C027

RSO-021, a First-in-class Covalent Inhibitor of Mitochondrial PRX3: From Bench to Bedside

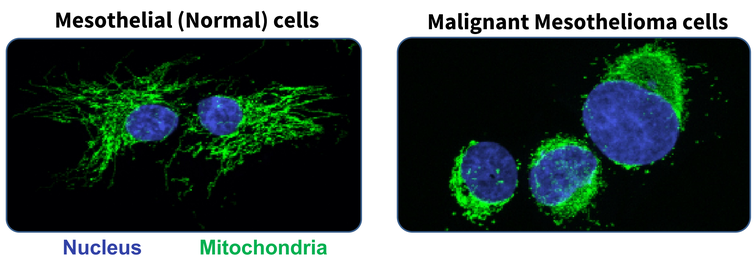
D. Fennell[1,2], S. Dulloo[1,2], P. Szlosarek[3], F. Thistlethwaite[4], S. Lord[5], N. Rahman[5], B. Cunniff[6], J. Dzialo[1], C. Poile[1], R. Panchal[2], J. Duncan[7], P. Graham[7], A. Bexon[7], G. Naumov[7], J. Spicer[8]

1. Mesothelioma Research Programme, University of Leicester, **2.** University Hospitals of Leicester NHS Trust, Leicester, UK, **3**. St. Bartholomew's Hospital NHS Trust, London, UK, **4**. The Christie NHS Foundation Trust, Manchester, UK, **5.** University of Oxford, Oxford, UK, **6.** University of Vermont, Larner College of Medicine, Burlington, VT, USA, **7.** RS Oncology LLC, Cambridge, MA, USA, **8.** Comprehensive Cancer Centre, Guy's Hospital, King's College London, London, UK

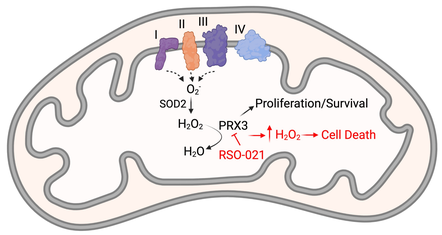
Background

Tumor cells generate elevated levels of reactive oxygen species (ROS) and therefore exhibit increased expression and activity of critical ROS scavenging pathways, including the mitochondrial peroxide scavenging enzyme peroxiredoxin 3 (PRX3).

- PRX3 is a peroxidase responsible for metabolizing ~ 90% of mitochondrial ROS, primarily H_2O_2.
- PRX3 transcript levels are upregulated, compared to normal tissues, in approximately 50% of cancers (data from the GEPIA2 database).
- Genetic knock down of PRX3 in human tumor cells results in sensitization to apoptosis.
- The mitochondria of malignant mesothelioma (MM) cells are structurally and functionally altered leading to disrupted metabolic function that supports tumor growth and can be therapeutically targeted (see figure below).

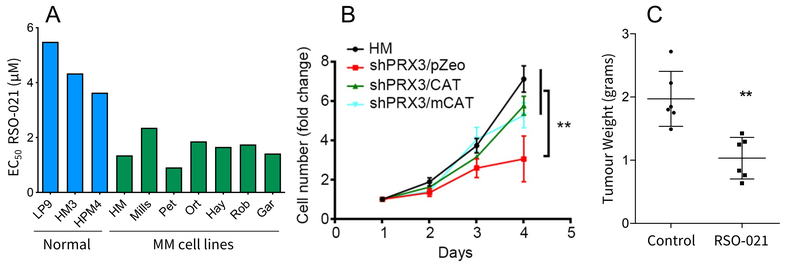


RSO-021 is a Novel Mitochondrial PRX3 Inhibitor



RSO–021 is a novel formulation of Thiostrepton (TS) for clinical development. RSO-021 is a covalent inhibitor that inactivates PRX3 peroxidase activity through direct adduction of active site cysteine residues, in turn, inducing oxidative stress to levels incompatible with tumor cell survival.

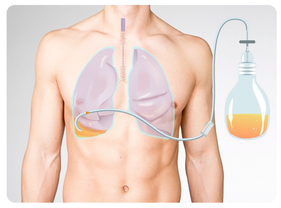
Pre-Clinical Rationale



A) EC_{50} of RSO-021 in normal mesothelial and various mesothelioma cell lines (varying BAP1 expression). **B)** PRX3 knock down with siRNA significantly reduces MM (HM cell line-pleural biphasic) proliferation (red). Co-expression of the H_2O_2 scavenger catalase rescues proliferation in cells lacking PRX3 expression (green and blue). **C)** Weight of residual tumors resected from mice harboring MM xenografts in the peritoneal cavity following four weeks of treatment with 20 mg/ml RSO-021 2x weekly. ** p<0.01

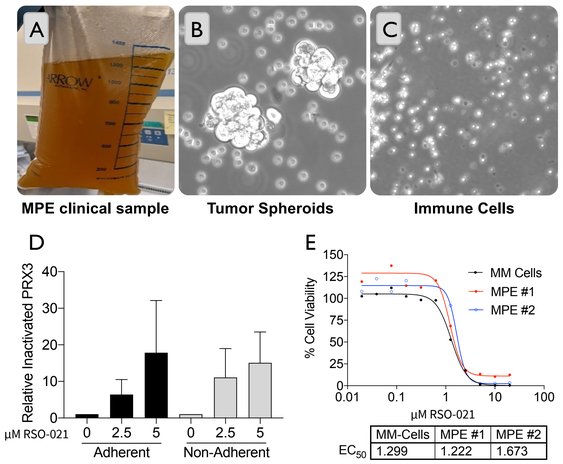
References: Cunniff B. *et al.* PLoS One, 2015, 10(5). & Nelson K.J. et al. Antioxidants (Basel), 2021, 10(2):150.

