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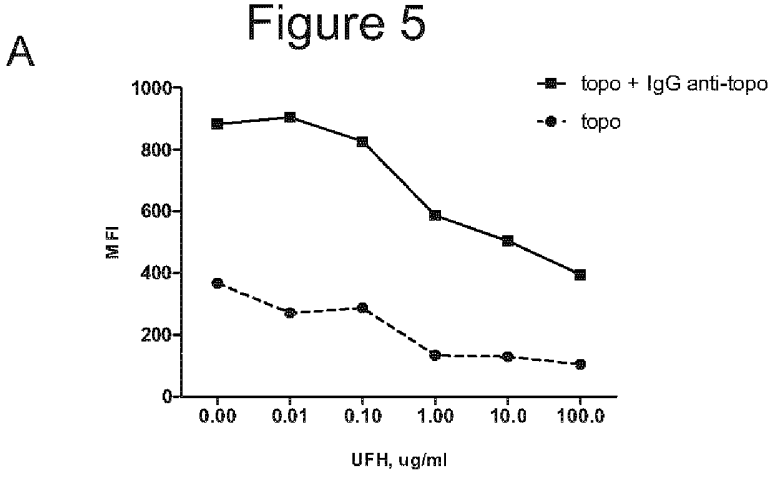
Figure 5



(57) Abstract: The present application shows that the topo/anti-topo immune complexes (IC) found in patients susceptible to systemic sclerosis (SSc) or afflicted with SSc mediate their action through binding heparan sulfate present on the surface of fibroblasts. Therefore, the present application provides a method for the prevention and/or treatment of SSc in a subject in need thereof and positive for anti-topo antibodies, wherein a preventive and/or therapeutic amount of a glycosaminoglycan compound is administered to prevent the docking of the topo/anti- topo IC on the surface of fibroblasts or to displace the topo/anti-topo IC from the surface of fibroblasts. Also provided are related methods for the determination of the usefulness of an agent for the prevention/treatment of SSc.

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GLYCOSAMINOGLYCAN-BASED THERAPY FOR THE PREVENTION/TREATMENT OF SYSTEMIC SCLEROSIS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. provisional patent application 61/485,301 filed on May 12, 2011. The content of this provisional patent application is incorporated herewith in its entirety.

BACKGROUND

Deposition of immune complexes (IC) is a prominent feature of several autoimmune diseases, including systemic lupus erythematosus (SLE), Sjögren syndrome and rheumatoid arthritis and is considered to mediate tissue injury and inflammation. Moreover, inhibition of IC binding to target cells has been shown to provide benefits in both *in vitro* and *in vivo* autoimmune models.

Autoantibodies to DNA topoisomerase I (anti-topo) are one of the major autoantibodies found in systemic sclerosis (SSc), an autoimmune disease characterized by inflammation, blood vessel damage and progressive interstitial and perivascular fibrosis. They are present in 20 to 30% of SSc patients and are associated with the diffuse form of the disease characterized by extensive cutaneous and lung fibrosis, and high mortality. Although anti-topo titers correlate with SSc activity and severity, their implication in the pathophysiology of SSc is still unclear. However, there is now growing evidence that they are not just silent markers of the disease.

A number of nuclear autoantigens, released from apoptotic or necrotic cells, have been found to bind to cell surfaces where they are targeted by autoantibodies (Henault et al., 2006; Robitaille et al., 2009; Jacob et al., 1989). One of these autoantigens is the nuclear enzyme topo, targeted in the diffuse form of the SSc. Previous reports have demonstrated that topo binds specifically to the surface of fibroblasts, which are key cells in fibrotic development, and recruits anti-topo autoantibodies from SSc patients, thus forming immune complexes on fibroblast surfaces. These fibroblasts were shown to induce adhesion and activation of monocytes (Henault et al., 2006). Thus, topo/anti-topo IC on fibroblast surfaces could contribute to the initiation of an inflammatory cascade.

There is thus a need to identify the cellular target responsible for the specific binding of topo to fibroblasts' external surfaces to inhibit such interaction and ultimately design a therapy for subjects susceptible to SSc or afflicted by SSc.

SUMMARY

The present application provides compounds useful in the treatment and/or prevention of SSc because they limit the association of topo/anti-topo immune complexes to the surface of fibroblasts, more specifically to the heparan sulfate present on the external surface of fibroblasts.

According to a first aspect, the present application provides a method for the prevention and/or treatment of systemic sclerosis (SSc) or a symptom thereof in a subject in need thereof and having anti-topoisomerase antibodies. Broadly, the method comprises administering a preventive and/or therapeutic amount of a glycosaminoglycan compound to the subject so as to prevent and/or treat SSc in the subject. In an embodiment, the glycosaminoglycan has (i) an hexosamine selected from the group consisting of α-D-N-acetylglucosamine (GlcNAc), α-D-N-sulfoglucosamine (GlcNS), α-D-N-acetylglucosamine-6-O-sulfate (GlcNAc(6S)), and α-D-N-sulfoglucosamine-6-O-sulfate (GlcNS(6S)); (ii) an hexose or hexuronic acid unit selected from the group consisting of β-D-glucuronic acid (GlcUA) and 2-O-sulfo-α-L-iduronic acid (IdoUA(2S)); and/or (iii) -4IdoUA(2S)α1-4GlcNS(6S)α1- as a predominant linkage between monomeric units. In another embodiment, the glycosaminoglycan is negatively charged. In yet another embodiment, the glycosaminoglycan is heparin. In still a further embodiment, the heparin is unfractionated heparin. In another embodiment, the glycosaminoglycan is derived from heparin, and still, in a further embodiment, the heparin is a low molecular weight heparin.

According to a second aspect, there is provided a glycosaminoglycan compound for the prevention of systemic sclerosis or a symptom thereof in a subject having anti-topoisomerase antibodies as well as a a glycosaminoglycan compound for the treatment of systemic sclerosis or a symptom thereof in a subject having anti-topoisomerase antibodies. Various embodiments of the glycosaminoglycan compounds that can be used have been described above and can be applied herewith.

According to a third aspect, there is provided the use of a glycosaminoglycan compound for the prevention of systemic sclerosis or a symptom thereof in a subject positive for anti-topoisomerase antibodies as well as the use of a glycosaminoglycan compound for the treatment of systemic sclerosis or a symptom thereof in a subject positive for anti-

topoisomerase antibodies. Various embodiments of the glycosaminoglycan compounds that can be used have been described above and can be applied herewith.

According to a fourth aspect, there is provided a method of characterizing an agent's ability to prevent systemic sclerosis (SSc) or a symptom thereof in a subject positive for anti-topoisomerase antibodies. Broadly, the method comprises combining the agent with an heparan sulfate derived from a fibroblast; combining the resulting mixture with an immune complex comprising a topoisomerase and an antibody specific for the topoisomerase; extracting a value of a binding level of the immune complex to the heparin sulfate in the presence of the agent; comparing the value of the binding level to a control value; and characterizing the agent. The agent is considered to have the ability to prevent SSc or the symptoms thereof in the subject when the measured value is lower than the control value. The agent is considered to lack the ability to prevent SSc or the symptoms thereof when the measured value is equal to or higher than the control value. In an embodiment, the heparan sulfate is presented on the surface of a fibroblast. In another embodiment, the topoisomerase is topoisomerase I. In still another embodiment, the method comprises contacting the immune complex and/or the agent with a fibroblast. In an embodiment, the immune complex is associated with a label, and , still in a further embodiment, the extracting comprises measuring the label associated with the immune complex. In an embodiment, the control value is at least one of the binding level of the immune complex in the absence of the agent, the binding level of the immune complex in the presence of a control agent that fails to prevent SSc or a symptom thereof in the subject and a pre-determined value of a binding level of the binding complex associated with a lack of prevention of SSc of a symptom thereof.

According to a fifth aspect, there is provided a method of characterizing an agent's ability to treat systemic sclerosis (SSc) or a symptom thereof in a subject having anti-topoisomerase antibodies. Broadly, the method comprises combining an immune complex comprising a topoisomerase and an antibody specific to the toposiomerase with an heparan sulfate derived from a fibroblast; combining the resulting mixture with the agent; extracting a value of a binding level of the immune complex to the heparin sulfate in the presence of the agent; comparing the value of the binding level to a control value; and characterizing the agent. The agent is characterized as having the ability to prevent and/or treat SSc in the subject when the value extracted is lower than the control value. The agent is characterized as lacking the ability to prevent and/or treat SSc when the value extracted is equal to or higher than the control value. In an embodiment, the heparan sulfate is presented on the surface of a

fibroblast. In another embodiment, the topoisomerase is topoisomerase I. In still another embodiment, the method comprises contacting the immune complex and/or the agent with a fibroblast. In an embodiment, the immune complex is associated with a label, and , still in a further embodiment, the extracting comprises measuring the label associated with the immune complex. In an embodiment, the control value is at least one of the binding level of the immune complex in the absence of the agent, the binding level of the immune complex in the presence of a control agent that fails to treat SSc or a symptom thereof and a pre-determined value of a binding level of the binding complex associated with a lack of treatment of SSc or a symptom thereof.

According to a sixth aspect, there is provided a screening system for characterizing an agent's ability to prevent and/or treat systemic sclerosis (SSc) or a symptom thereof in a subject having anti-topoisomerase antibodies. The screening system comprises a reaction vessel for combining the agent, an heparan sulfate derived from a fibroblast and an immune complex comprising a topoisomerase and an antibody specific for the topoisomerase; a processor in a computer system; a memory accessible by the processor; and at least one application coupled to the processor. The at least one application is configured for extracting a value of a binding level of the immune complex to the heparin sulfate in the presence of the agent; comparing the extracted value of the binding level to a control value; and characterizing the agent. The agent is characterized as having the ability to prevent and/or treat SSc in the subject when the extracted value of the binding level of the immune complex is lower than the control value. The agent is characterized as lacking the ability to prevent and/or treat SSc when the extracted value of the binding level of the immune complex is equal to or higher than the control value. In an embodiment, the heparan sulfate is presented on the surface of a fibroblast. In yet another embodiment, the topoisomerase is topoisomerase I. In still another embodiment, the screening vessel is for contacting the immune complex and the agent with a fibroblast. In another embodiment, the immune complex is associated with a label, and still in a further embodiment, the extracting step comprises measuring the label associated with the immune complex. In still another embodiment, the control value is at least one of the binding level of the immune complex in the absence of the agent, the binding level of the immune complex in the presence of a control agent that fails to prevent and/or treat SSc or a symptom thereof in the subject and a pre-determined value of a binding level of the binding complex associated with a lack of prevention and/or treatment of SSc or a symptom thereof.

According to a seventh aspect, the present application provides a software product embodied on a computer readable medium and comprising instructions for characterizing an agent's ability to prevent and/or treat systemic sclerosis or a symptom thereof in a subject having anti-topoisomerase antibodies. Broadly, the medium's instructions indicate a receiving module for receiving a value of a binding level of an immune complex comprising a topoisomerase and an antibody specific for the topoisomerase to an heparan sulfate derived from a fibroblast in the presence of the agent; a comparison module for determining if the value of the binding level of the immune complex in the presence of the agent is lower than, equal to or higher than a control value and generating a corresponding output; and a characterization module receiving the corresponding output from the comparison module and adapted to characterize the usefulness of the agent for preventing and/or treating SSc or the symptom thereof. The agent is characterized as having the ability to prevent and/or treat SSc or the symptom thereof in the subject when the value of the binding level of the immune complex is lower than the control value. The agent is characterized as lacking the ability to prevent and/or treat SSc in the subject when the value of the binding level of the immune complex is equal to or higher than the control value. In an embodiment, the heparan sulfate is presented on the surface of a fibroblast. In yet another embodiment, the topoisomerase is topoisomerase I. In still another embodiment, the immune complex is associated with a label. In a further embodiment, the extracting comprises measuring the label associated with the immune complex. In yet another embodiment, the control value is at least one of: the binding level of the immune complex in the absence of the agent, the binding level of the immune complex in the presence of a control agent that fails to prevent and/or treat SSc or a symptom thereof in the subject and a pre-determined value of a binding level of the binding complex associated with a lack of prevention and/or treatment of SSc or a symptom thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the nature of the invention, reference will now be made to the accompanying drawings, showing by way of illustration, a preferred embodiment thereof, and in which:

Fig. 1 illustrates how anti-topo autoantibodies from systemic sclerosis (SSc) patients increased topo binding to normal human dermal fibroblast (NHDF) surfaces. **A**, Results of flow cytometry analysis of topo-PE binding to NHDF with or without IgG purified from normal serum (left panel) or from anti-topo-positive SSc serum (right panel). The number of positive intact cells versus fluorescence intensity is presented. Controls were incubated with BSA-PE and are depicted as shaded area. Dotted lines represent topo-PE binding; solid lines

represent topo-PE/IgG binding. **B**, Time course of topo-PE binding to NHDF with or without anti-topo or normal IgG. Data are presented as the mean fluorescence intensity (MFI). Results are representative of at least 3 independent experiments. * = P≤ 0.0001 versus topo-treated cells. **C**, Results of flow cytometry analysis of topo-PE binding to NHDF with IgG from anti-topo-positive SSc sera (n=19), normal sera (n = 5), anti-Th/To positive SSc sera (n = 3), or anti-centromere (ACA)-positive SSc sera (n = 5). Data are presented as MFI. Topo binding to NHDF was significantly increased in the presence of IgG from anti-topo-positive SSc sera (* = P≤ 0.0001 versus normal and anti-topo-negative SSc sera).

