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(54) Title: METHODS OF TREATING INFECTIOUS DISEASES

(57) Abstract: The present invention relates to methods of treating an infection in a subject in need thereof. In particular, the present invention relates to treating an infection by administering a therapeutically effective amount of a BCL2 family inhibitor (e.g., a BH3 mimetic agent) alone or in combination with an anti-infective agent. The invention further relates to methods and compositions, including pharmaceutical compositions, containing both a BCL2 family inhibitor and an anti-infective agent. Also provided are kits containing both a BCL2 family inhibitor and an anti-infective agent either separately or combined into a single formulation.



METHODS OF TREATING INFECTIOUS DISEASES

FIELD OF THE INVENTION

The specification relates generally to the field of therapeutic agents. More particularly, the specification relates to methods and related compositions for preventing or treating infectious diseases.

BACKGROUND OF THE INVENTION

Infectious disease persists as a worldwide health challenge. The prevalence of pathogens (*e.g.*, antibiotic-resistant pathogenic bacteria) that are now resistant to previously successful treatments is high and increasing at an alarming rate. Indeed, antimicrobial resistance, largely driven by overuse of antibiotics, is now considered a global health threat. Thus, there is an urgent and ongoing need to identify new strategies for treating infections, particularly those that are easily spread and likely to reach epidemic scale.

SUMMARY OF THE INVENTION

The present inventors have found that BCL2 family inhibitors can be used to treat infections in a subject. Accordingly, in one aspect described herein is a method for treating an infection, comprising administering to a subject in need thereof a therapeutically effective amount of a BCL2 family inhibitor.

In some embodiments the infection to be treated is a bacterial infection. In some embodiments the bacterial infection is an infection by bacteria of one or more species of *Legionella*, *Mycobacterium*, *Salmonella*, *Staphylococcus*, pathogenic *E. coli*, or *Pseudomonas*. In some embodiments the bacterial infection includes infection by antibiotic-resistant bacteria. In other embodiments treatment of a subject suffering from a bacterial infection includes, in addition to treatment with a BCL2 family inhibitor, treatment with a bactericidal or bacteriostatic agent. In one embodiment, the bactericidal or bacteriostatic agent is administered contemporaneously with the BCL2 family inhibitor. In another embodiment the BCL2 family inhibitor is administered before the bactericidal or bacteriostatic agent. In a further embodiment the BCL2 family inhibitor is administered after the bactericidal or bacteriostatic agent.

In other embodiments the infection to be treated is a viral infection, in particular a viral infection other than a viral infection by an influenza virus. In some

embodiments the viral infection includes infection by one or more of, HSV, HTLV-1, HTLV-2, HIV, a respiratory virus, rhinovirus, or a coronavirus, or Ebola.

In further embodiments the infection to be treated is a fungal infection. In some embodiments the fungal infection is an infection by one or more of a yeast, *Aspergillus*,
5 *Pneumocystitis*, and *Cryptococcus*.

In some embodiments the infection to be treated is a protozoal infection. In some embodiments the protozoal infection is an infection by one or more of *Trypanosoma cruzi*, *Toxoplasma gondii*, *Cryptosporidium*, or *Encephalitozoon*.

In some embodiments the subject is suffering from tuberculosis, pneumonia,
10 Legionnaires' disease, or an acute respiratory infection.

In some embodiments subject to be treated is suffering from an infection that includes infection of macrophages. In one embodiment, the infected macrophages include alveolar macrophages.

In some embodiments the infection is an acute or subacute infection.

15 In some embodiments the subject to be treated for the infection was not diagnosed as suffering from a cancer.

In certain embodiments administration of the BCL2 family inhibitor occurs within a period from about 1 hours to about 72 hours, about 6 hours to about 48 hours , or about 6 hours to about 24 hours, following a diagnosis of the infection. In other
20 embodiments, the administration occurs no later than about 72 hours or about 48 hours following a diagnosis of the infection.

In some embodiments the subject to be treated for the infection is human. In other embodiments the subject to be treated is non-human. In some embodiments the non-human subject is a non-human primate, a rat, a mouse, a pig, a cow, a sheep, a
25 chicken, or a duck.

In an embodiment, the inhibitor at least targets one or more or all of BCL-XL, BCL-2, MCL-1 or A1 of the BCL2 family of proteins. In a preferred embodiment, the inhibitor at least targets BCL-XL. In an alternate embodiment, BCL2 is not the only BCL2 family member the inhibitor targets (i.e. the inhibitor is not BCL2 specific).

30 In an embodiment, the BCL2 family inhibitor is selected from, but not necessarily limited to, the group consisting of a BH3 mimetic agent, an RNAi against a BCL2 family member, and an antibody against a BCL2 family member. In an embodiment, the BCL2 family inhibitor is a BH3 mimetic agent.

In some embodiments the BH3 mimetic agent used in any aspect described
35 herein is a BH3 mimetic agent that inhibits BCL-XL. In one embodiment the BH3 mimetic agent is a selective inhibitor of BCL-XL. In some embodiments the BH3

mimetic agent in any aspect described herein is a peptide. In some embodiments the BH3 mimetic agent in any aspect described herein is a small molecule compound ("BH3 mimetic compound"). In some embodiments the BH3 mimetic compound is a compound having a structure selected from the group consisting of Formulas I-XII, as set forth herein.

In a related aspect described herein is the use of a BCL2 family inhibitor for the preparation of a medicament for treating an infection.

In another related aspect described herein is a BCL2 family inhibitor for use in the treatment of an infection.

In a further aspect described herein is a pharmaceutical composition containing a therapeutically effective amount of a BCL2 family inhibitor and a bactericidal or bacteriostatic agent.

In yet another aspect described herein is a kit when used for the treatment of a bacterial infection, the kit comprising a BCL2 family inhibitor. In some embodiments the kit includes, in addition to the BCL2 family inhibitor, a bactericidal or bacteriostatic agent.

Any embodiment herein shall be taken to apply *mutatis mutandis* to any other embodiment unless specifically stated otherwise. For instance, as the skilled person would understand examples of inhibitors and infections outlined above for the methods of the invention equally apply to the use, pharmaceutical compositions and kit of the invention.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

The invention is hereinafter described by way of the following non-limiting Examples and with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

Figure 1 - ABT-737 restricts *Legionella* burdens in BMDMs, *in vitro*, by inducing host cell death. a, b, c, DraG7 positive (dead) bone marrow-derived macrophages

(BMDMs) were quantified by live-cell imaging over 72 h, following infection with (a, c) $\Delta flaA$ or (b) $\Delta dotA$ *Legionella* at (a, b) MOI of 10 or (c) 25, and addition of 10 μ M ABT-737. Mean and standard deviation (SD) of three repeats are shown. Data representative of at least three independent experiments. d, Time-lapse images of immortalized C57BL/6 macrophages infected with $\Delta flaA$ expressing GFP, in the presence of Draq7 and ABT-737 (500 nM) or vehicle. Scale bar = 10 μ m. Infected macrophages showing apoptotic morphology were quantified 24 h post-infection in three independent samples (a total of 600 cells from triplicate experiments were analyzed). e, $\Delta flaA$ replication in BMDMs treated with 500 nM ABT-737 was examined by determining bacterial colony forming units (CFUs) at the indicated time points. Mean and standard error (SE) are shown (n = 3).

Figure 2 - Targeting BCL-XL, rather than BCL-2, limits intracellular *Legionella* loads. a, Left: flow cytometry analysis of cell death (propidium iodide staining) and bacterial load (GFP fluorescence) of $\Delta flaA^{GFP+}$ infected immortalized C57BL/6 macrophages treated with 500 nM BH3-mimetics, 48 h post-infection (MOI = 10). The percentages of cells in each quartile of a dot plot are shown. Right: schematic depicting the pro-survival BCL-2 family members targeted by the different BH3-mimetics used in this study (A-1155463 is abbreviated to 463). b, Live-cell microscopic analysis of Draq7-positive (dead) BMDMs measured for 72 h following infection with $\Delta flaA$ *Legionella* and treatment with BH3-mimetics (500 nM). Mean and SD of three repeats are shown. Data representative of at least three independent experiments. c, Bacterial burden (CFUs) of $\Delta flaA$ *Legionella* isolated from BMDMs treated with BH3 mimetics (500 nM) at 48 h post-infection are shown. Mean (line) and SE are shown (n = 3). d, Flow cytometric analysis of GFP⁺ macrophages infected with $\Delta flaA^{GFP+}$ and treated with different BH3-mimetic compounds at the indicated concentrations for 48 h. Mean of triplicate samples is shown. e, Bacterial burden (CFUs) of $\Delta flaA$ *Legionella* isolated from wild type (WT) and BCL-XL-deficient BMDMs. Mean and SE are shown (n = 3). f, g, Live-cell microscopic analysis of Draq7 positive (dead) BMDMs infected with $\Delta flaA$ and $\Delta dotA$. Mean and SD of three repeats are shown. Data representative of at least three independent experiments.

Figure 3 - Loss of BCL-XL induces apoptosis in *Legionella*-infected macrophages. a, Time course immuno-blot analysis for cleaved (i.e., activated) caspase-3 in wild type (WT) and BCL-XL-deficient BMDMs infected with $\Delta flaA$ or $\Delta dotA$ or b, A-1155463 (463)-treated WT BMDMs infected with $\Delta flaA$. Actin blots are loading controls. Data

representative of two independent experiments. **c**, Indirect immuno-fluorescence staining analysis of activated caspase-3 in BMDMs infected with *Legionella* expressing GFP. Scale bar = 10 μ m. >200 cells were analyzed. **d**, Δ *flaA* *Legionella* burden (CFUs) of infected BMDMs at 48 h post-infection following treatment with 500 nM of BH3-mimetic and 20 μ M of the pan-caspase inhibitor Q-VD-OPh (Q-VD). Mean (line) and SE are shown (n = 3). **e, f, g, h**, Live-cell microscopic analysis of Draq7-positive (dead) BMDMs infected with Δ *flaA* and treated with 500 nM of BH3-mimetic and 20 μ M Q-VD-OPh (Q-VD). Mean and SD of three repeats are shown. Data representative of at least three independent experiments. **i, j**, Bacterial burden (CFUs) of Δ *flaA* infected BMDMs 48 h post-infection. Mean (line) and SE are shown (n = 3). Dotted line indicates mean CFUs of untreated BMDMs at 6 h post-infection.

Figure 4 - BCL-XL expression is required for *Legionella* replication in lungs. a, Immuno-blot analysis of BCL-XL in BMDMs derived from wild type (WT) and three *Bcl-x^{fllox/fllox};ER-Cre* mice treated with tamoxifen. Ponceau staining as a loading control is shown. **b**, Lung bacterial burden (CFUs) of C57BL/6 (WT) and *Bcl-x^{fllox/fllox};ER-Cre* mice 48 h after intra-nasal infection. Mean (line) and detection limit (dotted line) are shown. **c, d**, Lung bacterial burden (CFUs) from ABT-263-treated (c) A/J mice after intra-nasal infection with 2.5×10^5 WT *L. pneumophila* at 48 h, or (d) C57BL/6 (WT) mice after intra-nasal infection with 1×10^5 *L. longbeachae* at 72 h post-infection. Mean (line) from two independent experiments and detection limit (dotted line) are shown. **e**, Mice survival curve following ABT-263 or DMSO (vehicle) treatment of *L. longbeachae*-infected C57BL/6 mice (n=5/treatment). **f**, Average body weight of each treatment group (from 4e) over the 7 days following infection (n=5/treatment).

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Figure 5 - ABT-737 protects BMDMs from *L. pneumophila*-induced cell death. a, b, Draq7 positive (dead) bone marrow-derived macrophages (BMDMs) were quantified over 72 h using time-lapse imaging in the presence of 10 μ M ABT-737 or vehicle (DMSO) (b) without infection or (a) after infection with wild type *L. pneumophila*, at an MOI of 10. Mean and SD are shown. Data representative of at least three independent experiments.

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Figure 6 - BCL-XL protects BMDMs from wild type *L. pneumophila*-induced death. a, Immuno-blot analysis of BCL-XL protein content in bone marrow derived macrophages (BMDMs) derived from *Bcl-x^{fllox/fllox};ER-Cre* mice, whereby progenitor cells were left untreated, or else treated with 4-hydroxytamoxifen (4-HT; 50 nM, 2 days

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post-harvesting) to induce deletion of the two floxed *Bcl-x* alleles. **b**, Live-cell microscopic analysis of Draq7 positive (dead) WT (C57BL/6) and BCL-XL-deficient BMDMs infected with WT *L. pneumophila*. Mean and SD are shown. Data representative of at least three independent experiments.

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Figure 7 - *L. pneumophila* infection of macrophages in culture depends on BCL-XL. Bacterial burden (CFUs) of $\Delta flaA$ *L. pneumophila* in WT (C57BL/6) and BCL-XL-deficient BMDMs isolated from tamoxifen-treated *Bcl-x^{flox/flox};ER-Cre* mice, at 48 h post-infection. Mean and SE are shown (n = 3).

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Figure 8 - BH-3 mimetics targeting BCL-XL inhibit replication of *Legionella longbeachae* in BMDMs. Bacterial burden (CFUs) of *Legionella longbeachae*-infected BMDMs treated with 500 nM of BH3-mimetic, or control vehicle, at 48 h post-infection. Dotted line shows infection level at 6 h. Mean and SE are shown (n = 3).

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Figure 9 - A BH3 mimetic having the structure of Formula (XIII) targets BCL-XL and inhibits replication of *Legionella longbeachae* in vivo. Bacterial burden (CFUs) of *Legionella longbeachae* in the lungs of infected mice treated with control (DMSO) or 50 mg/kg of a BCL-XL-selective BH3 mimetic (Formula (XIII)) 72 hours post-infection. Scatter plot denotes number of lung-derived CFUs in control (left; circles) or BH3 mimetic-treated animals (right; squares); n=5 per group.

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Figure 10 - A BCL-XL-Selective BH3 Mimetic Compound, A-1331852 (Formula XIII) is Effective in Treatment of a Model of Latent *Mycobacterium tuberculosis* infection. **a**, Schematic overview of an experiment in which mice, in a latent model of *M. tuberculosis* infection, were administered either vehicle or BCL-XL-selective BH3 mimetic compound, A-1331852 (Formula XIII). **b**, Scatter plot denotes number of lung-derived CFUs following the reactivation protocol in vehicle control (left) or A-1331852-treated animals (right); n=6 per group. **c**, Bar graph denoting the occurrence of reactivation or non-reactivation in vehicle-treated (left) or A-1331852-treated animals (right); n=6 per group.

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DETAILED DESCRIPTION OF THE INVENTION

General Techniques and Definitions

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Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of

ordinary skill in the art (*e.g.*, in cell culture, cell biology, molecular genetics, infectious disease especially acute infections, immunology, pharmacology, protein chemistry, and biochemistry).

Unless otherwise indicated, the cell culture and immunological techniques
5 utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, *A Practical Guide to Molecular Cloning*, John Wiley and Sons (1984), J. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), *Essential Molecular*
10 *Biology: A Practical Approach*, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), *DNA Cloning: A Practical Approach*, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (editors), *Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) *Antibodies: A Laboratory Manual*,
15 Cold Spring Harbour Laboratory, (1988), and J.E. Coligan et al. (editors) *Current Protocols in Immunology*, John Wiley & Sons (including all updates until present).

As used herein, the term about, unless stated to the contrary, refers to +/- 10%, more preferably +/- 5%, of the designated value.

Throughout this specification the word "comprise", or variations such as
20 "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used in this application, the term "or" is intended to mean an inclusive "or" rather than an exclusive "or." That is, unless specified otherwise, or clear from context,
25 "X employs A or B" is intended to mean any of the natural inclusive permutations. That is, if X employs A; X employs B; or X employs both A and B, then "X employs A or B" is satisfied under any of the foregoing instances. Further, at least one of A and B and/or the like generally means A or B or both A and B. In addition, the articles "a" and "an" as used in this application and the appended claims may generally be
30 construed to mean "one or more" unless specified otherwise or clear from context to be directed to a singular form.

The term "acute infection," as used herein, refers to an infection characterized by rapid onset of disease, a relatively brief period of symptoms, and resolution within days. It is usually accompanied by early production of the underlying infectious
35 pathogen and elimination of infection by the host immune system. The related term

“subacute,” refers to an infection that is not chronic and that runs a rapid and severe, but less than acute, course.

The term “anti-infective agent,” as used herein, refers to any agent (*e.g.*, a small molecule compound) that directly inhibits the ability of an infectious pathogen to infect, replicate, survive, or otherwise cause damage to its host. Examples of anti-infective agents include, but are not limited to, antibiotics, antiviral drugs, antifungal drugs, and antiprotozoal drugs. BCL2 family inhibitors, as used herein, are specifically excluded from being considered anti-infective agents.

The term “BCL2 family inhibitor,” as used herein, refers to any agent that inhibits the pro-survival activity of at least one member of the BCL2 protein family subgroup that includes BCL-2, BCL-XL, BCL-W, MCL-1, and A1/BFL1. Examples of types of BCL2 inhibitors include, but are not limited to, small molecule compound BH3 mimetics, peptides, RNAi, and antibodies.

The terms “BH3 mimetic agent,” or “BH3 mimetic” as used herein, refer to any agent (*e.g.*, a small molecule compound or a peptide) that interacts, in a manner analogous to a BH3 domain, with the hydrophobic groove of the prosurvival BCL-2 proteins (BCL-2, BCL-XL, BCL-W, MCL-1, and A1/BFL1) to antagonize their prosurvival (antiapoptotic) activity. Examples of BH3 only domain proteins include NOXA, BAD, and BIM.

The terms “co-administration” or the like, as used herein, are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are administered by the same or different route of administration or at the same or different time.

The terms “effective amount” or “therapeutically effective amount,” as used herein, refer to a sufficient amount of an agent or a compound being administered which will relieve to some extent one or more of the symptoms of the disease or condition being treated. The result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an “effective amount” for therapeutic uses is the amount of the composition including a compound as disclosed herein required to provide a clinically significant decrease in disease symptoms without undue adverse side effects. An appropriate “effective amount” in any individual case may be determined using techniques, such as a dose escalation study. The term “therapeutically effective amount” includes, for example, a prophylactically effective amount. An “effective amount” of a therapeutic agent disclosed herein is an amount effective to achieve a desired pharmacologic effect or therapeutic improvement without undue adverse side

effects. It is understood that "an effect amount" or "a therapeutically effective amount" can vary from subject to subject, due to variation in metabolism of the compound of any of age, weight, general condition of the subject, the condition being treated, the severity of the condition being treated, and the judgment of the prescribing physician.

- 5 By way of example only, therapeutically effective amounts may be determined by routine experimentation, including but not limited to a dose escalation clinical trial. Where more than one therapeutic agent is used in combination, a "therapeutically effective amount" of each therapeutic agent can refer to an amount of the therapeutic agent that would be therapeutically effective when used on its own, or may refer to a
10 reduced amount that is therapeutically effective by virtue of its combination with one or more additional therapeutic agents.

- The term "preferentially inhibits," and the like, as used herein, in reference to an inhibitor indicates that inhibition of the activity of one member of a protein family by an inhibitor may be significantly higher (e.g., 25%, 50%, 300%, 1000% higher) than
15 for another member of the same protein family, but does not necessarily mean that the inhibitor exclusively inhibits one and only one member of a protein family.

The term "small molecule," as used herein, refers to a chemical compounds or molecule having a molecular weight below 2000 daltons.

- The terms "treating" or "treatment," as used herein, refer to both direct
20 treatment of a subject by a medical professional (e.g., by administering a therapeutic agent to the subject), or indirect treatment, effected, by at least one party, (e.g., a medical doctor, a nurse, a pharmacist, or a pharmaceutical sales representative) by providing instructions, in any form, that (i) instruct a subject to self-treat according to a claimed method (e.g., self-administer a drug) or (ii) instruct a third party to treat a
25 subject according to a claimed method. Also encompassed within the meaning of the term "treating" or "treatment" are prevention or reduction of the disease to be treated, e.g., by administering a therapeutic at a sufficiently early phase of disease to prevent or slow its progression.

30 Methods of Treating Infections

- The methods described herein include treating an infection by administering a therapeutically effective amount a BCL2 family inhibitor, e.g., a BH3 mimetic agent. Various types of infectious pathogens, upon infection of a host cell, upregulate the pro-survival activity of one or more proteins in the BCL2 family to prevent the host cell
35 apoptosis, which would otherwise curtail the infectious cycle. Without being bound by theory, it is believed that by inhibiting the activity of pro-survival BCL2 proteins, and

particularly BCL-XL, in infected cells a BCL2 family inhibitor restores apoptosis in such cells thereby blocking the ability of certain pathogens to replicate and infect other cells.

In some embodiments the infection to be treated is a bacterial infection. Examples of bacterial infections to be treated by the methods described herein include, but are not limited to, infectious caused by one or more of *Legionella* (e.g., *L. pneumophila* and *L. longbeachae*), *Mycobacterium*, *Salmonella*, and *Pseudomonas*. Optionally, in addition to treating a subject suffering from a bacterial infection with a BCL2 family inhibitor, the subject may also be administered a bactericidal or bacteriostatic agent. Suitable examples of bactericidal agents include, but are not limited to, antibiotics such as aminoglycosides, ansamycins, carbapenems, cephalosporins, glycopeptides, lipopeptides, monobactams, nitrofurans, penicillins, quinolones, capreomycine, cycloserine, ethionamide, isoniazid, pyrazinamide, rifamycin, rifabutin, rifapentine, and streptomycin. Examples of bacteriostatic agents include, but are not limited to, bacteriostatic antibiotics such as lincosamides, macrolides, sulphonamides, tetracyclines, spectinomycin, trimethoprim, clindamycin, ethambutol, novobiocin, tigecycline, and, oxazolidinones.

In some embodiments, the bacterial infection to be treated includes infection by antibiotic-resistant bacteria, e.g., antibiotic resistant forms of any of *Staphylococcus aureus*, *Enterococcus*, and *Pseudomonas aeruginosa*, and *Achinobacter baumannii*.

In other embodiments, the infection to be treated is a viral infection other than an infection by an influenza virus. Exemplary viral infections to be treated by the methods described herein include, but are not limited to, infections by HSV, HTLV-1, HTLV-2, HIV, a respiratory virus, rhinovirus, or a coronavirus, or ebola

In further embodiments, the infection to be treated is a fungal infection. Such infections include, but are not limited to, yeast infections (e.g., *Candida* infections), *Aspergillus*, *Pneumocystitis*, and *Cryptococcus*.

In some embodiments, the infection to be treated includes a protozoal infection. Examples of such protozoal infections to be treated include, but are not limited to, infections by *Leishmania*, *Trypanosoma cruzi*, *Toxoplasma gondii*, and *Encephalitozoon*.

The skilled artisan will appreciate that depending on the type of infection, and particularly the speed of the replication cycle of the underlying pathogen, treatment with a BCL-2 family inhibitor can be initiated at different times following diagnosis of an infection in a subject. Accordingly, in some embodiments the administration occurs within a period ranging from about 30 minutes to about 72 hours following a diagnosis

of the infection, *e.g.*, about 45 minutes, 1 hours, 2 hours, 3 hours 6 hours, 8 hours, 12 hours, 18 hours, 24 hours, 36 hours, 50 hours, 60 hours, 65 hours, or another time period from about 30 minutes to about 72 hours following diagnosis of the infection. In one embodiment the administration occurs within a period from about 6 hours to about 48 hours following a diagnosis of the infection. In some embodiments administration of the BCL-2 family inhibitor occurs no later than about 30 minutes to about 60 hours following a diagnosis of the infection, *e.g.*, within about 45 minutes, 1 hours, 2 hours, 3 hours 6 hours, 8 hours, 12 hours, 18 hours, 24 hours, 36 hours, 48 hours, 50 hours, or another time period no later than about 30 minutes to about 60 hours following diagnosis of the infection. In one embodiment the administration occurs no later than about 48 hours following a diagnosis of the infection.

It will be appreciated that, depending on the particular infectious pathogen and its route of infection, one or more cell types become infected by the infectious pathogen. In some embodiments the subject to be treated is suffering from an infection that includes infection of macrophages. In one embodiment the infected macrophages are alveolar macrophages.

In some embodiments the subject to be treated as per any of the methods described herein, is a subject that was not diagnosed as suffering from a cancer. In some embodiments the subject to be treated is suffering from an acute or subacute infection. In some embodiments the subject to be treated is suffering from tuberculosis, pneumonia, or an acute respiratory infection.

Subjects that can be treated by the methods described herein include, but are not limited to, humans, non-human primates, pigs, cows, sheep, ducks, and chicken. In some embodiments the subject to be treated is a human patient.

