(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 August 2010 (05.08.2010)

PCT

(10) International Publication Number
WO 2010/086614 A1

(51) International Patent Classification:
A61K 31/683 (2006.01) *A61K 31/688* (2006.01)
A61K 31/685 (2006.01) *A61P 31/04* (2006.01)

(21) International Application Number:
PCT/GB2010/000146

(22) International Filing Date:
29 January 2010 (29.01.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0901423.4 29 January 2009 (29.01.2009) GB

(71) Applicant *(for all designated States except US)*: **THE SECRETARY OF STATE FOR DEFENCE** [GB/GB]; DSTL, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **ROWLAND, Caroline, Ann** [GB/GB]; DSTL, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). **HARTLEY, Margaret, Gillian** [GB/GB]; DSTL, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). **EYLES, James, Edward** [GB/GB]; DSTL, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB).

(74) Agent: **FARMSWORTH, Alastair, Graham**; D/IPR Formalities section, Poplar 2, MOD Abbey Wood #2218, Bristol BS348JH (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2010/086614 A1

(54) Title: TREATMENT

(57) Abstract: The present invention relates to a phosphoantigen for use as a medicament, particularly in the prophylaxis and/or treatment of infection by *Burkholderia*, including but not limited to *Burkholderia pseudomallei* and *Burkholderia mallei*; and a pharmaceutical composition comprising such phosphoantigens.

Treatment

The present disclosure relates to phosphoantigens for use in the prophylaxis and/or treatment of infection by *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp* for example *Burkholderia pseudomallei* infection i.e. melioidosis, and pharmaceutical compositions comprising said phosphoantigen in particular for use in the prophylaxis and/or treatment of said infections.

Burkholderia pseudomallei was first isolated by the British pathologist A. Whitmore from a 10 year old boy in Rangoon, Burma, who died of pneumonitis. Autopsy of further patients revealed tubercular lesions in the lung and abscesses on internal organs. Gram-negative rods were isolated in pure culture from the lesions, and identified by Whitmore as the causative agent of the disease. The glanders-like disease was subsequently named melioidosis and the pathogen *Bacterium whitmori.* After several changes of name, the pathogen was finally assigned to the *Burkholderia* genus, a decision based on several criteria including 16S RNA sequences and DNA homology. The *Burkholderia* genus consists of non-spore forming bacilli which are highly polymorphic. Only three species are pathogenic for man; *B. pseudomallei* and *Burkholderia mallei* are capable of causing disease in otherwise healthy individuals whereas *Burkholderia cepacia* causes infections in immunocompromised people and may be responsible for cepacia syndrome in patients with cystic fibrosis. *B. pseudomallei* is closely related to *B. mallei*, the aetiological agent of glanders. *B. mallei* is non-motile, while *B. pseudomallei* possesses polar flagellae and is motile.

B. pseudomallei has been isolated from water and soil in tropical countries, mainly between latitudes 20° North and South where the temperature does not fall below 4°C. Most of the cases of melioidosis are identified in people from these regions, and patients developing melioidosis in temperate, non-endemic areas have usually lived or travelled in endemic regions. Endemic countries include Thailand, Vietnam, Cambodia, Southern China and Northern Australia, and of these Thailand has the highest number of reported cases. Melioidosis is usually acquired by abrasion of the skin and infection by contaminated soil and water, and is thus frequently reported in rice farmers. Human-to-human and maternal transmission are rare. Inhalation of infectious dusts is another documented route of acquiring melioidosis. This was especially notable during the Vietnam War, when helicopter crewmen became infected. By 1973, following the withdraw of US forces from Vietnam, there had been 343 reported cases of melioidosis resulting in 36 deaths. Melioidosis in many cases formed a latent infection which only recrudesced a significant time after return to the US, giving rise to the name "time-bomb disease" among ex-servicemen.

Melioidosis is the name given to any infection caused by *B. pseudomallei.* The pathogen causes a wide spectrum of disease, ranging from the acute to chronic, and even latent infections which can last for decades. Melioidosis often presents with other conditions such

as diabetes which can cause immunosuppression in the individual. Almost all organs and systems in the body can be affected, including the lungs. In Thailand, melioidosis is a significant cause of morbidity and death. Death is due to septic shock, respiratory failure and organ failure, occurring within days of onset of acute melioidosis. Intra-venous ceftazidime and imipenem are the antibiotic therapies of choice, and if given early in infection can reduce mortality rates to approximately 40 %. *B. pseudomallei* is resistant to many of the other antibiotics currently available and relapse is common. The effectiveness of measures to reduce exposure to the causative organism have not been established and a vaccine is not available.

Given the debilitating nature of infection with *Burkholderia,* for example with *B. pseudomallei* , that no prophylactic treatment/vaccine is available which is suitable for use in the general public and the infections are difficult to treat, an effective prophylactic therapy and/or treatment of the infections would be useful.

The present disclosure provides a phosphoantigen for the prophylaxis and/or treatment of infection by *Burkholderia pseudomallei, Burkholderia mallei* and *Burkholderia spp,* for example, *Burkholderia pseudomallei* infection i.e. melioidosis.

Brief Description of the Figures

Figure 1 shows human PBMCs treated with certain phosphoantigens reduce intracellular *Burkholderia pseudomallei in vitro* PBMCs cultured with IPP+IL-2 and CHDMAPP+IL-2 kill intracellular *B. pseudomallei* 708a . The human monocytic cell line THP-1 was infected with *B. pseudomallei* and PBMCs cultured with either media alone, IL-2 alone, IPP+IL-2 or CHDMAPP+IL-2 were added to the infected cells.. Some phosphoantigen-cultured cells received additional phosphoantigen treatment with IPP (IPP+IL-2 +IPP) or CHDMAPP (CHDMAPP+IL-2 +CHDMAPP) 4 h prior to the infection assay. Data is represented as the fold decrease in intracellular *B. pseudomallei* numbers as a ratio of intracellular bacterial numbers retrieved from control wells containing THP-1 cells alone divided by intracellular bacterial numbers retrieved from wells containing cultured PBMCs for each individual. Data is retrieved from8 separate experiments with blood from 8 different individuals.

Figure 2 shows purified human gammadelta T cell activity against intracellular *Burkholderia pseudomallei* is enhanced by treatment with phosphoantigen CHDMAPPPurified human γδ T cells kill intracellular *B. pseudomallei* 708a *in vitro* which is enhanced by treatment with CHDMAPP. The human monocytic cell line THP-1 was infected with *B. pseudomallei* and media alone (THP-1 only) or purified human γδ T cells (obtained from one individual) were added at $1x10^6$ or $1x10^5$ cells/ml and were either unstimulated or treated with CHDMAPP 4 h prior to infection assay (+CHDMAPP). The graph shows the

number of intracellular *B. pseudomallei* cfu/ml retrieved under each condition as a mean of triplicate wells. Error bars indicate standard deviation.

Figure 3: Marmoset splenic cell preparations cultured with CHDMAPP+IL-2 kill intracellular *B. pseudomallei* 708a. The human monocytic cell line (THP-1) was infected with *B. pseudomallei*. Media alone (THP-1 only) or splenic cell preparations, cultured with IL-2 or CHDMAPP+IL-2 for 10 days, were added to infected THP-1 at $1x10^6$ cells/ml. The graph shows the number of intracellular *B. pseudomallei* (cfu/ml) retrieved under each condition as a mean of triplicate wells from 3 different individuals. Error bars indicate standard deviation.

