Therapeutic potential of novel PLK1 inhibitor CYC140 in esophageal cancer and acute leukemia

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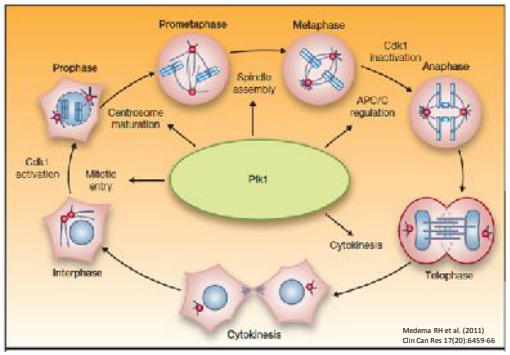
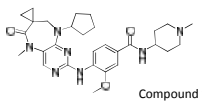
Background



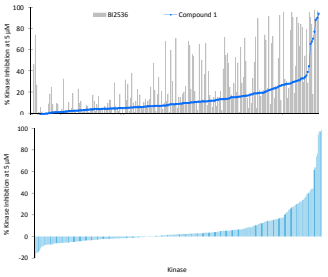
- PLK1, a serine / threonine kinase, is a key regulator of cell division controlling mitotic entry, centrosome maturation, bipolar spindle assembly, regulation of APC/C, mitotic exit and cytokinesis[1]
- PLK1 is frequently overexpressed in cancer, including esophageal cancer and acute leukemia, and elevated expression correlates with disease progression, invasiveness and poor prognosis[2-6]
- Cancer cell proliferation is blocked *in vitro* and *in vivo* by small-molecule PLK1 inhibitors or PLK1 antisense / siRNA[7]. PLK1 inhibitors cause mitotic arrest and subsequent induction of apoptosis in cancer cells[8].
- CYC140 is a selective and potent ATP-competitive inhibitor of PLK1, selected as a clinical candidate. CYC140 has completed IND-enabling studies. Here we present the biological characterization of CYC140, and examine its potential in preclinical models of esophageal cancer and acute leukemia, key target indications with unmet medical need.

CYC140 is selective for PLK1



Compound 1

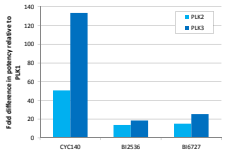
Using a *de novo* ligand design approach we generated a pyrimidodiazepinone scaffold. Compound 1 is an early lead from this chemical series[9]. CYC140 was selected as a development candidate following selection for drug-like properties[10].



Kinase selectivity of compound 1 (blue line) and BI2536 (grey bars) was evaluated in a 216-kinase panel at 5µM. IC_{50} values were then determined for kinases inhibited by compound 1 by more than 50%. For both compounds greatest potency was observed against PLK1 followed by PLK2 and 3. Compared with BI2536, compound 1 showed improved selectivity for PLK1 over PLK2 and 3, and also over the broader kinase panel.

Kinase selectivity of CYC140 was evaluated in a 283-kinase panel at 5µM. IC_{50} values were then determined for kinases inhibited by more than 50%. Compared to compound 1, CYC140 showed improved potency against PLK1, and further improved selectivity over PLK2 and 3 [10]. CYC140 is a selective PLK1 inhibitor (IC_{50} ~3nM), and was >50 fold more potent against PLK1 than other PLKs, and >100 fold less potent against non PLK kinases.

