CYC065, potential therapeutic agent for AML and MLL leukemia

Sheelagh Frame, Chiara Saladino, Susan Davis, David Blake & Daniella Zheleva. Cyclacel Ltd., Dundee, United Kingdom

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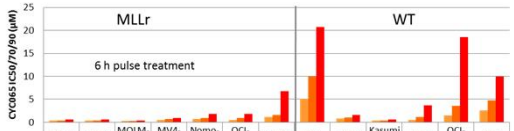
INTRODUCTION

- Chromosomal rearrangements involving the human mixedï lineage leukemia (MLL) gene at 11q23 are associated with the development of acute leukemia. Abnormalities in the MLL gene can be detected in *de novo* acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and acute lymphoblastic leukemia (ALL) in adults and children as well as in therapy-related AML, particularly after treatment with DNA topoisomerase II inhibitors
- MLL gene rearrangements are a strong predictor of adverse outcome, and innovative therapeutic strategies are urgently needed to improve prognosis
- Rearranged MLL (MLLr) interacts with the transcription complex including CDK9 and upregulates genes from the HOX family and MEIS1, which contributes to leukemic transformation.[1,2] Down-regulation of MEIS1 gene expression decreases proliferation and survival of MLL-related leukemia cells; targeting this gene may have therapeutic potential[3]
- Myeloid cell leukemia sequence-1 (Mcl-1), an anti-apoptotic protein related to Bcl-2, is overexpressed in AML and MLLr and plays a central role in survival and drug resistance.[4] Mcl-1 has a very short half-life and its levels can be downregulated by transcriptional inhibitors (e.g. inhibitors of CDK9/cyclin T). Targeting Mcl-1 may be a potential therapeutic strategy for AML and MLLr[5]
- The aim of this study was to explore the therapeutic potential of CYC065, a novel CDK2, 5, 9 inhibitor, for AML, ALL and MLL leukemia, in pre-clinical models

CYC065

- CYC065 is a second generation CDK inhibitor selected as a clinical development candidate; IND-enabling studies including toxicology completed - no unexpected findings
- Similar mechanism to Phase 2 CDKI, seliciclib; improved potency & pharmaceutical properties
- CYC065 selectively inhibits:
  - CDK2 (IC50 = 5 nM), which drives cell cycle transition and activates major DNA double-strand break repair pathways
  - CDK5 (IC50 = 21 nM), which drives metastatic spread (esp. in pancreatic and lung cancers)
  - CDK9 (IC50 = 26 nM), which regulates transcription of genes (incl. cyclins, Mcl-1, etc.) through phosphorylation of RNA polymerase II
- Causes apoptotic cell death of cancer cells at submicromolar concentrations
- Good pharmaceutical properties. High solubility and oral bioavailability; suitable for intravenous and oral administration
- Antitumor efficacy achieved in *in vivo* xenograft models with once a day oral dosing at well tolerated doses

Sensitivity of AML cell lines with different MLL status to CYC065



	EOL-1	ML-2	MOLM-13	MV4-11	Nomo-1	OCI-AML2	THP-1	HEL	HL60	Kasumi-1	KG-1	OCI-AML5	PL21
IC50	0.35	0.33	0.26	0.5	0.66	0.53	1.1	5.12	0.79	0.32	0.5	1.44	2.55
IC70	0.41	0.38	0.31	0.69	0.88	0.93	1.6	9.96	1.04	0.41	1.12	3.55	4.78
IC90	0.56	0.59	0.4	0.92	1.8	1.8	6.8	20.75	1.6	0.57	3.7	18.49	9.92
AraC IC50 (μM)	1.31	0.81	1.01	5.34	>10	>10	>10	0.62	1.91	3.85	1.23	0.17	1.08
MLLr	PTD	AF6	AF9	AF4	AF9	PTD	AF9	-	-	-	-	-	-

- MLL/AML cell lines are highly sensitive to CYC065. A 6 h pulse treatment at submicromolar or low micromolar concentration is sufficient to achieve 90% growth inhibition
- Cytosine arabinoside (AraC) was selected as a reference compound; AraC is widely used in the treatment of acute leukemia, and MLL-rearranged infant ALL cells are sensitive toward AraC *in vitro*[6]. AML cell lines with MLL rearrangements are less sensitive to AraC when compared to CYC065

1. Dou & Hess, Int J Haematol.2008, 87:10
2. Liedtke & Cleary, Blood, 2009, 113: 6061
3. Kumar et al. Blood, 2009, 113:1756
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5. Kasper et al, Blood Cancer J. 2012: 2: e60
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8. Wong et al. Genes Dev. 2007, 21:2762

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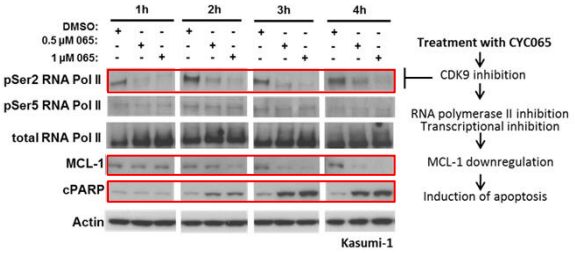
Sensitivity of ALL cell lines with different MLL status to CYC065

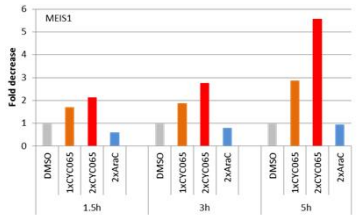
	Karpas-45	KOPN-8	RS4;11	SEM	CCRF-CEM	DND-41	Jurkat	MN-60	MOLT-3	EOL-1	HEL
IC50	0.508	0.38	0.162	0.444	0.586	0.624	5.647	1.898	0.505	0.274	2.912
IC70	0.893	0.485	0.253	0.758	0.733	1.206	8.422	7.334	0.63	0.314	3.685
IC90	2.153	0.716	0.657	2.043	1.051	3.672	15.134	30	0.907	0.389	5.859
MLLr	AFX	ENL	AF4	AF4	-	-	-	-	-	PTD	-

