Published in final edited form as:

Expert Rev Anti Infect Ther. 2010 March ; 8(3): 325–338. doi:10.1586/eri.10.4.

Present and future therapeutic strategies for melioidosis and glanders

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Abstract

Burkholderia pseudomallei and *Burkholderia mallei* are the causative agents of melioidosis and glanders, respectively. Both Gram-negative pathogens are endemic in many parts of the world. Although natural acquisition of these pathogens is rare in the majority of countries, these bacteria have recently gained much interest because of their potential as bioterrorism agents. In modern times, their potential destructive impact on public health has escalated owing to the ability of these pathogens to cause opportunistic infections in diabetic and perhaps otherwise immunocompromised people, two growing populations worldwide. For both pathogens, severe infection in humans carries a high mortality rate, both species are recalcitrant to antibiotic therapy – *B. pseudomallei* more so than *B. mallei* – and no licensed vaccine exists for either prophylactic or therapeutic use. The potential malicious use of these organisms has accelerated the investigation of new ways to prevent and to treat the diseases. The availability of several *B. pseudomallei* and *B. mallei* genome sequences has greatly facilitated target identification and development of new therapeutics. This review provides a compilation of literature covering studies in antimelioidosis

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Financial & competing interests disclosure

Steven W Dow and Herbert P Schweizer were supported by NIH-NIAID grant U54 AI065357. D Mark Estes and Alfredo G Torres were supported by U54 AI057156, N01-AI-30065 Part C (19) and 1U01AI082103-01. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

and antiglanders antimicrobial drug discovery, with a particular focus on potential novel therapeutic approaches to combat these diseases.

Keywords

antibiotics; antimicrobial compounds; *Burkholderia mallei*; *Burkholderia pseudomallei*

Melioidosis & glanders

Melioidosis and glanders are caused by the closely related species *Burkholderia pseudomallei* and *Burkholderia mallei*, respectively. *B. pseudomallei* is a Gram-negative, facultative aerobic bacillus that has been researched for its ability to survive intracellularly in host cells and produce acute and chronic infections. The bacterium is endemic to south east Asia and northern Australia, where melioidosis is an ongoing public health problem, especially during the rainy season [1–3]. The overall mortality rate in individuals infected with *B. pseudomallei* is 50% in northeast Thailand (35% in children) and 19% in Australia [1,2]. Although melioidosis is primarily found in south east Asia and Australia, mounting evidence indicates that *B. pseudomallei* is more widespread than previously thought [4]. Clinical manifestations of melioidosis are extremely diverse and vary from acute sepsis to chronic localized pathology to latent infection, which can reactivate decades later from an as yet unknown tissue reservoir. Depending on whether the disease is acute or chronic, melioidosis can mimic other infections, including glanders, typhoid fever, malaria, TB and bacterial sepsis [5,6]. Community-acquired infection with melioidosis probably occurs as a consequence of the bacterium in soil or water entering the host through cuts or skin abrasions, or via inhalation or ingestion. In small animal models of *B. pseudomallei* infection, the bacterium is much more infective by airborne or intranasal routes [7,8]. This appears to be consistent with the reported cases of melioidosis occurring in previously healthy US helicopter crews during the Vietnam War, possibly as a consequence of the inhalation of soil-derived dusts or water mist containing bacteria [9]. However, detailed information on the ease with which *B. pseudomallei* is able to infect healthy immunocompetent humans by the airborne route is not available.

Otherwise healthy individuals can develop melioidosis but the majority of community-acquired cases have some underlying immunosuppressive condition, particularly diabetes but also chronic renal disease, thalassemia or alcoholism [10]. In naturally occurring melioidosis, the mortality rate in acute cases can exceed 50% and prolonged treatment with antibiotics may result in only temporary control of the infection, with 10–15% of patients relapsing when antibiotic therapy is withdrawn. The treatment of melioidosis is often problematic because the bacteria are inherently resistant to many of the commercially available antibiotics and successful therapy often requires extended treatment regimens. Prophylactic strategies for human melioidosis do not exist.

The causative agent of glanders, *B. mallei*, is a Gram-negative, aerobic, spore-forming bacterium. Glanders is generally confined to equines in parts of the Middle East, Asia and South America [11–13]. In humans, it is primarily an occupational disease, affecting individuals in close contact with infected animals such as veterinarians, grooms and farmers. However, a number of laboratory-acquired cases of glanders have also been reported. Infection primarily results from the contamination of wounds, abrasions or breaks in mucous membranes. In equines and humans, *B. mallei* infection can present as either nasal–pulmonary infections (glanders) or cutaneous infections (farcy), and the disease may develop either acutely or chronically [14]. Like *B. pseudomallei*, there is considerable potential for this organism to be used as an agent of biological warfare or bioterrorism. It is

documented that during World War I, *B. mallei* was used to attack horses involved in the movements of the military and there is evidence that glanders was also used in World War II. It has also been reported that *B. mallei* was utilized as a bioweapon during the Soviet invasion and occupation of Afghanistan [15].

Glanders treatment & prophylaxis

The scarcity of glanders in most countries in the world over the past 50–60 years means there is scant knowledge, with the exception of a few principally Russian sources, of the antimicrobial susceptibility of *B. mallei*, particularly to modern antimicrobials [16–22]. Likewise, the *in vivo* efficacy of modern antimicrobials against *B. mallei* is not well known and few *in vitro* antibiotic susceptibility studies for *B. mallei* have been performed. The antibiotic susceptibility pattern of *B. mallei* is similar to that of *B. pseudomallei*, with *B. mallei* exhibiting resistance to a number of antibiotics [23]. In some studies, doxycycline showed good *in vitro* activity against *B. mallei* [23]. Both *B. mallei* and *B. pseudomallei* are sensitive to imipenem, and most strains are also susceptible to ceftazidime, ciprofloxacin and piperacillin [24]. Furthermore, *B. mallei* is susceptible to aminoglycosides because it lacks the AmrAB–OprA efflux pump, which confers high-level aminoglycoside resistance to *B. pseudomallei* [25].

There are little data on the efficacy of antibiotics for the treatment of human glanders but current treatment options include a mixed antibiotic regimen that is often only partially effective, and assumes an early and accurate diagnosis. Single-drug antibiotic therapy is only partially effective and combinations of antibiotics must be used for extended periods. For example, a researcher who developed glanders following accidental laboratory exposure was treated with imipenem and doxycycline for 2 weeks, followed by treatment with azithromycin and doxycycline for an additional 6 months [26]. An additional problem is that even more antibiotic-resistant variants of *B. mallei* have been reported [27] and surgical drainage of abscesses may be required in some cases. Glanders prophylaxis has not yet been studied in detail, although approaches similar to those recommended below for melioidosis may be feasible. Moreover, there is no prophylactic or therapeutic vaccine for *B. mallei* infection available for humans or animals [23].

Melioidosis therapy

Options for the treatment of melioidosis have traditionally been limited as *B. pseudomallei* exhibits resistance to diverse antibiotics, including first- and second-generation cephalosporins, penicillins, macrolides and aminoglycosides [28,29]. However, *B. pseudomallei* remains susceptible to some third-generation cephalosporins, carbapenems and some fluoroquinolones, as well as amoxicillin- β -lactamase inhibitor combinations (e.g., amoxicillin-clavulanic acid), chloramphenicol, tetracyclines and trimethoprim-sulfonamides (e.g., trimethoprim-sulfamethoxazole) *in vitro* [29,30]. The clinical efficacy of chloramphenicol, trimethoprim-sulfamethoxazole, doxycycline, imipenem, ciprofloxacin and ofloxacin has been evaluated for the treatment of melioidosis [31–35].

Owing to *B. pseudomallei*'s propensity to enter into prolonged latency, melioidosis therapy is biphasic, consisting of a short-term parenteral acute phase and a long-term oral eradication phase. Both of these will be reviewed in the following paragraphs. Generally speaking, acute -phase treatment involves bacteriocidal drugs with or without postantibiotic effects and oral eradication therapy bacteriostatic drugs. One of the difficulties in interpreting published clinical efficacy data is that studies with some drugs are supported by small trials only.

In humans, intravenous ceftazidime (with or without trimethoprim–sulfamethoxazole), amoxicillin–clavulanic acid, imipenem and cefoperazone–sulbactam have each been reported as effective treatments for acute disease, following randomized clinical trials [35]. Meropenem has also been used successfully [36]. Oral regimens comprising chloramphenicol, trimethoprim–sulfamethoxazole and doxycycline, or amoxicillin–clavulanic acid alone, have also been shown to be effective for eradication therapy following treatment for acute infection. Doxycycline has been used alone in the treatment of localized melioidosis, and in combination with other antimicrobials for systemic disease [28], and thus may have some utility as a prophylactic or immediate antimicrobial treatment for melioidosis in cases where trimethoprim–sulfamethoxazole may not be applicable, for instance in infections with trimethoprim-resistant isolates. Recent animal experimentation demonstrated the *in vivo* efficacy of doxycycline in pre- and postexposure prophylaxis [37].

An alternative protocol recommended for treatment of acute melioidosis infection included high-dose intravenous ceftazidime, or a carbapenem, for at least 10–14 days, followed by oral eradication therapy [36,38,39]. Treatment with ceftazidime, in a sequential open-label, randomized trial of ceftazidime against chloramphenicol–doxycycline–trimethoprim–sulfamethoxazole, has demonstrated a mortality benefit in severe disease and the use of ceftazidime was associated with a significant reduction in mortality in adult Thai melioidosis patients [40,41]. Ceftazidime-based regimens continue to be used for the intensive phase of melioidosis treatment, since studies have failed to demonstrate improved outcomes following initial treatment with amoxicillin–clavulanic acid, cefoperazone–sulbactam or imipenem [42].

