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Uridine Cytidine Kinase 2 as a Potential Biomarker for Treatment with RX-3117 in Pancreatic Cancer*

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Abstract. *Background/Aim: The novel cytidine analog RX-3117, which is activated by uridine-cytidine kinase 2 (UCK2), shows encouraging activity in pancreatic and bladder cancer Phase IIa studies. In this study we highlight the potential role of UCK2 as a biomarker for selecting patients for RX-3117 treatment. Patients and Methods: The online genomics analysis and visualization platform, R2, developed by the Oncogenomics department at the AMC (Amsterdam, The Netherlands) was used for in silico UCK2-mRNA correlation with overall survival of pancreatic cancer patients, while UCK2 protein expression was evaluated by immunohistochemistry on pancreatic tumor formalin-fixed-paraffin-embedded sections from independent pancreatic cancer patients. mRNA expression was also determined for SUIT-2, PANC-1 and PDAC-3. Lastly, the drug sensitivity to RX-3117 was investigated using the Sulforhodamine-B cytotoxicity assay. Results: The in silico data showed that a high UCK2-mRNA expression was correlated with a shorter overall survival in pancreatic cancer patients. Moreover, UCK2 protein expression was high in 21/25 patients, showing a significantly shorter mean. Overall Survival (8.4 versus 34.3 months, p=0.045). Sensitivity to RX-3117 varied between 0.6 and 11 μM. Conclusion: Pancreatic cancer cells are sensitive to pharmacologically achievable RX-3117 concentrations and UCK2 might be exploited as a biomarker for patient treatment selection.*

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In 2018, pancreatic cancer ranked as the fourth cause of cancer-related deaths in Europe (1) and its incidence rate continues to increase (2). The poor outcome of pancreatic cancer is multi-factorial. The majority of patients are diagnosed at advanced disease stage (2) due to its asymptomatic behavior, deeming patients ineligible for surgery. Treatment with radio- or chemo-therapy is therefore the sole option for these patients. Currently, the treatment regimen consists of a combination of: i) leucovorin, 5-fluorouracil, irinotecan and oxaliplatin (FOLFIRINOX) for patients with a good performance or ii) gemcitabine alone or with nab-paclitaxel for the remaining group. The FOLFIRINOX regimen has also significantly improved the survival of patients with resected pancreatic cancer compared to gemcitabine monotherapy, however, its toxicity is considerable (3, 4). In the case of gemcitabine regimens, gemcitabine resistance remains an important limitation, prompting studies for the development of novel therapeutics and biomarkers to help guide treatment decisions.

Fluorocyclopentenylcytosine (RX-3117) is a new antimetabolite that belongs to the same cytidine analogs subgroup as gemcitabine but differs structurally in the ribose-moiety, where it possesses a double bond and a fluoro group. Mechanistic studies have shown that RX-3117 is taken up by cells through the human equilibrative nucleoside transporter 1 (hENT1, SLC29A) (5, 6) and possibly the concentrative nucleoside transporter (hCNT1). Next, RX-3117 is activated by the specific tumor-expressed uridine-cytidine kinase 2 (UCK2) (7), followed by further metabolism of RX-3117 to the active di- and tri-phosphorylated metabolites that are incorporated into RNA and DNA and interfere with their synthesis. RX-3117 can also down-regulate DNA methyltransferase 1 (DNMT1), leading to hypomethylation and subsequent activation of tumor suppressor genes (8, 9). RX-3117 is currently in clinical trials (NCT02030067) as an orally administered monotherapy in patients with advanced bladder cancer and

solid tumors, including pancreatic cancer. Additionally, a combination of RX-3117 with Nab-paclitaxel (Abraxane®) is being evaluated in refractory pancreatic cancer patients (NCT03189914).

RX-3117 shows great potential as a new treatment for pancreatic cancer due to the ease in the route of administration (oral) and its activity in gemcitabine-resistant models. Preliminary results from clinical trials have shown that patients that did not respond to gemcitabine were indeed sensitive to RX-3117, summarized by Balboni *et al*. (6). These results prompted further ongoing clinical trials with RX-3117 as well studies on predictive biomarkers for patient selection (6).

