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Vaccine strategies against cystic fibrosis pathogens

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ABSTRACT

A great number of cystic fibrosis (CF) pathogens such as *Pseudomonas aeruginosa*, the *Burkholderia cepacia* and the *Mycobacterium abscessus* complex raised difficult therapeutic problems due to their intrinsic multi-resistance to numerous antibiotics. Vaccine strategies represent one of the key weapons against these multi-resistant bacteria in a number of clinical settings like CF. Different strategies are considered in order to develop such vaccines, linked either to priming the host response, or by exploiting genomic data derived from the bacterium. Interestingly, virulence factors synthesized by various pathogens might serve as targets for vaccine development and have been, for example, evaluated in the context of CF.

Keywords: cystic fibrosis, vaccine, *Mycobacterium abscessus*, *Pseudomonas aeruginosa*, *Burkholderia* spp.

Cystic fibrosis and microbial lung infections.

Cystic fibrosis (CF) is a disease which arises from a Mendelian defect due to a series of mutations in the *cftr* gene encoding the Cl^- channel¹. The resulting flaw in this protein is responsible for increasing the viscosity of the mucus, which promotes the accumulation and the attachment of bacteria to mucins. Chronic inflammation² and early bacterial infection maintain a vicious circle and are each responsible for the lung damage which ensues. Lung infections in CF patients represent the most frequent but also the more serious manifestations since they are responsible for more than 90% of CF patient deaths³. The microorganisms that may infect the respiratory system are bacteria, fungi and viruses. Bacterial colonization occurs very early in the natural history of the disease⁴. The first causative organisms are *Haemophilus influenzae* and *Staphylococcus aureus*. *S. aureus* is usually the first detected⁵ and its prevalence is rising⁶. Affinity of *S. aureus* for CF mucus contributes to persistent colonization and progressive pulmonary damage increasing the potential for further infections to set in, for example *Pseudomonas* spp.⁵. *Pseudomonas aeruginosa* colonization arises several months to several years after. Finally, several bacterial complexes are found responsible for severe infections in CF, in addition to be the most difficult to treat: the *Burkholderia cepacia* complex (Bcc) and the *Mycobacterium abscessus* complex, which has emerged recently as a threat in CF patients, and may present with *Mycobacterium avium*, the major non-tuberculous mycobacterium (NTM) present in CF lungs with a significant prevalence^{7,8}.

Opportunistic pathogens becoming untreatable weapons in CF patients

P. aeruginosa is the environmental opportunistic pathogen in CF patients. It is the most commonly isolated bacterium that infects individuals with CF, with colonization and chronic infections that may affect up to 80% of adult CF patients⁹. *P. aeruginosa* establishes a chronic

endobronchial infection which impacts on morbidity and mortality of CF patients. *P. aeruginosa* is also notable for its resistance to antibiotics, making it therefore a difficult to treat pathogen, which, once acquired, is rarely, if ever, eradicated. In addition, *P. aeruginosa* frequently colonized CF lungs as a biofilm, which reduces the patient's immune response and access by antibiotics¹⁰. A second opportunistic pathogen, represented as a complex is the *Burkholderia cepacia* complex (Bcc). It is composed of 18 species that are able to cause opportunistic and lethal infections CF patients¹¹. The two most clinically relevant species are *Burkholderia cenocepacia* and *Burkholderia multivorans*¹². These environmental, intracellular and biofilm-forming bacteria are extremely antibiotic resistant organism¹². Bcc infections are rarely cleared from CF patients once they are colonized, as observed in *P. aeruginosa* infections. The third antibiotic-resistant bacterium found in CF patients with frequency between 3 to 7%^{7,13} is *Mycobacterium abscessus*. It is a rapidly growing mycobacterium also existing as a complex: the *Mycobacterium abscessus* complex¹⁴, with two subspecies *M. abscessus abscessus* and *M. abscessus bolletii* respectively. *M. abscessus* is, within the group of rapid-growers, responsible for a broad spectrum of diseases in humans. Lung infections are frequent, with CF patients particularly susceptible^{7,13,15}, in addition to muco-cutaneous infections often of nosocomial origin¹⁶. Recent reports of human-to-human transmission in the context of CF care have been described^{17,18}. *M. abscessus* raises very challenging therapeutic issues because of its natural resistance to most available antibiotics^{19,20}. Severe, even fatal, infections in CF patients have been described due to therapeutic deadlock²¹. *M. abscessus* infection might represent a contraindication for lung transplantation in several countries²², leaving CF patients without therapeutic options.

