AGENT FOR TREATING DISEASE

The present invention is concerned with treatment of autoimmune diseases. The invention involves an agent such as a humanised monoclonal antibody with a long-term effect that may be administered to patients in therapy less frequently than previously described. It is particularly beneficial for patients having diseases or characteristics that cannot be removed in the short term, or require indefinite treatment for effective relief from symptoms. The invention envisages uses and methods of treatment employing the compositions and medicaments comprising the agent.

Autoimmunity is the failure of an organism to recognise its own constituent parts (down to sub-molecular levels) as "self", which results in an immune response against its own cells and tissues. Any disease that results from such an aberrant immune response is termed an autoimmune disease. Autoimmune diseases include multiple sclerosis (MS), rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, colitis ulcerosa, Crohn's disease, myasthenia gravis (MG), autoimmune polyglandular syndrome type II (APS-II), Hashimoto's thyreoiditis (HT), type-1 diabetes (T1D), systemic lupus erythematosus (SLE) and autoimmune lymphoproliferative syndrome (ALS).

Autoimmune disease occurs when T cells recognise and react to 'self' molecules, that is, molecules produced by the cells of the host. Activation of 'autoreactive' T cells by presentation of autoantigens processed by antigen presenting cells (APC) leads to their clonal expansion and migration to the specific tissues, where they induce inflammation and tissue destruction.

Normally, T cells are tolerant with regard to autologous tissue and only react on presentation of heterologous structures. Central tolerance and peripheral tolerance comprise the two mechanisms by which the immune system hinders autoreactive T cells from inducing their deleterious functions. Central tolerance is mediated through negative selection. This process entails the elimination, through clonal deletion of autoreactive T cells, during ontogenic development in the thymus.

Peripheral tolerance is the backup available if central tolerance fails and autoreactive cells escape the thymus. This mechanism of tolerance occurs continuously throughout life, keeping autoreactive cells in check through immune ignorance (anergy), peripheral deletion and/or active suppression.

T regulatory cells (Tregs, formerly also designated "suppressor cells") as part of active suppression maintain peripheral tolerance and regulate autoimmunity (Suri-Payer et al., J Immunol. 157: 1799-1805 (1996); Asano et al., J Exp. Med. 184:387-396 (1996); Bonomo et al., J. Immunol. 154: 6602-6611 (1995); Willerford et al., Immunity 3: 521-530 (1995); Takahashi et al., Int. Immunol. 10: 1969-1980 (1998); Salomon et al., Immunity 12: 431-440 (2000); Read et al., J Exp. Med. 192: 295-302 (2000). In general, regulatory T cells inhibit the activation and/or function of T helper type 1 (TH1) and TH2 effector cells. Dysregulation in Treg cell frequency or functioning can lead to debilitating autoimmune diseases (Baecher-Allan et al., Immunol. Review 212: 203-216 (2006); Shevach, Annu. Rev. Immunol. 18: 423-449 (2000); Salomon et al., Immunity 12: 431-440 (2000); Sakaguchi et al., Immunol. Rev. 182: 18- 32 (2001)).

Several subsets of regulatory T cells have been characterized. The family of Tregs consists of two key subsets: naturally arising e.g. $CD4^+CD25^+$ Tregs and peripherally induced, Tr1 and Th3 Tregs. Furthermore NKTregs and $CD8^+$ Tregs have been described in humans and rodents (Fehérvari et al., J. Clin. Investigation 114: 1209-1217 (2004)).

Thymus-derived Treg cells (naturally occurring $CD4^+CD25^+$Treg) are the main regulatory cells involved regulating autoimmunity or pathogenic immune responses.

i) they are $CD4^+$ T cells and constitute 5-10% of peripheral $CD4^+$ T cells

ii) they mature in the thymus

iii) they are generally characterized by the combined expression of the IL-2 receptor (CD25), the low molecular isoform of the CD45 molecule, CD152 (CTLA-4), and the transcription factor FoxP3.

The role of Tregs is exemplified best by experiments involving reconstitution of immunodeficient nude mice with $CD4^+$ cells that were depleted of $CD25^+$ cells. $CD4^+CD25^-$ reconstituted nude mice develop various organ-specific autoimmune diseases, such as gastritis, oophoritis, orchitis, and thyreoiditis (Suri-Payeret al.; J. Immunol. 160: 1212-1218 (1998)).

