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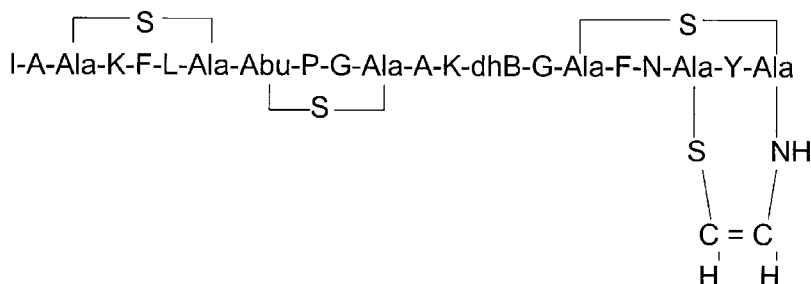
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FIGURE 1



(57) Abstract: The present invention lies in the field of the treatment and prophylaxis of infectious diseases. It relates to the prophylaxis and treatment of bacterial infections of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient, comprising the lantibiotic Gallidermin or a pharmaceutically active variant thereof as active substance, and related subject matters.

GALLIDERMIN TO TREAT INFECTIONS OF THE RESPIRATORY TRACT

FIELD OF THE INVENTION

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The present invention lies in the field of the treatment and prophylaxis of infectious diseases. It relates to the prophylaxis and treatment of bacterial infections of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient, comprising the lantibiotic Gallidermin or a pharmaceutically active variant thereof as active substance, and related subject matters.

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BACKGROUND OF THE INVENTION

15 Infectious diseases, especially those which are caused by gram-positive bacteria are still a severe danger for humans and animals. In the livestock industry they lead to severe losses. Important genera of gram-positive pathogenic bacteria are *Staphylococcus* and *Streptococcus*. For example the species *Staphylococcus aureus* and *Streptococcus pneumoniae* can cause life-threatening diseases of the lung, in
20 humans as well as in mammals like cattle and pigs. Other examples for critical pathogens of the human lung are *Haemophilus influenza* and *Moraxella catarrhalis*. Especially the growing rates of immunity against classical antibiotic treatments make it necessary to develop alternative concepts for therapies against such infections.

25 One possible route to address this aim may reside in the use of lantibiotics. This route has been chosen and further elaborated by the present invention.

Lantibiotics are a class of small peptidic, i.e. peptide or peptide-derived molecules that exert an antibiotic effect, especially on bacteria which effect can be bacteriostatic or
30 bacteriocidal. They are naturally produced by gram positive bacteria as gene encoded precursor peptides (e.g. *gdmA* as the gene for the Gallidermin precursor peptide) and undergo posttranslational modification of the primary transcript (prolantibiotic) to generate the mature, active peptide. Therefore many of them are characterized by significant posttranslational modifications, including the modification of amino acids
35 into unusual, sometimes even bridged thioether amino acids, like lanthionine and 3-

methyllanthionine. Members of this class include Subtilin, Nisin, Epidermin (= Staphylococcin 1580), Gallidermin (=Staphylococcin T; the leu-6 variant of Epidermin), Pep5, Ancovenin, Ro 09-0198, Cinnamycin and Duramycin. Pep5, Epidermin and Gallidermin are all naturally produced by microorganisms of the genus

5 Staphylococcus.

The modes of action exerted by the diverse lantibiotics are described by U.Pag and H.-G. Sahl (2002) in *Curr. Phar. Design*, vol. 8, 815-833. Lantibiotics are generally divided into two classes in respect to their mode of action: lantibiotics of class A (Nisin-
10 type) usually integrate into the plasma membrane and form pores which leads to a loss of low-molecular intracellular components and of the electric membrane potential. Lantibiotics of class B (Mersacidin-type or Cinnamycin-type) usually block the synthesis of peptidoglycan by binding tightly to the LIPID II and thus to a direct inhibition of the synthesis of the bacterial cell wall. The 4-ring lantibiotic Gallidermin,
15 like other 4-ring lantibiotics (Epidermin and Mutacin) is an example for a class A-type lantibiotic. Other examples are the 5-ring lantibiotics Nisin and Subtilin and the 3-ring lantibiotic Pep5.

The first descriptions of Gallidermin are the scientific publications from Kellner et al.,
20 *Eur. J. Biochem.*, 177:53-59 (1988) and from Schnell et al., *FEMS Microbiol. Lett.* 49:263-267 (1989). Today it is commonly accepted that Gallidermin comprises the structure that is represented by figures 1 and 2 of this application.

The natural variant of Gallidermin is described by Furmanek et al. (1999), *J. Appl. Microbiol.*, Vol. 87, 856-866. It exerts antimicrobial activity especially against the *Staphylococcus* species which could – as assumed by the authors – be used for the treatment of staphylococcal infections.

The use of Gallidermin for the treatment of bacterial infections of the skin is disclosed
30 by EP 342486 A1. There it is described as being particularly efficient against *Propionibacterium acnes*.

EP 427912 A1 discloses a method of inhibiting procaryotic microbial growth especially useful in the food industry which method involves adding a synergistically effective
35 combination of a lantibiotic and lysozyme to an environment in which such microbial

growth is to be inhibited. Preferred lantibiotics are selected from the group consisting of Nisin, Subtilin, Pep 5, Epidermin, Gallidermin, Cinnamycin, Ro09-0198, Duramycin or Ancovenin. According to a preferred method, the procaryotic microorganism is a gram-positive bacteria. Respective medical uses are not disclosed.

5

Pharmaceutical compositions containing bacteriocin are disclosed in WO 93/13793 A1. Such bacteriocins are selected from Nisin, Subtilin, Epidermin, Pep5, Ancovenin, Gallidermin, Duromycin and Cinnamycin. These compositions are described and/or claimed to be useful as antibacterial agents, especially with respect to infections of bacteria in the gastrointestinal tract of humans, e.g. infections caused by the gram-positive as well as gram-negative bacteria *Helicobacter*, *Escherichia*, *Salmonella*, *Bacillus*, *Clostridia*, *Bacteroides*, *Campylobacter* or *Yersinia*.

10

WO 94/28726 A2 describes pharmaceutical uses of the lantibiotic Duramycin, which facilitates the clearance of retained pulmonary secretions in a patient for example with cystic fibrosis. The lantibiotic is preferably administered topically to the respiratory epithelia, such as by generating an aerosol thereof which is then inhaled by the subject. Also disclosed is a method of combatting tuberculosis comprising administering a lantibiotic to a subject in need of such treatment. This is exemplified again by Duramycin.

15

20

The use of Gallidermin and/or Epidermin in the field of veterinary medicine is disclosed by WO 95/05844 A1. These lantibiotics are described to be useful for the prevention or treatment of bovine mastitis and for the sterilization of milk products without affecting microorganisms involved in the production of cheese and yoghurt. Relevant pathogens of mastitis are gram-positive *Streptococcus spp.* However, in the meantime Epidermin has found to be less active than Gallidermin in this technical field.

25

The use of lantibiotics (here called lanthocins) for killing antibiotic-resistant pathogenic bacteria, especially gram-positives is disclosed by WO 97/00694 A1. Claimed are especially Nisin, Subtilin, Epidermin, Gallidermin, Pep 5, Cinnamycin, Duramycin and Ancovenin, preferred Nisin, which is also supported by the examples.

30

Nisin is also described to be useful for preventing or treating infections by antibiotic-resistant or multidrug-resistant pathogenic bacterial strains of *Streptococcus*

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pneumoniae, by US patent US 5910479. The use of other lantibiotics like Gallidermin is also claimed but not exemplified. However, in the meantime Nisin has found to be less active than Gallidermin in this technical field.

- 5 The scientific publication from Kovács et al. (2006) in *J. Bacteriol.*, 188 (16), 5797-5805 provides information about the biochemical activity of cationic antimicrobial peptides like Nisin and Gallidermin and a way of resistance of bacteria against their activity. The authors describe that the incorporation of D-alanine in teichoic acids of Gram-positive bacteria like *S. pneumoniae* helps to protect them against the
10 destruction of their cells walls by Nisin or Gallidermin.

The biotechnological production of lantibiotics like Gallidermin is described by EP 342658 A1 and EP 543195 A2. They solve the problem of the posttranslational modifications of the respective peptides, especially of transforming the amionoacid
15 side chains into the structures which characterize the respective molecule by providing host cells which are able to perform these modifications. Recent application WO 2007/093548 A1 discloses methods for the production of lantibiotics, especially of Gallidermin which are based on the biotechnological production of Gallidermin and further comprised by appropriate downstream purification steps.

20

Though lantibiotics have been described in the state of the art as antibacterial agents in general there is still little experience in the selection of specific effects that could be exploited for certain pharmaceutical applications.

- 25 Additionally there is just little experience if lantibiotics can be used for the treatment of infections of the respiratory tract at all and how such a treatment could be facilitated.

The only example for a medical lantibiotic treatment of pulmonary diseases seems to be the disclosure of the above discussed application WO 94/28726 A2 describing the
30 use of Duramycin for the clearance of retained pulmonary secretions and of infections of *Mycobacterium tuberculosis*. The biochemical activity of Duramycin can be described as an inhibition of Phospholipase A2 which leads to a reduced water resorption from mucus and therefore to better secretion. However, the activity of Duramycin against gram-positive bacteria is not optimal and it is desirable to identify
35 lantibiotics with higher activity than Duramycin.

The scientific publication from Maher and McClean (2006) in *Biochem. Pharmacol.*, 71
(9), 1289-1298 describes a study of the effect of different antibacterial peptides
(Gallidermin, Vancomycin, Nisin A, Magainin I, Magainin II and Melittin) against
bacteria and of the cytotoxicity of these compounds against certain gastrointestinal
5 endothelial cells. These cells serve as a model system for the gastrointestinal tract.
The study aims at a treatment of clinical infections of the gastrointestinal tract. In this
specific environment, Gallidermin showed the best selective toxicity among the tested
peptides. However, this publication is silent about infections of other regions of the
patient's body, especially about the respiratory tract and does not provide any data
10 beyond the applied gastrointestinal cellular model.

Goldstein et al. (1998), *J. Antimicrob. Chemother.*, 42(2), 277-278 describe the activity
of a certain antibacterial peptide, i.e. of Nisin against *S. pneumoniae in vitro* and in a
mouse infection model. This model consists of a certain mouse strain that has been
15 infected intraperitoneally. This publication is also silent about infections of other
regions of the patient's body, especially about the respiratory tract and about the
efficacy of other antibacterial peptides.

For sake of completeness the use of Tobramycin should be mentioned here because
20 it is state of the art to use this agent for the treatment of infections of the respiratory
tract in the form of an aerosol. Chemically, Tobramycin belongs to the group of
aminoglycosids. However, this molecule exerts nephrotoxic and ototoxic side effects
which cause risks for the treatment of patients. It is therefore desirable to identify
antibiotic molecules for the treatment of infections of the respiratory tract which do not
25 exert these negative side effects.

From these explanations one can see that there is a need for further and alternative
therapies of bacterial infections caused by gram-positive bacteria and other
susceptible bacterial pathogens, especially those which show resistance against
30 known antibiotics or even a multiple resistance. This need exists especially for
infections of the respiratory tract of humans and of animals.

These infections lead to severe diseases of human patients as well as of animals, e.g.
bronchitis and pneumonia. In the field of veterinary medicine there is a major need for
35 cost efficient treatments of especially mammalian animals like dogs and cats, horses

as well as cattle and pigs which make up the largest and economically most important group of livestock animals.

5 SUMMARY OF THE INVENTION

As a solution for the above described needs, the present invention provides a pharmaceutical composition for the prophylaxis or treatment of a bacterial infection of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient, comprising Gallidermin or a pharmaceutically active variant thereof as active substance.

