The small molecule CDK 2 and 9 inhibitors CYC065 and CCT68127 are potent inhibitors of MYCN via transcriptional repression

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Introduction

Amplification of the *MYCN* oncogene is a common and tumour-specific genomic event in aggressive tumours, but is not effectively targeted by any existing clinical drug. Attempts to target MYC transcription factors using direct approaches have failed. The recent discovery that the cyclin-dependent kinase (CDK) -7 inhibitor THZ1 targets super enhancer-driven expression of transcription factors such as MYC and MYCN generated much excitement, although this compound is not a clinically viable inhibitor. We explored the ICR medicinal chemistry compound library of CDK inhibitors for small-molecules with selective activity against MYCN-dependent neuroblastoma cells. The orally bioavailable, tri-substituted purine CDK inhibitors CYC065 and CCT68127, analogues of seliciclib (CYC202, Cyclacel, Ltd), a clinical inhibitor of CDK2, 7 and 9, exhibited exquisite selectivity profiles concomitant with enhanced potency and selectivity for CDK9, a component of PTEFb (CDK9:cyclinT1) and a rate-limiting regulator of MYC transcription. The aim of this study was to explore the sensitivity of neuroblastoma cells *in vitro* and *in vivo* to CYC065 and CCT68127.

Methods

We assessed the activity of CYC065 and CCT68127 *in vitro* using a panel of neuroblastoma cells driven by endogenous, exogenous, or TET-regulatable expression of MYCN. The efficacy of CYC065 was evaluated *in vivo*, using subcutaneous xenograft models of both MYCN amplified (Kelly) and non-amplified neuroblastoma (SKNAS) and a Th-*MYCN* genetically-engineered murine model of neuroblastoma.

Results

Neuroblastoma cell lines were highly sensitive to both CYC065 and CCT68127 concomitant with *MYCN* amplification and expression levels and presence of a native MYCN promoter. Both small molecules blocked neuroblastoma cell proliferation, induced apoptosis and caused depletion of MYCN mRNA and protein within 1 hr, at the cellular Gi50, and in a time- and dose-dependent manner. Preclinical trials of CYC065 and CCT68127 in *MYCN*-dependent models of neuroblastoma resulted in significantly reduced tumour burdens and prolonged survival.

Discussion

We report that CYC065, a potent inhibitor of CDK9 and a drug that is currently in a phase I study in adult patients with advanced cancer, exhibits potent and selective activity against neuroblastoma

cells and models driven by expression of MYCN. CYC065 is a clinical-stage compound with selective activity for MYCN and should be further evaluated as a candidate targeted treatment for children with neuroblastoma and other MYCN-dependent malignancies.

The small molecule CDK2 and CDK9 inhibitors CYC065 and CCT68127 are potent inhibitors of MYCN via transcriptional repression

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INTRODUCTION

- Neuroblastoma is a common solid tumour that accounts for 15% of cancer deaths in children.
- Amplification of the *MYCN* oncogene is the most common genomic alteration in aggressive tumours and associated with poor clinical outcome.
- Although MYCN is expressed as a tumour-specific oncoprotein in poor-outcome neuroblastoma [1], making it a therapeutic target of great importance, no drugs with significant activity against MYCN or MYC are available for clinical use in cancer.
- Inactivation of CDK2 has been shown to be synthetically lethal to neuroblastoma cells with *MYCN* amplifycation and overexpression [2].
- The aim of this study was to explore the sensitivity of neuroblastoma cells *in vitro* and *in vivo* to tri-substituted purine CDK2/9 inhibitors (Cyclacel Ltd), CCT68127 and the clinical compound CYC065, which are analogues of Seliciclib (R-Roscovitine).

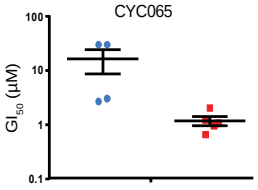
CONCLUSIONS

- Neuroblastoma cell lines with *MYCN* amplification and expression are highly sensitive to the CDK inhibitors, CYC065 and CCT68127.
- CYC065 and CCT68127 block neuroblastoma cell proliferation, induce apoptosis, inhibit MYCN transcription and downregulate MYCN protein.
- CYC065 and CCT68127 regress tumours and prolonged survival in MYCN-amplified neuroblastoma xenografts and the *Th*-MYCN GEMM of neuroblastoma.

The potent *in vitro* and *in vivo* anti-tumour activity suggest that CYC065 and CCT68127 may have therapeutic potential in neuroblastoma with amplification of the *MYCN* oncogene.