	IC_{50} (nM)		
	PLK1	PLK2	PLK3
CYC140	2.95	149	393
BI2536	1.56	21.7	28.8
BI6727	2.1	31.7	53.6



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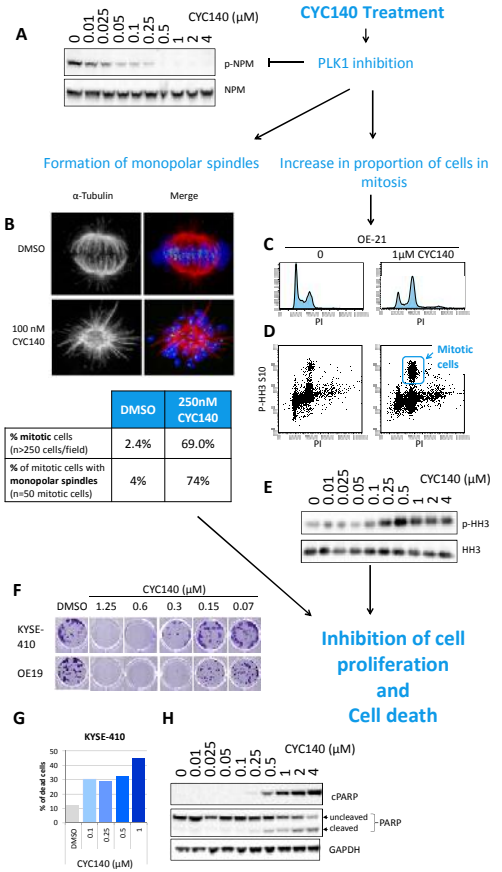
CYC140 mechanism of action

- **Treatment of proliferating cells with CYC140 results in reduced phosphorylation of the PLK1 substrate, pSer4-nucleophosmin (p-NPM), accumulation of cells in mitosis (p-HH3 S10 positive) and an increase in the proportion of mitotic cells with monopolar spindles, features consistent with PLK1 inhibition. This results in inhibition of cell proliferation and induction of cell death in malignant cell lines.**



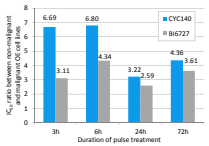
	DMSO	250nM CYC140
% mitotic cells (n>250 cells/field)	2.4%	69.0%
% of mitotic cells with **monopolar spindles** (n=50 mitotic cells)	4%	74%

The time-course of events following treatment with CYC140 can be followed through panels A-H. CYC140 showed a dose-dependent decrease in the PLK1 substrate, pSer4 of NPM 2h after compound treatment in KYSE-410 cells (**A**). To observe the subsequent effect of CYC140 on spindle morphology, HeLa cells were treated with compound for 24h then fixed and stained with antibodies against α-tubulin (red) and the centromeric marker CREST (green); DNA was stained with DAPI (blue) and images acquired by confocal microscopy at 100x (**B**). To observe the effect of CYC140 on cell cycle distribution, cells were treated with CYC140 for 6h, then grown in compound-free media until harvested for downstream analysis at 24h. Flow cytometry was performed on OE-21 cells to measure the effect of CYC140 (1µM) on the proportion of 4N cells (**C**) and p-histone H3 positive mitotic cells (**D**). An increase in the proportion of mitotic cells could also be demonstrated in KYSE-410 cells with an increase in the mitotic marker, pSer10 histone H3, by Western blotting at the same time-point (**E**). Clonogenic assays demonstrated potent growth inhibition by CYC140 (**F**); cells were treated with CYC140 for 6h then cultured in compound-free media for an additional 12 days, fixed and stained with 0.4% crystal violet solution. Cell death was assessed in KYSE-410 cells at 72h (following a 6h pulse treatment) using a Viacount assay (**G**) or by Western blotting for cleavage of PARP, an indicator of cell death (**H**).

CYC140 preferentially targets malignant cells

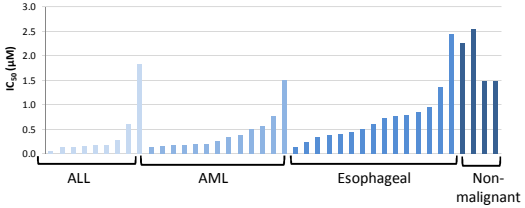
Esophageal cell lines were treated with CYC140 for the indicated time, then media was replaced with compound-free media. Cell viability was evaluated at 144h using a resazurin assay.
ESCC: Esophageal Squamous Cell Carcinoma, BAC: Barrett's Adenocarcinoma

IC_{50} (nM)			3h	6h	24h	72h
Non-malignant		CP-A	1050	1061	98	82
Malignant	ESCC	KYSE-410	188	167	27	21
	BAC	OE19	139	158	35	17
	ESCC	OE-21	180	154	55	27
	BAC	OE-33	76	62	15	14



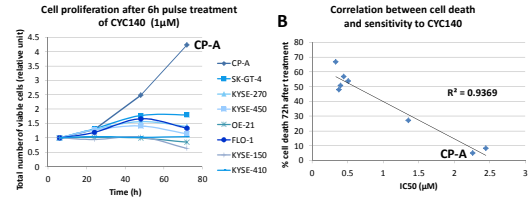
- **Short CYC140 treatments (3-6h) increase the differential cytotoxicity between malignant and non-malignant esophageal cell lines**

Cell lines derived from acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML) or esophageal cancer were treated with CYC140 for 6h then media was replaced with compound-free media. Cell viability was evaluated at 72h using a resazurin assay.