A further aspect of the present invention concerns a combined product, comprising a pharmaceutical composition according to the invention and a technical apparatus which allows the application of metered doses of the pharmaceutical composition.

A further aspect of the present invention concerns the respective uses of Gallidermin or a pharmaceutically active variant thereof as active substance for the manufacture of a pharmaceutical composition for the prophylaxis or treatment of a bacterial infection of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient.

A further aspect of the present invention concerns the respective methods for the prophylaxis or treatment of a bacterial infection of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient, wherein the method comprises administration of Gallidermin or a pharmaceutically active variant thereof as active substance to said patient.

A further aspect of the present invention concerns the respective uses of Gallidermin or a pharmaceutically active variant thereof as active substance for the prophylaxis or treatment of a bacterial infection of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient.

These and other aspects of the present invention are described herein literally, by examples and/or by reference to the following figures.

BRIEF DESCRIPTION OF THE DRAWINGS**Figure 1**

- 5 Structure of Gallidermin; Abbreviations of aminoacids:

Abbreviation	Meaning
I	isoleucine
A or Ala	alanine
K	lysine
F	phenylalanine
L	leucine
Abu	2-aminobutyric acid
P	proline
G	glycine
Dhb	2,3-dehydro-2-aminobutyric acid
N	asparagine
Y	tyrosine
- S -	thioether
- NH -	imino group
- CH = CH -	ethylene group

Figure 2

- 10 Chemical structure of Gallidermin

DETAILED DESCRIPTION OF THE PRESENT INVENTION

- 15 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as those commonly understood by one of ordinary skill in the art to which the invention pertains. Generally, the procedures for cell culture, infection, protein purification, molecular biology methods and the like are common methods used in the art. Such techniques can be found in reference manuals such as, for example,

Sambrook *et al.* (2001, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory Press); Ausubel *et al.* (1994, Current Protocols in Molecular Biology, Wiley, New York) and Coligan *et al.* (1995, Current Protocols in Protein Science, Volume 1, John Wiley & Sons, Inc., New York).

5

Nucleotide sequences are presented herein by single strand, in the 5' to 3' direction, from left to right, using the one letter nucleotide symbols as commonly used in the art and in accordance with the recommendations of the IUPAC-IUB Biochemical Nomenclature Commission (*Biochemistry*, 1972, vol. 11; pages 1726-1732).

10

All values and concentrations presented herein are subject to inherent variations acceptable in biological science within an error of $\pm 10\%$. The term "about" also refers to this acceptable variation.

15

One aspect of the present invention provides a pharmaceutical composition for the prophylaxis or treatment of a bacterial infection of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient, comprising Gallidermin or a pharmaceutically active variant thereof as active substance.

20

The term *Gallidermin* is illustrated by figures 1 and 2 of this application, both showing the wildtype molecule of Gallidermin as produced naturally by *Staphylococcus gallinarum* (DSM 4616) and disclosed in EP 342486 A2. In the case of doubt figures 1 and 2 of this present application define the wild type molecule Gallidermin according to the present invention.

25

It should be noted that the term Gallidermin according to the invention also comprises variants of Gallidermin that are additionally characterized by point mutations in one, two or three positions of the molecule shown in figures 1 and 2 which counts 22 amino acids in its wildtype form, some of them modified as explained in the above mentioned literature. Point mutations leading to Gallidermin variants according to the invention comprise the deletion, the insertion or the substitution of – in total – up to three amino acids of the wildtype molecule. In this sense the molecule Epidermin is also a variant of Gallidermin and is thus comprised by the definition *Gallidermin* or a pharmaceutically active variant according to the invention because it differs from the

35

wildtype Gallidermin in only one position, i.e. I instead of L in position 6.

Further pharmaceutically active variants according to the invention are exemplified by the scientific publication from B.Ottenwälder et al. (1995) in *Appl. Environ. Microbiol.*,
5 Nov. 1995, pages 3894-3903. Preferred variants according to the invention are variants L6V, Dhb14Dha, A12L and Dhb14P, as described by Ottenwälder et al. They can be derived from the wildtype Gallidermin by the substitution (exchange) of leucin in position 6 against valine, of Dhb in position 14 against Dha (2,3-didehydroalanine), of alanine in position 12 against leucine and Dhb in position 14 against proline, and
10 combinations of up to three of these exchanges. According to the invention these substitutions can be combined with other sequence variations as long as the total number of variations in comparison to the wildtype Gallidermin is not larger than 3.

Methods for the biotechnological production of Gallidermin are disclosed in
15 EP 342658 A1, EP 543195 A2 and WO 2007/093548 A1 (see above). Gallidermin and pharmaceutically active variants thereof comprised by the invention can be produced accordingly. Further ameliorations of that process might be developed in the future and can consequently be exploited for the production of Gallidermin and pharmaceutically active variants thereof according to the pending application.
20 Sequence variations can be introduced as described by Ottenwälder et al. or analogously.

Without wishing to be bound by this theory it is believed in the context of the present application that Gallidermin and other active variants according to the invention like
25 Epidermin can be ascribed to type-A lantibiotics which act on the bacterial membrane by pore formation. It is believed that such a pore leads to an efflux of low-molecular intracellular components and the depolarization of the cytoplasmic membrane resulting in an instant termination of essential biosynthetic processes. Gram-negative and mammalian cells are usually less affected by this mechanism which makes
30 lantibiotics according to the invention especially suitable for the treatment of infections caused by gram-positive bacteria. The proof for the efficacy of this treatment is given in the examples of this application.

Furthermore, some other not gram-positive bacteria could be identified which are
35 susceptible for Gallidermin and/or Gallidermin variants according to the invention.

Examples for such infectious bacterial pathogens are *Haemophilus influenzae*, *Branhamella* (= *Moraxella*) *catarrhalis* and *Helicobacter* (= *Campylobacter*) *pylori* (see examples of this application). Pharmaceutical compositions for the prophylaxis or treatment of a bacterial infection by such susceptible bacteria are therefore also

5 comprised by the invention.

The term *pharmaceutical composition* refers to all compositions of chemical substances that are suitable for the application to a human or non-human patient and thus for the treatment or prophylaxis of a disease. Ingredients for pharmaceutical

10 compositions are in principal known from the state of the art or can be derived from the ongoing progress in this technical field. They all belong to the claimed area as far as they are designed for for the prophylaxis or treatment of a bacterial infection of the respiratory tract and comprise Gallidermin or a pharmaceutically active variant thereof as active substance.

15 Basically all pharmaceutically active or supportive substances known or under development in this technical field additional to the substance(s) according to the invention are referred to by this feature.

20 Suitable preparations for administering the active substance(s) according to the invention include tablets, capsules, suppositories, solutions, etc. Of particular importance according to the invention is the administration of the compounds according to the invention by inhalation (see below). The proportion of pharmaceutically active compound or compounds should be in the range from 0.05 to

25 90% by weight, preferably 0.1 to 50% by weight of the total composition.

Suitable tablets may be obtained, for example, by mixing the active substance(s) with known excipients, for example inert diluents such as calcium carbonate, calcium phosphate or lactose, disintegrants such as corn starch or alginic acid, binders such

30 as starch or gelatine, lubricants such as magnesium stearate or talc and/or agents for delaying release, such as carboxymethyl cellulose, cellulose acetate phthalate, or polyvinyl acetate. The tablets may also comprise several layers.

Coated tablets may be prepared accordingly by coating cores produced analogously to

35 the tablets with substances normally used for tablet coatings, for example collidone or

shellac, gum arabic, talc, titanium dioxide or sugar. To achieve delayed release or prevent incompatibilities the core may also consist of a number of layers. Similarly the tablet coating may consist of a number of layers to achieve delayed release, possibly using the excipients mentioned above for the tablets.

5

Syrups or elixirs containing the active substance(s) or combinations thereof according to the invention may additionally contain a sweetener such as saccharine, cyclamate, glycerol or sugar and a flavour enhancer, e.g. a flavouring such as vanillin or orange extract. They may also contain suspension adjuvants or thickeners such as sodium
10 carboxymethyl cellulose, wetting agents such as, for example, condensation products of fatty alcohols with ethylene oxide, or preservatives such as p-hydroxybenzoates.

Solutions are prepared in the usual way, e.g. with the addition of isotonic agents, preservatives such as p-hydroxybenzoates, or stabilisers such as alkali metal salts of
15 ethylenediamine tetraacetic acid, optionally using emulsifiers and/or dispersants, whilst if water is used as the diluent, for example, optionally organic solvents may optionally be used as solvating agents or dissolving aids, and transferred into injection vials or ampoules or infusion bottles.

20 Capsules containing one or more active substances or combinations of active substances may for example be prepared by mixing the active substances with inert carriers such as lactose or sorbitol and packing them into gelatine capsules.

Suitable suppositories may be made for example by mixing with carriers provided for
25 this purpose, such as neutral fats or polyethyleneglycol or the derivatives thereof.

Excipients which may be used include, for example, water, pharmaceutically acceptable organic solvents such as paraffins (e.g. petroleum fractions), vegetable oils (e.g. groundnut or sesame oil), mono- or polyfunctional alcohols (e.g. ethanol or
30 glycerol), carriers such as e.g. natural mineral powders (e.g. kaolins, clays, talc, chalk), synthetic mineral powders (e.g. highly dispersed silicic acid and silicates), sugars (e.g. cane sugar, lactose and glucose), emulsifiers (e.g. lignin, spent sulphite liquors, methylcellulose, starch and polyvinylpyrrolidone) and lubricants (e.g. magnesium stearate, talc, stearic acid and sodium lauryl sulphate).

35

The preparations are administered by the usual methods, preferably by inhalation (see below). For oral administration the tablets may contain, apart from or additionally to the abovementioned carriers, additives such as sodium citrate, calcium carbonate and dicalcium phosphate together with various additives such as starch, preferably potato starch, gelatine and the like. Moreover, lubricants such as magnesium stearate, sodium lauryl sulphate and talc may be used at the same time for the tableting process. In the case of aqueous suspensions the active substances may be combined with various flavour enhancers or colourings in addition to the excipients mentioned above.

The dosage of the compound(s) according to the invention is naturally greatly dependent on the route of administration and the disease to be treated. The compound(s) according to the invention can be used effectively in the μg range or above, for example in the gram range. Particularly when administered by a method other than inhalation, the compounds according to the invention may be given in higher doses (in the range from 1 to 1000 mg, for example, although this does not imply any limitation).

E.g. for inhalation a solution of a Gallidermin variant according to the invention can be prepared that comprises this active variant in a concentration of between 0.1 and 10 mg/ml, and increasingly preferred between 0.25 and 7.5 mg/ml, between 0.5 and 5 mg/ml, between 0.75 and 2.5 mg/ml, mostly preferred between 0.9 and 1.1 mg/ml (see below).

Such a solution preferably further comprises an appropriate buffer, preferably in the weak acid pH range between 4 and 7, preferably 5 and 6 pH units. Examples for appropriate buffers are weak organic acids plus a salt of that same acid, e.g. lactic acid, maleic acid or acetic acid, plus the respective salt of sodium, potassium, magnesium or calcium or a phosphate buffer.

Such a solution preferably further comprises stabilisers to protect it physically, chemically and/or biologically. Such stabilizers are in principal known from the state of the art. Stabilisation against physical effects like freezing and thawing can be reached by e.g. polymeric substances like polyols. Stabilisation against chemical effects is reached by molecules which protect the chemical and structural identity of the Gallidermin molecule. These are for example protease inhibitors like benzamidin

hydrochloride, Phenylmethylsulfonyl fluoride (PMSF) and derivatives of boric acids, and reducing agents like dithiothreitol (DTT), β -Mercaptoethanol, Cystein or Glutathion to stabilize the disulfide bridges.

