CANCER
COMMUNICATIONS Open Access

LETTER TO THE EDITOR

Inhibition of glucuronidation in pancreatic cancer improves gemcitabine anticancer activity

Dear Editor,

Pancreatic ductal adenocarcinoma (PDAC) treatment is focused on two regimens. The polychemotherapy, FOLFIRINOX (folinic acid, fluorouracil, irinotecan, oxaliplatin), is used in patients with good health conditions [1], while gemcitabine, as monotherapy, in patients with poor health conditions [2–4]. Gemcitabine resistance-associated pathways have been targeted to sensitize cancer cells, but the results were disappointing. Using a transcriptomic bioinformatics analysis combined with biological validation, we showed that glucuronidation was associated with the gemcitabine resistance in PDAC, and its inhibition could switch tumors from resistant to sensitive.

To unravel the biological drivers of gemcitabine response in PDAC, we determined the transcriptomic dissimilarity between two preclinical models with defined gemcitabine sensitivity (Supplementary Figure S1A). Hierarchical clustering was applied to the expression matrix of 90 patient-derived xenografts (PDXs) and 38 patient-derived primary cell cultures (PDCs). Seven patients with high transcriptomic similarity (HTS) between both preclinical models were detected (Figure 1A). This was confirmed by Spearman's correlation, where HTS samples displayed a higher coefficient than the low transcriptomic similarity (LTS) group (0.48 ± 0.11 vs 0.13 ± 0.18, $P <$ 0.001, Figure 1B). Next, we defined a transcriptomic
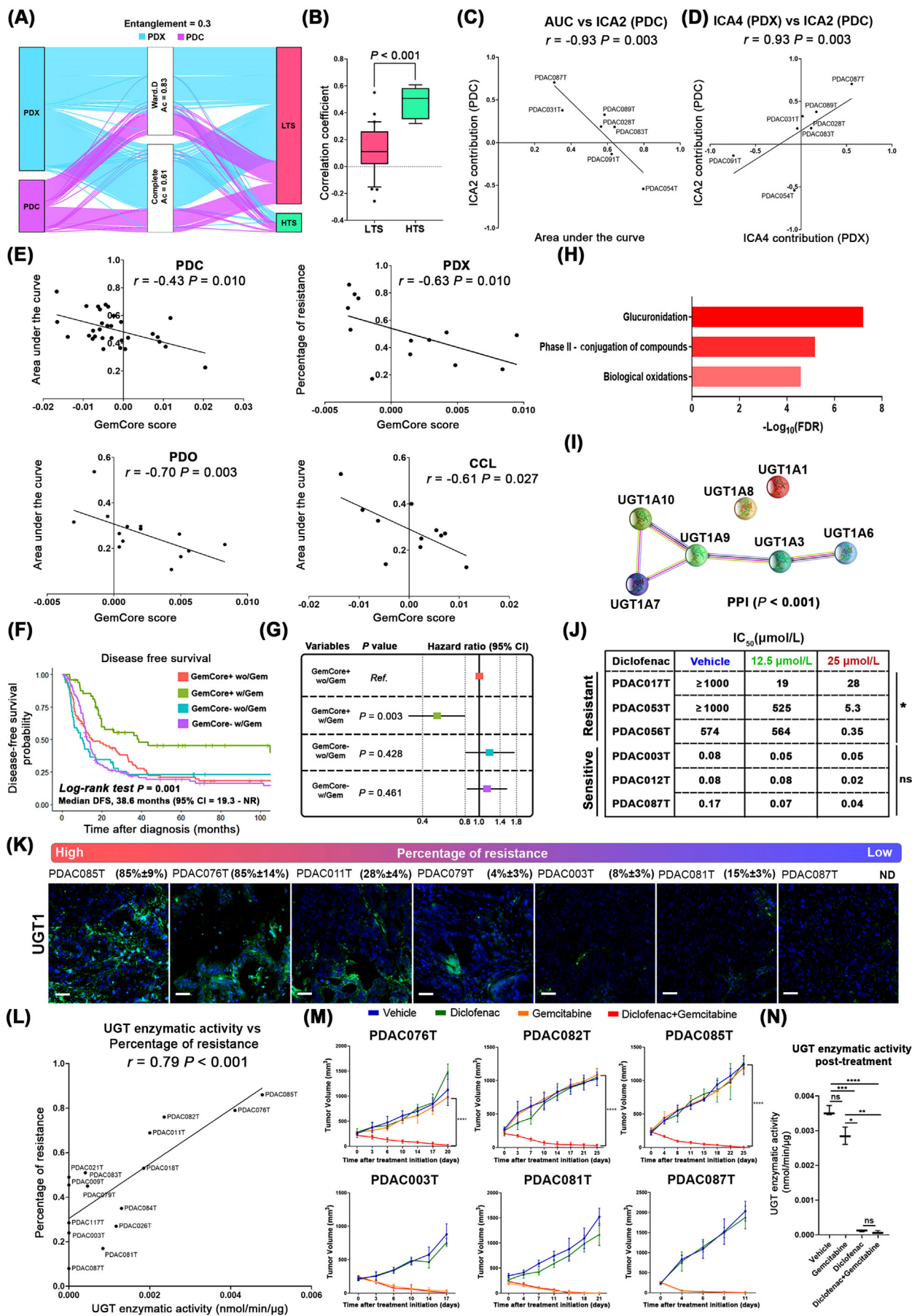
component that captured the biological mechanisms associated with gemcitabine response in the HTS group using independent component analysis (ICA). The selected component had to meet two conditions: (1) be able to capture PDC gemcitabine response and (2) have a homologous component into PDX biological latent spaces. ICA2 component displayed a high correlation with gemcitabine response in PDCs measured by the area under the curve (AUC, $r = -0.93$, $P = 0.003$, Figure 1C). Then, we determined the ICA2 inter-model stability into PDX biological components. ICA4 component extracted from the HTS-PDXs showed a high correlation with ICA2 ($r = 0.93$, $P = 0.003$, Figure 1D). The PDC-selected component was refined, reducing the gene number in intervals of 1 standard deviation until we achieved a higher correlation coefficient in the remaining 31 PDC samples ($r = -0.43$, $P = 0.010$, Figure 1E). This resulted in a reduced ICA2 component with 99 high contributive genes (GemCore). The GemCore score was validated in an independent cohort of 13 PDX samples ($r = -0.63$, $P = 0.010$, Figure 1E), 14 patient-derived organoids (PDOs, $r = -0.70$, $P = 0.003$, Figure 1E), and 11 commercial cell lines (CCL, $r = -0.61$, $P = 0.027$, Figure 1E).

