Cystic fibrosis infections: treatment strategies and prospects

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Abstract

Pseudomonas aeruginosa and Burkholderia cepacia are the two major Gram-negative rods that colonize/infect the lungs of patients with cystic fibrosis (CF). These organisms may cause progressive respiratory failure, although occasionally more rapid infections result in the ’Cepacia’ syndrome. Many antibiotics have been used against Pseudomonas and Burkholderia, but once chronic colonization has been established, eradication of these organisms is rare. Drug therapy for CF patients is compromised by a number of bacterial factors that render the infectious agents resistant to antibiotics, including efflux pumps that remove antibiotics, lack of penetration of antibiotics into bacterial biofilms, and changes in the cell envelope that reduce the permeability of antibiotics. Any combination of these mechanisms increases the likelihood of bacterial survival. Therefore, combinations of antibiotics or of antibiotic and nonantibiotic compounds are currently being tested against Pseudomonas and Burkholderia. However, progress has been slow, with only occasional combinations showing promise for the eradication of persistent Gram-negative rods in the airways of CF patients. This review will summarize the current knowledge of CF infections and speculate on potential future pathways to treat these chronic infections.

Cystic fibrosis (CF) and respiratory failure

CF is the most common lethal inherited disorder affecting Caucasians, with an incidence of approximately one in 2500 (Campana et al., 2004). CF results from mutations in the CF transmembrane conductance regulator gene (cftr). CFTR normally functions as a cAMP-regulated chloride channel, but also as a regulator of other channels (McAuley & Elborn, 2000; Dorwart et al., 2004). While the exact mechanisms by which defects in CFTR result in chronic suppurative lung disease are not yet clear, at least 90% of patients with CF die from respiratory failure. Defects in CFTR result in decreased chloride secretion into the airways and increased sodium absorption from the airways. The combination of these defects results in relative dehydration of the airway mucus, which in turn results in reduced mucociliary clearance, mucous retention, and chronic infection (Boucher, 2002).

While young patients with CF are generally infected with Haemophilus, later, Staphylococcus becomes a significant pathogen. Still later, Pseudomonas becomes the major pathogen (Gilligan, 1991; Fig. 1). Initially, Pseudomonas colonization occurs with nonmucoid strains; with time, most – but not all – of these strains switch on the mucoidy gene such that they show increased resistance to various antibiotics. Attempts at eradication of a mucoid Pseudomonas are generally less successful than attempts at eradication of early, nonmucoid variants of Pseudomonas aeruginosa. Given the importance of Pseudomonas and Burkholderia, this review will consider mainly Gram-negative rods and the many adaptations that fit them for persistence in the CF airway, together with antibiotic and other therapeutic options.

Over the past four decades, outcomes for patients with CF have improved dramatically, with medium survival now approximately 40 years. The mainstays of therapy for CF, such as nutritional support and mechanical mucociliary clearance, are now supplemented with aggressive antibiotic regimens intended to suppress or eradicate bacterial colonization, anti-inflammatory agents, and new approaches that improve mucociliary clearance. Many trials have shown utility with nebulized antibiotics such as
The problem of drug resistance

Progressive airway infection in CF subjects generally begins with an early *P. aeruginosa* infection that resembles bronchitis. This is often cleared by antibiotics, but subsequently replaced by new, more virulent, and resistant strains that can become chronic colonizers of the respiratory tract. These chronic infections are more difficult to eradicate with current therapies (Burns et al., 2001). *Pseudomonas* is the most common organism found in the CF airway (Gilligan, 1991; Lyczak et al., 2002; Gibson et al., 2003). In contrast, the *B. cepacia* complex is relatively less common, but may exhibit particular virulence in the CF airway. *Burkholderia* complex organisms are often more difficult to eradicate as the organism may be intrinsically resistant to multiple antibiotics, with susceptibility profiles that differ from those in non-CF patients (Moss, 1995; Govan & Deretic, 1996; Ramsey, 1996). As occasional individuals show a rapid deterioration following colonization with *Burkholderia*, the importance of this organism cannot be underestimated (Liou et al., 2001).

