SPECIFIC AIMS

High-risk types of human papillomaviruses (HPV) are responsible for virtually all cases of human cervical carcinoma, as well as an increasing number of other HPV-associated malignancies, including those of the head and neck, anus and vulva. One growing group of patients particularly affected by HPV includes those with compromised immune systems resulting from HIV infection, other diseases or medical treatments. Perhaps the most noteworthy advance in recent years has been the development of safe and effective vaccines targeted against HPV. However, these vaccines are not beneficial for patients who are already infected, appropriate for use in patients with compromised immune systems, or readily available in all developing countries. Once cancer has developed, current treatment options are relatively limited and focus on physically removing the cancer through surgery. Unfortunately, tumors frequently return, particularly following late-stage diagnosis and/or if the patient is immunocompromised. Chemo- and radio-therapies that rely on the induction of apoptosis in HPV^+ tumor cells are relatively ineffective, primarily due to the actions of a virus-encoded oncoprotein, E6, that subverts both intrinsic and extrinsic apoptotic pathways by accelerating the degradation of key molecular players. Therefore, new approaches that can eliminate HPV-containing cells, even in the absence of a functional adaptive immune system, must be developed.

To meet this need, we propose to combine spinacine, a small, naturally occurring molecule whose E6-inhibiting abilities were recently discovered by our laboratory, with existing therapeutic approaches that function by inducing apoptosis. Our laboratory and others have shown that high-risk versions of the HPV E6 oncoprotein induce resistance to both intrinsic and extrinsic apoptosis by mediating the rapid degradation of p53, caspase 8 and FADD [1-6]. The absence of these molecules in turn leads to the protection of infected cells from agents that would otherwise induce programmed cell death. To counter this, we searched for molecules that would inhibit the ability of E6 to bind to its cellular apoptotic partners by screening over 3000 compounds. Spinacine was selected as our lead candidate, because it is able to block the binding of E6 to both caspase 8 and E6AP, thereby sensitizing HPV^+ cells to apoptosis triggered by agents such as TRAIL (a ligand that selectively induces apoptosis in cancer cells) and chemotherapeutic drugs such as cisplatin and doxorubicin. As predicted by our model, spinacine restores cellular levels of caspase 8, FADD and p53. Together, these observations support the *scientific premise* of our proposal.

The *long-term goal* of our laboratory is to develop novel, effective therapies for patients suffering from HPV-associated malignancies, and the *overall objective* of this current application is to move our exciting *in vitro* and cellular observations into a mouse xenograft model. In this model, we will 1) Assess the toxicity of spinacine, and 2) Determine the *in vivo* effectiveness of a combinational therapy that pairs spinacine with an agent that induces apoptosis (TRAIL or cisplatin). The *conceptual framework* supporting this proposal states that by targeting the E6/apoptotic protein interactions with small, drug-like molecules such as spinacine, we will be able to increase cellular levels of p53, caspase 8 and FADD. This will sensitize cells to apoptosis, thereby enabling agents that induce apoptosis to eliminate or decrease the growth of HPV^+ tumors *in vivo*. All necessary assays, reagents and expertise are available to us, and for these reasons, we are well positioned to immediately undertake the work described in the following specific aims:

> **Specific Aim 1: Determine the toxicity of spinacine in mice.** We will assess the toxicity of spinacine in mice, defining the maximum tolerated dose and identifying the optimum dose with which to carry out experiments designed to test its efficacy.
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> **Specific Aim 2: Evaluate the ability of spinacine to synergize with TRAIL- and/or chemo-based therapies to reduce or eliminate HPV^+ tumor growth.** We will assess the ability of spinacine to synergize with hrTRAIL and/or the DNA damaging drug cisplatin to inhibit tumor growth in a xenograft model.

At the conclusion of this work, we will have 1) Determined the toxicity of the E6-inhibiting molecule spinacine in mice, and 2) Evaluated the effectiveness of combining spinacine with TRAIL- and cisplatin-based treatments in an animal model. This work has the potential to save the lives of patients suffering from HPV-associated malignancies.