Session PO.ET07.03 - Receptor Targeting and the Tumor Microenvironment

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3905 / 5 - Strategic combination of the cyclin-dependent kinase inhibitor CYC065 with venetoclax to target anti-apoptotic proteins in chronic lymphocytic leukemia

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Presenter/Authors

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Disclosures

R. Chen: ; Cyclacel Pharmaceuticals, Inc.. Y. Chen: None. S. Frame: ; Cyclacel Pharmaceuticals, Inc. D. Blake: ; Cyclacel Pharmaceuticals, Inc. . W.G. Wierda: None. D. Zheleva: ; Cyclacel Pharmaceuticals, Inc. W. Plunkett: ; Cyclacel Pharmaceuticals, Inc..

Abstract

CYC065 is a cyclin-dependent kinase (Cdk) inhibitor that is highly selective towards Cdk2 and Cdk9. In chronic lymphocytic leukemia (CLL), a disease that is addicted to the over-expression of anti-apoptotic proteins for survival, inhibition of Cdk9 by CYC065 reduced phosphorylation of the C-terminal domain of RNA polymerase II and blocked transcription. These actions depleted the intrinsically short-lived anti-apoptotic protein Mcl-1, but not Bcl-2, and induced apoptosis in CLL cells in vitro. The IC_{50} for CYC065-induced CLL cell death after a 24-hr incubation was 0.8 μM, a concentration that is achievable in the clinic at tolerated doses. CYC065 killed the CLL cells equally efficiently in the presence or absence of the human stromal cell line, StromaNKtert, and with or without a stimulation condition that mimics the lymphoid tissue microenvironment (anti-IgM, anti-CD-40, IL-4). Venetoclax, which specifically inhibits Bcl-2 function, is approved for treatment of CLL with del(17p); however upregulation of Mcl-1 is associated with resistance to venetoclax in the lymph nodes. Therefore, we hypothesized that the combination of CYC065 with venetoclax would target the parallel mechanisms that promote the survival control in CLL cells, and induce synergistic cell death by apoptosis. A time course study of the single agents showed that under conditions that mimic the lymph node microenvironment, cell death induction by venetoclax required 6-8 hr to reach the plateau of cell killing and maximal killing by CYC065 occurred after 24 hr, consistent with the different mechanisms of action of the two compounds. Following the removal of CYC065 or venetoclax after 4, 8, 12, or 24 hr incubations, there was no evidence for additional cell death after an additional 48 hr in drug-free medium regardless of the duration of drug incubation. Immunoblots showed recovery of RNA pol II phosphorylation, and restored Mcl-1 expression upon washout of CYC065. The reversible action of these compounds has potential implications for clinical scheduling combining these compounds. Median effect analysis indicated that CYC065 and venetoclax combined synergistically in CLL samples with or without 17p deletion. A dose reduction analysis confirmed mutual potentiation of each other when combined. Combination of IC_{50} concentrations of CYC065 and venetoclax for 24 hr was sufficient to decrease the viability of CLL cells by over 90% in the lymph node mimicking microenvironment. Thus, these data provided rationale for clinical combination of CYC065 and venetoclax in CLL. CYC065 is currently in a Phase I clinical trial in patients with advanced solid tumors (NCT02552953) using an intermittent dosing regimen which causes at least 24 hr Mcl-1 downregulation in patient PBMCs at well tolerated dose levels.

Strategic combination of the cyclin-dependent kinase inhibitor CYC065 with venetoclax to target anti-apoptotic proteins in chronic lymphocytic leukemia

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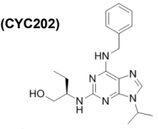
Introduction

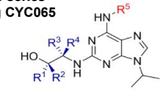
- CYC065 is a cyclin-dependent kinase (Cdk) inhibitor that is highly selective towards Cdk2 and Cdk9.
- In chronic lymphocytic leukemia (CLL), a disease that is addicted to the over-expression of anti-apoptotic proteins for survival, inhibition of Cdk9 by CYC065 reduced phosphorylation of the C-terminal domain of RNA polymerase II and blocked transcription. These actions depleted the intrinsically short-lived anti-apoptotic protein Mcl-1, but not Bcl-2, and induced apoptosis in CLL cells in vitro.
- Venetoclax (ABT-199), which specifically inhibits Bcl-2 function, is approved for treatment of CLL with del(17p); however upregulation of Mcl-1 is associated with resistance to venetoclax in the lymph nodes.

