Overcoming Melphalan Resistance in the Treatment of Multiple Myeloma

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INTRODUCTION

- Multiple myeloma (MM) is a cancer of the plasma cells in the bone marrow
- Melphalan is the mainstay of myeloma chemotherapy
- MM remains incurable- most patients tend to relapse
- Cancer cells develop resistance to different therapies by rewiring or altering their metabolism
- Understanding the metabolism of cancer cells and how it changes in cells that are resistant to various therapies is important

RESEARCH QUESTION

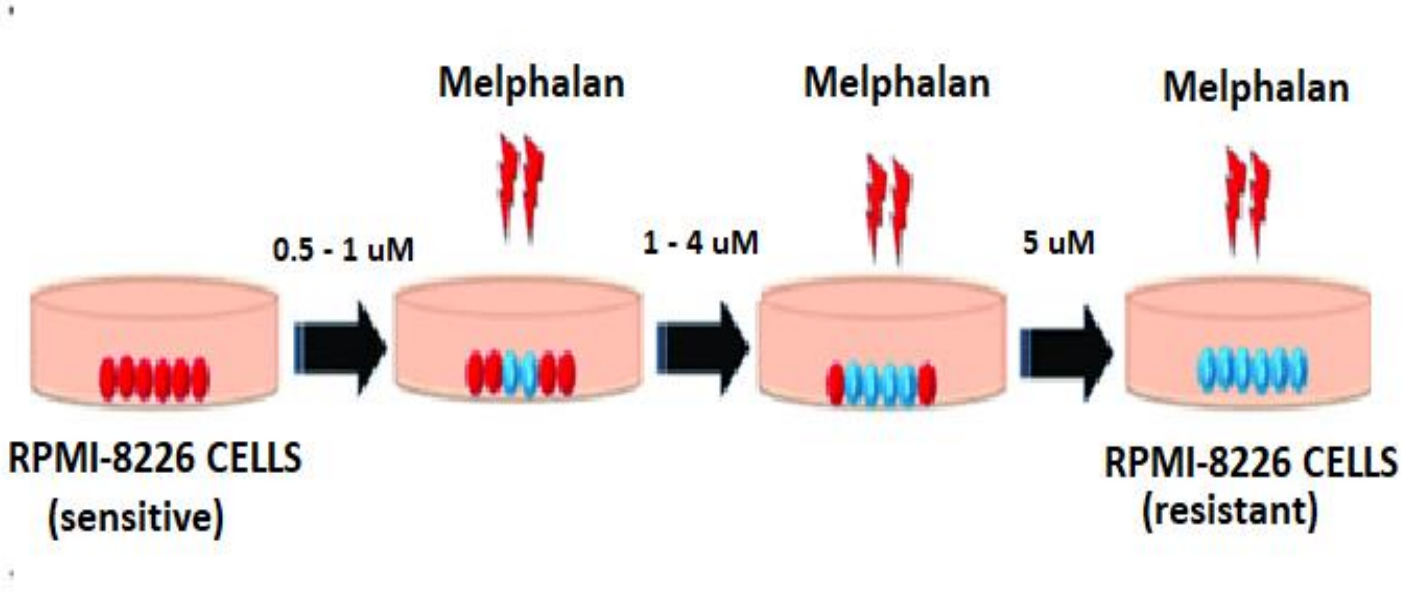
What are the differences in the metabolic pathways in melphalan resistant multiple myeloma cells compared to those that are sensitive to melphalan?

HYPOTHESIS

The metabolism of MM cells resistant to melphalan is different from those that are sensitive to melphalan

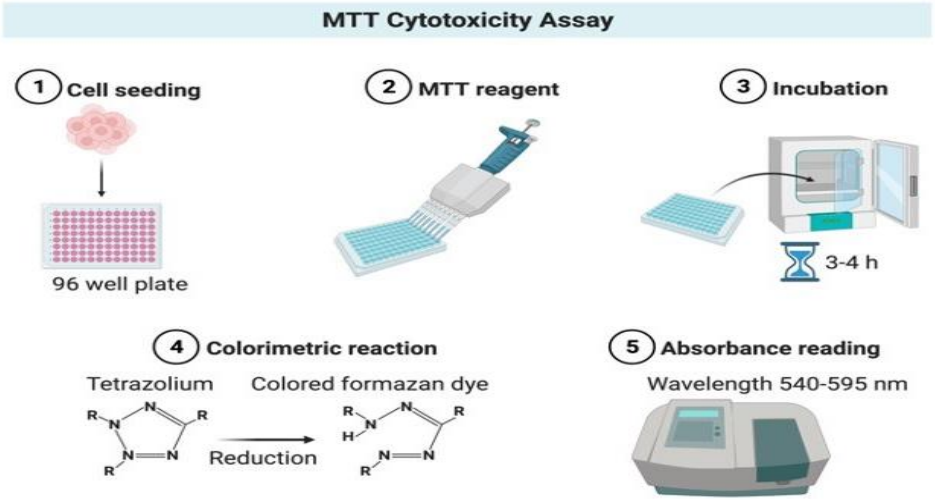
If these key metabolic pathway differences can be identified, they can potentially be overcome pharmacologically to improve the efficacy of melphalan in the treatment of this common and incurable cancer

