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DIVISION DE NUEVAS CREACIONES No. 05-011187- -00008-0000

Superintendencia de Industria y Comercio Fecha: 2009-01-15 16:44:26 Dep. 2020 NUECREACIONE
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Act. 467 SOLICIPRORROCA Folios: 2

Trªmite: 011
Evento: 379
Actuación: 467

Referencia : Expediente No. 05.011.187
Asunto : Solicitud de Patente para NUEVOS COMPOSICIONES
DE MEDICAMENTOS A BASE DE NUEVOS
ANTOCOLINERGICOS E INHIBIDORES DE EGFR-
QUINASA
Solicitante : BOEHRINGER INGELHEIM PHARMA GMBH & CO.
KG

Clase : A61 031/046, A61 031/047, A61 031/505
Fecha de Solicitud : 8 de febrero de 2005

1. Yo, Emilio FERRERO, como apoderado del solicitante, en el asunto antes

identificado, atentamente solicito a Usted se sirva concederme un plazo de treinta (30) dias, para atender el oficio No. 13287 notificado por fijación en lista del 17 de Octubre de
2008.

2. Presento junto con este memorial, el recibo expedido por el Fondo Especial

de la Superintendencia de Industria y Comercio, en donde costa el pago hecho por valor de $81.000 en conformidad con lo dispuesto en la resolución No. 55819 del 26 de diciembre de 2008.

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T.P.A. No. 31.929

NIT 860.041.367-3

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***** CUNSIGNACION *****

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CAYELIER ABO6ADUS CUNSISNACION BANCO DE BOGOTA 062754387 102218933 05/01/2009 89,730,020.00

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TOTAL $ 81,000.00

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(12) United States Patent
Nadel et al.

(54) IN VITRO AND IN VIVO ASSAY FOR AGENTS WHICH TREAT MUCUS
HYPERSECRETION

(75) Inventors: Jay A. Nadel; Klyoshl Thkeyama, both of San Francisco, CA (US)

(73) Assignee: The University of California, San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/375,597

(22) Filed: Aug. 17, 1999

Related U.S. Application Data
(60) Provisional application No. 60/097,023, filed on Aug. 18, 1998.

(51) hit. C1.7.............................A61K 49/00; C12Q 1/02; C12N 5/00; C12N 5/02

(52) U.S. Cl................................ 424/9.2; 435/29; 435/375

(58) Field of Search..................................424/1.49, 1.41, 424/1.57, 450, 9.2; 435/91.6, 7.21, 7.2,
325, 455, 375, 29; 514/2, 44; 530/388.24,
387.1; 536/23.1, 23.5, 24.5, 24.31, 24.33

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(10) Patent No.: US 692709747 B!
(45) Date of Patent: Aug. 7, 2001

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(57) ABSTRACT

Hypersecretion of mucus, in the lungs is inhibited by the administration of an epidermal growth factor receptor (EGF-R) antagonist. The EGF-R antagonist may be in the form of a small organic molecule, an antibody, or portion of an antibody that binds to and blocks the EGF receptor. The EGF-R antagonist is preferably administered by injection in an amount sufficient to inhibit formation of goblet cells in pulmonary airways. The degranulation of goblet cells that results in airway mucus production is thereby inhibited. Assays for screening candidate agents that inhibit goblet cell proliferation are also provided.

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5 Claims, 4 Drawing Sheets

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IN VITRO AND IN VIVO ASSAY FOR
AGENTS WHICH TREAT MUCUS
HYPERSECRETION

This applications claims benefit of U.S. Provisional application No. 60/097,023, filed Aug. 18, 1998.

GOVERNMENT RIGHTS

The United States Government may have certain rights in this application pursuant to Grant HL-24136 awarded by the National Institutes of Health Program.

FIELD OF THE INVENTION

This invention relates generally to the field of pulmonary treatment. More particularly, the invention relates to inhib-iting hypersecretion of mucus in lungs and airways by the administration of an EGF-R antagonist. In addition, this invention also relates to methods for the development or assessment of candidate agents capable of inhibiting hyper-secretion of mucus in the lungs.

