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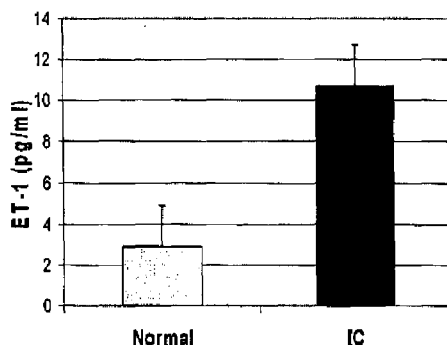
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(54) Title: METHOD OF DIAGNOSING AND TREATING INTERSTITIAL CYSTITIS

FIG. 1



(57) Abstract: A method for diagnosing, or differentially diagnosing, interstitial cystitis (IC) involves detecting or measuring increased expression of a biomarker Endothelin 1 (ET-I) in a biological sample from a mammalian subject, particularly in the urine or urothelial tissue. An increased level of expression of ET-I above the level of expression in the same sample of a healthy mammalian subject is an indication of a diagnosis of IC. Such diagnosis may further involve identify other clinical symptoms of IC. Additionally the method may use additional biomarkers, such as Hb-EFG, EGF, APF, IL-8, IL-6, and cGMP. Assay methods and diagnostic reagents and kits for such diagnosis are provided. Methods and compositions for treating IC by reducing the action, production or synthesis of ET-I in the urine or urothelium and/or inhibiting its binding to its ETA and/or ETB receptors are also provided.



METHOD OF DIAGNOSING AND TREATING INTERSTITIAL CYSTITIS

BACKGROUND OF THE INVENTION

5 Interstitial cystitis, also known as painful bladder syndrome, is a pathological condition that affects as many as 1 million patients in the U.S. with a high incidence in females¹. A heterogeneous array of symptoms varies from case to case and even in the same individual. Such symptoms can include mild discomfort, pressure, tenderness, intense pain in the bladder and pelvic area, increased urinary frequency/urgency, nocturia or a combination
10 of these symptoms¹². Pain may change in intensity as the bladder fills with urine or as it empties. People with interstitial cystitis also have a defect in the protective lining (transitional epithelium; also urothelium) of the bladder. The loss of the integrity of the urothelium is suspected to lead to leaky epithelium lining the bladder wall and allow toxic substances in urine to irritate the bladder wall.

15 The etiology of IC is complex including inflammation, infection, hormone involvement, autoimmunity, psychological effects, and genetic implications. The pathogenesis of IC involves inflammation, neural changes, urothelial dysfunctions, hypersensitivity, fibrosis, oxidative stress and apoptosis in the development and progression of this painful bladder syndrome. Tomazewski et al.² investigated the association between
20 the pathologic changes in the bladder biopsies with urinary bladder dysfunction among 204 IC patients enrolled in the Interstitial Cystitis Database (ICDB). The pathologic changes noted were increased number of mast cells in the lamina propria, loss of urothelium, granulation tissue and increased vascular density in the lamina propria, and submucosal hemorrhage. Thus, the lesion in the bladder appears to center in the epithelium and the
25 lamina propria of the bladder.

Patients with IC have substantially lower cystometric bladder volumes compared to normal women (265 ml versus 586 ml) and first voiding desire is at 74 ml compared to 315 ml for normal^{3,4}. This suggests that the mechanoreceptors and chemoreceptors in the bladder of IC patients may be altered, and this may trigger myelinated A- δ fibers or C-fibers found in
30 the detrusor smooth muscle or the submucosa in response to bladder distention^{5,6}. Although there are several hypotheses to account for the pathogenesis of IC, a widely accepted theory focuses on urothelial dysfunction. The urothelium, covered by proteoglycan and uroplakin, prevents the adhesion of infectious organisms, and the entrance of irritating urinary solutes into the interstitium⁷. A glycosylated frizzled-related peptide (APF) which inhibits the
35 proliferation of urothelial cell is secreted by urothelial cells from patients with IC⁸. A

defective urothelium is less protective, allowing urinary solutes like potassium to leak into the mucosa and submucosal region⁹. This can lead to activation of C-fibers, release of substance P and mast cell degranulation and subsequent injury to the submucosal tissue. These tissue changes lead to neuronal activation, leading to urinary urgency/frequency and pelvic pain associated with IC¹⁰.

Current diagnostic tests for IC include urinalysis for evidence of a urinary tract infection, urine culture and cytology, and potassium sensitivity tests. Still other diagnostic tests include cystoscopy, i.e., an examination of the bladder through a cystoscope inserted through the urethra. Additionally, cystodistension performed with anesthetics can determine the bladder's capacity. In males, a diagnostic procedure can include laboratory examination of prostate secretions.

It is difficult to make a clear diagnosis of IC because clinicians are hampered by the lack of biomarkers to either evaluate the symptoms, pathological changes, and pathogenesis of the condition and/or to provide appropriate treatments. Even when a mode of treatment is selected and tried, it is difficult to evaluate the effect of treatment for inducing beneficial changes in the bladder wall, the urothelium and the suburothelial regions, which are severely affected in IC. Most biomarkers for IC or related syndromes that were analyzed in the past were inflammatory cytokines (such as IL-1 β , IL-6, TNF α). Significant levels of IL-6 were found in the urine and bladder wall of patients in one study¹⁴. Another study^{34,35} suggested the difficulty of correlating urine biomarkers such as IL-6 and IL-8 and biopsy findings, although a strong association between urine IL-8 and bladder mast cell count was found.

The presence of an anti-proliferative factor (APF), a low molecular weight sialoglycopeptide, and potent inhibitor of the growth of normal urothelial cells and bladder cancer cells⁸ was documented in the urine of patients with IC. The specificity of APF in urine from patients with IC (versus normal controls and patients with a variety of other urogenital disorders) indicates that it may be a useful biomarker for IC. A recent publication⁴¹ referred to gene expression profiles of bladder tissues of patients with ulcerative IC. This study used very little urothelium because the urothelial layer was denuded due to ulceration.

There remains a need in the art for simpler, less invasive and more accurate diagnostic assays for IC, as well as for new and more effective treatments of this disorder.

SUMMARY OF THE INVENTION

In one aspect, a method is provided for diagnosing interstitial cystitis (IC), or differentially diagnosing IC from other infections or inflammatory conditions of the bladder

or urinary tract. The method includes measuring the level of expression of a biomarker Endothelin 1 (ET-1) in a biological sample from a mammalian subject, preferably a human subject. When compared to the level of expression of ET-1 in a healthy mammalian subject, an increased level of expression is an indication of a diagnosis of IC. More particularly, this method is demonstrated when the biological sample is urine or urothelial cells or urothelial tissue.

In another aspect, a diagnostic method for IC involves measuring the level of expression of ET-1 and at least one additional biomarker in the above-noted biological sample. The combined changes in expression of ET-1 and the additional biomarker from their respective levels of expression in a healthy mammalian subject is an indication or differential indication of a diagnosis of IC. Further differential diagnoses may be performed by combining the biomarker measurements with other clinical symptoms of IC.

In another aspect, a method is provided for monitoring progression of IC in a mammalian subject suffering from that disorder. In this method the level of expression of ET-1 in a biological sample from a mammalian subject having IC is measured and compared to the level of expression of ET-1 of a temporally earlier biological sample of the same subject. In this method, a decreased expression level of the ET-1, particularly in the urine or urethelium of the subject, compared to that in an earlier biological sample of the same subject is indicative of regression or improvement in the disorder. Conversely, an increase in ET-1 in the later sample is indicative of progression or increase in severity of the disorder. As one embodiment, this method can be applied to a subject being treated for IC. In this circumstance, the method enables a determination of the efficacy of the treatment.

In other aspects of the methods described herein, the methods may include a variety of known assay formats capable of identifying the presence of ET-1. In certain embodiments, such a method may employ use of certain machines or computer-programmed instruments that can transform detectable signals generated from diagnostic reagents complexed with the ET-1 present in the biological sample into numerical or graphical data useful in performing the diagnosis. In one embodiment, the diagnosis or differential diagnosis based on the identification or measurement of the ET-1 level in a subject's sample is associated with the presentation of certain clinical symptoms in a subject. In another embodiment, the diagnosis provides a quantitative assessment of the likelihood of IC occurrence in a subject that has not yet developed clinical symptoms of IC.

In another aspect, a diagnostic composition or kit for diagnosing or differentially diagnosing the occurrence, stage or progression of IC in a mammalian subject is provided. In

one aspect, the composition contains one or a plurality of polynucleotides immobilized on a substrate, wherein at least one polynucleotide is a genomic probe that hybridizes to ET-1 mRNA. In another aspect, the composition contains one or a plurality of PCR primer-probe sets, wherein at least one primer-probe set amplifies a polynucleotide (mRNA) sequence of ET-1. In another aspect, the composition contains one or a plurality of ligands, such as antibodies or fragments, wherein at least one ligand binds to ET-1 in a biological sample of a mammalian subject. In other embodiments, the other polynucleotides or other primer-probe sets or other ligands are designed to detect additional biomarkers. Such diagnostic compositions may also contain conventional labels which emit detectable signals when complexes with the ET-1 in the sample are formed.

In another aspect, a method for treating interstitial cystitis includes delivering to a mammalian subject in need thereof a therapeutic composition that inhibits the action of ET-1 or reduces the amount or production of ET-1, particularly in the bladder. In another aspect, a method for treating interstitial cystitis includes delivering to a mammalian subject in need thereof a therapeutic composition that inhibits the action or expression of ET-1 in the urothelial cells of the subject. In another aspect, a method for treating interstitial cystitis includes delivering to a mammalian subject in need thereof a therapeutic composition that inhibits the binding of ET-1 with its receptors (ET_A and ET_B) on urothelial cells. In still another aspect, a method for inhibiting urothelial cell proliferation includes delivering to a mammalian subject in need thereof an effective amount of a reagent that inhibits ET-1 production, synthesis or action in the bladder, e.g., an ET-1 antagonist, ET_A or ET_B receptor antagonist or a functional fragment thereof.

In yet another aspect, there is provided a use of ET-1 or a ligand for ET-1 or primer or probe capable of hybridizing to ET-1, or other biomarker noted below, in the diagnosis or assessment of differential diagnosis, or for monitoring the progression of IC in a subject.

In yet another aspect, there is provided a use of a composition that inhibits the action of ET-1 or the binding of ET-1 with its receptors, or reduces the amount or production of ET-1, in a method for treating interstitial cystitis.