Inclusion of the $CD4^+CD25^+$ subset into reconstitution experiments with nude mice prevents the onset of these diseases (Sakaguchi et al., J Immunol. 155: 1151-1164 (1995)). The protective value of $CD4^+CD25^+$ cells against organ-specific autoimmunity has also been shown in several other models of autoimmunity (e.g. autoimmune gastritis, prostatitis, oophoritis, glomerulonephritis, epidimytis and thyreoiditis) caused by neonatal thymectomy performed 3 days after birth (d3Tx) or inflammatory bowel disease caused by reconstitution of SCID mice with CD45RBhigh, $CD4^+CD25^-$ T cells. Administration of anti-CD25 antibody *in vivo* in mice also induces organ-localised autoimmune disease.

The discovery of the importance of the transcriptional regulator FoxP3 in mouse $CD4^+CD25^+$ T regulatory cell function and the previous observations that patients with IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance), a severe inflammatory disease similar to that seen in mice deficient in $CD4^+CD25^+$ regulatory cells (scurfy syndrome), have mutations in *FoxP3*, provided a direct correlation between an autoimmune animal model, mouse regulatory T cells, and a human autoimmune disease (Sakaguchi et al., J Immunol. 155: 1151-1164 (1995)).

The pharmaceutical mechanism of regulatory T cells is not fully clear. $CD4^+CD25^+$ Tregs inhibit polyclonal and antigen-specific T cell activation. The suppression is mediated by a cell contact-dependent mechanism that requires activation of $CD4^+CD25^+$ Tregs via the TCR but Tregs do not show a proliferative response upon TCR activation or stimulation with mitogenic antibodies (anergic) (Shevach, Nature Rev. Immunol 2 : 389 (2002). Once stimulated, they are competent to suppress in an antigen-independent manner the response of CD4+ T cells and CD8+ T cells as well as inhibit B-cell activation and their clonal expansion.

There are additional data indicating that suppressor activity of $CD4^+CD25^+$ Tregs partially also relies on anti-inflammatory cytokines like TGF-ß (Kingsley et al., J Immunol. 168: 1080 (2002); Nakamura et al., J Exp. Med. 194: 629-644 (2001)). The functional significance of TGF-ß secretion is furthermore supported by the findings that TGF-ß-deficient mice develop autoimmune disease and that administration of neutralizing antibodies to TGF-ß abrogates in vivo the prevention of autoimmunity or tolerance-inducing activity of $CD4^+$ T cells in some models.

Within the $CD4^+$ T cell subset at least 2 more different types of cells with suppressive function may exist, which are induced after exposure to specific, exogenous antigen (called 'adaptive or inducible regulatory T cells'): Type 1 T regulatory (Tr1) cells and Th3 cells. These cell types appear to be distinguishable from $CD4^+CD25^+$ Tregs based on their cytokine production profiles. However, the relationship between these different types is unclear and the modes of action are overlapping.

Tr1 cells were induced by repetitive stimulation of TCR in the presence of IL-10 and were shown to mainly down-regulate immune responses via the production of high levels of IL-10 together with moderate amounts of TGF-ß (Chen et al., J. Immunol. 171: 733-744 (2003)) .

Th3 cells (identified in a model of EAE after oral delivery of antigen) produce high amounts of TGF-ß and variable amounts of IL-4 and IL-10. IL-4, itself, was shown to be a key factor for the differentiation of Th3 cells, in contrast to Tr1 cells that are differentiated with IL-10 (Chen et al., Science 265:1237-1240 (1994)).

Suppression of T cell function by using immunosuppressive drugs is a principal therapeutic strategy that has been used successfully to treat autoimmune diseases. However these drugs induce a general immunosuppression due to their poor selectivity, resulting in inhibition of not only the harmful functions of the immune system, but also useful ones. As a consequence, several risks like infection, cancer and drug toxicity may occur.

Agents interfering with T cell function are therapeutic mainstays for various autoimmune diseases.

