Activity of RP7214, a novel, selective, and potent small molecule inhibitor of DHODH, in AML

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Introduction

Dihydroorotate dehydrogenase (DHODH), a rate limiting enzyme in pyrimidine synthesis, is overexpressed in certain cancers and impedes tumor cell proliferation *via* cell cycle arrest. RP7214 is a novel and potent DHODH inhibitor that inhibited DHODH activity in enzyme and PHA induced HWB/PBMC proliferation with IC_{50} and EC_{50} values of 7.8 & 2.5/0.60 nM respectively. The objective of this study was to evaluate the effect of RP7214 and in combination with Azacytidine or Venetolcax in preclinical models of AML.

	DHODH	HWB	PBMC
IC_{50}/EC_{50} (nM)	7.8	2.5	0.6

Table 1. DHODH enzyme activity was determined by its reduction of 2,6-dichloroindophenol during the oxidation of dihydroorotate using mitochondrial membrane preparations of U937 cells.

For EC_{50} determination, freshly isolated human PBMCs or HWB were treated for desired concentrations of the inhibitor and induced with 2 µM PHA. CD4+ cells were determined after 48 h by flow cytometry

Cell Line	-Uridine	+ Uridine
	GI_{50} (µM)	
U937	3.2	>10
THP-1	2.0	>10
HL-60	3.6	>10
MV411	2.6	>10

Table 2. Growth inhibition in AML cell lines. Cell lines were treated with RP7214 in presence (100 µM for U937, HL-60, and MV411 and 300 µM for THP-1) or absence of Uridine. ***RP7214 inhibited growth of AML cell lines with GI_{50} between 2–3.2 µM and addition of uridine caused rightward shift with GI_{50} > 10µM***

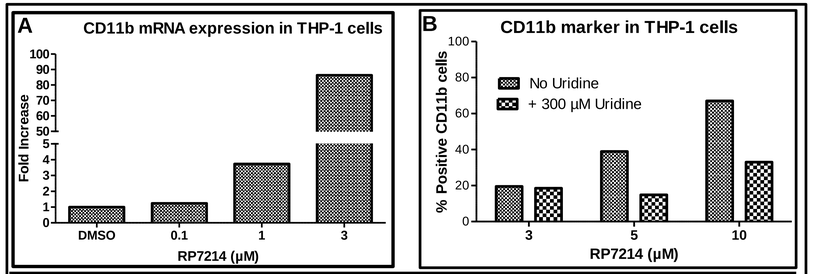


Figure 1. A. CD11b mRNA expression in THP-1 cells. THP-1 cells were plated in complete media at pre-determined density in 6-well plates and treated with RP7214 for 72 hours. ***RT-PCR analysis indicated an 80-fold increase in mRNA expression of the differentiation marker, CD11b, in THP-1 cells treated with 3 µM RP7214.***

B. THP-1 cells were plated in complete media at pre-determined density in 6-well plates and treated with RP7214 for 96 hours in presence or absence of uridine and analysed by flow cytometry for CD-11b expression***. Expression of the CD11b marker on THP-1 cell surface was enhanced by 40% following incubation with 5 µM compound; effect of which was reduced to 15% in the presence of 300 µM Uridine.***

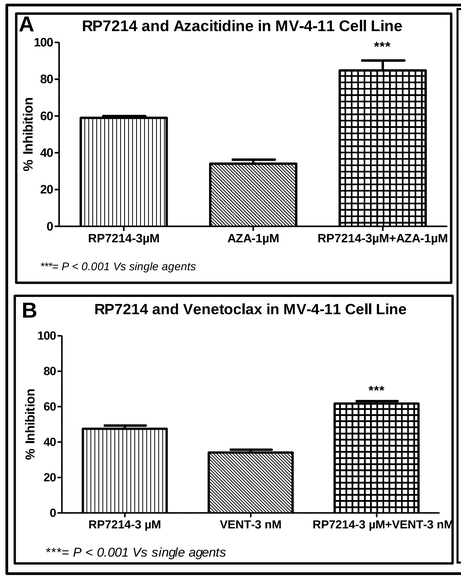
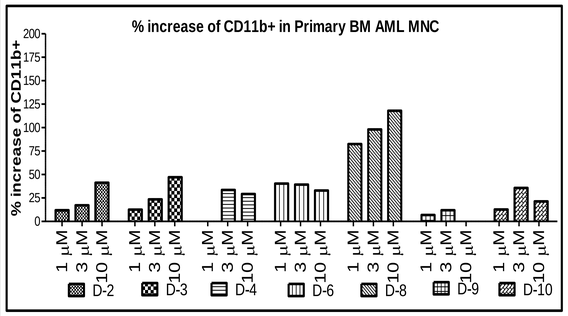


Figure 2. Growth inhibition in MV-4-11 Cell line. MV-4-11 cells were plated in complete media at pre-determined density in 96-well plates and treated with RP7214 or Azacitidine (AZA)/Venetoclax (VENT or combination of RP7214 with AZA/VENT for 72 hours and MTT assay was performed.