Additionally, GemCore was validated in two external cohorts of patients: (1) a subset of 80 patients treated with gemcitabine as an adjuvant from the cancer genome atlas (TCGA) and (2) 305 patients from the study by Nicolle et al. [5] where 170 patients were treated with adjuvant gemcitabine and 135 without a reported treatment. GemCore+ discriminated the higher survival of patients in the gemcitabine-treated group in the TCGA (Log-rank test $P = 0.035$ Supplementary Figure S1B) and Nicolle et al. cohorts (Log-rank test $P = 0.002$, Supplementary Figure S1C). The median overall survival (OS) of the TCGA GemCore+ patients was 24.6 months [95% confidence interval (CI) = 21.7 months-not reached], while in the Nicolle et al. cohort the median OS was not reached (95% CI = 50.6 months-not reached) for the GemCore+ patients treated with gemcitabine. The median disease-free survival (DFS) of the GemCore+ patients who received gemcitabine

(A) Entanglement = 0.3

(B) $P < 0.001$

(C) AUC vs ICA2 (PDC)
$r = -0.93 \, P = 0.003$

(D) ICA4 (PDX) vs ICA2 (PDC)
$r = 0.93 \, P = 0.003$

(E)
PDC $r = -0.43 \, P = 0.010$
PDX $r = -0.63 \, P = 0.010$
PDO $r = -0.70 \, P = 0.003$
CCL $r = -0.61 \, P = 0.027$

(F) Disease free survival
Log-rank test $P = 0.001$
Median DFS, 38.6 months (95% CI = 19.3 - NR)
GemCore+ wo/Gem
GemCore+ w/Gem
GemCore- wo/Gem
GemCore- w/Gem

(G)

Variables	P value	Hazard ratio (95% CI)
GemCore+ wo/Gem	Ref.	
GemCore+ w/Gem	$P = 0.003$	
GemCore- wo/Gem	$P = 0.428$	
GemCore- w/Gem	$P = 0.461$	

(H)
Glucuronidation
Phase II - conjugation of compounds
Biological oxidations
$-Log_{10}(FDR)$

(I) PPI ($P < 0.001$)

(J)

	IC$_{50}$(µmol/L)		
Diclofenac	Vehicle	12.5 µmol/L	25 µmol/L
Resistant PDAC017T	≥1000	19	28
PDAC053T	≥1000	525	5.3
PDAC056T	574	564	0.35
Sensitive PDAC003T	0.08	0.05	0.05
PDAC012T	0.08	0.08	0.02
PDAC087T	0.17	0.07	0.04

