Preclinical efficacy & mechanism of action of combination of nitric oxide donor and growth hormone-releasing hormone antagonists for castration-resistant prostate cancer

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Yash Soni[1]; Manish Kuchakulla[1]; Joshua M. Hare[2,3]; Ranjith Ramasamy[1,2#] , **Himanshu Arora[1,2,4#]**

[1]Department of Urology, Miller School of Medicine, University of Miami, Miami, Florida, USA

[2]The Interdisciplinary Stem Cell Institute, Miller School of Medicine, University of Miami, Miami, Florida, USA

[3]Department of Medicine, Cardiology Division, Miller School of Medicine, University of Miami, Miami, Florida, USA

[4]John P Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, Florida, USA

Disclosures

- American Cancer Society
- CTSI

Background

- Novel therapeutics are being explored for the treatment of castration resistant prostate cancer (CRPC)
- Nitric Oxide affects receptors for GnRH, LH and T through the HPG axis
- GHRH-R (receptor) has been explored as an approach to target prostate cancer
- S-Nitrosoglutathione (GSNO) is a nitric oxide donor
- MIA-602 is a GHRH antagonist

Objective

- To evaluate the impact of increased NO levels in the presence of GHRH antagonists on CRPC tumor progression

Methods

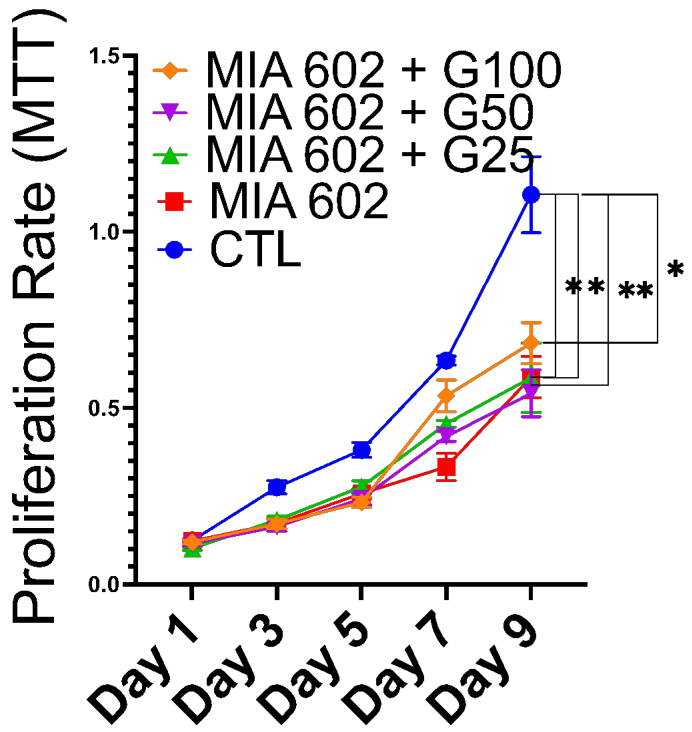
- MTT proliferation assay was performed using 22RV1 cells at 1 uM concentration of GHRHR antagonist (MIA-602) in the presence or absence of increasing concentration of GSNO (25, 50 or 100uM respectively).
- Expression of down terminal markers of CRPC (PSA, AR TMRPSS2, AR, ARV7 and pERK respectively) were checked after treating with MIA-602 at 1uM concentration, in the absence or presence of increasing concentration of GSNO (10, 25, 50 or 100uM respectively).
- In-vivo, CRPC murine models were treated in 3 groups:

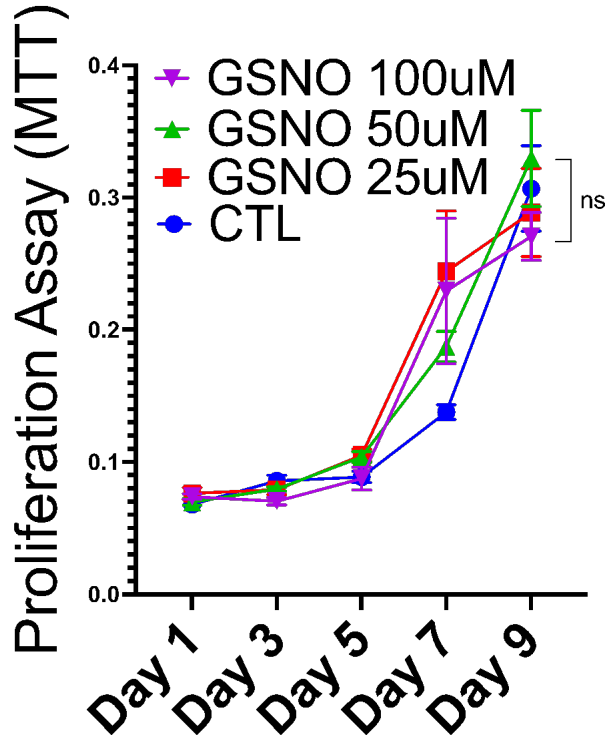
Group 1 - Vehicle control

Group 2 - GSNO treatment at the dosage of 10mg/kg/day intraperitonially (IP)