Whilst phosphoantigens have been suggested previously as potential adjuvants, there has been no suggestion that such compounds can be used in the treatment of a patient in their own right, that is, it has not been previously suggested that a treatment against any of the pathogens described herein could involve a phosphoantigen in the absence of an antigen, or if an antigen is present, that it is not pharmaceutically active in the prophylaxis and/or treatment of *Burkholderia* infection, or it is not present in sufficient amounts to be pharmaceutically active in the prophylaxis and/or treatment of *Burkholderia* infection. As used herein, "adjuvant" refers to a non-antigenic substance or substantially non-antigenic substance that is used in combination with an antigen for enhancing the immune response against the antigen. As used herein, "pharmaceutically active" means that a substance has a statistically significant measurable effect on the material/host to which is it is administered. The present invention is not intended to cover use of a phosphoantigen as an adjuvant in a medicament, such as in a medicament against any of the diseases mentioned herein. As used herein, "treatment" can mean prophylactic treatment (i.e. pre-treating) or treatment post-infection with a pathogen of the type disclosed herein. A treatment is considered successful if the symptoms of an infection are ameliorated or prevented.

The present invention therefore relates to priming an individual with phosphoantigen to provide beneficial therapeutic effects in relation to prevention of infection or amelioration of symptoms, after infection with a *Burkholderia* infection.

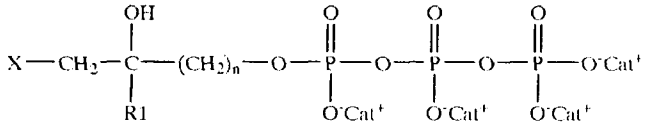
The Vγ9Vδ2 T cells stimulated by the phosphoantigen are thought to have an important role to play in the innate and adaptive immune response and may provide protective immunity (preventing infection of an individual) or ameliorate the symptoms of those infected as the body is stimulated to fight the infection.

Most bacteria produce phosphoantigens as intermediates of the DOXP metabolic pathway. Phosphoantigens are small molecular weight molecules with phosphorylated structures that selectively activate human or non-human primate T cells expressing Vγ9Vδ2 T cell receptors. Having said this it has not be shown definitively that *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp* produces a phosphoantigen.

Surprisingly, the use of phosphoantigens seem particularly effective in the prophylaxis and/or treatment of infection by *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp,* in particular *Burkholderia pseudomallei, i.e.* melioidosis. This may be related to the fact that the bacteria are intracellular pathogens.

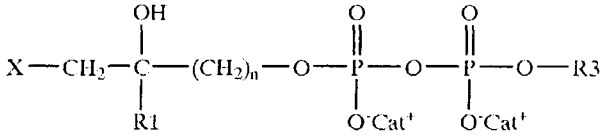
Phosphoantigens have been isolated from bacterial species such as TUBag1-4 from *M. Tuberculosis.*

Certain other phosphoantigens are known such as BrHPP and IHPP examples 1 and 2 respectively in US application publication No. 2004/0087555. The application relates to compounds of the following formula:

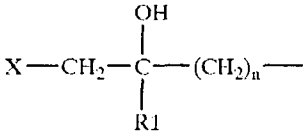


wherein:
X is a halogen selected from I, Br or Cl, R^1 is selected from methyl and ethyl, and Cat^+ is a cation, and n is an integer between 2 and 20.

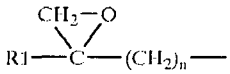
A particularly suitable structure therein is:



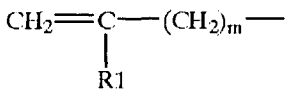
wherein X, R1 and Cat+ is as defined above and R3 is selected from:



;

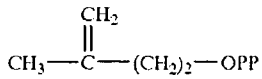
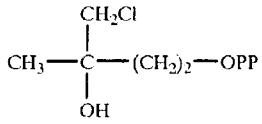
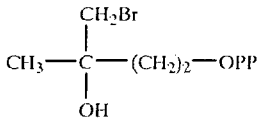
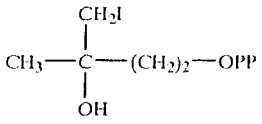
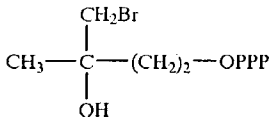
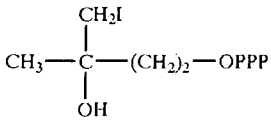
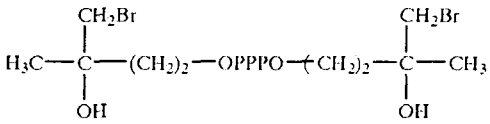
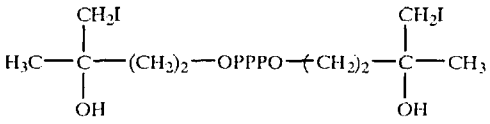


; and



.

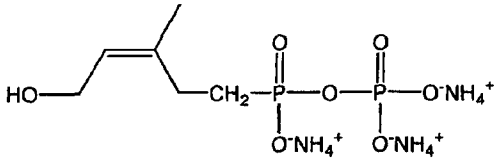
Below is a table of a number of phosphoantigens from said application.

Name	Abbreviation	MOLECULE Structure
isopentenyl pyrophosphate	IPP	
3-(chloromethyl)-3-butanol-1-yl diphosphate	ClHPP	
3-(bromomethyl)-3-butanol-1-yl diphosphate	BrHPP	
3-(iodomethyl)-3-butanol-1-yl diphosphate	IHPP	
3-(bromomethyl)-3-butanol-1-yl triphosphate	BrHPPP	
3-(iodomethyl)-3-butanol-1-yl triphosphate	IHPPP	
α, γ di-3-(bromomethyl)-3-butanol-1-yl triphosphate	di-BrHTP	
α, γ di-3-(iodomethyl)-3-butanol-1-yl triphosphate	di-IHTP	

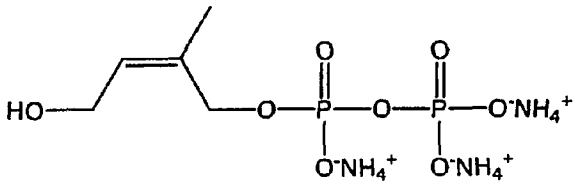
wherein is P phosphate.

Other compounds explicitly disclosed in the case include 3-(bromomethyl)-3-butanol-1-yl triphosphate (BrHPPP); 3-(iodomethyl)-3-butanol-1-yl triphosphate (IHPPP); uridine 5'-triphosphate gamma-[3-methyl-3butene-1-yl]; alpha, beta di[3-bromomethyl-3-butanol-1-yl]diphosphate.

