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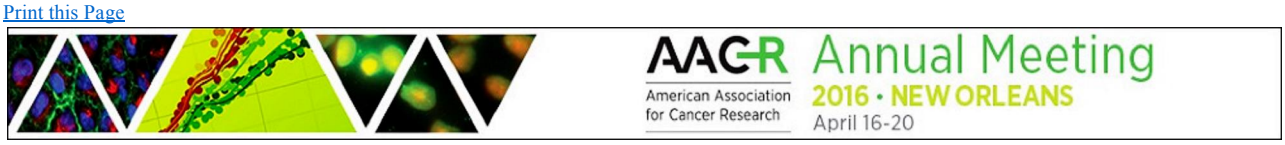

Presentation Abstract

Abstract Number:	1309
Presentation Title:	CYC065, a novel CDK2/9 inhibitor, is an effective inducer of cell death and synergizes with BCL2 and BET inhibitors in B-cell lymphoma, including double-hit lymphomas
Presentation Time:	Monday, Apr 18, 2016, 8:00 AM -12:00 PM
Location:	Section 18
Poster Board Number:	28
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Abstract Body:

Introduction: Double-hit lymphomas (DHL), defined by concurrent *MYC* and *BCL2* rearrangements, have poor prognosis compared to standard-risk diffuse large B cell lymphomas (DLBCL). Current treatment regimens involve multiple chemotherapies, but do not specifically exploit these molecular features of the disease.

Several studies have established that: (i) DLBCL show frequent overexpression of Mcl-1, an anti-apoptotic member of the Bcl-2 family (30% and 50% in the ABC and GCB subtypes, respectively), (ii) MYC-driven lymphomas are highly sensitive to depletion of Mcl-1, (iii) MYC overexpression and inhibition of CDK activity are synthetically lethal and (iv) resistance to BH3 mimetics targeting Bcl-2 can be conferred by upregulation of Mcl-1.

CYC065 is a specific and potent CDK2/9 inhibitor, currently in a Phase 1 trial in patients with advanced cancer (NCT02552953). The mechanism of action of CYC065 involves rapid reduction of the levels of both Mcl-1 and MYC, suggesting a therapeutic rationale for investigating this agent in DLBCL. This preclinical study explored the single agent activity of CYC065 in B cell lymphoma, and its potential to combine with other molecularly targeted agents of interest, including venetoclax (ABT-199, a Bcl-2 inhibitor), and BET inhibitors.

Methods: Single agent activity of CYC065 was explored using short pulse treatments (6-8 h) in B cell lymphoma cell lines. Viability and total cell number were assessed 24, 48 or 72 h following treatment. Levels of MYC, Bcl-2 and Mcl-1 were determined by Western blotting at baseline and following treatment with CYC065. Cell fate was examined by flow cytometry. CYC065 was combined with venetoclax or BET inhibitors ((+)-JQ-1, GSK525762 and OTX-015), which were administered concomitantly for a 6 h pulse or up to 72 h. Combination data were analyzed by the Chou & Talalay method.

Results: The median IC_{50} for CYC065 in 13 B cell lymphoma cell lines was 0.43 µM. No obvious difference was observed between ABC and GCB subtypes of DLBCL, and DHL DLBCL lines had similar IC_{50} values to non-DHL DLBCL lines (median 0.47 µM vs 0.29 µM; p=0.95). As expected from the target inhibitory profile, CYC065 caused a rapid decrease in the phosphorylation of S2 of the CTD of RNA polymerase II followed by downregulation of Mcl-1 and MYC, and rapid induction of apoptosis in sensitive cell lines. CYC065 had no impact on Bcl-2 levels. Combining CYC065 with venetoclax was highly synergistic in DHL lines (median CI values range from 0.1-0.8), and resulted in >90% cell death. The combination of CYC065 and BET inhibitors was also highly synergistic.

Conclusions: CYC065 targets key oncogenic and survival pathways in DLBCL. CYC065 is a potent and effective inducer of cell death and combines synergistically with Bcl-2 or BET inhibitors in B cell lymphoma cell lines, including DHLs, which represent an unmet clinical need.

