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14. ABSTRACT
The radiation therapy (RT) has proven to be effective at increasing survival of men with prostate cancer. However, the results are far from optimal, with 30-40% of men with intermediate to high risk prostate cancer failing within 5 years. We have been investigating agents that have the potential to enhance the cell killing effects of one or both of these treatments. NS-123 is a drug that we have identified as having such potential. The objective of any combination of therapeutic agents is to achieve an improved therapeutic gain. The therapeutic gain is a function of both the tumor and normal tissue response. There is no universally accepted measure of a therapeutic result: lifespan, duration of remission, quality of life are all important and reflect different facets of the total result. When therapies are compared, it is necessary to show that one treatment controls the disease better than another for a similar level of toxicity. We recently reported the results of preclinical studies on a novel radiosensitizer, 4'-bromo-3'-nitropropiophenone (NS-123) that we identified using a cell-based, high-throughput screening method. In these studies, NS-123 radiosensitized human lung adenocarcinoma, colon adenocarcinoma, and glioma cells. Recently, we have demonstrated that NS-123 also radiosensitizes prostate cancer cells. NS-123 appears to be a 'true' radiosensitizer as no overt toxicity was seen in any of the normal tissue models that we studied. Investigations into the mechanisms responsible for this radiosensitization suggest that NS-123 inhibits the DNA repair pathways, possible as a result of some upstream inhibition within the phosphatidylinositol-3-kinase/Akt pathway. NS-123 appears to sensitize prostate cancer cells (PC3 and DU145) with only a short exposure of 1 hr. Animal studies with daily treatment (50 mg/kg) showed no toxicity. We have generated tumors on the flanks of male mice and put them into one of four treatment groups (no treatment, RT, NS-123, NS-123+RT). RNA from these tumors is currently being analyzed.

15. SUBJECT TERMS
NS-123 radiosensitizes prostate cancer cells

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- **INTRODUCTION:**

We reported the results of preclinical studies on a novel radiosensitizer, 4'-bromo-3'-nitropropiophenone (NS-123) that we identified using a cell-based, high-throughput screening method.[1] In these studies, NS-123 radiosensitized human lung adenocarcinoma, colon adenocarcinoma, and glioma cells. As part of this project, we have also found that **NS-123 radiosensitizes prostate cancer cells**. Importantly, NS-123 appears to be a 'true' radiosensitizer as no overt toxicity was seen in any of the normal tissue models that we studied. Preclinical investigation of NS-123 has formed the basis for this research proposal. This update represents work that his team has collectively put together over the past 12 months.

- **BODY:**

The proposed experiments are to determine the efficacy of NS-123 as a radiosensitizing agent in the treatment of prostate cancer as well as providing a better understanding of the molecular mechanisms responsible for the control prostate cancer. The primary focus of the last 12 months has been to perform the necessary pre-clinical studies *in vivo* and *in vitro*. The ability to exploit this relationship is likely to have significant impact for the care of patients with prostate cancer. Assuming these results are as encouraging as the results previously published, we anticipate developing a clinical trial using this therapeutic rationale.

Specific Aim 1: Maximize the therapeutic gain obtained by combining NS-123 with RT±AD in prostate cancer cells *in vitro*.

The ability of NS-123 to act as a radiosensitizer was tested by clonogenic experiments in PC3 and DU145 human prostate cancer cell lines. These clonogenic experiments were designed to vary both the incubation time prior to irradiation (Pre-IR) and also the time post-irradiation (Post-IR) to determine which condition(s) is most effective at killing the cancer cells based on the Dose Enhancement Ratio (DER) at Survival Fractions of 0.1 and/or 0.001. The higher the DER, the more radiosensitivity observed. A summary of the results is presented in Table 1.

Table 1. Results of Clonogenic Assays (average of at least 2 experiments)

Cell Line	Pre-IR time (hr)	Post-IR time (hr)	NS-123 (μM)	DER at 0.1	DER at 0.01
DU145	1.0	0.0	20	1.37	
DU145	1.0	0.0	30	1.66	
DU145	**1.0**	**4.0**	**20**	**1.81**	
DU145	**1.0**	**4.0**	**30**	**2.16**	
DU145	1.0	24.0	20	1.55	
DU145	1.0	24.0	30	1.83	
DU145	4.0	0.0	20	1.26	
DU145	4.0	0.0	30	1.59	
DU145	4.0	4.0	20	1.24	
DU145	4.0	4.0	30	1.86	
DU145	16.0	0.0	20	1.06	
DU145	16.0	0.0	30	1.07	
DU145	16.0	4.0	20	1.03	
DU145	16.0	4.0	30	1.14	
DU145	24.0	0.0	20	1.02	
DU145	24.0	0.0	30	1.09	
PC3	1.0	0.0	20	1.10	1.03
PC3	1.0	0.0	30	0.95	0.98
PC3	1.0	4.0	20	1.28	1.34
PC3	1.0	4.0	30	1.27	1.29
PC3	4.0	0.0	20	1.05	1.05
PC3	4.0	0.0	30	1.27	1.12
PC3	4.0	4.0	20	1.15	
PC3	4.0	4.0	30	1.05	
PC3	16.0	0.0	20	0.99	0.99
PC3	16.0	0.0	30	1.00	0.96
PC3	16.0	4.0	20	1.06	1.02
PC3	16.0	4.0	30	1.02	0.98
PC3	24.0	0.0	20	0.93	0.91
PC3	24.0	0.0	30	0.91	
PC3	24.0	4.0	20	0.87	
PC3	24.0	4.0	30	0.76	