BACKGROUND OF THE INVENTION

In the conducting airways of the respiratory system, the mucociliary system serves as the primary defense mecha-nism to move inhaled particles or infectious agents out ofthe airways in the lungs. In addition, substances present in airway fluids serve to limit the toxicity of the particles and to inactivate infective agents. The physical mechanism of coughing serves to expel the mucus from the airway pas-sages (see e.g., "Foundations of Respiratory Care," Pierson and Kacmarek, eds. (1992) Churchill Livingstone Inc. New York, N.Y.; "Harrison’s Principles of Internal Medicine", Fauci et al., eds. (1997) 14th Edition, McGraw Hill, New York, N.Y.

The mucociliary system consists of ciliated epithelial cells, epithelial goblet cells, and serous and mucous cells located in submucosal glands. The cilia are surrounded by an aqueous layer (periciliary fluid) secreted into the lumen of the airway passage by the active transport of chloride and the passive movement of water across the epithelium. The cilia make contact with the mucus floating on this aqueous layer, and via a unidirectional propelling motion provide for movement of mucus toward the glottis (see Pierson and Kacmarek, supra and Fauci, et al, supra). Mucus is produced by the epithelial goblet cells and submucosal gland cells and is secreted into the lumen of the airway after degranulation. While mucus generally facilitates the clearance ofinhaled particles or infectious agents, hypersecretion of mucus in the airways may cause progressive airway obstruction. In peripheral airways, cough is ineffective for clearing secre-tions. Furthermore, because of their small dimensions, small airways containing many goblet cells are especially vulner-able to airway plugging by mucus. Airway hypersecretion affects a substantial number of individuals; it is seen in a variety of pulmonary diseases, such as chronic bronchitis, acute asthma, cystic fibrosis, and bronchiectasis. Hypersecretion ofmucus is the major symptom in patients with chronic obstructive pulmonary disease (COPD) and defines the condition (i.e. chrpnic cough and sputum production). This condition alone affects 14 million Ameri-cans and can cause progressive disability and death. It has been estimated that asthma affects at least 4% of the U.S. population and accounts for at least 2000 deaths annually (Pierson and I(ucmarek, supra). During an acute asthmatic event, the bronchial walls swell, mucus volume increases

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and bronchial smooth muscle contracts, resulting in airway narrowing. As a result of hypersecretion in acute asthma, extensive mucus plugging can be a major cause ofmorbidity and mortality.
Hypersecretion has also been implicated in cystic fibrosis, which is one of the most common, fatal, genetic diseases in the world. Cystic fibrosis is an autosomal recessive disease that causes the airway mucosal cell to become unresponsive to cyclic-AMP-dependent protein kinase activation of the 10 membrane chloride ion channels (Pierson and Kacmarek, supra and Fauci, et al., supra). The subsequent electrolyte imbalance reduces the level of hydration of the airway mucus, thus resulting in highly viscous mucus in the lungs of an individual afflicted with cystic fibrosis. Hypersecretion obstructs the air passages of individuals with cystic fibrosis, 15 further compromising lung function.
Other disease involving hypersecretion include chronic obstructive lung disorder (COPD). Oxidant stress plays an important role in the pathogenesis of COPD. Cigarette smoke, which generates oxygen free radicals, is strongly 20 implicated in the pathogenesis. Neutrophils are often seen at site ofinflammation in COPD, and interestingly, oxygen free radicals are known to be released by neutrophils during activation.

Mechanical intubation is often necessary in order to
25 provide assisted ventilation to patients with various pulmo-nary diseases. A tube is introduced via the oropharanx and placed in the trachea. To prevent leaking of air around the endotracheal tube, a balloon is inflated around the tube in the lower trachea, which may abrade the epithelium and cause 30 goblet cell metaplasia. Wounding of epithelium leads to repair processes, which can result in abundant mucus secre-tion. Such prolonged tracheal intubation in patients can lead to deleterious effects due to hypersecretion.
As a result of the high levels of mucus in the lungs of
35 patients with hypersecretoiy pulmonary diseases, mucosal clearance is reduced. Pathological agents such as bacteria, e.g. Pseudomonas aeruginosa, often establish colonies within the mucus, resulting in frequent lung infection. Classical modalities of treating individuals afflicted with
40 airway hypersecretion include antibiotic therapy, bronchodi-lators(e.g., methylxanthines, sympathomimetics with strong P2 adrenergic stimulating properties, anticholinergics), use of systemic or inhaled corticosteroids, primarily in asthma,

liquefaction of the mucus by oral administration of ’ expectorants, e.g. guaifenesin, and aerosol delivery of
"mucolytic" agents, e.g. water, hypertonic saline solution (see Harrison’s, supra). A newer therapy for cystic fibrosis is the administration of DNAse to target the DNA-rich mucus

or sputum (Shak, et al. (1990) Proc. Nat!. Aced. (USA) 50 87:9188-9192; Hubbard, R.C. et al (1991) N. EngL J. Med.
326:812). In addition, chest physical therapy consisting of percussion, vibration and drainage are also used to facilitate

clearance of viscous mucus. Lung transplantation may be a final option for those with severe pulmonary impairment.
55 Therefore, more efficacious or alternative therapy to target the mucosalsecretionsis needed. Specifically, there is a need for a specific modality that will reduce the formation of mucus secretions in the airways.
Relevant Literature