Figure 2 illustrates the effects of IgG anti-topo on topo binding on NHDF surfaces. **A**, Autoantibodies to topo were determined by ELISA as outlined in the Examples below in individual sera from 19 selected anti-topo-positive SSc patients, 8 anti-topo-negative SSc patients and 5 normal controls. Shown are the correlation between MFI of topo-PE/IgG binding to NHDF surfaces obtained by flow cytometry analysis and optical density (O.D) values obtained by ELISA with all selected patients, r = 0.86 and P < 0.0001 . **B**, The efficacy of anti-topo depletion was confirmed by immunoblotting on purified topo. **C** and **D**, The effects of whole IgG anti–topo on topo binding to NHDF surfaces were compared with those of the corresponding anti-topo-depleted IgG, as determined using fluorescence microspcopy (**C**) and flow cytometry analysis (**D**). In **C**, Topo-Cy3 and IgG anti-topo (left panel; red) or anti-topo depleted IgG (right panel) were added to live, adherent, unfixed and non-permeabilized NHDF for 30 minutes. Also shown are Hoechst 33342-stained nuclei (blue). Bar = 20 µm. Results are representative of 3 different experiments and different anti-topo-depleted IgG. In **D**, each data point represents the mean of triplicate determinations for each IgG preparation (n = 4). * = P≤ 0.0057 versus whole IgG anti–topo.

Figure 3 illustrates the interaction of topo and topo/anti-topo immune complexes with heparan sulfate (HS) chains on NHDF surfaces. Shown are results of flow cytometry analysis (**A**) and fluorescence microscopy (**B**) of topo and topo/anti-topo immune complexes binding to HS on NHDF. NHDF were pretreated or not with heparitinase (Hep) for one hour and incubated with topo-PE (**A**) or topo-Cy3 (**B**) and IgG purified from anti–topo–positive SSc sera for 30 minutes. **A**, The expression of HS on NHDF was demonstrated with an antibody specific to HS chains and a PE-conjugated secondary antibody. HS chains were lost after Hep treatment. The CD51 expression level, an unrelated cell surface molecule, did not change with Hep treatment. The number of positive intact cells versus MFI is presented for untreated and Hep-treated cells. Values are the mean and SEM. * = P≤ 0.007 versus untreated cells. Results are representative of 3 independent experiments. **B**, Topo-Cy3 (red)

and IgG purified from anti–topo–positive SSc sera were added to live, adherent, unfixed and non-permeabilized NHDF for 30 minutes (**A** and **B**). The expression of HS on NHDF was demonstrated with an antibody specific to HS chains and a FITC-conjugated secondary antibody (green) (**C** and **D**). Also shown are Hoechst 33342-stained nuclei (blue). Bar = 20 μm. Results are representative of 3 different experiments.

Figure 4 illustrates how unfractionated heparin (UFH) and low molecular weight heparin (LMWH) block the interaction of topo/IgG anti-topo immune complexes with HS chains on NHDF surfaces. Shown are results of flow cytometry analysis (**A** and **B**) and fluorescence microscopy (**C**) of the effects of UFH and LMWH (daltepatin, enoxaparin and fondaparinux) on topo/anti-topo immune complexes binding to NHDF. **A**, NHDF were incubated with topo-PE either alone or with IgG purified from anti-topo positive SSc serum for 30 minutes at 25°C. Where required, UHF (10 mg/ml) were added to the cells simultaneously with topo or topo/anti-topo IgG immune complexes. The number of positive intact cells versus fluorescence intensity is presented. Controls were incubated with BSA-PE and are depicted as shaded area. Solid black lines represent topo-PE binding; dotted black lines represent topo-PE binding treated with UFH; solid red lines represent topo-PE/IgG anti-topo binding; dotted red lines represent topo-PE/IgG anti-topo binding treated with UFH. **B**, NHDF were incubated with topo-PE/IgG anti-topo and with increasing concentrations of UFH or LMWH for 30 minutes. Data are presented as MFI. Results are representative of at least 3 independent experiments. **C**, NHDF were incubated with topo-Cy3/IgG anti-topo (red) simultaneously with UFH or dalteparin (10mg/ml) for 30 minutes. Also shown are Hoechst 33342-stained nuclei (blue). Bar = 20 μm. Results are representative of 3 different experiments.

Figure 5 illustrates the dissociation of topo/IgG anti-topo immune complexes from HS chains on NHDF surfaces by UFH and LMWH. **A**, NHDF were first incubated with topo-PE either alone or simultaneously with IgG anti-topo autoantibodies for 30 minutes, followed by a second incubation of increasing concentrations of UFH for another 30 minutes to disrupt topo-PE/IgG anti-topo immune complexes binding on NHDF surfaces. Data are presented as the MFI and are representative of at least 3 independent experiments. **B**, The disrupting effect of UFH on topo/anti-topo immune complexes binding to NHDF surfaces was compared to those of several LMWH. In **C**, the concentration of UFH and dalteparin used was 10 mg/ml and each data point represents the mean of duplicate determinations for each IgG preparation (n = 8). ** = $P \leq 0.0067$ and * = $P \leq 0.0179$ versus untreated.

Figure 6 illustrates the proposed signaling mechanism showing the pathogenic role of topo/IgG anti-topo immune complexes on fibroblasts as a contributor to the initiation of fibrosis in SSc patients (left) as well as the effects of its perturbation by UFH or LMWH treatments (right).

DETAILED DESCRIPTION

In accordance with the present invention, a therapy is provided for systemic sclerosis (SSc) patients based on the administration of agents capable of inhibiting the interaction between an immune complex (IC) and a fibroblast. This therapy is particularly useful when the IC are constituted by DNA topoisomerase I (topo) and their corresponding autoantibody (anti-topo). The topo/anti-topo IC can be found in different patients susceptible to or afflicted with SSc but are usually found in patients suffering from the diffuse form of SSc.

As known in the art, topo/anti-topo IC appear to contribute to the pathogenesis of SSc by binding to the surface of fibroblasts. The inhibition of this interaction would be beneficial for the prevention and treatment of SSc as well as to the alleviation of symptoms associated with SSc.

As shown herein, topo/anti-topo IC bind specifically to the heparan sulfate present on the surface of the fibroblast. The anti-topo autoantibody even increased the binding of topo to the surface of fibroblasts in a heparan-sulfate dependant manner. As also shown herein, agents have been identified of being capable of inhibiting the interaction between topo/anti-topo IC and fibroblast's surface heparan sulfate and even displacing the IC from the surface of the fibroblasts. The inhibition of such interaction between the IC and the fibroblast's heparan sulfate is thought to impede or limit the opsonization of fibroblasts, therefore inhibiting the induction of the pro-fibrotic and pro-inflammatory fibroblast phenotype and, ultimately, preventing fibrosis associated with SSc.

I – Method of prevention and treatment of SSc

The present application provides a method of treating and/or preventing SSc. Also referred to as scleroderma, SSc is a systemic autoimmune disease. SSc can be subcategorized into two forms, a first limited form and a second diffuse form, the latter affecting more tissues and organs as well as being more aggressive. One of the main aspects of the pathophysiology of this disease is the overproduction of collagen in connection with an autoimmune dysfunction. T cells accumulate in the skin and are thought to secrete cytokines and other proteins that stimulate collagen deposition. Stimulation of the fibroblast, through opsonization, and

monocyte recruitment, appears crucial to the disease process. Damage to endothelium is an early abnormality in the development of SSc and this also seems to be due to collagen accumulation by fibroblasts, although direct alterations by cytokines, platelet adhesion and a type II hypersensitivity reaction have similarly been implicated. Increased endothelin and decreased vasodilation have been documented.

The present application provides a method for treating/preventing SSc. As used herein, the terms “treating”, “treat” or “treatment” refer to the ability of the method to limit or stop the progression of the disease, alleviate symptoms associated with the disease (for example, skin thickening due to fibrosis), and, in certain instances, may also reverse a pathophysiologic damage associated with SSc (such as fibrosis). As used herein, the terms “preventing”, “prevent” or “prevention”, refer to the ability of the method to preclude the onset of SSc in the treated subject.

The present application also provides a method of preventing the onset of at least one symptom and/or limiting the severity of at least one symptom associated with SSc. As used in this context, the term “preventing” refers to the ability of the method to preclude the onset of at least one symptom of SSc. On the other hand, “limiting” indicates that the therapy is capable of alleviating at least one symptom associated with SSc, either by stopping or delaying the appearance of such symptoms, limiting the severity of such symptom, etc. The present method can be used to alleviate a single symptom or a combination of symptoms associated with SSc. The symptoms alleviated can concern a single organ or tissue or a plurality of organs and tissues.

SSc is a systemic disease and as such involves various organs and tissues. In the skin, SSc causes thickening, hardening, scarring and itching. Blood vessels (capillaries) may also become more visible. When large areas are affected, fat and muscle wastage may weaken limbs and affect appearance. Most patients also have vascular symptoms such as Raynaud's phenomenon, which leads to attacks of discoloration of the hands and feet in response to cold. Raynaud's usually affects the fingers and toes. SSc can cause painful ulcers on the fingers and toes which are known as digital ulcers. Calcinosis (deposition of calcium in lumps under the skin) is also common in SSc, and is often seen near the elbows, knees or other joints.

In the musculoskeletal system, the first symptoms that patients with SSc have are typically joint pains, which can progress to arthritis. Pain in tendons and muscles may also be present. Joint mobility, especially of the small joints of the hand, may be restricted by skin

thickening and calcinosis. Patients may develop muscle weakness due to myositis (inflammatory myopathy),.

Some impairment in lung function is almost universally seen in patients with diffuse SSc on pulmonary function testing. Deposits of collagen in the lungs leads to pulmonay fibrosis. Patients with anti-topo are at a significantly greater risk for severe pulmonary fibrosis, a potential cause of mortality. Some patients can develop pulmonary hypertension, *i.e.* elevation in the pressures of the pulmonary arteries. This can be progressive, and lead to right sided heart failure. The earliest manifestation of this may be a decreased diffusion capacity on pulmonary function testing. Other pulmonary complications in more advanced disease include aspiration pneumonia, pulmonary hemorrhage and pneumothorax.

In the digestive tract, peptic stricture can be observed and associated with chronic gastroesophageal reflux and/or dysphagia. Diffuse SSc can affect any part of the gastrointestinal tract. The most common manifestation in the esophagus is reflux esophagitis, which may be complicated by peptic stricturing, or benign narrowing of the esophagus. SSc can also decrease motility anywhere in the gastrointestinal tract due to fibrosis. As SSc progresses, decreased esophageal motility may worsen due to further progressive fibrosis (scarring). If this is left untreated, acid from the stomach can back up into the esophagus causing esophagitis, and GERD. The small intestine can also become involved, leading to bacterial overgrowth and malabsorption, of bile salts, fats, carbohydrates, proteins, and vitamins. The colon can be involved, and this involvement can cause pseudo-obstruction or ischemic colitis. Rarer complications include pneumatosis cystoides intestinalis, or gas pockets in the bowel wall, wide mouthed diverticula in the colon, and liver fibrosis. SSc may also be associated with gastric antral vascular ectasia (GAVE), also known as watermelon stomach.

Renal involvement, in SSc, is a poor prognostic factor and frequently a cause of death. The most important clinical complication of SSc involving the kidney is scleroderma renal crisis. Symptoms of renal crisis are malignant hypertension (high blood pressure, often with evidence of multiorgan acute damage to the kidneys, heart, brain and retina), hyperreninemia (high renin levels), azotemia (kidney failure with accumulation of waste products in the blood) and microangiopathic hemolytic anemia (destruction of red blood cells).

The therapy described herein is designed on the inhibition/limitation of the interaction between topo/anti-topo IC and the heparan sulfate present on the surface of fibroblasts. It is

known in the art that the association between topo/anti-topo IC with fibroblasts is pivotal to fibroblast opsonization, induction of pro-fibrotic and pro-inflammatory fibroblastic phenotype and, ultimately, to fibrosis. Therefore, by inhibiting or limiting the interaction between the topo/anti-topo IC and the heparan sulfate at the fibroblast's surface , the method described herein prevents the IC to mediate their immunomodulatory actions and limits the pathological consequences associated with SSc. The present application further supports the important role of the anti-topo autoantibody in the association of topo/antitopo IC to the fibroblast's surface. The present application also clearly shows that this association can either be prevented or reversed by a therapeutic agent and, ultimately prevent or halt the fibroblast's activation. Therefore, it is believed that the administration of an agent capable of preventing the topo/anti-topo IC from associating with the heparan sulfate on the surface of a fibroblast and/or capable of dissociating the topo/anti-topo IC from the heparan sulfate on the surface of the fibroblast will be useful for preventing, treating and/or alleviating the symptoms (at least one) associated with SSc.

