TOBACCO SETTLEMENT REVENUE OVERSIGHT COMMITTEE

TOBACCO SETTLEMENT REVENUE (TSR) FUNDING REQUEST

Name of entity requesting TSR funds: _New Mexico State University_

Name(s) of each program for which TSR funds will be used: _The protective effects of low-dose nicotine treatments on post-irradiation oxidative damage_

Description of each program, including its purpose: _Please see attached documents and

budget_

Have you requested TSR funds prior to this request? No

Have you received TSR funds prior to this request? No

If yes, in what fiscal years? _N/A_

What will you use the requested funds for? Please include goals and objectives.
The goal of this application is to investigate the potential of low doses of nicotine at elevating cellular defenses and lowering damage associated with cancer irradiation treatments. The objectives are to establish protective low doses of nicotine, arrive at benchmark levels of damage and damage reduction, and developed this approach into a potential treatment for patients of all ages who receive irradiation as part of their cancer treatment.

Is this a change from previous years' use? No
If yes, please describe the change and reason(s): _N/A_

Amount requested (Total amount, and amount for each program):
Total requested funds in this project are $306,542.

What other sources of funding are applied to this purpose?
None, this was a project specifically designed to improve cancer patient outcomes in New Mexico by taking advantage of nicotine and its stimulatory effects.

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COVER PAGE FOR NM TOBACCO SETTLEMENT GRANT APPLICATION

Project Title: The protective effects of low-dose nicotine treatments on post-irradiation oxidative damage.

	Name	Affiliation
Principal Investigator:	Giancarlo Lopez-Martinez	New Mexico State University, Biology

Total Budget Requested: $306,542

Period of Performance Requested: Start _July 1, 2017_ End _June 30, 2018_

PROJECT SUMMARY:

Radiotherapy for cancer leads to numerous and intensifying off-target effects. Even the most effective radiotherapy treatments will result in the production of free radicals, reactive oxygen species (ROS), and the ensuing oxidative damage (OxD). OxD is an imbalance between ROS production, including free radicals, and cellular antioxidants, which leads to damaged cellular components (lipids, proteins, DNA/RNA). Accumulated oxidative damage leads to age-related declines in health and physical activity (i.e. mobility), and immune-competence. In most cases, the strong side effects experienced by patients (i.e. fatigue, skin problems, infection, and new incidences of cancer), are due to post-irradiation oxidative damage. This is exacerbated by the fact that ROS do not have a single cause and arise from a multitude of sources (dehydration, starvation/poor nutrition, prolonged physical activity, pathogen infection, etc.), and potentially affect every aspect of organismal life (i.e. ROS leads to premature hearing loss in *humans*). In spite of the negative aspects of ROS, they are a beneficial and required part of immune responses and cellular communication pathways and are generated at low levels during hormesis. Hormesis occurs when an organism is exposed to a mild dose of a stressor, harmful/deadly at high doses, which triggers an adaptive protective response that improves survival (i.e., *human cells* experience increased proliferation and lifespan in response to low levels of gamma irradiation). Physiological conditioning hormesis, the use of pretreatments to trigger protective responses, has the potential to lower post-irradiation oxidative damage, therefore improving the effectiveness of radiotherapy. My previous work shows that environmental hormesis can lower post-irradiation oxidative damage, increase longevity, and improve sexual performance at old age in a model system, *Drosophila melanogaster*. The main goal of this project is to understand the hormetic potential of low-levels of nicotine at triggering chemical hormesis and lowering post-irradiation oxidative stress and damage. This intervention has the potential to improve the efficiency of radiotherapy by lowering side effects associated with oxidative damage. The project will i) investigate the potential of nicotine as a hormetic treatment by carefully studying the dose-response in flies, and ii) reveal the effects that nicotine hormesis has on post-irradiation oxidative damage. This project will provide funding for competitive training of students in a growing branch of biomedical research by teaching them highly desirable research, data analysis, and presentation skills. Using biochemistry, physiological assays, and molecular tools to study hormesis and post-irradiation oxidative damage, we will reveal the effects that nicotine hormesis can have during radiotherapy which may lead to the creation and study of clinical interventions that potentially take advantage of physiological conditioning hormesis to treat *humans* using nicotine patches.

I. SPECIFIC AIMS

Aim 1) Investigate the potential of low doses of nicotine at elevating cellular defenses (i.e. antioxidant capacity), and lowering oxidative damage. The outcome of this aim is to *identify at least two doses of nicotine that have the greatest increase in protection* by utilizing the highly repeatable total antioxidant capacity biochemical assay and using *Drosophila melanogaster* flies as a model system. There is preliminary data from Drosophila flies showing that low doses of nicotine improve Parkinson's disease symptoms in a hormetic framework. Doses will be evaluated biochemically (lipid, protein, and DNA damage), and molecularly (expression of candidate genes). The two doses found to have the most protective effects will be further investigated in Aim 2.

