

Unusual mechanism of resistance to the novel cytidine analog fluorocyclopentenylcytosine (RX-3117)



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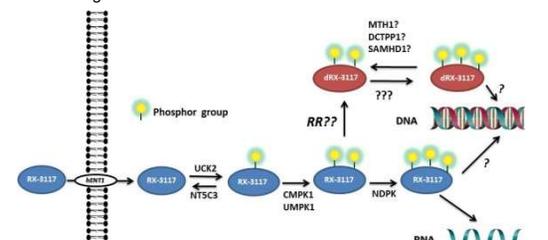
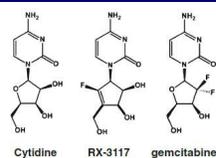
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PHARMACEUTICALS

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INTRODUCTION

- RX-3117 (fluorocyclopentenylcytosine) is a novel cytidine analog¹
- RX-3117 inhibits DNA methylation similar to azacytidine (aza-CR) and aza-deoxycytidine (aza-CdR)
- RX-3117 is incorporated into RNA and DNA²
- RX-3117 is active in cell lines and tumors resistant to gemcitabine^{2,3}



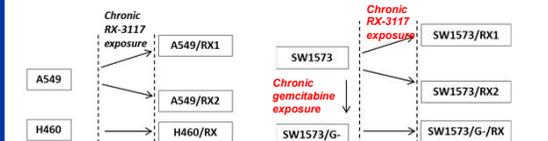
- RX-3117 is taken up by the human equilibrative nucleoside transporter (hENT) and activated by uridine-cytidine kinase 2 (UCK2) to RX-3117-MP⁴
- RX-3117 downregulates DNA methyltransferase 1 (DNMT1)^{1,2}
- RX-3117 is currently evaluated in a Phase IIIa trial in combination with Abraxane in first line metastatic pancreatic cancer patients (NCT03189914)³

AIM OF THE STUDY

Unravel the mechanism of acquired resistance to RX-3117

METHODS

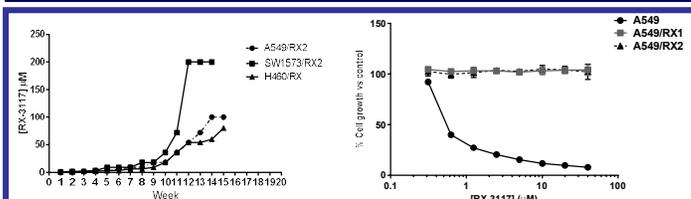
- Methods**
- Resistance was induced by exposure of the non-small lung cancer (NSCLC) cell lines A549 and SW1573, and the gemcitabine resistant variant SW1573/G to stepwise increasing concentration of RX-3117.
 - Drug sensitivity was assessed with the sulforhodamine B assay.
 - Transporters and activation- and deactivation by activity assays, RT-PCR, ICC and WB.
 - LC-MS/MS was used for RX-3117 Nucleotide accumulation.
 - NGS followed by differential expression analysis on the parental and corresponding RX-3117 resistant cells.



References

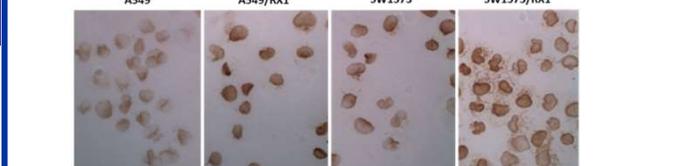
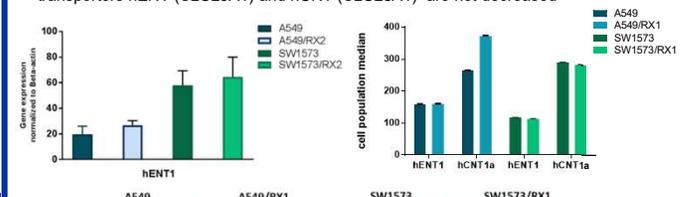
1. Choi W.J., et al., J. Med. Chem., 55; 4521-4525, 2012.
2. Peters G.J., et al. Invest New Drugs, 31; 1444-1457, 2013
3. Yang M.Y et al. Anticancer Research, 34; 6951-6959, 2014
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5. Balboni B et al, Exp Opin Invest Drug, 2019, in press

RESULTS

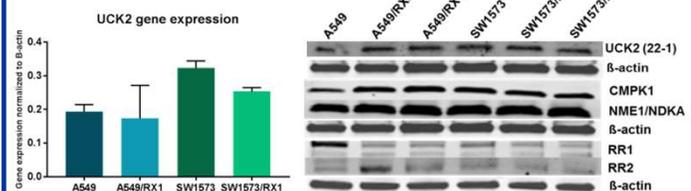


- Induction of resistance to RX-3117 by a stepwise increase of RX-3117
- Complete resistance was observed in A549 and SW1573 cells (exposure for 72 hr)
- Cross-resistance was observed against: Ethynylcytidine, 5-aza-cytidine, Cyclopentenylcytosine
- There was no cross resistance to gemcitabine

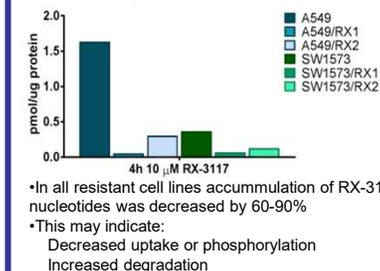
FACS analysis, immunocytochemistry and RT-PCR (not depicted) showed that the transporters hENT (SLC29A1) and hCNT (SLC28A1) are not decreased



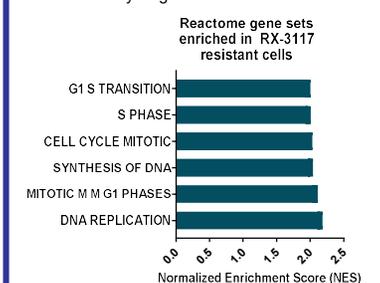
- RT-PCR revealed no decrease in the crucial activation enzyme UCK2 and UMPK (not shown)
- Western blots did not show a decrease in protein expression of UCK2
- Other nucleotides kinases (CMPK1, NDKA) were not decreased
- Ribonucleotide reductases (RR1 and RR2) showed an inconsistent pattern



Total phosphorylated RX-3117 metabolites



NGS sequencing and GSEA analysis showed an upregulation of important cellular pathways, such as cell cycle gene sets



Candidate genes upregulated in RX-3117 resistant cells. The heatmap shows the gene expression (z-score normalized) of the most occurring genes in "resistant" pathways