Group 3 - GSNO (10mg/kg/day IP) and MIA-602 (5ug/kg/day IP) combination treatment.

MIA-602 reduces the cell proliferation of 22RV1 cells



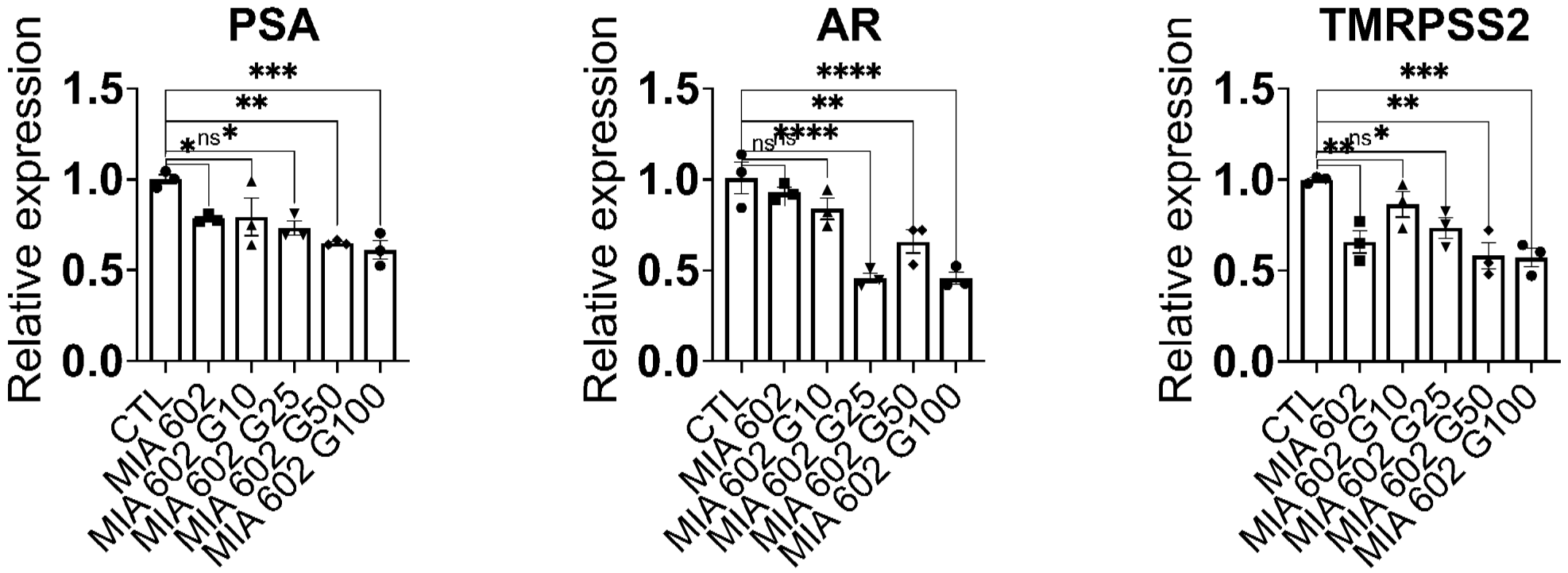


5000 22RV1 cells were seeded in 96 well plate and were treated with MIA-602 at 1uM concentration, in the absence or presence of increasing concentration of GSNO (from 25, 50 or 100uM respectively) (6 technical replicates per treatment condition). Proliferation rate was evaluated after 1, 3, 5, 7 and 9 days respectively.

