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(54) Title: IN VIVO IMAGING OF MATRIX METALLOPROTEINASES IN LUNG DISEASE

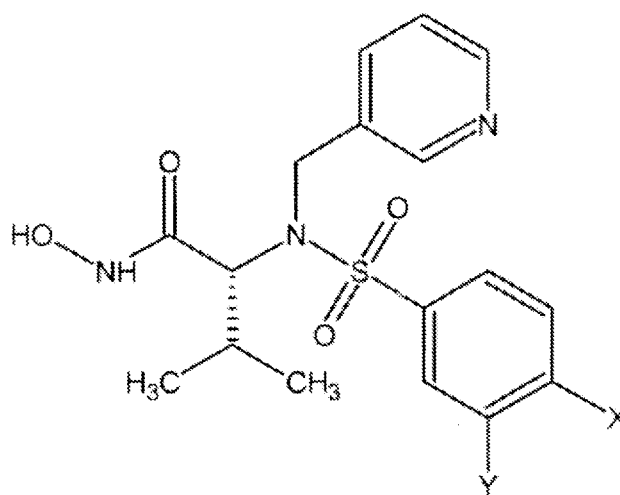


Fig. 2

(57) Abstract: The present invention provides a method of imaging a subject's lung which comprises contacting the subject's lung with a matrix metalloproteinase inhibitor labeled with a radioisotope under conditions such that the inhibitor binds to matrix metalloproteinase in the lung, and then imaging the radiolabeled inhibitor bound to matrix metalloproteinase in the subject's lung. The invention also provides Ro 32-3555 labeled with a radioisotope.



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IN VIVO IMAGING OF MATRIX METALLOPROTEINASES IN LUNG DISEASE

This application claims priority of U.S. Provisional Application No. 62/339,598, filed May 20, 2016, the contents of which are hereby incorporated by reference in its entirety.

Throughout this application, various publications are referenced. Full citations for these references are present immediately before the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

Background of the Invention

Proteases capable of digesting both collagen and elastin are secreted from inflammatory cells within the lungs of patients with chronic obstructive pulmonary disease (COPD); in addition, epithelial cells are known to produce collagenolytic enzymes (Imai 2001). The initiation and sustaining mechanism of destruction is multi-factorial and diverse from case to case, however connective tissue degradation is a common, unique and definite mechanism in emphysema development. Multiple MMPs have been implicated in the development of emphysema, including MMP-1, MMP-9, MMP-12, and MMP-13 (Goldklang 2013). MMP-1, a collagenase, is specifically expressed in lung epithelial cells and cigarette smoke directly induces MMP-1 in epithelial cells (Imai 2001, Foronjy 2003, Mercer 2009). An additional collagenase, MMP-13, is also increased in the epithelial cells and macrophages of patients with COPD (Lee 2009).

Due to the importance of metalloproteinase expression in the pathogenesis of a number of diseases radiolabeled metalloproteinase inhibitors have been developed for tumor imaging and for cardiovascular applications (Wagner 2009, Kopka 2004). The uptake of a ^{99m}Tc-labeled MMP inhibitor (RP805) was reported in atheroma of ApoE null mice (Tekabe 2010). All of the MMP inhibitors studied to date are broad based and were developed for the treatment of inflammatory and degenerative diseases. One of these drugs is a non-peptidyl broad-

spectrum MMP inhibitor CGS27023A with high affinities against MMPs-1, -2, -3, -7, -9, -12, and -13 (Kopka 2004, Faust 2009, Romero-Perez 2009, Ouchi 2008, Rao 2006).

Summary of the Invention

The present invention provides a method of imaging a subject's lung which comprises contacting the subject's lung with a matrix metalloproteinase inhibitor labeled with a radioisotope under conditions such that the inhibitor binds to matrix metalloproteinase in the lung, and then imaging the radiolabeled inhibitor bound to matrix metalloproteinase in the subject's lung.

The invention also provides Ro 32-3555 labeled with a radioisotope.

Brief Description of the Figures

Figure 1. Increased inflammation and MMP-9 expression in LPS exposed lungs. (A) Increased total BAL (bronchoalveolar lavage fluid) cell count. (B) Increased MMP-9 but not MMP-13 in LPS treated lungs.

Figure 2. Structure of CGS27023A and its derivative(s). (a) When X is MeO and Y is H the compound is in its non-derivative form, CGS27023A; (b) when X is OH and Y is H the compound is "CGS27023A with hydroxylation" or "HO-CGS27023A", in which an -OH group is present at point X.

Figure 3. MMP-1 inhibition by HO-CGS27023A. EnzoLifeSciences, MMP-1 drug discovery kit and inhibitors (N-Isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid (NNGH) or HO-CGS27023A) were incubated at 37°C for 30-60 minutes to allow inhibitor-enzyme reaction. After, fluorogenic substrate was added to detect MMP-1 activity. Data are presented as mean + SD. NNGH is a MMP inhibitor.

Figure 4. In vivo CGS/¹²³I imaging after intranasal LPS exposure shows abundant MMP uptake. (A) Sagittal, coronal, and transverse projections from merged SPECT/CT scans for a mouse exposed to LPS on top and one exposed to PBS (control) on bottom. Tracer uptake is shown and the scale bars are displayed on the right. There is uptake of tracer observed in both lung fields of the LPS exposed mouse and no observable uptake in the PBS exposed mouse. (B) Average (+SD) of 3 LPS and 2 PBS exposed mice with tracer uptake for both lungs quantified as %ID in the graph on the left and uptake in the excised lungs(%ID/g) on the right.

Figure 5. Blood pool clearance and biodistribution of CGS/¹²³I. (A) Blood pool clearance shows optimal imaging timing of 8 hours following injection (B) Organ biodistribution 8 hours after injection of radiotracer in WT mice shows very low tracer uptake in lungs and liver. (C) Full body scans in 2 views of WT mouse injected with CGS/¹²³I and imaged at 8 hours. Sagittal image (left) shows bladder and thyroid uptake; coronal images (right) show thyroid uptake but little uptake in other organs.

Figure 6. Increased CGS/¹²³I uptake in the lungs of smoke exposed and MMP-1 transgenic mice but not air exposed non-transgenic mice. (A) Representative images from wild-type room air control, wild-type smoke exposed and MMP-1 transgenic mice. (B) Quantitative lung uptake of CGS/¹²³I. There is increased uptake in the MMP-1 transgenic and a trend for increased uptake in smoke exposed wild-type mice as compared to control. Mean + SD, n=5 per group.

Figure 7. Gamma well counts in the MMP-1 transgenic mice. Mean + SD, p=0.08 for right lungs and p=0.06 for left lungs, n=3 per group.

Figure 8. Experimental groups and design for MMP-targeted imaging of MMP-1 and MMP-9 transgenic mice. 10 mice per group are imaged.

Figures 9: Increased MMP-13 in the setting of smoke exposure and influenza virus infection. Increased MMP-13 protein levels in the lungs (A) and BAL fluid (B) of smoke exposed and influenza infected mice (sacrificed day 15 post-infection). (C) MMP-13 expression peaks at day 7 following influenza infection, and expression then decreases further out from injury. (D) Increased lung destruction in smoke exposed and influenza infected mice (sacrificed day 15 post-infection). Data are presented as mean + S.E.M.

Figure 10: Groups and design for in vivo MMP imaging in a COPD exacerbation model. (A) Experimental flow. (B) Experimental groups.

Detailed Description of the Invention

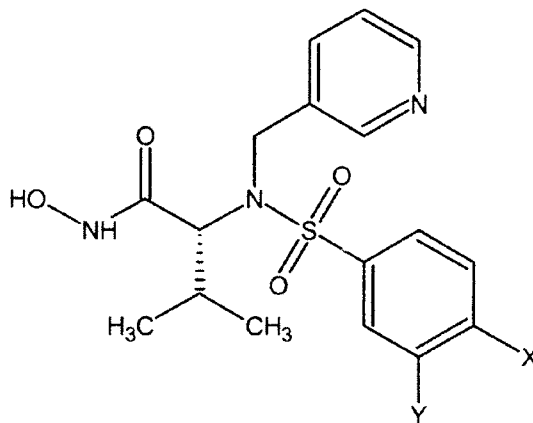
The present invention provides a method of imaging a subject's lung which comprises contacting the subject's lung with a matrix metalloproteinase inhibitor labeled with a radioisotope under conditions such that the inhibitor binds to matrix metalloproteinase in the lung, and then imaging the radiolabeled inhibitor bound to matrix metalloproteinase in the subject's lung.

In one embodiment, the matrix metalloproteinase inhibitor is Ro 32-3555 or a modified form of CGS27023A.

In some embodiments, the label is I-123.

In one embodiment, the matrix metalloproteinase inhibitor is Ro 32-3555. In another embodiment, the matrix metalloproteinase inhibitor is a modified form of CGS27023A.

