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Vaccines for the Prevention of Melioidosis and Glanders

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Abstract

Purpose of review—*Burkholderia pseudomallei*'s and *Burkholderia mallei*'s high rate of infectivity, limited treatment options, and potential use as biological warfare agents underscore the need for development of effective vaccines against these bacteria. Research efforts focused on vaccines against these bacteria are in pre-clinical stages, with no approved formulations currently on the market.

Recent findings—Several live attenuated and subunit vaccine formulations have been evaluated in animal studies, with no reports of significant long term survival after lethal challenge.

Summary—This review encompasses the most current vaccine strategies to prevent *B. pseudomallei* and *B. mallei* infections while providing insight for successful vaccines moving forward.

Keywords

vaccines; melioidosis; glanders; *Burkholderia pseudomallei*; *Burkholderia mallei*

Introduction

Melioidosis is an infectious disease caused by *Burkholderia pseudomallei* and which is endemic in many tropical regions of the world [1]. *B. pseudomallei*, and its relative *Burkholderia mallei*, the causative agent of glanders, are aerobic, gram-negative, motile bacilli classified by the US Centers for Disease Control and Prevention (CDC) as Tier 1 select agents due to high virulence when inhaled, and potential to cause large-scale illness if disseminated. Additionally, *Burkholderia* spp. have resistance to a wide range of antibiotics, including penicillin [2, 3], which adds additional concern surrounding the bacteria. Research efforts over the last decade have evaluated prophylactic and therapeutic treatment options to combat these emerging infectious diseases; however, to date, there are no licensed vaccines on the market. This is likely due to the difficulty in designing safe and effective vaccines that protect against both acute and chronic infections caused by these pathogens. In addition, *B.*

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Conflict of Interest

Monica M. Johnson and Kristy M. Ainslie each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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pseudomallei and *B. mallei* are biosafety level three (BSL3) agents that require additional laboratory safety precautions, further hampering vaccine development. This review will outline recent vaccine strategies to prevent infections by *B. pseudomallei*, and *B. mallei*, highlighting potential candidate vaccines that demonstrated significant protection in a model for melioidosis and glanders.

Diseases caused by *Burkholderia*

Members of the genus *Burkholderia* are ecologically diverse due to their large and dynamic genomes, allowing them to survive and persist in the environment and host cells alike [4]. *B. pseudomallei* is an obligate aerobe found at high concentrations in tropical regions with favorable moisture and heat conditions allowing for sustained growth [5]. Whereas *B. pseudomallei* is a soil-dwelling bacterium, *B. mallei* is an obligate animal pathogen that is non-motile [6]. Despite the differences in environmental location, they are both facultative intracellular bacteria that can survive and replicate in many cell types in the host. We next discuss how infection from these bacteria occur and highlight the challenges that must be overcome to formulate vaccines against these infectious agents.

Emergence of melioidosis is endemic in regions such as southeast Asia and northern Australia, and once patients are infected, the disease is difficult to treat do to its unique ability to infect organs and persist [7, 8]. Manifestation of symptoms depends greatly on the route of infection, with the most severe occurring due to the inhalational form of infection [9]. Acute infection with *B. pseudomallei* can lead to symptoms of severe pneumonia and rapid septicemia. Chronic infection can lie dormant for many years and upon re-activation, lead to fatal abscesses in multiple organs [10]. The difficulty in treating melioidosis is in part because *B. pseudomallei* is able to infect, replicate, and survive in a multitude of host cells, include monocytes [11, 12]. Once intracellular, the bacterium can evade endocytic vesicle transport and multiply in the cytoplasm. This advanced style of motility enables *B. pseudomallei* to move to different host cells and cause the formation of multi-nucleate giant cells (MNGC), resulting in toxicity and local necrosis [13]. As the infection moves to different organs such as the liver, spleen, and brain, the potential for a septic infection increases, as does the mortality due to infection [14–16].

Whereas melioidosis infection can occur via contact with infected soil or water, glanders is a zoonotic disease caused by *B. mallei* that requires contact with infected animals [17, 18]. However, like *B. pseudomallei*, *B. mallei* is concerning as a biothreat due to its ability to infect via inhalation resulting in high fatality. In fact, *B. mallei* was one of the first bioweapons utilized in the World Wars and is classified, along with *B. pseudomallei*, as a Tier 1 Select Agent by the CDC [19]. Although no cases of human glanders has been reported in the U.S. since the 1950's, the fatality rate of infection were reportedly high (40%), even when properly diagnosed and treated [1, 20].

Infections by *B. pseudomallei* and *B. mallei* are inherently difficult to treat due to their resistance to broad-spectrum antibiotics, mostly as a result of efflux pump mechanisms [21]. Additionally, *Burkholderia* are opportunistic pathogens that can lie dormant for many years, making the establishment of intracellular chronic infections hard to treat [11]. There is a

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crucial need for preventative measures, such as vaccines, to provide protection to individuals living in or visiting endemic areas. Although a number of vaccine candidates have been suggested, many fail to provide complete and sterilizing immunity against both acute and chronic infections. Summarized next are strategies utilizing various antigen and adjuvant combinations to confer protection against *Burkholderia* spp. infections.

Live-attenuated vaccines

Live attenuated vaccines (LAV) are known for their ability to elicit potent humoral and cell-mediated immune responses that can provide long lasting protection [22, 23]. Sequencing of the *Burkholderia* genome has allowed researchers to genetically alter genes identified as virulence factors and exploit the resultant mutated genes as promising candidates for LAVs. These mutations are largely cellular metabolism and transport, secretion system (SS) or virulence-associated factors.

The largest portion of research on LAV mutants is altering cellular metabolism and transport. Attenuated *Burkholderia* mutant strains lacking functional genes in amino acid synthesis [*purN* [24], *purM* [25, 24], *ilvI* [26–28], *aroC* [29], *asd* [30]], prokaryotic signaling [(*relA/spT* [31]], iron transport/intracellular survival (*tonB/hcp1* [32]], and iron transporter deficiency [*tonB* [33]] have all been evaluated for their protective potential and are outlined in Table 1. Of these mutated LAVs, most notably, mice vaccinated with the auxotrophic amino acid synthesis mutant strain *B. mallei (ilvI)* demonstrated a T helper cell (Th) type 1-like immune response resulting in significant protection against a lethal aerosol challenge with *B. mallei*, suggesting a prominent role for Th1-skewing immune responses [27]. Similarly, Haque et al., observed significant expression of cytokines IFN-γ, IL-12, and IL-18, as early mediators for protection, against acute infection in BALB/c mice following intraperitoneal (i.p.) vaccination with a *B. pseudomallei* auxotrophic *ilvI* mutant strain (*ilvI*/2D2) [28]. When severe combined immunodeficiency (SCID) mice received adoptively transferred T cells from 2D2-boosted mice, they were significantly protected from an i.p. challenge of *B. pseudomallei*. This would suggest that T cells are protective against melioidosis, independent of B cells [28]. However, the vaccine failed to protect completely, since 2D2-immunized mice demonstrated initial protection with a mean survival time of 52 days but mice began to succumb to infection 30 days post-challenge, with histopathological analysis revealing the presence of splenic abscesses. Using an auxotrophic *B. mallei* mutant deficient in an iron active transport system (*tonB*), vaccination of mice resulted in a significant increase in survival after an acute inhalation (i.n.) challenge with, separately, *B. mallei* (100%) and *B. pseudomallei* (75%) [33]. Moreover, serum from vaccinated mice exhibited *B. mallei*-specific immunoglobulin (Ig) G1, IgG2a, and IgM [33]. Since many of the virulence genes mentioned above are conserved among the *Burkholderia* genus, it would be interesting to evaluate their efficacy against other strains such as *B. cepacia*, the bacterium implicated as the causative agent of respiratory infections in patients with cystic fibrosis [34]. Infection/prevention models against *B. cepacia* is an area of research that has been largely ignored [35].

