Use of Zikani's Ribosome Modulating Agents (RMAs) for Treating Recessive Dystrophic & Junctional Epidermolysis Bullosa (RDEB & JEB) with Nonsense Mutations

Mei Chen, Yingping Hou, Michelle Hao, Soheil Aghamohammadzadeh, Esteban Terzo,

Roger Clark, Vijay Modur

Zikani Therapeutics, Boston, United States

University of Southern California, Los Angeles, United States

Background: Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a genetic skin condition characterized by skin tearing and unremitting blistering upon minimal trauma. Repeated blistering, fibrosis and scarring paves the way to a higher than 90% chance of developing aggressive squamous cell carcinoma by age 55. RDEB is caused by mutations in the *COL7A1* gene encoding collagen type VII (C7) the major component of anchoring fibrils mediating epidermis-dermis adherence. Almost 25% of RDEB patients carry nonsense *COL7A1*-mutations that lead to premature termination codons (PTC). Similarly, almost 80% of Junctional Epidermolysis Bullosa (JEB) cases are caused by mutations in the *LAMB3* gene encoding the β3 subunit of laminin 332 and approximately 95% of JEB associated *LAMB3* mutations are nonsense mutations. Currently there is an unmet need for the treatment of RDEB and JEB.

Zikani Therapeutics has discovered an array of macrocyclic compounds with ring structures similar to macrolide antibiotics that can facilitate readthrough activity of nonsense mutations in the *COL7A1* and *LAMB3* genes by acting as Ribosome Modulating Agents (RMAs). The medicinal chemistry synthetic advancements of these macrocyclic compounds have allowed targeting the human ribosome while preserving the structural elements responsible for safety and pharmacokinetic profile of clinically used macrolide antibiotics.

Methods: C7 expression was used as a measure of readthrough activity by immunoblot assays in two primary human fibroblasts from RDEB patients (R578X/R578X and R163X/R1683X-*COL7A1)*. Similarly immunoblot assays in C325X/c.629-12T>A-*LAMB3* keratinocytes were used to measure readthrough activity for JEB. The relative readthrough activity of each compound was measured relative to Gentamicin. An imaging-based fibroblast migration assay was used as an assessment of C7 functionality in RDEB-fibroblasts over 16-20 hrs. Incubation period for above experiments was 48 hrs for RDEB fibroblasts and 72 hours for JEB keratinocytes.

Results: 9 RMAs demonstrated increased protein expression in both patient RDEB fibroblasts. The highest readthrough activity at tested concentrations without cytotoxicities increased protein expression up to 179% of Gentamicin (400 µg/ml), with favored readthrough activity in R163X/R1683X-*COL7A1* fibroblasts. Concurrent with protein expression, fibroblast hypermotility phenotype observed in RDEB was rescued by reducing motility by ~35% to WT levels (same level as 690 µM Gentamicin treated cells). Laminin β3 expression was also showed to be increased by 6 RMAs in keratinocytes to 33-83% of (400 µg/ml) Gentamicin.

Conclusions: To date, 9 RMAs have been identified that enhance the expression of functional C7 in a mutation-dependent manner in two different RDEB patient fibroblast backgrounds (R578X/R578X and R163X/R1683X-*COL7A1)*. A further 6 RMAs have been identified that enhance the readthrough of C325X-*LAMB3* in JEB patient keratinocytes. Based on the clinical trial conducted by us with topical

gentamycin in 2017, Zikani's RMAs achieve clinically significant levels of read through for the treatment of recessive dystrophic and Junctional Epidermolysis Bullosa.

Key words:

Epidermolysis Bullosa, Nonsense mutation, Readthrough, Ribosome modulation