60 The use of EGF inhibitors to block the growth of cancer cells is reviewed by Levitski (1994) EurJ Biochem. 226(1): 1-13; Powis (1994) Pharmac. Ther. 62:57-95; Kondapaka and Reddy (1996) Mol. Cell Endocrin. 117:53-58.

65 SUMMARY OF THE INVENTION Hypersecretion of mucus in airways is an adverse symp-tom of a number of different pulmonary diseases. The

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secretion results from the degranulation of goblet cells, the proliferation of which is promoted by stimulation of epider-mal growth factor receptors (EGF-R). The present invention treats pulmonary hypersecretion by administering therapeu-tic amounts of EGF antagonists, preferably kinase inhibitors. The antagonists may be in the form of small molecules, antibodies, or portions of antibodies that bind to either EGF or its receptor. In another aspect ofthe invention, in vitro and in vivo methods predictive of the therapeutic potential of candidate agents to inhibit hypersecretion of mucus are to provided.
A primary object of the invention is to provide a method
of treating diseases involving hypersecretion of mucus in lungs.
Another object of the invention is to provide formulations ’ useful in the treatment of diseases that result in hypersecre-tion of mucus.

Yet another object of the invention is to provide an in vitro assay for the screening of candidate agents that inhibit 20 hypersecretion of mucus, where the method involves the steps of (i) contacting an in vitro model of goblet cell proliferation with EGF or the functional equivalent thereof; (ii) subsequently contacting the in vitro model with a candidate agent; and (iii) assessing goblet cell proliferation, 25 wherein inhibition of goblet cell proliferation is indicative of the candidate agent’s therapeutic potential.
Another object of the invention is to provide an in vivo assay for the screening of candidate agents that inhibit hypersecretion of mucus, where the method involves (i) 30 creating an animal model of hypersecretory pulmonary disease by inducing EGF-R, e.g. with tumor necrosis factor-alpha (TNF-a); (ii) stimulating the induced EGF-R with its ligand, e.g. transforming growth factor alpha (TGF-u) or EGF, to produce mucin producing goblet cells; (iii) treating 35 with a candidate agent; and (iv) assessing goblet cell pro-liferation or mucus secretion, wherein an inhibition of goblet cell proliferation or mucus secretion is indicative of the candidate agent’s therapeutic potential.
A further object of the invention is to provide in vitro and 40 in vivo assays for the screening of EGF-R antagonists that inhibit hypersecretion of mucus.
An advantage of the invention is that it provides a means
for preventing excessive formation of mucus in pulmonary airways. 45 A feature of the invention is that a range of different types
of antagonists can be used to block the effects of EGF and/or TGF-a and their interaction with EGF-R.

An aspect ofthe invention is formulations of EGF antago nists for reducing formation of mucus secretion in the airways of a mammalian patient, preferably a human patient. Another object of the invention is a method of pulmonary delivery of EGF antagonists for reducing mucus secretions in the airways of a mammalian patient, preferably a human 55 patient.
Another object ofthe invention is to provide a method for treating a range of different diseases which have as a symptom the excess formulation of mucus secretions in the airways. These diseases include, without limitation, chronic 60 bronchitis, acute asthma, cystic fibrosis, bronchiectasis, chronic obstructive lung disease, hypersecretion resulting from epithelial damage such as allergic stimuli or mechani-cal abrasions, and nasal hypersecretion.
These and other objects, advantages, and features of the 65 invention will become apparent to those persons skilled in the art upon reading the details of the treatment methods,

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and in vitro and in vivo assay methods, as more fully described below.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. IA is a western blot of EGF-R in NCI-H292 and in A431 cells, FIG. lB, immunocytochemical analysis with anti-EGF-R antibody in cultures of NCI-H292 cells. FIG. 1C, Northern analysis of EGF-R in NCI-H292 cells.
FIG. 2. Alcian blue/PAS staining of NCI-H292 cells for identification of mucin glycoproteins.
FIG. 3. Northern analysis for MUC5 gene expression in NCI-H292 cells.

