ASTRO'S 67TH ANNUAL MEETING

REDISCOVERING RADIATION MEDICINE AND EXPLORING NEW INDICATIONS

Rutherrin® Activated by Radiation Therapy Induces Synergistic Tumor Regression through Direct Destruction and Immune Activation in Multiple Preclinical Cancer Models

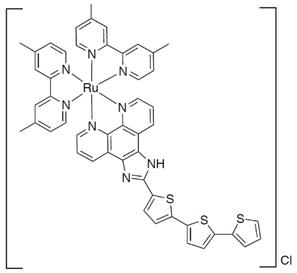
Mark Roufaiel [1], Pavel Kaspler [1], Roger DuMoulin-White [1], and Arkady Mandel [1]

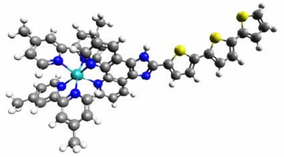
[1] Theralase Technologies Inc., Toronto, Ontario, Canada.

theraLASE® Destroying Cancer at the Speed of Light™

Background

Ruvidar® (TLD-1433) is a light-activated small molecule under clinical investigation for the treatment of adult patients with high-risk Bacillus Calmette Guerin (**"BCG"**)-Unresponsive Non-muscle Invasive Bladder Cancer (**"NMIBC"**) Carcinoma In-Situe (**"CIS"**). It binds to the iron transport glycoprotein transferrin to form the Rutherrin® formulation, which enhances drug uptake into cancer cells through transferrin receptors over-expressed on the cancer cell surface. Upon activation by light or radiation, Ruvidar® delivers a toxic burst of cytotoxic Reactive Oxygen Species (**"ROS"**) confined spatially and temporally to the irradiated region, destroying cancer cells, while sparing healthy cells.





Objective

The aim of this study is to evaluate the *in vitro* and *in vivo* efficacy of Rutherrin® when combined with radiation therapy, assessing tumour regression, survival, and immune response activation across multiple disease models, such as a subcutaneous colorectal model, orthotopic Glioblastoma Multiforme (**"GBM"**) or a lung orthotopic model.

Results: *In vitro* efficacy

- Multiple cells lines were pre-treated with +/- Rutherrin® before radiation.
- Cells were then incubated and viability was measured in a clonogenic assay.
- Rutherrin® induced a significant 2-log increase in cancer cell kill versus radiation alone (**Figure 1**).
- While radiation induces minor ROS production, the level was significantly increased when pre-treated with Rutherrin® (**Figure 2**).

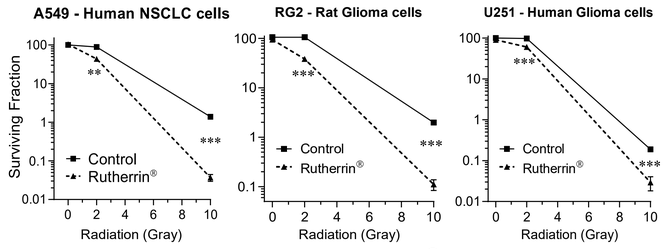


Figure 1: Cell kill with the addition of Rutherrin® to radiation treatment

Results: *In vitro* studies continued

- Rutherrin® increases ROS production with increasing radiation energy, likely due to increased Cherenkov light production.
- Scavenging ROS production inhibits the effect of Rutherrin®, suggesting oxidative stress as a key component of enhanced cytotoxicity.

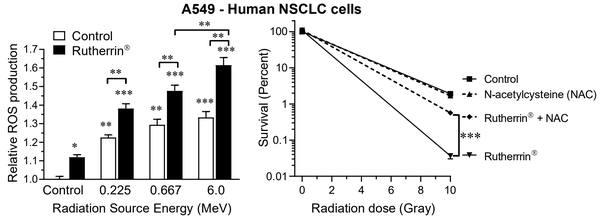


Figure 2: A) Rutherrin® increases Reactive Oxygen Species (ROS) production. B) Cell kill with or without scavenging ROS production using NAC.

Immunogenic Effects

- Rutherrin® + radiation destroyed cancer cells, secreted higher ATP levels, and expressed higher surface calreticulin levels, suggesting immunogenic cell death.
- Cells pretreated with Rutherrin® had significantly lower surface expression of immune checkpoint markers (e.g. CD47 and PD-L1).
- The combination treatment increased the expression of immune-related chemokines and cytokines, suggesting a stronger immune response induction.

