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


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INTRODUCTION

• RX-3117 (fluorocyclopentenylcytosine) is a novel cytidine analog.1
• RX-3117 inhibits DNA methyltransferase 1 (DNMT1) and activated by uridine-cytidine kinase 2 (UCK2) to RX-3117-MP.2
• RX-3117 is taken up by the human equilibrative nucleoside transporter (hENT) and hCNT (SLC28A1) are not decreased.3,4
• RX-3117 is currently evaluated in a Phase IIa trial in combination with gemcitabine.3
• RX-3117 downregulates DNA methyltransferase 1 (DNMT1)  and activated by uridine-cytidine kinase 2 (UCK2) to RX-3117-MP.2
• RX-3117 is incorporated into RNA and deoxycytidine (aza-CdR) to azacytidine (aza-CR) and aza-
• RX-3117 inhibits DNA methylation similar to 5-aza-cytidine (5aza-CR) and aza-

AIM OF THE STUDY

Unravel the mechanism of acquired resistance to RX-3117

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.
• Resistance was induced by exposure of the non-small lung cancer (NSCLC) cell lines A549 and SW1573, and SW1573 cells (exposure for 72 hr)
• Complete resistance was observed in A549 and SW1573 cells (exposure for 72 hr)
• Cross-resistance was observed against:
  - Ethynylcytidine
  - 5-aza-cytidine
  - Cyclpentenylcytosine
• There was no cross resistance to gemcitabine

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 Cyclpentenylcytosine
• There was no cross resistance to gemcitabine

• 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

CONCLUSIONS

• RX-3117 nucleotide accumulation is decreased in resistant cells
• This is not related to a decrease of the nucleoside transporters hENT1 and hCNT1
• Activation enzymes (UCK2, UMPK, CMPK, NDKA) are not decreased
• The degradation enzymes 5'-nucleotidase, MTH1 (NUDT1) and DCTP1 are increased in resistant variants

Resistance to RX-3117 is most likely related to an increased RX-3117 nucleotide breakdown, leading to cell cycle disturbance

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