Other aspects and advantages of the invention are described further in the following detailed description of the preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a bar graph showing the results of an ELISA assay for ET-1 in human urine. The concentration of ET-1 in the urine of female patients with IC (n=5; dark bar) is contrasted

with that of normal healthy women (n=4; light bar). The results show that the average ET-1 level is 2.9 ± 1.9 pg/ml in urine from the normal control group. The average ET-1 level increases to 10.7 ± 2.3 pg/ml in urine from IC patients. These data from five women with IC compared with that from four normal women, indicates that there is an increased amount of Endothelin in the urine of patients with IC.

Fig. 2A is a graph showing the effect of ET-1 on growth of cultured rabbit primary urothelial cells. The graph shows a 30-40% inhibition of growth of rabbit primary urothelial cells by ET-1 at 20 μ M.

Fig. 2B is a graph showing the effect of ET-1 on growth of cultured human primary urothelial cells. This graph shows the inhibition of 20 μ M ET-1 on the cell growth of cultured human urothelial cells and this inhibition is reversed by 1 μ M PD145065 (Sigma Chemicals, St. Louis, MO ; a global ET-1 receptor antagonist that inhibits both ET_A and ET_B). The fact that the ET-1 receptor antagonist abolishes the ET-1-induced anti-proliferative effect show that ET-1's effect is receptor-mediated.

Fig. 3 is a bar graph showing and quantifying newly synthesized DNA by detecting EdU, a nucleoside analog of thymidine that is incorporated in DNA during active DNA synthesis for both control urothelial cells and ET-1-treated urothelial cells. ET-1 treatment (10 nM, which is equal to 25 ng/ml) induced a significant decrease of EdU staining in cultured urothelial cells, which means less newly synthesized DNA in the presence of ET-1.

DETAILED DESCRIPTION OF THE INVENTION

In response to the need in the art, methods for diagnosing and/or monitoring the progress, or response to therapeutic regimen, of interstitial cystitis (IC) in a mammalian subject involve employing endothelin-1 (ET-1) as a biomarker. The invention provides a previously unrecognized correlation between urine/urothelial cell ET-1 levels in mammalian subjects and the occurrence or progression of interstitial cystitis. Compositions for performing such diagnostic methods and therapeutic methods for treating IC are also disclosed.

As evidenced in the examples below and summarized here, the inventors found that in patients clinically diagnosed with IC vs. normal controls, a correlation exists between elevations of ET-1 and IC that has not been shown before. Thus, ET-1, and particularly its level in the urine or expression level in urothelial cells of a subject suspected of having IC, serves as a biomarker for IC. It is also found that ET-1 lowers the proliferation of urothelial cells and the ET-1-induced inhibition of urothelial proliferation is reversed by a global ET-1

antagonist. It is further theorized that the high level of ET-1 in the urine and the urothelial cells prevents the proliferation and repair of urothelial lining, which loses urothelial integrity. Passage of the toxic solutes from the urine into the urothelium increases irritation and aggravation of the inflammatory process in IC.

5 A. *Diagnostic/Prognostic Methods for Interstitial Cystitis*

Novel methods for performing a diagnosis of interstitial cystitis (IC) in a mammalian subject or a differential diagnosis of IC versus other infections or inflammatory conditions of the bladder or urinary tract involves evaluating levels of endothelin-1 (ET-1) in a biological sample of a mammalian subject.

10 By the phrase “biological sample” is meant urine, urine containing shed urothelial cells or sedimented urothelial cells, or tissue biopsies from the urothelium. In certain embodiments, a sample may be another fluid or tissue containing urothelial cells, either benign or malignant cells. While the methods described herein may be operative in other biological samples, such as whole blood, serum or plasma, saliva, semen, and cellular
15 exudates from a mammalian subject, as well as tissue biopsies, it is more relevant to the loss of integrity of the urothelium to detect the biomarker in fluids or cells from the bladder cavity. Such samples may further be diluted with saline, buffer or a physiologically acceptable diluent. Alternatively, such samples are concentrated by conventional means.

 By the terms “patient” or “subject” as used herein means a mammalian animal,
20 including a human, a veterinary or farm animal, a domestic animal or pet, and animals normally used for clinical research. More specifically, the subject of these methods and compositions is a human. In one aspect of the methods described herein, the subject undergoing the diagnostic or therapeutic method is asymptomatic for IC. In another aspect, the subject undergoing the diagnostic or therapeutic methods described herein shows clinical
25 signs of IC.

 As used herein, the term “reference standard” refers to in one embodiment, one or more identical samples from the same subject, but collected at a different time, or in another embodiment, one or more samples from one or more different subjects who are healthy; or in another embodiment, to one or more samples from one or more different subjects who have
30 IC or another related disorder. In still another embodiment, the reference standard is a numerical average or average range of the expression of a biomarker in a defined population, rather than a single subject. For example, in one embodiment, a reference standard for a biomarker in a healthy subject is a numerical value or range for expression of that biomarker in a population of average healthy subjects. Likewise, in an alternative embodiment, a

reference standard for the expression of a biomarker in a subject with a disease at a certain stage of severity is a numerical value or range for the average expression of that biomarker in a population composed of multiple patients with the disease at a certain stage of severity.

The biomarker, Endothelin-1 (ET-1), is a potent vasoconstrictor peptide with 21 amino acid residues³¹. The nucleotide and amino acid sequences of ET-1 for humans and other mammals are known. See, e.g., GenBank database Accession Nos. NM_001955 and AAA52339 for the human nucleotide and amino acid sequences of the pre-protein and mature “big” and “small” forms of ET-1. Such sequences may also include known variants or mutations of ET-1. By “ET-1” as used herein is meant the small form of the mature protein, characterized in humans by the 21 amino acid sequence CSCSSLMDKECVYFCHLDIIW (SEQ ID NO: 1) or a functional fragment thereof. By the term “functional fragment” is meant any fragment of a nucleotide or amino acid sequence that shares the same biological function of the entire sequence.

The receptors for endothelin, ET_A and ET_B, originally found in the endothelium of blood vessels have been found in many other organs. ET-1 promotes the growth of various cell types, such as fibroblasts, mesangial cells, and endothelial cells³². On the other hand, ET-1 decreases human bronchial epithelial cell migration and proliferation³³. ET-1 has been shown to act as a potent growth factor for smooth muscle²⁰ and it has also been shown to have an effect on the contractile phenotype of smooth muscle as well²¹. In contrast to the mitogenic effect that ET-1 has on smooth muscle cells, it has been shown to have an antiproliferative effect on cultured urothelial cells (as shown in Figs. 2A, 2B and 3). ET-1 is released upon stretch²²⁻²⁴. In addition, ET-1 release is stimulated by cytokines^{25, 26}. ET-1 is also a mediator of pain. Increased levels of ET-1 have been associated with labor pain and angina¹⁴. In addition, abdominal constriction induced by ET-1 in rats was shown to be blocked by morphine¹⁸. Surprisingly, ET-1 has also been associated with analgesia¹⁹. It has been suggested that the two main endothelin receptor subtypes may determine the specific effect produced with activation of ET^A receptors triggering pain²¹ and activation of ET^B receptors producing analgesia^{19,20}.

Unless defined otherwise in this specification, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs and by reference to published texts.

In one aspect, a method for diagnosing, or differentially diagnosing, interstitial cystitis (IC) thus includes measuring the level of expression of ET-1 in a biological sample from a mammalian subject. In one embodiment, such a method involves contacting a sample

with a diagnostic reagent capable of forming a complex or association with the ET-1 in the sample; followed by detecting or measuring the level of the complex or association thus formed. Upon comparison with ET-1 levels in a biological sample healthy mammalian subject (or a reference standard developed from an average of ET-1 levels in a normal healthy mammalian population), an increased level of expression of ET-1 is an indication of a diagnosis of IC. In one aspect, the increased expression of ET-1 in the urine or a sample containing urothelial cells of a subject having at least one clinical symptom of IC provides a diagnosis of the disease. Thus, ET-1 is a newly found biomarker for IC. In another embodiment, ET-1 is employed in a differential diagnosis with other biomarkers or with the assessment of other clinical symptoms indicative of IC.

While such comparison can occur by direct comparison of the ET-1 measurement in the subject's biological sample with the expression levels in similar samples of one or more healthy subjects, it is more typical for a reference average expression level to be provided as a number or range. A level of expression of ET-1 in the subject's sample that is above the level of expression in a healthy mammalian subject (or reference average) is an indication of a diagnosis or a differential diagnostic indicator of IC.

Similarly, a method for monitoring progression of IC in a mammalian subject involves measuring the level of expression of ET-1 in a biological sample from a mammalian subject having IC over a given time period. In one embodiment, such a method involves contacting a sample with a diagnostic reagent capable of forming a complex or association with the ET-1 in the sample; followed by detecting or measuring the level of the complex or association thus formed. The expression level of ET-1 is then compared with the level of expression in one or more biological samples of the same subject assayed earlier in time, or before or during treatment. While such comparison can occur by direct comparison with one or more prior assessments of the same patient's status, it is also possible for a reference average expression level at specific stages or severities of the disease among other patients with IC to be provided as a reference number or profile. A decreased expression level of the ET-1 in the urine or urethelium of the subject compared to that of an earlier biological sample of the same subject or reference average is indicative of regression of said IC. A level of expression of the ET-1 in the subject's sample that is above the subject's prior level of expression (or reference average) is an indication of a poor prognosis or increasing severity of the disease. In circumstances under which the subject is being treated for IC, this method is useful to determine the degree of success or efficacy of a particular therapeutic regimen for IC. This method may indicate circumstances in which a change of therapy is necessary.

By the phrase "above the level of expression" is meant to refer to concentration of ET-1 when the protein's concentration generally in urine or another body fluid is measured. However, this phrase can also refer to relative increases in expression observed in subject's tissue from normal tissue by immunohistochemical (IHC) staining (see Example 2) as well as
5 by calculating the numerical concentration from the IHC results. Any method by which one of skill in the art may detect an increase or change in expression of the biomarker is encompassed by that phrase and by the methods described herein.

The measurement of ET-1 concentration, preferably in urine or urothelial biopsy tissue, serves as a biomarker for IC. According to the method of this invention, to determine
10 a diagnosis or to assess progression or response to treatment of IC, the level of ET-1 protein in a biological fluid of a mammalian subject is measured and compared to a reference standard of ET-1 levels in a normal healthy mammalian population. In one embodiment, such a method involves contacting a sample with a diagnostic reagent capable of forming a complex or association with the ET-1 in the sample; followed by detecting or measuring the
15 level of the complex or association thus formed. An elevated ET-1 level in the subject's biological sample compared to that standard can diagnose or aid in confirming diagnosis of IC obtained by other methods, e.g., clinical symptoms.

