Dihydroorotate Dehydrogenase Inhibition Reveals Metabolic Vulnerability in Chronic Myeloid Leukemia

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Background

The introduction of different generations of tyrosine kinase inhibitors (TKIs) has changed treatment outcomes in chronic myeloid leukemia (CML) and obtaining an operational cure is possible. However, not all patients fully benefit from TKI therapy, and TKI resistance and relapse after therapy discontinuation have been recorded. An alternative approach that can target CML clones irrespective of their state might help to increase the number of patients who can halt the therapy and have a better treatment outcome. Dihydroorotate dehydrogenase (DHODH) is a vital enzyme in the de novo pyrimidine biosynthesis pathway, and it is located in the inner mitochondria membrane. DHODH catalyzes the fourth step of the pyrimidine biosynthesis pathway by oxidation of dihydroorotate to orotate. Previously, a new and potent DHODH inhibitor called Meds433 was developed by our group. In the present study, we shed light on the role of DHODH inhibition in CML stem/progenitor cells and how it can be a promising approach in the treatment of CML.

Method

For this study, 60 BM and PB samples of newly diagnosed and 4 TKI-resistant CML patients were collected. After sorting CML CD34+ cells different experiments were performed.

Result

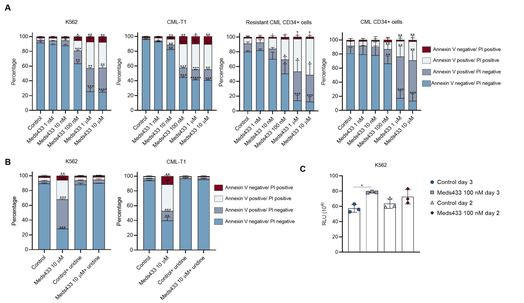


Figure 1. Meds433 induces apoptosis in CML cells. **A** represents apoptosis assay in CML cell lines and primary cells. **B** addition of uridine rescued the effect of Meds433 and shows its selectivity. **C** demonstrates uridine level after DHODH inhibition. RLU inversely correlates with uridine level.

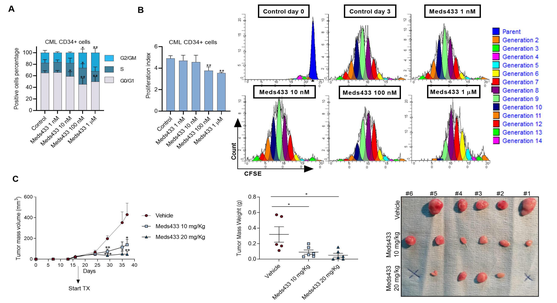


Figure 2. Meds433 reduces the proliferation of CML cells. **A** and **B** shows cell cycle arrest and reduction of proliferation in CML CD34+ cells treated with different concentrations of Meds433, respectively. **C** represents KU-812-derived (CML cell line) tumor mass volume and tumor weight after the treatment with 10 and 20 mg/kg Meds433, respectively.

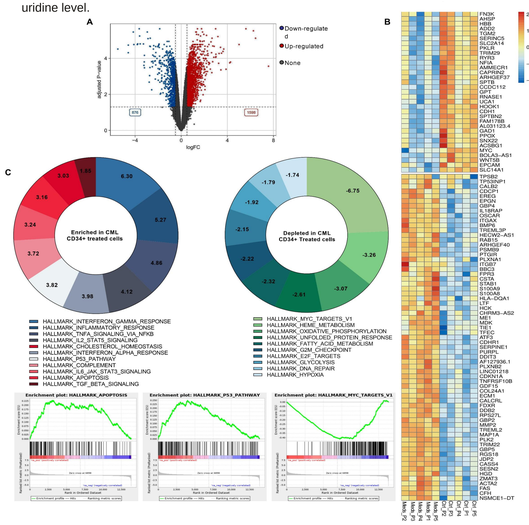


Figure 3. Meds433 changes transcriptome in CML CD34+ cells. **A** the volcano plot demonstrates the number of up-and down-regulated genes in CML CD34+ cells after treatment with 100 nM Meds433. **B** The top 100 differentially expressed genes are depicted in the heatmap, and **C** shows enriched, and depleted hallmark gene sets

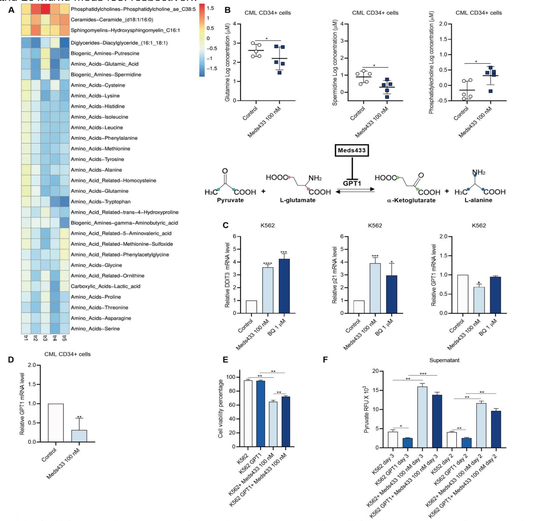
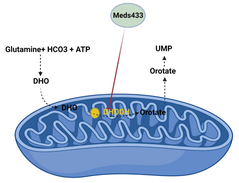


Figure 4. DHODH inhibition changes the metabolic profile in CML CD34+ cells. **A** and **B** shows altered metabolites of CML CD34+ treated with Meds433. **C** shows the relative mRNA level of DDIT3, p21, and GPT1 in K562 cells treated with Meds433 and brequinar (BQ). **D** shows the relative mRNA level of GPT1 in treated CML CD34+. **E** shows cell viability in K562 and GPT1 overexpressed K562, respectively. **F** demonstrates Pyruvate levels in the supernatant of K562 and GPT1 overexpressed K562.

Conclusion

There are CML patients who show resistance to TKI therapy and are prone to progress to more advanced phases of the disease. So, implementing an alternative approach for targeting TKIs insensitive cells would be of the essence. Here, we found that CML cells are vulnerable to DHODH inhibition mediated by Meds433, a new and potent DHODH inhibitor recently developed by our group. Meds433 significantly activates the apoptotic pathway and leads to the reduction of amino acids and induction of huge metabolic stress in CML CD34+ cells mainly through GPT1. Altogether, our study shows that DHODH inhibition is a promising approach for targeting CML stem/progenitor cells and may help more patients discontinue the therapy.



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