Bacterial secretion systems (SS) contain a number of proteins that interact with and hijack critical host proteins and pathways, allowing the pathogen to survive and propagate in the

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host environment [22]. Due to their prominent role in pathogenicity, SS proteins have been exploited in LAVs against *Burkholderia* spp. (Table 1; [36, 23, 37]). Memisevic et al., used a bioinformatics and a yeast two-hybrid approach to identify uncharacterized and novel virulence factors that were conserved between both *B. mallei* and *B. pseudomallei* [38, 39]. Using this information, they created three *B. mallei* mutant strains (BMAA0728, BMAA1865, BMAA0553), one of which (BMAA0728) lacks the virulence gene encoding components of a type VI SS [38]. As an LAV, this mutant strain (BMAA0728/*tssN*) demonstrated delayed MNGC formation *in vitro* [40]. Vaccination of BALB/c mice lead to significant survival (67%) 21 days post-infection in an aerosol challenge model of glanders; however, 100% mortality was observed at 60 days' post infection [40]. More recently, Hatcher et al., generated a *B. mallei* mutant strain deficient in both iron transport and a type VI SS protein (*tonB/hcp1/*CLH001). Immunization with CLH001 in both BALB/c and NSG (severely immunocompromised) mice resulted in complete protection (100%) against lethal i.n. challenge with *B. mallei* and reduced bacterial burden after 35 days following a high dose challenge [32]. Although the prevention of the chronic infection was not evaluated, this attenuated strain demonstrated potential to protect against glanders in a pre-clinical challenge model.

LAV candidates targeting various virulence factors have also been prepared and evaluated; however, all have failed to demonstrate sterilizing immunity against long-term infections, which are typically observed 30 days post-challenge [23, 41]. In addition to the economic burden associated with designing and manufacturing LAVs, there are significant safety concerns due to the ability of attenuated pathogens to transform into virulent strains, limiting therapeutic opportunities for elderly (>65 years) and immunocompromised individuals [42]. For these reasons, it is important to consider other vaccine formulations.

Subunit vaccines

A safer alternative to LAVs is a subunit protein vaccine, which uses an antigenic bacterial component to induce an immune response. Without the use of a live bacterium, subunit vaccines are typically formulated with immune stimulating adjuvants to boost cellular and humoral immunity. Several subunit vaccines against *Burkholderia* have been evaluated, and reviewed extensively [43, 23, 35, 44]. Secreted and membrane-associated proteins as well as polysaccharides are often used as antigens in subunit vaccines because they are abundantly expressed by gram-negative bacteria and are shown to be potent stimulators of host immune responses. For *Burkholderia* subunit vaccines, these include lipopolysaccharides (LPS) and outer membrane proteins and vesicles (OMP and OMV, respectively). LPS have shown to play an important role in the pathogenesis of *Burkholderia* infection and a number of LPS molecules have been identified in *B. pseudomallei* and *B. mallei*, which are therefore, a likely target for subunit vaccines [45]. As a potent inducer of host immune responses, LPS O-antigen has been shown to provide epitopes for the development of antigen-specific antibodies, and the lipid A region is a potent agonist for Toll-like receptors (TLRs) [46]. In an animal model for melioidosis, i.p. immunization with LPS isolated from *B. pseudomallei* induced predominantly IgM and IgG3 responses and afforded significant protection (50% at day 35) following an i.p. challenge with a lethal dose of *B. pseudomallei* [47]. This protection; however, was not observed in an aerosol challenge [47, 48]. Additionally, passive

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immunity was observed and provided protection (mean time of death- 29 days) following an i.p challenge of *B. pseudomallei* [48].

Studies have also investigated the immunogenic potential of *B. pseudomallei*-specific OMPs as a subunit antigen against *Burkholderia* infections. Casey et al., evaluated the surface protein ompW, which functions in the attachment of bacterium to host epithelial lining, as a subunit antigen because it is conserved among several *Burkholderia* spp. [49]. Purified ompW administered via i.p. injection with the adjuvant monophosphoryl lipid A (MPL) induced a potent Th1-skewing serological response and protected BALB/c mice (75% survival up to 21 days) against lethal i.p. challenge of *B. pseudomallei*; however, failed to surpass the efficacy of the live attenuated 2D2 vaccine control [49]. Using a different adjuvant, vaccination with recombinant omp85 antigen emulsified in Freund's complete adjuvant (CFA) triggered a Th2-type immune response resulting in reduced bacterial loads in organs (lung, liver and spleen) and 70% survival up to 15 days after lethal i.p. *B. pseudomallei* challenge [50]. Additionally, mouse sera from the rOMP85 immunization group promoted complement-mediated killing and opsonization of *B. pseudomallei* by human polymorphonuclear cells [50]. OMVs are secreted by bacteria and are enriched with multiple proteins, lipids, and polysaccharides and have demonstrated robust immunogenicity as vaccine antigens against lethal i.n. and i.p. *Burkholderia* challenges when administered with an aluminum hydroxide (alum) adjuvant [51, 52]. Intraperitoneal vaccination of *B. pseudomallei*-derived OMVs afforded significant protection (67% at 21 days) against acute septicemic *B. pseudomallei* infection (i.p. challenge) and induced robust OMV-, LPS-, and CPS-specific serum IgG and IgM antibody responses in mice [53]. Similarly, *B. pseudomallei*-derived OMVs administered by subcutaneous injection, along with an adjuvant (CpG oligodeoxynucleotides; TLR 9 agonist), in a non-human primate model (rhesus macaque) also resulted in the production of OMV-, LPS-, and CMP-specific IgG serotypes [52]. Since members of the *Burkholderia* genus express structurally similar polysaccharides and membrane proteins, CPS's, LPS's, OMP's, and OMV's all provide promising antigens that can be used as a multivalent vaccine strategy against melioidosis.

Other approaches to identify or isolate protein antigens from *B. pseudomallei* have also been employed and are outlined in Table 1 [54]. Notably, immunization with LolC (ATP-binding cassette (ABC) transporter-type lipoprotein-releasing protein) demonstrated significant humoral and cellular immunogenicity that resulted in 80% survival at 42 days when administered with the adjuvant MPL [55]. Additional antigens, such as FliC (the flagella major subunit), have been proposed as a potential subunit vaccine candidate due to its ability to elicit long-lasting immune memory [56]. Vaccination with PilV (type IV pilin protein) and the adjuvant CFA produced high levels of IgG, strongly biased toward IgG1, but was unable to protect BALB/c mice against lethal i.p. *B. pseudomallei* challenge following subcutaneous immunization [57]. More recently, Champion et al., identified a series of genes: BPSL1897 (uncharacterized protein), BPSL3369 (acetaldehyde dehydrogenase), and BPSL2287 (iron cluster assembly protein) expressed by the bacteria colonizing the spleens and lungs of chronically infected mice [58]. Following co-administration with these recombinant proteins and MPL, significant protection was observed for 42 days against lethal challenge [58].