The approach of using agents aiming at the activation of regulatory T cells for the therapy of autoimmune diseases have been up to now proven to be extremely difficult. Activation of Tregs via the TCR using the agonistic anti-CD3 antibody OKT-3 (Abramowicz et al, N Engl. J Med. 1992 Sep 3;327(10):736) or via the co-stimulatory molecule CD28 using the superagonistic anti-CD28 antibody TGN 1412 leads to complete depletion of the regulatory T cell population as well as other conventional T cells and the systemic induction and release of excessive amounts of pro-inflammatory cytokines including IFN-γ, TNF-α, IL-1 and IL-2, resulting in a clinically apparent cytokine release syndrome (CRS) in humans (Suntharalingam et al, N Engl. J Med. 2006 Sep 7;355(10):1018-28).

After the first two to three injections of 5 mg of the monoclonal antibody OKT3 most patients develop a cytokine release syndrome with high levels of tumour necrosis factor-alpha, interleukin-2, and gamma-interferon appearing within 1-2hrs in the circulation of kidney transplant recipients. (Abramowicz et al , Transplantation. 1989 Apr;47(4):606-8). This results in a narrow therapeutic window which limits the usefulness of this antibody in the treatment of autoimmune disease.

Treatment with a total dose of 5-10 mg of TGN1412 (0.1 mg anti-CD28 per kilogram of body weight) lead to a systemic inflammatory response with multiorgan failure within 90 minutes after receiving a single intravenous dose of the TGN 1412 (Suntharalingam et al, N Engl. J Med. 2006 Sep 7;355(10):1018-28).

It is generally agreed that CD4 T cells play a major part in initiating and maintaining autoimmunity. Accordingly, it has been proposed to use mAbs against CD4 T cells surface molecules, and in particular anti-CD4 mAbs, as immunosuppressive agents. Although numerous clinical studies confirmed the potential interest of this approach, they also raised several issues to be addressed in order to make anti-CD4 mAbs more suitable for use in routine clinical practice.

Several different mechanisms of action for CD4 mAbs have been proposed including: (1) antagonism of CD4-MHC II interactions resulting in inhibition of T cell activation, (2) CD4 receptor modulation as determined by a decrease in cell surface expression of CD4, (3)

partial signaling through the CD4 receptor in the absence of T cell receptor cross-linking which can suppress subsequent T cell activation and trigger CD4 T cell apoptotic death, (4) Fc-mediated complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC) leading to CD4 T cell depletion, and (5) stimulation of regulatory T cells.

Fc-mediated complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC) leading to CD4 T cell depletion is the main observed mechanism and is especially demonstrated for antibodies of the IgG1 subclass. Only a few CD4 antibodies have been attributed to the other mechanisms like TRX-1, TNX-355, IDEC-151, OKTcdr4A with only TRX-1 being an IgG1 (Schulze-Koops et al., J Rheumatol. 25(11): 2065-76 (1998); Mason et al., J Rheumatol. 29(2): 220-9 (2002); Choy et al., Rheumatology 39(10): 1139-46 (2000); Herzyk et al., Infect Immun. 69(2): 1032-43 (2001); Kon et al., Eur Respir J. 18(1): 45-52 (2001); Mourad et al., Transplantation 65(5): 632-41 (1998); Skov et al., Arch Dermatol. 139(11): 1433-9 (2003); Jabado et al., J Immunol. 158(1): 94-103 (1997)).

Dose-dependent depletion of $CD4^+$ T cells at "high" doses (multiple cycles with dosages > 100 mg) and transient sequestration (short-lived depletion) at "lower" doses (multiple cycles with dosages >10 mg), is observed with several CD4 antibodies (Mason et al., J. Rheumatol. 29 (2): 220-229 (2002); Kon et al., Eur. Respir J. 18(1):45-52 (2001)) and HuMax-CD4 (Skov et al., Arch Dermatol. 139(11): 1433-1439 (2003), Choy et al., Rheumatology 41 (10):1142-8 (2002)). Despite of their depletional activity, mAbs to CD4 failed to provide clinical benefit and consistent efficacy in autoimmune diseases investigated e.g. rheumatoid arthritis (Strand et al., Nature Reviews 6: 75-92 (2007)). Furthermore depletion of $CD4^+$ T cells is generally considered as scenario, which might cause a severe immunosuppression.

The B-F5 antibody (murine IgG1 anti-human CD4) was tested in different autoimmune diseases.

A small number of patients with severe psoriasis have been treated with the murine B-F5 antibody and some positive effects were described (Robinet et al., Eur J Dermatol 1996: 6: 141-6, and Robinet et al., J Am Acad Dermatol 1997; 36: 582-8).