Aim 2) Study the effect of nicotine hormesis on post-irradiation oxidative damage. The outcome of this aim is to identify the magnitude of the hormetic/protective effect of nicotine by looking at short and long-term post-irradiation effects. A combination of organismal assays (circadian activity, flight ability, starvation, reproduction, and longevity) aimed at measuring performance and biochemistry will be used to determine the full range of the protective effect of nicotine. Additionally, we will study the direct effects of nicotine hormesis on post-irradiation oxidative damage to lipids, proteins, and DNA associated with cancer irradiation treatments.

Aim 3) Future directions: translational application and transgenerational effects. In order to develop this work into a potential medical treatment, a broad look at the effect that nicotine hormesis has on the body is required. This will necessitate transcriptomic and proteomic approaches. We will take a big picture approach and look at all the genes and proteins expressed as a result of nicotine hormesis in order to identify pathways that in the future might be more easily triggered (i.e. without the need of nicotine). We will also look at the transgenerational effects of nicotine hormesis to assess whether offspring benefit from parental treatments, as we have shown in the past.

I. Research Strategy:

Significance: As effective as radiotherapy is at reducing cancer cell proliferation it comes with an array of short and long-term side effects due to off target effects of irradiation. Conventional therapies meant to suppress the off-target effects of radiotherapy have been less than effective, leaving ample potential for unconventional therapies. The oxidative damage generated by ionizing radiation, such as gamma and X-rays, will continue to damage cells, tissues, and organs long after the irradiation event has ended. Therapies targeting cancer irradiation recovery must have both strong immediate and long-term effects. Physiological conditioning hormesis is currently being used to increase recovery from heart attacks (McDonald et al. 2014), improve symptoms of Alzheimer's disease (Cuttler et al. 2016), neuroprotection (Stetler et al. 2014), and improve recovery from array of surgical procedures (Calabrese 2008). This work is significant because it explores the use of an already approved chemical therapy with multiple delivery methods (nicotine gum, patches, lozenges, nasal spray and inhalers) as a novel therapeutic clinical technology targeting post-irradiation damage in a cancer radiotherapy context.

Innovation: Currently nicotine is seen as an insecticide and a chemical responsible for smoking addiction. However, nicotine has great potential as a therapeutic drug given that nicotinic brain receptors are linked to attention, cognition, and focus. Recent work has shown that nicotine can improve various metrics of human performance including fine motor movements, response time, alertness, and memory (Heishman et al. 2010, Sarter 2015). This application seeks to further explore the potential hormetic effects of nicotine by looking at post-irradiation effects. Work suggesting that nicotine can protect against radiation is lacking, but there is some data showing that nicotine protects against motor deficit associated with Parkinson's disease in *Drosophila melanogaster* (Chambers et al 2013); an improvement which mechanistically is very similar to reducing oxidative damage associated with radiotherapy.

The novel use of nicotine as a therapeutic agent outside of addiction has great potential as a starting point for the potential use of plant-derived compounds for novel therapies. The real advantage of this work is the reduced investigation time required in the development of a therapy already approved for a different condition. The safety and side effects of nicotine have been extensively studied and any hormetic effect found here can be turned into therapy rather easily compared to the brand new development of a therapeutic treatment. By investigating the potential of nicotine at reducing off-target radiation effects we are not developing an entirely new treatment but extending the potential use of a treatment already commercially available.

Approach: The goal of this research plan is to elucidate the hormetic effects of nicotine on post-irradiation oxidative damage and to investigate the transgenerational effects of such a treatment.

Aim 1: Investigate the potential of low doses of nicotine at elevating cellular defenses (i.e. antioxidant capacity), and lowering oxidative damage.

Hypothesis: Nicotine hormetic treatment leads to an upregulation of protective compounds (stress genes, antioxidant defenses, and radioprotectants) that lower oxidative damage to lipids, proteins, and DNA/RNA.

Approach: We will test a series of doses of nicotine, (*S*)-3-[1-Methylpyrrolidin-2-yl]pyridine, for hormetic potential by exposing developing flies to a range of nicotine doses and studying various aspects of physiological performance. Previously, we have shown that different doses of anoxia (no oxygen), temperature, dehydration, and even ionizing radiation (X-rays and UV) have hormetic effects that improve treatment survival and organismal performance (i.e. activity and mobility; Elnitsky et al. 2009, Benoit et al. 2009, López-Martínez and Hahn 2012 and 2014, López-Martínez et al 2016a and López-Martínez et al 2016b).

