First-in-class Peroxiredoxin 3 (PRX3) inhibitor RSO-021 triggers Mesenchymal-to-Epithelial Transition in Mesothelioma

Terri Messier[1], Victoria Gibson[1], David J. Seward[1], Essa Y. Baitei[2], Peter Wells Jordan[2], Joanna Dzialo[2], Aleksandra Bzura[2], Charlotte Poile[2], Kudzayi Kutywayo[2], Apostolos Nakas[2], George N. Naumov[3], 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

Mesothelioma, a lethal cancer associated with asbestos exposure, lacks effective drug therapy particularly in the relapsed treatment setting. Epithelial-to-mesenchymal transition (EMT) confers an aggressive sarcomatoid phenotype capable of invasion, metastasis, and drug resistance. Pharmacological targeting of EMT could favorably alter the progression and treatment of mesothelioma. We identified a geospatial transcriptomic gradient between adjacent epithelioid and sarcomatoid regions in patient-derived biphasic mesotheliomas involving hypoxia tolerance, TGF-β, and EMT. Mesothelioma cells rely on buffering of mitochondrial oxidative stress through the expression and activity of mitochondrial peroxiredoxin 3 (PRX3), which is covalently inactivated by the first-in-class inhibitor RSO-021 (Thiostrepton, TS), now under investigation in the phase 1/2 MITOPE clinical trial (NCT05278975). To better understand the pharmacodynamics of TS, transcriptomic profiles of drug sensitive and resistant biphasic mesothelioma cell lines were generated. Differential analysis revealed that TS induced upregulation of ROS response and apoptosis signaling, while downregulating TGF-β and EMT markers. Additionally, we observed altered expression of the mesothelioma specific EMT genes Col5A, mesothelin, and VISTA. Resistance to TS conferred significant proliferative defects and was reversible upon TS removal, however, the downregulation of EMT genes was conserved, suggesting an irreversible mesenchymal-to-epithelial transition. Lastly, downregulation of EMT by TS was determined in a cohort of TS treated mesothelioma surgical explants. In summary, PRX3 is a clinically actionable drug target, inhibition of which correlates with EMT modulation.

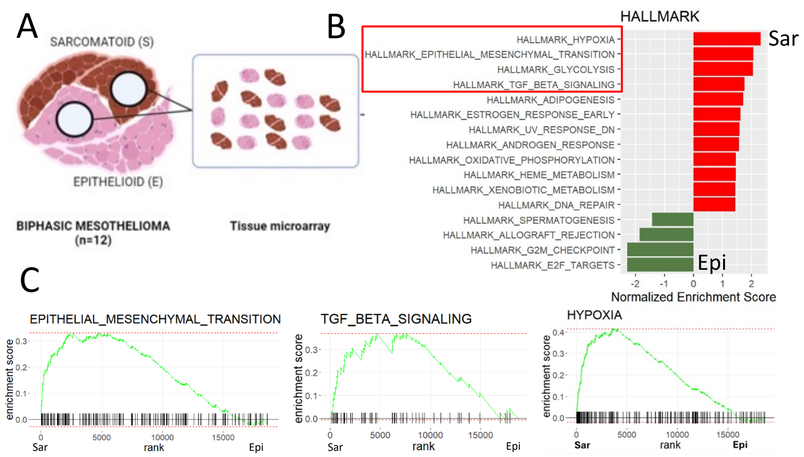

Figure 1: Characterizing the intra-tumor processes that underpin epithelioid (E) to sarcomatoid (S) transition. A) Spatial multiregional sampling of adjacent, intra-tumor E (7) vs S (12) regions from 7 patients with bi-phasic mesothelioma, was followed by transcriptome analysis of tumor regions. **B)** GSEA analysis comparing E and S transcriptomes. Red bars enriched in S regions. **C)** EMT, TGF-β, and Hypoxia pathways are enriched in sarcomatoid regions.

