphate; DCTD: deoxycytidine monophosphate deaminase; CDA: cytidinedeaminase; 5'-NT: 5'-nucleotidase; dFdUMP: 2'-deoxy-2',2'-difluorouridine monophosphate

Table 2. hENT1 expression in pancreatic cancer: results from the CONKO-001 trial (Abstract # 4124) [8].

Study arm	No. of patients	Disease free survival (DFS)		Overall survival (OS)	
		According to hENT1 expression		According to hENT1 expression	
Gemcitabine	88	12.9 months	[a]High: 11.5 months [b]Low: 13.2 months	22.7 months	[a]High: 19.7 months [b]Low: 24.4 months
p value / Hazard Ratio			p=0.5/HR:1.19		p=0.92/HR:1.03
Observation	68	6.2 months	[a]High: 5.9 months [b]Low: 6.2 months	19.1 months	[a]High: 20.4 months [b]Low: 17.7 months
p value / Hazard Ratio			p=0.83		p=0.65

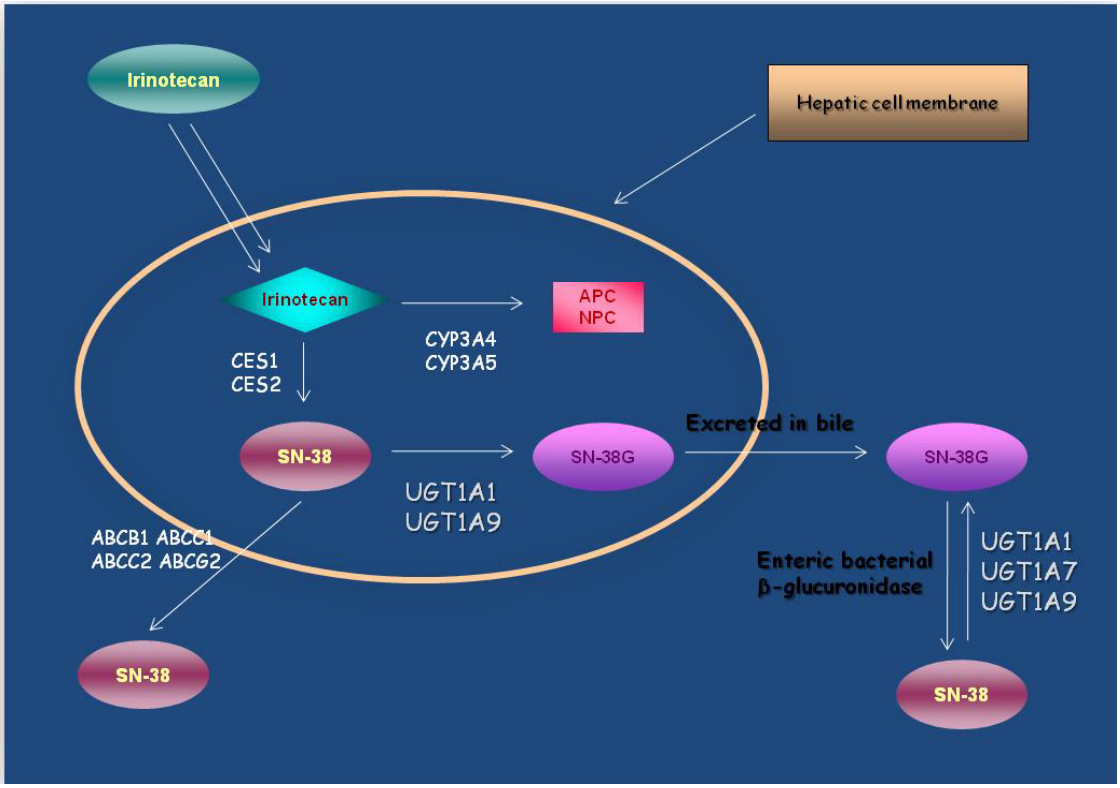

Figure 2. Metabolic pathway of irinotecan, a prodrug that is activated by carboxylesterase to the active metabolite SN-38. SN-38 is glucuronidated by uridinediphosphateglucuronosyl-transferases (UGTs). Especially, hepatic UGT1A1 and UGT1A9, as well as extrahepatic UGT1A7, are forming the inactive metabolite SN-38 glucuronide (SN-38G), which is eliminated in the bile.