In rheumatoid arthritis patients, the results observed in a placebo controlled trial with a daily dose of B-F5 did not indicate a significant improvement (Wendling et al. J Rheumato1;25(8):1457-61, 1998).

In multiple sclerosis (MS) patients, some positive effects were observed after a 10 days treatment in patients with relapsing-remitting forms, some of who were still relapse-free at the 6th month post-therapy (Racadot et al., J Autoimmun, 6(6):771-86, 1993). Similar effects were observed by Rumbach et al. (Mutt Scler;1(4):207-12, 1996).

In severe Crohn's disease, no significant improvement was observed in patients receiving B-F5 for 7 consecutive days or (Canva-Delcambre et al., Aliment Pharmacol Ther 10(5):721-7, 1996).

In prevention of allograft rejection, it was reported that B-F5 bioavailability was not sufficient to allow its use for prophylaxis of allograft rejection (Dantal et al. Transplantation, 27;62(10):1502-6, 1996).

It appears from the above that a first issue to be solved is the need for using longer term doses of mAb to obtain a clinical improvement. In general, the long term use of disease modifying therapeutics results in increased numbers of adverse events or loss of clinical effects, necessitating a dose increase ("dose-creep") to maintain response (Strand et al., Nature Reviews Drug Discovery 6: 75-92, (2007)) due to immunogenicity. The lesser and/or the less frequent disease modifying therapeutics have to be administered the lower those adverse events will be, plus there is an immediate beneficial effect for the patients requiring lower and/or less doses of the therapeutics.

Another drawback of therapy with monoclonal antibodies in humans is that these antibodies are generally obtained from mouse cells, and provoke anti-mouse responses in the human recipients. This not only results in a lower efficiency of the treatment and even more of any future treatment with mouse monoclonal antibodies, but also in an increased risk of anaphylaxis.

This drawback can, in principle, be avoided by the use of humanized antibodies, obtained by grafting the complementarity-determining regions (CDRs) of a mouse monoclonal antibody, which determine the antigen-binding specificity, onto the framework regions (FRs) of a human immunoglobulin molecule. The aim of humanization is to obtain a recombinant antibody having the same antigen-binding properties as the mouse monoclonal antibody from which the CDR sequences were derived, and far less immunogenic in humans.

In some cases, substituting CDRs from the mouse antibody for the human CDRs in human frameworks is sufficient to transfer the antigen-binding properties (including not only the specificity, but also the affinity for antigen). However, in many antibodies, some FR residues are important for antigen binding, because they directly contact the antigen in the antibody-antigen complex, or because they influence the conformation of CDRs and thus their antigen binding performance.

Thus, in most cases it is also necessary to substitute one or several framework residues from the mouse antibody for the human corresponding FR residues. Since the number of substituted residues must be as small as possible in order to prevent anti-mouse reactions, the issue is to determine which amino acid residue(s) are critical for retaining the antigen-binding properties. Various methods have been proposed for predicting the more appropriate sites for substitution. Although they provide general principles that may be of some help in the first steps of humanization, the final result varies from an antibody to another. Thus, for a given antibody, it is very difficult to foretell which substitutions will provide the desired result.

Previously the humanization of mouse B-F5 has been attempted, and success has been achieved in producing humanized B-F5 (hereinafter referred to as hB-F5) having similar CD4 binding properties to the parent mouse B-F5.

Thus, in WO 2004/083247, the humanised antibody BT061 (humanised B-F5, or simply hB-F5) has been found to be useful in treating autoimmune diseases, such as psoriasis and rheumatoid arthritis. This antibody is an antibody that is capable of activating CD4+CD25+ regulatory T cells. The patent application discloses compositions for parenteral administration, formulated to allow the administration of a dose of from 0.1-10 mg,

preferably from 1-5 mg. Dosage regimes envisaged are an intravenous 1 mg per day dose and a 5 mg every second day dose for rheumatoid arthritis patients over a period of 10 days.

The study was also described by Wijdenes et al., in an abstract and poster presented at the EULAR conference, June 2005. The treatment of 11 patients suffering from rheumatoid arthritis was disclosed. The patients were treated with 5 intravenous infusions of 5 mg BT061 every other day with concomitant treatment with 150 mg Diclophenac.