CYC065 is a second generation aminopurine Cdk inhibitor

Seliciclib (CYC202)



Analogue series including CYC065

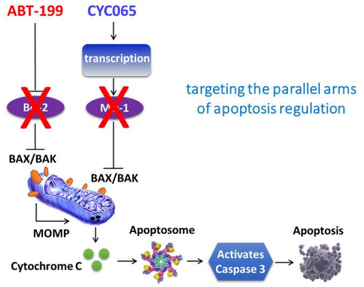


Inhibition profile of CYC065 toward the Cdks

Kinase	IC_{50}, nM
Cdk2/cyclin E	3.5
Cdk2/cyclin A	4.5
Cdk9/cyclin T	26.2
Cdk5/p35	20.5
Cdk7/cyclin H	193
Cdk4/cyclin D	232
Cdk1/cyclin B	578
Cdk6/cyclin D	> 10 000

Hypothesis

We hypothesize that the combination of CYC065 with venetoclax would target the parallel mechanisms that promote survival in CLL cells, and induce synergistic cell death by apoptosis.

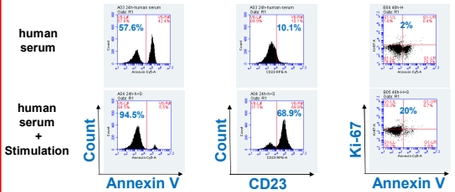


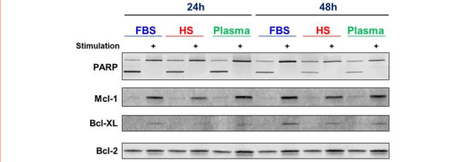
targeting the parallel arms of apoptosis regulation

Results

CLL culture condition to mimic the lymph node microenvironment

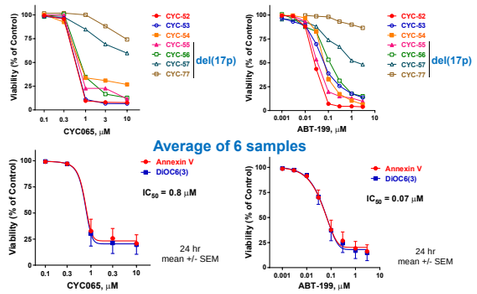
A stimulation mix consisting of anti-IgM, anti-CD40 and IL-4 induces CLL survival, activation and proliferation





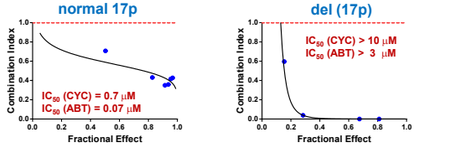
FBS: fetal bovine serum HS: human AB type serum Plasma: patient plasma

Dose response curves of CYC065 and ABT-199 in CLL



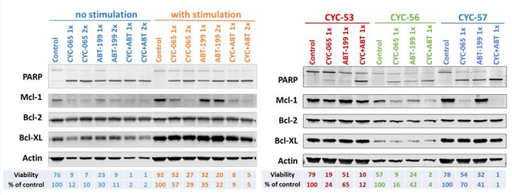
- Cell viability was measured by staining the CLL cells with either DiOC6(3)/PI or annexin V/PI. Both methods yielded the same IC_{50} values, indicating the cells died through the intrinsic pathways of apoptosis.
- Heterogeneity exists among CLL sample response to CYC065 and ABT-199.
- Certain del (17p) samples, including CYC-57 and CYC-77, appear to be less responsive to either ABT-199 or CYC065 .