Malignant Pleural Effusion



Small amounts of pleural effusion in the pleural space is physiologically normal. Mesothelioma and metastatic disease to the lungs often results in build-up of excess fluid (~15% of cancers). Malignant pleural effusion (MPE) is routinely drained using an intrapleural catheter.

PRX3 Inhibition in Malignant Pleural Effusion



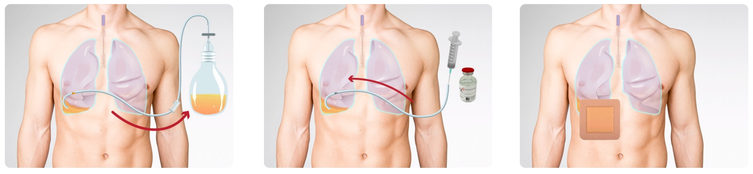
TS retains activity in patient derived malignant pleural effusions (MPE)

A) MPE collected from patients with metastatic disease. **B-C)** Adherent tumor spheroids and non-adherent immune cells grown in MPE supernatant. **D)** Relative PRX3 inactivation by RSO-021 in both tumor (adherent) and immune (non-adherent) cells. **E)** MPE derived tumor cells are equally sensitive to RSO-021 compared to established MM cell lines.

- MPE contains tumor & immune cells and makes a good translational sample.
- RSO-021 shows target inhibition in both tumor & immune cell MPE components.
- RSO-021 retains activity in a complex patient derived MPE.

RSO-021 Local Administration

RSO-021 will be administered once-a-week via an indwelling intrapleural catheter (IPC) until disease progression, unacceptable toxicity, withdrawal of consent or study termination. Prior to each dose patients will have pleural effusion drained to dryness per standard of care. After each administration the IPC is secured until next RSO-021 dosing time point.

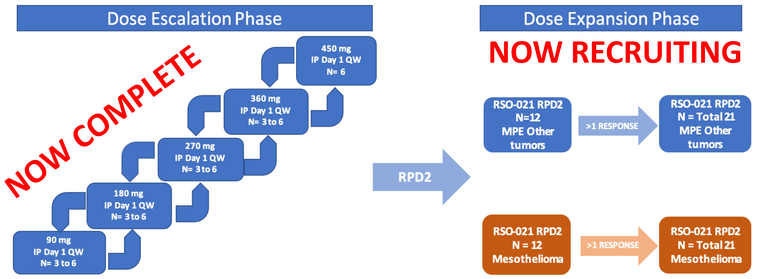


1. Drain pleural effusion to dryness

2. Administer RSO-021 via IPC

3. Secure IPC

MITOPE Phase 1/2 Study Design



Primary Objective:
To assess the safety, tolerability and toxicity profile of RSO-021 in patients with MPE from any solid tumor type and mesothelioma

Secondary Objectives:
- Establish systemic and local PK
- Preliminary anti-tumor activity
- Evaluate respiratory function
- Redox status in translational samples

Key Inclusion/Exclusion Criteria

Key Inclusion Criteria	Key Exclusion Criteria
Male or female ≥ 18 years old	Prior systemic anti-cancer or radiation therapy, or surgery within 3 weeks or 5 half-lives. Treatment with investigational product/device within 30 days.
ECOG performance status 0-1	Previous or concurrent malignancy (some exceptions).
Histological diagnosis of MPE caused by non-mesothelioma solid tumor (expansion cohort only) or mesothelioma.	Patients whose extent of tumor or loculations would render intrapleural administration incomplete and/or ineffective.
MPE must be considered the priority for symptom control.	Known hypersensitivity to the active ingredient/excipient.
Received at least 1 prior standard of care treatment regimen, with documented progression and no approved alternative available.	Any surgical or medical condition which is likely to interfere with the results of the study or pose an additional risk in participating.
Resolution of all acute reversible toxic effects of prior therapy to Grade ≤1	Active infection with human immunodeficiency virus (HIV).
Dose Escalation: Paraffin block of most recent biopsy Dose Expansion: Fresh tumor biopsy during screening and after third dose.	Active infection with hepatitis B; or hepatitis C in absence of sustained virologic response
Adequate organ function as defined by lab values	Pregnant or breast-feeding patients
Postmenopausal or surgically sterile, or be willing to practice highly effective methods of birth control	Symptomatic/unstable CNS tumor or metastases and/or carcinomatous meningitis
Willingness and ability to comply with schedule/procedures	Use of systemic corticosteroids within 15 days or other immunosuppressive drugs within 3 weeks.

Current Study Status

The MITOPE study initiated first patient treatment in March 2022 and has completed recruitment for the Phase 1 dose escalation portion of the trial. The dose expansion phase 2 is open for recruitment of patients at the following UK sites:

- Dr. D. Fennell – Leicester
- Dr. J. Spicer - Guy's Hospital, London
- Dr. S. Lord – Oxford
- Dr. F. Thistlethwaite - The Christie, Manchester
- Dr. K Blyth – Glasgow & Clyde Hospital, Scotland

Clinicians are encouraged to refer any eligible patients to the open sites. MITOPE trial is supported by Mesothelioma UK (www.mesothelioma.uk.com), NIHR (www.nihr.ac.uk) and clinicaltrials.gov: ***NCT05278975***

For more information scan the **QR code** or contact: **MITOPE@RSOncology.com**

Financial disclosures: The MITOPE study is sponsored by RS Oncology LLC.