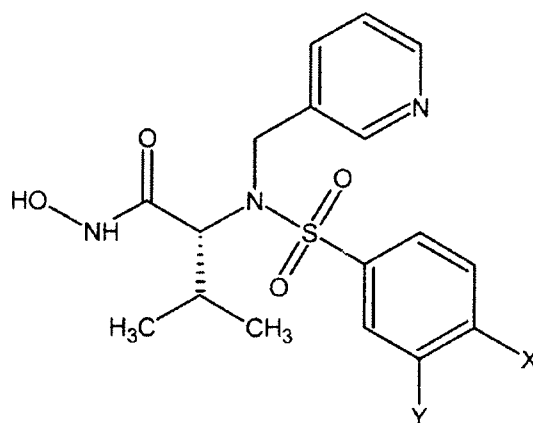
In an embodiment, the modified form of CGS27023A has the structure:



(1)

wherein X is OH and Y is H.

In another embodiment, The the matrix metalloproteinase inhibitor labeled with a radioisotope has the structure:



(1)

wherein X is OH and Y is I-123.

In some embodiments, the subject is suspected of being afflicted with a destructive lung disease. In other embodiments, the subject is afflicted with a destructive lung disease.

The destructive lung disease may be chronic obstructive pulmonary disease (COPD), lymphangioleiomyomatosis (LAM), idiopathic pulmonary fibrosis (IPF), or acute lung injury (ALI). The destructive lung disease may also be emphysema.

In some embodiments, the imaging is single-photon emission computed tomography (SPECT) imaging.

In one embodiment, the method further comprises administering to the subject an amount of the matrix metalloproteinase inhibitor labeled with a radioisotope.

In some embodiments, the method further comprises imaging the subject's lung with a single-photon emission computed tomography (SPECT) scanner. In another embodiment, the method further comprises imaging the subject's lung with a computed tomography (CT) scanner.

In some embodiments, the method further comprises imaging the subject's lung with a SPECT/CT scanner. In one embodiment, the SPECT/CT scanner is a nano-SPECT/CT scanner.

In one embodiment, the method further comprises determining the amount of matrix metalloproteinase (MMP) expression in the lung of the subject.

In some embodiments, the MMP expression is selected from one or more of the group consisting of MMP-1 expression, MMP-9 expression, MMP-12 expression, and MMP-13 expression.

In one embodiment, the method further comprises determining the amount of the label in the lung of the subject.

In some embodiments, the method further comprises determining if the subject is afflicted with destructive lung disease. In another embodiment, the method further comprises determining the degree of lung destruction in the subject.

In an embodiment, the presence of MMP expression in the subject's lung in an amount that is higher than a reference amount indicates that the subject is afflicted with a destructive lung disease.

In one embodiment, the reference amount is based on the amount of MMP expression in a healthy subject's lung.

In another embodiment, the presence of the label in the subject's lung in an amount that is higher than a reference amount indicates that the subject is afflicted with a destructive lung disease.

In one embodiment, the reference amount is based on the amount of the label in a healthy subject's lung.

In some embodiments, the amount of MMP expression present in the subject's lung determines the degree of lung destruction in the subject.

In one embodiment, the amount of the label present in the subject's lung determines the degree of lung destruction in the subject.

The invention also provides Ro 32-3555 labeled with a radioisotope.

In some embodiments, the radioisotope is I-123.

All combinations and subcombinations of the various elements described herein are within the scope of the invention.

This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

The present invention allows the detection of MMPs *in vivo* and provides a marker of disease activity. The methods allow one to (1) detect the disease at an early stage compared to available imaging techniques and pulmonary function testing and (2) target a relatively specific pathway with molecular lung imaging to directly visualize a process critical to disease initiation and progression.

The experiments herein support the detection of MMP production *in vivo* utilizing newly developed MMP inhibitor probes. The time point in which these pathways are activated following injury are defined and the imaging studies with structural and physiological changes observed in emphysema may be correlated. *In vivo* animal models are used to develop the MMP-inhibitor for SPECT-CT imaging and quantify the difference between an MMP signal in the smoke exposed lung and in the normal lung. A novel imaging modality to view lung destructive activity *in vivo* is established. The up regulation of MMP expression in transgenic models of emphysema is correlated with MMP expression in animals exposed to cigarette smoke. The chosen MMP imaging agent, CGS27023A, functions as a competitive inhibitor of multiple MMPs. However, CGS27023A has been modified with a hydroxylation step to be suitable for radioactive iodination, increasing the feasibility of *in vivo* imaging.

Example 1: Preliminary Studies

In vivo MMP imaging following LPS treatment

PBS (phosphate-buffered saline) control and LPS (lipopolysaccharide) treated C57Bl/6J mice were imaged for MMPs 24 hours after a single dose of intranasal LPS from *Escherichia coli* 0111:B4 (Sigma, 1.5 mg/kg in sterile PBS) to demonstrate support for *in vivo* SPECT-CT Imaging. Two PBS treated mice and three LPS mice were imaged. This model induces very robust inflammation (Figure 1A) and induction of MMP-9 but not MMP-13 (Figure 1B).

In vivo MMP imaging

Direct radio-iodination is used to label the relevant molecules with a gamma emitting radiotracer because of the small size of the molecules

(Kopka 2004). An F-18 label for PET imaging may also be used (Behrends 2015). CGS27023A, which has the structure shown in Figure 2(a), was modified by adding an hydroxyl group and radiolabeled to obtain HO-[¹²³I]I-CGS27023A (CGS/¹²³I) non-peptidyl hydroxamic acid as a radioligand for assessing MMP activity *in vivo* with SPECT imaging as previously reported (Kopka 2004). The chemical structure of HO-CGS27023A showing where the hydroxyl group was substituted is shown in Figure 2(b). Assays with this compound confirm that it is still a potent inhibitor of collagenase activity after this hydroxylation step (Figure 3).

Direct iodination with I-123 was performed according to the protocol as described in Markwell 1978. Briefly, the compound was dissolved in DMSO and PBS and carrier free Na ¹²³I was added to the solution (800 µCi of Na ¹²³I per 100 µg protein). The reaction was allowed to proceed for 15 min and the reaction vessel was removed to terminate the iodine oxidation. Doses of approximately 40 µCi CGS/¹²³I were drawn and injected into LPS or PBS treated mice.

Approximately one hour after femoral vein catheter placement and injection, mice underwent SPECT/CT scanning on a Bioscan nano-SPECT/CT (Mediso). At completion of scanning the mice were euthanized and lung tissue was taken for gamma well counting. The scan data was reconstructed and regions of interest were drawn around lung activity and uptake of probe measured in MBq (mCi) and divided by decay corrected injected dose (ID) to yield %ID. The lung tissue was weighed and counted in the gamma well counter for %ID/g tracer uptake. Figure 4 shows the results from these experiments. Based on visual assessment of the scans, there was small uptake in the thyroid gland and kidney uptake but no appreciable liver or heart uptake was observed.

These experiments demonstrate that one can synthesize the modified CGS27023A compound and successfully directly iodinate with I-123 and that once synthesized, this compound has an energy profile and half-life favorable for SPECT imaging.

Example 2:

The imaging studies of this example further demonstrate the clinical value of developing modified CGS27023A (HO- ^{123}I -CGS27023A, radioiodinated non-peptidyl hydroxamic acid) as a radioligand for assessing MMP lung activity. Utilizing both mouse smoke exposure models and the human MMP-1 emphysema transgenic mouse (D'Armiento 1992), SPECT/CT imaging was performed on 3 groups of mice (WT room air exposed, WT smoke exposed, and MMP-1 transgenic mice).

Direct iodination of modified CGS27023A (specifically, HO-CGS27023A) with I-123 was performed according to Markwell 1978. This iodinated modified CGS27023A formed is referred to herein as CGS/ ^{123}I . Briefly, the compound was dissolved in DMSO and PBS and added to pre-coated iodination tube. Carrier free Na ^{123}I was added to the solution (800 μCi of Na ^{123}I per 100 μg protein). The reaction was allowed to proceed for 15 min and the reaction vessel was removed to terminate the iodine oxidation.

Doses of approximately 0.150 mCi CGS/ ^{123}I were drawn and injected via a femoral vein catheter and mice were imaged on average 8 hours later. Initially 3 WT mice and 3 TG mice were injected with probe and blood samples taken via tail vein nicking for counting and blood pool clearance determination. The blood pool clearance data showed biexponential washout with $t_{1/2}$ of first component = 34 minutes and for the second component = 760 minutes (Figure 5(A)). Optimal imaging time determined from these curves was at approximately 8 hours after injection. Three additional WT mice underwent analysis for radiotracer biodistribution at 8 hours after injection, which showed very low tracer uptake in lungs and liver (Fig 5(B)). Full body scans in 2 views of WT mouse injected with CGS/ ^{123}I and imaged at 8 hours showed that excretion appears to be mainly via the kidneys at this time point based upon bladder activity. Without pre-administration of a thyroid blocking drug, it was expected to see thyroid uptake of a radioiodinated probe.