In some embodiments a BCL2 family inhibitor is selected from among a BH3 mimetic agent, an RNAi against a BCL2 family member, and an antibody against a BCL2 family member.

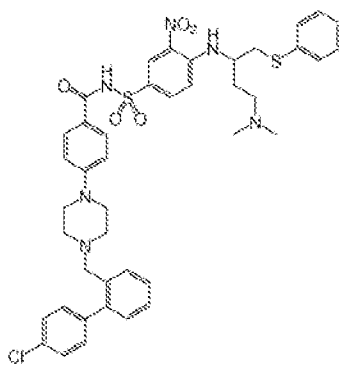
In some embodiments a BH3 mimetic agent suitable for treatment in a method described herein is a BH3 mimetic agent that inhibits the anti-apoptotic activity of BCL-XL, but can also inhibit the anti-apoptotic activity of other BCL-2 proteins, *e.g.*, BCL-2 and BCL-W. In other embodiments the BH3 mimetic agent to be used is a BCL-XL-selective inhibitor. In some embodiments, the BH3 mimetic agent inhibits BCL-2, BCL-W, and BCL-XL.

Suitable BH3 mimetic agents include, but are not limited to, small molecules, peptide mimetics (*e.g.*, terphenyl peptide mimetics), and peptides. In some

embodiments, a BH3 mimetic agent is a peptide, *e.g.*, a stapled BH3 peptide as described in, et al., Labelle et al. (2012); and Walensky et al. (2004).

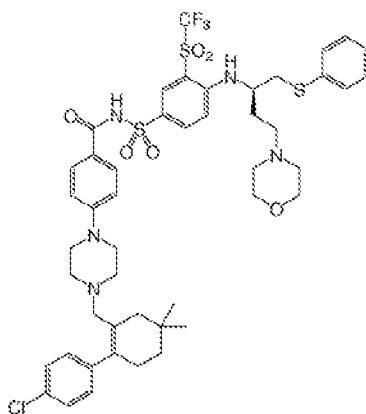
In other embodiments the BH3 mimetic agent to be used is a BH3 mimetic compound. Suitable BH3 mimetic agent compounds for the methods described herein include, but are not limited to, those disclosed in international patent publications WO2010080478, WO2005049594, WO2007040650, WO2010080503, WO2013055895, WO2013055897, and WO2014028381.

In particular embodiments a suitable BH3 mimetic compound is N-(4-(4-((4'-chloro-(1,1'-biphenyl)-2-yl)methyl)piperazin-1-yl)-benzoyl)-4-(((1R)-3-(dimethylamino)-1-((phenylsulfanyl)methyl)propyl)amino)-3-nitrobenzene-sulfonamide, otherwise known as ABT-737, the structure of which is shown below as Formula (I), or an analogue or derivative thereof:



Formula (I)-ABT-737

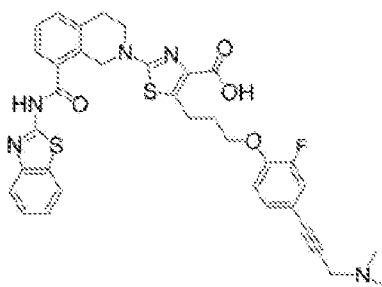
In other embodiments the BH3 mimetic compound to be used is N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(morpholin-4-yl)-1-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfonyl) benzenesulfonamide, otherwise known as ABT-263, having the structure of Formula (II), or an analogue or derivative thereof:



Formula (II)-ABT-263

In another embodiment the BH3 mimetic compound to be used is a BCL-XL-selective inhibitor compound A-1155463, having the structure of Formula (III), or an analogue or derivative thereof:

5

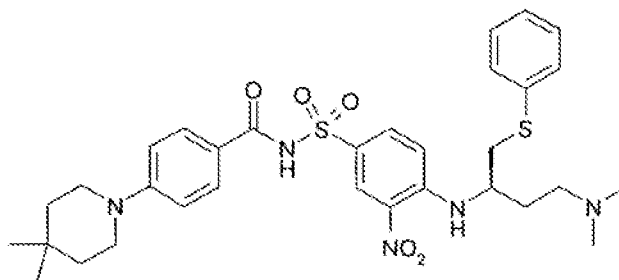


10

Formula (III)-A-1155463

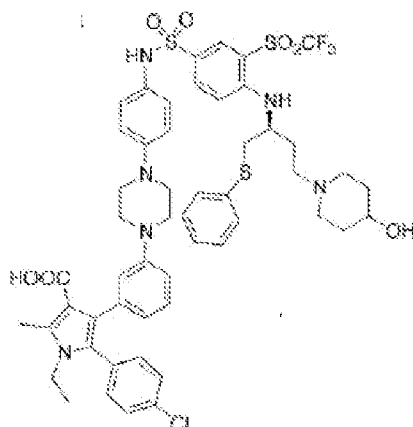
In other embodiments the BH3 mimetic compound to be used is A-385358, having the structure of Formula (IV), or an analogue or derivative thereof:

15

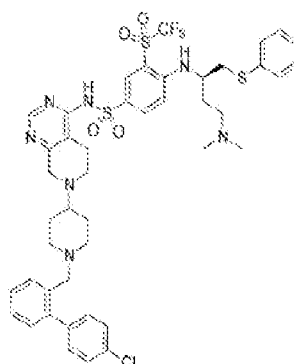
**Formula (IV)-A-385358**

In other embodiments the BH3 mimetic compound to be used is BM-957, having the structure of Formula (V), or an analogue or derivative thereof:

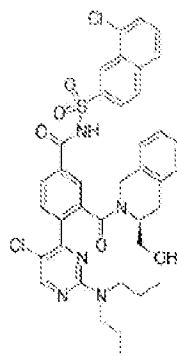
14

**Formula (V)-BM-957**

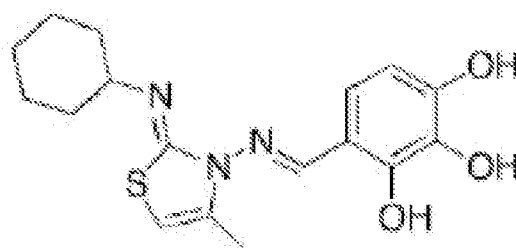
In other embodiments the BH3 mimetic compound to be used has the structure of Formula (VI), or an analogue or derivative thereof:

**Formula (VI)**

In other embodiments the BH3 mimetic compound to be used has the structure of Formula (VII), or an analogue or derivative thereof:

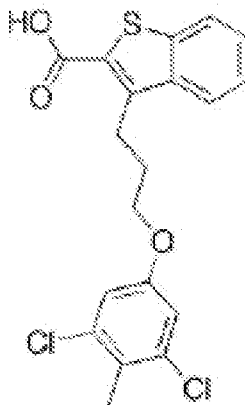
**Formula (VII)**

In other embodiments the BH₃ mimetic compound to be used is MIM-1, having the structure of Formula (VIII), or an analogue or derivative thereof:



Formula (VIII)-MIM-1

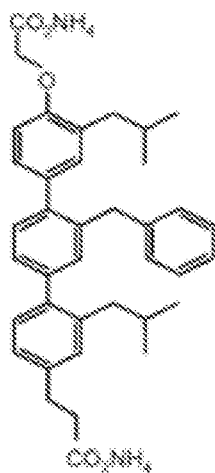
In other embodiments the BH₃ mimetic compound to be used has the structure of Formula (IX), or an analogue or derivative thereof:



Formula (IX)

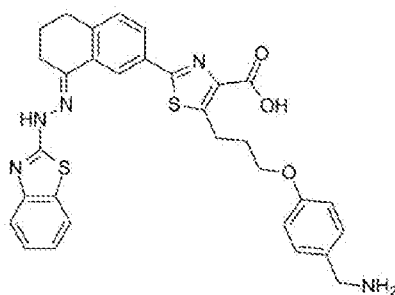
In other embodiments the BH₃ mimetic compound to be used is BH₃-M6, having the structure of Formula (X), or an analogue or derivative thereof:

16

**Formula (X)-BH3-M6**

In some embodiments the BH3 compound to be used is WEHI-539, having the structure of Formula (XI), or an analogue or derivative thereof:

5

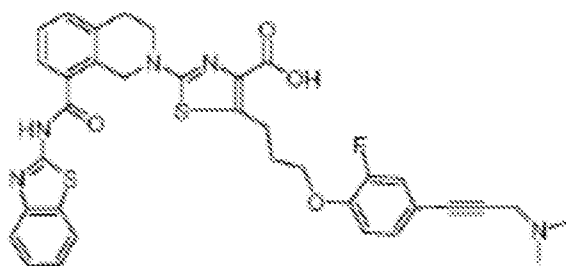


10

Formula (XI)-WEHI-539

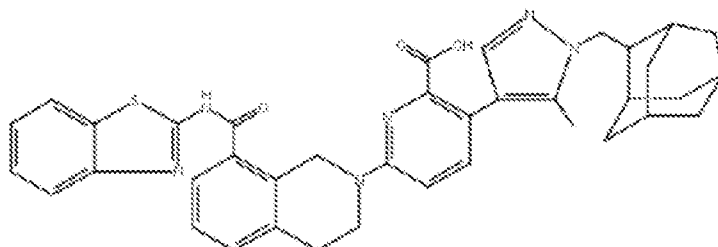
In further embodiments the BH3 compound to be used is a compound having the structure of Formula (XII), or an analogue or derivative thereof:

15

**Formula (XII)**

In further embodiments the BH3 compound to be used is the compound disclosed in example 3 of international patent publication WO2013055897 and having the structure of Formula (XIII), or an analogue or derivative thereof:

20

**Formula (XIII)**

RNA Interference (RNAi)

5 In some embodiments the BCL2 family inhibitor includes one or more RNAis against a BCL2 family inhibitor. In particular embodiments the RNAi is directed against BCL-XL.

 The terms "RNA interference", "RNAi" or "gene silencing" refer generally to a process in which a double-stranded RNA molecule reduces the expression of a nucleic acid sequence with which the double-stranded RNA molecule shares substantial or total
10 homology. However, it has more recently been shown that RNA interference can be achieved using non-RNA double stranded molecules (see, for example, US 20070004667).

 In some embodiments described herein a BCL2 family inhibitor comprises
15 nucleic acid molecules comprising and/or encoding double-stranded regions for RNA interference. The nucleic acid molecules are typically RNA but may comprise chemically-modified nucleotides and non-nucleotides.

 The double-stranded regions should be at least 19 contiguous nucleotides, for example about 19 to 23 nucleotides, or may be longer, for example 30 or 50
20 nucleotides, or 100 nucleotides or more. The full-length sequence corresponding to the entire gene transcript may be used. Preferably, they are about 19 to about 23 nucleotides in length.

 The degree of identity of a double-stranded region of a nucleic acid molecule to the targeted transcript should be at least 90% and more preferably 95-100%. The
25 nucleic acid molecule may of course comprise unrelated sequences which may function to stabilize the molecule.

 The term "short interfering RNA" or "siRNA" as used herein refers to a nucleic acid molecule which comprises ribonucleotides capable of inhibiting or down regulating gene expression, for example by mediating RNAi in a sequence-specific
30 manner, wherein the double stranded portion is less than 50 nucleotides in length, preferably about 19 to about 23 nucleotides in length. For example the siRNA can be a

nucleic acid molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siRNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary.

As used herein, the term siRNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid (siNA), short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, siRNA molecules as described herein can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siRNA molecules as described herein can result from siRNA mediated modification of chromatin structure to alter gene expression.

By "shRNA" or "short-hairpin RNA" is meant an RNA molecule where less than about 50 nucleotides, preferably about 19 to about 23 nucleotides, is base paired with a complementary sequence located on the same RNA molecule, and where said sequence and complementary sequence are separated by an unpaired region of at least about 4 to about 15 nucleotides which forms a single-stranded loop above the stem structure created by the two regions of base complementarity.

Included shRNAs are dual or bi-finger and multi-finger hairpin dsRNAs, in which the RNA molecule comprises two or more of such stem-loop structures separated by single-stranded spacer regions.

Once designed, the nucleic acid molecules comprising a double-stranded region can be generated by any method known in the art, for example, by *in vitro* transcription, recombinantly, or by synthetic means.

Modifications or analogs of nucleotides can be introduced to improve the properties of the nucleic acid molecules. Improved properties include increased nuclease resistance and/or increased ability to permeate cell membranes. Accordingly, the terms "nucleic acid molecule" and "double-stranded RNA molecule" includes

synthetically modified bases such as, but not limited to, inosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl-, 2-propyl- and other alkyl- adenines, 5-halo uracil, 5-halo cytosine, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiuracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl

5 adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thioalkyl guanines, 8-hydroxyl guanine and other substituted guanines, other aza and deaza adenines, other aza and deaza guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

RNA is used to target various BCL2 family members are known in the art, as exemplified without limitation, for BCL-XL, in Tsai et al. (2014); for BCL2 and BCL-W in Crawford et al. (2010); for MCL-1 in Keuling et al. (2009); and for A1/BFL1 in Ottina et al. (2012).

Antibodies

15 In some embodiments a BCL2 family inhibitor is an antibody against one or more BCL2 family members. In some embodiments the antibody inhibits BCL-XL as well at least one other BCL2 family member. In other embodiments the antibody preferentially inhibits BCL-XL. Preferably, the antibody is an antibody modified to penetrate or be taken up (passively or actively) in mammalian cells.

20 The term "antibody" as used herein includes polyclonal antibodies, monoclonal antibodies, bispecific antibodies, fusion diabodies, triabodies, heteroconjugate antibodies, chimeric antibodies including intact molecules as well as fragments thereof, and other antibody-like molecules. Antibodies include modifications in a variety of forms including, for example, but not limited to, domain antibodies including either the
25 VH or VL domain, a dimer of the heavy chain variable region (VHH, as described for a camelid), a dimer of the light chain variable region (VLL), Fv fragments containing only the light (VL) and heavy chain (VH) variable regions which may be joined directly or through a linker, or Fd fragments containing the heavy chain variable region and the CH1 domain.

30 A scFv consisting of the variable regions of the heavy and light chains linked together to form a single-chain antibody (Bird et al., 1988; Huston et al., 1988) and oligomers of scFvs such as diabodies and triabodies are also encompassed by the term "antibody". Also encompassed are fragments of antibodies such as Fab, (Fab')₂ and FabFc₂ fragments which contain the variable regions and parts of the constant regions.
35 Complementarity determining region (CDR)-grafted antibody fragments and oligomers of antibody fragments are also encompassed. The heavy and light chain components of

an Fv may be derived from the same antibody or different antibodies thereby producing a chimeric Fv region. The antibody may be of animal (for example mouse, rabbit or rat) or human origin or may be chimeric (Morrison et al., 1984) or humanized (Jones et al., 1986).

5 As used herein the term "antibody" includes these various forms. Using the guidelines provided herein and those methods well known to those skilled in the art which are described in the references cited above and in such publications as Harlow & Lane, *Antibodies: a Laboratory Manual*, Cold Spring Harbor Laboratory, (1988) the antibodies for use in the methods of the present invention can be readily made.

10 The antibodies may be Fv regions comprising a variable light (VL) and a variable heavy (VH) chain in which the light and heavy chains may be joined directly or through a linker. As used herein a linker refers to a molecule that is covalently linked to the light and heavy chain and provides enough spacing and flexibility between the two chains such that they are able to achieve a conformation in which they are
15 capable of specifically binding the epitope to which they are directed. Protein linkers are particularly preferred as they may be expressed as an intrinsic component of the Ig portion of the fusion polypeptide.

 In another embodiment, recombinantly produced single chain scFv antibody, preferably a humanized scFv, is used in the methods of the invention.

20 In one embodiment, the antibodies have the capacity for intracellular transmission. Antibodies which have the capacity for intracellular transmission include antibodies such as camelids and llama antibodies, shark antibodies (IgNARs), scFv antibodies, intrabodies or nanobodies, for example, scFv intrabodies and VHH intrabodies. Such antigen binding agents can be made as described by Harmsen and De
25 Haard (2007), Tibary et al. (2007), and Muyldermans (2001), and references cited therein. Yeast SPLINT antibody libraries are available for testing for intrabodies which are able to disrupt protein-protein interactions (see for example, Visintin et al. (2008) for methods for their production). Such agents may comprise a cell-penetrating peptide sequence or nuclear-localizing peptide sequence such as those disclosed in Constantini
30 et al. (2008). Also useful for in vivo delivery are Vectocell or Diato peptide vectors such as those disclosed in De Coupade et al. (2005) and Meyer-Losic et al. (2006).

 In addition, the antibodies may be fused to a cell penetrating agent, for example a cell-penetrating peptide. Cell penetrating peptides include Tat peptides, Penetratin, short amphipathic peptides such as those from the Pep-and MPG-families,
35 oligoarginine and oligolysine. In one example, the cell penetrating peptide is also conjugated to a lipid (C6-C18 fatty acid) domain to improve intracellular delivery

(Koppelhus et al., 2008). Examples of cell penetrating peptides can be found in Howl et al., (2007) and Deshayes et al. (2008). Thus, the invention also provides the therapeutic use of antibodies fused via a covalent bond (*e.g.* a peptide bond), at optionally the N-terminus or the C-terminus, to a cell-penetrating peptide sequence.

5 Antibodies which inhibit one or more of BCL-XL, BCL-2, BCL-W, MCL-1, or A1/BFL-1 activity are available from various sources such as Santa Cruz Biotechnology, and as exemplified for BCL-2 in Cohen-Saidon et al. (2003). In certain embodiments the antibody to be used is an antibody that inhibits the activity of BCL-XL. In some embodiments the antibody to be used is antibody that preferentially
10 inhibits BCL-XL.

Symptoms, diagnostic tests, and prognostic tests for various types of infections are known in the art. See, *e.g.*, Warrell et al. (2012).

The BCL2 family inhibitors and related compositions described herein can be used in the preparation of medicaments for the treatment of an infection according to
15 any of the methods described herein. Typically, a method for treating a subject suffering from an infection, includes administration of a pharmaceutical composition containing at least one BCL2 family inhibitor, or a pharmaceutically acceptable salt, pharmaceutically acceptable N-oxide, pharmaceutically active metabolite, pharmaceutically acceptable prodrug, or pharmaceutically acceptable solvate thereof, in
20 therapeutically effective amounts to said subject.

Treatment can be for prophylactic and/or therapeutic treatments. In therapeutic applications, a BCL2 family inhibitor, *e.g.*, a BH3 mimetic compound, is administered to cure or at least partially arrest the symptoms of a patient already suffering from and/or diagnosed as having an infection. Amounts effective for this use will depend on
25 the severity and course of the infection, previous therapy, the patient's health status, weight, response to the treatment, and the infectious agent's resistance to treatment. It is considered well within the skill of the art for one to determine such therapeutically effective amounts by routine experimentation (including, but not limited to, a dose escalation clinical trial).

30 In prophylactic applications, compositions containing a BCL2 family inhibitor are administered to a patient susceptible to or otherwise at risk of infection, for example, during or after recent travel to an epidemic zone. Such an amount is defined to be a "prophylactically effective amount or dose," *i.e.*, a dose sufficient to prevent or reduce the onset of infection. In this use, the precise amounts also depend on the
35 patient's state of health, weight, timing, etc. It is considered well within the skill of the

art for one to determine such prophylactically effective amounts by routine experimentation (*e.g.*, a dose escalation clinical trial).

In a case where a subject's status does improve, upon reliable medical advice, the administration of a BCL2 family inhibitor may be given continuously; alternatively, the dose of drug being administered may be temporarily reduced or temporarily suspended for a certain length of time (*i.e.*, a "drug holiday"). The length of the drug holiday can vary between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, or 60 days. The dose reduction during a drug holiday may be from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

The amount of a given BCL2 family inhibitor that will be suitable as a therapeutically effective dose will vary depending upon factors such as the particular BCL-2 family inhibitor, infection and its severity, the characteristics (*e.g.*, weight) of the subject or host in need of treatment, and the properties of the infectious pathogen (*e.g.*, its doubling time and drug resistance), but can nevertheless be routinely determined in a manner known in the art according to the particular circumstances surrounding the case, including, *e.g.*, the specific agent being administered, the route of administration, the condition being treated, and the subject or host being treated. In general, however, doses employed for adult human treatment will typically be in the range of 0.02-5000 mg per day, or from about 1-1500 mg per day. The desired dose may conveniently be presented in a single dose or as divided doses administered simultaneously (or over a short period of time) or at appropriate intervals, for example as two, three, four or more sub-doses per day.

The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are not uncommon. Such dosages may be altered depending on a number of variables, not limited to the activity of the BCL2 family inhibitor used, the type of infection to be treated, the mode of administration, the requirements of the individual subject, the severity of the infection or condition being treated, and the judgment of the practitioner.

Toxicity and therapeutic efficacy of such therapeutic regimens can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, the determination of the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic

index and it can be expressed as the ratio between LD₅₀ and ED₅₀. BCL2 family inhibitors, and particularly BH3 mimetic compounds, exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in human and non-human subjects.

- 5 The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with minimal toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

10 Combination Treatments

- BCL2 family inhibitors can also be used in combination with other agents used to treat infectious diseases (anti-infective agents), that are selected for their therapeutic value for the infection to be treated. In general, the compositions described herein and, in embodiments where combinational therapy is employed, other agents do not
- 15 necessarily have to be administered in the same pharmaceutical composition, and may, because of different physical and chemical characteristics, preferably be administered by different routes. The determination of the mode of administration and the advisability of administration, where possible, in the same pharmaceutical composition, is well within the knowledge of the skilled clinician. The initial administration can be
- 20 made according to established protocols known in the art, and then, based upon the observed effects, the dosage, modes of administration and times of administration can be modified by the skilled clinician.

- A BCL2 family inhibitor and an additional therapeutic agent may be administered concurrently (*e.g.*, simultaneously, essentially simultaneously or within
- 25 the same treatment protocol) or sequentially, depending upon the nature and phase of the infection, the condition of the patient, and the actual choice of therapeutic agents used. The determination of the order of administration, and the number of repetitions of administration of each therapeutic agent during a treatment protocol, is well within the knowledge of the skilled physician after evaluation of the disease being treated and
- 30 the condition of the patient.

- It is known to those of skill in the art that therapeutically-effective dosages can vary when the drugs are used in treatment combinations. Methods for experimentally determining therapeutically-effective dosages of drugs and other agents for use in combination treatment regimens are described in the literature. For example, the use of
- 35 metronomic dosing, *i.e.*, providing more frequent, lower doses in order to minimize toxic side effects, has been described extensively in the literature. Combination

treatment further includes periodic treatments that start and stop at various times to assist with the clinical management of the patient.

For combination therapies, dosages of co-administered therapeutic agents will of course vary depending on the type of co-agents employed, on the specific BCL2 family inhibitor, on the infection being treated and so forth

It is understood that the dosage regimen to treat, prevent, or ameliorate the condition(s) for which relief is sought, can be modified in accordance with a variety of factors. These factors include the disorder from which the subject suffers, as well as the age, weight, sex, diet, and medical condition of the subject. Thus, the dosage regimen actually employed can vary widely and therefore can deviate from the dosage regimens set forth herein.

The BCL2 family inhibitor and additional therapeutic agent which make up a combination therapy disclosed herein may be a combined dosage form or in separate dosage forms intended for substantially simultaneous administration. The pharmaceutical agents that make up the combination therapy may also be administered sequentially, with either therapeutic compound being administered by a regimen calling for two-step administration. The two-step administration regimen may call for sequential administration of the active agents or spaced-apart administration of the separate active agents. The time period between the multiple administration steps may range from, a few minutes to several hours, depending upon the properties of each pharmaceutical agent, such as potency, solubility, bioavailability, plasma half-life and kinetic profile of the pharmaceutical agent. Circadian variation of various physiological parameters may also be evaluated to determine the optimal dose interval.

In addition, administration or co-administration of a BCL2 family inhibitor for treatment of an infection may be used in combination with procedures that may provide additional or synergistic benefit to the patient. By way of example only, patients may undergo genetic testing to identify genetic variation in their own genome or a pathogen's genome so as to optimize treatment parameters, *e.g.*, the type of BCL2 family inhibitor to be administered, dosing regimen, and co-administration with anti-infective agents.

Initial administration can be via any route practical, such as, for example, an intravenous injection, a bolus injection, infusion over 5 minutes to about 5 hours, a pill, a capsule, inhaler, injection, transdermal patch, buccal delivery, and the like, or combination thereof. A compound should be administered as soon as is practicable after the onset of a disease or condition is detected or suspected, and for a length of time necessary for the treatment of the infection.