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CYC065, a novel CDK2/9 inhibitor, is an effective inducer of cell death and synergizes with BCL2 and BET inhibitors in B cell lymphoma, including double-hit lymphomas

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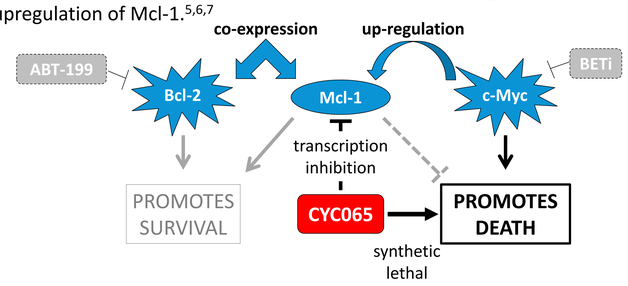
Abstract # 1309

Introduction

- Double-hit lymphomas (DHL), defined by concurrent *MYC* and *BCL2* rearrangements, have poor prognosis compared to standard-risk diffuse large B cell lymphomas (DLBCL). Current treatment regimens involve multiple chemotherapies, but do not specifically exploit these molecular features of the disease.
- Several studies have established that: (i) DLBCL show frequent overexpression of Mcl-1, an anti-apoptotic member of the Bcl-2 family (50% and 30% in the ABC and GCB subtypes, respectively[1]), (ii) MYC-driven lymphomas are highly sensitive to depletion of Mcl-1[2], (iii) MYC overexpression and inhibition of CDK activity show synthetic lethality[3,4] and (iv) resistance to BH3 mimetics targeting Bcl-2 can be conferred by upregulation of Mcl-1.[5,6,7]



- CYC065 is a specific and potent CDK2 and CDK9 inhibitor currently in first-in-human phase 1 clinical trial in patients with advanced cancer (NCT02552953); CDK2 IC_{50} = 5 nM, CDK9 IC_{50} = 26 nM.[8]
- CYC065 has good pharmaceutical properties including high solubility and oral bioavailability, and is suitable for intravenous and oral administration routes.
- CYC065 anti-cancer potential has been demonstrated in preclinical models including acute leukemias driven by MLL rearrangements/PTD[9], cyclin E amplified and drug resistant uterine and breast tumors[10,11], and *MYCN*-overexpressing neuroblastoma.[12]
- **<u>Study Aims:</u> Evaluate the single agent activity of CYC065 in B cell lymphoma, including DHL and triple-hit lymphoma (THL), and establish the potential to combine with other compounds which also target the specific molecular features of B cell lymphoma, including venetoclax (ABT-199) – a BH3 mimetic targeting Bcl-2, and BET inhibitors, which modulate MYC-dependent transcription.**

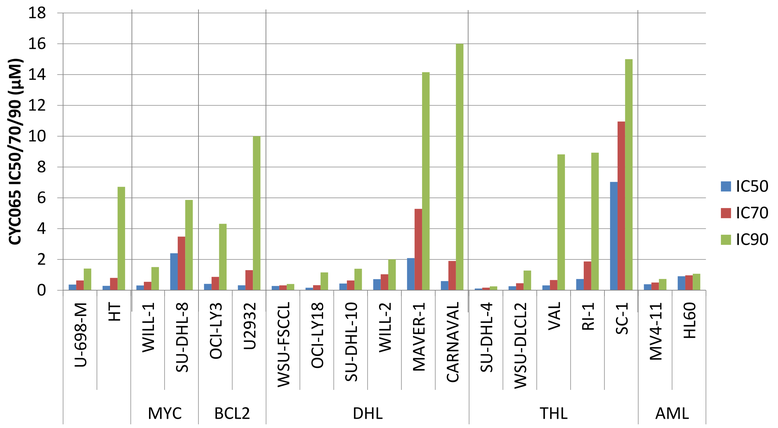
Cell Line	Tumor Type	MYC rearrangement	BCL2 rearrangement	BCL6 rearrangement
U-698-M	LBL	-	-	-
HT	GCB DLBCL	-	-	-
WILL-1	DLBCL	t(8;14); IgH-MYC	-	-
SU-DHL-8	GCB DLBCL	t(8;22); IgL-MYC	-	-
OCI-LY3	DLBCL	-	t(14;18) with copy number amplification of the BCL2 region	-
U-2932	ABC DLBCL	-	extensive genomic amplification of BCL2 region	-
WSU-FSCCL	B cell NHL	t(14;18) and t(8;11) with BCL2 and MYC rearrangements		-
OCI-LY18	DLBCL	t(8;18;14) leading to alterations of MYC, BCL2 and IgH genes		-
SU-DHL-10	GCB DLBCL	Concurrent rearrangements of IgH with MYC and BCL2		-
WILL-2	DLBCL	t(8;22) and t(14;18) affecting rearrangements of IgL-MYC and IgH-BCL2		-
MAVER-1	MCL	non-IgH rearrangement of MYC	amplification of BCL2 region	-
CARNAVAL	DLBCL	t(14;18) and t(14;18) affecting concurrent biallelic rearrangements of IgH with MYC and BCL2		-
SU-DHL-4	GCB DLBCL	Non-Ig-MYC rearrangement	t(14;18); BCL2 MBR-IgH	Noncanonical BCL6 rearrangement
WSU-DLCL2	DLBCL	Non-Ig MYC rearrangement	t(14;18); IgH-BCL2	Noncanonical BCL6 rearrangement
VAL	B-ALL	Carries three-way translocation t(8;14;18); overexpresses BCL2 & MYC		t(3;4); BCL6-TTF/RHOH
RI-1	ABC DLBCL	t(4;8); MYC rearrangement	BCL2 amplification	Noncanonical BCL6 rearrangement
SC-1	FL	t(8;14;18) translocation affecting genomic co-amplification of BCL2 & MYC; IgH-BCL2		BCL6-MBNL1 fusion

