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(54) **Title**: MIRNA EXPRESSION IN ALLERGIC DISEASE

(57) **Abstract**: Disclosed are methods for detecting an allergic lung disease that involve assessing the level of one or more microRNAs (miRNAs) in a biological sample, wherein the level of the one or more miRNAs in the biological sample compared to a reference level of the one or more miRNAs is indicative of allergic lung disease. Also disclosed are methods for the treatment or prevention of inflammatory or allergic lung disease that involve administration of a let-7 miRNA inhibitor as set forth herein, as well as biochips and kits that can be applied in the methods of the present invention.

DESCRIPTION

miRNA EXPRESSION IN ALLERGIC DISEASE

BACKGROUND OF THE INVENTION

This application claims priority to U.S. Application No. 61/176,824 filed on May 8, 2009, the entire disclosure of which is specifically incorporated herein by reference in its entirety without disclaimer.

This invention was made with government support under HL095382 and AI070973 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. Field of the Invention

The present invention relates generally to the fields of molecular biology, immunology, and the diagnosis and treatment of allergic lung disease. More particularly, it concerns particular miRNA and their application in the diagnosis and treatment of allergic lung disease.

2. Description of Related Art

The allergic lung diseases comprise a clinically heterogeneous group of disorders that relative to other chronic diseases afflict a disproportionately large number of persons in highly industrialized societies. By far the most prevalent allergic lung disease is allergic asthma. Asthma affects approximately 1 out of 8 Americans including children, making it one of the most common of chronic ailments (Wills-Karp, 1999). Despite major advances in the understanding of asthma pathophysiology, prognosis and treatments have changed little over the past decade. Clinical signs and symptoms of asthma, including episodic dyspnea, cough, and shortness of breath, are due to airway obstruction, which in turn is related to airway hyperresponsiveness (AHR), a physiological alteration in which the airway transiently constricts in response to a wide variety of provocative stimuli (Wills-Karp, 1999).

Airway obstruction in asthma is frequently observed in the context of local and systemic allergic inflammation that may include elevated total allergen-specific immunoglobulin E (IgE) levels and increased numbers of eosinophils and T helper type 2 (Th_2) cells, a terminally differentiated CD4+ T cell that secretes interleukins 4 (IL-4), IL-5, IL-6, IL-9 and IL-13 (Fahy *et al.*, 2000). Based on experimental studies of rodents, airway obstruction in the setting of allergic lung disease is largely mediated by the Th_2 cytokines IL-

4 and IL-13 (Corry *et al.*, 1996; Corry *et al.*, 1998; Grunig *et al.*, 1998). However, whereas IL-4 acts as a growth factor for Th_2 cells and immunoglobulin (Ig) E-secreting B cells, IL-13 acts on target lung tissues to induce AHR, goblet cell metaplasia and mucus hypersecretion (Corry, 1999). Despite the compartmentalized functional roles of IL-4 and IL-13, these cytokines are strongly related and signal through a common pathway that includes the alpha chain of the IL-4 receptor (IL-4Rα) (Zurawski *et al.*, 1993), the alpha 1 chain of the IL-13 receptor (IL-13Rα1) (Hilton *et al.*, 1996), the gamma chain of the IL-2 receptor (IL-2Rγ) (Russell *et al.*, 1993; Matthews *et al.*, 1995) and the transcription factor signal transducer and activator of transcription 6 (STAT6) (Hou *et al.*, 1994).

Other signaling pathways are equally important to the coordinated generation of Th_2 cells and other allergic effector cells. The transcription factors GATA3 and STAT6 are essential for stable Th_2 cell commitment and Th_2 cytokine secretion (Zheng and Flavell, 1997; Kishikawa *et al.*, 2001). In addition, co-stimulatory molecules are required for Th_2 cell development and experimental asthma, including tumor necrosis factor receptor super family-4 (Tnfrsf4; OX40) (Ohshima *et al.*, 1998; Salek-Ardakani *et al.*, 2003) and CD28 (Keane-Myers *et al.*, 1997; McArthur and Raulet, 1993). Thus, asthma-like disease in mice is mediated through a final common Th2 cell-dependent IL-13 signaling pathway in which STAT6 is activated through IL-4Rα and IL-13Rα1. Moreover, Th_2 cells arise through the same cytokine signaling pathway activated by IL-4 and additionally through a variety of co-stimulatory signaling pathways. Numerous additional signaling circuits, including epidermal growth factor (Takeyama *et al.*, 1999), thymic stromal lymphopoietin (Al-Shami *et al.*, 2005; Zhou *et al.*, 2005), IL-25 (Angkasekwinai *et al.*, 2007), histaminergic (Dunford *et al.*, 2006) and gamma amino butyric acid-(GABA)-ergic (Xiang *et al.*, 2007) pathways, complement protein 3a (C3a) (Drouin *et al.*, 2002), adenosine (Chunn *et al.*, 2001) and many others complement these core signaling mechanisms and significantly modify expression of allergic inflammation and the allergic lung disease phenotype.

MicroRNAs (miRNAs) are short, non-coding RNAs that target and silence protein coding genes through 3'-UTR elements. Relatively few miRNAs have been studied and an overall understanding of the importance of these regulatory transcripts in complex *in vivo* systems is lacking. Further, the precise role of miRNA in a variety of biological and developmental functions has not been fully elucidated. Because a single miRNA can typically affect the expression of several hundred different transcripts, predicting the function or *in vivo* effect of even a single miRNA can be particularly challenging. Clearly, there is a need for new and/or improved methods for diagnosing and treating allergic lung disease.

SUMMARY OF THE INVENTION

The present invention is based in part on the finding that substantial miRNA changes occur in the lung as a result of allergen challenge, and, further modulating or inhibiting the function of miRNA can result in the substantial inhibition of allergic and inflammatory responses *in vivo*. Specific miRNAs of potential disease relevance were observed to be down-regulated upon initial allergen challenge in a mouse model of allergic disease in humans. Certain aspects of the present invention are based in part on the finding of an additional layer of regulation involving post-transcriptional editing of multiple miRNAs that altered the target repertoire. As shown in the below examples, specific changes in miRNA expression were observed in the lung after allergen challenge *in vivo*. The inventors further discovered that inhibition of single or multiple let-7 miRNA, including, *e.g.*, mmu-mir-155, markedly inhibited inflammatory and allergic lung disease indices *in vivo*. Various aspects of the present invention relate to therapeutically treating an inflammatory or allergic lung disease via the inhibition of one or more let-7 miRNA.

Certain aspects of the present invention are based, in part, on the discovery that let-7 miRNA affect allergic and inflammatory responses in the lung. Multiple technologies were applied to globally analyze miRNA expression and function in allergic lung disease, an experimental model of asthma. Deep sequencing and microarray analyses of the mouse lung short RNAome revealed numerous extant and novel miRNAs and other transcript classes. Similar to mRNAs, lung miRNA expression changed dynamically during the transition from the naïve to the allergic state, suggesting numerous functional relationships. A possible role for miRNA editing in altering the lung mRNA target repertoire was also identified. Multiple members of the highly conserved let-7 miRNA family were the most abundant lung miRNAs, and it was confirmed *in vitro* that interleukin 13 (IL-13), a cytokine essential for expression for allergic lung disease, is regulated by mmu-let-7a. However, inhibition of let-7 miRNAs *in vivo* using a locked nucleic acid (LNA) profoundly inhibited production of allergic cytokines and the disease phenotype. These findings thus reveal unexpected complexity in the miRNAome underlying allergic lung disease and demonstrate a pro-inflammatory role for let-7 miRNAs. While certain aspects of the present invention relate to miRNA changes in the lung, it is nonetheless anticipated that similar changes in miRNA expression may result from exposure to an allergen or inflammatory stimuli in other tissues, including, for example, the skin, gastrointestinal tract (including esophagus, stomach, intestine, and colon), upper respiratory tract (*e.g.,* particularly the nasal sinuses), the eyes (*e.g.,* particularly the corneas,

sclerae and conjunctivae), liver and central nervous system (*e.g.,* particularly the brain and spinal cord).

Some aspects of the present invention involve methods for detecting an allergic or inflammatory lung disease, comprising assessing the level of one or more microRNAs (miRNAs) in a biological sample, wherein the level of the one or more miRNAs in the biological sample compared to a reference level of the one or more miRNAs is indicative of allergic or inflammatory lung disease. In certain embodiments, at least one of the one or more miRNAs comprises: (i) mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, mir-712, Asth-miR-1, or Asth-miR-2; (ii) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, or mir-467c; (iii) a sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, or 99% sequence identity to a sequence as set forth in (i); (iv) a sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, or 99% sequence identity to a sequence as set forth in (ii); (v) the complement of a sequence as set forth in (i) or (iii); or (vi) the complement of a sequence as set forth in (ii) or (iv); wherein a decrease in the expression level of one or more miRNAs from group (i), (iii) or (v), or an increase in the expression level of one or more miRNAs from group (ii), (iv) or (vi) in the biological sample compared to a reference level of the one or more miRNAs is indicative of allergic or inflammatory lung disease. The lung disease may be an allergic lung disease selected from the group consisting of asthma, hay fever, hypersensitivity pneumonitis, eosinophilic pneumonia (acute or chronic), Churg-Strauss Syndrome, allergic bronchopulmonary mycosis, and tropical eosinophilic pneumonia. In certain embodiments the allergic lung disease is asthma. The biological sample may comprise white blood cells or lung tissue. The method may further comprise obtaining a biological sample from a subject. In certain embodiments, more than one miRNAs is detected. In certain embodiments, the sequence of at least one miRNA is the complement of a sequence as set forth in (i) or (ii). At least one miRNA that is detected may or may not have a stem-loop structure. The method may further comprising detecting the presence or absence of one or more Piwi protein interacting RNAs (piRNAs).

Another aspect of the present invention relates to a biochip comprising an isolated nucleic acid comprising: (i) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-

5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712; (ii) a sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, or 99% sequence identity to a sequence as set forth in (i); (iii) the complement of a sequence as set forth in (i) or (ii); or (iv) a nucleic acid sequence comprising at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more contiguous nucleic acids of Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195); attached to said biochip. The biochip may comprise a plurality of nucleic acids as set forth in one or more of (i), (ii), (iii), and (iv).

Yet another aspect of the present invention involves methods of inhibiting a target gene in a cell, comprising contacting the cell with a nucleic acid in an amount sufficient to inhibit expression of the target gene, wherein the nucleic acid comprises: (i) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712; (ii) a sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, or 99% sequence identity to a sequence as set forth in (i); (iii) the complement of a sequence as set forth in (i) or (ii); or (iv) a nucleic acid sequence comprising at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more contiguous nucleic acids of Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195). The target gene may be an interleukin or a cytokine including, *e.g.*, GATA3, STAT6, IL13RA1, GATA3, CD4, ADRB2, JAK1, IL4, JAK1, IRAK1, STAT6, or IL13. The cell may be in a subject, such as a mammal. In certain embodiments, the subject is a human. The human may or may not have an allergic lung disease or may or may not be suspected of having an allergic lung disease. The allergic lung disease may be asthma. The cell may be a lung cell.

Another aspect of the present invention relates to methods of treating or preventing exacerbation of an allergic lung disease in a subject, comprising administering to said subject a pharmaceutically effective amount of a composition comprising a nucleic acid comprising: (i) mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, mir-712, Asth-miR-1, or Asth-miR-2; or (ii) a nucleic acid which selectively binds or

inhibits one or more of: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, or mir-467c. The nucleic acid may be a group (ii) nucleic acid, and nucleic acid may be chemically modified or comprise a nucleotide analog. In certain embodiments, the nucleic acid is selected from the group consisting of (5'-AACTATACAACCTACTACCTCA-3' (SEQ ID NO:246)), (5'-AACTATACAACCTCCTACCTCA-3' (SEQ ID NO:247)), and (5'-CAACCTACTACCTC-3' (SEQ ID NO:248)). The nucleic acid may be an LNA. The subject may be a mammal, such as a human. The allergic lung disease may be asthma, hay fever, or hypersensitivity pneumonitis. Said nucleic acid may comprise a phosphoramidate linkage, a phosphorothioate linkage, a phosphorodithioate linkage, or an O-methylphosphoroamidite linkage. Said nucleic acid may comprise one or more nucleotide analogs. In certain embodiments, the method further comprises administering to the subject one or more secondary forms of therapy for the treatment or prevention of allergic lung disease.

The therapeutic or preventive methods set forth herein may further involve administering to the subject one or more secondary forms of therapy for the treatment or prevention of allergic lung disease. Examples of secondary forms of therapy include a corticosteroid, a beta-2 adrenergic receptor agonist, a leukotrine modifier, an anti-immunoglobulin E (IgE) antibody, and a mast cell stabilizing agent.

The nucleic acid may optionally be included in a vector. For example, the vector may be a viral vector. Non-limiting examples of viral vectors include an adenovirus, an adeno-associated virus, a lentivirus, or a herpes virus. The vector may be a particular, such as a lipid-containing particle (*e.g.*, liposome).

Administration of the pharmaceutical compositions of the present invention may be by any method known to those of ordinary skill in the art. Non-limiting examples include via an aerosol, topically, locally, intravenously, intraarterially, intramuscularly, by lavage, or by injection into the thoracic cavity.

Yet another aspect of the present invention relates to kits comprising a biochip as set forth herein and one or more sealed containers. The kit may further comprise instructions for use of said biochip.

Some aspects of the present invention relate to kits comprising a sealed container comprising a nucleic acid, wherein said nucleic acid comprises: (i) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c,

mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712; (ii) a sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, or 99% sequence identity to a sequence as set forth in (i); (iii) the complement of a sequence as set forth in (i) or (ii); or (iv) a nucleic acid sequence comprising at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more contiguous nucleic acids of Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195). The kit may further comprise a set of primers specific for transcription or reverse transcription of one or more nucleic acid sequences as set forth in (i), (ii), (iii), or (iv). The kit may further comprise a biochip. The kti may further comprise instructions for use.

Yet another aspect of the present invention relates to kits comprising a sealed container comprising a set of primers specific for transcription or reverse transcription of a nucleic acid sequence, wherein said nucleic acid sequence comprises: (i) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712; (ii) a sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, or 99% sequence identity to a sequence as set forth in (i); (iii) the complement of a sequence as set forth in (i) or (ii); or (iv) a nucleic acid sequence comprising at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more contiguous nucleic acids of Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195).

Some aspects of the present invention relate to methods of treating an allergic or inflammatory lung disease in a subject comprising administering to the subject a let-7 miRNA inhibitor. In certain embodiments, the let-7 miRNA inhibitor is selected from the group consisting of siRNA, an antisense oligonucleotide, a locked nucleic acid (LNA), an antisense RNA, and a plasmid expressing an antisense RNA. The let-7 miRNA inhibitor may bind said miRNA under high stringency condiditons. In certain embodiments, the let-7 miRNA inhibitor is an LNA. In certain embodiments, the LNA comprises: (i) (5'-AACTATACAACCTACTACCTCA-3' (SEQ ID NO:246)), (5'-AACTATACAACCTCCTACCTCA-3' (SEQ ID NO:247)), or (5'-CAACCTACTACCTC-3' (SEQ ID NO:248)); (ii) a sequence having at least 80% sequence identity to a sequence as set

forth in (i); or (iii) the complement of a sequence as set forth in (i) or (ii). The let-7 miRNA inhibitor may be administered in a pharmaceutically acceptable composition. In certain embodiments, the let-7 miRNA inhibitor is administered orally, intravenously, via an aerosol, topically, locally, intravenously, intraarterially, intramuscularly, by lavage, or by injection into the thoracic cavity. The subject may be a mouse, a rat, a rodent, a cat, a horse, a goat, a sheep, a cow, a rabbit, a primate, or a human.

Yet another aspect of the present invention relates to an isolated nucleic acid comprising: (i) (5'-AACTATACAACCTACTACCTCA-3', SEQ ID NO:246), (5'-AACTATACAACCTCCTACCTCA-3' SEQ ID NO:247), or (5'-CAACCTACTACCTC-3' SEQ ID NO:248); (ii) a sequence having at least 80% sequence identity to (5'-AACTATACAACCTACTACCTCA-3' SEQ ID NO:246), (5'-AACTATACAACCTCCTACCTCA-3' SEQ ID NO:247), or (5'-CAACCTACTACCTC-3' SEQ ID NO:248); or (iii) the complement of a sequence as set forth in (i) or (ii); wherein the isolated nucleic acid can selectively bind a let-7 miRNA. In certain embodiments, the isolated nucleic acid selectively binds the let-7 miRNA under high stringency conditions. The nucleic acid may comprise a phosphoramidate linkage, a phosphorothioate linkage, a phosphorodithioate linkage, or an O-methylphosphoroamidite linkage, or other chemical modification. The nucleic acid may comprise one or more nucleotide analogs. In certain embodiments, the nucleic acid is a locked nucleic acid (LNA). The nucleic acid may be comprised in a pharmaceutically acceptable composition.

Another aspect of the present inveniton relates to an isolated nucleic acid selected from the group consisting of SEQ ID NO:285-322, or a complement thereof. The nucleic acid may be present on a biochip or a microarray.

Some aspects of the present invention relate to methods of screening for a modulator of an allergic or inflammatory lung response comprising: (a) contacting a lung cell with a candidate substance; and (b) measuring the expression level of one or more microRNAs (miRNAs) in the lung cell; wherein at least one of the one or more miRNAs comprises: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712, Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195); wherein an increase in the expression level of one or more of: mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-

452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, mir-712, Asth-miR-1, or Asth-miR-2 in the lung cell indicates that the modulator can inhibit an allergic or inflammatory lung response; and wherein a decrease in the expression level of one or more of: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c in the lung cell indicates that the modulator can inhibit an allergic or inflammatory lung response.

Yet another aspect of the present invention relates to methods of identifying a subject to receive an inhibitor of an allergic or inflammatory lung response comprising: measuring the expression level of one or more microRNAs (miRNAs) in a lung cell from the subject; wherein at least one of the one or more miRNAs comprises: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712, Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195); wherein an increase in the expression level of one or more of: mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, mir-712, Asth-miR-1, or Asth-miR-2 in the lung cell indicates that the subject may therapeutically benefit from said inhibitor; and wherein a decrease in the expression level of one or more of: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c in the lung cell indicates that the subject may therapeutically benefit from said inhibitor. The subject may be a human. The method may further comprises a method of personalizing a therapy for an allergic or inflammatory lung disease. Said measuring may be performed in a plurality of subjects. The method further comprises a method of identifying a sub-population of patients to receive said inhibitor; for example, these embodiments may be useful for identifying a sub-population which may particularly benefit from a therapy to treat an allergic or inflammatory lung disease.

Another aspect of the present invention relates to a transgenic mouse comprising a mutation in a let-7 miRNA, wherein the mutation prevents the expression of the let-7 miRNA, and wherein the mouse exhibits a reduced susceptibility to an allergic lung response. In certain embodiments, the let-7 miRNA is mir-155 (mouse miRNA-155).

Yet another aspect of the present invention relates to a progeny mouse of the mouse of claim 72, wherein the progeny mouse comprises a mutation in a let-7 miRNA, wherein the mutation prevents the expression of the let-7 miRNA, and wherein the progeny mouse exhibits a reduced susceptibility to an allergic lung response.

The reference level is a reference level of miRNA expression from a different subject or group of subjects. The reference level may be a reference level of expression of any of the aforementioned miRNAs from a subject known to be affected by an allergic lung disease or from a subject known to not be affected with an allergic lung disease. For example, the reference level may be the level of expression of one or more of the aforementioned miRNA species in one or more subjects with severe asthma. In other embodiments, the reference level is the level of expression of one or more of the aforementioned miRNA species in one or more subjects without asthma.

The reference level can be obtained from a single subject or from a group of subjects. The reference level of miRNA expression can be determined using any method known to those of ordinary skill in the art, such as any of the methods discussed above and elsewhere in this description. In some embodiments, the reference level is an average level of expression of any of the aforementioned miRNA obtained from a cohort of subjects with an allergic lung disease. The reference level may be a single value of miRNA expression, or it may be a range of values of miRNA expression. The reference level may also be depicted graphically as an area on a graph.

The subject may be any subject, such as an avian, an amphibian, or a mammal. Non-limiting examples of mammals include mice, rats, dogs, cats, horses, goats, sheep, cows, rabbits, primates, and humans. In particular embodiments, the subject is a patient that is suspected of having an allergic lung disease.

In particular embodiments, the level of more than one miRNA is assessed. The level of miRNA can be assessed by any method known to those of ordinary skill in the art. Non-limiting examples for assessing expression of miRNA are discussed in greater detail below.

"Allergic lung disease" as used herein refers to any disease of the lung that is associated with presence of eosinophils in the lung. Non-limiting examples of allergic lung disease include asthma, hay fever, hypersensitivity pneumonitis, eosinophilic pneumonia (acute or chronic), Churg-Strauss Syndrome, allergic bronchopulmonary mycosis, and tropical eosinophilic pneumonia. In specific embodiments, the allergic disease is asthma. "Asthma" is a common disorder in which chronic inflammation of the bronchial tubes (bronchi) makes them swell or construct, narrowing the airways. Asthma involves only the

bronchial tubes and does not affect the air sacs (alveoli) or the parenchyma of the lung. Airway constriction in asthma is due to three major processes acting on the bronchi: inflammation, bronchospasm, and mucus over-production. Various factors may precipitate an asthma attack in a subject, including allergies, infections, strong odors, fumes, and so forth.

"Biological sample" as used herein may mean a sample of biological tissue or fluid that comprises nucleic acids. Such samples include, but are not limited to, tissue or fluid isolated from subjects. Biological samples may also include sections of tissues such as biopsy and autopsy samples, frozen sections taken for histologic purposes, blood (such as white blood cells), plasma, serum, sputum, stool, tears, mucus, hair, and skin. Biological samples also include explants and primary and/or transformed cell cultures derived from animal or patient tissues. A biological sample may be provided by removing a sample of cells from an animal, but can also be accomplished by using previously isolated cells (*e.g.*, isolated by another person, at another time, and/or for another purpose), or by performing the methods described herein *in vivo*. Archival tissues, such as those having treatment or outcome history, may also be used. Tissue, such as lung tissue is specifically contemplated as a biological sample. Lung tissue may be obtained by any method known to those of ordinary skill in the art, such as via bronchoscopy or obtained at the time of thoracotomy.

The nucleic acids and miRNAs set forth herein may optionally include one or more phosphoramidate linkages, phosphorothioate linkages, phosphorodithioate linkages, or O-methylphosphoroamidite linkages. The nucleic acid may optionally include one or more nucleotide analogs. Non-limiting examples are discussed in greater detail in the specification below.

It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention.

The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device and/or method being employed to determine the value.

As used herein the specification, "a" or "an" may mean one or more, unless clearly indicated otherwise. As used herein in the claim(s), when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

The following figures form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIGS. 1A-D: Characterization and distribution of small RNAs in mouse lung. (FIG. 1A), Frequency of NGS-derived sequences as a function of nucleotide (nt) length. The 21-23 nt peak is typical for miRNAs. (FIG. 1B), Pie charts shows absolute numbers of sequenced transcripts from distinct lung RNA classes comparing allergen challenged to naïve mice. (FIG. 1C), Distribution of nucleotide modifications along the length of mature lung miRNAs comparing allergen challenged to naïve mice. (FIG. 1D) Editing of mmu-let-7a-1 as detected by NGS comparing allergen challenged to naïve lungs in which the ninth nucleotide of the seed sequence 'U' has been modified to 'G'. *: indicates canonical mature sequence (SEQ ID NOS:328-348).

FIGS. 2A-D: Gene and miRNA expression profiling of allergen challenged and naïve mouse lungs. (FIG. 2A) Heat map of genes (mRNAs) induced or repressed ($P<0.01$, fold>1.5) in allergen challenged versus naïve lung. (FIG. 2B) Validation of gene microarray findings by quantitative RT-PCR for selected genes. (FIG. 2C) Heat map of miRNAs induced or repressed ($P<0.01$, fold>1.5) in allergen challenged versus naïve lung. (FIG. 2D) Validation of miRNA microarray findings by quantitative RT-PCR for selected miRNAs. Bar graph data are presented as means ± SEM, N=3; *: $P < 0.05$.

FIGS. 3A-D: Inverse expression of IL13 and let-7a suggests a functional association. (FIG. 3A) The let-7a target sequence in the IL13 3'UTR is conserved across mammalia (Targetscan 5.1 (SEQ ID NOS:349-355)). (FIG. 3B) Mature let-7a sequence folded onto the mouse IL-13 3'UTR target site and predicted minimum free energy (mfe) value. (FIG. 3C, FIG. 3D) Quantitative RT-PCR analysis of IL13 and IFN-γ (FIG. 3C) and mmu-let-7a (FIG. 3D) transcripts from *in vitro* cultured Th1 and Th2 cells. Data are presented as means ± SEM, N=3; *: $P < 0.05$.

FIGS. 4A-I: IL13 expression is suppressed by let-7a. (FIG. 4A, FIG. 4B) let-7a suppresses mouse and human IL-13 in HEK293T cells. HEK293T cells were transfected with plasmids containing firefly luciferase under the control of the mouse (FIG. 4A) or human (FIG. 4B) IL-13 3'UTR or control 3'UTR and simultaneously with plasmids expressing pre-mmu-miR-705, scrambled pre-miR, or pre-let-7a (39, 117 or 350 ng) as indicated. After 2 days, gene expression was quantitated as firefly relative light units after normalizing for transfection efficiency based on Renilla luciferase activity (firefly/Renilla). (FIG. 4C, FIG. 4D) Anti-let-7a rescues mouse IL13 expression. HEK293T cells were transfected simultaneously with mouse (FIG. 4C) or human (FIG. 4D) IL13 3'UTR and pre-mmu-let-7a plasmids as in (FIG. 4A) and additionally scrambled, irrelevant (anti-mir-705) or anti-let-7a locked nucleic acids (LNA; 5.8, 17.5 and 52.5pmol). After 2 days, IL-13 expression was assessed as firefly/renilla relative light units. (FIGS. 4E-G) let-7a suppresses IL-13 gene expression in primary T cells. (FIG. 4E) Mouse splenic CD4+T cells were electroporated with FITC-labeled anti-mmu-let-7a LNAs (black curve) or sham (red curve) and the efficiency of transfection was assessed by flow cytometry. Additional T cells were transfected with control or anti-let-7a LNA (80 and 240 pmol) and relative expression of let-7a (FIG. 4F) and IL13 (FIG. 4G) transcripts were determined by RT-qPCR 24 hours later. (FIG. 4H) Editing of let-7a to let-7e reduces efficiency of targeting of IL13. HEK293T cells were transfected with mouse IL13 3'UTR-containing luciferase plasmid as in (FIG. 4A) and either plasmids for expression of let-7a or edited let-7a (U→G) and either scrambled or anti-let7a (U→G) LNA as indicated and the effect on IL13 gene expression was assessed as relative light units. (FIG. 4I) Pre-let7a (U→G) is fully processed to let-7e. RT-qPCR quantitation of let-7e or let-7a in HEK293T cells transfected with either pre-let-7a or pre-let-7a (U→G) expression plasmids. Data are presented as means ± SEM, N=3 or 4 replicates/condition; *: $P < 0.05$ for the indicated comparisons.

FIGS. 5A-E. Let-7 miRNAs are required for expression of allergic lung disease. (FIG. 5A) Protocol timeline for ovalbumin (OVA) immunization intraperitoneally (IP) and challenge intranasally (IN) and LNA administration intravenously (IV). (FIG. 5B) Anti-let-7 LNA suppresses T cell let-7 and IL-13 *in vivo*. RT-qPCR analysis of let-7a, IL-13 and IFN-γ transcripts in splenic CD4 T cells from mice treated under the indicated conditions. (FIG. 5C), Airway responsiveness as assessed by the change in respiratory system resistance (R_{RS}) in response to graded intravenous acetylcholine (Ach) challenge. *: $P < 0.05$ relative to naïve or OVA or OVA + Control LNA groups. (FIG. 5D), Total bronchoalveolar lavage fluid (BALF) inflammatory cells (eosinophils, macrophages, neutrophils, lymphocytes, total cells). (FIG. 5E), Bronchoalveolar lavage fluid levels of the indicated cytokines. *: $P < 0.05$ for the indicated comparisons. Data are presented as means ± SEM, N=5 mice per group.

FIGS. 6A-F: Novel miRNAs Asth-miR-1 and 2. Putative novel miRNAs discovered from illumina sequence data using the algorithm described in Methods. (FIG. 6A (SEQ ID NOS:356-372), FIG. 6B (SEQ ID NOS:373-401)), Sequences aligning with Asth-miR-1 from naïve and allergen challenged lung, respectively. The copy number of each sequence variant is shown at the end of the sequence. (FIG. 6C (SEQ ID NOS:402-417)) Predicted folded hairpin with mature Asth-miR-1 sequence marked in red. (FIG. 6D (SEQ ID NOS:418-422), FIG. 6E), Sequences aligning with Asth-miR-2 from naïve and allergen challenged lung (AC) with copy number of each sequence shown. (FIG. 6F) Folded hairpin and mature miRNA sequence marked in red. Pri-miRNA Asth-miR-2 is a single exon gene located in the intronic region of the mouse nucleolin gene.

FIG. 7: Novel miRNAs discovered from mouse T cells. Mature miRNA sequences are outlined in yellow and are depicted in the context of the putative pre-miRNA sequences. Criteria for determining new miRNAs are based on sequence, folding characteristics within the putative pre-miRNA and the minimum free energy (mfe) of the association (see Methods). Red and Blue arrows indicate putative Drosha/Pasha and Dicer cleavage sites, respectively (SEQ ID NOS:423-428).

FIGS. 8A-D: mmu-mir-155 is required for expression of allergic lung disease. Wild type and mir-155-/- mice were challenged intranasally over two weeks with an allergenic fungal proteinase (FP) or PBS and the effect on the asthma phenotype was determined. (FIG. 8A) airway responsivenessas assessed by the change in respiratory system resistance (RRS) in response to intravenous acetylcholine (Ach) challenge. (FIG. 8B) Total and differential cell counts in bronchoalveolar lavage (BAL) fluid for macrophages (mac), eosinophils (Eos),

neutrophils (Neu) and lymphocytes (Lymph). (FIG. 8C) Total IL-4- and interferon gamma (IFN-)- secreting cells detected from whole lung. (FIG. 8D) Concentration of selected cytokines in BAL fluid.

FIG. 9. Distribution of Small RNAs in Helper T Cells. The number of reads that exclusively mapped to one or a combination of three databases, miRNAs, piRNAs from T cell subsets.

FIGS. 10A-B: FIG. 10A, Highly expressed miRNAs including let -7 series miRNAT_H1 cells show increased expression of mmu-let-7c which is involved in CD4+ cell activation (Cobb *et al.* 2007; Li *et al.,* 2007). T_H2 cells show decreased expression of mmu-mir-181a which is involved in CD4+ T cell development (Cobb *et al.,* 2007; Li *et al.,*). FIG. 10B, Highly expressed miRNAs excluding let -7 series miRNA. T_H1 cells show increased mmu-mir-101a. The putative gene targets are: STAT6, GATA3, CD38, IL-4Rα. T_H2 cells show increased expression of mmu-mir-199a. The putative gene targets are: STAT6, GATA3, IL-4Rα, ICOS. miRNA expression confirmed by qRT-PCR.

