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(54) Title: METHOD OF TREATING MORPHEA

(57) Abstract: The present invention providesmethods of treating morphea with selective co-stimulation modulators binding to the CD80/CD86 ligands on an antigen-presenting cell.

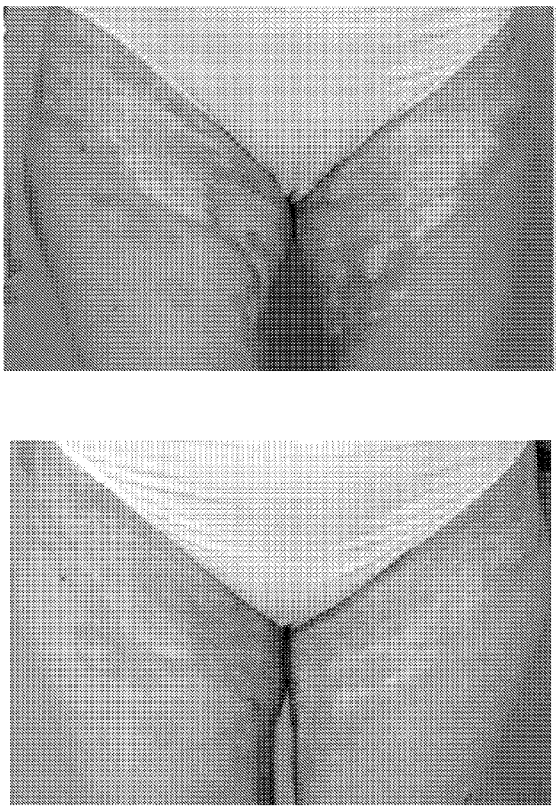


Fig. 1

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METHOD OF TREATING MORPHEA

Technical field of the invention

The present invention relates to the field of autoimmune diseases. In particular the present invention relates to methods of treating morphea with selective co-stimulation modulators binding to the CD80/CD86 ligands on an antigen-presenting cell.

Background of the invention

Morphea is an uncommon disorder. It is more frequent among women, and in young adults aged 20–40 years. The aetiology of morphea is unknown. It is believed that inflammatory processes in the skin induce increased synthesis of collagen from fibroblasts. At present, active superficial morphea can be treated with ultraviolet A1 (UVA1) with good results. Meanwhile, there is no efficient therapy for the profound, progressive and destructive morphea variants.

WO 93/00431 (Bristol-Myers Squibb) identifies the CTLA4 receptor as ligand for the B7 antigen. WO 93/00431 further provides a CTLA4 immunoglobulin fusion protein comprising the extracellular domain of the CTLA4 receptor. The recombinant chimeric fusion protein may be used to react with B7 positive cells to regulate immune responses mediated by T cell interactions with the B7 antigen positive cells.

In a later application, WO 01/92337, Bristol-Myers Squibb provides mutant molecules of the CTLA4 immunoglobulin fusion protein comprising the extracellular domain of the CTLA4 receptor. These mutant molecules bind to CD80 and/or CD86 with a greater avidity than CTLA4.

Summary of the invention

There is an unmet need for additional and alternative therapies for morphea. Thus, an object of the present invention relates to the provision of a medicament

for the treatment of morphea and methods of treating morphea using said medicament.

Thus, one aspect of the invention relates to method of treating or alleviating the symptoms of morphea comprising administration to a subject in the need thereof a therapeutically effective amount of a fusion protein, where said fusion protein is selected from the group consisting of:

(a) a fusion protein comprising a first amino acid sequence comprising an amino acid sequences corresponding to the extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and a second amino acid sequence comprising amino acid residues corresponding to the constant region of an immunoglobulin molecule,

(b) the fusion protein of (a) having one or more mutations (preferable point mutations) in said extracellular domain of CTLA4 and/or one or more mutations (preferable point mutations) in said constant region of an immunoglobulin molecule.

Another aspect of the present invention relates to the use of fusion protein as defined herein for the manufacture of a medicament for the treatment or alleviation of the symptoms of morphea.

Yet another aspect of the present invention is to provide the fusion protein as defined herein for use in the treatment or alleviation of the symptoms morphea.

Brief description of the figures

Figure 1 shows Patient No. 1 with disseminated morphea profunda (top picture) before treatment with abatacept and (bottom picture) after 16 treatments. For further details see Case 1 of Example 1.

Figure 2 shows Patient No. 2 with disseminated morphea profunda (top picture) before treatment with abatacept and (bottom picture) after 5 treatments. For further details see Case 2 of Example 1.

The present invention will now be described in more detail in the following.

Detailed description of the invention

Definitions

Prior to discussing the present invention in further details, the following terms and conventions will first be defined:

Sequence identity

As commonly defined "identity" is here defined as sequence identity between genes or proteins at the nucleotide or amino acid level, respectively.
Thus, in the present context "sequence identity" is a measure of identity between proteins at the amino acid level and a measure of identity between nucleic acids at nucleotide level. The protein sequence identity may be determined by comparing the amino acid sequence in a given position in each sequence when the sequences are aligned. Similarly, the nucleic acid sequence identity may be determined by comparing the nucleotide sequence in a given position in each sequence when the sequences are aligned.

To determine the percent identity of two nucleic acid sequences or of two amino acids, the sequences are aligned for optimal comparison purposes (e.g., gaps may be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total

# of positions (e.g., overlapping positions) x 100). In one embodiment the two sequences are the same length.

One may manually align the sequences and count the number of identical nucleic acids or amino acids. Alternatively, alignment of two sequences for the determination of percent identity may be accomplished using a mathematical algorithm. Such an algorithm is incorporated into the NBLAST and XBLAST programs. BLAST nucleotide searches may be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches may be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecule of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST may be utilised. Alternatively, PSI-Blast may be used to perform an iterated search which detects distant relationships between molecules. When utilising the NBLAST, XBLAST, and Gapped BLAST programs, the default parameters of the respective programs may be used. See http://www.ncbi.nlm.nih.gov. Alternatively, sequence identity may be calculated after the sequences have been aligned e.g. by the BLAST program in the EMBL database (www.ncbi.nlm.gov/cgi-bin/BLAST). Generally, the default settings with respect to e.g. "scoring matrix" and "gap penalty" may be used for alignment. In the context of the present invention, the BLASTN and PSI BLAST default settings may be advantageous.

The percent identity between two sequences may be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

Mutation

In the context of the present invention the term "mutation" refers to an alteration in the amino acid sequence of a reference (wildtype) polypeptide leading to a mutant polypeptide. The alteration is typically reflecting an underlying alteration in the nucleic acids sequence encoding wildtype (or reference) polypeptide. In the context of the present invention, when referring to a mutated extracellular domain of human CTLA4 the reference polypeptide is the extracellular domain of human CTLA4 as set forth in SEQ ID NO: SEQ #3.

Fusion protein

In the context of the present invention the term "fusion protein" or "chimeric fusion protein" refers to a polypeptide comprising a least two heterologous protein sequences (subunits) joined to form a single polypeptide sequence. Fusion proteins are designed and prepared using standard techniques known the skilled person in the art. Typically, the least two nucleic acids sequences encoding the protein subunit are ligated in frame to generate a nucleic acid sequence comprising an open reading frame encoding said fusion protein. The nucleic acid sequence may be employed to produce the fusion protein by recombinant expression in a suitable host, for example CHO cells.