As such, antibiotic treatment exemplifies a clear challenge now faced with these opportunistic pathogens. We demonstrated for example a significant link between previous intravenous antibiotic courses and the isolation of *M. abscessus* in CF patient lungs, underlining the role

of broad-spectrum antimicrobial therapy in the emergence of *M. abscessus* disease²³. And this is true for the continuous emergence of resistant *P. aeruginosa* or Bcc due to the repeated antibiotic therapeutic regimens given to CF patients²⁴. Emergence of multi-resistant bacteria leads to therapeutic impasses with severe and fatal infections²⁴.

Vaccine approaches

Pathogens can be divided into two groups according to whether vaccines exist against them or not. However, no human vaccine has been developed so far against the antibiotic-resistant pathogens described above. As such, the development of prophylactic or therapeutic vaccines is of extreme importance when confronted with bacteria of this high resistance..

When defining an appropriate target, making the correct choice and how such tools can be developed is a long and tedious process. Development of vaccine targets can profit from genome sequence comparisons²⁵, and, using a process known as reverse vaccinology, might introduce a novel strategy to identify target antigens that might serve as potential vaccines²⁵. We chose this strategy for the development of a first vaccine against *M. abscessus*²⁶ (see below). Indeed, the presence of specific genes in opportunistic pathogen genomes that are absent from saprophytic bacterial genomes belonging to the same genus represents the key to unravel virulence factors that can then be targeted using a vaccine approach. A better understanding of the “virulence” genes contributes greatly to the development of new control strategies against these microorganisms. In addition, the choice of a virulence factor as a vaccine target has been shown to be relevant in the scientific literature²⁵.

Vaccination can then be performed using recombinant proteins when expression and purification are possible; or using the expression of plasmid DNA with modified eukaryotic gene sequences, as has been performed with *M. abscessus*²⁶. In fact, in the different vaccine strategies, DNA vaccines exemplify one of these new strategies and has been used successfully in the context of infectious diseases²⁷. Over the last 15 years, DNA vaccines have

proved effective in animal models including candidates against HIV, malaria and influenza²⁸. DNA vaccines have been extensively evaluated in humans with a recent review identifying 72 Phase I, 20 Phase II and two Phase III human trials²⁹. *Mycobacteria*, such as *Mycobacterium tuberculosis*, have received particular attention in this respect^{30,31}. Finally, some common antigens might be present in a variety of different CF pathogens, and the development of a vaccine might have the potential for conferring cross-protection against several CF pathogens (see below).

Vaccines against opportunistic CF pathogens

Some infections^{32,33} frequently associated with impaired respiratory function in patients with cystic fibrosis are the subject of vaccine development: for example infections with *P. aeruginosa*.

The development of a vaccine against *P. aeruginosa* has so far mobilized many research teams, even though no human trials have been conducted yet. Numerous vaccine attempts have been made against this pathogen that is generally considered to be the most targeted among pathogens infecting cystic fibrosis patients, and in order to obtain a state of the art overview in this domain, you would do well to explore the following recent reviews³⁴⁻³⁷. Major target antigens include the O-glycosylated lipopolysaccharide, cell-surface alginate, flagella, components of the Type III secretion system and outer membrane proteins³⁸. The FliC flagellin protein widely considered as a virulence factor, has pro-inflammatory activity on respiratory epithelial cells. Flagellin has been one of the major targets for vaccines and therapeutic development especially for CF patients. For example, as early as 1995, *P. aeruginosa* flagella were developed as a vaccine against *P. aeruginosa* and a Phase I study demonstrated that intramuscular immunization in healthy human adults results in high and long-lasting serum IgG flagella antibody titers and IgG, IgA and secretory IgA isotypes in the secretory immune system³⁹. Then, in a phase III study, an immunization with a bivalent

