A multifunctional bispecific antibody against *Pseudomonas aeruginosa* as a potential therapeutic strategy

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Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative bacteria that is responsible for significant morbidity and mortality in human populations (1). It is a common cause of hospital-acquired and ventilator-associated pneumonia, opportunistic infections in immunosuppressed and burns patients, and most notably a frequent and troublesome bacteria in the airway of people with cystic fibrosis (1,2).

P. aeruginosa possesses several features that render it a formidable pathogen and a particularly challenging target to treat in patients (3). *P. aeruginosa* have a number of virulence factors and strategies to evade host-defense systems. Compounds such as exotoxin A, pyocyanin, phospholipase C, proteases and hydrogen cyanide are all released that cause host damage and subvert the host immune response (2). The bacteria are capable of entering different states including the formation of mucoid biofilm-forming colonies where they produce large quantities of alginate and are protected from both host-defense mechanisms and antibiotics (4). *P. aeruginosa* are also capable of quorum-sensing via a homoserine-L-lactone system (5).

In the lungs of people with cystic fibrosis it is known that genetically and phenotypically diverse populations of *P. aeruginosa* exist in chronic infection (6). In this situation a number of mutations leading to antimicrobial resistance appear, such as efflux pumps and hypermutability. The cystic fibrosis airway is particularly vulnerable to chronic infection with *P. aeruginosa* due to reduced mucociliary clearance, impaired innate immunity and abundance of extracellular DNA from necrotic neutrophils in mucus, which provides a support for the biofilm matrix and a hypoxic niche (2). *P. aeruginosa* infection is associated with significantly worse clinical outcomes in people with cystic fibrosis (7). Considerable efforts are therefore taken in clinical cystic fibrosis management to firstly prevent infection by avoiding contact with other patients, secondly in attempting to eradicate *P. aeruginosa* at the first isolation with aggressive antimicrobial regimens and thirdly in reducing associated morbidity in those that are chronically infected or colonised with the organism (8-10).

The use of antibiotics targeted against *P. aeruginosa* is a mainstay of cystic fibrosis treatment in the form of oral, intravenous or nebulised therapy. However, given that it is a life-long condition, problems with multiple drug resistance are often significant and eradication of *P. aeruginosa* infection becomes effectively impossible once it is chronically established in the majority of individual patients (11,12). Ultimately issues with pan-resistant *P. aeruginosa* are especially relevant in consideration of suitability of patients with advanced disease for lung transplantation where it is essential that an antimicrobial cocktail is available that will kill the bacteria in the immediate post-transplant phase when high levels of immunosuppression are required (13). Furthermore allergies to antibiotics are not uncommon in people with cystic fibrosis and may limit which antimicrobials can be prescribed (14).

These issues coupled with the relative dearth in the development of new antibiotics in general at present mean that alternative approaches to tackle bacterial infections are urgently required (15). The use of antibodies targeted against bacteria, so-called "passive immunisation", represent one such option. The general concept is not new and indeed dates back to the pre-antibiotic era

when hyper-immune serum was used to treat infections such as diphtheria and tetanus (16). In both of these examples serum was an effective treatment due to its ability to neutralise the toxins that are a key part of disease pathogenesis. Serum treatment was less effective against other bacteria such as pneumococcus or *Staphylococcus aureus*, reflecting more diverse associated pathophysiology and heterogeneity amongst the organisms themselves, and the subsequent advent of antibiotics made such approaches effectively redundant (16). By way of an example, the use of passive immunisation with palivizumab to protect against respiratory syncytial virus in high-risk infants during winter months is widely accepted (17).

In a paper by DiGiandomenico *et al*. published in *Science Translational Medicine* in November 2014, the authors report work performed by MedImmune to develop a multifunctional bispecific antibody against *P. aeruginosa* as a potential therapeutic and/or preventative strategy (18). Data is presented showing a positive protective effect of the antibody against *P. aeruginosa* infection in the lungs of mice (18).

The authors have previously developed monoclonal antibodies directed at epitopes of *P. aeruginosa* Psl, an exosaccharide required for biofilm formation that also reduces host phagocytic function, and the PcrV protein, which plays a key role in enhancing the type III secretion system and subsequent cytotoxicity by bacterial toxin injection into host cells (19). Other investigators have performed an early phase clinical study of an antibody, KB001, targeted against the PcrV protein and type III secretion system in *P. aeruginosa* in people with cystic fibrosis. The study was primarily designed around safety and pharmacodynamics but results suggested a trend towards reduced airway inflammation at 28 days but generated no statistically significant differences in *P. aeruginosa* density or clinically relevant outcomes (20).