Information from Drexler, Leukemia & Lymphoma 2015, 39:18, R.A.F. Macleod (DSMZ) personal communication and Sanchez-Izquierdo, Neoplasia 2003, 101:4539.

(1) Wenzel, Leukemia 2013, 27:1381 (2) Kelly, Genes Dev. 2014, 28:58 (3) Horiuchi, J Exp Med. 2012, 209:679 (4) Huang, Genes Dev. 2014, 28:1800 (5) Yecies, Blood 2010, 115: 3304 (6) Phillips, Blood Cancer J. 2015, 5, e368 (7) Li, Leukemia 2015, 29: 170 (8) Frame, AACR 2010, Abs. 3886 (9) Saladino, AACR 2015, Abs 1650 (10) Cocco, AACR 2015, Abs 3103 (11) Scaltriti, PNAS 2011, 9:3761 (12) Poon, 4th Neuroblastoma Society Symp. 2015.

AACR, 16-20 April 2016, New Orleans, LA.

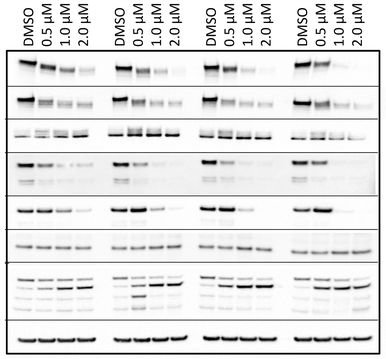
B cell lymphoma, including DHL/THL, are sensitive to CYC065

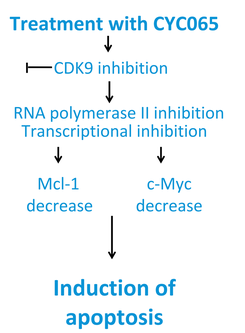


B cell lymphoma lines were exposed to an 8 h pulse of CYC065. Viable cell number was quantified at 72 h using a Viacount assay (PI exclusion), expressed relative to DMSO controls to calculate $IC_{50/70/90}$ values. NB: WSU-DLCL2 is reported to be CHOP resistant and exhibits the MDR phenotype; SU-DHL-8 is reported to be dependent on Bcl-x_L.

The majority of B cell lymphoma cell lines, including DHL & THL, are highly sensitive to a short pulse of CYC065. 15 of 17 B cell lymphoma cell lines tested, including DHL and THL, showed IC_{50} ≤2 μM, and 8 of these showed IC_{90} values ≤2 μM.

CYC065 mechanism of action in B cell lymphoma cell lines

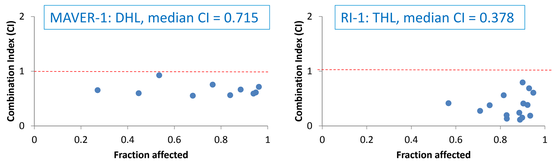




WILL-1 (*MYC* rearranged)

Concurring with its CDK9 inhibitory activity, CYC065 caused a rapid decrease in the phosphorylation of S2 of the CTD of RNA polymerase II, followed by downregulation of Mcl-1 and c-Myc and rapid induction of apoptosis. CYC065 had no impact on Bcl-2 levels. Similar findings were obtained in several additional B cell lymphoma lines, with the exception of SU-DHL-8 and SC-1 which, as expected from their sensitivity profile, showed no evidence of PARP cleavage.