vaccine for flagella subtypes significantly lowered the risk for initial *P. aeruginosa* infection in CF patients⁴⁰. Other proteins have been used in human trials: OprF and OprI that are outer membrane proteins are able to induce a specific antibody response in the lung after nasal and oral vaccinations, and are as such promising candidates for the development of anti-pseudomonas immunization⁴¹. Furthermore, a fusion protein of the *P. aeruginosa* OprF fragment, OprI, and FliC promoted the clearance of *P. aeruginosa* in a pulmonary challenge model⁴². Another study which also tested these two outer membrane proteins as mucosal vaccines, lead to the development of airway immunogenicity against the pathogen with superior efficacy compared to systemic vaccination⁴³. We can add to this, as more recently, among the latest antigens and strategies tested, an assay using the conserved surface exopolysaccharide alginate, a virulence factor produced by mucoid strains, has been tried in mice⁴⁴ and conferred protection after intranasal challenge. It was also efficacious as a therapeutic vaccine. Previously, an assay was performed in humans with O-polysaccharide conjugated to toxin A and vaccinated children developed less chronic *Pseudomonas* lung infections than non-vaccinated children^{45,46}. An element of alginate (polymannuronic acid) has also been conjugated to flagellin leading to protective efficacy in a mouse lung infection model⁴⁷. Some *P. aeruginosa* antigens conjugated to bovine serum albumin have also been tested in mice⁴⁸. However, despite more than 50 years of research efforts, a licensed vaccine against *P. aeruginosa* is still a long way off from being available for CF patients.

With reference to the *Burkholderia cepacia* complex (Bcc), several virulence factors associated with human infection were tested for their potential as vaccine candidates⁴⁹. Such an approach was undertaken by a group that sought immunoreactive proteins expressed by both *Burkholderia cenocepacia* and *Burkholderia multivorans*⁵⁰. A recent review summarizes in detail all vaccination experiments that have been undertaken against Bcc⁵¹. As a recent example, the flagellar protein FliC from *Burkholderia pseudomallei* and considered as a

virulence factor has been shown to confer protection in mice⁵². This study shows that the epitopes of interest in *B. pseudomallei* FliC cross-react with orthologous *B. multivorans* and *B. cenocepacia* FliC sequences suggesting protection can be conferred against members of the Bcc. Other proteins like OMPs from different *Burkholderia* species are also able to generate protection in mice^{53,54}.

Vaccination in the context of mycobacteria.

The most widely used global vaccine is the “Bacille Calmette et Guérin” (BCG) strain of *Mycobacterium bovis* used in the fight against tuberculosis, a disease caused by *Mycobacterium tuberculosis*, which presently kills 1.3 million individuals around the world every year⁵⁵. This technique harnessed the historically defined strategy developed by Jenner⁵⁶, by using the antigenic repertoire of a non-pathogenic strain for human, in order to confer protection against the human pathogen. Despite its widespread use, BCG is still a controversial vaccine, and its deficiencies have led to the development of new research axes, using either purified compounds or a DNA vaccines in a quest to improve anti-mycobacterial vaccines. Several mycobacterial proteins (Ag85A/B, 65-kDa heat shock protein, hsp65, 36-kDa proline-rich antigen, MPB83, MPB70, CFP-10 and ESAT-6) have been evaluated as DNA vaccines in experimental models⁵⁷⁻⁶³. By virtue of its strong capacity to induce CD4+-mediated Th1 and CD8+-mediated cytotoxic T-lymphocyte responses²⁵, DNA vaccine approaches are particularly attractive for their preventive and therapeutic activity against intracellular pathogens such as pathogenic mycobacteria⁶¹. The majority of these studies were conducted in the fields of human^{30,31,57,60,61} and bovine tuberculosis^{58,59}; and even in leprosy (immuno-dominant 35-kDa protein⁶⁴). Few DNA vaccines have been developed which are related to pathogenic or opportunistic NTM, for example *M. avium* (35-kDa protein⁶⁵; p85A-

EGFP, p65K-EGFP⁶⁶); *Mycobacterium ulcerans*⁶⁷ or *Mycobacterium marinum*^{62,63}. None were developed in the context of tackling infections in inherited diseases such as CF.

