A Combinatorial Approach for Targeted, Systemic Delivery using Small Molecules and Reversible Masking to Bypass Non-Specific Uptake *In Vivo*

Nancy Smyth Templeton[1], Qiaoyun Shi[2], Andrew T. Nguyen[3], Yu Angell[4], Defeng Deng[3], Chang-Rim Na[3], Kevin Burgess[4], David D. Roberts[5], F. Charles Brunicardi[3], Phillip B. Maples[1], John Nemunaitis[1]
[1]Gradalis Inc., 2545 Golden Bear Drive, Suite 110, Carrollton, TX; [2]Department of Molecular Physiology & Biophysics; [3]Department of Surgery, Baylor College of Medicine, One Baylor Plaza, Houston, TX; [4]Department of Chemistry, Texas A&M University, College Station, TX, [5]Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

We have developed a multi-disciplinary approach that brings molecular biology, delivery technology, and combinatorial chemistry together to create improved systemic, targeted delivery to target cells avoiding non-specific uptake *in vivo* using reversible masking. We initially used a well characterized model targeting the asialoglycoprotein receptor in the liver. Using our bilamellar invaginated vesicle (BIV) liposomal delivery system with reversible masking, we increased expression in the liver by 76-fold, nearly equaling expression in first-pass organs using non-targeted complexes, with no expression in other organs. We applied our proof-of-principle technology to provide efficient targeted delivery to human tumor vasculature. We achieved efficient targeted delivery by attachment of human tumor endothelium-specific ligands to the surface of our BIV complexes used in conjunction with reversible masking to bypass non-specific tissues and organs. We created *in vitro* human tumor vasculature models by co-culturing primary human umbilical vein endothelial cells (HUVEC) with human lung or pancreatic cancer cells. The model was confirmed by increased expression of tumor endothelial phenotypes including CD31 and VEGF-A, and prolonged survival of endothelial capillary-like structures. The co-cultures were used for high-throughput screening of a specialized small-molecule library to identify tumor endothelium-specific ligands. We identified small molecules that enhanced transfection efficiency of tumor endothelial cells, but not normal endothelial cells or cancer cells. Different small molecules are required to target delivery to human pancreatic or to human lung tumor endothelium. Intravenous injection of our targeted, reversibly masked complexes to human tumor endothelium-pancreatic tumor bearing mice specifically increased transfection to the tumor endothelium about 200-fold. Efficacy studies using our optimized targeted delivery of a plasmid encoding thrombospondin-1 eliminated tumors completely after five intravenous injections administered once every week. We have also identified different small molecules that target delivery to human breast, human lung or human pancreatic cancer cells. These small molecules are tumor type specific.