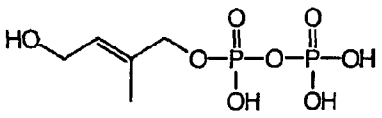
An alternative phosphoantigen is C-IPP



(3-methylbut-3-enyl pyrophosphonate);

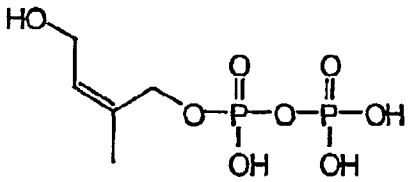


HIPP;



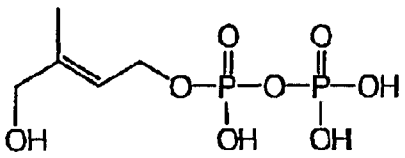
HTiglylPP

;



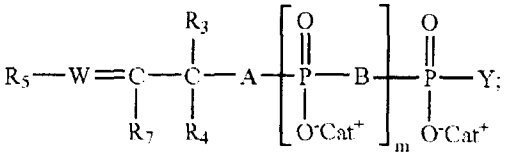
HAngelylPP

A particularly potent phosphoantigen is CHDMAPP:

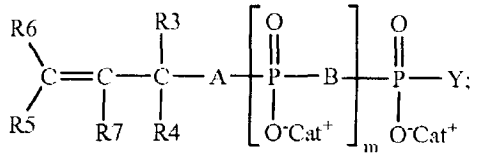


Certain phosphoantigens are described in published patent application publication number US2008/0207568 (Innate Pharma) which describes certain phosphoantigens of the formula:

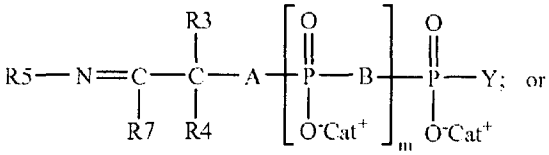
(I)



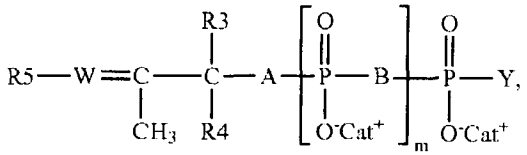
(II)



(III)



(V)



wherein
Cat+ represents one or more cations
m is an integer 1 to 3,
B is O, NH, CHF, CF_2 or CH_2 or any other isosteric group,
W is C-R6 or N,
R7 is a C_{1-3} alkyl group or any other isosteric group such as CF_3,
R3, R4 and R6 are independently selected from H, C_{1-3} alky or any other isosteric group such as CF_3,
R5 is selected from C_{2-3}acyl, aldehyde, a C_{1-3} alcohol or a C_{2-3} ester, and
Y=O$^-$Cat+ is as defined therein.

Any suitable phosphoantigen may be employed for the prophylaxis or treatment of said infection, including a synthetic and/or natural phosphoantigen. Particularly suitable phosphoantigens are described above, such as CHDMAPP and IPP.

Prophylaxis as employed herein refers to wherein the individual has a reduced risk of infection, i.e. invasion and multiplication by *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp,* in particular *Burkholderia pseudomallei.* In one embodiment the prophylactic treatment results in a reduced risk of developing melioidosis.

Reduced risk as employed herein refers to a 20, 30, 40, 50, 60, 70, 80% or more reduced risk of the developing the infection or a severe form of the infection compared to an individual who has not had any prophylactic treatment against the relevant pathogen.

Severe infection as employed herein is infection that under the present prescribing habits/protocols would warrant treatment with a combination of chloramphenicol, doxycycline and co-trimoxazole and would include persistent infection of the blood.

In one embodiment there is a provided a phosphoantigen for use in the treatment or prophylaxis of melioidosis.

Thus in one aspect, the disclosure provides a method of treating infections described herein, particularly melioidosis, comprising administering a therapeutically effective amount of the phosphoantigen prophylactically or to a patient infected by *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp,* in particular *Burkholderia pseudomallei.*

In one embodiment the phosphoantigen is administered before infection, for example 1, 2 or 3 days or a week or a month before exposure.

In one embodiment the phosphoantigen in given shortly after exposure, inoculation or infection, for example 1 hour to 3 days, such as 2 to 24 hours after.

In one embodiment the phosphoantigen is administered before exposure and shortly after exposure, particularly as *per* the time frames above.

In one embodiment the phosphoantigen is employed for the treatment of chronic infection, for example melioidosis, for example as a monthly treatment regime.

In one aspect there is provided use of a phosphoantigen for the manufacture of a medicament for the treatment of *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp* infection, in particular *Burkholderia pseudomallei* infection *i.e.* melioidosis.

In one embodiment a combination of a phosphoantigen and human interleukin-2 (IL-2) are employed.

The *in vitro* data with the relevant pathogens indicates that the bacterial load in a human monocytic cell line is reduced 10 to 1000 fold in the presence of human blood or purified γδ T cells treated with phosphoantigen and IL-2 *ex vivo*. Given that the cells employed in the *in vitro* assay are retrieved from the blood of individuals' in an approximation of the *in vivo* mechanism gives confidence that the phosphoantigens will work by the same mechanism *in vivo*.

In one embodiment the disclosure relates to a pharmaceutical composition comprising phosphoantigen in the presence or absence of IL-2, along with a pharmaceutically acceptable excipient, for example for the treatment or prophylaxis, in particular as described herein. The composition may be employed as described above for the phosphoantigen.

In one embodiment the phosphoantigen is employed in combination with IL-2 or an alternative or further cytokine such as IL-15, IL-21 interferon-γ and/or interferon-α.

In alternative embodiment the disclosure also relates to a composition comprising a phosphoantigen and an adjuvant and optionally in combination with IL-2 or an alternative or further cytokine such as IL-15, IL-21 interferon-γ and/or interferon-α.

The active components of the combinations of the disclosure may, for example be co-formulated if they stable when mixed together. Alternatively, the components may be formulated separately and co-administered, that is administered at the same time or approximately the same time, for example one immediately after the other. In a further alternative the components may be administered sequentially, that is to say with a delay between the administrations, for example a delay of 1 to 12 hours.

Formulations

The phosphoantigens or compositions according to the present disclosure maybe administered orally, topically, parenterally, transdermally, as a suppository or by any other pharmaceutically appropriate route.

Typical delivery routes include parenteral administration, e.g., intradermal, intramuscular or subcutaneous delivery. Other routes include oral administration, intranasal, intravaginal routes, intradermal and transdermal administration.

In one embodiment the phosphoantigen according to the disclosure is provided optionally in either as a lyophilized formulation for reconstitution later or as a liquid formulation.

Transdermal administration, such as by iontophoresis, may also be an effective method to deliver phosphoantigen to muscle. Epidermal administration may also be employed. Thus the disclosure also extends to delivery by a transdermal patch, which may be occlusive or non-occlussive.

The actives can also be formulated for administration via the nasal passages. Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 10 to about 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid for administration as, for example, nasal spray, nasal drops, or by aerosol administration by nebulizer, include aqueous or oily solutions of the active ingredient. For further discussions of nasal administration of AIDS-related vaccines, references are made to the following patents, U.S. Pat. Nos. 5,846,978, 5,663,169, 5,578,597, 5,502,060, 5,476,874, 5,413,999, 5,308,854, 5,192,668, and 5,187,074.

Compositions of use in the disclosure include liquid preparations, for an orifice, e.g., oral, nasal, anal, vaginal, etc. administration, such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. In compositions of the disclosure the relevant active ingredient may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like.

The active ingredients can be incorporated, if desired, into liposomes, microspheres or other polymer matrices (Felgner et al., U.S. Pat. No. 5,703,055; Gregoriadis, Liposome Technology, Vols. I to III (2nd ed. 1993), each of which is incorporated herein by reference). Liposomes, for example, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

Liposome carriers may serve to target a particular tissue or infected cells, as well as increase the half-life of the active. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the vaccine to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to, e.g., a receptor prevalent among lymphoid cells, such as monoclonal antibodies or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired immunogen of the disclosure can be directed to the site of lymphoid cells, where the liposomes then deliver the immunogen(s). Liposomes may be formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka, *et al.*, Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

The liposomes generally contain a neutral lipid, for example phosphatidylcholine, which is usually non-crystalline at room temperature, for example egg yolk phosphatidylcholine, dioleoyl phosphatidylcholine or dilauryl phosphatidylcholine.