Intravenous amoxicillin–clavulanic acid has been used in Thailand for the treatment of sepsis and melioidosis [43]. A clinical trial of this agent in an intensive therapy setting demonstrated a similar mortality but a higher treatment failure rate than traditional treatment, requiring a change in antibiotic regimen [44]. By contrast, use of oral amoxicillin–clavulanate (with supplemental amoxicillin) in maintenance therapy was associated with a higher relapse rate than conventional treatment with trimethoprim–sulfamethoxazole, doxycycline and chloramphenicol [31]. An ongoing issue is whether trimethoprim–sulfamethoxazole with doxycycline is more efficacious than trimethoprim–sulfamethoxazole alone, which is currently being addressed by clinical trials in Thailand. The use of doxycycline alone for eradication therapy is associated with unacceptable rates of relapse and treatment failure compared with the conventional regimen of trimethoprim–sulfamethoxazole, doxycycline and chloramphenicol. However, the three-drug protocol is associated with high rates of adverse events (32% in this trial) that may limit adherence [45]. Similarly, short-course ciprofloxacin–azithromycin therapy and prolonged quinolone monotherapy were also associated with high relapse rates [33,46]. These findings suggest that the failure to complete at least 12 weeks of therapy remains a significant determinant of relapse, reinforcing the necessity for strict adherence to duration-of-treatment protocols and the need to develop improved and/or better-tolerated treatment regimens.

Chloramphenicol was used until relatively recently in Thailand for the oral phase of melioidosis treatment, but is no longer used because the results of a clinical trial showed it to be unnecessary [47]. Rare exceptions to this may exist, for example, in patients with neurological involvement who are infected with an organism that is resistant to trimethoprim–sulfamethoxazole or in patients who cannot tolerate this drug. Chloramphenicol penetrates the brain well, while amoxicillin–clavulanic acid does not. Although neurological involvement occurs infrequently (1.5% of cases in Thailand) [48], and the chance of a patient developing neurological disease with a strain that is resistant to trimethoprim–sulfamethoxazole is very low, chloramphenicol should be reserved for two reasons: first, neurological involvement has been poorly studied in patients and animal

models and, second, recent animal experimentation data indicated that *B. pseudomallei* is found in the brain more readily and frequently than was previously thought [49].

Despite having good intracellular penetration and generally significant *in vitro* activities, treatment with quinolones such as ciprofloxacin has proven disappointing in practice but the cause(s) for this remain to be elucidated [33,46,50]. However, the ability of quinolones, and newer fluoroquinolones in particular, to accumulate within phagocytes suggests they may still prove useful for the treatment of melioidosis. Although widely tested *in vitro* [30,51,52] and, to a lesser extent, *in vivo* [53] in a murine melioidosis model, the fluoroquinolones have poor clinical efficacy and are currently not recommended for the treatment of melioidosis [50,54]. A comparison of ciprofloxacin and azithromycin for 12 weeks versus trimethoprim-sulfamethoxazole and doxycycline for 20 weeks demonstrated a relapse rate of 22 and 3%, respectively, in clinical studies [33]. Ciprofloxacin or ofloxacin given for a median of 15 weeks to treat 57 adult patients with melioidosis was associated with an unacceptably high failure rate of 29% [46]. Consistent with these clinical observations are the results of animal experiments where ciprofloxacin, gatifloxacin and moxifloxacin given for 14 days did not provide good postexposure protection (defined in terms of survival rates) in a murine melioidosis model [53].

Ciprofloxacin, either alone or in combination with other drugs, has been used in the treatment of melioidosis despite the apparent lack of efficacy in previous studies [46,55]. This usage is based on the idea that ciprofloxacin can penetrate to very high concentrations in phagocytic cells where *B. pseudomallei* resides, thereby allowing ciprofloxacin to achieve concentrations 4–12 times those that can be achieved in extracellular fluids [56,57]. Thus, with serum concentrations of ciprofloxacin of 2–3 mg/l achievable by standard oral dosing, theoretically, intracellular concentrations of up to 20 mg/l should be attained [58]. Furthermore, serum levels of ciprofloxacin of 9 mg/l can be achieved by intravenous infusion, albeit for short periods [58]. Although human clinical experience and animal experimentation proved disappointing thus far, it may be prudent to further explore and/or re-examine the *in vitro* and *in vivo* activities of some of the newer fluoroquinolones.

Based on the current state of knowledge and clinical experience, recommended first-line treatment for acute human melioidosis is intravenous ceftazidime or a carbapenem (i.e., meropenem) for at least 10–14 days, followed by oral eradication therapy consisting of trimethoprim-sulfamethoxazole with or without doxycycline for 12–20 weeks. The choice of oral therapy for patients not able to tolerate first-line therapy or when this is contraindicated for other reasons (e.g., childhood and pregnancy) is amoxicillin-clavulanic acid [59].

Melioidosis prophylaxis

Melioidosis prophylaxis has only been addressed in animal experiments, although postexposure prophylaxis following laboratory exposure to *B. pseudomallei* has been recommended previously [59], but there is no evidence for its efficacy in humans. A recent study assessed the efficacy of oral administration of the commonly used melioidosis drugs amoxicillin-clavulanic acid, doxycycline and trimethoprim-sulfamethoxazole for pre- and postexposure prophylaxis in a murine melioidosis model [34,37]. Of these antibiotics, trimethoprim-sulfamethoxazole proved the most effective pre- and postexposure prophylaxis, rescuing 100% of the animals when administered 48 h pre-exposure and within the first 24 h postinfection as assessed by survival rates and elimination of bacteria from the lung. In the same studies, doxycycline had significant efficacy but amoxicillin-clavulanic acid was only marginally effective. These data suggested that the recommended trimethoprim-sulfamethoxazole could indeed be used as a first-line agent for prevention and

treatment of acute and chronic disease. In a previous study, fluoroquinolones (ciprofloxacin, gatifloxacin and moxifloxacin) given for 14 days did not provide good postexposure protection (defined in terms of survival rates) to BALB/c mice following subcutaneous inoculation of *B. pseudomallei*, even when treatment was initiated 6 h postchallenge [53]. Ciprofloxacin and doxycycline given 48 h before or immediately after intraperitoneal bacterial challenge in a mouse model raised the median lethal dose, but *B. pseudomallei* was recovered from surviving animals, and relapse of infection was observed in some treated animals followed up for 5 weeks [34]. Use of chloramphenicol for prophylaxis has not been reported, and would be a drug of last choice given its considerable side-effect profiles.

Resistance to clinically used antibiotics

Although *B. pseudomallei* isolates resistant to clinically significant antibiotics can be readily obtained in the laboratory, resistance in clinical isolates is relatively rare. Furthermore, in most instances it remains unclear whether resistance in clinical isolates is the result of treatment or an intrinsic property of the infective organism. Clinical isolates resistant to ceftazidime and/or amoxicillin–clavulanic acid are rare (<0.2%) and primary resistance to carbapenem antibiotics has not yet been reported [54,60]. Clinical isolates tend to be more resistant to antibiotics used for oral eradication. Trimethoprim–sulfamethoxazole resistance in Thai isolates ranges from 13 to 16% [61,62] and 2.5% in Australia [63]. It is unclear why trimethoprim–sulfamethoxazole is geographically variable. Primary resistance to doxycycline in Australian and Thai isolates are about the same (<2%) [54,64]. Emergence of resistant isolates during treatment varies from isolated cases for ceftazidime or amoxicillin–clavulanate [64] to 7% for chloramphenicol [29]. It has been argued that the development of drug resistance during oral therapy may be highly pertinent for those patients who relapse with the same strain as relapse isolates tend to be resistant to single or multiple antibiotics [54,64]. *In vivo* and *in vitro* evidence indicates that drug exposure induces physiological changes – for example, filaments [54,65] or small colony variants that may render the bacteria more tolerant to antibiotics [66].

Novel therapeutics for glanders & melioidosis

Reappraisal of existing treatments & testing of novel antibiotics

Although there are effective antimicrobial treatment regimens currently available for treatment of several different *Burkholderia* infections, problems such as the prolonged length of treatment, the severe nature of the disease and the potential for deliberate aerosol delivery of infection highlight the need for new antiglander and antimelioidosis drugs. *B. mallei* and *B. pseudomallei* are very similar with respect to their physiology, biochemistry and, in many respects, their pathogenicity in animal models, particularly with respect to their intracellular survival within phagocytes [67]. It is therefore conceivable that many of the approaches aimed at discovery of new therapeutic interventions against glanders and melioidosis will be similar. However, *B. pseudomallei* is arguably the more recalcitrant of the two bacteria and therefore this organism will be the driver for development of new therapeutic strategies. Unfortunately, clinical experience with *B. pseudomallei* infections has shown that despite evidence of good *in vitro* activity, an antibiotic may be ineffective *in vivo* [29,46]. The arguments and caveats associated with the possible use of therapeutics for melioidosis would therefore most likely also apply to treatment of glanders.

Studies with existing & novel antibiotics—As mentioned before, *B. pseudomallei* is intrinsically resistant to a wide range of antimicrobial agents including β -lactam antibiotics, aminoglycosides and macrolides [29,68], and the few antibiotic susceptibility studies that have been performed with *B. mallei* indicated resistance to some of the same antibiotics [19,23]. Recent efforts have focused on assessing the *in vitro* susceptibilities of these

organisms to a wider variety of different antimicrobial agents and the evaluation of new agents in animal models of infection. A recent study indicated that lower minimal inhibitory concentrations were obtained with imipenem, ceftazidime, piperacillin, piperacillin/tazobactam, doxycycline and minocycline (the susceptibilities measured are consistent with the current recommendations for the treatment of infections caused by these pathogens), while fluoroquinolones and aminoglycosides had poor activities against *B. pseudomallei* [60]. A similar study with *B. mallei* demonstrated susceptibility to aminoglycosides, macrolides, quinolones, doxycycline, piperacillin, ceftazidime and imipenem [24]. Finally, a recent study by Karunakaran and Puthuchery demonstrated that imipenem, meropenem and trimethoprim-sulfamethoxazole have *in vitro* efficacy against *B. pseudomallei* and that moxifloxacin, ertapenem and azithromycin were not recommended antibiotics for treatment of melioidosis [69]. Overall, these results underline the significance of resistance in both species and the need for further investigation of this topic. For example, a recent clinical report indicated that a patient with a clonal infection of *B. pseudomallei* had subpopulations of the bacterium that exhibited ceftazidime and amoxicillin-clavulanic acid susceptibilities that differed among the different clinical subpopulations [70].