Imaging of smoke exposed and MMP-1 transgenic mice

Following biodistribution and blood pool clearance studies, imaging was performed on MMP-1 transgenic mice, mice that underwent 10 days of smoke exposure, and room air control mice. The MMP-1 transgenic

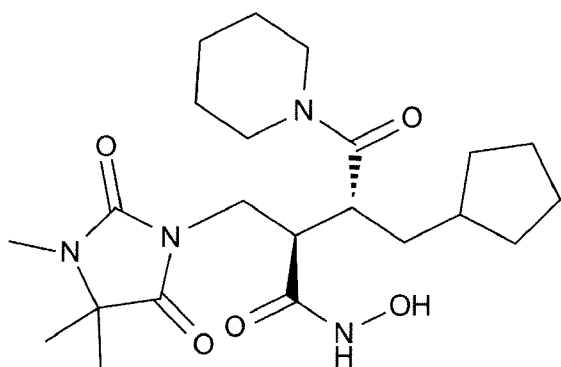
mouse imaged, line 50, is well described to have MMP-1 expression and activity within the lung, and develops emphysema spontaneously, in the absence of smoke or other inflammatory stimuli (D'Armiento 1992). Utilizing the Teague Enterprises Smoke Exposure System, mice were exposed five hours per day, five days per week, for two weeks to cigarette smoke (3R4F University of Kentucky Research Cigarettes) with a total particulate matter maintained at approximately 100 mg/m³. Littermate room air wild-type mice served as a control. The scan data was reconstructed with regions of interest drawn around lung activity and uptake of probe measured in MBq (mCi) and divided by decay corrected injected dose (ID) to yield %ID. The lung tissue was weighed and counted in the well counter for %ID/g tracer uptake. Examples of scans from the 3 groups of mice are provided in Figure 6. There is little or no uptake in the lungs of the room air exposed WT mouse while the smoke exposed WT mouse and the transgenic mouse show good lung uptake.

Quantification shows that uptake was significantly higher in the lungs of MMP-1 transgenic as compared to WT room air control mice ($p=0.03$ for both right and left lungs), and there was a trend to a significant increase when comparing WT room air control mice to smoke exposed WT mice (right lung $p=0.006$, left lung $p=0.07$). Gamma well counting for MMP-1 transgenic mice and litter mate wild-type control showed a trend toward an increased %ID/g tissue, primarily due to small sample size and large variance in the MMP-1 transgenic values (Figure 7).

These results show favorable parameters for CGS/¹²³I as a probe to image expression of MMPs in diseased lungs. I-123 is used routinely in clinical nuclear medicine studies. The half-life of I-123 of 13 hours allows time for blood pool clearance and has little uptake in normal lungs, or in the liver overlapping with the lower lobes. Probe uptake was increased in the MMP transgenic mice and during cigarette smoke exposure in these preliminary experiments. This data supports the other examples set forth in this application. It has been demonstrated that not only is it possible to detect MMPs under inflammatory conditions, but it is also possible to detect specific single MMP expression in a transgenic mouse without additional injury.

Example 3: Radioiodination of Ro 32-3555

The structure and chemical name of Ro 32-3555 (Cat. No. 2916) is below.



(α R, β R)- β -(Cyclopentylmethyl)-N-hydroxy- γ -oxo- α -[(3,4,4-trimethyl-2,5-dioxo-1-imidazolidinyl)methyl]-1-piperidinebutanamide.

The compound Ro 32-3555 is linked with Bolton-Hunter Reagent (Sulfo-SHPP) to introduce tyrosine-like residues for subsequent iodination. The conjugated compound is then radiolabeled with ^{123}I using the indirect method of Chizzonite. Briefly, Tris Iodination buffer is added to pre-coated Pierce Iodination Tubes. Carrier free $\text{Na } ^{123}\text{I}$ is added to the solution. The activated iodide is added to the solution and mixed and reacted for 6-9 min at room temperature. Scavenging buffer is added and the reaction mixture is incubated for additional 5 min. After addition of Tris/BSA to the solution, the iodinated solution is passed through a chromatography column. One cc sample is eluted from the column. Fractions containing the tracer are pooled and QC performed.

Example 4:

The procedures of Example 1 and Example 2 are performed, except that CGS/ ^{123}I is replaced by radiolabeled Ro 32-3555 (described in Example 3).

Radiolabeled Ro 32-3555 demonstrates similar biodistribution shown by CGS/ ^{123}I . Additionally, radiolabeled Ro 32-3555 is shown to have an energy profile and half-life favorable for SPECT imaging.

In the experiment having 3 groups of mice (WT room air exposed, WT smoke exposed, and MMP-1 transgenic mice) described in Example 2, SPECT/CT imaging shows similar results for mice administered radiolabeled Ro 32-3555 as those administered CGS/ ^{123}I . Thus,

radiolabeled Ro 32-3555, like CGS/¹²³I, may be used to detect specific single MMP expression in a transgenic mouse without additional injury.

Example 5: CGS/¹²³I radiotracer for in vivo imaging

In this example, the rapid blood pool clearance is confirmed and biodistribution in mice is completed.

Blood pool clearance, biodistribution and imaging

CGS/¹²³I is prepared as outlined in Example 1 above. Blood pool clearance and biodistribution studies are performed for CGS/¹²³I using 10 C57Bl/6J mice. A dose of approximately 40 microcuries (μCi) CGS/¹²³I (100 μL) is injected through a femoral vein catheter. Thereafter a small droplet of blood (about 10-20 μL) is obtained by tail vein nicking at 2, 5, 10, 15, 30, 60, 120, and 180 minutes. At 10, 30, 60, and 120 minutes, each mouse undergoes planar imaging in the anteroposterior position for a preset time of 10 minutes. At 180 minutes, mice undergo SPECT/CT imaging (Bioscan nanoSPECT/CT).

Image analysis

The SPECT/CT scans are reconstructed and processed using VivoQuant software (Invivo, Boston, MA). Regions of interest (ROIs) are drawn around each lung on serial 10 voxel thick transverse sections from apex to base, excluding the very basal segments in which up-scatter from below the diaphragm such as liver is seen. Activity for each transverse section for both right and left lungs are summed for each lung and expressed as mCi using a calibration factor. Total tracer uptake in mCi for each lung is divided by the decay corrected injected dose to yield the % injected dose (%ID). The animals are sacrificed, the lungs are removed and tissues are counted in the gamma well counter for %ID/gram of tissue.

Example 6: The use of CGS/¹²³I identifies MMP activity in MMP-1 and MMP-9 transgenic mice.

The ability of CGS/¹²³I to detect MMP expression in established transgenic mouse models is tested. Two animal models, which express different MMPs in different cell types, are utilized. The first MMP-1 transgenic develops emphysema after aging due to MMP-1 expression in the epithelial cells of the lung (D'Armiento1992, Foronjy 2003).

This model is ideal because mice do not have an ortholog of human MMP-1 (Carver 2015), which means that the littermate controls should not have any MMP expression (D'Armiento 1992). The second model expresses MMP-9 in the macrophages of the lung, which increases upon injury (Foronjy 2008, Mehra 2010). The experimental plan is outlined in Figure 8. MMP-1 and MMP-9 transgenic mice and their litter mate wild-type controls undergo CGS/¹²³I SPECT-CT imaging with a full assessment of MMP expression and activity following sacrifice. Ten (10) mice are analyzed per group.

Example 7: Imaging of an *in vivo* COPD exacerbation model with increased MMP-13 expression

It is well accepted that viral infections have significant consequences in smokers, particularly in patients with chronic obstructive pulmonary disease (COPD) and can cause severe disease exacerbations with loss of lung function (Wedzicha 2013, Donaldson 2002) and increased mortality (Gelbert 1990). MMP-13 levels were examined in an *in vivo* model system of acute viral exacerbations of COPD (Kang 2008). After two weeks of smoke exposure (Teague Enterprises Smoke Exposure System, 3R4F University of Kentucky Research Cigarettes, total particulate matter maintained at approximately 100 mg/m³), mice were anesthetized with isoflurane, and PR8 influenza virus administered by nasal aspiration. A dose of 0.05 LD₅₀ in C57Bl/6 was administered in 50 µl aliquots (Kang 2008). After an additional period of up to two weeks, mice were sacrificed, bronchoalveolar lavage was performed and tissue analyzed. For morphometric analysis, 20 pictures at 10x magnification were taken and destructive index calculated.

In this model, increased levels of MMP-13 protein (MMP-13 ELISA kit, Cloud-Clone Corp., Houston, TX) in whole lung homogenates were found (Figure 9(A)) and bronchoalveolar fluid (Figure 9(B)) at the terminal end point (day 15 after influenza infection) was found. MMP-13 RNA expression showed MMP-13 levels peaking at 7 days following influenza infection, and declining at subsequent examination on day 12 (Figure 9(C)).