Cytotoxic T lymphocyte associated antigen 4 (CTLA-4/CD152)

Cytotoxic T lymphocyte associated antigen 4 (CTLA-4/CLTA4/CD152) is homologous to CD28 and its gene maps to the same chromosomal band as the gene for CD28. CTLA-4 was identified by differential screening of a murine cytolytic T cell cDNA library. Human CTLA-4 was cloned in 1988. Expression was first reported using a soluble genetic fusion of the extracellular domain to an Fc domain and this fusion protein was shown to be a potent inhibitor of in vitro immune responses dependent upon cellular interactions between T and B lymphocytes (Linsley et al., J. Exp. Med. (1991) 174:561-569). The structure of CTLA-4 extracellular domain is characteristic of the immunoglobulin variable domain. The human CTLA-4 precursor sequence is identified by the Swiss-Prot primary accession number: P16410.

CTLA-4 appears to be critical for the negative regulation of T cell responses. Blockade of CTLA-4 has been found to remove inhibitory signals, while aggregation of CTLA-4 has been found to provide inhibitory signals that down-regulate T cell responses (Allison and Krummel, (1995) Science 270:932). The B7 molecules have a higher affinity for CTLA-4 than for CD28 (Linsley et al., (1991) J. Exp. Med. 174: 561-569) and B7-1 and B7-2 have been found to bind to distinct regions of the CTLA-4 molecule and have different kinetics of binding to CTLA-4 (Linsley et al., (1994) Immunity 1:793).

The importance of the B7:CD28/CTLA-4 co-stimulatory pathway has been demonstrated *in vitro* and in several *in vivo* model systems. Blockade of this co-stimulatory pathway results in the development of antigen specific tolerance in murine and human systems. Conversely, expression of B7 by B7 negative murine tumor cells induces T-cell mediated specific immunity accompanied by tumor rejection and long lasting protection to tumor challenge. Activation of CTLA-4, for example, transmits a negative signal to a T cell. Engagement of CTLA-4 inhibits IL-2 production and can induce cell cycle arrest. In addition, mice that lack CTLA-4 develop lymphoproliferative disease. The blockade of CTLA-4 with antibodies may remove an inhibitory signal, whereas aggregation of CTLA-4 with antibody transmits an inhibitory signal.

Since the leader sequence that is cleaved off CTLA-4 is not defined in the literature or public databases, at least two different numbering systems are possible. The CTLA-4 sequence can, inter alia, have Ala or with Met as the N-terminal amino acid residue of the protein. Accordingly, the amino acid sequence corresponding to the extracellular domain of human CTLA-4 as set forth in SEQ ID NO: 3 is having the Met at position 1. The alternative version of the extracellular domain of human CTLA-4 having the Ala as the N-terminal amino acid residue of the protein is provided with SEQ ID NO: 4. Reference to amino acid residues in the extracellular domain of human CTLA-4 is, unless otherwise indicates, made using the extracellular domain of human CTLA-4 as set forth in SEQ ID NO: 3 as reference molecule.

In lines with the above, SEQ ID NO: 5 provides CTLA4Ig having a Met as the N-terminal amino acid residue of the protein. The alternative version having the Ala as the N-terminal amino acid residue is provided with SEQ ID NO: 6.

SEQ ID NO: 7 provides the L104EA29Y variant having a Met as the N-terminal amino acid residue of the protein. The alternative version having the Ala as the N-terminal amino acid residue is provided with SEQ ID NO: 8.

SEQ ID NO: 9 provides the L104E variant having a Met as the N-terminal amino acid residue of the protein. The alternative version having the Ala as the N-terminal amino acid residue is provided with SEQ ID NO: 10.

The variants of the extracellular domain of human CTLA-4 having the sequences set forth in SEQ ID NO: 11, SEQ ID NO: 11. SEQ ID NO: 12. SEQ ID NO: 13. SEQ ID NO: 14 are in the form having a Met as the N-terminal amino acid residue of the protein. It follows the alternative version having Ala as the N-terminal amino acid residue may also be used.

Abatacept

CTLA-4-Ig (CTLA4Ig, abatacept, Orencia,) is the extracellular domain of CTLA-4 fused to the Fc of IgG1, the resulting soluble protein is a dimer with a molecular weight of approximately 92 kDa. It is being developed by Bristol-Myers Squibb Co (BMS) for rheumatoid arthritis (RA). Abatacept contains in its CDR3-like domain the amino acid hexapeptide motif MYPPPY, which is shared between CD28 and CTLA-4 and is necessary for binding to the B7 ligands. SEQ ID NO: 18 comprises the amino acid hexapeptide motif MYPPPY.

Mutation of the first tyrosine (Y) in the hexapeptide motif MYPPPY motif to alanine (A) abolishes binding to CD80, but also results in reduced binding to CD86, whereas a phenylalanine (F) substitution allows for retention of the full affinity for CD80 with a total loss of CD86 binding. Residues in the CDR3-like and CDR1-like regions are also important for the interaction of abatacept with its ligands.

Belatacept

A second generation form of abatacept has been, referred to as LEA-29Y (or belatacept), has being developed by Bristol-Myers Squibb as an immunosuppressant for transplantation. This mutant molecule (set forth in SEQ ID NO: 7) having glutamic acid (E) instead of leucine (L) at position 104 and tyrosine (Y) instead of alanine (A) at position 29 of the extracellular domain of CTLA4 (SEQ ID NO: 3) exhibits approximately 2-fold greater binding avidity for CD80 (B7-1) and approximately 4-fold greater binding avidity for CD86 (B7-2) than abatacept.

Morphea

Scleroderma encompasses a spectrum of disorders characterized by thickening of the skin and subcutaneous tissue. Two different clinical categories generally are identified: systemic sclerosis, in which visceral changes are present, and localized scleroderma (morphea), in which lesions are limited to the skin.

The aetiology of morphea is unknown. It is believed that inflammatory processes in the skin induce increased synthesis of collagen from fibroblasts.

Several types of localized sclerosis or morphea may be distinguished on the basis of clinical manifestations and levels of tissue involvement. However, morphea classification is hampered by common overlapping of the various types. A morphea classification have been proposed that includes the plaque (morphea en plaque, guttate morphea, atrophoderma of Pasini and Pierini, keloid morphea, and lichen sclerosus et atrophicus), generalized, bullous, linear (en coup de sabre, linear scleroderma, and progressive hemifacial atrophy), and deep (morphea profunda, eosinophilic fasciitis, and disabling pansclerotic morphea of children) types and their corresponding subtypes (in parenthesis).