Consequently, the present application provides a method of preventing and/or treating SSc in a subject in need thereof. In order to do so, an agent capable of preventing the topo/anti-topo IC from associating with the heparan sulfate on the surface of a fibroblast and/or capable of dissociating the topo/anti-topo IC from the heparan sulfate on the surface of a fibroblast is administered to the individual. This method likely prevents, treats SSc and/or alleviates at least one symptom associated with SSc in the subject. In a preferred embodiment, the subject that is being treated is positive for anti-topo autoantibodies. In a further embodiment, the agent that is being administered has been identified by the screening method described herein. In addition, in order to optimize therapy, it is possible to administer the agent only to subjects with anti-topo antibodies having already been diagnosed with a predisposition to SSc or having been diagnosed with SSc.

Diagnosis of SSc is usually achieved by clinical evaluation. In addition, the presence of autoantibodies is also useful in the diagnosis of SSc. Patients suffering from SSc may have autoantibodies specific for anti-topo, anti-Th/To, anti-centromere (anti-CENP-B) and anti-RNA polymerase III. Anti-Th/To antibodies, found in 3–5% of patients with systemic sclerosis (SSc), recognise a nucleolar 7–2/8–2 RNA-protein complex. They are associated with limited SSc (lSSc) with lung involvement and reduced survival comparing with anticentromere (ACA) (+) patients. However, clinical studies on anti-Th/To are limited due to poor availability of serological tests. Anti-centromere antibody is more common in limited SSc whereas, anti-topo is more common in diffuse SSc. In an embodiment, the subject is first diagnosed with

SSc before being treated by the method presented herewith. In addition, since Raynaud's phenomenon and the appearance of anti-topo antibodies typically precede the onset of other symptoms of SSc, often by several months or years, it might be advisable to determine if the subject with Raynaud's phenomenon is positive for anti-topo autoantibody before being treated with the agent. In yet a further embodiment, it may be advisable to treat a subject with Raynaud's phenomenon positive for anti-topo even though no diagnosis of SSc has yet been made in order to prevent the onset of SSc or limit the symptoms eventually associated thereto.

Up to date, there is no cure for SSc. In addition, no anti-fibrotic drug has been shown to be effective in SSc. This there is a major need for the identification of novel anti-fibrotic therapeutics. However, some drug-based therapies are used for alleviating the symptoms of SSc. For example, these drug-based therapies include, but are not limited to, ACE inhibitors which are used to treat SSc renal crisis. Other drugs include proton pump inhibitors, to treat GERD, and prednisone, to treat arthritis and myositis. The agents described herein can be administered in combination with these drug-based therapies. For example, The agents described herein can be administered prior to these other drug-based therapies, in conjunction with theses other drug-based therapies and/or after the administration of these other drug-based therapies.

For alleviating some symptoms of SSc, medical interventions are often required. These medical interventions include, but are not limited to insertion of a permanent pacemaker, lung and heart transplantation, dialysis, and renal transplantation. The agents described herein can be administered in combination with these medical interventions. For example, the agents described herein can be administered prior to these medical interventions, in conjunction with these medical interventions and/or after these medical interventions.

In an embodiment, the agent is a glycosaminoglycan. As shown in the present application, members of the glycosaminoglycan family are capable of preventing the association between topo/anti-topo IC and the heparan sulfate and even displace topo/anti-topo IC from the heparan sulfate. Glycosaminoglycans are unbranched polysaccharides consisting of a repeating disaccharide unit. The repeating unit consists of a hexose or a hexuronic acid linked to a hexosamine. Members of the glycosaminoglycan family vary in the type of hexosamine, hexose or hexuronic acid unit they contain (*e.g.* glucuronic acid, iduronic acid, galactose, galactosamine, glucosamine). They also vary in the geometry of the glycosidic linkage. Members of the glycoasminoglycan family include, but are not limited to chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin, heparan sulfate and hyaluronan. In the

therapeutic methods described herein, it is contemplated that a single type of glycosaminoglycan can administered. It is also contemplated that a mixture of different glycosaminoglycans can be administered in combination (simultaneously, sequentially, etc.).

Heparan sulfate. Proteoglycans (PG) are abundant molecules on the cell surfaces of numerous cells. They represent low affinity but high capacity receptors that facilitate the presentation of a ligand to its high affinity receptor, thus acting as coreceptors. They are composed of disaccharide repetitions with various sulphated regions and exhibit great molecular diversity which confers to them cell and tissue-specificity. The PG that most frequently act as coreceptors for a number of ligands are the heparan sulphate PG (HSPG) family. Heparan sulfate is highly similar in structure to heparin, however heparan sulfates disaccharide units are organised into distinct sulfated and non-sulfated domains. The hexuronic acid/hexose moieties composing heparan sulfate can be β-D-glucuronic acid (GlcUA), α-L-iduronic acid (IdoUA) and 2-O-sulfo-*α*-L-iduronic acid (IdoUA(2S)). The hexosamine moiety composing heparan sulfate can be α-D-N-acetylglucosamine (GlcNAc), α-D-N-sulfoglucosamin (GlcNS), α-D-N-acetylglucosamine-6-O-sulfate (GlcNAc(6S)) and α-D-N-sulfoglucosamine-6-O-sulfate (GlcNS(6S)). The predominant glycosidic linkage between monomeric subunits in heparan sulfate is -4GlcUAβ1-4GlcNAcα1-. The most common disaccharide unit within heparan sulfate is composed of a glucuronic acid (GlcA) linked to N-acetylglucosamine (GlcNAc) typically making up around 50% of the total disaccharide units.

Heparin. Heparin is a naturally-occurring polysaccharide. Natural heparin consists of molecular chains of varying lengths, or molecular weights. Chains of molecular weight from 5 000 to over 40 000 (more preferably 6 000 to 20 000) Daltons make up polydisperse pharmaceutical-grade heparin (also referred to as unfractionated heparin). Heparin is a highly acidic mucopolysaccharide formed of equal parts of sulfated D-glucosamine and D-glucuronic acid with sulfaminic bridges. More specifically, the hexosamine moiety composing heparin can be α-D-N-acetylglucosamine (GlcNAc), α-D-N-sulfoglucosamine (GlcNS), α-D-N-acetylglucosamine-6-O-sulfate (GlcNAc(6S)), and α-D-N-sulfoglucosamine-6-O-sulfate (GlcNS(6S)). The hexose/hexuronic acid unit composing heparin can be β-D-glucuronic acid (GlcUA) and 2-O-sulfo-α-L-iduronic acid (IdoUA(2S)). The predominant glycosidic linkage between monomeric subunits in heparin is -4IdoUA(2S)α1-4GlcNS(6S)α1-. The most common disaccharide unit within heparin is IdoA(2S)-GlcNS(6S) makes up 85% of heparins from beef lung and about 75% of those from porcine intestinal mucosa. Heparin can be derived from natural sources, mainly animal liver, intestine, lung or mast cells.

Heparin-derived products: low-molecular-weight heparins (LMWHs). Low-molecular-weight heparins (LMWHs) consist of only short chains of polysaccharide (Jestke et al., 2008). LMWHs are defined as heparin salts having an average molecular weight of less than 10 000 Da, 9 000 Da, 8 000 Da, 7 000 Da or even less than 6 000 Da. In an embodiment, at least 60% of all polymers of the LMWHs have a molecular weight of less than 10 000 Da, 9 000 Da, 8 000 Da, 7 000 Da or even less than 6 000 Da. In an embodiment, the LMWHs have a range of molecular weight having a lower limit of 2 000 Da, 3 000 Da or even 4 000 Da and an upper limit of at least 6 000 Da, 7 000 Da, 8 000 Da, 9 000 Da or even 10 000 Da. In another embodiment, the LMWHs have an average molecular weight of about 3 000 Da, 3 500 Da, 4 000 Da, 4 500 Da, 5 000 Da, 5 500 Da, or 6 500 Da (± 500 Da). In a preferred embodiment, the LMWHs has a range of molecular between 2 000 and 10 000 Da and an average molecular weight of about 5 000 Da (± 1 000 Da) These are obtained by various methods of fractionation or depolymerisation of polymeric heparin. Various methods of heparin depolymerisation are used in the manufacture of low-molecular-weight heparin. The most common LMWH are listed below:

Table. Biochemical properties of various LMWH

Process for making LMHW	Average MW or molecular mass	Generic Name of LMWH	Trade name of LMWH
Oxidative depolymerisation with hydrogen peroxide	6 kDa	ardeparin	Normiflo™
Deaminative cleavage with isoamyl nitrite	5 KDa	certoparin	Sandoparin™ Embolex™
Alkaline beta-eliminative cleavage of the benzyl ester of heparin	4 KDa	enoxaparin	Lovenox™ Clexane™ Indenox™
Oxidative depolymerisation with Cu^{2+} and hydrogen peroxide	3.1 KDa	parnaparin	Fluxum
Beta-eliminative cleavage by the heparinase enzyme	4.5 kDa	tinzaparin	Innohep™ Logiparin™
Deaminative cleavage	5.6 kDa 4.3 KDa	dalteparin(*) reviparin	Fragmin™ Clivarin™

Process for making LMHW	Average MW or molecular mass	Generic Name of LMWH	Trade name of LMWH
with nitrous acid	4.2 KDa 5.0 kDa	nadroparin certoparin	Fraxiparin™

(*) In Dalteparin, 14-26% of the polymer have a molecular weight higher than > 8000 Da.

LMWH can also be produced by deaminative cleavage with nitrous acid results in the formation of an unnatural anhydromannose residue at the reducing terminal of the oligosaccharides produced. This can subsequently be converted to anhydromannitol using a suitable reducing agent. Likewise both chemical and enzymatic beta-elimination result in the formation of an unnatural unsaturated uronate residue (UA) at the non-reducing terminal.

It is generally recognized in the art that the N-sulfate group content of heparin-derived products (such as LMWH) largely exceeds that of N-acetyl groups and the concentration of O-sulfate groups exceeds those of N-sulfate.

The main differences between heparin (i.e. “unfractioned heparin”) and LMWH include, but are not limited to:

- Average molecular weight: heparin is about 15 kDa and LMWH is about 5 kDa;
- No need for monitoring of the APTT coagulation parameter for LMWH in clinical practice although some LMWH may have effect on the activated cephalin time measurement;
- Possibly a smaller risk of bleeding, osteoporosis, heparin-induced thrombocytopenia for LMWH;
- The anticoagulant effects of heparin are typically reversible with protamine sulfate, while protamine is less effective on LMWH.
- LMWH has less of an effect on thrombin compared to heparin, but maintains the same effect on Factor Xa. However, the effect on thrombin and Factor Xa may vary from one LMWH to another.

Chemically synthesized LMWH. Fondaparinux (Arixtra™) is an anticoagulant medication chemically related to low molecular weight heparins. Fondaparinux is a synthetic

pentasaccharide. Its molecular mass is 1726.77 g/mol. In the present application, fondaparinux has been shown to lack the ability to prevent the association of the topo/anti-topo IC to the heparan sulfate or to displace the topo/anti-topo IC from the heparan sulfate.

Fondaparinux is very similar to known GAGs, the identity and sequence of its five monomeric sugar units contained is identical to a sequence of five monomeric sugar units that can be isolated after either chemical or enzymatic cleavage of the polymeric glycosaminoglycans heparin and heparan sulfate (HS). Fondaparinux has a relatively low molecular weight with an average molecular weight between 1.7 and 2 kDa. Fondaparinux differs from LMWH, heparin and heparan sulfate with respect to the identity of its reducing end (an O-methyl group). Further, fondaparinux binds with a higher affinity to antithrombin III (ATIII) when compared to heparin or LMWH (1 000 fold increase). As shown herewith, fondaparinux has been shown to have little to no effects to prevent the association of the topo/anti-topo IC to the heparan sulfate or to displace the topo/anti-topo IC from the heparan sulfate. Without wishing to be bound to theory, these results suggest that fondaparinux may be too small to interfere with the topo/anti-topo and heparan sulfate interaction. It can also suggest that the presence of an o-methyl group at its reducing end limits its ability to interfere with the topo/anti-topo and heparan sulfate interaction. It can further suggest that the carbohydrate moieties forming fondaparinux probably cannot bind to topo/anti-topo IC. Consequently, in an embodiment, fondaparinux is specifically excluded from the GAG compounds described here to be useful for the prevention/treatment of SSc or the alleviation of at least one symptoms associated thereto. In an embodiment, GAG compounds having an O-methyl group at their reducing end are also considered not to be useful for the treatment and/or prevention of SSc. In yet a further embodiment, GAG compounds having an increased affinity to ATIII when compared to heparin (at least 100 fold and preferably at least 1000 fold increase) are also considered not to be useful for the treatment and/or prevention of SSc.

Pharmaceutical compositions. The GAG-based agents presented herewith can be administered as a pharmaceutical composition. Such compositions usually comprise a “pharmaceutical carrier”. As used herein, a “carrier” or “pharmaceutical carrier” is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more active agent(s) to an animal (such as a human), and is typically liquid or solid. A pharmaceutical carrier is generally selected to provide for the desired bulk, consistency, etc., when combined with components of a given pharmaceutical composition, in view of the intended administration mode. Typical pharmaceutical carriers include, but are not limited to binding agents (e.g., pregelatinized maize starch,

polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycotate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc.).