Utilizing this smoke-virus model system, imaging as outlined above is undertaken to examine *in vivo* MMP-13 within the lungs of MMP-13 knockout and wild-type mice. This allows for (1) evaluation of the ability of this imaging probe to detect MMP-13 in a disease relevant animal model and (2) assessment of the specificity of the imaging probe for MMP-13 utilizing a unique MMP-13 knockout mouse (Takaishi 2008). Animal groups and experimental design are outlined in Figure 10. Animals are imaged at the time of peak MMP-13 expression (day 7), and a subset of animals recovered after imaging for sacrifice at the time of peak destruction (day 15). With this experimental design, not only is the ability of CGS/¹²³I to image MMP-13 at peak levels determined, but it is determined if MMP-13 imaging signal can predict the degree of lung destruction at a later time point.

Analysis of Inflammation, MMP Immunohistochemistry, Zymography and Lung Morphometry

At the time of sacrifice, the chest is opened and careful neck dissection is performed. A 20-gauge catheter is inserted into the trachea and secured with a silk suture. Bronchoalveolar lavage is performed with 1 mL sterile PBS, and following this, the left lung is removed for gamma well counting. The right lung is pressure perfused with 10% formalin to 25 cm H₂O for 20 minutes. Tissues are stored in formalin for at least 24 hours prior to paraffin embedding and sectioning (4 μm). Sections are stained with hematoxylin and eosin (H&E) for histological analysis including morphometry, immunohistochemistry and quantification of inflammatory cells. Immunohistochemistry for MMP-1 (human, in MMP-1 transgenic mouse only), -9, and -13 is performed on at least four sections from each mouse for correlation with imaging results. Morphometric analysis of the H&E stained lungs is performed as outlined above. Quantitative signals from the CGS/¹²³I imaging is correlated with measures of MMP expression and activity including immunohistochemistry, gelatin zymography, casein zymography, and collagenase activity assay. In addition, in particular for the smoke-influenza experiments, the signal of uptake 7 days post infection, the time of peak MMP-13 expression, is correlated with the degree of emphysema at the terminal endpoint.

Number of mice and statistical analysis

For each group of mice, a minimum of 8 mice are analyzed for imaging, the primary end point. An average for each type of measurements is calculated, with the standard deviation. The statistical analysis of the data is performed using the unpaired 2-tailed Student's t-test, with $p < .05$ considered significant. An identification of a 10-20% change in radiotracer uptake as a consequence of transgenic MMP expression or smoke and influenza exposure is preferred. In order to have an 80% power to detect a 20% difference in radiotracer uptake, a total of 8 animals is required in each subgroup ($\text{Alpha}=.05$, $1-\text{Beta}=.2$).

Results

The MMP-1 and MMP-9 transgenic mice have increased MMP signal on imaging as compared to their wild-type littermate controls (Table 1). In the smoke-influenza model system, wild-type mice have increased signal on imaging compared to baseline. This signal is absent or is reduced in the MMP13 knockout, depending on the potential induction of other MMPs in this model. This model system is able to detect differences between uninjured and injured mice.

Table 1. Signal strength of CGS/¹²³I in the MMP transgenic mice.

MMP Signal	Transgenic	Wild-Type
MMP-1 TG / WT	+	-
MMP-9 TG / WT	+	-
MMP-13 KO / WT Smoke-Influenza	-/+	+++

If the signal is not robust due to the low expression of MMP-9 in the MMP-9 macrophage mouse, a consequence may be that it takes a year for emphysema to develop in this model (Foronjy 2008). In this case, the animals are induced with an injury (Mehra 2010) increasing MMP-9 levels so as to detect a signal with imaging. An alternative is to perform quantitative studies by crossing the MMP-1 transgenic mice to generate homozygous MMP-1 transgenic mice, which would have double the production of MMP-1 (D'Armiento 1992). The CGS27023A is a broad-spectrum inhibitor, therefore the imaging is not specific to single MMPs.

Example 8:

The procedures of Examples 5, 6 and 7 are performed, except that CGS/¹²³I is replaced by radiolabeled Ro 32-3555 (described in Example 3). The results of radiolabeled Ro 32-3555 are similar to those of CGS/¹²³I, however, radiolabeled Ro 32-3555 is more selective.

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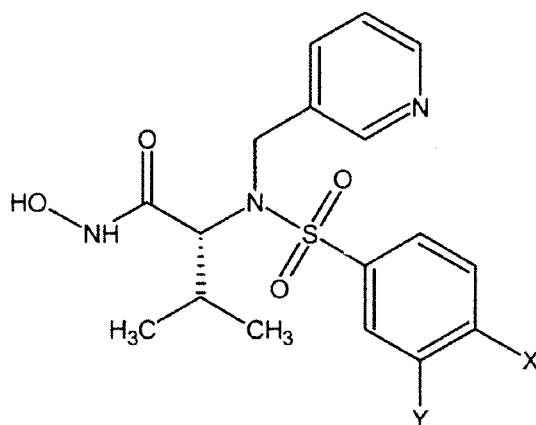
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What is claimed is:

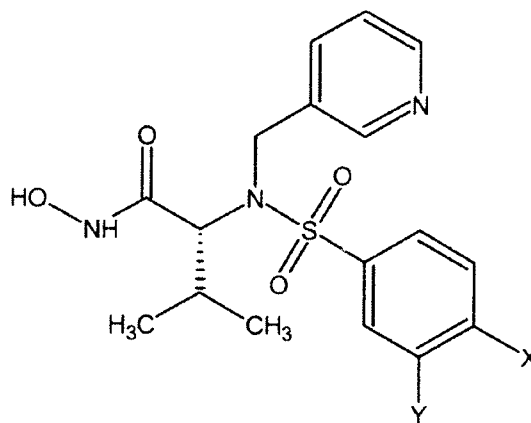
1. A method of imaging a subject's lung which comprises contacting the subject's lung with a matrix metalloproteinase inhibitor labeled with a radioisotope under conditions such that the inhibitor binds to matrix metalloproteinase in the lung, and then imaging the radiolabeled inhibitor bound to matrix metalloproteinase in the subject's lung.
2. The method of claim 1, wherein the matrix metalloproteinase inhibitor is Ro 32-3555 or a modified form of CGS27023A.
3. The method of claim 1 or 2, wherein the label is I-123.
4. The method of claim 2 or 3, wherein the matrix metalloproteinase inhibitor is Ro 32-3555.
5. The method of claim 2 or 3, wherein the matrix metalloproteinase inhibitor is a modified form of CGS27023A.
6. The method of claim 5, wherein the modified form of CGS27023A has the structure:



(1)

wherein X is OH and Y is H.

7. The method of claim 1, wherein the matrix metalloproteinase inhibitor labeled with a radioisotope has the structure:



(1)

wherein X is OH and Y is I-123.

8. The method of any one of claims 1-7, wherein the subject is suspected of being afflicted with a destructive lung disease.
9. The method of any one of claims 1-7, wherein the subject is afflicted with a destructive lung disease.
10. The method of any one of claims 8-9, wherein the destructive lung disease is chronic obstructive pulmonary disease (COPD), lymphangioleiomyomatosis (LAM), idiopathic pulmonary fibrosis (IPF), or acute lung injury (ALI).
11. The method of any one of claims 8-9, wherein the destructive lung disease is emphysema.
12. The method of any one of claims 1-11, wherein the imaging is single-photon emission computed tomography (SPECT) imaging.
13. The method of any one of claims 1-12, wherein the method further comprises administering to the subject an amount of the matrix metalloproteinase inhibitor labeled with a radioisotope.
14. The method of any one of claims 1-13, wherein the method further comprises imaging the subject's lung with a single-photon emission computed tomography (SPECT) scanner.
15. The method of any one of claims 1-14, wherein the method further comprises imaging the subject's lung with a computed tomography (CT) scanner.

16. The method of any one of claims 1-13, wherein the method further comprises imaging the subject's lung with a SPECT/CT scanner.
17. The method of claim 16, wherein the SPECT/CT scanner is a nano-SPECT/CT scanner.
18. The method of any one of claims 1-17, wherein method further comprises determining the amount of matrix metalloproteinase (MMP) expression in the lung of the subject.
19. The method of claim 18, wherein the MMP expression is selected from one or more of the group consisting of MMP-1 expression, MMP-9 expression, MMP-12 expression, and MMP-13 expression.
20. The method of any one of claims 1-19, further comprising determining the amount of the label in the lung of the subject.
21. The method of any one of claims 1-20, further comprising determining if the subject is afflicted with destructive lung disease.
22. The method of any one of claims 1-21, further comprising determining the degree of lung destruction in the subject.
23. The method of any one of claims 1-22, wherein the presence of MMP expression in the subject's lung in an amount that is higher than a reference amount indicates that the subject is afflicted with a destructive lung disease.
24. The method of claim 23, wherein the reference amount is based on the amount of MMP expression in a healthy subject's lung.
25. The method of any one of claims 1-22, wherein the presence of the label in the subject's lung in an amount that is higher than a reference amount indicates that the subject is afflicted with a destructive lung disease.
26. The method of claim 25, wherein the reference amount is based on the amount of the label in a healthy subject's lung.
27. The method of any one of claims 1-26, wherein the amount of MMP expression present in the subject's lung determines the degree of lung destruction in the subject.