One suitable reference standard is that established by measuring ET-1 values of a normal population sample. Thus, the standard is preferably provided by using the same assay
20 technique as is used for measurement of the subject's ET-1 levels, to avoid any error in standardization. As demonstrated in the examples below, the relative level of risk of IC can be determined based upon the increase of ET-1 as compared against the ET-1 levels of a population. As demonstrated by the examples below, the occurrence of IC is associated with increased levels of ET-1 in urine and increased expression of ET-1 in urothelial tissue.

As described above, "normal" levels of ET-1 in a population can vary based upon any
25 variables in an individual assay used for measurement and the standardization of reagents employed in such assay. Therefore, in one embodiment of this invention, i.e., that based upon the assay and antibody employed in the examples below, the "normal" level of urinary ET-1 ranges between and including the concentrations 1.0 and 4.0 pg/ml. For example, according
30 to the ELISA assay employed in the examples below, average levels of urinary ET-1 were 2.9 ± 1.9 pg/ml in urine from the normal control group. However, in another assay, the "normal" value may skew somewhat from the minimum average of 1.0 pg/ml or maximum average of 4.0 pg/ml. Increasingly sensitive assays may further affect the "normal" range of ET-1 in the biological samples of a population. However, other averages within the ranges may be

obtained for larger populations or populations of patients differing in other physiological characteristics, e.g., gender, weight, physical condition, etc. or for other types of biological samples.

Thus, for purposes of this invention when using an ELISA, particularly the ELISA
5 and reagents of the examples below, a diagnosis of IC may be obtained when a subject's urinary ET-1 is significantly elevated above 4.0 pg/ml. For example, the results of Example 3 demonstrate that the average urinary ET-1 concentration in IC patients was 10.7 ± 2.3 pg/ml. Thus, in one embodiment of the diagnostic method of this invention a diagnosis of IC may be made when a subject's urinary ET-1 level is greater than 4 pg/ml. In another embodiment of
10 the diagnostic method of this invention a diagnosis of IC may be made when a subject's urinary ET-1 level is greater than 5 pg/ml. In yet another embodiment of the diagnostic method of this invention a diagnosis of IC may be made when a subject's urinary ET-1 level is or greater than 6 pg/ml. In yet another embodiment of the diagnostic method of this invention a diagnosis of IC may be made when a subject's urinary ET-1 level is or greater
15 than 7 or 8 pg/ml. In yet another embodiment of the diagnostic method of this invention a diagnosis of IC may be made when a subject's urinary ET-1 level is or greater than 9 or 10 pg/ml, and so on.

Alternatively, a diagnosis of IC may be obtained when the elevation in ET-1 expression is noted in a sample containing shed or sedimented urothelial cells expressing ET-
20 1. Still alternatively, a diagnosis of IC may be obtained when the elevation in ET-1 expression is noted by a relative increase in expression of ET-1 in biopsy tissue samples, particularly urothelial cells of the bladder or urinary tract when contrasted with "normal" cells or tissues. See, e.g., Example 2 which demonstrates an increase in urothelial ET-1 observed by immunohistochemical microscopy.

25 In still a further embodiment of methods according to this invention, a differential diagnosis of IC in a subject may be performed by measuring ET-1 levels in combination with measuring one of more second or other IC biomarkers and/or coupled with clinical symptoms of IC. In one aspect, the diagnostic or disease monitoring methods described herein further include coupling the measurement of the biomarker ET-1 with measuring the level of
30 expression of at least one additional biomarker in a patient's sample. The combined changes in expression of ET-1 and the additional biomarker from their respective levels of expression in a healthy mammalian subject is an indication of a diagnosis or differential diagnosis, of IC. Among such additional biomarkers diagnostic or useful in a differential diagnosis of IC are urinary or urothelial-related markers, such as Hb-EFG, EGF, APF, IGF-1, IGFBP-3^{34,35}.

Other useful additional biomarkers are mast cell activation-related markers, such as histamine and methylhistamine^{34,35}. Still other additional biomarkers are inflammatory markers, such as IL-8, IL-6, IL-1, nitric oxide gas, and cGMP^{34,35}. Still other additional biomarkers are innervation-related markers, nerve growth factors and norepinephrin^{34,35}.

5 Correlation between the ET-1 level and a level indicative of IC for the second biomarker further confirms the diagnosis or evaluation of the severity of the IC. Thus the measurement of ET-1 may serve to confirm indications of IC provided by assays for other biomarkers. Alternatively the measurement of ET-1 may serve to more accurately diagnose IC than other biomarkers alone. Still alternatively, the clinical symptoms of IC, e.g., mild
10 discomfort, pressure, tenderness, intense pain in the bladder and pelvic area, increased urinary frequency/urgency, nocturia or a combination of these symptoms, when coupled with the ET-1 measurement provides a more accurate diagnosis of the disease.

As stated above, specific diagnostic methodology employed in the measurement of ET-1 in the biological sample includes measuring the ET-1 as ribonucleic acid (mRNA, i.e.,
15 measuring the transcription of ET-1) or protein (i.e., measuring translation of the protein) using conventional assay technologies. In one embodiment, the ET-1 expression is measured in the urothelial cells obtained from punch biopsies or from cells in the urine sediment (obtained after centrifugation) at the mRNA and protein levels, respectively by polymerase chain reaction and enzyme-linked immunosorbent assay (ELISA). The specific
20 methodologies that can be employed to perform the diagnostic methods described herein may be readily selected and adapted by one of skill in the art, including the exemplified ELISA assay for ET-1 in a urine sample and immunohistochemical staining of a urothelium sample.

In one embodiment, the ET-1 expression is measured in the urothelial cells obtained from punch

25 1. *Assays for Protein*

The measurement of the ET-1 protein (or any second biomarker) in the biological sample may employ as a diagnostic reagent any suitable ET-1 ligand, e.g., antibody (or antibody to any second biomarker) to detect the protein. Such antibodies may be presently extant in the art or presently used commercially, such as those available as part of the ASSAY
30 DESIGNS ET-1 assay kit, or may be developed by techniques now common in the field of immunology. As used herein, the term "antibody" refers to an intact immunoglobulin having two light and two heavy chains or any fragments thereof. Thus a single isolated antibody or fragment may be a polyclonal antibody, a high affinity polyclonal antibody, a monoclonal antibody, a synthetic antibody, a recombinant antibody, a chimeric antibody, a humanized

antibody, or a human antibody. The term "antibody fragment" refers to less than an intact antibody structure, including, without limitation, an isolated single antibody chain, a single chain Fv construct, a Fab construct, a light chain variable or complementarity determining region (CDR) sequence, etc. A recombinant molecule bearing the binding portion of an anti-ET-1 antibody, e.g., carrying one or more variable chain CDR sequences that bind ET-1, may also be used in a diagnostic assay of this invention. As used herein, the term "antibody" may also refer, where appropriate, to a mixture of different antibodies or antibody fragments that bind to ET-1. Such different antibodies may bind to a different portion of the ET-1 protein than the other antibodies in the mixture. Such differences in antibodies used in the assay may be reflected in the CDR sequences of the variable regions of the antibodies. Such differences may also be generated by the antibody backbone, for example, if the antibody itself is a non-human antibody containing a human CDR sequence, or a chimeric antibody or some other recombinant antibody fragment containing sequences from a non-human source. Antibodies or fragments useful in the method of this invention may be generated synthetically or recombinantly, using conventional techniques or may be isolated and purified from plasma or further manipulated to increase the binding affinity thereof. It should be understood that any antibody, antibody fragment, or mixture thereof that binds ET-1 or a particular sequence of ET-1 as defined above may be employed in the methods of the present invention, regardless of how the antibody or mixture of antibodies was generated.

Similarly, in suitable diagnostic reagents, the antibodies may be tagged or labeled with reagents capable of providing a detectable signal, depending upon the assay format employed. Such labels are capable, alone or in concert with other compositions or compounds, of providing a detectable signal. Where more than one antibody is employed in a diagnostic method, e.g., such as in a sandwich ELISA, the labels are desirably interactive to produce a detectable signal. Most desirably, the label is detectable visually, e.g. colorimetrically. A variety of enzyme systems operate to reveal a colorimetric signal in an assay, e.g., glucose oxidase (which uses glucose as a substrate) releases peroxide as a product that in the presence of peroxidase and a hydrogen donor such as tetramethyl benzidine (TMB) produces an oxidized TMB that is seen as a blue color. Other examples include horseradish peroxidase (HRP) or alkaline phosphatase (AP), and hexokinase in conjunction with glucose-6-phosphate dehydrogenase that reacts with ATP, glucose, and NAD⁺ to yield, among other products, NADH that is detected as increased absorbance at 340 nm wavelength.

Other label systems that may be utilized in the methods of this invention are detectable by other means, e.g., colored latex microparticles (Bangs Laboratories, Indiana) in

which a dye is embedded may be used in place of enzymes to provide a visual signal indicative of the presence of the resulting ET-1-antibody complex in applicable assays. Still other labels include fluorescent compounds, radioactive compounds or elements. Preferably, an anti-ET-1 antibody is associated with, or conjugated to a fluorescent detectable
5 fluorochromes, e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), coriophosphine-O (CPO) or tandem dyes, PE-cyanin-5 (PC5), and PE-Texas Red (ECD). Commonly used fluorochromes include fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), and also include the tandem dyes, PE-cyanin-5 (PC5), PE-cyanin-7 (PC7), PE-cyanin-5.5, PE-Texas Red (ECD), rhodamine, PerCP,
10 fluorescein isothiocyanate (FITC) and Alexa dyes. Combinations of such labels, such as Texas Red and rhodamine, FITC + PE, FITC + PECy5 and PE + PECy7, among others may be used depending upon assay method.

Detectable labels for attachment to diagnostic reagents useful in diagnostic assays of this invention may be selected from among numerous compositions known and readily
15 available to one skilled in the art of diagnostic assays. The anti-ET-1 antibodies or fragments useful in this invention are not limited by the particular detectable label or label system employed. Thus, selection and/or generation of suitable anti-ET-1 antibodies with optional labels for use in this invention is within the skill of the art, provided with this specification, the documents incorporated herein, and the conventional teachings of immunology.

20 Similarly the particular assay format used to measure the ET-1 in a biological sample may be selected from among a wide range of immunoassays, such as enzyme-linked immunoassays, such as those described in the examples below, sandwich immunoassays, homogeneous assays, immunohistochemistry formats, or other conventional assay formats. One of skill in the art may readily select from any number of conventional immunoassay
25 formats to perform this invention.

Other reagents for the detection of protein in biological samples, such as peptide mimetics, synthetic chemical compounds capable of detecting ET-1 may be used in other assay formats for the quantitative detection of ET-1 protein in biological samples, such as high pressure liquid chromatography (HPLC), immunohistochemistry, etc.