The emergence of pan-resistant Gram-negative pathogens has led to the recent development of new therapeutics based on proven drug targets and chemical entities. These include some newer fluoroquinolones, tetracyclines and β -lactam antibiotics. *B. pseudomallei* is susceptible to tigecycline *in vitro* [71] and tigecycline has been shown to be protective *in vivo* in an acute murine melioidosis model in combination with ceftazidime [72], but it has not yet been evaluated in clinical trials. Ceftobiprole is a novel parenteral cephalosporin whose broad spectrum of activity includes most clinically important Gram-negative bacteria. A recent study showed that the *in vitro* efficacy of ceftobiprole against *B. pseudomallei* is less than that of ceftazidime and that only 40% of Thai *B. pseudomallei* strains are susceptible to ceftobiprole [73]. BAL30072 is a new monobactam with activity against drug-resistant Gram-negative pathogens. We recently reported that BAL30072 has superior *in vitro* activity against *B. pseudomallei* when compared with ceftazidime, meropenem or imipenem [74].

The *in vitro* and *in vivo* susceptibilities of *B. mallei* to ceftazidime and levofloxacin were recently tested. Ceftazidime is a third-generation cephalosporin that is effective against *Pseudomonas* and is a first-line antibiotic for treatment of melioidosis. Levofloxacin is a quinolone antibiotic with high cell membrane permeability, which makes antibiotics in this class particularly effective against a number of intracellular pathogens [75]. A study was recently designed to assess the *in vitro* susceptibilities of these antibiotics with different modes of action and compared with their efficacy in macrophages or mice infected with *B. mallei* [75]. It was found that intranasal infection with *B. mallei* resulted in 90% death in nontreated control mice, while antibiotic treatment initiated 10 days postinfection proved to be effective *in vivo* with all antibiotic treated mice surviving to day 34 postinfection [75]. However, treatment with these antibiotics did not result in complete clearance of the bacterial infection and bacteria were found in lungs and spleens of the survivors. Nonetheless, this is the first study to demonstrate that both antibiotics had some utility for early treatment of glanders, including the ability for intracellular penetration and clearance of organisms *in vitro*, despite bacterial burdens recovered *in vivo* following intraperitoneal antibiotic treatment [75].

Animal models for evaluation of therapeutic interventions—As indicated above, clinical experience with *B. pseudomallei* infections has shown that despite good *in vitro* activity, an antibiotic may be ineffective *in vivo* [29,46]. The need for animal models of prophylactic antibiotic regimens has been prompted by the need for novel therapeutic regimens for accidental laboratory exposures or malicious releases during bioterrorism

events [34,37]. While animal models can indeed be used to predict clinical outcomes of therapeutic interventions, they still have significant shortcomings. Over the years, animal models that mimic disease manifestations (e.g., chronic vs acute in different mouse strains) and risk factors (e.g., diabetic rats) have been developed (reviewed in [76]). The main animal species that have been used in melioidosis research thus far are mice, hamsters and rats. Depending on the strain, routes of inoculation and dose, the host species differ widely in both susceptibility and pathogenesis of disease. Therefore, it is not yet clear which of the small animal models accurately reflects human disease and it is quite possible that different animal species and strains may be necessary for mimicking different aspects of human melioidosis. Large animal models have not yet been fully developed although it is quite clear that nonhuman primate models may be central for vaccine and therapeutic development and efficacy studies.

Immuno-antimicrobial therapy for *B. pseudomallei* infection—An alternative strategy to improve the efficacy of conventional antimicrobial therapy is to combine immunotherapy with antibiotic therapy. Such an approach has been evaluated for treatment of fungal infections, protozoal infections, and *Francisella* and *Mycobacterium* infection [77–79]. Previously, granulocyte colony-stimulating factor (G-CSF) therapy was suggested as an effective combination with antimicrobial therapy in *B. pseudomallei* infection, based on evidence that neutrophils were important for controlling *B. pseudomallei* pneumonic infection [80]. However, when this approach was evaluated *in vivo* in mice treated with G-CSF plus ceftazidime, the combined treatment was not found to be any more effective than treatment with either agent alone [81].

Recently, an *in vitro* screening assay was developed to identify cytokines and antimicrobial drugs that exhibited synergistic activity for controlling intracellular infection with *B. pseudomallei*. Using this assay, IFN- γ was identified as having particularly potent antibacterial activity against *B. pseudomallei* in infected macrophages when combined with certain classes of antimicrobials, including third-generation cephalosporins, potentiated penicillins and aminoglycosides [82]. Moreover, treatment of mice infected intranasally with a lethal dose of *B. pseudomallei* with subtherapeutic concentrations of ceftazidime and liposome–DNA immune stimulatory complexes generated significantly increased survival times and decreased bacterial burdens compared with treatment with either agent alone. Thus, immunotherapy may be used to increase the efficacy of conventional antimicrobial therapy for melioidosis, by decreasing the doses of antibiotics required for effective treatment or by shortening the duration of therapy required to eradicate residual bacteria.

Virulence factors as potential therapeutic targets

Capsule: The initial observations indicating that the *Burkholderia* extracellular capsule was important for full virulence were derived from subtractive hybridization analyses carried out between *B. pseudomallei* and a related avirulent organism, *Burkholderia thailandensis*. This led to the identification of a capsular polysaccharide in *B. pseudomallei*, which was found to be required for virulence in the Syrian hamster model of acute melioidosis [13]. Insertional inactivation of a glycosyltransferase gene resulted in a *B. pseudomallei* mutant strain that was 10,000-fold more attenuated for virulence than the wild-type strain. The capsule antigen identified as a virulence determinant was found to be the type I O-polysaccharide with the structure-(3)-2-O-acetyl-6-deoxy- β -D-manno-heptopyranose-(1) [83]. The role of the capsule in *B. pseudomallei* pathogenesis has been investigated and it was found that the addition of purified capsule polysaccharide increased the virulence of a capsule mutant strain in the hamster model [84]. Furthermore, the addition of purified *B. pseudomallei* capsule to serum bactericidal assays increased the survival of a serum-sensitive *B. pseudomallei* strain. Capsule production by *B. pseudomallei* contributed to reduced activation of the complement

cascade by reducing the levels of complement factor C3b deposition, thereby contributing to the persistence of bacteria in the blood of the infected host [84]. Because *B. pseudomallei* is known to be resistant to a number of antibiotics, there is a need for the development of therapeutic strategies targeting specific virulence factors [85]. Analysis of the genome sequence of this organism has revealed the presence of three other capsule gene clusters, namely the genes encoding for the biosynthesis of one of the capsular polysaccharides, which have been well characterized [83]. Taking into consideration the genetic and structural similarities that exist between characterized capsules of *B. mallei* and *B. pseudomallei*, it is feasible to investigate whether therapeutic compounds that target the proteins responsible for capsule biosynthesis may be able to protect against both pathogens.

Quorum sensing: Bacteria, particularly those growing in biofilms, communicate extensively with each other and employ a signaling mechanism to facilitate survival in hostile environments and to regulate a variety of bacterial functions essential for survival in a community. The notion that *Burkholderia* can signal each other and coordinate their virulence mechanisms against susceptible hosts is now well established [86,87]. These signaling networks represent a previously uncharacterized survival strategy in *B. mallei* and *B. pseudomallei* by which they evade antimicrobial defenses and overwhelm the host [88]. Quorum sensing (QS) molecules can regulate human transcriptional programs to the advantage of the pathogen [89]. In the case of *Burkholderia* species, they utilize QS systems (e.g., the CepIR or BpsIR QS systems) that rely on *N*-acyl-homoserine lactone or other related signaling molecules to regulate expression of virulence factors or other proteins associated with a variety of functions in a population-density-dependent manner [90,91]. These intricate and vital microbial communication systems are a weak point to exploit against *Burkholderia* because these systems are essential for full virulence in various infection models [89]. Therefore, development of novel therapeutics affecting the synthesis of QS signaling molecules or their regulatory components make logical approaches for intervention because they are essential and bacterial-specific targets.

The type III secretion system: Type III secretion is one of at least six different types of protein secretion employed by Gram-negative bacteria to transport proteins from the cytoplasm to the external milieu [92]. Type III secretion systems (TTSS) are responsible for delivering bacterial proteins, termed effectors, from the bacterial cytosol directly into the interior of host cells [93]. The TTSS is expressed predominantly by pathogenic bacteria and is usually used to introduce deleterious effectors into host cells, although plant and insect symbiotic bacteria also use the TTSS to interact with their hosts system [94]. Winstanley *et al.* reported the first TTSS gene cluster in *B. pseudomallei* [95]. The presence of this TTSS has been correlated with pathogenicity in *B. pseudomallei* [96,97]. An *in silico* study [98] demonstrated the presence of a TTSS analog in *B. mallei* and it was shown that this TTSS is essential for full virulence of *B. mallei in vitro* and in animal models of infection [96,97]. Recent progress in the development of bacterial live carrier vaccines has been made, using the TTSS for heterologous antigen (Ag) delivery [99] and by the utilization of type III secreted products as a subcutaneously administered bovine vaccine [100]. Many components of TTSSs reveal functional conservation probably due to the fact that shared type III genes were recruited by horizontal transfer during evolution [92]. Using this premise, Rosqvist *et al.* have shown that full-length wild-type *Yersinia* YopE can be secreted and translocated by *Salmonella enterica* serovar Typhimurium in a type III-dependent manner [101]. Further, it was demonstrated that *S. Typhimurium* allows secretion and translocation of chimeric YopE fused to large antigenic protein fragments of *Listeria monocytogenes*, which results in the induction of Ag-specific CD8 T-cell responses in orally vaccinated mice and animal protection against a virulent *L. monocytogenes* challenge [102]. In the case of the *B. mallei* TTSS, we have constructed a reporter system to characterize the TTSS and identified the secreted proteins associated with pathogenesis, which has provided crucial information

about the function of TTSS effectors in bacterial intracellular survival and invasion, and eventually, for the development of a candidate vaccine [103]. However, further studies are required to define the therapeutic role of the TTSS effector proteins and their use as components of candidate vaccines.