In order to fully quantify the protective effects of nicotine conditioning, we will evaluate appropriate doses and whether repeated applications over a specific time frame are required to maximize the efficacy of the treatment. Based on previous work we will target doses ranging from 3 to 12 µg/ml (Chambers et al. 2013). We will initially evaluate what age (larval, pupal, and/or adult), and delivery method (diet, cutaneous application, or injection) is ideal for maximizing the effect of nicotine on the flies. Because total antioxidant capacity strongly correlates with the hormetic potential of treatments (López-Martínez and Hahn 2012), we will use biochemical assays to determine the increase in total antioxidant capacity, the duration of that antioxidant elevation, and the oxidative damage to target cellular components.

We will use the Trolox-equivalent antioxidant capacity assay (TEAC; Re et al. 1999) to determine the potential of hormetic treatments at elevating cellular defenses. *D. melanogaster* flies will be used in all experiments proposed here. Flies will be exposed to treatments, and samples will be collected and frozen during a recovery series, after adult emergence, sexual maturity, and reproduction. Two doses that yield the highest TEAC will be examined for specific antioxidant enzyme expression (SOD1, SOD2, GPx, catalase, etc.) which are the main genes involves in lowering post-irradiation oxidative damage (López-Martínez, Hahn 2012).

Concurrently, those two doses will be tested for different markers of oxidative damage. Frozen samples will be used to quantify specific makers of oxidative damage to lipids, proteins, and DNA. The highly repeatable and informative TBARS assay (Ohkawa et al. 1979) will be used to quantify the extent of lipid oxidation damage. Oxidative damage to proteins will be quantified using the protein carbonyl assay (Levine et al. 1990). DNA damage, and potential repair, will be measured using the comet assay (Collins 2004). The outcome of this aim will be the identification of nicotine conditioning doses that confers the greatest increase in oxidative damage protection.

Aim 2: Study the effect of nicotine hormesis on post-irradiation oxidative damage.

Hypothesis: Nicotine hormesis will result in a lowering of post-irradiation oxidative damage that improves organismal performance over the short and long-term.

Approach: We will use the two nicotine doses known to elicit a protective cellular response to test for lowering post-irradiation oxidative damage. Biochemically assays of oxidative damage for lipids, proteins, and DNA (see Aim 1) will be use to assess whether the pretreatment with nicotine resulted in lowered oxidative damage after irradiation. But because oxidative damage is accumulated over time, it is quite possible that biochemical assays may not detect a reduction and performance assays would be required to quantify the full extent of the response.

We will measure immediate and long-term survival using two common assays my lab is very experience performing; starvation and longevity. Additionally we will look at climbing ability, flight ability, motility, circadian activity, mating, reproduction, and fecundity. In the past, we have had to design and vet our own performance assays and we are knowledgeable enough to do this if necessary. The data from these assays would elucidate the full effects that low-dose nicotine hormesis has on post-irradiation oxidative damage and organismal performance.

Aim 3) Future directions: translational application and transgenerational effects. In order to develop this work into a potential medical treatment, a broad look at the effect that nicotine hormesis has on the body is required. This will require transcriptomic and proteomic approaches. By taking a broad genetic view of all the genes expressed, we will be able to build a road map of the full protective effect of nicotine and potentially learn how to induce the protective effect in the absence of nicotine. We will additionally explore the effects of nicotine protection by looking at offspring and how they perform in organismal tests from Aim 2.

Budget

Personnel:
I request 2 months of salary support for myself during the summer. I also request funds for two graduate assistantships during the academic year, including tuition, and salary for two undergraduate students.

Travel:
I request funds to travel to two national-level professional meetings for me and three students.

Publication fees:
I request funds to publish at least two papers; one to be published in an open access journal.

Materials and Supplies:
I request funds for colony rearing and maintenance including rearing consumables (cages, vials, and bottles), and diet components. The cost of organismal performance assays will vary on whether new assays have to be designed, but I request funding for materials, paint, and tools. Additional material costs are associated with chemicals for biochemical and molecular assays, and laboratory consumables.

Equipment:
I request funds to purchase Trikinetics Drosophila Locomotor Activity Monitors and for two drosophila-specific incubators.

Bioinformatics costs: I request funds for transcriptomic and proteomic analysis of samples at an off-site facility.

Total costs: I request a total of **$306,542**.