FIG. 4A and 4B. Immunohistochemical analysis of EGF-R with an anti-EGF-R antibody in pathogen-free rats. FIG. 4A, TNFa-treated rats. FIG. 4B, ovalbumin-sensitized
rats.

FIG. 5 is a graph depicting the effect of EGF-R tyrosine kinase inhibitor (BIBX1522) on production of goblet cells (expressed as % of stained area of airway epithelium occu-pied by Alcian blue/PAS-positive stained cells).

DETAILED DESCRIPTION OF THE
PREFERRED EMBODIMENTS

Compositions and methods are provided for the treatment of airway mucus hypersecretion by administering therapeu-tic amounts ofEGF antagonists, preferably kinase inhibitors. The antagonists may be in the form of small molecules, antibodies, or portions of antibodies that bind to either EGF or its receptor. In airway hypersecretory diseases, e.g. chronic bronchitis, bronchiectasis, cystic fibrosis, acute asthma, COPD, etc., mucin synthesis in airways is increased and mucus hypersecretion occurs. The secreted mucus results in airway obstruction, an effect that causes death in these diseases.

Several causes of airway damage and inflammation are shown herein to induce the expression of the epidermal growth factor receptor in airway epithelial cells. After induc-tion of EGF-R, subsequent stimulation of EGF-R by both ligand-dependent and -independent mechanisms results in mucin production at both gene expression and protein levels. Selective inhibitors of EGF-R tyrosine kinase are demon-strated to block this mucin gene and protein expression. Without limiting the invention, it is suggested that an evolutionary sequence of goblet cell production may be based on the expression of EGF-R. Stimulation with TNFa induces intense EGF-R staining of non-granulated secretory cells; their subsequent activation by EGF-R ligands causes progressive staining for mucous glycoconjugates in the cytoplasm, and the cells become "pre-goblet" and then "goblet" cells. The data suggest that EGF-R activation promotes selective ceil differentiation, but not proliferation. Goblet cells are apparently derived from non-granulated secretory cells that express EGF-R and are stimulated by EGF-R ligands to produce mucins.
In addition to stimulation by cytokines, the EGF-R may be stimulated by other signaling mediators. For example, prolonged cigarette smoking is associated with progressive pathologic changes in peripheral airways, including goblet cell hyperplasia. Proinflammatory cytokine-activated neu-trophils and cigarette smoke are slown to cause mucin synthesis in human bronchial epithelial cells via ligand-independent activation ofEGF-R, implicating recruited neu-trophils and cigarette smoke as regulators of epithelial cell differàntiation that result in abnormal induction of mucin-producing cells in airways. Neutrophils activated by a van -

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ety of stimuli, including IL-8, N-formyl-methionyl-leucyl-phenylalanine,TNF-a, cigarette smoke or H202 upregulate mucin expression in epithelial cells, which synthesis is inhibited by EGF-R inhibitors. Neutrophils are also capable of producing the EGF-R ligands, EGF and TGFa. In addition, epithelial cells are sources of EGF-R ligands. Mechanical injury to airway epithelium can also cause hypersecretion, and be responsible for mucous plugging. Inhibitors of EGF-R tyrosine kinase serve to prevent mucous hypersecretion after tracheal intubation.
Epithelial damage is a common finding in studies of patients even with mild asthma, and the damage is increas-ingly related to worsening of clinical symptoms. Epithelial damage produced by the allergic response induces EGF-R activation, which results in abnormal goblet cell production. EGF-R is implicated in epithelial damage, for example the "airway remodeling" that occurs in asthma, repair and wound closure. Mechanical epithelial damage and epithelial injury in asthma may involve a similar EGF-R cascade, resulting in abnormal growth of epithelial secretory cells. Hypersecretion is also an important manifestation of inflammatory diseases of the nose. When nasal goblet cells are "challenged" by inducing goblet cell degranulation uti-lizing a neutrophil-dependent mechanism, expression of EGF-R and mucins are strongly upregulated. These events were associated with regranulation of the goblet cells. When inflammation, such as stimulation of neutrophil infiltration, causes goblet cell degranulation and mucin secretion, up-regulation and activation of EGF-R re-supplies the air-way epithelium with mucins.
Before the present methods oftreatment and formulations are described, it is to be understood that this invention is not limited to particular methods and formulations described, as such may, of course, vary. it is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.
Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or mate-rials in connection with the publications are cited.
The publications discussed herein are provided solely for their disclosure prior to the filing date of the present appli-cation. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual pub-lication dates which may need to be independently con-firmed.