UCK2 expression shows potential as a biomarker for patient treatment selection, since it plays an essential role for RX-3117 function, by catalyzing the first essential activation step of RX-3117 (7). Functionally, UCK2 belongs to the uridine cytidine kinase family, which is responsible for the phosphorylation of uridine and cytidine to their monophosphorylated forms, UMP and CMP, respectively. Structurally, UCK2 shows homology with isoform UCK1, however, UCK2 is only expressed in human placenta and testis (10). UCK2 overexpression has been found in several cancer types, such as colorectal cancer, breast cancer tissue, and glioblastoma, and recent studies reported UCK2 expression also in pancreatic cancer cells (11-13).

In this study we evaluated the expression and potential role of UCK2 in predicting the response in pancreatic cancer together with the effect of RX-3117 on immortalized and primary pancreatic cancer cells.

Table I. *Outcome according to clinical characteristics in the 25 PDAC patient samples collected in an independent study.*

Characteristics	N (%)	OS months Mean (95%CI)	*p*-Value
No. of patients			
All	25	14.0 (12.1-15.8)	
Age, years			
≤65	10	19.1 (14.3-23.9)	0.346
>65	15	25.6 (18.3-33.0)	
Gender			
Male	16	19.9 (14.6-25.2)	0.596
Female	9	24.9 (17.9-31.8)	
Resection status			
R0	12	26.5 (14.1-23.1)	0.236
R1	13	18.6 (11.2-31.0)	
Lymph node			
No	5	28.7 (19.6-37.8)	0.234
Yes	20	19.4 (16.0-22.9)	
Grading			
1-2	15	25.6 (19.4-31.7)	0.129
3	10	13.7 (11.1-16.3)	
UCK2 expression			
Low	4	34.3 (26.9-41.8)	0.045
High	21	18.4 (15.2-21.7)	

Patients and Methods

Evaluation of UCK2 mRNA expression in pancreatic cancer. The mRNA expression of UCK2 was evaluated using the web-based genomics analysis and visualization platform R2 (R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl)) on the TCGA dataset (Tumor pancreatic adenocarcinoma TCGA dataset 178-rsem-tcgars).

Patients. In this study we used tumor samples from 25 patients with pancreatic ductal adenocarcinoma (PDAC), who underwent surgical tumor resection with curative intent. All patients provided a written informed consent for the storage and IHC analysis of their specimens, according to the protocol approved by the Local Ethics Committee of the University of Pisa (#3909, 3rd July 2013).

Immunohistochemical evaluation of UCK2 expression in pancreatic cancer tissues. The protein expression of UCK2 was evaluated by immunohistochemistry (IHC) of Formalin-Fixed, Paraffin-Embedded (FFPE) tumor samples from the 25 PDAC patients. Representative areas of PDAC tumor specimens for each case were selected and prepared for IHC as previously described (14), using the polyclonal rabbit antibody UCK2 (YK-582, Abcam, Burlingame, CA, USA) at room temperature for 1 h (1:100 dilution, in phosphate buffered saline solution) and probed with biotinylated anti-rabbit and anti-mouse secondary antibody, plus a high-sensitivity streptavidin–HRP conjugate (Cell Marque, CytoScan™ , Rocklin, CA, USA) and counterstained with hematoxylin. For negative controls the UCK2 antibody was omitted.

Sections were semi-quantitatively scored for the percentage of cells with UCK2 staining as follows: i) 1+ (<25% staining of tumor cells), ii) 2+ (25–50%), iii) 3+ (50% to 75%). Additionally, the staining intensity was quantified as: i) 0 (negative), ii) 1+ (weak), iii) 2+ (intermediate), or iv) 3+ (strong). Intensity and extension values were added for final scoring (range 0–6), and the tissues with final scores of 4+, 5+ and 6+ were defined as "high expression" of UCK2, while scores below 4+ as "low expression". IHC staining was assessed by two independent investigators (FN and RC), who also examined the amount of tumor and tissue loss, as well as the background, and overall interpretability with respect to high background and low signal intensity.

Cell lines. The pancreatic cancer cell lines PANC-1 (ATCC® CRL-1469, Manassas, Virginia, United States), SUIT-2 (JCRB1094, Tokyo, Japan) and the primary cell line PDAC-3 (15) were cultured in RPMI (Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum (FBS) (Biowest, Nuaillé, France). The cells were grown at 37˚C, 5% CO_2 and were frequently tested for mycoplasma contamination.