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In addition to protein-based subunit antigens, glycol-conjugate-based vaccines, in which bacterial surface polysaccharides are chemically conjugated to a carrier protein, have been demonstrated to be highly effective subunit vaccines against *Burkholderia*. For example, type O-polysaccharide and CPS antigens covalently linked to carrier proteins demonstrated high IgG titers and protected mice from a lethal i.p. *B. pseudomallei* challenge [59, 60]. Furthermore, it was demonstrated that mice co-administrated with a capsular polysaccharide (CPS) glycoconjugate and the promising subunit antigen, LolC, exhibited higher survival rates following lethal challenge compared to single administration [59]. Although promising, many of the current glycoconjugate formulations do not include glycans from the native host since *B. pseudomallei* requires BSL3 facilities, limiting their accessibility. To address this hurdle, Garcia-Quintanilla et al. utilized a novel method for synthesizing glycoconjugates through exploitation of the protein glycosylation machineries of bacteria; however, purified OPS II glycoconjugate provided only partial protection (40% at day 12) against lethal i.n. challenge of *B. pseudomallei* [61]. Overall, subunit vaccines, although safer than LAVs, demonstrate only partial protection against *Burkholderia* spp. infections and unfortunately remain stagnant in an attempt at pre-clinical testing, with relatively little evaluation against glanders.

Micro- and nano-systems for improved vaccine formulations against *Burkholderia*

Advances in nanotechnology have provided a significant impact in vaccine development, particularly in the use of metal-, polymer- and lipid-based nanoparticles and nanoemulsions as formulations to enhance vaccine efficacy [62]. Gold nanoparticles (AuNPs) are promising candidates for biological applications due their unique physicochemical properties, biocompatibility, and ease of synthesis and conjugation [63]. In a vaccine model against glanders, AuNPs covalently coupled with protein antigens (TetHc, Hcp1 and FliC) and conjugated LPS (isolated from *B. thailandensis*) generated significantly higher antibody titers compared to LPS alone and resulted in improved, but incomplete protection against a lethal i.n. *B. mallei* challenge [64]. A similar study evaluated the protective efficacy of AuNP glycoconjugates (LPS- FliC) in a non-human primate model (rhesus macaque) using an aerosol *B. mallei* challenge. Vaccination with the AuNP formulation demonstrated protection and LPS-specific IgG titers on day 80 in three out of six vaccinated animals that survived [65].

Polymeric microparticle (MP) carriers, which can encapsulate multiple adjuvant and antigen components, provide many advantages over metal-based nanosystems because they are biodegradable, can be sized to passively target antigen presenting cells (APCs), and can serve as an immune depot. Vaccine carriers composed of poly-(lactide-co-glycolide) (PLGA) have been extensively characterized in animal models and are currently being evaluated in Phase I clinical trials (Selecta); however, they have not been utilized for vaccines against *Burkholderia* [66]. Our group utilizes the acid sensitive biopolymer, acetalated dextran (Ac-DEX), because it is immunologically inert, has triggered release in the low pH environments of the phagosome and lysosome, and can be formulated with tunable degradation for formation of a vaccine depot [67–70]. Ac-DEX MPs have been

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previously shown to enhance cross-presentation of subunit antigens leading to protection in an anthrax vaccine model [71, 72]. In a model against acute melioidosis, BALB/c mice were vaccinated subcutaneously with free whole killed *B. pseudomallei* cell lysate (antigen) and Ac-DEX MPs encapsulating the adjuvant resiquimod (TLR 7/8 agonist) [73]. One of the two studies presented followed a rapid immunization/challenge schedule (vaccination on day 0, boost on day 7, followed by challenge on day 14). Resiquimod MPs co-delivered with soluble lysate as the antigen elicited robust Th1- and Th2-type immune responses leading to significant delay to time of death (88% at day 14) compared to empty MP-treated mice when given a lethal i.p. *B. pseudomallei* challenge; however, only 16% of mice survived past 26 days post-challenge [73]. Additionally, *B. pseudomallei* was not detected in the liver and blood of several surviving mice (2/4) following lethal challenge in the lysate with resiquimod MP group. Moreover, a fraction of other surviving mice in all the groups vaccinated with encapsulated resiquimod (resiquimod MPs, lysate MPs co-delivered with resiquimod MPs, and resiquimod MPs with lysate absorbed on alum) had sterile blood and livers (~10% of total mice challenged in the group), with some mice in the alum and resiquimod MP group having sterile spleens. This suggests that a sterilizing immunity was achieved in a fraction of immunized mice. When this platform was expanded to a more traditional day 0 and 14 prime and boost with a 28-day challenge, somewhat similar trends in survival were observed, indicating that perhaps these formulations could be used effectively in a more rapid timeframe, such as post-exposure prophylaxis [73]. Although the vaccine lead to incomplete protection, likely due to lack of discrete antigens, Ac-DEX represents a novel delivery vehicle for a subunit vaccine against melioidosis.

Nanoemulsions (NE) are a clinically relevant adjuvant system that could be an alternative to alum for many subunit vaccines. Recently, the nanoemulsions MF59, which is a squalene-based oil-in water emulsion, was FDA-approved as an adjuvant for influenza vaccines (FLUAD, Novartis) [74]. The nanoscale size and the negative charge of the NE-antigen mixture allows the vaccine to penetrate mucosal layers and to facilitate cellular uptake, which makes it an ideal vehicle for vaccine carriers against aerosolized *Burkholderia* infections. Although NEs have not been used for *B. pseudomallei* or *B. mallei* vaccines, Makidon et al., co-administered a NE with the OMP antigen (OmpA) derived from *B. cepacia* and found that immunized mice were protected against pulmonary bacterial colonization after a *B. cenocepacia* or *B. multivorans* challenge [74]. Moreover, vaccination with the NE adjuvanted vaccine yielded a balanced Th1/Th2 cellular response and production of mucosal IgA [75].

Liposomes, a lipid bi-layer vessel, have been used as adjuvant complexes in *Burkholderia* vaccines. When used in a melioidosis vaccine, whole-killed *B. pseudomallei* administered with cationic liposomes ((2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium; DOTAP) ionically complexed with immunomodulatory CpG afforded complete protection (100%) for up to 30 days against a lethal *B. pseudomallei* challenge [76]. CpG is a synthetic oligodeoxynucleotide adjuvant that has been shown to promote a TLR9-mediated Th1 immune response when used in several bacterial vaccines [77, 78]. Henderson et al., evaluated the use of cationic liposomes complexed with non-coding plasmid DNA (CLDC) as a potential mucosal vaccine adjuvant [79]. Immunization with plasmid and heat-killed *B. pseudomallei* induced efficient uptake by DCs, potent mucosal IgA antibody responses,

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sustained antigen-specific CD8+ T cell responses, and afforded 100% protection (>40 days) from a lethal i.n. *B. pseudomallei* challenge [79].

