(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 November 2013 (21.11.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/171296 A1

(51) International Patent Classification:
*G01N 33/68* (2006.01) *A61K 39/395* (2006.01)

(21) International Application Number:
PCT/EP2013/060126

(22) International Filing Date:
16 May 2013 (16.05.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
12305548.5 16 May 2012 (16.05.2012) EP

(71) Applicants: **INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE)** [FR/FR]; 101, rue de Tolbiac, F-75013 Paris (FR). **UNIVERSITÉ PARIS DIDEROT - PARIS 7** [FR/FR]; 5, rue Thomas Mann, F-75205 Paris Cedex 13 (FR).

(72) Inventors: **BENSUSSAN, Armand**; U976, Hôpital Saint Louis, 1 Avenue Claude Vellefaux, Pavillon Bazin, F-75475 Paris (FR). **BOUAZIZ, Jean-David**; U976, Hôpital Saint Louis, 1 Avenue Claude Vellefaux, Pavillon Bazin, F-75475 Paris (FR). **TAZI, Abdellatif**; U976, Hôpital Saint Louis, 1 Avenue Claude Vellefaux, Pavillon Bazin, F-75475 Paris (FR). **BAGOT, Martine**; U976, Hôpital Saint Louis, 1 Avenue Claude Vellefaux, Pavillon Bazin, F-75475 Paris (FR). **SAUSSINE, Anne**; U976, Hôpital Saint Louis, 1 Avenue Claude Vellefaux, Pavillon Bazin, F-75475 Paris (FR).

(74) Agent: **HIRSCH, Denise**; Inserm Transfert, 7 rue Watt, F-75013 Paris (FR).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

*[Continued on next page]*

(54) Title: DIAGNOSTIC AND TREATMENT OF SARCOIDOSIS

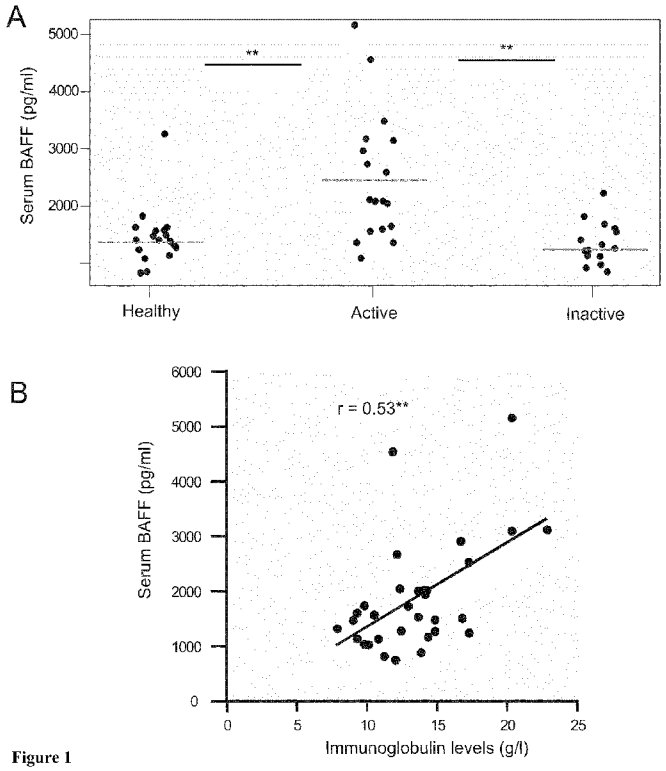



Figure 1

(57) Abstract: The present invention relates to a method for diagnosis active sarcoidosis in a patient comprising: measuring the expression level of B-cell activating factor (BAFF) in a sample obtained from said patient; and comparing said expression level to a threshold value. The invention also relates to a compound which inhibits the binding of BAFF to TACI, BCMA or BAFF-R or a compound which is an inhibitor of BAFF, TACI, BCMA or BAFF-R gene expression for use in the treatment of active sarcoidosis.

WO 2013/171296 A1

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published**:

— *with international search report (Art. 21(3))*

# DIAGNOSTIC AND TREATMENT OF SARCOIDOSIS

## FIELD OF THE INVENTION:

The present invention relates to a method for diagnosis active sarcoidosis in a patient comprising: measuring the expression level of B-cell activating factor (BAFF) in a sample obtained from said patient; and comparing said expression level to a threshold value. The invention also relates to a compound which inhibits the binding of BAFF to TACI, BCMA or BAFF-R or a compound which is an inhibitor of BAFF, TACI, BCMA or BAFF-R gene expression for use in the treatment of active sarcoidosis.

## BACKGROUND OF THE INVENTION:

Sarcoidosis is a multisystemic disease of unknown etiology (Iannuzzi MC et al, 2007) characterized by a disproportionate Th1 granulomatous immune response in the organs involved. Th1 lymphocytes predominantly secrete interleukin-2 and interferon gamma, enhance macrophage tumor necrosis factor (TNF) alpha production and amplify the local cellular immune response. Although innate and T cell immunity play key roles in the pathogenesis of sarcoidosis, several arguments suggest a potential involvement of humoral immune responses in this disease. For example, active sarcoidosis has been associated with plasmatic hypergammaglobulinemia, B cell accumulation has been shown in pulmonary lesions and a beneficial effect of anti-CD20 monoclonal antibody B cell-depleting therapy has been reported in select patients. B cell-activating factor from the TNF family (BAFF), also called BlyS (B lymphocyte stimulator), is a TNF superfamily member best known for its role in the survival and maturation of B cells (Mackay M et al, 2002). Increased blood levels of BAFF have been found in patients with a variety of autoimmune diseases, suggesting that excessive BAFF stimulation in humans contributes to the development of autoimmunity. For example, it has been demonstrated that BAFF plays a role in systemic lupus erythematosus (Cheema GS et al, 2001). However, no link has been demonstrated between BAFF and sarcoidosis.

## SUMMARY OF THE INVENTION:

The inventors analyzed BAFF levels in untreated patients with active chronic sarcoidosis and compared these results with healthy donors and inactive sarcoidosis patients. Increased circulating BAFF levels were found in active sarcoidosis patients and correlated with serum hypergammaglobulinemia.

Thus, the invention relates to a method for diagnosis active sarcoidosis in a patient comprising: measuring the expression level of B-cell activating factor (BAFF) in a sample obtained from said patient; and comparing said expression level to a threshold value.

The invention also relates to a compound which inhibits the binding of BAFF to TACI, BCMA or BAFF-R or a compound which is an inhibitor of BAFF, TACI, BCMA or BAFF-R gene expression for use in the treatment of active sarcoidosis.

## DETAILED DESCRIPTION OF THE INVENTION:

### ***Definitions:***

Throughout the specification, several terms are employed and are defined in the following paragraphs.