FIG. 11. Novel putative targets. Target Scan4.1 was used to determine the putative targets of the seed sequences the novel miRNAs. Novel miRNAs that target GATA3 in naïve and T_H1 cells were found. In T_H2 cells, BCL6 was a putative target. In addition many cell survival and apoptotic gene targets were identified in all of the subsets (SEQ ID NOS:429-434).

FIG. 12. Putative miRNA:mRNA Associations. Bioinformatics analysis of Illumina sequencing data and mRNA microarray chip (Illumina) of effector T cell subsets identify putative gene targets of sequenced mRNAs. Putative miRNA Regulation of Gilz in Helper T Cell Differentiation.

FIG. 13. miRNA Mechanism of Action. During normal T_H1 polarization T-bet, NFAT, NF-κIFN-γ are up-regulated, Gilz overexpression inhibits TCR/CD3-induced NF-κB activation and nuclear translocation (Ayroldi *et al.,* 2001) and contributes to CD4+ commitment toward T_H2 phenotype (Camarlle *et al.,* 2005). The T_H1-specific functional association data suggests the miRNA targeting Gilz suppress its function thus allowing T_H1 lineage commitment.

Description of Illustrative Embodiments

A. Definitions

"Subject" as used herein may mean fish, amphibians, reptiles, birds, and mammals, such as mice, rats, rabbits, goats, cats, dogs, cows, apes and humans.

"Attached" or "immobilized" as used herein to refer to a nucleic acid proble and a solid support may mean that the binding between the probe and the solid support is sufficient to be stable under conditions of binding, washing, analysis, and removal. The binding may be covalent or non-covalent. Covalent bonds may be formed directly between the probe and the solid support or may be formed by a cross linker or by inclusion of a specific reactive group on either the solid support or the probe or both molecules. Non-covalent binding may be one or more of electrostatic, hydrophilic, and hydrophobic interactions. Included in non-covalent binding is the covalent attachment of a molecule, such as streptavidin, to the support and the non-covalent binding of a biotinylated probe to the streptavidin. Immobilization may also involve a combination of covalent and non-covalent interactions.

"Complement" or "complementary" as used herein to refer to a nucleic acid may mean Watson-Crick (*e.g.*, A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

"Differential expression" may mean qualitative or quantitative differences in the temporal and/or cellular gene expression patterns within and among cells and tissue. Thus, a differentially expressed gene may qualitatively have its expression altered, including an activation or inactivation, in, *e.g.*, normal versus disease tissue. Genes may be turned on or turned off in a particular state, relative to another state thus permitting comparison of two or more states. A qualitatively regulated gene may exhibit an expression pattern within a state or cell type which may be detectable by standard techniques. Some genes may be expressed in one state or cell type, but not in both. Alternatively, the difference in expression may be quantitative, *e.g.*, in that expression is modulated, either up-regulated, resulting in an increased amount of transcript, or down-regulated, resulting in a decreased amount of transcript. The degree to which expression differs need only be large enough to quantify via standard characterization techniques such as expression arrays, quantitative reverse transcriptase PCR, northern analysis, and RNase protection.

"Gene" used herein may be a natural (*e.g.*, genomic) or synthetic gene comprising transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (*e.g.*, introns, 5'- and 3'-untranslated sequences). The coding region of a gene may be a nucleotide sequence coding for an amino acid sequence or a functional RNA, such as tRNA, rRNA, catalytic RNA, siRNA, miRNA or antisense RNA. A gene may also be an mRNA or cDNA corresponding to the coding regions (*e.g.*, exons and miRNA) optionally comprising 5'- or 3'-untranslated sequences linked thereto. A gene may also be an amplified

nucleic acid molecule produced *in vitro* comprising all or a part of the coding region and/or 5'- or 3'-untranslated sequences linked thereto.

"Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

"Label" as used herein may mean a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include .sup.32P, fluorescent dyes, electron-dense reagents, enzymes (*e.g.*, as commonly used in an ELISA), biotin, digoxigenin, or haptens and other entities which can be made detectable. A label may be incorporated into nucleic acids and proteins at any position.

"Nucleic acid" or "oligonucleotide" or "polynucleotide" used herein may mean at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions. Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine,

thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

A nucleic acid will generally contain phosphodiester bonds, although nucleic acid analogs may be included that may have at least one different linkage, *e.g.*, phosphoramidate, phosphorothioate, phosphorodithioate, or O-methylphosphoroamidite linkages and peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with positive backbones; non-ionic backbones, and non-ribose backbones, including those described in U.S. Pat. Nos. 5,235,033 and 5,034,506, which are incorporated by reference. Nucleic acids containing one or more non-naturally occurring or modified nucleotides are also included within one definition of nucleic acids. The modified nucleotide analog may be located for example at the 5'-end and/or the 3'-end of the nucleic acid molecule. Representative examples of nucleotide analogs may be selected from sugar- or backbone-modified ribonucleotides. It should be noted, however, that also nucleobase-modified ribonucleotides, *i.e.* ribonucleotides, containing a non-naturally occurring nucleobase instead of a naturally occurring nucleobase such as uridines or cytidines modified at the 5-position, *e.g.* 5-(2-amino)propyl uridine, 5-bromo uridine; adenosines and guanosines modified at the 8-position, *e.g.* 8-bromo guanosine; deaza nucleotides, *e.g.* 7-deaza-adenosine; O- and N-alkylated nucleotides, *e.g.* N6-methyl adenosine are suitable. The 2'-OH-group may be replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, NR.sub.2 or CN, wherein R is C.sub.1-C.sub.6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I. Modified nucleotides also include nucleotides conjugated with cholesterol through, *e.g.*, a hydroxyprolinol linkage as described in Krutzfeldt *et al.* (2005); Soutschek *et al.* (2004); and U.S. Patent Publication No. 20050107325, which are incorporated herein by reference. Modified nucleotides and nucleic acids may also include locked nucleic acids (LNA), as described in U.S. Patent Publication No. 2002/0115080, U.S. Patent 6,268,490, and U.S. Patent 6,770,748, which are incorporated herein by reference. LNA nucleotides include a modified extra methylene "bridge" connecting the 2' oxygen and 4' carbon of the ribose ring. The bridge "locks" the ribose in the 3'-endo (North) conformation, which is often found in the A-form of DNA or RNA. LNA nucleotides can be mixed with DNA or RNA bases in the oligonucleotide whenever desired. Such oligomers are commercially available from companies including Exiqon (Vedbaek, Denmark). Additional modified nucleotides and nucleic acids are described in U.S. Patent Publication Nos. 20050182005, which is incorporated herein by reference. Modifications of the ribose-phosphate backbone may be done for a variety of reasons, *e.g.*, to increase the stability and half-life of such molecules in physiological environments, to enhance diffusion

across cell membranes, or as probes on a biochip. Mixtures of naturally occurring nucleic acids and analogs may be made; alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made.

A nucleic acid may be used to therapeutically inhibit a let-7 miRNA. For example a nucleic acid comprising a seqence having at least 80%, 85%, 90%, 95%, or all of SEQ ID NO:246-248 may be used to inhibit the function of a let-7 miRNA *in vitro* or *in vivo*. As shown in the below examples, the inhibition of one or more let-7 miRNA (*e.g.*, mmu-mir-155) is sufficient to substantially inhibit allergic or inflammatory lung responses in vivo.

For example, in certain embodiments a complementary nucleic acid, such as a modified nucleic acid or an LNA, may be used to bind or suppress the function of one or more let-7 miRNA. As shown in the below examples, full-length LNAs anti-complementary to let-7a (5'-AACTATACAACCTACTACCTCA-3' (SEQ ID NO:246)) or let-7e (5'-AACTATACAACCTCCTACCTCA-3' (SEQ ID NO:247)) may be used to inhibit the function of these let-7a or let-7e, respectively. A truncated anti-let-7a,b,c.d LNA (*e.g.*, 5'-CAACCTACTACCTC-3' (SEQ ID NO:248)) may be used *in vitro* or *in vivo* to bind or inhibit the function of multiple miRNA, such as multiple let-7 miRNA. In certain embodiments, a LNA may be administered to a subject, such as a mouse, rat, primate, or human subject, to inhibit the function of one or more miRNA. As shown in the below examples, inhibition of the function of one or more let-7 miRNA (*e.g.*, mmu-mir-155, *etc.*) can result in a decrease in an inflammatory and/or allergic lung response. It is anticipated that the foregoing sequences do not need to be LNA; similar effect may be achieved using one or more of the foregoing sequences either alone or comprising one or more modification (*e.g.*, to reduce *in vivo* degradation, improve pharmacokinetics, *etc.*).

"Promoter" as used herein may mean a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter may comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter may also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter may be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter may regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of

promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV40 late promoter and the CMV IE promoter.

"Stringent hybridization conditions" used herein may mean conditions under which a first nucleic acid sequence will hybridize to a second nucleic acid sequence, such as in a complex mixture of nucleic acids. Stringent conditions are sequence-dependent and will be different in different circumstances. Stringent conditions may be selected to be about 5-10.degree. C. lower than the thermal melting point (T.sub.m) for the specific sequence at a defined ionic strength pH. The T.sub.m may be the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T.sub.m, 50% of the probes are occupied at equilibrium). Stringent conditions may be those in which the salt concentration is less than about 1.0 M sodium ion, such as about 0.01-1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30.degree. C. for short probes (*e.g.*, about 10-50 nucleotides) and at least about 60.degree. C. for long probes (*e.g.*, greater than about 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal may be at least 2 to 10 times background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5.times.SSC, and 1% SDS, incubating at 42.degree. C., or, 5.times.SSC, 1% SDS, incubating at 65.degree. C., with wash in 0.2.times.SSC, and 0.1% SDS at 65.degree. C.

"Substantially complementary" used herein may mean that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the complement of a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides, or that the two sequences hybridize under stringent hybridization conditions.

B. MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are short, non-coding RNAs that target and silence protein coding genes through 3'-UTR elements. Important roles for miRNAs in numerous biological processes have been established, but comprehensive analyses of miRNA function in complex diseases are lacking. MiRNAs are initially transcribed as primary miRNAs (pri-miRNAs) that are then cleaved by the nuclear RNAses Drosha and Pasha to yield precursor-miRNAs

(pre-miRNAs). These precursors are further processed by the cytoplasmic RNAse III dicer to form short double stranded miR-miR* duplexes, one strand of which (miR) is then integrated into the RNA Induced Silencing Complex (RISC) that includes the enzymes dicer and Argonaute (Ago). The mature miRNAs (~17-24nt) direct RISC to specific target sites located within the 3'UTR of target genes. Once bound to target sites, miRNAs represses translation through mRNA decay, translational inhibition and/or sequestration into processing bodies (P-bodies) (Eulalio *et al.*, 2008; Behm-Ansmant *et al.*, 2006; Chu and Rana, 2006). Recent estimates find that over 60% of protein coding genes carry 3'-UTR miRNA target sites (Friedman *et al.*, 2009). In this regard, miRNAs act as key regulators of processes as diverse as early development (Reinhart *et al.*, 2000), cell proliferation and cell death (Brennecke *et al.*, 2003), apoptosis and fat metabolism (Xu *et al.*, 2003), and cell differentiation (Chen, 2004; Dostie *et al.*, 2003). In addition, studies of miRNA expression in chronic lymphocytic leukemia (Calin *et al.*, 2008), colonic adenocarcinoma (Michael *et al.*, 2003), Burkitt's lymphoma (Metzler *et al.*, 2004), cardiac disease (Zhao *et al.*, 2007) and viral infection (Pfeffer *et al.*, 2004) suggest vital links between miRNA and numerous diseases.

MicroRNAs are highly conserved during evolution and yet subjected to post-transcriptional modification through RNA editing. RNA-dependent adenosine deaminase (ADAR)-mediated A-to-I editing has been shown to mediate nucleotide changes in some pre-miRNAs (Habig *et al.*, 2007; Bass, 2006). It has been previously shown that non-random nucleotide changes occurring in mouse ovary and pancreas miRNAs are enriched in nucleotides at the extreme 5' end and at nucleotide 9 (Reid *et al.*, 2008). U-to-G modifications at position 9 in mmu-let-7a can potentially modulate duplex stability and therefore regulate mRNA cleavage and decay (Reid *et al.*, 2008). Thus, transcriptional and post-transcriptional regulatory processes potentially powerfully influence the regulatory potential of miRNAs.

miRNAs thus far observed have been approximately 21-22 nucleotides in length and they arise from longer precursors, which are transcribed from non-protein-encoding genes. *See* review of Carrington *et al.* (2003). The precursors form structures that fold back on each other in self-complementary regions; they are then processed by the nuclease Dicer in animals or DCL1 in plants. miRNA molecules interrupt translation through precise or imprecise base-pairing with their targets.

miRNAs are involved in gene regulation. Some miRNAs, including *lin-4* and *let-7*, inhibit protein synthesis by binding to partially complementary 3′ untranslated regions (3′

UTRs) of target mRNAs. Others function like siRNA and bind to perfectly complementary mRNA sequences to destroy the target transcript.

Research on microRNAs is increasing as scientists are beginning to appreciate the broad role that these molecules play in the regulation of eukaryotic gene expression. The two best understood miRNAs, *lin-4* and *let-7*, regulate developmental timing in *C. elegans* by regulating the translation of a family of key mRNAs (reviewed in Pasquinelli, 2002). Several hundred miRNAs have been identified in *C. elegans*, *Drosophila*, mouse, and humans. As would be expected for molecules that regulate gene expression, miRNA levels have been shown to vary between tissues and developmental states. In addition, one study shows a strong correlation between reduced expression of two miRNAs and chronic lymphocytic leukemia, providing a possible link between miRNAs and cancer (Calin, 2002). Although the field is still young, there is speculation that miRNAs could be as important as transcription factors in regulating gene expression in higher eukaryotes.

There are a few examples of miRNAs that play critical roles in cell differentiation, early development, and cellular processes like apoptosis. *lin-4* and *let-7* both regulate passage from one larval state to another during *C. elegans* development (Ambros, 2003). *mir-14* and *bantam* are *drosophila* miRNAs that regulate cell death, apparently by regulating the expression of genes involved in apoptosis (Brennecke *et al.*, 2003, Xu *et al.*, 2003). miR-181 guides hematopoietic cell differentiation (Chen *et al.,* 2004). Enhanced understanding of the functions of miRNAs will undoubtedly reveal regulatory networks that contribute to normal development, differentiation, inter- and intra-cellular communication, cell cycle, angiogenesis, apoptosis, and many other cellular processes.

Certain embodiments of the present invention involve methods for diagnosing or treating an allergic lung disease in a subject that involves inhibiting the function or measuring expression, respectively, of one or more miRNA species in a sample from the subject. miRNA function can be inhibited, for example, by the administration of a complementary or substantially complementary nucleic acid (*e.g.*, a modified nucleic acid such as LNA, *etc.*). The miRNA species that may be used to diagnose or treat an allergic or inflammatory lung disease include species selected from the group shown in Table 1 below.

Table 1. Selected miRNA

miRNA	Sequence	SEQ.ID NO.
let-7a	UGAGGUAGUAGGUUGUAUAGU	SEQ. ID NO. 1
let-7b	UGAGGUAGUAGGUUGUGUGGUU	SEQ.ID NO. 2
let-7c	UGAGGUAGUAGGUUGUAUGGUU	SEQ.ID NO. 3
let-7d	AGAGGUAGUAGGUUGCAUAGU	SEQ.ID NO. 4
let-7d-3p	CUAUACGACCUGCUGCCUUUCU	SEQ.ID NO. 5
let-7e	UGAGGUAGGAGGUUGUAUAGU	SEQ.ID NO. 6
let-7f	UGAGGUAGUAGAUUGUAUAGU	SEQ.ID NO. 7
let-7g	UGAGGUAGUAGUUUGUACAGU	SEQ.ID NO. 8
let-7i	UGAGGUAGUAGUUUGUGCUGU	SEQ.ID NO. 9
miR-1	UGGAAUGUAAAGAAGUAUGUA	SEQ.ID NO. 10
miR-100	AACCCGUAGAUCCGAACUUGUG	SEQ.ID NO. 11
mir-101a	UACAGUACUGUGAUAACUGAAG	SEQ.ID NO. 12
mir-101b	UACAGUACUGUGAUAGCUGAAG	SEQ.ID NO. 13
mir-103	AGCAGCAUUGUACAGGGCUAUGA	SEQ.ID NO. 14
miR-106a	CAAAGUGCUAACAGUGCAGGUA	SEQ.ID NO. 15
miR-106b	UAAAGUGCUGACAGUGCAGAU	SEQ.ID NO. 16
mir-107	AGCAGCAUUGUACAGGGCUAUCA	SEQ.ID NO. 17
miR-10a	UACCCUGUAGAUCCGAAUUUGUG	SEQ.ID NO. 18
miR-10b	CCCUGUAGAACCGAAUUUGUGU	SEQ.ID NO. 19
miR-125a	UCCCUGAGACCCUUUAACCUGUG	SEQ.ID NO.20
miR-125b	UCCCUGAGACCCUAACUUGUGA	SEQ.ID NO. 21
miR-126-3p	UCGUACCGUGAGUAAUAAUGC	SEQ.ID NO. 22
mir-126-5p	CAUUAUUACUUUUGGUACGCG	SEQ.ID NO. 23
miR-127	UCGGAUCCGUCUGAGCUUGGC	SEQ.ID NO. 24
miR-128a	UCACAGUGAACCGGUCUCUUUU	SEQ.ID NO. 25

miRNA	Sequence	SEQ.ID NO.
miR-128b	UCACAGUGAACCGGUCUCUUUC	SEQ.ID NO. 26
miR-130a	CAGUGCAAUGUUAAAAGGGCAU	SEQ.ID NO. 27
mir-132	UAACAGUCUACAGCCAUGGUCG	SEQ.ID NO. 28
miR-133a	UUGGUCCCCUUCAACCAGCUGU	SEQ.ID NO. 29
miR-133b	UUGGUCCCCUUCAACCAGCUA	SEQ.ID NO. 30
miR-140-3p	UACCACAGGGUAGAACCACGG	SEQ.ID NO. 31
miR-141	UAACACUGUCUGGUAAAGAUGG	SEQ.ID NO. 32
miR-142-5p	CAUAAAGUAGAAAGCACUAC	SEQ.ID NO. 33
mir-143	UGAGAUGAAGCACUGUAGCUCA	SEQ.ID NO. 34
miR-145	GUCCAGUUUUCCCAGGAAUCCCUU	SEQ.ID NO. 35
mir-146	UGAGAACUGAAUUCCAUGGGUU	SEQ.ID NO. 36
mir-146b	UGAGAACUGAAUUCCAUAGGCU	SEQ.ID NO. 37
mir-148a	UCAGUGCACUACAGAACUUUGU	SEQ.ID NO. 38
mir-148b	UCAGUGCAUCACAGAACUUUGU	SEQ.ID NO. 39
miR-149	UCUGGCUCCGUGUCUUCACUCC	SEQ.ID NO. 40
mir-150	UCUCCCAACCCUUGUACCAGUG	SEQ.ID NO. 41
mir-151	CUAGACUGAGGCUCCUUGAGG	SEQ.ID NO. 42
mir-152	UCAGUGCAUGACAGAACUUGGG	SEQ.ID NO. 43
mir-155	UUAAUGCUAAUUGUGAUAGGGG	SEQ.ID NO. 44
miR-15a	UAGCAGCACAUAAUGGUUUGUG	SEQ.ID NO. 45
miR-15b	UAGCAGCACAUCAUGGUUUACA	SEQ.ID NO. 46
miR-16	UAGCAGCACGUAAAUAUUGGCG	SEQ.ID NO. 47
miR-17-5p	CAAAGUGCUUACAGUGCAGGUAGU	SEQ.ID NO. 48
miR-181a	AACAUUCAACGCUGUCGGUGAGU	SEQ.ID NO. 49
miR-181b	AACAUUCAUUGCUGUCGGUGGG	SEQ.ID NO. 50
miR-181c	AACAUUCAACCUGUCGGUGAGU	SEQ.ID NO. 51

miRNA	Sequence	SEQ.ID NO.
miR-182	UUUGGCAAUGGUAGAACUCACA	SEQ.ID NO. 52
miR-183	UAUGGCACUGGUAGAAUUCACUG	SEQ.ID NO. 53
miR-185	UGGAGAGAAAGGCAGUUC	SEQ.ID NO. 54
miR-187	UCGUGUCUUGUGUUGCAGCCGG	SEQ.ID NO. 55
miR-191	CAACGGAAUCCCAAAAGCAGCU	SEQ.ID NO. 56
miR-194	UGUAACAGCAACUCCAUGUGGA	SEQ.ID NO. 57
miR-195	UAGCAGCACAGAAAUAUUGGC	SEQ.ID NO. 58
mir-199a-3p	ACAGUAGUCUGCACAUUGGUUA	SEQ.ID NO. 59
mir-199a	CCCAGUGUUCAGACUACCUGUUC	SEQ.ID NO. 60
mir-199b	CCCAGUGUUUAGACUACCUGUUC	SEQ.ID NO. 61
miR-19b	UGUGCAAAUCCAUGCAAAACUGA	SEQ.ID NO. 62
miR-200a	UAACACUGUCUGGUAACGAUGU	SEQ.ID NO. 63
miR-200b	UAAUACUGCCUGGUAAUGAUGAC	SEQ.ID NO. 64
miR-200c	UAAUACUGCCGGGUAAUGAUGG	SEQ.ID NO. 65
miR-203	UGAAAUGUUUAGGACCACUAG	SEQ.ID NO. 66
miR-205	UCCUUCAUUCCACCGGAGUCUG	SEQ.ID NO. 67
miR-206	UGGAAUGUAAGGAAGUGUGUGG	SEQ.ID NO. 68
miR-20a	UAAAGUGCUUAUAGUGCAGGUAG	SEQ.ID NO. 69
miR-20b	CAAAGUGCUCAUAGUGCAGGUA	SEQ.ID NO. 70
miR-21	UAGCUUAUCAGACUGAUGUUGA	SEQ.ID NO. 71
miR-214	ACAGCAGGCACAGACAGGCAG	SEQ.ID NO. 72
miR-218	UUGUGCUUGAUCUAACCAUGU	SEQ.ID NO. 73
miR-22	AAGCUGCCAGUUGAAGAACUGU	SEQ.ID NO. 74
miR-221	AGCUACAUUGUCUGCUGGGUUU	SEQ.ID NO. 75
miR-222	AGCUACAUCUGGCUACUGGGUCUC	SEQ.ID NO. 76
miR-223	UGUCAGUUUGUCAAAUACCCC	SEQ.ID NO. 77

miRNA	Sequence	SEQ.ID NO.
miR-224	UAAGUCACUAGUGGUUCCGUUUA	SEQ.ID NO. 78
miR-23a	AUCACAUUGCCAGGGAUUUCC	SEQ.ID NO. 79
miR-23b	AUCACAUUGCCAGGGAUUACC	SEQ.ID NO. 80
miR-24	UGGCUCAGUUCAGCAGGAACAG	SEQ.ID NO. 81
miR-25	CAUUGCACUUGUCUCGGUCUGA	SEQ.ID NO. 82
miR-26a	UUCAAGUAAUCCAGGAUAGGC	SEQ.ID NO. 83
miR-26b	UUCAAGUAAUUCAGGAUAGGUU	SEQ.ID NO. 84
mir-27a	UUCACAGUGGCUAAGUUCCGC	SEQ.ID NO. 85
mir-27b	UUCACAGUGGCUAAGUUCUGC	SEQ.ID NO. 86
miR-28	AAGGAGCUCACAGUCUAUUGAG	SEQ.ID NO. 87
miR-29a	UAGCACCAUCUGAAAUCGGUU	SEQ.ID NO. 88
miR-29b	UAGCACCAUUUGAAAUCAGUGUU	SEQ.ID NO. 89
miR-29c	UAGCACCAUUUGAAAUCGGU	SEQ.ID NO. 90
miR-30a-3p	CUUUCAGUCGGAUGUUUGCAGC	SEQ.ID NO. 91
miR-30a-5p	UGUAAACAUCCUCGACUGGAAG	SEQ.ID NO. 92
miR-30b	UGUAAACAUCCUACACUCAGCU	SEQ.ID NO. 93
miR-30c	UGUAAACAUCCUACACUCUCAGC	SEQ.ID NO. 94
miR-30d	UGUAAACAUCCCCGACUGGAAG	SEQ.ID NO. 95
miR-30e	UGUAAACAUCCUUGACUGGA	SEQ.ID NO. 96
miR-30e-3p	CUUUCAGUCGGAUGUUUACAGC	SEQ.ID NO. 97
miR-31	AGGCAAGAUGCUGGCAUAGCUG	SEQ.ID NO. 98
miR-320	AAAAGCUGGGUUGAGAGGGCGAA	SEQ.ID NO. 99
miR-322	AAACAUGAAGCGCUGCAACA	SEQ.ID NO. 100
miR-324-3p	CCACUGCCCCAGGUGCUGCUGG	SEQ.ID NO. 101
miR-324-5p	CGCAUCCCCUAGGGCAUUGGUG	SEQ.ID NO. 102
miR-328	CUGGCCCUCUCUGCCCUUCCGU	SEQ.ID NO. 103

miRNA	Sequence	SEQ.ID NO.
miR-331	GCCCCUGGGCCUAUCCUAGAA	SEQ.ID NO. 104
miR-335	UCAAGAGCAAUAACGAAAAAUGU	SEQ.ID NO. 105
miR-341	UCGAUCGGUCGGUCGGUCAGU	SEQ.ID NO. 106
miR-342	UCUCACACAGAAAUCGCACCCGUC	SEQ.ID NO. 107
miR-345	UGCUGACCCCUAGUCCAGUGC	SEQ.ID NO. 108
miR-34a	UGGCAGUGUCUUAGCUGGUUGUU	SEQ.ID NO. 109
miR-34c	AGGCAGUGUAGUUAGCUGAUUGC	SEQ.ID NO. 110
miR-350	UUCACAAAGCCCAUACACUUUCA	SEQ.ID NO. 111
miR-351	UCCCUGAGGAGCCCUUUGAGCCUG	SEQ.ID NO. 112
miR-361	UUAUCAGAAUCUCCAGGGGUAC	SEQ.ID NO. 113
miR-365	UAAUGCCCCUAAAAAUCCUUAU	SEQ.ID NO. 114
miR-374-5p	AUAUAAUACAACCUGCUAAGUG	SEQ.ID NO. 115
miR-375	UUUGUUCGUUCGGCUCGCGUGA	SEQ.ID NO. 116
miR-379	UGGUAGACUAUGGAACGUAGG	SEQ.ID NO. 117
miR-422b	CUGGACUUGGAGUCAGAAGGCC	SEQ.ID NO. 118
miR-424	CAGCAGCAAUUCAUGUUUUGGA	SEQ.ID NO. 119
miR-429	UAAUACUGUCUGGUAAUGCCGU	SEQ.ID NO. 120
miR-434-3p	UUUGAACCAUCACUCGACUCC	SEQ.ID NO. 121
miR-449	UGGCAGUGUAUUGUUAGCUGGU	SEQ.ID NO. 122
miR-450	UUUUUGCGAUGUGUUCCUAAUA	SEQ.ID NO. 123
miR-451	AAACCGUUACCAUUACUGAGUU	SEQ.ID NO. 124
miR-455-3p	AUGCAGUCCACGGGCAUAUACACU	SEQ.ID NO. 125
miR-467a	AUAUACAUACACACACCUACAC	SEQ.ID NO. 126
miR-467b	AUAUACAUACACACACCAACAC	SEQ.ID NO. 127
miR-484	UCAGGCUCAGUCCCCUCCCGAU	SEQ.ID NO. 128
miR-486	UCCUGUACUGAGCUGCCCCGAG	SEQ.ID NO. 129

miRNA	Sequence	SEQ.ID NO.
miR-497	CAGCAGCACACUGUGGUUUGUA	SEQ.ID NO. 130
miR-501-3p	AAUGCACCCGGGCAAGGAUUUG	SEQ.ID NO. 131
miR-532	CAUGCCUUGAGUGUAGGACCGU	SEQ.ID NO. 132
miR-541	AAGGGAUUCUGAUGUUGGUCACA	SEQ.ID NO. 133
miR-652	AAUGGCGCCACUAGGGUUGUGCA	SEQ.ID NO. 134
miR-669c	AUAGUUGUGUGUGGAUGUGUGU	SEQ.ID NO. 135
miR-671	AGGAAGCCCUGGAGGGGCUGGAGG	SEQ.ID NO. 136
miR-672	UGAGGUUGGUGUACUGUGUGUG	SEQ.ID NO. 137
miR-674	GCACUGAGAUGGGAGUGGUGUA	SEQ.ID NO. 138
miR-674-3p	CACAGCUCCCAUCUCAGAACAA	SEQ.ID NO. 139
miR-676	CCGUCCUGAGGUUGUUGAGCU	SEQ.ID NO. 140
miR-689	CGUCCCCGCUCGGCGGGGUCC	SEQ.ID NO. 141
miR-690	AAAGGCUAGGCUCACAACCAAA	SEQ.ID NO. 142
mir-705	GGUGGGAGGUGGGGUGGGCA	SEQ.ID NO. 143
miR-709	GGAGGCAGAGGCAGGAGGA	SEQ.ID NO. 144
miR-720	AUCUCGCUGGGGCCUCCA	SEQ.ID NO. 145
miR-744	UGCGGGGCUAGGGCUAACAGC	SEQ.ID NO. 146
mir-762	GGGGCUGGGGCCGGGACAGAGC	SEQ.ID NO. 147
miR-805	GAAUUGAUCAGGACAUAGGG	SEQ.ID NO. 148
miR-92	UAUUGCACUUGUCCCGGCCUG	SEQ.ID NO. 149
miR-93	CAAAGUGCUGUUCGUGCAGGUAG	SEQ.ID NO. 150
miR-98	UGAGGUAGUAAGUUGUAUUGUU	SEQ.ID NO. 151
miR-99a	ACCCGUAGAUCCGAUCUUGU	SEQ.ID NO. 152
miR-99b	CACCCGUAGAACCGACCUUGCG	SEQ.ID NO. 153
mir-101a	UACAGUACUGUGAUAACUGAAG	SEQ ID. NO. 154
mir-101b	UACAGUACUGUGAUAGCUGAAG	SEQ ID NO: 155

miRNA	Sequence	SEQ.ID NO.
mir-103	AGCAGCAUUGUACAGGGCUAUGA	SEQ ID NO:156
mir-107	AGCAGCAUUGUACAGGGCUAUCA	SEQ ID NO:157
mir-146	UGAGAACUGAAUUCCAUGGGUU	SEQ ID NO:158
mir-146b	UGAGAACUGAAUUCCAUAGGCU	SEQ ID NO:159
mir-148a	UCAGUGCACUACAGAACUUUGU	SEQ ID NO:160
mir-148b	UCAGUGCAUCACAGAACUUUGU	SEQ ID NO:161
mir-152	UCAGUGCAUGACAGAACUUGGG	SEQ ID NO:162
mir-155	UUAAUGCUAAUUGUGAUAGGGG	SEQ ID NO:163
miR-181a	AACAUUCAACGCUGUCGGUGAGU	SEQ ID NO:164
miR-181b	AACAUUCAUUGCUGUCGGUGGG	SEQ ID NO:165
miR-181c	AACAUUCAACCUGUCGGUGAGU	SEQ ID NO:166
mir-199a-3p	ACAGUAGUCUGCACAUUGGUUA	SEQ ID NO:167
mir-199a	CCCAGUGUUCAGACUACCUGUUC	SEQ ID NO:168
mir-199b	CCCAGUGUUUAGACUACCUGUUC	SEQ ID NO:169
mir-27a	UUCACAGUGGCUAAGUUCCGC	SEQ ID NO:170
mir-27b	UUCACAGUGGCUAAGUUCUGC	SEQ ID NO:171
mir-705	GGUGGGAGGUGGGGUGGGCA	SEQ ID NO:172
miR-709	GGAGGCAGAGGCAGGAGGA	SEQ ID NO:173
mir-762	GGGGCUGGGGCCGGGACAGAGC	SEQ ID NO:174
mir-147	GUGUGCGGAAAUGCUUCUGCUA	SEQ ID NO:249
mir-135a	UAUGGCUUUUUAUUCCUAUGUGA	SEQ ID NO:250
mir-135b	UAUGGCUUUUCAUUCCUAUGUGA	SEQ ID NO:251
mir-683	CCUGCUGUAAGCUGUGUCCUC	SEQ ID NO:252
mir-130b	CAGUGCAAUGAUGAAAGGGCAU	SEQ ID NO:253
mir-615-5p	GGGGGUCCCCGGUGCUCGGAUC	SEQ ID NO:254
mir-142-3p	UGUAGUGUUUCCUACUUUAUGGA	SEQ ID NO:255

miRNA	Sequence	SEQ.ID NO.
mir-130b	CAGUGCAAUGAUGAAAGGGCAU	SEQ ID NO:256
mir-18b	UAAGGUGCAUCUAGUGCUGUUAG	SEQ ID NO:257
mir-340-5p	UUAUAAAGCAAUGAGACUGAUU	SEQ ID NO:258
mir-501-5p	AAUCCUUUGUCCCUGGGUGAAA	SEQ ID NO:259
mir-1191	CAGUCUUACUAUGUAGCCCUA	SEQ ID NO:260
mir-421	AUCAACAGACAUUAAUUGGGCGC	SEQ ID NO:261
mir-717	CUCAGACAGAGAUACCUUCUCU	SEQ ID NO:262
mir-467c	UAAGUGCGUGCAUGUAUAUGUG	SEQ ID NO:263
mir-681	CAGCCUCGCUGGCAGGCAGCU	SEQ ID NO:264
mir-880	UACUCCAUCCUCUCUGAGUAGA	SEQ ID NO:265
mir-1190	UCAGCUGAGGUUCCCCUCUGUC	SEQ ID NO:266
mir-671-3p	UCCGGUUCUCAGGGCUCCACC	SEQ ID NO:267
mir-1196	AAAUCUACCUGCCUCUGCCU	SEQ ID NO:268
mir-667	UGACACCUGCCACCCAGCCCAAG	SEQ ID NO:269
mir-452	UGUUUGCAGAGGAAACUGAGAC	SEQ ID NO:270
mir-483	AAGACGGGAGAAGAGAAGGGAG	SEQ ID NO:271
mir-743a	GAAAGACACCAAGCUGAGUAGA	SEQ ID NO:272
mir-485	AGAGGCUGGCCGUGAUGAAUUC	SEQ ID NO:273
mir-770-5p	AGCACCACGUGUCUGGGCCACG	SEQ ID NO:274
mir-483*	UCACUCCUCCCCUCCCGUCUU	SEQ ID NO:275
mir-193	AACUGGCCUACAAAGUCCCAGU	SEQ ID NO:276
mir-296-5p	AGGGCCCCCCCUCAAUCCUGU	SEQ ID NO:277
mir-715	CUCCGUGCACACCCCCGCGUG	SEQ ID NO:278
mir-712	CUCCUUCACCCGGGCGGUACC	SEQ ID NO:279

As shown in the below examples, certain miRNA were observed to be upregulated in the lung in response to an inflammatory or allergic challenge (*e.g.*, mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c), while other miRNA were observed to be downregulated in response to an inflammatory or allergic challenge (*e.g.*, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, mir-712).