Exemplary Therapeutic Agents for Use in Combination with a BCL2 family inhibitor

Where the subject is suffering from or at risk of suffering from a bacterial infection, a BCL2 family inhibitor can be used in combination with one or more bactericidal or bacteriostatic agents, including, but not limited to: gentamicin, tobramycin, streptomycin, doripenem, cefazolin, cefaclor, cefepime, ceftobiprole, vancomycin, oritavancin, clindamycin, daptomycin, azithromycin, telithromycin, furazolidone, linezolid, amoxicillin, amoxicillin/clavulanate, ciprofloxacin, gemifloxacin, sulfacetamide, minocycline, capreomycin, cycloserine, ethambutol, ethionamide, isoniazid, pyrazinamide, rifampicin, and rifabutin.

Where the subject is suffering from or at risk of suffering from a viral infection (other than infection by an *influenza* virus), a BCL2 family inhibitor can be used in combination with one or more anti-viral drugs, including, but not limited to: cidofovir, ribavirin, acyclovir, ganciclovir, vidarabine, abacavir, nevirapine, ritonavir, gemcitabine, and decitabine.

Where the subject is suffering from or at risk of suffering from a fungal infection, a BCL2 family inhibitor can be used in combination with one or more anti-fungal drugs including, but not limited to: candididin, fluconazole, tioconazole, voriconazole, abafungin, terbinafine, micafungin, and undecylenic acid.

Where the subject is suffering from or at risk of suffering from a protozoal infection, a BCL-2 family inhibitor can be used in combination with one or more anti protozoal drugs including, but not limited to: ambisome, amphotericin, lumenfantrine, chloroquine, daraprim, diloxanide furoate, doxycycline monohydrate, lariam, malarone, pentamidine isetionate, pentostam, proquanil hydrochloride, tinidazole, and wellvone.

Compositions*Pharmaceutical Agents/Formulations*

Also described herein are pharmaceutical compositions comprising therapeutically effective amounts of (i) a BCL2 family inhibitor, including, but not limited to, any BCL2 family inhibitor described herein; and (ii) an anti-infective agent.

In certain embodiments the BCL2 family inhibitor is a BH3 mimetic agent, an RNAi against a BCL2 family member, or an antibody against a BCL2 family member. In certain embodiments the pharmaceutical composition comprises a BH3 mimetic agent, including, but not limited to any BH3 mimetic agent described herein. In some embodiments the BH3 mimetic agent in the pharmaceutical composition preferentially inhibits BCL-XL relative to other BCL2 family members.

In some embodiments a pharmaceutical composition comprises a BCL2 family inhibitor and a bactericidal or bacteriostatic agent, including, but not limited to any bactericidal or bacteriostatic agent recited herein. In other embodiments the pharmaceutical composition comprises a BCL2 family inhibitor and an anti-viral agent, including, but not limited to, any antiviral agent recited herein. In further embodiments the pharmaceutical composition comprises a BCL2 family inhibitor and an antifungal agent including, but not limited to, any antifungal agent recited herein. In yet other embodiments the pharmaceutical composition comprises a BCL2 family inhibitor and an antiprotozoal agent including, but not limited to, any antiprotozoal agent recited herein.

Dosage Forms

The compositions described herein can be formulated for administration to a subject via any conventional means including, but not limited to, oral, parenteral (*e.g.*, intravenous, subcutaneous, or intramuscular), buccal, inhalation, intranasal, rectal or transdermal administration routes.

The pharmaceutical compositions described herein, which include a BCL2 family inhibitor (*e.g.*, a BH3 mimetic compound) and an anti-infective agent, can be formulated into any suitable dosage form, including but not limited to, aqueous oral dispersions, liquids, mists, gels, syrups, elixirs, slurries, suspensions and the like, for oral ingestion by a patient to be treated, solid oral dosage forms, aerosols, controlled release formulations, fast melt formulations, effervescent formulations, lyophilized formulations, tablets, powders, pills, dragees, capsules, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations, and mixed immediate release and controlled release formulations.

Pharmaceutical preparations for oral use can be obtained by mixing one or more solid excipient with one or more of the compounds described herein, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, for example, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methylcellulose, microcrystalline cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose; or others such as: polyvinylpyrrolidone (PVP or povidone) or calcium phosphate. If desired, disintegrating agents may be added, such as the cross-linked croscarmellose sodium, polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or
5 lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

10 In some embodiments, the solid dosage forms disclosed herein may be in the form of a tablet, (including a suspension tablet, a fast-melt tablet, a bite-disintegration tablet, a rapid-disintegration tablet, an effervescent tablet, or a caplet), a pill, a powder (including a sterile packaged powder, a dispensable powder, or an effervescent powder) a capsule (including both soft or hard capsules, *e.g.*, capsules made from animal-
15 derived gelatin or plant-derived HPMC, or "sprinkle capsules"), solid dispersion, solid solution, bioerodible dosage form, controlled release formulations, pulsatile release dosage forms, multiparticulate dosage forms, pellets, granules, or an aerosol. In other embodiments, the pharmaceutical formulation is in the form of a powder. In still other embodiments, the pharmaceutical formulation is in the form of a tablet, including but
20 not limited to, a fast-melt tablet. Additionally, pharmaceutical formulations described herein may be administered as a single capsule or in multiple capsule dosage form. In some embodiments, the pharmaceutical formulation is administered in two, or three, or four, capsules or tablets.

In some embodiments, solid dosage forms, *e.g.*, tablets, effervescent tablets,
25 and capsules, are prepared by mixing particles of a BCL2 family inhibitor and an anti-infective compound with one or more pharmaceutical excipients to form a bulk blend composition. When referring to these bulk blend compositions as homogeneous, it is meant that the particles of the active agents, are dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective
30 unit dosage forms, such as tablets, pills, and capsules. The individual unit dosages may also include film coatings, which disintegrate upon oral ingestion or upon contact with diluent. These formulations can be manufactured by conventional pharmacological techniques.

The pharmaceutical solid dosage forms described herein can include a BCL2
35 family inhibitor and an anti-infective agent (*e.g.*, a bacteriostatic antibiotic) as described herein and one or more pharmaceutically acceptable additives such as a

compatible carrier, binder, filling agent, suspending agent, flavoring agent, sweetening agent, disintegrating agent, dispersing agent, surfactant, lubricant, colorant, diluent, solubilizer, moistening agent, plasticizer, stabilizer, penetration enhancer, wetting agent, anti-foaming agent, antioxidant, preservative, or one or more combination thereof. In some embodiments, using standard coating procedures, such as those described in Remington's Pharmaceutical Sciences, 20th Edition (2000), a film coating is provided around the formulation of the BCL2 family inhibitor and the anti-infective agent. In one embodiment, some or all of the particles of these active agents are coated. In another embodiment some or all of the particles of the active agents are microencapsulated. In still another embodiment, the particles of the active agents are not microencapsulated and are uncoated.

Suitable carriers for use in the solid dosage forms described herein include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, sodium caseinate, soy lecithin, sodium chloride, tricalcium phosphate, dipotassium phosphate, sodium stearyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose, microcrystalline cellulose, lactose, mannitol and the like.

Suitable filling agents for use in the solid dosage forms described herein include, but are not limited to, lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrans, dextran, starches, pregelatinized starch, hydroxypropylmethylcellulose (HPMC), hydroxypropylmethylcellulose phthalate, hydroxypropylmethylcellulose acetate stearate (HPMCAS), sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

In order to release the BCL2 family inhibitor and anti-infective agent, from a solid dosage form matrix as efficiently as possible, disintegrants are often used in the formulation, especially when the dosage forms are compressed with binder. Disintegrants help rupturing the dosage form matrix by swelling or capillary action when moisture is absorbed into the dosage form. Suitable disintegrants for use in the solid dosage forms described herein include, but are not limited to, natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel[®], or sodium starch glycolate such as Promogel[®] or Explotab[®], a cellulose such as a wood product, methylcrystalline cellulose, *e.g.*, Avicel[®] PH101, Avicel[®] PH102, Avicel[®] PH105, Elcema[®] P100, Emcocel[®], Vivacel[®], Ming Tia[®], and Solka-Floc[®], methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked

sodium carboxymethylcellulose (Ac-Di-Sol[®]), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay
 5 such as Veegum[®] HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

Binders impart cohesiveness to solid oral dosage form formulations: for
 10 powder filled capsule formulation, they aid in plug formation that can be filled into soft or hard shell capsules and for tablet formulation, they ensure the tablet remaining intact after compression and help assure blend uniformity prior to a compression or fill step. Materials suitable for use as binders in the solid dosage forms described herein include, but are not limited to, carboxymethylcellulose, methylcellulose (*e.g.*, Methocel[®]),
 15 hydroxypropylmethylcellulose (*e.g.*, Hypromellose USP Pharmaccoat-603, hydroxypropylmethylcellulose acetate stearate (Aquate HS-LF and HS), hydroxyethylcellulose, hydroxypropylcellulose (*e.g.*, Klucel[®]), ethylcellulose (*e.g.*, Ethocel[®]), and microcrystalline cellulose (*e.g.*, Avicel[®]), microcrystalline dextrose, amylose, magnesium aluminum silicate, polysaccharide acids, bentonites, gelatin,
 20 polyvinylpyrrolidone/vinyl acetate copolymer, crospovidone, povidone, starch, pregelatinized starch, tragacanth, dextrin, a sugar, such as sucrose (*e.g.*, Dipac[®]), glucose, dextrose, molasses, mannitol, sorbitol, xylitol (*e.g.*, Xylitab[®]), lactose, a natural or synthetic gum such as acacia, tragacanth, ghatti gum, mucilage of isapol husks, starch, polyvinylpyrrolidone (*e.g.*, Povidone[®] CL, Kollidon[®] CL,
 25 Polyplasdone[®] XL-10, and Povidone[®] K-12), larch arabogalactan, Veegum[®], polyethylene glycol, waxes, sodium alginate, and the like.

In general, binder levels of 20-70% are used in powder-filled gelatin capsule formulations. Binder usage level in tablet formulations varies whether direct
 30 compression, wet granulation, roller compaction, or usage of other excipients such as fillers which itself can act as moderate binder. Formulators skilled in art can determine the binder level for the formulations, but binder usage level of up to 70% in tablet formulations is common.

Suitable lubricants or glidants for use in the solid dosage forms described herein include, but are not limited to, stearic acid, calcium hydroxide, talc, corn starch,
 35 sodium stearyl fumarate, alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, magnesium stearate, zinc

stearate, waxes, Stearowet[®], boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol or a methoxypolyethylene glycol such as Carbowax[™], PEG 4000, PEG 5000, PEG 6000, propylene glycol, sodium oleate, glyceryl behenate, glyceryl palmitostearate, glyceryl benzoate, magnesium or sodium lauryl sulfate, and the like.

Suitable diluents for use in the solid dosage forms described herein include, but are not limited to, sugars (including lactose, sucrose, and dextrose), polysaccharides (including dextrans and maltodextrin), polyols (including mannitol, xylitol, and sorbitol), cyclodextrins and the like.

Suitable wetting agents for use in the solid dosage forms described herein include, for example, oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, quaternary ammonium compounds (*e.g.*, Polyquat 10[®]), sodium oleate, sodium lauryl sulfate, magnesium stearate, sodium docusate, triacetin, vitamin E TPGS and the like.

Suitable surfactants for use in the solid dosage forms described herein include, for example, sodium lauryl sulfate, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, *e.g.*, Pluronic[®] (BASF), and the like.

Suitable suspending agents for use in the solid dosage forms described here include, but are not limited to, polyvinylpyrrolidone, *e.g.*, polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, polyethylene glycol, *e.g.*, the polyethylene glycol can have a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, vinyl pyrrolidone/vinyl acetate copolymer (S630), sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethylcellulose, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, *e.g.*, gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulose, such as, *e.g.*, sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

It should be appreciated that there is considerable overlap between additives used in the solid dosage forms described herein. Thus, the above-listed additives should be taken as merely exemplary, and not limiting, of the types of additives that can be included in solid dosage forms described herein. The amounts of such additives

can be readily determined by one skilled in the art, according to the particular properties desired.

In other embodiments, one or more layers of the pharmaceutical formulation are plasticized. Illustratively, a plasticizer is generally a high boiling point solid or liquid. Suitable plasticizers can be added from about 0.01% to about 50% by weight (w/w) of the coating composition. Plasticizers include, but are not limited to, diethyl phthalate, citrate esters, polyethylene glycol, glycerol, acetylated glycerides, triacetin, polypropylene glycol, polyethylene glycol, triethyl citrate, dibutyl sebacate, stearic acid, stearyl, stearate, and castor oil.

Compressed tablets are solid dosage forms prepared by compacting the bulk blend of the formulations described above. In various embodiments, compressed tablets which are designed to dissolve in the mouth will include one or more flavoring agents. In other embodiments, the compressed tablets will include a film surrounding the final compressed tablet. In some embodiments, the film coating can provide a delayed release of the BCL2 family inhibitor and/or the anti-infective agent. In other embodiments, the film coating aids in patient compliance (*e.g.*, Opadry[®] coatings or sugar coating). Film coatings including Opadry[®] typically range from about 1% to about 3% of the tablet weight. In other embodiments, the compressed tablets include one or more excipients.

A capsule may be prepared, for example, by placing the bulk blend of the formulation of a BCL2 family inhibitor and anti-infective agent inside of a capsule. In some embodiments, the formulations (non-aqueous suspensions and solutions) are placed in a soft gelatin capsule. In other embodiments, the formulations are placed in standard gelatin capsules or non-gelatin capsules such as capsules comprising HPMC.

In other embodiments, the formulation is placed in a sprinkle capsule, wherein the capsule may be swallowed whole or the capsule may be opened and the contents sprinkled on food prior to eating. In some embodiments, the therapeutic dose is split into multiple (*e.g.*, two, three, or four) capsules. In some embodiments, the entire dose of the formulation is delivered in a capsule form.

In various embodiments, the particles of the BCL2 family inhibitor and anti-infective agent and one or more excipients are dry blended and compressed into a mass, such as a tablet, having a hardness sufficient to provide a pharmaceutical composition that substantially disintegrates within less than about 30 minutes, less than about 35 minutes, less than about 40 minutes, less than about 45 minutes, less than about 50 minutes, less than about 55 minutes, or less than about 60 minutes, after oral administration, thereby releasing the formulation into the gastrointestinal fluid.

In another aspect, dosage forms may include microencapsulated formulations. In some embodiments, one or more other compatible materials are present in the microencapsulation material. Exemplary materials include, but are not limited to, pH modifiers, erosion facilitators, anti-foaming agents, antioxidants, flavoring agents, and carrier materials such as binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, and diluents.

Microencapsulated formulations of a BCL2 family inhibitor and anti-infective agent, may be formulated by methods known by one of ordinary skill in the art. Such known methods include, *e.g.*, spray drying processes, spinning disk-solvent processes, hot melt processes, spray chilling methods, fluidized bed, electrostatic deposition, centrifugal extrusion, rotational suspension separation, polymerization at liquid-gas or solid-gas interface, pressure extrusion, or spraying solvent extraction bath. In addition to these, several chemical techniques, *e.g.*, complex coacervation, solvent evaporation, polymer-polymer incompatibility, interfacial polymerization in liquid media, in situ polymerization, in-liquid drying, and desolvation in liquid media could also be used. Furthermore, other methods such as roller compaction, extrusion/spheronization, coacervation, or nanoparticle coating may also be used.

In some embodiments, the solid dosage formulations of the BCL2 family inhibitor and anti-infective agent, are plasticized (coated) with one or more layers. Illustratively, a plasticizer is generally a high boiling point solid or liquid. Suitable plasticizers can be added from about 0.01% to about 50% by weight (w/w) of the coating composition. Plasticizers include, but are not limited to, diethyl phthalate, citrate esters, polyethylene glycol, glycerol, acetylated glycerides, triacetin, polypropylene glycol, polyethylene glycol, triethyl citrate, dibutyl sebacate, stearic acid, stearyl, stearate, and castor oil.

In other embodiments, a powder including the formulations of the BCL2 family inhibitor and anti-infective agent, may be formulated to include one or more pharmaceutical excipients and flavors. Such a powder may be prepared, for example, by mixing the formulation and optional pharmaceutical excipients to form a bulk blend composition. Additional embodiments also include a suspending agent and/or a wetting agent. This bulk blend is uniformly subdivided into unit dosage packaging or multi-dosage packaging units.

In still other embodiments, effervescent powders are also prepared in accordance with the present disclosure. Effervescent salts have been used to disperse medicines in water for oral administration. Effervescent salts are granules or coarse powders containing a medicinal agent in a dry mixture, usually composed of sodium

bicarbonate, citric acid and/or tartaric acid. When salts of the compositions described herein are added to water, the acids and the base react to liberate carbon dioxide gas, thereby causing "effervescence." Examples of effervescent salts include, *e.g.*, the following ingredients: sodium bicarbonate or a mixture of sodium bicarbonate and sodium carbonate, citric acid and/or tartaric acid. Any acid-base combination that results in the liberation of carbon dioxide can be used in place of the combination of sodium bicarbonate and citric and tartaric acids, as long as the ingredients were suitable for pharmaceutical use and result in a pH of about 6.0 or higher.

In other embodiments, the formulations described herein, which include a BCL2 family inhibitor and anti-infective agent, are solid dispersions. Methods of producing such solid dispersions are known in the art and include, but are not limited to, for example, US 4,343,789, 5,340,591, 5,456,923, 5,700,485, 5,723,269, and US 2004/0013734. In still other embodiments, the formulations described herein are solid solutions. Solid solutions incorporate a substance together with the active agent and other excipients such that heating the mixture results in dissolution of the drug and the resulting composition is then cooled to provide a solid blend which can be further formulated or directly added to a capsule or compressed into a tablet. Methods of producing such solid solutions are known in the art and include, but are not limited to, for example, US 4,151,273, 5,281,420, and 6,083,518.

The pharmaceutical solid oral dosage forms including formulations described herein can be further formulated to provide a controlled release of the BCL2 family inhibitor and/or anti-infective agent. Controlled release refers to the release of one or more active agents from a dosage form in which they are incorporated according to a desired profile over an extended period of time. Controlled release profiles include, for example, sustained release, prolonged release, pulsatile release, and delayed release profiles. In contrast to immediate release compositions, controlled release compositions allow delivery of an agent to a subject over an extended period of time according to a predetermined profile. Such release rates can provide therapeutically effective levels of agent for an extended period of time and thereby provide a longer period of pharmacologic response while minimizing side effects as compared to conventional rapid release dosage forms. Such longer periods of response provide for many inherent benefits that are not achieved with the corresponding short acting, immediate release preparations.

In some embodiments, the solid dosage forms described herein can be formulated as enteric coated delayed release oral dosage forms, *i.e.*, as an oral dosage form of a pharmaceutical composition as described herein which utilizes an enteric

coating to affect release in the small intestine of the gastrointestinal tract. The enteric coated dosage form may be a compressed or molded or extruded tablet/mold (coated or uncoated) containing granules, powder, pellets, beads or particles of the active ingredient and/or other composition components, which are themselves coated or uncoated. The enteric coated oral dosage form may also be a capsule (coated or uncoated) containing pellets, beads or granules of the solid carrier or the composition, which are themselves coated or uncoated.

The term "delayed release" as used herein refers to the delivery so that the release can be accomplished at some generally predictable location in the intestinal tract more distal to that which would have been accomplished if there had been no delayed release alterations. In some embodiments the method for delay of release is coating. Any coatings should be applied to a sufficient thickness such that the entire coating does not dissolve in the gastrointestinal fluids at pH below about 5, but does dissolve at pH about 5 and above. It is expected that any anionic polymer exhibiting a pH-dependent solubility profile can be used as an enteric coating in the methods and compositions described herein to achieve delivery to the lower gastrointestinal tract. In some embodiments the polymers described herein are anionic carboxylic polymers.

In some embodiments, the coating can, and usually does, contain a plasticizer and possibly other coating excipients such as colorants, talc, and/or magnesium stearate, which are well known in the art. Suitable plasticizers include triethyl citrate (Citroflex 2), triacetin (glyceryl triacetate), acetyl triethyl citrate (Citroflex A2), Carbowax 400 (polyethylene glycol 400), diethyl phthalate, tributyl citrate, acetylated monoglycerides, glycerol, fatty acid esters, propylene glycol, and dibutyl phthalate. In particular, anionic carboxylic acrylic polymers usually will contain 10-25% by weight of a plasticizer, especially dibutyl phthalate, polyethylene glycol, triethyl citrate and triacetin. Conventional coating techniques such as spray or pan coating are employed to apply coatings. The coating thickness must be sufficient to ensure that the oral dosage form remains intact until the desired site of topical delivery in the intestinal tract is reached.

Colorants, detackifiers, surfactants, antifoaming agents, lubricants (*e.g.*, carnuba wax or PEG) may be added to the coatings besides plasticizers to solubilize or disperse the coating material, and to improve coating performance and the coated product.

In other embodiments, the BCL2 family inhibitor plus anti-infective agent formulations described herein are delivered using a pulsatile dosage form. A pulsatile dosage form is capable of providing one or more immediate release pulses at

predetermined time points after a controlled lag time or at specific sites. Pulsatile dosage forms may be administered using a variety of pulsatile formulations known in the art. For example, such formulations include, but are not limited to, those described in US 5,011,692, 5,017,381, 5,229,135, and 5,840,329. Other pulsatile release dosage forms suitable for use with the present formulations include, but are not limited to, for example, US 4,871,549, 5,260,068, 5,260,069, 5,508,040, 5,567,441 and 5,837,284. In one embodiment, the controlled release dosage form is pulsatile release solid oral dosage form including at least two groups of particles, (*i.e.* multiparticulate) each containing a formulation described herein. The first group of particles provides a substantially immediate dose of the BCL2 family inhibitor and anti-infective agent upon ingestion. The first group of particles can be either uncoated or include a coating and/or sealant. The second group of particles includes coated particles, which includes from about 2% to about 75%, from about 2.5% to about 70%, or from about 40% to about 70%, by weight of the total dose of the active agents in the formulation, in admixture with one or more binders. The coating includes a pharmaceutically acceptable ingredient in an amount sufficient to provide a delay of from about 2 hours to about 7 hours following ingestion before release of the second dose. Suitable coatings include one or more differentially degradable coatings such as, by way of example only, pH sensitive coatings (enteric coatings) such as acrylic resins either alone or blended with cellulose derivatives, *e.g.*, ethylcellulose, or non-enteric coatings having variable thickness to provide differential release of the formulation.

Many other types of controlled release systems known to those of ordinary skill in the art and are suitable for use with the formulations described herein. Examples of such delivery systems include, *e.g.*, polymer-based systems, such as polylactic and polyglycolic acid, polyanhydrides and polycaprolactone; porous matrices, nonpolymer-based systems that are lipids, including sterols, such as cholesterol, cholesterol esters and fatty acids, or neutral fats, such as mono-, di- and triglycerides; hydrogel release systems; silastic systems; peptide-based systems; wax coatings, bioerodible dosage forms, compressed tablets using conventional binders and the like. See, *e.g.*, Liberman et al. (1990); Singh et al. (2002); US 4,327,725, 4,624,848, 4,968,509, 5,461,140, 5,456,923, 5,516,527, 5,622,721, 5,686,105, 5,700,410, 5,977,175, 6,465,014 and 6,932,983.

In some embodiments, pharmaceutical formulations are provided that include particles of a BCL2 family inhibitor and anti-infective agent, and at least one dispersing agent or suspending agent for oral administration to a subject. The formulations may

be a powder and/or granules for suspension, and upon admixture with water, a substantially uniform suspension is obtained.

Liquid formulation dosage forms for oral administration can be aqueous suspensions selected from the group including, but not limited to, pharmaceutically acceptable aqueous oral dispersions, emulsions, solutions, elixirs, gels, and syrups. *See, e.g., Singh et al. (2002).*

The aqueous suspensions and dispersions described herein can remain in a homogenous state, as defined in The USP Pharmacists' Pharmacopeia (2005 edition, chapter 905), for at least 4 hours. The homogeneity should be determined by a sampling method consistent with regard to determining homogeneity of the entire composition. In one embodiment, an aqueous suspension can be re-suspended into a homogenous suspension by physical agitation lasting less than 1 minute. In another embodiment, an aqueous suspension can be re-suspended into a homogenous suspension by physical agitation lasting less than 45 seconds. In yet another embodiment, an aqueous suspension can be re-suspended into a homogenous suspension by physical agitation lasting less than 30 seconds. In still another embodiment, no agitation is necessary to maintain a homogeneous aqueous dispersion.