30 2. *Nucleic Acid Assay Formats*

Still other methods useful in performing the diagnostic steps described herein are known and well summarized in, e.g., US Patent No. 7,081,340. Such methods include methods based on hybridization analysis of polynucleotides, methods based on sequencing of polynucleotides, proteomics-based methods or immunochemistry techniques. The most

commonly used methods known in the art for the quantification of mRNA expression in a sample include northern blotting and *in situ* hybridization; RNase protection assays; and PCR-based methods, such as reverse transcription polymerase chain reaction (RT-PCR) or qPCR. Alternatively, antibodies may be employed that can recognize specific DNA-protein
5 duplexes. The methods described herein are not limited by the particular techniques selected to perform them. Exemplary commercial products for generation of reagents or performance of assays include TRI-REAGENT, Qiagen RNeasy mini-columns, MASTERPURE Complete DNA and RNA Purification Kit (EPICENTRE®, Madison, Wis.), Paraffin Block RNA Isolation Kit (Ambion, Inc.) and RNA Stat-60 (Tel-Test), the MassARRAY-based
10 method (Sequenom, Inc., San Diego, CA), differential display, amplified fragment length polymorphism (iAFLP), and BeadArray™ technology (Illumina, San Diego, CA) using the commercially available Luminex100 LabMAP system and multiple color-coded microspheres (Luminex Corp., Austin, Tex.) and high coverage expression profiling (HICEP) analysis.

The diagnostic methods described herein can employ contacting a patient's sample
15 with a diagnostic reagent, as described above, which forms a complex or association with the ET-1 in the patients' sample. Detection or measurement of the sample ET-1 may be obtained by use of a variety of apparatus or machines, such as computer-programmed instruments that can transform the detectable signals generated from the diagnostic reagents complexed with the ET-1 in the biological sample into numerical or graphical data useful in performing the
20 diagnosis. Such instruments may be suitably programmed to permit the comparison of the measured ET-1 in the sample with the appropriate reference standard and generate a diagnostic report or graph.

Such diagnostic methods useful herein include the methods described in the following Examples.

25 *B. Diagnostic Compositions and Reagents*

In conjunction with the performance of the various diagnostic techniques described herein, another aspect of the invention is a variant of diagnostic reagents employing the ET-1 biomarker. One embodiment of a diagnostic reagent comprises at least one polynucleotide immobilized on a substrate. The polynucleotide is a genomic probe that hybridizes to ET-1
30 cDNA and or ET-1 mRNA. The reagent can contain additional biomarkers useful as a genetic signature of IC. In one embodiment the reagent enables detection of changes in expression in ET-1 from that of a reference expression profile. In another embodiment, differences between the expression of ET-1 and additional biomarkers in a subject from that of the signature profile can indicate a diagnosis of IC.

Still another diagnostic reagent or composition or kit for diagnosing the occurrence, stage or progression of IC in a mammalian subject includes one or more PCR primer-probe sets that amplifies a polynucleotide sequence of ET-1. Another such composition or kit contains a second PCR primer-probe set that amplifies a polynucleotide sequence of a
5 "second" IC biomarker, such as any of those identified above. The diagnostic compositions of the invention can be presented in the format of a microfluidics card, a microarray, a chip or chamber that employs the PCR, RT-PCR or Q PCR techniques described above. In one aspect, such a format is a diagnostic assay using TAQMAN® Quantitative PCR low density arrays. When a biological sample from a selected subject is contacted with the primers and
10 probes in the diagnostic composition, PCR amplification of genes in the gene expression profile from the subject permits detection of changes in expression in the splicing factor genes in the gene expression profile from that of a reference gene expression profile. Significant changes in the gene expression indicating an increase in the expression level of ET-1 from that of the reference gene expression profile can correlate with a diagnosis of IC.

15 The selection of the polynucleotide sequences, their length and labels used in the composition are routine determinations made by one of skill in the art in view of the teachings of which genes can form the gene expression profiles suitable for the diagnosis and prognosis of IC. For example, useful primer or probe sequences can be at least 8, at least 10, at least 15, at least 20, at least 30, at least 40 and over at least 50 nucleotides in length. For example,
20 such probes and polynucleotides can be complementary to portions of the coding or non-coding strand of the nucleotide sequences encoding ET-1 (see GenBank sequences referenced above). The probes and primers can be at least 70%, at least 80%, at least 90%, at least 95%, up to 100% complementary to sequences encoding ET-1 or to regulatory sequences flanking the ET-1 coding sequences.

25 Such diagnostic reagents useful in the methods described herein include the reagents described in the following Examples.

Still another diagnostic reagent includes a composition or kit comprising at least one ligand that binds to an expression product of ET-1. In one embodiment the ligand is associated with a detectable marker. In another embodiment, the ET-1 is a protein and said
30 ligand is an antibody or functional fragment thereof, such as a Fab fragment, a complementarity determining region "CDR", an scFV, among other known sequences. Such reagents are useful in immunohistochemistry diagnostic methods. Antibodies or antisera, preferably polyclonal antisera, and most preferably monoclonal antibodies, or other protein-binding ligands specific for each factor 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 horseradish 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. Protocols and kits for immunohistochemical analyses are well known in the art and are commercially available. In still another embodiment, ET-1 is a nucleic acid sequence and said ligand is an antisense sequence or polynucleotide as discussed above.

Such diagnostic reagents and kits containing them are useful for the measurement and detection of ET-1 in the methods described herein for diagnosis/prognosis of IC. In such composition, the antibodies or peptides or nucleic acid sequences may be immobilized on suitable substrates, e.g., bound to an avidin-coated solid support, plates, sticks, or beads. Of course, other binding agents known to those of skill in the diagnostic assay art may also be employed for the same purposes. Other reagents include conventional diagnostic labels or label systems for direct or indirect labeling of the antibodies, peptides or nucleic acid sequences, with e.g., radioactive compounds, radioisotopes, such as ^{32}P , ^{125}I , technetium; fluorescent or chemiluminescent compounds, such as FITC, rhodamine or luciferin; and proteins such as biotin or enzymes and enzyme co-factors, such as alkaline phosphatase, β -galactosidase or horseradish peroxidase; and/or molecular labels such as FLAG, etc. Other elements of the label systems include substrates useful for generating the signals upon interaction with the other components of the label system, e.g., a streptavidin and horseradish peroxidase system. Any method known in the art for separately conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter et al, 1962 Nature 133:945; Pain et al 1981 J. Immunol., Meth. 40:219 and other conventional texts.

Alternatively, a diagnostic kit thus also contains miscellaneous reagents and apparatus for reading labels, e.g., certain substrates that interact with an enzymatic label to produce a color signal, etc., apparatus for taking blood samples, as well as appropriate vials and other diagnostic assay components.

C. *Therapeutic Methods and Reagents*

In still another aspect, methods for treating interstitial cystitis are provided. A method for treating interstitial cystitis involves delivering to a mammalian subject in need thereof a therapeutic composition that inhibits the action, production or synthesis of ET-1 in urine or urothelial cells of the subject. In another aspect, the method involves delivering to a mammalian subject in need thereof a therapeutic composition that inhibits the binding of ET-

1 with its receptors on urothelial cells of the subject. In another aspect, the method involves delivering to a mammalian subject in need thereof a therapeutic composition that inhibits the expression of ET-1 in the urothelial cells of the subject.

5 The expression of ET-1 and its action may be inhibited by antagonists to ET-1, antagonists to ET-1 receptors (ET_A and ET_B) and/or inhibition of its expression by the urothelial cells by RNA interference technology. The phrase "ET-1 antagonist" means an agent that inhibits the synthesis of ET-1 by the urothelial cells or an agent that binds ET-1 and forms a complex, which complex cannot bind to ET-1 receptors. The phrase "ET-1 receptor antagonist" means an agent that binds ET_A and/or ET_B, thereby preventing binding between
10 ET-1 and its receptors. Such antagonists may include, e.g., polynucleotides, siRNA fragments thereof, antibodies, fragments thereof and or small chemical molecules. In another aspect of the method, the composition is an ET-1 antagonist or ET-1 receptor antagonist that blocks the binding between ET-1 and its receptors. In one embodiment the composition down-regulates ET-1 expression by urothelial cells. In another embodiment, the down-
15 regulation is mediated by siRNA. In still another embodiment, the antagonist is an antibody or fragment thereof that binds ET-1 or an ET-1 receptor antibody or fragment thereof (anti-ET_A antibody or anti-ET_B antibody). The choice of antagonist, e.g., whether protein antagonist, such as an antibody or antibody fragment that binds ET-1, or a nucleic acid inhibitor, such as an anti-sense sequence, siRNA, or a small molecule antagonist that inhibits
20 the ET-1 or ET_A or ET_B receptors, may be selected by one of skill in the art.

In another therapeutic aspect, ET-1 has been shown to inhibit the proliferation of epithelial cells. Inhibition of urothelial proliferation by ET-1 requires the binding of ET-1 to its receptors (ET_A & ET_B) on the urothelial cells. Therefore, ET-1, produced by the urothelial cells or produced elsewhere and excreted in the urine collected in the bladder cavity itself
25 serves as an undesirable anti-proliferative agent. ET-1 prevents the repair and regeneration of urothelial cells and minimizes the urothelial barrier in certain conditions. Maintenance of the barrier is important for preventing the urinary solutes from diffusing into the bladder wall, and causing inflammatory changes and irritation common in IC.

Thus, in one embodiment, a therapeutic method involves inhibiting the production or
30 synthesis of ET-1, and/or ET-1 receptors, to decrease the action of ET-1, particularly in the bladder. This is accomplished by delivering to the target cell an effective amount of an antagonist of ET-1 or an antagonist of the ET-1 receptors that prevents binding between ET-1 and its receptors. In one embodiment, the delivery comprises administering a plasmid or viral vector comprising a polynucleotide encoding an antagonist of ET-1 or an antagonist of the

ET-1 receptors, or a functional fragment thereof under the control of a promoter operable in eukaryotic epithelial cells. In another embodiment, the delivery comprises administering an ET-1 antagonist or ET-1 receptor antagonist as a protein ectopically to the cell. In still a further embodiment, delivery occurs under conditions permitting the uptake of ET-1 antagonist or ET-1 receptor antagonist by the cell. In another embodiment, the ET-1 antagonist or ET-1 receptor antagonist is administered in an amount sufficient to prevent damage to the bladder wall. In another embodiment, the ET-1 antagonist or ET-1 receptor antagonist is administered in an amount to treat IC.