RESULTS

CYC065 and CCT68127 block cell proliferation

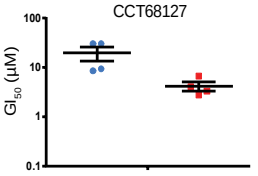


No MYCN expression

SK-N-SH	SK-N-AS	SH-SY5Y	SH-EP
>30	>30	2.69	3.02

MYCN expression

Kelly	BE(2)C	IMR32	L-AN-5	SH-EP-MYCN
1.22	2.03	0.65	0.95	1.06



No MYCN expression

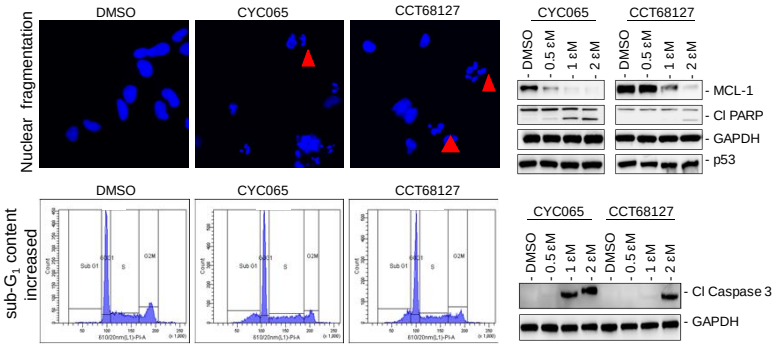
SK-N-SH	SK-N-AS	SH-SY5Y	SH-EP
>30	>30	9.33	8.46

MYCN expression

Kelly	BE(2)C	IMR32	L-AN-5	SH-EP-MYCN
3.30	6.60	2.73	NT	4.04

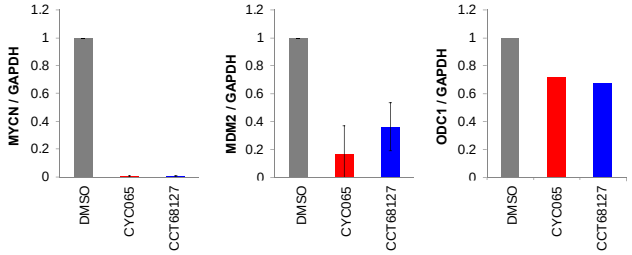
- Neuroblastoma cells with MYCN protein expression are highly sensitive towards CYC065 and CCT68127 after 8 hours exposure

CYC065 and CCT68127 induce apoptosis in neuroblastoma cells



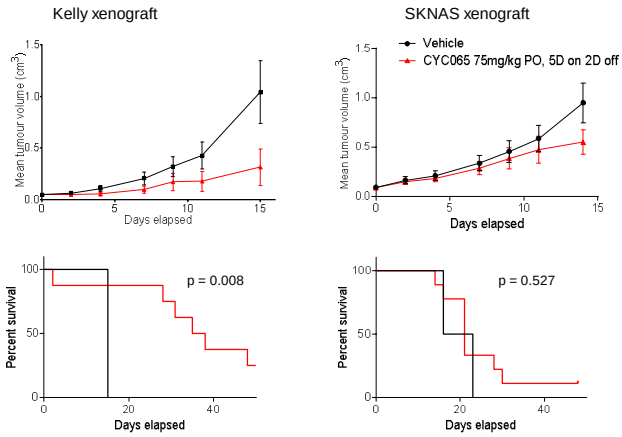
- CYC065 and CCT68127 induce nuclear fragmentation, PARP cleavage, Caspase-3 cleavage and increase sub-G_1 content of Kelly cells

CYC065 and CCT68127 inhibit MYCN transcription



- CYC065 and CCT68127 reduced the transcription of MYCN and its transcripts, MDM2 and ODC1, after treatment (1μM, 6-8 hours) in Kelly neuroblastoma cells (quantitative RT-PCR)

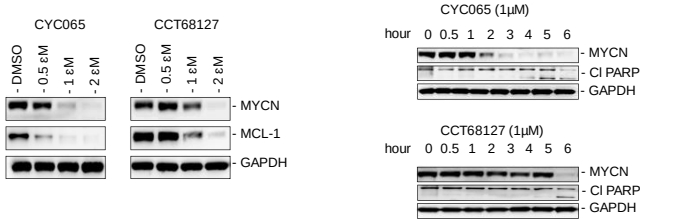
CYC065 induced significant growth inhibition and extended survival in *xenograft* models of neuroblastoma



Xenograft model	T/C ratio (Day 15)
Kelly	0.30
SKNAS	0.58

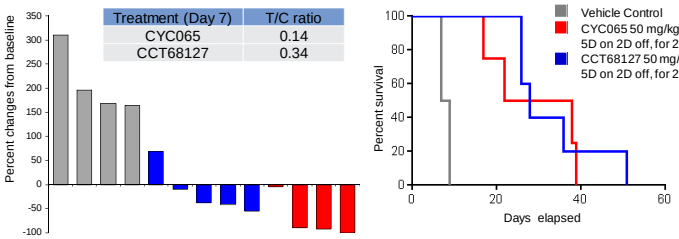
- CYC065 induced significant growth inhibition and extended survival in MYCN-amplified neuroblastoma (Kelly) xenografts

CYC065 and CCT68127 downregulate MYCN in a time and dose dependent manner



- Downregulation of MYCN protein was concomitant with PARP cleavage for Kelly treated with CYC065 and CCT68127

CYC065 and CCT68127 regress tumour and prolong survival in *Th*-MYCN GEMM of neuroblastoma



- CYC065 and CCT68127 cause significantly tumour regressesion and prolong survival in *Th*-MYCN GEMM of neuroblastoma

References. [1] Valentijn *et al.*, PNAS, 2012, **109**(47):19190-19195. [2] Molenaar *et al.*, PNAS, 2009, **106**(31):12968-12973.

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