I Care I Cure Proposal – Gershon

Project Title: Preclinical Development of ATR Inhibitor VE-822, Delivered Systemically in Nanoparticles, for Medulloblastoma Therapy

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Scientific Abstract

This proposal will investigate the therapeutic potential of targeting the DNA damage response protein ATR for medulloblastoma therapy, using a small molecule inhibitor, VE- 822, in novel, nanoparticle formulation (pVE-822). Radiation and chemotherapy significantly extend survival for most patients with medulloblastoma, but produce significant long-term neuro-cognitive side effects. Our rationale for targeting ATR derives from the phenotypes of humans with ATR mutation and mice with ATR conditional deletion. In both groups, disruption of ATR function causes failure of neural progenitors that may be cells of origin for medulloblastoma. Moreover, ATR function is required to maintain genomic stability, while medulloblastoma is highly sensitive to DNA-damaging therapies. Our preliminary studies show that pVE-822, administered IP into medulloblastoma-bearing SmoM2 mice, penetrates into tumors and markedly increases DNA damage. We now propose to test the hypotheses that ATR inhibition with pVE-822 will exert a significant anti-tumor effect using SmoM2 mice as a preclinical, primary tumor model of medulloblastoma (Aim 1), disrupt the tumor response to DNA damage, inducing genomic instability (Aim 2), and potentiate the effect of radiation therapy in our *in vivo* model (Aim 3).

This proposal will test both the novel approach of ATR inhibition, and the novel use of the polymeric micelle drug-delivery platform, to improve medulloblastoma therapy.

Impact Statement

Improved therapy is needed for medulloblastoma, the most common malignant brain tumor in children. Current radiation and chemotherapy, while effective for most patients, produces significant long-term toxicity, including cognitive impairment, severe weight loss, early strokes and growth failure. This therapy also fails 20% of patients, who develop incurable recurrence.

This proposal will test a novel approach to medulloblastoma therapy by targeting ATR, a protein that is essential to the genomic stability of proliferating cells, and that is particularly required by medulloblastoma cells of origin. To deliver the ATR inhibitor VE-822 across the blood brain barrier, we have developed a unique, nanoparticle formulation, pVE-822. We will test pVE-822 in transgenic, medulloblastoma-prone mice, a model in which tumors form with native vasculature, recapitulating the blood brain barrier that complicates therapy for brain tumor patients. This proposal will advance the development of new therapeutics for medulloblastoma by 1) testing the novel treatment approach of inhibiting ATR, and 2) showing the potential of nanoparticle polymeric micelles to deliver drugs into brain tumors.

Specific Aims

We will test the hypothesis that targeting the DNA repair protein ATR can enhance therapy for medulloblastoma, the most common malignant pediATRic brain tumor. Radiation and chemotherapy, which act by damaging DNA, confer long-term survival for most medulloblastoma patients. Improved treatment is needed, however, because many patients die of recurrence and survivors have life-long neuro-cognitive injury.

We focused on ATR as a target for medulloblastoma therapy because prior investigations in mice showed that neural progenitors that give rise to medulloblastoma require ATR during development. ATR prevents DNA damage during replication and responds to DNA damage by phosphorylating the checkpoint kinase Chk1 which induces G2 cell cycle arrest. This G2 checkpoint maintains genomic stability by preventing cells with damaged DNA from entering mitosis. Our preliminary studies show that conditional deletion of ATR promotes DNA damage in neural progenitors and prevents tumor formation when bred into in medulloblastoma-prone SmoM2 mice. We now propose to investigate the therapeutic potential of ATR inhibitor VE-822, using SmoM2 mice as a preclinical model.

To optimize delivery of VE-822 to brain tumors, we developed a nanoparticle formulation using the poly(2-oxazoline) (POx) micelle drug- delivery platform pioneered by Drs. Kabanov and Sokolsky (pVE-822; Fig 1). Our preliminary studies show that pVE-822 crosses the blood brain barrier when administered IP in mice, causing DNA damage in medulloblastomas without harming the normal brain (Fig 3). Our Specific Aims will determine optimal use of pVE-822 and its mechanism of effect.

We propose that pVE-822 will exert anti-tumor effects by directly increasing DNA damage and preventing a normal DNA damage response, thus increasing genomic instability and potentiating DNA-damaging therapies. We will determine the anti-tumor effect of pVE-822 in a genetically engineered mouse model of medulloblastoma (Aim 1), measure the effect on DNA damage response and genomic integrity (Aim 2), and determine the efficacy of combining pVE-822 with radiation (Aim 3).

SA1) Determine the maximum benefit of single-agent pVE-822. Nanoparticle-delivered pVE-822 is an entirely new agent that we have devised. We will establish the pharmacodynamics and pharmacokinetics of this agent, determining the maximum tolerated dose (1A), and measuring the tumor penetration of the drug in primary medulloblastomas in transgenic mice (1B,C). We will use this information to conduct a prospective pre-clinical trial of pVE-822 in transgenic, medulloblastoma-prone mice, measuring survival time and tumor growth (1D). This Aim will test the hypothesis that pVE-822 can produce a measurable anti-tumor effect in mice with primary medulloblastoma.

SA2) Determine the impact of ATR inhibition on tumor cell DNA. Aim 2 will test the hypothesis that ATR inhibition will produce intolerable genomic instability in tumor cells. In 2A, we will examine how ATR inhibition alters the DNA damage response by treating medulloblastoma-bearing mice with pVE-822 or vehicle and then measuring the phosphorylation of DNA-damage response proteins p53, Chk1 and 53BP1. We predict that pVE-822 will disrupt DNA repair by causing activation of p53 and 53BP1 without Chk1. In 2B, we will examine the effect of pVE-822 on cell cycle checkpoint, a known function of Chk1. Based on our prior data in ATR- deleted mice, we hypothesize that ATR inhibition will disrupt the G2-M checkpoint that typically prevents cells with DNA damage from undergoing mitosis. In 2C, we will analyze tumors from pVE-822-treated and control mice using cytogenetics and array CGH to detect changes in genomic stability, a potential consequence of aberrant DNA repair and checkpoint failure. These studies will examine the mechanism of effect of pVE-822.

SA3) Determine the added benefit of pVE-822 in SmoM2 mice treated with radiation therapy. Here we will directly test the hypothesis that pVE-822 will increase the efficacy of conventional therapy for medulloblastoma. Radiation is unlikely to be replaced as an upfront therapy for medulloblastoma because it is effective for most patients. If pVE-822 can increase the anti-tumor effect of radiation, it may be possible to reduce the dose of radiation patients receive, while increasing overall efficacy. We have developed a mouse model of radiation therapy by administering cranial x-ray beams to medulloblastoma-bearing SmoM2 mice. The therapeutic benefit of this treatment can be measured by comparing the survival of treated and untreated mice. We will administer pVE-822 or vehicle to mice with medulloblastoma undergoing radiation therapy and compare survival after treatment, to test how pVE-822 combines with standard therapy.