Generalized morphea is a form of localized scleroderma. When morphea skin plaques are very widespread, it is referred to as generalized morphea. The skin patches become very hard and dark and spread over large areas of the body. Underlying muscles are often affected, causing them to tighten and atrophy. Symptoms of generalized morphea are widespread morphea lesions over large areas of the body, which may sometimes cause limb contractures and atrophy. Antiphospholipid Syndrome Symptoms of Antiphospholipid Syndrome include deep vein thrombosis (DVT), chronic leg ulcers, recurrent miscarriages, headache, heart attacks, renal vein and artery thrombosis, pulmonary embolism, and pulmonary hypertension. It is identified by the presence of anticardiolipin antibodies, in a blood test. The same antibodies have been associated with generalized morphea scleroderma. Diagnosis of morphea is often confirmed with a skin biopsy, which is usually performed by a dermatologist. Morphea is usually diagnosed and treated by dermatologists.

Unilateral generalized morphea (UGM) is a rare variant of localized scleroderma. As the onset of UGM usually occurs in pediatric patients, pediatricians should be cognizant of the presentation of this uncommon condition.

Bullous morphea is a rare disease, which occurs most commonly on the lower extremities and inferior abdomen. Fewer than 100 cases have been reported in the world literature. While the etiology of bullous formation is not certain, lymphatic obstruction from the sclerodermatous process is considered the likeliest cause. It is also suggested that vascular changes like arteritis and phlebosclerosis play a role in bullae formation. This hypothesis gains credence from the hemorrhagic nature of many morphea bullae. Furthermore, a correlation between local trauma and blister formation has been found. In one report it was found that the eosinophil granule component major basic protein (MBP) in the base of morphea blisters. It was concluded that major basic protein is responsible for blister formation in at least some cases of morphea.

Linear scleroderma "en coup de sabre" (LSCS) and plaque morphea are both variants of localized scleroderma. LSCS presents as band-like sclerotic skin lesions, usually involving a single unilateral change in the frontoparietal area of the head. This is characterized by atrophy and a furrow of the skin. Progressive involution of the craniofacial bones may result in hemifacial atrophy, which appears to be identical to the Parry Romberg syndrome (idiopathic progressive facial hemiatrophy). A clear differentiation between Parry Romberg syndrome and LSCS is often not possible and the aetiology of the two conditions may be similar. Plaque morphea is characterized by circumscribed sclerotic plaques with ivory coloured centres and, in the active state of the disease, with violaceous borders. With further progression the centre becomes white or yellowed. Most commonly the lesions are single or few in number, but they may be multiple and are typically localized on the trunk and the extremities. Compared with plaque morphea, LSCS may be accompanied by neurological complications such as epileptic seizures and other focal neurological symptoms. Ipsilateral abnormalities of brain imaging have been reported in patients with LSCS.

Morphea Profunda is a generalized or, more rarely, localized sclerotic process that mainly involves the deep dermis and subcutaneous tissue but also the fascia and

superficial muscle. Clinically, plaques are mildly inflamed, hyperpigmented, symmetrical, and somewhat ill-defined. The skin feels thickened and bound down to the underlying fascia and muscle. Plaques are smooth and shiny, but areas of both dermal and subcutaneous atrophy may be present, particularly in chronic lesions. Clinical findings may be difficult to distinguish from generalized morphea. The limits between morphea profunda and generalized morphea are not clear and may well be part of a spectrum including generalized morphea and eosinophilic fasciitis. The overlaping extent of cutaneous involvement shown by these various conditions precludes their clinical or even histological distinction. Morphea profunda is more frequent in females than males. The onset of sclerosis is gradual and relatively rapid, usually occurring during a period of several months. Signs of acute inflammation, such as edema and erythema, are rarely observed. Increased physical exertion has been invoked as a possible precipitating factor.

Eosinophilic fasciitis is an uncommon disease characterized by a symmetrical, scleroderma-like thickening of the skin of the limbs. Most patients start with an edematous phase that may be accompanied by pitting edema of the extremities. Subsequently, the upper extremities medial aspects typically show skin dimpling resulting from focal dermis-to-fascia tethering caused by sclerotic bands striding the panniculus, which is followed by a final stage consisting of induration and tightening of the skin. Lesions of morphea on different parts of the body are present in a significant proportion of cases (30%). Usually, these lesions are not synchronous with fasciitis and appear either before or after fascial inflammation. Hypergammaglobulinemia, peripheral eosinophilia, and increased ESR are features of eosinophilic fasciitis but, because they may be transient, normal laboratory findings do not rule out the diagnosis. Similarly, although eosinophilia may be seen on histological examination of the fascia (often in association with peripheral eosinophilia), its presence is not required for the diagnosis. Extracutaneous involvement most commonly presents as synovitis or tenosynovitis, arthritis, contractures, or carpal tunnel syndrome. Although uncommon and mild, other manifestations of visceral disease in eosinophilic fasciitis are pulmonary, muscle, esophageal, and even cardiac abnormalities.

Disabling pansclerotic morphea of children is an aggressive mutilating variant of morphea with onset before the age of 14 years, although an adult onset has been

described. This condition involves all layers of the skin, extending through the dermis and subcutaneous tissues to involve muscle, tendon, and bone. Typically, sclerotic plaques develop on the extensor aspects of the extremities and trunk to progressively involve the entire skin, including the neck, face, and scalp, with sparing of the fingertips and toes and lack of Raynaud's phenomenon. Extensive, severe, unilateral lesions of pansclerotic morphea also have been described. Painful ulcerations and contracture deformities, often severe and disabling, occur as a result of pansclerotic changes. Soft-tissue calcification and subsequent ulceration are recognized complications posing significant management difficulties. Squamous cell carcinoma arising in long-standing pansclerotic morphea has been occasionally reported. Frequent laboratory findings include peripheral eosinophilia, hypergammaglobulinemia, and increased ESR. ANA test results are sometimes positive. Finally, some visceral complications such as abnormal pulmonary function test results, myopathic electromyographic alterations related to the areas of sclerosis, and reduced esophageal motility have been reported.

The treatment of localized scleroderma is still unsatisfactory. Unlike other less aggressive forms of localized scleroderma (plaque morphea), deep types of morphea showing evidence of progressive inflammatory and fibrotic lesions require treatment with anti-inflammatory drugs or other disease-modifying agents. Although their action mechanism is incompletely understood, daily antimalarial agents (chloroquine or hydroxychloroquine sulfate) are being used, presumably for their immunomodulating properties. Sometimes, in addition to antimalarials, more aggressive treatment may be necessary, including systemic corticosteroids (prednisone at a dose of approximately 1 mg/kg).
Other pharmacologic agents used include vitamin E, vitamin D3 (oral calcitriol), aminobenzoate potassium, penicillin, retinoids, gamma interferon, cytotoxic agents (D-penicillamine, methotrexate, cyclosporine, cyclophospamide, azathioprine), and bosentan (endothelin receptor antagonist). UVA irradiation, in the form of bath and oral psoralen, as well as high- and low-dose UVA1 therapy, has been applied to the treatment of localized scleroderma, even in extensive and deep forms such as pansclerotic morphea, with beneficial responses. UVA irradiation have been postulated to increase the activity of metalloproteinases and

modulate the expression of cytokines participating in connective tissue remodelling.