The antibody described in this study is not disclosed to be suitable for use in higher doses or longer term doses, and it is still desirable to find treatments at longer term doses so as to treat a greater number of patients.

$CD4^+CD25^+$ regulatory T cells are able to maintain peripheral tolerance and regulate autoimmunity by inhibiting the proliferation and cytokine production both of antigen specific $CD4^+$ and $CD8^+$ T cells.

Previous studies in mice demonstrated that murine $CD4^+CD25^+$ T cells suppress both proliferation and IFN-γ production by $CD8^+$ T cells induced either by polyclonal or Ag-specific stimuli. Furthermore $CD4^+CD25^+$ T cells inhibit the activation of $CD8^+$ responders by inhibiting both IL-2 production and up-regulation of IL-2Rα-chain (CD25) expression (Piccirillo et al., J. Immunol. 167: 1137-1140 (2001)). In contrast to regulatory T cells, conventional T cells express CD25 only upon activation by an immunogen stimulus.

In humans, $CD4^+CD25^+$ T cells preactivated by anti-CD3 or anti-CD28 antibody affect $CD8^+$ T cells by leading to reduced proliferation in response to polyclonal and allogeneic stimulation and reduced production of IFN-γ. Suppressed CD8+ T cells keep their anergic phenotype despite multiple re-stimulations with their cognate antigen and IL-2 for up to 3 weeks. (Camara et al., Eur. J. Immunol. 33:3473 – 3483 (2003), Dieckman et al., Immunology.115(3):305-14 (2005)).

Having regard to the above prior art, it is an aim of the present invention to treat patients having autoimmune diseases with lesser and/or lower doses. In particular, it is an aim of the

present invention to find treatments that may be applied to patients at low frequency during a long term therapy, in order to minimize the number of injections needed to obtain a clinical benefit.

Astonishingly, the inventors have observed that BT061 specifically stimulates $CD4^+CD25^+$ Tregs which suppress the proliferation of CD8+ T cells by inhibiting the production of IL-2 and IFN-γ by alloreactive $CD8^+$ T cells. Furthermore BT061 pre-activated $CD4^+CD25^+$ Tregs render suppressed $CD8^+$ T cells unable to express CD25 upon re-stimulation for up to 10 days enabling for the first time a long-term therapeutic effect in treating patients with this antibody, as CD25 expression is one of the hallmarks of activation of such CD8+ T cells. The failure to express CD25 thus results in a reduction of unwanted activation of the immune system.

Accordingly, the invention provides a pharmaceutical composition for treating an autoimmune disease comprising a pharmaceutically acceptable carrier and an agent capable of activating CD4+CD25+ regulatory T cells, wherein the composition is to be administered to a subject at most every 3 days.

Preferably, the period between administrations is at least 4 weeks, at least 12 weeks, at least twenty four weeks, at least 6 calendar months or at least a year. In one embodiment the treatment period is greater than one year and the composition is administered yearly over a period of 1, 2, 3, 4 or 5 years.

In particular, the composition is to be administered most every 6 days, preferably at most every 10 days. Typically the medicament may be administered to a subject every 3-31 days, more preferably every 3-10 days, or every 3-6 days.

The long term nature of the therapy is not especially limited, provided that it is longer than the time required for immediate/acute treatment of the patient concerned. However, periods of at least 6-months, 1 year, 18 months, 2 years and 5 years are envisaged, as well as indefinite periods (e.g. the lifetime of the patient, or until the patient is cured, or displays no symptoms).

When treating patients with an agent capable of activating CD4+CD25+ regulatory T cells both an immediate effect and a long-term therapeutic effect can be demonstrated. Without being bound by theory, this is believed to be due to the fact that agent stimulates CD4+CD25+ Tregs which not only directly inhibit CD8+ T cells but also render CD8+ T cells unable to express CD25 upon re-stimulation. It is thought that long-term effects are further mediated by a combination of effectiveness at low levels and a slow decline of the effective concentration of antibody given in a clinical setting, resulting in extended periods at an effective level of the agent.