In one embodiment the pharmaceutical formulation comprising the phosphoantigen is buffered, for example to a pH in the range 6.8 to 7.8.

In one embodiment the pharmaceutical formulation comprises a stabilizer, for example human serum albumin, normal serum albumin and/or human plasma protein fraction.

In some embodiments optionally the formulation may comprise an adjuvant, for example a known adjuvant formulation may be used to reconstitute the formulation.

Antigens employed in the disclosure may be mixed or adsorbed with adjuvants, which include but are not limited to alum, muramyl dipeptide and saponins such as Quil A. This may further boost the immune system's ability to deal with the infection.

Particular adjuvants are those selected from the group of metal salts, oil in water emulsions, Toll like receptors agonist, (in particular Toll like receptor 2 agonist, Toll like receptor 3 agonist, Toll like receptor 4 agonist, Toll like receptor 7 agonist, Toll like receptor 8 agonist and Toll like receptor 9 agonist), saponins or combinations thereof. The level of free antigen in a given formulation may be increased by, for example, formulating the composition in the presence of phosphate ions, such as phosphate buffered saline, or by increasing the ratio of antigen to metal salt. In one embodiment the adjuvant does not include a metal salt as sole adjuvant. In one embodiment the adjuvant does not include a metal salt.

In an embodiment the adjuvant is a Toll like receptor (TLR) 4 ligand, for example an agonist such as a lipid A derivative, in particular monophosphoryl lipid A or more specifically 3-deacylated monophoshoryl lipid A (3D-MPL).

3-Deacylated monophosphoryl lipid A is known from US patent No. 4,912,094 and UK patent application No. 2,220,211 (Ribi) and is available from Ribi Immunochem, Montana, USA.

3D-MPL is sold under the trademark MPL® by Corixa corporation and primarily promotes CD4+ T cell responses with an IFN-g (Th_1) phenotype. It can be produced according to the methods disclosed in GB 2 220 211 A. Chemically it is a mixture of 3 -deacylated monophosphoryl lipid A with 3, 4, 5 or 6 acylated chains. Generally in the compositions of the present disclosure small particle 3D-MPL is used. Small particle 3D-MPL has a particle size such that it may be sterile- filtered through a 0.22μm filter. Such preparations are described in International Patent Application No. WO 94/21292.

Synthetic derivatives of lipid A are known and thought to be TLR 4 agonists including, but not limited to:
OM174 (2-deoxy-6-O-[2-deoxy-2-[(R)-3-dodecanoyloxytetra-decanoylamino]-4-O-phosphono-β-D-glucopyranosyl]-2-[(R)-3-hydroxytetradecanoyl amino]-α-D-glucopyranosyldihydrogenphosphate), (WO 95/14026).
OM 294 DP (3S, 9 R) -3-[(R)-dodecanoyloxytetradecanoylamino]-4-oxo-5-aza-9(R)- [(R)-3 -hydroxytetradecanoylaminodecan- 1, 10-diol, 1,10-bis(dihydrogenophosphate) (WO 99/64301 and WO 00/0462).

OM 197 MP-Ac DP (3S, 9R)-3-[(R)-dodecanoyloxytetradecanoylamino]-4-oxo-5-aza-9-[(R)-3-hydroxytetradecanoylamino] decan-1, 10-diol, 1 -dihydrogenophosphate 10-(6-aminohexanoate) (WO 01/46127).

Typically when 3D-MPL is used the antigen and 3D-MPL are delivered with alum or presented in an oil in water emulsion or multiple oil in water emulsions. The incorporation of 3D-MPL is advantageous since it is a stimulator of effector T-cell responses. Alternatively the 3D-MPL may be formulated as liposomes.

Other TLR4 ligands which may be used are alkyl glucosaminide phosphates (AGPs) such as those disclosed in WO 98/50399 or US 6303347 (processes for preparation of AGPs are also disclosed), or pharmaceutically acceptable salts of AGPs as disclosed in US 6764840. Some AGPs are TLR4 agonists, and some are TLR4 antagonists. Both are thought to be useful as adjuvants.

Another immunostimulant for use in the present disclosure is Quil A and its derivatives. Quil A is a saponin preparation isolated from the South American tree Quilaja Saponaria Molina and was first described as having adjuvant activity by Dalsgaard *et al.* in 1974 ("Saponin adjuvants", Archiv. fur die gesamte Virusforschung, Vol. 44, Springer Verlag, Berlin, p243-254). Purified fragments of Quil A have been isolated by HPLC which retain adjuvant activity without the toxicity associated with Quil A (EP 0 362 278), for example QS7 and QS21 (also known as QA7 and QA21). QS21 is a natural saponin derived from the bark of Quillaja saponaria Molina which induces CD8+ cytotoxic T cells (CTLs), Th_1 cells and a predominant IgG2a antibody response.

Particular formulations of QS21 have been described which further comprise a sterol (WO 96/33739). The ratio of QS21:sterol will typically be in the order of 1:100 to 1:1 weight to weight.

Generally an excess of sterol is present, the ratio of QS21:sterol being at least 1:2 w/w. Typically for human administration QS21 and sterol will be present in a vaccine in the range of about 1 μg to about 100 μg, such as about 10 μg to about 50 μg per dose.

A formulation comprising QS21 and liposomes may be prepared, for example containing a charged lipid, which increases the stability of the lipsome-QS21 structure for liposomes composed of saturated lipids. In these cases the amount of charged lipid is often 1-20% w/w, such as 5-10%. The ratio of sterol to phospholipid is 1-50% (mol/mol), such as 20-25%. These compositions may contain MPL (3-deacylated mono-phosphoryl lipid A, also known as 3D-MPL).

The saponins may be separate in the form of micelles, mixed micelles (generally, but not exclusively with bile salts) or may be in the form of ISCOM matrices (EP 0 109 942), liposomes or related colloidal structures such as worm-like or ring- like multimeric complexes or lipidic/layered structures and lamellae when formulated with cholesterol and lipid, or in the form of an oil in water emulsion (for example as in WO 95/17210). The

saponins may often be associated with a metallic salt, such as aluminium hydroxide or aluminium phosphate (WO 98/15287).

Usually, the saponin is presented in the form of a liposome, ISCOM or an oil in water emulsion.

Immunostimulatory oligonucleotides may also be used. Examples of oligonucleotides for use in adjuvants of the present disclosure include CpG containing oligonucleotides, generally containing two or more dinucleotide CpG motifs separated by at least three, more often at least six or more nucleotides. A CpG motif is a cytosine nucleotide followed by a guanine nucleotide. The CpG oligonucleotides are typically deoxynucleotides. In one embodiment the internucleotide in the oligonucleotide is phosphorodithioate, or more preferably a phosphorothioate bond, although phosphodiester and other internucleotide bonds are within the scope of the disclosure. Also included within the scope of the disclosure are oligonucleotides with mixed internucleotide linkages. Methods for producing phosphorothioate oligonucleotides or phosphorodithioate are described in US 5,666,153, US 5,278,302 and WO 95/26204.

