Abstract No: 7004

CT7439 an oral first-in class selective CDK12/13 Inhibitor and Cyclin K degrader: Mechanistic profiling and combination efficacy in an ovarian cancer model

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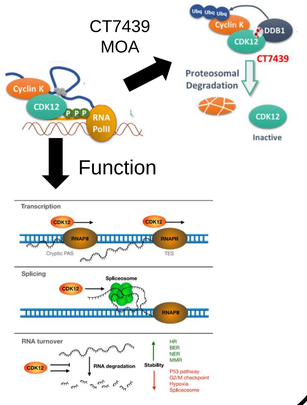
Introduction

Cyclin-dependent Kinases 12/13 (CDK12/13) play a crucial role in transcription, translation, mRNA splicing, cell cycle regulation, and DNA damage repair. Dysregulation of CDK12/13 has been implicated in tumorigenesis, and genetic alterations affecting CDK12 have been identified in multiple cancer types, including breast cancer, ovarian cancer, gastric cancer, and prostate cancer.

CT7439 is a small molecule kinase inhibitor of cyclin-dependent kinases CDK12/CDK13 that received FDA IND approval in 2023. In addition to inhibiting CDK12/13, CT7439 acts as a monovalent glue degrader of cyclin K (CCNK), the cyclin required for CDK12/13 function. The potency and functional activity of CT7439 is therefore determined by this dual mode of action and not solely by its kinase inhibition.

Figure adapted from Magnuson et al 2022



Proteomics confirms selective Cyclin K degradation and reduction in DNA repair proteins

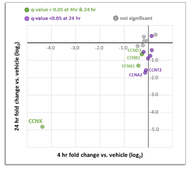
Method: OV-90 cells were incubated in vehicle or 30 nM CT7439 for 4 or 24 hr in triplicate and proteins analysed by DIA (data-independent acquisition) mass spectrometry of all detectable peptides. In total an average of 9565 proteins were quantified per sample. Statistical analysis was performed to identify altered levels of proteins in response to CT7439 compared to vehicle. The distribution of response to CT7439 for proteins within a specific GO term were compared to the responses of all proteins detected in that condition.

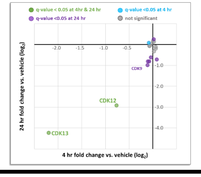
A. Cyclin K is the only cyclin strongly degraded in response to CT7439

17 members of the cyclin family were detected at both 4 and 24 hr timepoints. Cyclin K shows a selective acute and sustained reduction upon exposure to CT7439.

B. CDK12 and CDK13 protein levels are also reduced upon treatment with CT7439

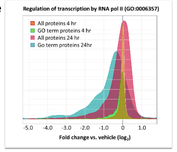
17 members of the CDK family were detected and statistically significant reductions were observed in both CDK12 and CDK13.





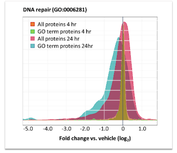
C. RNA transcription proteins are acutely regulated by CT7439

Proteins involved in the regulation of RNA transcription by RNA polymerase II (645 detected proteins; GO term GO0006357) show reduced levels after 4 hr (q-value < $1x10^{-15}$) which becomes more pronounced at 24 hr (q-value < $1x10^{-15}$) as a consequence of CDK12/13 regulating transcription.



D. DNA repair genes show reduced protein levels after 24 hr exposure to CT7439

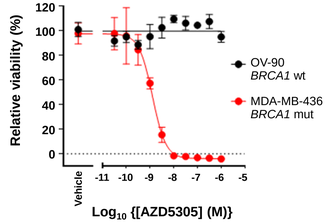
DNA repair proteins (250 detected proteins; GO term GO0006281) shows a significant reduction in their levels after 24 hr (q-value = $2x10^{-9}$), but not 4 hr (q-value = 0.21), demonstrating reduced DNA repair capacity treated cells.