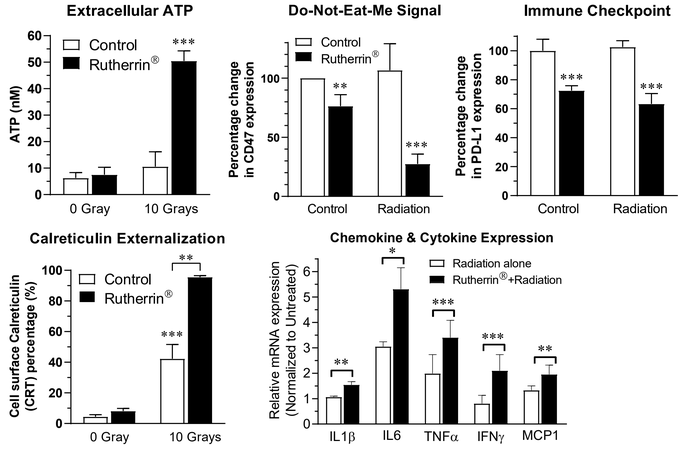


Figure 3: A) & D) Immunogenic cell death markers (ATP and Calretinculin) analysis. B) & C) Immune checkpoint makers (CD47 and PD-L1) expression. E) Chemokine and cytokine gene expression.

Sub-Cutaneous colorectal CT26 Mouse Model

- Mice pretreated with Rutherrin® showed 3 times more regression versus radiation alone.
- Upon rechallenge of treated mice, higher protective immunity was also observed.

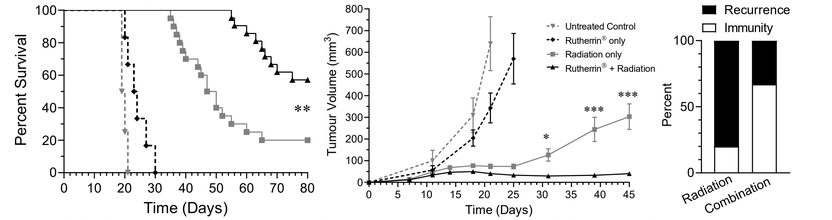


Figure 4: A) Kaplan-Meier survival analysis; B) Tumour volume quantification; C) Assessment of memory immune response induction through rechallenge with fresh cancer cells.

Orthotopic GBM RG2 Rat Model

- Rutherrin® effectively crosses the blood brain barrier leading to high selectivity (>10 times drug in GBM compared to normal brain).
- Rutherrin® pretreatment – combined with fractionated radiation – increased efficacy by increasing tumour kill, causing a delay in tumour growth, and improved overall survival.

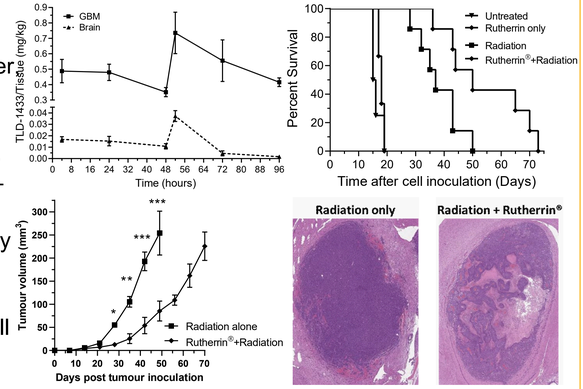


Figure 5: Drug concentration in GBM or normal brain overtime (Drug injected at 0 and 48 hours). B) & C) Kaplan-Meier survival and tumour volume analyses. D) Representative H&E histology cross-section in the center of the tumour

Orthotopic Lung Cancer Mouse Model

- Higher drug uptake observed in tumour samples (higher retention / slower clearance).
- Rutherrin® combination with radiation delayed tumour growth and prolonged survival.

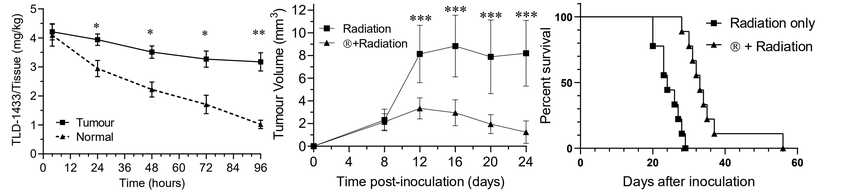


Figure 6: A) Drug concentration in tumour or normal lung samples. B) & C) Tumour volume and Kaplan-Meier survival.