(K) Percentage of resistance — High / Low
PDAC085T (85%±9%) PDAC076T (85%±14%) PDAC011T (28%±4%) PDAC079T (4%±3%) PDAC003T (8%±3%) PDAC081T (15%±3%) PDAC087T ND
UGT1

(L) UGT enzymatic activity vs Percentage of resistance
$r = 0.79 \, P < 0.001$

(M) Vehicle / Diclofenac / Gemcitabine / Diclofenac+Gemcitabine
PDAC076T, PDAC082T, PDAC085T, PDAC003T, PDAC081T, PDAC087T

(N) UGT enzymatic activity post-treatment

in the Nicolle et al. cohort (38.6 months, 95% CI = 19.3 months-not reached) was higher than the other groups ($P = 0.001$, Figure 1F). Cox proportional hazard model for overall survival showed a strong interaction between the GemCore stratification and gemcitabine treatment. TCGA GemCore- patients displayed a hazard ratio (HR) of 2 (95% CI = 1.10-3.90, $P = 0.035$, Supplementary Figure S1D), and in the Nicolle et al. cohort GemCore+ patients treated with gemcitabine showed a HR for OS of 0.45 (95% CI = 0.27-0.73, $P = 0.001$, Supplementary Figure S1E) and for DFS of 0.5 (95% CI = 0.32-0.79, $P = 0.003$, Figure 1G). In contrast with previous reports [5–7], GemCore+ patients were detected in both classical and basal-like subtypes and were distributed across the pancreatic adenocarcinoma molecular gradient (PAMG) in TCGA (Supplementary Figure S1F-G) and Nicolle et al. cohorts (Supplementary Figure S1H-I). No association was found between the gemcitabine response and the mutational profile of KRAS and TP53 (Supplementary Table S1). To identify the biological mechanisms associated with the gemcitabine response, we analyzed the pathways related to the gemcitabine-resistant phenotype. Pathway enrichment analysis on the GemCore revealed a significant role of detoxification pathways (Figure 1H-I) as determinants of gemcitabine response. Among them, glucuronidation was the only pathway with potential targets able to be modulated (Supplementary Table S2).

UGT1A expression and enzymatic activity were measured in 14 PDCs by immunofluorescent and fluorometric assays, respectively. PDCs showed high intra- and inter-heterogeneity in the percentage of positive cells for the UGT1A family, ranging from 90% to not detectable (Supplementary Figure S2A). UGT1A enzymatic activity was correlated positively with the percentage of positive cells ($r = 0.82$, $P < 0.001$, Supplementary Figure S2B) and the gemcitabine response ($r = 0.76$, $P < 0.001$, Supplementary Figure S2C), suggesting an association between the UGT1A family and the PDC resistant profile. The association between UGT1A and gemcitabine resistance was evaluated in vitro using two specific UDP-glucuronosyltransferase (UGT) inhibitors, demethylzeylasteral [8] (T-96) and hecogenin [9]. Fifteen PDCs were assessed in a cotreatment setting with two non-cytotoxic concentrations for each UGT inhibitor (Supplementary Figure S2D). T-96 and hecogenin displayed a strong sensitization effect on gemcitabine at the highest concentration of the inhibitors (25 and 12.5 μmol/L, respectively), and this effect was negatively correlated with the PDC UGT activity for both T-96 ($r = -0.80$, $P < 0.001$, Supplementary Figure S3A) and hecogenin ($r = -0.76$, $P < 0.001$, Supplementary Figure S3B). Because T-96 and hecogenin are not used in the clinics, and their UGT inhibition effect is isoform-dependent, we studied the effect of diclofenac, an unspecific pan-UGT inhibitor broadly utilized clinically [10]. Diclofenac's sensitization effect on gemcitabine was assessed in a subset of six (3 resistant and 3 sensitive) PDCs. Diclofenac improved the gemcitabine cytotoxic activity in the resistant PDCs ($P = 0.011$) but not in the sensitive ones (Figure 1J, Supplementary Figure S3C). These in vitro observations were validated in PDXs, where the UGT