While the presence of NTM is demonstrated in 7 to 13% of CF patients⁸, infections by *M. tuberculosis* are rare in CF patients^{68,69}. BCG remains a currently recommended vaccine in children at risk of exposure to TB; and as a consequence of partial efficacy against NTM, continues to be recommended in CF children. The two mycobacteria that together are responsible for the majority of infections in cystic fibrosis patients are *M. avium* and *M. abscessus*, a slow and a rapid growing mycobacteria (SGM and RGM), respectively, and to our knowledge, no vaccine approach against these bacteria has been considered in the context of CF. As mentioned above, development of vaccine targets can benefit from knowledge derived from genome sequence comparison²⁵. Genome sequence comparison between the *M. abscessus* genome and *Mycobacterium chelonae*, *Mycobacterium smegmatis* genomes (two rapid growing NTM, which are less or non-pathogenic respectively), allowed the unraveling of several key virulence factors¹⁹. As described in each genome sequences, several of these virulence factors were acquired by horizontal gene transfer, from non-fermenting Gram negative bacteria such as those found in CF patient lungs: *P. aeruginosa* and Bcc¹⁹. Among others we characterized MAB_0555, a phospholipase C (PLC) with the highest homology with PLC-N from *P. aeruginosa*⁷⁰. We have demonstrated the impact of MAB_0555 PLC in the virulence of *M. abscessus* in mice⁷⁰, when pre-cultivated on amoeba. We have also shown that the recombinant protein or the plasmid encoding PLC conferred protection, after an aerosol or IV challenge (Figure 1), in $\Delta 508$ or CF mice⁷¹ only²⁶. The two formulations (Figure 1) gave quite similar results, namely a diminution of bacterial load in lungs three weeks after a *M. abscessus* aerosol challenge²⁶. PLC are also present in other CF pathogens like *P. aeruginosa* and the immune cross reactivity between *M. abscessus* PLC and *P. aeruginosa* PLC could lead to a vaccine protective against both mycobacterial and Gram negative

infection in CF patients, as we were able to show the recognition of the MAB_0555 PLC by sera from CF patients only infected by *P. aeruginosa*. This approach is currently underway in our laboratory with other target designs recently unraveled⁷².

Conclusion

Vaccination is effective in preventing infections and we advocate their use in patients with CF, specifically for the prevention of respiratory infections. In addition to the traditional vaccination schedules,⁷³ evaluation studies to demonstrate the immunological and clinical efficacy of novel vaccines against multi-resistant bacteria remain necessary in this particular patient population.

REFERENCES

1. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; 245:1066–73; PMID:2475911
2. Ratjen FA. Cystic fibrosis: pathogenesis and future treatment strategies. *Resp Care* 2009; 54:595- 602; PMID:19393104
3. Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. *N Engl J Med* 2005; 352:1992–2001; PMID:15888700
4. May JR, Herrick NC, Thompson D. Bacterial Infection in cystic fibrosis. *Arch Dis Child* 1972; 47:908-13; PMID: 4630478
5. Marks MI. Clinical significance of *Staphylococcus aureus* in cystic fibrosis. *Infection* 1990; 18:53-6; PMID: 2107147
6. LiPuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 2010; 23:299- 323; PMID:20375354
7. Roux AL, Catherinot E, Ripoll F, Soismier N, Macheras E, Ravilly S, et al. Multicenter study of prevalence of nontuberculous mycobacteria in patients withcystic fibrosis in France. *J Clin Microbiol* 2009; 47:4124–8; PMID:19846643
8. Olivier KN, Weber DJ, Lee JH, Handker A, Tudor G, Molina PL, et al. Nontuberculous mycobacteria. *Am J Respir Crit Care Med* 2003; 167:835– 40; PMID:12433669
9. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 2003; 168:918-51; PMID:14555458
10. Drenkard, E. Antimicrobial resistance of *Pseudomonas aeruginosa* biofilms. *Microb. Infect* 2003; 5:1213-1219; PMID:14623017