Multidrug resistance (MDR), as defined by the CF Foundation (http://www.cff.org/), is the resistance of an organism to two out of three different classes of antibiotics, namely, β-lactams, aminoglycosides, or quinolones. In one study of aminoglycoside-modifying enzymes in 1075 non-CF *P. aeruginosa* strains, at least one enzyme was found in 74% of isolates and only 26% were found to have impermeability as the sole mechanism of resistance to amikacin (Hurley et al., 1995). However, in CF isolates, impermeability is by far the most prevalent mechanism of resistance to amikacin (Hurley et al., 1995; MacLeod et al., 2000). Estimates suggest that 25–45% of adult CF patients are chronically infected with multiresistant bacteria within their airways (Lechtzin et al., 2006).

The *P. aeruginosa* PA01 genome sequence has revealed at least 12 efflux pumps of the resistance nodulation cell division (RND) family (Lomovskaya & Watkins, 2001; Piddock, 2006). These efflux pump-related mechanisms present new challenges to the treatment of infections (Gillis et al., 2005). Disruption of the outer membrane by aminoglycosides competing with divalent cations for lipopolysaccharide-binding sites is well documented. This disruption is overcome by the presence of excess (8 mM) Ca2+ or Mg2+, causing a marked reduction in susceptibility to several aminoglycosides by up to 64-fold in several strains of *P. aeruginosa* (Mao et al., 2001). This same study reported the surprising finding that this antagonism only occurred in strains that contained a functional MexXY-OprM efflux pump. Interestingly, despite azithromycin showing promise in the treatment of chronic CF infections (Jaffe et al., 1998; Wolter et al., 2002; Saiman et al., 2003), there was a correlation between the presence of the MexCD-OprJ RND...
efflux pump and biofilm-specific azithromycin resistance (Gillis et al., 2005). Finally, a recent study has identified a novel P. aeruginosa efflux pump that mediates biofilm-specific resistance to tobramycin, gentamicin, and ciprofloxacin (Zhang & Mah, 2008). It is now generally accepted that a synergistic interaction between efflux due to tripartite RND pumps extruding antibiotics, and decreased uptake through the outer membrane, results in increased resistance (Zgurskaya & Nikaido, 2000). Beyond efflux-mediated resistance to antibiotics, a link is now established between efflux pumps and resistance to host natural substances such as defence molecules bile, and hormones and processes such as colonization and persistence in the host (Piddock, 2006).

**Biofilms and adaptive genetic changes**

Natural biofilms nearly always harbour a multitude of microbial species and multiple genetic strains of the same species—the region around a single tooth, for example, may be sheathed in a biofilm community of several hundred species (Paster et al., 2001). Even in a single-species biofilm, strain diversification is normal (Kolter & Greenberg, 2006). Such diversification of either species or strains, or both, may be due to adaptations to layered microniches within a single biofilm with a scaffolding matrix of polysaccharides, proteins, or even nucleic acids. The change from a nomadic to a sessile lifestyle is triggered by an increase in cyclic-di-GMP, accompanied by an inverse regulation of genes involved in virulence, motility, and matrix components (Kolter & Greenberg, 2006). In addition to the common CF pathogens P. aeruginosa, B. cepacia complex, Staphylococcus aureus, and Haemophilus influenzae, other bacterial colonizers that invade the CF airways and contribute to morbidity, including Stenotrophomonas maltophilia, Alcaligenes xylosoxidans, and Klebsiella spp. and pathogenic viruses and fungi are also common (Harrison, 2007). The prevalence of major bacterial respiratory infections by age group in CF subjects is depicted in Fig. 1. It is clear from a number of studies that coinfecting species interact, both synergistically and antagonistically, and evolve in response to selection pressures exerted by these interactions and by the host environment (Harrison, 2007). Coinfections probably result from sequential, rather than simultaneous, colonization, but it is clear from the succession of species in patients over time that P. aeruginosa is the predominant infectious agent. Although there is some evidence of synergistic interactions between P. aeruginosa and other coinfecting species, antagonism is far more likely. For example, once established, P. aeruginosa can lyse the cells of S. aureus and other Gram-positive bacteria (Palmer et al., 2005).