GAG-based agents disclosed herewith may be administered with a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such compositions to patients. Although oral administration is preferred, any appropriate route of administration may be employed, for example, subcutaneous, topical, intra-dermal, intravenous, parenteral, intramuscular, intraperitoneal, intranasal, or aerosol administration. Therapeutic formulations may be in the form of liquid solutions or suspension; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found in, for example, Remington: The Science and Practice of Pharmacy, (19th ed.) ed. A.R. Gennaro AR., 1995, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for agonists of the invention include ethylenevinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, (e.g. lactose) or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

In addition, the GAG-based agent is preferentially administered in a “pharmaceutically effective amount”, “preventive effective amount” or “therapeutically effective amount”. These terms refer to an amount (dose) effective in treating a patient, preventing the appearance of SSc and/or alleviating the symptoms associated with SSc. It is also to be understood herein that a “pharmaceutically effective amount” may be interpreted as an amount giving a desired

therapeutic effect, either taken in one dose or in any dosage or route, taken alone or in combination with other therapeutic agents.

A therapeutically effective amount or dosage of a GAG-based agent, may range from about 0.001 to 30 mg/kg body weight, with other ranges of the invention including about 0.01 to 25 mg/kg body weight, about 0.025 to 10 mg/kg body weight, about 0.3 to 20 mg/kg body weight, about 0.1 to 20 mg/kg body weight, about 1 to 10 mg/kg body weight, 2 to 9 mg/kg body weight, 3 to 8 mg/kg body weight, 4 to 7 mg/kg body weight, 5 to 6 mg/kg body weight, and 20 to 50 mg/kg body weight. In other embodiments, a therapeutically effective amount or dosage may range from about 0.001 to 50 mg total, with other ranges of the invention including about 0.01 to 10 mg, about 0.3 to 3 mg, about 3 to10 mg, about 6 mg, about 9 mg, about 10 to 20 mg, about 20-30 mg, about 30 to 40mg, and about 40 to 50 mg. Those skilled in the art appreciate that heparin and its derivatives are usually administered as International Units (IU) of anti-Xa activity as set forth in the World Health Organization Reference Standards. For example, one mg of enoxaparin is equal to 100 IU of anti-Xa activity.

II – Tools for determining the usefulness of an agent in the prevention/treatment of SSc

The present application specifically shows that the interaction between topo/anti-topo IC and the heparan sulfate found on the surface of fibroblasts is central to the pathophysiology of SSc. As such, agents capable for limiting this interaction (either by preventing it from happening or by displacing the IC) will also provide to be useful for the prevention and/or treatment of SSc. Accordingly, the present application does provide a screening method and related products for assessing the usefulness of an agent in the treatment of SSc by determining its ability to prevent the association of the topo/anti-topo IC to the heparan sulfate and/or to displace the topo/anti-topo IC from the heparan sulfate.

The screening method enables the characterization of an agent ability to prevent/treat SSc or symptoms associated thereto. When the screening assay is used to determine the ability of an agent to prevent SSc or a symptom associated thereto, the ability of the agent to prevent the association of the topo/anti-topo IC to the heparan sulfate should be assessed. In order to do so, the agent is combined with an heparan sulfate derived from a fibroblast. Then, an topo/anti-topo IC is added to the mixture to determine if the agent is able to prevent (and if so to which extent) the binding of the topo/anti-topo IC to the heparan sulfate. In order to make such determination, the binding level of the topo/anti-topo IC to the heparan sulfate is compared to a control value. If the binding level of the topo/anti-topo IC in the presence of the agent is lower than the control value, then the agent is characterized as being useful for

the prevention of SSc or a symptom associated thereto. However, if the binding level of the topo/anti-topo IC in the presence of the agent is equal to or higher than the control value, then the agent is characterized as not being useful for the prevention of SSc or a symptom associated thereto.

When the screening assay is used to determine the ability of an agent to treat SSc or alleviate a symptom associated thereto, the ability of the agent to displace the topo/anti-topo IC from the heparan sulfate should be assessed. In order to do so, the topo/anti-topo is combined with an heparan sulfate derived from a fibroblast. Then, the agent is added to the mixture to determine if it is capable to displace (and if so to which extent) the bound topo/anti-topo IC from the heparan sulfate. In order to make such determination, the binding level of the topo/anti-topo IC to the heparan sulfate is compared to a control value. If the binding level of the topo/anti-topo IC in the presence of the agent is lower than the control value, then the agent is characterized as being useful for the treatment of SSc or a symptom associated thereto. However, if the binding level of the topo/anti-topo IC in the presence of the agent is equal to or higher than the control value, then the agent is characterized as not being useful for the treatment of SSc or a symptom associated thereto.

The screening methods described herein can be applied to determine the usefulness of any agent or compound, irrespective of its physico-chemical nature. It is also contemplated that some agents will be useful for the prevention of SSc or a symptom associated thereto but not for the treatment of SSc or a symptom associated thereto and *vice versa*. It is further contemplated that other agents will be more useful for the prevention of SSc or a symptom associated thereto than for the treatment of SSc or a symptom associated thereto and *vice versa*. It is also further contemplated that some agents will be useful for the prevention of SSc or a symptom associated thereto as well as for the treatment of SSc or a symptom associated thereto.

Heparan sulfate are present on the surface of every cell and their physico-chemical characteristic are cell-type specific. As shown herein, heparan sulfate presented on the surface of fibroblasts are involved in the association of the topo/anti-topo IC and these cells. Consequently, it is important that the heparan sulfate used in the screening method described herein be identical or sufficiently similar to those present on the surface of fibroblasts. Such heparan sulfate may be chemically synthesized or derived from fibroblasts (for example retrieved from a fibroblast or a fibroblast's membrane or a portion thereof). Alternatively or optionally, a biological active fragment of an heparan sulfate maybe used, i.e. such fragment should retain the ability to bind the topo/anti-topo IC. In an embodiment, the

heparan sulfate is presented on the surface of a cell. In another embodiment, the heparan sulfate may be immobilized on a synthetic surface to facilitate high-throughput screening.

In some embodiment, an *in vitro* environment (also referred to as a reaction vessel) can be created where the agent is combined with the heparan sulfate and/or the topo/anti-topo IC reagent. The contact between the agent and the heparan sulfate and/or the topo/anti-topo IC reagent must be made under conditions suitable and for a period of time that will enable the agent to interaction with the heparan sulfate and/or the topo/anti-topo IC reagent and possible alter the binding between the heparan sulfate and/or the topo/anti-topo IC reagent. Suitable *in vitro* environments can include, for example, a cell-free environment where a heparan sulfate or a biologically active fragment thereof is combined in a reaction media comprising the appropriate reagents to enable the assessment of the binding level between the heparan sulfate and/or the topo/anti-topo IC (buffers, substrates, additives, etc.).

Another suitable *in vitro* environment for the assay described herewith is a cultured cell or a fragment of the membrane of such cell. The cultured cell should express an heparan sulfate on its surface and such heparan sulfate should be identical or substantially similar to the heparan sulfate present on the surface of fibroblast. The heparan sulfate should be able to bind to the topo/anti-topo IC. In an embodiment, the cultured cell can be a fibroblast, such as, for example, a skin or a lung fibroblast. Such cell can be, for example, a primary cell line, an immortalized cell line or a transformed cell line. Optionally, a fragment of the membrane of the cultured cell can be used. The cellular membrane used should present an heparan sulfate on its surface and such heparan sulfate should be identical or substantially similar to the heparan sulfate present on the surface of fibroblast. The heparan sulfate should be able to bind to the topo/anti-topo IC.

A further embodiment of the reaction vessel is a non-human animal (also referred to as an animal model). If the characterization of the agent occurs in an animal model, then the agent is administered to the animal (such as a rodent). Various dosage and modes of administration maybe used to fully characterize the agent's ability to prevent and/or treat SSc or a symptom associated thereto. The non-human animal can be, for example, a mouse, a rat, a pig, monkey, etc.

Once the agent has been combined with the heparan sulfate and/or the topo/anti-topo IC, a measurement or value of a binding level of the topo/anti-topo IC to the heparan sulfate is made. This assessment may be made directly in a reaction vessel (by using a probe) or on a sample from the reaction vessel. The measurement of binding level can also be made by

using a label (or a combination of labels), for example on the topo/anti-topo IC and/or heparan sulfate, and determining the presence or absence as well as relative intensity of the label. Such determination may be made, for example, by using fluorescent cell counting, mass spectrometry and/or antibody-based methods (such as ELISA).

The measuring step can rely on the addition of a quantifier specific to the parameter to be assessed (binding level). The quantifier can either specifically bind the topo/anti-topo IC-heparan sulfate complex or lack the ability to specifically bind to the topo/anti-topo IC-heparan sulfate complex. The amount of the quantifier that specifically binds (or that does not bind) to the topo/anti-topo IC-heparan sulfate complex will be determined to provide a measurement of the binding level between the topo/anti-topo IC-heparan sulfate complex. In another embodiment, the quantifier can be modified in the presence or absence of the topo/anti-topo IC-heparan sulfate complex. In this specific instance, the amount of modified (or unmodified) quantifier will be determined to provide a measurement of the parameter of the binding level between the topo/anti-topo IC-heparan sulfate complex. In an embodiment, the signal measured from the quantifier can be provided by a label that is either directly or indirectly linked to a quantifier.

Once the measurement has been made, its value can be extracted, and the binding level obtained is compared to a control value. The control value may be the binding level of the topo/anti-topo IC to the heparan sulfate in the absence of the agent. The control value can also be the binding level of the topo/anti-topo IC to the heparan sulfate in the presence of a control agent that fails to prevent/treat SSc or a symptom associated thereto. Such control agents include, but are not limited to, a pharmaceutically acceptable excipient. The control level can also be a predetermined value of the binding level of the topo/anti-topo IC to the heparan sulfate associated with a lack of prevention and/or treatment of SSc.

In an embodiment, the comparison can be made by an individual. In another embodiment, the comparison can be made in a comparison module. Such comparison module may comprise a processor and a memory card to perform an application. The processor may access the memory to retrieve data. The processor may be any device that can perform operations on data. Examples are a central processing unit (CPU), a front-end processor, a microprocessor, a graphics processing unit (PPU/VPU), a physics processing unit (PPU), a digital signal processor and a network processor. The application is coupled to the processor and configured to determine the effect of the agent on the binding level of the topo/anti-topo IC with respect to the control value. An output of this comparison may be transmitted to a display device. The memory, accessible by the processor, receives and stores data, such as

measured parameters of the binding level of the topo/anti-topo IC or any other information generated or used. The memory may be a main memory (such as a high speed Random Access Memory or RAM) or an auxiliary storage unit (such as a hard disk, a floppy disk or a magnetic tape drive). The memory may be any other type of memory (such as a Read-Only Memory or ROM) or optical storage media (such as a videodisc or a compact disc). Once the comparison between the binding level of the topo/anti-topo IC and the control value is made, then it is possible to characterize the agent's ability to treat and/or prevent SSc or a symptom associated thereto.

The present application also provides a screening system for characterizing an agent's ability to prevent and/or treat SSc or a symptom associated thereto in a subject. This screening system can have a reaction vessel for combining the agent, the topo/anti-topo IC and the heparan sulfate, a processor in a computer system, a memory accessible by the processor and an application coupled to the processor. The application or group of applications is (are) configured for receiving a value of binding level of the topo/anti-topo IC to the heparan sulfate in the presence of the agent; comparing the binding level in the presence of the agent to a control value and/or characterizing the agent's ability to prevent/treat SSc or a symptom associated thereto. If the binding level of the topo/anti-topo IC in the presence of the agent is lower than the control value, then the agent is characterized as being useful for the prevention/treatment of SSc or a symptom associated thereto. However, if the binding level of the topo/anti-topo IC in the presence of the agent is equal to or higher than the control value, then the agent is characterized as not being useful for the prevention/treatment of SSc or a symptom associated thereto.

The present application further provides a software product embodied on a computer readable medium. This software product comprises instructions for characterizing an agent's ability prevent and/or treat SSc or a symptom associated thereto. The software product comprises a receiving module for receiving a value of a binding level of the topo/anti-topo IC in the presence of the agent in a reaction vessel; a comparison module receiving input from the measuring module for determining if the value of the binding level of the topo/anti-topo in the presence of the agent is lower than, equal to or higher than a control value; a characterization module receiving input from the comparison module for identifying the usefulness of the agent for preventing/treating SSc or a symptom associated thereto. The comparison module and characterization module may each comprise a processor, a memory accessible by the processor to perform an application. If the binding level of the topo/anti-topo IC in the presence of the agent is lower than the control value, then the agent is

characterized as being useful for the prevention/treatment of SSc or a symptom associated thereto. However, if the binding level of the topo/anti-topo IC in the presence of the agent is equal to or higher than the control value, then the agent is characterized as not being useful for the prevention/treatment of SSc or a symptom associated thereto.

In an embodiment, an application found in the computer system of the screening system is used in the comparison module. A measuring module extracts/receives information from the reaction vessel with respect to the binding level of the topo/anti-topo IC. The receiving module is coupled to a comparison module which receives the value(s) of the binding level of the topo/anti-topo IC and determines if this value is lower than, equal to or higher than a control value. The comparison module can be coupled to a characterization module.