CREATING A MELPHALAN-RESISTANT CELL LINE



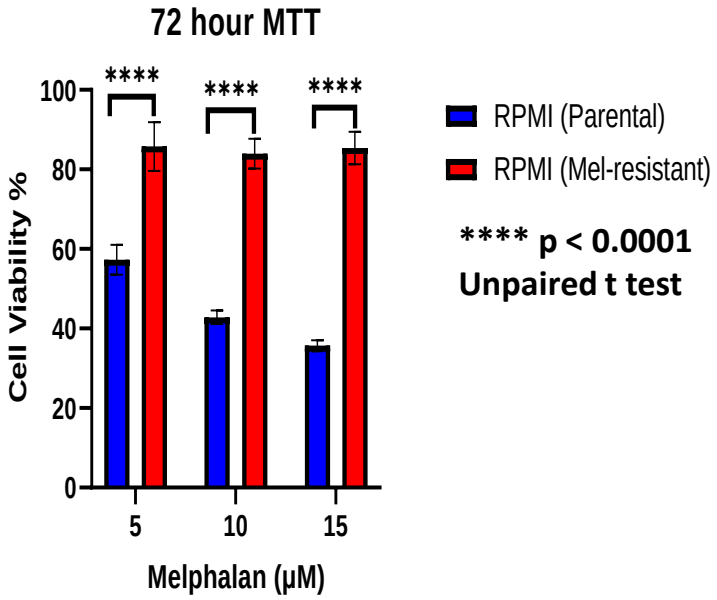
- Isogenic melphalan-resistant MM cell lines created to serve as an *in vitro* model for melphalan resistance
- Parent cell line RPMI-8226 (B lymphocytes isolated from peripheral blood of a patient with plasmacytoma in 1966) exposed to escalating concentrations of melphalan

MTT CYTOTOXICITY ASSAY FOR ASSESSMENT OF CELL VIABILITY

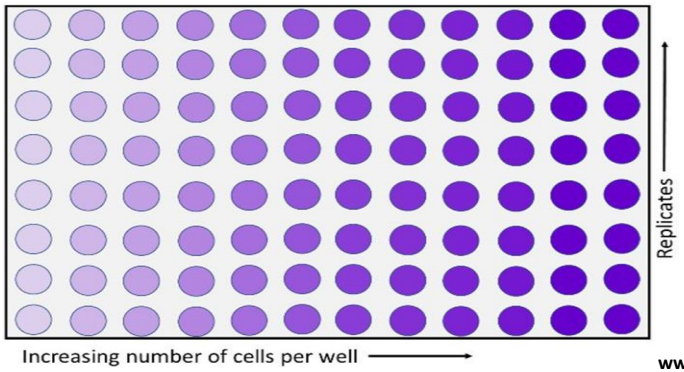


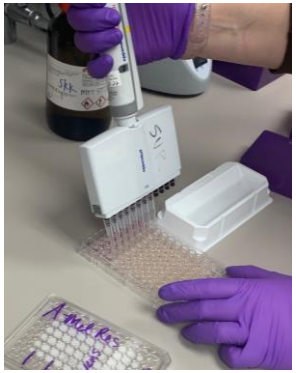
https://theferalexplorer.wordpress.com/2019/07/05/understanding-mtt-assay/