C. Methods for Analyzing Expression of miRNA and Gene Expression

Some embodiments of the methods of the present invention involve analysis of miRNA expression or gene expression. Methods for analyzing gene expression or expression of miRNA include, but are not limited to, methods based on hybridization analysis of polynucleotides, sequencing of polynucleotides, and analysis of protein expression such as proteomics-based methods. Commonly used methods for the quantification of mRNA expression in a sample include northern blotting and in situ hybridization (Parker and Barnes, 1999), RNAse protection assays (Hod, 1992), and PCR-based methods, such as reverse transcription polymerase chain reaction (RT-PCR) (Weis *et al.*, 1992). In some embodiments, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. Representative methods for sequencing-based gene expression analysis include Serial Analysis of Gene Expression (SAGE), and gene expression analysis by massively parallel signature sequencing (MPSS).

1. PCR-Based Methods

Gene expression or miRNA expression can be analyzed using techniques that employ PCR. PCR is useful to amplify and detect transcripts from a sample. RT-PCR is a sensitive quantitative method that can be used to compare mRNA levels in different samples (*e.g.*, endomyocardial biopsy samples) to examine gene expression signatures.

To perform RT-PCR, mRNA is isolated from a sample. For example, total RNA may be isolated from a sample of lung tissue. mRNA may also be extracted, for example, from frozen or archived paraffin-embedded and fixed tissue samples. Methods for mRNA extraction are known in the art. See, *e.g.*, Ausubel *et al.* (1997). Methods for RNA extraction from paraffin embedded tissues are disclosed, for example, in Rupp and Locker, 1987, and

De Andres *et al.*, 1995. Purification kits for RNA isolation from commercial manufacturers, such as Qiagen, can be used. Other commercially available RNA isolation kits include MasterPure.TM. Complete DNA and RNA Purification Kit (EPICENTRE.TM., Madison, Wis.), and, Paraffin Block RNA Isolation Kit (Ambion, Inc.). Total RNA from tissue samples can be also isolated using RNA Stat-60 (Tel-Test) or by cesium chloride density gradient centrifugation.

RNA is then reverse transcribed into cDNA. The cDNA is amplified in a PCR reaction. A variety of reverse transcriptases are known in the art. For example, extracted RNA can be reverse-transcribed using a GeneAmp RNA PCR kit (Perkin Elmer, Calif., USA), following the manufacturer's instructions. The derived cDNA can then be used as a template in the subsequent PCR reaction.

For quantitative PCR, a third oligonucleotide, or probe, is used to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and typically is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative analysis.

RT-PCR can be performed using commercially available equipment, such as an ABI PRISM 7700.TM. Sequence Detection System (Perkin-Elmer-Applied Biosystems, Foster City, Calif., USA), or Lightcycler.RTM. (Roche Molecular Biochemicals, Mannheim, Germany). Samples can be analyzed using a real-time quantitative PCR device such as the ABI PRISM 7700.TM. Sequence Detection System.TM.

A variation of the RT-PCR technique is real time quantitative PCR, which measures PCR product accumulation through a dual-labeled fluorigenic probe, such as a TaqMan.TM. probe. Real time PCR is compatible both with quantitative competitive PCR, where internal competitor for each target sequence is used for normalization, and with quantitative comparative PCR using a normalization gene contained within the sample, or a housekeeping gene for RT-PCR.

Gene expression may be examined using fixed, paraffin-embedded tissues as the RNA source or fresh tissue such as tissue obtained from a biopsy of pulmonary tissue. Examples of

methods of examining expression in fixed, paraffin-embedded tissues, are described, for example, in Godfrey *et al.*, 2000; and Specht *et. al.*, 2001.

Another approach for gene expression analysis employs competitive PCR design and automated, high-throughput matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS detection and quantification of oligonucleotides. This method is described by Ding and Cantor, 2003. See also the MassARRAY-based gene expression profiling method, developed by Sequenom, Inc. (San Diego, Calif.).

Additional PCR-based techniques for gene expression analysis include, *e.g.*, differential display (Liang and Pardee, 1992); amplified fragment length polymorphism (iAFLP) (Kawamoto *et al.*, 1999); BeadArray.TM. technology (Illumina, San Diego, Calif.; Oliphant *et al.*, 2002; Ferguson *et al.*, 2000); BeadsArray for Detection of Gene Expression (BADGE), using the commercially available Luminex100 LabMAP system and multiple color-coded microspheres (Luminex Corp., Austin, Tex.) in a rapid assay for gene expression (Yang *et al.*, 2001); and high coverage expression profiling (HiCEP) analysis (Fukumura *et al.*, 2003).

2. Microarrays

Other techniques for examining gene expression in a sample involve use of microarrays. Microarrays permit simultaneous analysis of a large number of gene expression products. Typically, polynucleotides of interest are plated, or arrayed, on a microchip substrate. The arrayed sequences are then hybridized with nucleic acids (*e.g.,* DNA or *RNA*) from cells or tissues of interest. The source of mRNA typically is total RNA. If the source of mRNA is lung tissue, mRNA can be extracted.

In various embodiments of the microarray technique, probes to at least 10, 25, 50, 100, 200, 500, 1000, 1250, 1500, or 1600 polynucleotides are immobilized on an array substrate. The probes can include DNA, RNA, copolymer sequences of DNA and RNA, DNA and/or RNA analogues, or combinations thereof.

In some embodiments, a microarray includes a support with an ordered array of binding (*e.g.*, hybridization) sites for each individual polynucleotide of interest. The microarrays can be addressable arrays, such as positionally addressable arrays where each probe of the array is located at a known, predetermined position on the solid support such that the identity of each probe can be determined from its position in the array.

Each probe on the microarray can be between about 10-50,000 nucleotides in length. The probes of the microarray can consist of nucleotide sequences of any length. An array can

include positive control probes, such as probes known to be complementary and hybridizable to sequences in the test sample, and negative control probes such as probes known to not be complementary and hybridizable to sequences in the test sample.

Methods for attaching nucleic acids to a surface are well-known in the art. Methods for immobilizing nucleic acids on glass are described (Schena *et al*, 1995; DeRisi Shalon *et al.*, 1996). Techniques are known for producing arrays with thousands of oligonucleotides at defined locations using photolithographic techniques are described by Fodor *et al.*, 1991; Pease *et al.*, 1994; Lockhart *et al.*, 1996; U.S. Pat. Nos. 5,578,832; 5,556,752; and 5,510,270). Other methods for making microarrays have been described. See, *e.g.*, Maskos and Southern, 1992. Any type of array may be used in the context of the present invention.

3. Serial Analysis of Gene Expression (SAGE)

Gene expression or miRNA expression in samples may also be determined by serial analysis of gene expression (SAGE), which is a method that allows the simultaneous and quantitative analysis of a large number of gene transcripts, without the need of providing an individual hybridization probe for each transcript (see Velculescu *et al.*, 1995; and Velculescu *et al.*, 1997). Briefly, a short sequence tag (about 10-14 nucleotides) is generated that contains sufficient information to uniquely identify a transcript, provided that the tag is obtained from a unique position within each transcript. Then, many transcripts are linked together to form long serial molecules, that can be sequenced, revealing the identity of the multiple tags simultaneously. The expression pattern of a population of transcripts can be quantitatively evaluated by determining the abundance of individual tags, and identifying the gene corresponding to each tag.

4. Protein Detection Methodologies

Immunohistochemical methods are also suitable for detecting the expression of the genes. Antibodies, most preferably monoclonal antibodies, specific for a gene product are used to detect expression. The antibodies can be detected by direct labeling of the antibodies themselves, for example, with radioactive labels, fluorescent labels, hapten labels such as, biotin, or an enzyme such as horse radish peroxidase or alkaline phosphatase. Alternatively, unlabeled primary antibody is used in conjunction with a labeled secondary antibody, comprising antisera, polyclonal antisera or a monoclonal antibody specific for the primary antibody. Immunohistochemistry protocols and kits are well known in the art and are commercially available.

Proteomic methods can allow examination of global changes in protein expression in a sample. Proteomic analysis may involve separation of individual proteins in a sample by 2-D gel electrophoresis (2-D PAGE), and identification of individual proteins recovered from the gel, such as by mass spectrometry or N-terminal sequencing, and analysis of the data using bioinformatics. Proteomics methods can be used alone or in combination with other methods for evaluating gene expression.

In various aspects, the expression of certain genes in a sample is detected to provide clinical information, such as information regarding prognosis. Thus, gene expression assays include measures to correct for differences in RNA variability and quality. For example, an assay typically measures and incorporates the expression of certain normalizing genes, such known housekeeping genes. Alternatively, normalization can be based on the mean or median signal (Ct) of all of the assayed genes or a large subset thereof (global normalization approach). In some embodiments, a normalized test RNA (*e.g.*, from a patient sample) is compared to the amount found in a sample from a patient with left ventricular dysfunction. The level of expression measured in a particular test sample can be determined to fall at some percentile within a range observed in reference sets.

D. Kits

The technology herein includes kits for evaluating miRNA or gene expression in samples. A "kit" refers to a combination of physical elements. For example, a kit may include, for example, one or more components such as probes, including without limitation specific primers, antibodies, a protein-capture agent, a reagent, an instruction sheet, and other elements useful to practice the technology described herein. These physical elements can be arranged in any way suitable for carrying out the invention.

Kits for analyzing RNA expression may include, for example, a set of oligonucleotide probes for detecting expression of a gene or a miRNA (*e.g.*, from Table 1). The probes can be provided on a solid support, as in an array (*e.g.*, a microarray), or in separate containers. The kits can include a set of oligonucleotide primers useful for amplifying a set of genes described herein, such as to perform PCR analysis. Kits can include further buffers, enzymes, labeling compounds, and the like. Any of the compositions described herein may be comprised in a kit. In a non-limiting example, an individual miRNA is included in a kit. The kit may further include water and hybridization buffer to facilitate hybridization of the two strands of the miRNAs. The kit may also include one or more transfection reagents to facilitate delivery of the miRNA to cells.

A kit for analyzing protein expression can include specific binding agents, such as immunological reagents (*e.g.,* an antibody) for detecting protein expression of a gene of interest. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there is more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a single vial. The kits of the present invention also will typically include a means for containing the nucleic acids, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, such as a sterile aqueous solution.

However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

The container means will generally include at least one vial, test tube, flask, bottle, syringe and/or other container means, into which the nucleic acid formulations are placed, preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

The kits of the present invention will also typically include a means for containing the vials in close confinement for commercial sale.

Such kits may also include components that preserve or maintain the miRNA or that protect against its degradation. Such components may be RNAse-free or protect against RNAses. Such kits generally will comprise, in suitable means, distinct containers for each individual reagent or solution.

A kit will also include instructions for employing the kit components as well the use of any other reagent not included in the kit. Instructions may include variations that can be implemented.

It is contemplated that such reagents are embodiments of kits of the invention. Such kits, however, are not limited to the particular items identified above and may include any reagent used for the manipulation or characterization of miRNA.

E. Vectors for Cloning, Gene Transfer and Expression

Within certain embodiments expression vectors are employed to express a nucleic acid of interest, such as a miRNA that inhibits the expression of a particular gene. Expression requires that appropriate signals be provided in the vectors, and which include various regulatory elements, such as enhancers/promoters from both viral and mammalian sources that drive expression of the genes of interest in host cells. Elements designed to optimize messenger RNA stability and translatability in host cells also are defined. The conditions for the use of a number of dominant drug selection markers for establishing permanent, stable cell clones expressing the products are also provided, as is an element that links expression of the drug selection markers to expression of the polypeptide.

1. Regulatory Elements

Throughout this application, the term "expression construct" is meant to include any type of genetic construct containing a nucleic acid coding for a gene product in which part or all of the nucleic acid encoding sequence is capable of being transcribed. The transcript may be translated into a protein, but it need not be. In certain embodiments, expression includes both transcription of a gene and translation of mRNA into a gene product. In other embodiments, expression only includes transcription of the nucleic acid encoding a gene of interest.

In certain embodiments, the nucleic acid encoding a gene product is under transcriptional control of a promoter. A "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. The phrase "under transcriptional control" means that the promoter is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the gene.

The term promoter will be used here to refer to a group of transcriptional control modules that are clustered around the initiation site for RNA polymerase II. Much of the thinking about how promoters are organized derives from analyses of several viral promoters, including those for the HSV thymidine kinase (*tk*) and SV40 early transcription units. These studies, augmented by more recent work, have shown that promoters are composed of discrete functional modules, each consisting of approximately 7-20 bp of DNA, and containing one or more recognition sites for transcriptional activator or repressor proteins.

At least one module in each promoter functions to position the start site for RNA synthesis. The best known example of this is the TATA box, but in some promoters lacking a

TATA box, such as the promoter for the mammalian terminal deoxynucleotidyl transferase gene and the promoter for the SV40 late genes, a discrete element overlying the start site itself helps to fix the place of initiation.

Additional promoter elements regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the tk promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either co-operatively or independently to activate transcription.

In other embodiments, the human cytomegalovirus (CMV) immediate early gene promoter, the SV40 early promoter, the Rous sarcoma virus long terminal repeat, rat insulin promoter and glyceraldehyde-3-phosphate dehydrogenase can be used to obtain high-level expression of the coding sequence of interest. The use of other viral or mammalian cellular or bacterial phage promoters which are well-known in the art to achieve expression of a coding sequence of interest is contemplated as well, provided that the levels of expression are sufficient for a given purpose.

By employing a promoter with well-known properties, the level and pattern of expression of the protein of interest following transfection or transformation can be optimized. Further, selection of a promoter that is regulated in response to specific physiologic signals can permit inducible expression of the gene product. Tables 2 and 3 list several regulatory elements that may be employed, in the context of the present invention, to regulate the expression of the gene of interest. This list is not intended to be exhaustive of all the possible elements involved in the promotion of gene expression but, merely, to be exemplary thereof.

Enhancers are genetic elements that increase transcription from a promoter located at a distant position on the same molecule of DNA. Enhancers are organized much like promoters. That is, they are composed of many individual elements, each of which binds to one or more transcriptional proteins.

The basic distinction between enhancers and promoters is operational. An enhancer region as a whole must be able to stimulate transcription at a distance; this need not be true of a promoter region or its component elements. On the other hand, a promoter must have one or more elements that direct initiation of RNA synthesis at a particular site and in a particular

orientation, whereas enhancers lack these specificities. Promoters and enhancers are often overlapping and contiguous, often seeming to have a very similar modular organization.

Below is a list of viral promoters, cellular promoters/enhancers and inducible promoters/enhancers that could be used in combination with the nucleic acid encoding a gene of interest in an expression construct (Table 2 and Table 3). Additionally, any promoter/enhancer combination (as per the Eukaryotic Promoter Data Base EPDB) could also be used to drive expression of the gene. Eukaryotic cells can support cytoplasmic transcription from certain bacterial promoters if the appropriate bacterial polymerase is provided, either as part of the delivery complex or as an additional genetic expression construct.

TABLE 2

Promoter and/or Enhancer

Promoter/Enhancer	References
Immunoglobulin Heavy Chain	Banerji *et al.,* 1983; Gilles *et al.*, 1983; Grosschedl *et al.*, 1985; Atchinson *et al.*, 1986, 1987; Imler *et al.*, 1987; Weinberger *et al.*, 1984; Kiledjian *et al.*, 1988; Porton *et al.*; 1990
Immunoglobulin Light Chain	Queen *et al.*, 1983; Picard *et al.*, 1984
T-Cell Receptor	Luria *et al.*, 1987; Winoto *et al.*, 1989; Redondo *et al.*; 1990
HLA DQ a and/or DQ β	Sullivan *et al.*, 1987
β-Interferon	Goodbourn *et al.*, 1986; Fujita *et al.*, 1987; Goodbourn *et al.*, 1988
Interleukin-2	Greene *et al.*, 1989
Interleukin-2 Receptor	Greene *et al.*, 1989; Lin *et al.*, 1990
MHC Class II 5	Koch *et al.*, 1989
MHC Class II HLA-DRa	Sherman *et al.*, 1989
β-Actin	Kawamoto *et al.*, 1988; Ng *et al.*; 1989
Muscle Creatine Kinase (MCK)	Jaynes *et al.*, 1988; Horlick *et al.*, 1989; Johnson *et al.*, 1989
Prealbumin (Transthyretin)	Costa *et al.*, 1988
Elastase I	Ornitz *et al.*, 1987
Metallothionein (MTII)	Karin *et al.*, 1987; Culotta *et al.*, 1989
Collagenase	Pinkert *et al.*, 1987; Angel *et al.*, 1987a
Albumin	Pinkert *et al.*, 1987; Tronche *et al.*, 1989, 1990
α-Fetoprotein	Godbout *et al.*, 1988; Campere *et al.*, 1989
t-Globin	Bodine *et al.*, 1987; Perez-Stable *et al.*, 1990
β-Globin	Trudel *et al.*, 1987
c-fos	Cohen *et al.*, 1987
c-HA-*ras*	Triesman, 1986; Deschamps *et al.*, 1985
Insulin	Edlund *et al.*, 1985
Neural Cell Adhesion Molecule (NCAM)	Hirsh *et al.*, 1990

TABLE 2

Promoter and/or Enhancer

Promoter/Enhancer	References
α_1-Antitrypain	Latimer *et al.*, 1990
H2B (TH2B) Histone	Hwang *et al.*, 1990
Mouse and/or Type I Collagen	Ripe *et al.*, 1989
Glucose-Regulated Proteins (GRP94 and GRP78)	Chang *et al.*, 1989
Rat Growth Hormone	Larsen *et al.*, 1986
Human Serum Amyloid A (SAA)	Edbrooke *et al.*, 1989
Troponin I (TN I)	Yutzey *et al.*, 1989
Platelet-Derived Growth Factor (PDGF)	Pech *et al.*, 1989
Duchenne Muscular Dystrophy	Klamut *et al.*, 1990
SV40	Banerji *et al.*, 1981; Moreau *et al.*, 1981; Sleigh *et al.,* 1985; Firak *et al.*, 1986; Herr *et al.*, 1986; Imbra *et al.*, 1986; Kadesch *et al.*, 1986; Wang *et al.*, 1986; Ondek *et al.*, 1987; Kuhl *et al.*, 1987; Schaffner *et al.*, 1988
Polyoma	Swartzendruber *et al.*, 1975; Vasseur *et al.*, 1980; Katinka *et al.*, 1980, 1981; Tyndell *et al.*, 1981; Dandolo *et al.*, 1983; de Villiers *et al.*, 1984; Hen *et al.*, 1986; Satake *et al.*, 1988; Campbell and/or Villarreal, 1988
Retroviruses	Kriegler *et al.*, 1982, 1983; Levinson *et al.*, 1982; Kriegler *et al.*, 1983, 1984a, b, 1988; Bosze *et al.*, 1986; Miksicek *et al.*, 1986; Celander *et al.*, 1987; Thiesen *et al.*, 1988; Celander *et al.*, 1988; Choi *et al.*, 1988; Reisman *et al.*, 1989
Papilloma Virus	Campo *et al.*, 1983; Lusky *et al.*, 1983; Spandidos and/or Wilkie, 1983; Spalholz *et al.*, 1985; Lusky *et al.*, 1986; Cripe *et al.*, 1987; Gloss *et al.*, 1987; Hirochika *et al.*, 1987; Stephens *et al.*, 1987
Hepatitis B Virus	Bulla *et al.*, 1986; Jameel *et al.*, 1986; Shaul *et al.*, 1987; Spandau *et al.*, 1988; Vannice *et al.*, 1988
Human Immunodeficiency Virus	Muesing *et al.*, 1987; Hauber *et al.*, 1988; Jakobovits *et al.*, 1988; Feng *et al.*, 1988; Takebe *et al.*, 1988; Rosen *et al.*, 1988; Berkhout *et al.*, 1989; Laspia *et al.*, 1989; Sharp *et al.*, 1989; Braddock *et al.*, 1989

TABLE 2

Promoter and/or Enhancer

Promoter/Enhancer	References
Cytomegalovirus (CMV)	Weber *et al.*, 1984; Boshart *et al.*, 1985; Foecking *et al.*, 1986
Gibbon Ape Leukemia Virus	Holbrook *et al.*, 1987; Quinn *et al.*, 1989

TABLE 3

Inducible Elements

Element	Inducer	References
MT II	Phorbol Ester (TFA) Heavy metals	Palmiter *et al.*, 1982; Haslinger *et al.*, 1985; Searle *et al.*, 1985; Stuart *et al.*, 1985; Imagawa *et al.*, 1987, Karin *et al.*, 1987; Angel *et al.*, 1987b; McNeall *et al.*, 1989
MMTV (mouse mammary tumor virus)	Glucocorticoids	Huang *et al.*, 1981; Lee *et al.*, 1981; Majors *et al.*, 1983; Chandler *et al.*, 1983; Ponta *et al.*, 1985; Sakai *et al.*, 1988
β-Interferon	poly(rI)x poly(rc)	Tavernier *et al.*, 1983
Adenovirus 5 E2	ElA	Imperiale *et al.*, 1984
Collagenase	Phorbol Ester (TPA)	Angel *et al.*, 1987a
Stromelysin	Phorbol Ester (TPA)	Angel *et al.*, 1987b
SV40	Phorbol Ester (TPA)	Angel *et al.*, 1987b
Murine MX Gene	Interferon, Newcastle Disease Virus	Hug *et al.*, 1988
GRP78 Gene	A23187	Resendez *et al.*, 1988
α-2-Macroglobulin	IL-6	Kunz *et al.*, 1989
Vimentin	Serum	Rittling *et al.*, 1989
MHC Class I Gene H-2κb	Interferon	Blanar *et al.*, 1989
HSP70	ElA, SV40 Large T Antigen	Taylor *et al.*, 1989, 1990a, 1990b
Proliferin	Phorbol Ester-TPA	Mordacq *et al.*, 1989
Tumor Necrosis Factor	PMA	Hensel *et al.*, 1989
Thyroid Stimulating Hormone α Gene	Thyroid Hormone	Chatterjee *et al.*, 1989

Of particular interest are muscle specific promoters, and more particularly, cardiac specific promoters. These include the myosin light chain-2 promoter (Franz *et al.*, 1994; Kelly *et al.*, 1995), the alpha actin promoter (Moss *et al.*, 1996), the troponin 1 promoter

(Bhavsar *et al.*, 1996); the Na^+/Ca^{2+} exchanger promoter (Barnes *et al.*, 1997), the dystrophin promoter (Kimura *et al.*, 1997), the alpha7 integrin promoter (Ziober and Kramer, 1996), the brain natriuretic peptide promoter (LaPointe *et al.*, 1996) and the alpha B-crystallin/small heat shock protein promoter (Gopal-Srivastava, 1995), alpha myosin heavy chain promoter (Yamauchi-Takihara *et al.*, 1989) and the ANF promoter (LaPointe *et al.*, 1988).

Where a cDNA insert is employed, one will typically desire to include a polyadenylation signal to effect proper polyadenylation of the gene transcript. The nature of the polyadenylation signal is not believed to be crucial to the successful practice of the invention, and any such sequence may be employed such as human growth hormone and SV40 polyadenylation signals. Also contemplated as an element of the expression cassette is a terminator. These elements can serve to enhance message levels and to minimize read through from the cassette into other sequences.

2. Selectable Markers

In certain embodiments of the invention, the cells contain nucleic acid constructs of the present invention, a cell may be identified *in vitro* or *in vivo* by including a marker in the expression construct. Such markers would confer an identifiable change to the cell permitting easy identification of cells containing the expression construct. Usually the inclusion of a drug selection marker aids in cloning and in the selection of transformants, for example, genes that confer resistance to neomycin, puromycin, hygromycin, DHFR, GPT, zeocin and histidinol are useful selectable markers. Alternatively, enzymes such as herpes simplex virus thymidine kinase (*tk*) or chloramphenicol acetyltransferase (CAT) may be employed. Immunologic markers also can be employed. The selectable marker employed is not believed to be important, so long as it is capable of being expressed simultaneously with the nucleic acid encoding a gene product. Further examples of selectable markers are well known to one of skill in the art.

3. Multigene Constructs and IRES

In certain embodiments of the invention, the use of internal ribosome binding sites (IRES) elements are used to create multigene, or polycistronic, messages. IRES elements are able to bypass the ribosome scanning model of 5′ methylated Cap dependent translation and begin translation at internal sites (Pelletier and Sonenberg, 1988). IRES elements from two members of the picanovirus family (polio and encephalomyocarditis) have been described (Pelletier and Sonenberg, 1988), as well an IRES from a mammalian message (Macejak and

Sarnow, 1991). IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message.

Any heterologous open reading frame can be linked to IRES elements. This includes genes for secreted proteins, multi-subunit proteins, encoded by independent genes, intracellular or membrane-bound proteins and selectable markers. In this way, expression of several proteins can be simultaneously engineered into a cell with a single construct and a single selectable marker.

4. Delivery of Expression Vectors

There are a number of ways in which expression vectors may introduced into cells. In certain embodiments of the invention, the expression construct comprises a virus or engineered construct derived from a viral genome. One of the preferred methods for *in vivo* delivery involves the use of an adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express an antisense polynucleotide that has been cloned therein. In this context, expression does not require that the gene product be synthesized.

The expression vector comprises a genetically engineered form of adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kB, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kB (Grunhaus and Horwitz, 1992). In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titer, wide target cell range and high infectivity. Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham *et al.,* 1977). Since the E3 region is dispensable from the adenovirus genome (Jones

and Shenk, 1978), the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham and Prevec, 1991).

The adenovirus may be replication-defective or replication-competent. The adenovirus may be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain the conditional replication-defective adenovirus vector for use in the present invention. This is because Adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

Adenovirus is easy to grow and manipulate and exhibits broad host range *in vitro* and *in vivo*. This group of viruses can be obtained in high titers, *e.g.*, 10^9-10^{12} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells.

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. Other viral vectors may be employed as expression constructs in the present invention. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Baichwal and Sugden, 1986; Coupar *et al.,* 1988) adeno-associated virus (AAV) (Ridgeway, 1988; Baichwal and Sugden, 1986; Hermonat and Muzycska, 1984), lentivirus, and herpesviruses may be employed.

With the recognition of defective hepatitis B viruses, new insight was gained into the structure-function relationship of different viral sequences. *In vitro* studies showed that the virus could retain the ability for helper-dependent packaging and reverse transcription despite the deletion of up to 80% of its genome (Horwich *et al.,* 1990).

In order to effect expression of sense or antisense gene constructs, the expression construct must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. One mechanism for delivery is via viral infection where the expression construct is encapsidated in an infectious viral particle.

Several non-viral methods for the transfer of expression constructs into cultured mammalian cells also are contemplated by the present invention. These include calcium

phosphate precipitation (Graham and Van Der Eb, 1973; Chen and Okayama, 1987; Rippe *et al.*, 1990) DEAE-dextran (Gopal, 1985), electroporation (Tur-Kaspa *et al.*, 1986; Potter *et al.*, 1984), direct microinjection (Harland and Weintraub, 1985), DNA-loaded liposomes (Nicolau and Sene, 1982; Fraley *et al.*, 1979) and lipofectamine-DNA complexes, cell sonication (Fechheimer *et al.,* 1987), gene bombardment using high velocity microprojectiles (Yang *et al.*, 1990), and receptor-mediated transfection (Wu and Wu, 1987; Wu and Wu, 1988). Some of these techniques may be successfully adapted for *in vivo* or *ex vivo* use.