In another embodiment, an application found in the computer system of the screening system is used in the characterization module. The comparison module is coupled to the characterization module which receives the comparison and determines the agent's ability prevent/treat SSc (or a symptom associated thereto) based on this comparison. When the comparison indicates that the agent is capable of lowering the binding level of the topo/anti-topo IC with respect to the control value, the agent is then characterized as being able to prevent/treat SSc. When the comparison indicates that the agent is capable of augmenting or does not alter the binding level of topo/anti-topo IC with respect to the control value, the agent is then characterized as being unable to prevent and/or treat SSc or a symptom associated thereto.

In a further embodiment, the receiving module, comparison module and characterization module are organized into a single discrete system. In another embodiment, each module is organized into different discrete system. In still a further embodiment, at least two modules are organized into a single discrete system.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I - MATERIALS AND METHODS

Cell culture and reagents. Normal human dermal fibroblasts (NHDF) from adults and their culture media were from Lonza (Walkersville, MD). NHDF were cultured in fibroblast basal medium supplemented with 2% fetal bovine serum (FBS), 5 µg/ml of bovine insulin, 1 ng/ml of human fibroblast growth factor B, 50 µg/ml of gentamicin sulfate and 100 µg/ml penicillin-streptomycin (Wisent Inc., St-Bruno, Canada). Cells were used at passage 5. Cells were

grown at 37°C in air with 5% CO_2. Subculturing was achieved before confluency, using trypsin and trypsin-neutralizing solution (Lonza). DNA topoisomerase I (topo) was obtained from Immunovision (Springdale, AR). All the experiments were done using topo purified from rabbit's thymus. Recombinant human topo produced using the baculovirus/insect cell expression system and obtained from Diarect (Freiburg, Germany) was also tested in many experiments with similar results (data not shown). Each topo preparation was tested for the presence of endotoxin and analyzed for purity upon receipt by gel electrophoresis and immunoblotting. Mouse monoclonal antibody against topo was purchased from USBiological (Massachussetts, MA). Heparitinase (Hep) and monoclonal anti-heparan sulphate (HS) chain antibody (F58-10E4) were obtained from Seikagaku Corporation (Tokyo, Japan). Anti-CD51 antibody (P2W7) was obtained from R&D systems (Minneapolis, MN). Unfractionated heparin (UFH) was from Sigma (Oakville, Ontario, Canada). Low molecular weight heparins (LMWH): dalteparin (Fragmin™; Pfizer Canada), enoxaparin (Lovenox™; Sanofi Aventis) and fondaparinux (Arixtra™; Glaxosmithkline) were purchased directly from Notre-Dame Hospital's pharmacy (Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada).

Antibody purification. Human IgG were purified as described in Robitaille et al. (2009). Briefly, total IgG was purified from sera by affinity chromatography using the Nab Protein G Spin Chromatography Kit (Pierce, Rockford, IL) following the manufacturer's instructions. Final IgG concentrations were determined with the Bradford dye-binding procedure. Anti-topo IgG was depleted as previously described with minor changes. Briefly, individual whole IgG preparation from sera of anti-topo positive SSc patients were incubated for several passages on nitrocellulose membranes with adsorbed topo. Anti-topo IgG depletion was confirmed by immunoblotting on purified topo after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SSc patients were selected from a French-Canadian cohort with SSc diagnosed at the Connective Tissue Diseases and Vascular Medicine Clinics of Notre-Dame Hospital, Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada. All patients fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) preliminary criteria for the classification of SSc (Scuseel-Lonzettti et al, 2002; Subcommittee for scleroderma criteria, 1980). Sera were collected as previously described (4).

Flow cytometry. Topo or bovine serum albumin (BSA) was coupled to the fluorochrome PE using the Lightning-Link PE Conjugation Kit™ from Innova Biosciences (Babraham, Cambridge, UK) according to manufacturer's instructions. Cells were grown until confluence,

detached with phosphate buffered saline (PBS)/2% trypsin and washed once with PBS. Topo binding was assessed by incubating cells with topo-PE (1.0 µg/ml) for 30 minutes at 25°C in PBS/3% BSA, cells were washed with PBS and fixed with 1% paraformaldehyde (PFA) for 5 minutes at 4°C. As needed, total IgG purified from normal subjects and SSc patients (100 µg/ml), mouse monoclonal anti-topo antibody (50 µg/ml), or anti-topo depleted IgG (100 µg/ml) were added to the cells simultaneously with topo-PE. Where required, cells were preincubated with Hep (0.05 U/ml) for 1 hour at 37°C and the presence of HS chains on the cell surface was detected with an anti-heparan sulfate antibody (1/100). As needed, NHDF were incubated with either UFH or LMWH at the indicated concentrations simultaneously with topo alone or topo/anti-topo autoantibodies for the competition assays or after 30 minutes for the dissociation assays. Cell permeability was assessed in the presence of 7-aminoactinomycin D (Sigma), and permeable cells were gated out. Fluorescence was detected on a FACScan and analyzed by CellQuest™ software (BD Biosciences, San Jose, CA).

Immunofluorescence assays. Topo or BSA were coupled to the fluorochrome Cy3™ using the Lightning-Link Cy3™ Conjugation Kit from Innova Biosciences according to manufacturer's instructions. Cells were grown to confluence on glass coverslips in a 12-well plate and incubated with topo-Cy3™ (1 µg/ml) for 30 minutes at 37°C in complete medium, cells were washed once with PBS and fixed with 1% PFA for 5 minutes at 4°C. Where required cells were pretreated with Hep (0.05 U/ml; 1 hour) and/or incubated with total IgG (100 µg/ml) purified from normal subjects and SSc patients or anti-topo depleted IgG for 30 minutes at 37°C. As needed, NHDF were incubated with either the UFH or LMWH at 10 µg/ml. Nuclei were stained with Hoescht 33342 diluted in PBS-3% BSA. Cells were examined at a magnification of 40X with an Eclipse E600™ fluorescence microscope (Nikon, Melville, NY) using NIS-elements 3.0 Ar™ software (Nikon, Melville, NY) with a photometrics HQ2 coolSNAP™ camera (Tucson, AZ).

Topo ELISA. The 96 well microtiter Immulon IIB™ plates were coated with topo at 350 ng/well in coating buffer (15 mM Na_2CO_3 + 35 mM $NaHCO_3$, pH 9.6) for 1 hour at 37°C. After extensive washes with PBS-0,5% Tween-20, wells were blocked with blocking buffer (PBS-foetal bovin serum 2%-BSA 1%-0,5% Tween-20) for 2 hours at 37°C. IgG purified form SSc patients and normal controls were added (10 µg/ml) in blocking buffer for 1 hour at 37°C and antibody binding was revealed with horseradish peroxidase–conjugated goat anti-human IgG (Jackson ImmunoResearch, West Grove, PA) and o-phenylenediamine/citrate substrate

solution. The optical density (OD) at 490 nm was read in an MRX Revelation™ Microplate Reader (Dynex, Chantilly, VA). Samples were tested in triplicate.

Statistical analysis. Student's unpaired 2-tailed t-test was used for all statistical analysis. Statistical tests were performed using GraphPad Prism 5.0™ software (GraphPad Software, San Diego, CA). To investigate the association between OD and mean fluorescence intensity (MFI), a Pearson correlation coefficient was used to estimate an univariate linear model. A robust sandwich estimators of the covariance matrix was used to produce valid P values (Zeilis, 2006; R Development Core Team, 2010) .

EXAMPLE II – IDENTIFICATION OF THE TARGET FOR TOPO ON THE SURFACE OF FIBROBLASTS

The material and methods used in the Example are described in Example I.

Anti-topo autoantibodies from systemic sclerosis (SSc) patients amplify topo binding to normal human dermal fibroblast (NHDF) surfaces. Previous work has demonstrated that topo/anti-topo IC bound to the surface of fibroblasts contribute to the initiation of an inflammatory cascade. Analysis to characterize the interaction of topo and anti-topo autoantibodies with NHDF surfaces was undertaken in order to elucidate the putative pathogenic role of topo/anti-topo IC in SSc.

The ability of fluorescently conjugated-topo (topo-PE) to bind to fibroblast surfaces was first evaluated and compared with that of topo-PE after incubation with anti-topo antibodies, presumably in the form of IC. NHDF were incubated with topo-PE, either alone or simultaneously with whole IgG purified from anti-topo positive SSc sera or with IgG purified from normal sera and examined for fluorescence emitted from PE by flow cytometry. As shown in Fig. 1A, topo-PE (dotted lines) strongly stained fibroblasts, as compared with BSA-PE staining (shaded area), used as a negative PE-labeled protein control. Topo-PE binding to NHDF increased when anti-topo autoantibodies from SSc patients were present (Fig. 1A; right panel). In contrast, IgG purified from normal sera had no effect on topo binding. (Fig. 1A; left panel). As shown in Fig. 1B, the amplifying effects of anti-topo autoantibodies on topo binding was detectable as early as 5 minutes after adding the reagents to the NHDF and slowly reached a maximum after 1-2 hours of incubation. No amplifying activity was observed when normal IgG were used even after 2 hours of incubation. Similar results were obtained with IgG purified from all 19 anti-topo-positive SSc sera tested (Fig. 1C). None of the IgG from normal sera (n = 5), anti-Th/To positive SSc sera (n = 3), or anti-centromere (ACA)-

positive SSc sera (n = 5) tested had any effect on topo binding to NHDF surfaces (Fig. 1C). Similar results were obtained with normal lung fibroblasts (data not shown).

Negative controls were performed by incubating NHDF with equal concentrations of BSA-PE combined with anti-BSA antibody, presumably also in the form of IC. A second negative control was used, human heat-aggregated gamma globulins as an Fc receptor blocking agent added prior to incubation with topo/anti-topo IC. Negative results obtained with both controls indicated that the amplification of topo binding was specific to topo/anti-topo IC and was not Fc receptor-mediated (data not shown). Similar results were obtained with normal lung fibroblasts (data not shown).

To evaluate the potential association between anti-topo antibody titers in whole IgG purified from anti-topo-positive SSc sera (OD) from ELISA and their respective amplifying effects on topo-PE binding to NHDF surfaces (MFI) from flow cytometry, univariate linear regression analysis was performed. As shown in Fig. 2A, there was a strong correlation between these two variables ($P < 0.0001$) and, with a Pearson correlation coefficient of 0,86, these results strongly suggest that the observed increased binding of topo to NHDF surfaces seen in Fig. 1 is directly associated with the presence and titers of anti-topo autoantibodies in whole IgG preparations. Similar results were obtained with normal lung fibroblasts (data not shown).

To further confirm that the amplifying effects observed with topo/anti-topo IC were dependent upon the presence of anti-topo autoantibodies, anti-topo antibody depletion was performed. The efficacy of anti-topo depletion was first controlled by immunoblotting on purified topo. As shown in Fig. 2B, all of the anti-topo-depleted IgG tested had lost their reactivity against topo as compared with the corresponding whole IgG. The amplifying efficacy of these 2 groups of IgG on topo binding to NHDF surfaces was compared by fluorescence microscopy (Fig. 2C). Here, topo directly coupled to a fluorochrome (topo-Cy3) was added to live, adherent, unfixed and non-permeabilized NHDF for 30 minutes simultaneously with whole IgG anti-topo or with their corresponding anti-topo depleted IgG. As shown in Fig. 2C, in the presence of topo/anti-topo IC (left panel), topo-Cy3 displayed a uniform and strong fluorescent pattern over the cell surface while the use of anti–topo depleted IgG preparations strongly diminished the fluorescent intensity of topo-Cy3 bound to NHDF (right panel). Fig. 2D shows that all of the anti-topo-depleted IgG preparations tested revealed a significant loss in their amplifying activity on topo-Cy3 binding as compared with their corresponding whole IgG preparations (n = 4, SSc patients). Taken together, these data strongly suggest that anti-topo antibodies from SSc patients amplify topo binding to NHDF surfaces. Similar results were obtained with normal lung fibroblasts (data not shown).

Topo binds to NHDF surfaces by interacting with heparan sulfate (HS) chains. Based on the ability of topo to bind heparin and because HSPG act as coreceptors for several proteins, the possibility was investigated that topo uses HS chains as coreceptors on NHDF. First, NHDF were incubated for one hour in the presence or absence of heparitinase (Hep), a specific enzyme that cleaves the HS chains from proteoglycans, prior to incubation with topo or topo/anti-topo IC. The efficacy of HS chain removal by Hep treatment was confirmed with HS chain-specific antibody and appeared to be nearly complete (Fig. 3A). As shown in Fig. 3A, degradation of HS chains with Hep also lead to an important and significant decrease of topo and topo/anti-topo IC binding to the cell surface. The cell surface expression of CD51, which does not bear HS chains and should therefore not be sensitive to Hep treatment, was used as a negative control. As shown in Fig. 3A, the presence of CD51 at the cell surface was not affected by Hep treatment. The presence of HS chains and the ability of topo/anti-topo IC binding following exposure of NHDF to Hep were also investigated by fluorescence microscopy. As shown in Fig. 3B, HS chain expression (green) and topo/anti-topo IC binding (red) decreased following Hep treatment (panels b and d, respectively) as compared with untreated cells (panels a and c). Taken together, these data show that topo and topo/anti-topo IC bind to HS chains on NHDF, thus suggesting that HSPG act as coreceptors. Experiments to identify the ultimate cell surface receptor for topo on NHDF are currently in progress in our laboratory. Similar results were obtained with normal lung fibroblasts (data not shown).