Inference:

- *GSNO did not interfere with ability of MIA compounds towards reducing cell proliferation.*

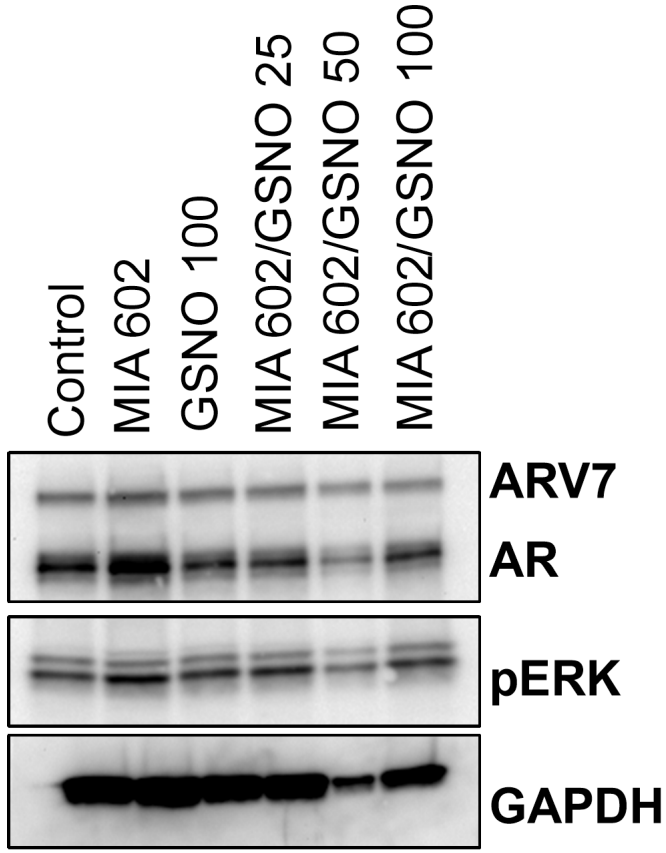
Increasing concentration of NO potentiates the effects of MIA-602 on PSA, AR and TMRPSS2



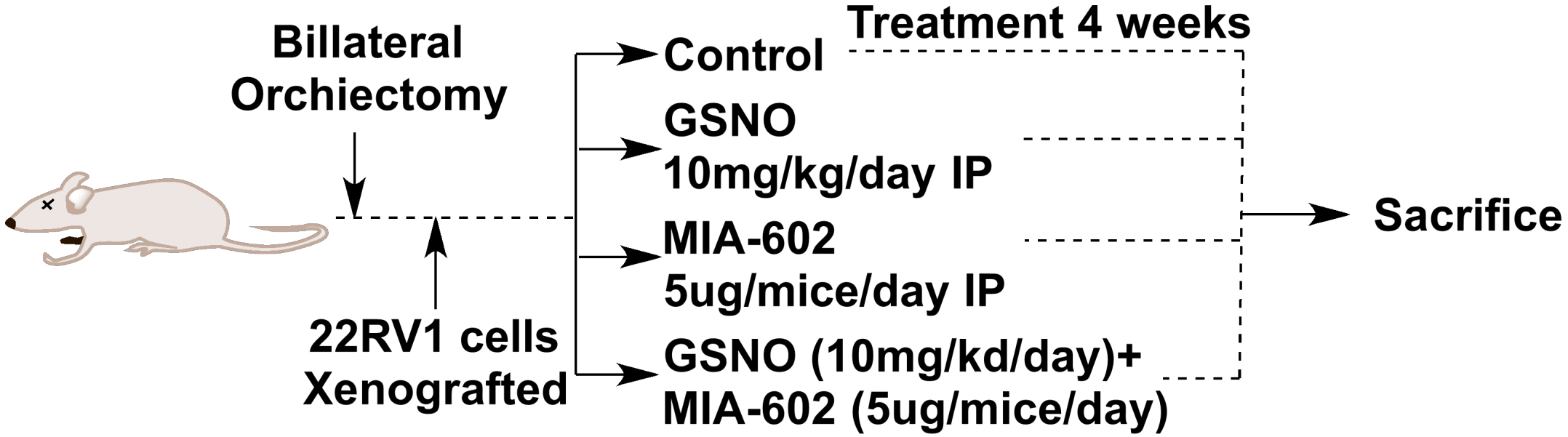
350,000 22RV1 cells were seeded in 6 well plate. The cells were treated with MIA-602 at 1uM concentration in the absence or presence of increasing concentration of GSNO (from 10, 25, 50 or 100uM respectively). 48 hours post treatment, RNA was isolated, cDNA was made, and qPCR was performed to evaluate the down terminal markers of CRPC (PSA, AR and TMRPSS2)

MIA-602 in combination with GSNO decreased the expression of AR, ARV7 and pERK

22RV1 cells were seeded in 6 well plates, The cells were treated with MIA 602 at 1uM concentration, in the absence or presence of increasing concentration of GSNO (from 10, 25, 50 or 100uM respectively). 48 hours post treatment, total protein was isolated, and western blotting was performed to evaluate the down terminal markers of CRPC (AR, ARV7 and pERK)