Once the expression construct has been delivered into the cell the nucleic acid encoding the gene of interest may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the gene may be stably integrated into the genome of the cell. This integration may be in the cognate location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

In yet another embodiment of the invention, the expression construct may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well. Dubensky *et al.* (1984) successfully injected polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Neshif (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a gene of interest may also be transferred in a similar manner *in vivo* and express the gene product.

In still another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.,* 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et*

al., 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

In a further embodiment of the invention, the expression construct may be entrapped in a liposome. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, 1991). Also contemplated are lipofectamine-DNA complexes.

Liposome-mediated nucleic acid delivery and expression of foreign DNA *in vitro* has been very successful. Wong *et al.,* (1980) demonstrated the feasibility of liposome-mediated delivery and expression of foreign DNA in cultured chick embryo, HeLa and hepatoma cells. Nicolau *et al.,* (1987) accomplished successful liposome-mediated gene transfer in rats after intravenous injection.

Other expression constructs which can be employed to deliver a nucleic acid encoding a particular gene into cells are receptor-mediated delivery vehicles. These take advantage of the selective uptake of macromolecules by receptor-mediated endocytosis in almost all eukaryotic cells. Because of the cell type-specific distribution of various receptors, the delivery can be highly specific (Wu and Wu, 1993).

In other embodiments, the delivery vehicle may comprise a ligand and a liposome. For example, Nicolau *et al.* (1987) employed lactosyl-ceramide, a galactose-terminal asialganglioside, incorporated into liposomes and observed an increase in the uptake of the insulin gene by hepatocytes. Thus, it is feasible that a nucleic acid encoding a particular gene also may be specifically delivered into a cell type by any number of receptor-ligand systems with or without liposomes. For example, epidermal growth factor (EGF) may be used as the receptor for mediated delivery of a nucleic acid into cells that exhibit upregulation of EGF receptor. Mannose can be used to target the mannose receptor on liver cells. Also, antibodies to CD5 (CLL), CD22 (lymphoma), CD25 (T-cell leukemia) and MAA (melanoma) can similarly be used as targeting moieties.

In a particular example, the oligonucleotide may be administered in combination with a cationic lipid. Examples of cationic lipids include, but are not limited to, lipofectin, DOTMA, DOPE, and DOTAP. The publication of WO/0071096, which is specifically

incorporated by reference, describes different formulations, such as a DOTAP:cholesterol or cholesterol derivative formulation that can effectively be used for gene therapy.

In certain embodiments, gene transfer may more easily be performed under *ex vivo* conditions. *Ex vivo* gene therapy refers to the isolation of cells from an animal, the delivery of a nucleic acid into the cells *in vitro*, and then the return of the modified cells back into an animal. This may involve the surgical removal of tissue/organs from an animal or the primary culture of cells and tissues.

F. Clinical Information

1. Definitions

"Treatment" and "treating" as used herein refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject for the purpose of obtaining a therapeutic benefit of a disease or health-related condition.

The term "therapeutic benefit" or "therapeutically effective" as used throughout this application refers to anything that promotes or enhances the well-being of the subject with respect to the medical treatment of this condition. This includes, but is not limited to, a reduction in the frequency or severity of the signs or symptoms of a disease.

"Prevention" and "preventing" are used according to their ordinary and plain meaning to mean "acting before" or such an act. In the context of a particular disease or health-related condition, those terms refer to administration or application of an agent, drug, or remedy to a subject or performance of a procedure or modality on a subject for the purpose of blocking the onset of a disease or health-related condition.

The term "compound" refers to any chemical entity, pharmaceutical, drug, and the like that can be used to treat or prevent a disease, illness, sickness, or disorder of bodily function. Compounds comprise both known and potential therapeutic compounds. A compound can be determined to be therapeutic by screening using the screening methods of the present invention. A "known therapeutic compound" refers to a therapeutic compound that has been shown (*e.g.*, through animal trials or prior experience with administration to humans) to be effective in such treatment. In other words, a known therapeutic compound is not limited to a compound efficacious in the treatment of asthma.

A "sample" is any biological material obtained from an individual. For example, a "sample" may be a blood sample or a lung tissue sample.

2. Dosage

A pharmaceutically effective amount of a therapeutic agent as set forth herein is determined based on the intended goal, for example inhibition of cell death. The quantity to be administered, both according to number of treatments and dose, depends on the subject to be treated, the state of the subject, the protection desired, and the route of administration. Precise amounts of the therapeutic agent also depend on the judgment of the practitioner and are peculiar to each individual.

For example, a dose of the therapeutic agent may be about 0.0001 milligrams to about 1.0 milligrams, or about 0.001 milligrams to about 0.1 milligrams, or about 0.1 milligrams to about 1.0 milligrams, or even about 10 milligrams per dose or so. Multiple doses can also be administered. In some embodiments, a dose is at least about 0.0001 milligrams. In further embodiments, a dose is at least about 0.001 milligrams. In still further embodiments, a dose is at least 0.01 milligrams. In still further embodiments, a dose is at least about 0.1 milligrams. In more particular embodiments, a dose may be at least 1.0 milligrams. In even more particular embodiments, a dose may be at least 10 milligrams. In further embodiments, a dose is at least 100 milligrams or higher.

In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, *etc.*, can be administered, based on the numbers described above. Dosages of nucleic acid or LNA which may be used include, for example, about from 10-100 mg (LNA or nucleic acid)/g body weight, about 25-75 mg (LNA or nucleic acid)/g body weight, about mg (LNA or nucleic acid)/g body weight, or any range derivable therein. A dosage of about 50 mg (LNA or nucleic acid)/g mouse

body weight was observed to be effective to substantially inhibit allergic or inflammatory lung responses in mice *in vivo*.

The dose can be repeated as needed as determined by those of ordinary skill in the art. Thus, in some embodiments of the methods set forth herein, a single dose is contemplated. In other embodiments, two or more doses are contemplated. Where more than one dose is administered to a subject, the time interval between doses can be any time interval as determined by those of ordinary skill in the art. For example, the time interval between doses may be about 1 hour to about 2 hours, about 2 hours to about 6 hours, about 6 hours to about 10 hours, about 10 hours to about 24 hours, about 1 day to about 2 days, about 1 week to about 2 weeks, or longer, or any time interval derivable within any of these recited ranges.

In certain embodiments, it may be desirable to provide a continuous supply of a pharmaceutical composition to the patient. This could be accomplished by catheterization, followed by continuous administration of the therapeutic agent. The administration could be intra-operative or post-operative.

G. Pharmaceutical Compositions and Routes for Administration to Patients

Some embodiments of the present invention involve administration of pharmaceutical compositions. Where clinical applications are contemplated, pharmaceutical compositions will be prepared in a form appropriate for the intended application. Generally, this will involve preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

One will generally desire to employ appropriate salts and buffers in preparing compositions of therapeutic agents. Buffers also will be employed when recombinant cells are introduced into a patient. Aqueous compositions of the present invention comprise an effective amount of the therapeutic agent, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. The phrases "pharmaceutically acceptable" or "pharmacologically acceptable" refers to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" includes solvents, buffers, solutions, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like acceptable for use in formulating pharmaceuticals, such as pharmaceuticals suitable for administration to humans. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients of the present

invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions, provided they do not inactivate the therapeutic agents of the compositions.

The active compositions of the present invention may include classic pharmaceutical preparations. Administration of these compositions according to the present invention may be via any common route so long as the target tissue is available via that route. Administration may be by any method known to those of ordinary skill in the art, such as intravenous, intradermal, subcutaneous, intramuscular, intraperitoneal or intrathecal injection, or by direct injection into cardiac tissue. Other modes of administration include oral, buccal, and nasogastric administration. The active compounds may also be administered parenterally or intraperitoneally. Such compositions would normally be administered as pharmaceutically acceptable compositions, as described *supra*. In particular embodiments, the composition is administered to a subject using a drug delivery device. For example, the drug delivery device may be a catheter or syringe. In some embodiments, the composition is applied as a coating to a medical device, such as a stent.

By way of illustration, solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include, for example, sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Generally, these preparations are sterile and fluid to the extent that easy injectability exists. Preparations should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Appropriate solvents or dispersion media may contain, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial an antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for

example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions may be prepared by incorporating the active compounds in an appropriate amount into a solvent along with any other ingredients (for example as enumerated above) as desired, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the desired other ingredients, *e.g.*, as enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation include vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient(s) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

For oral administration the therapeutic agents of the present invention generally may be incorporated with excipients. Any excipient known to those of ordinary skill in the art is contemplated.

The compositions of the present invention generally may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include, for example, acid addition salts (formed with the free amino groups of the protein) derived from inorganic acids (*e.g.*, hydrochloric or phosphoric acids, or from organic acids (*e.g.*, acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups of the protein can also be derived from inorganic bases (*e.g.*, sodium, potassium, ammonium, calcium, or ferric hydroxides) or from organic bases (*e.g.*, isopropylamine, trimethylamine, histidine, procaine and the like.

Upon formulation, solutions are preferably administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations may easily be administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution generally is suitably buffered and the liquid diluent first rendered isotonic for example with sufficient saline or glucose. Such aqueous solutions may be used, for example, for intravenous, intramuscular, subcutaneous and intraperitoneal administration. Preferably, sterile aqueous media are employed as is known to those of skill in the art, particularly in light of the present disclosure. By way of illustration, a single dose may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in

dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

H. Combined Therapy

In another embodiment, it is envisioned to use an miRNA or an miRNA inhibitor as set forth herein in combination with other therapeutic modalities. Thus, in addition to the therapies described above, one may also provide to the patient more "standard" pharmaceutical cardiac therapies. Examples of other therapies include, without limitation, other pharmaceutical therapies of asthma or other allergic lung disease.

The other therapeutic modality may be administered before, concurrently with, or following administration of the miRNA The therapy using miRNA may precede or follow administration of the other agent(s) by intervals ranging from minutes to weeks. In embodiments where the other agent and the miRNA are administered separately, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that each agent would still be able to exert an advantageously combined effect. In such instances, it is contemplated that one would typically administer the miRNA and the other therapeutic agent within about 12-24 hours of each other and, more preferably, within about 6-12 hours of each other, with a delay time of only about 12 hours being most preferred. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

It also is conceivable that more than one administration of an miRNA, or the other agent will be desired. In this regard, various combinations may be employed. By way of illustration, where the miRNA is "A" and the other agent is "B", the following permutations based on 3 and 4 total administrations are exemplary:

A/B/A B/A/B B/B/A A/A/B B/A/A A/B/B B/B/B/A B/B/A/B
A/A/B/B A/B/A/B A/B/B/A B/B/A/A B/A/B/A B/A/A/B B/B/B/A
A/A/A/B B/A/A/A A/B/A/A A/A/B/A A/B/B/B B/A/B/B B/B/A/B

Other combinations are likewise contemplated. Non-limiting examples of pharmacological agents that may be used in the present invention include any

pharmacological agent known to be of benefit in the treatment of asthma. Examples include inhaled corticosteroids, long-activing beta-2 agonists (such as salmetrol and formoterol), leukotriene modifiers such as montelukast, zafirlukast, and zileuton, cromolyn and nedocromil, theophylline, short-acting beta-2 agonists such as albuterol, ipratropium, and oral and intravenous corticosteroids. Further examples include immunotherapy and anti-IgE monoclonal antibodies, such as omalizumab.

I. Biochips

A biochip is also provided. The biochip may comprise a solid substrate comprising an attached nucleic acid sequence that is capable of hybridizing to an miRNA sequence described herein. "Probe" as used herein may mean an oligonucleotide capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. Probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. There may be any number of base pair mismatches which will interfere with hybridization between the target sequence and the single stranded nucleic acids described herein. However, if the number of mutations is so great that no hybridization can occur under even the least stringent of hybridization conditions, the sequence is not a complementary target sequence. A probe may be single stranded or partially single and partially double stranded. The strandedness of the probe is dictated by the structure, composition, and properties of the target sequence. Probes may be directly labeled or indirectly labeled such as with biotin to which a streptavidin complex may later bind. The probes may be capable of hybridizing to a target sequence under stringent hybridization conditions. The probes may be attached at spatially defined address on the substrate. More than one probe per target sequence may be used, with either overlapping probes or probes to different sections of a particular target sequence. The probes may be capable of hybridizing to target sequences associated with a single disorder.

The probes may be attached to the biochip in a wide variety of ways, as will be appreciated by those in the art. The probes may either be synthesized first, with subsequent attachment to the biochip, or may be directly synthesized on the biochip.

The solid substrate may be a material that may be modified to contain discrete individual sites appropriate for the attachment or association of the probes and is amenable to at least one detection method. Representative examples of substrates include glass and modified or functionalized glass, plastics (including acrylics, polystyrene and copolymers of

styrene and other materials, polypropylene, polyethylene, polybutylene, polyurethanes, TeflonJ, *etc.*), polysaccharides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses and plastics. The substrates may allow optical detection without appreciably fluorescing.

The substrate may be planar, although other configurations of substrates may be used as well. For example, probes may be placed on the inside surface of a tube, for flow-through sample analysis to minimize sample volume. Similarly, the substrate may be flexible, such as a flexible foam, including closed cell foams made of particular plastics.

The biochip and the probe may be derivatized with chemical functional groups for subsequent attachment of the two. For example, the biochip may be derivatized with a chemical functional group including, but not limited to, amino groups, carboxyl groups, oxo groups or thiol groups. Using these functional groups, the probes may be attached using functional groups on the probes either directly or indirectly using a linkers. The probes may be attached to the solid support by either the 5' terminus, 3' terminus, or via an internal nucleotide.

The probe may also be attached to the solid support non-covalently. For example, biotinylated oligonucleotides can be made, which may bind to surfaces covalently coated with streptavidin, resulting in attachment. Alternatively, probes may be synthesized on the surface using techniques such as photopolymerization and photolithography

J. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

Materials and Methods

Mice and allergen challenge. All experiments were performed in accordance with institutional and United States National Institutes of Health guidelines. Allergen challenge of C57BL/6 mice was performed with an allergenic fungal proteinase and ovalbumin as previously described (Kheradmand *et al.*, 2002).

Preparation of short-RNA transcripts for Illumina sequencing. Short RNA transcripts of <60 nucleotide length were gel purified after running 10 mg of total RNA on 15% TBE-Urea polyacrylamide gel. A synthetic 26-residue adapter RNA oligonucleotide (5' GUU CAG AGU UCU ACA GUC CGA CGA UC 3' (SEQ ID NO:280)) was ligated to the 5' and of the small-RNAs. The ligated small-RNA was gel purified to remove un-ligated free adapter. A synthetic 22-residue 3' adapter with inverted dideoxythymidine added at the 3' end (5' p UCG UAU GCC GUC UUC UGC UUG idT 3' (SEQ ID NO:281)) was ligated to the 5' ligated small-RNA and gel purified. The resultant RNA library was reverse transcribed and amplified by PCR for 15 cycles using adapter-specific primers. The PCR products were sequenced using Illumina (Solexa)-based Next Generation Sequencing.

Small RNA Mapping and Classification. After filtering for the Illumina small RNA adapter sequences, the reads were mapped to the reference mouse genome (NCBI Build 37, UCSC mm9) using the Pash software package as previously described (Coarfa & Milosavljevic. 2008, Kalafus *et al.* 2004).

Novel miRNA discovery. All small RNA sequences that failed to align with a known miRNA, piRNA or snoRNA were passed through a novel miRNA discovery platform as described in Supplementary Experimental Procedures.

Microarray analyses: llumina Sentrix Universal-12 Mouse v2 Gene Expression BeadChip Array (45281 transcripts) was used for gene profiling, and Illumina Mouse v2 MicroRNA Expression BeadChip Array (611 miRNAs) was used for miRNA profiling. The gene array data generated were quantile normalized (using software kindly provided by Dr. Kerby Shedden). Significantly regulated genes and miRNAs were identified by comparing allergen challenged with naïve using t-test (log-transformed data) and fold change (ratio of averages of the two groups). Java TreeView (Saldanha, 2004) represented expression patterns as color maps, where gene and miRNA values were centered on the median expression of the naïve group.

Isolation, culture, and transfection of CD4+T cells from spleen. Mouse spleens were collected and CD4 T cells isolated by immunomagnetic selection. Th1 and Th2 cells were differentiated as previously described (Grunig *et al.*, 1998).

Nucleofection of *in vitro* anti-let-7a LNAs in to CD4 T cells was performed by using mouse T cell nucleofector kit (Lonza, Walkersville, MD) according to the manufacturer's protocol and the cells were cultured for 48 hours. 80 and 240 pmol of anti-let-7a LNAs and 240 pmol of scrambled LNA were used for transfection. For determining the efficiency of transfection, cells that were transfected with flourescein labeled LNAs were nucleofected in to CD4 T cells and subjected to flow cytometry after 48 hours.

For RNA extraction, cells were homogenized in Trizol and total RNA was isolated using miRNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's protocol.

In vitro validation. HEK293T cells were used for co-transfection of plasmids expressing miRNAs, 3'UTR of target genes and anti-miRNA or control LNAs. Briefly, HEK293T cells that were cultured in 24-well plates were co-transfected with plasmids expressing IL-13 3'UTR (350-ng) or control 3'UTR (350-ng) and/or, mouse/human let-7a (350 or 117 or 39-ng) or mouse let-7a(U→G) (350-ng), or mouse miR-705 (350-ng) or scrambled miRNA (350-ng) and/or mouse/human anti-let-7a LNA (52.5, 17.5 and 5.8 pmol) or scrambled LNA (52.5 pmol) or anti-miR-705 LNA (52.5 pmol) or mouse anti-let-7e (let-7a (U→G)) LNA (52.5 pmol). Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used as transfection reagent according to the manufacturer's protocol. Firefly and Renilla Luciferase light units were measured after 2 days of co-transfection by using Dual-Luciferase Reporter Assay System (Promega, Madison, WI) with the help of FLOU star OPTIMA microplate reader (Bmg Labtech, Cary, NC).

In vivo transfection and allergy induction. For *in vivo* LNA experiments, female Balb/c mice between 5-8 weeks were used. Mice were sensitized with 50 μL of chicken ovalbumin and alum by intraperitonial injection twice (day zero and 7) at one-week intervals. On days 15 and 17, LNAs prepared in 0.9% saline were injected intravenously into mice through the tail vein. On days 16, 17 and 18 mice were intranasally challenged with chicken ovalbumin (25 μg in 50 μL PBS) before analysis on day 19 (FIG. 5A).

Quantitation of allergic lung disease. 24 hours after the final allergen challenge, the allergic lung disease phenotype was analyzed as previously described (Kheradmand *et al.*, 2002).

Quantitative PCR. Quantitative PCR of miRNAs and mRNAs were performed by using Taqman miRNA expression and gene expression assays, respectively (Applied Biosystems, Foster City, CA). PCR data was analyzed by using delta delta Ct method of relative quantification. For microRNA expression, either snoRNA202 and RNU48 were used as endogenous controls and for gene expression, GAPDH was used as the endogenous control.

Statistical analysis. For all statistical analyses, ANOVA with post hoc Tukey tests or t-tests were used. Statistical significance were calculated with P-value <0.05.

Mice and allergen challenge. Prior to the intranasal administration of allergens, female C57BL/6 mice between 4 and 7 weeks of age, were anesthetized in an airtight chamber purged with a 3.2% isoflurane in oxygen vapor mixture for 10 minutes to achieve deep general anesthesia. Anesthetized mice received intranasally 45 mL chicken ovalbumin (22.5 mg) and 9mL of protease derived from *Aspergillus melleus* (formerly *A. oryzae;* Sigma chemical company, St. Louis, MO*;* 7 mg) in PBS by applying droplets to the nares with a pipette. Allergen challenged mice received intranasal allergen on a schedule of every alternate day for eight total challenges and lungs were removed 24 hours after the final challenge. Lungs were perfused with ice cold, sterile normal saline to remove blood and collected in Trizol (Invitrogen, Carlsbad, CA). Total RNA was extracted by chloroform-ethanol method.

Small RNA Mapping and Classification. After filtering for the Illumina small RNA adapter sequences, the reads were mapped to the reference mouse genome (NCBI Build 37, UCSC mm9) using the Pash software package (Kalafus *et al.*, 2004). Pash anchoring used contiguous seeds of size 11 and masked out 5% of the genome containing highly repetitive sequences. The mapping results were uploaded to Genboree (www.genboree.com) for visualization. Reads with mappings that overlap miRNAs (miRBase version 14.0), piRNAs (piRNABank - pirnabank.ibab.ac.in), snoRNAs (RNAdb - jsm-research.imb.uq.edu.au/rnadb), genes (UCSC Genes track genome.ucsc.edu), or repeats (UCSC Repeat Masker track - genome.ucsc.edu) were identified. In the case of repeats each mapping is associated with specific types of repeat such as LINEs, SINEs, DNA or RNA.

Novel miRNA discovery. All small RNA sequences that failed to align with a known miRNA, piRNA or snoRNA were passed through a novel miRNA discovery platform. Each sequence was first mapped on the reference genome sequence (mm9) and 100 bases of flanking the sequence on either side were extracted to find the putative hairpin. The extracted sequence was then folded using the Vienna RNA folding package (Zuker and Jacobson, 1998). This provides the secondary RNA structure and associated minimum free energy

(mfe) structure of each occurrence of the original small RNA sequence on the reference genome. To determine if a structure forms a plausible miRNA hairpin, a multi-stage "folding filter" was applied. This folding filter enforces three minimally restrictive miRNA hairpin rules: 1) The putative miRNA sequence must rest on one side of a single hairpin. Any more complicated structure involving the miRNA sequence is rejected since the definition of a miRNA requires that it form a simple, single-hairpin precursor. 2) The putative miRNA sequence must bind relatively tightly within the hairpin. Since miRNA biogenesis dictates that the precursor will be edited down to a short double stranded RNA involving the miRNA, it is understood that the miRNA sequence must bind relatively tightly within the hairpin. Following this, the inventors edited the folded MCE-MIR plus flanking sequence down to just the subsequence involved in the hairpin structure itself. It is important to edit and refold to ensure that the hairpin predicted is stable on its own, and not artificially stabilized by nearby structural elements. After editing and refolding the inventors checked to see if the refolded sequence met the final rule, 3) the putative hairpin must have a miRNA-appropriate energy (free energy below -20 kcal/mol). A small RNA sequence was identified as a putative novel miRNA if all these criteria were met.

Plasmids and Locked Nucleic Acids (LNA). LNAs were purchased from Exiqon (Woburn, MA). For *in vitro* transfection, full-length LNAs anti-complementary to let-7a (5'-AACTATACAACCTACTACCTCA-3' (SEQ ID NO:246)) and let-7e (5'-AACTATACAACCTCCTACCTCA-3' (SEQ ID NO:247)) were used together with control LNAs anti-mmu-mir-705 (5'-TGCCCACCCCACCTCCCAC-3' (SEQ ID NO:282)) and scrambled LNA (5'-AGAGCTCCCTTCAATCCAAA-3' (SEQ ID NO:283)). For *in vivo* transfections, a truncated anti-let-7a,b,c.d LNA (5'-CAACCTACTACCTC-3' (SEQ ID NO:248)) was used together with scrambled LNA (5'-AGAGCTCCCTTCAAT-3' (SEQ ID NO:284)). MicroRNA and 3'UTR expression clones (let-7a: MmiR3368-MR01; mmu-mir-705: MmIR3181-MR01; scrambled miRNA: CmIR001-MR01; IL13: MmiT027416-MT01; scrambled 3'UTR: CmiT000001-MT01 were purchased from Genecopoeia (Rockville, MD).

Isolation, culture, and transfection of CD4+T cells from spleen. Mouse spleens were collected and CD4 T cells isolated by immunomagnetic selection (Miltenyi). Briefly, suspensions of splenocytes were prepared by lightly pushing spleens through 40-um nylon strainers (BD Bioscinces, Durham, NC). CD4+T lymphocytes were purified by using CD4 (L3T4) MicroBeads (Milteny Biotech, Auburn, CA) according to the manufacturer's protocol. For Th1 and Th2 differentiation, CD4+T cells were added to 96-well plates that were coated with anti-CD3 (1 μg/mL) antibodies. While all the cells received anti-CD28 (1-

μg/mL) and IL-2 (50 U/mL) antibodies, for Th1 differentiation, IL-12 (40-ng/mL) and anti-IL-4 (10-μg/mL) were added, whereas, for Th2 differentiation, IL-4 (20-ng/mL), anti-IFN-γ (10-μg/mL) and anti-IL-12 (10-μg/mL) were added and the cells were cultured for two weeks.

Nucleofection of *in vitro* anti-let-7a LNAs in to CD4 T cells was performed by using mouse T cell nucleofector kit (Lonza, Walkersville, MD) according to the manufacturer's protocol and the cells were cultured for 48 hours. 80 and 240 pmol of anti-let-7a LNAs and 240 pmol of scrambled LNA were used for transfection. For determining the efficiency of transfection, cells that were transfected with flourescein labeled LNAs were nucleofected in to CD4 T cells and subjected to flow cytometry after 48 hours.

For RNA extraction, cells were homogenized in Trizol and total RNA was isolated using miRNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's protocol.

Quantitation of allergic lung disease. 24 hours after the final allergen challenge, the allergic lung disease phenotype was analyzed as previously described (Kheradmand *et al.*, 2002). Briefly, mice were anesthetized with etomidate and placed on a mechanical ventilator inside a custom-designed rodent plethysmograph. Airway hyperresponsiveness (AHR) was assessed by determining the change in respiratory system resistance (R_{RS}) induced by provocative challenge with graded intravenous acetylcholine (Ach; dose expressed as mg/g body weight) as described (Kheradmand *et al.*, 2002). Bronchoalveolar lavage fluid (BALF) was collected by instilling and withdrawing 1.6 ml of sterile phosphate buffered saline (PBS) through the tracheal cannula in two aliquots of 0.8 ml. BALF total and differential cell counts were performed using a standard hemocytometer and H&E staining of cytospin slides as described (Kheradmand *et al.*, 2002). Quantitation of cytokines from BAL fluid was performed by bead-assisted analysis (MILLIPLEX MAP Kit Mouse Cytokine / Chemokine Immunoassay; Millipore, Billerica, MA, USA) using a Bioplex analyzer (BioRad, Hercules, CA) according to the manufacturers' protocols. CD4 T cells from spleen were isolated and total RNA was extracted as described for lung.

EXAMPLE 2

PRO-INFLAMMATORY ROLE FOR LET-7 MICRORNAS IN EXPERIMENTAL ASTHMA

miRNAs dominate the lung short RNAome and several are edited to alter the target repertoire. The lung short RNAomes of naïve and allergen challenged mice were

characterized using the Genboree platform for mapping NGS data from the < 60 nucleotide lung RNA fraction derived under each condition. This analysis revealed significant differences in the length distribution of small RNAs in naïve and allergen challenged lungs (FIG. 1A). The 21-23 nt RNA fraction was highly enriched in allergic as compared to naïve lung, whereas in the latter an increase in the 31-33 nt small RNA fraction was observed. miRNAs numerically dominated the short RNAome of both naïve and allergen-challenged animals, but numerous additional transcript classes were detected (Figure 1B). Of particular interest were the Piwi-interacting RNAs (piRNAs) which previously were believed to be expressed only in haploid (gonadal) tissues of mammals (Xu *et al.*, 2008).

NGS identified a total of 405 distinct miRNAs (> 10 copies of complete sequences identified each) in naïve and 328 miRNAs in allergen challenged mice (Tables 4 and 5). Let-7 family miRNAs were dominant, comprising 58% and 64% of total lung miRNAs from naïve and allergen challenged lungs, respectively (Tables 4 and 5). Among these, let-7f was most abundant in both naïve and allergen challenged lungs.

Table 4: Distribution of sequence reads aligning with miRNAs in Naive Lung.