Isocitrate lyase inhibitors as novel antimicrobials—Isocitrate lyase (ICL) catalyzes the first step in the glyoxylate shunt, a carbon assimilatory pathway that allows the net synthesis of C4 dicarboxylic acids from C2 compounds such as acetate. For example, in *Mycobacterium tuberculosis*, the glyoxylate cycle is comprised of a single gene encoding malate synthase, but two genes encoding ICL [104]. Although deletion of *icl1* (encodes an enzyme closely related to ICLs in other eubacteria) or *icl2* (a protein product more homologous to eukaryotic ICLs) ICL isoforms in the glyoxylate pathway had little effect on bacterial growth in macrophages and mice, deletion of both genes resulted in complete impairment of intracellular replication and rapid elimination from the lungs [105]. The feasibility of targeting ICL1 and ICL2 for chemical inhibition was shown using a dual-specific ICL inhibitor, which blocked growth of *M. tuberculosis* on fatty acids and in macrophages [105]. Furthermore, the structure of ICL from *M. tuberculosis* has been solved in complex with the inhibitors 3-nitropropionate and 3-bromopyruvate [106]. As the intracellular pathogen *B. pseudomallei* causes lung pathology similar to that caused by *M. tuberculosis* [107], owing to the absence of ICL orthologs in mammals, and because ICL is a persistence factor for both *B. pseudomallei* and *M. tuberculosis*, the requirement of fatty acid metabolism for persistent *B. pseudomallei* infections was recently evaluated [108]. It was found that in contrast to *M. tuberculosis*, the *B. pseudomallei* ICL is considered a persistence factor, but this is not due to an inability to metabolize fatty acids, and the mutation of this gene results in very different clinical outcomes during animal infections [105,109]. It has been demonstrated that the mutation of ICL-1 in *M. tuberculosis* prevents the establishment of a chronic infection and results in a decrease in virulence [109]. By contrast, the mutation of ICL in *B. pseudomallei* prevents the establishment of a chronic infection and results in an increase in virulence [108]. Therefore, the authors of the paper indicated that caution should be used in the development of ICL inhibitors as novel antimicrobials because the inhibition of ICL does not always result in a decrease in virulence. The inhibition of ICL activity during a chronic *B. pseudomallei* infection resulted in an overwhelming acute infection. However, it was observed that treatment with itaconic acid plus ceftazidime reduced bacterial loads during chronic pulmonary *B. pseudomallei* infections more so than treatment with ceftazidime alone [108]. These results suggest that ICL inhibitors should not be developed solely as antimicrobials but could be used in combination with conventional antibiotics to treat not only *B. pseudomallei* infection but also *M. tuberculosis* infections.

Evaluation of granulysin antimicrobial activities against *B. mallei*—Granulysin is a broad-spectrum antimicrobial peptide with activities against both Gram-positive and Gram-negative bacteria [110–115]. Granulysin is a member of the saposin-like family of proteins; the most primitive member of the family is an amoebapore [114]. Granulysin is expressed by a variety of effector cell populations including CD4⁺ T cells, CD8⁺ T cells, $\gamma\delta$ T-cell receptor (TCR)⁺ cells and natural killer (NK) cells [110–114]. Granulysin expression can be used to identify bactericidal or bacteriostatic effector cells *in vivo* and can be tracked through a variety of mechanisms at both the transcriptional and post-transcriptional levels following vaccination and infection. Moreover, granulysin delivered in the appropriate context may serve as a therapeutic agent for *Burkholderia* infection given its activity against *B. mallei*. The broad-spectrum antimicrobial activity of granulysin-like molecules suggests an important mechanism by which granule exocytosis of T lymphocytes and NK cells may inhibit bacterial infection, in addition to killing of infected phagocytes. The direct antibacterial activity of granulysin-like molecules is particularly important against pathogens

such as *Burkholderia* species, in which killing of the infected cell does not necessarily result in killing of the pathogenic organism [115]. A significant reduction of *B. mallei* viability was observed following exposure to 10 and 100 μ M of human granulysin peptide, indicating lytic activity [115]. Peptide mapping studies have characterized a core amino acid region including residues in helix 2 through helix 3 as the biologically active site for granulysin-induced bacterial lysis [110,116–118]. The cationic properties of granulysin result from an abundance of arginine and lysine residues within the active site of the molecule responsible for antimicrobial activities. Arginine is the predominant positively charged amino acid residue in granulysin.

CpG DNA as a vaccine conjugate against *Burkholderia*—Due to high levels of antibiotic resistance, vaccination is a promising tool for prevention of melioidosis and glanders. Use of CpG in conjunction with vaccines will substantially strengthen vaccine development platforms. With no licensed vaccine available for either *B. pseudomallei* or *B. mallei*, these bacteria present a significant threat as a potential agent for bioterrorism [14,119]. Different CpG oligodinucleotide (CpG ODN) motifs are known to influence both the adaptive immune response in specific ways and to enhance resistance to infection in many animal models via Toll-like receptor (TLR)9 stimulation [120–122]. B-class CpG ODN strongly induces B-cell proliferation to plasma cells and plasmacytoid dendritic cell (pDC) development in humans while C-class CpG ODN exerts the same effects as B-class CpG ODN to a lesser extent as well as inducing IFN- α production [123,124]. The correlates for protection mediated by the adaptive immune response during *B. pseudomallei* infection are not fully characterized. TLR9 activated B cells and pDC express high levels of costimulatory molecules and secrete type I-promoting cytokines and chemokines [121,122]. Studies of different CpG ODN motifs in a mouse model of *B. pseudomallei* and *B. mallei* infection offer a unique opportunity to both evaluate the effectiveness of different CpG motifs for use as a future adjuvant in vaccine development and elucidate correlates of adaptive protection.

Recently, the effects of different CpG classes on survival following lethal challenge with *B. pseudomallei* and *B. mallei* were examined. Prior studies have shown that the use of B-class CpG in conjunction with immunogenic proteins against *B. pseudomallei* offers greater protection to mice than protein alone [125]. B-class CpG ODNs have been shown to be 90% effective in preventing lethality from intraperitoneal *B. pseudomallei* challenge [126,127]. In addition, mice immunized with DCs that were pre-exposed to *B. pseudomallei* and B-class CpG gain greater protection from lethal challenge than mice receiving DCs unexposed to B-class CpG [128]. However, the utility of B-class CpG against aerosol challenge and the efficacy of B-class CpG as compared with C-class CpG remain unknown.

The results indicate that prior administration of either B- or C-class CpG provides strong protection against *B. pseudomallei* and *B. mallei* aerosol challenge. B-class-CpG-primed mice had a much lower level of colony forming units (CFUs) in the spleen, indicating a role for B cells in preventing pathogenesis following infection. We show that prior administration of either B- or C-class CpG provides strong protection against *B. pseudomallei* or *B. mallei* challenge via the respiratory route (Figures 1 & 2). Only B-class-CpG primed mice had a major reduction in bacterial burden in the spleen as well as providing 80% protection, suggesting a primary role for B cells in preventing pathogenesis following infection. We have identified similar results using loss of B-cell function analysis following vaccination with heat-killed *B. mallei* with rapid progression to death in either an acute (BALB/c) or chronic (C57BL/6) model of infection [129]. In addition, the data strongly support the use of CpG adjuvant for vaccine development platforms against *B. pseudomallei* or *B. mallei* infection. More recently, mucosally delivered immunotherapy has also been used to generate protection against pneumonic *B. pseudomallei* infection. These studies used

cationic liposomes to deliver noncoding plasmid DNA to the airways of mice, as incorporation of DNA or CpG ODN into liposomes has been shown to markedly potentiate the immune stimulatory properties of these TLR9 agonists [130]. Mice treated with liposome–DNA complexes shortly before or after lethal challenge with *B. pseudomallei* had significantly increased survival times compared with control mice [131]. Protection elicited by mucosal immunotherapy with liposome–DNA complexes was found to be dependent on IFN- γ production and on NK cells, but to be independent of T-cell responses. Although mice were protected from acute challenge, they were not protected from developing chronic disease, particularly splenic abscesses.

Use of TLR9 agonists as vaccine adjuvants for *Burkholderia* vaccines: CpG ODN and liposome–DNA complexes have also been shown to be very effective vaccine adjuvants [121]. The adjuvant activity of these TLR9 agonists stems directly from their potent activation of innate immune responses, particularly Th1 responses. Thus, vaccines prepared using CpG ODN adjuvants, or liposome–DNA adjuvants, elicit strong cellular immune responses dominated by IFN- γ production.

The correlates that protection mediated by the adaptive immune response during *B. pseudomallei* infection has not been fully characterized. B cells and DCs activated by CpG ODN and liposome–DNA complexes express high levels of costimulatory molecules and secrete type I-promoting cytokines and chemokines [121,122]. Therefore, vaccination studies were conducted using CpG ODN or liposome–DNA complexes as adjuvants for parenteral or mucosal vaccines against *B. mallei* or *B. pseudomallei*.

Prior studies have shown that the use of B-class CpG in conjunction with immunogenic proteins against *B. pseudomallei* offers greater protection to mice than protein alone [125]. B-class CpG have been shown to be 90% effective in preventing lethality from intraperitoneal *B. pseudomallei* challenge [126,127]. Additionally, mice immunized with DCs that were pre-exposed to *B. pseudomallei* and B-class CpG gain greater protection from lethal challenge than mice receiving DCs unexposed to B-class CpG [128]. However, the utility of B-class CpG against aerosol challenge and the efficacy of B-class CpG as compared with C-class CpG remains unknown.

