Session PO.ET06.06 - Targeting Protein Kinases and DNA Repair

4178 / 1 - The novel PLK1 inhibitor, CYC140: Identification of pharmacodynamic markers, sensitive target indications and potential combinations

April 4, 2017, 1:00 - 5:00 PM | Section 7

Presenter/Authors

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Abstract

Introduction: CYC140 is a selective and potent ATP-competitive inhibitor of PLK1, which has completed IND-enabling studies. The aim of this translational project was to inform the clinical development path of CYC140. Esophageal cancer was investigated as a potential target indication based on unmet medical need and the observation that PLK1 is frequently overexpressed in esophageal tumors and carries a poor prognosis.

Experimental procedures: The anticancer activity of CYC140 was examined across multiple tumor types to identify sensitive target indications or tumor subsets. 6 h pulse exposure to CYC140 was used to determine sensitivity in a panel of over 250 cancer cell lines, including 15 esophageal cancer cell lines, using CellTiter Glo and resazurin-based assays. Candidate pharmacodynamic markers were examined in malignant and non-malignant cells. Drug combination testing was undertaken in several esophageal cell lines using approved and targeted agents. Solid tumor and leukemia xenograft models were performed to assess CYC140 dosing schedules and efficacy.

Summary: Inhibition of PLK1 by CYC140 perturbs the entry into and exit from mitosis. In malignant cells, CYC140 treatment leads to the appearance of mitotic cells with monopolar spindles and a persistent increase in the proportion of cells in G2 and M phase, resulting in complete growth inhibition and induction of cell death. In non-malignant cells, the growth arrest is transient, and cells resume cycling once compound is removed. Short (6 h) pulse treatments of CYC140 maximise the difference in cellular response between malignant esophageal cell lines and cells of a non-malignant origin. In the esophageal cell line panel, CYC140 cellular IC_{50} correlates with induction of apoptosis. The effect of CYC140 on pharmacodynamic markers of PLK-1 activity such as phospho-nucleophosmin and phospho-histone H3 was characterized in malignant and non-malignant cells. Several promising combinations of CYC140 with targeted agents were identified, including EGFR inhibitors, HDAC inhibitors and PI3K pathway inhibitors, and CYC140 can also be combined with cytotoxic agents approved for use in esophageal cancer, such as cisplatin or irinotecan. CYC140 anti-tumor efficacy was demonstrated in solid tumor and leukemic xenograft models with responses including tumor regression and tumor-free cures.

Conclusions: CYC140 is a promising anti-cancer agent with potent anti-proliferative activity and therapeutic potential in a variety of cancers, including esophageal cancer and acute leukemia. The mode of action of CYC140 is consistent with PLK1 inhibition and cell death is preferentially triggered in sensitive malignant cells. Suitable pharmacodynamic markers and several promising combinations have been identified that could assist clinical development of CYC140.

The novel PLK1 inhibitor, CYC140: Identification of pharmacodynamic markers, sensitive target indications and potential combinations

Abstract # 4178

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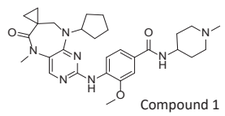
Background

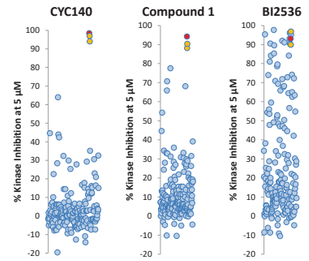
- CYC140 is a selective and potent ATP-competitive inhibitor of PLK1, which has completed IND-enabling studies
- The mechanism of action of CYC140 is consistent with PLK1 inhibition, causing an increase in the proportion of 4N cells in G2 and mitosis, and the appearance of mitotic cells with a monopolar spindle, leading to growth inhibition and cell death
- Short (6 h) CYC140 treatment maximizes cytotoxicity to malignant cells compared with non-malignant cells, suggesting the potential to achieve a therapeutic window
- CYC140 treatment is efficacious in esophageal cancer and acute leukemia mouse xenograft models
- In this study we sought to identify sensitive target indications, suitable pharmacodynamic markers and promising combinations with relevant cytotoxics or targeted agents

CYC140 is selective for PLK1



Compound 1

A PLK1 inhibitory pyrimidodiazepinone series, represented by early lead Compound 1, was assessed for PLK1 potency and selectivity, cellular activity and drug-like properties, resulting in selection of CYC140 as a development candidate.