### *Diagnostic method*

The invention relates to a method for diagnosis active sarcoidosis in a patient comprising a step consisting of detecting B-cell activating factor (BAFF) expression in a sample obtained from said patient.

As used herein, the term "BAFF" has its general meaning in the art and denotes a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. This cytokine is a ligand for three receptors: TNFRSF13B/TACI, TNFRSF17/BCMA, and TNFRSF13C/BAFF-R (see for example Cancro P. Michael et al., 2009). This cytokine is expressed in B cell lineage cells, and acts as a potent B cell activator. It has been also shown to play an important role in the proliferation and differentiation of B cells. An exemplary sequence for human BAFF protein is deposited in the UniProtKB/Swiss-Prot database under accession numbers Q9Y275.

In a one embodiment, the sarcoidosis may be a pulmonary sarcoidosis, a skin sarcoidosis, a joint sarcoidosis, a heart sarcoidosis, a peripheral nervous system sarcoidosis, a central nervous system sarcoidosis, a liver sarcoidosis, an eye sarcoidosis, an upper respiratory tract sarcoidosis or a gastro intestinal tract sarcoidosis.

In a one embodiment, the sarcoidosis is an active chronic sarcoidosis.

Typically, the sample according to the invention may be a blood, plasma, serum sample. In a particular embodiment, said sample is blood.

The term "detecting" as used above includes qualitative and/or quantitative detection (measuring levels) with or without reference to a control. Typically BAFF expression may be measured for example by enzyme-labeled and mediated immunoassays (such as ELISA) performed on the sample.

Particularly, the invention relates to a method for diagnosis of active sarcoidosis in a patient comprising a step a) consisting of measuring BAFF expression level in a sample obtained from said patient. Preferably, the method of the invention further comprises a step of comparing the BAFF expression level obtained in step a) to a threshold value.

The “control” may be a healthy subject, i.e. a subject who does not suffer from any sarcoidosis. The control may also be a subject suffering from inactive sarcoidosis. Preferably, said control is a healthy subject.

Detection of BAFF expression in the sample may be performed by measuring the level of BAFF protein. In the present application, the “level of BAFF protein” means the quantity or concentration of said BAFF protein.

Such methods comprise contacting a sample with a binding partner capable of selectively interacting with BAFF protein present in the sample. The binding partner is generally an antibody that may be polyclonal or monoclonal, preferably monoclonal.

The presence of the protein can be detected using standard electrophoretic and immunodiagnostic techniques, including immunoassays such as competition, direct reaction, or sandwich type assays. Such assays include, but are not limited to, Western blots; agglutination tests; enzyme-labeled and mediated immunoassays, such as ELISAs; biotin/avidin type assays; radioimmunoassays; immunoelectrophoresis; immunoprecipitation, etc. The reactions generally include revealing labels such as fluorescent, chemiluminescent,

radioactive, enzymatic labels or dye molecules, or other methods for detecting the formation of a complex between the antigen and the antibody or antibodies reacted therewith.

The aforementioned assays generally involve separation of unbound protein in a liquid phase from a solid phase support to which antigen-antibody complexes are bound. Solid supports which can be used in the practice of the invention include substrates such as nitrocellulose (e. g., in membrane or microtiter well form); polyvinylchloride (e. g., sheets or microtiter wells); polystyrene latex (e.g., beads or microtiter plates); polyvinylidine fluoride; diazotized paper; nylon membranes; activated beads, magnetically responsive beads, and the like.

More particularly, an ELISA method can be used, wherein the wells of a microtiter plate are coated with a set of antibodies against the proteins to be tested. A sample containing or suspected of containing the marker protein is then added to the coated wells. After a period of incubation sufficient to allow the formation of antibody-antigen complexes, the plate(s) can be washed to remove unbound moieties and a detectably labeled secondary binding molecule is added. The secondary binding molecule is allowed to react with any captured sample marker protein, the plate is washed and the presence of the secondary binding molecule is detected using methods well known in the art.

Various immunoenzymatic staining methods are known in the art for detecting a protein of interest. For example, immunoenzymatic interactions can be visualized using different enzymes such as peroxidase, alkaline phosphatase, or different chromogens such as DAB, AEC, or Fast Red; or fluorescent labels such as FITC, Cy3, Cy5, Cy7, Alexafluors, etc. Counterstains may include H&E, DAPI, Hoechst, so long as such stains are compatable with other detection reagents and the visualization strategy used. As known in the art, amplification reagents may be used to intensify staining signal. For example, tyramide reagents may be used. The staining methods of the present invention may be accomplished using any suitable method or system as would be apparent to one of skill in the art, including automated, semi-automated or manual systems.

The method of the invention may comprise a further step consisting of comparing BAFF expression with a control reference.

The invention thus relates to a method for diagnosis active sarcoidosis in a patient comprising determining the expression level of BAFF in a sample obtained from said patient and comparing said expression level to a threshold value. As used herein, "expression level of BAFF" refers to an amount or a concentration of a transcription product, for instance mRNA coding for BAFF, or of a translation product, for instance the protein BAFF. Typically, a level

of mRNA expression can be expressed in units such as transcripts per cell or nanograms per microgram of tissue. A level of a polypeptide can be expressed as nanograms per microgram of tissue or nanograms per milliliter of a culture medium, for example. Alternatively, relative units can be employed to describe an expression level.

In a one embodiment, when the measure of BAFF protein is performed by ELISA, the expression level of BAFF protein in a patient suffering of active sarcoidosis is increased by at least 40%, preferably by at least 50%; preferably by at least 60 %, preferably by at least 70%, preferably by at least 80%, more preferably by at least 90%, even more at least 100% compared to a control reference. In other words, preferably, when BAFF protein is measured by ELISA, the quantity of BAFF protein in a patient suffering of active sarcoidosis is increased by at least 40%, preferably by at least 50%; preferably by at least 60 %, preferably by at least 70%, preferably by at least 80%, more preferably by at least 90%, even more at least 100% compared to a control reference.

Typically, a "threshold value", "threshold level" or "cut-off value" can be determined experimentally, empirically, or theoretically. A threshold value can also be arbitrarily selected based upon the existing experimental and/or clinical conditions, as would be recognized by a person of ordinary skilled in the art. Preferably, the person skilled in the art may compare the expression levels of BAFF obtained according to the method of the invention with a defined threshold value.

Preferably, said threshold value is the mean expression level of BAFF of a population of healthy individuals. As used herein, the term "healthy individual" denotes a human which is known to be healthy, i.e. which does not suffer from active sarcoidosis, has never been subjected to such active sarcoidosis, and does not need any medical care.

