**Pharmacodynamics and Protein Expression of RheoSwitch® Inducible Gene Programs**

**Abstract**

Over the past decade, immunotherapies have emerged as promising means to fight cancer. It is currently well accepted that combining multiple immunotherapeutic modalities will likely be necessary to achieve optimal tumor control and to develop a deeper impact on cancer treatment. Toward that goal, and in an effort to validate the feasibility and expression capacity of multiplexed gene constructs that intrinsically express these immunotherapeutics—such as IL-12, human IFN-γ, and a CTLA-4 decoy (CTLA-4 DCY)—in single, dual, or triple combinations. In all constructs, the three effector proteins were expressed under the control of Intragen's RheoSwitch Therapeutic System® (RTS®) activated by an orally available small molecule activator (LA). A videolibrary (also known as hRNS-100®) Expression of the three genes of interest (G0) was driven by distinct RTS® promoter variants, which allow for conditional gene expression following oral treatment with vehicle. Seven plasmids were constructed, three encoding the each G0 alone, three containing combinations of two G0, and one encoding all three G0. Expression of all G0 was evaluated in vitro in HEK293 and HEK293-IFNα cells for expression and function, and in vivo following administration through a single oral treatment and subsequent evaluation into mice pre-exposed to the respective full dose of vehicle or vehicle administered with vehicle, and were then evaluated for the single or concomitant expression and function of the encoded G0. Treatment with the full dose of vehicle or low dose of vehicle and multi-effector plasmids peaked increased levels of IL-12B, hIFN-γ, and CTLA-4 DCY when compared with vehicle. In contrast, in vivo expression was seen in cell surface supernatants or in the absence of the activator G0, regardless of ITS-100® expression. Treatment with full single G0 plasmids were functionally active in cell-based assays—hIL-12, hIFN-γ expression from Mtb IL-10, hIFN-γ enhanced JAK-STAT activation, and CTLA-4 DCY-IND binding to CTLA-4. Taken together, these results show for the first time the feasibility of synergistic expression of multiple therapeutic effects from a single RTS® regulated multiplexed construct in vivo. The in vivo studies also highlight the potential of an EDB to generate therapeutics for targeted delivery of single or multiple RTS-regulated cancer immunotherapeutics. Additionally, use of these novel regulated immunotherapeutics approaches could potentially be translated into an effective clinical regimen for a variety of cancers.

**Introduction**

• Immunotherapies targeting cancer have proven successful. Combining multiple immunomodulatory modalities should have a synergetic effect over the monotherapy alone.

• Constituent IL-12, IFN-γ and anti-CTLA-4 antibodies (clones) have been demonstrated to be efficacious. However, the success of these approaches has been limited by toxicity.

• The RheoSwitch Therapeutic System® (RTS®) technology represents a novel regulated gene-expression system utilizing the constitutive IκB, videolibrary (hRNS-100®), on an orally available small molecule activator. This combination permits the controlled clinical production of the target of interest thereby markedly reducing systemic toxicity.

• In this study, we evaluated the use of the RTS expression platform for the expression of single, dual, or triple combination constructs to validate the feasibility of the RTSS regulated multiplexed constructs.

**RheoSwitch® Proteins and Activator Ligand Controls**

Type of Interactions Evaluated

- Inducible Gene Program
- Timing and Level of Target Gene Expression
- Concomitant In Vitro Expression and Function of hIL-12B, hIFN-γ, and CTLA-4 DCY from a Single Multi-Effector Plasmid

**Concomitant In Vitro Expression and Function of hIL-12B, hIFN-γ, and CTLA-4 DCY from a Single Multi-Effector Plasmid**

**In Vitro Expression of hIL-12, hIFN-γ, and CTLA-4 DCY following Intramuscular Electroporation in Mice**

**In Vivo Expression of hIL-12, hIFN-γ, and CTLA-4 DCY from Single and Multi-Effector Plasmids following Intramuscular Electroporation in Mice**

**Conclusions**

- These data also highlight the potential of novel regimens as an effective clinical regimen for the treatment of cancer.