Definitions

By "epidermal growth factor" or "EGF" is meant a protein or portion thereof having biological activity charac-terized by mitogenic activity on epithelial cells (e.g., Cohen (1986) Biosciences Reports 6(12):1017; Aaronson, S. A., "Growth Factors and Cancer," Science (1991) 254:1146-1153). Exemplary is the human epidermal growth factor, for example as described by Urdea et al. (1983) Proc. Nat. Aced. Sci. 80:7461-7465.

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Of particular interest for the purposes of this invention is the mitogenic activity of EGF on goblet cells. Also intended to be encompassed by this definition are proteins of portions thereof which are the functional equivalent of EGF in terms of the biological response elicited by EGF.
By "epidermal growth factor receptor" or "EGF-R" is meant a protein a portion thereof capable of binding EGF protein or a portion thereof. Exemplary is the human epi-dermal growth factor receptor (see Ullricb et al. (1984) 10 Nature 309:418-425; Genbank accession number NM_005228). Preferably, the binding of the EGF ligand activates the EGF-R (e.g. resulting in activation of intrac-ellular mitogenic signaling, autophosphorylation ofEGF-R). One of skill in the art will appreciate that other ligands, in addition to EGF, may bind to EGF-R and activate the EGF-R. Examples of such ligands include, but are not limited to, TGF-a, betacellulin, amphiregulin, heparin-binding EGF (IIB-EGF) and neuregulin (also known as hergiilin) (Strawn and Shawver (1998) Exp.-Opin. Invest.

20 Drugs 7(4)553-573, and "The Protein Kinase Facts Book: Protein Tyrosine Kinases" (1995) Hardie, et al. (eds.), Aca-demic Press, New York, N.Y.).
By "EGF-R antagonist" is meant any agent capable of directly or indirectly inhibiting the effect of EGF-R, par-
25 ticularly the effect of EGF-R on goblet cell proliferation or
hypersecretion of mucus by goblet cells. EGF-R can be activated through ligand-dependent and ligand-independent mechanisms, resulting in either autophosphorylation or trans-phosphorylation, respectively. EGF-R antagonists of 30 interest may inhibit either or both of these mechanisms. For example, binding of TNF-a to the EGF-R results in a ligand-dependent phosphorylation, which may be blocked by an antibody that binds EGF-R, thereby preventing the interaction of EGF with a ligand that would activate the EGF 35 receptor. Examples of such antibodies are described by Goldstein et al. (1995) Gun. Cancer Res. 1:1311-1318; Lorimer et al. (1995) Clin. CancerRes. 1:859-864; Schmidt and Wels (1996) Br J. Cancer 74:853.-862. Small molecule tyrosine kinase inhibitors are also effective as EGF-R
40 antagonists.
Alternatively, it is shown that compounds such as oxygen free radicals stimulate a trans-phosphorylation of the EGF-R, resulting in ligand-independent activation of the receptor. Other means of activating EGF-R by transphosphorylation 45 include ultraviolet and osmotic stress, stimulation of G-proten coupled-receptor by endothelin-1, lysophospha-tidic acid and thrombin, ml muscarinic acetylcholine receptor, and human growth hormone. Antagonists of this ligand-independent mechanism include anti-oxidants, such as super oxide dismutase, DMSO, DMTU, ascorbic acid, and the like.

An EGF-R antagonist may be an antibody that binds to a factor that stimulates EGF production or EGF-R production, thereby inhibiting promotion of goblet cell proliferation by 5,5 EGF (i.e. an inhibitor of the phosphorylation cascade that phosphorylates EGF-R). For example, a fusion protein of TGFa-Pseudomonas exotoxin 40 is described by Arteaga et al. (1995) Cancer Res. 54:4703-4709.
In a preferred embodiment, the EGF-R antagonist is an
60 inhibitor of the tyrosine kinase activity of EGF-R, particu-larly small molecule inhibitors having selective action on EGF-R as compared to other tyrosine kinasespreferred small molecules block the natural EGF receptor in a mammal, preferably a human and have a molecular weight
65 of less than 1 kD.
Inhibitors of EGF and EGF-R include, but are not limited
to, tyrosine kinase inhibitors such as quinazolines, such as