	Naiive Lung		
miRNA_miRBase 14.0	Exact Match to miRNA (+/-4)	Exact Match to miRNA	Match to miRNA with 1-3 mismatches
mmu-let-7a	31636	21224	11584
mmu-let-7b	25572	11208	26577
mmu-let-7c	75537	49150	28947
mmu-let-7d	10833	7107	2780
mmu-let-7e	2756	1726	816
mmu-let-7f	83754	61224	16859
mmu-let-7g	13090	10580	3197
mmu-let-7i	4550	3223	6453
mmu-mir-1	27914	24720	3803
mmu-mir-100	104	52	38
mmu-mir-101a	5412	747	3492
mmu-mir-101b	1647	168	565
mmu-mir-103	5301	2960	2806
mmu-mir-106a	10	10	31
mmu-mir-106b	198	85	46
mmu-mir-107	1103	305	62
mmu-mir-10a	3341	406	1118

mmu-mir-10b	16	0	0
mmu-mir-1195	0	0	5
mmu-mir-1197	0	0	74
mmu-mir-125a-3p	5	0	0
mmu-mir-125a-5p	102	5	7
mmu-mir-125b-5p	326	212	86
mmu-mir-126-3p	1114	404	0
mmu-mir-126-5p	245	206	295
mmu-mir-127	54	43	504
mmu-mir-1274a	0	0	1598
mmu-mir-128	696	336	207
mmu-mir-129-5p	0	0	5
mmu-mir-130a	520	490	280
mmu-mir-130b	73	73	20
mmu-mir-132	5	5	13
mmu-mir-133a	130	0	20
mmu-mir-133b	2	0	0
mmu-mir-136	0	0	200
mmu-mir-138	0	0	1365
mmu-mir-139-5p	10	0	49
mmu-mir-140	9	9	6
mmu-mir-141	20	13	0
mmu-mir-142-3p	234	9	0
mmu-mir-142-5p	1507	417	278
mmu-mir-143	11123	4546	8044
mmu-mir-145	5739	2901	2970
mmu-mir-146a	733	298	700
mmu-mir-146b	472	80	290
mmu-mir-148a	266	201	142
mmu-mir-148b	160	138	105
mmu-mir-149	0	0	30
mmu-mir-150	71	34	10
mmu-mir-151-3p	81	25	0
mmu-mir-151-5p	306	146	37
mmu-mir-152	1030	918	1145
mmu-mir-155	13	6	0
mmu-mir-15a	210	44	156
mmu-mir-15b	491	244	23
mmu-mir-16	1784	1096	461
mmu-mir-17	94	65	32
mmu-mir-181a	6060	1436	1762
mmu-mir-181b	1422	196	816
mmu-mir-181c	65	13	28
mmu-mir-181d	406	129	216
mmu-mir-182	12	7	22
mmu-mir-1839-3p	6	0	0

mmu-mir-1839-5p	952	466	626
mmu-mir-185	680	567	234
mmu-mir-186	50	15	0
mmu-mir-187	7	7	29
mmu-mir-1899	0	0	2859
mmu-mir-18a	12	0	0
mmu-mir-1903	0	0	5
mmu-mir-191	2225	662	747
mmu-mir-192	549	232	188
mmu-mir-193	204	168	56
mmu-mir-1930	0	0	14
mmu-mir-1934	0	0	14
mmu-mir-1937a	478	36	491
mmu-mir-1937b	478	134	0
mmu-mir-1938	0	0	9
mmu-mir-1939	0	0	802
mmu-mir-194	14	0	0
mmu-mir-1940	12	7	0
mmu-mir-1944	6	6	0
mmu-mir-1947	0	0	54
mmu-mir-195	418	46	180
mmu-mir-1950	0	0	353
mmu-mir-1955	0	0	22
mmu-mir-1956	0	0	73
mmu-mir-1957	58	0	876
mmu-mir-1959	1148	0	4505
mmu-mir-1961	59	0	23
mmu-mir-1965	0	0	17
mmu-mir-1967	0	0	6
mmu-mir-1968	0	0	177
mmu-mir-196a	0	0	10
mmu-mir-1971	0	0	10
mmu-mir-1983	0	0	99
mmu-mir-199a-3p	5820	2082	0
mmu-mir-199a-5p	38	4	0
mmu-mir-199b	5820	2082	0
mmu-mir-19b	64	24	6
mmu-mir-200a	593	169	210
mmu-mir-200b	291	135	131
mmu-mir-200c	326	146	86
mmu-mir-201	0	0	23
mmu-mir-203	85	33	60
mmu-mir-205	57	17	5
mmu-mir-206	13	13	0
mmu-mir-208a	14	14	17
mmu-mir-20a	42	30	17

mmu-mir-20b	20	12	0
mmu-mir-21	5169	1521	902
mmu-mir-210	21	16	6
mmu-mir-2135	70	0	7
mmu-mir-2137	0	0	10
mmu-mir-2138	1342	0	0
mmu-mir-214	30	19	38
mmu-mir-2140	649	0	0
mmu-mir-2141	0	0	428
mmu-mir-2142	7892	30	1514
mmu-mir-2143	9	0	0
mmu-mir-2144	49	0	0
mmu-mir-2145	1959	110	59
mmu-mir-2146	777	0	221
mmu-mir-215	5	0	19
mmu-mir-218	8	8	0
mmu-mir-2182	0	0	5
mmu-mir-219	25	6	0
mmu-mir-22	1292	966	236
mmu-mir-221	781	142	470
mmu-mir-222	274	90	84
mmu-mir-223	44	17	45
mmu-mir-224	14	0	25
mmu-mir-23a	3567	517	1845
mmu-mir-23b	1501	265	1293
mmu-mir-24	2666	1402	6994
mmu-mir-25	812	601	321
mmu-mir-26a	5440	4044	4841
mmu-mir-26b	2097	263	923
mmu-mir-27a	1490	287	769
mmu-mir-27b	912	319	929
mmu-mir-28	38	11	0
mmu-mir-293	0	0	6
mmu-mir-296-5p	9	9	0
mmu-mir-298	13	0	5
mmu-mir-29a	11214	8326	3098
mmu-mir-29b	524	344	60
mmu-mir-29c	1106	812	229
mmu-mir-302a	0	0	11
mmu-mir-30a	4981	953	2831
mmu-mir-30b	260	227	34
mmu-mir-30c	498	90	124
mmu-mir-30d	1813	284	857
mmu-mir-30e	327	29	353
mmu-mir-31	186	42	42
mmu-mir-32	5	0	0

mmu-mir-320	1430	849	1547
mmu-mir-322	379	73	98
mmu-mir-323-5p	0	0	19
mmu-mir-324-5p	22	22	0
mmu-mir-326	6	6	0
mmu-mir-33	196	139	70
mmu-mir-331-3p	86	49	0
mmu-mir-331-5p	0	0	58
mmu-mir-335-5p	82	23	13
mmu-mir-338-3p	6	0	0
mmu-mir-339-5p	22	8	5
mmu-mir-340-5p	143	109	7
mmu-mir-341	0	0	16
mmu-mir-342-3p	212	120	0
mmu-mir-345-3p	29	0	0
mmu-mir-345-5p	14	0	0
mmu-mir-34a	29	16	11
mmu-mir-34b-3p	46	21	0
mmu-mir-34b-5p	385	228	11
mmu-mir-34c	8789	3831	3359
mmu-mir-350	53	16	0
mmu-mir-351	12	0	0
mmu-mir-361	20	14	47
mmu-mir-362-3p	26	13	0
mmu-mir-362-5p	7	0	0
mmu-mir-363	0	0	12
mmu-mir-365	28	28	0
mmu-mir-370	0	0	10
mmu-mir-374	104	83	6
mmu-mir-375	307	259	69
mmu-mir-376a	8	8	0
mmu-mir-378	1462	588	1316
mmu-mir-379	12	12	11
mmu-mir-382	0	0	7
mmu-mir-411	15	7	0
mmu-mir-423-3p	39	30	0
mmu-mir-423-5p	895	577	174
mmu-mir-425	20	12	6
mmu-mir-429	111	50	27
mmu-mir-448	0	0	35
mmu-mir-449a	205	176	26
mmu-mir-449c	30	0	0
mmu-mir-450a-5p	20	20	0
mmu-mir-450b-3p	10	10	0
mmu-mir-451	89	28	14
mmu-mir-453	0	0	49

mmu-mir-455	41	9	8
mmu-mir-466a-3p	5	0	0
mmu-mir-466b-3-3p	1	0	0
mmu-mir-466b-3p	5	0	0
mmu-mir-466c-3p	5	0	0
mmu-mir-466e-3p	5	0	0
mmu-mir-467a	24	18	0
mmu-mir-467b	24	0	0
mmu-mir-467c	0	0	7
mmu-mir-467e	12	12	0
mmu-mir-470	0	0	13
mmu-mir-471	0	0	4928
mmu-mir-484	23	23	0
mmu-mir-485	0	0	120
mmu-mir-486	22	11	23
mmu-mir-490	7	0	0
mmu-mir-491	0	0	11
mmu-mir-494	0	0	36
mmu-mir-497	207	53	46
mmu-mir-499	0	0	38
mmu-mir-500	12	7	6
mmu-mir-501-3p	6	6	0
mmu-mir-503	189	158	0
mmu-mir-504	0	0	9
mmu-mir-532-3p	27	18	0
mmu-mir-532-5p	106	91	0
mmu-mir-541	8	0	0
mmu-mir-542-3p	24	8	0
mmu-mir-542-5p	6	6	0
mmu-mir-546	0	0	14
mmu-mir-574-3p	49	25	0
mmu-mir-582-5p	5	0	0
mmu-mir-592	0	0	30
mmu-mir-598	27	8	0
mmu-mir-615-5p	0	0	247
mmu-mir-652	96	50	668
mmu-mir-654-5p	0	0	22
mmu-mir-665	0	0	275
mmu-mir-668	7	0	210
mmu-mir-669a	15	6	0
mmu-mir-669c	54	18	0
mmu-mir-669h-5p	0	0	35
mmu-mir-672	31	31	0
mmu-mir-674	111	25	91
mmu-mir-676	33	7	5
mmu-mir-685	0	0	6

mmu-mir-690	290	26	55
mmu-mir-695	0	0	5
mmu-mir-697	0	0	10
mmu-mir-700	0	0	20
mmu-mir-703	0	0	9
mmu-mir-707	0	0	6
mmu-mir-708	13	6	0
mmu-mir-709	12	0	19
mmu-mir-715	0	0	27
mmu-mir-717	0	0	9
mmu-mir-718	0	0	9
mmu-mir-720	47	26	206
mmu-mir-744	425	329	439
mmu-mir-760	0	0	7
mmu-mir-763	0	0	10
mmu-mir-7a	46	8	0
mmu-mir-805	645	36	70
mmu-mir-871	0	0	6
mmu-mir-872	90	24	34
mmu-mir-876-5p	0	0	5
mmu-mir-879	9	0	0
mmu-mir-92a	330	14	267
mmu-mir-92b	178	25	170
mmu-mir-93	323	204	109
mmu-mir-96	7	7	11
mmu-mir-98	96	74	27
mmu-mir-99a	561	234	345
mmu-mir-99b	622	400	468

Table 5: Distribution of sequence reads aligning with miRNAs in Allergen Challenged Lung.

	Allergen Challenged Lung		
miRNA_miRBase 14.0	Exact Match to miRNA (+/-4)	Exact Match to miRNA	Match to miRNA with 1-3 mismatches
mmu-let-7a	182234	131808	38610
mmu-let-7b	117273	53199	85788
mmu-let-7c	289933	197288	68360
mmu-let-7d	37016	26666	6792
mmu-let-7e	17466	11810	4586
mmu-let-7f	279496	215576	33450
mmu-let-7g	65409	52382	11034
mmu-let-7i	39647	29829	36366
mmu-mir-1	22694	20666	1435
mmu-mir-100	284	165	108

mmu-mir-101a	8739	1254	3475
mmu-mir-101b	3571	304	1117
mmu-mir-103	26674	16416	9071
mmu-mir-106b	120	44	40
mmu-mir-107	4218	1215	269
mmu-mir-10a	10134	1442	1714
mmu-mir-10b	38	0	0
mmu-mir-1198	22	15	14
mmu-mir-1199	0	0	11
mmu-mir-122	169	67	37
mmu-mir-125a-3p	9	0	0
mmu-mir-125a-5p	1159	89	243
mmu-mir-125b-3p	11	5	0
mmu-mir-125b-5p	1477	964	512
mmu-mir-126-3p	1842	719	0
mmu-mir-126-5p	634	546	330
mmu-mir-127	309	238	726
mmu-mir-1274a	0	0	671
mmu-mir-128	316	194	73
mmu-mir-1306	0	0	12
mmu-mir-130a	666	636	221
mmu-mir-130b	47	47	5
mmu-mir-132	13	13	21
mmu-mir-133a	20	0	0
mmu-mir-134	5	5	0
mmu-mir-135b	37	18	6
mmu-mir-136	5	0	84
mmu-mir-138	17	6	783
mmu-mir-139-3p	43	5	0
mmu-mir-139-5p	54	8	129
mmu-mir-140	10	10	0
mmu-mir-141	84	7	18
mmu-mir-142-3p	221	10	0
mmu-mir-142-5p	3935	476	813
mmu-mir-143	28345	10993	8529
mmu-mir-144	5	5	0
mmu-mir-145	622	311	159
mmu-mir-146a	1623	679	1205
mmu-mir-146b	12481	2284	7623
mmu-mir-147	73	73	46
mmu-mir-148a	1158	878	297
mmu-mir-148b	445	381	207
mmu-mir-150	121	73	25
mmu-mir-151-3p	325	89	0
mmu-mir-151-5p	868	415	73
mmu-mir-152	4141	3863	2432

mmu-mir-154	15	15	0
mmu-mir-155	52	11	0
mmu-mir-15a	146	62	41
mmu-mir-15b	384	121	14
mmu-mir-16	1002	688	277
mmu-mir-17	67	39	7
mmu-mir-181a	8536	1832	1929
mmu-mir-181b	2092	242	1253
mmu-mir-181c	262	29	7
mmu-mir-181d	810	254	380
mmu-mir-182	135	24	54
mmu-mir-183	26	9	5
mmu-mir-1839-3p	16	0	0
mmu-mir-1839-5p	5300	2581	2347
mmu-mir-184	121	102	51
mmu-mir-185	1874	1506	751
mmu-mir-186	145	38	9
mmu-mir-187	25	15	96
mmu-mir-188-5p	0	0	12
mmu-mir-1892	0	0	5
mmu-mir-1893	0	0	10
mmu-mir-1899	0	0	155
mmu-mir-18a	6	0	0
mmu-mir-1901	0	0	5
mmu-mir-191	4431	1677	1528
mmu-mir-192	1731	715	493
mmu-mir-193	103	93	22
mmu-mir-1930	0	0	23
mmu-mir-1937a	280	12	429
mmu-mir-1937b	280	184	0
mmu-mir-1939	0	0	759
mmu-mir-193b	15	10	0
mmu-mir-194	46	22	0
mmu-mir-1940	0	0	5
mmu-mir-195	306	21	135
mmu-mir-1950	0	0	16
mmu-mir-1955	0	0	16
mmu-mir-1957	145	0	914
mmu-mir-1959	1060	0	762
mmu-mir-1961	31	0	6
mmu-mir-1964	21	21	19
mmu-mir-1971	0	0	48
mmu-mir-199a-3p	37851	15036	5
mmu-mir-199a-5p	140	16	14
mmu-mir-199b	37851	15036	0
mmu-mir-19b	42	16	7

mmu-mir-200a	2960	787	857
mmu-mir-200b	1625	758	661
mmu-mir-200c	1313	478	348
mmu-mir-203	487	236	174
mmu-mir-205	5	0	0
mmu-mir-206	13	13	0
mmu-mir-20a	27	21	5
mmu-mir-21	100689	36933	13096
mmu-mir-210	43	35	0
mmu-mir-2137	0	0	29
mmu-mir-2138	18	0	21
mmu-mir-214	57	29	154
mmu-mir-2142	187	0	5
mmu-mir-2143	66	0	0
mmu-mir-2145	45	0	0
mmu-mir-215	45	29	6
mmu-mir-218	6	6	6
mmu-mir-219	20	4	0
mmu-mir-22	2014	1020	409
mmu-mir-221	10783	2524	5125
mmu-mir-222	1817	319	728
mmu-mir-223	106	47	118
mmu-mir-224	66	0	0
mmu-mir-23a	8912	451	3880
mmu-mir-23b	3751	298	2291
mmu-mir-24	2304	1604	5714
mmu-mir-25	5357	4348	1265
mmu-mir-26a	6472	5310	7339
mmu-mir-26b	4047	420	1193
mmu-mir-27a	4159	142	672
mmu-mir-27b	4614	1266	2122
mmu-mir-28	221	50	6
mmu-mir-296-3p	10	0	0
mmu-mir-298	142	0	88
mmu-mir-299	10	5	0
mmu-mir-29a	28931	23179	3891
mmu-mir-29b	1093	798	94
mmu-mir-29c	903	806	107
mmu-mir-301a	8	0	0
mmu-mir-30a	32244	7522	9016
mmu-mir-30b	407	388	55
mmu-mir-30c	954	140	183
mmu-mir-30d	13360	1354	3750
mmu-mir-30e	1615	93	1100
mmu-mir-31	375	105	100
mmu-mir-32	13	0	5

mmu-mir-320	8614	4994	5928
mmu-mir-322	154	46	0
mmu-mir-324-5p	29	23	0
mmu-mir-326	10	10	0
mmu-mir-328	17	17	17
mmu-mir-329	5	5	0
mmu-mir-33	608	459	101
mmu-mir-331-3p	38	26	0
mmu-mir-331-5p	0	0	106
mmu-mir-335-5p	295	102	7
mmu-mir-337-5p	12	0	0
mmu-mir-338-3p	17	0	0
mmu-mir-339-5p	40	6	5
mmu-mir-340-5p	1424	1012	119
mmu-mir-341	9	0	20
mmu-mir-342-3p	430	209	0
mmu-mir-342-5p	34	12	0
mmu-mir-345-3p	187	0	0
mmu-mir-345-5p	28	0	0
mmu-mir-34a	74	30	0
mmu-mir-34b-3p	104	53	0
mmu-mir-34b-5p	774	486	21
mmu-mir-34c	18302	12303	4428
mmu-mir-350	40	15	0
mmu-mir-351	179	10	192
mmu-mir-361	49	29	81
mmu-mir-362-3p	162	81	0
mmu-mir-362-5p	30	0	0
mmu-mir-365	44	38	0
mmu-mir-370	0	0	23
mmu-mir-374	90	75	33
mmu-mir-375	815	679	280
mmu-mir-376a	29	21	0
mmu-mir-377	0	0	6
mmu-mir-378	3852	1881	2372
mmu-mir-379	84	51	114
mmu-mir-382	116	78	0
mmu-mir-411	59	25	5
mmu-mir-421	16	6	5
mmu-mir-423-3p	312	196	0
mmu-mir-423-5p	6395	4001	1228
mmu-mir-425	44	19	0
mmu-mir-429	363	101	68
mmu-mir-433	33	28	11
mmu-mir-434-3p	27	17	0
mmu-mir-449a	1158	969	159

mmu-mir-449c	124	0	0
mmu-mir-450a-5p	74	46	0
mmu-mir-450b-3p	66	50	0
mmu-mir-451	167	39	0
mmu-mir-452	5	0	0
mmu-mir-453	0	0	6
mmu-mir-455	74	16	0
mmu-mir-466h	0	0	5
mmu-mir-467a	12	12	0
mmu-mir-467b	12	0	0
mmu-mir-467c	7	0	0
mmu-mir-467e	7	7	0
mmu-mir-471	0	0	171
mmu-mir-484	42	35	6
mmu-mir-485	9	0	91
mmu-mir-486	59	44	21
mmu-mir-490	10	0	6
mmu-mir-491	0	0	13
mmu-mir-494	0	0	530
mmu-mir-495	0	0	5
mmu-mir-497	529	171	106
mmu-mir-500	22	15	5
mmu-mir-501-3p	161	52	0
mmu-mir-503	1493	1243	103
mmu-mir-504	0	0	22
mmu-mir-532-3p	8	8	0
mmu-mir-532-5p	822	736	146
mmu-mir-541	196	21	61
mmu-mir-542-3p	213	86	0
mmu-mir-542-5p	18	18	0
mmu-mir-543	10	0	0
mmu-mir-574-3p	49	44	0
mmu-mir-574-5p	38	6	14
mmu-mir-582-3p	17	0	0
mmu-mir-582-5p	11	5	0
mmu-mir-598	109	27	13
mmu-mir-615-5p	0	0	223
mmu-mir-652	293	47	479
mmu-mir-665	6	0	20
mmu-mir-669c	271	138	19
mmu-mir-669h-5p	0	0	30
mmu-mir-672	841	678	91
mmu-mir-674	251	48	116
mmu-mir-676	149	32	0
mmu-mir-691	0	0	6
mmu-mir-693-5p	0	0	5

mmu-mir-695	0	0	43
mmu-mir-697	0	0	11
mmu-mir-700	0	0	49
mmu-mir-708	16	8	0
mmu-mir-720	90	55	126
mmu-mir-744	3138	2406	2165
mmu-mir-760	0	0	8
mmu-mir-762	0	0	18
mmu-mir-7a	320	72	36
mmu-mir-805	1239	216	380
mmu-mir-872	428	122	38
mmu-mir-879	5	0	0
mmu-mir-9	6	6	0
mmu-mir-92a	856	38	825
mmu-mir-92b	626	48	460
mmu-mir-93	282	221	67
mmu-mir-96	14	14	0
mmu-mir-98	960	753	136
mmu-mir-99a	2572	1414	1137
mmu-mir-99b	4013	2562	2350

Upon mapping of sequences to the miRBase-14.0 pre-miRNA database, allowing for 1-4 mismatches in the aligned reads to a given pre-miRNA, several miRNAs were detected that were post-transcriptionally modified (edited) in at least one position of the seed sequence. The distribution of nucleotide changes in relation to position for all miRNAs in naive and allergen challenged lungs are shown in FIG. 1C. When the normalized numbers of nucleotide modifications were compared, the miRNA mmu-mir-101a showed a 10% increase in the number of 8th nucleotide modifications from C- to- U in naïve lungs as compared to allergen challenged. Using the TargetScan 5.1 algorithm, it was observed that the target repertoire of the modified mir-101a species had been re-directed to be identical to that of mmu-mir-144. Among several predicted changes in the target repertoire, this edit potentially enhances affinity for several allergy-related genes, including GATA and CD28 (Das, Chen, Yang, Cohn, Ray and Ray 2001; Keane-Myers, Gause, Linsley, Chen and Wills-Karp 1997). However, in keeping with prior observations from pancreatic tissue and mouse ovary (Reid *et al.*, 2008), post-transcriptional modifications were particularly common in the let-7 family of microRNAs. The most common such modification was a U to G change at position 9 (let-7a(9U→G)), which was detected by comparison of let-7a sequences with pre-mmu-let-7a (FIG. 1D). This post-transcriptional modification effectively converts let-7a to let-7e, which largely

shares the same targets (TargetScan 5.1). Thus, post-transcriptional editing of multiple lung miRNAs occurs, potentially altering the target repertoire for some miRNAs.

Using a novel miRNA discovery bioinformatics platform (Gu *et al.*, 2008) the inventors further identified 25 putative novel miRNAs from naïve and allergen challenged lungs (Tables 6 and 7). Two miRNAs, Asth-miR-1 and Asth-miR-2, were highly expressed in naïve and possibly down regulated following allergen challenge (FIG. 6).

Table 6

Putative Mature Mir(pmm)	SEQ ID NO:	Forward Sequence	Reverse Sequence	Hairpin	Exact to Hairpin	Exact to Plus 4	Exact to pmm
1	285	TGAAGCGCGGGTA	GTTAGTGATGATCAATAAA (SEQ ID NO:290)	8497-13-91-1:R:mm9;-35.100	337	144	91
2	286	GAAGGAACTACAAGACAGCT		12510-20-5-1:R:mm9;-27.900	5	5	5
3	287	CCCGGGTTTCGGCACCA		6746-17-208-1:R:mm9;-97.000	1004	622	208
4	288	TAACAGGTCTGTGA		9953-14-132-1:R:mm9;-29.900	920	593	132
5	289	AGATTGATTGTTAAGCTGAAA		5224-21-16-1:R:mm9;-34.700	117	65	16
6	291	TGGGCTACACATTTT		13900-15-19-1:R:mm9;-33.300	101	47	19
7	292	AGCGATTTGTCTGG		2678-14-206-1:R:mm9;-26.100	1466	1093	206
7.1	293	AGCGATTTGTCTGG		2678-14-206-2:R:mm9;-28.300	1245	1093	206
8	294	GCATTGGTGGTTCAGT		9415-16-876-1:R:mm9;-27.800	3274	2760	876
9	295	CGCAGTTTTATCCGGTA		7178-17-39-1:R:mm9;-26.630	327	84	39
9.1	296	CGCAGTTTTATCCGGTA		7178-17-39-3:R:mm9;-28.300	244	69	39
10	297	GCGTTGGTGGTATAGTGGTGA		14359-21-372-1:R:mm9;-42.900	1538	1538	372
11	298	GGCTCCATAGCTCAGGG		10646-17-57-1:R:mm9;-27.600	106	106	57
12	299	GAGCACCCCATTGGCTACCCAC		13020-22-6-1:R:mm9;-77.240	12	12	6
13	300	GAAGATTAGCATGGCCCCTG		12450-20-13-1:R:mm9;-25.300	74	29	13
14	301	TGGATATGATGACTGA		14705-16-25-1:R:mm9;-55.300	127	58	25

15	302	GAAGGGCAAAAGCTCGCTTGATCTTGA	6423-27-14-1:R:mm9;-26.400	57	52	14
16	303	GTATGTGCTTGGCTGAGGA	15270-19-138-1:R:mm9;-33.000	388	326	138
17	304	CCCGGGTTTCGGCACCA	6746-17-208-5:R:mm9;-98.500	1004	622	208
18	305	ATCGTAATCTGAGCCGA	15481-16-28-1:R:mm9;-40.200	299	50	28
18.1	306	ATCGTAATCTGAGCCGA	5243-17-33-1:R:mm9;-35.400	52	33	33
19	307	TACCATGATCACGA	10320-14-14-1:R:mm9;-39.200	59	14	14
20	308	CTAAAATTGGAACGATACAGA	11096-21-10-1:R:mm9;-41.900	45	35	10
21	309	CGGAACTGAGGCCATGA	7495-17-68-1:R:mm9;-40.800	519	389	68

Table 7

	SEQ ID NO:	Forward Sequence	Reverse Sequence	Hairpin	Exact to Hairpin	Exact to Plus 4	Exact to pmm
1	310	GCTAAGCAGGGTCGGGCCTGGTTA	GTCTACGGCCATACCACCCTGAA (SEQ ID NO:311)	3470-24-25-1:R:mm9;-30.300	57	47	25
2	312	ACGGGAGGGCCGGGCGGCGAG		1267-21-5-1:R:mm9;-70.300	5	5	5
3	313	GCATTGGTGGTTCAGTGGTAGAATTC		1764-26-285-1:R:mm9;-27.800	968	960	285
4*	314	GCGTTGGTGGTATAGTGGTGA		5553-21-19-1:R:mm9;-42.900	88	88	19
5	315	GGTGGTGCAGGCAGGAGAGCCA		6579-22-5-1:R:mm9;-77.240	5	5	5
6	316	TGGACACTGGAGAGAGAGCTTT		12241-22-7-1:R:mm9;-37.300	7	7	7
7	317	GACTGCTGATCCGGGTGATGCGAA		3164-24-5-1:R:mm9;-39.300	5	5	5
8*	318	TGGATATGATGACTG		3675-15-16-1:R:mm9;-55.300	51	37	16
9	319	GGGGGTATAGCTC		2283-13-62-1:R:mm9;-47.200	184	109	62
10*	320	CCCGGGTTTCGGCACCA		1641-17-34-1:R:mm9;-97.000	82	75	34
11	321	GACGAGGTGGCCGAG		2115-15-19-1:R:mm9;-39.900	86	66	19
12	322	TTGGGCAGAGGAGGCAGGGACA		13492-22-10-1:R:mm9;-32.100	10	10	10

*: also found in naïve lung RNA

Identification of relevant miRNA-mRNA functional pairs. Deep sequencing is capable of identifying and enumerating both known and novel miRNAs as well as other classes of short transcripts, but the sensitivity of this technique for detecting and quantitating all known transcripts in complex samples such as lungs remains unknown. To circumvent this potential limitation of NGS, mRNA and miRNA microarray analyses were performed using total RNA from naive and allergen challenged mouse lungs and validated findings for selected genes using quantitative PCR (FIGS. 2A-D). A total of 195 genes were upregulated and 281 genes were downregulated in allergen challenged lungs relative to naïve (FIG. 2A). In addition to numerous immunoglobulin genes, the most highly induced genes included EAR11, an eosinophil-associated ribonuclease (Cormier *et al.*, 2001), Gob-5 (CLCA3), a gene with uncertain function linked to allergic disease (Nakanishi *et al.*, 2001), Ym2 (CHI3L4), a chitinase-like molecule that is induced by IL-4 (Webb *et al.*, 2001), and matrix metalloproteinase 12 (MMP12), an IL-13-inducible proteinase that is required for allergen-induced airway eosinophilia (Pouladi *et al.*, 2004). Enhanced expression of IL-4 and other Th2 cytokine transcripts was also detected in allergen-challenged lungs as expected, with the notable exception of IL-13.

Conversely, genes that were most prominently downregulated with allergen challenge included contractile proteins (alpha 1 actin (ACTA1); troponin C (TNNC2)), chemokines (CXCL14), ARNTL (BMAL1), a CLOCK-associated gene linked to glucose metabolism (Rudic *et al.*, 2004), IFITM6 (fragilis5), and lysozyme. qRT-PCR analysis of selected genes validated mRNA transcripts that were either up- or downregulated (FIG. 2B). In contrast to microarray results, IL-13 transcripts were clearly markedly enhanced by allergen challenge as assessed by qRT-PCR (FIG. 2B). Moreover, the enhanced presence of both IL-13 transcript and protein in allergic lungs has been repeatedly documented (Arima *et al.* 2002; Corry *et al.*, 1996; Grunig *et al.* 1998; Huang *et al.*, 1995; Kasaian *et al.* 2007), indicating that the inability to detect this transcript by microarray was spurious. These studies thus confirm that numerous allergy-related genes are upregulated in lungs following allergen challenge.

Microarray analyses further identified numerous miRNAs that were significantly up- and down-regulated with allergen challenge (FIG. 2C). Expression of the most abundant miRNA transcripts, most notably let-7 miRNAs, did not change with allergen challenge. qRT-PCR again verified trends in expression of selected miRNAs that changed significantly and it was confirmed that let-7a transcripts were not altered by allergen challenge (FIG. 2D). Based on Targetscan 5.1 predictions, numerous miRNAs were identified from these analyses that putatively target genes of relevance to the asthma phenotype (Table 9). For example, a

potential target of mir-135a, which was significantly up-regulated in asthmatic mice, is signal transducer and activator of transcription 6 (STAT6), a transcription factor that is required for Th2 responses and experimental asthma (Kuperman *eet al.*, 1998).

Table 9: Lung miRNAs and potential targets with relevance to allergic disease

MicroRNA	Target gene	Gene name	Context Score*
mmu-mir-712	GATA3	GATA binding protein 3	-0.12
mmu-mir-699	STAT6	signal transducer and activator of transcription 6	-0.26
mmu-mir-743a	IL13RA1	interleukin 13 receptor, alpha 1	-0.4
mmu-mir-1196	GATA3	GATA binding protein 3	-0.33
mmu-mir-709	CD4	CD4	-0.23
mmu-mir-717	ADRB2	adrenergic, beta-2-, receptor	-0.33
mmu-mir-142-5p	JAK1	Janus kinase 1	-0.16
mmu-mir-340-5p	IL4	interleukin 4	-0.25
mmu-mir-340-5p	JAK1	Janus kinase 1	-0.26
mmu-mir-146b	IRAK1	interleukin-1 receptor-associated kinase 1	-0.91
mmu-mir-135a	STAT6	signal transducer and activator of transcription 6	-0.45
mmu-let-7	IL13	interleukin 13	–

* Derived from TargetScan 5.1.

IL-13 is a target gene of let-7a. Subsequent efforts were focused on the abundant and extremely conserved let-7 miRNA family, the function of which in mammals remains largely undefined. The let-7 family target recognition sequence in the IL13 3' UTR is highly conserved across mammalian species (FIG. 3A). Moreover, all mouse let-7 miRNAs (mmu-let-7a-i; mmu-mir-98) are predicted to target IL-13 (TargetScan 5.1). To verify this, the inventors first folded the mature let-7a-1 miRNA sequence against the mouse IL-13 3'UTR target sequence. This comparison revealed a high degree of complementarity characterized by a very low mean free energy value of -30.4 kcal/mol (FIG. 3B).

Lung IL-13 transcripts were markedly enhanced with allergen challenge (FIG. 2B) whereas total lung let-7a transcripts did not change (FIG. 2D), which failed to support a functional relationship between IL-13 and let-7a. However, Th2 cells are the predominant

source of lung IL-13 following allergen challenge and represent a small (0.01-0.1%) fraction of total lung cells following allergen challenge in this model. Both IL-13 and mmu-let-7a transcripts in Th2 cells derived from naïve mouse CD4 T cells were quantitated. Similar to lung, let-7 miRNAs were the most abundant miRNA transcripts in T helper cells. As expected, IL-13 transcripts were markedly enhanced whereas interferon gamma (IFN-γ) transcripts were suppressed in Th2 relative to Th1 cells (FIG. 3C), but in contrast to lung, mmu-let-7a transcripts were markedly suppressed in Th2 cells, an inverse association with IL-13 that did suggest a functional interaction (FIG. 3D).

