Disperazol: treating *Pseudomonas aeruginosa* catheter associated urinary tract biofilm infections.

Michael Graz[1,3], Katrine Qvortrup[2], Claus Moser[1], Tim Tolker-Nielsen[1], Michael Givskov[1,3]

[1] Costerton Biofilm Center, University of Copenhagen, Denmark; [2] Department of Chemistry, Technical University of Denmark, Denmark; [3] Disperazol Pharma APS, Humlebaek, Denmark

Disperazol is a small molecule, to be co-administered with antibiotics for treating recalcitrant *Pseudomonas aeruginosa* biofilm infections. Our primary indication is hospital-acquired, catheter associated urinary tract infections (CAUTI). About 75% of hospital acquired UTIs are due to biofilms on catheters which are installed in up to 25% of hospitalised patients. 23% of these biofilms are positive for *P.aeruginosa*, indicating that *P.aeruginosa* CAUTIs have become a significant healthcare burden.

In response to external cues, biofilm bacteria can turn from sessile into a planktonic life-mode and vice-versa. Within the bacteria, high levels of c-di-GMP sustain biofilm formation, whereas low c-di-GMP activates several processes causing biofilms to dismantle and liberate antibiotic susceptible bacteria. In contrast to their planktonic counterparts, biofilm bacteria show a remarkable capacity to withstand multiple classes of antibiotics. Our focused approach to mitigate biofilm infections is to drug the c-di-GMP signalling pathway in *P.aeruginosa*.

We identified BifA of *P.aeruginosa* as a new antimicrobial target. A recent breakthrough with the Jenal laboratory in Basel revealed the mechanism of Disperazol's action; it's a ligand that activates the phosphodiesterase activity of BifA, one of 41 enzymes modulating intracellular c-di-GMP levels in response to external cues. We have confirmed that Disperazol exhausts the c-di-GMP pool and prevents *P.aeruginosa* from assuming the biofilm life mode.

Furthermore, combining biofilm-dismantling with antibiotic treatment, bacterial susceptibility to conventional antibiotics is significantly increased. In a murine CAUTI model utilising *P.aeruginosa*-coated catheters, a combination of orally administered 3µg Disperazol/g BW and 1µg Ciprofloxacin/g BW proved to be 100-fold more efficient than oral Ciprofloxacin alone.

The efficacy of Disperazol is not affected by the (i) Ciprofloxacin-induced efflux pump *mexAB* including regulatory defects caused by *mexS, mexT, mexE or nfxB* mutations; (ii) nor by high Calcium concentration in CF lungs; and (iii) nor by alginate overproducing strains from CF sufferers.

Go/No-go toxicity studies indicates that Disperazol is safe: with minimal plasma protein binding; no effect on cellular ATP levels; and minimal effect on various Cytochrome isoforms.

We are raising $6 million to validate Disperazol's efficacy in a non-rodent model, and progress to a Phase 1 first in human trial in 2025.