NAMPT as a Potential Biomarker for Daporinad Treatment: Analyzing the Effects of Overexpression and Knockdown in Prostate Cancer Cell Lines

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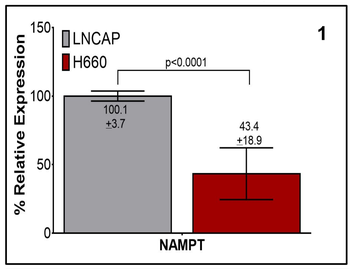
I. Background

- Neuroendocrine prostate cancer (NEPC) is an aggressive form of late stage prostate cancer that typically arises from androgen deprivation therapy (ADT) resistance.[1]
- Daporinad (FK866) is an investigational drug that inhibits nicotinamide phosphoribosyltransferase (NAMPT), decreasing the biosynthesis of nicotinamide adenine dinucleotide (NAD+). This alters energy reserves in metabolically active tumor cells and induces tumor cell apoptosis.[2]
- Daporinad was selected in our laboratory as a potential treatment for NEPC using computational dose-response modeling.[3]
- NCI-H660 is a NEPC strain that expresses lower NAMPT levels compared to LNCAP, which is a non-NEPC strain that expresses higher NAMPT levels (fig. 1).
- NCI-H660 is more sensitive to daporinad treatment than LNCAP (fig. 2).
- **Objective**: To determine if there is a relationship between NAMPT expression levels and prostate cancer cell sensitivity to daporinad treatment, verifying the computational results.
- **Hypothesis:** Knocking down the NAMPT gene will result in decreased prostate cancer cell viability after daporinad treatment, and overexpressing NAMPT will result in cellular resistance to daporinad.



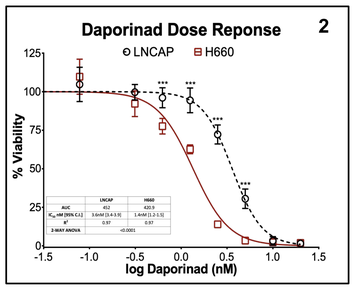


Fig. 1. NAMPT Expression in LNCAP and NCI-H660 Lines
Relative NAMPT expression was quantified using the ΔΔCt method. NCI-H660 displayed a significantly lower NAMPT expression compared to LNCAP (p<0.001) relative to the control.
Fig. 2. Daporinad Dose Response in NCI-H660 and LNCAP
LNCAP is less sensitive to daporinad treatment compared to NCI-H660 (daporinad IC_{50} LNCAP = 3.6 nM, daporinad IC_{50} NCI-H660 = 1.4 nM).

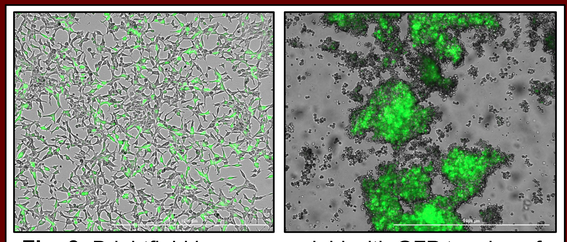
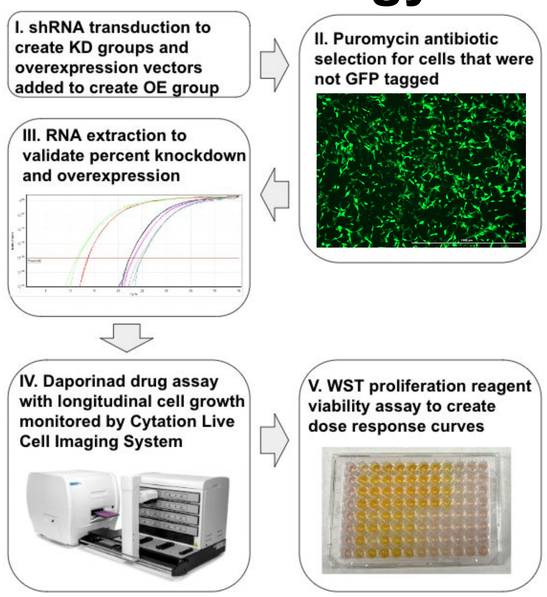


Fig. 3. Brightfield images overlaid with GFP tagging of LNCAP (left) and NCI-H660 (right).

II. Methodology



Cell Culture

- LNCAP: Adherent cell model grown in RPMI 1640 media with 10% fetal bovine serum (FBS).
- NCI-H660: Suspension cell model grown in HITES media (5% FBS). Both lines were cultured at 37°C in a 5% CO_2 incubator.

Antibiotic Selection

- Flasks with an estimated 20% or more cells missing the GFP label (fig. 3) were treated with 1µg/mL Puromycin in RPMI 1640 media for 3-5 days.

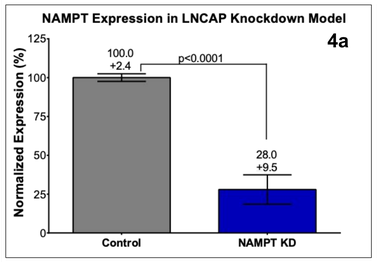
RNA Extraction and NAMPT Expression

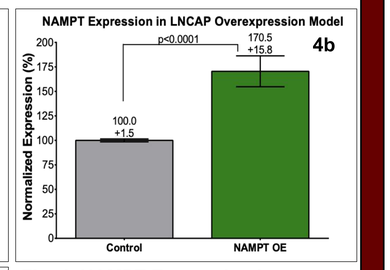
- RNA extraction of harvested cells from each model was performed using the Quick-RNA MiniPrep Plus Kit (Zymo Research). Validation of overexpression and knockdown was quantified by RT-PCR.