Combining antigen delivery (e.g., nanoparticles, biopolymers, liposomes) with potent adjuvant stimulation represent promising vaccine models against melioidosis, specifically in their ability to more efficiently deliver vaccine components and generate a broader immune response.

Conclusions

Despite the many strategies explored in the development of a vaccine against *Burkholderia,* all have failed to induce complete and sterilizing immunity over an extended period of time. Most of the reported studies observed short-term survival followed by death in response to a lethal challenge of *B. pseudomallei* or *B. mallei* (Table 1). Although no studies demonstrated complete sterilizing immunity, several promising LAV and subunit vaccines against *Burkholderia* have been proposed. Most notably, a mutant strain deficient in iron transport and a type IV SS [*tonB/hcp1*) [32]] and several mutant strains lacking functional virulence factor genes [39, 40] demonstrated significant protection against a lethal *Burkholderia* challenge. Although LAVs are potent stimulators of immunity, safety concerns make them unlikely candidates for clinical applications; therefore, subunit vaccines become more practical. However, subunit vaccines are poorly immunogenic and mostly require co-administration with an adjuvant. LolC, the leading subunit *Burkholderia* antigen to date, conferred significant protection when administered with the adjuvant, MPL [55]. Promising advances in vaccine development have utilized novel formulations involving emulsions, polymeric particles, liposomes, and metal-based nanoparticles. A notable vaccine candidate uses the cationic liposomal formulation with a plasmid ionically absorbed to the surface to serve as an adjuvant. When co-administered with heat-killed *B. pseudomallei*, a strong CD8+ immune response was generated and 100% survival past 40 days was reported following an i.n. *B. pseudomallei* challenge [79]. However, future work needs to be done in this field as only a handful of nano and micro-particle formulated vaccine candidates have been reported.

For vaccine candidates to progress beyond pre-clinical stages of development, standardization in experimental design needs to be taken into account. Inconsistency across studies has prevented researchers from moving forward in deciding candidate vaccines for evaluation in non-human primates, in addition to the uncertainty regarding which NHP model is best for *Burkholderia* infection [80]. Route of vaccine administration and bacterial challenge seem to be major factors influencing protective immunity against *Burkholderia* infections. While the i.p. route is used most commonly in experimental mouse models because of its ease of administration, aerosol, i.n., or subcutaneous challenges could be considered a more physiologically relevant model for melioidosis. An aerosol challenge is certainly deemed a more rigorous challenge route; however, in addition to variable dosing, it requires specialized equipment at a BSL3 facility, which significantly restricts availability. The variable dose of a variety of bacterial strains also makes it difficult to compare studies. For example, it has been reported that a *B. pseudomallei* challenge dose required for a rapidly fatal infection is significantly higher via the subcutaneous route compared to the

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inhalation route [81]. A careful evaluation and reporting of LD_{50}'s for each challenge strain and route can help to mitigate these differences. Moreover, relevant animal models need to be taken into account when evaluating vaccine efficacy. Most common strains of mice reported in studies are BALB/c and C57BL/6, which have demonstrated differential susceptibility to *B. pseudomallei* infection, with C57BL/6 being more resistant [82]. Since BALB/c mice are classically a model of acute infection, with C57BL/6 modeling more chronic infection, evaluation in both strains would be warranted in a rigorous vaccine study. Additionally, no studies have evaluated vaccine efficacy in models with underlying conditions such as diabetes and immunosuppression, which make up a large population of susceptible individuals in endemic regions and would certainly alter vaccine efficacy.

Because the field of *Burkholderia* vaccines has not advanced much beyond preliminary studies looking at vaccine efficacy, it has been difficult to thoroughly evaluate the mechanism of immune responses. Studies into CD8+ and CD4+ T cell response are not well reported because immunological tools like immunodominant peptides or tetramers have yet to be resolved for any of the antigens prepared. Perhaps a more in-depth analysis of epitopes could provide knowledge that will allow researchers to design better antigens that improve immunogenicity, with the potential to provide protection over the course of long-term infection. The work of Champion et al., evaluating expression markers in the bacteria of chronically infected mice [58] is a step in the right direction to create vaccines to combat long-term infection, approaching the problem as an immunologist rather than a microbiologist could further lead new information on vaccine improvement. A collaborative effort from microbiologists and immunologists, as well as vaccine formulation scientists, could work to overcome this difficult-to-prevent infection. By uniting these areas of knowledge, research efforts should be able to focus on developing a broad-spectrum vaccine that confers long-lasting immunity to multiple strains of *Burkholderia* responsible for lethal melioidosis and glanders.

References

Papers of particular interst, published recently, have been highlighted as: ** Of major importance

1. Cheng, AC., Dance, DA., Currie, BJ. Euro Surveill. Vol. 3. Sweden: 2005. Bioterrorism, Glanders and melioidosis; p. E1-2.author reply E1–2
2. Biological and chemical terrorism: strategic plan for preparedness and response. Recommendations of the CDC Strategic Planning Workgroup. MMWR Recomm Rep. 2000; 49(Rr-4):1–14.
3. Chaowagul W. Recent advances in the treatment of severe melioidosis. Acta Trop. 2000; 74(2–3): 133–7. DOI: 10.1016/S0001-706X(99)00062-5 [PubMed: 10674641]
4. Woods, DE., Sokol, PA. The Prokarytotes. New York, NY: Springer; 2006. The genus Burkholderia.
5. Stopnisek N, Bodenhausen N, Frey B, Fierer N, Eberl L, Weisskopf L. Genus-wide acid tolerance accounts for the biogeographical distribution of soil Burkholderia populations. Environ Microbiol. 2014; 16(6):1503–12. DOI: 10.1111/1462-2920.12211 [PubMed: 23945027]
6. Georgiev, VS. National Institute of Allergy and Infectious Diseases, NIH: Volume 2: Impact on Global Health. New York, NY: Springer Science & Business Media; 2009.
7. Currie BJ, Fisher DA, Howard DM, Burrow JNC, Selvanayagam S, Snelling PL, et al. The epidemiology of melioidosis in Australia and Papua New Guinea. Acta Tropica. 2000; 74(2–3):121–7. DOI: 10.1016/S0001-706X(99)00060-1 [PubMed: 10674639]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