Silver carbene compounds—Silver has been used for many years in a variety of forms for its antimicrobial properties. The activity of silver against Gram-positive and Gram-negative bacteria is well established [132–135]. In the presence of oxygen, Ag⁺ ions are produced, which possess microbicidal activities [132–136]. In *in vitro* studies of antimicrobial activity against *B. mallei* and *B. pseudomallei* we evaluated silver carbene compounds (SCCs). Minimum inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of the methylated caffeine silver acetate complex SCC1, as well as of SCC5, SCC10, SCC12 and SCC22, against two potential respiratory pathogens, *B. pseudomallei* K96243 and *B. mallei* ATCC23344, are shown in Table 1 [136]. The MICs and MBCs for all silver compounds against *B. pseudomallei* were 4–6 and 6–10 $\mu\text{g/ml}$ or higher, respectively. In the case of *B. mallei*, the MICs and MBCs of all compounds were 1–4 and 6 $\mu\text{g/ml}$, respectively [136]. These data suggested that *B. mallei* is more susceptible to silver carbene complexes, especially SCC1 and SCC22, than *B. pseudomallei*. The MIC₉₀ of SCC1 and SCC22 against *B. pseudomallei* were 8 and 6 $\mu\text{g/ml}$, respectively. Based on these results, it appeared that SCC5 may be the best candidate to treat *B. pseudomallei* infection (Table 1). Whereas SCC5 and SCC22 were prime candidates for *B. mallei* as their MIC₅₀ values were only 2 $\mu\text{g/ml}$ for both complexes [136]. A caveat regarding the use of silver compounds for therapy development is that most metal compounds are not approved for systemic administration but only for use in coatings of implanted devices. Thus, their use for melioidosis and glanders treatment would be limited.

Expert commentary

At present, we can still use antibiotics to treat *Burkholderia* infections, particularly melioidosis in endemic areas or glanders in case of a bioterrorist attack. However, management of melioidosis continues to represent a challenge for physicians, mainly due to poorly understood intrinsic antibiotic resistance and tolerance, and the persistence of these infections in susceptible individuals, frequently leading to clinical therapeutic failures. Development of improved *in vitro* and *in vivo* models to accelerate decisions on moving new therapeutic candidates forward into clinical testing is crucial to supply those compounds to endemic areas or affected populations in the case of a biological attack. With few exceptions, newer generations of antibiotics do not appear to offer significant advantages over the traditional arsenal of antimicrobial agents. For patients suffering from a chronic infection, very few clinical options exist. Therefore, it is critical to develop effective pre- and postexposure treatment approaches combining current antibiotics and novel therapeutic agents to shorten treatment of melioidosis or glanders. Whether combination therapy offers an advantage over antibiotic monotherapy requires further studies, including more randomized trials and a more careful assessment of synergy and antagonism issues when using drug combinations. How to achieve the latter is still a matter of debate [137]. For clinical trials, partnerships need to be developed to assure that new therapeutic compounds can be safely and ethically tested in endemic areas. With a current limited pipeline of new classes of antimicrobial agents, the availability of sequenced bacterial genomes has opened the possibility of identifying new targets; however, most of these targets are still under preliminary investigation. Other remaining challenges include understanding of *in vitro* versus *in vivo* discrepancies. For example, questions such as why fluoroquinolone shows such disappointing *in vivo* efficacies and why third-generation cephalosporins differ in their *in vivo* activities remain to be answered.

Five-year view

In many parts of the world, recognition of the potential use of *B. mallei* and *B. pseudomallei* as bioterrorism agents has led to an increase of funds into research into these important pathogens. As a result, our knowledge on the biology and pathogenesis of these bacteria is rapidly increasing. It can be anticipated that over the next 5 years some of this knowledge will be exploited to arrive at better strategies for glanders and melioidosis management. Development of new animal models, especially nonhuman primate and/or other large animal models meeting the US FDA two-animal rule, will accelerate the testing of new compounds and re-evaluation of existing drug regimens. This in turn will assist in transitioning some new drug candidates into clinical trials in endemic areas. Supporting development of new therapeutic strategies will include efforts aimed at understanding mechanisms of drug resistance and/or tolerance. A major challenge and consideration in developing new melioidosis therapies is to keep them applicable and affordable in endemic areas.

Key issues

- Mixed antibiotic regimens are required to treat glanders and melioidosis patients, but treatment is only partially effective.
- Ceftazidime or a carbapenem followed by trimethoprim–sulfamethoxazole with or without doxycycline is the most common recommended treatment for acute human melioidosis.
- BAL30072 is a new monobactam with superior activity against Gram-negative pathogens, including *Burkholderia pseudomallei*.

- Little data on the efficacy of antibiotics for the treatment of human glanders are available but ceftazidime and levofloxacin have shown some utility.
- The availability of several *B. pseudomallei* and *Burkholderia mallei* genome sequences has greatly facilitated target identification and development of new therapeutics.
- Granulysin and silver carbene compounds delivered in the appropriate context may serve as a therapeutic option for *Burkholderia* infection given its activity against these pathogens.
- CpG in conjunction with vaccines will substantially strengthen vaccine development platforms due to its properties as an adjuvant and antimicrobial therapy against *Burkholderia*.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Wiersinga WJ, van der Poll T, White NJ, Day NP, Peacock SJ. Melioidosis: insights into the pathogenicity of *Burkholderia pseudomallei*. *Nat. Rev. Microbiol* 2006;4:272–282. [PubMed: 16541135]
2. Cheng AC, Currie BJ. Melioidosis: epidemiology, pathophysiology, and management. *Clin. Microbiol. Rev* 2005;18:383–416. [PubMed: 15831829] • A current comprehensive review about melioidosis and its clinical management.
3. Currie BJ, Jacups SP. Intensity of rainfall and severity of melioidosis, Australia. *Emerg. Infect. Dis* 2003;9:1538–1542. [PubMed: 14720392]
4. Currie BJ, Dance DA, Cheng AC. The global distribution of *Burkholderia pseudomallei* and melioidosis: an update. *Trans. R. Soc. Trop. Med. Hyg* 2008;102:S1–S4. [PubMed: 19121666]
5. Koponen MA, Zlock D, Palmer DL, Merlin TL. Melioidosis: forgotten, but not gone. *Arch. Intern. Med* 1991;151:605–608. [PubMed: 2001144]
6. Chaowagul W, Suputtamongkol Y, Dance DA, Rajchanuvong A, Pattara-arechachai J, White NJ. Relapse in melioidosis: incidence and risk factors. *J Infect. Dis* 1993;168:1181–1185. [PubMed: 8228352]
7. Holden MT, Titball RW, Peacock SJ, et al. Genomic plasticity of the causative agent of melioidosis, *Burkholderia pseudomallei*. *Proc. Natl Acad. Sci. USA* 2004;101:14240–14245. [PubMed: 15377794] •• First report of the complete genome sequence of *Burkholderia pseudomallei*.
8. Gan YH, Chua KL, Chua HH, et al. Characterization of *Burkholderia pseudomallei* infection and identification of novel virulence factors using a *Caenorhabditis elegans* host system. *Mol. Microbiol* 2002;44:1185–1197. [PubMed: 12068805] • The authors use the *Caenorhabditis elegans* model to identify novel virulence determinants of *B. pseudomallei*.
9. Howe C, Sampath A, Spotnitz M. The *pseudomallei* group: a review. *J Infect. Dis* 1971;124:598–606. [PubMed: 5001752]
10. Ip M, Osterberg LG, Chau PY, Raffin TA. Pulmonary melioidosis. *Chest* 1995;108:1420–1424. [PubMed: 7587451]
11. Arun S, Neubauer H, Gürel A, et al. Equine glanders in turkey. *Vet. Rec* 1999;144:255–258. [PubMed: 10209817]
12. Lehave O, Aizenstien O, Katz LH, Hourvitz A. Glanders – a potential disease for biological warfare in humans and animals. *Harefuah* 2002;141:88–91. [PubMed: 12170562]
13. Reckseidler S, DeShazer D, Sokol PA, Woods DE. Detection of bacterial virulence genes by subtractive hybridisation: identification of capsular polysaccharide of *Burkholderia pseudomallei*

as a major virulence determinant. *Infect. Immun* 2001;69:34–44. [PubMed: 11119486] • The authors use the subtractive hybridization method for the identification of *B. pseudomallei* virulence factors.