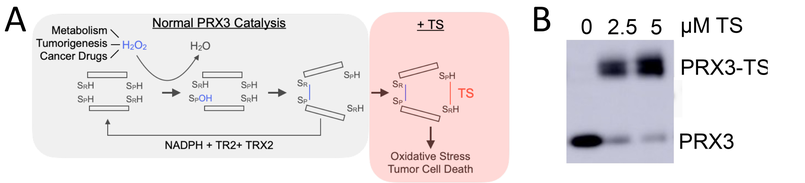

Figure 2: RSO-021 (Thiostrepton, TS) is a covalent inhibitor of mitochondrial Peroxiredoxin 3 (PRX3). The proposed MOA of TS. PRX3 is the primary mitochondrial peroxidase required for H_2O_2 clearance from the mitochondria induced by metabolic, tumorigenic, and drug treatment inputs. During the metabolism 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)** TS-dependent modifications are visualized by reducing SDS-PAGE and western blotting where a dose-dependent irreversible increase in modified PRX3 (PRX3-TS) is present.

Results

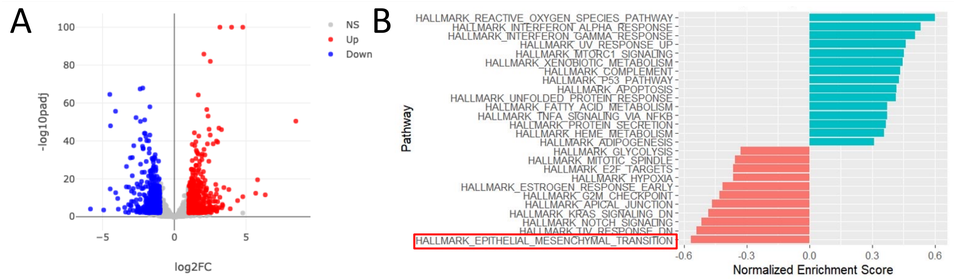

Figure 3. RNA-Sequencing of TS treated Meso cells. A) RNA-Seq was conducted on human biphasic mesothelioma cell lines treated with TS for 18hrs and compared to untreated cells (n = 3 biological replicates) **B)** GSEA analysis comparing Ctrl to TS treated cell lines. Red bars are hallmarks enriched in Ctrl cells.

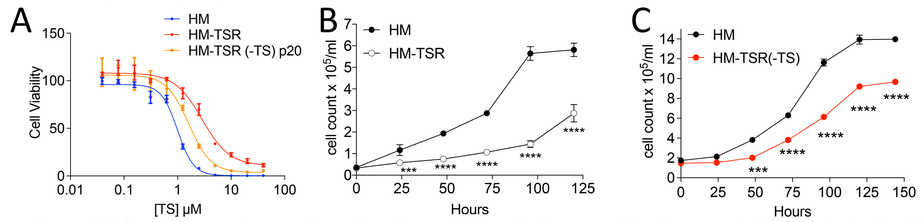

Figure 4. TS-resistance slows cell proliferation and is partially reversible. To generate TS-resistant cells, HM cells were dosed with TS by repetitive drug treatment with a short 24-48hr recovery period. TS concentration was gradually increased by 1.5-fold until a concentration was reached that no longer provided viable cells. Development of a tolerant cell line at 5 µM occurred after 8 months. **A)** Cell viability assay of HM, HM-TSR (TS-resistant), and HM-TSR cells removed from TS pressure for 20 passages. Note; partial resistance of HM-TSR cells is reversible when pressure is removed. **B)** Cell growth analysis of HM and HM-TSR cells over 125 hours (n = 4). **C)** HM-TSR cells removed from TS pressure (HM-TSR (-TS)) retain a proliferation defect (n = 4).