To determine if IL-13 is a genuine target of mmu-let-7a, plasmids expressing the pre-miRNA for mmu-let-7a and a luciferase gene containing the IL-13 3'UTR were co-transfected into HEK293T cells. In a dose-dependent manner, mmu-let-7a suppressed luciferase production, whereas neither a scrambled miRNA nor an irrelevant miRNA (mir-705) had any effect (FIG. 4A). Further, scrambled or anti-let-7a locked nucleic acids (LNA) (ref) representing the entire reverse complement of mmu-let-7a were transfected into these cells. Again in a dose dependent manner, anti-let-7a LNAs progressively reversed the suppressive effect of mmu-let-7a on luciferase production (FIG. 4B). Identical experiments were performed using the human IL-13 3'UTR, human let-7a (hsa-let-7a, which is identical to mmu-let-7a) and the same LNAs and produced identical results (Figs. 4C, D). Together, these studies indicated that both human and mouse IL-13 are targets of let-7a and that this miRNA can be specifically inhibited by an LNA.

These findings were next confirmed in primary murine CD4+ T cells. The majority (>80%) of T helper cells could be transfected with anti-let-7a LNA (FIG. 4E), which by RT-qPCR reduced let-7a transcripts >90% at the highest LNA dose given (FIG. 4F). This was accompanied by a 2.5-fold greater increase in CD4 T cell IL-13 transcripts following activation (FIG. 4G). Together, these findings confirm that IL-13 is regulated by let-7a and demonstrate the utility of LNAs for the specific inhibition of miRNAs in primary T cells.

Finally, this *in vitro* system was used to compare native let-7a and let-7a(9U→G) for their ability to silence IL-13 expression. Despite having identical affinities for the IL-13 3'UTR recognition site (Targetscan 5.1), let-7a(9U→G) (let-7e) was less efficient in suppressing IL-13 expression relative to let-7a (FIG. 4H). The let-7a(9U→G) pre-miRNA as used in these studies is not identical to the let-7e pre-miRNA, raising the possibility that the let-7a(9U→G) pre-miRNA was not properly processed into mature let-7e. However in separate transfection experiments, it was confirmed by qRT-PCR that mature let-7e was fully

processed from the let-7a(9U→G) pre-miRNA, as was mature let-7a from let-7a pre-miRNA (FIG. 4I). Thus, editing of let-7a to let-7a(9U→G) creates let-7e, which is less efficient at suppressing IL-13 expression.

Pro-inflammatory role of let-7 miRNAs *in vivo*. In addition to IL-13, let-7 miRNAs are predicted to inhibit other genes of interest in asthma, including the beta-2-adrenergic receptor (β_2-AR; ADRB2), a catecholamine receptor that is required for expression of experimental allergic lung disease (refs). However, the entire let-7 miRNA family is predicted to regulate over 800 conserved targets (TargetScan 5.1). It was reasoned that the overall *in vivo* function of mmu-let-7a, or indeed any miRNA, cannot alone be predicted from *in silico* analysis of the target repertoire even combined with knowledge of individually validated targets. Thus, to begin to assess overall function of let-7 miRNAs *in vivo*, allergen immunized mice were systemically administered either a scrambled or an anti-let-7 LNA that is the reverse complement of the first 14 nucleotides (5') of let-7a, b, c and d. LNAs were administered before intranasal allergen challenge, but after allergen sensitization, to determine their effect on the effector phase of the disease (FIG. 5A). The specificity of this *in vivo* protocol was first evaluated, and it was observed that anti-let-7 LNA, but not a scrambled LNA, reduced let-7a transcripts in splenic CD4 T cells (FIG. 5B). However, unlike the immediate effect of anti-let-7a on T cells transfected *in vitro* (FIG. 4F, G), after 3 days of allergen challenge *in vivo*, splenic CD4 T cell IL-13 transcripts were reduced, whereas transcripts of an unrelated gene, IFN-γ, were unaffected (FIG. 5B).

The discrepancy in expression of the same target gene observed with immediate (FIGS. 4A-I) and delayed (FIGS. 5A-E) administration of an anti-let 7 LNA was unexpected and suggested that secondary or even tertiary effects of let-7 inhibition arise over time *in vivo* to suppress inflammatory gene expression. To determine if this anti-inflammatory effect is physiologically significant, the effect of anti-let-7 miRNAs on the allergic lung disease phenotype was determined. Two canonical features of this phenotype are airway hyperreactivity, which was determined in anesthetized, mechanically ventilated animals as the change in respiratory system resistance (R_{RS}) induced by graded injections of acetylcholine; and recruitment to the airways of inflammatory cells. As expected, scrambled LNA had no effect on these asthma-related parameters (FIG. 5C, D). In contrast, anti-let-7 LNA markedly suppressed both hyperresponsiveness to acetylcholine and lung inflammation, especially eosinophil recruitment to the airways. Analysis of airway cytokines confirmed that anti-let-7, but not control LNA significantly inhibited secretion of canonical Th2 cell

cytokines including IL-4, IL-5 and IL-13 (FIG. 5E). In contrast, neither LNA influenced secretion of IFN-γ, ruling out a possible anti-viral response triggered by the exogenous LNAs. Thus, in contrast to expectations from analysis of individual gene targets *in vitro*, *in vivo* suppression of let-7 miRNAs revealed the pro-inflammatory role of select members of this miRNA family in allergic lung disease.

Using a combination of high-resolution miRNA microarrays and NGS together with detailed bioinformatic analyses, a whole genome view of major families of short transcripts and the RNAome of the lung in its naïve state and the changes it undergoes in response to challenge with a potent respiratory allergen are presented here. Lung miRNAs demonstrated profound changes in overall abundance, sequence, and composition of individual species. Many new miRNAs have been discovered through this effort and it was determined that let-7 microRNAs are the most abundant of all miRNAs in mouse lung. Although the majority of prior studies suggested a dominant anti-inflammatory role for miRNAs in immunity, *in vivo* analyses revealed a potent pro-inflammatory role for let-7 miRNAs in allergic lung disease. Together, these results constitute an important miRNA database and provide unique insight into the control of allergic inflammation.

Emerging evidence suggests that miRNA function is highly nuanced and can range from straightforward silencing to fine-tuning of gene expression (Reid *et al.*, 2008). A striking finding of this study is that miRNA editing potentially represents a new dimension of this essential function. Previously, miRNAs of the let-7 family were observed to be extensively edited in cells derived from human and mouse pancreas and ovary and the current study extends this finding to the lung (Reid *et al.*, 2008). It is shown here that relatively under-represented, non-let-7 miRNAs show similar editing. The C-to-U modification of mmu-mir-101a effectively converts the seed sequence to that of mmu-mir-144, with significant potential alterations in the target repertoire. Moreover, this data demonstrates that conversion of let-7a to let-7e (let-7a(9U→G)) reduces the ability of mmu-let-7a to regulate established targets such as IL-13. All let-7 miRNAs are predicted to target the same genes and let 7a-and let-7e appear to target IL-13 with identical affinity (TargetScan 5.1). These studies therefore indicate that subtleties exist with respect to the efficiency of target suppression relevant to position 9 nucleotides that are not accounted for by current prediction algorithms. Further analysis of the effect of miRNA edits, both naturally occurring and induced, on target regulation will be useful in refining the accuracy of target predictions.

Many of the novel miRNAs presented herein are homologous to transcripts previously identified from humans, zebra fish and mice as piRNAs. Some of the novel transcripts exceed the typical length of miRNAs (~22nt), *e.g.*, Asth-miR-1 consists of 26 nt. However, the genomic context of all novel putative miRNAs permits the formation of a stable pre-miRNA duplex that may serve as a substrate for the nuclear Drosha/Pasha microprocessor required for miRNA biogenesis. Because piRNA precursors do not form such duplexes, and indeed the biogenesis of piRNAs remains uncertain (Kim *et al.*, 2009), these novel sequences are most appropriately classified as miRNAs.

Identified herein are numerous miRNAs from mouse lung with potential relevance to the control of allergic inflammation as suggested by a limited analysis of the target repertoire. The data indicates a highly complex role played by miRNAs in this disease model. For the current study, additional effort ws focused on understanding the global significance of let-7 miRNAs to the control of allergic lung inflammation. This large miRNA family was chosen because of the high degree of conservation of family members across metazoans and unexpectedly robust expression in both T cells and lung that suggested a conserved and likely critical function (Lee and Ambros 2001). Let-7 miRNAs and the let-7 processing regulator Lin28 (Viswanathan *et al.*, 2008) have previously been identified as regulators of developmental timing, morphogenesis and cancer (Hammell *et al.*, 2009; Iliopoulos *et al.*, 2009; Viswanathan *et al.* 2009). However, the miRNA-controlled cellular circuitry involved in development and oncongenesis overlaps with programs governing inflammation (Davidson-Moncada *et al.*, 2010; Iliopoulos *et al.*, 2009), suggesting that a regulatory role for let-7 miRNAs in lung inflammation was possible.

Although as predicted IL-13 is regulated by let-7a, given the more than 800 predicted targets of let-7 miRNAs, the inventors reasoned that the effects of let-7 inhibition in a complex *in vivo* model of inflammation could not be predicted based on target validation alone. Indeed, neither the failure of lung IL-13 and let-7a transcript expression to correlate inversely nor the suppressive effect of let-7a on T cell IL-13 transcripts predicted the requisite role of let-7 miRNAs in allergic lung disease. These findings emphasize the difficulty in predicting miRNA function in complex *in vivo* systems and indicate that the primary effects of let-7 inhibition on target gene expression translate over time into dominant secondary effects that ultimately suppress inflammation. The large size of the let-7 target repertoire and such secondary effects precluded precise identification of the pro-inflammatory mechanism coordinated by let-7 miRNAs, an effort made more complex by the recent discoveries that let-

7 miRNAs can either promote or suppress target gene expression by binding either canonical or non-canonical mRNA elements (Lytle *et al.*, 2007; Vasudevan, Tong and Steitz 2007).

Assessing the biological function of let-7 miRNAs *in vivo* is challenging. In addition to targeting essentially the same ~820 mRNAs, the nine known let-7 miRNAs derive from 12 genetic loci (three exist as duplicate miRNA genes), effectively precluding a direct family-wide gene silencing approach through homologous recombination. For this study, LNAs were used since the safety, efficacy and specificity of which have been demonstrated both *in vitro* and *in vivo* (Elmen *et al.* 2008; Lanford *et al.*, 2010; Wahlestedt *et al.* 2000). LNAs have the additional advantage over alternate gene silencing approaches that potentially toxic transfection vehicles (viruses, polyethyleneimine, etc.) are not required for *in vivo* use (Stein *et al.* 2010). The present studies confirm the specificity of LNAs used *in vitro* and *in vivo* and no toxicity was observed in mice receiving either control or anti-let-7 LNAs. These studies therefore support the therapeutic application of anti-let-7 LNAs in asthma and possibly other allergic conditions specifically to target let-7 and potentially numerous other miRNAs.

In summary, a variety of genomic approaches were used to demonstrate that numerous miRNAs and other short transcripts are expressed in mouse lung and undergo marked changes in abundance during the transition from the naïve state to allergic lung disease. Selected miRNAs undergo editing, creating potentially novel means for regulating the target repertoire and numerous novel miRNAs were identified. miRNAs of interest to allergic disease were identified, and it was demonstrated that the most abundant lung miRNAs, from the let-7 family, are required to support allergic lung disease.

EXAMPLE 3

EFFECTS OF INHIBITION OF LET-7 MIRNA -155 *IN VIVO*

This Example describes the effects of the inhibition of mmu-mir-155 (mouse miRNA 155) in mice; as shown below, the data indicates that miRNA-155 is required for the expression of allergic lung responses *in vivo*. Novel miRNAs from mouse T cells were also identified.

Materials and Methods

Mice. Four to six-week-old female Balb/c and C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained at the Transgenic Mouse Facility (TMF) at Baylor College of Medicine (BCM) and treated in accordance with the

institutional and federal guidelines of BCM and the National Institutes of Health (NIH), respectively.

$CD4^+$ T cell Isolation and In Vitro Differentiation. Mice were anesthetized with a single intraperitoneal (IP) dose of pentobarbital sodium. Following cervical dislocation the spleens were aseptically removed and a single-cell suspension was obtained by gently pressing spleens through a 40 μm nylon mesh cell strainer (BD Falcon, San Jose, CA) placed inside one well of a six-well cell culture plate containing 3 ml of complete media (CM): 1640 RPMI supplemented with 10% FBS, 1% glutamine (100X) in 0.85% NaCl (Invitrogen, Carlsbad, CA) 1% antibiotic-antimycotic (100X) liquid: 10,000 units penicillin (base), 10,000 μg streptomycin, 25 μg amphotericin B/ml utilizing penicillin G (sodium salt), streptomycin sulfate and amphotericin B as Fungizone® Antimycotic in 0.85% saline (Invitrogen, Carlsbad,CA). The single-cell suspension was transferred and re-filtered through the mesh nylon cell strainer into a 50-ml conical vial (BD Falcon, San Jose, CA). The single well is washed thoroughly with an additional 3 ml of supplemented complete media and also filtered through the mesh cell strainer. Isolated splenocytes were collected by centrifugation at 1200 rpm for 5 min at 4°C. The red blood cells were lysed after re-suspending cells in 5 ml of ACK lysing buffer for 3 min at room temperature (RT). The buffer was neutralized with 5 ml of complete media. The resulting splenocytes were passed through a second 40 μm nylon mesh cell strainer and washed with complete media, pelleted and resuspended in 10 mls of complete media. The total cell number was determined, the cells were washed again and resuspended in 90 μl of degassed labeling buffer (solution containing PBS (phosphate buffered saline), pH 7.2, 0.5% BSA (bovine serum albumin) and 2mM EDTA (ethylenediaminetetraacetic acid)) and 10 μl of $CD4^+$ (L3T4) microbeads (Miltenyi Biotec, Auburn, CA) per 10^7 total cells. The splenocytes were incubated on ice for 30 minutes. Subsequently, cells were washed with 10 ml of labeling buffer, spun at 1200 rpm for 5 min and resuspended in 500 μl of labeling buffer. The splenocytes were added to a prepared MACS LS column. After washing the column 3 times with 3 ml of labeling buffer, the column was removed from the magnetic field, 5 ml of buffer was added to the column and the $CD4^+$ T cells were eluted from the column with the supplied plunger. The cells are counted, pelleted and resuspended in complete media at a concentration of 4 $x10^6$ cells per 10 ml. The purified CD4+ T cells were incubated in complete media. For T_H1 polarizing conditions, cells were cultured with: IL-12 (2ng), IFN-γ (100U) and anti-IL-4 (11B11, 10ug) and for T_H2 polarizing conditions, the cells were cultured with: IL-4 (200U), IL-6 (100U), anti-IFN-γ

(AN18, 5ug) and anti-IL-12 (clone C17.8, 2μg). All T cells were stimulated twice (day 0 and day 8) with 5 μg/ml plate-bound CD3e antibody (clone 145-2C11, BD Pharmingen), 5μg/ml soluble CD28 antibody (clone 37.51, BD Pharmingen) and IL-2 (20U) in flat-bottom 96-well cell culture plates (Corning) in tandem with polarizing conditions. Naïve T cells were not stimulated and total RNA was extracted immediately. Supernatants were collected for IL-4 and IFN-γ analysis.

Total RNA Isolation. The $CD4^+$ T cells (naïve and differentiated subsets) were homogenized in Trizol® Reagent using 1 ml of reagent per 5-10 x 10^6 cells (Invitrogen, Carlsbad, CA) and either frozen at -80°C for later extraction or immediate extraction of total RNA by adding chloroform per 1 ml of Trizol® Reagent. The RNA was precipitated from the colorless aqueous phase using 0.5 ml of isopropyl alcohol per 1 ml Trizol® Reagent used. The precipitated RNA was collected by centrifugation at 12,000 x g for 10 min at 4°C. The RNA was washed with 1 ml 75% ethanol per 1 ml Trizol® Reagent used. The RNA was re-dissolved in nuclease-free water. RNA isolated from freshly sorted $CD4^+$ naïve (~ 18 million cells) or T_H1 and T_H2 cell differentiation experiments from material pooled from two independent 96-well plates each with ~18 million cells.

Isolation and Enrichment of Small RNAs. The small RNA fraction (<200nt) was isolated from the $CD4^+$ T cells with the Pure Link™ miRNA Isolation Kit (Invitrogen, Carlsbad, CA). To completely dissociate the nucleoprotein complexes the cells were lysed using 1 ml of Trizol® Reagent per 5-10 × 10^6 cells and incubated at room temperature for 5 min. Per 1 ml of Trizol® Reagent 200 μl of cholorofom was added. The total lysate was shaken by hand for 15 seconds and incubated for 2-3 min. The mixture was centrifuged at 12,000 × g at 4°C for 15 min and separated into a lower phenol-chloroform phase, interphase and colorless upper aqueous phase. The upper aqueous phase containing the RNA was collected and mixed with 100% ethanol to a final concentration of 35%. The lysate-ethanol mixture was added to a Spin Cartridge (provided in the kit) and centrifuged at 12,000 × g for 1 min at room temperature. The flow-through was retained and mixed with ethanol for a final concentration of 70%. The mixture was then added to a second Spin Cartridge and centrifuged at 12,000 × g for 1 min at room temperature. The flow-through was discarded and the column-bound small RNA molecules were washed twice with 500 μl Wash Buffer (provided in the kit) and centrifuged the twice at 12,000 × g for 1 min at room temperature and the flow-through was discarded. To remove any residual Wash Buffer the Spin Cartridge is centrifuged for 2-3 min at maximum speed at room temperature. The RNA is eluted after

added 50 µl of sterile, RNAse-free water to the Spin Cartridge, incubated at room temperature for 1 min and collected at maximum speed for 1 min at room temperature. The RNA was stored at 80°C or immediately submitted to the Microarray Core Facility (Baylor College of Medicine, Houston, TX) to assess quality and concentration. RNA quality of all samples was determined on an Aglient 2100 Bioanalyzer (quality parameters: ribosomal RNA concentration, DNA contamination, RNA integrity, and overall quality, Quantum Analytics, Inc., Foster City, CA) and the concentration was also measured by Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). Purified RNA was kept in nuclease-free water at -80°C.

RNA End Modification and Amplification (REMA). Denaturing 15% TBE-UREA polyacrylamide gels (Invitrogen, Carlsbad, CA) were used to further isolate short RNAs. An equal volume of enriched short RNA samples (10 µl containing 2-5 mg RNA) from naïve, T_H1 and T_H2 samples and gel loading buffer (Invitrogen, Carlsbad, CA) was heated to 65°C for 5 min and loaded onto the gel. A 10 base pair DNA ladder (Invitrogen, Carlsbad, CA) was loaded into an additional well of the gel. After running the gel for 1 hr at 200V the short RNA bands (15-65nt) that corresponded to 5-55 base pairs on the DNA ladder were excised from the gel, extracted from the polyacrylamide, precipitated and washed. In separate reactions, synthetic RNA adapter oligonucleotides were added to the 5' (5' GUU CAG AGU UCU ACA GUC CGA CGA UC 3' (SEQ ID NO:280)) and 3'(5' p-UCG UAU GCC GUC UUC UGC UUG-idT 3' (SEQ ID NO:281)) ends of the short RNAs in the presence of RNase inhibitor buffer and ATP. To remove the un-ligated adapter sequences after the addition of the 5' adapter, the ligated short RNAs were gel purified on a 15% polyacrylamide gel (Invitrogen, Carlsbad, CA) and excised from the gel based on the DNA ladder bands corresponding to 30-90 base pairs (RNA equivalent 40-100 nt). Similar steps were performed to ligate and gel purify the 3' adapter to the ligated 5'adapter RNA. The RNA was excised from a 10% TBE-Urea gel based on the DNA ladder bands corresponding to 50 -120 base pairs (RNA equivalent 60 -130 nt). Using a 3'adapter sequence specific primer (5' CAA GCA GAA GAC GGC ATA CGA 3' (SEQ ID NO:325)). The resulting ligated short RNA sequences were reverse transcribed and PCR amplified for 15 cycles using 5' and 3' adapter specific primers (Forward primer-5' AAT GAT ACG GCG ACC ACC GAC AGG TT CAG AGT TCT ACA GTC CGA 3' (SEQ ID NO:326); reverse primer-5' CAA GCA GAA GAC GGC ATA CGA 3' (SEQ ID NO:327)). The sequences were identified using Illumina-based Next Generation Sequencing.

*Novel MiRNA Discovery Strategy.*The unique sequences that did not map to known miRNA precursors were subjected to a novel miRNA discovery pipeline previously described (Creighton *et al.*, 2009). The small RNA sequences were mapped to the whole genome and the sequences that map exactly are retained (including 100 bases flanking each side). These putative miRNA hairpin sequences are folded with Vienna package (Hofacker, 2009) and those structures that meet Ambros criteria are filtered for single-loop hairpins with the putative miRNA on one side of the hairpin and have a minimum free energy of <-25 kcal/mol (Ambros *et al.*, 2003). The hairpins are then trimmed to include only the putative precursor. Subsequently, they are refolded and filtered again using Ambros criteria. The hairpins that are produced are considered novel miRNA hairpin precursors containing mature miRNA sequences found in the small-RNAome of a sample.

Mice and allergen challenge. Female C57BL/6 mice between 4 and 7 weeks of age were anesthetized in an airtight chamber purged with a 3.2% isoflurane in oxygen vapor mixture for 10 minutes to achieve deep general anesthesia. Anesthetized mice received intranasally 45 mL chicken ovalbumin (22.5 mg) and 9mL of protease derived from *Aspergillus melleus* (formerly *A. oryzae;* Sigma chemical company, St. Louis, MO*;* 7 mg) in PBS by applying droplets to the nares with a pipette. Allergen challenged mice received intranasal allergen on a schedule of every alternate day for eight total challenges

Quantitation of allergic lung disease. 24 hours after the final allergen challenge, mice were anesthetized with etomidate and placed on a mechanical ventilator inside a custom-designed rodent plethysmograph. Airway hyperresponsiveness (AHR) was assessed by determining the change in respiratory system resistance (R_{RS}) induced by provocative challenge with graded intravenous acetylcholine (Ach; dose expressed as mg/g body weight) as described (Kheradmand *et al.*, 2002). Bronchoalveolar lavage fluid (BALF) was collected by instilling and withdrawing 1.6 ml of sterile phosphate buffered saline (PBS) through the tracheal cannula in two aliquots of 0.8 ml. BALF total and differential cell counts were performed using a standard hemocytometer and H&E staining of cytospin slides as described (Kheradmand *et al.*, 2002). Quantitation of cytokines from BAL fluid was performed by bead-assisted analysis (MILLIPLEX MAP Kit Mouse Cytokine / Chemokine Immunoassay; Millipore, Billerica, MA, USA) using a Bioplex analyzer (BioRad, Hercules, CA) according to the manufacturers' protocols. CD4 T cells from spleen were isolated and total RNA was extracted as described for lung.

Results

Novel miRNAs from mouse T cells. We sequences short RNAs from mouse T cells, comparing naïve to T_H1 and T_H2 cells. These findings revealed the presence of numerous miRNAs, especially members of the let-7 miRNA family (LM Batts, DB Corry, manuscript in preparation). Six novel miRNA were discovered and assigned according to the novel miRNA discovery platform (FIG. 7 and Methods). FIG. 7 illustrates six novel miRNAs placed in the context of their putative pre-miRNAs.

Mmu-mir-155 is required for expression of allergic lung disease. wild type and mice deficient in mmu-mir-155 were challenged intranasally with a fungal derived allergenic proteinase (FP) to determine the requirement of this miRNA for allergic lung disease. Mir-$155^{-/-}$ mice failed to develop airway hyperreactivity as assessed by the change in respiratory system resistance in response to Ach challenge, whereas wild type mice developed robust airway hyperreactivity in comparison to PBS-challenged control animals (FIG. 2A). Furthermore, mir-$155^{-/-}$ mice manifested reduced airway eosinophilia, an important marker of allergic inflammation, and failed to recruit to the lungs IL-4-secreting cells, including T_H2 cells (FIG. 2B, C). The lack of allergic cytokine secretion was further confirmed by analysis of bronchoalveolar lavage fluid, which showed robust IL-4 secretion into the in by wild type mice, but little or no IL-4 secretion in mir-155-deficient animals (FIG. 2D).

The data above indicates that mir-155 is required for expression of asthma-like disease in mice. These findings support the idea that inhibition of mir-155 may be therapeutic in persons with asthma.

EXAMPLE 4

Changes in miRNA Expression in Lung Following Allergen Challenge

Asthma is an allergic disease that results in the obstruction of airways as a result of goblet cell hyperplasia and airway hyper-reactivity (AHR). Two novel miRNAs Asth-miR-1 and Asth-miR-2 were identified. Asth-miR-1 is predicted to target Toll-like receptor (TLR) adaptor TIRAP and IRAK which associates to activate NF-Kb, AP-1 and IRFs, and under some conditions, induce allergic lung disease. Asth-miR-2 is predicted to target ryanodine receptor 2 (RyR) that mediates Ca2+ release that induces airway smooth muscle contraction and bronchoconstriction. The integration of Asth-miR-1 and Asth-miR-1 2 with current therapies can potentially significantly enhance the efficacy and specificity of drugs used to combat asthma.

EXAMPLE 5

MicroRNA Profiles of CD4+ Helper T Cell Subsets Methods Isolated CD4+

T cells from spleens of wildtype (WT) Balb/c mice(MACS system). Total RNA was isolated and from Naïve, TH1 and TH2 cells. Naïve cells were polarized using appropriate TH1 (anti-CD3, anti-CD28, IL-2, IFN-γ, anti-IL-4, IL-12) or TH2 (anti-CD3, anti-CD28, IL-2, anti-IFN-γ, IL-4, IL-6) skewing conditions for 10 days. Small RNA transcripts were sequenced using Next Generation Sequencing Technology (Solexa). mRNA expression was determined by microarray chip (Illumina).

Results

Results are depicted in FIG. 9, FIG. 10, FIG. 11, FIG. 12, and FIG. 13.

Small RNA Transcript Sequencing Reveals Novel MiRNAs Naïve Cells:

-5'-GGGATGTAGCTCAGTGGTAG-3' (SEQ ID NO:241) = BCL2

-5'-GTTGGTGGAGCGATTTGTCTGG-3' (SEQ ID NO:242)= GATA3

TH1 Cells:

-5'-AAGCAGGGTCGGGCCTGGTTA-3' (SEQ ID NO:243) = GATA3

-5'-CTTCTGATCGAGGCCCAGCCCGT-3' (SEQ ID NO:244) = IL-6

TH1 Cells:

-5'-GGGGGTGTAGCTCAGTGGTA-3' (SEQ ID NO:245) = BIK

* * *

All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. A method for detecting an allergic or inflammatory lung disease, comprising assessing the level of one or more microRNAs (miRNAs) in a biological sample, wherein the level of the one or more miRNAs in the biological sample compared to a reference level of the one or more miRNAs is indicative of allergic or inflammatory lung disease.

2. The method of claim 1, wherein at least one of the one or more miRNAs comprises:

(i) mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, mir-712, Asth-miR-1, or Asth-miR-2;

(ii) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, or mir-467c;

(iii) a sequence that has at least 80% sequence identity to a sequence as set forth in (i);

(iv) a sequence that has at least 80% sequence identity to a sequence as set forth in (ii);

(v) the complement of a sequence as set forth in (i) or (iii); or

(vi) the complement of a sequence as set forth in (ii) or (iv);

wherein a decrease in the expression level of one or more miRNAs from group (i), (iii) or (v), or an increase in the expression level of one or more miRNAs from group (ii), (iv) or (vi) in the biological sample compared to a reference level of the one or more miRNAs is indicative of allergic or inflammatory lung disease.

3. The method of claim 1, wherein the lung disease is an allergic lung disease selected from the group consisting of asthma, hay fever, hypersensitivity pneumonitis, eosinophilic pneumonia (acute or chronic), Churg-Strauss Syndrome, allergic bronchopulmonary mycosis, and tropical eosinophilic pneumonia.

4. The method of claim 3, wherein the allergic lung disease is asthma.

5. The method of claim 1, wherein the biological sample comprises white blood cells or lung tissue.

6. The method of claim 1, further comprising obtaining a biological sample from a subject.

7. The method of claim 1, wherein more than one miRNAs is detected.

8. The method of claim 1, wherein the sequence of at least one miRNA that is detected has at least 85% sequence identity to a sequence as set forth in (i).

9. The method of claim 8, wherein the sequence of at least one miRNA that is detected has at least 90% sequence identity to a sequence as set forth in (i).

10. The method of claim 9, wherein the sequence of at least one miRNA that is detected has at least 95% sequence identity to a sequence as set forth in (i).

12. The method of claim 1, wherein the sequence of at least one miRNA is the complement of a sequence as set forth in (i) or (ii).

13. The method of claim 1, wherein at least one miRNA that is detected has a stem-loop structure.

14. The method of claim 1, further comprising detecting the presence of one or more Piwi protein interacting RNAs (piRNAs).

15. A biochip comprising an isolated nucleic acid comprising:

(i) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712;

(ii) a sequence that has at least 80% sequence identity to a sequence as set forth in (i);

(iii) the complement of a sequence as set forth in (i) or (ii); or

(iv) a nucleic acid sequence comprising at least 10 contiguous nucleic acids of Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195);

attached to said biochip.

16. The biochip of claim 15, wherein the biochip comprises a plurality of nucleic acids as set forth in one or more of (i), (ii), (iii), and (iv).

17. A method of inhibiting a target gene in a cell, comprising contacting the cell with a nucleic acid in an amount sufficient to inhibit expression of the target gene, wherein the nucleic acid comprises:

(i) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712;

(ii) a sequence that has at least 80% sequence identity to a sequence as set forth in (i);

(iii) the complement of a sequence as set forth in (i) or (ii); or

(iv) a nucleic acid sequence comprising at least 10 contiguous nucleic acids of Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195).

18. The method of claim 17, wherein the target gene is an interleukin or a cytokine.

19. The method of claim 17, wherein the target gene is selected from the group consisting of GATA3, STAT6, IL13RA1, GATA3, CD4, ADRB2, JAK1, IL4, JAK1, IRAK1, STAT6, and IL13.

20. The method of claim 17, wherein the cell is in a subject.

21. The method of claim 20, wherein the subject is a mammal.

22. The method of claim 21, wherein the mammal is a human.

23. The method of claim 22, wherein the human has an allergic lung disease or is suspected of having an allergic lung disease.

24. The method of claim 23, wherein the allergic lung disease is asthma.

25. The method of claim 17, wherein the cell is a lung cell.

26. A method of treating or preventing exacerbation of an allergic lung disease in a subject, comprising administering to said subject a pharmaceutically effective amount of a composition comprising a nucleic acid comprising:

(i) mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, mir-712, Asth-miR-1, or Asth-miR-2; or

(ii) a nucleic acid which selectively binds or inhibits one or more of: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, or mir-467c.

27. The method of claim 26, wherein the nucleic acid is a group (ii) nucleic acid, and wherein the nucleic acid is chemically modified or comprises a nucleotide analog.

28. The method of claim 27, wherein the nucleic acid is selected from the group consisting of (5'-AACTATACAACCTACTACCTCA-3' (SEQ ID NO:246)), (5'-AACTATACAACCTCCTACCTCA-3' (SEQ ID NO:247)), and (5'-CAACCTACTACCTC-3' (SEQ ID NO:248)).