14. Whitlock GC, Estes DM, Torres AG. Glanders: off to the races with *Burkholderia*. *FEMS Microbiol. Lett* 2007;277:115–122. [PubMed: 18031330] •• A current comprehensive review about *Burkholderia mallei* pathogenesis.
15. Dance, DAB. Melioidosis and glanders as possible biological weapons. In: IW, Fong; K, Alibek, editors. *Bioterrorism and Infectious Agents. A New Dilemma for the 21st Century*. USA: Springer; 2005. p. 99-145.
16. Ipatenko NG. Study of the bacteriostatic and bactericidal properties of certain antibiotics. *Trudy Moskovskoi Veterinarnoi Akademii* 1972;61:142–148.
17. Al-Izzi SA, Al-Bassam LS. *In vitro* susceptibility of *Pseudomonas mallei* to antimicrobial agents. *Comp. Immunol. Microbiol. Infect. Dis* 1989;12:5–8. [PubMed: 2776405]
18. Antonov IV, Iliukhin VI, Popovtseva LD, Batmanov VP. Sensitivity of *Pseudomonas* to currently used antibacterial drugs. *Antibiot. Khimioter* 1991;36:14–16. [PubMed: 2053815]
19. Batmanov VP. Sensitivity of *Pseudomonas mallei* to fluoroquinolones and their efficacy in experimental glanders. *Antibiot. Khimioter* 1991;36:31–34. [PubMed: 1781709]
20. Batmanov VP. Treatment of experimental glanders with combinations of sulfazine or sulfamonomethoxine with trimethoprim. *Antibiot. Khimioter* 1993;38:18–22. [PubMed: 8031190]
21. Batmanov VP. Sensitivity of *Pseudomonas mallei* to tetracyclines and their effectiveness in experimental glanders. *Antibiot. Khimioter* 1994;39:33–37. [PubMed: 7857158]
22. Manzeniuk IN, Dorokhin VV, Svetoch EA. The efficacy of antibacterial preparations against *Pseudomonas mallei* in *in-vitro* and *in-vivo* experiments. *Antibiot. Khimioter* 1994;39:26–30. [PubMed: 7979790]
23. Kenny DJ, Russell P, Rogers D, Eley SM, Titball RW. *In vitro* susceptibilities of *Burkholderia mallei* in comparison to those of other pathogenic *Burkholderia* spp. *Antimicrob. Agents Chemother* 1999;43:2773–2775. [PubMed: 10543761]
24. Heine HS, England MJ, Waag DM, Byrne WR. *In vitro* antibiotic susceptibilities of *Burkholderia mallei* (causative agent of glanders) determined by broth microdilution and E-test. *Antimicrob. Agents Chemother* 2001;45:2119–2121. [PubMed: 11408233]
25. Nierman WC, DeShazer D, Kim HS, et al. Structural flexibility in the *Burkholderia mallei* genome. *Proc. Natl Acad. Sci. USA* 2004;101:14246–14251. [PubMed: 15377793] •• First report of the complete genome sequence of *B. mallei*.
26. Srinivasan A, Kraus CN, DeShazer D, et al. Glanders in a military research microbiologist. *N. Engl. J. Med* 2001;345:256–258. [PubMed: 11474663]
27. Manzeniuk IN, Stepanshin IG, Svetoch EA. Evaluation of the potential of chemotherapy of glanders caused by *Pseudomonas mallei* strains resistant to sulfanilamides and trimethoprim. *Antibiot. Khimioter* 1994;39:30–34.
28. Leelarasamee A, Bovornkitti S. Melioidosis: a review and update. *Rev. Infect. Dis* 1989;11:413–425. [PubMed: 2665001]
29. Dance DAB, Wuthiekanun V, Chaowagul WI, White NJ. The antimicrobial susceptibility of *Pseudomonas pseudomallei*. Emergence of resistance *in-vitro* and during treatment. *J. Antimicrob. Chemother* 1989;24:295–309. [PubMed: 2681116]
30. Ashdown LR. *In-vitro* activities of the newer β -lactam and quinolone agents against *Pseudomonas pseudomallei*. *Antimicrob. Agents Chemother* 1988;32:1435–1436. [PubMed: 3196005]
31. Rajchanuvong A, Chaowagul W, Suputtamongkol Y, Smith MD, Dance DA, White NJ. A prospective comparison of co-amoxiclav and the combination of chloramphenicol, doxycycline and co-trimoxazole for the oral maintenance treatment of melioidosis. *Trans. R. Soc. Trop. Med. Hyg* 1995;89:546–549. [PubMed: 8560537]
32. Simpson AJH, Suputtamongkol Y, Smith MD, et al. Comparison of imipenem and ceftazidime as therapy for severe melioidosis. *Clin. Infect. Dis* 1999;29:381–387. [PubMed: 10476746]
33. Chetchotisakd P, Chaowagul W, Mootsikapun P, Budhsarawong D, Thinkamrop B. Maintenance therapy of melioidosis with ciprofloxacin plus azithromycin compared with co-trimoxazole plus doxycycline. *Am. J. Trop. Med. Hyg* 2001;64:24–27. [PubMed: 11425157]

34. Russell P, Eley SM, Ellis J, et al. Comparison of efficacy of ciprofloxacin and doxycycline against experimental melioidosis and glanders. *J. Antimicrob. Chemother* 2000;45:813–818. [PubMed: 10837435]
35. White NJ. Melioidosis. *Lancet* 2003;361:1715–1722. [PubMed: 12767750]
36. Cheng AC, Fisher DA, Anstey NM, Stephens DP, Jacups SP, Currie BJ. Outcomes of patients with melioidosis treated with meropenem. *Antimicrob. Agents Chemother* 2004;48:1763–1765. [PubMed: 15105132]
37. Sivalingam SP, Sim SH, Jasper LC, et al. Pre- and post-exposure prophylaxis of experimental *Burkholderia pseudomallei* infection with doxycycline, amoxicillin/ clavulanic acid and co-trimoxazole. *J. Antimicrob. Chemother* 2008;61:674–678. [PubMed: 18192684] • Treatment of melioidosis with important implications on the management of laboratory accidents or following an intentional release of *B. pseudomallei*.
38. Dance DA. Melioidosis. *Curr. Opin. Infect. Dis* 2002;15:127–132. [PubMed: 11964912]
39. Chaowagul W. Recent advances in the treatment of severe melioidosis. *Acta Trop* 2000;74:133–137. [PubMed: 10674641]
40. White NJ, Dance DA, Chaowagul W, Wattanagoon Y, Wuthiekanun V, Pitakwatchara N. Halving of mortality of severe melioidosis by ceftazidime. *Lancet* 1989;2(8665):697–701. [PubMed: 2570956]
41. Sookpranee M, Boonma P, Susaengrat W, Bhuripanyo K, Punyagupta S. Multicenter prospective randomized trial comparing ceftazidime plus co-trimoxazole with chloramphenicol plus doxycycline and co-trimoxazole for treatment of severe melioidosis. *Antimicrob. Agents Chemother* 1992;36:158–162. [PubMed: 1590682]
42. Samuel M, Ti TY. Interventions for treating melioidosis. *Cochrane Database Syst. Rev* 2001;2 CD001263.
43. Dance DA, Wuthiekanun V, Chaowagul W, White NJ. The activity of amoxycillin/ clavulanic acid against *Pseudomonas pseudomallei*. *J. Antimicrob. Chemother* 1989;24:1012–1014. [PubMed: 2621173]
44. Suputtamongkol YA, Rajchanuwong W, Chaowagul DA, et al. Ceftazidime vs. amoxicillin/ clavulanate in the treatment of severe melioidosis. *Clin. Infect. Dis* 1994;19:846–853. [PubMed: 7893868]
45. Chaowagul W, Simpson AJ, Suputtamongkol Y, Smith MD, Angus BJ, White NJ. A comparison of chloramphenicol, trimethoprim-sulfamethoxazole, and doxycycline with doxycycline alone as maintenance therapy for melioidosis. *Clin. Infect. Dis* 1999;29:375–380. [PubMed: 10476745]
46. Chaowagul W, Suputtamongkul Y, Smith MD, White NJ. Oral fluoroquinolones for maintenance treatment of melioidosis. *Trans. R. Soc. Trop. Med. Hyg* 1997;91:599–601. [PubMed: 9463680]
47. Chaowagul W, Chierakul W, Simpson AJ, et al. Open-label randomized trial of oral trimethoprim-sulfamethoxazole, doxycycline, and chloramphenicol compared with trimethoprim-sulfamethoxazole and doxycycline for maintenance therapy of melioidosis. *Antimicrob. Agents Chemother* 2005;49:4020–4025. [PubMed: 16189075] • Describes a randomized trial conducted in Northeast Thailand demonstrating that trimethoprim-sulfamethoxazole and doxycycline is as effective as, and better tolerated than, the conventional four-drug regimen.
48. Limmathurotsakul D, Chaowagul W, Wongsrikaew P, et al. Variable presentation of neurological melioidosis in Northeast Thailand. *Am. J. Trop. Med. Hyg* 2007;77:118–120. [PubMed: 17620641]
49. Owen, SJ.; Batzloff, M.; Chehrehasa, F., et al. Nasal inoculation of mice: colonisation, lymphoid tissue, and a direct route to the brain; Presented at: International Symposium on Inhalation Animal Models for Melioidosis and Glanders; Baltimore, MD, USA: 2009 Feb 25.
50. Ashdown LR, Currie BJ. Melioidosis: when in doubt leave the quinolone alone! *Med. J. Aust* 1992;157:427–428. [PubMed: 1280318]
51. Winton MD, Everett ED, Dolan SA. Activities of five new fluoroquinolones against *Pseudomonas pseudomallei*. *Antimicrob. Agents Chemother* 1988;32:928–929. [PubMed: 3415212]
52. Ho PL, Cheung TK, Kinoshita R, et al. Activity of five fluoroquinolones against 71 isolates of *Burkholderia pseudomallei*. *J. Antimicrob. Chemother* 2002;49:1042–1044. [PubMed: 12039903]

53. Steward J, Piercy T, Lever MS, et al. Comparison of gatifloxacin, moxifloxacin and ciprofloxacin for treatment of experimental *Burkholderia pseudomallei* infection. *J. Antimicrob. Chemother* 2005;55:523–527. [PubMed: 15731198]
54. Wuthiekanun V, Peacock SJ. Management of melioidosis. *Expert Rev. Anti Infect. Ther* 2006;4:445–455. [PubMed: 16771621] • Comprehensive review about management of melioidosis.
55. Lumbiganon P, Sookpranee T. Ciprofloxacin therapy for localized melioidosis. *Ped. Infect. Dis* 1992;11:418–419.
56. Koga H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes. *Antimicrob. Agents Chemother* 1987;31:1904–1908. [PubMed: 3439800]
57. Chateau MT, Caravano R. Rapid fluorometric measurement of the intracellular concentration of ciprofloxacin in mouse peritoneal macrophages. *J. Antimicrob. Chemother* 1993;31:281–287. [PubMed: 8463173]
58. LeBel M. Ciprofloxacin: chemistry, mechanism of action, resistance, antimicrobial spectrum, pharmacokinetics, clinical trials, and adverse reactions. *Pharmacotherapy* 1988;8:3–33. [PubMed: 2836821]
59. Peacock SJ, Schweizer HP, Dance DAB, et al. Consensus guidelines on the management of accidental laboratory exposure to *Burkholderia pseudomallei* and *Burkholderia mallei*. *Emerg. Infect. Dis* 2008;14(7):e2. [PubMed: 18598617] • Description of the current guidelines on the management of accidental laboratory *B. pseudomallei* exposures.
60. Thibault FM, Hernandez E, Vidal DR, Girardet M, Cavallo JD. Antibiotic susceptibility of 65 isolates of *Burkholderia pseudomallei* and *Burkholderia mallei* to 35 antimicrobial agents. *J. Antimicrob. Chemother* 2004;54:1134–1138. [PubMed: 15509614] • The authors performed a susceptibility analysis of *Burkholderia* strains to a large number of antibiotics.
61. Lumbiganon P, Tattawasatra U, Chetchotisakd P, Wongratanaheewin S, Thinkhamrop B. Comparison between the antimicrobial susceptibility of *Burkholderia pseudomallei* to trimethoprim–sulfamethoxazole by standard disk diffusion method and by minimal inhibitory concentration determination. *J. Med. Assoc. Thailand* 2000;83:856–860.
62. Wuthiekanun V, Cheng AC, Chierakul W, et al. Trimethoprim/sulfamethoxazole resistance in clinical isolates of *Burkholderia pseudomallei*. *J. Antimicrob. Chemother* 2005;55:1029–1031. [PubMed: 15886263]
63. Piliouras P, Ulett GC, Ashhurst-Smith C, et al. A comparison of antibiotic susceptibility testing methods for cotrimoxazole with *Burkholderia pseudomallei*. *Int. J. Antimicrob. Agents* 2002;19:427–429. [PubMed: 12007851]
64. Jenney AW, Lum G, Fisher DA, Currie BJ. Antibiotic susceptibility of *Burkholderia pseudomallei* from tropical northern Australia and implications for therapy of melioidosis. *Int. J. Antimicrob. Agents* 2001;17:109–113. [PubMed: 11165114]
65. Chen K, Sun GW, Chua KL, et al. Modified virulence of antibiotic-induced *Burkholderia pseudomallei* filaments. *Antimicrob. Agents Chemother* 2005;49:1002–1009. [PubMed: 15728895]
66. Haussler S, Rohde M, Steinmetz I. Highly resistant *Burkholderia pseudomallei* small colony variants isolated *in vitro* and in experimental melioidosis. *Med. Microbiol. Immunol* 1999;188:91–97. [PubMed: 10753061]
67. Pruksachartvuthi S, Aswapokee N, Thankerngpol K. Survival of *Pseudomonas pseudomallei* in human phagocytes. *J. Med. Microbiol* 1990;31:109–114. [PubMed: 2304065]
68. Tribuddharat C, Moore RA, Baker P, Woods DE. *Burkholderia pseudomallei* class a β -lactamase mutations that confer selective resistance against ceftazidime or clavulanic acid inhibition. *Antimicrob. Agents Chemother* 2003;47:2082–2087. [PubMed: 12821450]
69. Karunakaran R, Puthucheary SD. *Burkholderia pseudomallei*: *in vitro* susceptibility to some new and old antimicrobials. *Scand. J. Infect. Dis* 2009;39:858–861. [PubMed: 17852912]
70. Sam I-C, See KH, Puthucheary SD. Variations in ceftazidime and amoxicillin–clavulanate susceptibilities within a clonal infection of *Burkholderia pseudomallei*. *J. Clin. Microbiol* 2009;47:1556–1558. [PubMed: 19297597]