11. Lipuma J. Update on the *Burkholderia cepacia* complex. *Curr Opin Pulm Med* 2005; 11:528–33; PMID:16217180
12. Caraher E, Duff C, Mullen T, Mc Keon S, Murphy P, Callaghan M, et al. Invasion and biofilm formation of *Burkholderia dolosa* is comparable with *Burkholderia cenocepacia* and *Burkholderia multivorans*. *J Cyst Fibros* 2007; 6:49-56; PMID:16781896
13. Olivier KN, Weber DJ, Wallace Jr RJ, Faiz AR, Lee JH, Zhang Y, et al. Nontuberculous mycobacteria I: multicenter prevalence study in cystic fibrosis. *Am J Respir Crit Care Med* 2003; 167:828–34 ; PMID:12433668
14. Leao SC, Tortoli E, Euzeby JP, Garcia MJ. Proposal that *Mycobacterium massiliense* and *Mycobacterium bolletii* be united and reclassified as *Mycobacterium abscessus* subsp. *bolletii* comb. nov., designation of *Mycobacterium abscessus* subsp. *abscessus* subsp. nov. and emended description of *Mycobacterium abscessus*. *IntJ Syst Evol Microbiol* 2011; 61:2311–3; PMID:21037035
15. Pierre-Audigier C, Ferroni A, Sermet-Gaudelus I, Le Bourgeois M, Offredo C, Vu-Thien H, et al. Age-related prevalence and distribution of nontuberculous mycobacterial species among patients with cystic fibrosis. *J Clin Microbiol* 2005; 43:3467–70; PMID:16000480
16. Wallace RJ Jr, Brown BA, Griffith DE. Nosocomial outbreaks/pseudo-outbreaks caused by nontuberculous mycobacteria. *Annu Rev Microbiol* 1998; 52:453-90; PMID:9891805
17. Aitken ML, Limaye A, Pottinger P, Whimbey E, Goss CH, Tonelli MR, et al. Respiratory outbreak of *Mycobacterium abscessus* subspecies *massiliense* in a lung transplant and cystic fibrosis center. *Am J Respir Crit Care Med* 2012; 185:231–2; PMID:22246710
18. Bryant JM, Grogono DM, Greaves D, Foweraker J, Roddick I, Inns T, et al. Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. *Lancet* 2013; 381:1551–60; PMID:23541540

19. Ripoll F, Pasek S, Schenowitz C, Dossat C, Barbe V, Rottman M, et al. Nonmycobacterial virulence genes in the genome of the emerging pathogen *Mycobacterium abscessus*. PLoS ONE 2009; 4:e5660; PMID:19543527
20. Nash KA, Brown-Elliott BA, Wallace Jr RJ. A novel gene, *erm(41)*, confers inducible macrolide resistance to clinical isolates of *Mycobacterium abscessus* but is absent from *Mycobacterium chelonae*. Antimicrob Agents Chemother 2009; 53:1367–76; PMID: 19171799
21. Sanguinetti M, Ardito F, Fiscarelli E, La Sorda M, D'Argenio P, Ricciotti G, et al. Fatal pulmonary infection due to multidrug-resistant *Mycobacterium abscessus* in a patient with cystic fibrosis. J Clin Microbiol 2001;39:816–9; PMID:11158161
22. Taylor JL, Palmer SM. *Mycobacterium abscessus* chest wall and pulmonary infection in a cystic fibrosis lung transplant recipient. J Heart Lung Transplant 2006; 25:985–8; PMID:16890122
23. Catherinot E, Roux AL, Vibet MA, Bellis G, Ravilly S, Lemonnier L, et al. *Mycobacterium avium* and *Mycobacterium abscessus* complex target distinct cysticfibrosis patient subpopulations. J Cyst Fibros 2013;12:74–80; PMID:22857820
24. Lopez AD, Murray CC. The global burden of disease, 1990–2020. Nat Med 1998; 4:1241–3; PMID:9809543
25. Hoft DF, Brusica V, Sakala IG. Optimizing vaccine development. Cell Microbiol 2011 13:934-942; PMID:21631691
26. Le Moigne V, Rottman M, Goulard C, Barteau B, Poncin I, Soismier N, Canaan S, Pitard B, Gaillard JL, Herrmann JL. Bacterial phospholipases C as vaccine candidate antigens against cystic fibrosis respiratory pathogens: the *Mycobacterium abscessus* model. Vaccine 2015; 33:2118-24; PMID: 25804706