Typically, the skilled person in the art may determine the expression level of BAFF in a biological sample, preferably blood, of 100 individuals known to be healthy. The mean value of the obtained expression levels is then determined, according to well known statistical analysis, so as to obtain the mean expression level of BAFF. Said value is then considered as being normal and thus constitute a threshold value. By comparing the expression levels of BAFF to this threshold value, the physician is then able to diagnose active sarcoidosis. Indeed, by comparing the expression level of BAFF obtained in a biological sample, preferably blood, of a given subject to a threshold value, one can easily determine whether said subject suffers from active sarcoidosis or not.

Accordingly, the physician would be able to adapt and optimize appropriate medical care of a subject in a critical and life-threatening condition suffering from active sarcoidosis.

The determination of said prognosis is highly appropriate for follow-up care and clinical decision making.

Therefore, the invention is drawn to a method for diagnosis active sarcoidosis in a patient comprising the following steps:

a) determining the level of expression of BAFF in a sample obtained from said patient;

b) determining the mean expression level of BAFF in a biological sample of a population of healthy individuals, preferably 100 healthy individuals; and

c) a step of comparing the expression level of BAFF obtained of a) to the mean expression level of BAFF obtained in b).

The present invention also relates to kits for the diagnosis of active sarcoidosis, comprising means for detecting BAFF expression.

According to the invention, the kits of the invention may comprise an anti-BAFF protein antibody; and another molecule coupled with a signalling system which binds to said BAFF protein antibody.

Typically, the antibodies or combination of antibodies are in the form of solutions ready for use. In one embodiment, the kit comprises containers with the solutions ready for use. Any other forms are encompassed by the present invention and the man skilled in the art can routinely adapt the form to the use in immunohistochemistry.

The present invention also relates to BAFF gene or protein as a biomarker for the diagnosis of active sarcoidosis.

In another embodiment, the invention relates to an in vitro method for monitoring a patient's response sarcoidosis treatment which comprises a step of measuring the level of BAFF protein, in a sample from a patient.

Thus, the present invention provides for the use of BAFF protein as a biomarker for the monitoring of anti sarcoidosis therapies.

According to the invention, the expression level of BAFF protein may be determined to monitor a patient's response to sarcoidosis treatment.

***Therapeutic methods***

A second aspect of the invention relates to a compound which inhibits the binding of BAFF to TACI, BCMA or BAFF-R or a compound which is an inhibitor of BAFF, TACI, BCMA or BAFF-R gene expression for use in the treatment of active sarcoidosis.

As used herein, the term "TACI" has its general meaning in the art and refers to a transmembrane receptor protein found predominantly on the surface of B cells, which are an important part of the immune system. TACI is a lymphocyte-specific member of the tumor necrosis factor (TNF) receptor superfamily.

As used herein, the term "BCMA" has its general meaning in the art and refers to member of the TNFR superfamily expressed on B cells.

As used herein, the term "BAFF-R" has its general meaning in the art and refers to a receptor expresses on all mature B cells.

In one embodiment, the compound according to the invention may bind to BAFF, TACI, BCMA or BAFF-R and block the binding of BAFF on TACI, BCMA or BAFF-R and block its physiological effects. To identify a compound able to block the interaction between BAFF, TACI, BCMA or BAFF-R, a test may be used. For example, the compound to test will compete with the binding of BAFF labelled with a flurochrom (as fluorescein isothiocyanate) on TACI, BCMA or BAFF-R transfected cell lines. The inhibition of the binding will then analyzed by flow cytometry.

Typically, the compound according to the invention includes but is not limited to a small organic molecule, an antibody, and a polypeptide.

In one embodiment, the compound according to the invention may be a low molecular weight compound, e. g. a small organic molecule (natural or not).

The term "small organic molecule" refers to a molecule (natural or not) of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e. g., proteins, nucleic acids, etc.). Preferred small organic molecules range in size up to about 10000 Da, more preferably up to 5000 Da, more preferably up to 2000 Da and most preferably up to about 1000 Da.

In one embodiment, the compound according to the invention is an antibody. Antibodies directed against BAFF, TACI, BCMA or BAFF-R can be raised according to known methods by administering the appropriate antigen or epitope to a host animal selected, e.g., from pigs, cows, horses, rabbits, goats, sheep, and mice, among others. Various adjuvants known in the art can be used to enhance antibody production. Although antibodies useful in practicing the invention can be polyclonal, monoclonal antibodies are preferred. Monoclonal antibodies against BAFF, TACI, BCMA or BAFF-R can be prepared and isolated using any technique that provides for the production of antibody molecules by continuous cell lines in culture. Techniques for production and isolation include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975); the human B-cell hybridoma technique (Cote et al., 1983); and the EBV-hybridoma technique (Cole et al. 1985). Alternatively, techniques described for the production of single chain antibodies (see e.g., U.S. Pat. No. 4,946,778) can be adapted to produce anti- BAFF, anti-TACI, anti-BCMA or anti-BAFF-R single chain antibodies. Coumpounds useful in practicing the present invention also include anti-BAFF, anti-TACI, anti-BCMA or anti-BAFF-R antibody fragments including but not limited to F(ab')2 fragments, which can be generated by pepsin digestion of an intact antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab and/or scFv expression libraries can be constructed to allow rapid identification of fragments having the desired specificity to BAFF, TACI, BCMA or BAFF-R.

Humanized anti-BAFF, anti-TACI, anti-BCMA or anti-BAFF-R antibodies and antibody fragments therefrom can also be prepared according to known techniques. "Humanized antibodies" are forms of non-human (e.g., rodent) chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (CDRs) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human

immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Methods for making humanized antibodies are described, for example, by Winter (U.S. Pat. No. 5,225,539) and Boss (Celltech, U.S. Pat. No. 4,816,397).

Then, for this invention, neutralizing antibodies of BAFF, TACI, BCMA or BAFF-R are selected.

In one embodiment, the compound according to the invention is an anti-BAFF antibody.

In a particular embodiment, the antibody according to the invention may be the Belimumab (see for example Espinosa G. et al, 2010 or Liu Zheng et al., 2011).

In a particular embodiment, the antibody according to the invention may be an antibody according to Scholz L. Jean et al., 2008.

In a particular embodiment, the antibody according to the invention may be the LY2127399 (see for example Davidson A 2010).

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application WO0043032.

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application WO2006025345.

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application WO2006025345.

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application CN101851291.

In another embodiment, the compound according to the invention is an anti-TACI antibody.

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application WO02066516.

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application WO2004011611.

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application WO0160397.

In another embodiment, the compound according to the invention is an anti-BCMA antibody.

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application WO02066516.

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application WO0124811.

In a particular embodiment, the antibody according to the invention may be an antibody according to the patent application WO0160397.

In another embodiment, the compound according to the invention is an anti-BAFF-R antibody.