Thus, in one embodiment a therapeutic composition includes a plasmid or viral vector comprising a polynucleotide encoding ET-1 antagonist or an ET-1 receptor antagonist thereof under the control of a promoter operable in eukaryotic epithelial cells in a pharmaceutically acceptable vehicle or carrier. The selection of a suitable plasmid backbone or viral vector, of which many are well known in the art and commercially available may be done by a person of skill in the art. Examples of such vectors include recombinant adenoviral vectors, herpes simplex virus (HSV)-based vectors, adeno-associated viral (AAV) vectors, hybrid adenoviral/AAV vectors, recombinant retroviruses or lentiviruses which are constructed to carry or express a selected nucleic acid composition of interest, modified vaccinia virus Ankara (MVA), Vaccinia, Adeno-associated virus (AAV), Alphavirus etc.. Retrovirus vectors that can be employed include those described in EP 0 415 731; International Patent Publication Nos. WO 90/07936; WO 94/03622; WO 93/25698; and WO 93/25234; U.S. Pat. No. 5, 219,740; International Patent Publication Nos. WO 93/11230 and WO 93/10218; GB Patent No. 2,200,651; and EP 0 345 242, among others. Alphavirus-based vectors may also be used as the nucleic acid molecule encoding the transgene. Such vectors can be constructed from a wide variety of alphaviruses, including, for example, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532). Representative examples of such vector systems include those described in U.S. Pat. Nos. 5,091,309; 5,217,879; and 5,185,440; and International Patent Publication Nos. WO 92/10578; WO 94/21792; WO 95/27069; WO 95/27044; and WO 95/07994. Such selection of vector and expression system is not believed to limit this invention.

In another embodiment, a therapeutic composition for administration to a human subject for treatment of a disease related to expression of ET-1 includes siRNA, that downregulates the production of ET-1 by urothelial cells, under the control of a promoter

operable in eukaryotic epithelial cells in a pharmaceutically acceptable vehicle or carrier. In another embodiment, a therapeutic composition for administration to a human subject for treatment of a disease related to expression of ET-1 includes an ET-1 antagonist under the control of a promoter operable in eukaryotic epithelial cells in a pharmaceutically acceptable vehicle or carrier.

In still another embodiment, a pharmaceutical composition for administration to a human subject for treatment of a disease related to IC or expression of ET-1 contains an effective amount of an antagonist of ET-1 or an ET_A or ET_B antagonist in protein form in a pharmaceutically acceptable vehicle. This composition may be infused intravesically to the bladder cavity to minimize the cardiovascular effect of ET-1 antagonists.

As defined herein, pharmaceutically acceptable vehicles or carriers suitable for use in these compositions are well known to those of skill in the art and may be readily selected by same. In one embodiment, a preferred pharmaceutical carrier contains water for injection with mannitol added for tonicity at a concentration of about 45 mg/mL. Other possible carriers include, without limitation, and depending upon pH adjustments, buffered water, buffered saline, such as 0.8% saline, phosphate buffer, 0.3% glycine, hyaluronic acid, alcoholic/aqueous solutions, emulsions or suspensions. Other conventionally employed diluents, adjuvants and excipients, may be added in accordance with conventional techniques. Preservatives and other additives such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like may also be provided in the pharmaceutical carriers. The preparation of these pharmaceutically acceptable compositions, from the above-described components, having appropriate pH isotonicity, stability and other conventional characteristics is within the skill of the art. See, *e.g.*, texts such as Remington: The Science and Practice of Pharmacy, 21st ed, Lippincott Williams & Wilkins, publ., 2005; and The Handbook of Pharmaceutical Excipients, 6th edit., eds. R. C. Rowe *et al*, APhA Publications, 2009.

As used herein, the term "effective amount" or "pharmaceutically effective amount" refers to the amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal, individual or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes one or more of the following: (1) preventing the disease; *e.g.*, preventing a disease, condition or disorder in an individual that may be predisposed to the disease, condition or disorder but does not yet experience or display the pathology or symptomology of the disease; (2) inhibiting the disease; *e.g.*, inhibiting a disease, condition or disorder in an individual that is experiencing or

displaying the pathology or symptomology of the disease, condition or disorder (*i.e.*, arresting or slowing further development of the pathology and/or symptomology); and (3) ameliorating the disease; *e.g.*, ameliorating a disease, condition or disorder in an individual that is experiencing or displaying the pathology or symptomology of the disease, condition or disorder (*i.e.*, reversing the pathology and/or symptomology). For example, an effective amount, when administered to a subject to treat IC, is an amount of a therapeutic sufficient to inhibit, slow, reduce, or eliminate inflammation, bladder pain and sensitivity and reduce ET-1 levels to a normal range. For example, an effective amount of an ET-1 receptor antagonist or ET-1 antagonist as a therapeutic is an amount sufficient to increase proliferation of urothelial cells in the bladder.

The amounts of ET-1 antagonists or ET-1 receptor antagonists in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1 mg/mL, usually at or at least about 2 mg/mL to as much as 20 mg/mL, or alternatively up to 50 mg/mL or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. Dosages will also be adjusted for the severity of disease, type of disease, and physical condition of the subject as determined by a physician.

A human unit dose form is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (see, *e.g.*, Remington's Pharmaceutical Sciences, 17th Edition, A. Gennaro, Editor, Mack Publishing Co., Easton, Pa., 1985).

Similarly as therapeutic compositions, the mode of administration for *in vivo* administration may be selected by one of skill in the art, and can include any suitable route. Such routes may be selected from, *e.g.*, oral, intravenous (*i.v.*), infusion, parenteral (aside from *i.v.*, such as intralesional, intraperitoneal and subcutaneous injections), intraperitoneal, transdermal (including all administration across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues). Other routes of administration are also feasible and include, without limitation, liposome-mediated delivery or implant delivery.

30 D. Screening Assays for the Identification of Useful Compounds

As disclosed herein, Endothelin-1 is a potential target for development of a therapeutic to treat interstitial cystitis. Antagonists to ET-1 or to its receptors are potentially useful as a therapeutic to prevent binding between ET-1 and its receptors and reduce symptoms associated with IC and enable the cells in the urothelial lining to repair themselves.

In another aspect, a method for screening a test compound or molecule for use in treating IC includes contacting a test compound with a mammalian urothelial cell expressing ET-1 or the receptors ET_A and/or ET_B; and measuring the effect of the test compound on the expression level of ET-1 or its receptors. A decrease in the normal expression of ET-1 by the cell indicates a compound or molecule useful in therapy of IC. Thus, ET-1 and/or its receptors may also be used in the screening and development of chemical compounds, small molecules, nucleic acid sequences, such as cDNAs, or proteins which affect the expression of ET-1, and thus have utility as therapeutic drugs for the treatment of IC. In one embodiment, a test compound, including small molecules, peptides or polypeptides, nucleotide sequences, such as cDNAs or miRNAs, are selected for testing. The selected test compound is contacted or exposed to a mammalian cell expressing ET-1 or its receptors. After suitable culture conditions, e.g., 37°C for about 48-60 hours, the effect of the compound on the expression level of the gene product is assessed by any suitable means. The effect of the compound to alter the normal expression of ET-1 is related to its impact on the expression level of the gene product or receptors by said cell. Where the effect is that the compound allows maintenance of normal expression levels or decreases elevated levels of ET-1, allowing normal expression of ET-1, that test compound is indicated to be useful as a potential therapeutic compound in the treatment of IC. Where the effect is that the compound increases the expression level of ET-1, the compound is indicated to have a potential adverse effect on inflammatory conditions of the bladder, such as IC.

A high throughput splicing assay used to identify compounds that change expression of ET-1 by urothelial cells may also be used to screen compounds. In one embodiment such an assay involves contacting in each individual well of a multi-well plate a different selected test compound (e.g., nucleotide sequence, amino acid sequence, small molecules, etc) with a mammalian urothelial cell that expresses a normal level of ET-1 or ET-1 receptors. In one embodiment that cell is transfected with a minigene that expresses luciferase (or another marker gene) only when said cell expresses ET-1 at a certain level. After the compound has been exposed to the expressing cell under appropriate culture conditions, the level of the marker gene (or luminescence) is conventionally measured. A change in the expression of the ET-1 level normally expressed by the cell caused by any of the test compounds is correlated with the expression or lack of expression of the marker in each well. For example, where such an assay is utilized to screen test compounds, the maintenance or a decrease in expression level of ET-1 is indicative that the compound has a potential therapeutic effect. A

result in which there is an increase in expression level of ET-1 is indicative that the compound has a potential inflammatory or adverse effect on IC.

Other conventional assays and techniques also exist for the identification and development of compounds and drugs which impact the expression or activity of ET-1, such as methods described in publications referenced herein. Such other assay formats may be used and the assay formats are not a limitation.

E. Examples

The examples that follow do not limit the scope of the embodiments described herein. Our preliminary studies on bladder from IC patients showed upregulation of ET-1 in the bladder epithelium and submucosal mesenchymal cells when compared to that of normal. Urothelial cells cultured from IC patients also revealed an overexpression of ET-1 compared to those cells cultured from normal individuals. One skilled in the art will appreciate that modifications can be made in the following examples which are intended to be encompassed by the spirit and scope of the invention.

EXAMPLE 1 – GENERAL EXPERIMENTAL METHODS

A. Statistics

Data are described appropriately before proceeding to analysis. Biomarker expression and all other continuous data are presented as sample means ($\pm$ SEM). Graphical methods are used to examine the distribution and quality of the data. Group comparisons are performed using ANOVA. Categorical variables are summarized by proportions and compared across groups using standard chi-square tests of association. Exact p-values from Fisher's exact test are calculated for these tests. Logistic regression models are used to identify which patient demographics, study sites, and other clinical factors are predictive of particular phenotypes. In addition, statistical tests for univariate associations between clinical phenotypes and biomarkers are computed using Statistical analysis software (SAS; SAS, Cary, NC). Adjustments for clinical sites are made for most analyses. Separate multivariable predictive models are developed for each clinical phenotype incorporating the statistically significant biomarkers identified in the univariate analyses. After adjusting for statistically significant clinical variables, such as age, menopausal status, gender, and BMI, the selected biomarkers are entered sequentially in a forward selection model, with adjustments for cluster effects among subjects within clinical centers.

Unless mentioned otherwise, data are expressed as mean $\pm$ standard error. Sample size calculation are based on preliminary data suggesting a difference between normal control

and IC patients (or vulvodynia, or prostatitis) with $\alpha=0.05$ and power=0.8. Data from western, Real-time PCR and Northern are quantified and statistically analyzed. Observations from these three groups are compared using a one-way ANOVA (analysis of variance), followed by Bonferroni corrections for multiple comparison procedures. Differences are considered
5 significant at $P < 0.05$. (JMP ® 7, SAS, Cary, NC).

B. Fixation and Embedding:

Bladder tissues are fixed in 4% paraformaldehyde in PBS (pH 7.4) at 4°C for 2 hours. Tissues are washed three times in PBS for 30 minutes each, dehydrated in an ascending ethanol series (30, 50, 70, 85, 95 and 100%), infiltrated into Technovit 7100 (Hereaus Kulzer
10 GmbH & Co. KG, Wehrheim, Germany) overnight, and embedded in Technovit 7100 at room temperature by adding the polymerization agent provided in the kit.