Throughout the years, many different treatments have been tried for morphea including topical, intra-lesional, and systemic corticosteroids. Antimalarials such as hydroxychloroquine or chloroquine have been used. Immunomodulators such as methotrexate, topical tacrolimus, and penicillamine have been tried. Ultraviolet A (UVA) light, with or without psoralens have also been tried. Overall the effect these treatments on controlling the disease activity have not been significant for the profound, progressive and destructive morphea.

The inventors of the present invention have surprisingly found that a fusion protein comprising a first domain corresponding to the extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and a second domain corresponding to the constant region of an immunoglobulin molecule (exemplified by abatacept) may be successfully applies in the treatment or alleviating the symptoms of localized scleroderma (also referred to as morphea). The detailed description of the treatment of two patients with abatacept is provided in Example 1.

The present invention employs recombinant chimeric fusion protein comprising a first protein domain comprising an amino acid sequences corresponding to the extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4) joined to a second protein domain comprising amino acid residues corresponding to the constant region of an immunoglobulin molecule. The first protein domain is positioned N-terminal to the second protein domain in the fusion protein. The domain may be joined directly or separate by a spacer (or junction sequence). The fusion protein employed by the present invention is preferably obtained by recombinant expression using a mammalian producer cell. It follows that in order to obtained high amount of said fusion protein, the fusion protein is expressed as an immature fusion protein having a signal peptide sequence located at the N-terminal end of the fusion protein. For secretion into the medium, the fusion protein may be expressed with an upstream signal that is cleaved as the protein is secreted. Various signal peptides sequences may be employed for example the oncostatin M signal peptide as disclosed in WO 93/00431, which is hereby

incorporated by reference. The choice of signal peptides sequences may depend on the expression system applied for recombinant production of the fusion protein. The signal peptide is cleaved of the immature fusion protein (post-translational processing) to obtain the mature fusion protein. The exact position of the cleavage may vary slightly (one or two amino acids). Further, the mature fusion protein may undergo further post-translational processing step which removes one or more amino acid residues from the N-term of the cleavage product. The mature form of the recombinant chimeric fusion protein is preferably used by the method of the present invention. The person skilled in the art in familiar with the art recombinant expression of proteins such as the above mentioned chimeric fusion proteins. Further, WO 93/00431 and WO 01/92337 provide methods that may be used for producing the fusion proteins used by the present invention. WO 93/00431 and WO 01/92337 are hereby incorporated by reference.

Thus, one aspect of the invention relates to method of treating or alleviating the symptoms of morphea comprising administration to a subject in the need thereof a therapeutically effective amount of a fusion protein, where said fusion protein is selected from the group consisting of:

(a) a fusion protein comprising a first amino acid sequence comprising an amino acid sequences corresponding to the extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and a second amino acid sequence comprising amino acid residues corresponding to the constant region of an immunoglobulin molecule,

(b) the fusion protein of (a) having one or more mutations (preferably point mutations) in said extracellular domain of CTLA4 and/or one or more mutations (preferably point mutations) in said constant region of an immunoglobulin molecule.

Fusion protein used by the method of the present invention may be a fusion protein sharing a high sequence identity with the fusion protein set forth in SEQ ID NO: 5 (abatacept). Thus, in one embodiment of the present invention, said fusion protein is having at least 90% sequence identity to the fusion protein set

forth SEQ ID NO: 5, such as at least 95% sequence identity, for example at least 97% sequence identity, such as at least 98% sequence identity, for example at least 99% sequence identity sequence identity to the fusion protein set forth SEQ ID NO: 5. The size of fusion protein is comparable to or essentially the same as that of the fusion protein set forth SEQ ID NO: 5. In one embodiment, the size of said fusion protein is in the range of 330 to 390 amino acids, such 340 to 380 amino acids. The fusion proteins are functionally equivalent to the fusion protein set forth in SEQ ID NO: 5 (abatacept) in the sense that they recognise and bind CD80 and/or CD86.

The present invention provides the method described herein for the treatment of the various subtypes of morphea. Thus, in one embodiment of the present invention said morphea is selected from the group consisting of plaque morphea, generalized morphea, bullous morphea, linear morphea and deep morphea. Thus, in another embodiment of the present invention said morphea is selected from the group consisting of en plaque, guttate morphea, atrophoderma of Pasini and Pierini, keloid morphea, lichen sclerosus et atrophicus, en coup de sabre, linear scleroderma, progressive hemifacial atrophy, morphea profunda, eosinophilic fasciitis, and disabling pansclerotic morphea of children. In a preferred embodiment, said morphea is morphea profunda.

Extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4)

In the context of the present invention the term "extracellular domain of CTLA4" refers to a portion of CTLA4 that recognizes and binds CD80 and/or CD86. The extracellular domain includes fragments or derivatives of CTLA4 that bind CD80 and/or CD86. As discussed above the CTLA-4 sequence can, inter alia, have Ala or with Met as the N-terminal amino acid residue of the protein. Accordingly, the extracellular domain of CTLA4 may be provided in alternative version of the extracellular domain of human CTLA-4 having, inter alia, Ala or with Met as the N-terminal amino acid residue of the protein.

The extracellular domain preferably originates from a primate or more preferably a human cytotoxic T-lymphocyte-associated protein 4 (CTLA4). Thus, in one

embodiment of the present invention said cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is of human origin.

In a preferred embodiment, the extracellular domain is the extracellular domain of the human cytotoxic T-lymphocyte-associated protein 4 (CTLA4) set forth in SEQ ID NO: 1.

The fusion protein may be provided in the formulation available as abatacept (CTLA-4-Ig, Orencia,) or Belatacept (trade name Nulojix). Thus, in a preferred embodiment of the present invention, said fusion protein is provided as abatacept (CTLA-4-Ig, Orencia,). In another embodiment, said fusion protein is provided as Belatacept (trade name Nulojix).

In one embodiment of the present invention, said extracellular domain comprises the amino acid sequence set forth in SEQ ID NO: 3. In a preferred embodiment, said extracellular domain is having or essentially having the amino acid sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 4.

In another embodiment of the present invention, said fusion protein is having one or more mutations (preferably point mutations) in at least one region of said extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4), wherein said region is having an amino acid sequence selected from the group consisting of SEQ ID NO: 17 and SEQ ID NO: 18. In one embodiment, said fusion protein is having two, three, four or five mutations in the extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4).

In a further embodiment of the present invention, wherein said extracellular domain comprises the amino acid sequence set forth in SEQ ID NO: 3, wherein the sequence of SEQ ID NO: 3 have been mutated by one or more amino acid substitutions, wherein said amino acid substitution is independently selected from the group consisting of Ser25, Ala29, Thr30, Glu31, Arg33, Tyr103, Leu104 and Gly105. The above fusion proteins are disclosed in further details in WO 93/00431 and WO 01/92337, which are incorporated by reference.

The junction region of an immunoglobulin molecule

The fusion protein comprising a said first amino acid sequence and a second amino acid sequence described herein may comprise a junction (spacer) sequence separating the two subunits. Thus, in one embodiment of the method of the present invention, the said fusion protein further comprises a junction sequence located between said first amino acid sequence and said second amino acid sequence. The length of the junction sequence may vary, e.g. the junction sequence may have a length of 50 amino acids or less, such as 40 amino acids or less, for example 30 amino acids or less, such as 20 amino acids or less, for example 10 amino acids or less, such as 5 amino acids or less. In one embodiment, the junction amino acids sequence consists of a single amino acid. In a further embodiment, said junction amino acids sequence is a glutamine residue.