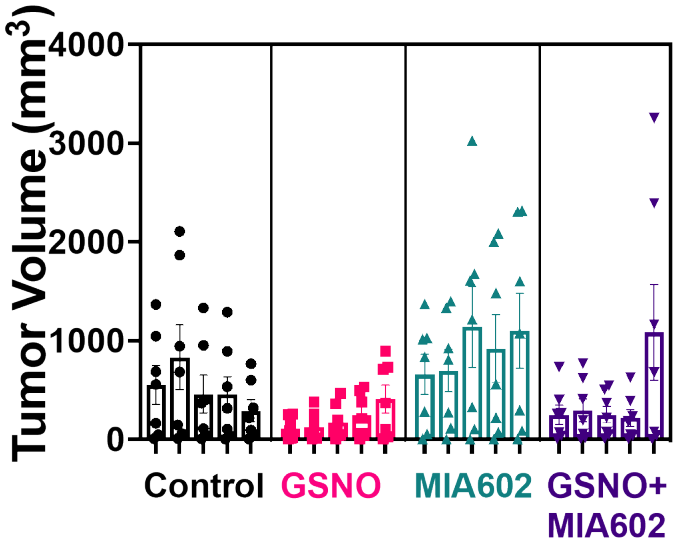


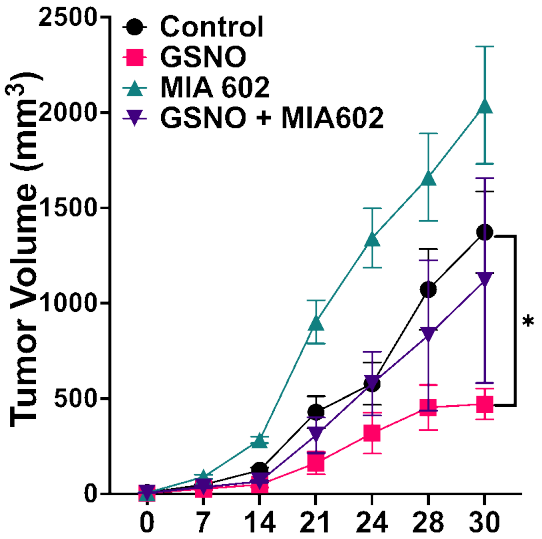
Animal Plan

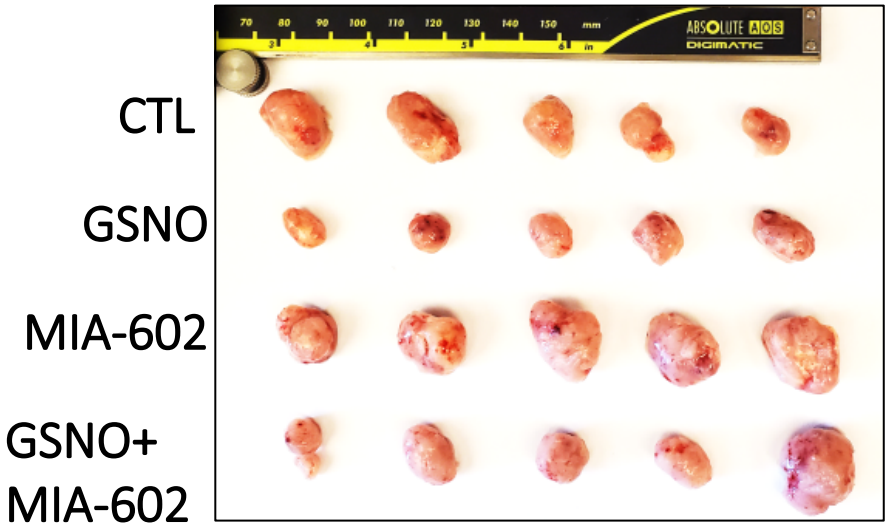


- We received MIA-602 at a concentration of 50ug/ml.
- 5ug (100ul) was injected IP per mice everyday for 30 days.

GSNO and MIA-602 combination showed a significant decrease in tumor volume and weight compared to the control







Take Home Points

- GSNO + MIA-602 combination decreases CRPC cell proliferation tumor burden
- GSNO does not impede the effects of MIA-602 on 22rv1 cell proliferation
- MIA-602 only in the presence of increased NO reduces expression of terminal markers of CRPC
- Further studies with active NO donors and GHRHR antagonists are in process to establish the preclinical efficacy and mechanism of action of the combination against CRPC

Thank you!

- For more information contact: hxa287@med.miami.edu