Effects of RSO-021 on cytokine profiles of malignant pleural effusions from patients enrolled in the MITOPE phase 1/2 clinical trial

Terri Messier[1], Molly Scotland[2], George N. Naumov[3], Sean Dulloo[2], Dean A. Fennell[2] and Brian Cunniff[1]

1. University of Vermont, Larner College of Medicine, Department of Pathology and Laboratory Medicine. 2. University Hospitals of Leicester, Leicester UK. 3. RS Oncology, LLC

Background

Tumor cells generate increased levels of reactive oxygen species (ROS) that promote growth and survival through activation of redox dependent signaling pathways. To survive under increased ROS levels tumor cells, including mesotheliomas, must increase antioxidant expression, activity and reliance on critical ROS scavenging pathways, including the mitochondrial peroxiredoxin 3 (PRX3) system. PRX3 is a critical ROS scavenging enzyme localized to the mitochondrial matrix. Our group has characterized the mechanism of action of the PRX3 inhibitor and pro-oxidant therapeutic thiostrepton (TS). TS covalently crosslinks the peroxidatic and resolving cysteines of PRX3, leading to increased levels of mitochondrial ROS, resulting in tumor cell death. TS is the active pharmaceutical ingredient of RSO-021, a new covalent inhibitor of PRX3 currently being tested in the MITOPE phase 1/2 clinical trial in patients with malignant pleural effusion (MPE) due to advanced solid tumors or mesothelioma (NCT05278975). RSO-021 is administered locally to patients through an indwelling pleural catheter (IPC) weekly. TS targets PRX3 in both adherent tumor cells and in non-adherent immune cell populations in a dose-dependent manner when tested ex vivo. Using MPE drainages from patients enrolled in the MITOPE trial we evaluated cytokine levels pre and 1 day and 7 days post initial administration of RSO-021. Cytokines were measured in decellularized MPE drainages using Human Cytokine ELISA plates manufactured by Signosis, Inc.

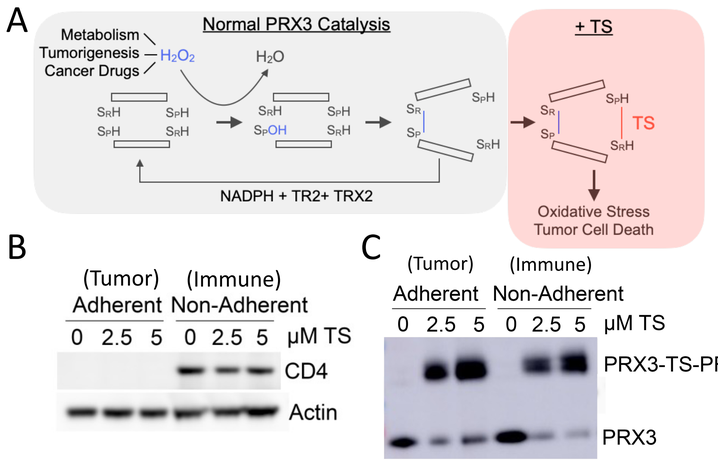

Figure 1: Thiostrepton (TS) is a covalent inhibitor of mitochondrial Peroxiredoxin 3 (PRX3). A) The proposed mechanism of action (MOA) of TS. PRX3 is the primary mitochondrial peroxidase required for H_2O_2 clearance from the mitochondria induced by metabolic, tumorigenic, and drug treatment inputs. During the metabolism of H_2O_2, PRX3 forms an intramolecular disulfide bond that orients the second active site for TS-dependent covalent crosslinking, inactivating the protein leading to increased oxidative stress and tumor cell death. **B)** Adherent and non-adherent MPE cells, cultured in decellularized MPE supernatant were collected after 24 hours incubation with indicated concentrations of TS *ex vivo*. Protein lysates were generated in RIPA buffer and subjected to SDS-PAGE. The CD4 T-cell co-receptor was only detected in the non-adherent cell population. **C**) TS retains activity in MPE cells cultured in MPE fluid. The TS-dependent PRX3-TS-PRX3 covalent crosslink is present in adherent and non-adherent cell populations.