Statistical significance of distributions assessed using the Kruskal-Wallis test

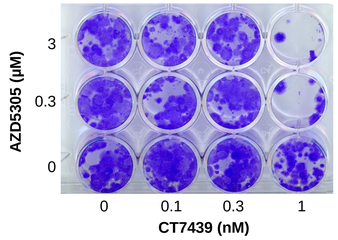
CT7439 sensitises BRCA wt cells to AZD5305, a PARP-1 selective inhibitor

AZD5305 is inactive in BRCA1 wt OV-90 (2D assay)



Viability of *BRCA1* wt, OV-90 or *BRCA1* mut, MDA-MB-436 cells treated with AZD5305 over 10 days.

CT7439 sensitises OV-90 to AZD5305 (colony assay)



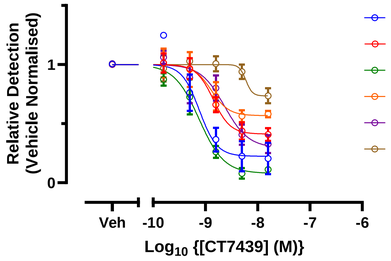
Colony formation assay of OV-90 cells treated with AZD5305 and/or CT7439 for 27 days.

Methods

AZD5305 viability assessment: OV-90 or MDA-436 cells were treated over 10 days with AZD5305 and viability was assessed by CellTiter-Glo assay.

AZD5305/CT7439 colony formation assay: 750 OV-90 cells were seeded per well of a 12 well plate and cultured in the presence of AZD5305 and/or CT7439 with media/drug replenishment every 3 or 4 days. Upon reaching confluency (27 days), cells were fixed in methanol and stained with crystal violet solution.

CT7439 potently regulates transcription of DNA repair genes in ovarian cancer cell line



Methods

OV-90 cells were treated with CT7439 for 4hrs and harvested.

For Cyclin K protein expression, cells were lysed and analysed by Western blot. Relative Cyclin K expression was calculated by normalising to expression of GAPDH.

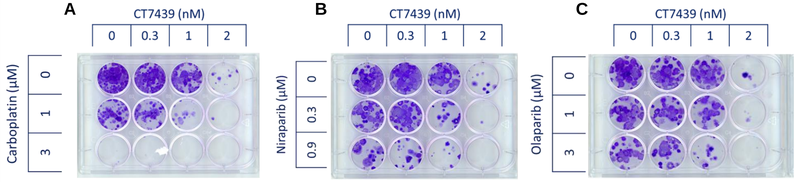
For gene expression, cDNA was generated by use of TaqMan Fast Advanced Cells-to-CT Kit. qPCR was run using Taqman probes (*B2M* as a housekeeper gene) and relative expression calculated by ΔΔCt method relative to vehicle treated control.

Data shown are compiled from 3 biological replicates

DNA damage repair gene and Cyclin K protein expression are reduced in CT7439 treated OV-90 cells after 4 hr, whereas expression of the CDK9 gene target, *MCL1*, is relatively unaffected.

Calculated IC_{50} values: Cyclin K: 0.42 nM, *BRCA1:* 1.45 nM, *ERCC4 :* 0.79 nM, *RAD51C:* 1.21 nM, *POLQ:* 2.46 nM and *MCL1*: >20 nM.

Colony formation shows synergy of CT7439 with Carboplatin, Niraparib or Olaparib



Colony formation assays of OV-90 cells treated with Carboplatin (A), Niraparib (B) or Olaparib (C) and/or CT7439.

Methods

For CT7439 colony formation assays: 750 OV-90 cells were seeded per well of a 12 well plate and cultured in the presence of Carboplatin, Niraparib or Olaparib and/or CT7439 with media/drug replenishment every 3 or 4 days. Upon reaching confluency (25 days), cells were fixed in methanol and stained with crystal violet solution.

Summary of CT7439

- CT7439 is an orally bioavailable selective CDK12/13 inhibitor and degrader of CCNK.
- Expression of multiple DDR proteins are down regulated by CT7439.
- CT7439 demonstrates clinical potential for monotherapy use in solid tumours and as a combination therapy with agents known to cause a DNA damage response.
- First in patient studies ongoing.

Reference

Magnuson *et al.* iScience 2022;25(9):105030.
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