- 5 If the endogenous surfactant production by the patient is affected by a disease, a pharmaceutical composition according to the invention can comprise one or more biocompatible surfactants to optimize the spreading of the active substance.

- 10 If the endogenous surfactant production by the patient is less severely affected by a disease, the pharmaceutical compositions according to the invention are preferably substantially free from surfactants, especially when they are designed for the administration by inhalation.

- 15 The term *patient* is to be understood in its broadest sense and thus includes all animals that comprise a respiratory tract that can be infected by bacteria. Preferably a patient according to the invention is a bird or a human or other mammal, more preferably human or a mammal that selected from the species of industrial importance like chicken or pig.

- 20 The term *respiratory tract* is to be understood in its broadest sense, comprising all organs that are involved in the oxygen supply and carbon dioxide detoxication of the body of a human or animal, esp. of a bird, mammal or human. The elements of the respiratory tract comprise nose, oral cavity, pharynx, larynx, trachea, bronchial tubes and the lungs.

- 25 The term *treatment* comprises all sorts of application of a pharmaceutical composition in order to inhibit the disease-causing activity of the relevant bacterial agent against the infected patient.

- 30 The term *prophylaxis* comprises all sorts of treatment of a patient that are applied before an infection of the respiratory tract by the relevant bacterial agent has taken place in order to protect against such an infection.

- 35 In a preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that Gallidermin or the pharmaceutically active variant thereof

is the wildtype Gallidermin or a variant Gallidermin with one, two or three point mutations in comparison to the wildtype Gallidermin, preferably the wildtype Gallidermin, Gallidermin L6V, Gallidermin A12L, Gallidermin Dhb14P or Gallidermin Dhb14Dha.

5

Such preferred pharmaceutically active variants are disclosed for example by the above discussed publication from Ottenwälder et al. Therein, Gallidermin L6V is described as a variant with an enhanced antimicrobial activity. Gallidermin A12L, Gallidermin Dhb14P are disclosed as variants with high resistance against proteolytic degradation. Gallidermin Dhb14Dha also exerts antimicrobial activity and is additionally characterized by a higher sensitivity against tryptic cleavage.

10

According to the present invention, the most effective Gallidermin variants against certain subsets of bacterial populations are selected in different scenarios of infections with respect to the genus and/or species of the bacteria causing the infection, to the species of the patient and/or to the exact part of the respiratory tract to be treated.

15

In a preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that the infected respiratory tract comprises at least partially the lung.

20

As defined above the elements of the respiratory tract comprise nose, oral cavity, pharynx, larynx, trachea, bronchial tubes and the lungs.

Because especially infections of the lung can lead to acute life threatening diseases it is highly important to protect the patient against such an infection and/or to treat such an infection efficiently. Additionally such infections can be epidemic in certain populations, which further stresses the importance that a pharmaceutical composition according to the invention be at hand in short time.

25
30

Examples for such infections are influenza epidemics. For example the bacterial species *Haemophilus influenzae*, formerly called Pfeiffer's bacillus or *Bacillus influenzae*, is a non-motile gram-negative coccobacillus first described in 1892 by Richard Pfeiffer during an influenza pandemic.

35

Another example of such life-threatening, human epidemic infections mostly of the lung is tuberculosis which is caused by gram-positive *Mycobacterium tuberculosis*. Related species infect e.g. bovine (*M. bovis*) and poultry (*M. avium-intracellulare*).

- 5 Another gram-negative infectious bacteria genus is *Branhamella* (=Moraxella), esp. the species *Branhamella catarrhalis* which can cause e.g. laryngitis, bronchitis, pneumonia or bronchopneumonia.

- The efficiency of Gallidermin against *Haemophilus influenzae* as well as against
10 *Branhamella* (=Moraxella) *catarrhalis* is demonstrated by example 9 of this application thus proving the susceptibility even of gram-negative bacteria against Gallidermin. Example 10 proves an antibiotic effect of Gallidermin against *Mycobacterium tuberculosis*.

- 15 In a preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that the gram-positive bacteria or other susceptible bacterial pathogens belong to a genus selected from *Bacillus*, *Branhamella* (=Moraxella), *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Propionibacterium*, *Haemophilus*, *Listeria*, *Micrococcus*, *Neisseriaceae*, *Mycobacterium*, *Helicobacter* or *Mycoplasma*,
20 specifically to a species selected from *Branhamella catarrhalis* (=Moraxella *catarrhalis*), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Corynebacterium diphtheriae*, *Haemophilus influenza*, *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Mycoplasma pneumoniae*, *Mycoplasma mycoides*, *Mycoplasma hyopneumoniae*, *Mycoplasma gallisepticum* or *Mycoplasma hominis*.

25

In preferred embodiments multiple infections by more than one of these pathogens are treated at the same time, increasingly preferred the infectious by at least 2, 3, 4, 5, 6, 7, 8, 9 and 10 of these pathogens. The same applies to prophylactic treatments.

- 30 *Bacillus* species' comprise pathogenic and non-pathogenic gram-positive species'. Pathogenic bacillus species' are e.g. *B. anthracis* and *B. cereus*, the treatment of / protection against those species' therefore characterizes preferred embodiments of the pending application.

- 35 The treatment of / protection against *Haemophilus*, esp. *Haemophilus influenzae*,

Branhamella (=Moraxella), esp. *Branhamella* (=Moraxella) *catarrhalis* and Mycobacterium, esp. *Mycobacterium tuberculosis* has already been discussed above.

Another genus of relevant, gram-positive pathogenic infectious bacteria is

- 5 Staphylococcus. Staphylococcus species, esp. *S. aureus* can cause infections of the skin as well as of mucous membranes, esp. of the nose and of the pharynx. Other pathogenic species are e.g. *S. intermedius*, *S. epidermidis* and *S. saprophyticus*. The efficiency of Gallidermin against *Staphylococcus aureus* as a representative from this group of pathogens is demonstrated by the examples of this application.

10

Another genus of relevant, gram-positive pathogenic infectious bacteria is

Streptococcus. E.g. *S. pneumoniae* is a causative of human pneumonia; *S. pyogenes* is a causative of tonsillitis, pharyngitis, scarlet fever and rheumatoid fever; *S. agalactiae* can cause e.g. meningitis; *S. dysgalactiae* and *S. uberis* can cause

15 bovine mastitis; *S. milleri*, *S. mutans* and *S. salivarius* are causatives of infections in the oral cavity and of the teeth.

Another genus of relevant, gram-positive pathogenic infectious bacteria is

- Corynebacterium. Esp. the species *Corynebacterium diphtheriae* is known as a
- 20 pathogenic bacteria causing diphtheria, an infectious disease esp. of the tonsils, and the mucous membranes of the nose and the pharynx. *C. bovis* is known as another agent to cause bovine mastitis.

Further gram-positive pathogenic infectious bacteria, relevant for the pending

- 25 application are: Propionibacterium, Listeria (e.g. *L. monocytogenes*) and Micrococcus. Further gram-negative pathogenic infectious bacteria, relevant for the pending application are Neisseriaceae, esp. *N. catarrhalis* which can cause infections esp. of the mouth and pharynx.

30 Another genus of relevant susceptible, gram-negative pathogenic infectious bacteria is Helicobacter (formerly Campylobacter). Esp. the species *Helicobacter pylori* is a known causative as infections in humans and animals, esp. infections of the gastric mucous membrane. Examples 13 and 14 of this application prove the antimicrobial activity of Gallidermin against this pathogen. The minimal inhibition concentration

- 35 (MIC) and minimal bactericidal concentration (MBC) of Gallidermin against *H. pylori*

have been determined as being 8 µg/ml Gallidermin and 16 µg/ml Gallidermin, respectively. However more refined results might be possible with more sensitive methods. The shown assays are also applicable to the other pathogenes discussed above, if accomodated to the respective growing conditions of these diverse species.

5

According to the invention, the genus *Mycoplasma* is assigned to the bacteria, though it might also be classified separately, close to virus. This genus comprises pleomorphous microorganisms, with species sizes ranging between 125 nm and 150 µm. Mycoplasmas lack a cell wall but are characterized by a thin cell membrane (of usually about 10 nm) which is also susceptible to Gallidermin and Gallidermin variants according to the invention.

The species *Mycoplasma pneumoniae* is a causative of an atypical pneumoniae of humans. *Mycoplasma mycoides* is the causative of bovine pleuropneumonia which infection can lead to severe damages of cattle herds. Both, *M. pneumoniae* and *M. mycoides* are thus called pleuropneumonia-like organisms (PPLO). *Mycoplasma hyopneumoniae* is a swine pathogen, also infecting esp. the lungs. *Mycoplasma gallisepticum* causes subacute to chronic infections of the respiratory tract (sinusitis) of birds, esp. of chicken. *Mycoplasma hominis* is a related human pathogen, mainly infecting the throat or the urogenital tract.

Further exemplary pathogens of the respiratory tract effectively cobatted by a pharmaceutical according to the invention are Chlamydia spp. (on the one side *Ch. psittaci* = *Ch. ornithosis* and *Ch. trachomatis* as mainly human pathogens, and on the other side *Ch. abortus* and *Ch. pecorum* as mainly goat and sheep pathogens), Legionella spp. (*L. pneumophila*, *L. bozemanii*, *L. micdadei*, *L. dumoffii*) and Coxiella spp. (*C. burneti*).

In a preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that the patient to be treated is a bird or a mammal, preferably chicken (*Gallus gallus*), human (*Homo sapiens*), bovine (*Bos bovis*), pig (*Sus domesticus*), sheep (*Ovis*, esp. *Ovis aries*), goat (*Capra*, esp. *Capra aegagrus*), horse (*Equus*), cat (*Felidae*), dog (*Canidae*), more preferably human (*Homo sapiens*).

The non-human species selected here are of high economic importance which made it necessary to define alternative ways for treatment and prophylaxis against infections of strains that might no longer be susceptible to a treatment with other antibiotics. Untreated, such infections of livestock could lead to severe economical damage.

5

Highest priority, however, has the treatment and prophylaxis of humans. This preference is also due to the fact that several pathogenes exemplified above cause human diseases, alternative treatment and prophylaxis methods for which had to be found.

10

In a preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that is prepared to be administered topically to the lungs, preferably by inhalative administration, more preferred by the use of a technical apparatus which allows the application of metered doses of the pharmaceutical composition.

15

Topical application of Gallidermin or the selected Gallidermin variant according to the invention has got the advantage that this anti-microbial agent gets in direct contact to the infectious bacteria or that there is just a short distance of diffusion left for getting in contact with the bacteria. This allows that the total amount of this antimicrobial agent in the pharmaceutical composition to be applied can be relatively low in comparison to a composition administered orally because it is not diluted by the circulation the blood in the whole body. Additional side effects of the agent in the body of the patient are then kept relatively low.

25

As can be seen from examples 11 and 12 both intravenous and inhalative administration are possible ways to treat patients. However, in this case, the animals treated by inhalative administration showed less signs of side effects of the applied Gallidermin variant. Which makes this form of direct topical application more favorable.

30

Without wishing to be bound by this theory the difference in side-effect profile between intravenous and inhaled applications of Gallidermin seems to be in favor of a selective basophile stimulation instead of a mast cell stimulation. In healthy animals, basophiles stay in the blood while mast cells are located in the tissue. The lung plays the role of a

35

barrier against inhaled Gallidermin which therefore cannot reach the systemic circulation and does not stimulate the basophiles.