In addition to the additives listed above, the liquid formulations can also include inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, sodium lauryl sulfate, sodium docusate, cholesterol, cholesterol esters, taurocholic acid, phosphatidylcholine, oils, such as cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Intranasal Formulations

Intranasal formulations are known in the art and are described in, for example, US 4,476,116, 5,116,817 and 6,391,452. Formulations prepared according to these and other techniques well-known in the art are prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. *See, for example, Ansel, H. C. et al. (1995).* Preferably these compositions and formulations are prepared with suitable nontoxic pharmaceutically acceptable ingredients. These ingredients are known to those skilled in the preparation of nasal dosage forms and some of these can be found in

REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 21st edition, 2005, a standard reference in the field. The choice of suitable carriers is highly dependent upon the exact nature of the nasal dosage form desired, *e.g.*, solutions, suspensions, ointments, or gels. Nasal dosage forms generally contain large amounts of water in addition to the active ingredient. Minor amounts of other ingredients such as pH adjusters, emulsifiers or dispersing agents, preservatives, surfactants, gelling agents, or buffering and other stabilizing and solubilizing agents may also be present. The nasal dosage form should be isotonic with nasal secretions.

For administration by inhalation, formulations of a BCL2 family inhibitor and an anti-infective agent may be in the form of an aerosol, a mist, or a powder. Pharmaceutical compositions described herein are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, such as, by way of example only, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound described herein and a suitable powder base such as lactose or starch.

Buccal Formulations

Buccal formulations are known in the art and are described in, for example, US 4,229,447, 4,596,795, 4,755,386, and 5,739,136. In addition, the buccal dosage forms described herein can further include a bioerodible (hydrolysable) polymeric carrier that also serves to adhere the dosage form to the buccal mucosa. The buccal dosage form is fabricated so as to erode gradually over a predetermined time period, wherein the delivery of the active agents is provided essentially throughout. Buccal drug delivery, as will be appreciated by those skilled in the art, avoids the disadvantages encountered with oral drug administration, *e.g.*, slow absorption, degradation of active agents by fluids present in the gastrointestinal tract and/or first-pass inactivation in the liver. With regard to the bioerodible (hydrolysable) polymeric carrier, it will be appreciated that virtually any such carrier can be used, so long as the desired drug release profile is not compromised, and the carrier is compatible with the active agents, and any other components that may be present in the buccal dosage unit. Generally, the polymeric carrier comprises hydrophilic (water-soluble and water-swellingable) polymers that adhere to the wet surface of the buccal mucosa. Examples of polymeric carriers useful herein include acrylic acid polymers and co, *e.g.*, those

known as "carbomers". Other components may also be incorporated into the buccal dosage forms described herein include, but are not limited to, disintegrants, diluents, binders, lubricants, flavoring, colorants, preservatives, and the like. For buccal or sublingual administration, the compositions may take the form of tablets, lozenges, or
5 gels formulated in a conventional manner.

Transdermal Formulations

Transdermal dosage formulations of a BCL2 family inhibitor and anti-infective agent described herein may incorporate certain pharmaceutically acceptable
10 excipients which are conventional in the art. In one embodiment the transdermal formulations described herein include at least three components: (1) a formulation of a BCL2 family inhibitor and an anti-infective agent; (2) a penetration enhancer; and (3) an aqueous adjuvant. In addition, transdermal formulations can include additional components such as, but not limited to, gelling agents, creams and ointment bases, and
15 the like. In some embodiments, the transdermal formulation can further include a woven or non-woven backing material to enhance absorption and prevent the removal of the transdermal formulation from the skin. In other embodiments, the transdermal formulations described herein can maintain a saturated or supersaturated state to promote diffusion into the skin.

20 Formulations suitable for transdermal administration of compounds described herein may employ transdermal delivery devices and transdermal delivery patches and can be lipophilic emulsions or buffered, aqueous solutions, dissolved and/or dispersed in a polymer or an adhesive. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents. Still further, transdermal
25 delivery of the compounds described herein can be accomplished by means of iontophoretic patches and the like. Additionally, transdermal patches can provide controlled delivery of active agents. The rate of absorption can be slowed by using rate-controlling membranes or by trapping the compound within a polymer matrix or gel. Conversely, absorption enhancers can be used to increase absorption. An
30 absorption enhancer or carrier can include absorbable pharmaceutically acceptable solvents to assist passage through the skin. For example, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound optionally with carriers, optionally a rate controlling barrier to deliver the compound to the skin of the host at a controlled and predetermined rate over a prolonged period of
35 time, and means to secure the device to the skin.

Transdermal formulations may be administered using a variety of devices which have been described in the art. For example, such devices include, but are not limited to, US 3,598,122, 3,598,123, 3,710,795, 3,731,683, 3,742,951, 3,814,097, 3,921,636, 3,972,995, 3,993,072, 3,993,073, 3,996,934, 4,031,894, 4,060,084, 5 4,069,307, 4,077,407, 4,201,211, 4,230,105, 4,292,299, 4,292,303, 5,336,168, 5,665,378, 5,837,280, 5,869,090, 6,923,983, 6,929,801 and 6,946,144.

Injectable Formulations

Formulations suitable for intramuscular, subcutaneous, or intravenous 10 injection may include physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents, or vehicles including water, ethanol, polyols (propyleneglycol, polyethylene-glycol, glycerol, cremophor and the like), suitable 15 mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. Formulations suitable for subcutaneous injection may also contain additives such as preserving, wetting, emulsifying, and 20 dispensing agents. Prevention of the growth of microorganisms can be ensured by various antibacterial and antifungal agents, such as parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, 25 such as aluminum monostearate and gelatin.

For intravenous injections, compounds described herein may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the 30 formulation. Such penetrants are generally known in the art. For other parenteral injections, appropriate formulations may include aqueous or nonaqueous solutions, preferably with physiologically compatible buffers or excipients. Such excipients are generally known in the art.

Parenteral injections may involve bolus injection or continuous infusion. 35 Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The pharmaceutical composition

described herein may be in a form suitable for parenteral injection as a sterile suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The pharmaceutical compositions described herein may be in unit dosage forms suitable for single administration of precise dosages. In unit dosage form, the formulation is divided into unit doses containing appropriate quantities of one or more compound. The unit dosage may be in the form of a package containing discrete quantities of the formulation. Non-limiting examples are packaged tablets or capsules, and powders in vials or ampoules. Aqueous suspension compositions can be packaged in single-dose non-reclosable containers. Alternatively, multiple-dose reclosable containers can be used, in which case it is typical to include a preservative in the composition. By way of example only, formulations for parenteral injection may be presented in unit dosage form, which include, but are not limited to ampoules, or in multi-dose containers, with an added preservative.

Kits

Also are provided herein are kits for use in the therapeutic applications described herein. Such kits include at least a BCL2 family inhibitor and instructions for use of the BCL2 family inhibitor for treatment of an infection according to any of the methods described herein. In some embodiments the BCL2 family inhibitor is a BH3 mimetic agent. In particular embodiments the BH3 mimetic agent inhibits BCL-XL. In some embodiments the BH3 mimetic agent inhibits BCL-XL preferentially relative to other BCL2 family members. In some embodiments a kit containing the BCL2 family inhibitor also includes an anti-infective agent.

In some embodiments the kit includes a BCL2 family inhibitor and bacteriostatic or bactericidal agent (*e.g.*, an antibiotic). In other embodiments the kit includes a BCL2 family inhibitor and an anti-viral agent. In further embodiments the kit includes a BCL2 family inhibitor and antifungal agent. In yet other embodiments the kit includes a BCL2 family inhibitor and an antiprotozoal agent.

Optionally, such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) including one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers can be formed from a variety of materials such as glass or plastic.

A kit will typically may include one or more additional containers, each with one or more of various materials (such as reagents, optionally in concentrated form, and/or devices) desirable from a commercial and user standpoint for use of a compound described herein. Non-limiting examples of such materials include, but not limited to, buffers, diluents, filters, needles, syringes; carrier, package, container, vial and/or tube labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions for performing at least one of the treatment methods described herein is typically also included.

A label can be on or associated with the container. A label can be on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label can be associated with a container when it is present within a receptacle or carrier that also holds the container, *e.g.*, as a package insert. A label can be used to indicate that the contents are to be used for treatment of an infection (*e.g.*, a bacterial infection). The label can also indicate directions for use of the contents, such as in the methods described herein.

EXAMPLES

Example 1 - Materials and Methods

Bacterial Strains

L. pneumophila 130b sero-group 1 (ATCC BAA-74) is a spectinomycin-resistant clinical isolate from the Wadsworth Veterans Administration Hospital, Los Angeles, CA (Edelstein et al, 1986). The avirulent $\Delta dotA$ and the flagellin-deficient $\Delta flaA$ strains are deletion mutants of *L. pneumophila* 130b (Harding et al, 2013). GFP was expressed constitutively using the plasmid pMMB207C. *L. pneumophila* 130b and *L. longbeachae* NSW-150, a serogroup 1 clinical isolate from Australia, were grown

from -80°C frozen stocks on buffered charcoal-yeast extract (BCYE) agar at 37°C for 48 h before each infection. For *Legionella* strains expressing GFP, chloramphenicol was used at $6\text{ }\mu\text{g.mL}^{-1}$. To determine bacterial numbers before infection, *Legionella* were re-suspended in PBS and the optical density at 600 nm (OD_{600}) determined, whereby an OD_{600} of 1 equaled 10^9 bacteria/mL.

Cell Culture

Murine bone marrow-derived macrophages (BMDMs) were obtained from femora and tibiae of female 6-8 week-old C57BL/6 mice, or from mice of the indicated genotypes. Macrophages were cultured in RPMI 1640 medium supplemented with 15% fetal bovine serum (Serana), 20% L-cell-conditioned medium (containing macrophage colony-stimulating factor), and 100 U/mL of penicillin-streptomycin (Sigma) in bacteriological dishes for 12 days, at $37^{\circ}\text{C} + 5\% \text{CO}_2$. Medium was replaced after one week. For infections, BMDMs were gently scraped from plates using a cell scraper (BD Falcon) and washed 3 times in PBS, before seeding into tissue culture-treated plates. Immortalised C57BL/6 mouse derived macrophages (Bauernfeind et al, 2009) were cultured in RPMI 1640 supplemented with 10 % fetal bovine serum, at 37°C and 5 % CO_2 .

Live Cell Imaging to Determine Macrophage Viability

Cells were seeded at a density of 5×10^5 cells/mL in 96-well tissue culture plates and cell death was determined essentially as described in (Croker et al, 2011). Cells were stained with $1\text{ }\mu\text{M}$ Cell Tracker Green (CTG) (Invitrogen) for 20 min in serum-free RPMI 1640. Medium was then replaced with RPMI 1640 supplemented with 15 % FBS and 10 % L-cell-conditioned medium. Cells were stained with $0.6\text{ }\mu\text{M}$ Draq7 (Abcam) or $1\text{ }\mu\text{g/mL}$ propidium iodide (Invitrogen). Cells were infected at a MOI between 10 (or as indicated) in triplicate biological repeats, and then treated with BH3 mimetics (Abbvie) at 500 nM (or at the indicated concentrations), and the pan-caspase inhibitor Q-VD-OPh (R&D Systems Biology) at $20\text{ }\mu\text{M}$. Before imaging, 50 μL of mineral oil (Sigma) was added to each well to prevent evaporation. Experiments were performed on a Leica AF6000 LX epi-fluorescence microscope equipped with an incubator chamber set at $37^{\circ}\text{C} + 5\% \text{CO}_2$ and an inverted, fully-motorized stage driven by Leica Advanced Suite Application software. Time-lapse images were acquired with bright-field, GFP, TxRed, and Y5 filters every hour for up to 72 h using a $10\times/0.8\text{-NA}$ objective. To determine the percentages of dead cells, images were analyzed in ImageJ and in MetaMorph[®] (Molecular Devices) using a

custom-made journal suite incorporating the count nuclei function to segment and count the number of CTG and Draq7 positive cells (adapted from Croker et al, 2011). The data of percentage Draq7-positive cells were analyzed in Excel and GraphPad Prism.

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Quantification of Legionella Infection

To determine bacterial burdens, macrophages were seeded at a density of 2.5×10^5 cells/mL into 12-well tissue culture plates and infected with *Legionella* strains expressing GFP at an MOI of 50. After 2 h, cells were washed $3 \times$ in PBS, medium replaced, and cells were then treated with BH3 mimetics [500 nM] and Q-VD-OPh [20 μ M]. At the indicated time points, cells were removed from the plates and stained with 1 μ g/mL propidium iodide (PI) (Invitrogen) before GFP and PI fluorescence were determined by flow cytometric analysis (BD FACSCalibur™). Cells were gated by forward and side scatter, and channels F1 and F3 were used to detect GFP and PI fluorescence, respectively. 10,000 events/sample were counted. Weasel software (WEHI) was used for the analysis. Alternatively, cells were lysed in 0.05 % digitonin for 5 min at room temperature and serial dilutions of the cell lysates and the corresponding culture media were plated on BCYE agar plates, and bacterial colonies counted after 72 h at 37 °C.

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Mice Infections and Tamoxifen Treatment

6-8 week-old male or female C57BL/6 and A/J mice (or mice of the indicated genotypes), in groups of five or more, were anesthetized by 4 % isoflurane inhalation and infected intra-nasally with either 2.5×10^6 *L. pneumophila*, or 1×10^5 *L. longbeachae*, in 50 μ L of sterile PBS. In some instances, after intra-nasal infection, mice were injected intra-peritoneally (i.p.) with 50 mg per kg body weight of ABT-263 (Selleckchem) in 50 μ L DMSO, or with DMSO (vehicle, control) only. For the ABT-263 studies, the infecting and analyzing investigator was blinded towards which group received the BH3 mimetic (all other studies were not blinded). For CFUs, at 6 or 48 h following infection, both lung lobes were removed and homogenized for 30 sec in PBS at 30,000 rpm using the Omni Tissue Master homogenizer. Serial dilutions of the lung homogenates were plated onto BCYE agar plates and bacterial colonies were counted after 72 h at 37 °C to determine CFUs. For the survival curve, mice were weighed daily and euthanized after greater than 15% weight loss, according to the animal ethics guidelines. Cre-mediated *Bcl-xl* deletion was induced by 3 doses of 200mg/kg

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tamoxifen (Sigma, T5648) dissolved in peanut oil/10% ethanol at 80 mg/ml administered on three separate days by oral gavage using bulb-tipped feeding needles.

Immunoblot Analysis

5 2.5×10^5 cells were lysed in 120 μ L SDS-loading dye, boiled for 5 min and samples analyzed by 12 % SDS-PAGE. After transfer to nitrocellulose membranes (Millipore), membranes were blocked with 5 % skim milk in T-BST (Tween-20, Tris-buffer) for 1 h at room temperature. Membranes were probed with anti-cleaved caspase-3 antibody (CST #9964), anti- β -actin antibody (Millipore #04-1116), or anti-
10 F1- β antibody (Monash Antibody Facility), (β -actin and F1- β were used as loading controls) and re-suspended in T-BST and 5 % skim milk overnight at 4 °C. After washing, membranes were probed with secondary goat anti-rabbit IgG (Life Technologies) and goat anti-mouse IgG (Life Technologies) antibodies conjugated to HRP (1:20,000 dilution in T-BST + 5 % skim milk). Membranes were developed with
15 the luminol-based enhanced chemiluminescence (ECL) and exposed to film (Kodak). Scanned images were processed in Adobe Photoshop.

Immunofluorescence Staining

Macrophages were seeded onto glass coverslips in 24-well plates and infected
20 with *Legionella* strains expressing GFP at an MOI of 10. BH3 mimetics [500 nM] were added to appropriate wells, 2 h after infection. At specific time-points, cells were fixed with 4 % PFA for 15 min, washed three times with PBS and treated with 50 mM NH_4Cl for 10 min. Cells were then permeabilized in 0.1 % Triton-X 100 in PBS for 5 min on ice and blocked in PBS + 1 % BSA overnight at 4 °C, before incubation with
25 anti-cleaved caspase-3 antibody (CST #9964) [1:400] for 30 min. After 3 $\times$ washes, cells were incubated with goat anti-rabbit IgG antibodies coupled to Alexa Fluor 594 (Life Technologies) for 30 min. Cells were mounted in oil (Dako) containing 10 μ g/mL Hoechst 33342 (Life Technologies) and imaged on an Olympus epi-fluorescence microscope using a 60x oil objective (0.8 NA), and analyzed in ImageJ.

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Statistical Analysis

For all *in vitro* data, the inventors performed 2-way ANOVA before using Tukey's post-hoc test for pair-wise comparisons. For Figure 4b the Mann-Whitney U test was used. For the analysis in Figs. 4c and d, the inventors bootstrapped the
35 independent samples to test for the difference between CFUs in treated and untreated mice. This confirmed that CFUs were significantly greater in the non-treatment

condition (99 % CI for difference between the two sample groups). In all cases, the lower bound of the confidence interval for the difference between conditions was substantially different to zero (in the expected direction), confirming that the conditions were significantly different, $p < 0.01$.

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Example 2 - The BH3 Mimetic Compound ABT-737 Inhibits Induces Apoptosis of Legionella-Infected Bone Marrow-Derived Macrophages

To investigate the role of BCL-2 in *L. pneumophila* infection the inventors used the flagellin-deficient $\Delta flaA$ mutant, as $\Delta flaA$ -infected BMDMs remain viable for up to 10 20 h post-infection, allowing intracellular replication (Figure 1a). This mimics wild-type *Legionella* infection in permissive human macrophages (Miao et al, 2010; Ren et al, 2006; Molofsky et al, 2006; Zamboni et al, 2006; and Amer et al, 2006). Subsequent macrophage death occurred in ~80 % of BMDMs by 60 h post-infection (Figure 1a), reflecting cycles of bacterial release and re-infection, and continual 15 bacterial replication at a low initial multiplicity of infection.

The BH3 mimetic compound, ABT-737, antagonizes pro-survival BCL-2 family members to cause cell death in some cells (Lessene et al, 2008). Unexpectedly, when $\Delta flaA$ *L. pneumophila*-infected BMDMs were treated with ABT-737 the majority of macrophages (~65 %) were protected from the extensive cell death that occurred in 20 untreated cells 60 h post infection (Figure 1a). Similarly, ABT-737 treatment protected BMDMs from extensive cell death after infection with wild type *L. pneumophila* (Figure 5b). In both cases ABT-737 treatment resulted in 30-35% BMDM death (Figure 1a and Figure 5b). Significantly, uninfected BMDMs remained viable and ABT-737 treatment did not induce significant death in these macrophages (Figure 5a), 25 or in BMDMs infected with the avirulent $\Delta dotA$ *L. pneumophila* strain lacking a functional T4SS (Figure 1b), nor did it alter *Legionella* axenic growth (data not shown).

The inventors hypothesized that ABT-737 might induce selective killing of the initial *Legionella*-harboring macrophages (~30-40% of cells) shortly after bacterial invasion. Consistent with this idea, at higher infection rates, ABT-737 treatment 30 resulted in the killing of ~80 % of BMDMs in culture, and this occurred faster than in infected macrophages that had not been exposed to ABT-737 (Figure 1c). Moreover, live-cell imaging of immortalized C57BL/6 (iBl/6) macrophages after infection with GFP-expressing $\Delta flaA$ *L. pneumophila* demonstrated that ABT-737 treatment rapidly induced apoptotic morphology (plasma membrane ruffling and blebbing) followed by 35 plasma membrane rupture (determined by DraQ7 staining) only in GFP+ macrophages (Figure 1d). In the absence of ABT-737 this apoptotic morphology was not observed

and $\Delta flaA$ *L. pneumophila* did not induce significant macrophage death within 12 h, despite robust intracellular bacterial replication, as reflected by increasing GFP fluorescence (Figure 1d). In contrast, in the presence of ABT-737, GFP fluorescence (reflecting bacterial replication) failed to increase over the 48 h following infection (Figure 1d).

To examine directly whether ABT-737 treatment reduces intracellular *L. pneumophila* loads, the inventors measured colony forming units (CFUs). $\Delta flaA$ *L. pneumophila* assumes exponential growth approximately 6 h post-infection (Figure 1e). While ABT-737 treatment did not affect the initial macrophage infection rate within this time frame (Figure 1e), it prevented any subsequent (24 and 48 h post-infection) increase in intracellular $\Delta flaA$ *L. pneumophila* burden (Figure 1e). Collectively, these data demonstrate that ABT-737 treatment specifically induces the death of macrophages containing virulent *Legionella*, and that this limits the intracellular bacterial burden.

Example 2 - The BH3 Mimetic Compound ABT-737 Targets BCL-XL

ABT-737 targets BCL-2, BCL-W and BCL-XL (Figure 2a). To gain insight into which BCL-2 member protects *Legionella*-infected macrophages from death, the inventors utilized BH3-mimetics that specifically bind to BCL-2 or BCL-XL. Surprisingly, the BCL-2-specific antagonist, ABT-199, failed to clear GFP-expressing $\Delta flaA$ *L. pneumophila* in iBL/6 and BMDM host cells (Figure 2a, b and c). In contrast to ABT-199, the BCL-XL-specific inhibitor A-1155463, which does not antagonize BCL-2 or BCL-W significantly (Tao et al, 2014), mimicked the effects of ABT-737. Specifically, like ABT-737, A-1155463 treatment caused the loss of GFP signal in iBL/6 macrophages after infection with GFP-expressing $\Delta flaA$ *L. pneumophila*, and resulted in a >100-fold CFU reduction in BMDMs 48 h post infection (Figure 2a and c). Consequently, A-1155463 protected macrophages from extensive *Legionella*-mediated killing (Figure 2a and b). Remarkably, A-1155463 was effective in limiting $\Delta flaA$ *Legionella* burdens even at low nanomolar concentrations, whereas ABT-199 was inefficient at doses lower than 5 μ M (Figure 2d).

To genetically confirm a role for BCL-XL in promoting *Legionella* infection, the inventors generated BCL-XL-deficient BMDMs. Constitutive loss of BCL-XL causes embryonic lethality around E14 due to excessive apoptosis of erythroid and neuronal progenitors (Motoyama et al, 1995). Thus, the inventors treated bone marrow progenitor cells from Bcl-x^{flax/flax};ER-Cre mice, which contain two floxed *Bcl-x* alleles (Wagner et al, 2000) and express the Cre-recombinase estrogen receptor fusion protein

(Rosa26-Cre-ERT2), with 4-hydroxytamoxifen (4-HT) to induce deletion of BCL-XL (referred to as *Bcl-x^{-/-}*). This resulted in a >90 % reduction of BCL-XL protein expression in differentiated BMDMs (Figure 6a). Importantly, genetic deletion of *Bcl-x* mimicked BCL-XL antagonism by BH3-mimetic compounds. First, the initial infection of *Bcl-x^{-/-}* BMDMs with Δ *flaA* *L. pneumophila* was not altered when compared to wild type macrophages, but subsequent *Legionella* burdens decreased over time (Figure 2e). Second, *Bcl-x* gene deletion protected the bulk of the macrophage population (~80 %; *i.e.*, those not initially infected) from Δ *flaA* *L. pneumophila*-induced killing when macrophages were infected at a low MOI (Figure 2f). Third, *Bcl-x^{-/-}* BMDMs were resistant to wild type *L. pneumophila*-induced cell death (Figure 6b). As expected, *Bcl-x^{-/-}* BMDMs remained viable after infection with the avirulent Δ *dotA* *L. pneumophila* strain (Figure 2g). Collectively, these data demonstrate that BCL-XL is uniquely required in *Legionella*-infected macrophages to sustain host cell survival and allow efficient intracellular bacterial replication.

BCL-XL inhibits the mitochondrial apoptotic pathway that culminates in the activation of the apoptotic executioner caspase-3 (Strasser et al, 2011). Consistent with this, Δ *flaA*, but not Δ *dotA*, *L. pneumophila*-infected *Bcl-x^{-/-}* BMDMs showed increased caspase-3 activation compared to wild type BMDMs, as measured by immunoblotting or staining of the active caspase-3 p17/p19 fragment (Figs. 3a and c). Increased caspase-3 activation was also detected in Δ *flaA* *L. pneumophila*-infected wild type BMDMs treated with A-1155463 (Figure 3b). In contrast, comparatively less caspase-3 activation was detected in Δ *flaA*-infected wild type macrophages that had been treated with vehicle control (Figure 3b).

These data suggest that caspase mediated apoptosis following BH3-mimetic treatment prevents intracellular *Legionella* growth. Therefore to test if *Legionella* growth can be restored in the absence of BCL-XL activity by inhibiting apoptosis, the inventors treated Δ *flaA* *L. pneumophila*-infected macrophages with ABT-737, ABT-199, or A-1155463 in the presence of the pan-caspase inhibitor compound Q-VD-OPh (Q-VD). By itself, Q-VD had no effect on the intracellular burden of Δ *flaA* *L. pneumophila* (Figure 3d) but, remarkably, it restored bacterial numbers in ABT-737- and A-1155463-treated wild type BMDMs (Figure 3d), and also in *Bcl-x^{-/-}* BMDMs (Figure 3i). The replication of Δ *flaA* *L. pneumophila* results in eventual macrophage lysis that is not blocked by Q-VD and, therefore, is likely to occur independently of caspase function (Figure 3e). Paradoxically then, Q-VD efficiently restored the ability of Δ *flaA* *L. pneumophila* to replicate and eventually kill BMDMs in the presence of either of the BCL-XL-targeting BH3-mimetics, ABT-737 or A-1155463 (Figs. 3g and

h). As expected, Q-VD had little effect on bacterial load or host cell death in $\Delta flaA$ *L. pneumophila*-infected wild type BMDMs treated with ABT-199 (Figs. 3d and f).