In the 2014 paper DiGiandomenico *et al*. hypothesised that combining mechanisms of action against both PsI and PcrV in one monoclonal antibody would be more practical than co-administration of two antibodies and potentially more efficacious. The authors therefore constructed bispecific antibodies targeting both Psl and PcrV with varying intermolecular distances between antigen binding sites. *In vitro* assessment of opsonophagocytosis and inhibition of cytotoxicity and attachment of *P. aeruginosa* to epithelial cells, showed one particular construct, BiS4αPa, to provide better protection than other constructs with differing interparatopic distances (BiS2αPa and BiS3αPa), individual mAbs for Psl and PcrV and the combination of these mAbs. This was attributed to the optimal interparatopic distance provided by BiS4αPa to allow simultaneous binding at both anti-Psl and anti-PcrV sites (18).

In vivo assessments of prophylactic protection conferred by BiS4αPa against *P. aeruginosa* strains were made using a murine lethal acute pneumonia model. Significantly improved protection for acute pneumonia was found with lower concentrations of BiS4αPa administered 24 h prior to infection with the highly pathogenic 6206 and the multi-drug resistant 6077 *P. aeruginosa* strains in comparison to either monoclonal antibodies to Psl or PcrV alone. Therapeutic effects were also evident when BiS4αPa was administered an hour post infection. Using this model, administration of BiS4αPa resulted in reduced bacterial dissemination and histological evidence of lung injury following infection with each of these strains (18).

Opsonophagocytic activity and anti-cytotoxic effects against clinical isolates of *P. aeruginosa* following protection with BiS4αPa in this model were also significantly better than those seen with an immunoglobulin G control. However, information regarding how this effect compared with individual or the combination of mAbs against Psl and PcrV is not included. Furthermore prophylactic and therapeutic administration of BiS4αPa against *P. aeruginosa* strains with multi-drug resistance (with the exception of resistance to colistin) in this acute pneumonia model prevented lethality. BiS4αPa was also found to show dose dependent protective and therapeutic effects in comparison to an immunoglobulin G control in multiple other models including immunocompromised pneumonia, thermal injury and bacteraemia (18).

To ascertain the mechanism of superior anti-cytotoxic BiS4αPa action, the bispecific antibody construct was modified further by firstly replacing the anti-Psl binding unit with negative control sequence, secondly replacing the anti-PcrV binding unit with a negative control sequence, and thirdly the inclusion of a point mutation to reduce opsonophagocytic killing function without affecting PcrV and Psl binding. *In vitro* assessment of these modified constructs together with investigation in the acute pneumonia model suggested that potentiated effects of BiS4αPa were due to the anti-cytotoxic activity enhanced by its action against Psl, in addition to its opsonophagocytic killing and inhibition of cell attachment (18).

To determine the benefits of BiS4αPa use in conjunction with existing antibiotic therapy sub-therapeutic doses of ciprofloxacin or meropenem were administered with sub-prophylactic doses of BiS4αPa 24 h pre-infection in the

murine acute pneumonia model. All mice were still alive 120 h post-infection following combination therapy, whereas those who received either BiS4αPa or either antibiotic in isolation died from infection. Combination therapy of BiS4αPa with tobramycin against the tobramycin-resistant *P. aeruginosa* strain 6077 showed improved survival and a significantly lower bacterial burden in the murine pneumonia model. These findings suggest that in this model, there are advantages of complementing antibiotic therapy with this bispecific construct, particularly in the context of drug resistant strains of *P. aeruginosa* (18).

This work has generated a multi-mechanistic clinical candidate known as MEDI3902 for potential use in the treatment or prevention of *P. aeruginosa* infections. The authors also comment that multifunctional antibodies may be a promising platform for targeting other antibiotic-resistant pathogens (18).

Such approaches are complex however and are not without associated problems and possible limitations. The first and most obvious issue is that the antibody remains at an early-stage of development and assessment of its efficacy. The work described in the paper was performed in a murine acute model of pneumonia that arguably does not replicate the situation in humans during *P. aeruginosa* pulmonary infection. Careful and cautious evaluation of the antibody in man is required. There are several examples in the literature of antibacterial antibodies that have shown promising efficacy in pre-clinical animal models only to be proven ineffective in human studies (16,21-23). In particular the airway in people with cystic fibrosis, where *P. aeruginosa* is such a clinical problem, is notably more complex, with a combination of defective host defense, challenging physiology and adaptation of *P. aeruginosa* to facilitate chronic infection (2).

Other important factors include safety concerns about the administration of biological therapies and risk of potentially significant allergic reactions, administration involving injections and high financial cost. With such specific treatment clearly accurate and rapid microbiological diagnosis is an essential requirement that is often challenging in current clinical practice. In addition most antibiotics provide some spectrum of coverage of multiple bacteria that is not likely to be provided by a targeted antibody.

Despite these concerns the development of multifunctional antibacterial antibodies represents an exciting new therapeutic approach that may potentially address an area of great clinical need. In future years as knowledge of bacterial pathogenesis advances along with refinements in monoclonal antibody design and manufacturing plus development of rapid molecular diagnostics in clinical microbiology the relevance and importance of antibody-based approaches is only likely to increase (16).

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Footnote

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