SN-38: 7-ethyl-10-hydroxycamptothecin; CES1,2 carboxyesterase 1 and 2; SN-38G: SN-38 glucuronide; CYP3A4, CYP3A5: cytochrome P-450 3A4 and 3A5; APC: 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyl-oxycamptothecin; NPC: 7-ethyl-10-[4-amino-1-piperidino] carbonyloxycamptothecin); ABC: adenosine triphosphate-binding cassette B1, C1, C2, G2.

Table 3. Results from UGT1A1 genotype-guided dosing study of mFOLFIRINOX (mFOLFIRINOX) in previously untreated patients with advanced gastrointestinal malignancies (Abstract #4125) [9].

UGT1A1 genotype	Initial IRI dose (mg/m^2)	No. of DLT/patients	No. of DLT events
***1/*1**	180	2/15	Neutropenic fever (n=2)
***1/*28**	135	2/16	Grade 3 fatigue (n=2) Diarrhea (n=1)
***28/*28**	90	3/9	Neutropenic fever (n=2) Grade 3 abdominal pain (n=1)

Table 4. Results of treatment response and PGx prediction (Abstract #4130) [13].

Chemotherapy (No. of pts)	Treatment response/PGx prediction		p value, Hazard Ratio
	Sensitive	Resistant	
First line (n=35)			
PFS (months)	10.4	3.6	p=0.0001, HR 0.14
OS (months)	17.2	8.3	p=0.0249, HR 0.29
Second line (n=15)			
PFS (months)	5.7	2.5	p=0.027, HR 0.15
OS (months)	8.6	3.4	p=0.020, HR 0.14

via members of the UDP-glucuronosyl transferase family (UGTs), with the UGT1A1 felt to be the main member involved (Figure 2). UGT1A1 polymorphisms, with the most common variant being UGT1A1*28 genotype, in clinical studies, mostly involving colorectal cancer patients, has been associated with diminished enzyme activity, which in turn, affects SN-38 degradation, resulting to increased SN-38 bioavailability and thus dose-dependent toxicity [11, 12]. Sharma et al. investigated, whether modifying dosing of FOLFIRINOX (mFOLFIRINOX), based on UGT1A1*28 genotyping analysis in previously untreated patients with advanced pancreatic and other gastrointestinal malignancies, could lead to toxicity prevention [9]. The secondary objective was to assess the objective response rates (ORR; by RECIST 1.1) in the study population. 40 patients (pts) were evaluable for tolerability: 19 pancreatic cancer (PC) pts, 14 biliary tract cancer (BTC) pts, and 7 gastric cancer (GC) pts. In an attempt to establish the toxicity profile of irinotecan-based chemotherapy, patients with *1/*1, *1/*28 and *28/*28 genotype were treated with initial dose of irinotecan at 180, 135, and 90 mg/m^2, respectively, administered every 14 days. Prophylactic pegfilgrastim was not allowed in cycle 1 (28 days), unless clinically indicated. Table 3 summarizes the results of the study. Neutropenic fever was the most common dose limiting toxicity (DLT) (4/7 pts; 57%). Regarding objective response rates (ORR), 35 pts were evaluable for response. ORR: 10/18 (56%) PC pts; 4/13 (31%) BTC pts and 3/4 (75%) GCpts. The authors concluded that modified dosing of FOLFIRINOX is tolerable in homozygous UGT1A1*1/*1 pts at the standard irinotecan dose of 180 mg/m^2, in heterozygous UGT1A1 *1/*28 pts at a reduced dose of 135 mg/m^2, while pts lacking this allele (UGT1A1*28/*28) cannot tolerate a more reduced dose of 90 mg/m^2.