Example 3 - Selective Targeting of BCL-XL Reduces Bacterial Load *In Vivo* and Extends Survival

To test whether selective targeting of BCL-XL reduces bacterial load in the lungs *in vivo*, the inventors treated >10-week-old $Bcl-x^{flx/flx};ER-Cre$ mice with tamoxifen to generate BCL-XL deficient mice. These mice contained normal numbers of bone marrow progenitor cells, which generated BMDMs that lacked detectable levels of BCL-XL protein (Figure 4a), and showed reduced bacterial loads 48 h after $\Delta flaA$ *L. pneumophila* infection (Figure 7a). Next, tamoxifen-treated or untreated $Bcl-x^{flx/flx};ER-Cre$ mice were intranasally infected with $\Delta flaA$ *L. pneumophila*, and the bacterial burden in the lungs determined at 48 h post-infection.

As expected, untreated $Bcl-x^{flx/flx};ER-Cre$ mice showed a similar burden of $\Delta flaA$ *L. pneumophila* as C57BL/6 (wild type control) mice, after 48 h (Figure 4b). In contrast, bacterial burden was significantly ($p < 0.01$) reduced in tamoxifen-treated $Bcl-x^{flx/flx};ER-Cre$ infected mice (Figure 4b). Strikingly, four of 11 mice failed to produce culturable $\Delta flaA$ *L. pneumophila*, even after extended incubation. Tamoxifen treatment did not affect $\Delta flaA$ *L. pneumophila* load in the absence of ER-Cre, nor did ER-Cre expression itself have any effect on bacterial loads (Figure 4b).

To examine whether pharmacological targeting of BCL-XL reduces *Legionella* lung burden, the inventors infected A/J mice, which are susceptible to wild type *L. pneumophila*. Immediately after intranasal infection, mice were treated with a single clinically relevant (50 mg/kg body weight) dose of the orally available analogue of ABT-737 (ABT-263). This resulted in a significant reduction ($p < 0.01$) in the numbers of bacteria in the lungs at 48 h post-infection (Figure 4c). Given that wild type *L. pneumophila* causes a self-limiting infection even in susceptible A/J mice (Molofsky et al, 2006), the inventors also tested whether targeting BCL-XL would affect *Legionella longbeachae*, which grows in mouse lungs unrestrained (Gobin et al, 2009; and Asare et al, 2007). *L. longbeachae* causes lethal pneumonia by employing a different set of T4SS effectors (Cazalet et al, 2010). Despite this, ABT-737 and A-1155463 both prevented increased intracellular loads of *L. longbeachae* in BMDMs (Figure 8a). Administration of ABT-263 effectively controlled the burden of *L. longbeachae* in the lungs of C57BL/6 mice, and in four out of ten mice the inventors failed to culture any CFUs after 72 h of infection (Figure 4d). Furthermore, a single dose of 50 mg/kg ABT-263 completely rescued C57BL/6 mice from lethal *L. longbeachae* infections,

whereas all mice treated with vehicle rapidly lost body weight and succumbed within 96 h after infection (Figs. 4e and 4f). Collectively, these data extend our *in vitro* observations by demonstrating that intracellular pathogens require host cell BCL-XL pro-survival activity to sustain high bacterial loads in animal hosts.

5 Conjointly, our results demonstrate that apoptosis-inducing agents, originally designed to induce cancer cell death and currently in clinical trials for their efficacy as anti-cancer treatments (Lessene et al, 2008), can be potent antimicrobial compounds. As the threat to human health posed by drug-resistant bacteria looms large (Boucher et al, 2009), this discovery promises a novel therapeutic strategy for the control of
10 intracellular pathogens.

Example 4 - A BCL-XL-Selective BH3 Mimetic Compound, Reduces Bacterial Load *In Vivo* and Extends Survival

Eight week-old female C57BL/6 mice were infected intranasally with 10^5 WT
15 *Legionella longbeachae* bacteria and received either a single dose of 50 mg/kg of a BCL-XL-selective BH3 mimetic compound (Formula XIII), i.p., or a DMSO (control) injection. Mice were culled 72 h post-infections, lungs extracted, and serial dilutions plated onto BCYE plates, and incubated for three days at 37 C prior to colony counting. Similar to ABT-263, BCL-XL inhibition caused a 100 fold reduction in bacterial
20 counts.

Example 5 - A BCL-XL-Selective BH3 Mimetic Compound, A-1331852 (Formula XIII) is Effective in Treatment of a Model of Latent *Mycobacterium tuberculosis* infection

25 C57BL6 mice were infected using an inhalation exposure system with a low dose of M.tb (70-100 CFUs). At three weeks, they were commenced on isoniazid (0.1mg/L) and rifampicin (0.075mg/L) *ad libitum* for a total of 12 weeks. Mice were rested for two weeks, prior to treatment with A-1331852 at 25 mg/kg daily via gavage for 10 days. After a week of rest, mice were then injected intraperitoneally with
30 dexamethasone 120 µg for 6 doses. Mice were then sacrificed after one week for CFU calculations.

In the latency model, following reactivation with dexamethasone, no mice in the A-1331852 treated group reactivated, in comparison to 50% in the vehicle arm (Figure 10). The results show that BH3 mimetic compounds can be useful in treating latent
35 M.tb infection.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as
5 illustrative and not restrictive.

All publications cited herein are hereby incorporated by reference in their entirety. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet.
10 Reference thereto evidences the availability and public dissemination of such information.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of
15 these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

This application claims priority to Australian Provisional Patent Application No. 2015900554 entitled "Methods of Treating Infectious Diseases" filed on 18
20 February 2015, the content of which is incorporated by reference herein in its entirety.

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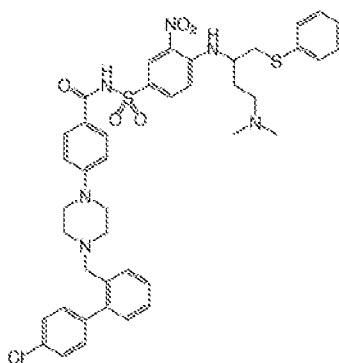
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CLAIMS

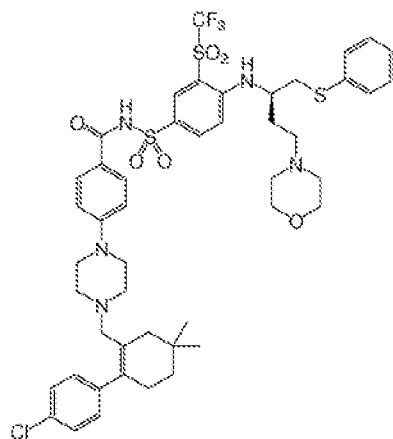
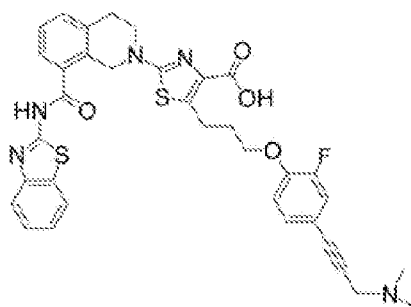
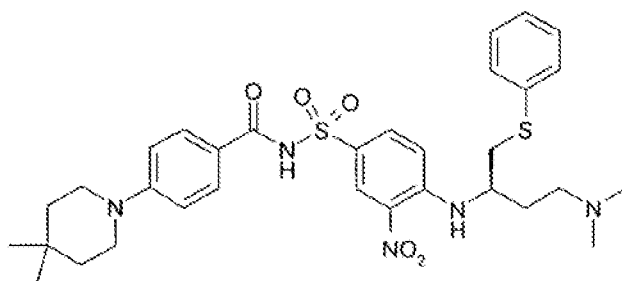
1. A method for treating an infection, comprising administering to a subject in need thereof a therapeutically effective amount of a BCL2 family inhibitor.
2. The method according to claim 1, wherein the infection is a bacterial infection.
3. The method according to 2, wherein the bacterial infection is an infection by bacteria of one or more species of *Legionella*, *Mycobacterium*, *Salmonella*, *Staphylococcus*, pathogenic *E. coli*, or *Pseudomonas*.
4. The method according to claim 2 or claim 3, further comprising administration of a bactericidal or bacteriostatic agent.
5. The method according to any one of claims 2-4, wherein the bacterial infection is an infection by antibiotic-resistant bacteria.
6. The method according to claim 1, wherein the infection is a viral infection.
7. The method according to claim 6, wherein the viral infection is an infection by HSV, HTLV-1, HTLV-2, HIV, a respiratory virus, rhinovirus, a coronavirus or ebola.
8. The method according to claim 1, wherein the infection is a fungal infection.
9. The method according to claim 8, wherein the fungal infection is an infection by a yeast, *Aspergillus*, *Pneumocystitis*, or *Cryptococcus*.
10. The method according to claim 1, wherein the infection is a protozoal infection.
11. The method according to claim 10, wherein the protozoal infection is an infection by *Trypanosoma cruzi*, *Toxoplasma gondii*, *Cryptosporidium*, or *Encephalitozoon*.
12. The method according to any one of claims 1 to 11, wherein the infection comprises infection of macrophages.

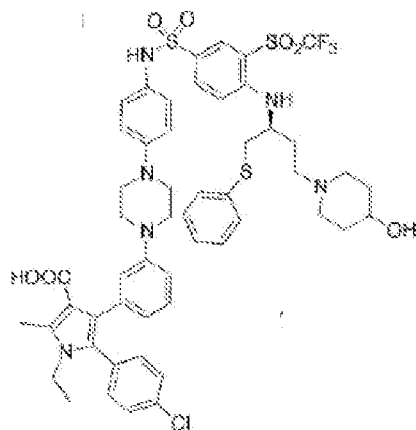
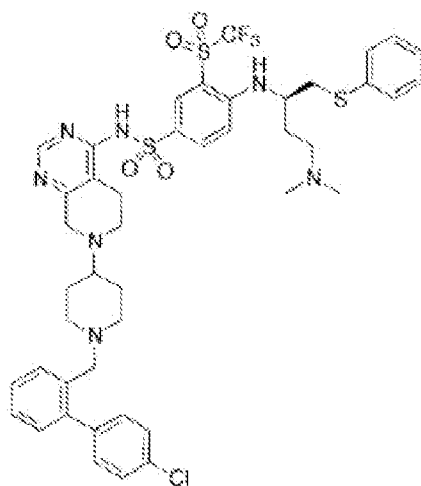
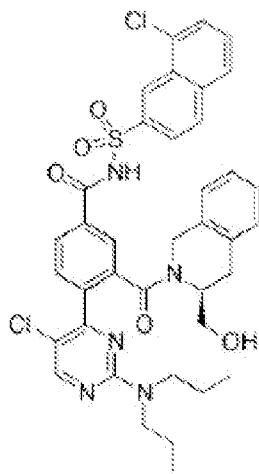
13. The method according to claim 12, wherein the macrophages comprise alveolar macrophages.
14. The method according to any one of claims 1 to 13, wherein the infection is an acute or subacute infection.
15. The method according to claim 1, wherein the subject is suffering from tuberculosis, pneumonia, Legionnaires' disease, or an acute respiratory infection.
16. The method according to any one of claims 1 to 15, wherein the BCL2 family inhibitor inhibits BCL-XL.
17. The method according to claim 16, wherein the BCL2 family inhibitor is a BCL-XL-selective inhibitor.
18. The method according to any one of claims 1 to 17, wherein the BCL2 family inhibitor is selected from the group consisting of a BH3 mimetic agent, an RNAi against a BCL2 family member, and an antibody against a BCL2 family member.
19. The method according to claim 18, wherein the BCL2 family inhibitor is a BH3 mimetic agent.
20. The method according to claim 19, wherein the BH3 mimetic agent is a compound selected from the group consisting of:

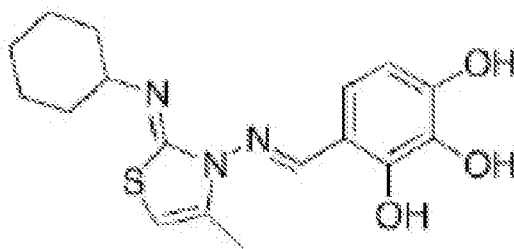
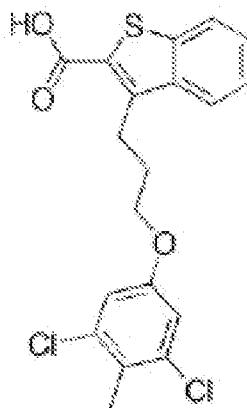
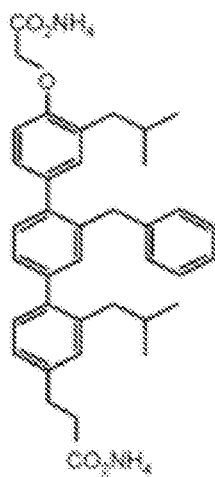


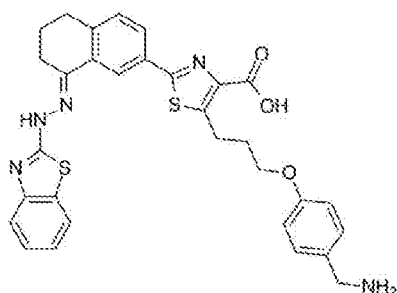
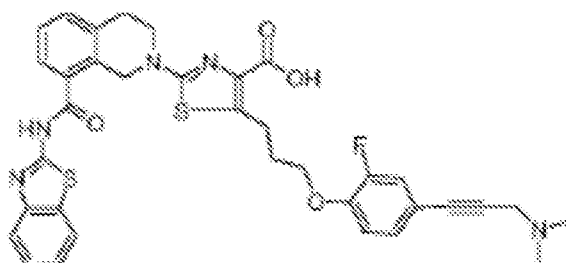
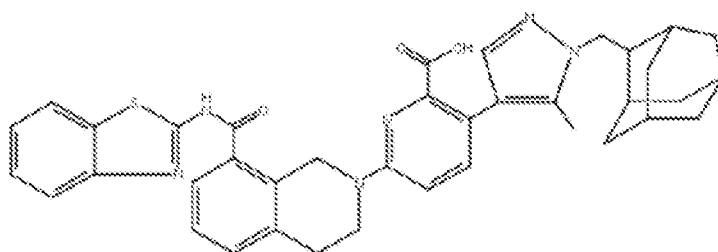
Formula (I) (ABT-737),

55

**Formula (II)-ABT-263,****Formula (III)-A-1155463,****Formula (IV)-A-385358,**

**Formula (V)-BM-957,****Formula (VI),****Formula (VII),**

**Formula (VIII)-MIM-1,****Formula (IX),****Formula (X)-BH3-M6,**

**Formula (XI)-WEHI-539,****Formula (XII), and****Formula (XIII).**

21. The method according to any one of claims 1 to 20, wherein the subject to be treated was not diagnosed as suffering from a cancer.
22. The method according to any one of claims 1 to 21, wherein the administration occurs within a period from about 6 hours to about 48 hours following a diagnosis of the infection.

23. The method according to any one of claims 1 to 22, wherein the administration occurs no later than about 48 hours following a diagnosis of the infection.
24. The method according to any one of claims 1 to 23, wherein the subject is human.
25. Use of a BCL2 family inhibitor for the preparation of a medicament for treating an infection.
26. A BCL2 family inhibitor for use in the treatment of an infection.
27. A pharmaceutical composition comprising therapeutically effective amounts of a BCL2 family inhibitor and a bactericidal or bacteriostatic agent.
28. A kit when used for the treatment of an infection, the kit comprising a BCL2 family inhibitor.
29. The kit according to claim 28 further comprising a bactericidal or bacteriostatic agent.

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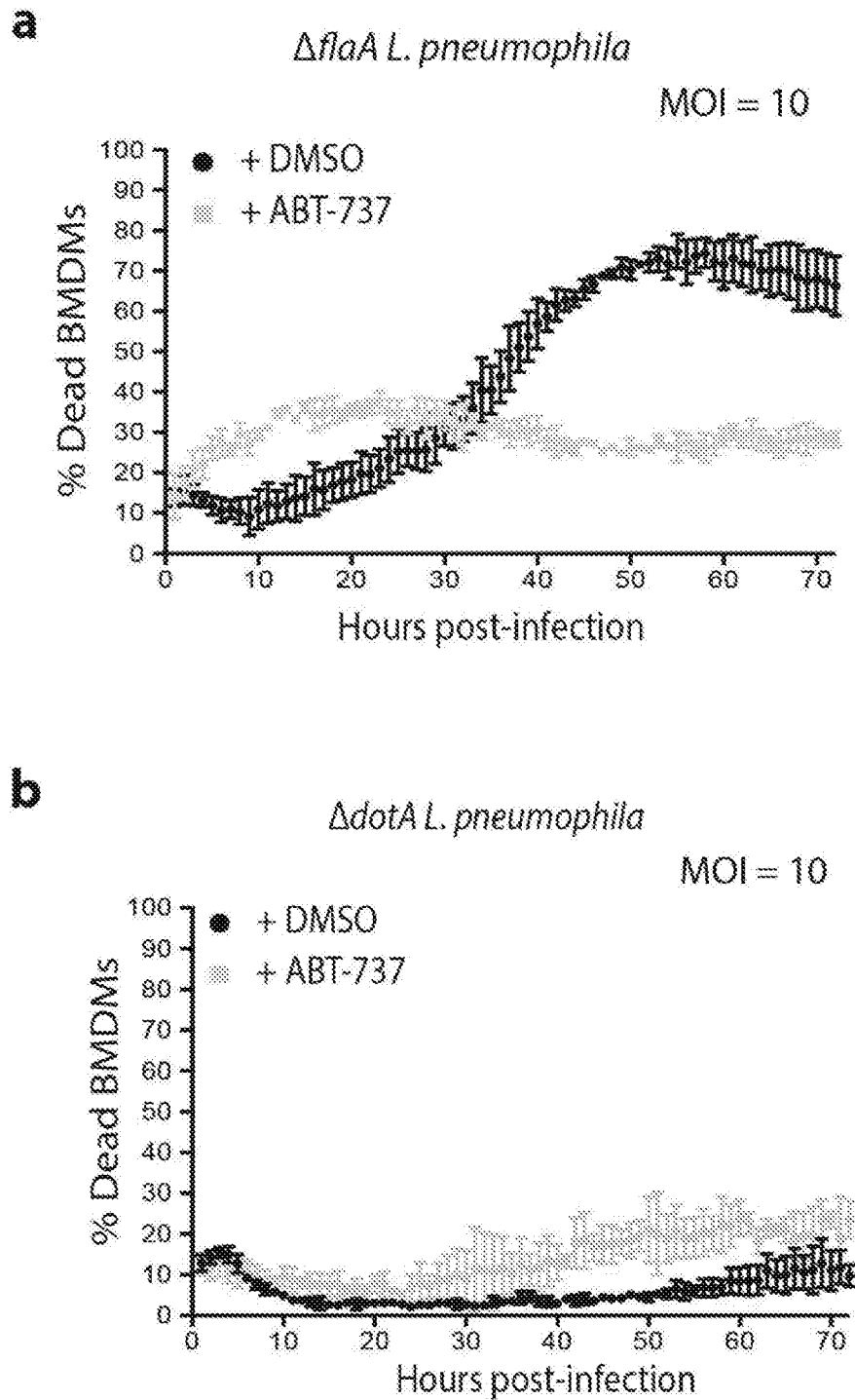


FIGURE 1a-b

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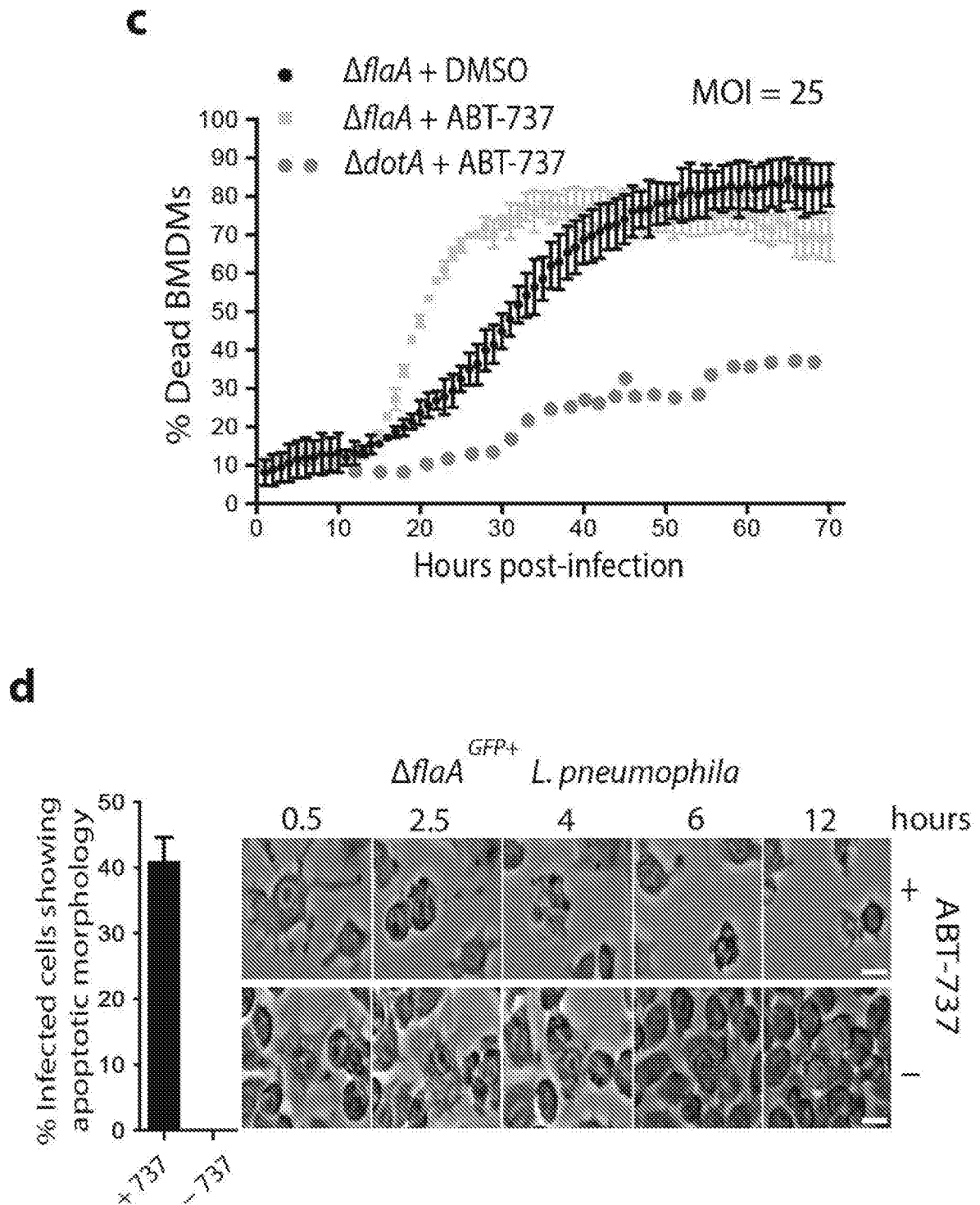