RNA-sequencing analysis. RNA-sequencing analyses for PDAC-3 and PANC-1 were performed, as described by Firuzi *et al*. (16). Briefly, the raw data were preprocessed for quality filtering and adapter trimming using FASTX Toolkit version 0.7 (http://hannonlab.cshl.edu/fastx_toolkit/) and subsequently mapped to the Human genome (GRCh38) using STAR alignment tool version 2.5.3a (https://github.com/alexdobin/STAR). We obtained ~90% of

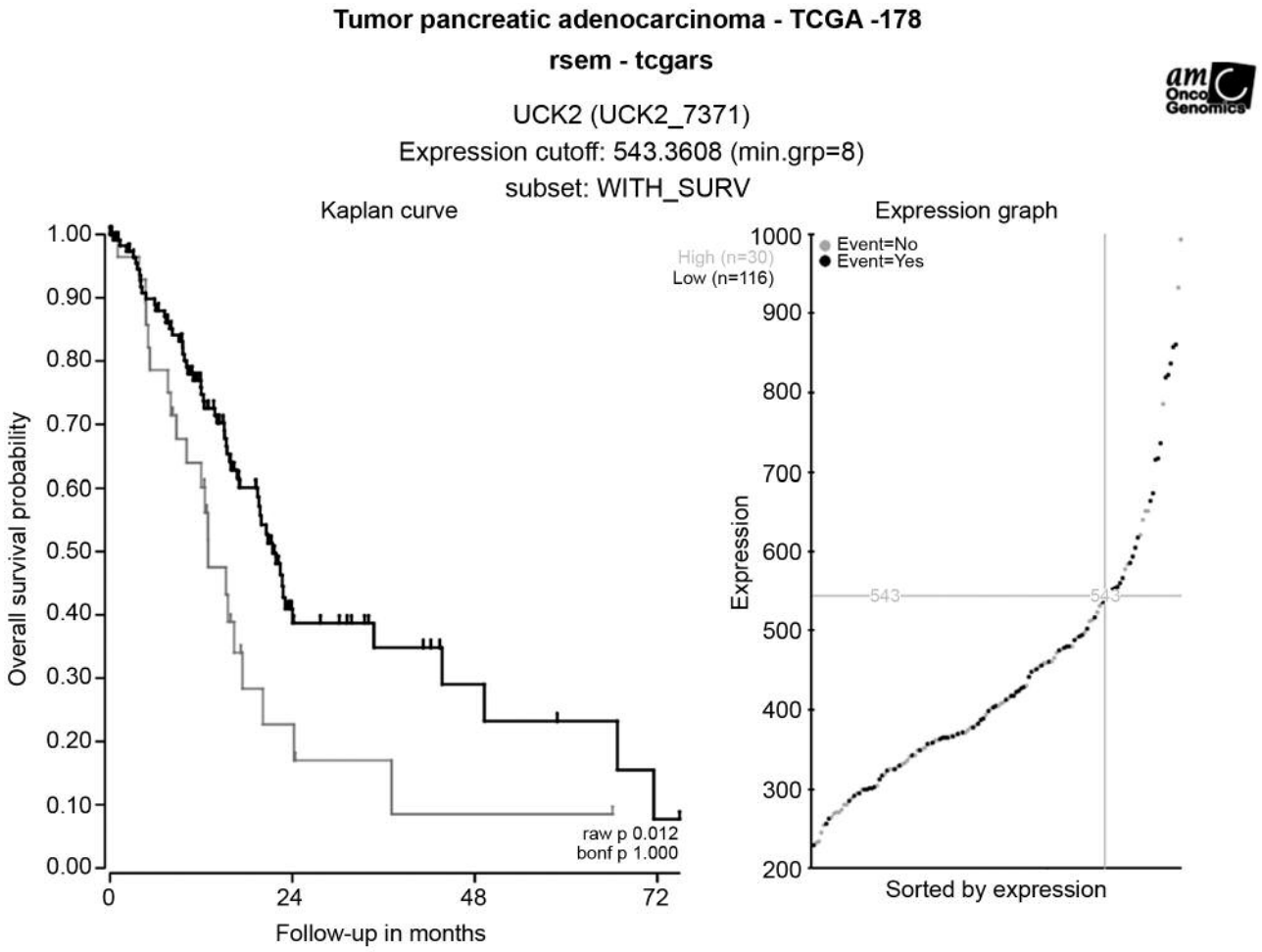


Figure 1. *Correlation of UCK2 mRNA expression with prognosis in pancreatic cancer patients in the TCGA database (Dataset Tumor Pancreatic adenocarcinoma – TCGA -178, analysed with rsem-tcgars, with scan cut-off modus at expression 543.3608). Kaplan-Meier curve of UCK2 mRNA expression with high (n=30; lower curve) and low (n=116, upper curve) expression, (left) and the expression per patient (grey=alive, black=deceased) produced with the R2 online genomics and visualization platform (right). Thirty-two samples were omitted from the analysis due to missing survival data.*