C. Sectioning and Immunostaining:

Semithin sections (2-3 μm) are prepared using a LKB rotary retracting microtome with a tungsten carbide blade. For histological study, slides are stained with hematoxylin and
15 eosin. Deparaffinized sections are washed in phosphate buffered saline (PBS) and blocked with 1% BSA for 1 hour. Then, the sections are incubated with the primary antibody at 4°C. Slides are washed three times for 5 minutes each in PBS and incubated with Texas Red-conjugated antimouse IgG (Sigma) diluted at 1:250 for 2 to 4 hours at room temperature, washed three times in PBS and mounted in Aqua-Poly/Mount medium. Sections are examined
20 with a fluorescence microscope (Nikon E800) equipped for confocal microscopy.

D. RNA extraction and Real time PCR:

Total RNA are isolated from frozen tissue using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. RNA concentrations are measured by UV spectrophotometer. 1.0 μg of total RNA are reverse transcribed with oligo (dT) primer
25 (Promega, Madison, WI) and M-MLV reverse transcriptase (GIBCO, Gaithersburg, MD) at 37°C for 1 hour. Then the cDNA are heated to 90°C for 5 min to inactivate the reverse transcriptase. 1 μL of this reverse transcription reaction are subjected to real time PCR amplification in the ABI 7500 Fast System (Applied Biosystems, CA). The following reaction components are prepared: 1 μL TaqMan Assay (20X), 1 μL RT, 8 μL water and 10 μL
30 universal mix (Applied Biosystems). GAPDH and p-actin are used as internal control for target genes expression.

E. Protein expression and Western blotting:

Total extractable protein are isolated from frozen tissues in 1% SDS extraction buffer (100 mg tissue/ml) containing 20% glycerol, 50 mM Tris -HCl (pH 6.8), 0.5% (v/v) Tween-

20 and protease inhibitors (0.5mM PMSF, 2 μ M pepstatin, 2 μ M antipain and 0.1 mg/ml trypsin inhibitor). Protein concentration in the supernatant are measured using the Bio- Rad DC protein assay kit. 20 μ g of total extract protein are loaded on SDS - PAGE gels. Then the protein are transferred to Immobilon - P membrane with transfer buffer [25mM Tris , 96 mM
5 glycine plus 50 mg SDS and 0.5 ml -mercaptoethanol in 1 liter buffer] for myosin and transfer buffer [25 mM Tris, 192 mM glycine and 20% (v/v) methanol] for other small proteins overnight at 4°C. Then membranes are blocked in 5% non-fat milk for 1 hour and incubated with primary antibody for 2 hour at room temperature. Then the membranes are washed with TBST buffer (20mM Tris, 500 mM NaCl , 0.05 % Tween 20) 3 times and incubated with
10 second antibody for 1hour. After three additional TBST buffer washes, substrates in the membrane can be visualized by using ECL kit (Amersham Pharmacia Biotech). Band intensities are scanned and analyzed using Fujifilm IAS-3000 imager and MultiGauge v3.0 Software program (Fuji Photo Film, Japan). Standard curves for all proteins analyzed are constructed to establish the linear concentration ranges.

15 F. *Immunostaining:*

Cross-sections (5 micron) of tissue sections are made from the paraffin blocks. Tissue sections are deparaffinized and put into descending grades of alcohol and then blocked 30 minutes in 1 % BSA solution. After blocking, sections are incubated for 2 hours at room temperature with the primary antibodies specific for target proteins. After washing 3 times in
20 PBST, the sections are then treated with second antibody according to primary antibody, then washed again 3 times with PBST. Finally slides are mounted with a drop of mounting medium (Aqua-Mount, Lerner Labs; Pittsburgh, PA) and viewed under a Nikon (Melville, NY) Eclipse 800 fluorescence microscope. Images are captured using a RT Slider SPOT camera (Diagnostic Instruments; Sterling Heights, MI) and Image-Pro Plus software from
25 Media Cybernetics (Silver Spring, MD). A negative control, in which only the secondary antibody is added, is used for all samples.

G. *In situ hybridization:*

Tissue are fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB) (pH 7.4) (4% PFA) at 4°C overnight. After fixation, tissue is embedded into paraffin. Cross-sections
30 are cut at 5 μ m. Probes of sense and antisense digoxigenin (DIG)-labeled RNA strands are transcribed in vitro from target sequences using an RNA labeling kit (Boehringer). In situ hybridization is performed as follows: Sections are deparaffinized, hydrated and treated with proteinase K (Boehringer, 10 mg/ml) and then hybridized using sense or antisense DIG-labeled RNA probe at 60°C for 18-24 h. Hybridization signals are then detected by using

alkaline phosphatase-conjugated anti-DIG antibody (Boehringer) and NBT as the chromogen. Each *in situ* hybridizations are performed in duplicate and slides are visualized and photographed using a Nikon Eclipse E800 microscope and Spot digital camera.

5 EXAMPLE 2 - THE EXPRESSION OF ET-1 IN THE URINARY BLADDER EPITHELIUM

The expression of ET-1 in the urothelium in the bladder biopsies from IC patients is analyzed at the mRNA and protein level by RT-PCR (and Real-time PCR) and Western blot analysis, respectively. The relative increase in the expression of the endothelin at the mRNA level is assessed by quantitative real-time PCR using the published procedure³⁷.

- 10 Immunofluorescence microscopy is used for *in situ* localization of the endothelin expression to the urothelial cell layer. For immunofluorescence microscopy, formalin-fixed tissue sections of bladder biopsies previously obtained for the ICDB (Interstitial Cystitis Database) were prepared as described above. The detailed procedure for performing the immunofluorescence microscopy is provided in Example 1. Briefly, Anti-ET-1 antibody
15 (Calbiochem, San Diego, CA; cat. #CP44) is used at a dilution of 1:400 and anti-mouse IgG-Cy3 secondary antibody (Sigma, St. Louis, MO; C-2181) at a dilution of 1:200. The cellular localization of ET-1 in the bladders from both control and IC patients is determined. Sections are double immunostained using antibody to actin conjugated to FITC, followed by antibody against ET-1, to aid in determining whether the submucosal smooth muscle cells also express
20 ET-1 and if this expression is increased in IC.

- Results from the immunofluorescence microscopic analyses provide information concerning the *in situ* localization of the ET-1 in the tissue (e.g., whether there is more in the urothelium compared to the smooth muscle layer). One photomicrograph of a histological section (5 micron in thickness) was taken from the bladder of a woman with interstitial
25 cystitis who died of other causes (figure not shown). Immunofluorescence microscopy was used for *in situ* localization of the endothelin expression in the urothelial cell layer using a commercially available antibody against ET-1 as discussed in Example 1. At several areas, the urothelium was denuded although it was preserved in some areas. This preliminary experiment revealed the presence of ET-1 in the urothelium and the submucosal
30 mesenchymal. The collagenous connective tissue (asterisks) and red blood cells in the capillaries in section from IC showed autofluorescence. This tissue section from the IC patient showed intense immunofluorescence compared to a section taken from the normal region of the bladder cystectomy for a healthy patient, using the procedures of Example 1 below (figure not shown), and also revealed hyperemia and hemorrhage. The preliminary

results of these analyses demonstrate that Endothelin-1 (ET-1) is highly expressed in the urothelium of patients with interstitial cystitis (IC) compared to normal patient bladder urothelium.

5 In another experiment, human urothelial cells from a biopsy of a normal woman from which cells have been sub-cultured and sub-cloned several times to obtain a homogeneous population of epithelial cells. These cells have become a stable cell line that maintains the epithelioid phenotype and expresses uroplakin. Karyotype analysis revealed that the cells are tetraploid but maintain stable chromosome numbers (79 chromosomes) and urothelial phenotype. These cells express uroplakin protein when stained with Uroplakin II stain and
10 DAPI, which stains nuclei (figure not shown) and confirms the normal urothelial cell phenotype. The cultured urothelial cells also express both Endothelin-1 receptor A and Endothelin-1 receptor B with appropriate staining (figures not shown).

In still another experiment, rabbit and human urothelial cells were cultured. Human urothelium cell culture was performed and it was observed that the cells maintain normal
15 morphology after multiple passages. Sufficient number of rabbit urothelial cells were obtained from primary cultures. These cells can be used as primary culture or as one passage for the proliferation assay. Under 100X magnification (photograph not shown) the rabbit urothelial cells after one passage show epithelioid morphology and express uroplakin II and endothelin receptors. Similarly under 200X magnification (photograph not shown) human
20 urothelial cells after 20 passages show epithelioid morphology and express uroplakin II and endothelin receptors.

In another experiment, human urothelium cells from a biopsy of a normal woman have been sub-cultured and sub-cloned several times until the cell culture has now become a stable cell line with tetraploid chromosomes. These cells express uroplakin protein using
25 Uroplakin II staining and DAPI, which stains nuclei (photograph not shown). These observations confirm normal urothelial cell phenotype. In addition, cultured urothelial cells express both Endothelin-1 receptor A and Endothelin-1 receptor B (photographs not shown).

EXAMPLE 3: EXPRESSION OF ET-1 IN THE URINE OF PATIENTS WITH IC

30 Endothelin expression is quantitated at the protein level by enzyme linked immunosorbent assay (ELISA). ELISA is performed using the human big Endothelin-1 (ET-1) Enzyme Immunometric Assay kit (Assay Designs, Inc., Ann Arbor, Michigan) to determine human big ET-1 peptide levels in urine samples from five female IC patients. The normal control group was urine samples from four normal healthy females. The ET-1 primary

antibody shows little to no cross-reactivity with related endothelin peptides including ET-2 and ET-3. The immunoassay kit has a sensitivity as low as 0.14 pg/ml. Because the two-site "Sandwich" ELISA assays require two antibodies, they can be more specific than RIA assays⁴⁰.

5 Briefly the appropriate number of wells in a 96 well microtiter plate coated with rabbit antibody specific to human ET-1 are washed twice with wash buffer containing phosphate buffered saline with detergent. Next 100 μ L of either standard, clarified urine, or plasma is added, the plate mixed, sealed and incubated at 37°C for 1 hour. Then the wells are washed for a total of 7 times with wash buffer and inverted and tapped on a paper towel. Next
10 100 μ L of rabbit antibody to human IgG conjugated with horseradish peroxidase is added and incubated at 37°C for 30 minutes at room temperature in the dark. The reaction is stopped with a solution of 1N sulfuric acid in water. The plate is read in a microplate reader at 450 nm with correction at 590 nm.