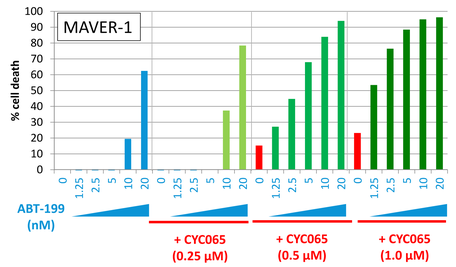
CYC065 and venetoclax combine effectively in B cell lymphoma



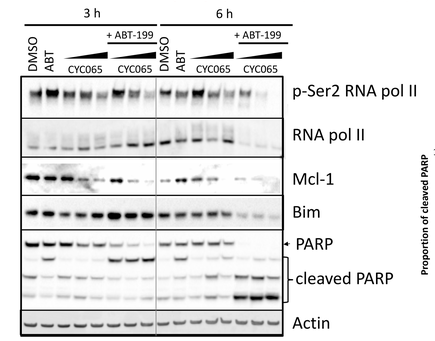
DHL/THL lines were exposed to a 6 h pulse of CYC065 (0.25, 0.5, 1.0 μM) or ABT-199 (1.25 – 20 nM MAVER-1 or 12.5 – 200 nM RI-1) or both compounds concomitantly, and then placed in fresh media.

Total viable cell number was calculated relative to DMSO controls and used to obtain combination index (CI) values according to the method of Chou & Talalay. CI values less than 0.9 are indicative of synergy. Sequential schedules were also highly effective at inducing cell death.

Short pulse of CYC065 plus venetoclax causes synergistic induction of apoptosis



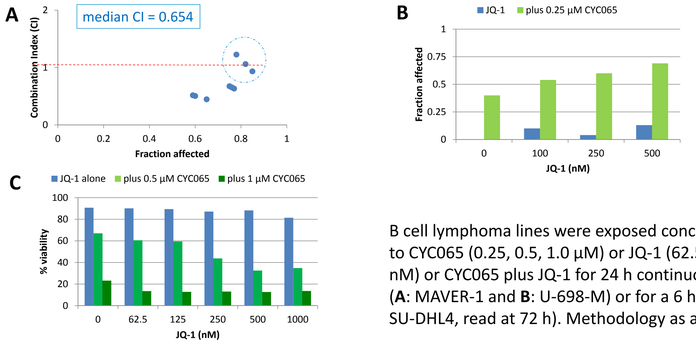
MAVER-1 cells were treated with a 6 h pulse of either CYC065 or ABT-199 or the combination, and then placed in fresh media. Percentage cell death was quantified at 24 h using a Viacount assay.



MAVER-1 cells were treated with either CYC065 (0.25, 0.5, 1.0 μM) or ABT-199 (5 nM) or the combination for 3 h or 6 h. Lysates were analyzed for the expression of various proteins. The proportion of PARP cleavage with each treatment is indicated in the graphs.

In MAVER-1 (a less sensitive DHL example) the combination of CYC065 with venetoclax potentiates the effect of CYC065 alone, causes Bim accumulation and results in a robust and synergistic increase in apoptosis. The coordinated upregulation of pro-apoptotic BH3-only proteins, such as Bim, and decrease in anti-apoptotic counterparts leads to complete eradication of cells in culture.

CYC065 and BET inhibitors combine effectively in B cell lymphoma



B cell lymphoma lines were exposed concomitantly to CYC065 (0.25, 0.5, 1.0 μM) or JQ-1 (62.5 - 1000 nM) or CYC065 plus JQ-1 for 24 h continuously (**A**: MAVER-1 and **B**: U-698-M) or for a 6 h pulse (**C**: SU-DHL4, read at 72 h). Methodology as above.

The addition of BET inhibitors was synergistic (CI ≤ 1) at suboptimal concentrations of CYC065 and additive with optimal levels of CYC065 (data points within circled area). Similar results were obtained with additional B cell lymphoma cell lines and with the clinical BETi GSK525762.

Conclusions

- **CYC065 targets key oncogenic and survival pathways in DLBCL; inhibition of CDK9-mediated transcription leads to robust decrease in key pro-survival and oncogenic regulators, including Mcl-1 and c-Myc, triggering rapid induction of apoptosis.**
- **B cell lymphomas - including DHL and THL - are sensitive to CYC065 short pulse treatment using CYC065 exposures shown to be achievable and well tolerated in preclinical species.**
- **The majority of B cell lymphoma lines tested are sensitive to single agent CYC065, the remaining cell lines are highly sensitive to the combination with venetoclax.**
- **Markers to stratify patients predicted to benefit from CYC065 alone - or from the combination with venetoclax - have been established and are being evaluated further.**