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Presentation Abstract

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Presentation Title:	Understanding the pathways involved in the repair of CNDAC induced DNA damage
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Poster Section:	22
Poster Board Number:	6
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Abstract Body:

CNDAC is the active metabolite of sapacitabine, which is currently being evaluated in Phase II clinical trials for acute myeloid leukaemia, myelodysplastic syndrome and non small cell lung cancer. CNDAC (2'-*C*-Cyano-2'-deoxy-β-D-*arabino*-pentafuranosylcytosine) was designed as a novel cytosine analogue with a unique mechanism of action. The presence of the cyano-group within the ribose moiety of the molecule causes the formation of single-stranded DNA strand breaks, following incorporation of CNDAC into an extending DNA chain. These breaks are difficult to repair and are processed into double-strand DNA breaks that activate the dsDNA damage checkpoint. As a consequence of this unique mechanism of action, CNDAC arrests cells in the G2/M phase of the cell cycle in contrast to other nucleoside agents such as cytarabine and gemcitabine which cause an S-phase arrest. As such CNDAC may have unique therapeutic applications as a single agent in certain tumour types as well as in combination with other agents compared with standard nucleoside analogues.

In order to identify options for maximising that activity of CNDAC, two approaches were taken to evaluate the repair mechanisms involved for CNDAC induced DNA damage. First a small scale siRNA screen was used to identify genes that were synthetically lethal with CNDAC. Ten targets were initially selected; prioritising genes known to be involved in DNA repair and including BRCA, ATM, CHK and ERCC1. The most dramatic increase in CNDAC sensitivity was seen when BRCA2 was targeted by siRNA, indicating that the homologous recombination DNA repair pathway is involved in the repair of CNDAC induced DNA damage. The second approach involved a cytotoxicity screen evaluating synergy with commercially available agents that either target DNA repair or induce DNA damage themselves. The most promising combinations were then followed up with flow cytometry analysis to examine the induction of cell death. Using this approach the best synergy was detected between CNDAC and either the ATM inhibitor, KU55933 or the CHK inhibitor PF477736. Again these data indicate that the homologous recombination pathway is involved in the repair of CNDAC induced DNA damage.

These data suggest that it would be feasible to explore the clinical use of sapacitabine in diseases where the homologous recombination DNA repair pathway is compromised, such as BRCA deficient tumours. Data from these ongoing studies will be presented.

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Abstract # 3502

UNDERSTANDING THE PATHWAYS INVOLVED IN THE REPAIR OF CNDAC INDUCED DNA DAMAGE

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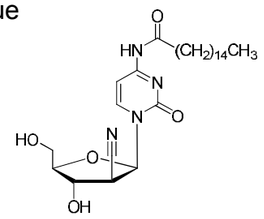
Sapacitabine Key Facts

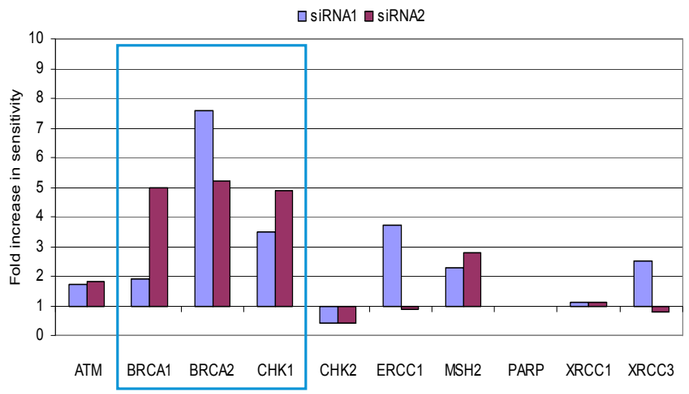
- Orally available 2′-deoxycytidine analogue
- Converted to CNDAC *in vivo*
- Incorporation into DNA results in β-elimination reaction that introduces DNA strand breaks
- CNDAC-induced DNA damage is repaired by homologous recombination
- Phase II clinical trials ongoing in AML, MDS and NSCLC
- Preclinical evaluation of targeted development opportunities in solid tumor backgrounds that exploit the molecule's mechanism of DNA damage



