4.RDEB.RAC Scientific Abstract A.Lane

Gene Transfer for Recessive Dystrophic Epidermolysis Bullosa (RDEB)

Epidermolysis bullosa (EB) is a family of inherited genetic blistering skin disorders. Children born lacking normal type VII collagen develop a severe, scarring EB subtype, recessive dystrophic epidermolysis bullosa (RDEB), which produces painful blisters and wounds on skin and mucous membranes. Type VII collagen normally anchors the epidermis (outer layer of skin) to the dermis (inner layer of skin). These open wounds are extremely painful for these individuals. RDEB subjects have a shortened life expectancy with early death from infection, organ failure or squamous cell carcinoma (SCC) associated with complications of their chronic wounds. Current therapy for RDEB consists of only palliative wound care and pain control. There are no therapies available that alter the course or severity of the disease.

In past and current studies, we have demonstrated that genetically corrected keratinocytes or fibroblasts can correct human EB skin tissues grafted onto immune deficient mice. The purpose of this study is to treat the skin surface of patients who have RDEB with their own keratinocytes which have been genetically engineered with a durable retroviral vector expressing type VII collagen. We expect that these grafts will provide long term healing of the wounds that previously would never heal.

Depending on the mutation, some RDEB patients may express the amino-terminal fragment of type VII collagen (NC1) and we call these patients NC1[+]. RDEB patients who do not express NC1 we call NC1[-]. Our group has documented an increased risk of development of SCC in patients that are NC1[+]. In addition, epidermolysis bullosa acquisita (EBA) is an acquired autoimmune blistering disease where auto antibodies develop to the NC1 portion of the anchoring fibrils. We expect that NC1[+] RDEB subjects will not develop EBA to keratinocytes grafts that express type VII collagen and that these grafts will cause very little increase in the risks of SCC on NC1[+] subjects.

Between two to ten RDEB subjects 18 years old or older will be recruited for this trial. RDEB will be confirmed by immunofluorescent (IF) and electron microscopic (EM) analysis of the subject's skin as well as by genetic confirmation. Culture of the subject's keratinocytes will confirm NC1[+] status. Gene transfer will be accomplished on the subject's cultured keratinocytes using a recombinant retroviral vector pLZRSE-Col7A1 containing the full-length human Col7A1 cDNA. The type VII expressing keratinocytes grafts will be grown into epithelial sheets which will be placed on non-healing wounds on the subject's trunk. Approximately two to four 50 cm^2 epithelial sheets will be used in a single grafting session.

The endpoint of this study will be to document engraftment of genetically engineered keratinocytes. We have defined the first clinically significant endpoint as a detectable expression of type VII collagen at the basement membrane of grafted wounds, expression of type VII collagen in the grafted keratinocytes and presence of anchoring fibrils. The sites selected for initial grafting will be sites

that are usually protected from trauma. During this trial we do not intend to induce injury to the experimental sites in order to confirm improved epidermal adhesion. We define normalization of the BMZ by positive IF for type VII collagen along the basement membrane zone (BMZ) and in grafted keratinocytes and by appearance of anchoring fibrils on EM. The subject's grafts will be biopsied at 12 weeks and 6 months in order to evaluate the BMZ.

This trial will be accomplished as a Phase I or Phase I/II trial dependent upon GMP production of the retroviral vector and/or human keratinocytes. Each subject will be closely observed for potential skin cancer development and/or expansion from the grafted areas to the larger wound site or onto non-blistered or non-ulcerated skin.