

**NIH application: Scientific Abstract/draft 2.0**  
**REGENT I**

The REGENT I gene transfer protocol employs a DNA plasmid vector encoding the gene for inducible nitric oxide synthase (iNOS), one of three widely-studied isoforms. In this protocol, nitric oxide synthase is delivered intravascularly to inhibit the formation of neointimal lesions. The plasmid vector, pAH9 is derived from pcDNA3 (Invitrogen) and carries a cDNA encoding human iNOS under the control of the constitutive promoter CMV-immediate early promoter/enhancer. [At the 3' end of the iNOS encoding region are the BGH-polyadenylation signal and the partially deleted polyadenylation signal of SV40. The pAH9 vector also carries a kanamycin resistance gene.]

The pAH9 plasmid is expressed in *Escherichia coli*, purified using current Good Manufacturing Practices, and formulated as a DC-30 lipoplex (CAR-MP583). Data from the literature indicate that cationic liposomes similar to the ones used for manufacturing CAR-MP583 have been used in 2 clinical pilot studies. CAR-MP583 is administered via the Infiltrator® device following balloon angioplasty (with or without stenting). The Infiltrator®, designed specifically for percutaneous drug delivery, is an investigational device currently being used in two clinical studies in the United States under investigator-sponsored INDs. CAR-MP583 is being developed as a treatment for restenosis following standard balloon angioplasty procedures. The intended treatment group is patients requiring balloon angioplasty for coronary artery stenosis.

Injury to the endothelium plays an essential role in the pathogenesis of vascular disease and induces the local expression of mitogens and chemotactic factors that stimulate vascular smooth muscle and leucocyte migration and proliferation. A deficiency in nitric oxide (NO) production or bioactivity and/or excess of growth promoting factors favors the development of vascular lesions.

Pharmacological and gene transfer studies demonstrated that NO inhibits smooth muscle cell proliferation. Restoration of the beneficial effects of NO activity ("gain of function") may be of potential therapeutic benefit in several forms of cardiovascular disease. Thus, the overexpression of nitric oxide synthase (NOS) with subsequent NO generation may represent an ideal tool for cardiovascular gene therapy, since an endogenous factor normally present within the vascular wall (*i.e.*, NO) functions as the therapeutic product.

The sponsor has developed an advanced model of vascular injury (stent-induced intimal proliferation) in porcine coronary arteries and has demonstrated that liposome-mediated gene transfer of iNOS (CAP-MP583) can inhibit stent-induced intimal lesion formation by 40-50%. In this model, local gene transfer was accomplished using the iNOS gene construct complexed with cationic liposomes delivered by means of the Infiltrator® device which enables local delivery of a therapeutic substance directly into the vascular wall.

Two toxicity studies in rats given iNOS lipoplexes intravenously provided little classical toxicological information. These studies were conducted to demonstrate the "worst case scenario" of administering the entire dose directly into the circulation. There were no deaths, no clinical signs, no effects on clinical biochemistry or urinalysis tests and the few histopathology findings were not reproducible nor were they seen in both genders. Although no frank toxicity was demonstrated, this "worst case" appears not to present serious risk.

In studies with over 100 minipigs treated by means of the Infiltrator device, no unscheduled deaths have occurred. Among the 27 minipigs in the pivotal toxicology study, occasional intramural hematoma was seen immediately after local treatment and scarring occurred at later evaluation times. This supports the assertion that the Infiltrator® device presents no greater risk than that of alternative angioplasty devices. The histopathology findings in coronary arteries are consistent with those typically seen after catheter intervention in native, healthy coronary artery tissue. These data are best viewed as descriptive of the process of trauma commonly induced by the interventional device, pharmacological activity of CAR-MP583 immediately thereafter, and resolution within six weeks after

transfection. These effects on coronary artery morphology are predictable, dose related, and transient. Other histological effects were largely resolved within six weeks.

Biodistribution into the site of local application has been demonstrated by polymerase chain reaction (PCR) analysis, and distribution into other tissues has been found to be virtually nonexistent. When seen, PCR signals were very low, but detectable with one exception: in the Day 3 coronary arteries of minipigs given 10 µg CAR-MP583 strong PCR signals were seen. This is not surprising in light of the low doses of plasmid DNA administered and their local delivery directly into the intimal lining. This also is consistent with the histopathology findings in rats and minipigs.

The above data provide the rational basis for the doses selected for the REGENT I (**Restenosis Gene Therapy Trial I**) clinical trial. The purpose of REGENT I is to obtain safety and tolerability information about CAR-MP583. REGENT I will study a maximum of four doses of CAR-MP583, with up to 3 applications of the dose in the target lesion. Based upon the nonclinical dose-response data, the anticipated human dose is in the 2 µg range. In animal studies, the maximum feasible dose of 10 µg has shown no predictable toxicity or tissue target for accumulation.

Doses selected for REGENT I (0.5, 2.0, 5.0, and 10.0 µg) begin at a 20-fold margin below the maximum feasible dose. A safety committee comprising experienced cardiologists and interventionalists will adjudicate all adverse events. An independent data safety monitoring board comprising gene therapy clinical trial experts will evaluate all serious adverse events. Dose-escalation in REGENT I will be carefully controlled. The primary endpoint of this trial is MACE, defined as a combined clinical endpoint of death, Q wave or non-Q wave MI, emergent bypass surgery, or repeat target lesion revascularization 30 days after balloon angioplasty, treatment with iNOS-lipoplex gene therapy and stent placement.