Examples of oligonucleotides are as follows: TCC ATG ACG TTC CTG ACG TT (CpG 1826)
TCT CCC AGC GTG CGC CAT (CpG 1758)
ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG
TCG TCG TTT TGT CGT TTT GTC GTT (CpG 2006)
TCC ATG ACG TTC CTG ATG CT (CpG 1668) TCG ACG TTT TCG GCG CGC GCC G (CpG 5456), the sequences may contain phosphorothioate modified internucleotide linkages. Alternative CpG oligonucleotides may comprise one or more sequences above in that they have inconsequential deletions or additions thereto.

The CpG oligonucleotides may be synthesized by any method known in the art (for example see EP 468520). Conveniently, such oligonucleotides maybe synthesized utilising an automated synthesizer.

Examples of a TLR 2 agonist include peptidoglycan or lipoprotein. Imidazoquinolines, such as Imiquimod and Resiquimod are known TLR7 agonists. Single stranded RNA is also a known TLR agonist (TLR8 in humans and TLR7 in mice), whereas double stranded RNA and poly IC (polyinosinic-polycytidylic acid - a commercial synthetic mimetic of viral RNA) are exemplary of TLR 3 agonists. 3D-MPL is an example of a TLR4 agonist whilst CpG is an example of a TLR9 agonist.

An immunostimulant may alternatively or in addition be included. In one embodiment this immunostimulant will be 3-deacylated monophosphoryl lipid A (3D-MPL).

Adjuvants combinations include 3D-MPL and QS21 (EP 0 671 948), oil in water emulsions comprising 3D-MPL and QS21 (WO 95/17210, WO 98/56414), or 3D-MPL formulated with other carriers (EP 0 689 454) including liposomes. Other adjuvant systems comprise a combination of 3D-MPL, QS21 and a CpG oligonucleotide as described in US 6558670 and US 6544518.

In one aspect the adjuvant comprises 3D-MPL. In one aspect the adjuvant comprises QS21. In one aspect the adjuvant comprises CpG. In one aspect the adjuvant comprises QS21 and 3D-MPL. In one aspect the adjuvant comprises QS21, 3D-MPL and CpG. In one aspect the adjuvant is formulated as an oil in water emulsion.

In one aspect the adjuvant is formulated as liposomes.

The amount of 3D-MPL used is generally small, but depending on the vaccine formulation may be in the region of 1 to 1000μg per dose, generally 1 to 500μg per dose, and more such as between 1 to 100μg per dose (10, 20, 30, 40, 50, 60, 70, 80 or 90μg per dose).

The amount of CpG or immunostimulatory oligonucleotides in the adjuvants or vaccines of the present disclosure is generally small, but depending on the vaccine formulation maybe in the region of 1 to 1000μg per dose, generally 1 to 500μg per dose, and more such as between 1 to 100μg per dose (10, 20, 30, 40, 50, 60, 70, 80 or 90μg per dose).

The amount of saponin for use in the adjuvants of the present disclosure may be in the region of 1 to 1000μg per dose, generally 1 to 500μg per dose, more such as 1 to 250μg per dose, and more specifically between 1 to 100μg per dose (10, 20, 30, 40, 50, 60, 70, 80 or 90μg per dose).

Thus in one embodiment there is provided a formulation comprising phosphoantigen and MPL, LPS or a derivative thereof.

In one embodiment there is provided a formulations comprising phosphoantigen and QS21.

In one embodiment there is provided a formulation comprising phosphoantigen and CpG.

Thus in one embodiment there is provided a formulation comprising phosphoantigen and MPL and QS21. Thus in one embodiment there is provided a formulation comprising phosphoantigen and MPL and CpG. Thus in one embodiment there is provided a formulation comprising phosphoantigen and QS21 and CpG. Thus in one embodiment there is provided a formulation comprising phosphoantigen and MPL, QS21 and CpG.

Adjuvant as employed herein is not intended to refer to the phosphoantigen element of the composition.

In one embodiment the formulation/composition is a vaccine.

Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller *et al.*, University Park Press, Baltimore, Maryland, U.S.A., 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877.

In one embodiment the formulation is provided as a formulation for topical administrations including inhalation.

Suitable inhalable preparations include inhalable powders, metering aerosols containing propellant gases or inhalable solutions free from propellant gases. Inhalable powders according to the disclosure containing the active substance may consist solely of the abovementioned active substances or of a mixture of the abovementioned active substances with physiologically acceptable excipient.

These inhalable powders may include monosaccharides (e.g. glucose or arabinose), disaccharides (e.g. lactose, saccharose, maltose), oligo- and polysaccharides (e.g. dextranes), polyalcohols (e.g. sorbitol, mannitol, xylitol), salts (e.g. sodium chloride, calcium carbonate) or mixtures of these with one another. Mono- or disaccharides are preferably used, the use of lactose or glucose, particularly but not exclusively in the form of their hydrates.

Particles for deposition in the lung require a particle size less than 10 microns, such as 1-9 microns for example from 0.1 to 5 μm, in particular from 1 to 5 μm. The particle size of the active (that is the antigen is of primary importance).

The propellent gases which can be used to prepare the inhalable aerosols are known in the art. Suitable propellent gases are selected from among hydrocarbons such as n-propane, n-butane or isobutane and halohydrocarbons such as chlorinated and/or fluorinated derivatives of methane, ethane, propane, butane, cyclopropane or cyclobutane. The abovementioned propellent gases may be used on their own or in mixtures thereof.

Particularly suitable propellent gases are halogenated alkane derivatives selected from among TG 11, TG 12, TG 134a and TG227. Of the abovementioned halogenated hydrocarbons, TG134a (1,1,1,2-tetrafluoroethane) and TG227 (1,1,1,2,3,3,3-heptafluoropropane) and mixtures thereof are preferred according to the invention.

The propellent-gas-containing inhalable aerosols may also contain other ingredients such as cosolvents, stabilisers, surface-active agents (surfactants), antioxidants, lubricants and means for adjusting the pH. All these ingredients are known in the art.

The propellant-gas-containing inhalable aerosols according to the invention may contain up to 5 % by weight of active substance. Aerosols according to the invention contain, for example, 0.002 to 5 % by weight, 0.01 to 3 % by weight, 0.015 to 2 % by weight, 0.1 to 2 % by weight , 0.5 to 2 % by weight or 0.5 to 1 % by weight of active.

In one embodiment of the disclosure the phosphoantigen is employed in a prime boost regime, as the priming and/or boosting dose. Priming in this context refers to priming the immune system for a response, such as Vγ9Vδ2 T cell proliferation and/activation. Boosting refer to increasing or sustaining the immune response of said priming.

Whilst not wishing to be bound by theory if the appropriate number of doses is exceeded in a relatively short period of time then it is possible induce anergy in the immune system, which is undesirable and should be avoided.
In the one embodiment the dose is in the range 1pg to 1000μg per Kg, such as 1ng to 10 μg per Kg.

In one embodiment the disclosure provides use of a phosphoantigen for enhancing protective host immune responses specifically those mediated by Vγ9δ2 T cells to enable to the host to fight infection with *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp* infection, in particular *Burkholderia pseudomallei* infection.

In one embodiment the disclosure provides a method of stimulating an immune response to fight *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp* infection, in particular *Burkholderia pseudomallei* infection.

In one embodiment the bioburden is lowered *in vivo* after administration of a phosphoantigen of the disclosure.

In one aspect the disclosure provides a method of treating cepacia syndrome in cystic fibrosis patients by administering a therapeutically effective amount of a phosphoantigen or composition described herein.

The disclosure also relates to use of said phosphoantigens and composition for the treatment of cepacia syndrome in cystic fibrosis patients.

