Fluorocyclopentenylcytosine (RX-3117) is activated by uridine-cytidine kinase 2, a potential biomarker

Godefridus J. Peters[1], Joris R Julsing[1], Kees Smid[1], Daniel de Klerk[1], Dzjemma Sarkisjan[1], Mi Young Yang[2], Young Bok Lee[2], Deog Joong Kim[2],

[1]*Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands;* [2]*Rexahn Pharmaceuticals, Inc., Rockville, MD, USA*

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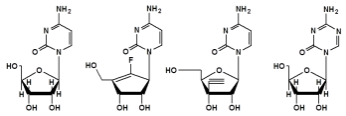
INTRODUCTION

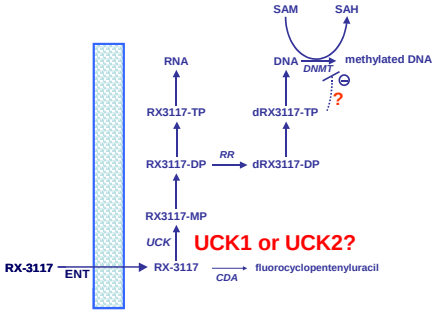
- RX-3117 (fluorocyclopentenylcytosine) is a novel cytidine analog[1]
- RX-3117 resembles ethynylcytidine (Ecyd) and azacytidine (aza-CR)
- RX-3117 is incorporated into RNA and DNA



- RX-3117 is taken up by the equilibrative nucleoside transporter (ENT)
- Preliminary data indicated that it inhibits DNA methyltransferase (DNMT) [1,2]
- RX-3117 is active in cell lines and tumors resistant to gemcitabine[2,3]
- RX-3117 is metabolized by an uridine-cytidine kinase (UCK)
- There are two forms: UCK1 and UCK2

AIMS OF THE STUDY

1. **Is RX-3117 activated by UCK1 or UCK2?**
2. **What is the expression and activity of UCK1 and UCK2 in model systems?**
3. **Can UCK2 be detected by immunohistochemistry?**
4. **Is UCK activity related to RX-3117's efficacy?**

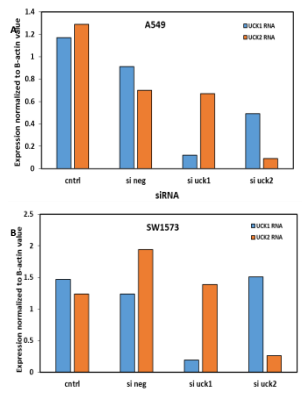


MODEL SYSTEMS

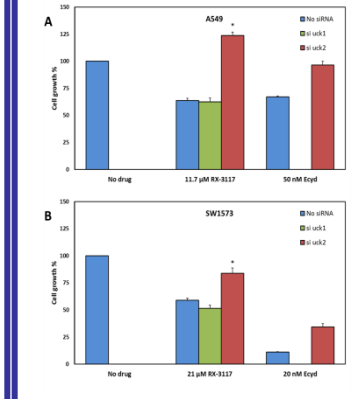
In vitro models:
- A549 and SW1573 non-small cell lung cancer (NSCLC) cell lines
- Cells were cultured in DMEM plus 10% fetal bovine serum
- A panel of various cell lines with different UCK activity

In vivo models:
- a panel of cells from various xenografts
- 3 xenograft models with different sensitivity to RX-3117 and UCK activity

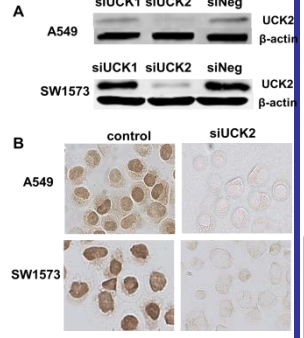
RESULTS



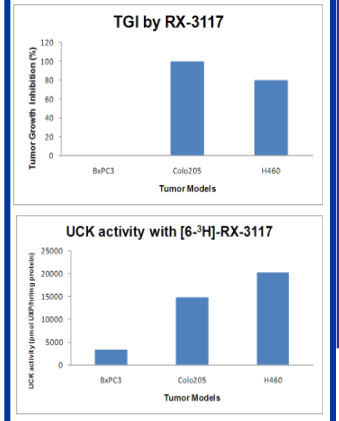
UCK1 and UCK2 mRNA levels 72 hr after siRNA transfection. **(A)** UCK1 and UCK2 mRNA levels after transfection in A549 cells. **(B)** UCK1 and UCK2 mRNA levels after transfection in SW1573 cells. Values are from one representative experiment of multiple performed experiments.