Comparing the viability of the 2 cell lines



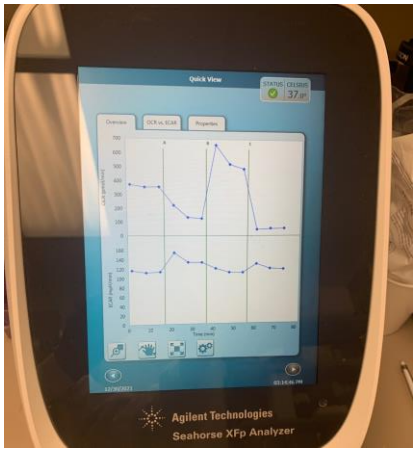
The steps in the MTT assay

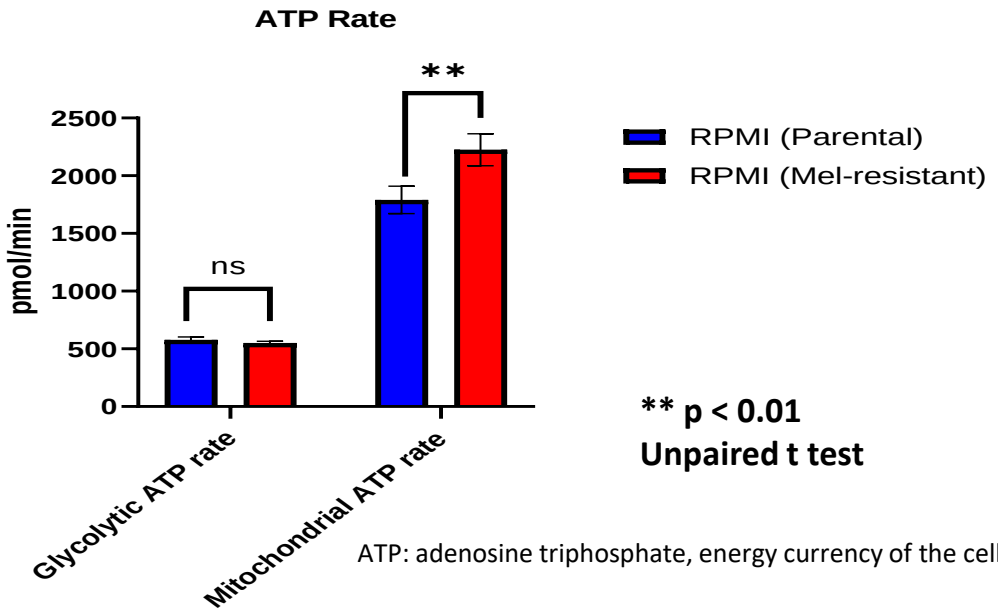




www.sigmaaldrich.com/...cell-proliferation-kit-i-mtt

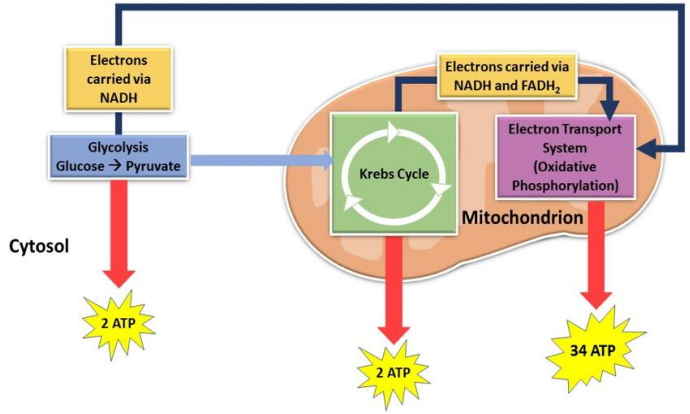
DIFFERENCES IN THE BIOENERGETIC PATHWAYS IN THE SENSITIVE AND RESISTANT CELL LINES WERE STUDIED USING SEAHORSE XF