27. Tighe H, Corr M, Roman M, Raz E. Gene vaccination: plasmid DNA is more than just a blueprint. *Immunol Today* 1998; 19:89-97; PMID:9509764
28. Klinman DM, Klaschik S, Tross D, Shirota H, Steinhagen F. FDA guidance on prophylactic DNA vaccines: analysis and recommendations. *Vaccine* 2010; 28:2801–05; PMID:19941989
29. Kutzler MA, Weiner DB. DNA vaccines: ready for prime time? *Nat Rev Genet* 2008; 9:776– 88; PMID:18781156
30. Tascon RE, Colston MJ, Ragno S, Stavropoulos E, Gregory D, Lowrie DB. Vaccination against tuberculosis by DNA injection. *Nat Med* 1996; 2:888–92; PMID:8705858
31. Huygen K, Content J, Denis O, Montgomery DL, Yawman AM, Deck RR, et al. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine. *Nat Med* 1996; 2:893–8; PMID:8705859
32. Pillarisetti N, Williamson E, Linnane B, Skoric B, Robertson CF, Robinson P, et al. Infection, inflammation, and lung function decline in infants with cystic fibrosis. *Am J Respir Crit Care Med* 2011; 184:75-81; PMID:21493738
33. Sagel SD, Gibson RL, Emerson J, McNamara S, Burns JL, Wagener JS, et al. Impact of pseudomonas and staphylococcal infection on inflammation and clinical status in young children with cystic fibrosis. *J Pediatr* 2009; 154:183- 8; PMID:18822427
34. Grimwood K, Kyd JM, Owen SJ, Massa HM, Cripps AW. Vaccination against respiratory *Pseudomonas aeruginosa* infection. *Hum Vaccin Immunother* 2015; 11:14-20; PMID: 25483510
35. Savoia D. New perspectives in the management of *Pseudomonas aeruginosa* infections. *Future Microbiol* 2014; 9:917-28; PMID:25156380

36. Johansen HK, Gøtzsche PC. Vaccines for preventing infection with *Pseudomonas aeruginosa* in cystic fibrosis. *Cochrane Database Syst Rev* 2013; 6:CD001399; PMID: 23771707
37. Sharma A, Krause A, Worgall S. Recent developments for *Pseudomonas* vaccines. *Hum Vaccin* 2011; 7:999-1011; PMID:21941090
38. Pier G. Application of vaccine technology to prevention of *Pseudomonas aeruginosa* infections. *Expert Rev Vaccines* 2005; 4:645-56; PMID:16221066
39. Döring G, Pfeiffer C, Weber U, Mohr-Pennert A, Dorner F. Parenteral application of a *Pseudomonas aeruginosa* flagella vaccine elicits specific anti-flagella antibodies in the airways of healthy individuals. *Am. J. Respir. Crit. Care Med* 1995; 151, 983–985; PMID:7697276
40. Döring G, Meisner C, Stern M; Flagella Vaccine Trial Study Group. Flagella vaccine trial study, a double-blind randomized placebo-controlled phase III study of a *Pseudomonas aeruginosa* flagella vaccine in cystic fibrosis patients. *Proc Natl Acad Sci USA* 2007; 104:11020–11025; PMID:17585011
41. Bumann D, Behre C, Behre K, Herz S, Gewecke B, Gessner JE, et al. Systemic, nasal and oral live vaccines against *Pseudomonas aeruginosa*: a clinical trial of immunogenicity in lower airways of human volunteers. *Vaccine* 2010; 28:707-13; PMID:19887136
42. Weimer ET, Lu H, Kock ND, Wozniak DJ, Mizel SB. A fusion protein vaccine containing OprF epitope 8, OprI, and type A and B flagellins promotes enhanced clearance of nonmucoid *Pseudomonas aeruginosa*. *Infect. Immun* 2009; 77:2356–66; PMID:19349426
43. Sorichter S, Baumann U, Baumgart A, Walterspacher S, von Specht BU. Immune responses in the airways by nasal vaccination with systemic boosting against *Pseudomonas aeruginosa* in chronic lung disease. *Vaccine* 2009; 27:2755-9; PMID:19366571

