Therapeutic activity of thiostrepton in patient-derived malignant pleural effusions

Brian Cunniff,1 Terri Messier,1 Roxana del Rio-Guerra2 Anthony Kopecky3, Geoff Sriver2, and George N. Naumov4
1. University of Vermont, Larner College of Medicine, Department of Pathology and Laboratory Medicine. 2. University of Vermont, Larner College of Medicine, Department of Surgery. 3. University of Vermont Medical Center, Department of Interventional Radiology. 4. RS Oncology, LLC

Background

Malignant pleural effusion (MPE) is a common complication of many tumor types, arising at the onset of terminal cancer with a median survival of 5-12 months. Historically, we have characterized and evaluated the activity of thiostrepton (TS) on malignant and immune cell types present in MPE samples from patients diagnosed with primary lung, breast, prostate, pancreatic and ovarian cancers. TS targets mitochondrial peroxiredoxin 3 (PRX3) and disrupts redox homeostasis preferentially in malignant tissues, presenting a promising therapeutic approach currently under investigation in the MITOPE clinical trial.

Thiostrepton Anti-Cancer MOA

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 peroxiredoxin required for H2O2 clearance from the mitochondria induced by metabolic, tumorigenic, and drug treatment inputs. During the metabolism of H2O2, PRX3 forms an intramolecular disulfide bond that results in the second active site for TS-dependent covalent crosslinking, inactivating the protein leading to increased oxidative stress and tumor cell death. B) Western blot of protein from malignant Mesothelioma (MM) cells (HM, cell line) treated with 5 or 10 µM TS for 24 hours. TS induces a covalent modification to PRX3 (antibody reactive band at ~33 kD, PRX3-3TS-PRX3) which is the dimetric species of PRX3. C) Dose-response curves of normal (blue) and cancer (black) cell lines to TS. D) Weight of residual tumors injected with mice harboring MM xenografts in the peritoneal cavity following 4 weeks of treatment with 20 mg/ml TS 2x weekly.

Methods

The MITOPE phase 1/2 clinical trial is designed to assess the safety, tolerability, and activity of RSO-023 (TS as AF) in patients with malignant pleural effusion (MPE) arising from metastatic disease or malignant mesothelioma (MM). In this study, the activity of TS in MPE derived from patients with primary lung, breast, prostate, pancreatic and ovarian cancers. This is the first study assessing the effect of TS on patient-derived MPE fluid cellular content.

MPE samples were collected, with Institutional Review Board approval, from 11 patients at the University of Vermont Medical Center via thoracoscopy. Cellular material from MPE was phenotyped by flow cytometry for the identification of resident cell populations. The ability of TS to inhibit its primary molecular target, mitochondrial PRX3, was evaluated in cells derived from MPE samples. The amount of PRX3 inhibited and cell viability was quantified following treatment of MPE samples with increasing concentrations of TS.

Results

Figure 2: Collection and processing of malignant pleural effusions (MPE). A) Summary of patient-derived MPE samples. Tumor type of the MPE and 0.2-1.4 liter of MPE was collected by thoracoscopy and immediately transported to the lab for processing. B) MPE samples were centrifuged at 1000×g for 10 minutes to separate MPE cellular content from the supernatant. Total cell counts were conducted. C) MPE cells were cultured in MPE supernatant for 24-48 hours prior to analysis of cellular material by flow cytometry or treatment with thiostrepton to assess responses (Figure 5). Tumor cells grew either as an adherent monolayer (left) or tumor spheroids (middle). Immune cells were present as non-adherent cells (right).

Figure 3: Phenotypic characterization of MPE. A) MPE sample pH was assessed pH ranged from 7.0 - 7.25. B) For each sample, 1 x 10⁶ cells were resuspended in 1x PBS and incubated 20 min/4°C with Live/Dead Blue Viability dye. Cells were washed 2x with 1x PBS-1% FBS (FC buffer). Cells were resuspended in 50 µl of FC buffer and treated with Fc and monocyte block (Human TruStain FcX and True-Stain Monocyte blockers) as per manufacturers’ instructions.

Figure 4: Primary immune cells identified in MPE. The antibody cocktail was prepared in 50 ul of Brilliant Stain Buffer and added to the sample. Samples were incubated with the antibody cocktail in the dark for 30 min at 4°C, then washed twice with FC buffer. All samples were acquired on Cytolkr Aurora using SpectroFlo 2.1 version. Subsequently, flow cytometric analysis was performed using FlowJo.

Figure 5: Thiostrepton retains target activity and cytotoxicity against adherent and non-adherent (immune) MPE cells. A) Adherent and non-adherent MPE cells, cultured in deacellulized MPE supernatant were collected after 24 hours incubation with indicated concentrations of TS. Protein lysates were generated in RIPA buffer and submitted to SDS-PAGE. The CD3 T-cell co-receptor was only detected in the non-adherent cell population. B) TS retains activity in MPE cells cultured in MPE fluid. The TS-dependent PRX3-3TS-PRX3 covalent crosslink is present in adherent and non-adherent cell populations. C) Densitometry quantification of PRX3-3TS-PRX3 (dimer) to PRX3 (monomer) ratio (n=5 samples). D) Cell viability assay of human malignant mesothelioma (MM) Cells, HM cell line and cells derived from PE #1 and PE #2 treated with increasing concentrations of TS. The IC₅₀ values (concentration of TS required to kill 50% of cells) are similar between MM cells in culture and MPE-derived cells.

Conclusions

MPE cellular component derived from patients diagnosed with lung, breast, prostate, pancreatic or ovarian cancer is primarily composed of CD45+CD3+CD4+ T cells. TS covalently adducted and inhibited mitochondrial PRX3 in both adherent, malignant, (CD4-) and non-adherent, immune, (CD4+) cell populations in a dose-dependent manner. These results indicate that TS retains anti-cancer activity in MPE fluid and targets cellular components of both MM and immune origin. This study, for the first time, demonstrates the ability of TS to inhibit mitochondrial PRX3 and induce cell death in patient-derived malignant effusions.

Acknowledgements

We thank all the patients who participated in this study and the Interventional Radiology clinical staff. This study was approved by the UVM Institutional Review Board (Study 0001156).

Funding and Financial Disclosures

This work was funded by RS Oncology, LLC through a Sponsored Research Agreement to The University of Vermont. B.C. and G.N. are paid consultants and equity holders in RS Oncology, LLC.

Scan me to learn more!