In a particular embodiment, the antibody according to the invention may be an antibody according to Ramanujam Meera et al., 2006.

In a particular embodiment, the antibody according to the invention may be an antibody according to Rauch Melanie et al., 2009.

In one embodiment, the compound according to the invention is an aptamer. Aptamers are a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide or oligopeptide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Such ligands may be isolated through Systematic Evolution of Ligands by EXponential enrichment

(SELEX) of a random sequence library, as described in Tuerk C. and Gold L., 1990. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., 1999. Peptide aptamers consists of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods (Colas et al., 1996).

Then, for this invention, neutralizing aptamers of BAFF, TACI, BCMA or BAFF-R are selected.

In one embodiment, the compound according to the invention is a polypeptide.

In a particular embodiment the polypeptide is a functional equivalent of TACI, BCMA or BAFF-R. As used herein, a "functional equivalent" of TACI, BCMA or BAFF-R is a compound which is capable of binding to BAFF, thereby preventing its interaction with TACI, BCMA or BAFF-R. The term "functional equivalent" includes fragments, mutants, and muteins of TACI, BCMA or BAFF-R. The term "functionally equivalent" thus includes any equivalent of TACI, BCMA or BAFF-R obtained by altering the amino acid sequence, for example by one or more amino acid deletions, substitutions or additions such that the protein analogue retains the ability to bind to BAFF. Amino acid substitutions may be made, for example, by point mutation of the DNA encoding the amino acid sequence.

Functional equivalents include molecules that bind BAFF and comprise all or a portion of the extracellular domains of TACI, BCMA or BAFF-R. Typically, said functional equivalents may be the extracellular domains of TACI, BCMA or BAFF-R expressed as Fc fusion protein. For example, fusion proteins may be composed of the extracellular ligand binding portion of TACI which blocks activation of TACI by BAFF (e.g. Atacicept, Merck) or a fusion protein composed of the extracellular ligand-binding portion of BAFF-R which blocks activation of BAFF-R by BAFF (e.g. BR3-Fc, Biogen and Genentech, see for example Vugmeyster Yulia et al., 2006). Such fusion proteins can be generated using methods known in the art, such as recombinant DNA technology as is described in details herein below.

In one embodiment, the polypeptide according to the invention is able to treat active sarcoidosis through its properties of decoy receptor.

By "decoy receptor", is meant that the polypeptide according to the invention trap BAFF and prevent its physiological effects on TACI, BCMA or BAFF-R.

The functional equivalents include soluble forms of TACI, BCMA or BAFF-R. A suitable soluble form of these proteins, or functional equivalents thereof, might comprise, for example, a truncated form of the protein from which the transmembrane domain has been removed by chemical, proteolytic or recombinant methods.

Preferably, the functional equivalent is at least 80% homologous to the corresponding protein. In a particular embodiment, the functional equivalent is at least 90% homologous as assessed by any conventional analysis algorithm such as for example, the Pileup sequence analysis software (Program Manual for the Wisconsin Package, 1996).

The term "a functionally equivalent fragment" as used herein also may mean any fragment or assembly of fragments of TACI, BCMA or BAFF-R that binds to BAFF. Accordingly the present invention provides a polypeptide capable of inhibiting binding of TACI, BCMA or BAFF-R to BAFF, which polypeptide comprises consecutive amino acids having a sequence which corresponds to the sequence of at least a portion of an extracellular domain of TACI, BCMA or BAFF-R, which portion binds to BAFF. In one embodiment, the polypeptide corresponds to an extracellular domain of TACI, BCMA or BAFF-R. In another embodiment, the polypeptide corresponds to the extracellular domains of TACI, BCMA or BAFF-R expressed as Fc fusion protein.

Functionally equivalent fragments may belong to the same protein family as the TACI, BCMA or BAFF-R identified herein. By "protein family" is meant a group of proteins that share a common function and exhibit common sequence homology. Homologous proteins may be derived from non-human species. Preferably, the homology between functionally equivalent protein sequences is at least 25% across the whole of amino acid sequence of the complete protein. More preferably, the homology is at least 50%, even more preferably 75% across the whole of amino acid sequence of the protein or protein fragment. More preferably, homology is greater than 80% across the whole of the sequence. More preferably, homology is greater than 90% across the whole of the sequence. More preferably, homology is greater than 95% across the whole of the sequence.

In one embodiment, the polypeptide according to the invention may be also a functional equivalent of BAFF. As used herein, a “functional equivalent” of BAFF is a compound which is capable of binding to TACI, BCMA or BAFF-R, thereby preventing its interaction with the natural ligand BAFF. The term "functional equivalent" includes fragments, mutants, and muteins of BAFF. The term "functionally equivalent" thus includes any equivalent of BAFF obtained by altering the amino acid sequence, for example by one or

more amino acid deletions, substitutions or additions such that the protein analogue retains the ability to bind to TACI, BCMA or BAFF-R. Amino acid substitutions may be made, for example, by point mutation of the DNA encoding the amino acid sequence. A compound as explained in the patent applications WO2004081043 or WO2006034106 may be used.

The polypeptides of the invention may be produced by any suitable means, as will be apparent to those of skill in the art. In order to produce sufficient amounts of BAFF, TACI, BCMA or BAFF-R or functional equivalents thereof for use in accordance with the present invention, expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the polypeptide of the invention. Preferably, the polypeptide is produced by recombinant means, by expression from an encoding nucleic acid molecule. Systems for cloning and expression of a polypeptide in a variety of different host cells are well known.

When expressed in recombinant form, the polypeptide is preferably generated by expression from an encoding nucleic acid in a host cell. Any host cell may be used, depending upon the individual requirements of a particular system. Suitable host cells include bacteria mammalian cells, plant cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells. HeLa cells, baby hamster kidney cells and many others. Bacteria are also preferred hosts for the production of recombinant protein, due to the ease with which bacteria may be manipulated and grown. A common, preferred bacterial host is E coli.

In specific embodiments, it is contemplated that polypeptides used in the therapeutic methods of the present invention may be modified in order to improve their therapeutic efficacy. Such modification of therapeutic compounds may be used to decrease toxicity, increase circulatory time, or modify biodistribution. For example, the toxicity of potentially important therapeutic compounds can be decreased significantly by combination with a variety of drug carrier vehicles that modify biodistribution. In example adding dipeptides can improve the penetration of a circulating agent in the eye through the blood retinal barrier by using endogenous transporters.

A strategy for improving drug viability is the utilization of water-soluble polymers. Various water-soluble polymers have been shown to modify biodistribution, improve the mode of cellular uptake, change the permeability through physiological barriers; and modify the rate of clearance from the body. To achieve either a targeting or sustained-release effect,

water-soluble polymers have been synthesized that contain drug moieties as terminal groups, as part of the backbone, or as pendent groups on the polymer chain.