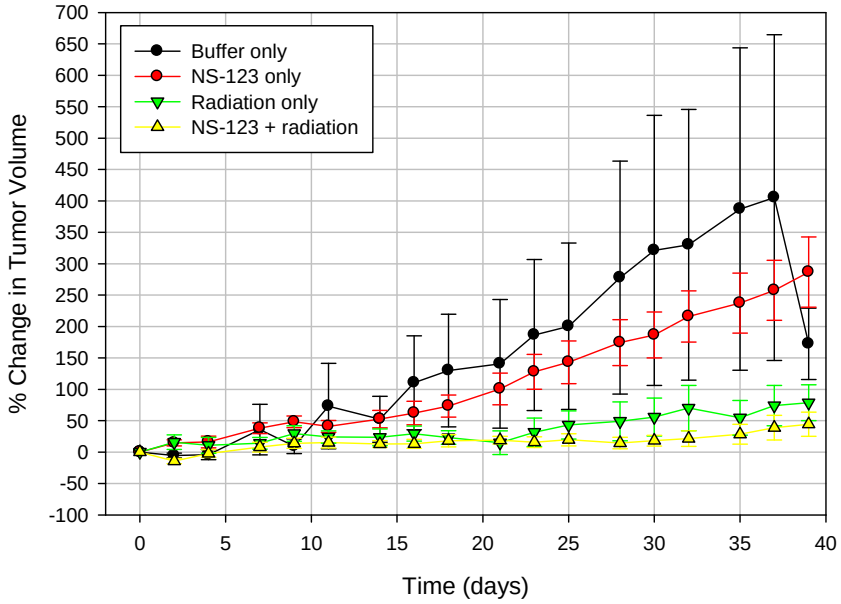
NS-123 (both at 20 and 30 μM) had more effectiveness as a radiosensitizer in the DU145 cell line compared to PC3. In the DU145 experiments, NS-123 best promoted radiosensitivity when the pre-IR time was short (4 hours or less). The

post-IR time did not appear to be a factor. Though not as dramatic, the same trend was observed in the PC3 clonogenic experiments.

There appears to be some radiosensitivity associated with NS-123, however a better understanding of the mechanism is needed to ensure that the treatment is optimized.

Specific Aim 2: Determine if NS-123 can be integrated into current prostate cancer treatment paradigms to produce an increase in the therapeutic gain *in vivo*. We have *in vivo* studies to investigating the radiosensitization of NS-123 in male adult nude (nu/nu) mice with implanted tumor cells. Based on results of **Aim 1**, 50 mg/kg of NS-123 was administered 1 hour prior to irradiation of the tumors. Subcutaneous tumors were established by injecting 5 x 10^5 PC3 cells in the flanks of nude mice. After time, tumors were allowed to grow and the mice were randomized into four experimental groups. These groups were: DMSO alone, NS-123 alone, Radiation alone (DMSO + RT), and NS-123 + RT; 9-10 mice were in each group. Tumor size was assessed with caliper measurements three times/week, and tumor volume calculated from the formula TV= Pi/6 x 1.69 x (LxW) raised to the 1.5 power.[3] Treatments were started when tumor volumes were approximately 50 mm^3.

Figure 1. Results of NS-123 investigated in Xenograft tumor model.



In this experiment, NS-123 showed some activity but did not reveal any definite radiosensitization. RNA analysis (microarrays using the Illumina HT-12 chips) of the tumors specimens is in progress.

- **KEY RESEARCH ACCOMPLISHMENTS:**
  - NS-123 radiosensitzes prostate cancer cells with a variety of treatment schedules. Radiosentiziation was identified with lower doses of NS-123 and with administration only 1 hr prior to irradiation.

- **REPORTABLE OUTCOMES:**
  - An abstract entitled “Preclinical testing of a novel small molecule radiosensitizer of prostate cancer cells” was presented at the 2012 Sylvester Cancer Center Annual Retreat.

- **CONCLUSION:**

Dr. Lally’s laboratory has been established and his research team is now in place. A manuscript on NS-123 was submitted for publication. Unfortunately, the manuscript was rejected but is under revision for submission in another journal. The work completed thus far has been very important and has helped define the animal experiments. These

studies are now underway to determine the *in vivo* radiosensitization potential of NS-123. Understanding of the molecular response may reveal the mechanisms involved.

- **REFERENCES:** List all references pertinent to the report using a standard journal format (i.e. format used in *Science, Military Medicine,* etc.).

1. Lally, B.E., et al., *Identification and biological evaluation of a novel and potent small molecule radiation sensitizer via an unbiased screen of a chemical library.* Cancer Res., 2007. **67**(18): p. 8791-8799.
2. Morgan, P.B., et al., *Radiation dose and late failures in prostate cancer.* Int J Radiat Oncol Biol Phys, 2007. **67**(4): p. 1074-81.
3. Feldman et al, *J Applied Quant Methods,* Vol. 4, 2009, equation #6

- **APPENDICES:**
  1. IACUC Approval
  2. UM Comparative Pathology Laboratory Accession