reads mapped to the Human Genome per sample. Gene counts in Fragments Per Kilobase of transcript per Million mapped reads (FPKM) normalization were computed using CuffLinks algorithm and plots were generated with R version 3.5.0. SUIT-2 expression data was downloaded from the Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccle).

Sulforhodamine-B cytotoxicity assay. PANC-1, SUIT-2 and PDAC-3 cells were seeded at a density of 3000 cells per well in flat bottomed 96 well plates (VWR, Dublin, Ireland) to perform the Sulforhodamine B (SRB) assay, as described by Keepers *et al*. (17). A representative curve for each cell line is given as mean±SEM, performed in triplicates, and the 50% inhibitory concentration (IC_{50}) values are calculated and depicted as mean±SEM, with a minimum of two experiments.

Statistics. All experiments were performed in triplicates and repeated at least two times. Data were analyzed by Student's-*t*-test or two-way ANOVA with multiple comparisons, followed by the Tukey's multiple comparison. The mRNA and protein expression data were generated blinded to clinical outcomes and the stratification was adopted at the end of the study. Associations between clinicopathological features were evaluated by Fisher and chi-square tests, while correlation with outcome was evaluated using the Kaplan-Meier curve and log-rank method, using SPSS-24 (IBM, Chicago, IL, USA). Statistical significance was set at $p<0.05$.

Results

High expression of UCK2 mRNA is significantly correlated with shorter overall survival. Applying the online genomics and visualization platform R2 developed by the Oncogenomics department at the AMC (Amsterdam, the Netherlands) on the publicly available TCGA dataset (Tumor pancreatic adenocarcinoma TCGA dataset 178-rsem-tcgars), it was found that in a cohort of 178 patients, 30 patients were classified with high UCK2 mRNA