Polyethylene glycol (PEG) has been widely used as a drug carrier, given its high degree of biocompatibility and ease of modification. Attachment to various drugs, proteins, and liposomes has been shown to improve residence time and decrease toxicity. PEG can be coupled to active agents through the hydroxyl groups at the ends of the chain and via other chemical methods; however, PEG itself is limited to at most two active agents per molecule. In a different approach, copolymers of PEG and amino acids were explored as novel biomaterials which would retain the biocompatibility properties of PEG, but which would have the added advantage of numerous attachment points per molecule (providing greater drug loading), and which could be synthetically designed to suit a variety of applications.

Those of skill in the art are aware of PEGylation techniques for the effective modification of drugs. For example, drug delivery polymers that consist of alternating polymers of PEG and tri-functional monomers such as lysine have been used by VectraMed (Plainsboro, N.J.). The PEG chains (typically 2000 daltons or less) are linked to the a- and e-amino groups of lysine through stable urethane linkages. Such copolymers retain the desirable properties of PEG, while providing reactive pendent groups (the carboxylic acid groups of lysine) at strictly controlled and predetermined intervals along the polymer chain. The reactive pendent groups can be used for derivatization, cross-linking, or conjugation with other molecules. These polymers are useful in producing stable, long-circulating pro-drugs by varying the molecular weight of the polymer, the molecular weight of the PEG segments, and the cleavable linkage between the drug and the polymer. The molecular weight of the PEG segments affects the spacing of the drug/linking group complex and the amount of drug per molecular weight of conjugate (smaller PEG segments provides greater drug loading). In general, increasing the overall molecular weight of the block co-polymer conjugate will increase the circulatory half-life of the conjugate. Nevertheless, the conjugate must either be readily degradable or have a molecular weight below the threshold-limiting glomular filtration (e.g., less than 60 kDa).

In addition, to the polymer backbone being important in maintaining circulatory half-life, and biodistribution, linkers may be used to maintain the therapeutic agent in a pro-drug form until released from the backbone polymer by a specific trigger, typically enzyme activity in the targeted tissue. For example, this type of tissue activated drug delivery is particularly useful where delivery to a specific site of biodistribution is required and the therapeutic agent is released at or near the site of pathology. Linking group libraries for use in activated drug

delivery are known to those of skill in the art and may be based on enzyme kinetics, prevalence of active enzyme, and cleavage specificity of the selected disease-specific enzymes. Such linkers may be used in modifying the protein or fragment of the protein described herein for therapeutic delivery.

In another embodiment, the compound according to the invention is an inhibitor of BAFF, TACI, BCMA or BAFF-R gene expression.

Small inhibitory RNAs (siRNAs) can also function as inhibitors of BAFF, TACI, BCMA or BAFF-R expression for use in the present invention. BAFF, TACI, BCMA or BAFF-R gene expression can be reduced by contacting a subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that BAFF, TACI, BCMA or BAFF-R gene expression is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g. see for example Tuschl, T. et al. (1999); Elbashir, S. M. et al. (2001); Hannon, GJ. (2002); McManus, MT. et al. (2002); Brummelkamp, TR. et al. (2002); U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836).

Ribozymes can also function as inhibitors of BAFF, TACI, BCMA or BAFF-R gene expression for use in the present invention. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of BAFF, TACI, BCMA or BAFF-R mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

Both antisense oligonucleotides and ribozymes useful as inhibitors of BAFF, TACI, BCMA or BAFF-R gene expression can be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramadite chemical synthesis. Alternatively, anti-sense RNA molecules can be generated by in vitro or in vivo transcription of DNA sequences encoding the RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the invention can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.

Antisense oligonucleotides siRNAs and ribozymes of the invention may be delivered in vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the antisense oligonucleotide siRNA or ribozyme nucleic acid to the cells and preferably cells expressing BAFF, TACI, BCMA or BAFF-R. Preferably, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the the antisense oligonucleotide siRNA or ribozyme nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rouse sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses (e.g., lentivirus), the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered

retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, 1990 and in Murry, 1991).

Preferred viruses for certain applications are the adeno-viruses and adeno-associated viruses, which are double-stranded DNA viruses that have already been approved for human use in gene therapy. The adeno-associated virus can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as, heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well known to those of skill in the art. See e.g. Sambrook et al., 1989. In the last few years, plasmid vectors have been used as DNA vaccines for delivering antigen-encoding genes to cells in vivo. They are particularly advantageous for this because they do not have the same safety concerns as with many of the viral vectors. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUCl9, pRC/CMV, SV40, and pBlueScript. Other plasmids are well known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA. Plasmids may be delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA plasmid can be injected by intramuscular, eye, intradermal, subcutaneous, or other routes. It may also be administered by intranasal sprays or drops, rectal suppository and orally. It may also be administered into the epidermis or a mucosal surface using a gene-gun. The plasmids may be given in an aqueous solution, dried onto gold particles or in association with another

DNA delivery system including but not limited to liposomes, dendrimers, cochleate and microencapsulation.

In a particular embodiment, the antisense oligonucleotide, siRNA, shRNA or ribozyme nucleic acid sequence is under the control of a heterologous regulatory region, e.g., a heterologous promoter. The promoter may be specific for Muller glial cells, microglia cells, endothelial cells, pericyte cells and astrocytes For example, a specific expression in Muller glial cells may be obtained through the promoter of the glutamine synthetase gene is suitable. The promoter can also be, e.g., a viral promoter, such as CMV promoter or any synthetic promoters.

Another object of the invention relates to a method for treating active sarcoidosis comprising administering to a subject in need thereof a therapeutically effective amount of a compound which inhibits the binding of BAFF to TACI, BCMA or BAFF-R or a compound which is an inhibitor of BAFF, TACI, BCMA or BAFF-R gene expression for use in the treatment of active sarcoidosis.

In a one embodiment, said compound is a monoclonal antibody anti-BAFF.

In one embodiment, the compound according to the invention is able to induce the inactivation of active sarcoidosis in subject in need thereof. In other word, the compound according to the invention is able to induce the passage from active state to inactive state of sarcoidosis in subject in need thereof.

***Therapeutic composition***

Another object of the invention relates to a therapeutic composition comprising a compound according to the invention for use in the treatment of active sarcoidosis.

In a one embodiment, the sarcoidosis may be a pulmonary sarcoidosis, a skin sarcoidosis, a joint sarcoidosis, a heart sarcoidosis, a peripheral nervous system sarcoidosis, a central nervous system sarcoidosis, a liver sarcoidosis, an eye sarcoidosis, an upper respiratory tract sarcoidosis or a gastro intestinal tract sarcoidosis.

Any therapeutic agent of the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

"Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc.