It will be appreciated from the above regimes that the inventors have found that, surprisingly, the humanised antibody BT061 (humanised B-F5, or simply hB-F5) does not substantially modulate nor induce release of pro-inflammatory cytokines, as compared to other T cell interacting antibodies (e.g. anti-CD3 antibodies), but stimulates CD4+CD25+ Tregs which directly inhibit CD8+ T cells plus also renders CD8+ T cells unable to express CD25 upon re-stimulation. Based on the inventors' investigation, dosages may be administered at larger intervals and for a far longer period than disclosed previously in WO 2004/083247.

The concentration of the agent is not especially limited, provided that it is present in a concentration that is appropriate for the patient. However, for high doses preferably, the concentration of the agent is from 10 to 150 mg/ml, 15 to 150 mg/ml, from 15 to 100 mg/ml, from 15 (or greater than 10) to 75 mg/ml, or from 20 to 60 mg/ml. Most preferably, the concentration of the agent is (approximately) any one of 10 mg/ml, 12.5 mg/ml, 20 mg/ml, 25 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml or 100 mg/ml.

In alternative preferred embodiments for lower doses, the concentration of the agent is from 0.1 μg/ml to 30 mg/ml or, 0.1 to 1000 μg/ml, and more preferably from 1-500 μg/ml and 2-250 μg/ml. Most preferably, the concentration of the agent is (approximately) any one of 15 μg/ml, 25 μg/ml, 125 μg/ml, 250 μg/ml, or 500 μg/ml, 1 mg/ml, 12.5 mg/ml or 25 mg/ml. The invention also provides the use of an agent as defined herein for the manufacture of a medicament for treating autoimmune disease wherein the agent is to be administered to a subject in a dosage regimen described herein. Further the invention provides an agent as

defined herein for use in the treatment of autoimmune disease wherein the agent is to be administered to a subject in a dosage regimen described herein.

The dosage volume applied to a subject using the composition is not especially limited, provided that it is delivered over a long term compared to regimes already known, and is therefore suitable for treating individuals who may benefit from long term therapy such as, but not limited to, severe cases with a long history of the disease and insufficient response to current therapies. The concentration of the agent within the dosage volumes can be varied in order to provide the required doses, which are described in this application.

The dosage volume will vary depending on the method of administration. Parenteral administration is preferred. Examples of parenteral administration are intramuscular administration, intravenous administration or subcutaneous administration. Where the composition is to be administered by intravenous infusion the dosage volume may be from 0.1 or 0.5 ml up to 500 ml, preferably between 15 and 25 ml, and typically about 20 ml. Where the composition is to be administered by subcutaneous or intramuscular injection, the dosage volume may be between 0.1 to 3 ml, preferably between 0.5 and 1.5 ml, and typically about 1 ml.

However, in some embodiments the composition may be provided in concentrated form and diluted to the strength required for the individuals concerned. Preferably, in these situations the composition is provided in relatively small volumes of about 1, 2, 3, 4 or 5 ml. In alternative embodiments, the composition is provided at the required strength and dosage volume described above (i.e. ready for administration). In one specific embodiment the pharmaceutical compositions for subcutaneous administration are provided in a ready for administration form which does not require dilution so that they can be easily administered by non-medical personnel.

As has been mentioned previously, it was not known that the agents capable of treating autoimmune diseases could be administered in the long term therapies at low frequencies that are envisaged by the present invention. Whilst known doses of the agents capable of treating autoimmune disease are effective in some individuals or disease types, the realisation that it

may be effective for longer periods of time and tolerated for longer periods has opened up the way for more effective treatment of some autoimmune diseases and classes of patients.

The invention will be illustrated by way of example only, with reference to the following Figures, in which:

Figure 1 shows the effect of BT061 on the cytokine synthesis of whole-blood cultures from 3 healthy donors. The cultures were stimulated in separate experiments with 4 different types of activators: CD3=anti-CD3 antibodies; LPS lipopolysaccharide; PHA=phytohaemagglutinin + anti-CD28 antibodies; SEB=staphylococcal enterotoxin B + anti-CD28 antibodies. Different cytokines were determined to measure effects on various leukocyte sub-populations: Treg cells (CD3: TGF-β, IL-10); monocytes/macrophages (LPS: IL-10, TNFα, IL-1β); Th2 cells (PHA: IL-4, IL-5, IL-13); Th1 cells (SEB: IL-2, IFNγ);