*Pseudomonas aeruginosa* grows in the airways of CF patients in a sessile biofilm matrix (Costerton et al., 1999; Singh et al., 2000; Mah & O’Toole, 2001; Hall-Stoodley et al., 2004), and these bacteria may be as much as 1000 times more resistant than their planktonic counterparts (Nickel et al., 1985; Drenkard, 2003; Moskowitz et al., 2004; Fux et al., 2005; Aaron, 2007). Bacteria in biofilms are in a relatively hypoxic environment, are slow growing, and reach cell densities of up to $10^9$ CFU mL$^{-1}$ in the sputa of CF patients (Ramsey et al., 1999). As with clinical treatments for all types of infections, prolonged and frequent courses of oral, aerosolized, or parenteral antibiotics often lead to the emergence and proliferation of multiresistant pathogens (Saiman, 2007). One adaptation of P. aeruginosa that improves its tendency to form biofilms is encoded by the aminoglycoside response regulator (arr) gene (Hoffman et al., 2005). This gene expresses an inner membrane phosphodiesterase, whose substrate is cyclic-di-GMP, a regulator of cell surface adhesiveness. These investigators propose that subinhibitory levels of aminoglycoside actually induce biofilm formation in P. aeruginosa by upregulating cyclic-di-GMP levels and that this mechanism evolved originally to counter antibiotic production by soil bacteria. Although far less prevalent or persistent than *P. aeruginosa*, methicillin-resistant *S. aureus* can occur frequently in CF patients. In one notable study (Molina et al., 2008), 93 resistant *S. aureus* isolates were recovered from 18 patients and most of these were capable of biofilm formation. However, while > 80% of these isolates were resistant to aminoglycoside antibiotics, they were all susceptible to linezolid, quinupristin/dalfopristin, and co-trimoxazole.

The most commonly observed phenotypic changes in CF bacterial pathogens over time are antibiotic resistance, mucoidy, a loss of cell motility, the appearance of ‘small colony variants’, increased mutation rate, and decreased production of virulence factors (Smith et al., 2006; Harrison, 2007). The last of these is thought to aid in immune invasion. *Pseudomonas aeruginosa* undergoes substantial genetic changes during chronic infection of CF airways (Foweraker et al., 2005; Smith et al., 2006), diversifying into
strains exhibiting different characteristics from environmental isolates. Multiple strains of *Pseudomonas* also grow, so that the typical sputum of patients with CF may contain at least 12 different colony types of *P. aeruginosa* with 12 unique resistance patterns (reviewed in Aaron, 2007). That the same traits are acquired suggests that there is a conserved pattern of adaptation to the airways of CF patients (Smith et al., 2006), giving rise to clonal *P. aeruginosa* lineages that persist for years. One such mutation is in the lasR gene that is found in many CF isolates and that confers increased resistance to β-lactams, which are commonly used to treat CF respiratory exacerbations (Salunkhe et al., 2005). LasR is a transcriptional regulator that is involved in quorum-sensing systems based on homoserine lactones (Schuster & Greenberg, 2006; Venturi, 2006).

Many virulence factors, including biofilm development, are quorum-sensing regulated (Whiteley et al., 1999; Wilcox et al., 2008). In CF patients chronically infected with *P. aeruginosa*, treatment with the macrolide antibiotic azithromycin improves the clinical outcome, although others argue that the anti-inflammatory effects of azithromycin are more important. Several studies indicate that azithromycin may exert its beneficial action by impeding quorum sensing, thereby reducing the pathogenicity of *P. aeruginosa*, especially when grown in an anaerobic culture (Hill et al., 2005).

