C042

# Inhibition of Dihydroorotate Dehydrogenase (DHODH) by RP7214 attenuates growth and promotes differentiation of AML cell lines

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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 single-agent activity of RP7214 on AML cell differentiation and growth.

| | DHODH) | HWB | PBMC |
|---|---|---|---|
| $IC_{50}$ (nM) | 7.8 | 2.5 | 0.6 |
| $EC_{50}$ (nM) | - | 2.5 | |

**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.

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***

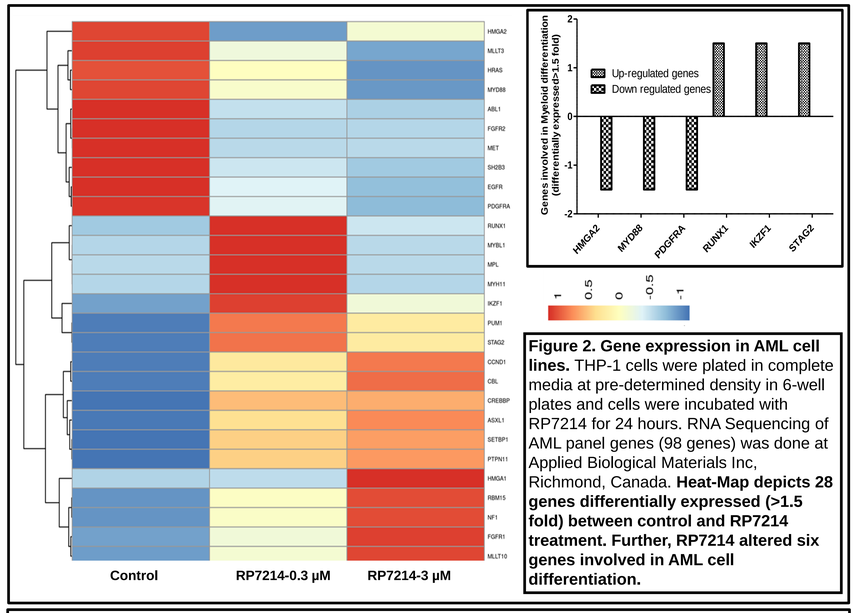


**Figure 2. Gene expression in AML cell lines.** THP-1 cells were plated in complete media at pre-determined density in 6-well plates and cells were incubated with RP7214 for 24 hours. RNA Sequencing of AML panel genes (98 genes) was done at Applied Biological Materials Inc, Richmond, Canada. **Heat-Map depicts 28 genes differentially expressed (>1.5 fold) between control and RP7214 treatment. Further, RP7214 altered six genes involved in AML cell differentiation.**

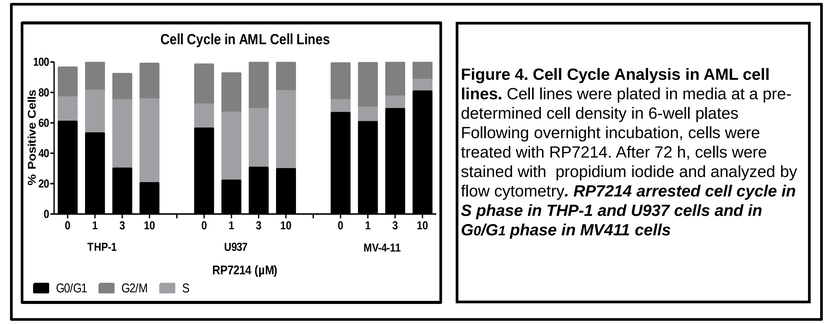


**Figure 4. Cell Cycle Analysis in AML cell lines.** Cell lines were plated in media at a pre-determined cell density in 6-well plates Following overnight incubation, cells were treated with RP7214. After 72 h, cells were stained with propidium iodide and analyzed by flow cytometry. ***RP7214 arrested cell cycle in S phase in THP-1 and U937 cells and in G0/G1 phase in MV411 cells***