Daporinad Drug Assay

- Daporinad was added in serial dilutions between 0.08 nM and 20 nM. Longitudinal cell growth was measured during >72 hour drug exposure time on Cytation Live Cell Imaging System.

III. Results





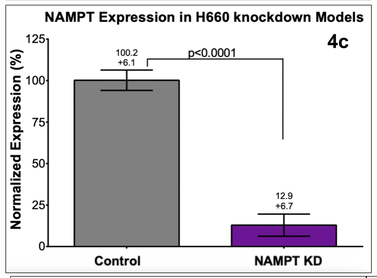
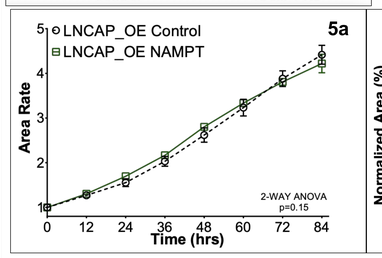


Fig. 4. NAMPT Expression in Different Transduction Models
Fig. 4a: NAMPT expression measured after the addition of lentiviral shRNA vectors targeting NAMPT or a scramble control. Knockdown decreased NAMPT expression by 69.5%. **Fig. 4b:** Overexpression vectors added to LNCAP resulted in a 70.5% increase in NAMPT expression. **Fig. 4c:** 87.3% knockdown was achieved in the NCI-H660 line.



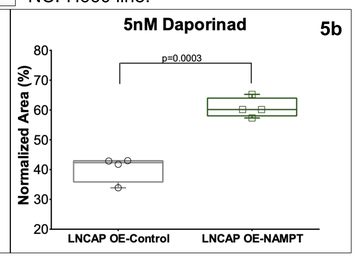


Fig. 5. LNCAP Overexpression Model
Fig. 5a: Depicted is the longitudinal growth data under normal growth conditions (no drug exposure). NAMPT overexpression in LNCAP had no effect on growth rate. **Fig. 5b:** NAMPT overexpression significantly increased cell resistance to 5nM daporinad treatment.

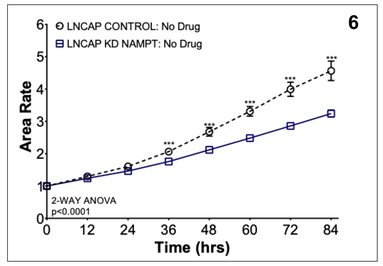


Fig. 6. LNCAP Knockdown Longitudinal Growth
The LNCAP control and NAMPT knockdown groups were measured on the Cytation Imaging system. NAMPT knockdown in LNCAP displayed a significantly decreased growth rate compared to the control. This decreased growth rate did not affect overall cell viability.

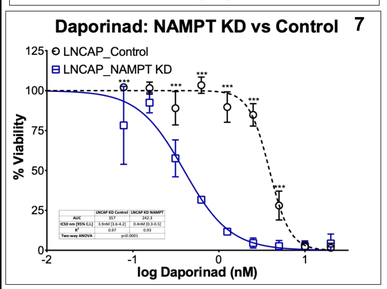


Fig. 7. NAMPT Knockdown Displayed Increased Sensitivity to Daporinad
NAMPT knockdown in LNCAP resulted in a significant decrease in percent viability after daporinad treatment (daporinad IC_{50} control = 3.9 nM, daporinad IC_{50} NAMPT KD = 0.4 nM). The LNCAP control and NAMPT knockdown groups were also measured on the Cytation Imaging system, which produced dose-response curves with normalized area that concurred with the result shown.

IV. Conclusions

- This experiment supported the hypothesis that knocking down NAMPT expression in LNCAP would increase cell sensitivity to daporinad treatment, and overexpression would drive resistance.
- Knockdown of NAMPT expression slowed cell growth, but had no effect on cell viability.
- Overexpression of NAMPT expression in LNCAP had no effect on growth rate, but drove resistance to daporinad treatment.
- shRNA added to the NCI-H660 model was successful in knocking down NAMPT expression. Daporinad drug assays with the NCI-H660 models are ongoing.
- Cancer cell expression of NAMPT may be a suitable indicator for daporinad response and aid in prostate cancer treatment selection.

V. Future Directions

- An additional experiment could verify that overexpression in the NCI-H660 model drives resistance to daporinad treatment.
- Alternative models of NEPC could be explored, such as DU-145, which is also an adherent cell line.
- Verification of knockdown and overexpression on a protein level for both cell lines is recommended for future experimentation. This could be accomplished through a Western Blot analysis.

VI. Acknowledgements

Thank you to Dr. Adam Lee and Dr. R. Stephanie Huang for mentorship and guidance throughout this project. We would also like to thank the University of Minnesota SCoPE (Summer College of Pharmacy Experiences) program for funding and academic support.

VII. References

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3. Zhang et al. (2023). Computational drug discovery for castration-resistant prostate cancers through in vitro drug response modeling. Proc Natl Acad Sci U S A. 2023 Apr 25;120(17):e2218522120. doi: 10.1073/pnas.2218522120. Epub 2023 Apr 17. PMID: 37068243.