The inhalative administration method has been developed especially for the topical
5 application of agents to the lungs and can thus be appropriate for the administration of
Gallidermin or a pharmaceutically active variant thereof as active substance to the
organ to be treated. For this purpose the active substance has to be made available in
forms suitable for inhalation. Inhalable preparations according to the invention include
10 inhalable powders, propellant-containing metered dose aerosols or propellant-free
inhalable solutions. Inhalable powders according to the invention containing
Gallidermin or a pharmaceutically active variant thereof as active substance may
consist of the active substances on their own or of a mixture of the active substances
with physiologically acceptable excipients. Within the scope of the present invention,
the term carrier may optionally be used instead of the term excipient. Within the scope
15 of the present invention, the term propellant-free inhalable solutions also includes
concentrates or sterile inhalable solutions ready for use. The preparations according to
the invention may contain the active substances and optional further active
substances either together in one formulation or in two separate formulations. These
formulations which may be used within the scope of the present invention are
20 described in more detail below.

Inhalable powders according to the invention may contain Gallidermin or a
pharmaceutically active variant thereof as active substance on its own or in admixture
with suitable physiologically acceptable excipients.

25

If the active substance(s) are present in admixture with physiologically acceptable
excipients, the following physiologically acceptable excipients may be used to prepare
these inhalable powders according to the invention: monosaccharides (e.g. glucose or
arabinose), disaccharides (e.g. lactose, saccharose, maltose), oligo- and
30 polysaccharides (e.g. dextran), polyalcohols (e.g. sorbitol, mannitol, xylitol), salts (e.g.
sodium chloride, calcium carbonate) or mixtures of these excipients with one another.
Preferably, mono- or disaccharides are used, while the use of lactose or glucose is
preferred, particularly, but not exclusively, in the form of their hydrates.

Within the scope of the inhalable powders according to the invention the excipients have a maximum average particle size of up to 250 μm , preferably between 10 and 150 μm , most preferably between 15 and 80 μm . It may sometimes seem appropriate to add finer excipient fractions with an average particle size of 1 to 9 μm to the excipient mentioned above. These finer excipients are also selected from the group of possible excipients listed hereinbefore. Finally, in order to prepare the inhalable powders according to the invention, micronised active substance preferably with an average particle size of 0.5 to 10 μm , more preferably from 1 to 6 μm , is added to the excipient mixture. Processes for producing the inhalable powders according to the invention by grinding and micronising and by finally mixing the ingredients together are known from the prior art. The inhalable powders according to the invention may be prepared and administered either in the form of a single powder mixture which contain Gallidermin or a pharmaceutically active variant thereof as active substance and an optional further active substance together or in the form of separate inhalable powders which contain only one of them separately.

The inhalable powders according to the invention may be administered using inhalers known from the prior art. Inhalable powders according to the invention which contain one or more physiologically acceptable excipients in addition to the active substance according to the invention may be administered, for example, by means of inhalers which deliver a single dose from a supply using a measuring chamber as described in US 4570630A, or by other means as described in DE 3625685 A. The inhalable powders according to the invention which contain the active substance(s) optionally in conjunction with a physiologically acceptable excipient may be administered, for example, using the inhaler known by the name Turbuhaler[®] or using inhalers as disclosed for example in EP 237507 A. Preferably, the inhalable powders according to the invention which contain physiologically acceptable excipient in addition to the active substance(s) are packed into capsules (to produce so-called inhalettes) which are used in inhalers as described, for example, in WO 94/28958 A.

A particularly preferred inhaler for using the pharmaceutical combination according to the invention in inhalettes is shown in Figure 1 of WO 03/087097 A1 to which reference is hereby made. This inhaler (Handyhaler[®]) for inhaling powdered pharmaceutical compositions from capsules is characterised by a housing 1 containing two windows 2, a deck 3 in which there are air inlet ports and which is

provided with a screen 5 secured via a screen housing 4, an inhalation chamber 6 connected to the deck 3 on which there is a push button 9 provided with two sharpened pins 7 and movable counter to a spring 8, and a mouthpiece 12 which is connected to the housing 1, the deck 3 and a cover 11 via a spindle 10 to enable it to be flipped open or shut, as well as airholes 13 for adjusting the flow resistance.

If the inhalable powders according to the invention are packed into capsules (inhalers) for the preferred use described above, the quantities packed into each capsule should be 1 to 30 mg per capsule. These capsules contain the possible separate doses mentioned hereinbefore for each single dose.

In an alternative, not less preferred form **propellant gas-driven inhalation aerosols** containing the active substances according to the invention can be applied. Inhalation aerosols containing propellant gas according to the invention may contain the active substances dissolved in the propellant gas or in dispersed form. Gallidermin or a pharmaceutically active variant thereof as active substance and the optional further active substance may be present in separate formulations or in a single preparation, in which they are either both dissolved, both dispersed or only one component is dissolved and the other is dispersed. The propellant gases which may be used to prepare the inhalation aerosols according to the invention are known from the prior art. Suitable propellant gases are selected from among hydrocarbons such as n-propane, n-butane or isobutane and halohydrocarbons such as fluorinated derivatives of methane, ethane, propane, butane, cyclopropane or cyclobutane. The propellant gases mentioned above may be used on their own or in mixtures thereof. Particularly preferred propellant gases are halogenated alkane derivatives selected from TG11, TG12, TG134a (1,1,1,2-tetrafluoroethane) and TG227 (1,1,1,2,3,3,3-heptafluoropropane) and mixtures thereof, of which the propellant gases TG134a, TG227 and mixtures thereof are preferred.

The propellant-driven inhalation aerosols according to the invention may also contain other ingredients such as co-solvents, stabilisers, surfactants, antioxidants, lubricants and pH adjusters. All these ingredients are known in the art.

The inhalation aerosols containing propellant gas according to the invention may contain up to 5 wt.-% of each active substance. Aerosols according to the invention

contain, for example, 0.002 to 5 wt.-%, 0.01 to 3 wt.-%, 0.015 to 2 wt.-%, 0.1 to 2 wt.-%, 0.5 to 2 wt.-% or 0.5 to 1 wt.-% of each active substance.

If the active substances are present in dispersed form, the particles of active
5 substance preferably have an average particle size of up to 10 μm , preferably from 0.1 to 6 μm , more preferably from 1 to 5 μm .

The propellant-driven inhalation aerosols according to the invention mentioned above may be administered using inhalers known in the art (MDIs = metered dose inhalers).

10 Accordingly, in another aspect, the present invention relates to pharmaceutical compositions in the form of propellant-driven aerosols as hereinbefore described combined with one or more inhalers suitable for administering these aerosols. In addition, the present invention relates to inhalers which are characterised in that they contain the propellant gas-containing aerosols described above according to the
15 invention. The present invention also relates to cartridges fitted with a suitable valve which can be used in a suitable inhaler and which contain one of the above-mentioned propellant gas-containing inhalation aerosols according to the invention. Suitable cartridges and methods of filling these cartridges with the inhalable aerosols containing propellant gas according to the invention are known from the prior art.

20 In an alternative, not less preferred form **propellant-free inhalable solutions or suspensions** containing the active substances according to the invention can be applied.

25 Propellant-free inhalable solutions and suspensions according to the invention contain, for example, aqueous or alcoholic, preferably ethanolic solvents, optionally ethanolic solvents mixed with aqueous solvents. If aqueous/ethanolic solvent mixtures are used the relative proportion of ethanol compared with water is not limited but preferably the maximum is up to 70 percent by volume, more particularly up to 60
30 percent by volume of ethanol. The remainder of the volume is made up of water. The solutions or suspensions containing the active substance(s) according to the invention separately or together, are adjusted to a pH of 2 to 7, preferably 2 to 5, using suitable acids and/or the respective acids which have already formed an acid addition salt with one of the active substances. Of the organic acids, ascorbic acid, fumaric acid and
35 citric acid are preferred. If desired, mixtures of the above acids may be used,

particularly in the case of acids which have other properties in addition to their acidifying qualities, e.g. as flavourings, antioxidants or complexing agents, such as citric acid or ascorbic acid, for example.

- 5 According to the invention editic acid (EDTA) or one of the known salts thereof, sodium editate, can be added as a stabiliser or complexing agent.

Co-solvents and/or other excipients may be added to the propellant-free inhalable solutions according to the invention. The terms excipients and additives in this context
10 denote any pharmacologically acceptable substance which is not an active substance but which can be formulated with the active substance or substances in the pharmacologically suitable solvent in order to improve the qualitative properties of the active substance formulation. Preferably, these substances have no pharmacological effect or, in connection with the desired therapy, no appreciable or at least no
15 undesirable pharmacological effect. The excipients and additives include, for example, surfactants, stabilisers, complexing agents, antioxidants and/or preservatives which guarantee or prolong the shelf life of the finished pharmaceutical formulation, flavourings, vitamins and/or other additives known in the art. The additives also include pharmacologically acceptable salts such as sodium chloride as isotonic
20 agents.

The preferred excipients include antioxidants such as ascorbic acid, for example, provided that it has not already been used to adjust the pH, vitamin A, vitamin E, tocopherols and similar vitamins and provitamins occurring in the human body.
25

Preservatives may be used to protect the formulation from contamination with pathogens. Suitable preservatives are those which are known in the art, particularly cetyl pyridinium chloride, benzalkonium chloride or benzoic acid or benzoates such as sodium benzoate in the concentration known from the prior art. The preservatives
30 mentioned above are preferably present in concentrations of up to 50mg/100ml, more preferably between 5 and 20mg/100ml.

Preferred formulations contain, in addition to the solvent water and the active substance(s), only benzalkonium chloride and sodium editate. In another preferred
35 embodiment, no sodium editate is present.

The propellant-free inhalable solutions according to the invention are administered in particular using inhalers of the kind which are capable of nebulising a small amount of a liquid formulation in the therapeutic dose within a few seconds to produce an aerosol
5 suitable for therapeutic inhalation. Within the scope of the present invention, preferred inhalers are those in which a quantity of less than 100 μ l, preferably less than 50 μ l, more preferably between 20 and 30 μ l of active substance solution can be nebulised in preferably one spray action to form an aerosol with an average particle size of less than 20 μ m, preferably less than 10 μ m, in such a way that the inhalable part of the
10 aerosol corresponds to the therapeutically effective quantity.

An apparatus of this kind for propellant-free delivery of a metered quantity of a liquid pharmaceutical composition for inhalation is described for example in International Patent Application WO 91/14468 A and also in WO 97/12687 A (cf. in particular
15 Figures 6a and 6b). The nebulisers (devices) described therein are known by the name Respimat[®].

This nebuliser (Respimat[®]) can advantageously be used to produce the inhalable aerosols according to the invention containing the combination of active substance(s).
20 Because of its cylindrical shape and handy size of less than 9 to 15 cm long and 2 to 4 cm wide, this device can be carried at all times by the patient. The nebuliser sprays a defined volume of pharmaceutical formulation using high pressures through small nozzles so as to produce inhalable aerosols.

25 The preferred atomiser essentially consists of an upper housing part, a pump housing, a nozzle, a locking mechanism, a spring housing, a spring and a storage container, characterised by

- a pump housing which is secured in the upper housing part and which comprises at one end a nozzle body with the nozzle or nozzle arrangement,
- 30 - a hollow plunger with valve body,
- a power takeoff flange in which the hollow plunger is secured and which is located in the upper housing part,
- a locking mechanism situated in the upper housing part,
- a spring housing with the spring contained therein, which is rotatably mounted
35 on the upper housing part by means of a rotary bearing,

- a lower housing part which is fitted onto the spring housing in the axial direction.

