Intramuscular Electroporation of an Optimized Rheoswitch-Regulated Interferon Plasmid Transgene Shows Long Term Persistence In Vivo: Implications for Therapy of Cancer

P Agarwal, T Chan, J Rothb, C Reed, B Merenick, JA Barrett, F Khali, H Youssefian, R Herbstreit, and WE Fogler

Theewoo Corporation, Germantown, MD: ZlPHERG Oncology Inc, Boston, MA

Abstract
Background: Regulated IFNα plasmids were approved in early 2011 for malignant melanoma despite significant side effects in clinical trials. Alternate approaches to utilize the antitumor properties of IFNα plasmid vectors may be necessary. We have engineered an inducible expression platform activated by a small molecule activator ligand (AL). The unique feature of this optimized plasmid was assessed in a B16F10 mouse model in vivo following a single intramuscular (IM) administration via electroporation.

Materials and Methods: Optimization of IFNα controllable DNA vectors for mouse or human use was accomplished by maximizing interferon regulatory domain and secretion signal peptide, derived from Intrexon’s Ultrabac® plasmid. To determine plasmid transfection efficiency, plasmid DNA was transferred into C2C12 myoblasts and HT1080 fibrosarcoma cells by electroporation using a BioRad GenePulser Xcell electroporation apparatus. The DNA complexes were co-transfected with a firefly luciferase vector at a ratio of 1:1, respectively. Transfection efficiency was determined by a luminescence-based assay (Promega’s Dual-Luciferase® Reporter Assay System).

Results: Inducible expression was observed in the presence of AL at 24 h. Results are shown as the ratio of luciferase activity in the presence of AL to luciferase activity in the absence of AL (vehicle control). The AL was found to be biologically active in a cell line that expresses the IFNα receptor. In vivo, the single intramuscular administration of IFN plasmid in mice resulted in sustained high levels of secreted protein in the presence of AL, which was electroplated into the muscle using an electroporation apparatus. Optimal AL concentrations resulted in sustained, continuous levels of IFNα expression. In the absence of AL, the IFNα levels were significantly reduced.

Conclusions: Our results demonstrate that the inducible delivery of IFN plasmid DNA can result in sustained high levels of IFNα expression at levels that can be therapeutically relevant. The results indicate that the combined use of inducible plasmid DNA and AL can be used to achieve sustained IFNα expression in vivo.

Vector Optimization Enabled by the Ultravector Platform Increases Regulated Plasma IFNα Expression In Vivo

Introducing the Rheoswitch Protein and Activator Ligand Controls Timing and Level of Target Gene Expression

Figure 1: The Rheoswitch Therapeutic System (RTS) includes expression plasmids containing three basic components: (1) an inducible expression vector, (2) a constitutively expressed factor and a co-expression partner, and (3) a Rheoswitch activator ligand (AL). In the absence of AL, the factor generally suppresses expression in the presence of AL, the system changes conformation and provides a dose-dependent “on” signal to target genes.

Figure 2: Inducible expression was observed in the presence of AL at 24 h. Results are shown as the ratio of luciferase activity in the presence of AL to luciferase activity in the absence of AL (vehicle control). The AL was found to be biologically active in a cell line that expresses the IFNα receptor. In vivo, the single intramuscular administration of IFN plasmid in mice resulted in sustained high levels of secreted protein in the presence of AL, which was electroplated into the muscle using an electroporation apparatus. Optimal AL concentrations resulted in sustained, continuous levels of IFNα expression. In the absence of AL, the IFNα levels were significantly reduced.

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Single Intramuscular Administration of pRTS-mIFNα Leads to Sustained Regulated Plasma IFNα Expression In Vivo

generation of Systemic and Intratumoral mIFNα Following IM administration with pRTS-mIFNα

Figure 3: C2C12 cells were infected with adenovirus particles at an MOI of 1000. After 24 h, the infected cells were harvested and subjected to RT-PCR analysis. The results are shown as the ratio of IFNα expression in the presence of AL to IFNα expression in the absence of AL. The AL was found to be biologically active in a cell line that expresses the IFNα receptor. In vivo, the single intramuscular administration of IFN plasmid in mice resulted in sustained high levels of secreted protein in the presence of AL, which was electroplated into the muscle using an electroporation apparatus. Optimal AL concentrations resulted in sustained, continuous levels of IFNα expression. In the absence of AL, the IFNα levels were significantly reduced.

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