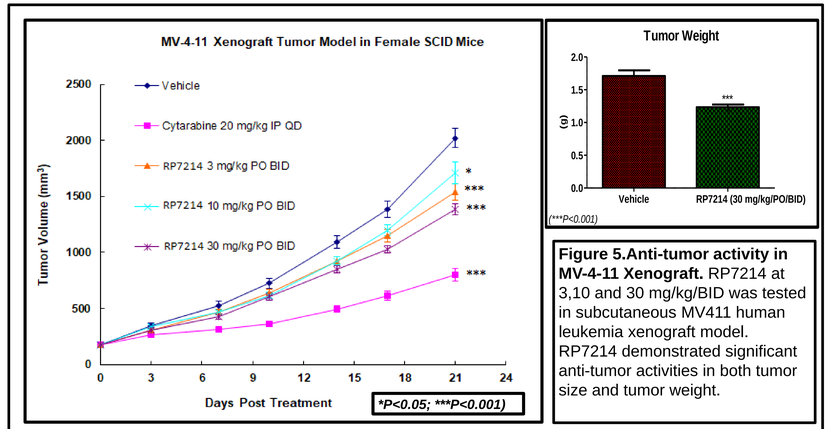


**Figure 5.Anti-tumor activity in MV-4-11 Xenograft.** RP7214 at 3,10 and 30 mg/kg/BID was tested in subcutaneous MV411 human leukemia xenograft model. RP7214 demonstrated significant anti-tumor activities in both tumor size and tumor weight.

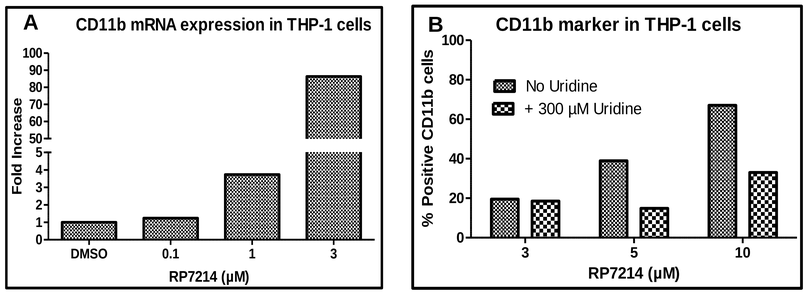





**Figure 1. A. CD11b mRNA expression in THP-1 cells.** THP-1 cells were plated in complete media at pre-determined density in 6-well plates and treated with RP7214 for 72 hours. ***RT-PCR analysis indicated an 80-fold increase in mRNA expression of the differentiation marker, CD11b, in THP-1 cells treated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

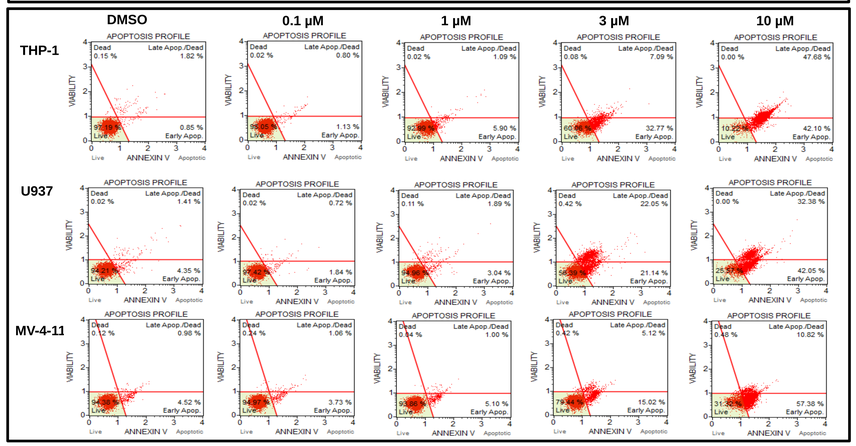


**Figure 4. Apoptosis in AML cell lines.** For measurement of apoptotic activity, cells were treated with desired concentrations of RP7214 for 72 h and stained with Annexin and propidium iodide (PI). ***Data demonstrated an increase in apoptotic population upon addition of 3 and 10 µM RP7214 in all the cell lines tested.***

## SUMMARY & CONCLUSIONS

- RP7214 is a potent and novel DHODH inhibitor in AML acting via suppression of cellular pyrimidine pools and promoting differentiation to fully mature cells.
- Demonstrated growth inhibitory activity in AML cell lines and induction of myeloid differentiation gene expression.
- Demonstrated anti-tumor activities in both tumor size and tumor weight in MV411 human leukemia xenograft model.
- The compound is currently being evaluated in IND-enabling tolerability studies with Phase-1 trial in AML expected to commence in 2020

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