29. The method of claim 28, wherein the nucleic acid is a LNA.

30. The method of claim 26, wherein the subject is a mammal.

31. The method of claim 30, wherein the mammal is a human.

32. The method of claim 30, wherein the allergic lung disease is asthma, hay fever, or hypersensitivity pneumonitis.

33. The method of claim 32, wherein the allergic lung disease is asthma.

34. The method of claim 26, wherein said nucleic acid comprises a phosphoramidate linkage, a phosphorothioate linkage, a phosphorodithioate linkage, or an O-methylphosphoroamidite linkage.

35. The method of claim 26, wherein said nucleic acid comprises one or more nucleotide analogs.

36. The method of claim 26, further comprising administering to the subject one or more secondary forms of therapy for the treatment or prevention of allergic lung disease.

37. The method of claim 36, wherein the secondary form of therapy is selected from the group consisting of a corticosteroid, a beta-2 adrenergic receptor agonist, a leukotrine modifier, an anti-immunoglobulin E (IgE) antibody, or a mast cell stabilizing agent.

38. The method of claim 26, wherein said nucleic acid is comprised in a vector.

39. The method of claim 38, wherein said vector is a viral vector.

40. The method of claim 39, wherein said viral vector is an adenovirus, an adeno-associated virus, a lentivirus, or a herpes virus.

41. The method of claim 26, wherein said vector comprises a lipid.

42. The method of claim 41, wherein said lipid is comprised in a liposome.

43. The method of claim 26, wherein the pharmaceutically effective amount of said composition is administered via an aerosol, topically, locally, intravenously, intraarterially, intramuscularly, by lavage, or by injection into the thoracic cavity.

44. A kit comprising a biochip as set forth in any of claims 15 or 16 and one or more sealed containers.

45. The kit of claim 44, further comprising instructions for use of said biochip.

46. A kit comprising a sealed container comprising a nucleic acid, wherein said nucleic acid comprises:

(i) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712;

(ii) a sequence that has at least 80% sequence identity to a sequence as set forth in (i);

(iii) the complement of a sequence as set forth in (i) or (ii); or

(iv) a nucleic acid sequence comprising at least 10 contiguous nucleic acids of Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195).

47. The kit of claim 46, further comprising a set of primers specific for transcription or reverse transcription of one or more nucleic acid sequences as set forth in (i), (ii), (iii), or (iv).

48. The kit of claim 46, further comprising a biochip.

49. The kit of claim 38, further comprising instructions for use.

50. A kit comprising a sealed container comprising a set of primers specific for transcription or reverse transcription of a nucleic acid sequence, wherein said nucleic acid sequence comprises:

(i) mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712;

(ii) a sequence that has at least 80% sequence identity to a sequence as set forth in (i);

(iii) the complement of a sequence as set forth in (i) or (ii); or

(iv) a nucleic acid sequence comprising at least 10 contiguous nucleic acids of Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195).

51. A method of treating an allergic or inflammatory lung disease in a subject comprising administering to the subject a let-7 miRNA inhibitor.

52. The method of claim 51, wherein the let-7 miRNA inhibitor is selected from the group consisting of siRNA, an antisense oligonucleotide, a locked nucleic acid (LNA), an antisense RNA, and a plasmid expressing an antisense RNA.

53. The method of claim 52, wherein the let-7 miRNA inhibitor is an LNA.

54. The method of claim 53, wherein the LNA comprises:

(i) (5'-AACTATACAACCTACTACCTCA-3' (SEQ ID NO:246)), (5'-AACTATACAACCTCCTACCTCA-3' (SEQ ID NO:247)), or (5'-CAACCTACTACCTC-3' (SEQ ID NO:248));

(ii) a sequence having at least 80% sequence identity to a sequence as set forth in (i); or

(iii) the complement of a sequence as set forth in (i) or (ii).

55. The method of claim 52, wherein the let-7 miRNA inhibitor is administered in a pharmaceutically acceptable composition.

56. The method of claim 55, wherein the let-7 miRNA inhibitor is administered orally, intravenously, via an aerosol, topically, locally, intravenously, intraarterially, intramuscularly, by lavage, or by injection into the thoracic cavity.

57. The method of claim 56, wherein the subject is a mouse, a rat, a rodent, a cat, a horse, a goat, a sheep, a cow, a rabbit, a primate, or a human.

58. An isolated nucleic acid comprising:

(i) (5'-AACTATACAACCTACTACCTCA-3', SEQ ID NO:246), (5'-AACTATACAACCTCCTACCTCA-3' SEQ ID NO:247), or (5'-CAACCTACTACCTC-3' SEQ ID NO:248);

(ii) a sequence having at least 80% sequence identity to (5'-AACTATACAACCTACTACCTCA-3' SEQ ID NO:246), (5'-AACTATACAACCTCCTACCTCA-3' SEQ ID NO:247), or (5'-CAACCTACTACCTC-3' SEQ ID NO:248); or

or (iii) the complement of a sequence as set forth in (i) or (ii);

wherein the isolated nucleic acid can selectively bind a let-7 miRNA.

59. The method of claim 58, wherein said nucleic acid comprises a phosphoramidate linkage, a phosphorothioate linkage, a phosphorodithioate linkage, or an O-methylphosphoroamidite linkage.

60. The method of claim 59, wherein said nucleic acid comprises one or more nucleotide analogs.

61. The isolated nucleic acid of claim 58, wherein the nucleic acid comprises a chemical modification .

62. The isolated nucleic acid of claim 58, wherein the nucleic acid is a locked nucleic acid (LNA).

63. The isolated nucleic acid of claim 58, wherein the nucleic acid is comprised in a pharmaceutically acceptable composition.

64. An isolated nucleic acid selected from the group consisting of SEQ ID NO:285-322, or a complement thereof.

65. The isolated nucleic acid of claim 64, wherein the nucleic acid is present on a biochip or a microarray.

66. A method of screening for a modulator of an allergic or inflammatory lung response comprising:

(a) contacting a lung cell with a candidate substance; and

(b) measuring the expression level of one or more microRNAs (miRNAs) in the lung cell; wherein at least one of the one or more miRNAs comprises: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-

715, or mir-712, Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195);

wherein an increase in the expression level of one or more of: mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, mir-712, Asth-miR-1, or Asth-miR-2 in the lung cell indicates that the modulator can inhibit an allergic or inflammatory lung response; and

wherein a decrease in the expression level of one or more of: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c in the lung cell indicates that the modulator can inhibit an allergic or inflammatory lung response.

67. A method of identifying a subject to receive an inhibitor of an allergic or inflammatory lung response comprising:

measuring the expression level of one or more microRNAs (miRNAs) in a lung cell from the subject; wherein at least one of the one or more miRNAs comprises: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c, mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, or mir-712, Asth-miR-1 (SEQ ID NO:187), Asth-miR 2 (SEQ ID NO:189), or Asth-miR-5 (SEQ ID NO:195);

wherein an increase in the expression level of one or more of: mir-681, mir-880, mir-1190, mir-709, mir-671-3p, mir-1196, mir-667, mir-452, mir-483*, mir-331-3p, mir-743a, mir-485, mir-30c-1*, mir-770-5p, mir-483, mir-193, mir-296-5p, mir-715, mir-712, Asth-miR-1, or Asth-miR-2 in the lung cell indicates that the subject may therapeutically benefit from said inhibitor; and

wherein a decrease in the expression level of one or more of: mir-147, mir-135a, mir-135b, mir-683, mir-130b, mir-1, mir-615-5p, mir-142-3p, mir-689, mir-130b, mir-155, mir-146b, mir-18b, mir-340-5p, mir-501-5p, mir-1191, mir-421, mir-146b*, mir-717, mir-467c in the lung cell indicates that the subject may therapeutically benefit from said inhibitor.

68. The method of claim 67, wherein the subject is a human.

69. The method of claim 67, wherein the method further comprises a method of personalizing a therapy for an allergic or inflammatory lung disease.

70. The method of claim 67, wherein said measuring is performed in a plurality of subjects.

71. The method of claim 70, wherein the method further comprises a method of identifying a sub-population of patients to receive said inhibitor.

72. A transgenic mouse comprising a mutation in a let-7 miRNA, wherein the mutation prevents the expression of the let-7 miRNA, and wherein the mouse exhibits a reduced susceptibility to an allergic lung response.

73. The transgenic mouse of claim 72, wherein the let-7 miRNA is mir-155 (mouse miRNA-155).

74. A progeny mouse of the mouse of claim 72, wherein the progeny mouse comprises a mutation in a let-7 miRNA, wherein the mutation prevents the expression of the let-7 miRNA, and wherein the progeny mouse exhibits a reduced susceptibility to an allergic lung response.

FIG. 1A

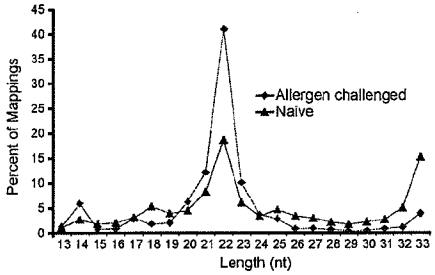


FIG. 1B

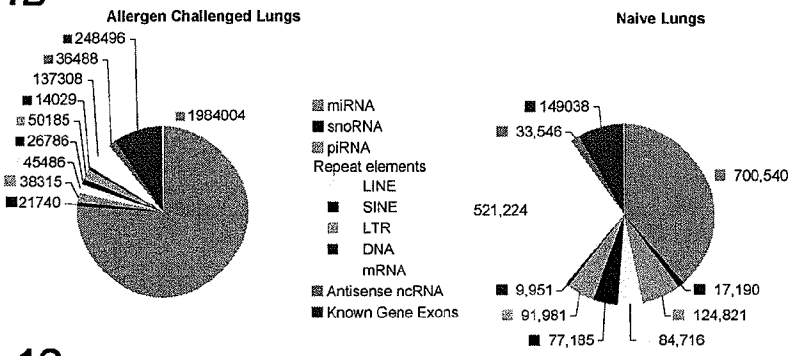


FIG. 1C

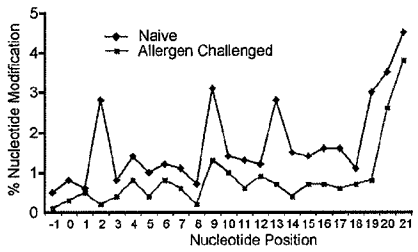


FIG. 1D

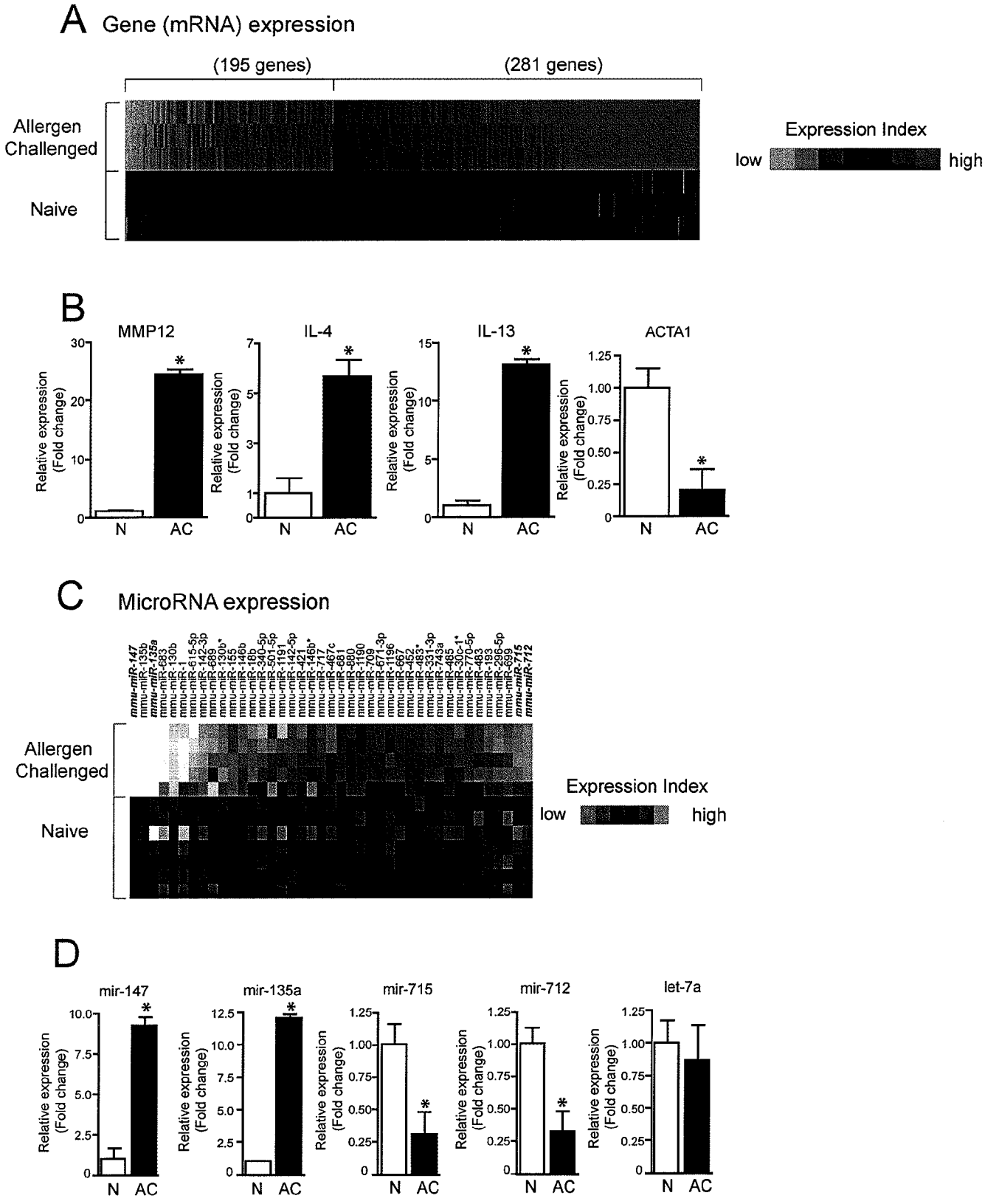
let-7a pre-miRNA sequence: TTCACTGTGGGATGAGGTAGTAGGTTGTATAGTTTTAGGGTCACACCCACCACTGGGAGATAACTATACAATCTACTGTCTTTCCTAAGGTGAT

let-7a mature sequence: **********TGAGGTAGTAGGTTGTATAGTT**********

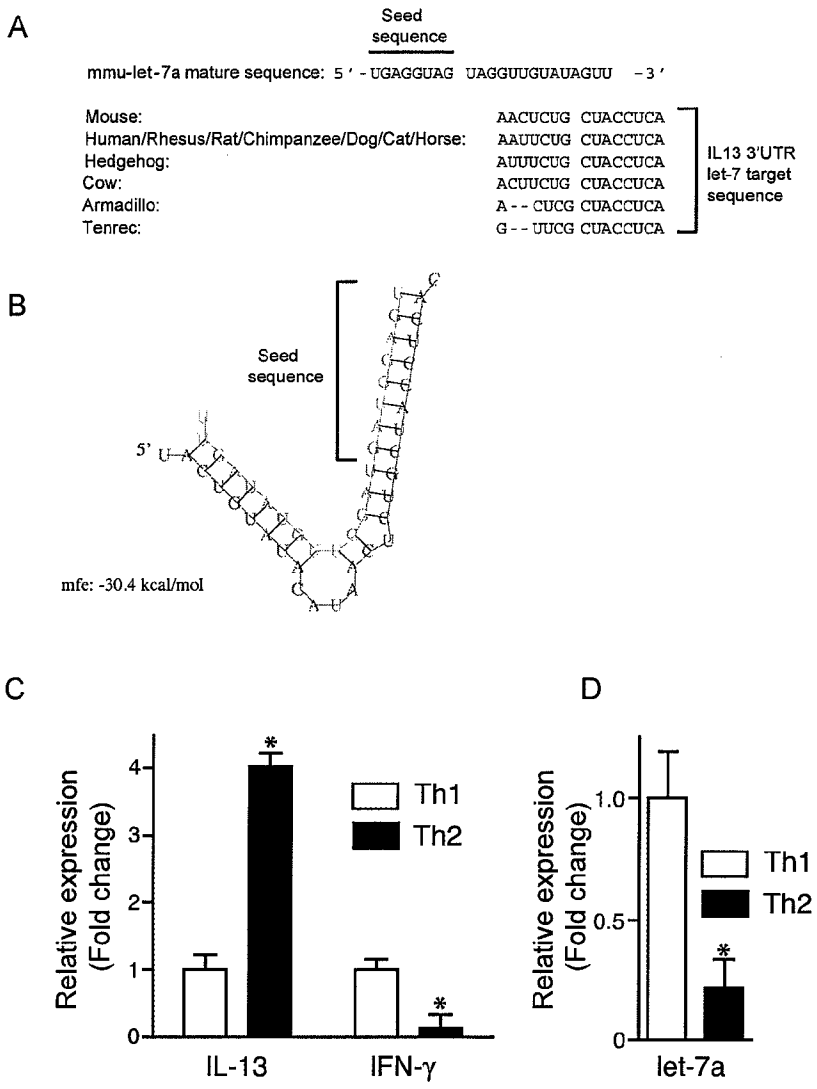
```
Allergen challenged                              # Sequenced transcripts
...........TGAGGTAGTAGGTTGTATAGTT.....................* 65924
...........TGAGGTAGgAGGTTGTATAGTTT..................... 3450
...........TGAGGTAGgAGGTTGTATAGTTTT.................... 284
...........TGAGGTAGgAGGTTGTATAGTTaT.................... 241
...........TGAGGTAGgAGGTTGTATAGTTTTt................... 32
...........TGAGGTAGgAGGTTGTATAGTTTaA................... 32
...........GAGGTAGgAGGTTGTATAGTTT...................... 23
...........TGAGGTAGgAGGTTGTATAGTTaTT................... 14
..........AGGTAGgAGGTTGTATAGTTT........................ 8
...........TGAGGTAGgAGGTTGTATAGTTTTtt.................. 8
...........TGAGGTAGgAGGTTGTATAGTaaT.................... 7
...........TGAGGTAGgAGGgTGTATAGTTT...................... 7

Naive
...........TGAGGTAGTAGGTTGTATAGTT.....................* 10617
...........TGAGGTAGgAGGTTGTATAGTTT..................... 510
...........TGAGGTAGgAGGTTGTANAGTTT..................... 199
...........TGAGGTAGgAGGTTGTATAGTTTT.................... 46
...........TGAGGTAGgAGGTTGTANAGTTaT.................... 17
...........TGAGGTAGgAGGTTGTANAGTTTT.................... 15
..........GAGGTAGgAGGTTGTATAGTTT....................... 9
```

FIGS. 1A-D

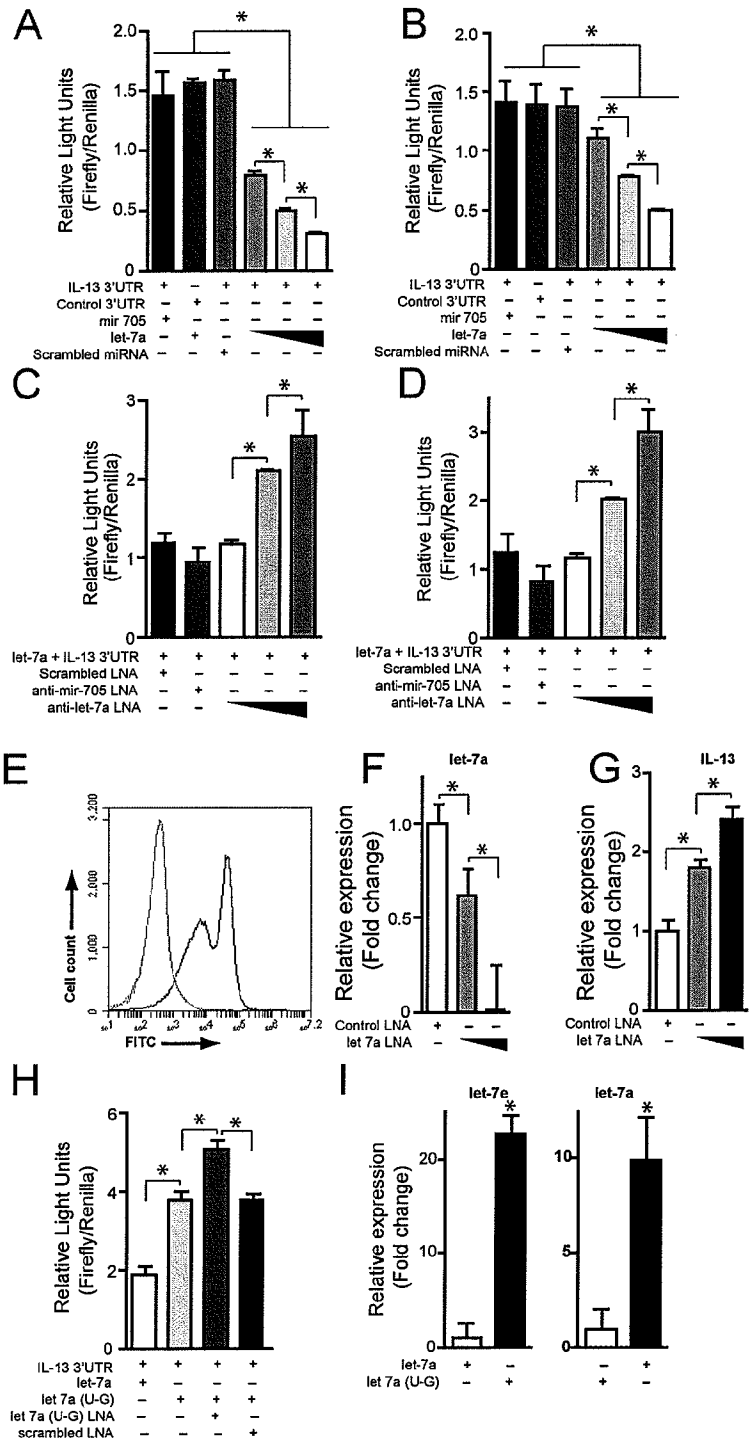
FIGS. 2A-D


A
Gene (mRNA) expression
(195 genes)
(281 genes)
Allergen Challenged
Naive
Expression Index
low
high
B
MMP12
IL-4
IL-13
ACTA1
Relative expression (Fold change)
N
AC
C
MicroRNA expression
Allergen Challenged
Naive
Expression Index
low
high
D
mir-147
mir-135a
mir-715
mir-712
let-7a
Relative expression (Fold change)
N
AC

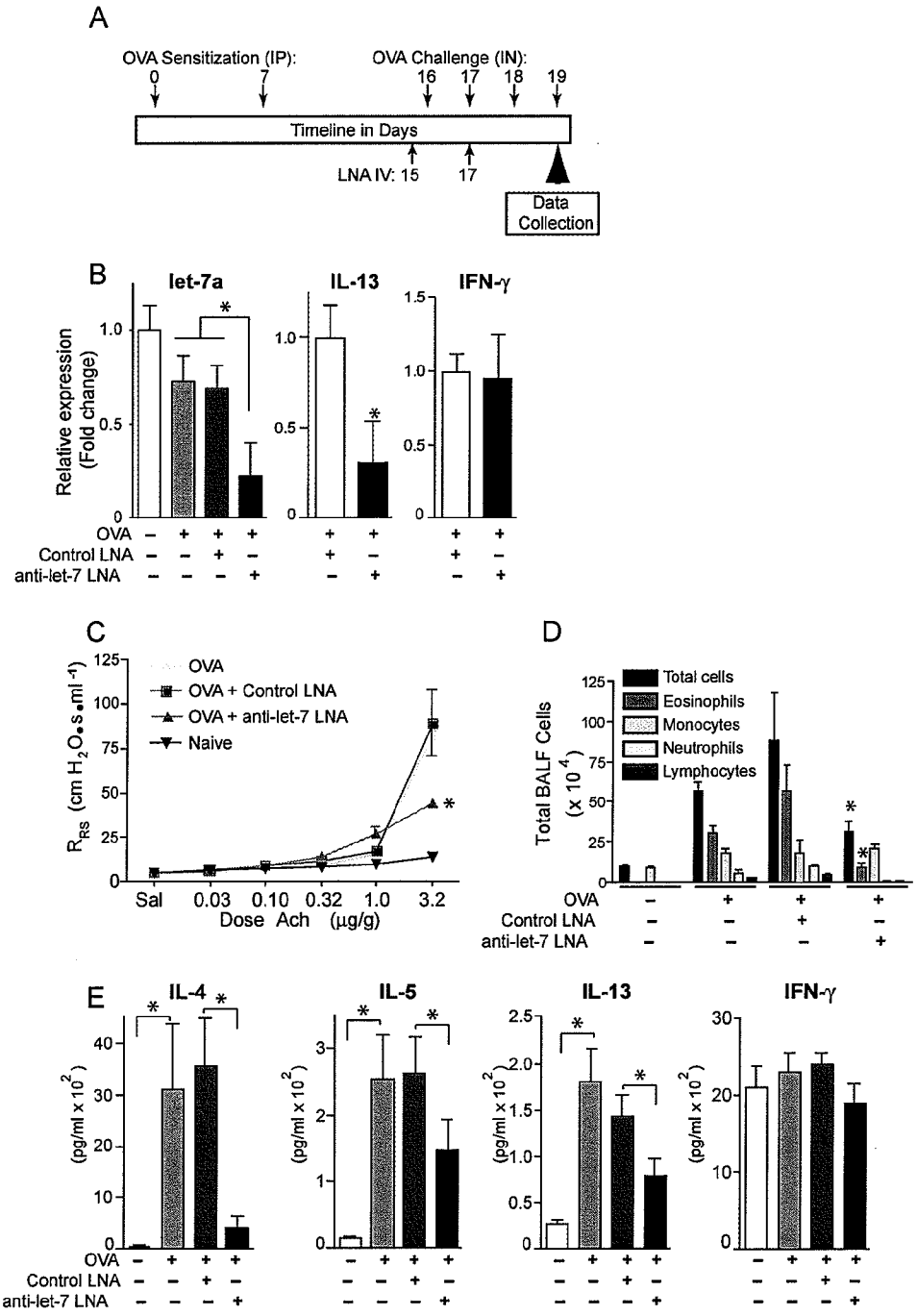
FIGS. 3A-D


A
Seed sequence
mmu-let-7a mature sequence: 5'-UGAGGUAG UAGGUUGUAUAGUU -3'
Mouse: AACUCUG CUACCUCA
Human/Rhesus/Rat/Chimpanzee/Dog/Cat/Horse: AAUUCUG CUACCUCA
Hedgehog: AUUUCUG CUACCUCA
Cow: ACUUCUG CUACCUCA
Armadillo: A--CUCG CUACCUCA
Tenrec: G--UUCG CUACCUCA
IL13 3'UTR let-7 target sequence
B
Seed sequence
5'
mfe: -30.4 kcal/mol
C
Relative expression (Fold change)
Th1
Th2
IL-13
IFN-γ
D
Relative expression (Fold change)
Th1
Th2
let-7a

FIGS. 4A-I


A
Relative Light Units (Firefly/Renilla)
IL-13 3'UTR
Control 3'UTR
mir 705
let-7a
Scrambled miRNA
B
Relative Light Units (Firefly/Renilla)
IL-13 3'UTR
Control 3'UTR
mir 705
let-7a
Scrambled miRNA
C
Relative Light Units (Firefly/Renilla)
let-7a + IL-13 3'UTR
Scrambled LNA
anti-mir-705 LNA
anti-let-7a LNA
D
Relative Light Units (Firefly/Renilla)
let-7a + IL-13 3'UTR
Scrambled LNA
anti-mir-705 LNA
anti-let-7a LNA
E
Cell count
FITC
F
let-7a
Relative expression (Fold change)
Control LNA
let 7a LNA
G
IL-13
Relative expression (Fold change)
Control LNA
let 7a LNA
H
Relative Light Units (Firefly/Renilla)
IL-13 3'UTR
let-7a
let 7a (U-G)
let 7a (U-G) LNA
scrambled LNA
I
let-7e
let-7a
Relative expression (Fold change)
let-7a
let 7a (U-G)

FIGS. 5A-E


A
OVA Sensitization (IP):
OVA Challenge (IN):
0
7
16
17
18
19
Timeline in Days
LNA IV: 15
17
Data Collection
B
let-7a
IL-13
IFN-γ
Relative expression (Fold change)
OVA
Control LNA
anti-let-7 LNA
C
OVA
OVA + Control LNA
OVA + anti-let-7 LNA
Naive
Sal
0.03
0.10
0.32
1.0
3.2
Dose Ach (μg/g)
D
Total cells
Eosinophils
Monocytes
Neutrophils
Lymphocytes
Total BALF Cells
E
IL-4
IL-5
IL-13
IFN-γ
(pg/ml x 10²)

FIGS. 6A-F

A

4317-20-67-3:R:mm9;-27.800-Asth-miR-1 (Naïve)

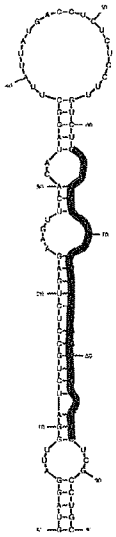
Canonical sequence: GCATTGGTGGTTCAGTGGTAGAATTC	Copy No.
GCATTGGTGGTTCAGTGGTAGAATTC	285
GCATTGGTGGTTCAGTGGTAGAA	145
GCATTGGTGGTTCAGTGGTAGA	116
GCATTGGTGGTTCAGTGGTA	67*
GCATTGGTGGTTCA	60
GCATTGGTGGTTCAGTGGTAGAAT	55
GCATTGGTGGTTCAG	54
GCATTGGTGGTTCAGTGGTAGAATT	44
GCATTGGTGGTTCAGTGG	38
GCATTGGTGGTTCAGTGGTAG	31
GCATTGGTGGTTCAGT	29
GCATTGGTGGTTCAGTGGTAGAATTCT	13
GCATTGGTGGTTCAGTG	9
TGGTAGAATTCTCGCCTGC	8
GCATTGGTGGTTCAGTGGT	7
TGGTTCAGTGGTAGAA	7

B

14511-20-513-3:R:mm9;-27.800– Asth-miR-1 (AC)

Canonical sequence: GCATTGGTGGTTCAGTGGTAGAATTC	Copy No.
GCATTGGTGGTTCAGT	876
GCATTGGTGGTTCAGTGGTA	513*
GCATTGGTGGTTCA	491
GCATTGGTGGTTCAGTG	378
GCATTGGTGGTTCAG	306
GCATTGGTGGTTCAGTGGTAGAATTC	255
GCATTGGTGGTTCAGTGNTA	174
GCATTGGTGGTTCAGTGGTAGA	115
GCATTGGTGGTTCAGTGG	110
GCATTGGTGGTTCAGTGNTAGAATTC	91
GCATTGGTGGTTCAGTGGTAGAA	65
GCATTGGTGGTTCAGTGGT	63
GCATTGGTGGTTCAGTGNTAGA	41
GCATTGGTGGTTCAGTGN	38
GCATTGGTGGTTCAGTGGTAGAAT	35
GCATTGGTGGTTCAGTGNT	28
GCATTGGTGGTTCAGTGNTAGAA	25
GCATTGGTGGTTCAGTGGTAGAATTCT	16
GCATTGGTGGTTCAGTGGTAG	14
GCATTGGTGGTTCAGTGGTAGAATT	13
GCATTGGTGGTTCAGTGNTAGAAT	11
GCATTGGTGGTTCAGTGNTAG	10
GCATTGGTGGTTCAGTGNTAGAATT	9
ATTGGTGGTTCAGTGGTA	7
GCATTGGTGGTTCAGTGNTAGAATTCT	7
TTGGTGGTTCAGTGGTA	6
ATTGGTGGTTCA	5
ATTGGTGGTTCAGT	5