EXAMPLES
Materials and methods

Preparation of Peripheral Blood Mononuclear Cells (PBMCs)

Human blood (60ml) was collected into BD Vacutainer CPT sodium citrate tubes and centrifuged (25min; 1500g (no brake)). Buffy coats containing PBMCs were removed and centrifuged (300g; 15min). The PBMC pellet was resuspended in 10ml medium (RPMI +10% Foetal calf serum (FCS), 200U/ml penicillin, 200µg/ml streptomycin and L-glutamine (10mM)). PBMCs were counted with trypan blue, centrifuged (300g; 15min) and re-suspended in appropriate media to $1x10^6$ cells/ml.

Culture of PBMCs with Phosphoantigen + IL-2

PBMCs were cultured for 10-14 days in media [RPMI1640, 200U/ml penicillin, 200µg/ml streptomycin and L-glutamine (10mM) and 10% Foetal Calf Serum (FCS) from selected sources] containing the synthetic phosphoantigens isopentenyl pyrophosphonate (IPP) (3µg/ml) (Sigma, UK) or CHDMAPP (113nM) (Innate Pharma, France) in the presence of human recombinant interleukin-2 (IL-2) (Sigma, UK) (100U/ml) to allow specific proliferation of Vγ9Vδ2 T cell populations. PBMCs were also cultured with either IL-2 (100U/ml) only or media alone. PBMCs were cultured under sterile conditions in $25cm^3$ cell culture flasks and media containing IL-2 (100U/ml) was added to flasks every 3-4 days (excepting PBMCs treated with media only) and were supplemented with fresh media without IL-2 where necessary.

Preparation of marmoset splenic cell suspensions

Spleens, removed posthumously from marmosets, were mashed through 40µm cell sieves and red blood cells were lysed using 0.85% ammonium chloride. Cells were centrifuged at 300g for 10 min and cells were resuspended in 10 ml medium (RPMI +10% Foetal calf serum (FCS), 200 U/ml penicillin, 200 µg/ml streptomycin and L-glutamine (10mM)). Splenic cell suspensions were counted with trypan blue, centrifuged (300g; 10min) and resuspended in appropriate media to $1x10^6$ cells/ml.

Culture of marmoset splenic cell suspensions with CHDMAPP + *IL-2*

Splenic cells were cultured for 10 days in media [RPMI1640, 200 U/ml penicillin, 200 µg/ml streptomycin and L-glutamine (10 mM) and 10% Foetal Calf Serum (FCS) from selected sources] containing the synthetic phosphoantigen CHDMAPP (113 nM) (Innate Pharma, France) and human recombinant interleukin-2 (IL-2) (Sigma, UK) (100 U/ml) to allow specific proliferation of Vγ9Vδ2 T cell populations. Cells were also cultured with IL-2 (100 U/ml) only. Cells were cultured under sterile conditions in $25cm^3$ cell culture flasks and media containing IL-2 (100 U/ml) was added to flasks every 3-4 days. Cells were supplemented with fresh media without IL-2 where necessary.

THP-1 human monocyte cell line

The non-adherent human monocytic cell line THP-1 (ATCC®) (ECACC, UK) was grown in RPMI, 10% Foetal calf serum (FCS) and L-glutamine (10 mM) all obtained from Sigma, UK

in cell culture flasks under sterile conditions. Prior to infection, THP-1 cells were removed from cell culture flasks and centrifuged (300g ;15min). Cells were resuspended in medium without antibiotics, counted and readjusted to required cell concentration ($2x10^6$cells/ml). THP-1 cells (1 ml/well) were plated into 24 well plates and activated with PMA (phorbol 12-myristate 13-acetate; Sigma, UK) at a final concentration of 10ng/ml and were incubated overnight (37°C; 5%CO_2) to produce an adherent monolayer. Media was removed from wells prior to infection and replaced with fresh media (900µl) without antibiotics.

Growth of B. pseudomallei strain 708a

All work with *B. pseudomallei* was performed at ACDP containment level 3. A viable frozen culture of *B. pseudomallei* 708a, a clinically isolated gentamycin-sensitive strain, was thawed and was streaked across dried L-agar and incubated overnight at 37°C; CO_2. The bacterial culture was then harvested with a sterile loop into sterile PBS to give a uniform, cloudy suspension with an OD_{450nm} reading between 0.3 to 0.4 of approximately $5x10^8$ cfu/ml. A one in 10 dilution of *B. pseudomallei* 708a suspension was performed prior to infection of THP-1 cells.

Intracellular infection of THP-1 monolayer with B. pseudomallei 708a

B. pseudomallei 708a suspension (100 µl/ml) was added to the THP-1 monolayer and incubated (30 min; 37°C; CO_2). Media was then removed and fresh media with 30 µg/ml gentamycin was added to kill any extracellular bacteria and were reincubated for a further 30 min. Cultured PBMCs ($1x10^5$ cells/ml) or marmoset splenic cell suspensions ($1x10^6$ cells/ml) were resuspended to an appropriate cell concentration in media containing 10µg/ml gentamycin or media only containing 10 µg/ml gentamycin were added to the infected THP-1 monolayer and incubated in a sealed container (with CO_2 packet) in 37°C incubator for 24 h. Supernatants were removed from wells following incubation and stored at -20°C. In order to retrieve intracellular bacteria, THP-1 were lysed using sterile distilled water (1 ml), pipetting up and down several times to disrupt cells. The lysed cell suspension (100 µl) was added to PBS (900 µl) to prevent further cell lysis. Droplets of the lysed cell suspension (3x20 µl) stabilised in PBS were placed onto duplicate dried L-agar plates and incubated for 2-3 days (37°C; 5% CO_2). Viable colonies were then counted to determine the number of viable intracelullar bacteria.

Statistical analysis

Data retrieved from infection assays with human PBMCs was analysed using a paired Student's t-test followed by a Bonferroni's multiple comparison correction. Data retrieved from infection assays with marmoset cell suspensions was analysed using a one-way ANOVA followed by Bonferroni's multiple comparison tests.

Results

Human PBMCs cultured with phosphoantigen kill intracellular B. pseudomallei

The effect of human blood, cultured with IPP+IL-2 or CHDMAPP+IL-2, on intracellular growth of *B. pseudomallei 708a* was investigated. The data is presented as a fold decrease in bacterial numbers for each individual experiment (n=8). (Figure 1). A significant reduction in intracellular bacterial numbers was observed in the presence of PBMCs cultured with both CHDMAPP+IL-2 or IPP+IL-2 in comparison with PBMCs cultured with media alone ($p<0.01$). In addition to this, a significant reduction in intracellular *B. pseudomallei* was observed following treatment with CHDMAPP+IL-2 ($p<0.01$) or IPP+IL-2 ($p<0.05$) in comparison with PBMCs treated with IL-2 alone.

The effect of additional treatment with either IPP or CHDMAPP following culture,4 hours prior to use in the infection assay, was also investigated. Addition of CHDMAPP (113 nM) toCHDMAPP+IL-2 cultured PBMCs enhanced the reduction in intracellular bacterial numbers when compared with CHDMAPP+IL-2 alone.

Purified human γδ T cells kill intracellular B. pseudomallei

In order to determine if γδ T cells were important in the reduction of intracellular bacterial numbers following treatment with phosphoantigen, the ability of purified peripheral human γδ T cells to reduce *B. pseudomallei* numbers was investigated. Purified γδ T cells ($1x10^6$ and $1x10^5$ cells/ml) reduced intracellular bacterial numbers significantly ($p<0.01$). Treatment of purified γδ T cells with CHDMAPP 4 hours prior to use in the killing assay significantly enhanced the reduction in bacterial numbers at $1x10^6$ cells/ml leading to a 1000-fold reduction in bacterial numbers (Figure 2).

