The CDK2/9 inhibitor fadraciclib in Richter transformation

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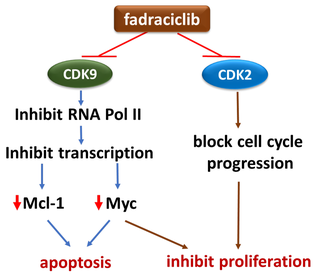
Introduction

- Richter transformation (RT), characterized by the transformation of chronic lymphocytic leukemia (CLL) to an aggressive large-cell lymphoma, remains a challenge for CLL therapy.
- Genetic aberrations, such as Myc activation, TP53 abnormality, NOTCH1 gene mutation or loss of CDKN2A are associated with uncontrolled RT cell proliferation and escaping of apoptosis.
- Among those changes, gain of Myc activity, found in around 70% of RT, plays a nexus role in the pathogenesis of RT. As a transcription factor, Myc activation has a profound effect on cell survival, proliferation, adhesion and metabolism. Because of its essential role in lymphoma transformation and aggressiveness, Myc is a vital target for therapy toward RT.
- Fadraciclib (CYC065) is a second-generation CDK inhibitor with selective potency toward CDK2 and CDK9. We have shown previously that by inhibiting CDK9-mediated transcription, fadraciclib reduced expression of the short-lived anti-apoptotic protein Mcl-1 to initiate apoptosis in primary CLL cells.
- Like Mcl-1, Myc is a quintessential example of an oncoprotein with rapid turnover in both its mRNA and protein, therefore a promising target of CDK9 inhibition.
- **We proposed that fadraciclib would target both the survival and proliferation pathways of RT pathogenesis through collaborative inhibition of Mcl-1, Myc and CDK2.**



Inhibition profile of fadraciclib

Kinase	IC_{50}, nM
CDK2/cyclin A	4.5
CDK5/p25	20.5
CDK9/cyclin T	26.2
CDK7/cyclin H	193
CDK4/cyclin D	232
CDK1/cyclin B	578
Cdk6/cyclin D	> 10,000

Methods:

RT cell line:

- **HPRT1:** a RT-DLBCL cell line derived from a RT patient.

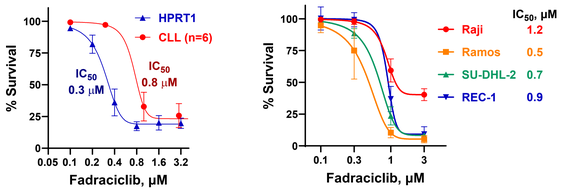
Lymphoma cell lines with Myc amplification:

- **SU-DHL-2:** DLBCL cell line
- **Raji and Ramos:** Burkitt's lymphoma lines
- **REC-1:** Mantle Cell Lymphoma line (NOTCH1 activation)

Results

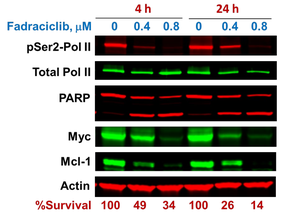
Induction of cell death by fadraciclib

A. Dose dependent induction of cell death by fadraciclib



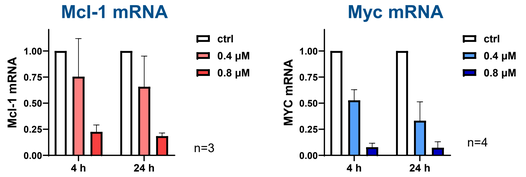
- Cells were incubated with increasing concentrations of fadraciclib.
- Viabilities were measured at 24h by Annexin V/PI staining.
- The HPRT1 cells were more sensitive to fadraciclib than the primary CLL cells.

B. Inhibition of CDK9 by fadraciclib reduced RNA Pol II activity and decreased protein levels of Mcl-1 and Myc in the HPRT1 cells.



- HPRT1 cells were incubated with fadraciclib at 0.4 and 0.8 mM for 4 and 24h.
- Viabilities were measured by Annexin V/PI staining.
- Inhibition of RNA Pol II phosphorylation on Ser-2 sites in its C-terminal domain was shown by immunoblotting.
- Fadraciclib reduced both Mcl-1 and Myc protein levels.
- PARP cleavage indicated initiation of apoptosis in the cell.
- Similar results were observed in the lymphoma cell lines (not shown).

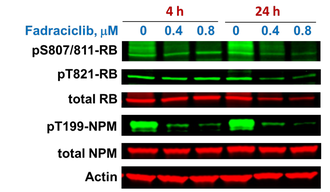
C. Fadraciclib reduced Mcl-1 and Myc mRNA in the HPRT1 cells.



- mRNA levels of Mcl-1 and Myc were measured by real-time RT-PCR.
- Similar results were observed in the lymphoma cell lines (not shown).

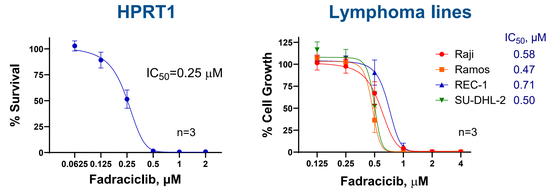
Inhibition of proliferation by fadraciclib

A. Fadraciclib inhibited CDK2 activity.

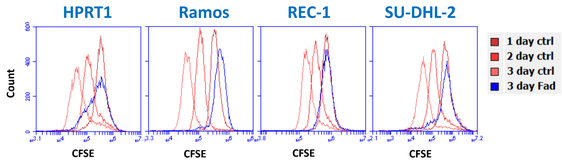


- RB and NPM are CDK2 substrates that regulate cell cycle progression.
- Reduced phosphorylation of both RB and NPM indicated inhibition of CDK2 activity by fadraciclib.

B. Fadraciclib inhibited cell proliferation.

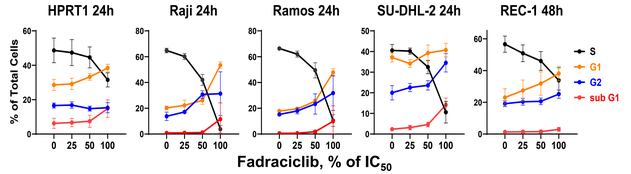


- Cells were seeded on 96-well plates with increasing conc. of fadraciclib.
- Cell proliferation was measured by the CellTiter-Glo 2.0 kit after 3 days.



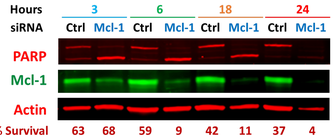
- Cells were stained with 5mM CFSE on day 0, before incubation with 1x IC50 concentrations of fadraciclib.
- At day 1, 2, 3, CFSE intensity were measured by flow cytometry.
- Fadraciclib blocked cell division in both the HPRT1 and the lymphoma lines.

C. Fadraciclib blocked cell cycle progression.



- Cells were incubated with 25%, 50% and 100% of IC50 concentrations of fadraciclib for inhibition of proliferation for each line.
- Cell cycle status was determined by Click-iT™ Plus EdU assay.
- DNA were stained with 7-AAD.
- Fadraciclib reduced S-phase population and arrested cells at G1.

Mcl-1 is essential for the survival of the HPRT1 cells



Specific knocking down of Mcl-1 by siRNA induced apoptosis in the HPRT1 cells.

Summary

1. By inhibiting both CDK9 and CDK2, fadraciclib blocked cell proliferation and induced apoptosis, both in the Richter cell line HPRT1, as well as in Myc dependent lymphoma cell lines.
2. These data provide rationale for investigation of clinical trial of fadraciclib in Richter transformation of CLL and in lymphoma.

References

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