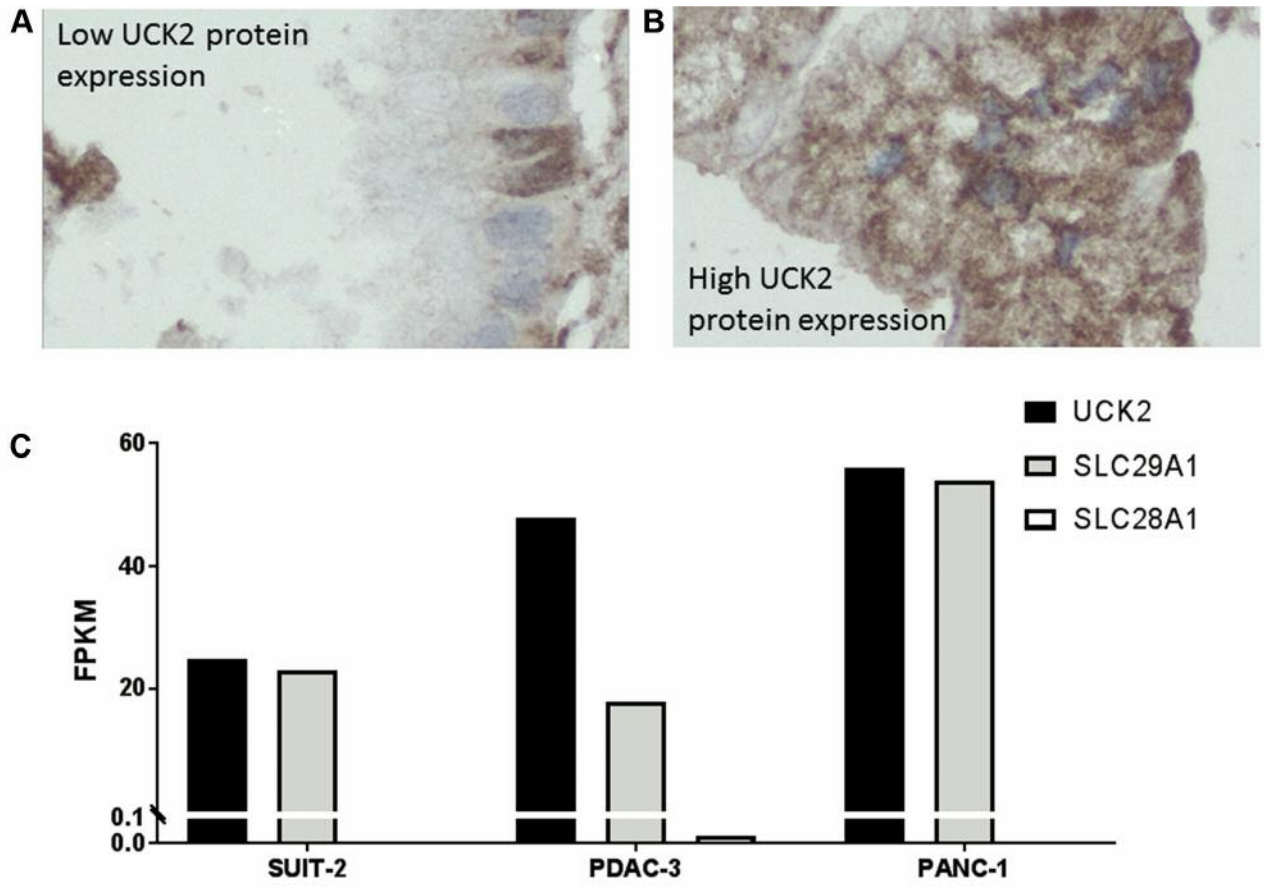
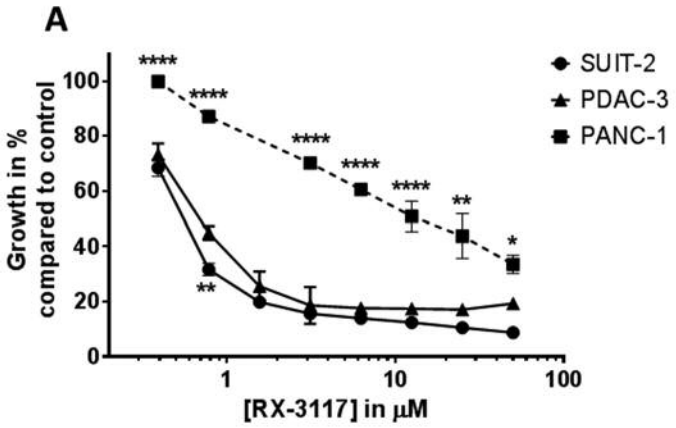


Figure 2. *UCK2 is overexpressed in human tumor tissue and pancreatic cancer cell lines. Evaluation of UCK2 protein expression in PDAC tissues. Representative images at high magnification (40x magnification) of pancreatic tumor tissues with low (A) and high (B) UCK2 protein expression, as detected in the glandular area of two PDAC specimens. (C) UCK2 mRNA expression in cell lines along with hENT1 (SLC29A1) and hCNT1 (SLC28A1) expression, as assessed by RNA-sequencing. The y-axis is plotted in two segments to visualize hCNT1 expression, which is <0.1 FPKM.*

expression, 116 with a low UCK2 mRNA expression and 32 samples were excluded due to missing survival data. In the computed Kaplan-Meier curve (Figure 1), it is shown that a high UCK2 mRNA level is significantly (p=0.012) correlated with a poor overall survival probability compared to a low expression.

UCK2 is highly expressed in human tissue and correlated with shorter survival. In a panel of 25 FFPE sections of pancreatic ductal adenocarcinoma patients, UCK2 showed a variable expression pattern, classified as 'low' or 'high' staining, as shown in representative images in Figures 2A and B. The majority of the patients (21/25) were classified as 'high expression' (score >3). Conversely, the remaining 4 patients were scored below 3. UCK2 expression was significantly correlated with OS (Table I). Patients with high UCK2 expression had an overall survival (OS) of 18.4 [95% confidence interval (CI), 15.2-21.7] months, while remaining patients had an OS of 34.3 (95%CI, 26.9-41.8) months (p=0.045). A trend towards a significant correlation was reported for grading. However, there were no differences in UCK2 expression levels in relation to all the clinicopathological parameters, such as age, sex, resection status, the presence of lymph nodes and tumor grading, as evaluated by Fisher and chi-square tests.