FIGURE 1 Derivation and validation of a gemcitabine-response component. (A). PDX and PDC agglomerative hierarchical clustering of transcriptomic data unravel 7 patients with high transcriptomic similarity (HTS, PDAC028T, PDAC031T, PDAC054T, PDAC083T, PDAC087T, PDAC089T and PDAC091T). (B) Boxplot of Spearman correlation coefficients shows a high correlation between PDXs and the corresponding PDCs in the HTS patients (0.48 ± 0.11) compared with the low transcriptomic similarity group (LTS, $n = 31$), which displayed a coefficient of 0.13 ± 0.18 ($P < 0.001$). (C) Identification of ICA2 component as gemcitabine-response component by independent component analysis on the 7 HTS PDCs and Spearman correlation with the AUC as selection parameter ($r = -0.93$, $P = 0.003$). (D) ICA2 sample contribution correlates positively with the ICA4 component extracted from the 7 HTS PDXs ($r = 0.93$, $P = 0.003$). (E) Scatterplots representing the correlation between the projected ICA2 and the response to gemcitabine measured by AUC, with Spearman test in an independent cohort of 31 PDCs ($r = -0.43$, $P = 0.010$), 13 PDXs ($r = -0.63$, $P = 0.010$), 14 PDOs ($r = -0.70$, $P = 0.003$) and 11 CCLs ($r = -0.61$, $P = 0.027$). (F) Nicolle et al., cohort ($n = 305$) showed a significant patient stratification following the GemCore signature for disease-free survival (DFS). GemCore+ patients treated with gemcitabine showed a median DSF (38.6 months, 95% CI = 19.93-Not reached) higher than other groups (G) Nicolle et al. cohort GemCore+ patients treated with gemcitabine showed a DFS hazard ratio of 0.5 (95% CI = 0.32-0.79, $P = 0.003$). (H) GemCore pathway enrichment analysis shows an association between drug detoxification mechanisms, such as glucuronidation (FDR < 0.001), phase II conjugation of compounds (FDR < 0.001), and biological oxidations (FDR < 0.001) with gemcitabine resistance. (I) GemCore String enrichment analysis shows significant protein-protein interactions (PPI) between the UGT proteins (J) The IC_{50} after the treatment with diclofenac, observing a significant reduction in the IC_{50} in the resistant PDCs at 25 μmol/L. (K) UGT1A expression measured by immunofluorescence in a set of PDX samples distributed along the gemcitabine response profile, displaying a range from 85% to no detectable number of cells. Scale bar: 200 μm. (L) Spearman correlation of UGT activity and percentage of resistance in a set of fifteen PDXs ($r = 0.79$, $P < 0.001$). (M) Tumor growth curves for six PDXs (3 resistant and 3 sensitive) treated with vehicle, gemcitabine, diclofenac, or diclofenac plus gemcitabine. Resistant PDXs (PDAC076T, PDAC082T, and PDAC085T) treated with diclofenac and gemcitabine show a significant reduction in tumor volume compared with gemcitabine alone. (N) Box plot of UGT activity levels in resistant PDXs at the end time-point. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Results are shown as means $\pm$ SD. Differences between two groups are calculated by the Mann-Whitney test. Multi-group comparison is calculated by the Kruskal-Wallis test following Dunn's test

activity was correlated with the percentage of positive cells ($r = 0.96$; $P < 0.001$, Figure 1K, Supplementary Figure S4) and response to gemcitabine (Figure 1L). We observed a reduction in tumor volume in the resistant PDXs co-treated with gemcitabine and diclofenac compared with gemcitabine alone ($P < 0.001$ at the endpoint, Figure 1M). The tumor volume reduction was associated with a decrease in the UGT enzymatic activity in the gemcitabine-resistant PDXs (Figure 1N).These in vitro and in vivo results demonstrated that UGT activity was associated with gemcitabine resistance in PDAC, promoting faster elimination of gemcitabine. Additionally, UGT inactivation switches PDAC gemcitabine response from resistant to sensitive. However, we cannot discard that the observed effect could be influenced by the diclofenac activity on cyclooxygenase-1 and -2, which are the main targets of this drug.

In conclusion, we found that glucuronidation was one of the main mechanisms involved in gemcitabine resistance in PDAC. Moreover, we proposed the combination of diclofenac and gemcitabine as a transferable clinical strategy to increase the gemcitabine anticancer activity in resistant PDAC.

AUTHOR CONTRIBUTIONS

ND and JI designed the study and obtained funding. NAF, AMA, BC, MB, OG, and JR performed the experiments and analyzed the data. NAF, AMA, EC, and JI constructed the figures and wrote the manuscript. All authors discussed the results and suggested revisions. All authors read and approved the final version of the manuscript. The corresponding author is responsible for all aspects of this work, including data analysis and presentation.

ACKNOWLEDGMENTS

COMPETING INTERESTS

ND and JI are funders of PredictingMed (Marseille, France). MB is employed by PredictingMed. All remaining authors have declared no conflicts of interest.