A. Combination treatment of RP7214 with AZA demonstrated > 25% growth inhibition (P < 0.001) compared to single agent of RP7214 and AZA in MV-4-11 cell line

B. Combination treatment of RP7214 with VENT demonstrated > 14% growth inhibition (P < 0.001) compared to single agent of RP7214 and VENT in MV-4-11 cell line



Differentiation of primary BM AML MNC Samples				
	% increase in of CD11b+			
Donor	10 µM	3 µM	1 µM	Response
D2	41.3	17.2	12.0	Sensitive
D3	47.1	23.6	12.6	Sensitive
D4	29.3	33.6	0	Sensitive
D6	33.0	39.3	40.3	Sensitive
D8	118.0	98.1	82.5	Sensitive
D9	0	12.0	6.8	Moderately Sensitive
D10	21.3	35.7	12.8	Sensitive

Figure 3. Increase of CD11b+ in Primary BM AML MNC. Human AML BM cells from 7 patients were cultured for four days in a liquid–based 96-well plate format using myeloid expansion medium in the presence of RP7214 to evaluate its effects on AML cell differentiation surface markers by flow cytometry analysis**.** Response of primary BM cells from AML patients to RP7214 was classified into three categories: sensitive if any of the myeloid markers CD11b increased ≥15%; moderately sensitive if the markers increased ≥5%, but <5%, resistant if the markers increased by <5%. ***RP7214 increased CD11b+ population by > 15% in 6 out of 8 primary AML BM MNC samples***

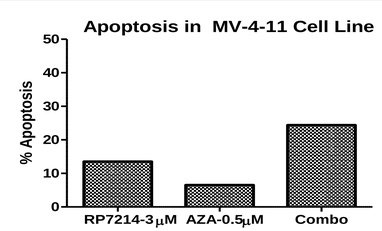
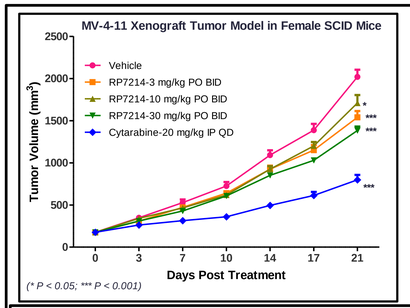


Figure 4. Apoptosis in MV-4-11 cells. For measurement of apoptotic activity, cells were treated with RP7214-3 µM and Azacytidine-0.5 µM for 72 h and stained with Annexin & PI and analyzed on Muse cell Analyzer. ***Data demonstrated the combination of 3 µM RP7214 + 0.5 µM Azacytidine led to 25% increase in the number of apoptotic cells***



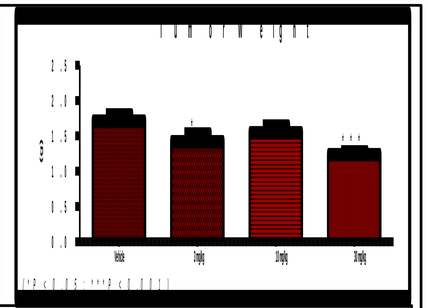


Figure 5. Anti-tumor activity in MV-4-11 Xenograft. RP7214 at 3,10, 30 mg/kg/PO and Cytarabine (Cyt) at 20 mg/kg/IP, were tested in a subcutaneous MV-4-11 human leukemia xenograft model. ***RP7214 at 3, 10, and 30 mg/kg BID demonstrated significant anti-tumor activities in both tumor size and tumor weight in subcutaneous MV-4-11 human leukemia xenograft model***

SUMMARY & CONCLUSIONS

- Inhibition of DHODH by RP7214 represents a unique therapeutic strategy in AML that accentuates the effect of approved and standard of care drugs such as Azacytidine or Venetoclax
- RP7214 potentiated the activity of Azacytidine or Venetoclax in reducing the cell growth.
- RP7214 as a single agent induced differentiation in BM AML MNC by increasing the CD11b+. Additionally, it demonstrated anti-tumor activity manifested by a reduction in both tumor size and tumor weight in MV-4-11 human leukemia xenograft model
- An open label study to evaluate the clinical activity of RP7214, in combination with Azacitidine in patients with Myelodysplastic Syndrome, Chronic Myelomonocytic Leukemia and Acute Myeloid Leukemia, is currently in progress.

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