The level of ET-1 in normal human urine is estimated to be in the range of 1.0 to 4.0
15 pg/ml, which falls easily in the linear range of this assay. The data from this analysis shows that urine of patients with IC demonstrates an elevation of ET-1 (Fig. 1). The average ET-1 level is 2.9 ± 1.9 pg/ml in urine from the normal control group, while the average ET-1 level increased to 10.7 ± 2.3 pg/ml in urine from the five IC patients. These data indicate that there is a greater than three-fold increase of Endothelin-1 in the urine of patients with IC than in the
20 urine of healthy women. These data demonstrate a correlation between elevations of urinary ET-1 and IC that has not been shown before; thus a determination of urinary ET-1 serves as a biomarker for the diagnosis of IC.

An increase in ET-1 levels in urine or an increased level of ET-1 in the bladder tissue suggests an upregulation of the endothelin signal transduction pathway in the urothelium
25 associated with IC. An increase in the level of ET-1 in either the urothelial extract or in the urine of IC patients compared to normal controls suggests that the mature ET-1 is being synthesized at a higher rate in the IC patients. Without being bound by theory, the mechanistic basis of this increase could be due to an upregulation of endothelin converting enzyme (ECE) which converts the ET-1 precursor known as Big ET-1 to the mature 21 amino
30 acid ET-1 peptide, etc.

EXAMPLE 4: EFFECT OF ET-1 ON THE PROLIFERATION OF THE UROTHELIAL CELLS

The overexpression of ET-1 in urothelial cells cultured from IC patients (Example 2) may have an inhibitory effect on the proliferation of urothelial cells, similar to that on

epithelial proliferation in the lung. Upregulation of ET-1 in cells in the submucosa would lead to proliferation of these cells associated with remodeling of the bladder wall. On the other hand, upregulation of the ET-1 in the urothelial cells would lead to inhibition of urothelial proliferation if the ET-1's effect on the urothelium is similar to respiratory epithelium. Thus, it is important to determine whether ET-1 also plays a role in the turnover of urothelial cells, at least in some subsets of IC patients.

Experiment A:

The epithelial cell culture and proliferation assay used for these experiments is described. Briefly, explanted epithelial cells propagated from fresh biopsy specimens are grown in DMEM-F12 with 10% heat-inactivated fetal bovine serum, 1% antibiotic/antimycotic, 1% L-glutamine, 1.0 unit/ml insulin (all from Sigma), and 5 µg/ml human epidermal growth factor at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Cultured cells are characterized to possess urothelial phenotype by binding AE-1/AE₃ pancytokeratin antibodies (Signet Laboratories, Dedham, MA). Cell proliferation is assessed also by ³H-thymidine incorporation and by cell counting using a haemocytometer. ET-1 samples are diluted at varying concentrations in serum-free MEM (containing only glutamine and antibiotics) and applied to cells. Control cultures receive only the vehicle. Cells are fed with serum-free medium for the proliferation assay.

Cultures are exposed to 1 µCi per well ³H-thymidine (1 Ci = 37 GiBg) for 4 hrs and trypsinized, sedimented by brief centrifugation (3,000 rpm for 10 min) and the cell pellets are washed with a buffer containing cold thymidine and the radioactivity determined using a scintillation counter. The ³H-thymidine incorporation is expressed as a mean decrease in cpm of >2 SD from the mean of control cells for each plate. In addition, the ³H-thymidine treated cell cultures are fixed in 5% buffered formalin. Cultures are washed with ascending grades of ethanol (70%, 95% and 100%) and air dried. Dry cultures are coated with NTB2 autoradiographic emulsion (KODAK) in the dark room, air dried, stored in a dark box and exposed at 4°C for 2 weeks. After developing the autoradiographs in D-19 (15°C) and fixation, culture plates are stained with H & E. Cultures are examined under a light microscope and the number of cells showing silver grains in the nuclei from 10 selected fields on the culture plate are counted. The data are expressed as % of cell incorporating ³H-thymidine.

A concentration response curve is prepared using varying concentrations of ET-1. Decreased ³H-thymidine incorporation is plotted as a function of the ET-1 concentrations.

Determining the cellular proliferation by autoradiography confirms the increased thymidine incorporation determined from the total radioactivity in the cell pellets.

Decreased ^3H -thymidine incorporation by the urothelial cells in the presence of ET-1 indicates an anti proliferative effect for ET-1. In order to determine whether this effect is specific for urothelial cells, cultures for fibroblasts and detrusor smooth muscle cells established in our laboratory from human bladder biopsies are used as controls.

ET-1 inhibits the proliferation of urothelial cell cultured from both human and rabbit bladder. When cultured rabbit primary urothelial cells are treated with ET-1 at 20 μM , a 30-40% inhibition of growth of the cells is demonstrated (Fig. 2A). When cultured human primary urothelial cells are treated with 20 μM ET-1, the cell growth is inhibited. However, this inhibition is reversed by treating the cells with 1 μM PD145065 (a global ET-1 receptor antagonist that inhibits both ET_A and ET_B ; see Fig. 2B). The fact that the ET-1 receptor antagonist abolishes the ET-1-induced anti-proliferative effect shows that ET-1's effect is receptor-mediated. These data confirm that ET-1 has an inhibitory effect on urothelial proliferation.

The results of this assay demonstrate that ET-1 overexpression inhibits proliferation of urothelial cells in IC. This indicates that agents that inhibit the expression, production or synthesis of ET-1 can be used therapeutically to repair bladder wall linings.

Experiment B:

Click-iT EdU assay (Invitrogen) was used to detect and quantify newly synthesized DNA. EdU is a nucleoside analog of thymidine and is incorporated in DNA during active DNA synthesis. In Click-iT assay, EdU can be detected by green fluorescent dye and nuclei can be detected by blue fluorescent dye. Using fluorescent microplate reader (Synergy2, BioTek) both green and blue fluorescent signal were detected and quantified as shown in the bar graph of Fig. 3). Phase image and merged EdU and nucleic staining images for both control and ET-1 treated urothelial cells (figures not shown) demonstrated that the ET-1 treatment (10 nM, which is equal to 25 ng/ml) induced a significant decrease of EdU staining in cultured urothelial cells, which means less newly synthesized DNA in the presence of ET-1.

An increase in the level of ET-1 in the urine of IC patients over levels in controls suggests that the mature ET-1 is synthesized at a higher rate in the IC patients. The increase may be due to up-regulation of endothelin converting enzyme (ECE), which converts the ET-1 precursor known as Big ET-1 to the mature 21 amino acid ET-1 peptide. The results from the immunofluorescence microscopy analyses provide information concerning the *in situ*

localization of the ET-1 in the tissue (e.g., greater expression in the epithelial compared to the smooth muscle layer or in different cells in the transitional epithelium, such as, basal cells versus intermediate or umbrella cells). An increase in ET-1 levels in urine or an increased level of ET-1 in the bladder tissue suggests up-regulation of the endothelin signal transduction pathway in the urothelium in IC. If ET-1A and ET-1B receptors are over-expressed in urothelial and sub-urothelial tissues from patients with symptoms associated with IC (when compared to control patients but not in tissues that do not contribute to a particular phenotype), then these biomarkers are specific for the IC clinical phenotype and/or of subtype of IC.

The ET-1 biomarker, found to correlate with disease presence, is a diagnostic tool and also a tool to follow disease progression, especially the changes in the integrity of the urothelium and subsequent pathologic insult to the suburothelial tissue including the nerve terminals. This biomarker provides insight into therapeutic targets for disease treatment. Specifically, the signaling mechanisms responsible for transcription of this biomarker suggests a means by which the disease progresses and by which prevention can be achieved. In one embodiment, if urinary ET-1 is increased in any of the non-IC lower urinary tract disorders, one may correlate the ET-1 urinary biomarker with clinical signs of IC, and also with the presence of one or more of the pro-inflammatory cytokines (such as IL1 β , IL-6 or IL-8) which has been associated with IC.

Decreased 3H-thymidine incorporation by urothelial cells in the presence of ET-1 indicates an anti-proliferative effect of ET-1. To determine whether this effect is specific for urothelial cells, cultures for fibroblasts and detrusor smooth muscle cells established in our laboratory from human bladder biopsies are used as controls. Proliferation assays in the presence of ET-1 receptor antagonists demonstrate that the observed effect on proliferation is in fact mediated *via* ET-1 binding to its receptors.

All documents identified of listed herein, as well as the specification of provisional US patent application No. 61/246,273, are incorporated herein by reference. While various embodiments in the specification or claims are presented using "comprising" language, under various circumstances, a related embodiment may also be described using "consisting of" or "consisting essentially of" language. It is to be noted that the term "a" or "an", refers to one or more, for example, "a reagent," is understood to represent one or more reagents. As such, the terms "a" (or "an"), "one or more," and "at least one" are used interchangeably herein. While the invention has been described with reference to specific embodiments, it is

appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A method for diagnosing, or differentially diagnosing, interstitial cystitis (IC) comprising:
measuring the level of expression of a biomarker Endothelin 1 (ET-1) in a biological sample from a mammalian subject, wherein an increased level of expression of ET-1 above the level of expression in a healthy mammalian subject is an indication of a diagnosis of IC.
2. The method according to claim 1 wherein said biological sample is urine or a sample containing urothelial cells from said subject.
3. The method according to claim 1, wherein the mammalian subject providing the biological sample has clinical symptoms selected from the group consisting of: mild discomfort in the bladder, pressure in the bladder, tenderness of the bladder, intense pain in the bladder and pelvic area, increased urinary frequency, increased urinary urgency, and nocturia.
4. The method according to claim 2, wherein the biological sample is urine and the level of expression of ET-1 is increased above 4 pg/ml.
5. The method according to claim 1, wherein said measuring step comprising measuring the ET-1 as ribonucleic acid (mRNA) or protein.
6. The method according to claim 1, further comprising measuring the level of expression of at least one additional biomarker of IC in said sample, wherein the combined changes in expression of ET-1 and the additional biomarker from their respective levels of expression in a healthy mammalian subject is an indication of a diagnosis of IC.
7. The method according to claim 5, wherein said additional IC biomarker is selected from the group consisting of HB-EFG, EGF, APF, IL-8, IL-6, and cGMP.
8. The method according to claim 1, wherein said measuring comprises conducting an enzyme-linked immunosorbent assay for ET-1 in the biological sample.

