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#293

Screening the druggable genome for synthetic lethal interactions with the CHK1 inhibitor PNT737 (SRA737)

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1. Introduction

Check point kinase 1 (CHK1) is a key regulator of the cell cycle, DNA damage repair and DNA replication.

CHK1 inhibition sensitises cancer cells to genotoxic agents and recent studies have indicated that CHK1 inhibitors could be used as single agents to treat cancers with high levels of replication stress.

We have recently described the discovery of a highly selective and orally bioavailable CHK1 inhibitor, SRA737 (previously PNT737/CCT245737), that not only has potent anti-tumour activity in combination with standard-of-care genotoxic agents but also as a single agent in defined tumour types.[1,2] SRA737 is currently being evaluated in two clinical trials in patients with advanced cancer.[3]

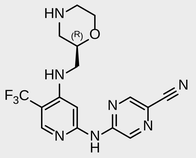


Figure 1: Structure of SRA737 (PNT737)

2. Aim

To identify those gene products whose loss is synthetically lethal with CHK1 inhibition in cancer cells in order to identify patient populations likely to be sensitive to single agent CHK1 inhibition or to identify novel combinations utilising CHK1 inhibitors.

3. Hit identification

We used the Dharmacon druggable human genome siRNA library to conduct a synthetic lethal screen with cell lines with low sensitivity to SRA737; non-small cell lung cancer (NSCLC) cell line A549 and colon cancer cell line SW620.

We identified *POLA1, POLE* and *POLE2* siRNA as hits in both A549 lung and SW620 colon human cancer cell lines.

All three belong to the B- family of DNA polymerases (α, δ and ε) which function at the replication fork; Pol δ and ε have also been implicated in other synthetic DNA processes.

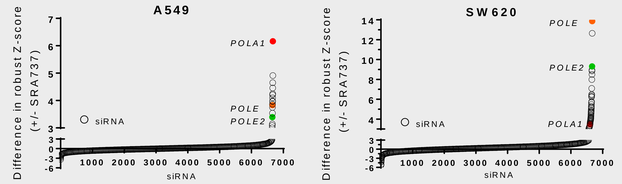


Figure 2: Summary of siRNA screen hits. Data presented as difference in robust Z-score between treated and untreated conditions; the greater the difference in robust Z-score, the greater the difference in cell viability. n≥3

4. Hit validation

POLA1, POLE and *POLE2* were validated as hits in A549 and SW620 and additional NSCLC and colon cancer cell lines using individual Qiagen siRNAs.

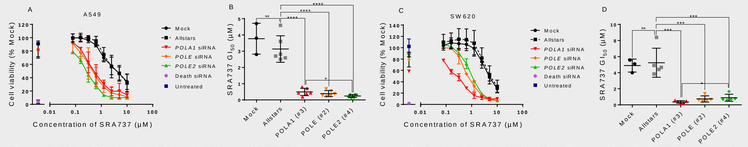


Figure 3: Hit validation in A549 and SW620. SRA737 dose-response curves in A549 (A) and SW620 (C) cells transfected with Allstars negative siRNA, *POLA1, POLE* or *POLE2* siRNA (SRB) (n=3). SRA737 GI_{50} determinations calculated from dose-response curves in A549 (B) and SW620 (D) cells.

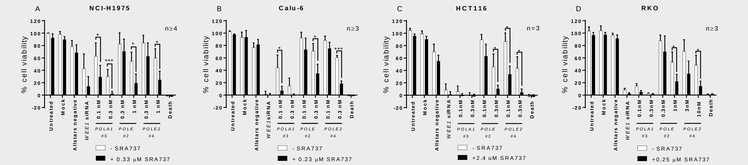
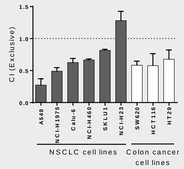


Figure 4 : Hit validation in additional human NSCLC and colon cancer cell lines. (A-D) Cells were transfected with either 25 nM Allstars negative control, *WEE1* siRNA, death siRNA or individual Qiagen *POLA1, POLE* or *POLE2* siRNA at a range of concentrations for 48 h. Mock cells treated with 0.2 % HiPerFect alone. Cells were then treated with optimum non-lethal concentration of SRA737 for 82 h, n≥3. Statistical analysis used unpaired students t-test *p<0.05, ***p<0.001.

