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# Pyrvinium selectively induces apoptosis of lymphoma cells through impairing mitochondrial functions and JAK2/STAT5

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ABSTRACT

Targeting mitochondrial respiration has emerged as an attractive therapeutic strategy in blood cancer due to their unique metabolic dependencies. In this study, we show that pyrvinium, a FDA-approved anthelmintic drug, selectively targets lymphoma T-cells though inhibition of mitochondrial functions and JAK2/STAT5. Pyrvinium induces apoptosis of malignant T-cell line Jurkat and primary T-cells from lymphoma patients while sparing T-cells from healthy donors. Increased level of active caspase-3 and decreased levels of Bcl-2 and Mcl-1 were also observed in Jurkat and lymphoma T-cells but not normal T-cells treated with pyrvinium. In addition, pyrvinium impairs mitochondrial functions by inhibit mitochondrial respiration, suppressing mitochondrial respiratory complex I activity, increasing ROS and decreasing ATP levels. However, the effects of pyrvinium were abolished in mitochondrial respiration-deficient Jurkat $\rho^0$ cells, confirming that pyrvinium acts on lymphoma T-cells via targeting mitochondrial respiration. We further show that lymphoma T-cells derived from patients depend more on mitochondrial respiration than normal T-cells, and this explains the selective toxicity of pyrvinium in lymphoma versus normal T-cells. Finally, we demonstrate that pyrvinium also suppresses JAK2/STAT5 signaling pathway in Jurkat cells. Our study suggests that pyrvinium is a useful addition to T-cell lymphoma treatment, and emphasizes the potential therapeutic value of the differences in the mitochondrial characteristics between malignant and normal T-cells in blood cancer.



## 1. Introduction

Lymphoma is one of the most common hematological malignances. T-cell lymphoma is a subtype of non-Hodgkin's lymphoma, which is more clinically aggressive with little understanding of molecular pathogenesis and poor cure rate [1]. Recent studies identified that the active JAK2-STAT signaling contributes to T-cell lymphoma development and propose the use of JAK inhibitors in T-cell lymphoma treatment [2,3]. Nevertheless, novel therapeutic strategies which can either independently or in combination with clinically available chemotherapies, are required for its better management with T-cell lymphoma.

Targeting oxidative phosphorylation has emerged as an intriguing therapeutic strategy in blood cancer due to their unique metabolic dependencies [4,5]. In contrast to normal hematopoietic cells, acute myeloid leukemia cells depend more on mitochondrial respiration rather than glycolysis to meet energy demands and maintain survival [6]. Inhibition of oxidative phosphorylation preferentially exhibits selective toxicity against blood cancer cells but not normal hematopoietic cells [6,7]. It has also been reported that there is increase in mitochondrial biogenesis and oxidative stress in murine T-cell lymphomas, which can be exploited therapeutically [8].

Although pyrvinium is an anthelmintic drug, several studies have highlighted pyrvinium as a novel type of anti-cancer drug. It inhibits growth of a large panel of tumor cell lines but has minimal cytotoxicity to normal fibroblast cells [9–14]. In addition, pyrvinium is extremely toxic to tumor cells in hypoglycemic condition [12,15]. When pyrvinium is combined with conventional

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chemotherapeutic drugs, the combination showed significant enhanced anti-cancer effects [10,12]. The mechanism of action of pyrvinium seems to be in a cancer cell-type specific manner. Thorne et al. have reported that pyrvinium inhibits growth of colon cancer cells through activation of casein kinase 1α (CK1α) and inhibition of β-catenin [14]. Harada et al. and Wei et al. demonstrated that pyrvinium inhibits proliferation of myeloma and leukemia cells by suppressing mitochondrial respiration [11,16]. Other reported mechanisms involved targeting unfolded protein response [12] and autophagy [10].

In this study, we investigated the effect of pyrvinium in lymphoma using T-cell line Jurkat and primary T-cells derived from T-cell lymphoma patients as well as healthy donors. We found that pyrvinium selectively induces apoptosis of lymphoma T-cells while sparing normal T-cells. We show that the pro-apoptotic effect of pyrvinium in T-cell lymphoma is attributed to its inhibition of mitochondrial respiration, accompanied by the suppression of JAK2/STAT5 and Akt signaling pathways. Importantly, we show that compared to normal T-cells, lymphoma T-cells have higher levels of mitochondrial respiration and cellular ATP. Finally, we demonstrate the possible connection between mitochondrial respiration and JAK2/STAT5 and Akt signaling pathways.

## 2. Materials and methods

### 2.1. Primary cells and drug

Lymphoma T-cells and normal T-cells were purified using Pan T-cell isolation kit (Miltenyi Biotec, Germany) from peripheral blood of lymphoma patients and healthy donors seen at JingZhou Hospital, Tongji Medical College, Huazhong University of Science and Technology (HUST), respectively. Written informed consents were obtained from all patients under protocols approved by the institutional review board. Lymphoma and normal T-cells were cultured using T-Cell Expansion Kit (Miltenyi Biotec, Germany). Pyrvinium (P0027, Sigma, US) was dissolved in DMSO.