The pharmaceutical compositions of the invention can be formulated for a topical, oral, intranasal, parenteral, intraocular, intravenous, intramuscular or subcutaneous administration and the like.

Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The doses used for the administration can be adapted as a function of various parameters, and in particular as a function of the mode of administration used, of the relevant pathology, or alternatively of the desired duration of treatment.

In addition, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; time release capsules; and any other form currently can be used.

Pharmaceutical compositions of the present invention may comprise a further therapeutic active agent. The present invention also relates to a kit comprising a compound according to the invention and a further therapeutic active agent.

In one embodiment said therapeutic active agent is nonsteroidal anti-inflammatory drugs, systemic and topic steroids, hydroxychloroquine, nivaquine, methotrexate, thalidomide, cyclophosphamide, azathioprine, leflunomide, cyclines, anti-tumor necrosis factor (TNF)-α reagents (infliximab, adalimumab and etanercept), anti-interleukin 12/23 reagents (ustekinumab).

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

**FIGURES:**

**Figure 1:** Increased serum BAFF levels in patients with active chronic sarcoidosis correlates with serum hypergammaglobulinemia. (A) Increased serum BAFF levels in active sarcoidosis patients. Scatter plots show the serum BAFF concentrations in 18 healthy donors (healthy), 18 active chronic sarcoidosis patients (active) and 15 inactive sarcoidosis patients (inactive). Each dot represents individual subjects, and horizontal bars represent the group means. Significant differences between the means of patient groups and healthy controls are indicated: **, $p<0.01$. (B) BAFF levels correlate with serum hypergammaglobulinemia in sarcoidosis. The graph shows a linear regression analysis of BAFF levels (pg/ml) versus serum immunoglobulin levels (g/l) in sarcoidosis patients. Each dot represents individual subjects. The Pearson's correlation coefficient is represented by "r". The significance of the strength of the linear relationship is indicated: **, $p<0.01$.

| | | Healthy | Active sarcoidosis | Inactive sarcoidosis |
|---|---|---|---|---|
| **Number** | | 18 | 18 | 15 |
| **Gender, M:F** | | 10/8 | 9/9 | 7/8 |
| **Age, yr** | | 41.9 ± 3.2 (18-61) | 49.1 ± 3.2 (27-68) | 49.6 ± 2 (37-66) |
| **Caucasian/Asian/Afrocaribbean** | | Not available | 9/0/9 | 7/0/8 |
| **Lung§** | | | 16 (89%) | 15 (100%) |
| **Radiographic stage I/II/III/IV** | | | *2/8/1/5* | *3/6/1/5* |
| **Skin§** | | | 10 (56%) | 13 (87%) |
| **Joints§** | | | 5 (27%) | 4 (27%) |
| **Upper respiratory tract§** | | | 6 (33%) | 3 (20%) |
| **Gastro intestinal tract§** | | | 1 (6%) | 1 (7%) |
| **Eyes§** | | | 4 (22%) | 1 (7%) |
| **Nerves§** | | | 1 (6%) | 2 (13%) |
| **Heart§** | | | 0 | 1 (7%) |
| **Gammaglobulins levels (N=6.4-13.0g/l)** | | | 15.2 ± 0.81g/l | 11.4 ± 0.65g/l * |
| **Treatment** | **None** | | 18 (100%) | 4 (27%) |
| | **Prednisone** | | 0 | 10 (67%) |
| | **Methotrexate** | | 0 | 6 (40%) |
| | **Infliximab** | | 0 | 3 (20%) |

**Table 1:** Demographic, clinical and biological characteristics of 18 healthy donors and 18 active and 15 inactive chronic sarcoidosis patients. M=male, F=female, values are given as mean ± SEM, * $p<0.05$ compared with active sarcoidosis, §Visceral sarcoidosis lesions that occurred at any time of the patient's history.

**EXAMPLE:**

**<u>Material & Methods</u>**

**Patients**

All patients' blood and skin samples were obtained after written informed consent in accordance with the Declaration of Helsinki and approval by our institutional review board (CPP, University Paris 12, France). Thirty-three patients who had clinical and histological features consistent with chronic sarcoidosis were prospectively included in this study. Sarcoidosis was diagnosed according to the guidelines of the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders statement on sarcoidosis (23). Patients with Löfgren's syndrome were not recruited because this acute form of sarcoidosis spontaneously recovers in most cases and may have a different immune response. Patients with active sarcoidosis had a progressive disease based on clinical, radiological and lung function evaluations, as previously defined by Boudoin and du Bois (24). All active sarcoidosis patients had a disease that required the initiation of systemic therapy, including corticosteroids, methotrexate or infliximab. Patients with inactive sarcoidosis had no clinical sign of disease activity and stable chest X-ray lung abnormalities that occurred either spontaneously or during the tapering phase of systemic treatment. Blood samples were collected from active sarcoidosis patients before any treatment (n=18) and from patients with inactive disease (n=15). Blood samples from age- and sex-matched control healthy donors (n=18) were collected at the "French Blood Center" (Saint Louis Hospital, Paris, France).

**Antibodies and immunofluorescence analysis**

Peripheral blood mononuclear cells were purified using centrifugation over a Ficoll-Hypaque density gradient, with >98% cell viability. The anti-human fluorochrome-conjugated antibodies used included CD19 (SJ25C1), CD24 (ML5), CD38 (HB7) (all from BD Bioscience), CD27 (0323, eBioscience), IgD (IgD26, Miltenyi Biotec), CD20 (B9E9, Beckmann Coulter) and isotype-matched control antibodies. Cells were analyzed using CyAn flow cytometers (Beckmann Coulter).

**Immunohistochemical staining**

Fixed and paraffin-embedded sarcoid skin biopsies were analyzed for CD20 expression (CD20 mAb, clone L26, DAKO, dilution 1/400) as previously described (25).

**Skin mononuclear cell isolation**

Skin samples were mechanically dissociated, treated with 1 mg/ml collagenase Ia (Sigma, Roche, 2 mg/ml, 37°C, 30 min) and filtered before flow cytometry analysis.

**BAFF enzyme-linked immunosorbent assay**

Serum BAFF levels were quantified using the human BAFF/Blys Quantikine® ELISA immunoassay (R&D Systems) according to the manufacturer's instructions. Briefly, blood was collected in a serum separator tube. Samples were allowed to clot for 30 min before centrifugation for 15 min at 1,000 x g. The centrifuged serum was immediately stored in aliquots at -80°C and used in duplicate after the first thaw.

**Statistical analysis**

The data are shown as the mean (±SEM). The differences between active and inactive groups of sarcoidosis patients and healthy controls were determined using the non-parametric Mann-Whitney test. The correlation between biological parameters was determined using the Pearson correlation test. P values <0.05 were considered statistically significant.