One study that tested 12 antibiotics for quorum-sensing inhibiting activity found that azithromycin, cefazidime, and ciprofloxacin decreased the expression of a range of quorum-sensing-regulated virulence factors, suggesting that the underlying mechanism may be mediated by changes in membrane permeability, thereby influencing the flux of quorum-sensing homoserine lactone (Skindersoe et al., 2008). Somewhat surprisingly, a long-term study found that 68 mutations accumulated in *P. aeruginosa* over 7.5 years in one CF patient (Smith et al., 2006). Many of the lost functions related to the expression of virulence (invasive) factors that are normally targets for the immune system could confer a selective advantage for chronic infections. Interestingly, a recent study showed that although azithromycin exhibited bactericidal activity against *P. aeruginosa* biofilms, resistant mutants were readily selected and these mutants frequently showed cross-resistance to the unrelated antipseudomonal agents such as ciprofloxacin or cefazime, but hypersusceptibility to imipenem or tobramycin (Mulet et al., 2009).

### Synergy and antagonism

Drug combinations are classified as synergistic, additive, or antagonistic, according to whether the effect of the drugs is larger than, equal to, or smaller than the effect predicted by their individual activities. Synergy of an antibiotic combination occurs when the minimal inhibitory concentration (MIC) of an antibiotic is decreased at least fourfold when combined with another antibiotic (Burns et al., 1999).

Following the recent development by clinical laboratories to test multiple combinations of antibiotics in the laboratory, a recent trial was undertaken that did not show any clinical benefit (Aaron, 2007; reviewed in Middleton, 2007). Also, as CF patients are generally infected with hypermutable strains of *P. aeruginosa*, synergy testing is exposed to constantly changing susceptibility patterns (Oliver et al., 2000; Macia et al., 2005).

Nevertheless, the accepted treatment of a pulmonary CF exacerbation consists of two parenteral agents from different antibiotic classes to delay the emergence of resistance (Govan & Deretic, 1996; Gibson et al., 2003). The hypermutability of *P. aeruginosa* CF strains is likely to be a key factor in the development of resistance and adaptation for long-term persistence (Macia et al., 2005).

Another important aspect of antagonism in CF infections caused by *P. aeruginosa* and *B. cepacia* in particular relates to cation stabilization of the outer membrane and ion flux across the inner membrane. Antagonism of aminoglycosides by divalent cations is well documented for *P. aeruginosa* (Medeiros et al., 1971; Zemelis & Jackson, 1973; D’Amato et al., 1975). This antagonism is probably due to the disruption of the outer membrane by aminoglycosides competing with divalent cations for lipopolysaccharide-binding sites. Divalent cations are known to antagonize aminoglycosides binding to lipopolysaccharides in the cell envelope. Polycations such as polyamines at millimolar concentrations increase the MICs of aminoglycosides, β-lactams, and other antibiotics (Kwon & Lu, 2006), probably due to inhibition of drug binding to lipopolysaccharides in the cell envelope (Zemelis & Jackson, 1973; Thompson et al., 1985; Cohn & Aronoff, 1989).