FIGURE 1c-d

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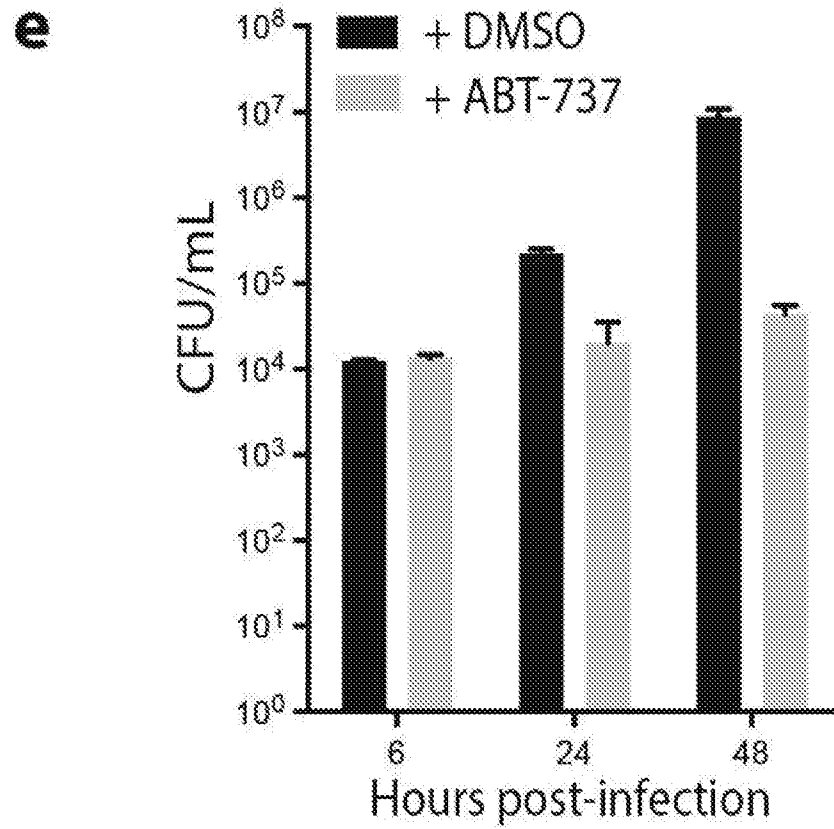


FIGURE 1e

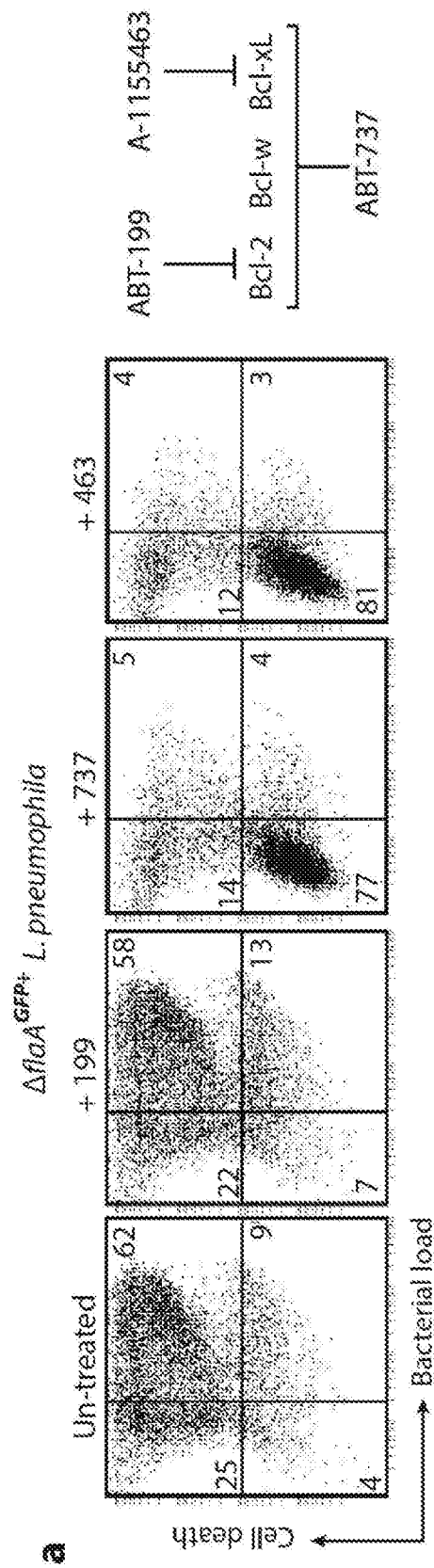
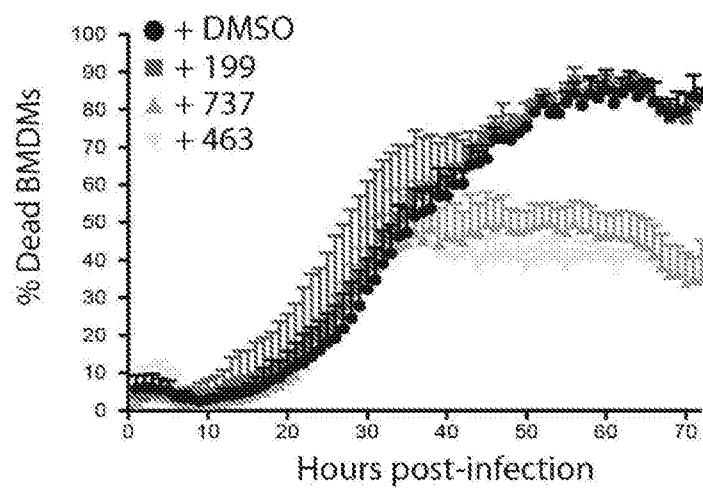
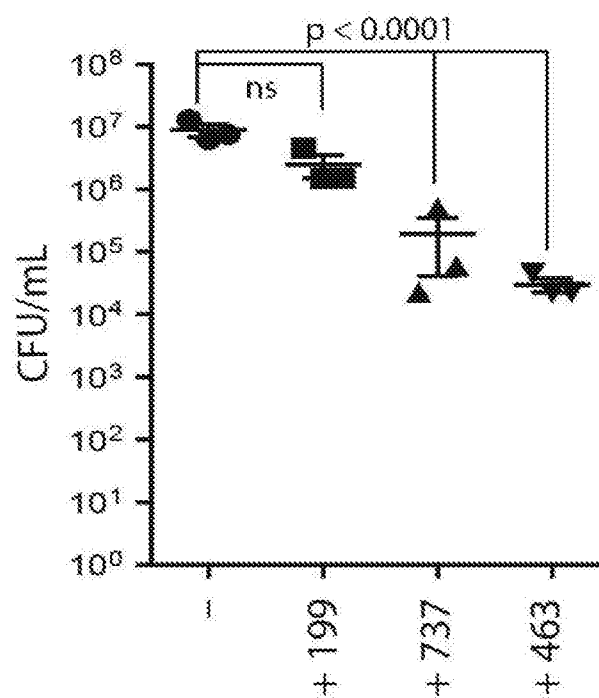


FIGURE 2a

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b*ΔflaA L. pneumophila***c***ΔflaA L. pneumophila***FIGURE 2b-c**

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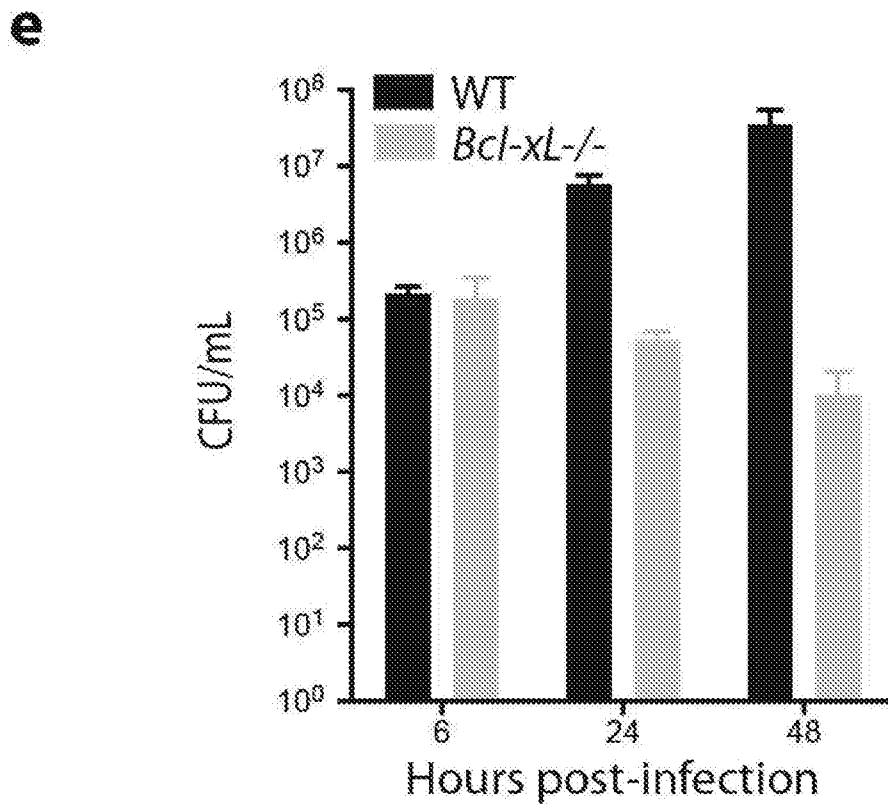
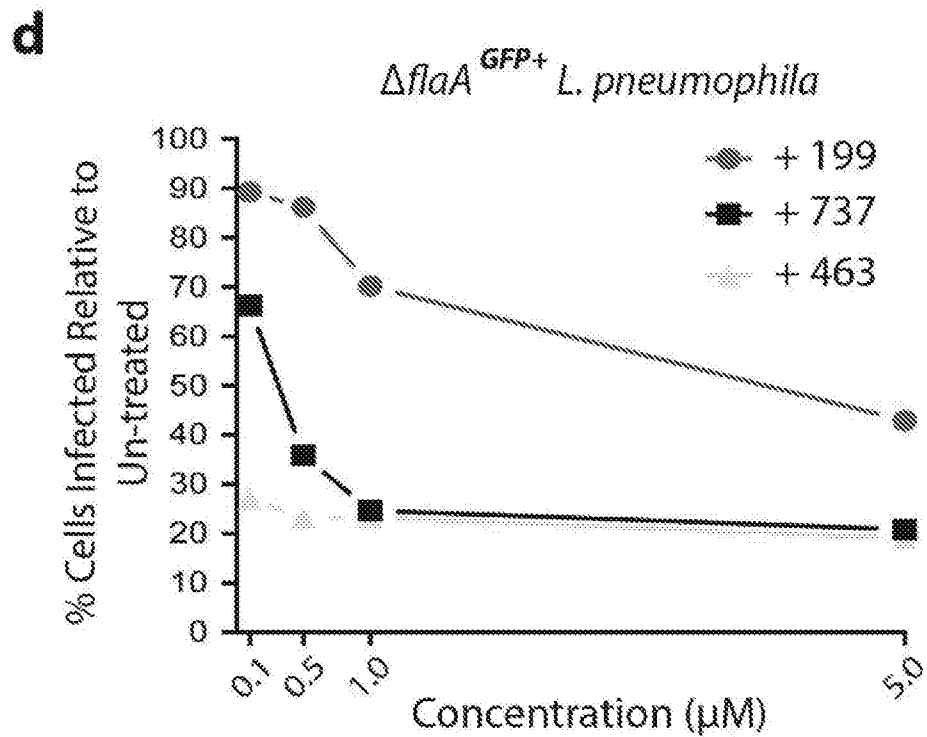
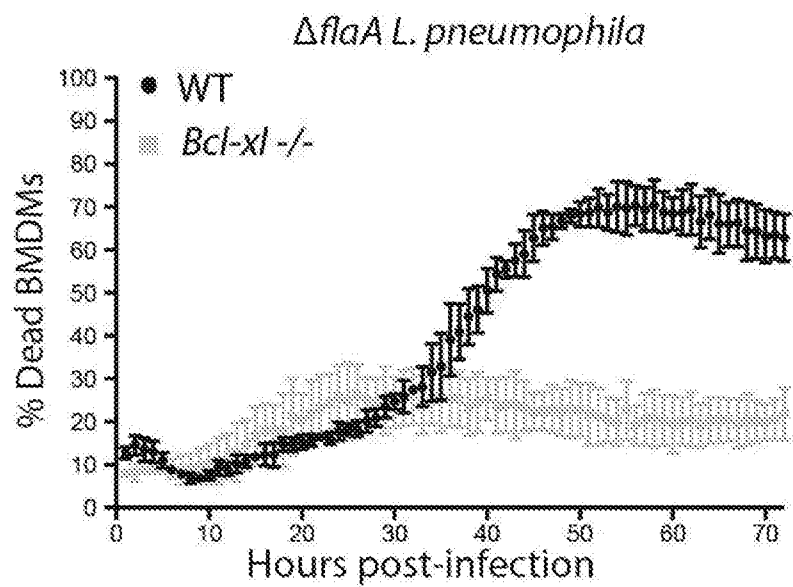
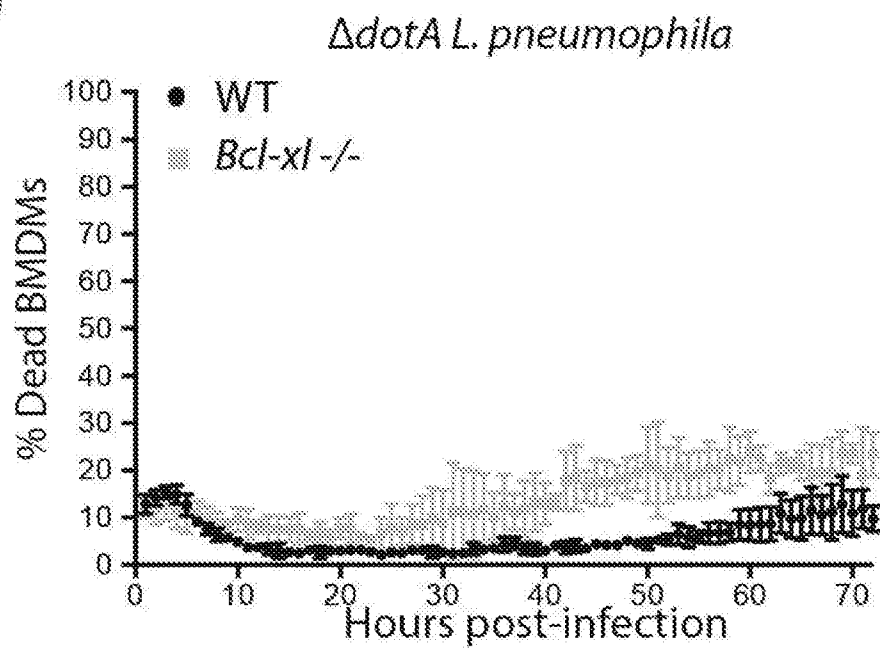


FIGURE 2d-e

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f**g****FIGURE 2f-g**

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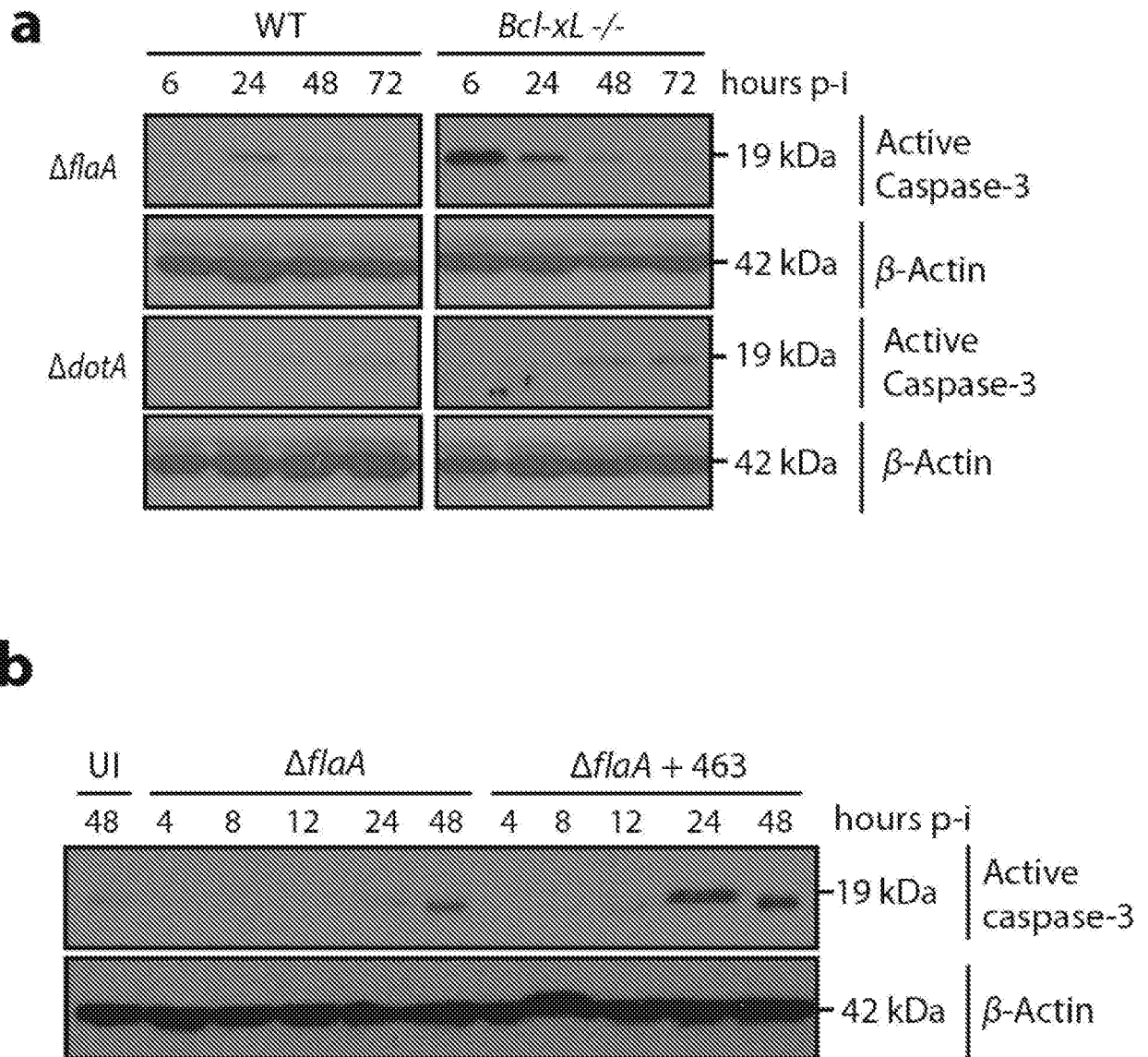


FIGURE 3a-b

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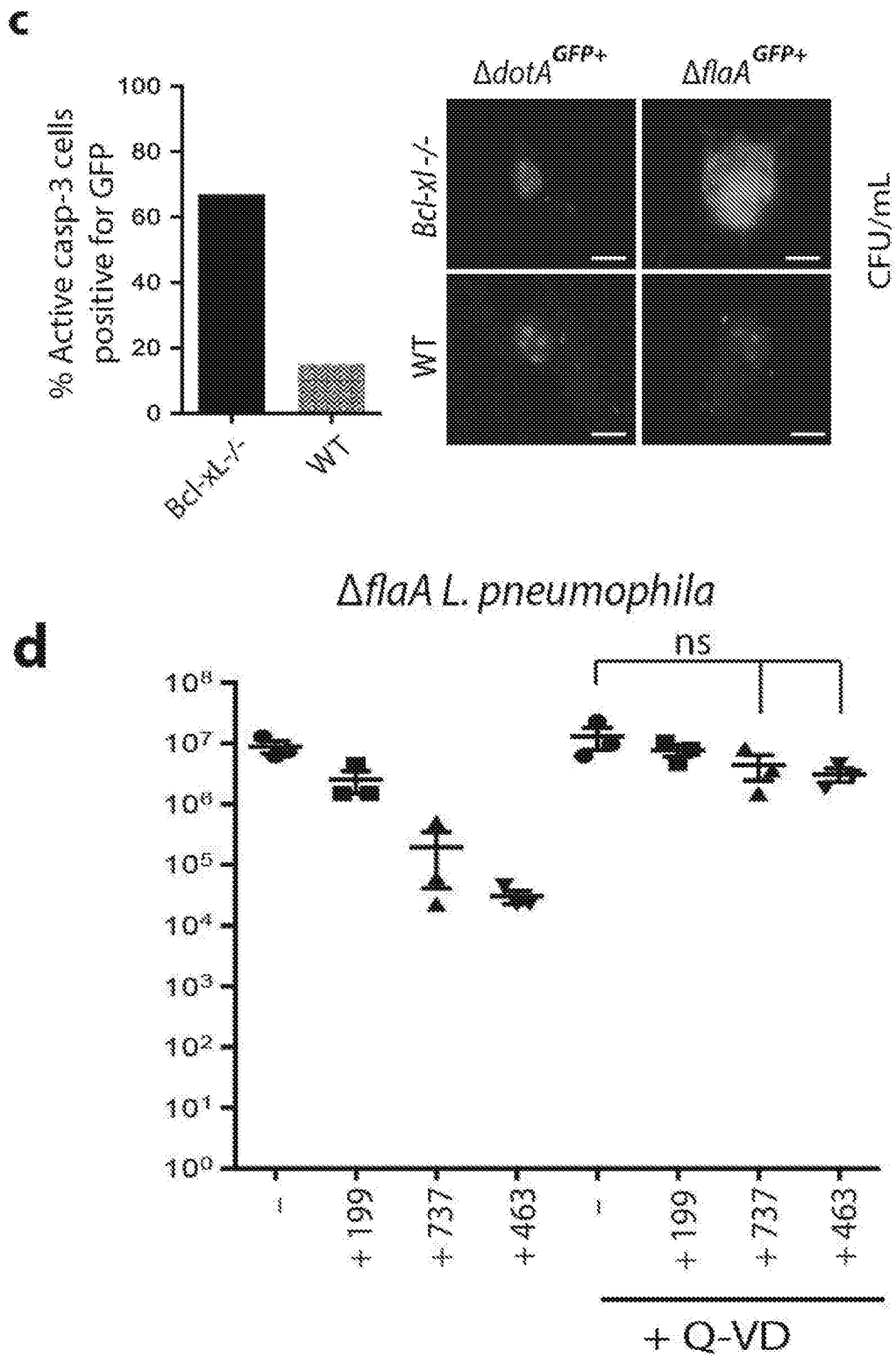


FIGURE 3c-d

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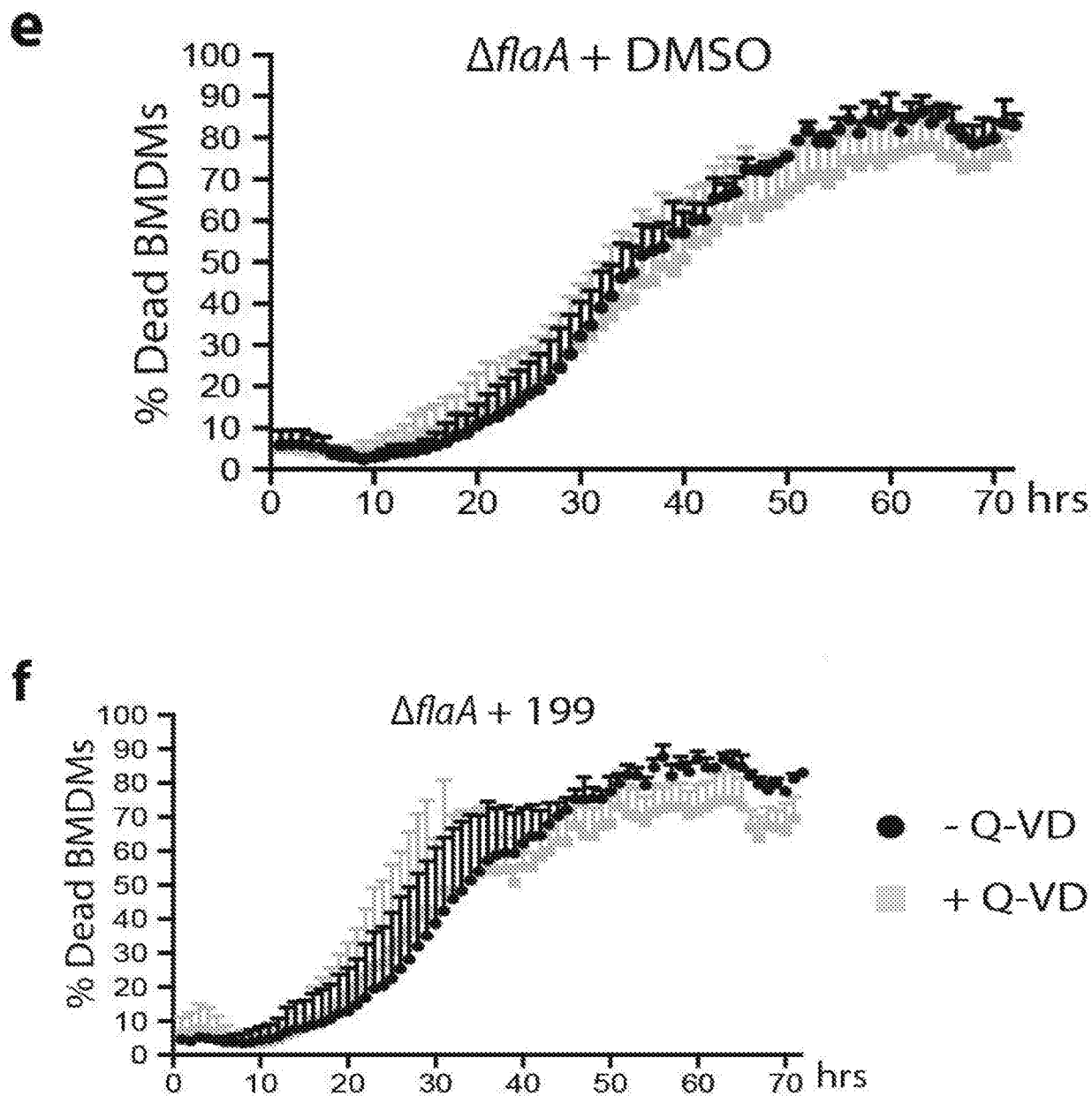