44. Farjah A, Owlia P, Siadat SD, Mousavi SF, Ardestani MS, Mohammadpour HK. Immunological evaluation of an alginate-based conjugate as a vaccine candidate against *Pseudomonas aeruginosa*. *APMIS* 2015; 123:175-83; PMID:25470757
45. Zuercher AW, Horn MP, Que JU, Ruedeberg A, Schoeni MH, Schaad UB, et al. Antibody responses induced by long-term vaccination with an octovalent conjugate *Pseudomonas aeruginosa* vaccine in children with cystic fibrosis. *FEMS Immunol Med Microbiol* 2006; 47:302-8; PMID:16831219
46. Zuercher AW, Imboden MA, Jampen S, Bosse D, Ulrich M, Chtioui H, et al. Cellular immunity in healthy volunteers treated with an octavalent conjugate *Pseudomonas aeruginosa* vaccine. *Clin Exp Immunol* 2006; 143:132-8; PMID:16367944
47. Campodónico VL, Llosa NJ, Bentancor LV, Maira-Litran T, Pier GB. Efficacy of a conjugate vaccine containing polymannuronic acid and flagellin against experimental *Pseudomonas aeruginosa* lung infection in mice. *Infect Immun* 2011; 79:3455-64; PMID: 21628521
48. Rodrigues NF, van Tilburg Bernardes E, Rocha RP, da Costa LC, Coutinho AC, et al. Bovine serum albumin nanoparticle vaccine reduces lung pathology induced by live *Pseudomonas aeruginosa* infection in mice. *Vaccine* 2013; 31:5062-6; PMID: 24021308
49. Casey WT, McClean S. Exploiting molecular virulence determinants in *Burkholderia* to develop vaccine antigens. *Curr Med Chem* 2015; 22:1719-33; PMID:25850766
50. Shinoy M, Dennehy R, Coleman L, Carberry S, Schaffer K, Callaghan M, et al. Immunoproteomic analysis of proteins expressed by two related pathogens, *Burkholderia multivorans* and *Burkholderia cenocepacia*, during human infection. *PLoS One* 2013; 8:e80796; PubMed PMID: 24260482

51. 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; 5:3:5; PMID:23386999
52. 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; 193:6041-9; PMID:25392525
53. 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; 199:81-92; PMID: 19967396
54. Bertot GM, Restelli MA, Galanternik L, Aranibar Urey RC, Valvano MA, Grinstein S. Nasal immunization with *Burkholderia multivorans* outer membrane proteins and the mucosal adjuvant adamantylamide dipeptide confers efficient protection against experimental lung infections with *B. multivorans* and *B. cenocepacia*. *Infect Immun* 2007; 75:2740-52; PMID: 17296759
55. World Health Organization. Global tuberculosis report 2013. www.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf
56. Jenner E. The Three Original Publications on Vaccination Against Smallpox. 1798, 1799, 1800. *The Harvard Classics*. 1909–14
57. Montgomery DL, Huygen K, Yawman AM, Deck RR, Dewitt CM, Content J, Liu MA, et al. Induction of humoral and cellular immune responses by vaccination with M. tuberculosis antigen 85 DNA. *Cell Mol Biol (Noisy-le-grand)* 1997; 43:285-92; PMID:9193782