Tobramycin uptake in *E. coli* is regulated by the electrical potential component of the proton motive force and is probably mediated by a voltage-gated channel in the cytoplasmic membrane that is inhibited by compounds that depolarize the membrane potential (Leviton et al., 1995). Na+, K+, and Cl− are ubiquitous in nature, so much so that elaborate pumping mechanisms for maintaining homeostasis are present in all bacteria. The use of drugs may disturb ions in constant flux, and vice versa. An Na+–antagonizing effect on tobramycin MICs has been demonstrated (Medeiros et al., 1971; Cohn & Aronoff, 1989; Treerat et al., 2008), as has a similar, but not yet defined, effect of K+ antagonism of tobramycin MICs for *P. aeruginosa* and *B. cenocepacia* (Treerat et al., 2008). The ChaA Na+, K+/H+ antiporter enables *E. coli* to adapt to salinity stress and to maintain Na+ and K+ homeostasis (Radchenko et al., 2006). This pump almost certainly has homologues in other Gram-negative bacteria such as *P. aeruginosa* and *B. cepacia*, and would explain, at least in part, the antagonism of these ions.
to tobramycin. Amiloride, N-amidino-3,5-diamino-6-chloropyrazine carboxamide, blocks Na\(^+\) channels in eukaryotic cell membranes (Kleyman & Cragoe, 1988). Sodium ion antagonism of tobramycin uptake in Gram-negative bacteria can be reversed by amiloride and homologues, enabling lower tobramycin MICs (Treerat et al., 2008).

**Chemotherapeutic strategies – one or more antibiotics**

Some authors have suggested that antimicrobial cycling in nosocomial settings, in which the empiric use of two or more classes of antibiotics is alternated over a time scale of months to years, may slow the evolution and spread of multiresistant pathogenic bacteria. However, mathematical modelling predicts that cycling is unlikely to be effective and may even hinder resistance control as the frequency, spread, and persistence of resistant strains may be stochastic or asymmetric and therefore evade cycling schemes (Bergstrom et al., 2004). Bacterial resistance to antibiotics is the bane of clinicians (Alekshun & Levy, 2007). Even when antibiotics are used to treat susceptible infections, systemic subinhibitory concentrations trigger the hormetic effects of considerable transcriptional activation and global responses in bacterial cells (Davies et al., 2006). Hormetic effects describe biological responses to environmental signals or stresses that are characterized by biphasic dose-response relationships exhibiting low-dose stimulation and high-dose inhibition.

There is a well-noted discrepancy between *in vitro* antibiotic susceptibility of *P. aeruginosa* and the clinical response to particular antibiotics (Hancock & Speert, 2000). Thus, *in vitro* testing of clinical isolates is limited by the absence of sputum and biofilms in the CF lung that increase antibiotic resistance. Large *in vitro* studies that show, for example, inhibition of 30–40% of *P. aeruginosa* and *B. cepacia* CF isolates by doripenem or meropenem (Chen et al., 2005; Traczewski & Brown, 2006) are unlikely to produce anything like these levels of susceptibility in vivo. Combinations of antibiotics are used to overcome treatment failures with single antibiotics and the most common pairing is a β-lactam with an aminoglycoside for which increased efficacy has been demonstrated in CF patients (Aronoff & Klinger, 1984; Regelmann et al., 1990; Smith et al., 1999; Lang et al., 2000). The last of these studies demonstrated that empirically chosen combinations of antibiotics produced many *in vitro* antagonistic results in multiresistant *P. aeruginosa* CF isolates. When a rapid antibiotic susceptibility test was used to screen 75 of these isolates from 44 patients, using double and triple antibiotic combinations, the best result of 96% killing was achieved with meropenem and high-dose tobramycin. Other antibiotic combinations that used tobramycin or meropenem with various β-lactams and quinolones were less successful, and adding a third antibiotic did not significantly improve bacterial killing. A large study that used 23 antibiotic combinations to test for synergy on 2621 *B. cepacia* CF isolates collected over an 8-year period found antibiotic synergies only rarely, ranging from 1 to 15% of strains per antibiotic combination (Zhou et al., 2007).

