Scientific Abstract

We are developing Vesicular Stomatitis Virus (VSV) as an oncolytic vector for the treatment of cancer. VSV, a Rhabdovirus, is a negative strand RNA virus, which replicates in the cytoplasm and is highly lytic, with no known transforming capabilities. VSV is arthropod-borne, and the primary hosts are rodents, cattle, horses and swine. Human infection results in very mild, flu-like consequences, although a case of pediatric encephalitis possibly associated with VSV has been reported. Moreover, following intra-cerebral injections of macaques neuro-virulence was observed. VSV is exquisitely sensitive to the antiviral actions of alpha/beta interferons as evidenced by the finding that mice harboring a defective IFN system become highly sensitive to normally innocuous exposure to VSV. However, many tumor cells have defects in their IFN response pathways and are non-responsive to exogenous IFN. Therefore, VSV replicates to high levels in many transformed cells. In contrast, normal cells are sensitive to the anti-viral interferons produced upon infection and infection is rapidly extinguished. As a result, VSV has been shown to be a potent oncolytic agent against a variety of both human and murine tumors, of several different histological types, via intratumoral or systemic routes. A recent protocol submitted to the RAC by Dr Savio Woo and colleagues from Mount Sinai School of Medicine proposed the testing of an attenuated VSV (VSV(MD51)) expressing M3 a chemokine-binding protein from gammaherpesvirus-68. In that protocol, the VSV is attenuated from the wild type virus by inclusion of the MD51 mutation in the viral matrix protein; in addition, the M3 transgene is included in the virus to inhibit immune cell accumulation in infected tumors and, therefore, allow greater spread of the virus. In this current protocol, we have attenuated the VSV in a different way. Thus, to exploit further the lack of responsiveness of tumor cells to anti-viral interferons, we reasoned that VSV engineered to over-express the anti viral interferon, IFN-β, would induce an anti-viral 'cytokine cloud' locally at the site of ongoing viral replication within a tumor. These cytokine clouds would further protect normal cells from VSV lysis, without, in theory, affecting the oncolytic potency of the virus on (interferon-non-responsive) tumor cells. In addition, production of recombinant IFN-β would be expected to stimulate a potent anti-tumor host defense. In this respect, our pre-clinical data of VSV-IFN-β virotherapy in murine models of HCC have shown that a). VSV expressing a biologically active IFN-β transgene (murine IFN-β is oncolytic against tumor cells derived from hepatocellular carcinomas, but not against normal hepatocytes; b). VSV expressing the human IFN-β (hIFN-β) has direct oncolytic activity against human HCC xenografts *in vivo* separate from the biological activity of the encoded IFN-β transgene; and c). biologically active (m)IFN-β significantly enhances the anti tumor activity of VSV-IFN-β as an oncolytic agent. Moreover, as a result of recommendations from the FDA, we have evaluated the toxicity, and biodistribution, of VSV-hIFN-β following injection into the left lateral lobe of the normal rat liver as well as following injection into orthotopic rat hepatocellular carcinoma tumors. We have also generated recombinant VSV expressing the rat IFN-β transgene to assess the potential toxicity from expression of the virally encoded rIFN-β gene in rats. Based on the results of these studies, *we propose here to develop VSV expressing human IFN-β (VSV-hIFN-β) as an oncolytic agent for clinical development for the treatment of hepatocellular carcinoma via direct intratumoral injection.* In order to balance efficacy with toxicity, and upon the advice of the FDA, we are proposing to apply a safety level of 2 logs below the dose at which no toxicity was observed in our toxicology studies and to start our clinical protocol at a dose of 10^5 $TCID_{50}$. In addition to safety, we will monitor virus levels and serum IFN-β as markers of gene transfer. In summary, we believe that VSV-IFN-β represents a highly potent anti tumor agent through <u>both</u> direct oncolysis of target tumor cells, <u>and</u> through the immune stimulatory properties of IFN-β. In addition, the inclusion of the human IFN-β gene will further attenuate the virus by ensuring a protective anti viral 'blanket' around infected tumors which will spare normal cells from toxicity.