The hollow plunger with valve body corresponds to a device disclosed in
5 WO 97/12687 A. It projects partially into the cylinder of the pump housing and is axially movable within the cylinder. Reference is made in particular to Figures 1 to 4, especially Figure 3, and the relevant parts of the description. The hollow plunger with valve body exerts a pressure of 5 to 60 Mpa (about 50 to 600 bar), preferably 10 to 60 Mpa (about 100 to 600 bar) on the fluid, the measured amount of active substance
10 solution, at its high pressure end at the moment when the spring is actuated. Volumes of 10 to 50 μ l are preferred, while volumes of 10 to 20 μ l are particularly preferred and a volume of 15 μ l per spray is most particularly preferred.

The valve body is preferably mounted at the end of the hollow plunger facing the valve
15 body.

The nozzle in the nozzle body is preferably microstructured, i.e. produced by microtechnology. Microstructured nozzle bodies are disclosed for example in WO 94/07607 A2; reference is hereby made to the contents of this specification,
20 particularly Figure 1 therein and the associated description.

The nozzle body consists for example of two sheets of glass and/or silicon firmly joined together, at least one of which has one or more microstructured channels which connect the nozzle inlet end to the nozzle outlet end. At the nozzle outlet end there is
25 at least one round or non-round opening 2 to 10 μ m deep and 5 to 15 μ m wide, the depth preferably being 4.5 to 6.5 μ m while the length is preferably 7 to 9 μ m.

In the case of a plurality of nozzle openings, preferably two, the directions of spraying of the nozzles in the nozzle body may extend parallel to one another or may be
30 inclined relative to one another in the direction of the nozzle opening. In a nozzle body with at least two nozzle openings at the outlet end the directions of spraying may be at an angle of 20 to 160° to one another, preferably 60 to 150°, most preferably 80 to 100°. The nozzle openings are preferably arranged at a spacing of 10 to 200 μ m, more preferably at a spacing of 10 to 100 μ m, most preferably 30 to 70 μ m. Spacings of

50 μm are most preferred. The directions of spraying will therefore meet in the vicinity of the nozzle openings.

5 The liquid pharmaceutical preparation strikes the nozzle body with an entry pressure of up to 600 bar, preferably 200 to 300 bar, and is atomised into an inhalable aerosol through the nozzle openings. The preferred particle or droplet sizes of the aerosol are up to 20 μm , preferably 3 to 10 μm .

10 The locking mechanism contains a spring, preferably a cylindrical helical compression spring, as a store for the mechanical energy. The spring acts on the power takeoff flange as an actuating member the movement of which is determined by the position of a locking member. The travel of the power takeoff flange is precisely limited by an upper and lower stop. The spring is preferably biased, via a power step-up gear, e.g. a helical thrust gear, by an external torque which is produced when the upper housing
15 part is rotated counter to the spring housing in the lower housing part. In this case, the upper housing part and the power takeoff flange have a single or multiple V-shaped gear.

20 The locking member with engaging locking surfaces is arranged in a ring around the power takeoff flange. It consists, for example, of a ring of plastic or metal which is inherently radially elastically deformable. The ring is arranged in a plane at right angles to the atomiser axis. After the biasing of the spring, the locking surfaces of the locking member move into the path of the power takeoff flange and prevent the spring from relaxing. The locking member is actuated by means of a button. The actuating button
25 is connected or coupled to the locking member. In order to actuate the locking mechanism, the actuating button is moved parallel to the annular plane, preferably into the atomiser; this causes the deformable ring to deform in the annular plane. Details of the construction of the locking mechanism are given in WO 97/20590 A.

30 The lower housing part is pushed axially over the spring housing and covers the mounting, the drive of the spindle and the storage container for the fluid.

When the atomiser is actuated the upper housing part is rotated relative to the lower housing part, the lower housing part taking the spring housing with it. The spring is
35 thereby compressed and biased by means of the helical thrust gear and the locking

mechanism engages automatically. The angle of rotation is preferably a whole-number fraction of 360°, e.g. 180°. At the same time as the spring is biased, the power takeoff part in the upper housing part is moved along by a given distance, the hollow plunger is withdrawn inside the cylinder in the pump housing, as a result of which some of the fluid is sucked out of the storage container and into the high pressure chamber in front of the nozzle.

If desired, a number of exchangeable storage containers which contain the fluid to be atomised may be pushed into the atomiser one after another and used in succession.

10 The storage container contains the aqueous aerosol preparation according to the invention.

The atomising process is initiated by pressing gently on the actuating button. As a result, the locking mechanism opens up the path for the power takeoff member. The biased spring pushes the plunger into the cylinder of the pump housing. The fluid leaves the nozzle of the atomiser in atomised form.

Further details of construction are disclosed in WO 97/12683 A and WO 97/20590 A, to which reference is hereby made.

20 The components of the atomiser (nebuliser) are made of a material which is suitable for its purpose. The housing of the atomiser and, if its operation permits, other parts as well, are preferably made of plastics, e.g. by injection moulding. For medical purposes, physiologically safe materials are used.

25 Figures 2a/b of WO 03/087097 A1 to which reference is hereby made show the nebuliser (Respimat®) which can advantageously be used for inhaling the aqueous aerosol preparations according to the invention. Figure 2a shows a longitudinal section through the atomiser with the spring biased while Figure 2b shows a longitudinal section through the atomiser with the spring relaxed. The upper housing part (51) contains the pump housing (52) on the end of which is mounted the holder (53) for the atomiser nozzle. In the holder is the nozzle body (54) and a filter (55). The hollow plunger (57) fixed in the power takeoff flange (56) of the locking mechanism projects partially into the cylinder of the pump housing. At its end the hollow plunger carries the valve body (58). The hollow plunger is sealed off by means of the seal (59). Inside the

30

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upper housing part is the stop (60) on which the power takeoff flange abuts when the spring is relaxed. On the power takeoff flange is the stop (61) on which the power takeoff flange abuts when the spring is biased. After the biasing of the spring the locking member (62) moves between the stop (61) and a support (63) in the upper
5 housing part. The actuating button (64) is connected to the locking member. The upper housing part ends in the mouthpiece (65) and is sealed off by means of the protective cover (66) which can be placed thereon. The spring housing (67) with compression spring (68) is rotatably mounted on the upper housing part by means of the snap-in lugs (69) and rotary bearing. The lower housing part (70) is pushed over
10 the spring housing. Inside the spring housing is the exchangeable storage container (71) for the fluid (72) which is to be atomised. The storage container is sealed off by the stopper (73) through which the hollow plunger projects into the storage container and is immersed at its end in the fluid (supply of active substance solution). The spindle (74) for the mechanical counter is mounted in the covering of the spring
15 housing. At the end of the spindle facing the upper housing part is the drive pinion (75). The slider (76) sits on the spindle.

An example for such a nebulizer (Respimat[®]) is also disclosed in Dalby, R., et al. (2004), *Int. J. Pharm.*, vol. 283, pages 1-9.

20

The nebuliser described above is suitable for nebulising the aerosol preparations according to the invention to produce an aerosol suitable for inhalation.

If the formulation according to the invention is nebulised using the method described
25 above (Respimat[®]) the quantity delivered should correspond to a defined quantity with a tolerance of not more than 25%, preferably 20% of this amount in at least 97%, preferably at least 98% of all operations of the inhaler (spray actuations). Preferably, between 5 and 30 mg of formulation, most preferably between 5 and 20 mg of formulation are delivered as a defined mass on each actuation.

30

However, the formulation according to the invention may also be nebulised by means of inhalers other than those described above, e.g. jet stream inhalers or other stationary nebulisers.

Accordingly, in a further aspect, the invention relates to pharmaceutical formulations in the form of propellant-free inhalable solutions or suspensions as described above combined with a device suitable for administering these formulations, preferably in conjunction with the Respimat®.

5

Accordingly the physicochemical parameter of the pharmaceutical compositions according to the invention, especially the viscosity of the respective solution or dispersion, and the technical design of the apparatus are co-ordinated to each other in order to allow the desired distribution of particle sizes. Such variations can be performed by a person skilled in the art of designing pharmaceuticals and/or pharmaceutical devices.

10

Accordingly to the explanations above, in a preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that the technical apparatus for the inhalative administration is selected from a dry powder inhaler or a nebulizer, preferably a nebulizer.

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Example 12 of the pending application illustrates the successful use of the apparatus Respimat® for the topical application of a composition according to the invention to guinea pigs as representatives of mammals, esp. of non-human patients.

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Accordingly to the explanations above, in a preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that it is prepared to be administered in the form of an inhalable powder with a maximum average particle size of up to 250 µm, preferably between 10 and 150 µm, most preferably between 15 and 80 µm or in the form of a micronised inhalable powder with an average particle size of 0.5 to 10 µm, more preferably from 1 to 6 µm.

25

Accordingly to the explanations above, in an equally preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that it is prepared to be administered in the form of propellant gas-driven inhalation solution or aerosol or in the form of a propellant-free inhalable solution or aerosol containing each active substance in a concentration of 0.002 to 5 wt.-%, and increasingly preferred 0.01 to 3 wt.-%, 0.015 to 2 wt.-%, 0.1 to 2 wt.-%, 0.5 to 2 wt.-%, and mostly preferred 0.5 to 1 wt.-% of each active substance.

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Accordingly to the explanations above, in an equally preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that it is prepared to be administered in the form of a propellant gas-driven inhalation

5 suspension or in the form of a propellant-free inhalable suspension containing the active substances in dispersed form with the particles of active substance in an average particle size of up to 10 μm , preferably from 0.1 to 6 μm , more preferably from 1 to 5 μm .

10 In a preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that the inhalative administration of Gallidermin or the pharmaceutically active variant thereof is to be administered to reach dissolved concentrations on the airway surface of the subject of from 0.125 to 256 $\mu\text{g/ml}$.

15 This concentration range has been found to be effective for treating the respiratory tract (examples 11 and 12) and applies especially to the organ to be treated, mostly preferred to the lung.

In a preferred embodiment a pharmaceutical composition according to the invention is
20 characterized by the fact that the therapeutically effective amount of Gallidermin or the pharmaceutically active variant thereof is to be administered between 0.01 and 1.2 mg per kg body weight of the patient.

This concentration range has been found to be effective for treating the respiratory
25 tract (examples 11 and 12).

In a preferred embodiment a pharmaceutical composition according to the invention is characterized by the fact that it comprises a second or further pharmaceutically active substance, preferably a further pharmaceutically active substance selected from the
30 group of antibacterial or antiviral substances, more preferred another pharmaceutically active variant of Gallidermin according to the invention.

The state of the art comprises a large quantity of pharmaceutical compositions for the treatment of the respiratory tract. Accordingly it is within the ambit of the invention to
35 combine the pharmaceutically active substance according to the invention with a

second or further pharmaceutically active substances to one pharmaceutical composition in order to apply them at once, whenever advantageous.

Such a combined pharmaceutical composition can be prepared by the manufacturer of
5 the fully formulated pharmaceutical or by a pharmacist shortly before the treatment or prophylaxis of the patient.

Preferably such a further pharmaceutically active substance is selected from the group of antibacterial or antiviral substances in order to broaden the scope of antimicrobial
10 activity of the pharmaceutical composition according to the invention. For example other antibiotics for combating infections of gram-positive, of gram-negative or of viruses can be used for such a combination. A combination therapy with different antibiotics, including Gallidermin or variants thereof is thus possible. This allows the treatment of multiple infection of the same organ, caused by more than one pathogen.
15 Also the combination of three or more antimicrobial activities can be advantageous.

Preferably such other agent is an peptide or enzyme with antibiotic or bacteriostatic activity, especially Lysozyme.