Approach taken to identify genes involved in CNDAC induced DNA repair

- Ten genes that have central roles in DNA damage sensing and repair were selected for evaluation
- At least two independent siRNAs were tested for each gene
- The effect of siRNA targeted knockdown on CNDAC sensitivity was evaluated in standard 96-well cytotoxicity assays
- Performed in 3 cell lines with suitable control compounds
- Cells were seeded in 6-well plates and allowed to settle for 2 h
- Cells were transfected with target siRNA or GL3 control siRNA
- After 24 h cells were trypsinised and replated in 96-well plates (4000/well). Samples were collected for extraction of RNA for qPCR and protein for western blot analysis
- Cells were allowed to settle for 2 h then incubated with CNDAC for 72 h
- The effect on viability was estimated using an MTT assay

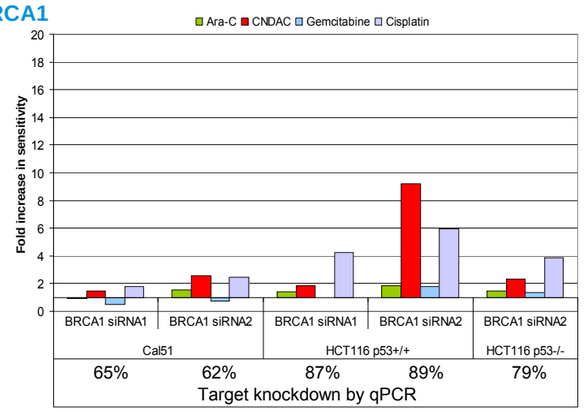
CNDAC/siRNA synthetic lethality screen



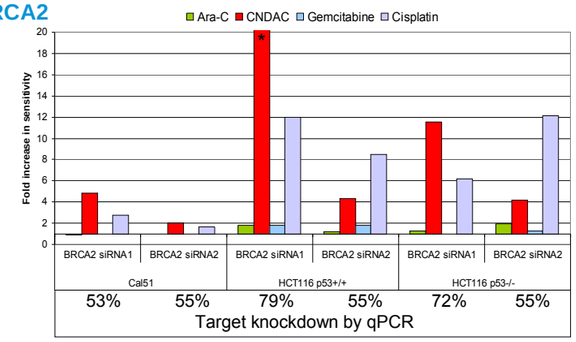
- The effect of gene knockdown on CNDAC sensitivity was assessed in HCT116 cells. Genes showing the greatest effect (BRCA1, BRCA2 and CHK1; boxed) were selected for follow up

Evaluation of the role of BRCA1 & BRCA2 in sensitivity to CNDAC

BRCA1



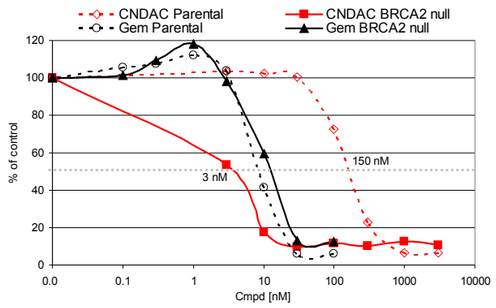
BRCA2



siRNA knockdown experiments were performed in three different cell lines. The effects on target gene expression were quantified using qPCR. Four compounds were evaluated, including three nucleoside analogues. Cisplatin was included as a positive control that has increased cytotoxicity in cells lacking BRCA function. * Actual value: 28-fold.

