Beyond Antibiotics: New Horizons in Treating *Burkholderia* Species Infections

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(See the article by Greenberg et al, on pages 1822–1830.)

Antibiotic-resistant strains of pathogenic bacteria have always been an issue, but in recent times the need for new antimicrobial treatments has never been more serious. Resistance is on the rise, while antibacterial discovery and development are on the decline [1]. The urge for new treatment modalities that go beyond antibiotics is clearly illustrated in the care of patients with chronic granulomatous disease and cystic fibrosis (CF), who are often colonized with gram-negative *Burkholderia* species that are intrinsically antibiotic resistant [2, 3]. In patients with CF, the vicious cycle of infection, sputum retention, and inflammation perpetuates itself, because host defense proteins released by neutrophils and macrophages stimulate mucus secretion and subsequent breakdown [2]. Multidrug resistance of *Burkholderia cepacia* complex (Bcc), which is associated with a rapid decline in pulmonary function and increased mortality, has been found to be present in up to one-fifth of patients with CF [2, 4]. In this issue of the Journal, Greenberg and colleagues [5] provide us with exciting and promising data on the use of antisense molecules as new therapeutics for *Burkholderia* species infections.

In this elegant study, the researchers targeted a gene called acyl carrier protein (*acpP*), which is known to be of importance for growth in Bcc, by making use of phosphorodiamidate morpholino oligomer (PMO) antisense technology. Peptide-conjugated PMOs (PPMOS) are water-soluble single-stranded DNA analogues that are resistant to degradation by ribonucleases and able to enter cells readily in order to bind to messenger RNA in a sequence-specific manner to prevent translation [6]. Sequence-specific approaches to inhibit bacterial replication are attractive in part because of the low likelihood of target sequence homology between pathogen and host, thus minimizing the potential for toxicity from cross-reactivity [6]. Greenberg et al [5] synthesized PPMOs directed against *acpP* of Bcc and tested their antimicrobial potency in vitro and in vivo.

The results are exciting. Excellent efficacy of AcpP PPMO is demonstrated in vitro, in neutrophil-killing assays, and most importantly in vivo in a murine model of Bcc infection. In a mouse model of chronic granulomatous disease, AcpP PPMO–treated mice show a nearly 80% reduction in mortality, compared with water-treated controls after an intraperitoneal challenge with *Burkholderia multivorans*. Importantly, mice treated with AcpP PPMO that did survive this experiment showed only limited organ damage, as demonstrated by histopathological analysis. It must be emphasized, however, that if this approach is to move forward, future critically important murine studies should include investigations on the route of infection (intranasal or systemic), route of PMO administration (pulmonary or intravenous), and dosing schemes as well as the co-administration of antibiotics to better mimic the clinical scenario.

The possible clinical implications of the current positive findings on antisense PPMO gene-targeted therapy go well beyond the care of patients with chronic granulomatous disease and CF. The present findings in Bcc infection of Greenberg et al [5] are consistent with previous positive studies on the efficacy of PPMOs against other pathogens. PPMOs are now known to be capable of viral replication inhibition—leading in some cases to increased survival of experimentally infected mice—in poliovirus, coxsackievirus B3, dengue virus, West Nile virus, Venezuelan equine encephalitis virus, respiratory syncytial virus, Ebola virus, and influenza A virus infection [6]. Furthermore, antisense molecules have been shown to be effective against bacterial infection in vitro with *Salmonella enterica*, *Mycobacterium smegmatis*, and *Klebsiella pneumoniae* [7–9] and in vivo in a murine model of intra-peritoneally injected *Escherichia coli* infection [10]. Now Greenberg et al [5] extend...
these findings toward the *Burkholderia* genus. Because acpP is well conserved across members of the *Burkholderia* genus, AcpP PPMO treatment will most probably also work for other *Burkholderia* species, such as the recognized biological threat agents *Burkholderia pseudomallei* and *Burkholderia mallei* [11].

Previous studies on PPMOs as a strategy to treat infectious diseases have focused mainly on their potential to inhibit viral and bacterial replication. In theory, however, antisense-based therapy could be used to silence any gene of interest. For example, in a mouse model of Duchenne muscular dystrophy a PPMO that ingeniously delivered a splice-switching oligonucleotide that could specifically restore dystrophin in these mice caused long-term improvement in the cardiac hypertrophy and diastolic dysfunction associated with Duchenne disease [12]. In the case of *Burkholderia* species infections, it is tempting to assess the feasibility of this method to target bacterial functions essential for infection, such as the putative virulence factors lipopolysaccharide, flagella, and type III secretion systems (TTSS) [11].

Possible competitors of PPMO-based therapies could include monoclonal antibodies that specifically target bacterial virulence factors. In a murine model of *Pseudomonas aeruginosa* infection, a pathogen closely related to the *Burkholderia* genus and another important pathogen in patients with CF, it was recently shown that instillation of a single dose of a human antibody Fab fragment that binds to the PcrV protein of the *P. aeruginosa* TTSS provided protection against lethal pulmonary challenge of *P. aeruginosa* and led to a substantial reduction of pulmonary bacterial counts [13]. This treatment modality is currently being tested in 2 independent randomized, placebo-controlled clinical trials in which the safety, pharmacokinetics, and efficacy of recombinant human Fab antibody directed against the PcrV protein of the *P. aeruginosa* TTSS are being evaluated in patients using mechanical ventilators in an intensive care unit who are colonized with *P. aeruginosa* and in CF patients infected with *P. aeruginosa* (ClinicalTrials.gov identifiers NCT00 691587 and NCT00638365, respectively). The first results of the study among patients using mechanical ventilators who are colonized with *P. aeruginosa* are promising: a reduction in *P. aeruginosa* pneumonia and a trend toward lower mortality was seen in the patients who were treated with the anti-TTSS antibody [14]. The potential clinical benefit of monoclonal antibodies directed against bacterial virulence factors lipopolysaccharide, flagella, and TTSS is underscored by a recent published multicenter randomized controlled trial of 2 novel neutralizing fully human monoclonal antibodies against *Clostridium difficile* toxins A and B for the secondary prevention of *C. difficile* infection [15]. In the intention-to-treat analysis, recurrent infection developed in 7 (7%) of 101 patients in the antibody group, compared with 25 (25%) of 99 patients in the control group. In both mentioned trials, the monoclonal antibodies were not immunogenic and had an adverse event profile similar to that of the placebo [14, 15]. In conclusion, the present data constitute a proof of principle for the use of PPMOs as potential therapeutics in *Burkholderia* species infections. The use of antisense PPMOs that specifically target bacterial genes known to be essential for growth or virulence or the possible future development of monoclonal antibodies directed against bacterial virulence factors could well be the beginning of a new era of novel therapeutics and immunotherapy for the management of *Burkholderia* species infections.

References