8. Limmathurotsakul D, Wongratanacheewin S, Teerawattanasook N, Wongsuvan G, Chaisuksant S, Chetchotisakd P, et al. Increasing Incidence of Human Melioidosis in Northeast Thailand. 2010; doi: 10.4269/ajtmh.2010.10-0038
9. Lafontaine ER, Zimmerman SM, Shaffer TL, Michel F, Gao X, Hogan RJ. Use of a safe, reproducible, and rapid aerosol delivery method to study infection by Burkholderia pseudomallei and Burkholderia mallei in mice. PLoS One. 2013; 8(10):e76804.doi: 10.1371/journal.pone.0076804 [PubMed: 24098563]
10. Ngauy V, Lemeshev Y, Sadkowski L, Crawford G. Cutaneous melioidosis in a man who was taken as a prisoner of war by the Japanese during World War II. J Clin Microbiol. 2005; 43(2):970–2. DOI: 10.1128/jcm.43.2.970-972.2005 [PubMed: 15695721]
11. Harley VS, Dance DA, Drasar BS, Tovey G. Effects of Burkholderia pseudomallei and other Burkholderia species on eukaryotic cells in tissue culture. Microbios. 1998; 96(384):71–93. [PubMed: 10093229]
12. Silva EB, Dow SW. Development of Burkholderia mallei and pseudomallei vaccines. Front Cell Infect Microbiol. 2013; 3:10.doi: 10.3389/fcimb.2013.00010 [PubMed: 23508691]
13. Kespichayawattana W, Rattanachetkul S, Wanun T, Utaisincharoen P, Sirisinha S. Burkholderia pseudomallei induces cell fusion and actin-associated membrane protrusion: a possible mechanism for cell-to-cell spreading. Infect Immun. 2000; 68(9):5377–84. DOI: 10.1128/IAI.68.9.5377-5384.2000 [PubMed: 10948167]
14. Howe C, Miller WR. Human glanders; report of six cases. Ann Intern Med. 1947; 26(1):93–115. DOI: 10.7326/0003-4819-26-1-93 [PubMed: 20278465]
15. Friedland JS, Suputtamongkol Y, Remick DG, Chaowagul W, Strieter RM, Kunkel SL, et al. Prolonged elevation of interleukin-8 and interleukin-6 concentrations in plasma and of leukocyte interleukin-8 mRNA levels during septicemic and localized Pseudomonas pseudomallei infection. Infect Immun. 1992; 60(6):2402–8. [PubMed: 1375198]
16. Lauw FN, Simpson AJ, Prins JM, Smith MD, Kurimoto M, van Deventer SJ, et al. Elevated plasma concentrations of interferon (IFN)-gamma and the IFN-gamma-inducing cytokines interleukin (IL)-18, IL-12, and IL-15 in severe melioidosis. J Infect Dis. 1999; 180(6):1878–85. DOI: 10.1086/315155 [PubMed: 10558944]
17. Boerner P. A preliminary report on the work by the Imperial Health Care Office leading to discovery of the glanders bacillus. Dtsch Med Wochenschr. 1882:707–8.
18. Sharrer GT. The great glanders epizootic, 1861–1866: a Civil War legacy. Agric Hist. 1995; 69(1):79–97. doi:http://www.jstor.org/stable/3744026. [PubMed: 11639801]
19. Wheelis M. First shots fired in biological warfare. Nature. 1998; 395(6699):213.doi: 10.1038/26089 [PubMed: 9751039]
20. Van Zandt KE, Greer MT, Gelhaus HC. Glanders: an overview of infection in humans. Orphanet J Rare Dis. 2013; 8:131.doi: 10.1186/1750-1172-8-131 [PubMed: 24004906]
21. Mima T, Schweizer HP. The BpeAB-OprB efflux pump of Burkholderia pseudomallei 1026b does not play a role in quorum sensing, virulence factor production, or extrusion of aminoglycosides but is a broad-spectrum drug efflux system. Antimicrob Agents Chemother. 2010; 54(8):3113–20. DOI: 10.1128/aac.01803-09 [PubMed: 20498323]
22. Galyov EE, Brett PJ, DeShazer D. Molecular insights into Burkholderia pseudomallei and Burkholderia mallei pathogenesis. Annu Rev Microbiol. 2010; 64:495–517. DOI: 10.1146/annurev.micro.112408.134030 [PubMed: 20528691]
23. Choh LC, Ong GH, Vellasamy KM, Kalaiselvam K, Kang WT, Al-Maleki AR, et al. Burkholderia vaccines: are we moving forward? Front Cell Infect Microbiol. 2013; 3:5.doi: 10.3389/fcimb.2013.00005 [PubMed: 23386999]
24. Breitbach K, Kohler J, Steinmetz I. Induction of protective immunity against Burkholderia pseudomallei using attenuated mutants with defects in the intracellular life cycle. Trans R Soc Trop Med Hyg. 2008; 102(Suppl 1):S89–94. DOI: 10.1016/s0035-9203(08)70022-1 [PubMed: 19121696]
25. Silva EB, Goodyear A, Sutherland MD, Podnecky NL, Gonzalez-Juarrero M, Schweizer HP, et al. Correlates of immune protection following cutaneous immunization with an attenuated

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Burkholderia pseudomallei vaccine. Infect Immun. 2013; 81(12):4626–34. DOI: 10.1128/iai.00915-13 [PubMed: 24101688]

26. Atkins T, Prior RG, Mack K, Russell P, Nelson M, Oyston PC, et al. A mutant of Burkholderia pseudomallei, auxotrophic in the branched chain amino acid biosynthetic pathway, is attenuated and protective in a murine model of melioidosis. Infect Immun. 2002; 70(9):5290–4. DOI: 10.1128/IAI.70.9.5290-5294.2002 [PubMed: 12183585]
27. Ulrich RL, Amemiya K, Waag DM, Roy CJ, DeShazer D. Aerogenic vaccination with a Burkholderia mallei auxotroph protects against aerosol-initiated glanders in mice. Vaccine. 2005; 23(16):1986–92. DOI: 10.1016/j.vaccine.2004.10.017 [PubMed: 15734072]
28. Haque A, Chu K, Easton A, Stevens MP, Galyov EE, Atkins T, et al. A live experimental vaccine against Burkholderia pseudomallei elicits CD4+ T cell-mediated immunity, priming T cells specific for 2 type III secretion system proteins. J Infect Dis. 2006; 194(9):1241–8. DOI: 10.1086/508217 [PubMed: 17041850]
29. Srilunchang T, Proungvitaya T, Wongratanacheewin S, Strugnell R, Homchampa P. Construction and characterization of an unmarked aroC deletion mutant of Burkholderia pseudomallei strain A2. Southeast Asian J Trop Med Public Health. 2009; 40(1):123–30. [PubMed: 19323044]
30. Norris MH, Propst KL, Kang Y, Dow SW, Schweizer HP, Hoang TT. The Burkholderia pseudomallei Deltaasd mutant exhibits attenuated intracellular infectivity and imparts protection against acute inhalation melioidosis in mice. Infect Immun. 2011; 79(10):4010–8. DOI: 10.1128/iai.05044-11 [PubMed: 21807903]
31. Muller CM, Conejero L, Spink N, Wand ME, Bancroft GJ, Titball RW. Role of RelA and SpoT in Burkholderia pseudomallei virulence and immunity. Infect Immun. 2012; 80(9):3247–55. DOI: 10.1128/iai.00178-12 [PubMed: 22778096]
32**. Hatcher CL, Mott TM, Muruato LA, Sbrana E, Torres AG. Burkholderia mallei CLH001 Attenuated Vaccine Strain Is Immunogenic and Protects against Acute Respiratory Glanders. Infect Immun. 2016; 84(8):2345–54. This study demonstrated the efficacy of a live attenuated vaccine against *Burkholderia mallei* infection utilizing a mutant strain deficient in iron aquistion and a type six secretion system component. DOI: 10.1128/iai.00328-16 [PubMed: 27271739]
33. Mott TM, Vijayakumar S, Sbrana E, Endsley JJ, Torres AG. Characterization of the Burkholderia mallei tonB Mutant and Its Potential as a Backbone Strain for Vaccine Development. PLoS Negl Trop Dis. 2015; 9(6):e0003863.doi: 10.1371/journal.pntd.0003863 [PubMed: 26114445]
34. LiPuma JJ. Assessing Airway Microbiota in Cystic Fibrosis: What More Should Be Done? J Clin Microbiol. 2015; 53(7):2006–7. DOI: 10.1128/jcm.01218-15 [PubMed: 25972422]
35. Pradenas GA, Ross BN, Torres AG. Burkholderia cepacia Complex Vaccines: Where Do We Go from here? Vaccines (Basel). 2016; 4(2)doi: 10.3390/vaccines4020010
36. Stevens MP, Haque A, Atkins T, Hill J, Wood MW, Easton A, et al. Attenuated virulence and protective efficacy of a Burkholderia pseudomallei bsa type III secretion mutant in murine models of melioidosis. Microbiology. 2004; 150(Pt 8):2669–76. DOI: 10.1099/mic.0.27146-0 [PubMed: 15289563]
37. Druar C, Yu F, Barnes JL, Okinaka RT, Chantratita N, Beg S, et al. Evaluating Burkholderia pseudomallei Bip proteins as vaccines and Bip antibodies as detection agents. FEMS Immunol Med Microbiol. 2008; 52(1):78–87. DOI: 10.1111/j.1574-695X.2007.00345.x [PubMed: 17995960]
38. Memisevic V, Zavaljevski N, Pieper R, Rajagopala SV, Kwon K, Townsend K, et al. Novel Burkholderia mallei virulence factors linked to specific host-pathogen protein interactions. Mol Cell Proteomics. 2013; 12(11):3036–51. DOI: 10.1074/mcp.M113.029041 [PubMed: 23800426]
39. Memisevic V, Zavaljevski N, Rajagopala SV, Kwon K, Pieper R, DeShazer D, et al. Mining host-pathogen protein interactions to characterize Burkholderia mallei infectivity mechanisms. PLoS Comput Biol. 2015; 11(3):e1004088.doi: 10.1371/journal.pcbi.1004088 [PubMed: 25738731]
40. Bozue JA, Chaudhury S, Amemiya K, Chua J, Cote CK, Toothman RG, et al. Phenotypic Characterization of a Novel Virulence-Factor Deletion Strain of Burkholderia mallei That Provides Partial Protection against Inhalational Glanders in Mice. Front Cell Infect Microbiol. 2016; 6:21.doi: 10.3389/fcimb.2016.00021 [PubMed: 26955620]

