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A COVALENT CDK7 INHIBITOR OVERCOMES VENETOCLAX RESISTANCE AND INDUCES APOPTOSIS VIA C-MYC/P53 AXIS IN ACUTE MYELOID LEUKEMIA

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INTRODUCTION

- Acute Myeloid Leukemia (AML) is a heterogeneous disease and shows poor clinical outcome.
- Improved understanding of AML biology led to multiple new therapeutic options for patients with AML. Despite that, most patients still succumb to the disease.
- Therefore, additional therapeutic options that can be more specific and less toxic are urgently needed.

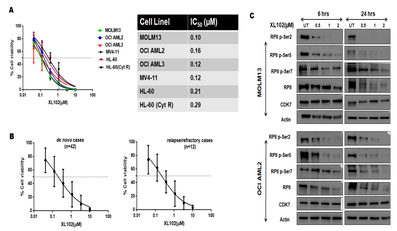
- CDK7 is required for initiation of transcription. Since oncogenes have high transcriptional rate, they are more susceptible to CDK7 inhibition than normal cells.
- The small molecule inhibitors aiming at restoring apoptosis in leukemic cells have shown encouraging activity in early clinical trials and some of these drugs including Venetoclax (specific BCL2 inhibitor) are currently being evaluated in randomized controlled clinical trials.
- **Targeting BCL2 with Venetoclax and CDK7 with novel CDK7 inhibitor XL102 will improve AML clinical outcome.**

METHOD

- Antiproliferative effects on primary myeloid blasts from 54 genetically heterogeneous cohort of AML patients and leukemic cell lines were analyzed.
- CDK7 and Myc CRISPR/Cas9 knockout using lentiV2-Cas9PURO vector was performed.
- NOD/SCID orthotopic and subcutaneous xenografts of AML were used to evaluate anti-leukemic activity of XL102.
- RNA sequencing of paired Venetoclax-sensitive (Ven^S) and resistant (Ven^R) AML cells was performed.

RESULTS

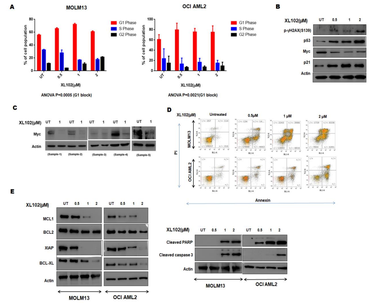
XL102 decreases cell viability in leukemic cell lines and primary myeloid blast leading to suppression of transcription



Cell Line	IC_{50} (μM)
MOLM13	0.10
OCI AML2	0.16
OCI AML3	0.12
MV4-11	0.12
HL-60	0.21
HL-60 (Cyt R)	0.29

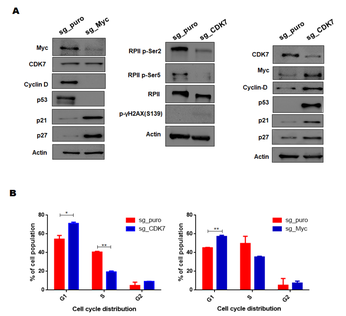
(A & B) XL102 selectively inhibited cell viability in leukemic cell lines as well as primary myeloid blast examined by CTG assay in different AML cell lines after 72 h of drug treatment. (C) XL102 inhibited CTD phosphorylation of RPII in AML cells in dose dependent manner

XL102 leads to cell cycle arrest and mitochondrial mediate apoptosis



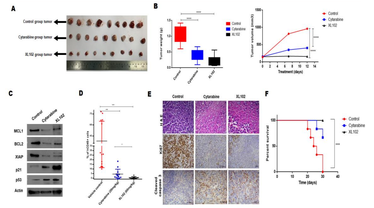
(A) Cell cycle analysis shows increase in population of cells in G1 phase. (B) Dose dependent decrease in expression of Myc and increase in p53, p21 and γH2AX after 6hrs of XL102 treatment in leukemic cells. (C)) Decrease in expression of Myc in patient derived AML blasts after treatment. (D) Change in expression of anti-apoptotic protein after 24hrs of drug treatment.

Knockout of CDK7 and Myc by CRISPR/Cas9 inhibits cell cycle progression in vitro



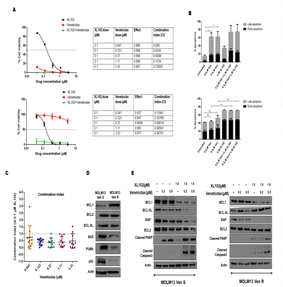
(A) CRISPR/Cas9 mediated knockout of Myc and CDK7 and downstream effect of expression of cell cycle proteins (B) Cell cycle suppression in Myc^{KO} and $CDK7^{KO}$ cells in comparison to control.

XL102 has shown potent anti-AML activity in vivo using subcutaneous as well as orthotopic mouse models.



(A) Representative image of tumor from mice treated with vehicle control, Cytarabine and XL102 (B) Mean tumor weight (in grams) and volume of mice treated with vehicle control, Cytarabine and XL102 (n=9 in each group). (C) Determination of apoptosis and cell cycle markers from tumor excised from xenografts. (D) Decrease in expression of hCD45 in bone marrow of treated animal groups in comparison to control. (E) H&E and immunohistochemistry staining of Ki67 and CC3 of spleen sections from vehicle or drug treated animals.. (G) Kaplan-Meier curve showing overall survival of mice transplanted with MOLM13 AML cells and treated with Vehicle, Cytarabine or XL102.

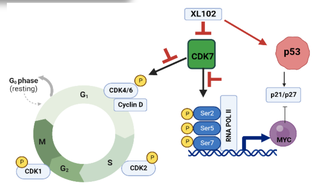
XL102 and Venetoclax demonstrates synergistic anti-leukemic activity in Venetoclax resistant AML cells



(A) Combination index (CI) values of combinatorial treatment with XL102 and Venetoclax in MOLM13 parental and MOLM13 Venetoclax resistant cells. (B)Apoptosis determination using Annexin V-FITC/PI (C) Combination index values of 10 AML blast samples. (D) Basal level expression of apoptotic proteins and p53 in MOLM13 Venetoclax sensitive versus resistant cells. (E) Change in expression of antiapoptotic protein after combinatorial treatment with XL102 and Venetoclax.

CONCLUSION

In conclusion, our study highlights that novel compound, XL102 induces apoptosis in AML cells via CDK7/Myc/p53 axis and has the potential to overcome the Venetoclax resistance. Thus, co-targeting of BCL2 using Venetoclax and CDK7 inhibitor using XL102 can improve clinical outcomes and can work as an alternative to conventional AML treatment



Here we report the anti-leukemic effects of a novel, highly specific, orally bioavailable covalent inhibitor of CDK7, XL102, and delineate the molecular basis for its efficacy in AML. XL102 is currently in clinical trials to treat various solid tumors (NCT04726332).

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CONTACT INFORMATION

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