UCK2 mRNA is expressed in pancreatic cancer cell lines and in primary cells. Based on our RNA-sequencing data, the Cancer Cell Line Encyclopedia and appropriate analyses, it was found that UCK2 mRNA is expressed in all three examined cell lines, with the highest FPKM score in PANC-1 cells (Figure 2C). Moreover, the transporter hENT1 (SLC29A1), which is responsible for the cellular uptake of RX-3117, was shown to be overexpressed to the same extent as UCK2. Interestingly, hCNT1, the human concentrative nucleoside transporter 1 (SLC28A1) that can also mediate gemcitabine's cellular uptake, is expressed at very low to undetectable levels in these cell lines.



Cell line	IC_{50} in µM ± SEM
SUIT-2	0.59 ± 0.03
PDAC-3	0.75 ± 0.06
PANC-1	11.0 ± 1.9

Figure 3. *Pancreatic cancer cells are sensitive to RX-3117. (A) Representative growth inhibition curves of pancreatic cancer cells SUIT-2, PDAC-3 and PANC-1 treated with RX-3117, plotted as mean±SEM of one experiment performed in triplicates and repeated at least twice. Significance is indicated as *p<0.05, **p<0.01, ****p<0.0001 at the top of the graph for the comparison of PANC-1 with PDAC-3, and at the bottom for the PDAC-3 compared to SUIT-2. (B) Is showing the corresponding IC_{50} values given as mean±SEM of at least two experiments.*

Pancreatic cancer cells are sensitive to RX-3117. The SUIT-2, PDAC-3 and PANC-1 cells showed different sensitivities to RX-3117 (Figure 3). Both SUIT-2 and PDAC-3 cells, despite the first being immortalized and the second a primary cell line, show a similar sensitivity to RX-3117, in a low micromolar range; instead, PANC-1 cells were more than 11-fold less sensitive ($p<0.002$; Student's *t*-test). Importantly, all these IC_{50} values are within clinically achievable plasma concentrations of patients treated with RX-3117 (6).

Discussion

Tumoral UCK2 expression correlated with poor prognosis of cancer patients with different cancer types, including pancreatic cancer (11, 18). UCK2 up-regulation, but neither UCK1 nor the isoform UCKL1, is also proposed as an indicator for a poor prognosis in hepatocellular carcinoma (HCC) (18). In HCC cells overexpressing UCK2, the migratory, invasive and proliferative potential of the cells increases and is inverted when UCK2 is knocked-down (19). Interestingly, Zhou *et al*., have linked the promotion of metastasis by UCK2 to the Stat3 pathway (20). A relatively unexplored feature of UCK2, a link to apoptosis induction needs to be investigated more extensively in pancreatic cancer.

The role of UCK2 in the aggressiveness of tumors can be considered as a potential marker for RX-3117 sensitivity, since this drug is activated selectively by UCK2 and not UCK1 (7). Our present and earlier data (6, 7) and analyses of publicly available databases show that UCK2 is expressed in many cancer cell lines and tissues Moreover, pancreatic cancer cells, including a primary cell line, are sensitive to RX-3117 in a concentration range that has been achieved in the current treatment protocol (6). Initial analysis of UCK2 expression of circulating tumor cells (CTC) in the ongoing Phase IIa studies have shown that evaluation of UCK2 and hENT1 is feasible during a clinical study (21) and could obviate the need for evaluating tissue expression in unresectable tumors. However, correlation between CTC and tumor tissue expression of UCK2 and/or ENT1 needs to be formally established. The present data on tumor specimens of patients underline the potential role of UCK2 in tumors and the feasibility of analysing UCK2 as a predictive biomarker for patients who would benefit from RX-3117 treatment. Naturally, future clinical studies should aim to validate the role of UCK2 in the antitumor activity of RX-3117, and ultimately consider the use of UCK2 as a potential prospective biomarker of both poor prognosis and potential response to RX-3117 treatment, together with the expression of hENT1.

Conflicts of Interest

GJP has received research funding and conference reimbursement from Rexahn Pharmaceuticals, Rockville, MD, USA.

