

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

Godefridus J. Peters<sup>1</sup>, Joris R Julsing<sup>1</sup>, Kees Smid<sup>1</sup>, Daniel de Klerk<sup>1</sup>, Dzemmas Sarkisjan<sup>1</sup>, Mi Young Yang<sup>2</sup>, Young Bok Lee<sup>2</sup>, Deog Joong Kim<sup>2</sup>,

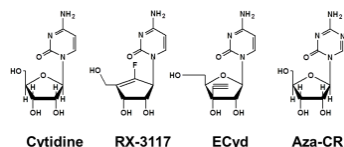
<sup>1</sup>Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands; <sup>2</sup>Rexahn Pharmaceuticals, Inc., Rockville, MD, USA

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## INTRODUCTION

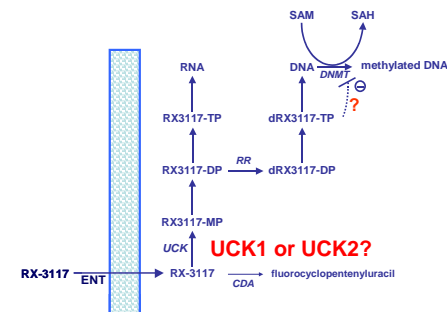
- RX-3117 (fluorocyclopentenylcytosine) is a novel cytidine analog<sup>1</sup>
- RX-3117 resembles ethynylcytidine (Ecyd) and azacytidine (aza-CR)
- RX-3117 is incorporated into RNA and DNA



- 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<sup>2,3</sup>
- RX-3117 is metabolized by an uridine-cytidine kinase (UCK)
- There are two forms: UCK1 and UCK2

## AIMS OF THE STUDY

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

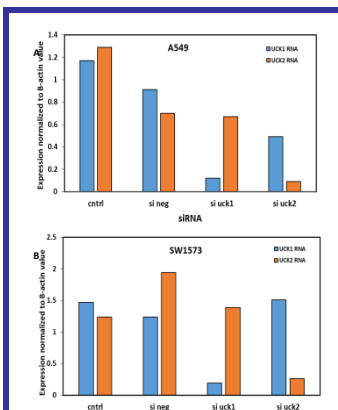


## MODEL SYSTEMS

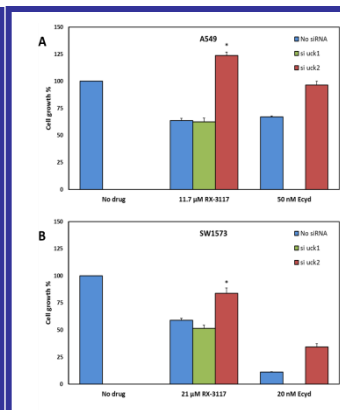
- In vitro models:**
- A549 and SW1573 non-small cell lung cancer (NSCLC) cell lines
  - Cells were cultured in DMEM plus 10% fetal bovine serum
  - A panel of various cell lines with different UCK activity

- 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.



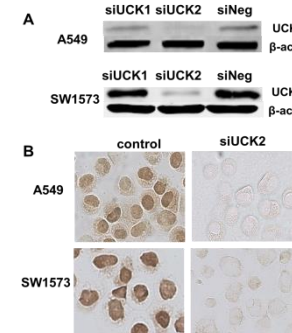
Effect of UCK1 and UCK2 down regulation on RX-3117 cytotoxicity in A549 (A) and SW1573 (B) cells. Values are means ± SEM (n=6). Values were normalized to 100% for untreated cells. Drugs were added 24 hr after transfection. Cells were exposed to drugs for 48 hr. Ecyd was used as a positive control. The 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 (\*).

## METHODS

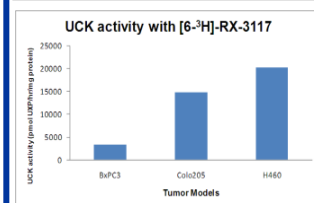
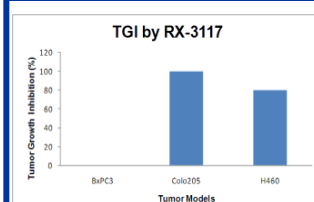
- UCK activity was measured in 8000g supernatants from cell extracts and tumor homogenates by using radioactive [<sup>3</sup>H]-RX-3117 and [<sup>2-14</sup>C]-uridine<sup>2</sup>
- RNA was isolated by Trizol, reverse transcribed to cDNA
- UCK1 and 2 expression were measured by qRT-PCR using a Light-Cycler 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<sup>4</sup>
- 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](mailto:gj.peters@vumc.nl)

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



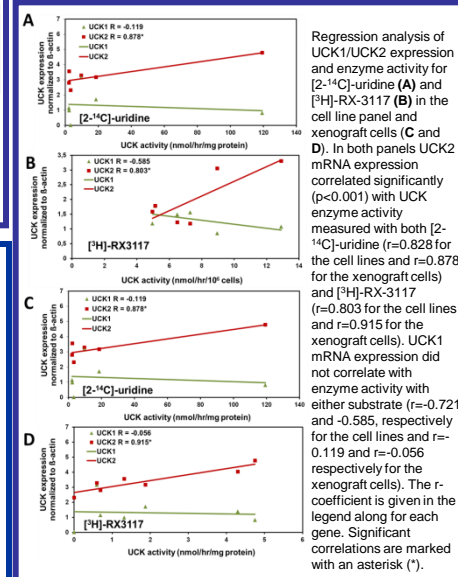
Validation of UCK2 down regulation. Silencing of UCK2, measured by western blotting (A) and immunocytochemistry (B).



## 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



## References

- Choi W.J., et al., J. Med Chem, 55 (2012) 4521-4525.
- Peters G.J., et al. Invest New Drugs, 31 (2013) 1444-1457
- Yang M.Y et al. Anticancer Research, 34 (2014) 6951-6959.
- Keepers Y.P., et al Eur J Cancer, 27 (1991) 897-900

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).<sup>3</sup>