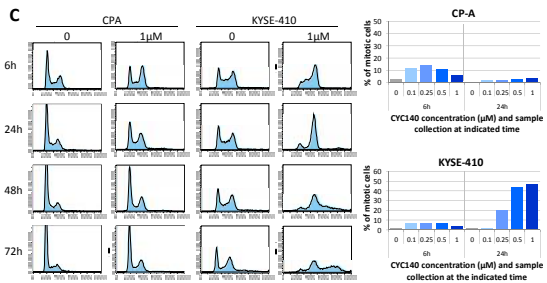


- **Non-malignant cell lines are more resistant than the majority of malignant cell lines**

Esophageal cell lines were treated with CYC140 for 6h; cells were then grown in compound-free media for up to 72h. Cell proliferation (**A**) and proportion of dead cells (**B**) were measured by flow cytometry using Viacount assay. Cell cycle distribution (**C**) was analyzed by flow cytometry using PI staining at the indicated time point.



- **CYC140 preferentially inhibits cell proliferation and induces cell death in malignant cell lines**

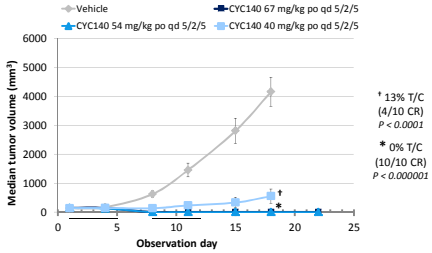


- **Both malignant and non-malignant cell lines show an increase in the 4N population (left; 1 µM CYC140 at 6h & 24h) and specifically the mitotic population (right) after 6h pulse treatment with CYC140. However, the arrest is only transient in non-malignant cells but persists, and accumulates, in malignant cell lines, leading to prolonged growth inhibition, and cell death**

Potent anti-tumor activity in xenografts

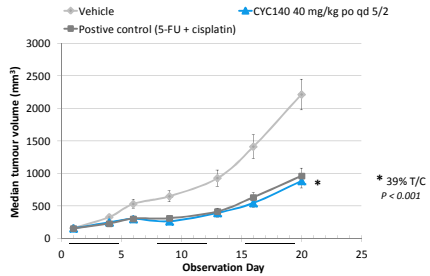
The *in vivo* anti-tumour efficacy of CYC140 has been evaluated in preclinical xenograft models of acute leukemia and solid tumors, including esophageal cancer

HL60 promyelocytic leukemia xenograft



- **Cures of all mice were observed at doses above 40 mg/kg**
- **The dose and regimen were well tolerated without significant loss in body weight**

OE19 esophageal xenograft



- **40 mg/kg CYC140 is equally active as positive control of weekly 5-FU 60 mg/kg i.p. and cisplatin 3 mg/kg i.v. in this esophageal model**

Conclusions

- **Potent and selective inhibitors of the mitotic kinase PLK1 have been synthesized and characterized; CYC140 has good drug-like properties and was selected as a clinical development candidate**
- **CYC140 is a selective PLK1 inhibitor, which causes an increase in the proportion of 4N cells in G2 and mitosis, appearance of mitotic cells with a monopolar spindle, leading to growth inhibition and cell death, as expected for a PLK1 inhibitor**
- **pHH3 – a mitotic marker, cPARP – an indicator of apoptotic cell death, and PLK1 substrates such as nucleophosmin are all useful pharmacodynamic markers for this compound**
- **CYC140 preferentially induces growth inhibition and cell death in malignant versus non-malignant cells; short treatment (6h) maximizes cytotoxicity to malignant cells compared with non-malignant cells, suggesting the potential to achieve a therapeutic window**
- **CYC140 treatment was efficacious in esophageal solid tumor and acute leukemia mouse xenograft models**
- **CYC140 is a promising anti-cancer agent with potent anti-proliferative activity and therapeutic potential in a variety of tumor indications, including esophageal cancer and acute leukemia.**

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