- CNDAC sensitivity was enhanced in the absence of either BRCA1 or especially BRCA2
- No BRCA dependence was seen for either gemcitabine or cytarabine, highlighting the differences in DNA damage response for these agents

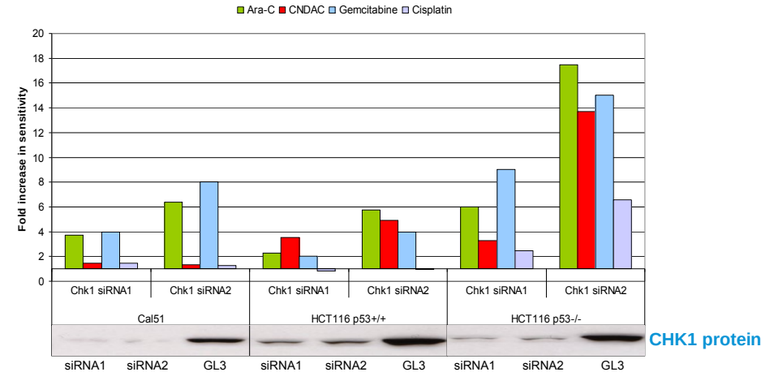
BRCA2 colony forming assay



DLD-1 parental cells (BRCA2 WT) were seeded at 150 cells per well and the isogenic BRCA2 null cells were seeded at 1500 cells per well in 24-well plates and allowed to adhere overnight. Cells were incubated for 10 days at 37ºC 5% CO_2 in a humidified atmosphere in the presence of a semi-log dilution series of CNDAC or gemcitabine. Colonies were fixed using Carnoy's Fixative (75% methanol, 25% acetic acid) for 5 minutes, and allowed to air dry. Colonies were stained using 0.4% (w/v) crystal violet for 2 minutes. Plates were scanned to provide a visual representation. Stain was then solubilised with 10% acetic acid, transferred to a 96-well plate and absorbance units measured using a BMG FLUOstar plate reader. This work was performed at Hypoxium Ltd.

- Clearly there was an enhanced sensitivity to CNDAC in the BRCA2 null cell line (~50 fold) which was not seen for gemcitabine

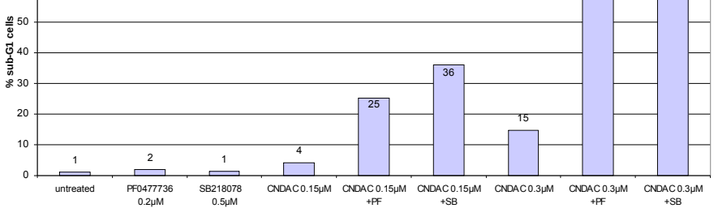
Evaluation of the role of CHK1 in sensitivity to CNDAC



siRNA knockdown experiments were performed in three different cell lines. The effects on target gene expression were assessed using western blot analysis.

- In HCT116 cells CNDAC sensitivity was enhanced in the absence of CHK1
- Enhanced sensitivity was also seen for both gemcitabine and cytarabine

Synergistic induction of cell death by combining CNDAC with CHK1 inhibitors



HL-60 cells were treated with PF0477736 (0.2 μM), SB218078 (0.5 μM), CNDAC (0.15 or 0.3 μM), or concomitant combinations of these agents for 48 h. Samples were collected for flow cytometry to examine the cell cycle profile and induction of cell death. % sub-G1 values are shown.

- CHK inhibition by either PF0477736 or SB218078 abrogated the CNDAC-induced G2 checkpoint leading to synergistic increases in cell death

Summary

- Sapacitabine is a novel, orally available, cell cycle modulating DNA damaging agent being evaluated as a single agent in hematological and solid tumor Phase II trials
- CNDAC induced DNA damage is repaired by homologous recombination
- Cytotoxicity of CNDAC is enhanced in the absence of either BRCA1 or BRCA2, in contrast to the other nucleoside analogues tested (gemcitabine and cytarabine). Similar enhanced sensitivity is seen for both CNDAC and cisplatin, suggesting clinical development options for sapacitabine in BRCA deficient tumors such as triple negative breast or ovarian cancer
- Cytotoxicity of CNDAC (as well as gemcitabine and cytarabine) is enhanced in HCT116 cells by depletion of CHK1. This is confirmed by the fact that combinations of CNDAC with CHK1 inhibitors are highly synergistic, suggesting additional clinical development options for sapacitabine in such combinations

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