Effect of UCK1 and UCK2 down regulation on RX-3117 cytotoxicity in A549 **(A)** and SW1573 **(B)** cells. Values are means ± SEM (n=6). Values were normalized to 100% for untreated cells. Drugs were added 24 hr after transfection. Cells were exposed to drugs for 48 hr. ECyd was used as a positive control. The protective effect of siUCK2 was considered significant in both A549 (p=0.004) and SW1573 cells (p=0.003). Significance is marked with an asterisk (*).



Validation of UCK2 down regulation. Silencing of UCK2, measured by western blotting **(A)** and immunocytochemistry **(B)**.



Antitumor effect of RX-3117 (upper panel) represented as TGI (total growth inhibition in xenografts of pancreatic cancer (BXPC3), colon cancer (Colo205) and lung cancer (NCI-H460). The insensitive BxPC3 had the lowest enzyme activity (lowest panel).[3]

METHODS

- UCK activity was measured in 8000g supernatants from cell extracts and tumor homogenates by using radioactive [^{3}H]-RX-3117 and [2-^{14}C]-uridine[2]
- RNA was isolated by Trizol, reverse transcribed to cDNA
- UCK1 and 2 expression were measured by qRT-PCR using a Light-Cycler 2.0
- siRNA against UCK1 and UCK2 were obtained from Dharmacon
- Cells were transfected using Dharmafect
- Cytotoxicity to RX-3117 and Ecyd was measured by adding the drugs 24 hr after siRNA treatment, exposed to the drugs for 48 h and cytotoxicity was evaluated by SRB assays[4]
- Immunocytochemistry was measured a polyclonal rabbit antibody (YK-582) (1:200 dilution) and visualized by DAB staining.
- For Western blots, the same antibody (1:1000) was used and visualized using goat anti-rabbit InfraRedDye using an Odyssey InfraRed imager.
- Statistics were done using the Student's t-test and Pearson correlation.

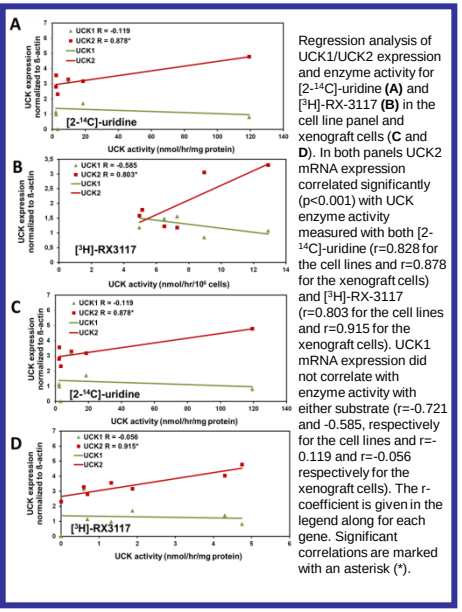
Correspondence: gj.peters@vumc.nl

For further information about RX-3117 and Rexahn Pharmaceuticals, Inc., please contact Dr. DJ Kim:
kimdj@rexahn.com, (240) 268-5300 X 306

CONCLUSIONS

- **RX-3117 is activated by UCK2**
- **UCK2 can be detected by a specific UCK2 antibody**
- **In tumor tissues UCK2 expression relates to UCK activity**
- **RX-3117 has a high antitumor activity in tumors with a high UCK activity**

RESULTS



Regression analysis of UCK1/UCK2 expression and enzyme activity for [2-^{14}C]-uridine **(A)** and [^{3}H]-RX-3117 **(B)** in the cell line panel and xenograft cells (**C** and **D**). In both panels UCK2 mRNA expression correlated significantly ($p<0.001$) with UCK enzyme activity measured with both [2-^{14}C]-uridine ($r=0.828$ for the cell lines and $r=0.878$ for the xenograft cells) and [^{3}H]-RX-3117 ($r=0.803$ for the cell lines and $r=0.915$ for the xenograft cells). UCK1 mRNA expression did not correlate with enzyme activity with either substrate ($r=-0.721$ and -0.585, respectively for the cell lines and $r=-0.119$ and $r=-0.056$ respectively for the xenograft cells). The r-coefficient is given in the legend along for each gene. Significant correlations are marked with an asterisk (*).

References

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2.Peters G.J., et al. Invest New Drugs, 31 (2013) 1444-1457
3.Yang M.Y et al. Anticancer Research, 34 (2014) 6951-6959.
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