Facile discovery of Ribosome Modulating Agents (RMAs) for Treating Cystic Fibrosis Class I Nonsense Mutations

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Background: Approximately 10% of patients diagnosed with cystic fibrosis (CF) harboring Class I CFTR mutations have nonsense mutations resulting in protein truncation with lack of functional CFTR ultimately leading to impaired lung and digestive capacity. Almost 40% of class I patients harbor one allele of the G542X-*CFTR* mutation. The remaining 60% harbor either W1282 (20%), R553X (18%), R116X (5%) or ultra-rare (18%) mutations. These patients have few if any clinical options resulting in a high unmet need as clinical trials so far have had limited success.

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 CFTR gene 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: Zikani RMA compounds were tested on five different *in vitro* and *ex vivo* assays in stable cell lines and primary (Human Bronchial Epithelial (HBE)) cells including a dual reporter (16HBE, G542X-*CFTR*), ELISA (HEK293, G542X-*CFTR*) and immunoblotting (HEK293, G542X-*CFTR*) alongside a functional membrane depolarization FLIPR assay (HEK293, G542X-*CFTR* minigene). Active compounds were confirmed in Ussing chamber (HBE, G542X/F508del, G542X/G542X) and intestinal organoid swelling (G542X/F508del, G542X/G542X) assays.

Results: To date, a total of 266 RMAs have been tested, of which, 46 were found to be active on at least one assay (17% hits). RMAs showing readthrough activity in at least one of the biochemical assays (28 hits, 10%) or the membrane depolarization assay (19 hits, 7%), were tested in the Ussing chamber assay to confirm CFTR readthrough activity in combination with a CFTR potentiator (1uM VX-770). 2 compounds have thus far shown activity in the Ussing chamber assay in HBEs with G542X/F508del-*CFTR* background (1% of tested compounds). At E_{MAX}, 33 µM ZKN-0214 has shown efficacy that is similar to 250 µM G418 (widely accepted as the most potent readthrough agent), At 30uM, ZKN-292 exceeds 100 µM G418 activity by 41% and has almost 80% of the activity on the G542X allele that VX-809 has on correction of F508del allele. These compounds were specific to the G542X mutant allele as the show no activity in F508del/F508del homozygous HBEs.

Conclusions: Directed discovery of RMA represents a novel and efficient method to identify novel read through agents with significant activity on clinically validated *ex vivo* Ussing chamber assays with 2 of Zikani's RMAs (ZKN-0214 and ZKN-0292). These compounds show efficacy similar to G418 (acting on the G542X allele) or VX-809 (acting on the F508del allele) suggesting potential for clinical application. As part of continued efforts, lead optimization is currently ongoing to improve efficacy after which seamless transition to IND studies are expected due to their potential for high oral bioavailability, predictable PK and safety profile.

Key words: Cystic Fibrosis, Nonsense mutation, Readthrough, Ribosome modulation