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PD 153035, 4-(3-chloroanilino) quinazoline, or CP-358,774, pyridopyrimidines, pyrimidopyrimidines, pyrrolopyrimidines, such as COP 59326, COP 60261 and CGP 62706, and pyrazolopyrimidines(Shawn and Shawver, supra.), 4-(phenylamino)-7H-pyrrolo[2,3-d]pyrimidines (Traxler et aL, (1996) J. Med. Chem 39:2285-2292), cur-cumin (diferuloyl methane) (Laxmin arayana, et al, (1995), Carcinogen 16:1741-1745), 4,5-bis (4-fluoroanilino) phthalimide (Buchdunger et al. (1995) Clin. Cancer Res. 1:813-821; Dinney et al (1997) Clin. Cancer Res. 3:161-168); tyrphostins containing nitrothiophene moieties (Brunton et al. (1996) Anti Cancer Druc Design 11:265-295); the protein kinase inhibitor ZD-1839 (AstraZeneca); CP-358774 (Pfizer, inc.); PD-0183805 (Warner-Lambert); or as described in International patent application W099/09016 (American Cyanamid); W098/43960 (American Cyanamid); W097/38983 (Warener Labert); W099/06378 (Warner Lambert); W099/06396 (Warner Lambert); W096/30347 (Pfizer, Inc.); W096/33978 (Zeneca); W096/33977 (Zeneca); and W096/33980) Zeneca; all herein incorporated by reference; or antisense molecules.

By "inhibiting" is meant decreasing, neutralizing, attenu-ating or preventing the proliferation of goblet cells, degranu-lation of goblet cells or hypersecretion of mucus by goblet cells.
The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylacticin terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein

covers any treatment of a disease in a mammal, particularly a human, and includes:
(a) preventing the disease or symptom from occurring in a subject who may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease or symptom, i.e., arresting its development; or
(c) relieving the disease or symptom, i.e., causing regres-sion of the disease or symptom. The invention is directed toward treating patients with pulmonary or airway disease and is particularly directed toward treat-ing patients’ hypersecretion of mucus, i.e. preventing, inhibiting or relieving hypersecretion of mucus. In terms of treating symptoms, the invention is directed toward decreasing mucus or sputum in the airways, inhibiting infection by pathological organisms, allevi-ating cough, and preventing hypoxia due to airway plugging.
More specifically "treatment" is intended to mean pro-viding a therapeutically detectable and beneficial effect on a patient suffering from a pulmonary disease involving hyper-secretion of mucus.

Still more specifically "treatment" shall mean preventing, alleviating, and/or inhibiting hypersecretion of mucus with a compound selected from the group consisting of EGF and/or EGF-R antagonists such as antibodies, protein tyrosine kinase inhibitors and antisense molecules and the like. An alternative treatment may comprise prevention of EGF-R expression in airway, thereby blocking the pathway at an earlier stage. For example, reagents that block binding ofTNFa to its receptor may prevent upregulation of EGF-R. Treatment includes preventing or inhibiting infections by pathological agents caused by and/or related to hypersecre-tion of mucus.