20 In another preferred mode such a further pharmaceutically active substance is selected from the group of the other pharmaceutically active variants of Gallidermin according to the invention. In doing so the specific advantages of the diverse variants of Gallidermin, esp. those defined above, can be combined with each other.

25 A further aspect of the present invention concerns a combined product, comprising a pharmaceutical composition according to the invention and a technical apparatus which allows the application of metered doses of the pharmaceutical composition.

All explanations made above for the pharmaceutical composition as well as for the
30 diverse technical apparatus' apply to this aspect of the present invention accordingly. It is intended to optimize the interaction of the pharmaceutical composition according to the invention (e.g. in the form of a dry powder or a solution) and of the apparatus (e.g. a dry powder inhaler or a nebulizer) in order to combat the infections of the respective part of the respiratory tract, esp. by the discussed bacteria effectively. For this purpose
35 all single features described above can be combined by a person skilled in the art.

A further aspect of the present invention resides in the use of Gallidermin or a pharmaceutically active variant thereof as active substance for the manufacture of a pharmaceutical composition for the prophylaxis or treatment of a bacterial infection of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient.

All explanations made above for the pharmaceutical composition apply to this aspect of the present invention accordingly.

A further aspect of the present invention resides in a method for the prophylaxis or treatment of a bacterial infection of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient, wherein the method comprises administration of Gallidermin or a pharmaceutically active variant thereof as active substance to said patient.

All explanations made above apply to this aspect of the present invention accordingly. For example a combined product can be applied for this method, which combined product comprises a pharmaceutical composition according to the invention and a technical apparatus which allows the application of metered doses of the pharmaceutical composition.

A further aspect of the present invention resides in the use of Gallidermin or a pharmaceutically active variant thereof as active substance for the prophylaxis or treatment of a bacterial infection of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient.

All explanations made above apply to this aspect of the present invention accordingly. For example a combined product can be applied for this use, which combined product comprises a pharmaceutical composition according to the invention and a technical apparatus which allows the application of metered doses of the pharmaceutical composition.

The following examples are intended to further explain the underlying invention without any limitation with respect to the scope of protection.

EXAMPLES

5 Example 1

Protection assay with epithelial cells

The effect of a lantibiotic like Gallidermin can be tested on epithelial cells like human epithelial cells, e.g. 293T cells (originally isolated from human kidney; available from
10 Invitrogen GmbH, Karlsruhe, Germany) or e.g. bovine epithelial cells. They can be cultivated in the medium Dulbecco's modified Eagle minimal essential Medium (DMEM; available from Invitrogen GmbH), plus 10% calf serum (CS); bovine epithelial cells can be cultivated in DMEM, plus 10% CS plus 5 µg/ml Insulin and 1 µg/ml hydrocortisone.

15

For the assays standard mikrotiter plates with 24 wells (working volume 1 ml) are to be covered with 10 µg/ml poly-lysine. Then cells are applied at a density of about 1×10^5 per well and incubated over night at 37°C. It is recommended to wash settled bovine epithelial cells at least once, around 1 h before infection with DMEM and cover them
20 with 1 ml invasion medium (DMEM plus insuline plus hydrocortisone, without CS). 293T cells do not have to be treated in this way before the infection.

Then these cells can be infected with infectious bacteria like *Staphylococcus aureus* that due to their life cycle invade the cells. Appropriate concentrations of these
25 bacteria can be chosen as e.g. a MOI (multiplicity of infection; relation of cells vs. infectious bacteria) of 2 or of 20 or any other value in between. This is followed by an invasion phase of about 2h. Then still extracellular bacteria in the supernatant are removed and fresh medium containing 20 µg/ml Lysostaphin and 50 µg/ml Gentamicin added in order to kill still remaining extracellular bacteria. This is followed by further
30 45 min of incubation. After removal, medium containing the lantibiotic to be tested, e.g. Gallidermin at diverse concentration rates (e.g. 50 µg/ml) in 1 ml of fresh medium is to be added to the test wells and the controls.

Then the cells can be harvested, e.g. for taking a time kinetics after 2, 4, 6 and 24 h
35 (or any other time value that seems to appropriate). For the harvesting the medium is

taken off and the cell lawn washed gently off with a solution of 1 % saponin (in distilled water). They are resuspended in 1 ml 1% saponin and incubated at 37°C for at least 2 h. Dilutions of these suspensions can be plated on Tryptic Soy Broth (TSB), agar plates;(Difco, BBL, Detroit, Michigan, USA) and then incubated for example over
 5 night at 37°C. On the next day (for the *hemB* mutants after 48 h) the value of CFU derived from the intracellularly surviving bacterial cells is determined. This can be interpreted as a value for the rate of effectiveness of the protection against the respective bacteria, exerted by the applied lantibiotic.

10

Example 2

Protection assay with human epithelial cells against an infection with *S. aureus*

According to the protocol of example 1 human epithelial cells 293T in several parallel
 15 arrays have been infected with a strain of *S. aureus* as an example of pathogenic gram-positive bacteria at concentration values of 2 and of 20 MOI. The analyzed lantibiotic Gallidermin has been applied at a concentration value of 50 µg/ml of the infection medium. Probes have been taken after 2, 4, 6 and 24h of incubation. The result of the time kinetics with the MOI of 20 is given in table 1; the result of the time
 20 kinetics with the MOI of 2 is given in table 2.

Table 1: Time kinetics of an infection of 293T cells by *S. aureus* with an MOI of 20, treated with 50 µg/ml Gallidermin (probe) and control without Gallidermin; values are given in 1,000,000 CFU of intracellularly
 25 surviving bacteria.

	control	probe
2h	175	-
4h	310	15
6h	330	28
24h	270	18

Table 2: Time kinetics of an infection of 293T cells by *S. aureus* with an MOI of 2, treated with 50 µg/ml Gallidermin (probe) and control without Gallidermin; values are given in 1,000 CFU of intracellularly surviving
 30

bacteria.

	control	probe
2h	7,5	-
4h	15,5	-
6h	8,5	0,3
24h	7	1,0

These experiments show that an incubation with Gallidermin significantly decreases
 5 the survival rate of gram-positive bacteria after the infection of human epithelial cells
 and subsequent treatment with Gallidermin (compare probe vs. control).

The kinetics reveal that this effect is maximal after in incubation with Gallidermin for 2
 to 6 h.

10

After 24 h of incubation with Gallidermin a small number of still surviving bacterial cells
 remained. However, this effect is also seen after incubation with other antibiotics like
 Ciprofloxacin, Tetracyclin, Vancomycin and Flucloxacillin (data not shown).

15

Example 3

Protection assay with human epithelial cells to test the adaptation of the infectious bacteria

20 As a modification of the experiment performed in example 2 it was now tested if the
 bacterial cells were able to acquire a resistancy against the Gallidermin treatment.

For this purpose, after 24 h incubation with (probe) and without (control) Gallidermin,
 the cells have been plated on agar plates containing 1, 2 or 4 µg/ml Gallidermin.

25

After incubation over night 200 colonies appeared on the probe plate containing
 1 µg/ml Gallidermin; the respective control showed a tight bacterial lawn. On the plates
 containing 2 or 4 µg/ml Gallidermin no bacterial growth could be detected.

30 This leads to the conclusion that a minor adaptation of *S. aureus* against the treatment

of Gallidermin takes place. But the development of a resistance could not be seen.

Example 4

5 Protection assay with bovine epithelial cells against an infection with *S. aureus*

Example 2 has been repeated with bovine epithelial MAC-T cells and with the same *S. aureus* strain as in example 2, which were applied at a concentration value of 20 and of 2 MOI. Again, Gallidermin has been applied at a concentration value of 50 µg/ml of the infection medium. Probes have been taken after 2, 4, 6 and 24h of incubation. The result of the time kinetics with the MOI of 20 is given in table 3, the result with the MOI of 2 is given in table 4.

15 **Table 3:** Time kinetics of an infection of MAC-T cells by the tested *S. aureus* strain with an MOI of 20, treated with 50 µg/ml Gallidermin (probe) and control without Gallidermin; values are given in 1,000,000 CFU of intracellularly surviving bacteria.

	control	probe
2h	200	25
4h	275	90
6h	325	145
24h	180	75

20 **Table 4:** Time kinetics of an infection of MAC-T cells by the tested *S. aureus* strain with an MOI of 2, treated with 50 µg/ml Gallidermin (probe) and control without Gallidermin; values are given in 1,000 CFU of intracellularly surviving bacteria.

	control	probe
2h	1300	45
4h	1700	340
6h	2450	770
24h	1200	250

This result confirms the results of the therapeutic and protective effect exerted onto human cells, as seen in example 2. After an infection with an MOI of 20 after 2h of incubation around 88% of invaded *S. aureus* cells are eliminated; the respective
5 elimination values for 4, 6 and 24h can be calculated as around 67%, 55% and 58%, respectively.

The respective values for an MOI of 2 can be estimated as 97%, 80%, 69% and 79%, respectively. Which means that the efficiency of the Gallidermin treatment is higher at
10 smaller infection rates.

As a conclusion one can state that Gallidermin is also effective in the protection of bovine epithelial cells.

15

Example 5

Protection assay with human epithelial cells against an infection with a highly intracellularly persisting *S. aureus* strain

20 From clinical diagnosis certain mutants of *S. aureus* are known which are phenotypically characterized by the growth in small colonies and an up to sixfold extended generation time. They are characterized by mutations in the electron transport chain, e.g. in the gene *hemB*. However, they can cause severe infections because they are able to resist in host cells without destroying them (Proctor, R.A.,
25 and Peters, G.; 1998: "Small colony variants in staphylococcal infections: diagnostic and therapeutic implications"; *Clin. Infect. Dis.*, vol. 27, 419-422). They are also characterized by a decreased sensitivity against the treatment with antibiotics, for example against aminoglycosides and Trimethoprim/Sulfamethoxazol (von Eiff, C., Proctor, R.A., and Peters, G.; 2000: "Staphylococcus aureus small colony variants:
30 formation and clinical impact"; *Int. J. Clin. Pract. Suppl.*, 44-49), leading to chronically persisting infections.

For this example a *hemB*-deletion strain of *S. aureus* (described in von Eiff, C., Heilmann, C., Proctor, R.A., Woltz, C., Peters, G., and Gotz, F.; 1997: "A site-directed
35 Staphylococcus aureus hemB mutant is a small-colony variant which persists

intracellularly"; *J. Bacteriol.*, vol. 179, 4706-4712) has been used: Strain I10 which is derived from wildtype strain 8325-4.

Now example 2 has been repeated, with the use of human epithelial cells 293T and
 5 *S. aureus* I10, at a concentration values of 20 MOI. Gallidermin has been applied at a concentration value of 50 µg/ml of the infection medium. Probes have been taken again after 2, 4, 6 and 24h of incubation. The result of the time kinetics is given in table 5.

10 Table 5: Time kinetics of an infection of 293T cells by *S. aureus* I10 with an MOI of 20, treated with 50 µg/ml Gallidermin (probe) and control without Gallidermin; values are given in 1,000 CFU of intracellularly surviving bacteria.

	control	probe
2h	70	10
4h	82	38
6h	100	52
24h	72	18

15

From this experiment one can see that an incubation with Gallidermin significantly decreases the survival rate of highly intracellularly persisting gram-positive bacteria after the infection of human epithelial cells and subsequent treatment with Gallidermin (compare probe vs. control).

20

In this case the maximum relative effect has been reached after 24h of incubation.

Example 6

25 Biofilm assay

This test is an adapted version of the one described in Rachid, S., Ohlsen, K., Wallner, U., Hacker, J., Hecker, M., and Ziebuhr, W. (2000): Alternative transcription factor sigma(B) is involved in regulation of biofilm expression in a *Staphylococcus*
 30 *aureus* mucosal isolate. *J. Bacteriol.*, Vol. 182, 6824-6826.