Marmoset splenic cells cultured with phosphoantigen kill intracellular B. pseudomallei

Marmoset splenic cells from 3 individuals were cultured with CHDMAPP+IL-2 or IL-2 alone and the effect of these cultured cells on growth of intracellular *B. pseudomallei 708a* in THP-1 cells was investigated. A 2-3 log reduction in intracellular bacterial numbers was observed in the presence of cells treated with CHDMAPP+IL-2 in comparison with cells cultured with IL-2 alone ($p<0.001$).

Claims

1. A phosphoantigen for the prophylaxis and/or treatment of *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp* infection.

2. A phosphoantigen according to claim 1, for the prophylaxis and/or treatment of *Burkholderia pseudomallei* infection.

3. A phosphoantigen for according to claim 2 for the prophylaxis or treatment of melioidosis.

4. A phosphoantigen according to any one of claims 1 to 3, wherein the phosphoantigen is IPP or CHDMAPP.

5. A phosphoantigen according to claim 1, where in the phosphoantigen is BrHPP or IHPP.

6. A pharmaceutical composition comprising a phosphoantigen and a pharmaceutical acceptable excipient.

7. A pharmaceutical composition according to claim 6, wherein the excipient is an adjuvant.

8. A pharmaceutical composition according to claim 6 or 7, for the prophylaxis and/or treatment of *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp* infection

9. A pharmaceutical composition according to claim 8, for the prophylaxis and/or treatment of *Burkholderia pseudomallei* infection.

10. A pharmaceutical composition according to claim 9, for the prophylaxis and/or treatment of melioidosis.

11. A method of treatment comprising administering a therapeutically effective amount of a phosphoantigen or a pharmaceutical composition comprising same prophylactically or as treatment to a patient in need thereof, for *Burkholderia pseudomallei, Burkholderia mallei* and/or *Burkholderia spp* infection.

12. A method according to claim 11, for the prophylaxis or treatment for *Burkholderia pseudomallei* infection.

13. A method according to claim 11, wherein the pharmaceutical composition does not further comprise an antigen that is pharmaceutically active in the prophylaxis and/or treatment of *Burkholderia* infection.

14. A pharmaceutical composition comprising a phosphoantigen for the prophylaxis and/or treatment of *Burkholderia* infection wherein the composition does not further comprise an antigen that is pharmaceutically active in the prophylaxis and/or treatment of *Burkholderia* infection.

15. The pharmaceutical composition of claim 14 wherein the *Burkholderia* infection is Burkholderia *pseudomallei* and/or *Burkholderia mallei*.

16. The pharmaceutical composition of claim 14 for the prophylaxis or treatment of melioidosis.

17. The pharmaceutical composition of any one of claims 14 to 16 wherein the phosphoantigen is IPP or CHDMAPP.

18. The pharmaceutical composition of any one of claims 14 to 16 wherein the phosphoantigen is BrHPP or IHPP.

19. Use of the composition recited in any one claims 14 to 18 for the manufacture of a medicament for the prophylaxis and/or treatment of *Burkholderia* infection.

20. The use of claim 19 wherein the *Burkholderia* is *Burkholderia pseudomallei* and/or *Burkholderia mallei*.

Fig.1.

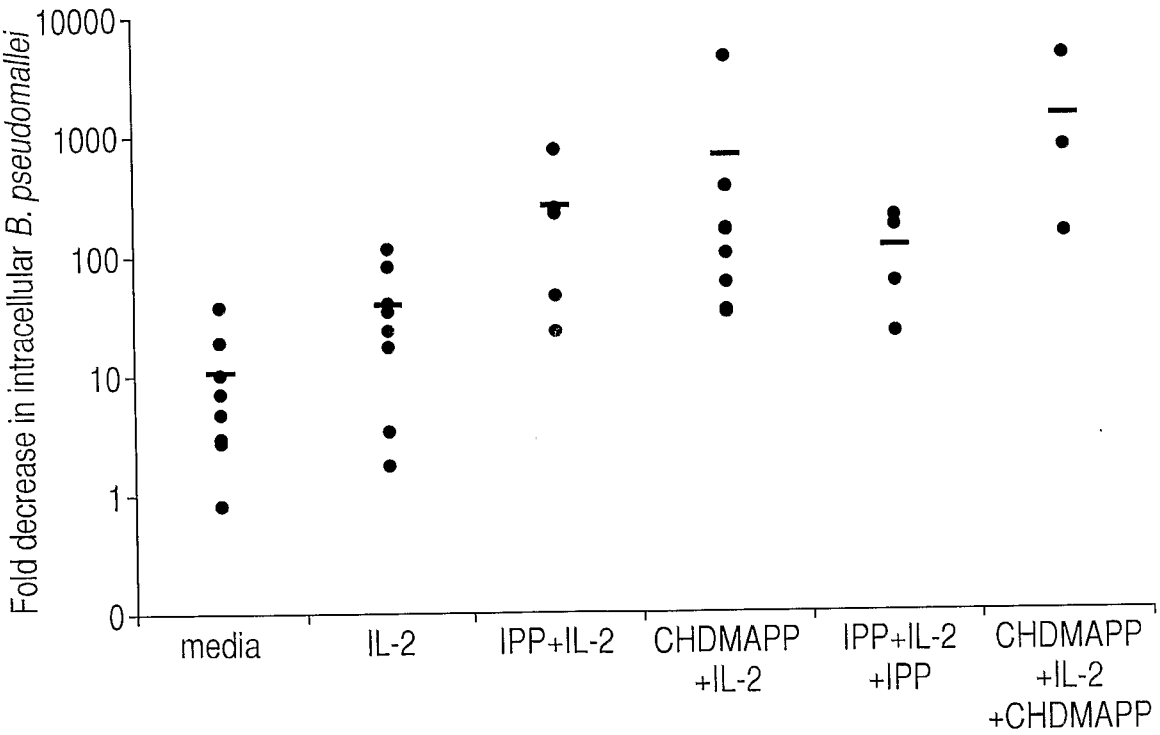

Fold decrease in intracellular B. pseudomallei
10000
1000
100
10
1
0
media
IL-2
IPP+IL-2
CHDMAPP +IL-2
IPP+IL-2 +IPP
CHDMAPP +IL-2 +CHDMAPP

Fig.2.