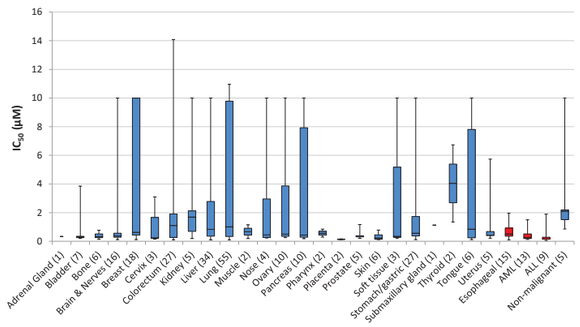
Kinase inhibition by CYC140, Compound 1 and BI2536 were compared in kinase panels with 192 kinases in common. CYC140 and Compound 1 showed greatest inhibition of PLK1 (red circles) followed by PLK2 and PLK3 (orange circles). Compared to BI2536, CYC140 and Compound 1 showed greatly improved selectivity for PLK1 over PLK2 and PLK3, and also over the broader kinase panel.

IC_{50} (nM)

	PLK1	PLK2	PLK3
CYC140	2.95	149	393
BI2536	1.56	21.7	28.8
BI6727	2.10	31.7	53.6

CYC140 is a potent PLK1 inhibitor (IC_{50} ~3nM) and is >50 fold more potent against PLK1 than other PLKs, and >100 fold less potent against non PLK kinases.

Esophageal cancer, AML and ALL are sensitive target indications



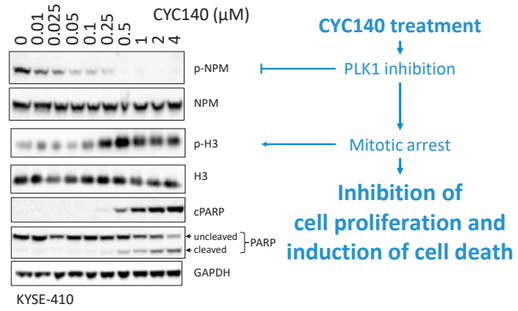
CYC140 cytotoxicity in a panel of 300 cell lines

Cell lines were treated with CYC140 for 6 h, cell viability was assessed at 72 h, and IC_{50} values were determined. A box and whisker plot shows the tumor types tested, and the number of cell lines in each group in parentheses. Where an IC_{50} value was not reached the maximum concentration tested (10 µM) is plotted.

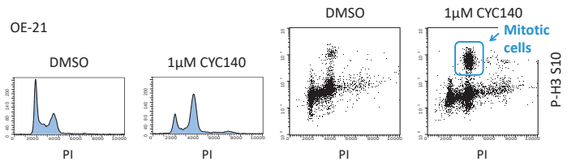
In contrast to non-malignant cell lines (median = 2.1 µM), 65% of malignant cell lines are sensitive to CYC140 (IC_{50} < 1 µM).

Sensitive tumor indications (n ≥5 and >80% samples with IC_{50} <1 µM); bladder (6/7), bone (6/6), brain & nerves (14/16), prostate (4/5), skin (6/6), uterus (4/5), esophageal cancer (13/15) and acute leukemias (20/22). Performed by Crown Biosciences and Cyclacel.

CYC140 mechanism of action and pharmacodynamic markers

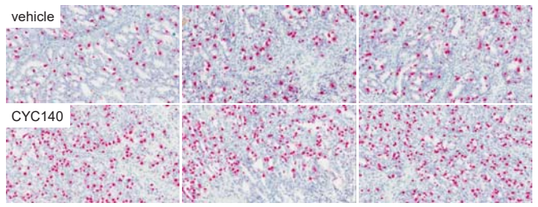


KYSE-410 cells were treated with CYC140 for 6 h, then compound was removed and replaced with compound-free media for the remainder of the experiment. Cells were lysed 2 h (NPM), 24 h (H3) or 72 h (PARP) after the start of treatment. Expression was monitored by Western blotting.