71. Thamlikitkul V, Trakulsomboon S. *In vitro* activity of tigecycline against *Burkholderia pseudomallei* and *Burkholderia thailandensis*. *Antimicrob. Agents Chemother* 2006;50:1555–1557. [PubMed: 16569883]
72. Feterl M, Govan B, Engler C, et al. Activity of tigecycline in the treatment of acute *Burkholderia pseudomallei* infection in a murine model. *Int. J. Antimicrob. Agents* 2006;28:460–464. [PubMed: 17046208]
73. Thamlikitkul V, Trakulsomboon S. *In vitro* activity of ceftobiprole against *Burkholderia pseudomallei*. *J. Antimicrob. Chemother* 2008;61:460–461. [PubMed: 18096556]
74. Mima, T.; Desarbres, E.; Page, MGP.; Schweizer, HP. *In vitro* activity of BAL30072 against *Burkholderia pseudomallei*; Presented at: 49th Interscience Conference on Antimicrobial Agents and Chemotherapy; 12–15 September; San Francisco, CA, USA. 2009. (Abstract F1–1478)
75. Judy BM, Whitlock GC, Torres AG, Estes DM. Comparison of the *in vitro* and *in vivo* susceptibilities of *Burkholderia mallei* to ceftazidime and levofloxacin. *BMC Microbiol* 2009;9:88. [PubMed: 19426516]
76. Titball RW, Russell P, Cuccui J, et al. *Burkholderia pseudomallei*: animal models of infection. *Trans. R. Soc. Trop. Med. Hyg* 2008;102:S111–S116. [PubMed: 19121670] • A current comprehensive review about animal models to test *B. pseudomallei* virulence.
77. Onyeji CO, Bui KQ, Nicolau DP, Nightingale CH, Bow L, Quintiliani R. Influence of adjunctive interferon- γ on treatment of gentamicin- and vancomycin-resistant *Enterococcus faecalis* infection in mice. *Int. J. Antimicrob. Agents* 1999;12:301–309. [PubMed: 10493606]
78. Onyeji CO, Nicolau DP, Nightingale CH, Bow L. Interferon- γ effects on activities of gentamicin and vancomycin against *Enterococcus faecalis* resistant to the drugs: an *in vitro* study with human neutrophils. *Int. J. Antimicrob. Agents* 1999;11:31–37. [PubMed: 10075275]
79. Pammit MA, Budhavarapu VN, Raulie EK, et al. Intranasal interleukin-12 treatment promotes antimicrobial clearance and survival in pulmonary *Francisella tularensis* subsp. *novicida* infection. *Antimicrob. Agents Chemother* 2004;48:4513–4519. [PubMed: 15561819]
80. Cheng AC, Stephens DP, Anstey NM, et al. Adjunctive granulocyte colony-stimulating factor for treatment of septic shock due to melioidosis. *Clin. Infect. Dis* 2004;38:32–37. [PubMed: 14679445]
81. Powell K, Ulett G, Hirst R, et al. G-CSF immunotherapy for treatment of acute disseminated murine melioidosis. *FEMS Microbiol. Lett* 2003;224(2):315–318. [PubMed: 12892898]
82. Propst KL, Troyer RM, Kellihan LM, Schweizer HP, Dow SW. Immunotherapy markedly increases the effectiveness of antimicrobial therapy for treatment of *Burkholderia pseudomallei* infection. *Antimicrob. Agents Chemother.* 2010 (In Press).
83. Perry MB, MacLean LL, Schollaardt T, Bryan LE, Ho M. Structural characterization of the lipopolysaccharide O antigens of *Burkholderia pseudomallei*. *Infect. Immun* 1995;63:3348–3352. [PubMed: 7543882]
84. Reckseidler-Zenteno SL, DeVinney R, Woods DE. The capsular polysaccharide of *Burkholderia pseudomallei* contributes to survival in serum by reducing complement factor C3b deposition. *Infect. Immun* 2005;73:1106–1115. [PubMed: 15664954]
85. Reckseidler-Zenteno SL, Moore R, Woods DE. Genetics and function of the capsules of *Burkholderia pseudomallei* and their potential as therapeutic targets. *Mini Rev. Med. Chem* 2009;9:265–271. [PubMed: 19200030] •• Description and use of *B. pseudomallei* virulence factors as anti-infective agents.
86. Ulrich RL, Deshazer D, Hines HB, Jeddelloh JA. Quorum sensing: a transcriptional regulatory system involved in the pathogenicity of *Burkholderia mallei*. *Infect. Immun* 2004;72:6589–6596. [PubMed: 15501791]
87. Ulrich RL, Deshazer D, Brueggemann EE, Hines HB, Oyston PC, Jeddelloh JA. Role of quorum sensing in the pathogenicity of *Burkholderia pseudomallei*. *J. Med. Microbiol* 2004;53:1053–1064. [PubMed: 15496380]
88. Eberl L. Quorum sensing in the genus *Burkholderia*. *Int. J. Med. Microbiol* 2006;296:103–110. [PubMed: 16490397]

89. Sokol PA, Malott RJ, Riedel K, Eberl L. Communication systems in the genus *Burkholderia*: global regulators and targets for novel antipathogenic drugs. *Future Microbiol* 2007;2:555–563. [PubMed: 17927476] • Linked quorum sensing to regulation of virulence genes in *Burkholderia*.
90. Sokol PA, Sajjan U, Visser MB, Ginges S, Forstner J, Kooi C. The CepIR quorum-sensing system contributes to the virulence of *Burkholderia cenocepacia* respiratory infections. *Microbiology* 2003;149:3649–3658. [PubMed: 14663096]
91. Kiratisin P, Sanmee S. Roles and interactions of *Burkholderia pseudomallei* BpsIR quorum-sensing system determinants. *J. Bacteriol* 2008;190:7291–7297. [PubMed: 18757538]
92. Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev* 1998;62:379–433. [PubMed: 9618447]
93. Yahr TL. A critical new pathway for toxin secretion? *N. Engl. J. Med* 2006;355:1171–1172. [PubMed: 16971725]
94. Viprey V, Del Greco A, Golinowski W, Broughton WJ, Perret X. Symbiotic implications of type III protein secretion machinery in *Rhizobium*. *Mol. Microbiol* 1998;28:1381–1389. [PubMed: 9680225]
95. Winstanley C, Hales BA, Hart CA. Evidence for the presence in *Burkholderia pseudomallei* of a type III secretion system-associated gene cluster. *J. Med. Microbiol* 1999;48:649–656. [PubMed: 10403415]
96. Stevens MP, Friebel A, Taylor LA, et al. A *Burkholderia pseudomallei* type III secreted protein, BopE, facilitates bacterial invasion of epithelial cells and exhibits guanine nucleotide exchange factor activity. *J. Bacteriol* 2003;185:4992–4996. [PubMed: 12897019]
97. Winstanley C, Hart CA. Presence of type III secretion genes in *Burkholderia pseudomallei* correlates with Ara(-) phenotypes. *J. Clin. Microbiol* 2000;38:883–885. [PubMed: 10655407]
98. Rainbow L, Hart CA, Winstanley C. Distribution of type III secretion gene clusters in *Burkholderia pseudomallei*, *B. thailandensis* and *B. mallei*. *J. Med. Microbiol* 2002;51:374–384. [PubMed: 11990489]
99. Russmann H, Shams H, Poblete F, Fu Y, Galan JE, Donis RO. Delivery of epitopes by the *Salmonella* type III secretion system for vaccine development. *Science* 1998;281:565–568. [PubMed: 9677200]
100. Potter AA, Klashinsky S, Li Y, et al. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine* 2004;22:362–369. [PubMed: 14670317]
101. Rosqvist R, Hakansson S, Forsberg A, Wolf-Watz H. Functional conservation of the secretion and translocation machinery for virulence proteins of yersiniae, salmonellae and shigellae. *EMBO J* 1995;14:4187–4195. [PubMed: 7556059]
102. Russmann H, Igwe EI, Sauer J, Hardt WD, Bubert A, Geginat G. Protection against murine listeriosis by oral vaccination with recombinant *Salmonella* expressing hybrid Yersinia type III proteins. *J. Immunol* 2001;167:357–365. [PubMed: 11418671]
103. Whitlock GC, Estes DM, Young GM, et al. Construction of a reporter system to study *Burkholderia mallei* type III secretion and identification of the BopA effector protein function in intracellular survival. *Trans. R. Soc. Trop. Med. Hyg* 2008;102:S127–S133. [PubMed: 19121674]
104. Fleischmann RD, Alland D, Eisen JA, et al. Whole-genome comparison of *Mycobacterium tuberculosis* clinical and laboratory strains. *J. Bacteriol* 2002;184:5479–5490. [PubMed: 12218036]
105. Munoz-Elias EJ, McKinney JD. *Mycobacterium tuberculosis* isocitrate lyase 1 and 2 are jointly required for *in vivo* growth and virulence. *Nat. Med* 2005;11:638–644. [PubMed: 15895072]
106. Sharma V, Sharma S, Hoener zu Bentrop K, et al. Structure of isocitrate lyase, a persistence factor of *Mycobacterium tuberculosis*. *Nat. Struct. Biol* 2000;7:663–668. [PubMed: 10932251]
107. van Schaik E, Tom M, DeVinney R, Woods DE. Development of novel animal infection models for the study of acute and chronic *Burkholderia pseudomallei* pulmonary infections. *Microbes Infect* 2008;10:1291–1299. [PubMed: 18707015] •• Describes the development of new animal models of acute and chronic *B. pseudomallei*.