** p < 0.01
Unpaired t test

ATP: adenosine triphosphate, energy currency of the cell

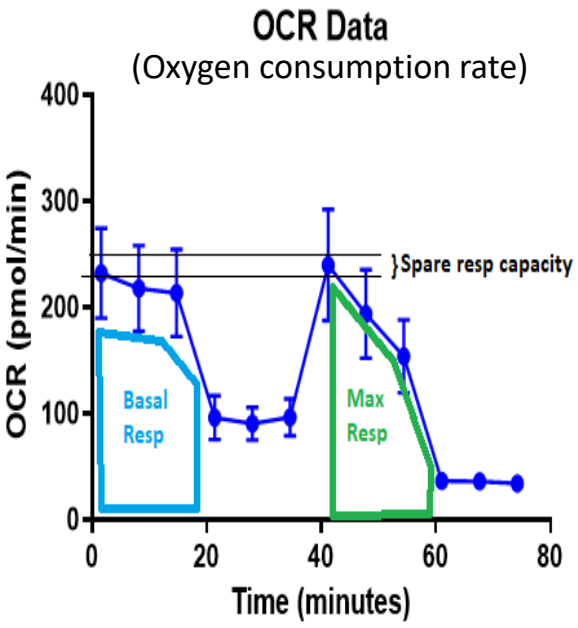


Melphalan-resistant cells showed a much greater mitochondrial capacity

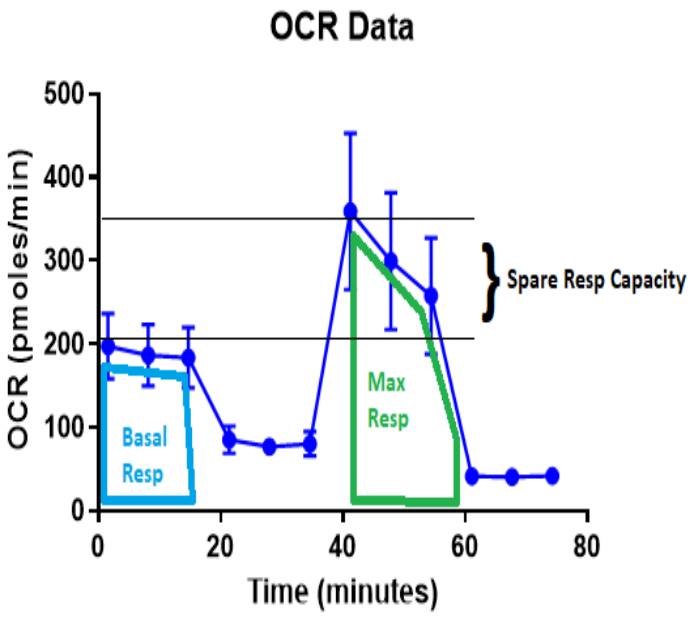
https://chem.libretexts.org/Courses/Saint_Marys_College_Notre_Dame_IN/CHEM_342%3A_Bio-inorganic_Chemistry/Readings/Metals_in_Biological_Systems_(Saint_Mary%27s_College)/Cytochrome_Oxidase

ASSESSING THE OXIDATIVE CAPACITY IN MELPHALAN-RESISTANT CELLS

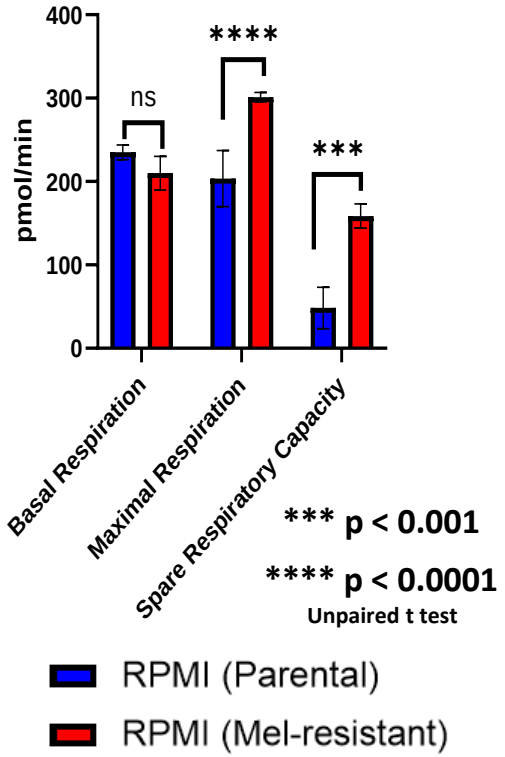
RPMI (parental)



RPMI (Mel-resistant)