### 2.2. Generation of mitochondrial DNA-deficient $\rho^0$ cell line

Human Jurkat cells were purchase from American Type Culture Collection (ATCC) and grown in RPMI1640 medium containing 4 mM L-glutamine (Life Technologies, CA, US) and 10% fetal bovine serum (FBS) (Hyclone, UK). Mitochondria DNA-deficient Jurkat $\rho^0$ was established according to the method previously described [17]. Briefly, Jurkat cells were growing in RPMI1640 medium containing 10% FBS, 2 μg/ml ethidium bromide (EtBr), 4 mM L-glutamine, 50 μg/ml uridine, 100 μg/ml sodium pyruvate (Sigma, MO, US) for 50 days, and thereafter maintained in above media without EtBr.

### 2.3. Measurement of apoptosis

Cells were treated with pyrvinium for 72 h prior to staining with Annexin V-FITC and propidium iodide (PI). The stained cells were analyzed by flow cytometry using Beckman Coulter FC500. The percentage of Annexin V-positive cells was determined using CXP analysis software.

### 2.4. Western blotting

Whole protein from cells were lysed by RIPA lysis buffer (Life Technologies Inc, US). Equal amount of total proteins were resolved using denaturing SDS–PAGE and analyzed by WB using antibodies against β-catenin, Axin (BD Transduction Labs, US), β-actin, Mcl-1, Bcl-2, active caspase-3 (Santa Cruz Biotechnology, US), and t-STAT5, p-STAT5 (T694), p-JAK2 (T1007/1008), t-JAK2 (Cell Signalling Technologies, US).

### 2.5. Metabolic assays

Cell was treated with DMSO or pyrvinium for 24 h prior to the following metabolic assays. Oxygen consumption rate (OCR) was measured using the Seahorse XF24 extracellular flux analyzer as previously described [18]. Briefly, five replicate wells of $5 \times 10^4$ drug-treated cells were seeded in 24-well XF24 well plates coated with BD Cell-Tak (BD Biosciences, MA, US). Seeded cells were then equilibrated to the un-buffered medium at 37 °C in a $CO_2$-free incubator for pH stabilization and transferred to the Seahorse XF24 extracellular flux analyzer. Analyses were performed under basal condition as well as after injection of oligomycin (OLI, 1 μg/ml), carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP, 0.4 μM) and antimycin A and rotenone combination (A&R, 2.5 μM and 2.5 μM) according to the manufacturer's instructions. For measuring intracellular reactive oxygen species (ROS) levels, cells were stained with redox-sensitive probe carboxy-H2DCFDA in PBS buffer. Labeled cells were re-suspended in PI buffer and analyzed using Beckman Coulter FC500 and FlowJo software. ATP levels were measured by CellTiter-Glo Luminescent Cell Viability Assay (Promega, WI, US) according to the manufacturer's instructions.

### 2.6. Measurement of mitochondrial respiratory complex activity

Cells were treated with pyrvinium for 24 h prior to complex activity measurement. Mitochondrial respiratory complex I, II, IV and V activities were measured using Mitochondrial Complex I, II, IV and V Activity Assay Kits (Novagen, US) according to manufacturers' instructions.

### 2.7. Statistical analyses

All data are expressed as mean and standard deviation (SD) to indicate data variability. Statistical analyses were performed by unpaired Student's t test. A p-value $<0.05$ was considered statistically significant.

## 3. Results

### 3.1. Pyrvinium selectively induces apoptosis of lymphoma T-cells while sparing normal T-cells

We investigated the effects of pyrvinium on apoptosis in human lymphoma T-cell line Jurkat, primary lymphoma T-cells derived from fifteen lymphoma patients as well as normal T-cells derived from fifteen healthy donors (Clinical and biological information of patients and healthy donors are summarized in Supplementary Tables S1 and S2). We found that pyrvinium-induced apoptosis of Jurkat cells in a dose-dependent manner as shown by flow cytometry of Annexin V staining (Fig. 1A and B). An important feature of targeted therapy is the ability to be selective in retaining activity against lymphoma cells while sparing normal cells. We observed that pyrvinium effectively induced apoptosis in lymphoma T-cells (Fig. 1C). However, the same concentration of pyrvinium either did not or induced apoptosis to a significantly less extent than in normal T-cells (Fig. 1C), indicating that pyrvinium exhibits selective toxicity against lymphoma versus normal T-cells. Consistent with the flow cytometry study, enhanced active caspase-3 activity and decreased levels of anti-apoptotic Bcl-2 and Mcl-1 were also demonstrated in Jurkat and lymphoma T-cells but not normal T-cells exposed to pyrvinium (Fig. 1D), further indicating the selective pro-apoptotic effects of pyrvinium in lymphoma T-cells.