**Results**

**Patient characteristics**

The demographic, clinical and biological characteristics of the 18 patients with active chronic sarcoidosis and the 15 patients with inactive sarcoidosis are summarized in Table I. None of the patients in the active sarcoidosis group were on steroid therapy or immunosuppressive drugs at the time of sampling. Serum gammaglobulin levels (normal range 6.4-13.0 g/l) were significantly higher in the patients with active sarcoidosis (mean 15.2 ± 0.8 g/l) compared with the group of patients with inactive disease (mean 11.4 ± 0.6 g/l, p <0.05).

**Increased serum BAFF levels in patients with active chronic sarcoidosis**

Increased circulating BAFF levels have been detected in several human inflammatory diseases, such as systemic lupus erythematosus (SLE), and may account for disease activity and severity (Cheema GS et al, 2001). We evaluated the serum BAFF levels in healthy individuals and active and inactive sarcoidosis patients. As shown in Fig. 1A, active sarcoidosis patients had significantly increased serum BAFF levels (2,343 ± 1,079 pg/ml) compared with healthy controls (1,352 ± 526 pg/ml; p<0.01). Inactive sarcoidosis patients had circulating BAFF levels comparable to healthy donors (1,239 pg/ml ± 376; NS, inactive

versus healthy; $p<0.01$ inactive versus active). BAFF levels were strongly correlated with serum hypergammaglobulinemia in patients with sarcoidosis ($r= 0.53$; $p=0.0015$; Fig 1B).

## REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

Cancro P. Michael, D'Cruz P. David, and Khamashta A. Munther. The role of B lymphocyte stimulator (BLyS) in systemic lupus erythematosus. J. Clin. Invest. 119:1066–1073, 2009.

Cheema GS, Roschke V, Hilbert DM, Stohl W. Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. Arthritis Rheum. 2001; 44(6):1313-9.

Davidson A. Targeting BAFF in autoimmunity. Curr Opin Immunol. 2010 Dec;22(6):732-9. Epub 2010 Oct 21.

Espinosa G, Cervera R. Belimumab, a BLyS-specific inhibitor for the treatment of systemic lupus erythematosus. Drugs Today (Barc). 2010 Dec;46(12):891-9.

Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N. Engl. J. Med 2007; 357(21):2153-65.

Liu Zheng, Anne Davidson. BAFF inhibition: A new class of drugs for the treatment of autoimmunity. 2011 Elsevier.

Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. Nat. Rev. Immunol. 2002; 2(7):465-75.

Ramanujam Meera, Xiaobo Wang, Weiqing Huang, Zheng Liu, Lena Schiffer, Haiou Tao, Daniel Frank, Jeffrey Rice, Betty Diamond, Karl O.A. Yu, Steven Porcelli, and Anne Davidson. Similarities and differences between selective and nonselective BAFF blockade in murine SLE. J. Clin. Invest. doi:10.1172/JCI26385, 2006.

Rauch Melanie, Roxane Tussiwand, Nabil Bosco, Antonius G. Rolink. Crucial Role for BAFF-BAFF-R Signaling in the Survival and Maintenance of Mature B Cells. PLoS ONE May 2009. Volume 4. Issue 5. e5456.

Scholz L. Jean, Jenni E. Crowley, Mary M. Tomayko, Natalie Steinel, Patrick J. O'Neill, William J. Quinn III, Radhika Goenka, Juli P. Miller, Yun Hee Cho, Vatana Long, Chris Ward, Thi-Sau Migone, Mark J. Shlomchik, and Michael P. Cancro. BLyS inhibition eliminates primary B cells but leaves natural and acquired humoral immunity intact. PNAS. October 7, 2008. vol. 105. no. 40. 15517–15522.

Vugmeyster Yulia et al. A Soluble BAFF Antagonist, BR3-Fc, Decreases Peripheral Blood B Cells and Lymphoid Tissue Marginal Zone and Follicular B Cells in Cynomolgus Monkeys. American Journal of Pathology, Vol. 168, No. 2, February 2006.

## CLAIMS:

1. A method for diagnosis active sarcoidosis in a patient comprising :

   - measuring the expression level of B-cell activating factor (BAFF) in a sample obtained from said patient; and
   - comparing said expression level to a threshold value.

2. The method according to claim 1, wherein said threshold value is the mean expression level of BAFF of a population of healthy individuals, preferably 100 healthy individuals.

3. The method according to claim 1 or 2, wherein said sample is selected in the group consisting of blood, plasma, serum.

4. The method according to any one of claims 1 to 3, wherein BAFF expression level is measured by quantifying the level of BAFF protein in the sample.

5. The method according to claim 4, wherein the quantification of the level of BAFF protein is performed by using a set of antibodies directed against BAFF.

6. The method according to claim 5, wherein the quantification of the level of BAFF protein is performed by enzyme-linked immunosorbent assay (ELISA).

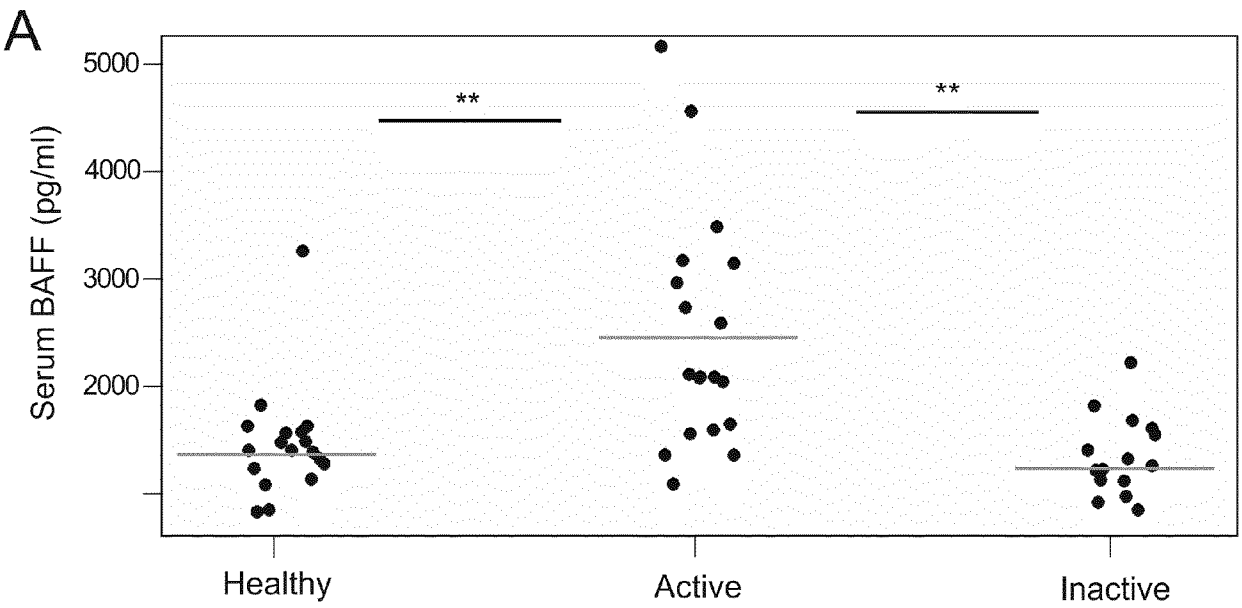
7. A compound which inhibits the binding of BAFF to TACI, BCMA or BAFF-R or a compound which is an inhibitor of BAFF, TACI, BCMA or BAFF-R gene expression for use in the treatment of active sarcoidosis.