EXAMPLE III –PREVENTION OF BINDING OF TOPO ON THE SURFACE OF FIBROBLASTS

The material and methods used in the Example are described in Example I.

Heparin inhibits binding of topo and topo/anti-topo IC to HS on NHDF surfaces. It has been demonstrated that heparin, a highly-sulfated glycosaminoglycan, can inhibit topo enzymatic activity *in vitro*. These observations thus led to the hypothesis that heparin can inhibit binding of topo to HS and prevent the accumulation of topo/anti-topo IC at the surface of fibroblasts. In order to verify this hypothesis, competition assays were performed between topo or topo/anti-topo IC and heparin. Here, topo-PE either alone or with whole IgG purified from anti-topo positive SSc sera were added to NHDF simultaneously with unfractionated heparin (UFH) and cells were examined by flow cytometry. As shown in Fig. 4A, the amount of topo and topo/anti-topo IC that bound to HS on NHDF was markedly reduced in the presence of UFH (black and red dotted lines, respectively) as compared with untreated cells (black and red thick lines), thus suggesting that UFH could be used to prevent the binding of topo/anti-

topo IC to fibroblasts. Similar results were obtained with normal lung fibroblasts (data not shown).

Fractionated heparin, or low molecular weight heparin (LMWH), is a product of the breakdown of UFH. LMWH has a similar mechanism of action as UFH and since several LMWH products are commercially available, their efficacy to inhibit topo/anti-topo IC binding to NHDF surfaces was investigated. As shown in Fig. 4B, the inhibiting effect of UFH on topo/anti-topo IC binding to NHDF was concentration-dependent with a maximal activity at 1 µg/ml and was comparable to those obtained with the two LMWH tested. Indeed, binding of topo/anti-topo IC to NHDF was reduced in a dose-dependent manner by dalteparin™ and enoxaparin™, reaching approximately 95 % inhibition at concentrations of 10,0 µg/ml. In contrast, fondaparinux™, a fully synthetic pentasaccharide, had little or no effect on the binding of topo/anti-topo IC to NHDF, suggesting that this molecule is completely inactive as a competitor. The effect of heparin and its derivatives on IC binding was next evaluated by fluorescence microscopy and again, like UFH, dalteparin™ reduced the staining of topo/anti-topo IC on NHDF as compared with untreated cells (Fig. 4C). Similar results were obtained with enoxaparin™ (data not shown). Taken together, these results indicate that LMWH have a similar mechanism of action as UFH, suggesting that dalteparin and enoxaparin can also be used as powerful inhibitors of topo/anti-topo IC binding to NHDF surfaces. Similar results were obtained with normal lung fibroblasts (data not shown).

EXAMPLE IV– DISPLACEMENT OF BINDING OF TOPO ON THE SURFACE OF FIBROBLASTS

The material and methods used in the Example are described in Example I.

Heparin dissociates topo and topo/anti-topo IC bound to HS on NHDF surfaces. Since SSc is a disease that often goes undiagnosed and because anti-topo autoantibodies are produced very early during its development, it became of interest to test whether heparin or its derivatives could dissociate topo or topo/anti-topo IC bound to NHDF surfaces. Topo-PE either alone or simultaneously with anti-topo autoantibodies were incubated with NHDF for 30 minutes, unbound molecules washed away, and increasing concentrations of UFH subsequently added. As seen in Fig. 5A, dissociation of both topo alone or topo/anti-topo IC from HS chains on NHDF increased in a dose-dependent manner in response to UFH. A similar dose-dependent effect with LMWH dalteparin™ and enoxaparin™ was observed on topo/anti-topo IC binding (Fig. 5B). Again, fondaparinux™ only caused very little dissociation of topo/anti-topo IC from NHDF. Dissociation assays were similarly performed with IgG

purified from 8 anti-topo-positive SSc sera tested with both UFH and dalteparin at concentrations of 10 µg/ml (Fig. 5C). Similar results were obtained with enoxaparin™ (data not shown). These findings suggest that UFH as well as dalteparin™ and enoxaparin™ could be used to remove topo/anti-topo IC bound to NHDF surfaces. Similar results were obtained with normal lung fibroblasts (data not shown).

Previous work has demonstrated that the nuclear autoantigen topo binds specifically to the surface of fibroblasts and recruits anti-topo autoantibodies which lead to the adhesion and activation of monocytes. Such an activation of the immune system may contribute to the pathogenesis of SSc via initiation of the fibrotic cascade typical of SSc pathogenesis.

As shown herein, anti-topo autoantibodies amplify topo binding to fibroblast surfaces. This amplification was specific for topo/anti-topo IC and strongly correlated with the presence and titers of anti-topo autoantibodies. This is consistent with other findings demonstrating that anti-topo autoantibody titers are correlated with the severity and activity of the disease and that they are associated with the worst form of SSc. Moreover, these data are also consistent with a recent paradigm stating that the pathogenicity of autoantibodies is amplified when autoantigens are accessible for IC formation.

Binding of topo to fibroblast surfaces involves a membrane receptor and results presented herein identified the membrane partner responsible for topo and consequently topo/anti-topo IC specific binding to fibroblast surfaces. The presence of HS chains on HSPG was shown to be necessary for topo and topo/anti-topo IC binding. Binding to proteoglycans on cell surfaces is a well-known process used by many proteins. Indeed, HSPG represent low affinity but high capacity coreceptors that facilitate the presentation of a ligand to its high affinity signalling receptor. Hence, HSPG present on the surface of fibroblasts are instrumental in the process leading to the putative initiation of the inflammatory cascade.

Since topo was shown to interact with HS chains, heparin, a glycosaminoglycan analogue of HS, was proposed to interfere with topo binding and consequently with the accumulation of topo/anti-topo IC on fibroblast surfaces. The presence of UFH as well as the two LMWH tested, namely dalteparin™ and enoxaparin™, markedly competed with topo/anti-topo IC binding to HS chains on fibroblast surfaces. Furthermore, topo/anti-topo IC bound to HS chains could be dissociated from fibroblast surfaces by UFH and LMWH. In contrast to UFH and LMWH, fondaparinux™, a completely synthetic molecule, caused very little inhibition and dissociation of topo/anti-topo IC binding suggesting that this molecule is inactive in our model.

In Figure 6, a mechanism is proposed for the pathogenic role of topo/anti-topo IC binding to fibroblast surfaces as contributor to the initiation of fibrosis in SSc patients (left) as well as the effects of its perturbation by UFH or LMWH treatments (right). Topo, originally released from apoptotic endothelial cells, binds to HSPG on bystander fibroblasts. In the presence of anti-topo autoantibodies, topo binding to HSPG is amplified (left panel). Topo/anti-topo IC accumulation on the cell surface induces adhesion and activation of monocytes, and would thus be associated with the amplification of the immune response, due to proinflammatory cytokines released by activated monocytes, and to fibrosis, due to local secretion of profibrotic cytokines by activated fibroblasts. Hence, the presence of anti-topo in SSc patients would be directly associated with increased immune responses and could drive the disease toward a more severe and unremitting fibrotic phenotype, as observed *in vivo*. In contrast, as illustrated by the right panel, the presence of UFH or LMWH is predicted to inhibit topo binding, either by the prevention of topo/anti-topo IC binding to HS chains or even by their dissociation, which would result in the inhibition of the inflammatory cascade by blocking the activation of the immune system and possibly the resulting fibrosis.

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While the invention has been described in connection with specific embodiments thereof, it will be understood that the scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

WHAT IS CLAIMED IS:

1. A method for the prevention and/or treatment of systemic sclerosis (SSc) or a symptom thereof in a subject in need thereof and having anti-topoisomerase antibodies, said method comprising administering a preventive and/or therapeutic amount of a glycosaminoglycan compound to the subject so as to prevent and/or treat SSc in the subject.

2. The method of claim 1, wherein the glycosaminoglycan has :

   - an hexosamine selected from the group consisting of α-D-N-acetylglucosamine (GlcNAc), α-D-N-sulfoglucosamine (GlcNS), α-D-N-acetylglucosamine-6-O-sulfate (GlcNAc(6S)), and α-D-N-sulfoglucosamine-6-O-sulfate (GlcNS(6S));
   - an hexose or hexuronic acid unit selected from the group consisting of β-D-glucuronic acid (GlcUA) and 2-O-sulfo-α-L-iduronic acid (IdoUA(2S)); and/or
   - -4IdoUA(2S)α1-4GlcNS(6S)α1- as a predominant linkage between monomeric units.

3. The method of claim 1 or 2, wherein the glycosaminoglycan is negatively charged.

4. The method of any one of claims 1 to 3, wherein the glycosaminoglycan is heparin.

5. The method of claim 4, wherein the heparin is unfractionated heparin.

6. The method of any one of claims 1 to 3, wherein the glycosaminoglycan is derived from heparin.

7. The method of claim 6, wherein the heparin is a low molecular weight heparin.

8. A glycosaminoglycan compound for the prevention of systemic sclerosis (SSc) or a symptom thereof in a subject having anti-topoisomerase antibodies.

9. A glycosaminoglycan compound for the treatment of systemic sclerosis (Ssc) or a symptom thereof in a subject having anti-topoisomerase antibodies.

10. The glycosaminoglycan compound of claim 8 or 9, wherein the glycosaminoglycan has :

- an hexosamine selected from the group consisting of α-D-N-acetylglucosamine (GlcNAc), α-D-N-sulfoglucosamine (GlcNS), α-D-N-acetylglucosamine-6-O-sulfate (GlcNAc(6S)), and α-D-N-sulfoglucosamine-6-O-sulfate (GlcNS(6S));
- an hexose or hexuronic acid unit selected from the group consisting of β-D-glucuronic acid (GlcUA) and 2-O-sulfo-α-L-iduronic acid (IdoUA(2S)); and/or
- -4IdoUA(2S)α1-4GlcNS(6S)α1- as a predominant linkage between monomeric units.

11. The glycosaminoglycan compound of any one of claims 8 to 10 being negatively charged.

12. The glycosaminoglycan of any one of claims 8 to 11 being heparin.

13. The glycosaminoglycan of claim 12 being unfractionated heparin.

14. The glycosaminoglycan of any one of claims 8 to 11 being derived from heparin.

15. The glycosaminoglycan of claim 14 being a low molecular weight heparin.

16. Use of a glycosaminoglycan compound for the prevention of systemic sclerosis (Ssc) or a symptom thereof in a subject positive for anti-topoisomerase antibodies.

17. Use of a glycosaminoglycan compound for the treatment of systemic sclerosis (Ssc) or a symptom thereof in a subject positive for anti-topoisomerase antibodies.

18. The use of claim 16 or 17, wherein the glycosaminoglycan has :

- an hexosamine selected from the group consisting of α-D-N-acetylglucosamine (GlcNAc), α-D-N-sulfoglucosamine (GlcNS), α-D-N-acetylglucosamine-6-O-sulfate (GlcNAc(6S)), and α-D-N-sulfoglucosamine-6-O-sulfate (GlcNS(6S));

- an hexose or hexuronic acid unit selected from the group consisting of β-D-glucuronic acid (GlcUA) and 2-O-sulfo-α-L-iduronic acid (IdoUA(2S)); and/or
- -4IdoUA(2S)α1-4GlcNS(6S)α1- as a predominant linkage between monomeric units.

19. The use of any one of claims 16 to 18, wherein the glycosaminoglycan is negatively charged.

20. The use of any one of claims 16 to 19, wherein the glycosaminoglycan is heparin.

21. The use of claim 20, wherein the glycosaminoglycan is unfractionated heparin.

22. The use of any one of claims 16 to 19, wherein the glycosaminoglycan is derived from heparin.

23. The use of claim 22, wherein the heparin is a low molecular weight heparin.

24. A method of characterizing an agent's ability to prevent systemic sclerosis (SSc) or a symptom thereof in a subject having anti-topoisomerase antibodies, said method comprising :

(a) combining the agent with an heparan sulfate derived from a fibroblast;

(b) combining the mixture of step (a) with an immune complex comprising a topoisomerase and an antibody specific for the topoisomerase;

(c) extracting a value of a binding level of the immune complex to the heparin sulfate in the presence of the agent;

(d) comparing the value of the binding level of step (c) to a control value; and

(e) characterizing the agent as :

i. having the ability to prevent SSc or the symptom thereof in the subject when the value extracted in step (c) is lower than the control value; and

ii. lacking the ability to prevent SSc or the symptom thereof when the value extracted in step (c) is equal to or higher than the control value.

25. The method of claim 24, wherein the heparan sulfate is presented on the surface of a fibroblast.

26. The method of claim 24 or 25, wherein the topoisomerase is topoisomerase I.

27. The method of any one of claims 24 to 26, wherein said step (a) and/or step (b) comprises contacting the immune complex and/or the agent with a fibroblast.