Variant fusion proteins

The fusion protein use by the method of the present invention may be provided in the form of a molecule comprising a mutated CTLA4 molecule, such a molecule comprising a mutated extracellular domain of CTLA4, that binds CD80 and/or CD86 preferably with higher avidity to CD80 and/or CD86 than abatacept (SEQ ID NO: 5). Examples of such molecules are disclosed in WO 01/92337. In a preferred embodiment, the fusion proteins are soluble proteins.

In a preferred embodiment of the present invention, said fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 5. In another preferred embodiment, said fusion protein is having or essentially having the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6. The application of the abatacept (SEQ ID NO: 5) in the method of treating morphea is disclosed in Example 1.

In one embodiment, said fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 7. In another embodiment, said fusion protein is having or essentially having the amino acid sequence set forth in SEQ ID NO: 7 (referred to as the L104EA29Y variant) or SEQ ID NO: 8.

In further embodiment, said fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 9. In yet a further embodiment, said fusion protein is having or essentially having the amino acid sequence set forth in SEQ ID NO: 9 (referred to as the L104E variant) or SEQ ID NO: 10.

In one embodiment, said cytotoxic T-lymphocyte-associated protein 4 (CTLA4) comprises an amino acid selected from the group consisting of SEQ ID NO: 11 (referred to as the L104D variant), SEQ ID NO: 12 (referred to as the L104EA29L variant), SEQ ID NO: 13 (referred to as the L104EA29W variant) and SEQ ID NO: 14 (referred to as the L104EA29T variant). In another embodiment, said extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is having or essentially having an amino acid selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

The constant region of an immunoglobulin molecule

The second subunit the fusion protein (the second amino acid sequence and Ig tail of the fusion protein) comprises amino acid residues corresponding to the constant region of an immunoglobulin molecule. Preferably, the Ig constant region is a human or monkey Ig constant region, e.g., human C(gamma)1, including the hinge, CH2 and CH3 regions. Modifications to the immunoglobulin molecule may be introduced to avoid unintended disulphide bridge formation and to reduce constant region-mediated biological effector functions and complement activation. Ways of providing such modified immunoglobulin molecule are disclosed in the prior art (e.g. in U.S. Patent Nos: 5,637,481; and 6,132,992).

The fusion protein used by the method of the present invention as described herein comprises a second amino acid sequence comprising amino acid residues corresponding to the constant region of an immunoglobulin molecule. In one embodiment of the present invention, said constant region of an immunoglobulin molecule comprises a hinge, CH2 and CH3 regions of an immunoglobulin molecule. In another embodiment, said constant region of an immunoglobulin is a human or monkey immunoglobulin constant region or a fragment thereof. In a further embodiment, said constant region of an immunoglobulin molecule comprises one or more mutations to reduce effector function. In yet a further

embodiment, said constant region of an immunoglobulin molecule comprises a hinge and any or all of the cysteine residues within the hinge are substituted with serine. In one embodiment, said fusion protein is having two, three, four or five mutations in the second amino acid sequence comprising amino acid residues corresponding to the constant region of an immunoglobulin molecule.

In a preferred embodiment, said second amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO: 2. In another embodiment, said second amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 16.

Further properties of the fusion protein used by the method of the invention

In a preferred embodiment of the present invention, said fusion protein is soluble.

In another embodiment, said fusion protein has a great avidity to CD86 and/or CD80 than the fusion protein having the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6.

In one embodiment, said fusion protein has a slower dissociation rate from binding CD86 than the fusion protein having the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6. In another embodiment, said fusion protein further comprises an amino acid sequence which alters the solubility, affinity or valency of said extracellular domain of CTLA4. In a further embodiment, said fusion protein is a selective co-stimulation modulator binding to the CD80/CD86 ligands on an antigen-presenting cell.

The skilled person in the art will know how to assess the above mentioned properties, for example by using in vitro assay. Examples of such assay are disclosed in WO 93/00431 and WO 01/92337.

The fusion protein used by the method of the present invention may be provided as a homodimer consisting of two identical fusion proteins as described herein, which are covalently linked by one disulphide bond. Thus, in one embodiment of

the present invention, said fusion protein is provided in the form of a dimer of said fusion protein.

Pharmaceutical composition

The fusion protein used by the present invention may be provided in the form of a pharmaceutical composition. The pharmaceutical compositions may be provided in a variety of dosage forms, which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or micro-vesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application. The most effective mode of administration and dosage regimen for the compositions of this invention depends upon the severity and course of the disease, the patients' health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the compositions should be titrated to the individual patient.

The pharmaceutical compositions also preferably include suitable carriers and adjuvants which include any material which when combined with the molecule of the invention (e.g., a soluble CTLA4 mutant molecule, such as, L104EA29Y or L104E) retains the molecule's activity and is non-reactive with the subject's immune system. Examples of suitable carriers and adjuvants include, but are not limited to, human serum albumin; ion exchangers; alumina; lecithin; buffer substances, such as phosphates; glycine; sorbic acid; potassium sorbate; and salts or electrolytes, such as protamine sulfate. Other examples include any of the standard pharmaceutical carriers such as a phosphate buffered saline solution; water; emulsions, such as oil/water emulsion; and various types of wetting agents. Other carriers may also include sterile solutions; tablets, including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods. Such compositions may also be formulated within various

lipid compositions, such as, for example, liposomes as well as in various polymeric compositions, such as polymer microspheres.

The pharmaceutical compositions used by the method of the present invention can be administered using conventional modes of administration including, but not limited to, intravenous (i.v.) administration, intraperitoneal (i.p.) administration, intramuscular (i.m.) administration, subcutaneous administration, oral administration, administration as a suppository, or as a topical contact, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

The fusion protein may be administered to a subject in an amount and for a time (e.g. length of time and/or multiple times) sufficient to block endogenous B7 (e.g., CD80 and/or CD86) molecules from binding their respective ligands, in the subject. Blockage of endogenous B7/ligand binding thereby inhibits interactions between B7-positive cells (e.g., CD80- and/or CD86-positive cells) with CD28- and/or CTLA4-positive cells. Dosage of a therapeutic agent is dependent upon many factors including, but not limited to, the type of tissue affected, the type of morphea being treated, the severity of the disease, a subject's health, and a subjects response to the treatment with the agents. Accordingly, dosages of the agents can vary depending on the subject and the mode of administration. The soluble CTLA4 mutant molecules may be administered in an amount between 0.1 to 20.0 mg/kg weight of the patient/day, preferably between 0.5 to 10.0 mg/kg/day. Administration of the pharmaceutical compositions of the invention can be performed over various times, preferably every two weeks for the first month and monthly thereafter.

In one embodiment, the fusion protein provided in the form of a pharmaceutical composition can be administered for one or more hours. In addition, the administration can be repeated depending on the severity of the disease as well as other factors as understood in the art.