FUNDING

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the local ethics committee (South Mediterranean Personal Protection Committee) following patient informed consent collection. The PaCaOmics study was registered at www.clinicaltrials.gov with registration number NCT01692873. PDAC samples were collected between January 2012 and December 2015. All experimental procedures on animals were approved by the Marseille Ethics Committee number 14 (C2EA-14).

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

TCGA cohort was downloaded with TCGA biolinks R package. Nicolle et al. cohort was requested directly to the authors, and CEL files from CCL were downloaded from the ArrayExpress database (E-MTAB-3610).

Nicolas Alejandro Fraunhoffer[1,2]
Analía Meilerman Abuelafia[1]
Brice Chanez[1]
Martin Bigonnet[1,4]
Odile Gayet[1]
Julie Roques[1]
Eduardo Chuluyan[2,3]
Nelson Dusetti[1] (ORCID)
Juan Iovanna[1] (ORCID)

[1]Cancer Research Center of Marseille, Inserm, Paoli-Calmettes Institut, Aix-Marseille University, Scientific and Technological Park of Luminy, Marseille 13288, France
[2]Buenos Aires University, Center for Pharmacological and Botanical Studies, Faculty of Medicine, National Council for Scientific and Technical Research, Buenos Aires, C1121ABG, Argentina
[3]Buenos Aires University, Faculty of Medicine, Department of Microbiology, Parasitology and Immunology, Buenos Aires C1121ABG, Argentina
[4]PredictingMed, Scientific and Technological Park of Luminy, Marseille 13288, France

Correspondence:
Juan Iovanna, Cancer Research Center of Marseille, Inserm, Paoli-Calmettes Institut, Aix-Marseille University, Scientific and Technological Park of Luminy, Marseille, 13288, France.
Email: juan.iovanna@inserm.fr

Nelson Dusetti, Email: nelson.dusetti@inserm.fr

ORCID

Nelson Dusetti https://orcid.org/0000-0002-6161-8483
Juan Iovanna https://orcid.org/0000-0003-1822-2237

References

1. Neoptolemos JP, Kleeff J, Michl P, Costello E, Greenhalf W, Palmer DH. Therapeutic developments in pancreatic cancer: Current and future perspectives. Nat Rev Gastroenterol Hepatol. 2018;15.(6):333–48.
2. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased Survival in Pancreatic Cancer with nab-Paclitaxel plus gemcitabine. N Engl J Med. 2013;369.(18):1691–703.
3. Burris HA, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first- line therapy for patients with advanced pancreas cancer: A randomized trial. J Clin Oncol. 1997;15.(6):2403–13.
4. Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: A randomized controlled trial. J Am Med Assoc. 2007;297.(3):267–77.
5. Nicolle R, Gayet O, Duconseil P, Vanbrugghe C, Roques J, Bigonnet M, et al. A transcriptomic signature to predict adjuvant gemcitabine sensitivity in pancreatic adenocarcinoma. Ann Oncol. 2021;32.(2):250–60.
6. Rashid NU, Peng XL, Jin C, Moffitt RA, Volmar KE, Belt BA, et al. Purity Independent Subtyping of Tumors (PurIST), A Clinically Robust, Single-sample Classifier for Tumor Subtyping in Pancreatic Cancer. Clin Cancer Res. 2020;26.(1):82–92.
7. Martinelli P, Carrillo-De Santa Pau E, Cox T, Sainz B, Dusetti N, Greenhalf W, et al. GATA6 regulates EMT and tumour dissemination, and is a marker of response to adjuvant chemotherapy in pancreatic cancer. Gut. 2017;66.(9):1665–76.
8. Zhao JW, Wang GH, Chen M, Cheng LH, Ji XQ. Demethylzeylasteral exhibits strong inhibition towards UDP- glucuronosyltransferase (UGT) 1A6 and 2B7. Molecules. 2012;17(8):9469–75.
9. Uchaipichat V, Mackenzie PI, Elliot DJ, Miners JO. Selectivity of substrate (trifluoperazine) and inhibitor (amitriptyline, androsterone, canrenoic acid, hecogenin, phenylbutazone, quinidine, quinine, and sulfinpyrazone) "probes" for human UDP-glucuronosyltransferases. Drug Metab Dispos. 2006;34.(3):449–53.
10. Uchaipichat V, Mackenzie PI, Guo XH, Gardner-Stephen D, Galetin A, Houston JB, et al. Human UDP-glucuronosyltransferases: Isoform selectivity and kinetics of 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. Drug Metab Dispos. 2004;32.(4):413–23.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.