FIGURE 3e-f

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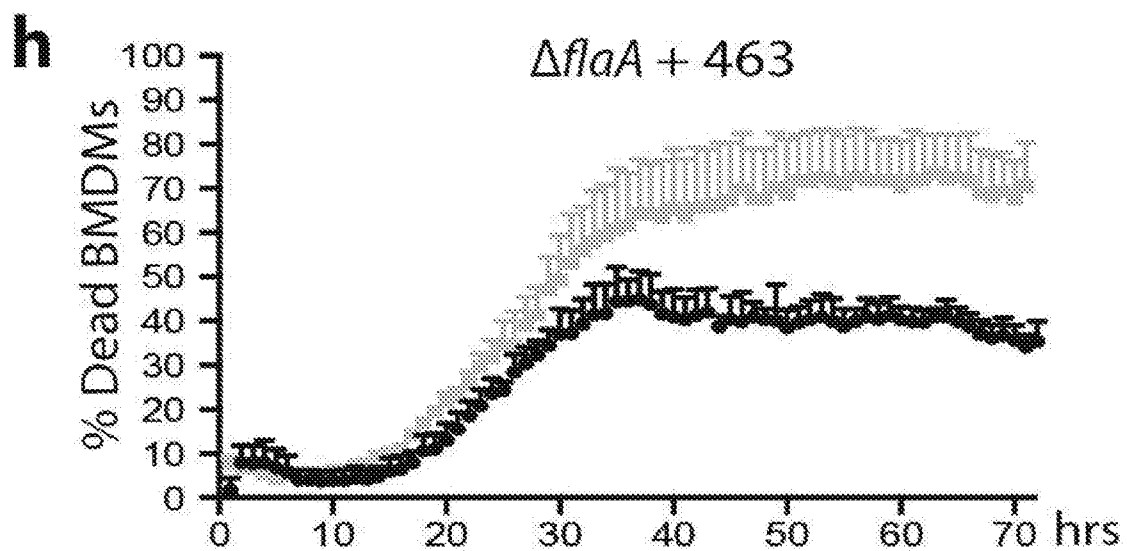
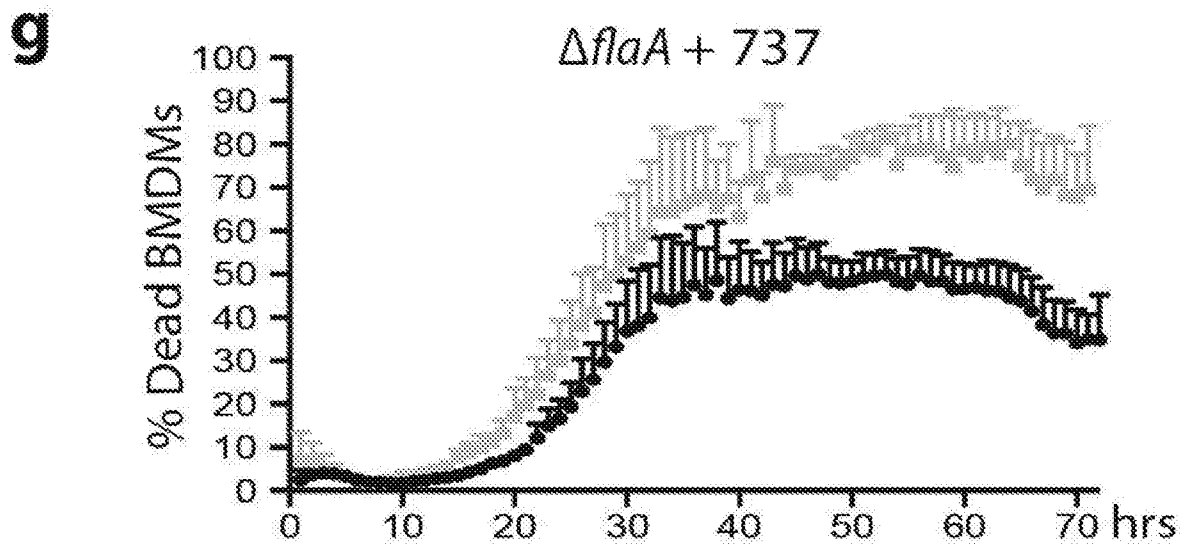
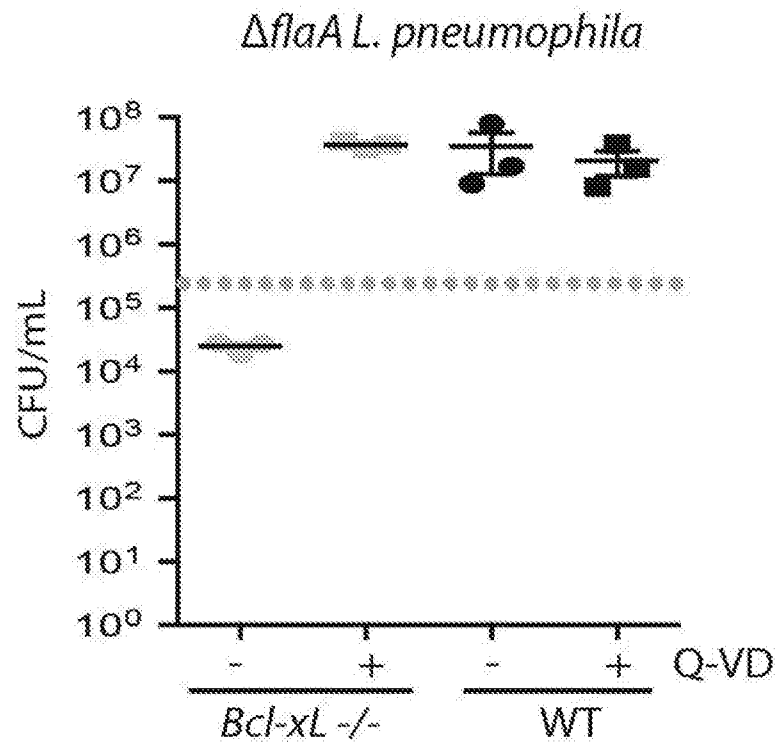


FIGURE 3g-h

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i



j

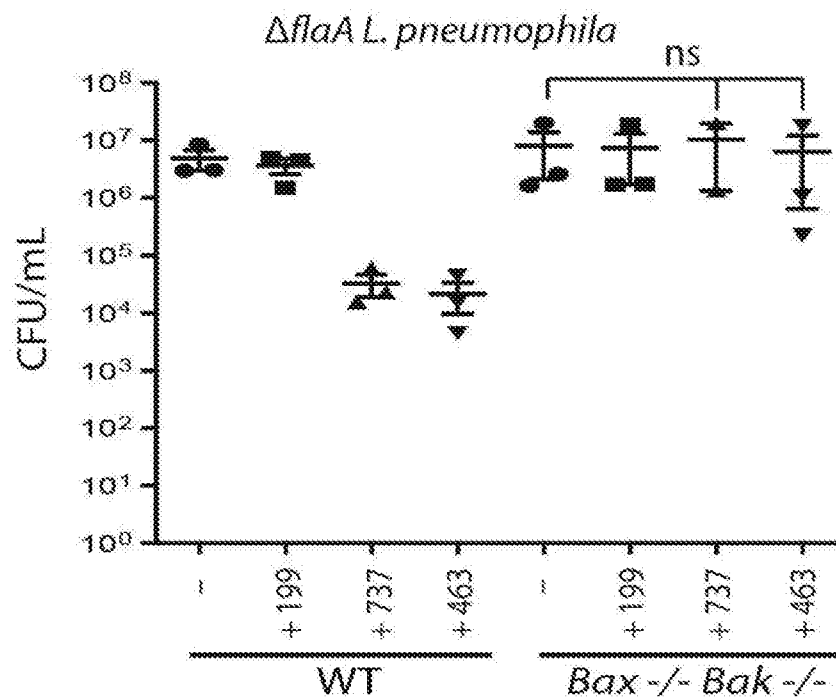
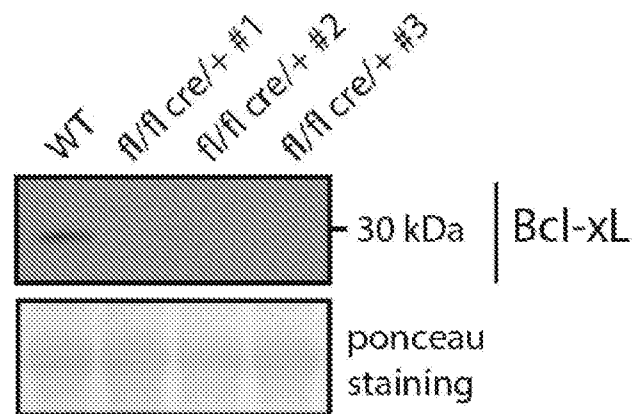
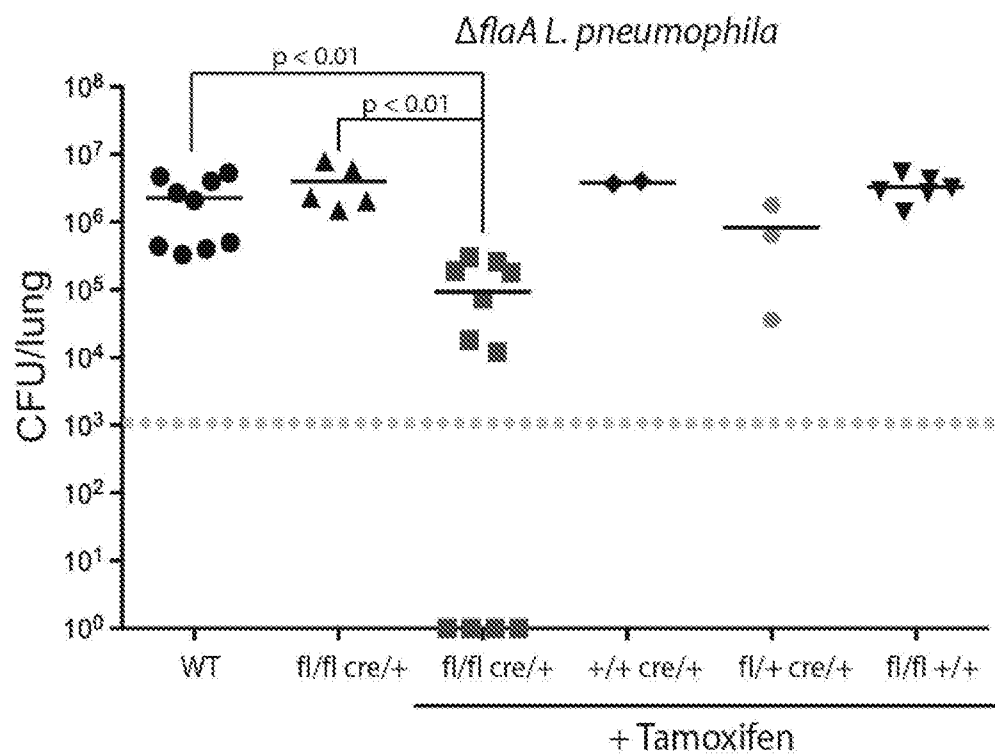


FIGURE 3i-j

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a**b****FIGURE 4a-b**

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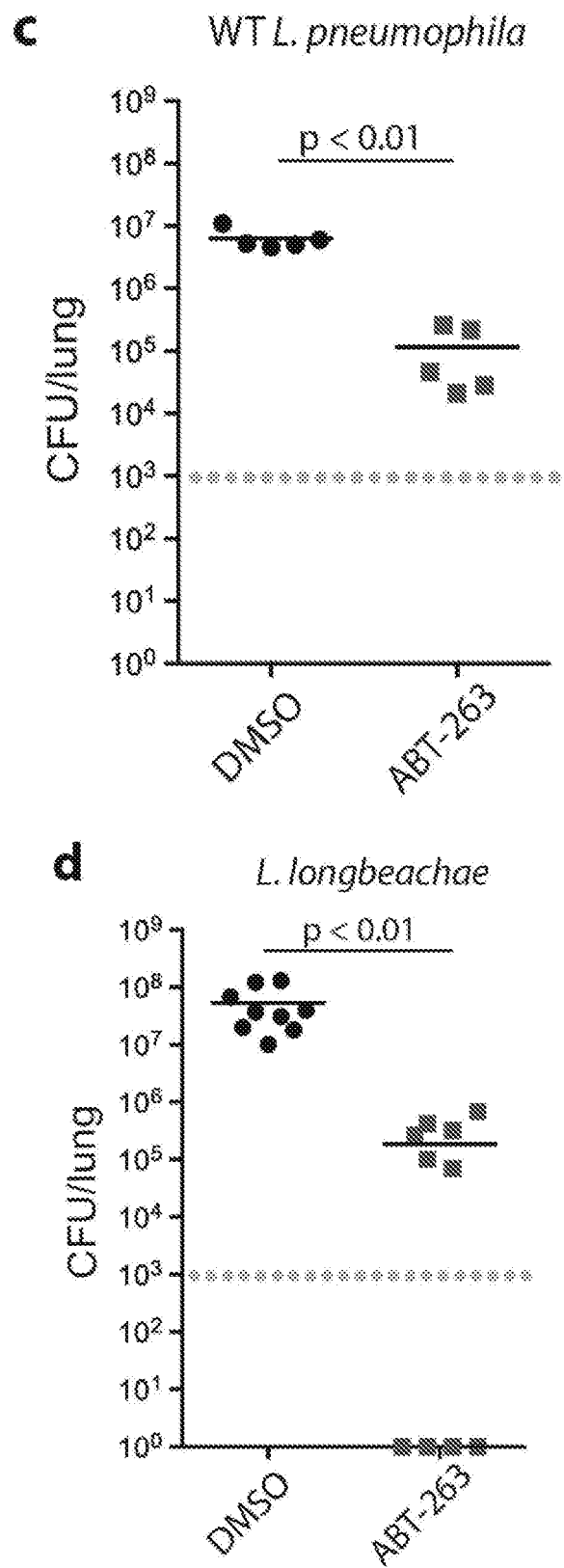


FIGURE 4c-d

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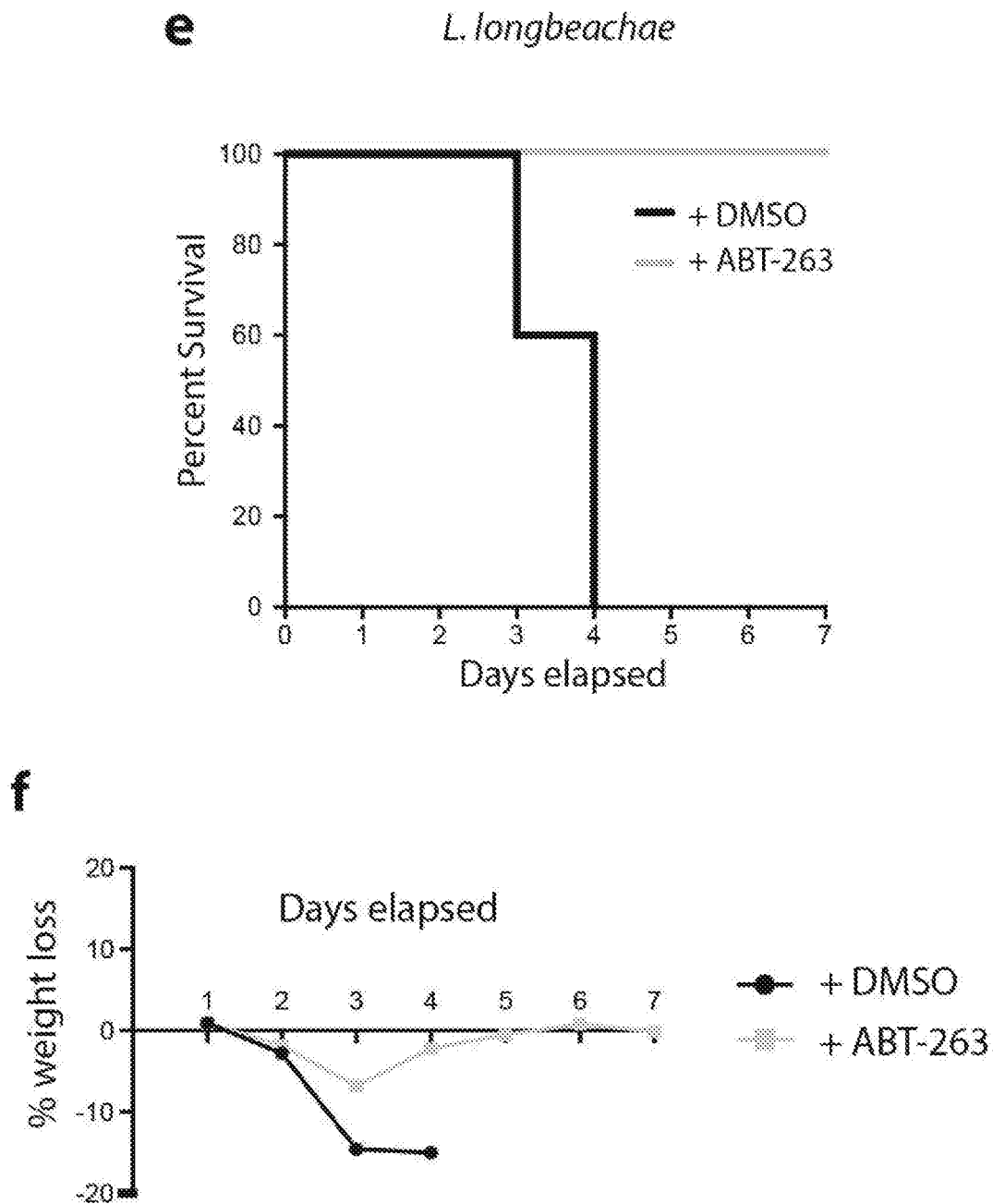
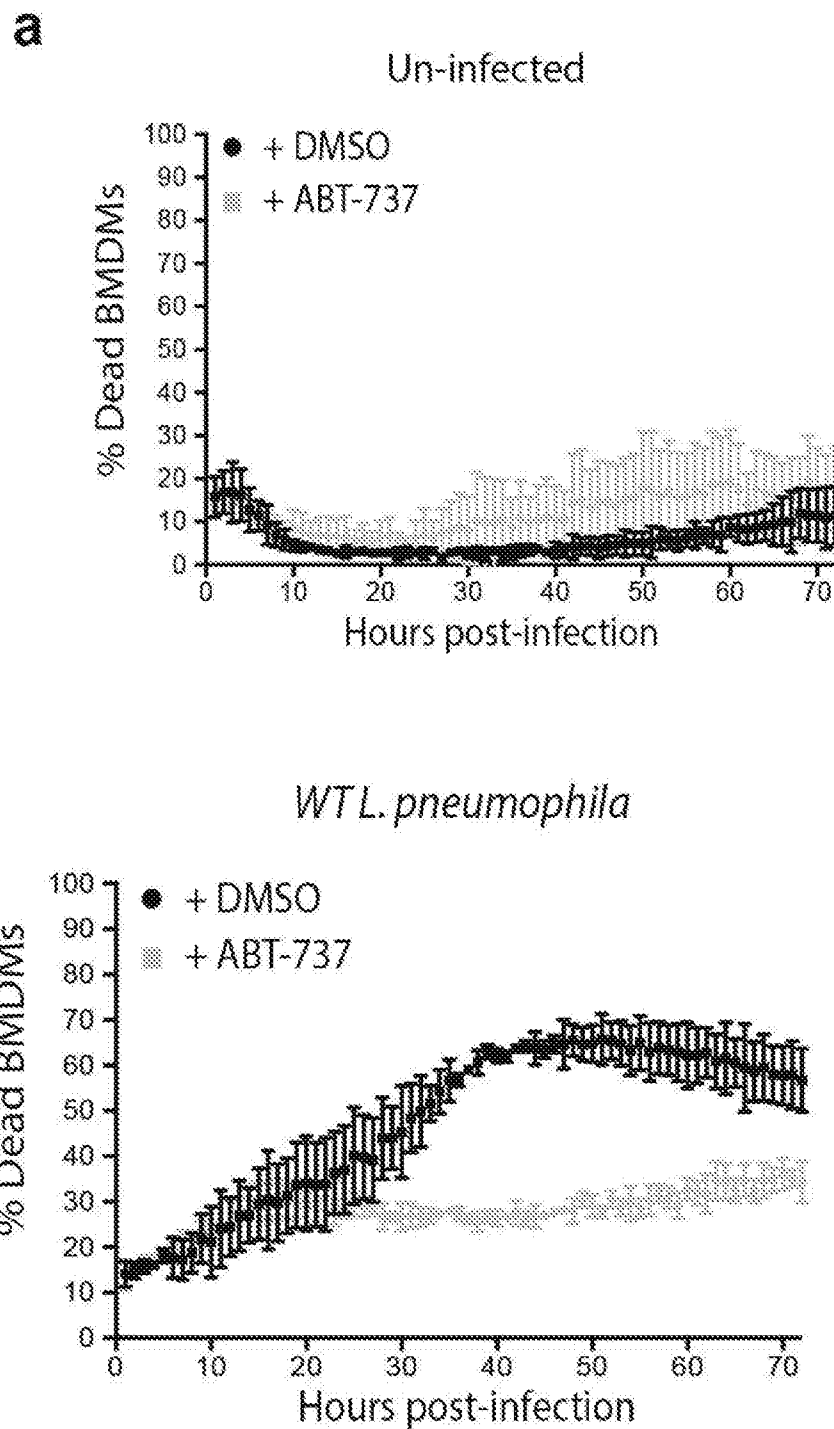
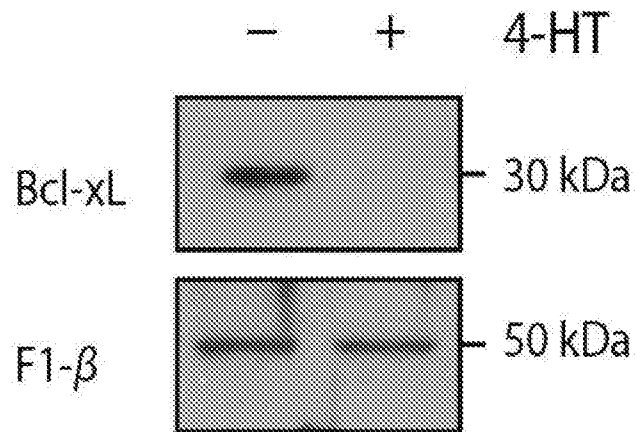
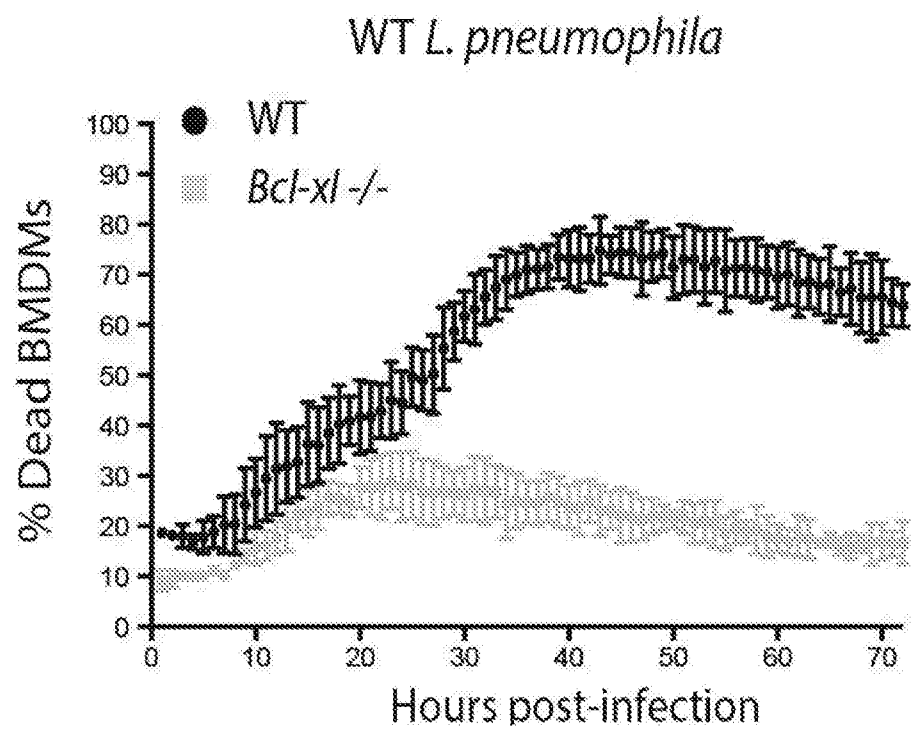