5. Combination studies



Aphidicolin inhibits the B-family DNA polymerases. *POLA1, POLE* and *POLE2* were also validated as hits using the selective tool compound aphidicolin.

Combination indices (CI) for SRA737 and aphidicolin were calculated using the Chou and Talalay method. CI < 1 indicates synergy, >1 antagonism and =1 additivity.[4,5]

The combination was robustly synergistic (CI≤0.8) in 8 out of 9 cancer cell lines tested.

Figure 5: Summary of CI's in panel of NSCLC and colon cancer cell lines. Cells were plated in 96 well plates and incubated for 36 h at 37 ºC. Plates were then treated with SRA737, aphidicolin or a combination of both at a 1:1 ratio of the GI_{50}s (previously determined) and incubated for 4 doubling times at 37 ºC. CI values; mean ± SD n≥3.

Conclusions

Inhibition of B-family DNA polymerases is synthetically lethal with CHK1 inhibition in cancer cell lines

Our data support the case for the use of the clinically relevant combination of SRA737 and gemcitabine, as gemcitabine is metabolised it is incorporated into DNA, inhibiting the B-family DNA polymerases.[6]

It will now be important to establish if subsets of colon and endometrial cancers with mutations in their POLE proofreading domain are sensitive to CHK1 inhibitors.

6. Replication stress response

Phosphorylation of RPA32 is a reported biomarker of replication stress.

PhosphoRPA32 levels, seen as band shift of total-RPA32, were higher in cells treated with a combination of *POLA1*, *POLE* and *POLE2* siRNA and SRA737, in comparison to cells treated with the siRNA or drug alone.

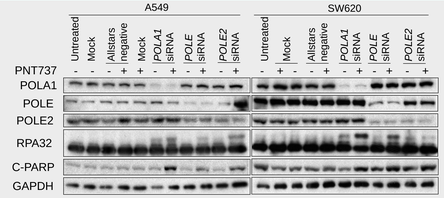


Figure 6: Replication stress biomarkers increase with combination of siRNA and SRA737 treatment. Cells were transfected with 0.1 nM (A549) or 1 nM (SW620) *POLA1* #3, *POLE* #2 or *POLE2* #4 siRNA for 48 h. Allstars negative control at 25 nM, mock treated with lipid only. Cells were then treated with 0.4 or 0.8 μM SRA737, A549 and SW620 respectively, for 24 h. GAPDH used as loading control. n=2.

7. DNA damage: γH2AX immunofluorescence

Combination of SRA737 and aphidicolin increases DNA damage compared to single agent.

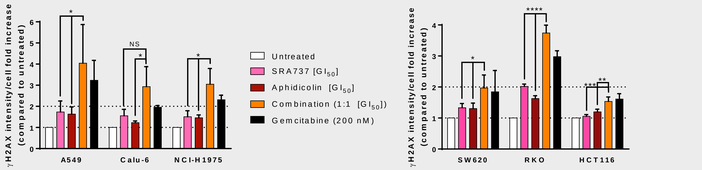


Figure 7: γH2AX intensity in cancer cells treated with SRA737, aphidicolin or combination. Cells were plated in 96 well plates, incubated for 48 h, then treated for 24 h. Cells were fixed and permeabilised and incubated with γH2AX primary antibody (1:500). Cells were imaged on the IN Cell and foci were analysed on IN Cell analyser workstation 3.7.2. (mean ± SD, n≥3).

Contact

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