Pharmacogenomic Modeling of Pancreatic Cancer (PDAC) for Prediction of Chemotherapy Response and Resistance in Second-Line Treatment Setting

Treatment selection based on individual's pharmacogenomic profile is a challenging approach in pancreatic cancer. Sangar et al. presented the results regarding pharmacogenomic profiling (PGx) of circulating tumor and invasive cells (CTICs) to predict effective treatment in the second-line setting [13].

In 50 patients with inoperable pancreatic adenocarcinoma, tumor progenitor cells were isolated from peripheral blood prior to first line and second line cytotoxic chemotherapy. After mRNA isolation, gene expression profiling was performed and correlated with pharmacogenomic data regarding chemosensitivity, obtained from the pancreatic cancer cell lines NCI-60, and with clinical data of response. Pathway analysis and specific gene expression differences were identified between responders and non-responders as well as between short and long survivors.

35 patients presented with disease response and 15 patients with disease progression after first line treatment. In these 15 patients, evidence of chemoresistance was observed when tumor progenitor cells extraction and analysis was repeated at disease progression. The results suggest that patients treated with cytotoxins, who were predicted to be effective, had a significantly better clinicopathological outcome against patients predicted to be ineffective, both in the first- and second-line setting (Table 4).

Furthermore, pathway analysis suggested that CTIC SMAD4, ATM and XPO1 expression levels were associated with disease progression in the first-line setting, while deregulation in 32 gene pathways was found in CTICs at disease progression.

The authors concluded that tumor progenitor cells isolation and gene expression profiling might be a useful predictive biomarker in patients with inoperable pancreatic cancer both in the first- and in the second-line setting. Performing pharmacogenomic studies in pancreatic cancer are feasible and may demonstrate potential pathways of treatment resistance.

Discussion

Numerous studies to identify biomarkers that are likely to be used for anticipating the clinical outcome of anticancer drugs in pancreatic cancer have been published. As the prognosis of the disease is poor, early evaluation of treatment effectiveness and early intervention in these patients is of great importance for prolonging survival as well as improving quality of life.

Surprisingly, hENT1 expression did not seem to predict gemcitabine sensitivity in patients treated in the adjuvant setting. Except for nucleoside transporters, it may be that enzymes involved in gemcitabine metabolism, including dCK, CDA, RRM1 may mediate resistance to gemcitabine [2, 14]. In addition, genetic polymorphisms of nucleoside transporter andgemcitabine-metabolizing genes may have a role in modifying response to gemcitabine, obscuring the effect of hENT1 expression.

Regarding irinotecan toxicity, there are comprehensive data to suggest that UGT1A1*28 may provide a genetic marker that patients can be screened for prior to irinotecan therapy and/or dose selection. However, there are still questions remaining, since although screening for this allele can identify patients at risk, the lack of UGT1A1*28 does not preclude the chances of a patient experiencing severe toxicity [5]. A study has also suggested that epigenetic factors, such as methylation, may also play a role in altering UGT1A1 expression [10]. Further analysis, particularly of the pharmacodynamic genes, will hopefully identify the genetic basis of response to irinotecan. Furthermore, monitoring circulating tumor and invasive cells (CTICs) may help gain early insight into treatment choice and effectiveness. Additionally, CTIC gene expression and mutational status may predict potential resistance to treatment [15].

Concluding, validation of a variety of genomic biomarkers would help the clinician to apply a more personalized

approach to administer systemic therapy in order to ensure increased efficacy whilst minimizing toxicity. Bringing the gap between the bench and bedside is a difficult task, and large prospective clinical studies are still warranted to confirm the role of these determinants as predictive markers of response in routine practice.

Conflict of Interest

The authors have no potential conflicts of interest.

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