9. The method according to claim 1, wherein said measuring comprising performing an immunohistochemical staining of the urothelial biopsy sample.
10. A method for monitoring progression of interstitial cystitis (IC) in a mammalian subject comprising measuring the level of expression of ET-1 in a biological sample from a mammalian subject having a clinical symptom of IC, wherein a decreased expression level of the ET-1 in the urine or urethelium of said subject compared to that of an earlier biological sample of the same subject is indicative of regression of said IC.
11. The method according to claim 10, wherein said subject is being treated for IC and wherein the method enables a determination of the efficacy of the treatment.
12. A diagnostic reagent or kit for diagnosing the occurrence, stage or progression of interstitial cystitis (IC) in a mammalian subject comprising at least one of (a) a polynucleotide immobilized on a substrate, wherein said polynucleotide is a genomic probe that hybridizes to ET-1 cDNA or mRNA or (b) a PCR primer-probe set that amplifies a polynucleotide sequence of ET-1 mRNA.
13. The reagent or kit according to claim 12, wherein said reagent (a) or (b) enables detection of changes in expression in ET-1 in urothelial cells sedimented from the urine or in urethelium from that of a reference expression profile, said changes correlated with the diagnosis, stage or progression of IC.
14. The reagent or kit according to claim 12, further comprising at least one additional polynucleotide reagent or PCR primer set for detection of an additional biomarker selected from the group consisting of HB-EFG, EGF, APF, IL-8, IL-6, and cGMP.
15. The reagent according to any one of claims 12 through 14, wherein said reagent or kit comprises a microarray, a microfluidics card, a computer-readable chip or chamber.
16. A method for treating interstitial cystitis comprising delivering to a mammalian subject in need thereof a therapeutic composition selected from the group consisting of:

- (a) a composition that reduces the amount or concentration of ET-1 in urine of said subject;
- (b) a composition that inhibits the binding of ET-1 with its receptors on urothelial cells; and
- (c) a composition that inhibits the expression of ET-1 by the urothelial cells of the subject.

17. The method according to claim 16, wherein said composition is an inhibitor of ET-1 synthesis by urothelial cells.

18. The method according to claim 16, wherein said composition is an ET-1 receptor antagonist.

19. The method according to claim 16, wherein said composition blocks binding between ET-1 and its receptors.

20. The method according to claim 16, wherein said composition down-regulates ET-1 expression by urothelial cells.

21. The method according to claim 20, wherein said down-regulation is mediated by siRNA directed against the ET-1 mRNA.

22. The method according to claim 16, wherein said antagonist is selected from the group consisting of an antibody or fragment thereof that binds ET-1 or its receptors ET_A or ET_B.

23. A method for increasing urothelial cell proliferation comprising delivering to a mammalian subject in need thereof an effective amount of a reagent that inhibits the synthesis, production or action of ET-1 in the bladder.

24. The method according to claim 23, wherein said reagent is an ET-1 antagonist or ET-1 receptor antagonist that is capable of inhibiting binding between ET-1 and its receptors.

FIG. 1

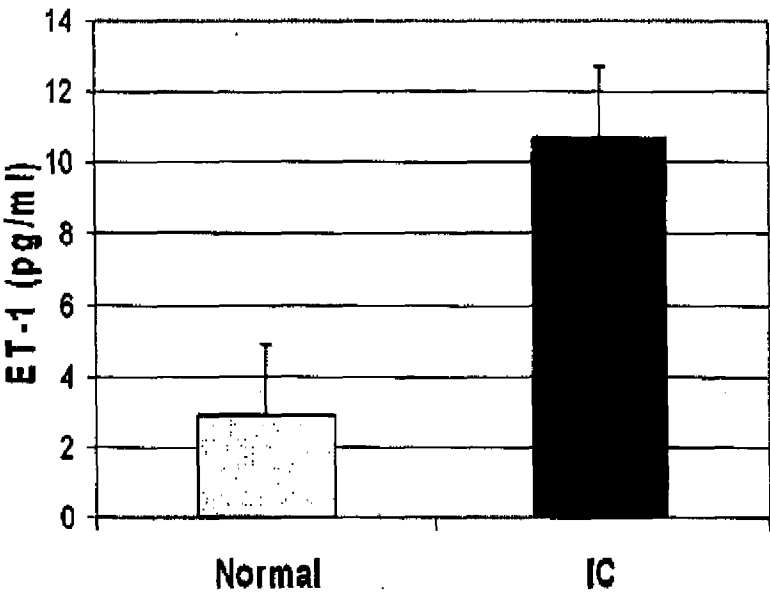


FIG. 2A

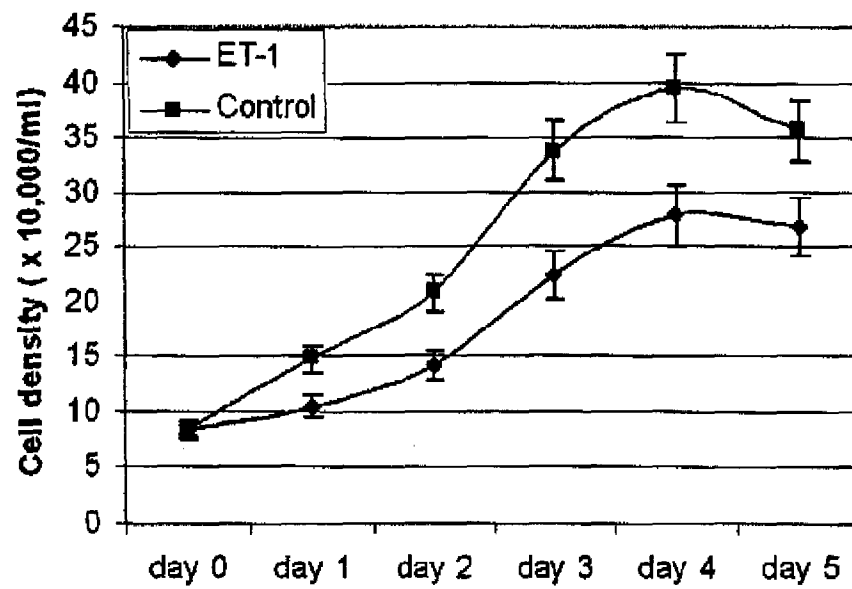


FIG. 2B

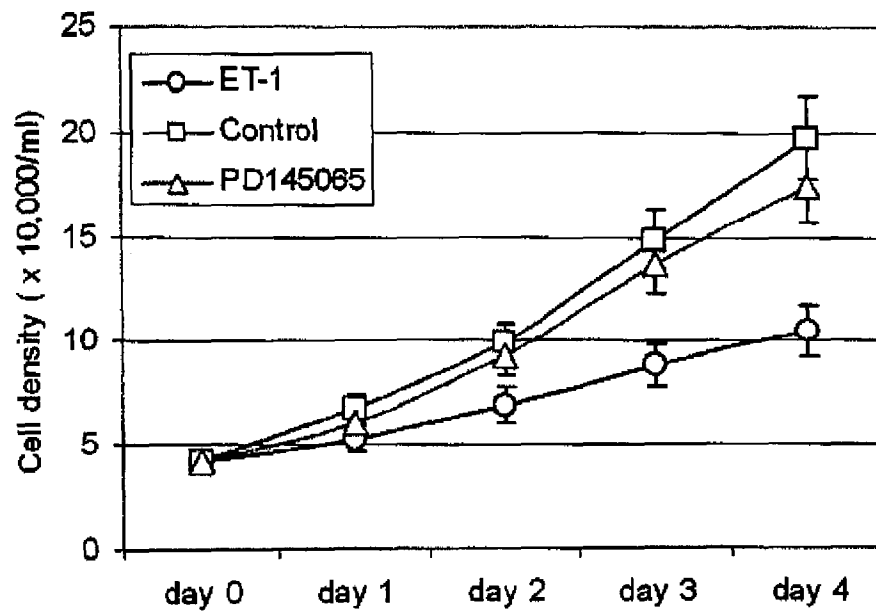
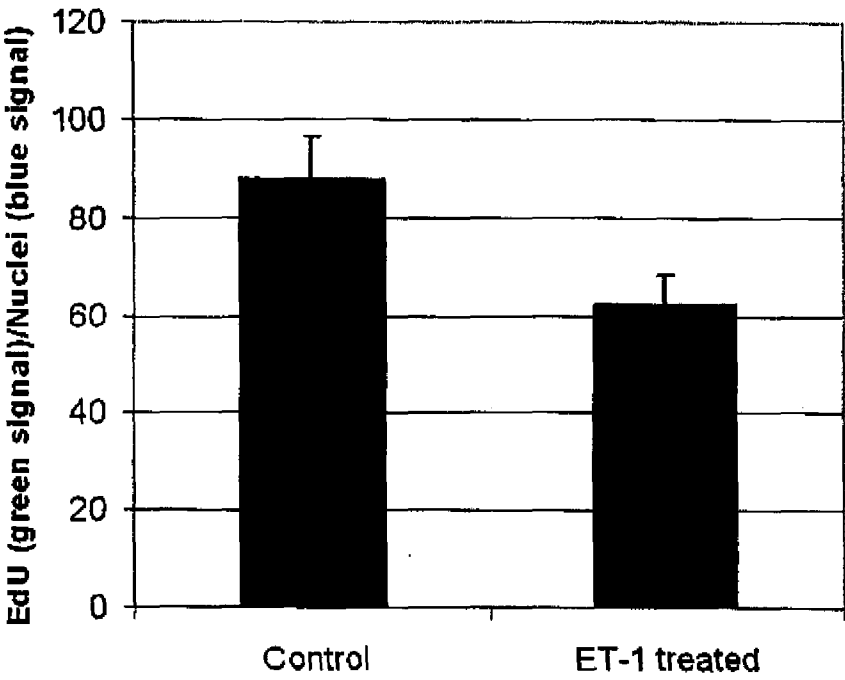


FIG. 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/049914

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 G01N33/68

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)
G01N C12Q

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, FSTA, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/069906 A2 (CELLEGY PHARMA INC [US]; MAK VIVIEN H W [US]; GRAYSON STEPHEN [US]) 12 September 2002 (2002-09-12) paragraph [0014]	1-11
X	US 2003/143544 A1 (MCCARTHY JEANETTE [US]) 31 July 2003 (2003-07-31) paragraph [0015]	12-15



Further documents are listed in the continuation of Box C.



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

4 January 2011

Date of mailing of the international search report

17/03/2011

Name and mailing address of the ISA/

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Authorized officer

Lunter, Pim

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/049914

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.:
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.:

1-15

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: 1-15

Method for diagnosing interstitial cystitis; method for
monitoring progression of interstitial cystitis; kit
therefor

2. claims: 16-22

Method of treating interstitial cystitis

3. claims: 23, 24

Method for increasing urothelial cell proliferation

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2010/049914

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02069906	A2	12-09-2002	CA 2440141 A1 12-09-2002
			EP 1383502 A2 28-01-2004
			JP 2005504721 T 17-02-2005
			KR 20100036390 A 07-04-2010
			MX PA03007960 A 04-12-2003

US 2003143544	A1	31-07-2003	NONE