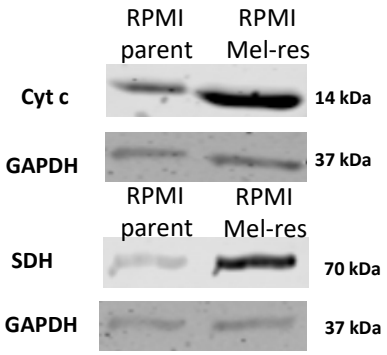


Mitochondrial Energetics



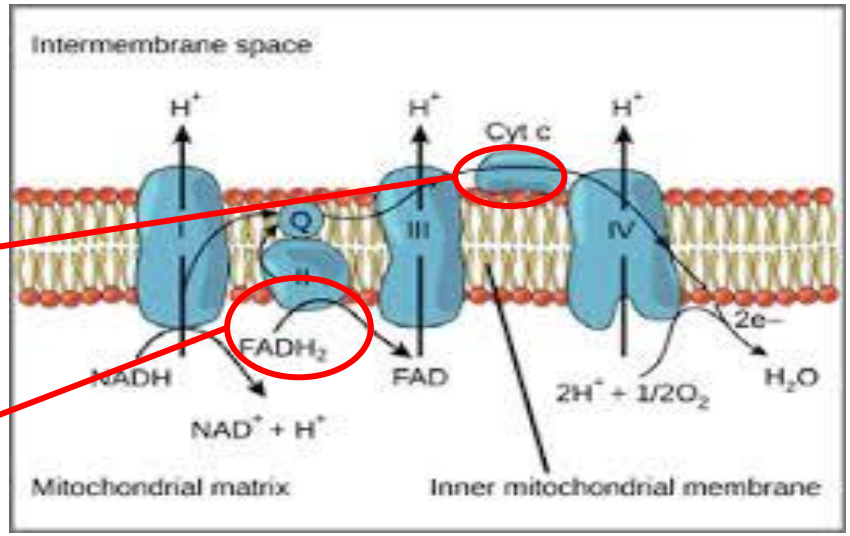
Melphalan-resistant cells had a much higher ability to generate energy via mitochondrial pathways

MITOCHONDRIAL BIOMASS IN MELPHALAN RESISTANT CELLS



Cytochrome c

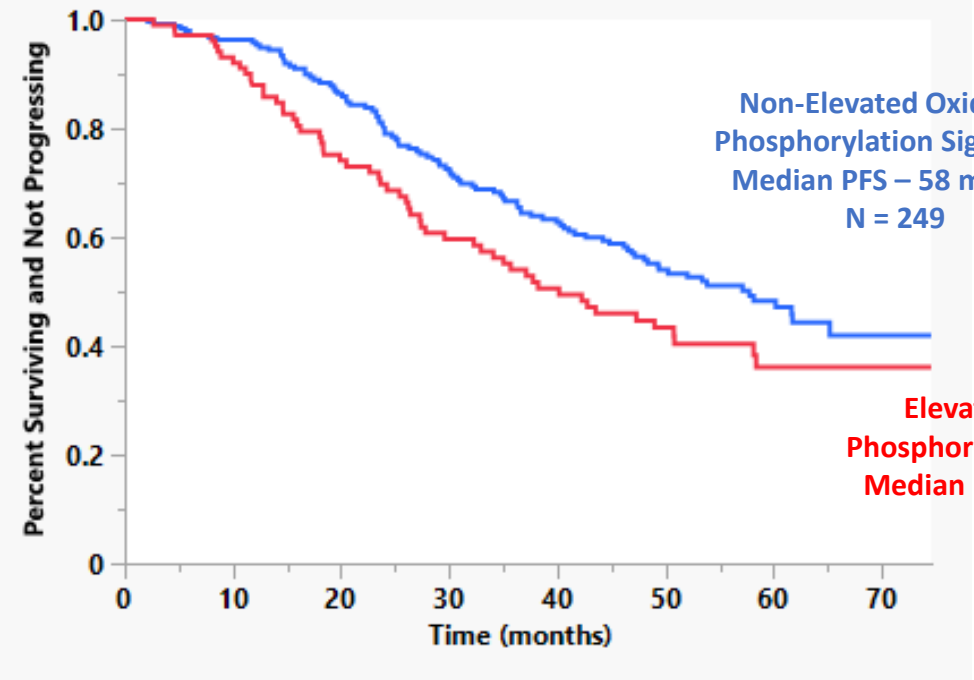
Succinate dehydrogenase (SDH)



https://courses.lumenlearning.com/cuny-kbcc-microbiologyhd/chapter/cellular-respiration/

- Mitochondrial proteins extracted using a cell lysate solution and amount estimated with Western blot technique
- Melphalan resistant cells have a higher mitochondrial biomass than sensitive cells

COMPARISON OF CLINICAL OUTCOMES IN MYELOMA PATIENTS WITH DIFFERENCES IN MITOCHONDRIAL ACTIVITY



Progression free survival (PFS) of patients with MM who had a stem cell transplant with high dose melphalan

Elevated oxidative phosphorylation capacity (greater mitochondrial activity) translates to a poorer survival in patients with newly diagnosed multiple myeloma

CONCLUSIONS

- **A greater mitochondrial capacity translates to a far greater ability to generate energy** by a cell for a given amount of source of energy
- This may be one of the mechanisms by which myeloma cells escape the stress and toxicity of melphalan
- This finding was validated by showing worse outcomes in myeloma patients with elevated mitochondrial oxidative phosphorylation activity
- Drugs that inhibit the mitochondrial capacity can be used with melphalan to improve its sensitivity in myeloma cells

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All graphics created by Isha Kapoor under the supervision of mentor unless stated otherwise.