**Alternative strategies – antibiotic and nonantibiotic**

Combinations of different antimicrobial agents with nonantibiotic compounds have been tested *in vitro* as alternative therapies against *P. aeruginosa* and *B. cepacia*, with mixed results. Nonantibiotics tested included polyethylenimine (Khalil et al., 2008), theophylline and theobromine (Rajyaguru & Muszynski, 1998), bismuth-thiols (Veloira et al., 2003), the sodium channel blocker amiloride (Cohn et al., 1988; Middleton et al., 2005; Treerat et al., 2008), the calcium channel blocker verapamil (Cohn et al., 1995), the bronchodilator salbutamol (Vaisman et al., 1987; Brand, 2000; Ramagopal & Lands, 2000), the cardiovascular drug amiodipine (Asok Kumar et al., 2004), the amiloride homologues (N,N-hexamethylene)amiloride, (N-methyl-N-isobutyl)amiloride, and benzamil (Cohn et al., 1992; Treerat et al., 2008), gallium (Kaneko et al., 2007), and farnesol (Jabra-Rizk et al., 2006).

Polyethylenimine is a weakly basic, aliphatic, polycationic, nontoxic polymer (10 kDa) that is – like polymyxin – a permeabilizer of Gram-negative bacteria (Helander et al., 1997), but has no bactericidal or bacteriostatic activity below 1 μM. A recent *in vitro* study demonstrated excellent synergy of polyethylenimine with different families of antibiotics against a clinical isolate of *P. aeruginosa* (Khalil et al., 2008). When polyethylenimine was used at a subinhibitory 0.25 μM in combination with β-lactams, cephalosporins, novobiocin, or chloramphenicol, it reduced the MICs to these antibiotics against the *P. aeruginosa* isolate by 1.5–56-fold. However, the MICs of macrolides, tetracyclines, or fluoroquinolones were unaffected, and the MICs of aminoglycosides, polymyxins, or vancomycins increased by 1.2–4.8-fold. Polyethylenimine is presumed to work by binding strongly to lipopolysaccharides, leading to disorganization of the outer membrane and facilitating antibiotic penetration. The antagonistic effect of polyethylenimine combined with classes of antibiotics that are hydrophilic and carry positive charge, such as aminoglycosides, could probably be explained by competition between polyethylenimine and these antibiotics for the same lipopolysaccharide-binding sites (Khalil et al., 2008).

Unlike *P. aeruginosa* and *E. coli*, whose outer membranes are disrupted and partially permeabilized by aminoglycosides, EDTA, and polymyxin B (Moore & Hancock, 1986), *B. cepacia* is unaffected, indicating that the membrane differs significantly in composition, structure, and/or
function from that in *P. aeruginosa* and *E. coli* (Rajyaguru & Muszynski, 1998). The susceptibility of *B. cepacia* to antibiotics is therefore not enhanced by cationic drugs as occurred for other organisms. Therefore, it is of interest to note the finding that the susceptibility of *B. cepacia* to gentamicin, amikacin, azithromycin, and ceftazidime, but not kanamycin or ampicillin, was reduced by fourfold in combination with the cationic compounds theophylline or theobromine at 0.2 mM (Rajyaguru & Muszynski, 1998).

The bismuth-thiols show *in vitro* synergy with tobramycin against the *B. cepacia* complex, and possess the added advantages of antibiofilm activity and micromolar MICs (Veloira et al., 2003). However, bismuth-thiols would need to be assessed before being administered to the airways. Amiloride–tobramycin *in vitro* studies (Cohn et al., 1988, 1995) showed moderately successful synergistic effects – up to fourfold lower tobramycin MICs – but in < 50% of clinical strains of *Pseudomonas* spp. and *B. cepacia* complex tested. Amiloride homologues have been tested against 20 *P. aeruginosa* CF isolates, with results indicating benzamil MICs at twofold lower than amiloride MICs, and other substituted amiloride homologues at fourfold lower MICs than those of amiloride (Cohn et al., 1992). A small clinical trial (Middleton et al., 2005) showed that nebulized amiloride–tobramycin eradicated the *B. cepacia* complex from the sputum samples of three of four CF patients. However, this therapy did not eradicate mucoid *P. aeruginosa* strains, presumably due to these isolates being tobramycin-resistant and amiloride not exerting enough synergistic pressure. A recent *in vitro* study that tested tobramycin in combination with amiloride, salbutamol, verapamil, or amlodipine showed – as in the earlier studies (Cohn et al., 1988, 1992, 1995) – mostly additive MICs against CF strains of *P. aeruginosa* and *B. cenocepacia* (Treerat et al., 2008). Much more promising was the combination of tobramycin and benzamil, which produced MICs up to 32-fold lower against four *P. aeruginosa* CF isolates, all of which were tobramycin resistant and two of which had mucoid phenotypes. Amiloride homologues such as benzamil, which are nontoxic at clinical levels, may therefore be among the most promising candidates for adjunctive therapy in treating pulmonary infections in CF patients.