RSO-021 Drug Product:
Micellar solution packaged in 30ml glass vial at 3mg/ml Thiostrepton

MITOPE
ClinicalTrials.gov Identifier: NCT05278975

RSO–021 is a novel formulation of Thiostrepton (TS) for clinical development. The primary objective of the MITOPE Phase 1/2 trial is to evaluate the efficacy and safety of RSO-021 in participants with Malignant Pleural Effusion Due to Advanced/Metastatic Solid Tumors Including Mesothelioma.

Results

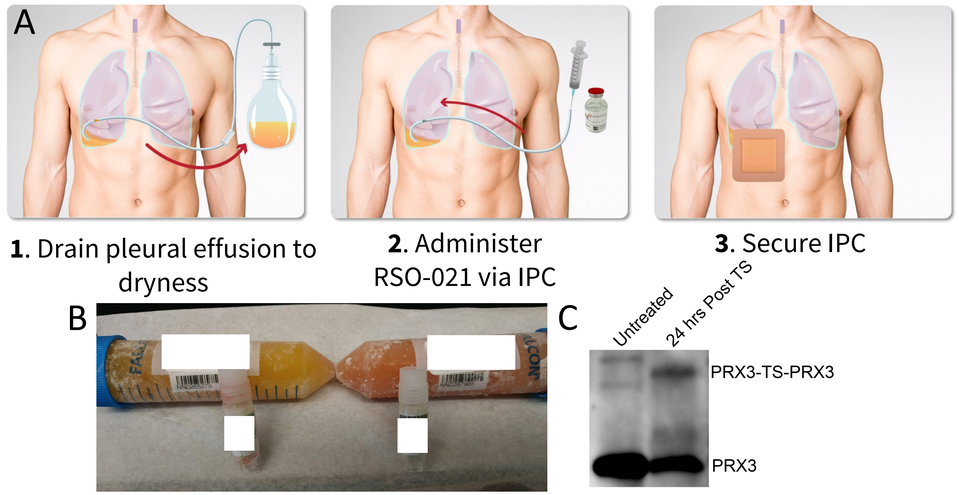

Figure 2. A) RSO-021 is administered once-a-week via an indwelling intrapleural catheter (IPC) until disease progression, unacceptable toxicity, withdrawal of consent or study termination. Prior to each dose patients will have pleural effusion drained to dryness per standard of care. After each administration, the IPC is secured until the next RSO-021 dosing time point. **B**) Pleural drainages (decellularized MPE and pelleted cell material) are collected from patients for translational research. **C**) PRX3 crosslinking by RSO-021 is retained in cellular material derived from patient samples following 24 hours of RSO-021 exposure.