Authors' Contributions

BEH, EG and GJP designed the study and wrote the manuscript. BEH, JI performed the cytotoxicity studies, GM the NGS data analyses, CR and NP the IHC and EG the patient data analysis.

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References

1 Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, Gavin A, Visser O and Bray F: Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer *103*: 356-387, 2018. PMID: 30100160. DOI: 10.1016/j.ejca.2018.07.005

2 Siegel RL, Miller KD and Jemal A: Cancer Statistics. CA Cancer J Clin *69(1)*: 7-34, 2019. PMID: 30620402. DOI: 10.3322/caac.21551

3 Caparello C, Meijer LL, Garajova I, Falcone A, Le Large TY, Funel N, Kazemier G, Peters GJ, Vasile E and Giovannetti E: FOLFIRINOX and translational studies: Towards personalized therapy in pancreatic cancer. World J Gastroenterol *22(31)*: 6987-7005, 2016. PMID: 27610011. DOI: 10.3748/wjg.v22. i31.6987

4 Conroy T, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul JL, Choné L, Francois E, Artu P, Biagi JJ, Lecomte T, Assenat E, Faroux R, Ychou M, Volet J, Sauvanet A, Breysacher G, Di Fiore F, Cripps C, Kavan P, Texereau P, Bouhier-Leporrier K, Khemissa-Akouz F, Legoux JL, Juzyna B, Gourgou S, O'Callaghan CJ, Jouffroy-Zeller C, Rat P, Malka D, Castan F and Bachet JB: FOLFIRINOX or gemcitabine as adjuvant therapy for pancreatic cancer. N Eng J Med *379(25)*: 2395-2406, 2018. PMID: 30575490. DOI: 10.1056/NEJMoa1809775

5 Peters GJ, Smid K, Vecchi L, Kathmann I, Sarkisjan D, Honeywell RJ, Losekoot N, Ohne O, Orbach A, Blaugrund E, Jeong LS, Lee YB, Ahn CH and Kim DJ: Metabolism, mechanism of action and sensitivity profile of fluorocyclopentenylcytosine (RX-3117; TV-1360). Invest New Drugs *31(6)*: 1444-1457, 2013. PMID: 24048768. DOI: 10.1007/s10637-013-0025-x

6 Balboni B, El Hassouni B, Honeywell RJ, Sarkisjan D, Giovannetti E, Poore J, Heaton C, Peterson C, Benaim E, Lee YB, Kim DJ and Peters GJ: RX-3117 (fluorocyclopentenyl cytosine): a novel specific antimetabolite for selective cancer treatment. Expert Opin Investig Drugs *28(4)*: 311-322, 2019. PMID: 30879349. DOI: 10.1080/13543784.2019.1583742

7 Sarkisjan D, Julsing JR, Smid K, De Klerk D, Van Kuilenburg ABP, Meinsma R, Lee YB, Kim DJ and Peters GJ: The cytidine analog fluorocyclopentenylcytosine (RX-3117) is activated by uridine-cytidine kinase 2. PLoS One *11(9)*: 1-16, 2016. PMID: 27612203. DOI: 10.1371/journal.pone.0162901

8 Sarkisjan D, Pronk F, Kuin R, El Hassouni B, Lee YB, Kim DJ, Dijk M, Geerke DP and Peters GJ: A novel mechanism of DNA methyltransferase (DNMT1) downregulation by epigenetic modulation with RX-3117 (fluoropentenylcytosine). Proc AACR 109th Annual Meeting; Chicago, IL, USA, abstract #5861, 2018.

9 Sarkisjan D, Steenbergen RDM, Cloos J and Peters GJ: Re-emerging antimetabolites with novel mechanism of action with respect to epigenetic regulation: Basic aspects. *In*: Chemotherapy for Leukemia: Novel Drugs and Treatment. Chapter 18. (Ed: T. Ueda), Springer Nature, Singapore Pte Ltd. 311-326, 2017. DOI: 10.1007/978-981-10-3332-2_18

10 Van Rompay AR, Norda A, Lindén K, Johansson M and Karlsson A: Phosphorylation of uridine and cytidine nucleoside analogs by two human uridine-cytidine kinases. Mol Pharmacol *59(5)*: 1181-1186, 2001. PMID: 11306702. DOI: 10.1124/mol. 59.5.1181