- MLL/ALL cell lines are highly sensitive to CYC065
- 8 ï 10 h pulse treatment at submicromolar or low micromolar concentration is sufficient to achieve 90% growth inhibition; 10 h pulse treatment of the most sensitive and resistant AML cell lines is shown for comparison

CYC065 mechanism of action in leukemia cell lines

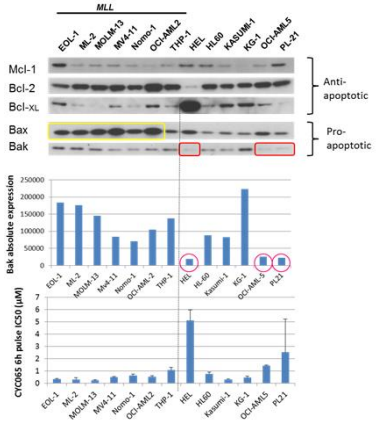


CYC065 inhibits MLL-driven gene expression



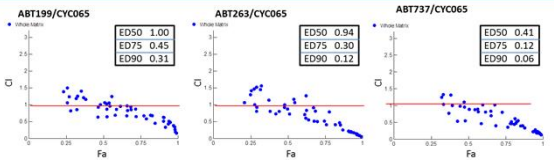
- MV4-11 cells were treated with 1 and 2 times the IC_{50} values determined in a 72 h Alamar Blue cytotoxicity assay
- Gene expression level was normalised using the expression of B2M (housekeeping gene)
- Rapid, dose-dependent inhibition of MEIS1 gene expression was observed in all cell lines with MLL rearrangements
- MEIS1 down-regulation in MV4-11 cells is associated with inhibition of cell growth[3]

Bcl2-family member protein level correlates with sensitivity to CYC065



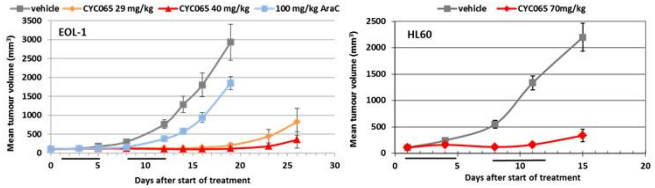
- AML cell lines with MLLr express high levels of Bax, which could be a reason for their apoptotic response following CYC065-mediated Mcl-1 down-regulation
- In AML cell lines with WT MLL, IC50/70/90 values are correlated with Bcl_{XL} (Pearson correlation coefficient 0.6-0.9) and inversely correlated with Bak (Pearson correlation coefficient approximately -0.6)
- Bcl_{XL} and Mcl-1 have an overlapping function in sequestering Bak; depletion of Mcl-1 releases Bak to induce apoptosis; however overexpression of Bcl_{XL} can compensate for loss of Mcl-1, sequestering Bak, and preventing apoptosis
- Resistant cells have negligible levels of Bak; loss of Bak expression prevents efficient induction of apoptosis
- Further exploration of these markers in an expanded cell line panel and primary AML is merited

Combining CYC065 with $Bcl2/Bcl_{XL}$ inhibitors is synergistic



- THP-1 cells were treated with 7x7 concentration matrix for 72 h & cell viability determined by Alamar Blue assay
- Top concentrations: CYC065 (0.8 μM), ABT199 (0.5 μM), ABT263 (1 μM) and ABT737 (4 μM); dilutions: 1:1.2 (CYC065) or 1:2 ($Bcl2/Bcl\text{-}_{XL}$ inhibitors)
- Combination Index (CI) values were calculated using the method of Chou & Talalay.[7] CI values less than 1 are indicative of synergy, less than 0.3 ï strong synergy. CI values at ED50, 75 and 90 are shown in the associated tables
- THP-1 data is representative of data obtained across several cell lines; the combination of CYC065 with the Bcl2/$Bcl\text{-}_{XL}$ inhibitors was synergistic in all tested leukemia cell lines ï AML (THP-1 & HEL) and ALL (Jurkat & SEM)

Potent anti-tumor activity of CYC065 in AML xenograft models



- CYC065 po, qd days 1-5 and 8-12 or AraC 100 mg/kg ip, qd days 1-5 (a standard optimised dosing regimen for this model)
- EOL-1: median TGI achieved on day 19 was 97 & 95% for 40 mg/kg & 29 mg/kg CYC065, respectively, and 41% for AraC
- HL60: 90% TGI achieved on day 11

CONCLUSIONS

- **AML and ALL cell lines with MLL rearrangements are highly sensitive to CYC065; a 6 – 10 h exposure is sufficient to significantly inhibit the sensitive MLLr and WT AML. Pre-clinical data have shown that such exposure is achievable and well tolerated**
- **The pro-apoptotic mechanism of CYC065 includes inhibition of phospho-RNAP II, transcription and Mcl-1 down-regulation**
- **CYC065 inhibits MLL-driven transcription. The effect on MEIS1 may be of particular importance as this gene is a rate-limiting determinant of MLL leukemia stem cell biology[8]**
- **The levels of Bak and Bcl_{XL} may be predictive for response of AML with WT MLL to CYC065. Further exploration of these potential stratification markers is required**
- **The potent *in vitro* and *in vivo* anti-tumor activity suggests that CYC065 may have therapeutic potential in AML and MLL leukemia**