For the treatment of a bacterial lawn with an agent like the lantibiotic Gallidermin the respective cells to be treated are grown in a overnight culture in an appropriate medium. Then they are then diluted in fresh medium, e.g. in the dilution rates 1 : 100; 1 : 1000 and 1 : 10000. From these dilutions probes of 200µl each are transferred into the wells of 96 well microtiterplates (working volume 200 µl). For the sake of a solid statistical result is is recommended to run several identical probes in parallel, e.g. 8 wells in one row of the microtiterplate. Then these plates are incubated again, e.g. for 20 - 24 h at 37°C.

Afterwards the medium is taken off and the settled lawn washed about 3 times with 1 x PBS (pH 7,0) (Phosphate buffered saline; 0.02 mol/l sodium phosphate buffer, 0.15 mol/l NaCl, pH 7.0.). The remaining bacteria are heat fixed (incubation for 30 min at 80 -100°C) and stained with 150 µl saturated crystal violet (Serva, Heidelberg, Germany; Cat. no. 27335) in water for 5 min. The excess crystal violet is washed off through rinsing with water and dried through gentle pushing of the microtiterplate. The stained biofilm can then be solved on 150 µl EtOH (90 - 100%); the parallel probes can then be pooled and the optical density be measured by use of a photometer.

Example 7

The effect of Gallidermin on the biofilm development

The effect of Gallidermin on the development of a biofilm has been assessed with the strongly biofilm building strain *S. aureus* 325. 9 overnight cultures have been run in parallel with increasing concentrations of gallidermin, which have then been analyzed as described in example 6. The result is shown in table 6.

Table 6: Effect of Gallidermin on the development of a biofilm by *S. aureus* 325, treated with increasing Gallidermin concentrations; the control has been run without Gallidermin; values are given in % of the control.

Concentration of Gallidermin [ng/ml]	Biofilm formation [% of control]
---	-------------------------------------

1,95	99
3,9	85
7,8	83
15,6	82
31,3	62
62,5	30
125	2
250	1
500	0

This experiment proves that an incubation with Gallidermin significantly affects the possibility of bacteria to build up a biofilm.

5

Example 8

Mikrobouillon dilution test for the determination of the MIC value

To test the viability of bacterial cells the MIC (minimum inhibiting concentration) value can be determined, which value is defined as the minimal concentration of antibiotic leading to growth inhibition of 50% or 90 % over the time span tested (MIC (50) or MIC (90) rsp.). The test starts with an overnight culture of the respective bacteria (like Streptococci or Staphylococci) in Mueller-Hinton-Medium medium (Merck, Darmstadt, Germany; art. no. 1.05396.0500). On the following day this culture is used to inoculate fresh medium at an initial bacterial concentration of 1% which then is let grown until the logarithmic phase (in most cases an optical density of around $OD_{(600nm)} \leq 0,5$) which equals about $0.5 - 1 \times 10^8$ CFU/ml. This suspension is now diluted by the factor of 1:500 and 100 μ l of this dilution are mixed with decreasing concentrations of Gallidermin dissolved in Mueller-Hinton-Medium or medium to fill the wells of 96 well microtiterplates. In doing so each well is inoculated with about $1-2 \times 10^5$ CFU/ml per well.

After 18 h the OD at 600 nm of the culture broth is determined and compared with the controls without antibiotic. The growth inhibition in % represents the sensitivity of bacterial strains against antibiotic substances, e.g. Gallidermin. By plotting the concentrations of the antibiotic vs. the inhibition of growth (%), the MIC(90) can be

determined graphically.

Example 9

5 MIC value of different bacteria in the presence of Gallidermin

To test the effectiveness of Gallidermin against further human pathogenic, not gram-positive bacteria the MIC value has been determined as defined in example 8. As a control the substance Tobramycin has been used. This compound is described to be effective in human therapy in the treatment of infections of the respiratory tract caused by the pathogens tested below, and is described to be applicable directly into the respiratory tract by a spray inhaler. The result is summarized in table 7.

15 Table 7: Sensitivity of bacterial strains against Gallidermin and against Tobramycin, measured as MIC value.

Organism	MIC Gallidermin [µg/ml]	MIC Tobramycin [µg/ml]
<i>Haemophilus influenzae</i> Hib	8	1
<i>Haemophilus influenzae</i> Rd	8	2
<i>Branhamella catarrhalis</i> 9143	0,125	0,125
<i>Branhamella catarrhalis</i> 1194	0,125	0,0625

It can be seen that the MIC values of *Haemophilus influenzae* against Gallidermin lie in the same concentration range as those against Tobramycin and that Gallidermin is a potent antibiotic against *Branhamella catarrhalis*. Another pathogen expected to show comparable results is *Moraxella*, e.g. *Moraxella lacunata*.

Example 10

25 Growth curve of *Mycobacterium tuberculosis* with in the presence of Gallidermin

To assay the antibiotic effect of Gallidermin against the pathogen *Mycobacterium tuberculosis* two growth curves of this bacterium in the presence of Gallidermin and

without Gallidermin have been measured.

For this purpose cells of a strain of *M. tuberculosis* (e.g. commercially available at DSMZ, No. 44158) have been used to inoculate an appropriate volume of an appropriate medium, e.g. Löwenstein-Jensen Medium (comprising 2.5 g KH₂PO₄, 0.24 g MgSO₄, 0.6 g Mg-Citrate, 3.6 g L-Asparagine, 30 g Potato flour, 0.4 g Malachite green in 600 ml distilled water; 12 ml Glycerol and 1000 ml fresh egg mixture (yolk and whites); commercially available under medium no. 354 at DSMZ). Two probes have been run in parallel: the culture without antibiotic (control) and the same culture plus 200 µg/ml of Gallidermin. Probes have been taken after 0, 1, 2, 3, and 6 days and plated as described in example 1 to determine the CFU value which equals the number of living cells. The result is presented in table 8.

Table 8: Growth curves of *M. tuberculosis* in the presence of 200 µg/ml of Gallidermin and without Gallidermin (control).

Time [days]	Probe: Living cells / with Gallidermin [CFU/ml]	Control: Living cells / without Gallidermin [CFU/ml]
0	1×10^7	1×10^7
1	9×10^6	3×10^7
2	$1,5 \times 10^6$	5×10^7
3	3×10^5	4×10^7
6	5×10^4	1×10^8

It can be seen that Gallidermin is a potent antibiotic which effectively inhibits the growth of the pathogen *M. tuberculosis*.

Example 11

Intravenous application of Gallidermin for the treatment of guinea pigs

To assess side effects of an administration of Gallidermin male and female Dunkin-Harley guinea pigs are obtained from the Experimental Animal Breeding Centre of Harlan Winkelmann (Germany). After fasting overnight, but with free access to

drinking water, animals with body weight of 400-500 g are used.

- Gallidermin has been dissolved in a vehicle containing 10 mM acetate, 3% mannitol and 3% sucrose in distilled water, pH 5, at concentrations permitting intravenous
- 5 administrations of 0.1- 1 mg/kg. After anaesthetization of the guinea pigs this solution of Gallidermin was injected intravenously in a cumulative fashion from 0.1 mg/kg to 1 mg/kg, in doses of 0.1 mg/kg each hour. The resulting bronchospasm as well as the corresponding heart beat rate have been measured as given in table 9.

- 10 **Table 9:** Effect of Gallidermin on overflow in anaesthetized guinea pigs; the measured bronchospasm is given in ml X 10 of the increase in overflow; the corresponding heart beat rate increase is given in additional beats per minute (bpm), on top of the basal rate of 189 ± 16 .

Dose [mg/kg i.v.]	Bronchospasm [ml X 10]	Heart beat rate increase [bpm]
vehicle	0 ± 0	-12.2 ± 3.3
0.1	0 ± 0	-12.8 ± 2.3
0.2	2 ± 1.9	-7.8 ± 9.1
0.3	8.2 ± 1.8	15.6 ± 5.7
0.4	40.6 ± 5.8	36.6 ± 16.2
0.5	45 ± 0	49.2 ± 14.9
1	45 ± 0	56.4 ± 10.6

- 15 In additional experiments (not shown) it could be proven that these effects could be blocked by pretreatment of histamine-1 antagonists such as ketotifen (Sigma) and chlorpheniramine (Sigma), both dissolved in physiological saline allowing intravenous administration of 3 mg/kg. These data clearly show that Gallidermin applied
- 20 intravenously to guinea pigs can cause the (side-)effects of bronchospasm and tachycardia that are most probably due to the activation of histamine-1 receptors located on heart and lung tissues.

25 **Example 12**

Inhalative application of Gallidermin for the treatment of guinea pigs

To assess side effects of an inhalative administration of Gallidermin Dunkin-Harley guinea pigs, as described in example 11 have been used again.

5

Gallidermin has been dissolved again in a vehicle containing 10 mM acetate, 3% mannitol and 3% sucrose in distilled water, pH 5, at concentrations permitting inhaled administrations of 10 - 300 µg/kg per actuation (10 µl) with a soft mist inhaler (Respimat®; described in *Int. J. Pharmaceutics* (2004), vol. 283, pages 1-9). The

10 inhaled administrations of 600 - 1200 µg/kg are performed by giving 2 - 4 actuations of 300 µg/kg (10 µl). The control group receives the vehicle in the same conditions (1 - 4 actuations of 10 µl). After anaesthetization of the guinea pigs this solution of Gallidermin was injected inhaled in a cumulative fashion from 10 µg/kg to 1.2 mg/kg.

15 The resulting bronchospasm as well as the corresponding heart beat rate have been measured as given in table 10.

20 Table 10: Effect of gallidermin on overflow in anaesthetized guinea pigs; the measured bronchospasm is given in ml X 10 of the increase in overflow; the corresponding heart beat rate increase is given in additional beats per minute (bpm), on top of the basal rate of 189 ± 16.

Dose [µg/kg i.h.]	Bronchospasm [ml X 10]	Heart beat rate increase [bpm]
vehicle	0 ± 0	-5 ± 2
10	0 ± 0	-7 ± 1
30	0 ± 0	-6.5 ± 6
100	0 ± 0	-6 ± 3
300	0 ± 0	-7 ± 7
600	0 ± 0	-14.5 ± 6
900	0 ± 0	-15.5 ± 6
1200	0 ± 0	-14.5 ± 9.3

These data clearly show that Gallidermin applied inhalatively to guinea pigs does not cause the (side-)effects of bronchospasm and tachycardia. On the opposite, Gallidermin inhaled induced a slight decrease in heart rate of 14 - 15 beats per minutes at the doses between 0.6 and 1.2 mg/kg. This decrease in heart rate was not
5 dose-dependent and lasted for less than 1 minute. The vehicle tested in the same conditions did not produce any change in overflow and also induced a slight decrease in heart rate of 5 - 6 beats per minute lasting less than 1 minute.

Surprisingly, Gallidermin inhaled up to 1.2 mg/kg (4 fold above the first intravenous
10 dose which induced side-effects) did not produce neither bronchospasm nor tachycardia. Only a minor decrease in heart rate was recorded between 0.6 and 1.2 mg/kg i.h. This slight side effect proved at least that the compound was well absorbed in the lung.

15 Aiming at a medical use of Gallidermin these data support the inhalative application of this lantibiotic over a systemic application.