28. The method of any one of claims 24 to 27, wherein said immune complex is associated with a label.

29. The method of claim 28, wherein said extracting comprises measuring the label associated with the immune complex.

30. The method of any one of claims 24 to 29, wherein the control value is at least one of : the binding level of the immune complex in the absence of the agent, the binding level of the immune complex in the presence of a control agent that fails to prevent SSc or the symptom thereof in the subject and a pre-determined value of a binding level of the binding complex associated with a lack of prevention of SSc of the symptom thereof.

31. A method of characterizing an agent's ability to treat systemic sclerosis (SSc) or a symptom thereof in a subject having anti-topoisomerase antibodies, said method comprising :

(a) combining an immune complex comprising a topoisomerase and an antibody specific to the toposiomerase with an heparan sulfate derived from a fibroblast;

(b) combining the mixture of step (a) with the agent;

(c) extracting a value of a binding level of the immune complex to the heparin sulfate in the presence of the agent;

(d) comparing the value of the binding level of step (c) to a control value; and

(e) characterizing the agent as :

i. having the ability to prevent and/or treat SSc or the symptom thereof in the subject when the value extracted in step (c) is lower than the control value; and

ii. lacking the ability to prevent and/or treat SSc or the symptom thereof when the value extracted in step (c) is equal to or higher than the control value.

32. The method of claim 31, wherein the heparan sulfate is presented on the surface of a fibroblast.

33. The method of claim 31 or 32, wherein the topoisomerase is topoisomerase I.

34. The method of any one of claims 31 to 33, wherein said step (a) and/or step (b) comprises contacting the immune complex and/or the agent with a fibroblast.

35. The method of any one of claims 31 to 34, wherein said immune complex is associated with a label.

36. The method of claim 35, wherein said extracting comprises measuring the label associated with the immune complex.

37. The method of any one of claims 31 to 36, wherein the control value is at least one of : the binding level of the immune complex in the absence of the agent, the binding level of the immune complex in the presence of a control agent that fails to treat SSc or the symptom thereof and a pre-determined value of a binding level of the binding complex associated with a lack of treatment of SSc or the symptom thereof.

38. A screening system for characterizing an agent's ability to prevent and/or treat systemic sclerosis (SSc) or a symptom thereof in a subject having anti-topoisomerase antibodies, said screening system comprising :

(a) a reaction vessel for combining the agent, an heparan sulfate derived from a fibroblast and an immune complex comprising a topoisomerase and an antibody specific for the topoisomerase;

(b) a processor in a computer system;

(c) a memory accessible by the processor; and

(d) at least one application coupled to the processor and configured for :

i. extracting a value of a binding level of the immune complex to the heparin sulfate in the presence of the agent;

ii. comparing the value of the binding level of step (i) to a control value; and

iii. characterizing the agent as :

- having the ability to prevent and/or treat SSc or the symptom thereof in the subject when the value of the binding level of the immune complex extracted in step (i) is lower than the control value; and

- lacking the ability to prevent and/or treat SSc or the symptom thereof when the value of the binding level of the immune complex extracted in step (i) is equal to or higher than the control value.

39. The screening system of claim 38, wherein the heparan sulfate is presented on the surface of a fibroblast.

40. The screening system of claim 38 or 39, wherein the topoisomerase is topoisomerase I.

41. The screening system of any one of claims 38 to 40, wherein said screening vessel is for contacting the immune complex and the agent with a fibroblast.

42. The screening system of any one of claims 38 to 41, wherein said immune complex is associated with a label.

43. The screening system of claim 42, wherein said extracting comprises measuring the label associated with the immune complex.

44. The screening system of any one of claims 38 to 43, wherein the control value is at least one of : the binding level of the immune complex in the absence of the agent, the binding level of the immune complex in the presence of a control agent that fails to prevent and/or treat SSc or the symptom thereof in the subject and a pre-determined value of a binding level of the binding complex associated with a lack of prevention and/or treatment of SSc or the symptom thereof.

45. A software product embodied on a computer readable medium and comprising instructions for characterizing an agent's ability to prevent and/or treat systemic sclerosis (SSc) or a symptom thereof in a subject having anti-topoisomerase antibodies, said instructions comprising :

(a) a receiving module for receiving a value of a binding level of (i) an immune complex comprising a topoisomerase and (ii) an antibody specific for the topoisomerase to an heparan sulfate derived from a fibroblast in the presence of the agent;

(b) a comparison module for determining if the value of the binding level of the immune complex in the presence of the agent is lower than, equal to or higher than a control value and generating a corresponding output;

(c) a characterization module receiving the corresponding output from the comparison module and adapted to characterize the usefulness of the agent for preventing and/or treating SSc, wherein :

- the agent is characterized as having the ability to prevent and/or treat SSc or the symptom thereof in the subject when the value of the binding level of the immune complex is lower than the control value; and

- the agent is characterized as lacking the ability to prevent and/or treat SSc or the symptom thereof in the subject when the value of the binding level of the immune complex is equal to or higher than the control value.

46. The software product of claim 45, wherein the heparan sulfate is presented on the surface of a fibroblast.

47. The software product of claim 45 or 46, wherein the topoisomerase is topoisomerase I.

48. The software product of any one of claims 45 to 47, wherein said immune complex is associated with a label.

49. The software product of claim 48, wherein said extracting comprises measuring the label associated with the immune complex.

50. The software product of any one of claims 45 to 49, wherein the control value is at least one of : the binding level of the immune complex in the absence of the agent, the binding level of the immune complex in the presence of a control agent that fails to prevent and/or treat SSc or the symptom thereof in the subject and a pre-determined value of a binding level of the binding complex associated with a lack of prevention and/or treatment of SSc or the symptom thereof.

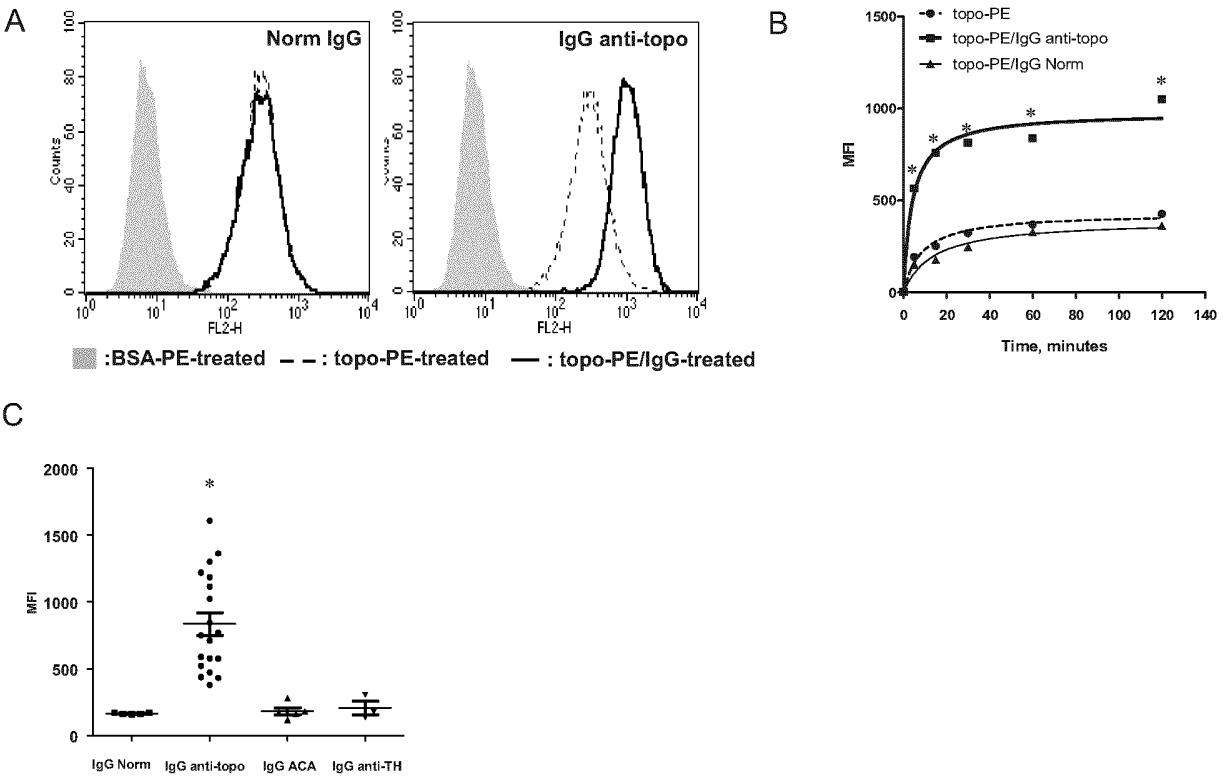
A
Norm IgG
IgG anti-topo
Counts
FL2-H
:BSA-PE-treated
: topo-PE-treated
: topo-PE/IgG-treated
B
topo-PE
topo-PE/IgG anti-topo
topo-PE/IgG Norm
MFI
Time, minutes
C
MFI
IgG Norm
IgG anti-topo
IgG ACA
IgG anti-TH

Figure 1

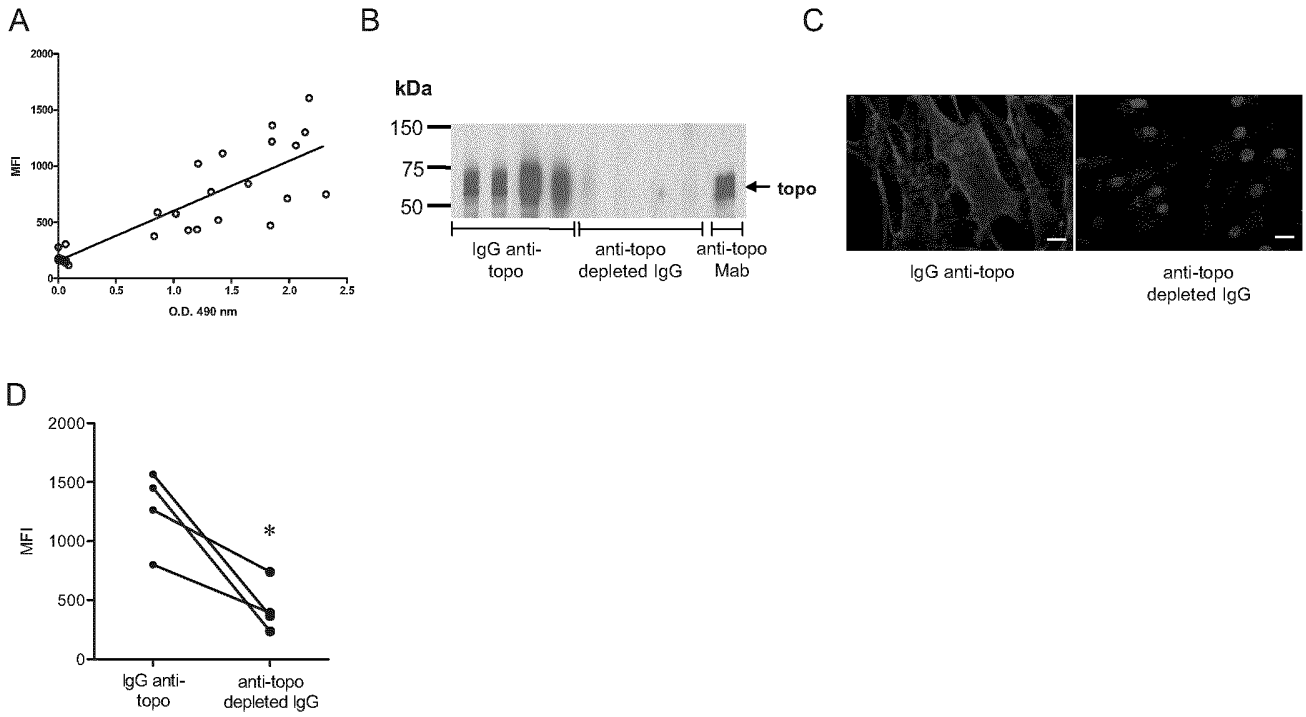
A
2000
1500
1000
500
0
MFI
0.0
0.5
1.0
1.5
2.0
2.5
O.D. 490 nm
B
kDa
150
75
50
topo
IgG anti-topo
anti-topo depleted IgG
anti-topo Mab
C
IgG anti-topo
anti-topo depleted IgG
D
2000
1500
1000
500
0
MFI
*
IgG anti-topo
anti-topo depleted IgG