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41. Scott AE, Laws TR, D'Elia RV, Stokes MG, Nandi T, Williamson ED, et al. Protection against experimental melioidosis following immunization with live Burkholderia thailandensis expressing a manno-heptose capsule. Clin Vaccine Immunol. 2013; 20(7):1041–7. DOI: 10.1128/cvi.00113-13 [PubMed: 23677322]
42. Galen JE, Curtiss R 3rd. The delicate balance in genetically engineering live vaccines. Vaccine. 2014; 32(35):4376–85. DOI: 10.1016/j.vaccine.2013.12.026 [PubMed: 24370705]
43. Casey WT, McClean S. Exploiting molecular virulence determinants in Burkholderia to develop vaccine antigens. Curr Med Chem. 2015; 22(14):1719–33. DOI: 10.2174/0929867322666150408111304 [PubMed: 25850766]
44. Bondi SK, Goldberg JB. Strategies toward vaccines against Burkholderia mallei and Burkholderia pseudomallei. Expert Rev Vaccines. 2008; 7(9):1357–65. DOI: 10.1586/14760584.7.9.1357 [PubMed: 18980539]
45. Moustafa DA, Scarff JM, Garcia PP, Cassidy SK, DiGiandomenico A, Waag DM, et al. Recombinant Salmonella Expressing Burkholderia mallei LPS O Antigen Provides Protection in a Murine Model of Melioidosis and Glanders. PLoS One. 2015; 10(7):e0132032.doi: 10.1371/journal.pone.0132032 [PubMed: 26148026]
46. West TE, Ernst RK, Jansson-Hutson MJ, Skerrett SJ. Activation of Toll-like receptors by Burkholderia pseudomallei. BMC Immunol. 2008; 9:46.doi: 10.1186/1471-2172-9-46 [PubMed: 18691413]
47. Ngugi SA, Ventura VV, Qazi O, Harding SV, Kitto GB, Estes DM, et al. Lipopolysaccharide from Burkholderia thailandensis E264 provides protection in a murine model of melioidosis. Vaccine. 2010; 28(47):7551–5. DOI: 10.1016/j.vaccine.2010.08.058 [PubMed: 20837078]
48. Nelson M, Prior JL, Lever MS, Jones HE, Atkins TP, Titball RW. Evaluation of lipopolysaccharide and capsular polysaccharide as subunit vaccines against experimental melioidosis. 2004; doi: 10.1099/jmm.0.45766-0
49**. Casey WT, Spink N, Cia F, Collins C, Romano M, Berisio R, et al. Identification of an OmpW homologue in Burkholderia pseudomallei, a protective vaccine antigen against melioidosis. Vaccine. 2016; 34(23):2616–21. This study demonstrated a potent cellular immune response to a subunit vaccination, which resulted in signficant protectection against lethal *Burkholderia pseudomallei* challenge in vivo. DOI: 10.1016/j.vaccine.2016.03.088 [PubMed: 27091689]
50. Su YC, Wan KL, Mohamed R, Nathan S. Immunization with the recombinant Burkholderia pseudomallei outer membrane protein Omp85 induces protective immunity in mice. Vaccine. 2010; 28(31):5005–11. DOI: 10.1016/j.vaccine.2010.05.022 [PubMed: 20546831]
51. Nieves W, Heang J, Asakrah S, Honer zu Bentrup K, Roy CJ, Morici LA. Immunospecific responses to bacterial elongation factor Tu during Burkholderia infection and immunization. PLoS One. 2010; 5(12):e14361.doi: 10.1371/journal.pone.0014361 [PubMed: 21179405]
52. Petersen H, Nieves W, Russell-Lodrigue K, Roy CJ, Morici LA. Evaluation of a Burkholderia pseudomallei Outer Membrane Vesicle Vaccine in Nonhuman Primates. Procedia Vaccinol. 2014; 8:38–42. DOI: 10.1016/j.provac.2014.07.007 [PubMed: 25165491]
53. Nieves W, Petersen H, Judy BM, Blumentritt CA, Russell-Lodrigue K, Roy CJ, et al. A Burkholderia pseudomallei outer membrane vesicle vaccine provides protection against lethal sepsis. Clin Vaccine Immunol. 2014; 21(5):747–54. DOI: 10.1128/cvi.00119-14 [PubMed: 24671550]
54. Chin CY, Tan SC, Nathan S. Immunogenic recombinant Burkholderia pseudomallei MprA serine protease elicits protective immunity in mice. Front Cell Infect Microbiol. 2012; 2:85.doi: 10.3389/fcimb.2012.00085 [PubMed: 22919676]
55. Harland DN, Chu K, Haque A, Nelson M, Walker NJ, Sarkar-Tyson M, et al. Identification of a LolC homologue in Burkholderia pseudomallei, a novel protective antigen for melioidosis. Infect Immun. 2007; 75(8):4173–80. DOI: 10.1128/iai.00404-07 [PubMed: 17517877]
56. Musson JA, Reynolds CJ, Rinchai D, Nithichanon A, Khaenam P, Favry E, et al. CD4+ T cell epitopes of FliC conserved between strains of Burkholderia: implications for vaccines against melioidosis and cepacia complex in cystic fibrosis. J Immunol. 2014; 193(12):6041–9. DOI: 10.4049/jimmunol.1402273 [PubMed: 25392525]