Synergistic combination of CYC065 and ABT-199



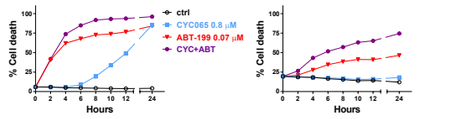
Fraction Affected	Combination Index	DRI CYC065	DRI ABT-199
0.5	0.6	3.3	3.5
0.75	0.5	3.9	4.0
0.9	0.4	4.7	4.5
0.99	0.3	7.0	5.9

Fraction Affected	Combination Index	DRI CYC065	DRI ABT-199
0.5	0.003	660	965
0.75	0.000	2.8e+4	4492
0.9	4.9e-5	1.2e+6	2.1e+4
0.99	1.7e-6	9.7e+9	6e+5



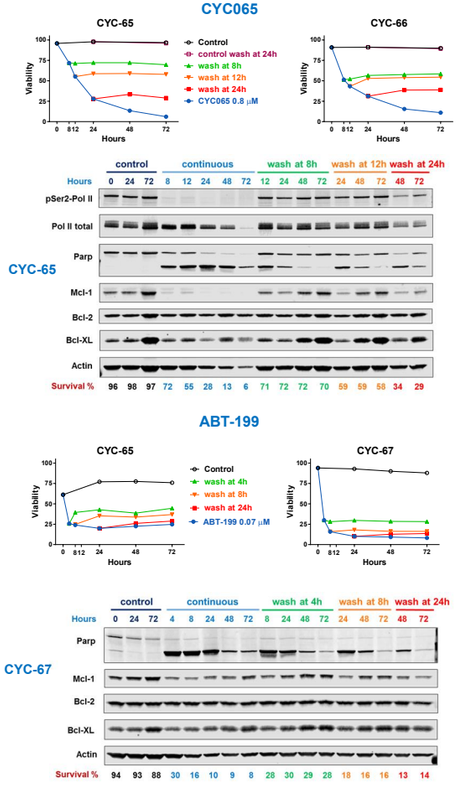
- Median effect analysis indicated that CYC065 and ABT-199 combined synergistically in CLL samples with or without 17p deletion (represented by two samples).
- Dose reduction analysis confirmed mutual potentiation of each other when combined.
- Strong synergic effect was achieved in CLL samples with 17p depletion, that were resistant to each single drug.
- Immunoblots showed further decrease of Mcl-1 with the combination. (1x and 2x indicates 1x and 2x IC_{50} concentration).

Time course of CYC065 and ABT-199 induced apoptosis



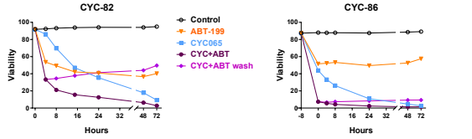
- The kinetics of cell death in response to CYC065 or ABT-199 was different, consistent with their different mechanism of action.
- Maximal cell death induced by ABT-199 plateaued at 6-8 h, while CYC065 required Ó24h to reach maximum killing.

Reversibility of the actions of CYC065 and ABT-199



- CLL cells were incubated with CYC065 or ABT-199 either continuously, or for the indicated time, were then washed and incubated in drug-free media.
- Cell viability was measured by annexin V/PI staining.
- There was no additional cell death following the removal of CYC065 or ABT-199.
- Immunoblots showed recovery of RNA pol II phosphorylation and Mcl-1 expression following removal of CYC065.

Reversibility following the removal of CYC065 / ABT-199 combination



- There was no additional cell death following the removal of CYC065 and ABT-199 combination, indicating both drugs need be present to kill CLL cells.

Conclusions:

- Stimulation conditions that mimic the lymphoid tissue microenvironment (with anti-IgM, anti-CD40 and IL-4) enhance survival and induce B cell activation and proliferation.
- CYC065 and ABT-199 combine synergistically, including samples that are intrinsically resistant to each individual compound.
- Following removal of CYC065 or ABT-199 alone or in combination, there is no evidence for the occurrence of additional cell death, indicating that an adequate exposure time must be maintained to maximize the induction of cell death.

Indication:

These data provide preclinical evidence for the clinical combination of CYC065 and venetoclax in CLL.

References

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