C



D

22996-23-6-1:R:mm9;-55.300– Asth-miR-2 (naïve)

Canonical sequence: TGGATATGATGACTGATTACCTGAGA	Copy No.
.....TGGATATGATGACTGA	25
.....TGGATATGATGACTGATTACCTGA	19
.....TGGATATGATGACTG	18
.....TGGATATGATGACTGATTACCTGAGA	11
..... GGATATGATGACTGATTACCTGAGAAA	8
.....TGGATATGATGACTGAT	8
.....TGGATATGATGACTGATNACCTGA	8
.....GGATATGATGACTGATTACCTGAGA	7
..... TGGATATGATGACTGATTAC	7
..... TGGATATGATGACTGATTACCTGAGAA	7
..... GGATATGATGACTGATTACCTGAGAA	6
..... TGGATATGATGACTGATTACCTG	6
..... TGGATATGATGACTGATNACCTGAGA	5
..... TGGATATGATGACTGATNACCTGAGAA	5
..... TGGATATGATGACTGATTACCT	5

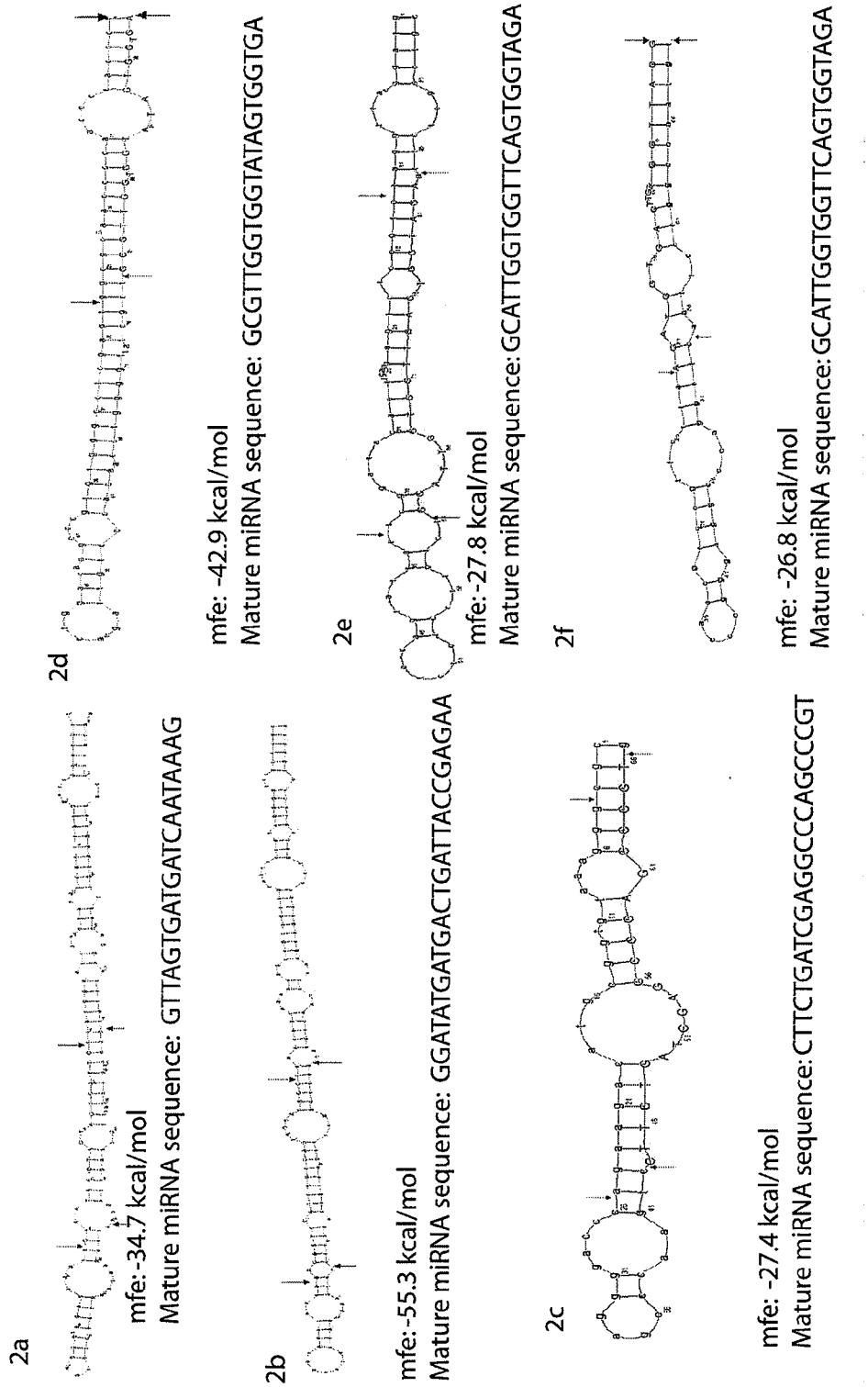
E

3217-26-14-1:R:mm9;-55.300– Asth-miR-2 (AC)

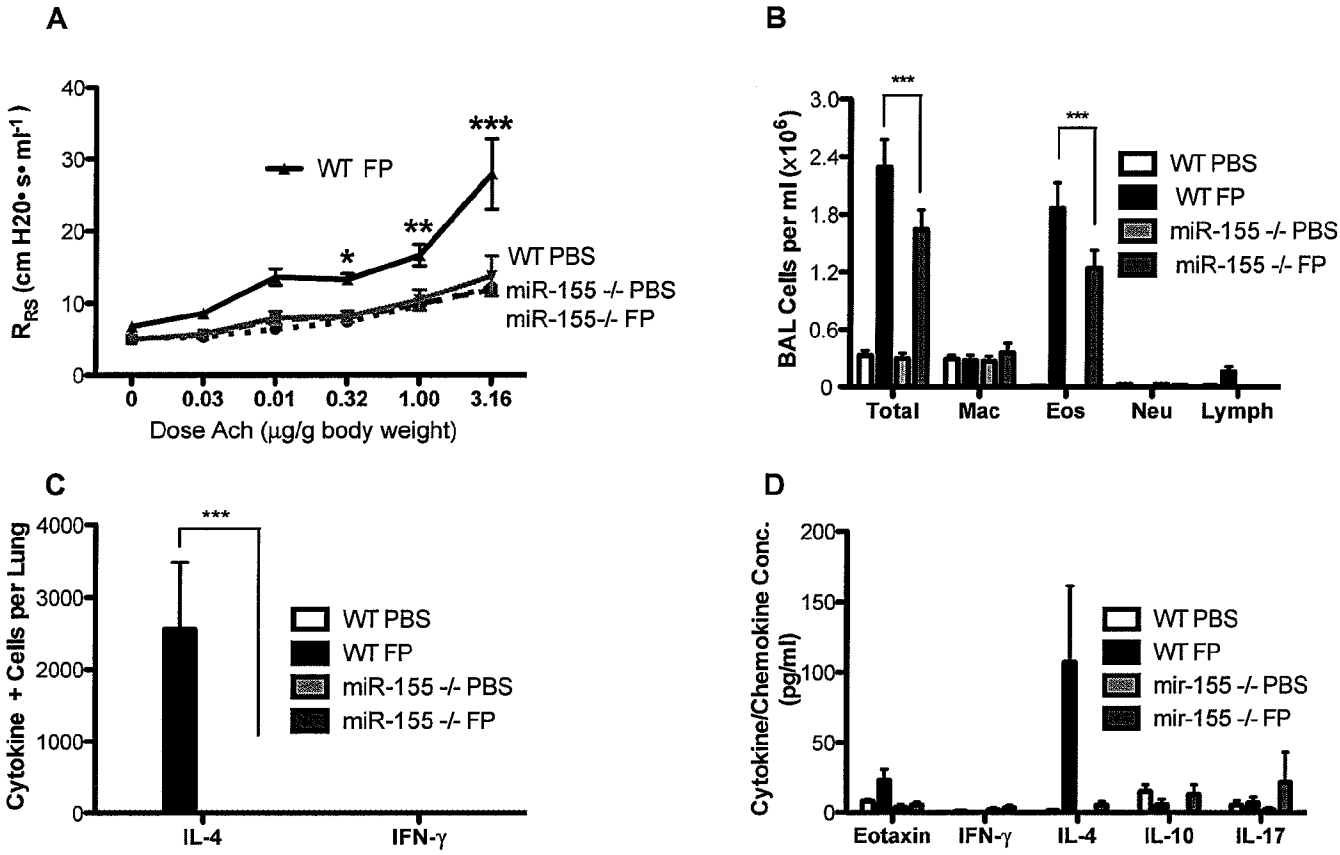
Canonical sequence: TGGATATGATGACTGATTACCTGAGA	Copy No.
..... TGGATATGATGACTG	16
.....TGGATATGATGACTGATTACCTGAGA	14*
.....TGGATATGATGACT	13
.....TGGATATGATGACTGA	8

F

FIG. 7


2a
mfe: -34.7 kcal/mol
Mature miRNA sequence: GTTAGTGATGATCAATAAAG
2b
mfe: -55.3 kcal/mol
Mature miRNA sequence: GGATATGATGACTGATTACCGAGAA
2c
mfe: -27.4 kcal/mol
Mature miRNA sequence: CTTCTGATCGAGGCCCAGCCCGT
2d
mfe: -42.9 kcal/mol
Mature miRNA sequence: GCGTTGGTGGTATAGTGGTGA
2e
mfe: -27.8 kcal/mol
Mature miRNA sequence: GCATTGGTGGTTCAGTGGTAGA
2f
mfe: -26.8 kcal/mol
Mature miRNA sequence: GCATTGGTGGTTCAGTGGTAGA

FIGS. 8A-D


A
B
C
D
WT FP
WT PBS
miR-155 -/- PBS
miR-155-/- FP
Dose Ach (µg/g body weight)
BAL Cells per ml (x10^6)
Total
Mac
Eos
Neu
Lymph
Cytokine + Cells per Lung
IL-4
IFN-γ
Cytokine/Chemokine Conc. (pg/ml)
mir-155 -/- PBS
mir-155 -/- FP
Eotaxin
IL-10
IL-17

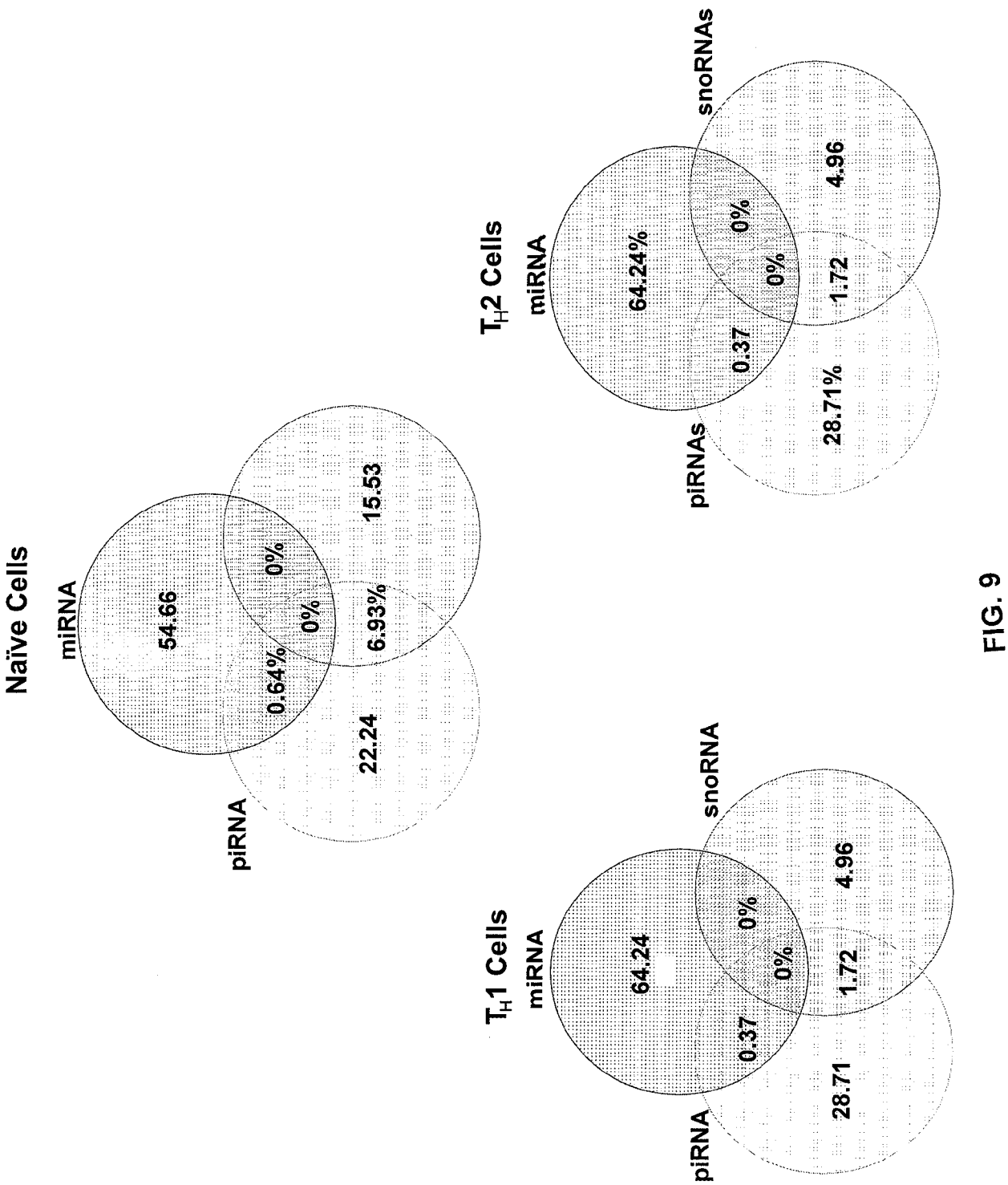

Naïve Cells
miRNA
54.66
0%
0%
0.64%
15.53
6.93%
22.24
piRNA
T_H1 Cells
miRNA
64.24
snoRNA
0%
0%
0.37
4.96
1.72
28.71
piRNA
T_H2 Cells
miRNA
64.24%
snoRNAs
0%
0%
0.37
4.96
1.72
28.71%
piRNAs

FIG. 9

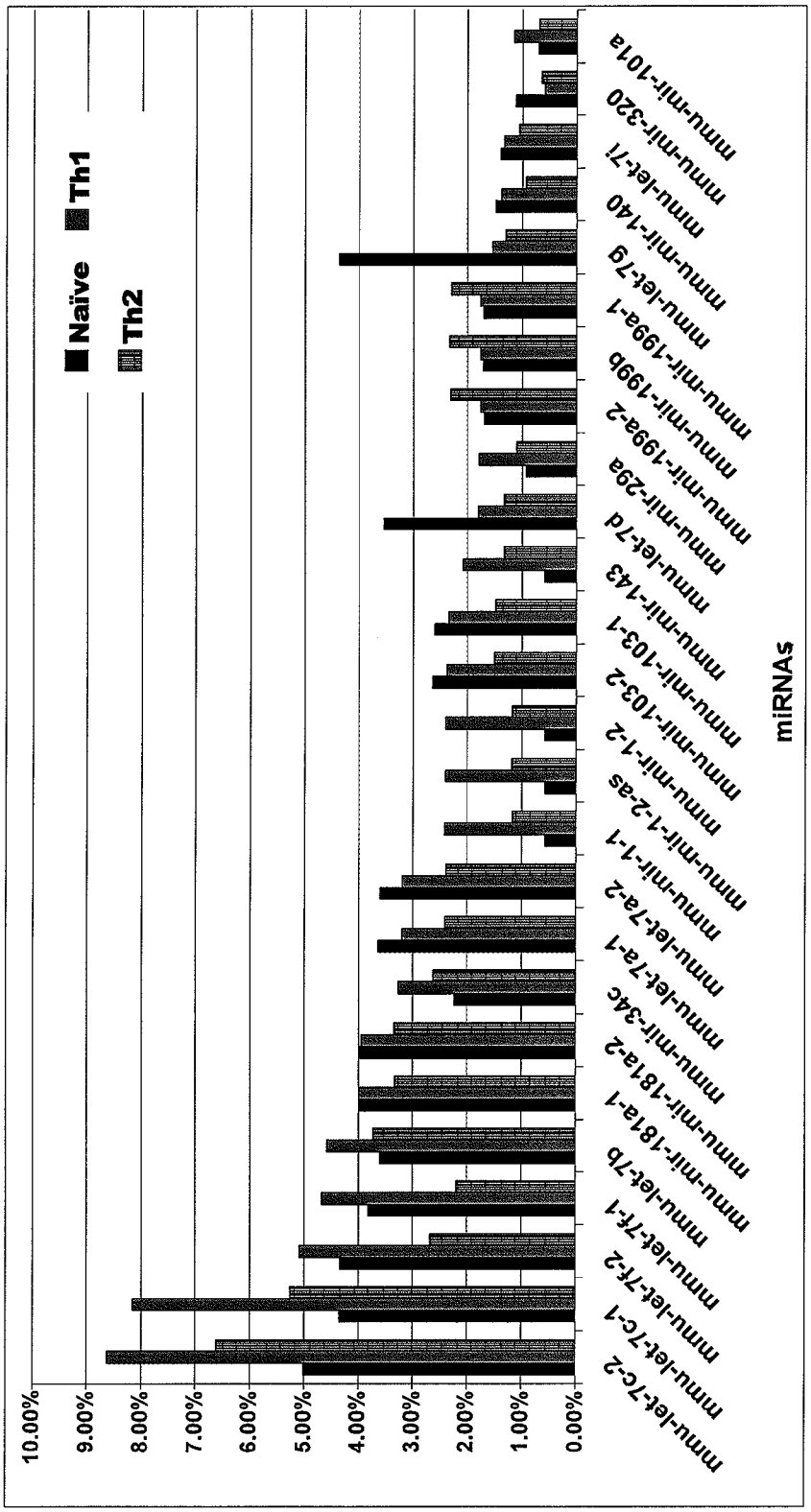

Naïve
Th1
Th2
10.00%
9.00%
8.00%
7.00%
6.00%
5.00%
4.00%
3.00%
2.00%
1.00%
0.00%
mmu-let-7c-2
mmu-let-7c-1
mmu-let-7f-2
mmu-let-7f-1
mmu-let-7b
mmu-mir-181a-1
mmu-mir-181a-2
mmu-mir-34c
mmu-let-7a-1
mmu-let-7a-2
mmu-mir-1-1
mmu-mir-1-2-as
mmu-mir-1-2
mmu-mir-103-2
mmu-mir-103-1
mmu-mir-143
mmu-let-7d
mmu-mir-29a
mmu-mir-199a-2
mmu-mir-199b
mmu-mir-199a-1
mmu-let-7g
mmu-mir-140
mmu-let-7i
mmu-mir-320
mmu-mir-101a
miRNAs

FIG. 10A

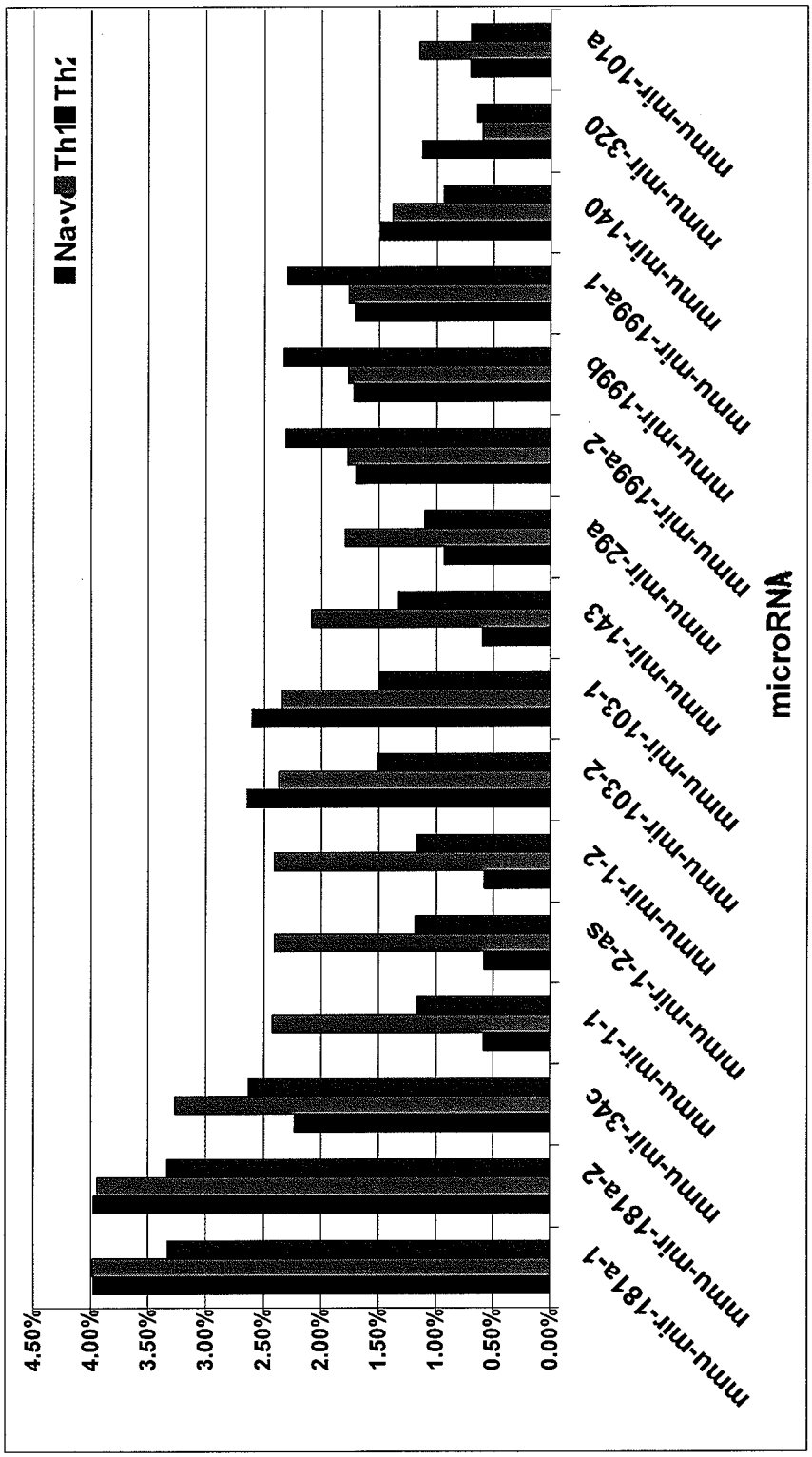

Naïve Th1 Th2
4.50%
4.00%
3.50%
3.00%
2.50%
2.00%
1.50%
1.00%
0.50%
0.00%
mmu-mir-181a-1
mmu-mir-181a-2
mmu-mir-34c
mmu-mir-1-1
mmu-mir-1-2as
mmu-mir-1-2
mmu-mir-103-2
mmu-mir-103-1
mmu-mir-143
mmu-mir-29a
mmu-mir-199a-2
mmu-mir-199b
mmu-mir-199a-1
mmu-mir-140
mmu-mir-320
mmu-mir-101a
microRNA

FIG. 10B

Naïve Cells:

- 5'-G**GGATGTA**GCTCAGTGGTAG-3' ⇒ BCL2
- 5'-G**TTGGTGG**AGCGATTTGTCTGG-3' ⇒ GATA3

T_H1 Cells:

- 5'-A**AGCAGG**GTCGGGCCTGGTTA-3' ⇒ GATA3
- 5'-C**TTCTGAT**CGAGGCCCAGCCCGT-3' ⇒ IL-6

T_H2 Cells:

- 5'-G**GGGGTGT**AGCTCAGTGGTA-3' ⇒ BIK
- 5'-**GGGGATGTAGCTCAGTGGTA**-3' ⇒ BCL6

FIG. 11

TH1 Specific (miRNA up:mRNA down)	
Sequenced miRNATranscripts	**Gene Target**
mmu-miR-143	TSC22 domain family 3 **(Gilz)**
mmu-miR-15b	
mmu-miR-16	
TH2 Specific (miRNA down:mRNA up)	
Sequenced miRNA Transcripts	**Gene Target**
mmu-miR-145	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog **(c-maf)**

FIG. 12

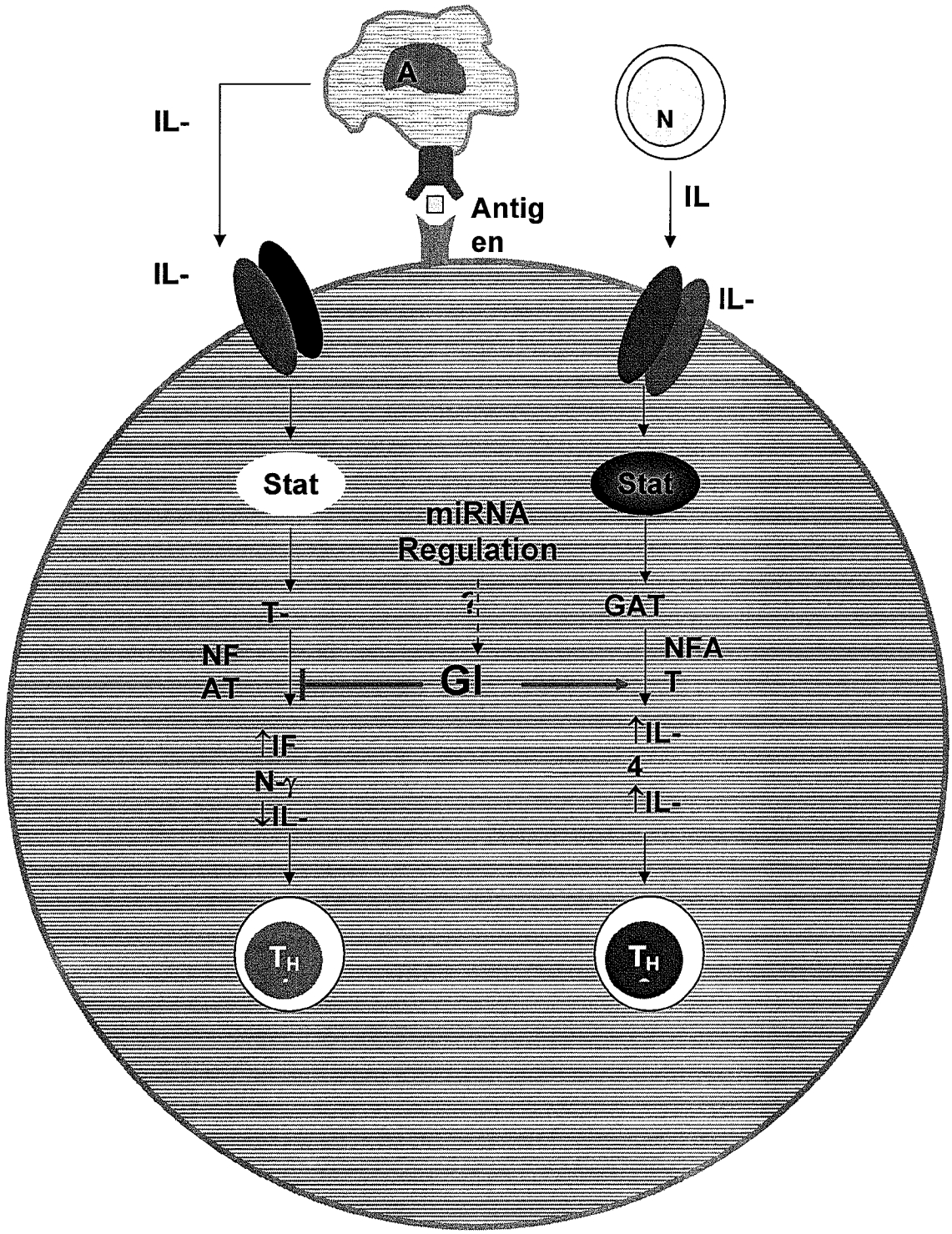

A
N
IL-
IL-
Antig
en
IL
IL-
Stat
Stat
miRNA
Regulation
?
T-
GAT
NF
AT
GI
NFA
T
IF
N-γ
IL-
IL-
4
IL-
TH
TH

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/034119

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 C12N15/113
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q C12N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/147974 A1 (UNIV SOUTH FLORIDA [US]; MOHAPATRA SHYAM S [US]; WANG JIA-WANG [US]) 4 December 2008 (2008-12-04) claims 1-2	1-63, 66-74
X	WO 2007/095614 A2 (UNIV LOUISVILLE RES FOUND [US]; WANG EUGENIA [US]) 23 August 2007 (2007-08-23) sequence 1991	15,16, 46-49
A	US 2006/019286 A1 (HORVITZ H R [US] ET AL HORVITZ H ROBERT [US] ET AL) 26 January 2006 (2006-01-26) the whole document	1-50, 66-71

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2010	11/10/2010

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/034119

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/142567 A2 (KAROLINSKA INST INNOVATIONS AB [SE]; PIVARCSI ANDOR [SE]; SONKOLY ENIK) 27 November 2008 (2008-11-27) the whole document	1-50, 66-71
X	WO 2009/012468 A2 (UNIV COLORADO [US]; PORT JONATHAN DAVID [US]; SUCHAROV CARMEN [US]; BR) 22 January 2009 (2009-01-22) sequence 50	58
X	WO 2007/143097 A1 (THIRD WAVE TECH INC [US]; ALLAWI HATIM T [US]; LYAMICHEV VICTOR [US]) 13 December 2007 (2007-12-13) sequence 4	58
X	THAI TO-HA ET AL: "Regulation of the germinal center response by microRNA-155" SCIENCE (WASHINGTON D C), vol. 316, no. 5824, April 2007 (2007-04), pages 604-608, XP002601769 ISSN: 0036-8075 the whole document	72-74
A	WO 2006/137941 A2 (AMBION INC [US]; BROWN DAVID [US]; FORD LANCE [US]; CHENG ANGIE; JARVI) 28 December 2006 (2006-12-28) the whole document	1-63, 66-74
A	WO 2008/073922 A2 (ASURAGEN INC [US]; JOHNSON CHARLES D [US]; BYROM MIKE [US]; BADER ANDR) 19 June 2008 (2008-06-19) the whole document	1-63, 66-74

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/034119

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-63, 66-74(all partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-50, 66-71(all partially)

methods to detect an allergic/inflammatory lung disease, methods to inhibit a target gene in a cell, methods of treating an allergic lung disease, methods to screen for a modulator of an allergic or inflammatory lung response, methods of identifying a subject to receive an inhibitor of an allergic or inflammatory lung response, and products, all based on miRNA mir-681, also indicated as SEQ ID NO: 264

2-50. claims: 1-74(partially)

same as above for all remaining miRNA's mentioned in claims 1-74

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2010/034119

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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