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57. Sangdee K, Waropastrakul S, Wongratanachewin S, Homchampa P. Heterologously type IV pilus expressed protein of Burkholderia pseudomallei is immunogenic but fails to induce protective immunity in mice. Southeast Asian J Trop Med Public Health. 2011; 42(5):1190–6. [PubMed: 22299445]

58**. Champion OL, Gourlay LJ, Scott AE, Lassaux P, Conejero L, Perletti L, et al. Immunisation with proteins expressed during chronic murine melioidosis provides enhanced protection against disease. Vaccine. 2016; 34(14):1665–71. This study evaluated the use of genes expressed by the bacteria during chronic infection as potential subunit antigens for a vaccine candidate against *Burkholderia pseudomallei* infection. DOI: 10.1016/j.vaccine.2016.02.038 [PubMed: 26917010]

59. Scott AE, Burtnick MN, Stokes MG, Whelan AO, Williamson ED, Atkins TP, et al. Burkholderia pseudomallei capsular polysaccharide conjugates provide protection against acute melioidosis. Infect Immun. 2014; 82(8):3206–13. DOI: 10.1128/iai.01847-14 [PubMed: 24866807]

60. Burtnick MN, Heiss C, Roberts RA, Schweizer HP, Azadi P, Brett PJ. Development of capsular polysaccharide-based glycoconjugates for immunization against melioidosis and glanders. Front Cell Infect Microbiol. 2012; 2:108.doi: 10.3389/fcimb.2012.00108 [PubMed: 22912938]

61. Garcia-Quintanilla F, Iwashkiw JA, Price NL, Stratilo C, Feldman MF. Production of a recombinant vaccine candidate against Burkholderia pseudomallei exploiting the bacterial N-glycosylation machinery. Front Microbiol. 2014; 5:381.doi: 10.3389/fmicb.2014.00381 [PubMed: 25120536]

62. Krishnamachari Y, Geary SM, Lemke CD, Salem AK. NANOPARTICLE DELIVERY SYSTEMS IN CANCER VACCINES. Pharm Res. 2011; 28(2):215–36. DOI: 10.1007/s11095-010-0241-4 [PubMed: 20721603]

63. Shah M, Badwaik VD, Dakshinamurthy R. Biological applications of gold nanoparticles. J Nanosci Nanotechnol. 2014; 14(1):344–62. DOI: 10.1166/jnn.2014.8900 [PubMed: 24730267]

64. Gregory AE, Judy BM, Qazi O, Blumentritt CA, Brown KA, Shaw AM, et al. A gold nanoparticle-linked glycoconjugate vaccine against Burkholderia mallei. Nanomedicine. 2015; 11(2):447–56. DOI: 10.1016/j.nano.2014.08.005 [PubMed: 25194998]

65. Torres AG, Gregory AE, Hatcher CL, Vinet-Oliphant H, Morici LA, Titball RW, et al. Protection of non-human primates against glanders with a gold nanoparticle glycoconjugate vaccine. Vaccine. 2015; 33(5):686–92. DOI: 10.1016/j.vaccine.2014.11.057 [PubMed: 25533326]

66. Smith DM, Simon JK, JRB. Applications of nanotechnology for immunology. Nature Reviews Immunology. 2013; 13:592–605. DOI: 10.1038/nri3488

67. Bachelder EM, Beaudette TT, Broaders KE, Dashe J, Fréchet JM. Acetal-derivatized dextran: an acid-responsive biodegradable material for therapeutic applications. J Am Chem Soc. 2008; 130(32):10494–5. DOI: 10.1021/ja803947s [PubMed: 18630909]

68. Broaders KE, Cohen JA, Beaudette TT, Bachelder EM, Fréchet JM. Acetalated dextran is a chemically and biologically tunable material for particulate immunotherapy. Proc Natl Acad Sci U S A. 2009; 106(14):5497–502. DOI: 10.1073/pnas.0901592106 [PubMed: 19321415]

69. Kauffman KJ, Do C, Sharma S, Gallovic MD, Bachelder EM, Ainslie KM. Synthesis and characterization of acetalated dextran polymer and microparticles with ethanol as a degradation product. ACS Appl Mater Interfaces. 2012; 4(8):4149–55. DOI: 10.1021/am3008888 [PubMed: 22833690]

70. Chen N, Collier MA, Gallovic MD, Collins GC, Sanchez CC, Fernandes EQ, et al. Degradation of acetalated dextran can be broadly tuned based on cyclic acetal coverage and molecular weight. Int J Pharm. 2016; 512(1):147–57. DOI: 10.1016/j.ijpharm.2016.08.031 [PubMed: 27543351]

71. Gallovic MD, Schully KL, Bell MG, Elberson MA, Palmer JR, Darko CA, et al. Acetalated Dextran Microparticulate Vaccine Formulated via Coaxial Electrospray Preserves Toxin Neutralization and Enhances Murine Survival Following Inhalational Bacillus Anthracis Exposure. Adv Healthc Mater. 2016; 5(20):2617–27. DOI: 10.1002/adhm.201600642 [PubMed: 27594343]

72. Schully KL, Sharma S, Peine KJ, Pesce J, Elberson MA, Fonseca ME, et al. Rapid vaccination using an acetalated dextran microparticulate subunit vaccine confers protection against triplicate challenge by bacillus anthracis. Pharm Res. 2013; 30(5):1349–61. DOI: 10.1007/s11095-013-0975-x [PubMed: 23354770]

73**. Schully KL, Bell MG, Prouty AM, Gallovic MD, Gautam S, Peine KJ, et al. Evaluation of a biodegradable microparticulate polymer as a carrier for Burkholderia pseudomallei subunit vaccines in a mouse model of melioidosis. Int J Pharm. 2015; 495(2):849–61. Study reports the use of a biodegradable microparticle delivery vehicle for enhanced immune stimulation during vaccination against melioidosis. DOI: 10.1016/j.ijpharm.2015.09.059 [PubMed: 26428631]

74. Vogel FR, Caillet C, Kusters IC, Haensler J. Emulsion-based adjuvants for influenza vaccines. Expert Rev Vaccines. 2009; 8(4):483–92. DOI: 10.1586/erv.09.5 [PubMed: 19348563]

75. Makidon PE, Knowlton J, Groom JV 2nd, Blanco LP, LiPuma JJ, Bielinska AU, et al. Induction of immune response to the 17 kDa OMPA Burkholderia cenocepacia polypeptide and protection against pulmonary infection in mice after nasal vaccination with an OMP nanoemulsion-based vaccine. Med Microbiol Immunol. 2010; 199(2):81–92. DOI: 10.1007/s00430-009-0137-2 [PubMed: 19967396]