In one embodiment of the present invention, the fusion protein (e.g. provided in the form of a pharmaceutical composition described herein) is administered by repeated intravenous injection at a frequency of every two weeks for the first month and monthly thereafter.

In another embodiment of the present invention, the fusion protein (e.g. provided in the form of a pharmaceutical composition described herein) is being administered in an amount of between 0.1 to 20.0 mg/kg weight of the patient/day.

Medical uses

The present invention further provides the fusion protein described herein for use in the manufacture of a medicament for the treatment of localized scleroderma.

Thus, a further aspect of the present invention relates to the use of a fusion protein selected from the group consisting of:
(a) a fusion protein comprising a first amino acid sequence comprising an amino acid sequences corresponding to the extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and a second amino acid sequence comprising amino acid residues corresponding to the constant region of an immunoglobulin molecule,
(b) the fusion protein of (a) having one or more point mutations in said extracellular domain of CTLA4 and/or one or more point mutations in said constant region of an immunoglobulin molecule,
for the manufacture of a medicament for the treatment or alleviation of the symptoms of morphea.

In one embodiment, said morphea is selected from the group consisting of plaque morphea, generalized morphea, bullous morphea, linear morphea and deep morphea. In another embodiment, said morphea is selected from the group consisting of en plaque, guttate morphea, atrophoderma of Pasini and Pierini, keloid morphea, lichen sclerosus et atrophicus, en coup de sabre, linear scleroderma, progressive hemifacial atrophy, morphea profunda, eosinophilic fasciitis, and disabling pansclerotic morphea of children.

It should be noted that the fusion protein described herein and used by the method of the present invention may are also used for the also used for the

manufacture of a medicament for the treatment or alleviation of the symptoms of localized scleroderma.

Yet a further aspect relates to a fusion protein selected from the group consisting of:

(a) a fusion protein comprising a first amino acid sequence comprising an amino acid sequences corresponding to the extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and a second amino acid sequence comprising amino acid residues corresponding to the constant region of an immunoglobulin molecule,

(b) the fusion protein of (a) having one or more point mutations in said extracellular domain of CTLA4 and/or one or more point mutations in said constant region of an immunoglobulin molecule,

for use in the treatment or alleviation of the symptoms morphea.

In one embodiment, said morphea is selected from the group consisting of plaque morphea, generalized morphea, bullous morphea, linear morphea and deep morphea. In another embodiment, said morphea is selected from the group consisting of en plaque, guttate morphea, atrophoderma of Pasini and Pierini, keloid morphea, lichen sclerosus et atrophicus, en coup de sabre, linear scleroderma, progressive hemifacial atrophy, morphea profunda, eosinophilic fasciitis, and disabling pansclerotic morphea of children.

It should be noted that embodiments and features described in the context of one of the aspects of the present invention also apply to the other aspects of the invention.

All patent and non-patent references cited in the present application, are hereby incorporated by reference in their entirety.

The invention will now be described in further details in the following non-limiting examples.

Examples

In the below example, the inventors provide the clinical data from the treatment of two patients suffering from chronic and progressive disseminated morphea profunda. The patients were received treatment with Abatacept, which is a recombinant fusion protein that selectively inhibits T-cell activation via competitive binding to CD80 or CD86, where T cells are involved in the disease pathophysiology. It is approved for the treatment of rheumatoid arthritis.

CASE REPORTS

Case 1

The first patient, an otherwise healthy 47-year-old woman, had first presented with clinical morphea lesions when she was 22 years old. A punch biopsy showed typical histological findings of morphea. The patient had no other organ-specific manifestations. Routine blood evaluation was normal. Antinuclear antibody (ANA) was positive. SSA, SSB, Scl-70, ENA, anti-cardiolipin and RNA-polymerase were all negative.

Previously, because of exacerbations in the disease, the patient had been treated with penicillamine, prednisolone, cyclosporine, high-potency corticosteroid ointment under occlusion, methotrexate, UVA1 (50 treatments), antimalarials and mycophenolate mofetil, in addition to physiotherapy. None of these treatments had any convincing effect on controlling disease activity.

In May 2009, the patient had a further exacerbation of the disease, with new lesions and severe pruritus.

Clinically, she had disseminated morphea, mainly on the extremities, involving the skin over the joints. Some lesions were new and active yellow-white lesions with a lilac ring; others were older and more sclerotic and atrophic with hyperkeratotic changes. Extensive post-inflammatory hyperpigmentation was also seen (Fig. 1).

A punch biopsy from an active lesion on the left thigh confirmed the diagnosis and showed a primarily lymphocytic inflammatory infiltrate around the superficial and deep blood vessels. Furthermore, inflammation at the junction between the dermis and the subcutaneous fat was observed, and the dermal collagen fibres were thickened.

Before treatment with abatacept, screening tests for hepatitis and tuberculosis were negative. X-ray of the thorax was normal.

Following oral and written informed consent, the patient was treated with 750 mg abatacept intravenously on days 1, 15, and 30, and thereafter every 4–6 weeks, according to her weight (66 kg). She has thus far received 20 treatments. During the treatment period, she developed hypertension, which is a known side-effect of abatacept. At the same time, she was treated with 15 mg prednisolone, which was carefully tapered to 0 before the 11th treatment with abatacept. After treating the hypertension with calcium antagonists and diuretics, her blood pressure was stable. Furthermore, control blood tests were normal during treatment.

The treatment with abatacept was well tolerated. The patient felt less itchy, and the joint motion was increased. The disease activity was reduced, both when evaluating the whole body and the single lesions. The erythema around the lesions decreased (Figure 1), and the older lesions became softer. The effect of the treatment was scored by modified Rodnan skin score (Clements et. al 1995). This total skin thickness score is commonly used as an outcome measure in trials of systemic sclerosis. Skin thickness is assessed by clinical palpation of 17 body areas on the front side on a 0–3 scale (normal, mild, moderate and severe). The modified Rodnan skin score is derived by summation of the scores from all 17 body areas. Previously, assessment of the skin score has been sufficiently reproducible to be included as a measure of treatment outcome (Clements et. al 1995). Our first patient had a modified Rodnan skin score of 18 before treatment start, and a score of 2 after 20 treatments. The evaluations were performed by BSG and ABO, separately or together (Table 1).

Since the clinical response has been good and the patient has had no severe adverse events, the treatment is continuing. The time intervals between treatments have been increased to 6 weeks. The effect on the skin and joints is monitored.

Table 1: Modified Rodnan skin score before and during treatment with abatacept in patient no. 1

Abatacept treatments, *n*	**Time, months**	**mRodman skin score**
0		18
4	2	15.5

7	5	8.5
9	7	9
13	10	9.5
15	12	7.5
20	19	2

Case 2

The second patient, a 38-year-old woman developed diffuse progressive morphea profunda, when she was 8 years old. The skin on her shoulders, back and right lower leg has been mainly affected. As a result, the right leg is rotated outwards and the flexion of the right knee and foot is reduced. No other organ-specific involvements have been observed. All blood samples have been normal. Previously, because of disease activity, the patient has been treated with penicillamine, prednisolone, cyclosporine and UVA1 (31 treatments).