The transition metal gallium could be a promising new therapeutic agent that exploits stresses imposed on bacteria by the *in vivo* environment or host defences. Iron is essential for bacterial growth and for biofilm formation. Gallium has an ionic radius similar to iron, and can substitute for iron in many biological processes, giving it the ‘Trojan-horse’ property of inhibiting many iron-dependent redox reactions, as gallium cannot be reduced (Kaneko et al., 2007). Gallium is internalized by *P. aeruginosa*, interferes with iron signaling, and decreases bacterial iron uptake. At low concentrations, Ga(NO₃)₂ inhibits *P. aeruginosa* growth and biofilm formation and kills planktonic and extant biofilm bacteria *in vitro*, and was effective in two murine lung infection models. Gallium is also active against MDR bacteria and could be a promising candidate for combination therapy with tobramycin. Gallium is already FDA approved for intravenous use as a contrast agent and is therefore likely to be clinically appropriate for use in treating CF infections. This promising study is subject to a number of caveats by the authors (Kaneko et al., 2007), which, while not precluding the development of gallium therapy, requires much additional experimentation.

Farnesol is a quorum-sensing molecule with antimicrobial properties. Synergy testing of farnesol (100 mM) and gentamicin (2.5 × MIC) was performed on static biofilms of methicillin-resistant *S. aureus* (Jabra-Rizk et al., 2006), with the result that the drug combination was able to reduce bacterial numbers by > 2 log units. Another recent study (Borriello et al., 2006) yielded interesting *in vitro* results that showed up to 2 logs of enhanced killing of the *P. aeruginosa* strain PAO1 by tobramycin or ciprofloxacin when arginine or nitrate was used as an adjuvant. However, although the effect only occurred against bacteria grown under anaerobic conditions, this included mature biofilms that may exist under essentially hypoxic conditions in the CF lung. In addition to those discussed above, other synergy studies have shown promise against CF infections (Chen et al., 2004; Drago et al., 2005; Jacqueline et al., 2005; Snydman et al., 2005). Another recent study (timurkaynak et al., 2006; Chan et al., 2007; Chin et al., 2008), but nothing to suggest that combination antibiotic therapy has a sustainable future in the clinical treatment of the common pathogens. In a further sign that novel approaches are being addressed, a recent study (LiPuma et al., 2009) tested a surfactant-stabilized oil–in–water nanoemulsion, named NB-401, against 150 CF respiratory tract bacterial isolates, of which 75 were *Burkholderia* and the other 75 included *Pseudomonas*, *Achromobacter*, *Pandoraea*, *Ralstonia*, *Stenotrophomonas*, and *Acinetobacter*. Nearly one-third of the isolates were MDR. Testing was extended to a subset of biofilm-grown strains and planktonic strains in a medium containing 43% CF sputum. Although the biofilms and sputum antagonized the nanoemulsion by decreasing its activity to minimum bactericidal concentrations 2–32-fold greater than the respective concentrations without sputum, NB-401 remained bactericidal to all planktonic and sessile bacteria.