108. van Schaik EJ, Tom M, Woods DE. *Burkholderia pseudomallei* isocitrate lyase is a persistence factor in pulmonary melioidosis: implications for the development of isocitrate lyase inhibitors as novel antimicrobials. *Infect. Immun* 2009;77:4275–4283. [PubMed: 19620343]
109. McKinney JD, Höner zu Bentrup K, Muñoz-Elías EJ, et al. Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* 2000;406:735–738. [PubMed: 10963599]
110. Endsley JJ, Furrer JL, Endsley MA, et al. Characterization of bovine homologues of granulysin and NK-lysin. *J. Immunol* 2004;173:2607–2614. [PubMed: 15294977]
111. Stenger S, Rosat JP, Bloom BR, Krensky AM, Modlin RL. Granulysin: a lethal weapon of cytolytic T cells. *Immunol. Today* 1999;20:390–394. [PubMed: 10462738]
112. Krensky AM, Clayberger C, Krensky AM, Clayberger C. Granulysin: a novel host defense molecule. *Am. J. Transplant* 2005;5:1789–1792. [PubMed: 15996224]
113. Krensky AM, Clayberger C. Biology and clinical relevance of granulysin. *Tiss. Ant* 2009;73:193–198.
114. Linde CM, Grundstrom S, Nordling E, et al. Conserved structure and function in the granulysin and NK-lysin peptide family. *Infect. Immun* 2005;73:6332–6339. [PubMed: 16177304]
115. Endsley JJ, Torres AG, Gonzales CM, et al. Comparative antimicrobial activity of granulysin against bacterial biothreat agents. *Open Microbiol. J* 2009;3:92–96. [PubMed: 19587798]
116. Andreu D, Carreno C, Linde C, Boman HG, Andersson M. Identification of an anti-mycobacterial domain in NK-lysin and granulysin. *Biochem. J* 1999;344:845–849. [PubMed: 10585872]
117. Ernst WA, Thoma-Uszynski S, Teitelbaum R, et al. Granulysin, a T cell product, kills bacteria by altering membrane permeability. *J. Immunol* 2000;165:7102–7108. [PubMed: 11120840]
118. Wang Z, Choice E, Kaspar A, et al. Bactericidal and tumoricidal activities of synthetic peptides derived from granulysin. *J. Immunol* 2000;165:1486–1490. [PubMed: 10903754]
119. Wheelis M. First shots fired in biological warfare. *Nature* 1998;395:213. [PubMed: 9751039]
120. Horn PA, Thomasson BM, Wood BL, Andrews RG, Morris JC, Kiem H-P. Distinct hematopoietic stem/progenitor cell populations are responsible for repopulating NOD/SCID mice compared with nonhuman primates. *Blood* 2003;102:4329–4335. [PubMed: 12816869]
121. Vollmer J, Krieg AM, Vollmer J, Krieg AM. Immunotherapeutic applications of CpG oligodeoxynucleotide TLR9 agonists. *Adv. Drug Deliv. Rev* 2009;61:195–204. [PubMed: 19211030]
122. Krieg AM, Krieg AM. Therapeutic potential of Toll-like receptor 9 activation. *Nat. Rev. Drug Discov* 2006;5:471–484. [PubMed: 16763660]
123. Waag DM, McCluskie MJ, Zhang N, Krieg AM. A CpG oligonucleotide can protect mice from a low aerosol challenge dose of *Burkholderia mallei*. *Infect. Immun* 2006;74:1944–1948. [PubMed: 16495571] • First report demonstrating protection with CpG against *B. mallei* infection.
124. Utasincharoen P, Kespichayawattana W, Anuntagool N, et al. CpG ODN enhances uptake of bacteria by mouse macrophages. *Clin. Exp. Immunol* 2003;132:70–75. [PubMed: 12653838]
125. Elvin SJ, Healey GD, Westwood A, Knight SC, Eyles JE, Williamson ED. Protection against heterologous *Burkholderia pseudomallei* strains by dendritic cell immunization. *Infect. Immun* 2006;74:1706–1711. [PubMed: 16495542]
126. Chen YS, Hsiao YS, Lin HH, Liu Y, Chen YL. CpG-modified plasmid DNA encoding flagellin improves immunogenicity and provides protection against *Burkholderia pseudomallei* infection in BALB/c mice. *Infect. Immun* 2006;74:1699–1705. [PubMed: 16495541]
127. Wongratanacheewin S, Kespichayawattana W, Intachote P, et al. Immunostimulatory CpG oligodeoxynucleotide confers protection in a murine model of infection with *Burkholderia pseudomallei*. *Infect. Immun* 2004;72:4494–4502. [PubMed: 15271908]
128. Coenye T, Vandamme P. Overrepresentation of immunostimulatory CpG motifs in *Burkholderia* genomes. *J. Cyst. Fibros* 2005;4:193–196. [PubMed: 15963770]
129. Whitlock GC, Lukaszewski RA, Judy BM, Paessler S, Torres AG, Estes DM. Host immunity in the protective response to vaccination with heat-killed *Burkholderia mallei*. *BMC Immunol* 2008;9:55. [PubMed: 18823549] • Describes the immune responses to vaccination with *B. mallei*.

130. Harland DN, Chu K, Haque A, et al. Identification of a LolC homologue in *Burkholderia pseudomallei*, a novel protective antigen for melioidosis. *Infect. Immun* 2007;75:4173–4180. [PubMed: 17517877]
131. Goodyear A, Kelliher L, Bielefeldt-Ohmann H, et al. Protection from pneumonic infection with *Burkholderia* species by inhalational immunotherapy. *Infect. Immun* 2009;77:1579–1588. [PubMed: 19179415]
132. Kascatan-Nebioglu A, Melaiye A, Hindi K, et al. Synthesis from caffeine of a mixed *N*-heterocyclic carbene-silver acetate complex active against resistant respiratory pathogens. *J. Med. Chem* 2006;49:6811–6818. [PubMed: 17154511]
133. Cannon CL, Hogue LA, Vajravelu RK, et al. *In vitro* and murine efficacy and toxicity studies of nebulized SCC1, a methylated caffeine-silver(I) complex, for treatment of pulmonary infections. *Antimicrob. Agents Chemother* 2009;53:3285–3293. [PubMed: 19451294] • Discusses the effectiveness of silver compounds to treat agents causing pulmonary infections.
134. Hindi KM, Ditto AJ, Panzner MJ, et al. The antimicrobial efficacy of sustained release silver-carbene complex-loaded l-tyrosine polyphosphate nanoparticles: characterization, *in vitro* and *in vivo* studies. *Biomaterials* 2009;30:3771–3779. [PubMed: 19395021]
135. Hindi KM, Panzner MJ, Tessier CA, et al. The medicinal applications of imidazolium carbene-metal complexes. *Chem. Rev* 2009;109:3859–3884. [PubMed: 19580262]
136. Wright BD, Panzner MJ, Deeraksa A, et al. Synthesis and *in vitro* efficacy studies of silver carbene complexes on biosafety level 3 bacteria. *Eur. J. Inorganic Chem* 2009;13:1739–1745. • Several silver compounds are used against a variety of biosafety level 3 pathogens.
137. Odds FC. Synergy, antagonism, and what the checkerboard puts between them. *J. Antimicrob. Chemother* 2003;52:1. [PubMed: 12805255]

Figure 1.

Comparative activities of B- and C-class CpGs administered 48 h prior to infection with *Burkholderia pseudomallei* BALB/c mice were inoculated with 2.4×10^3 colony-forming units of strain K96243 per mouse via the intranasal route.

Figure 2.
Bacterial loads in various tissues at 28 days postinfection with *Burkholderia pseudomallei*.

Table 1

Minimum inhibitory concentrations and minimal bactericidal concentrations of SCC1, SCC5, SCC10, SCC12 and SCC22.

Species	Strain	Compound	MIC (µg/ml)	MBC (µg/ml)
<i>Burkholderia pseudomallei</i>	K96243	SCC1	6	>10
		SCC5	4	6
		SCC10	6	10
		SCC12	6	10
		SCC22	4	>10
<i>Burkholderia mallei</i>	ATCC23344	SCC1	4	6
		SCC5	1	6
		SCC10	2	6
		SCC12	2	6
		SCC22	1	6

MBC: Minimal bactericidal concentration; MIC: Minimum inhibitory concentration; SCC: Silver carbene compound.

Adapted with permission from [136].