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By "antibody" is meant an immunoglobulin protein that is capable of binding an antigen. Antibody as used herein is meant to include antibody fragments, e.g. F(ab’)2, Fab’, Fab, capable of binding the antigen or antigenic fragment of $ interest. Preferably, the binding of the antibody to the antigen inhibits the activity of EGF or EGF-R.
The term "humanized antibody" is used herein to describe complete antibody molecules, i.e. composed of two com-plete light chains and two complete heavy chains, as well as
10 antibodies consisting only of antibody fragments, e.g. Fab,
Fab’, F (ab’) 2, and Fv, wherein the CDRs are derived from a non-human source and the remaining portion of the Ig molecule or fragment thereof is derived from a human antibody, preferably produced from a nucleic acid sequence
15 encoding a human antibody.
The terms "human antibody" and "humanized antibody"
are used herein to describe an antibody ofwhich all portions of the antibody molecule are derived from a nucleic acid sequence encoding a human antibody. Such human antibod-20 ies are most desirable for use in antibody therapies, as such antibodies would elicit little or no immune response in the human patient.
The term "chimeric antibody" is used herein to describe
an antibody molecule as well as antibody fragments, as 7.5 descri,ed above in the definition of the term "humanized antibody." The term "chimeric antibody" encompasses humanized antibodies. Chimeric antibodies have at least one portion of a heavy or light chain amino acid sequence derived from a first mammalian species and another portion 30 of the heavy or light chain amino acid sequence derived from a second, different mammalian species. Preferably, the variable region is derived from a non-human mammalian species and the constant region is derived from a human species. Specifically, the chimeric antibody is preferably 35 produced from a nucleotide sequence from a non-human mammal encoding a variable region and a nucleotide sequence from a human encoding a constant region of an antibody.
By "binds specifically" is meant high avidity and/or high
40 affinity binding of an antibody to a specific polypeptide.
Antibody binding to its epitope on a specific polypeptide is stronger than binding of the same antibody to any other epitope, particularly those which may be present in mol-ecules in association with, or in the same sample, as the
45 specific polypeptide of interest. Antibodies that bind spe-
cifically to a polypeptide of interest may be capable of binding other polypeptides at a weak, yet detectable, level, e.g. 10% or less of the binding shown to the polypeptide of interest. Such weak binding, or background binding, is 50 readily discernible from the specific antibody binding to the compound or polypeptide of interest, e.g. by use of appro-priate controls.
By "detectably labeled antibody", "detectably labeled anti-EGF" or "detectably labeled anti-EGF fragment" is
55 meant an antibody (or antibody fragment that retains binding
specificity), having an attached detectable label. The detect-able label is normally attached by chemical conjugation, but where the label is a polypeptide, it could alternatively be attached by genetic engineering techniques. Methods for 60 production of detectably labeled proteins are well known in the art. Detectable labels may be selected from a variety of such labels known in the art, but normally are radioisotopes, fluorophores, enzymes, e.g. horseradish peroxidase, or other moieties or compounds that either emit a detectable signal
65 (e.g. radioactivity, fluorescence, color) or emit a detectable
signal after exposure of the label to its substrate. Various detectable label/substrate pairs (e.g. horseradish peroxidase/

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diaminobenzidine, avidin/streptavidin, luciferase/luciferin), methods for labeling antibodies, and methods for using labeled antibodies to detect an antigen are well known in the art (for example, see Harlow and Lane, eds. (Antibodies: A Laboratory Manual (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

Therapeutic Methods

The present invention provides a method of treating pulmonary hypersecretion by administering therapeutic amounts of EGF-R antagonists. Any disease and particularly and pulmonary disease characterized by hypersecretion of mucus or accumulation of pathological levels ofmucus may be treated by the methods described herein. Examples of pulmonary hypersecretory diseases that may be treated by this method include, but are not limited to, chronic obstruc-tive lung diseases, such as chronic bronchitis, inflammatory diseases such as asthma, bronchiectasis, pulmonary fibrosis, COPD, diseases of nasal hypersecretion, e.g. nasal allergies, and other hypersecretory diseases. Genetic diseases such as cystic fibrosis, Kartagener syndrome, alpha-1-antitrypsin deficiency, familial non-cystic fibrosis mucus inspissation of respiratory tract, are intended to be included as well. Antagonists that directly target EGF or EGF-R are pre-ferred. However, one of skill in the art will appreciate that any factor or cell involved in the biological cascade that results in EGF-R promoting goblet cell proliferation may be targeted for inhibition, e.g. TGF-a antagonists. Without being bound by theory, a cascade begins during an inflam-matory response when cells such as mast cells or neutrophils release TNF-a, which then promotes EGF-R expression. Stimulation of EGF-R, e.g. by its ligand EGF, in turn triggers goblet cell proliferation. Thus, any cells or factors involved in the cascade, such as in the TNF-a pathway, may be targeted for antagonist activity.
The EGF-R antagonist administered in the therapeutic method may be in any form. By way of example, the EGF-R antagonist may be in the form of a small molecule (i.e., antisense oligonucleotide, tyrosine kinase inhibitor, etc.), antibodies or portion of antibodies that bind to EGF, TGFct or EGF-R.