Figure 2

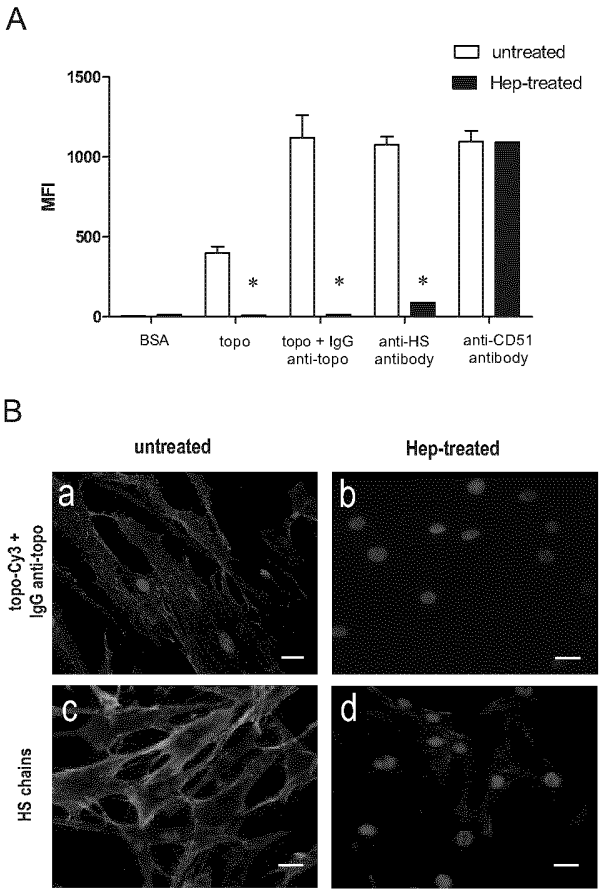
A
untreated
Hep-treated
1500
1000
500
0
MFI
*
*
*
BSA
topo
topo + IgG anti-topo
anti-HS antibody
anti-CD51 antibody
B
untreated
Hep-treated
topo-Cy3 + IgG anti-topo
HS chains
a
b
c
d

Figure 3

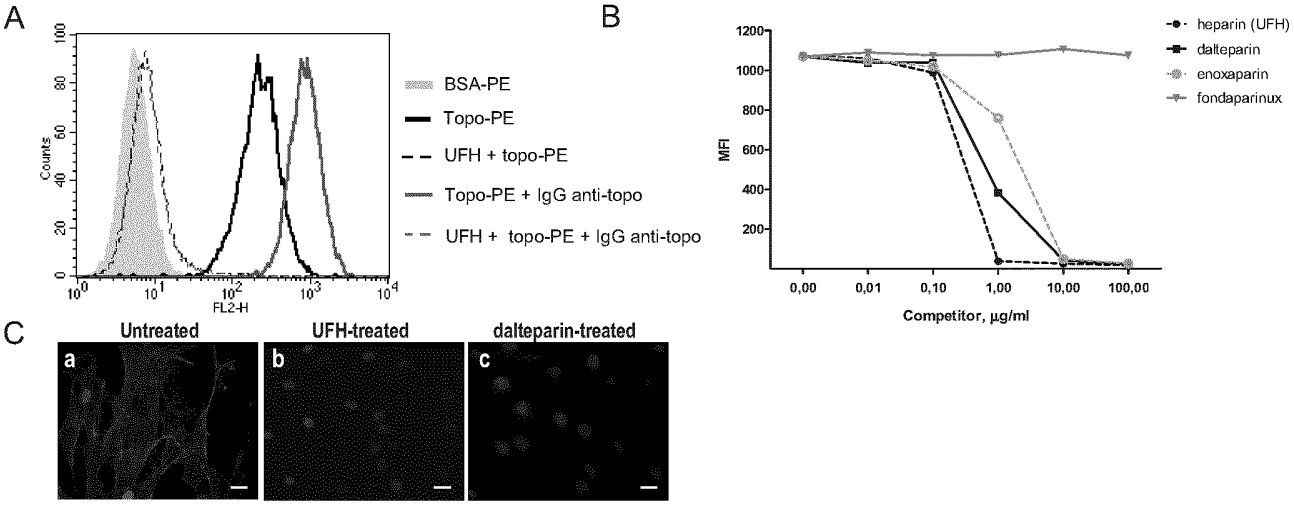
A
Counts
FL2-H
BSA-PE
Topo-PE
UFH + topo-PE
Topo-PE + IgG anti-topo
UFH + topo-PE + IgG anti-topo
B
MFI
Competitor, µg/ml
heparin (UFH)
dalteparin
enoxaparin
fondaparinux
C
Untreated
UFH-treated
dalteparin-treated
a
b
c

Figure 4

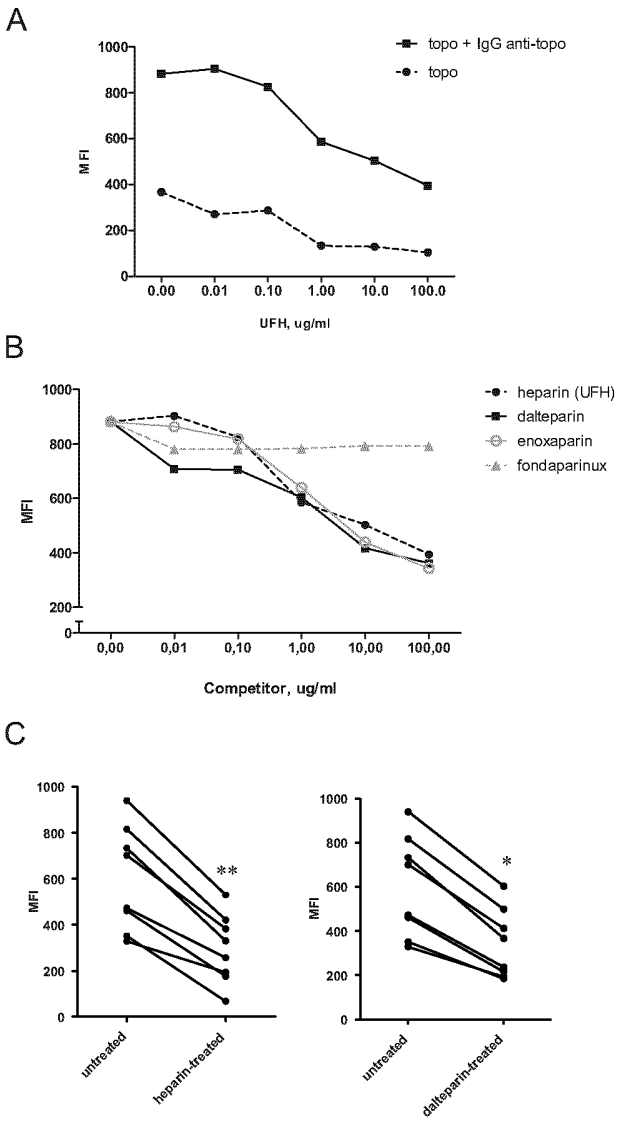
A
topo + IgG anti-topo
topo
MFI
1000
800
600
400
200
0
0.00
0.01
0.10
1.00
10.0
100.0
UFH, ug/ml
B
heparin (UFH)
dalteparin
enoxaparin
fondaparinux
MFI
1000
800
600
400
200
0
0,00
0,01
0,10
1,00
10,00
100,00
Competitor, ug/ml
C
MFI
**
untreated
heparin-treated
MFI
*
untreated
dalteparin-treated

Figure 5

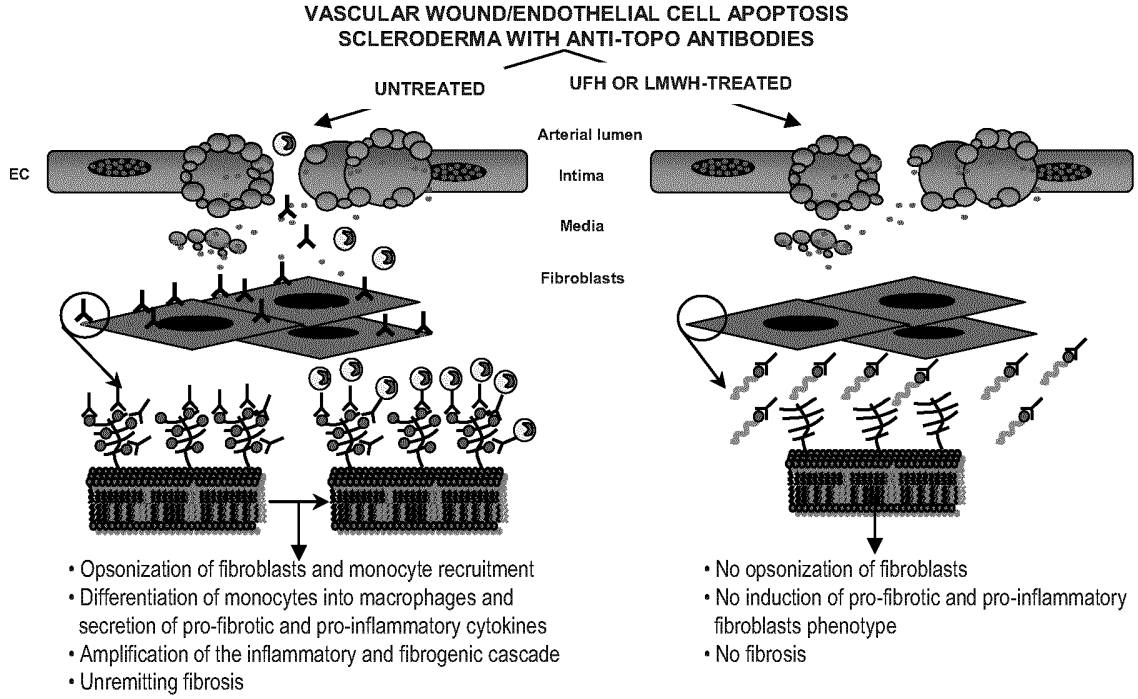
VASCULAR WOUND/ENDOTHELIAL CELL APOPTOSIS
SCLERODERMA WITH ANTI-TOPO ANTIBODIES
UNTREATED
UFH OR LMWH-TREATED
EC
Arterial lumen
Intima
Media
Fibroblasts
• Opsonization of fibroblasts and monocyte recruitment
• Differentiation of monocytes into macrophages and secretion of pro-fibrotic and pro-inflammatory cytokines
• Amplification of the inflammatory and fibrogenic cascade
• Unremitting fibrosis
• No opsonization of fibroblasts
• No induction of pro-fibrotic and pro-inflammatory fibroblasts phenotype
• No fibrosis

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/050309

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 31/726*** (2006.01) , ***A61K 31/727*** (2006.01) , ***A61P 37/06*** (2006.01) , ***G01N 33/15*** (2006.01) , ***G01N 33/50*** (2006.01) , ***G06F 19/00*** (2011.01), *C12N 9/90* (2006.01) , *C12Q 1/533* (2006.01)

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)

A61K* (2006.01)

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

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

Canadian Patent Database, EPOQUE (Epodoc, English Full-Text), Scopus; Keywords used: systemic sclerosis, scleroderma, glycosaminoglycan, heparin, heparan, chondroitin, dermatan, hyaluronan, keratan and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DENTON, C.P. ET AL. "Long-term low molecular weight heparin therapy for severe Raynaud's phenomenon: a pilot study", *Clinical and Experimental Rheumatology* (2000), 18:499-502 ISSN: 0392-856X see entire document	1-23
X	FERRAO A.V. ET AL. "The effect of heparin on cell proliferation and type-I collagen synthesis by adult human dermal fibroblasts", *Biochimica et Biophysica Acta* (1993) 1180:225-230 ISSN: 0925-4439 see entire document	1-23
X	CHODOROWSKA, G. "Results of long-term heparin treatment of patients with acroscleroderma", *Wiadomosci Lekarskie* (1984) 37:1765-1769 ISSN: 0043-5147 see entire document	1-23

[X] 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	Date of mailing of the international search report
01 August 2012 (01-08-2012)	08 August 2012 (08-08-2012)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer
Dana Eisler (819) 994-3466

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/050309

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the 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. [X] Claim Nos. : 1-7

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1-7 are directed to a method of treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search (Rule 39.1(iv), PCT). However, this Authority has carried out a search based on the alleged therapeutic effects of the defined glycosaminoglycan.

2. [ ] Claim 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. [ ] Claim 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 Extra 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 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 claim Nos. :

4. [X] 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 claim Nos. : 1-23

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.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRINBERG, N.B. ET AL. "Experience in long term treatment of systemic sclerodermia on a dispensary level", *Terapevticheskii Arkhiv* (1976) 48:115-119 ISSN: 0040-3660 see entire document, especially page 118.	1-23
A	HU, P.Q. ET AL. "Correlation of serum anti-DNA topoisomerase I antibody levels with disease severity and activity in systemic sclerosis", *Arthritis & Rheumatism* (2003) 48:1363-1373 ISSN 1529-0131	1-23

The present claims are directed to a plurality of alleged inventions as outlined in the following groups:

Group A: Claims 1-23 are directed to the treatment of systemic sclerosis with a glycosaminoglycan; and

Group B: Claims 24-50 are directed to a method of screening for compounds suitable to prevent or treat systemic sclerosis, and to computer systems and software used in the screening method.

The subject matter of Group A is directed to the use of a glycosaminoglycan for the treatment of systemic sclerosis, while the subject matter of Group B is directed to a general method of screening for compounds that might be suitable for the treatment or prevention of systemic sclerosis. The only feature in common between these groups is a compound for treating systemic sclerosis. However, compounds for treating or preventing systemic sclerosis are part of common general knowledge in the art. As such, the subject matter defined in these groups is not so linked as to form a single general inventive concept as required by PCT Rule 13.