CYC140 increases 4N population and mitotic cell number *in vitro*.

OE-21 cells were treated with CYC140 for 6 h and assayed for DNA content and phospho-histone H3 at 24 h by flow cytometry assays. This approach could form the basis of a clinical pharmacodynamic assay in circulating leukemic or tumor cells.



phospho-histone H3 IHC at 24 h post-treatment

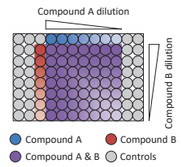
CYC140 increases OE19 mitotic cell number and cell death *in vivo*.

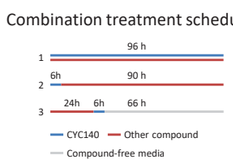
OE19 tumor-bearing mice (n=3) were administered a single oral dose of CYC140 (80 mg/kg). Tumor samples were collected at 24 h, 48 h or 72 h and prepared for IHC with a phospho-histone H3 antibody to monitor the proportion of cells in mitosis. CYC140 increased the proportion of cells in mitosis at 24 h (19.7%) compared to vehicle (14.5%; p=0.01; images above). In addition, there was evidence of increased tumor necrosis at subsequent time points, 48 h and 72 h (data not shown).

Xenografts performed by Crown Biosciences, tumor analysis by Mosaic Laboratories.

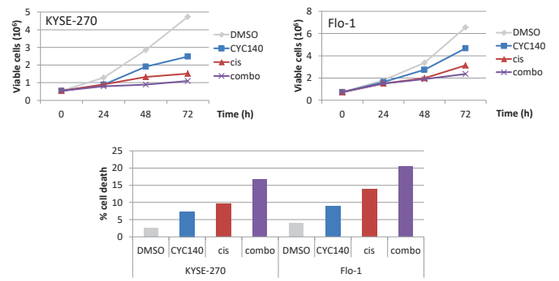
CYC140 combination screen

CYC140 was evaluated against a panel of 7 esophageal cancer cell lines in combination with 40 cytotoxics and targeted agents. A 7x7 concentration matrix was tested using one of three schedules shown and viability was assessed by resazurin-based assay at 96 h. Confirmatory tests used Viacount flow cytometry assay (dye exclusion) at 72 h.





CYC140 combinations with cytotoxic agents

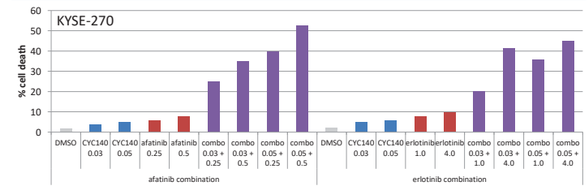


CYC140/cisplatin combination reduces cell number and increases cell death.

KYSE-270 and Flo-1 esophageal cells were treated with DMSO, CYC140 (0.03 µM), cisplatin (1 µM KYSE-270; 2.5 µM Flo-1) or a concomitant combination for 72 h. Total viable cell number was calculated at 24 h, 48 h and 72 h (upper panel) and the proportion of cell death was calculated at 72 h (lower panel). In all cases, the combination (purple) resulted in greater growth inhibition and cell death.

Similar results were obtained with irinotecan/SN38 and CYC140 (data not shown).

CYC140 combinations with targeted agents

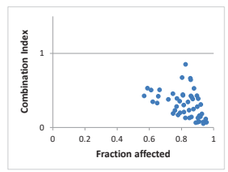


EGFR inhibitor combinations with CYC140 are strongly synergistic.

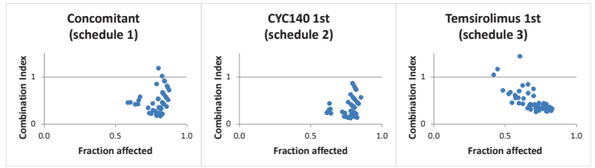
Synergy was observed when CYC140 was combined with four EGFR inhibitors in any of 3 schedules of administration in all 7 esophageal cancer cell lines. Representative data for KYSE-270 afatinib and erlotinib are shown. Similar results were obtained with gefitinib and lapatinib.

