Project Title: A Pilot Study of the Restoration of Full-Length Type VII Collagen in RDEB Patients with Nonsense Mutations After Topical, Intradermal and Intravenous Gentamicin Treatment

HSIRB Title: Gentamicin Therapy for Recessive Dystrophic Epidermolysis Bullosa Patients With Nonsense Mutations

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Brief Title: Gentamicin for RDEB

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Statistical Analysis Plan

This pilot, proof-of-concept, study is an un-blinded, single cohort, open study of 7 RDEB patients with proven nonsense mutations in their *COL7A1* gene. All of the patients have had their keratinocytes and fibroblasts placed into culture, and the presence of gentamicin in the cultures induced read-through of their nonsense mutation and the production of full-length type VII collagen (C7) *in vitro*. They all have proven diminished C7 and anchoring fibrils (AFs) in the dermal-epidermal junction of their skin. This is a unique and ideal cohort for the questions that are posed in this grant proposal. The expression of C7 and AFs will be measured in biopsy specimens taken before and at 30, and 90 days after start of treatment with topical, intradermal, and intravenous gentamicin. Quantitative C7 expression by IF will be performed by computer-assisted image analysis using NIH Image J software as previously described. The patients' results will compared against their baseline expression and the expression of C7 in normal human skin, a positive control run with each experiment. AFs will be assessed by immuno-electron microscopy and enumeration of AFs as performed in our previous study (Woodley DT et al., JAMA). The results of C7 and AF expression levels and changes (as well as other biological measures reflecting the effects of gentamicin [e.g. changes in the sizes of lesions] and clinical (EBDASI scores) and patient reports will be summarized with plots to display trends over time and standard descriptive statistics (point estimates and associated 95% confidence intervals). The co-primary efficacy endpoints will be (1) “C7 success” defined as developing a C7 expression level that is at least 35% of the normal control and (2) “AF success” defined as developing an AF expression level that is at least 35% of the normal control. Historical data indicate that the likelihood of an increase of 35% or greater is extremely, extremely unlikely; hence such an increase in C7 or AF expression will be attributed to gentamicin activity. At the 30- and 90-day assessments, the number of patients with a successful increase in C7 expression and in AF expression will be reported, as well as the mean/median changes and associated 95% confidence intervals. These analyses will be descriptive; the purpose is to document large, biologically/clinically important changes and to use this information to guide the design of future studies.

Safety parameters including audiometry and standard routine laboratory tests for blood, liver, and kidney function will be performed and the normal limits are well established. Adverse events and toxicities will be recorded for all patients and scored using the MedDRA 19.0 criteria; in particular, based on known toxicities associated with gentamicin, calculated creatinine clearance and pure tone audiometry will be measured as described above, and based on these, “stop criteria” will be used to stop treatment. All patients who begin treatment with gentamicin will be accounted for in all analyses.