Small Molecule EGF-R Antagonists

Tyrosine kinase inhibitors that act on the EGF receptor, and that are selective for the EGF-R, are known in the art, and may be used in the subject methods. Examples are described above, and of such may include B1BX1522 (Boehringer Ingelheim, Inc., Ingelheim, Germany); CGP59326B (Novartis Corporation, Basel, Switzerland); 4-aminoquinazoline EGF-R inhibitors (described in U.S. Pat. No. 5,760,041); substituted styrene compounds which can also be a naphthalene, an indane or a benzoxazine; including nitrile and molononitrile compounds (described in U.S. Pat. No. 5,217,999); the inhibitors disclosed in U.S. Pat. No. 5,773,476; potato carboxypeptidase inhibitor (PCI), a 39-amino acid protease inhibitor with three disulfide bridges, (Blanco-Aparicio et al. (1998) J Blol Chem 273 (20):12370-12377); bombesin antagonist RC-3095 (Szepeshazi et al. (1997) Proc Nat! Acad Sci USA 94:10913-10918) etc. Other tyrosine kinase inhibitors include quinazolines, such as PD 153035, 4-(3-chloroanilino)quinazoline, or CP-358,774, pyridopyrimidines, pyrimidopyrimidines, pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706, and pyrazolopyrimidines (Shawn and Shawver, supra.), 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines

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(Traxier et al. (1996) J. Meat Chem 39:2285-2292), cur-cumin (Korutla et al. (1994) Biochim Biophys Acta 1224:597-600); (Laxmin arayana (1995), Carcinogen 16:1741-1745); etc.
5 Preferred tyrosine kinase inhibitors are selective for EGF
receptor, ie. the EGF-R is inhibited to a greater degree than other cell surface receptors having tyrosine kinase activity. Selectivity is enhanced by the methods of formulation and drug delivery, e.g. where the inhibitor is preferentially 10 delivered to inflamed airways, etc.
Typical dosages for systemic administration range from
0.1 jug to 100 milligrams per kg weight of subject per administration. Those of skill will readily appreciate that dose levels can vary as a function of the specific compound,
15 the severity of the symptoms and the susceptibility of the
subject to side effects. Some of the specific compounds are more potent than others. Preferred dosages for a given compound are readily determinable by those of skill in the

art by a variety of means. A preferred means is to measure 20 the physiological potency of a given compound, for example

with the in vitro and in vivo tests described herein.

Antibodies as EGF-R Antagonists

25 Antibodies as EGF-R antagonists are of particular interest
(e.g. Viloria, et al., American Journal of Pathology 151:1523). Antibodies to EGF or EGF-R are produced by immunizing a xenogeneic immunocompetent mammalian host, including murine, rodentia, lagomorpha, ovine, 30 porcine, bovine, etc. with EGF or EGF-R or portions thereof. Preferably human EGF or EGF-R or portions thereof are used as the immunogen. The choice of a par-ticular host is primarily one of convenience. Immunizations are performed in accordance with conventional techniques, 35 where the immunogen may be injected subcutaneously, intramuscularly, intrapentoneally, intravascularly, etc. into the host animal. Normally, from about 1.0 mg/kg to about 10 mg/kg of EGF or EGF.R intraperitoneally every other day will be used as an immunogen. The injections may be with
40 or without adjuvant, e.g. complete or incomplete Freund’s
adjuvant, specol, alum, etc. After completion of the immu-nization schedule, the antiserum may be harvested in accor-dance with conventional waysto provide polyclonal antisera specific for EGF or the EGF-R.
45 Either monoclonal or polyclonal antibodies, preferably
monoclonal antibodies, are produced from the immunized animal. Polyclonal antisera may be harvested from serum by conventional methods from the animals after completion of the immunization schedule. For production of monoclonal antibodies, lymphocytes are harvested from the appropriate lymphoid tissue, e.g. spleen, draining lymph node, etc., and fused with an appropriate fusion partner, usually a myeloma line, producing a hybridoma secreting a specific monoclonal antibody. Screening clones of hybridomas for the antigenic
55 specificity of interest is performed in accordance with con-
ventional methods.

Of particular interest are antibodies, preferably mono-clonal antibodies, that bind to EGF-R or EGF so as to inhibit binding of EGF to EGF-R, e.g. an antibody that specifically
eo binds to the extracellular domain of EGF-R thereby pre-
venting binding of EGF. Such antibodies may be made by conventional methodology described above, or are commer-cially available. Examples of antibodies that would function as an EGF antagonist include, but are not limited to, the neutralizing anti-EGF-R monoclonal antibody C225 (Kawamoto et al. (1983) Proc. Nat’!. Acad. Sc!. (USA) 80:1337-1341; Petit et al. (1997) J. Path. 151:1523-153,