11 Shimamoto Y, Koizumi K, Okabe H, Kazuno H, Murakami Y, Nakagawa F, Matsuda A, Sasaki T and Fukushima M: Sensitivity of human cancer cells to the new anticancer Ribo-nucleoside TAS-106 is correlated with expression of uridine-cytidine kinase 2. Japanese J Cancer Res *93(7)*: 825-833, 2002. PMID: 12149149. DOI: 10.1111/j.1349-7006.2002.tb01325.x

12 van Kuilenburg AB and Meinsma R: The pivotal role of uridine-cytidine kinases in pyrimidine metabolism and activation of cytotoxic nucleoside analogues in neuroblastoma. Biochim Biophys Acta - Mol Basis Dis *1862(9)*: 1504-1512, 2016. PMID: 27239701. DOI: 10.1016/j.bbadis.2016.05.012

13 Xu SG, Yan PJ and Shao ZM: Differential proteomic analysis of a highly metastatic variant of human breast cancer cells using two-dimensional differential gel electrophoresis. J Cancer Res Clin Oncol *136(10)*: 1545-1556, 2010. PMID: 20155427. DOI: 10.1007/s00432-010-0812-0

14 Giovannetti E, Wang Q, Avan A, Funel N, Lagerweij T, Lee JH, Caretti V, van der Velde A, Boggi U, Wang Y, Vasile E, Peters GJ, Wurdinger T and Giaccone G: Role of CYB5A in pancreatic cancer prognosis and autophagy modulation. J Natl Cancer Inst *106(1)*: djt346, 2014. PMID: 24301457. DOI: 10.1093/jnci/ djt346

15 Funel N, Giovannetti E, Del Chiaro M, Mey V, Pollina LE, Nannizzi S, Boggi U, Ricciardi S, Del Tacca M, Bevilacqua G, Mosca F, Danesi R and Campani D: Laser microdissection and primary cell cultures improve pharmacogenetic analysis in pancreatic adenocarcinoma. Lab Investig *88(7)*: 773-784, 2008. PMID: 18490900. DOI: 10.1038/labinvest.2008.40

16 Firuzi O, Che PP, El Hassouni B, Buijs M, Coppola S, Löhr M, Funel N, Heuchel R, Carnevale I, Schmidt T, Mantini G, Avan A, Saso L, Peters GJ and Giovannetti E: Role of c-MET inhibitors in overcoming drug resistance in spheroid models of primary human pancreatic cancer and stellate cells. Cancers (Basel) *11(5)*: 638, 2019. PMID: 31072019. DOI: 10.3390/cancers11050638

17 Keepers YP, Pizao PE, Peters GJ, van Ark-Otte J, Winograd B and Pinedo HM: Comparison of the sulforhodamine B protein and tetrazolium (MTT) assays for in vitro chemosensitivity testing. Eur J Cancer Clin Oncol *27(7)*: 897-900, 1991. PMID: 1834124. DOI: 10.1016/0277-5379(91)90142-Z

18 Yu S, Li X, Guo X, Zhang H, Qin R and Wang M: UCK2 upregulation might serve as an indicator of unfavorable prognosis of hepatocellular carcinoma. IUBMB Life *71(1)*: 105-112, 2019. PMID: 30304569. DOI: 10.1002/iub.1941

19 Huang S, Li J, Tam NL, Sun C, Hou Y, Hughes B, Wang Z, Zhou Q, He X, and Wu L. Uridine-cytidine kinase 2 upregulation predicts poor prognosis of hepatocellular carcinoma and is associated with cancer aggressiveness. Mol Carcinog *58(4)*: 603-615, 2019. PMID: 30556610. DOI: 10.1002/mc.22 954

20 Zhou Q, Jiang H, Zhang J, Yu W, Zhou Z, Huang P, Wang J and Xiao Z: Uridine-cytidine kinase 2 promotes metastasis of hepatocellular carcinoma cells *via* the stat3 pathway. Cancer Manag Res *10*: 6339-6355, 2018. PMID: 30568496. DOI: 10.2147/CMAR.S182859

21 Kim DJ, Yang J, Wu W, Davis DW and Eisen A: Phenotyping pancreatic cancer CTCs as biomarkers for RX-3117 clinical trials. Proc AACR 110th Annual Meeting Atlanta, GA, USA, abstract #428, 2019.

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