FIGURE 4e-f

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**FIGURE 5**

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a**b****FIGURE 6**

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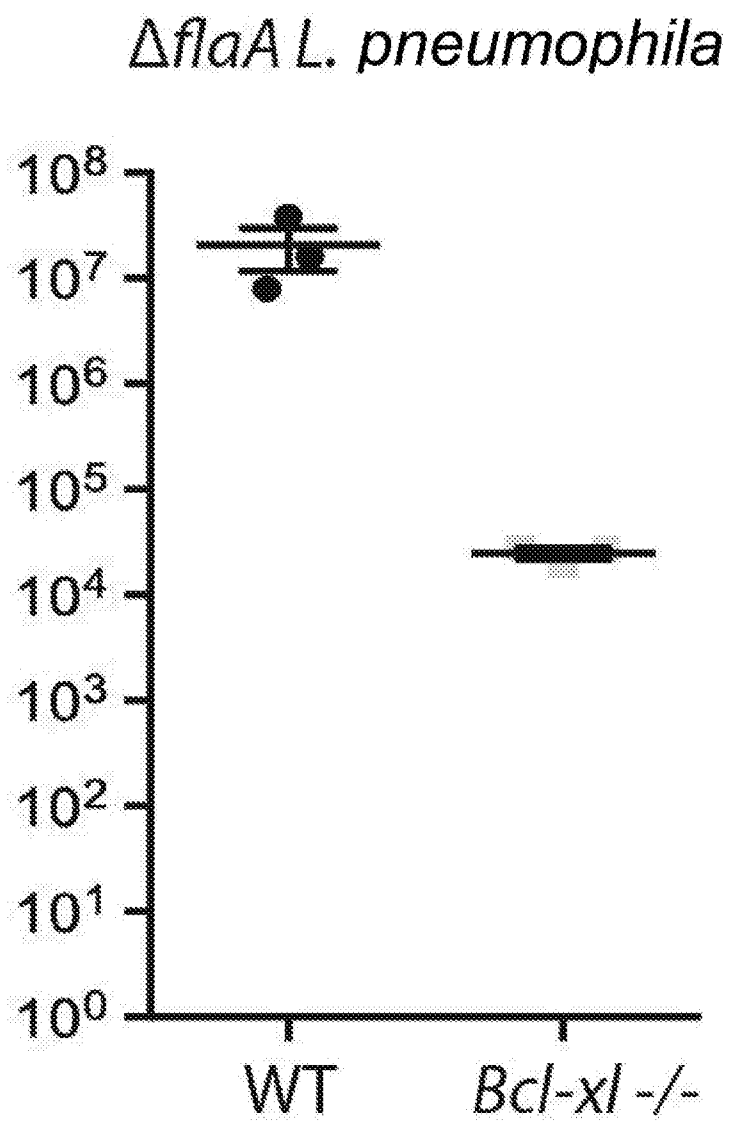


FIGURE 7

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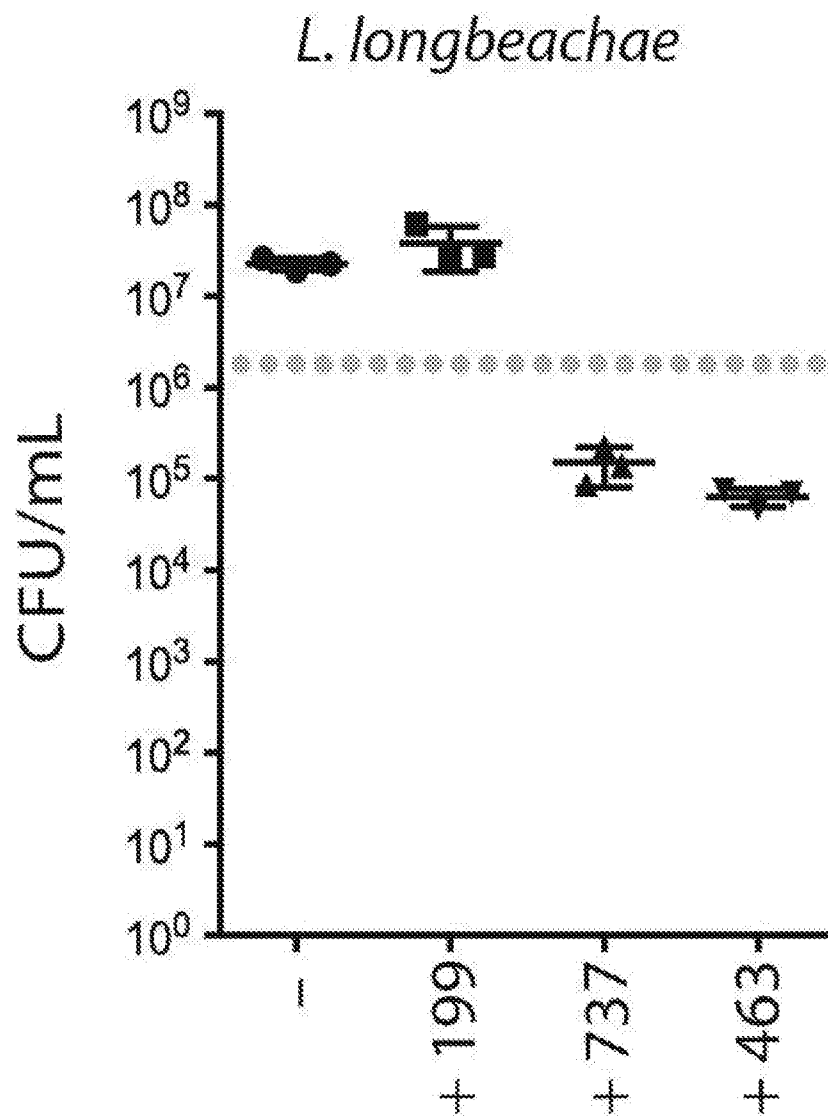


FIGURE 8

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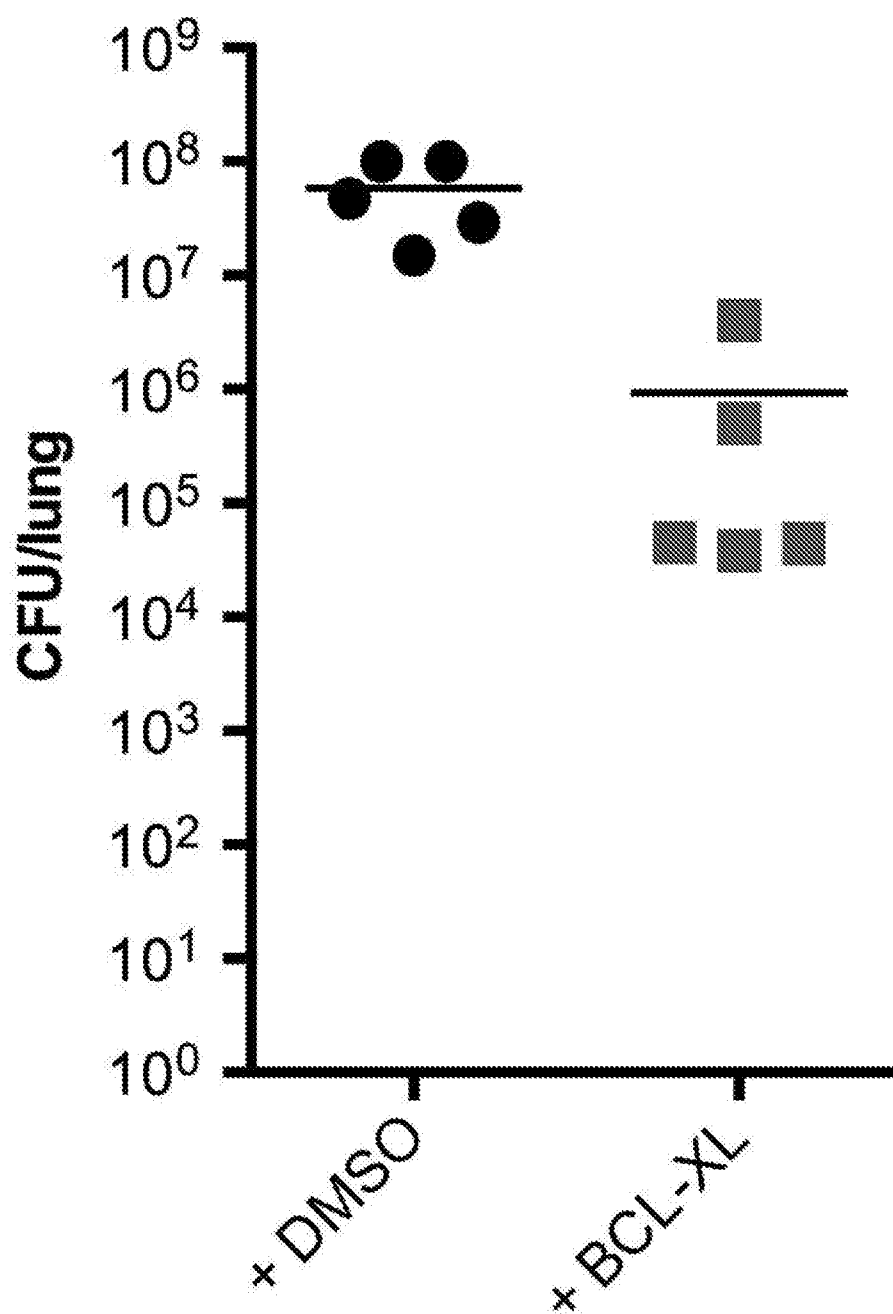


FIGURE 9

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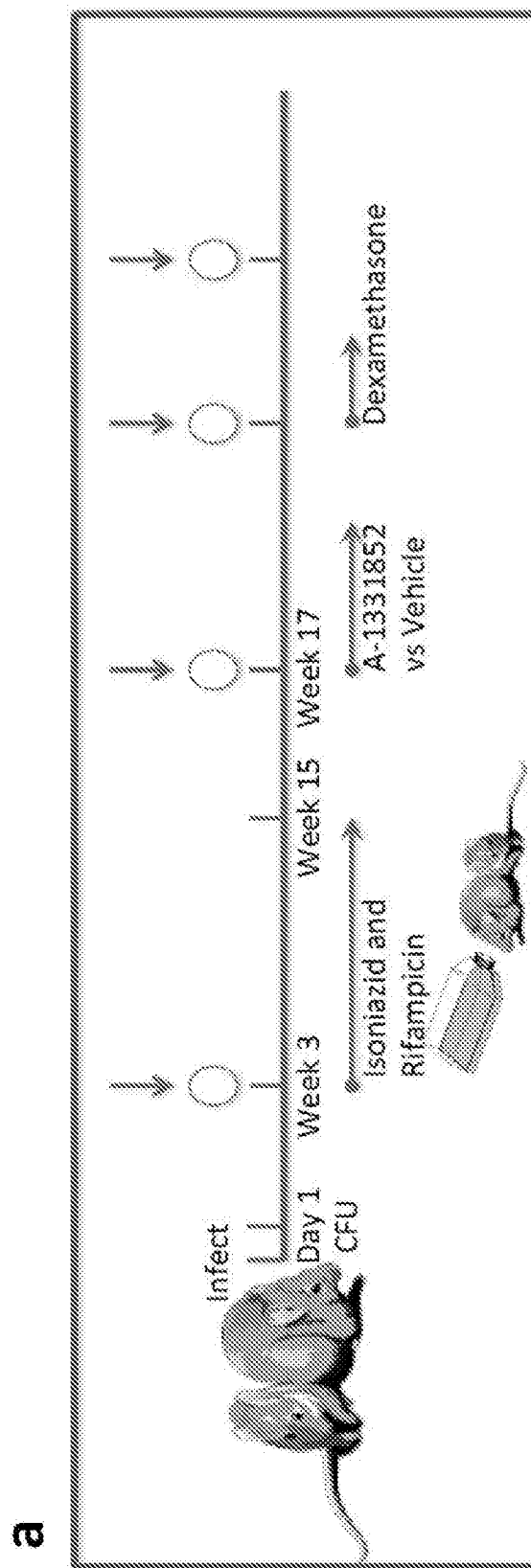


FIGURE 10a

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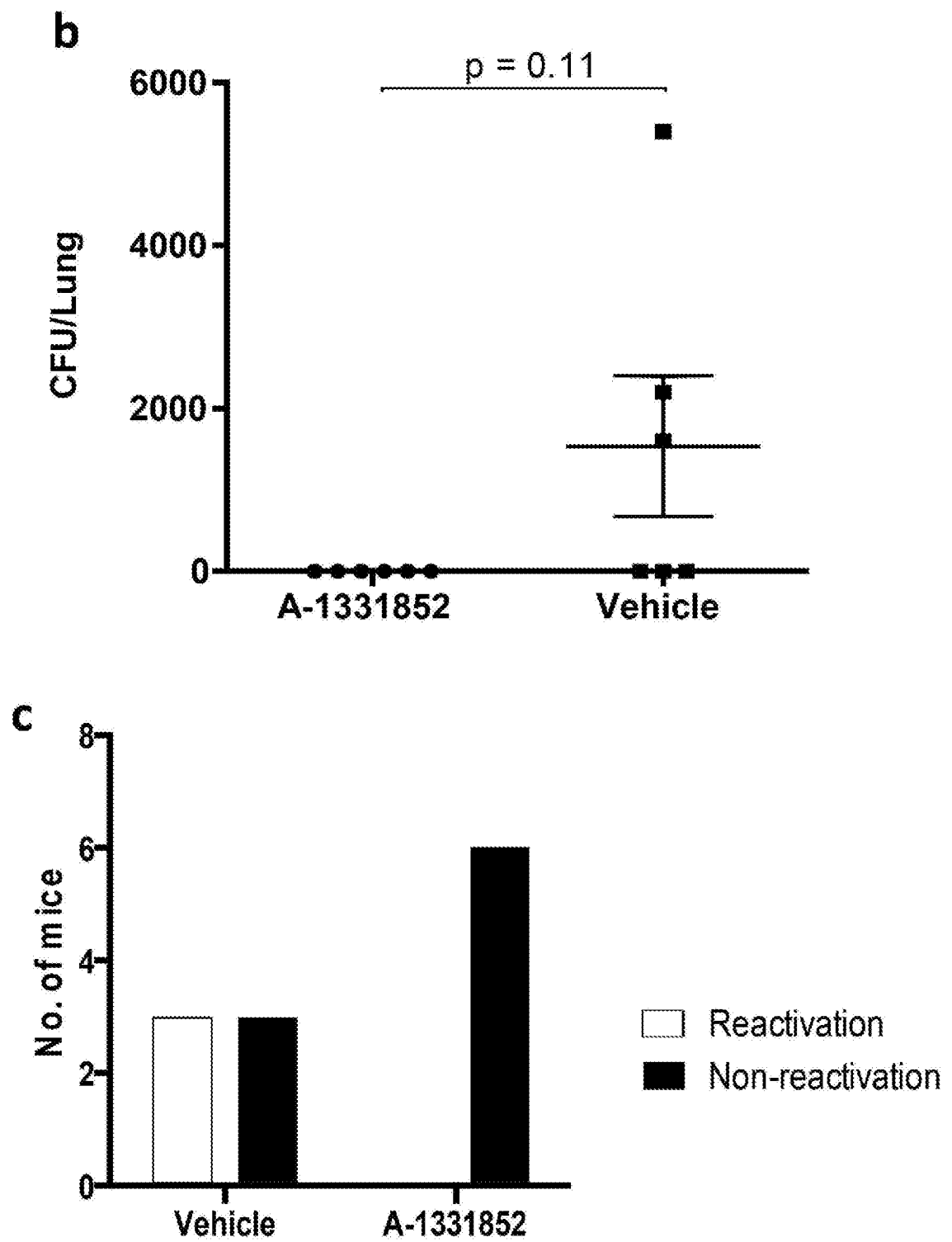


FIGURE 10b-c

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2016/050105

A. CLASSIFICATION OF SUBJECT MATTER

[See Supplemental Sheet]

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)

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 practicable, search terms used)

Applicant and Inventor Names searched at Patentscope and in internal databases provided by IP Australia

Epoque: WPIAP, Medline, Epodoc and keywords: Bcl-2 inhibitor, Bcl-XL inhibitor, A1 inhibitor, Mcl-1 inhibitor, BFL-1 inhibitor, Bcl-W inhibitor, BH3 mimetic, ABT 737, ABT 263, A 115463, BM 957, MIM-1, WEHI 539, genasense, G3139, venetoclax, ABT 199, obatoclax, GX 15070, bacteria, legionella, mycobacteria, salmonella, staphylococcus, pseudomonas, E coli, pneumonia, tuberculosis and related terms

STN: CAplus, Medline, EMBASE, Biosis: CAS numbers 852808-04-9, 923564-51-6, 1235034-55-5, 406228-55-5, 1391107-54-2, 509102-00-5, 475272-36-7, 1431866-33-9 and keywords: BH3 mimetic, ABT 737, ABT 263, A 115463, BM 957, MIM-1, WEHI 539, genasense, G3139, venetoclax, ABT 199, obatoclax, GX 15070, bacteria, legionella, mycobacteria, salmonella, staphylococcus, pseudomonas, E coli, pneumonia, tuberculosis and related terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 2 May 2016	Date of mailing of the international search report 02 May 2016
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Leah Walker AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262256170

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2016/050105

Supplemental Box – IPC Marks

*A61K 31/495 (2006.01)**A61K 31/5377 (2006.01)**A61K 31/47 (2006.01)**A61K 31/451 (2006.01)**A61K 31/496 (2006.01)**A61K 31/519 (2006.01)**A61K 31/426 (2006.01)**A61K 31/381 (2006.01)**A61K 31/428 (2006.01)**A61P 31/00 (2006.01)**A61P 31/12 (2006.01)**A61P 31/18 (2006.01)**A61P 31/16 (2006.01)**A61P 31/22 (2006.01)**A61P 31/10 (2006.01)**A61P 33/00 (2006.01)**A61P 31/06 (2006.01)*

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2016/050105
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/094708 A2 (DANA-FARBER CANCER INSTITUTE, INC.) 04 August 2011 See abstract, page 27 line 14 to page 28 line 28, page 37 line 5 to page 39 line 2, page 42 line 26 to page 43 line 3, Tables, especially Table 1-M on page 94 and Table 1-N on page 96	1-5, 12-29
X	WO 2010/068684 A2 (DANA FARBER CANCER INSTITUTE, INC.) 17 June 2010 See abstract, page 2 line 7 to page 5 line 10, page 29 lines 3-18	1-5, 18, 19, 21-29
X	LUCAS, C.D. et al. "Downregulation of Mcl-1 has anti-inflammatory pro-resolution effects and enhances bacterial clearance from the lung" Mucosal Immunology (2014) vol. 7, no. 4, pages 857-868 See page 858 left column paragraph 2, right column lines 38-41	1-5, 12-15, 18, 21-23, 25
X Y	DHIMAN, R. et al. "Inhibition of bfl-1/A1 by siRNA inhibits mycobacterial growth in THP-1 cells by enhancing phagosomal acidification" Biochimica et Biophysica Acta (2008) vol. 1780, pages 733-742 See abstract, page 8733 right column last paragraph See abstract, page 8733 right column last paragraph	1-5, 12-15, 18, 21-29 1-5, 12-25, 27-29
X	KOZIEL, J. et al. "The role of Mcl-1 in S. aureus-induced cytoprotection of infected macrophages" Mediators of Inflammation (2013) pages 1-12 See abstract, page 1 left column first paragraph, paragraph bridging pages 6 and 7	1-5, 12-15, 18, 21-29
X Y	WO 2014/028381 A1 (ABBVIE, INC.) 20 February 2014 S. aureus is disclosed to be a major cause of pneumonia (see page 1 left column first paragraph). S. aureus is disclosed to be a major cause of pneumonia (see page 1 left column first paragraph).	26 1-5, 12-25, 27-29
X Y	WO 2013/055897 A1 (ABBVIE INC.) 18 April 2013 See abstract, page 4 line 11 to page 14 line 30 See abstract, page 4 line 11 to page 14 line 30	26 1-5, 12-25, 27-29
X Y	WO 2013/055895 A1 (ABBVIE INC.) 18 April 2013 See abstract, page 4 line 10 to page 20 line 19 See abstract, page 4 line 10 to page 20 line 19	26 1-5, 12-25, 27-29
X Y	WO 2010/080503 A1 (GENENTECH, INC. et al.) 15 July 2010 See abstract, paragraph [0012], Table 1 See abstract, paragraph [0012], Table 1	26 1-5, 12-25, 27-29
X Y	WO 2010/080478 A1 (GENENTECH, INC. et al.) 15 July 2010 See page 2 line 1 to page 141 line 2 See page 2 line 1 to page 141 line 2	26 1-5, 12-25, 27-29
X Y	WO 2007/040650 A2 (ABBOTT LABORATORIES) 12 April 2007 See abstract, paragraph [0012], Table 1 See abstract, paragraph [0012], Table 1	26 1-5, 12-25, 27-29
X Y	WO 2005/049594 A1 (ABBOTT LABORATORIES) 02 June 2005 See abstract, page 1 lines 1-16, page 2 line 5 to page 8 line 33 See abstract, page 1 lines 1-16, page 2 line 5 to page 8 line 33	26 1-5, 12-25, 27-29

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2016/050105
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X L	WO 2000/004901 A1 (THOMAS JEFFERSON UNIVERSITY) 03 February 2000 See abstract, page 65 lines 17-30 Lack of Unity a posteriori	26
X Y	LABELLE, J. L. "A stapled BIM peptide overcomes apoptotic resistance in hematologic cancers" The Journal of Clinical Investigation (2012) vol. 122, no. 6, pages 2018-2031 See abstract, Figure 1 See abstract, Figure 1	26 1-5, 12-19, 21-25, 27-29
X Y	WALENSKY, L. D. et al. "Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix" Science (2004) vol. 305, no. 5689, pages 1466-1470 See abstract, Figure 2 See abstract, Figure 2	26 1-5, 12-19, 21-25, 27-29
A	TSUJIMOTO, Y. "Role of Bcl-2 family proteins in apoptosis: apoptosomes or mitochondria?" Genes to Cells (1998) vol. 3, 697-707 See abstract and page 698 paragraph bridging left and right columns	
P,X	WO 2015/126912 A1 (H. LEE MOFFITT CANCER CENTER AND RESEARCH INSTITUTE, INC. & CHONGQING ZEIN PHARMACEUTICAL CO., LTD) 27 August 2015 See abstract, page 12 line 1 to page 26 line 20, page 38 lines 20-28, page 39 line 1 to page 41 line 17, page 41 line 32 to page 42 line 7, Example 3, Tables 2-A and 2-B, Example 4, Tables 3-A to 3-E	1-5, 12-17, 21-29

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
2-5, 27, 29 (entirely) and 1, 12-26, 28 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 2-5, 27, 29 (entirely) and 1, 12-26, 28 (partially) are directed to methods, uses, kits and compositions comprising BCL2 family inhibitors for treating bacterial infections. The feature of BCL2 family inhibitors for treating bacterial infections is specific to this group of claims.
- Claims 6, 7 (entirely) and 1, 12-26, 28 (partially) are directed to methods, uses, kits and compositions comprising BCL2 family inhibitors for treating viral infections. The feature of BCL2 family inhibitors for treating viral infections is specific to this group of claims
- Claims 8, 9 (entirely) and 1, 12-26, 28 (partially) are directed to methods, uses, kits and compositions comprising BCL2 family inhibitors for treating fungal infections. The feature of BCL2 family inhibitors for treating fungal infections is specific to this group of claims
- Claims 10, 11 (entirely) and 1, 12-26, 28 (partially) are directed to methods, uses, kits and compositions comprising BCL2 family inhibitors for treating protozoal infections. The feature of BCL2 family inhibitors for treating protozoal infections is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is BCL2 family inhibitors for treating infections. However this feature does not make a contribution over the prior art because it is disclosed in:

WO 2000/004901 A1

This document discloses that viruses have developed mechanisms to circumvent the normal regulation of apoptosis in virus-infected cells, and these mechanisms have implicated BCL-2, and therefore teaches the use of small molecule inhibitors of BCL-2 function to treat viral infection, to induce apoptosis of virally infected cells (see abstract, page 65 lines 17-30).

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2016/050105	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
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