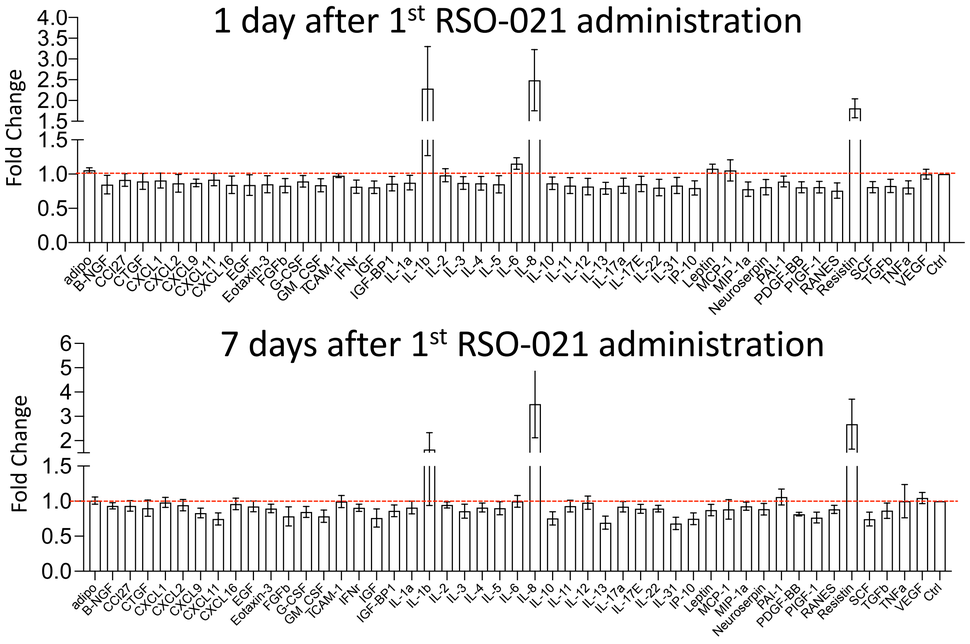

Figure 3: Measurement of Cytokine levels in MPE from patients in the MITOPE trial. Cytokine levels were measured in decellularized MPE drainages from 6 patients enrolled in the MITOPE trial at C1D1 (Pre-RSO-021), C1D2 (1 day post RSO-021) and C1D8 (7 days post RSO-021) using pre-coated Human Cytokine ELISA plates manufactured by Signosis, Inc. Data presented as fold change normalized to levels at C1D1.

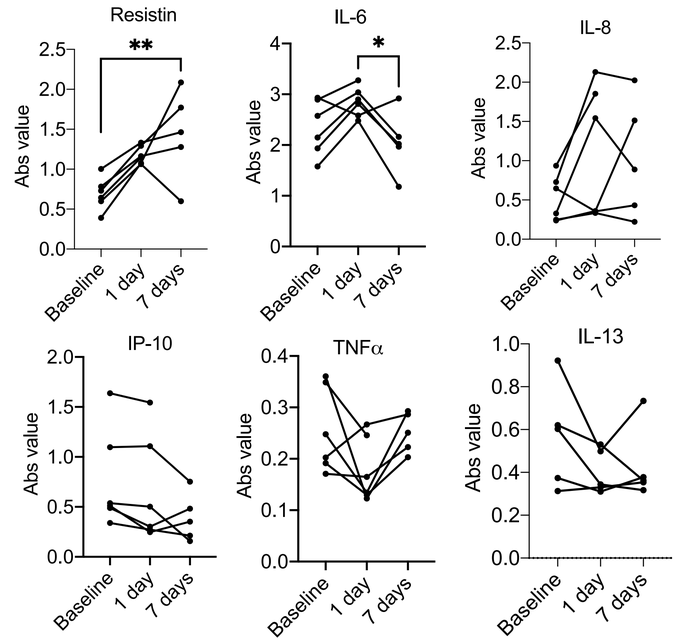

Figure 4: Intra-patient changes in specific cytokine levels at baseline (C1D1,Pre-RSO-021), Day 1 (C1D2) and day 7 (C1D8) post RSO-021 1st treatment. Absolute values as determined following ELISA are shown to highlight both inter- and intra-patient heterogeneity and dynamics over time, respectively. Consistent dynamics for Resisitn and IL-6 are observed while consistent trends are observed for IL-8, IP-10, TNFα and IL-13. ELISAs run on 6 patient derived PE.

Conclusions and MITOPE Trial Status

RSO-021 lead to increases in IL8 and Resistin that persisted for 7 days following administration. IL-6 levels significantly increased 1 day post administration before returning to baseline or below after 7 days. These changes to the tumor microenvironment may confer immune modulation contributing to tumor control. Ongoing research using patient samples and advanced ex vivo models will delineate the role of these cytokines.

The presented strategies and findings are being further explored using patient samples from the ongoing MITOPE trial. The United Kingdom-based multicenter study met its primary objective of evaluating the safety and tolerability of RSO-021. The phase 2 portion of the study is actively recruiting in the UK with expected expansion to US and EU sites.

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

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