76. Puangpetch A, Anderson R, Huang YY, Saengsot R, Sermswan RW, Wongratanacheewin S. Comparison of the protective effects of killed Burkholderia pseudomallei and CpG oligodeoxynucleotide against live challenge. Vaccine. 2014; 32(45):5983–8. DOI: 10.1016/j.vaccine.2014.08.035 [PubMed: 25223269]

77. Puangpetch A, Anderson R, Huang YY, Sermswan RW, Chaicumpa W, Sirisinha S, et al. Cationic liposomes extend the immunostimulatory effect of CpG oligodeoxynucleotide against Burkholderia pseudomallei infection in BALB/c mice. Clin Vaccine Immunol. 2012; 19(5):675–83. DOI: 10.1128/cvi.05545-11 [PubMed: 22441390]

78. McCluskie MD, Heather J. Oral, intrarectal and intranasal immunizations using CpG and non-CpG oligodeoxynucleotides as adjuvants. 2000; 19(4–5):413–22. DOI: 10.1016/S0264-410X(00)00208-5

79. Henderson A, Propst K, Kedl R, Dow S. Mucosal immunization with liposome-nucleic acid adjuvants generates effective humoral and cellular immunity. Vaccine. 2011; 29(32):5304–12. DOI: 10.1016/j.vaccine.2011.05.009 [PubMed: 21600950]

80**. Limmathurotsakul D, Funnell SG, Torres AG, Morici LA, Brett PJ, Dunachie S, et al. Consensus on the development of vaccines against naturally acquired melioidosis. Emerg Infect Dis. 2015; 21(6) Consensus report following an international meeting held in the United Kingdom (2014) evaluating potential candidate vaccines against naturally aquired melioidosis. The meeting was attended by international vaccine developers, researchers, and government health officials. doi: 10.3201/eid2106.141480

81. Titball RW, Russell P, Cuccui J, Easton A, Haque A, Atkins T, et al. Burkholderia pseudomallei: animal models of infection. Trans R Soc Trop Med Hyg. 2008; 102(Suppl 1):S111–6. DOI: 10.1016/s0035-9203(08)70026-9 [PubMed: 19121670]

82. Liu B, Koo GC, Yap EH, Chua KL, Gan YH. Model of differential susceptibility to mucosal Burkholderia pseudomallei infection. Infect Immun. 2002; 70(2):504–11. DOI: 10.1128/IAI.70.2.504-511.2002 [PubMed: 11796576]

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Table 1

Melioidosis and glanders vaccines under pre-clinical development

Vaccine	Route of administration (vaccine/challenge)	Challenge model (strain)	Survival (%) post challenge[a]	Reference
Live attenuated:				
purN mutant (*purN*)	i.n./i.p.	*B. pseudomallei* (E8)	100% at day 9	[24]
purM mutant strain (*purM*/Bp82)	SubQ/i.n.	*B. pseudomallei* (1026b)	100% (C57BL/6) and 60% (BALB/c) at day 60	[25]
ilvI mutant (*ilvI*/2D2)	i.p./i.p.; aerosol/aerosol	*B. pseudomallei* (576); *B. mallei* (ATCC 23344)	30% at day 70; 80% at day 35; 50% at day 30	[28]; [26]; [27]
aroC mutant (*aroC*/A2)	i.p./i.p.	*B. pseudomallei* (A2)	80% (C57BL/6) and 0% (BALB/c) at 5 months	[29]
asd mutant (*asd*)	i.n./i.n.	*B. pseudomallei* (1026b)	100% at day 80 (acute infection); 0% at day 60 (chronic infection)	[30]
r*elA/spT* mutant (*relA/spT*)	i.n./i.n.	*B. pseudomallei* (576)	100% at day 30 (C57BL/6)	[31]
tonB/hcp1 mutant (*tonB/hcp1* CLH001)	i.n./i.n.	*B. mallei* (CSM001); *B. mallei* (ATCC 23344)	100% survival at day 35	[32]
tonB mutant (*tonB*/TMM001)	i.n./i.n.	*B. mallei* (CSM001) & *B. pseudomallei* (K96243)	100% at day 28; 75% at day 36	[33]
tssN mutant strain (tssN/BMAA0728)	aerosol/aerosol	*B. mallei* (ATCC 23344)	67% at day 21	[40]
Capsular polysaccharide (CPS)- expressing mutant *B. thailandensis* strain	i.p./i.p.	*B. pseudomallei* (K96243)	100% at day 35	[41]
Subunit:				
Lipopolysaccharide (LPS)	i.p./i.p.; i.p./aerosol	*B. pseudomallei* (K96243); *B. pseudomallei* (NCTC 4845)	50% at day 35; NS at day 5	[47]; [48]
Purified outer membrane protein W (ompW)	i.p./i.p.	*B. pseudomallei* (576)	75% at day 21	[49]
Purified outer membrane protein 85 (omp85)	i.p./i.p;	*B. pseudomallei* (D286)	70% at day 15	[50]
Purified outer membrane vesicles (OMVs)	i.p./i.p.	*B. pseudomallei* (K96243)	67% at day 21	[53]
Purified MprA protein (SmBpF4)	i.p./i.p.	*B. pseudomallei* (D286)	100% at day 25	[54]
Purified LolC protein	SubQ/i.p.	*B. pseudomallei* (K96243)	80% at day 42	[55]
Purified PilV protein	SubQ/SubQ	*B. pseudomallei* (G207)	NS	[57]
Purified proteins BPSL1897, BPSL3369, BPSL2287	i.p./i.p.	*B. pseudomallei* (K96243)	75% at day 40	[58]
CPS and O-polysaccharide glycoconjugate (CPS2B1)	subQ/i.p.	*B. pseudomallei* (K96243)	90% at day 21	[59]
OPS II glycoconjugate	i.p./i.n.	*B. pseudomallei* (K96243)	40% at day 14	[61]

Vaccine	Route of administration (vaccine/challenge)	Challenge model (strain)	Survival (%) post challenge[a]	Reference
Gold nanoparticles (AuNPs) glycoconjugates (TetHc, Hcp1, FliC)	i.n./i.n.	*B. mallei* (ATCC 23344)	90% (TetHc; Hcp1) and 60% (FliC) at day 21	[64]
AuNP glycoconjugates (LPS-FliC)	aerosol/aerosol	*B. mallei* (ATCC 23344)	50% at day 80 (Rhesus macaque)	[65]
Polymer-encapsulated adjuvant and whole killed *B. pseudomallei*	SubQ/i.p.	*B. pseudomallei* (1026b)	88% at day 14	[73]
Cationic liposomes complexed with adjuvant and whole killed *B. pseudomallei*	i.m./i.p.	*B. pseudomallei* (1026b)	100% at day 30	[77]
Cationic liposomes complexed with plasmid DNA and whole killed *B. pseudomallei*	i.n./i.n.	*B. pseudomallei* (1026b)	100% at day 40	[79]

[a]Challenge model is BALB/c unless otherwise indicated

Intranasal: i.n.;

Intraperitoneal: i.p.;

subcutaneous: subQ;

Intramuscular: i.m.;

NS: no survival

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