28. The method of any one of claims 1-26, wherein the amount of the label present in the subject's lung determines the degree of lung destruction in the subject.
29. Ro 32-3555 labeled with a radioisotope.
30. The labeled Ro 32-3555 of claim 29 wherein the radioisotope is I-123.

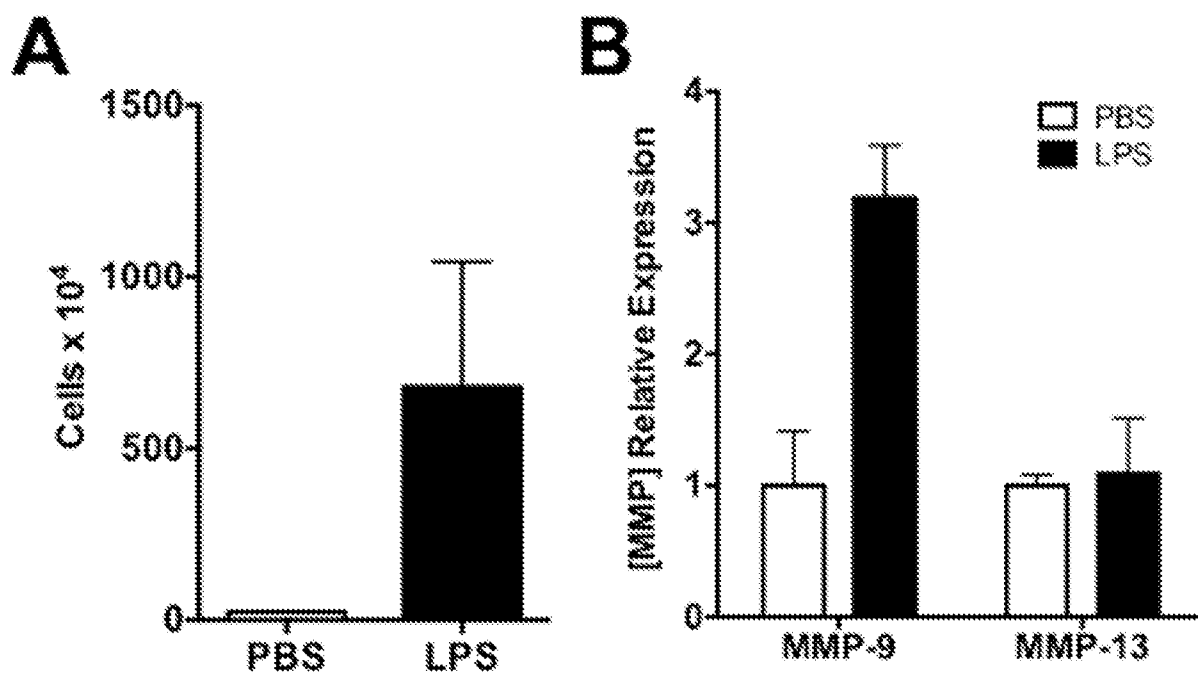


Fig. 1

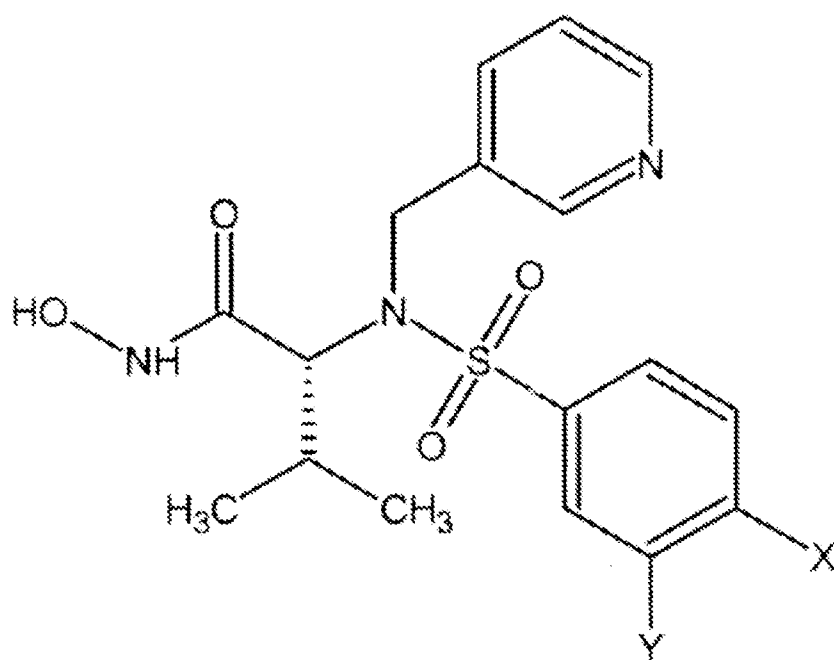


Fig. 2

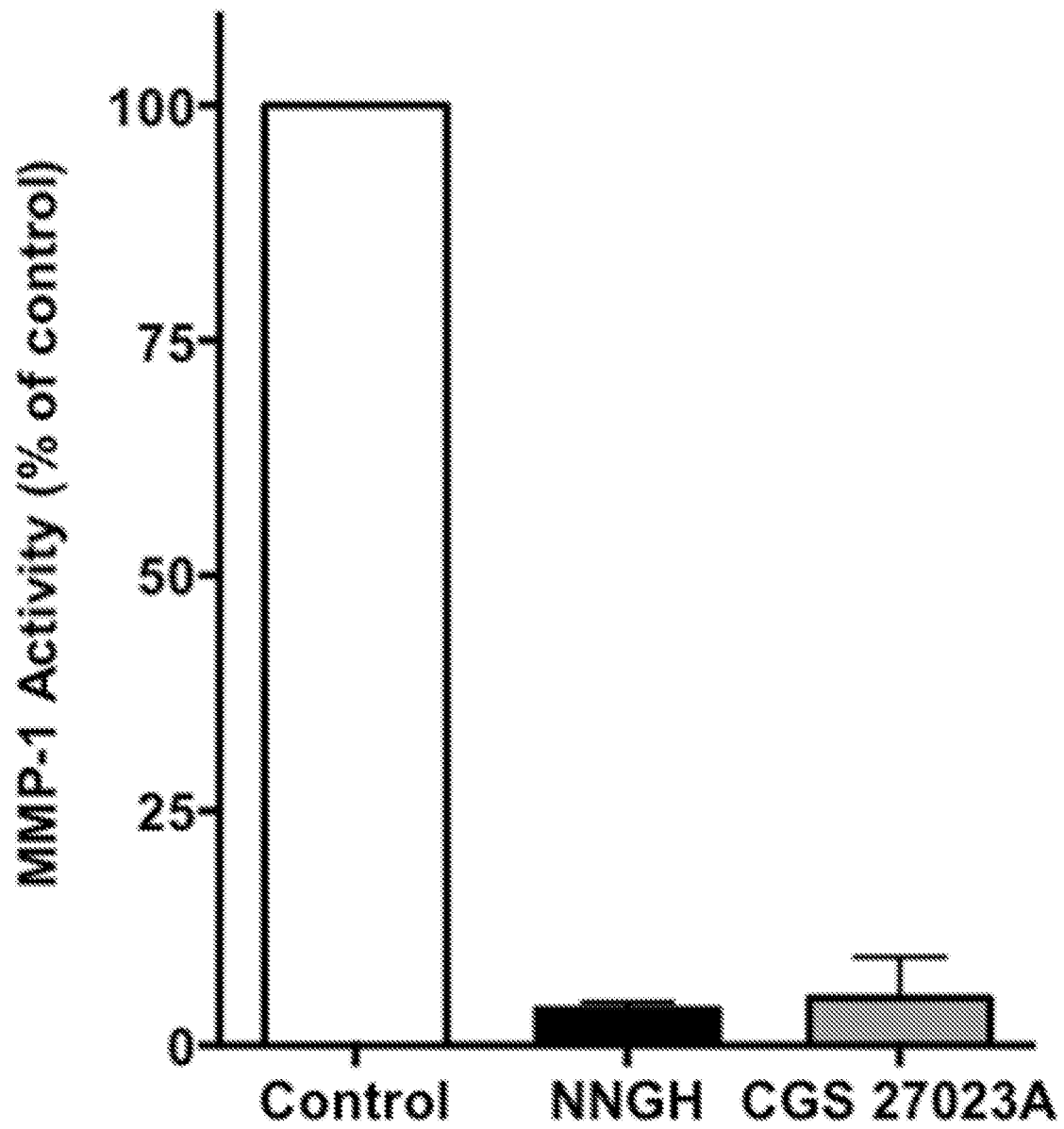


Fig. 3

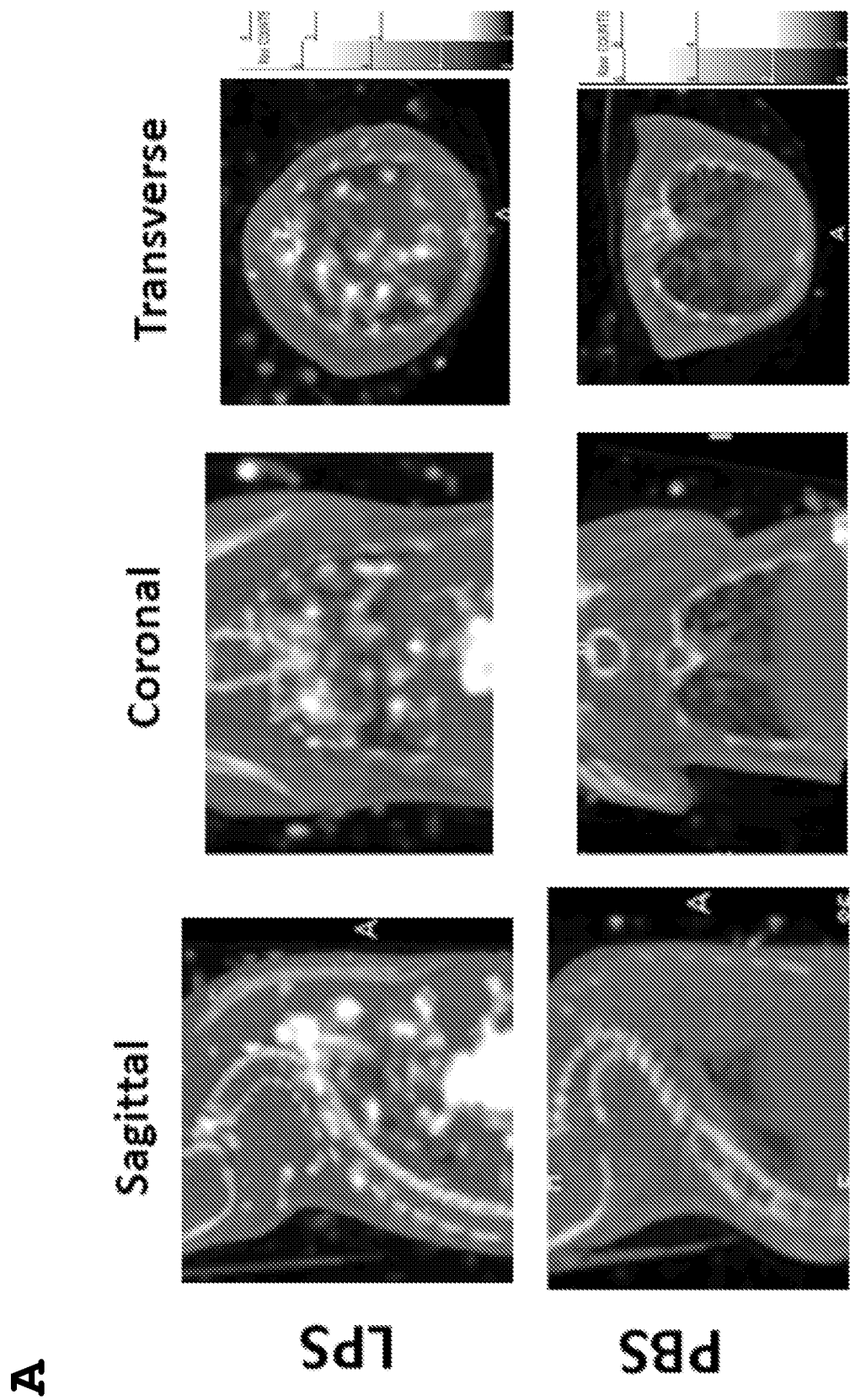


Fig 4

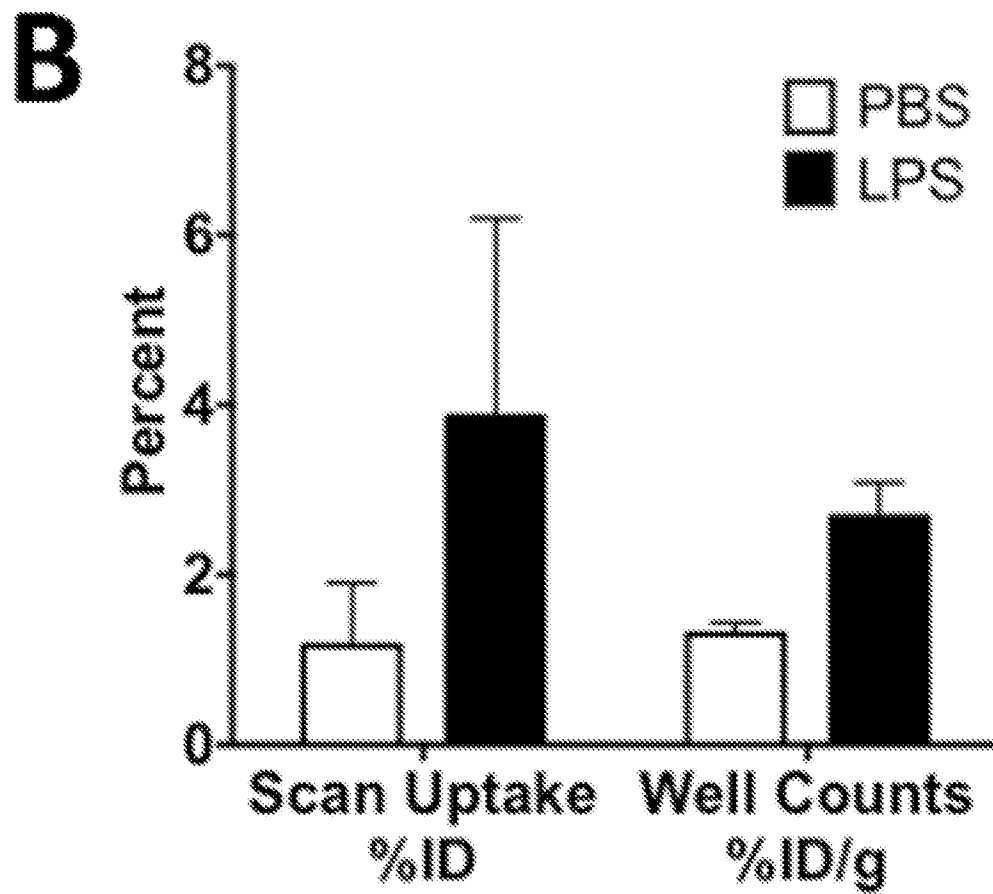


Fig. 4 (continued)