Above, KYSE-270 cells were treated with sub-therapeutic doses of CYC140 (0.03 or 0.05 µM), afatinib (0.25 or 0.5 µM) or erlotinib (1.0 or 4.0 µM) alone or in concomitant combination for 72 h resulting in synergistic increases in cell death. Similar synergy was observed in KYSE-450 and SK-GT-4 cell lines. KYSE-270 and KYSE-450 both have mutations in EGFR; SK-GT-4 is insensitive to all 3 compounds as single agents.

A representative Fa-CI plot of the afatinib combination in KYSE-270 (right) shows all CI values are <1, indicative of synergy.



KYSE-270 CYC140/afatinib



SK-GT-4

CYC140 combinations with inhibitors of the PI3K pathway are strongly synergistic in cells with PIK3CA mutations

Representative Fa-CI plots for the combination of temsirolimus and CYC140 in SK-GT-4 cells are shown above. The combination appears to show schedule-independence, with the majority of all CI values below 1, indicative of synergy. SK-GT-4 has a mutation in PIK3CA making it highly sensitive to temsirolimus. Similar results were obtained with OAC-P4C which is also highly sensitive to temsirolimus. In addition, both cell lines are highly sensitive to MK-2206, a PKB inhibitor, which is also synergistic in combination with CYC140 (data not shown).

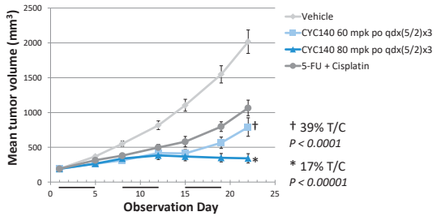
CYC140 can be effectively combined with approved cytotoxics including cisplatin and irinotecan, and is highly synergistic in combination with several targeted agents, including EGFR inhibitors and inhibitors of the PI3K pathway, including temsirolimus (CCI-779; mTOR) and MK-2206 (PKB).

Potent anti-tumor activity in xenografts

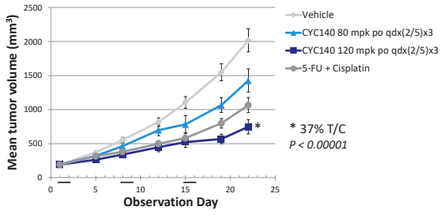
CYC140 demonstrates potent, dose dependent anti-tumor efficacy at well tolerated doses in preclinical xenograft models of acute leukemia and solid tumors, including esophageal cancer.

OE19 esophageal xenograft

5 day schedule



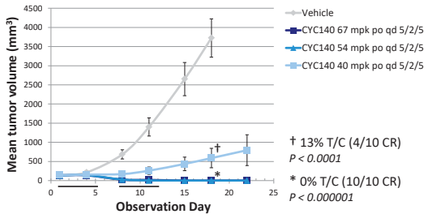
2 day schedule



CYC140 shows superior efficacy to a positive control of weekly 5-FU 60 mg/kg i.p. and cisplatin 3 mg/kg i.v. in this esophageal model.

Xenografts performed by Crown Biosciences.

HL60 promyelocytic leukemia xenograft



Cures of all mice were observed at doses of 54 mg/kg or 67 mg/kg.

Xenografts performed by Southern Research.

Conclusions

- CYC140 is a potent, selective PLK1 inhibitor, which has completed IND-enabling studies
- CYC140 has been evaluated in a panel of 300 malignant and non-malignant cell lines, identifying sensitive target indications including acute leukemia and esophageal cancer
- Consistent with its mechanism of action, CYC140 increased mitotic cell number and cell death in preclinical cancer models
- CYC140 can be combined positively with cytotoxics such as cisplatin and irinotecan
- CYC140 is strongly synergistic in combination with EGFR or PI3K pathway inhibitors, resulting in enhanced growth suppression and increased induction of cell death
- CYC140 shows potent, dose dependent anti-tumor efficacy in xenograft models of acute leukemia and esophageal cancer
- CYC140 is a promising anti-cancer agent with potent anti-proliferative activity and therapeutic potential in several tumor indications including esophageal cancer and acute leukemia

AACR Annual Meeting, April 1-5, 2017, Washington, D.C.

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