Example 13

20 **Short-time killing assay to test the principal antimicrobial activity of Gallidermin against *Helicobacter pylori***

This short-time assay has been applied as a first assay to test the existence of an antimicrobial activity of Gallidermin against *Helicobacter pylori* in general. For this
25 purpose a strain of *H. pylori*, e.g. *H. pylori* DSM 4867 (commercially available at DSMZ, i.e. Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; Braunschweig, Germany) is to be precultivated for 24 h on a blood agar plate (e.g. medium 420; commercially available at DSMZ) at 37°C under microaerophilic conditions. Such conditions are described e.g. in Cottet.S et al. (2002), *J. Biol. Chem.*,
30 vol. 277(37), pages :33978 to 33986. According to this teaching human epithelial cells (Caco-2), infected by and supporting the growth of *H. pylori* can be grown as polarized monolayers on filter supports, inserted in a vertical position into diffusion chambers equilibrated with air and 5% CO₂ at their basolateral surface (aerophilic conditions) and 5% CO₂, 5% O₂, 90% N₂ (microaerophilic conditions) in the apical compartment.

35

Then the bacteria are washed with physiologic salt solution and diluted to ca. 1×10^8 bacteria per ml in physiologic salt solution. Then Gallidermin or the respective variant to be tested is to be added in diverse concentrations over a broad range, e.g. at 31.3; 62.5; 125 and 250 $\mu\text{g/ml}$, and incubated for 1 h at room temperature.

5

After this incubation the bacteria from these different probes are plated on agar plates, preferably on blood agar plates again, and bread for 24 h at 37°C.

Such a test led to the results shown in table 11:

10

Table 11: Antimicrobial activity of Gallidermin against *Helicobacter pylori* after 1 h incubation at different concentrations; measured by subsequent plating.

Concentration of Gallidermin [$\mu\text{g/ml}$]	Surviving bacteria [bacteria $\times 10^7$ / ml]
0	6.6 ± 1.4
31.3	8.3 ± 1.2
62.5	7.7 ± 0.2
125	3.9 ± 1.2
250	0.3 ± 0.1

15 This test proves an antimicrobial activity of Gallidermin against *Helicobacter pylori* already after 1 h incubation. A concentration of 250 $\mu\text{g/ml}$ Gallidermin resulted in a reduction of more than 90%.

20 **Example 14**

Minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) of Gallidermin against *Helicobacter pylori*

Like in the example before a strain of *H. pylori* has been precultivated for 24 h on a

25 blood agar plate at 37°C under microaerophilic conditions.

Then appropriate liquid media (e.g. medium 420; commercially available at DSMZ) have been prepared by the addition of Gallidermin in concentrations of 2, 4, 8, 16, 32, and 64 µg/ml (each in duplicate). Then equal amounts of bacteria (in this case 1×10^8 per ml) have been applied and the probes were cultivated over night at 37°C under
5 microaerophilic conditions.

On the next day a visual inspection of the clouding took place. It was found that cultures with 2 and 4 µg/ml Gallidermin showed an intensive clouding, but none of the other cultures. Consequently, the tested strain possesses a minimal inhibition
10 concentration (MIC) of 8 µg/ml Gallidermin.

For the determination of the minimal bactericidal concentration (MBC) probes from these 6 liquid cultures were spread on blood agar plates, each in duplicate; additionally 5 cm long stripes of each culture were plated together on one plate.
15

As a result it was found that the growth of colonies was dependent on the Gallidermin concentration of the overnight culture: at a concentration of 8 µg/ml 3-5 colonies were found on one plate; at a concentration of 16 µg/ml and more no colonies were detected. This leads to the calculation of a minimal bactericidal concentration (MBC) of
20 Gallidermin against *H. pylori* of 16 µg/ml. This result could be verified by two repeats of this experiment.

This result proves again the antimicrobial activity of Gallidermin against *Helicobacter pylori* with a minimal inhibition concentration (MIC) of 8 µg/ml Gallidermin and a
25 minimal bactericidal concentration (MBC) of Gallidermin against *H. pylori* of 16 µg/ml.

A more detailed differentiation is possible by analogous testings of more cultures with more differentiated concentration steps of the applied lantibiotic.

CLAIMS

1. A pharmaceutical composition for the prophylaxis or treatment of a bacterial infection of the respiratory tract caused by gram-positive bacteria or other susceptible bacterial pathogens in a patient, comprising Gallidermin or a pharmaceutically active variant thereof as active substance.
2. A pharmaceutical composition according to claim 1, wherein Gallidermin or the pharmaceutically active variant thereof is the wildtype Gallidermin or a variant Gallidermin with one, two or three point mutations in comparison to the wildtype Gallidermin, preferably the wildtype Gallidermin, Gallidermin L6V, Gallidermin A12L, Gallidermin Dhb14P or Gallidermin Dhb14Dha.
3. A pharmaceutical composition according to claim 1 or 2, wherein the infected respiratory tract comprises at least partially the lung.
4. A pharmaceutical composition according to one of claims 1 to 3, wherein the gram-positive bacteria or other susceptible bacterial pathogens belong to a genus selected from *Bacillus*, *Branhamella* (=Moraxella), *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Propionibacterium*, *Haemophilus*, *Listeria*, *Micrococcus*, *Neisseriaceae*, *Mycobacterium*, *Helicobacter* or *Mycoplasma*, specifically to a species selected from *Branhamella catarrhalis* (=Moraxella catarrhalis), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Corynebacterium diphtheriae*, *Haemophilus influenza*, *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Mycoplasma pneumoniae*, *Mycoplasma mycoides*, *Mycoplasma hyopneumoniae*, *Mycoplasma gallisepticum* or *Mycoplasma hominis*.
5. A pharmaceutical composition according to one of claims 1 to 4, wherein the patient to be treated is a bird or a mammal, preferably chicken (*Gallus gallus*), human (*Homo sapiens*), bovine (*Bos bovis*), pig (*Sus domesticus*), sheep (*Ovis*), goat (*Capra*), horse (*Equus*), cat (*Felidae*), dog (*Canidae*), more preferably human (*Homo sapiens*).
6. A pharmaceutical composition according to one of claims 1 to 5, which is prepared to be administered topically to the lungs, preferably by inhalative

administration, more preferred by the use of a technical apparatus which allows the application of metered doses of the pharmaceutical composition.

7. A pharmaceutical composition according to claim 6, wherein the technical apparatus is selected from a dry powder inhaler or a nebulizer, preferably a nebulizer.
8. A pharmaceutical composition according to claims 6 or 7, wherein the pharmaceutical composition is prepared to be administered in the form of an inhalable powder with a maximum average particle size of up to 250 μm , preferably between 10 and 150 μm , most preferably between 15 and 80 μm or in the form of a micronised inhalable powder with an average particle size of 0.5 to 10 μm , more preferably from 1 to 6 μm .
9. A pharmaceutical composition according to claims 6 or 7, wherein the pharmaceutical composition is prepared to be administered in the form of propellant gas-driven inhalation solution or aerosol or in the form of a propellant-free inhalable solution or aerosol containing each active substance in a concentration of 0.002 to 5 wt.-%, and increasingly preferred 0.01 to 3 wt.-%, 0.015 to 2 wt.-%, 0.1 to 2 wt.-%, 0.5 to 2 wt.-%, and mostly preferred 0.5 to 1 wt.-% of each active substance.
10. A pharmaceutical composition according to claims 6 or 7, wherein the pharmaceutical composition is prepared to be administered in the form of a propellant gas-driven inhalation suspension or in the form of a propellant-free inhalable suspension containing the active substances in dispersed form with the particles of active substance in an average particle size of up to 10 μm , preferably from 0.1 to 6 μm , more preferably from 1 to 5 μm .
11. A pharmaceutical composition according to one or more of claims 6 to 10, wherein the inhalative administration of Gallidermin or the pharmaceutically active variant thereof is to be administered to reach dissolved concentrations on the airway surface of the subject of from 0.125 to 256 $\mu\text{g/ml}$.
12. A pharmaceutical composition according to one or more of claims 1 to 11, wherein the therapeutically effective amount of Gallidermin or the

pharmaceutically active variant thereof is to be administered between 0.01 and 1.2 mg per kg body weight of the patient.

13. A pharmaceutical composition according to one or more of claims 1 to 12,
comprising a second or further pharmaceutically active substance, preferably a
5 further pharmaceutically active substance selected from the group of
antibacterial or antiviral substances, more preferred another pharmaceutically
active variant of Gallidermin as defined in claims 1 or 2.
14. Combined product, comprising a pharmaceutical composition according to one
or more of claims 1 to 13 and a technical apparatus which allows the
10 application of metered doses of the pharmaceutical composition.
15. Use of Gallidermin or a pharmaceutically active variant thereof as active
substance for the manufacture of a pharmaceutical composition for the
prophylaxis or treatment of a bacterial infection of the respiratory tract caused
by gram-positive bacteria or other susceptible bacterial pathogens in a patient.
- 15 16. A method for the prophylaxis or treatment of a bacterial infection of the
respiratory tract caused by gram-positive bacteria or other susceptible bacterial
pathogens in a patient, wherein the method comprises administration of
Gallidermin or a pharmaceutically active variant thereof as active substance to
said patient.
- 20 17. Use of Gallidermin or a pharmaceutically active variant thereof as active
substance for the prophylaxis or treatment of a bacterial infection of the
respiratory tract caused by gram-positive bacteria or other susceptible bacterial
pathogens in a patient.

FIGURE 1

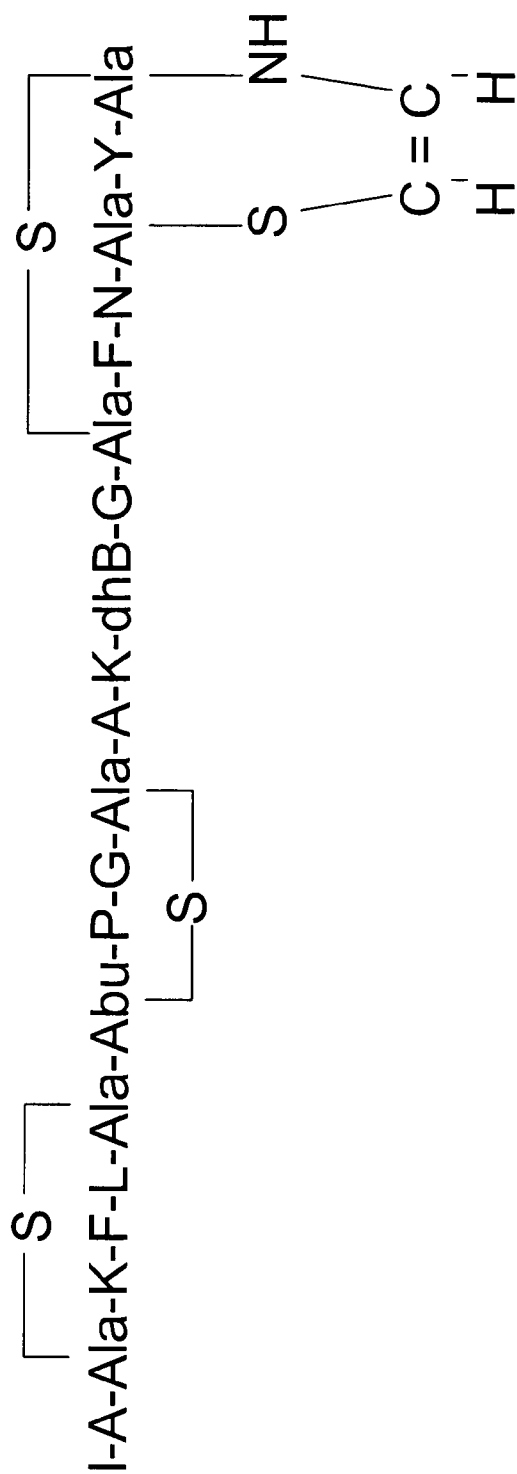


FIGURE 2