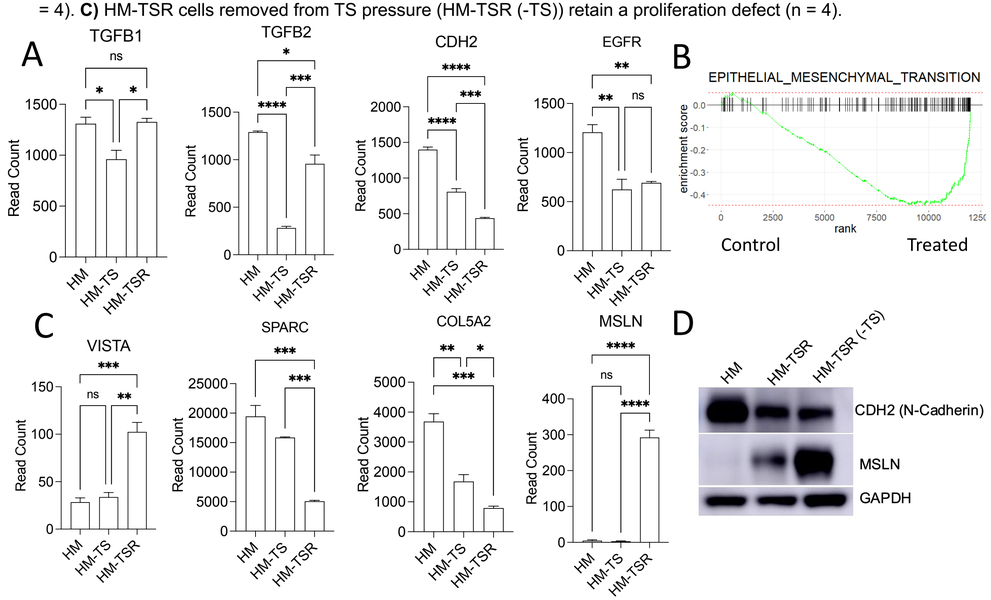

Figure 5. Specific changes in gene expression by TS treatment and TS-resistance. A) RNA-Seq showing canonical EMT genes are downregulated by TS and in TSR cells, normalized read counts. **B)** EMT gene signature is enriched in control samples compared to HM-TSR samples. **C)** Previously described EMT genes associated with mesothelioma are modulated by TS and in TSR cells. **D)** Protein expression of N-cadherin and MSLN in HM-TSR cells and HM-TSR cells that have been removed from TS pressure for 20 passages. Note changes to protein expression are maintained.

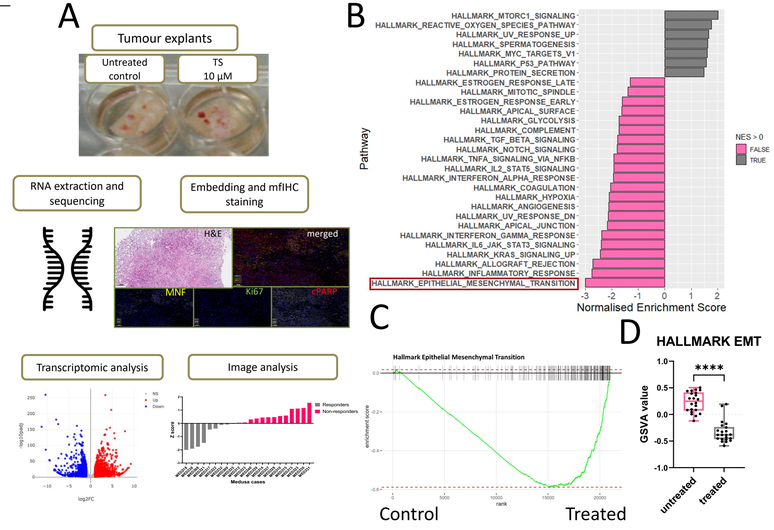

Figure 6. Epithelial mesenchymal transition genes are downregulated in patient derived explants (PDEs) treated with TS. A) Schematic of experiments conducted with PDEs. **B)** Gene set enrichment analysis (GSEA) method for Hallmark collection revealed that gene set related to EMT is enriched in untreated explants compared to those treated with 10µM of TS. **C)**. Enrichment plot for Hallmark EMT gene set. **D)** Comparison of values for Hallmark EMT gene set for untreated PDEs and those treated with 10µM of TS obtained by gene set variation analysis (GSVA) confirmed that genes associated with EMT are enriched in control samples.

Conclusions and MITOPE Trial Status

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.

- Dr. James Spicer - Guy's Hospital, London
- Prof. Dean Fennell – Leicester
- Dr. Simon Lord – Oxford
- Dr. Fiona Thistlethwaite - The Christie, Manchester
- Dr. Kevin Blyth - QE University Hospital, Glasgow
- Dr. Kevin Franks - St James Hospital, Leeds
- Dr. Peter Szlosarek - St Barts Hospital, London
- Dr. Sanjay Popat- Royal Marsden Hospital, London
- Dr. Nick Maskell - Southmead Hospital, Bristol
- Dr. Avinash Aujayeb - North Tyneside Hospital, Northumbria

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

Funding and Financial Disclosures

This work was funded by RS Oncology, LLC through a Sponsored Research Contract to The University of Vermont and the University of Leicester. T.M., B.C, G.N.N,, and D.A.F. are paid consultants and B.C. and G.N.N. are equity holders in RS Oncology, LLC. The MITOPE study is sponsored by RS Oncology LLC.