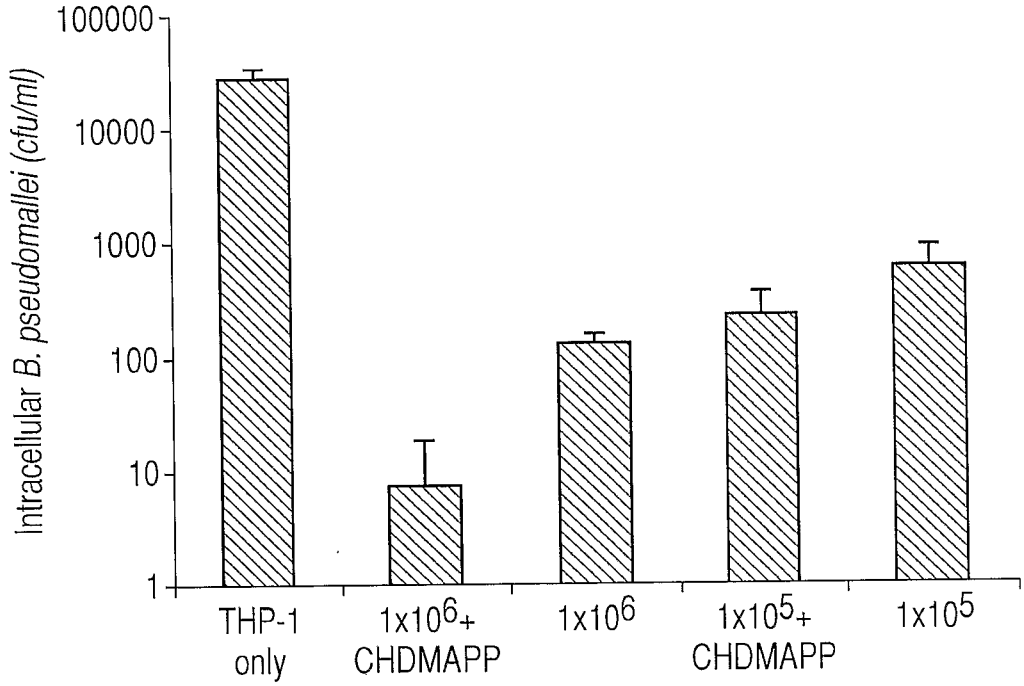
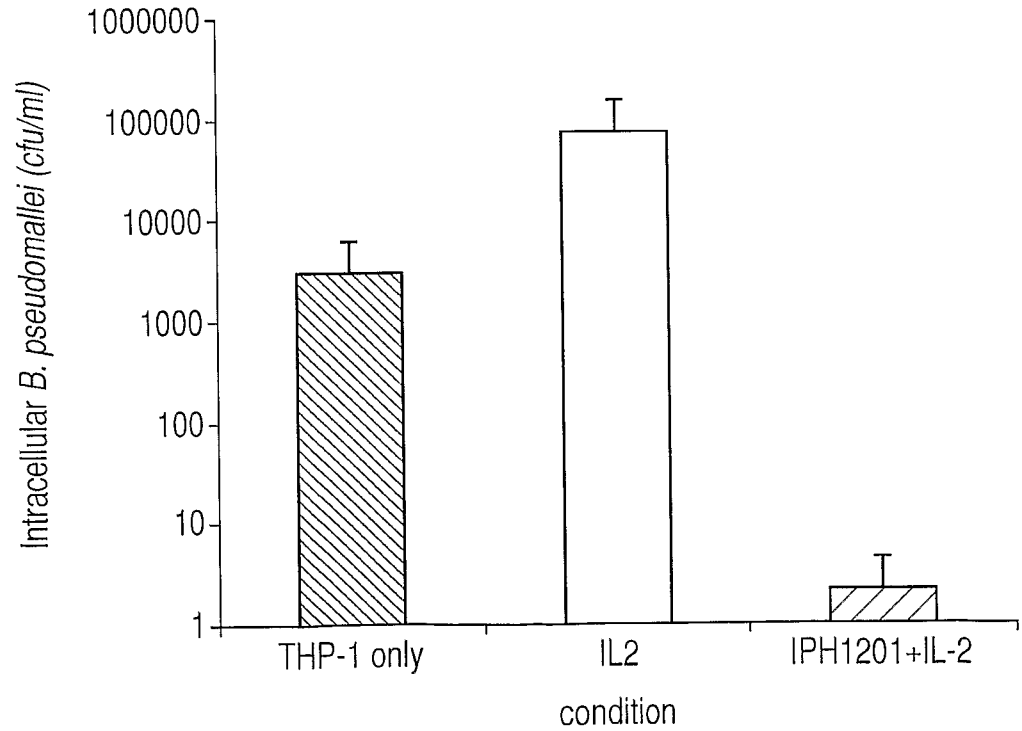

100000
10000
1000
100
10
1
Intracellular B. pseudomallei (cfu/ml)
THP-1 only
1x10^6+ CHDMAPP
1x10^6
1x10^5+ CHDMAPP
1x10^5

Fig.3.


1000000
100000
10000
1000
100
10
1
Intracellular B. pseudomallei (cfu/ml)
THP-1 only
IL2
IPH1201+IL-2
condition

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/000146

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/683 A61K31/685 A61K31/688 A61P31/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 7 399 756 B2 (JOMAA HASSAN [DE] ET AL) 15 July 2008 (2008-07-15) column 11, lines 40-50; claims 13-15, 21 claims 1-12	6,7 1-20
X	US 2008/207568 A1 (BELMANT CHRISTIAN [FR]) 28 August 2008 (2008-08-28) claims 1-50 paragraphs [0258] - [0261] paragraph [0265]	6,7
X	US 2004/087555 A1 (BELMANT CHRISTIAN [FR] ET AL) 6 May 2004 (2004-05-06) paragraphs [0094], [101] paragraphs [0103], [104]	6,7

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2010	12/07/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kerkmann, Miren

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/000146

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROWLAND CAROLINE A ET AL: "Critical role of type 1 cytokines in controlling initial infection with Burkholderia mallei" INFECTION AND IMMUNITY, vol. 74, no. 9, September 2006 (2006-09), pages 5333-5340, XP002588964 ISSN: 0019-9567 * abstract page 5339, column 1, paragraph 4 page 5339, column 2, paragraph 3	1-20
A	GOSSMAN, WILLIAM ET AL: "Quantitative Structure-Activity Relations for .gamma..delta. T Cell Activation by Phosphoantigens" JOURNAL OF MEDICINAL CHEMISTRY , 45(22), 4868-4874 CODEN: JMCMAR; ISSN: 0022-2623, 2002, XP002588965 * abstract page 4872, column 2, last paragraph	1-20
A	CASETTI, RITA ET AL: "Drug-Induced Expansion and Differentiation of V.gamma.9V.delta.2 T Cells In Vivo: The Role of Exogenous IL-2" JOURNAL OF IMMUNOLOGY , 175(3), 1593-1598 CODEN: JOIMA3; ISSN: 0022-1767, 2005, XP002588966 * abstract	1-20
A	GAUTHIER Y P ET AL: "Protease production by Burkholderia pseudomallei and virulence in mice." ACTA TROPICA 5 FEB 2000 LNKD-PUBMED:10674652, vol. 74, no. 2-3, 5 February 2000 (2000-02-05), pages 215-220, XP002588967 ISSN: 0001-706X * abstract	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2010/000146

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 7399756 B2	15-07-2008	AT 411805 T	15-11-2008
		CA 2453817 A1	06-02-2003
		CN 1533279 A	29-09-2004
		DK 1408984 T3	09-02-2009
		WO 03009855 A2	06-02-2003
		EP 1408984 A2	21-04-2004
		ES 2315411 T3	01-04-2009
		JP 2005508305 T	31-03-2005
		PT 1408984 E	26-12-2008
		US 2008249067 A1	09-10-2008
		US 2006030546 A1	09-02-2006
US 2008207568 A1	28-08-2008	AU 2006228435 A1	05-10-2006
		CA 2602223 A1	05-10-2006
		CN 101146575 A	19-03-2008
		EA 200702029 A1	28-02-2008
		EP 1863572 A2	12-12-2007
		WO 2006103568 A2	05-10-2006
		JP 2008534489 T	28-08-2008
		KR 20070113314 A	28-11-2007
US 2004087555 A1	06-05-2004	NONE	