Because of exacerbation of the morphea profunda with progression of inflammation and fibrosis in the old lesions, after giving birth to her third child, the patient was treated with 7.5 mg prednisolone daily together with abatacept. The patient had symmetrical severe deep fibrotic lesions on her shoulders, upper arms, buttock and right thigh, with a modified Rodnan skin score of 13. The deep fibrotic lesions were atrophic and hyperpigmented, with a typical lilac ring in the periphery of the lesions (Figure 2).

Before treatment with abatacept, a screening test for tuberculosis was positive. As a child, she had undergone Calmette vaccination against tuberculosis. For safety, she was treated for 4 weeks with prophylactic rifampicin and isoniazid.

The treatment, abatacept 500 mg, was given intravenously on days 1, 15, and 30 and thereafter every 4 weeks according to her weight (58 kg). The patient received a total of five treatments. She experienced an impressive improvement, and reported better movement of the shoulders, hips and knees. She could walk longer distances and had no adverse effects. In addition, it was possible to stop the prednisolone treatment after 4 treatments of abatacept. On evaluation after 12 weeks the inflammatory lesions had disappeared. After 7 months, the modified Rodnan skin score was 6 (Figure 2).

Unfortunately, abatacept treatment had to be stopped after 2.5 months, as the patient was diagnosed with breast cancer. The cancer is not believed to be related to the treatment with abatacept, but as a precaution it has been reported to the

Danish Medicines Agency.

DISCUSSION

This case report describes the first two patients with disseminated morphea profunda treated with abatacept. Morphea profunda is a rare disease and often runs a progressive course with physical and psychological sequelae. Until now, no treatment has been proven effective on active disease or been able to soften old sclerotic lesions.

The first patient is very satisfied with the abatacept treatment. She feels less itchy, has better joint movements and the treatment is well tolerated. The modified Rodnan skin score has reduced significantly during treatment. Thus, the disease activity has been diminished both when evaluating the whole body and the erythema around the single lesions. Even the older lesions feel softer on examination. Furthermore, it has been possible to taper the prednisolone treatment.

The second patient was also satisfied, as abatacept improved her joint movements and walking distance. This patient was also able to stop prednisolone treatment. However, this patient had to stop after 5 treatments with abatacept. After the treatment was stopped, a further softening effect on the old lesions was observed.

Thus, the inventors report here the first cases of diffuse morphea profunda, treated successfully with abatacept. The mechanism is unknown, but the expansion of T cells in the affected skin and circulation from patients with systemic sclerosis suggests antigen-specific activation. Th-17 and regulatory T-cells, which are subsets of effector T-cells, are key regulators of inflammation in several autoimmune diseases.

References

Clements P, Lachenbruch P, Siebold J, White B, Weiner S, Martin R, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. J Rheumatol 1995; 22: 1281–1285.

Daoud MS, et al. Bullous morphea: clinical, pathologic, and immunopathologic evaluation of 13 cases. J Am Acad Dermatol 30:937, 1994.

Linsley et al., J. Exp. Med. (1991) 174:561-569

Allison and Krummel, (1995) Science 270:932

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WO 01/92337 (Bristol-Myers Squibb).

Claims

1. A method of treating or alleviating the symptoms of morphea comprising administration to a subject in the need thereof a therapeutically effective amount of a fusion protein, where said fusion protein is selected from the group consisting of:

(a) a fusion protein comprising a first amino acid sequence comprising an amino acid sequences corresponding to the extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and a second amino acid sequence comprising amino acid residues corresponding to the constant region of an immunoglobulin molecule,

(b) the fusion protein of (a) having one or more point mutations in said extracellular domain of CTLA4 and/or one or more point mutations in said constant region of an immunoglobulin molecule.

2. The method of claim 1, wherein the size of said fusion protein is in the range of 340 to 380 amino acids and said fusion protein is having a 90% sequence identity to the fusion protein set forth SEQ ID NO: 5.

3. The method according any of the preceding claims, wherein said morphea is selected from the group consisting of plaque morphea, generalized morphea, bullous morphea, linear morphea and deep morphea.

4. The method according any of the preceding claims, wherein morphea is selected from the group consisting of en plaque, guttate morphea, atrophoderma of Pasini and Pierini, keloid morphea, lichen sclerosus et atrophicus, en coup de sabre, linear scleroderma, progressive hemifacial atrophy, morphea profunda, eosinophilic fasciitis, and disabling pansclerotic morphea of children.

5. The method according any of the preceding claims, wherein said cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is of human origin.

6. The method according any of the preceding claims, where said extracellular domain is the extracellular domain of the human cytotoxic T-lymphocyte-associated protein 4 (CTLA4) set forth in SEQ ID NO: 1.

7. The method according any of the preceding claims, where said extracellular domain comprises the amino acid sequence set forth in SEQ ID NO: 3.

8. The method according any of the preceding claims, where said extracellular domain is having the amino acid sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 4.

9. The method according to any of the preceding claims, wherein said fusion protein is having one or more point mutations in at least one region of said extracellular domain of a cytotoxic T-lymphocyte-associated protein 4 (CTLA4), wherein said region is having an amino acid sequence selected from the group consisting of SEQ ID NO: 17 and SEQ ID NO: 18.

10. The method according to any of the preceding claims, wherein said extracellular domain comprises the amino acid sequence set forth in SEQ ID NO: 3, wherein the sequence of SEQ ID NO: 3 have been mutated by one or more amino acid substitutions, wherein said amino acid substitution is independently selected from the group consisting of Ser25, Ala29, Thr30, Glu31, Arg33, Tyr103, Leu104 and Gly105.

11. The method according any of the preceding claims, wherein said fusion protein further comprises a junction sequence located between said first amino acid sequence and said second amino acid sequence.

12. The method according to claim 11, wherein said junction amino acids sequence is a glutamine residue.

13. The method according any of the preceding claims, wherein said fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 5.

14. The method according any of the preceding claims, wherein said fusion protein is having the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6.

15. The method according any of the preceding claims, wherein said fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 7.

16. The method according any of the preceding claims, wherein said fusion protein is having the amino acid sequence set forth in SEQ ID NO: 7 or SEQ ID NO: 8.

17. The method according any of the preceding claims, wherein said fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 9.

18. The method according any of the preceding claims, wherein said fusion protein is having the amino acid sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 10.

19. The method according any of the preceding claims, wherein said cytotoxic T-lymphocyte-associated protein 4 (CTLA4) comprises an amino acid selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

20. The method according any of the preceding claims, wherein said constant region of an immunoglobulin molecule comprises a hinge, CH2 and CH3 regions of an immunoglobulin molecule.

21. The method according any of the preceding claims, wherein said constant region of an immunoglobulin is a human or monkey immunoglobulin constant region or a fragment thereof.

22. The method according any of the preceding claims, wherein said constant region of an immunoglobulin molecule comprises one or more mutations to reduce effector function.

23. The method according any of the preceding claims, wherein said constant region of an immunoglobulin molecule comprises a hinge and any or all of the cysteine residues within the hinge are substituted with serine.