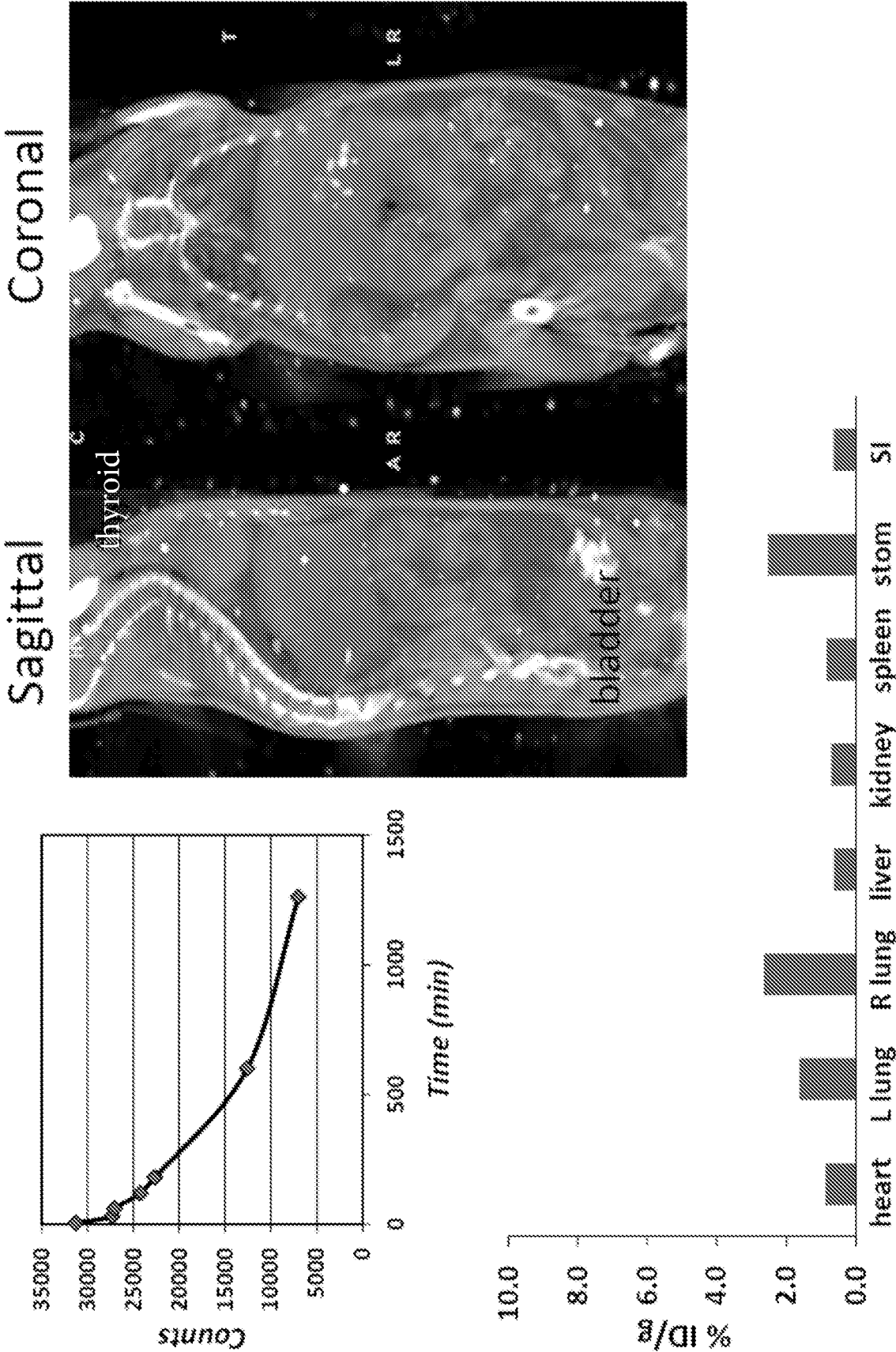


Fig. 5

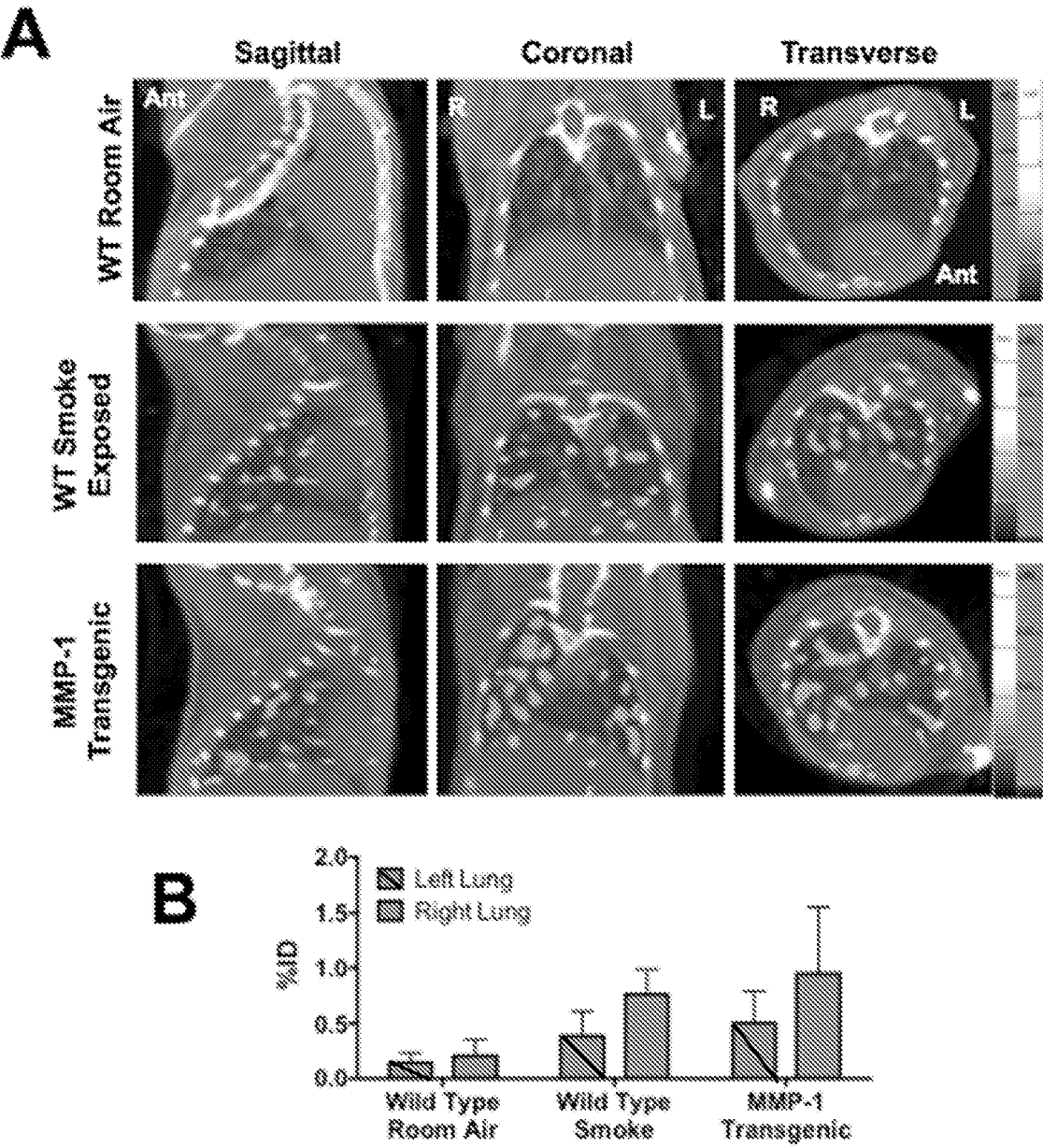


Fig. 6

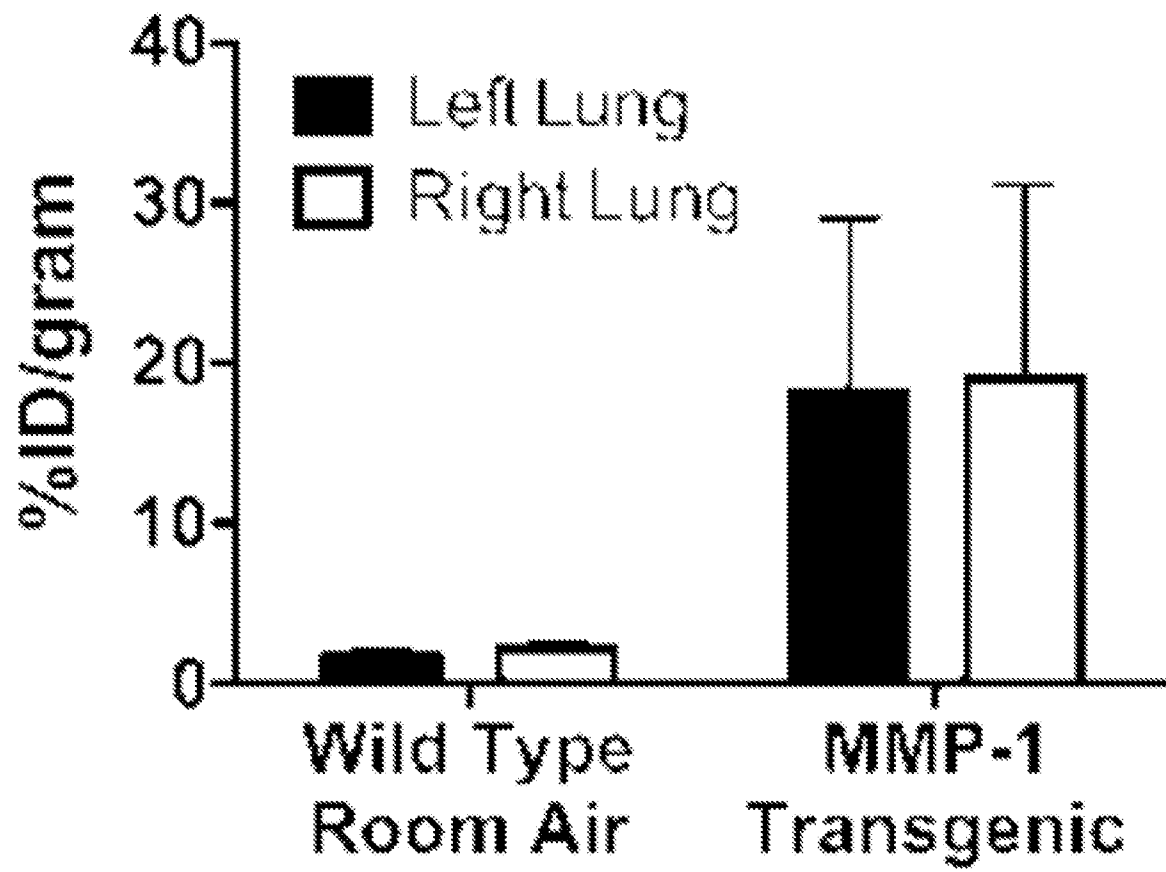


Fig. 7

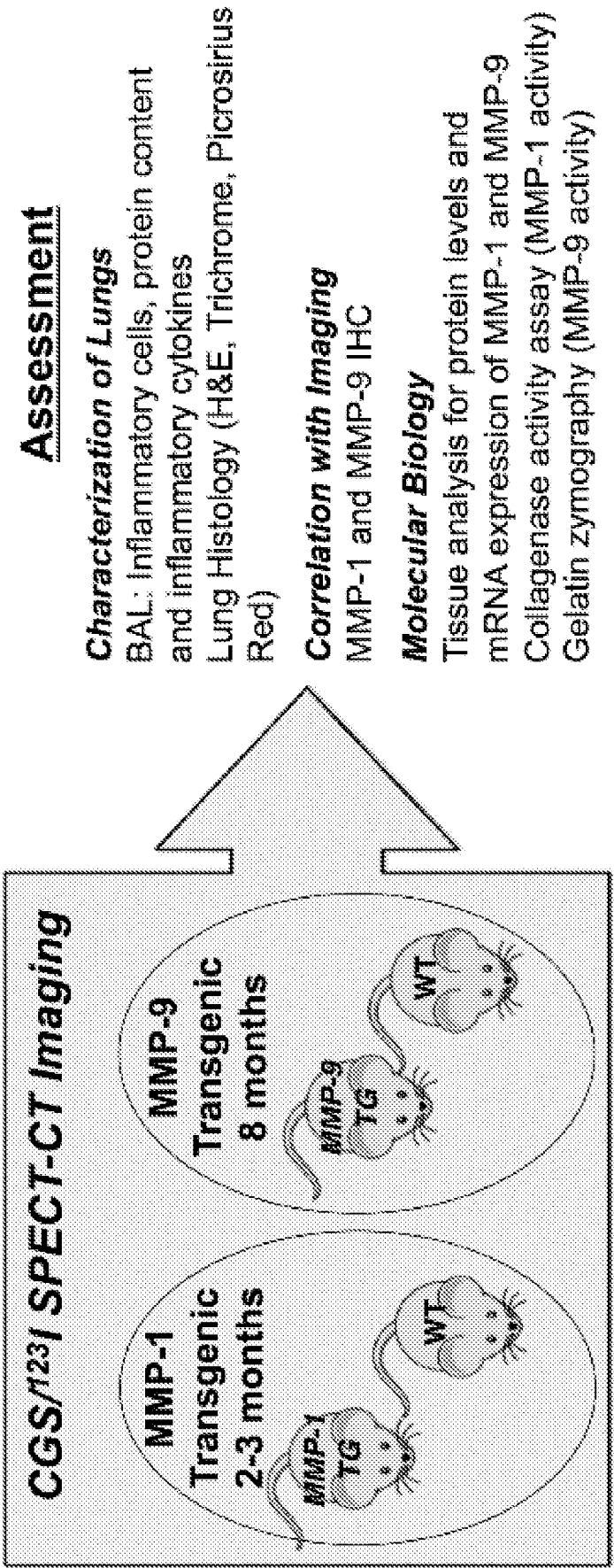


Fig. 8

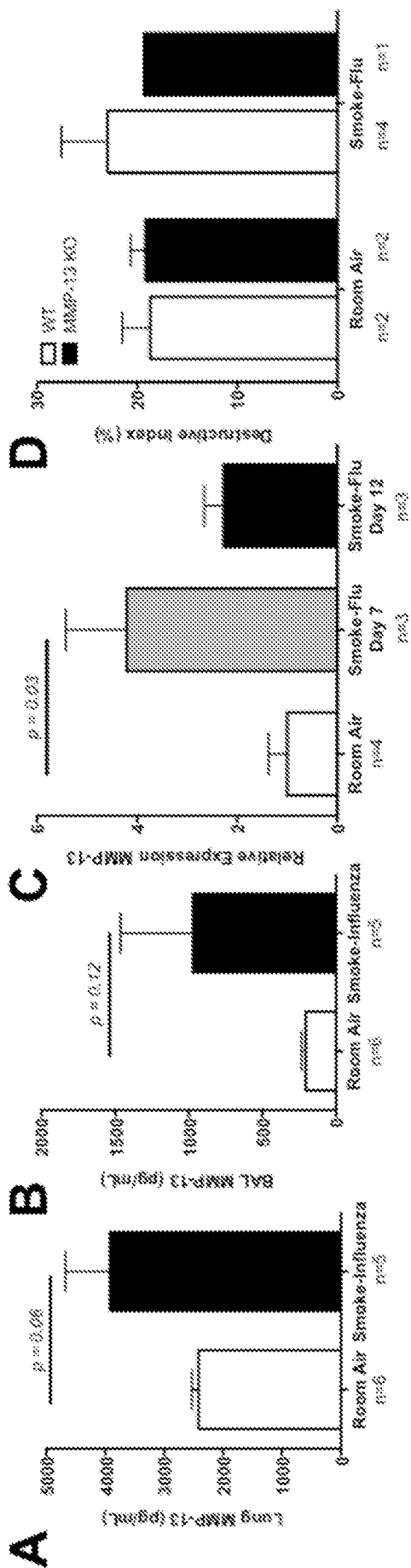


Fig. 9

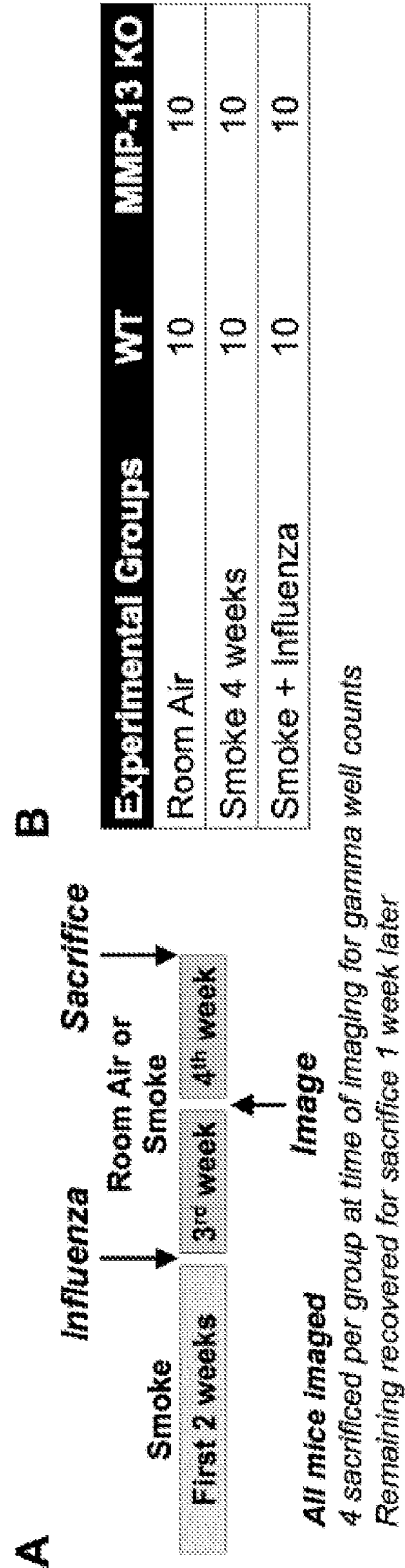


Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/33600

A. CLASSIFICATION OF SUBJECT MATTER
 IPC - A61K 51/12, 51/08; C07C 259/04 (2017.01)
 CPC - A61K 51/12, 51/08; C07C 259/04

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)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	~ Synthesis of Radiolabeled Biphenylsulfonamide Matrix Metalloproteinase Inhibitors as New Potential PET Cancer Imaging Agents (FEI, X et al) Bioorganic and Medicinal Chemistry Letters, 27 November 2002; figure 1, page 2217	29 --- 30
Y	~ Scintigraphic Imaging of Matrix Metalloproteinase Activity in the Arterial Wall In Vivo (SCHAFERS, M et al) Circulation, 11 March 2004; figures 1c and 1d, page 2555	1-2, 3/1-2, 7, 30
Y	~ The role of collagenases in experimental pulmonary fibrosis (OUCHI, H et al) Pulmonary Pharmacology and Therapeutics, 27 October 2007; pages 404-405	1-2, 3/1-2, 7

☐ 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 application or patent 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

28 July 2017 (28.07.2017)

Date of mailing of the international search report

22 AUG 2017

Name and mailing address of the ISA/

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 P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/33600

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.: 4-6, 8-28
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:

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

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.