8. The compound according to claim 7 wherein said compound is a monoclonal antibody anti-BAFF.

9. A pharmaceutical composition for the treatment of active sarcoidosis comprising a compound according to claims 7 or 8 and a pharmaceutically acceptable carrier.

10. A method for treating active sarcoidosis comprising administering to a subject in need thereof a therapeutically effective amount of a compound which inhibits the binding of BAFF to TACI, BCMA or BAFF-R or a compound which is an inhibitor of BAFF,

TACI, BCMA or BAFF-R gene expression for use in the treatment of active sarcoidosis.


A
Serum BAFF (pg/ml)
5000
4000
3000
2000
**
**
Healthy
Active
Inactive

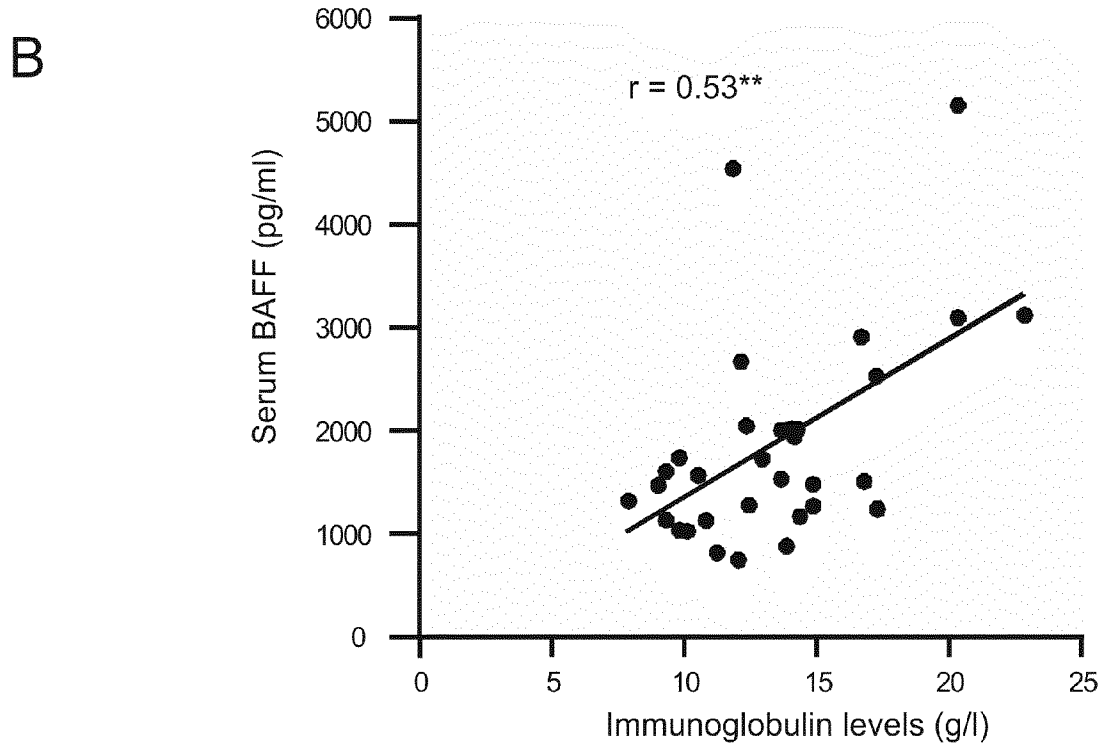

B
r = 0.53**
Serum BAFF (pg/ml)
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
Immunoglobulin levels (g/l)

**Figure 1**

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/060126

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 A61K39/395
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N A61K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | LAVIE F ET AL: "Expression of BAFF (BLyS) in T cells infiltrating labial salivary glands from patients with Sjögren's syndrome", JOURNAL OF PATHOLOGY, JOHN WILEY & SONS LTD, GB, vol. 202, no. 4, 1 April 2004 (2004-04-01), pages 496-502, XP002475876, ISSN: 0022-3417, DOI: 10.1002/PATH.1533 abstract; figure 1 | 1-10 |
| X | US 2008/220003 A1 (SCHNATBAUM KARSTEN [DE] ET AL) 11 September 2008 (2008-09-11) paragraph [0417] - paragraph [0423] | 7-10 |

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier 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 |
|---|---|
| 9 July 2013 | 16/07/2013 |

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

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/060126

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | JOHANSEN J S ET AL: "Increased serum YKL-40 in patients with pulmonary sarcoidosis-a potential marker of disease activity?", RESPIRATORY MEDICINE, BAILLIERE TINDALL, LONDON, GB, vol. 99, no. 4, 1 April 2005 (2005-04-01), pages 396-402, XP004780205, ISSN: 0954-6111, DOI: 10.1016/J.RMED.2004.09.016 abstract<br>-----<br>ANNE SAUSSINE ET AL: "Active Chronic Sarcoidosis is Characterized by Increased Transitional Blood B Cells, Increased IL-10-Producing Regulatory B Cells and High BAFF Levels", PLOS ONE, vol. 7, no. 8, 22 August 2012 (2012-08-22) , page e43588, XP055037513, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0043588 the whole document<br>----- | 1-10 |
| X,P | | 1-10 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/060126

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 2008220003 A1 | 11-09-2008 | AR 057041 A1 | 14-11-2007 |
| | | AU 2006254313 A1 | 07-12-2006 |
| | | BR PI0610875 A2 | 03-08-2010 |
| | | CA 2607862 A1 | 07-12-2006 |
| | | CN 101189209 A | 28-05-2008 |
| | | EP 1739078 A1 | 03-01-2007 |
| | | EP 1885691 A1 | 13-02-2008 |
| | | JP 2008545727 A | 18-12-2008 |
| | | KR 20080015401 A | 19-02-2008 |
| | | US 2008220003 A1 | 11-09-2008 |
| | | WO 2006128670 A1 | 07-12-2006 |
| | | ZA 200708824 A | 30-07-2008 |