24. The method according any of the preceding claims, wherein said second amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO: 2.

25 The method according any of the preceding claims, wherein said second amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 16.

26. The method according to any of the preceding claims, wherein said fusion protein is soluble.

27. The method according to any of the preceding claims, wherein the fusion protein has a great avidity to CD86 and/or CD80 than the fusion protein having the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6.

28. The method according to any of the preceding claims, wherein the fusion protein has a slower dissociation rate from binding CD86 than the fusion protein having the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6.

29. The method according to any of the preceding claims, wherein said fusion protein further comprises an amino acid sequence which alters the solubility, affinity or valency of said extracellular domain of CTLA4.

30. The method according to any of the preceding claims, wherein said fusion protein is a selective co-stimulation modulator binding to the CD80/CD86 ligands on an antigen-presenting cell.

31. The method according to any of the preceding claims, wherein said fusion protein is provided in the form of a dimer of said fusion protein.

32. The use of fusion protein as defined in any of the preceding claims for the manufacture of a medicament for the treatment or alleviation of the symptoms of morphea.

33. The use of previous claim 32, wherein said morphea is selected from the group consisting of plaque morphea, generalized morphea, bullous morphea, linear morphea and deep morphea.

34. The use protein of previous claim 33, wherein morphea is selected from the group consisting of en plaque, guttate morphea, atrophoderma of Pasini and Pierini, keloid morphea, lichen sclerosus et atrophicus, en coup de sabre, linear scleroderma, progressive hemifacial atrophy, morphea profunda, eosinophilic fasciitis, and disabling pansclerotic morphea of children.

35. The fusion protein as defined in any of the preceding claims for use in the treatment or alleviation of the symptoms morphea.

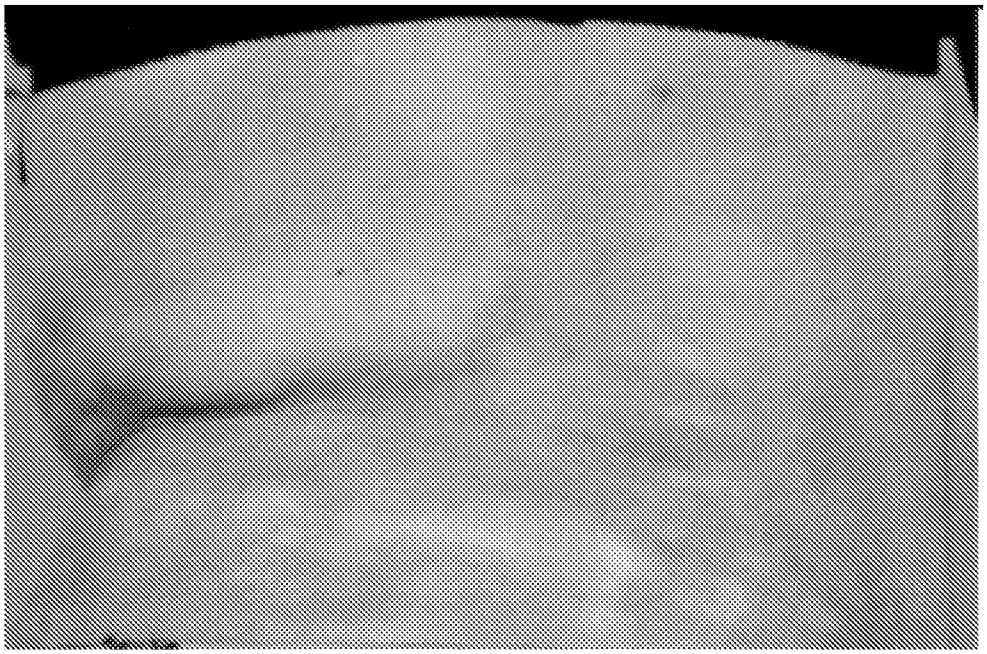
36. The fusion protein of previous claim 35, wherein said morphea is selected from the group consisting of plaque morphea, generalized morphea, bullous morphea, linear morphea and deep morphea.

37. The fusion protein of previous claim 36, wherein morphea is selected from the group consisting of en plaque, guttate morphea, atrophoderma of Pasini and Pierini, keloid morphea, lichen sclerosus et atrophicus, en coup de sabre, linear scleroderma, progressive hemifacial atrophy, morphea profunda, eosinophilic fasciitis, and disabling pansclerotic morphea of children.





Fig. 1



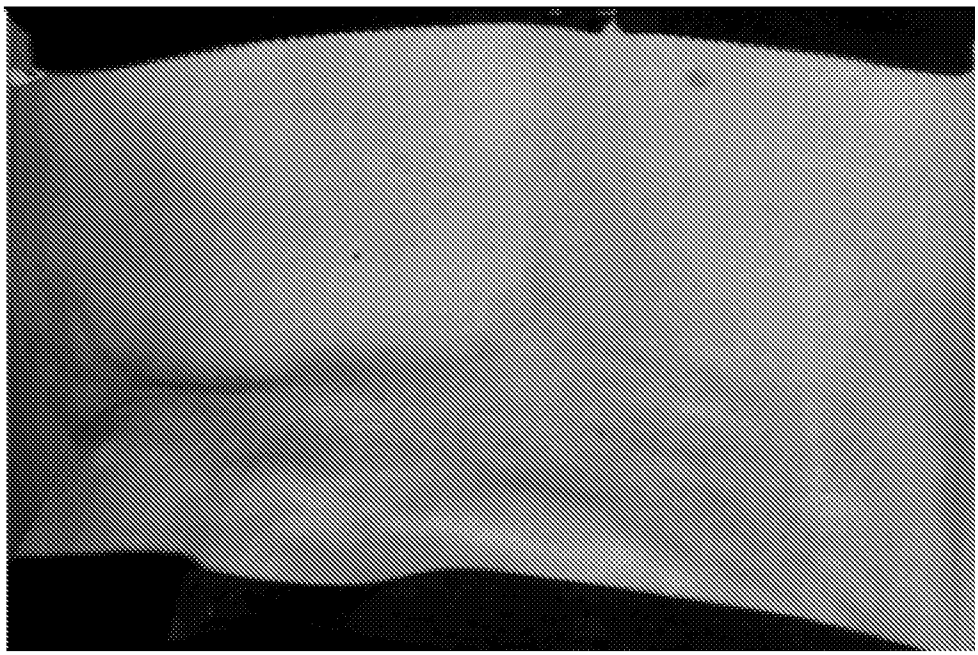


Fig. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2011/050289

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47 C07K14/705 C07K16/28
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)
C07K

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, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Anonymous: "A Pilot Study to Evaluate the Safety and Efficacy of Abatacept in Patients With Systemic Sclerosis", ClinicalTrials.gov archive NCT00442611, 1 March 2007 (2007-03-01), XP002674062, Retrieved from the Internet: URL:http://clinicaltrials.gov/archive/NCT00442611/2007_03_01 [retrieved on 2012-04-16] the whole document ----- -/--	1-37

[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

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"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
18 April 2012	31/05/2012

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

4

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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