**Prospects**

Recurrent infection with antibiotic-resistant pathogens is chiefly responsible for the morbidity and mortality seen in CF patients (Lyczak et al., 2002; Chmiel & Davis, 2003). These problems might only be solved by developing new antibiotics and/or alternative therapies. Unfortunately, apart from the
introduction of new-generation antibiotics from existing drug classes, the development of entirely new antibiotic classes has been largely abandoned, with only linezolid and daptomycin being introduced in the past four decades (Wenzel et al., 2005). Tigecycline is – like most ‘new’ antibiotics – a modified version of an existing drug, namely the tetracycline analogue, minocycline (Petersen et al., 1999). The disadvantage of this new analogue is that resistance from the upregulation of chromosomally encoded efflux pumps in several Gram-negative bacteria, including P. aeruginosa, has emerged, even during phase III clinical trials (Livermore, 2005). A new natural antibiotic, platensimycin, from Streptomyces platensis, blocks fatty acid synthesis, and has broad potent activity against Gram-positive pathogens, including methicillin-resistant S. aureus and vancomycin-resistant Enterococci (Wang et al., 2006). Is it prescience or pessimism that predicts that bacterial resistance will emerge to this antibiotic, just as it has for all those that have come before it? What, therefore, is the future for antibacterial chemotherapy?

New or modified antibiotics, or the reintroduction of older ones in disuse, will always be needed for treating nosocomial infections, but the problems caused by intractable infections, such as mucoid P. aeruginosa in the CF lung, methicillin-resistant S. aureus, or vancomycin-resistant Enterococci, cannot be overcome at present by traditional antibiotic monotherapy or combination antibiotic therapy, both of which are controversial areas in the treatment of respiratory infections in CF (Elphick & Tan, 2005). Nevertheless, antibiotic monotherapy might still be an option under some circumstances. For example, an old antibiotic colistin was used freely and effectively against Gram-negative bacteria in the preaminoglycoside era, but from

Fig. 2. The Cystic Fibrosis Foundation dynamic ‘pipeline’ of potential CF therapies, currently in development, as of April 2009. Reproduced with permission from the CF Foundation (www.cff.org/treatments/pipeline/).
the early 1970s, it has been essentially supplanted by the less toxic aminoglycosides and other antipseudomonal agents (reviewed in Li et al., 2005). However, colistin remains very effective against MDR organisms such as P. aeruginosa, Acinetobacter spp., and Klebsiella spp., with resistance to colistin occurring in < 3% of Gram-negative bacteria. Colistin is used frequently via nebulization route, as it exhibits little toxicity via this route, in contrast to the toxicity found in parenteral use.

One novel idea for the future involves programmed cell death, for which systems have been identified in various bacteria (reviewed in Engelberg-Kulka et al., 2004). The elucidation of X-ray crystal structures of suicide module protein components will hold promise for the rational chemical design of new compounds that will directly activate chromosome programmed cell death systems by interacting with their components. In response to the threat of extinction, microorganisms have found genetic and biochemical means of resistance to all natural and synthetic antimicrobial agents, heralding an urgent need for alternatives such as reintroducing old antibiotics, designing new drugs to trigger bacterial programmed cell death, or finding the most effective synergistic treatments with antibiotic/nonantibiotic combinations.

Current therapies are still mainly restricted to an alleviation of symptoms and care for patients remains complex and the burden of therapy is high (Proesmans et al., 2008). Nevertheless, a number of treatment strategies are being pursued beyond purely antibiotic therapies, the rationale being that CF is a complex disease that requires more than a single approach to alleviate the causes and symptoms of CF and the complications that accompany them. These potential therapies are intended to target problems in the airways and the complications that accompany them. These potential therapies are intended to target problems in the airways and the complications that accompany them. These potential therapies are intended to target problems in the airways and the complications that accompany them. These potential therapies are intended to target problems in the airways and the complications that accompany them.

Potential therapies in various stages of development (Fig. 2).

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Cystic fibrosis infections: treatments and prospects


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