58. Chambers MA, Vordermeier H, Whelan A, Commander N, Tascon R, Lowrie D, et al. Vaccination of mice and cattle with plasmid DNA encoding the *Mycobacterium bovis* antigen MPB83. Clin Infect Dis 2000; Suppl 3:S283-7. PMID:10875801
59. Vordermeier HM, Whelan A, Cockle PJ, Farrant L, Palmer N, Hewinson RG. Use of synthetic peptides derived from the antigens ESAT-6 and CFP-10 for differential diagnosis of bovine tuberculosis in cattle. Clin Diagn Lab Immunol 2001; 8:571-8; PMID:11329460
60. Huygen K. On the use of DNA vaccines for the prophylaxis of mycobacterial diseases. Infect Immun 2003; 71:1613-21; PMID:12654772
61. Romano M, Huygen K. DNA vaccines against mycobacterial diseases. Expert Rev Vaccines 2009; 8:1237-50; PMID:19722896
62. Oksanen KE, Halfpenny NJ, Sherwood E, Harjula SK, Hammarén MM, Ahava MJ, et al. An adult zebrafish model for preclinical tuberculosis vaccine development. Vaccine 2013; 31:5202-9; PMID:24055305
63. Feng G, Jiang Q, Xia M, Lu Y, Qiu W, Zhao D, et al. Enhanced immune response and protective effects of nano-chitosan-based DNA vaccine encoding T cell epitopes of Esat-6 and FL against *Mycobacterium tuberculosis* infection. PLoS One 2013; 8:e61135; PMID:23637790
64. Martin E, Roche PW, Triccas JA, Britton WJ. DNA encoding a single mycobacterial antigen protects against leprosy infection. Vaccine 2001; 19:1391-6. PMID:11163661
65. Martin E, Kamath AT, Triccas JA, Britton WJ. Protection against virulent *Mycobacterium avium* infection following DNA vaccination with the 35-kilodalton antigen is accompanied by induction of gamma interferon-secreting CD4(+) T cells. Infect Immun 2000; 68:3090-6; PMID:10816448

66. Velaz-Faircloth M, Cobb AJ, Horstman AL, Henry SC, Frothingham R. Protection against *Mycobacterium avium* by DNA vaccines expressing mycobacterial antigens as fusion proteins with green fluorescent protein. *Infect Immun* 1999, 67:4243-50; PMID:10417198
67. Tanghe A, Content J, Van Vooren JP, Portaels F, Huygen K. Protective efficacy of a DNA vaccine encoding antigen 85A from *Mycobacterium bovis* BCG against Buruli ulcer. *Infect Immun* 2001; 69:5403-11; PMID:11500410
68. Morand PC, Burgel PR, Carlotti A, Desmazes-Dufeu N, Farhi D, Martin C, et al. Mediastinal tuberculosis in an adult patient with cystic fibrosis. *J Clin Microbiol* 2011; 49:750-1; PMID:21106788
69. Asherova IK, Feigelson J, Vasilyeva LA, Gabitov VJ. Cystic fibrosis complicated by multidrug-resistant tuberculosis. *Acta Paediatr* 2006; 95:1513-4; PMID:17062491
70. Bakala N'Goma JC, Le Moigne V, Soismier N, Laencina L, Le Chevalier F, Roux AL, et al. *Mycobacterium abscessus* Phospholipase C expression is induced during coculture within amoebae and enhances *M. abscessus* virulence in mice. *Infect Immun* 2015; 83:780-91; PMID:25486995
71. van Doorninck JH, French PJ, Verbeek E, Peters RH, Morreau H, Bijman J, et al. A mouse model for the cystic fibrosis delta F508 mutation. *EMBO J* 1995; 14:4403-11; PMID:7556083
72. Roux AL, Ray A, Pawlik A, Medjahed H, Etienne G, Rottman M, et al. Overexpression of proinflammatory TLR-2-signalling lipoproteins in hypervirulent mycobacterial variants. *Cell Microbiol* 2011; 13:692-704; PMID:21143571
73. Malfroot A, Adam G, Ciofu O, Döring G, Knoop C, Lang AB, et al. Immunisation in the current management of cystic fibrosis patients. *J Cyst Fibros* 2005; 4:77-87; PMID:15978534