Figure 2 shows the effect of BT061 in human whole-blood cultures (donors with rheumatoid arthritis) on cytokine synthesis triggered by different stimulants;

Figure 3 shows the nucleotide sequence encoding the mouse B-F5 V_H region (SEQ ID No: 5);

Figure 4 shows the nucleotide sequence encoding the mouse B-F5 V_k region (SEQ ID No: 6);

Figure 5 shows the nucleotide sequence (SEQ ID No: 3) of a fragment of the plasmid encoding the V_H region of humanized BF-5. The sequence encoding the V region is underlined and the corresponding polypeptide sequence (SEQ ID No: 17) is indicated below the nucleotide sequence;

Figure 6 shows the nucleotide sequence (SEQ ID No: 4) of a fragment of the plasmid encoding the V_K regions of humanized BF-5. The sequence encoding the V region is underlined and the corresponding polypeptide sequence (SEQ ID No: 2) is indicated below the nucleotide sequence;

Figure 7 shows that BT061 (anti-CD4 mAb)-activated CD25+ Tregs suppress cytokine synthesis and CD25 re-expression of CD8+ T cells. Top: CD4-activated CD25+ Tregs suppress cytokine synthesis of co-activated CD8+ T cells. Bottom: CD4- activated CD25+ Tregs suppress CD25 re-expression of CD8+ T cells. The dot plots show gated CD8+ T cells only. A representative result of three is shown. Numbers indicate percentages of positive cells.

Figures 8A and 8B respectively show the TFNα and IL-6 release observed in a clinical trial with BT061 (single intravenous infusion or subcutaneous injection) in healthy volunteers in comparison to the levels reported with the anti-CD3 monoclonal antibodies. Dose levels and time to recovery are included in the figures. Results for TRX4 indicated in Figures as "2)" reported in Keymeulen et al., 2005 N. Engl. J. Med. Type 1 Diabetes patients. Results for Teplizumab indicated in Figures as "3)" reported in Herold et al., 2002 N. Engl. J. Med. Type I Diabetes patients. Normal values indicated in Figures as "4)" reported in Straub et al., 2007, Athr. & Rheumat. "*)" represents a single dose, "**)" represents a cumulative dose injected until peak concentration was reached.

Figure 9 shows IL-2 and IFN-γ plasma levels after administration of a single intravenous or subcutaneous dose of BT061 in healthy volunteers. ULN = upper limit of normal; LLN = lower limit of normal.

Figure 10 shows a kinetic of CD4 cell counts (cells per ml of plasma) in volunteers treated with a single intravenous dose of BT061. Mean values of 3 patients per dose group are shown. Dotted lines indicate the upper limit of normal (ULN) and the lower limit of normal (LLN).

Figure 11 shows a kinetic of CD4 cell counts (cells per ml of plasma) in volunteers treated with a single subcutaneous dose of BT061. Mean values of 3 patients per dose group are shown. Dotted lines indicate the upper limit of normal (ULN) and the lower limit of normal (LLN).

Figure 12 parts A to H provide graphs showing data from the clinical trials with psoriasis patients of dose group I as described in Example 8, in which patients are treated with a 0.5 mg intravenous injection of BT061 or a placebo. Parts A to H of Figure 8 provide graphs of the PASI scores of patients 1 to 8 of dose group I, respectively.

Figure 13 parts A to H provide graphs showing data from the clinical trials with psoriasis patients of dose group II as described in Example 8, in which patients are treated with a 2.5 mg intravenous injection of BT061 or a placebo. Parts A to H of Figure 9 provide graphs of the PASI scores of patients 1 to 8 of dose group II, respectively.

Figure 14 parts A and B provide photographs from the clinical trial with psoriasis patients as described in Example 8. The photographs are of the same patient who was a member of dose group II. The photograph shown in part A was taken prior to treatment. The photograph shown in part B was taken 28 days after treatment.

Figure 15 provides results for the clinical trial with rheumatoid arthritis patients as described in Example 9. The figure shows a bar chart of the percentage of patients from the dose groups receiving 1.25 mg, 6.25 mg, 12.5 mg and 25 mg subcutaneous BT061 achieving at least an ACR20 response. Six patients in each group received the antibody dose while two patients received a placebo.

Figure 16A and 16B provides results from the clinical trial with rheumatoid arthritis patients as described in Example 9. Figure 16A shows a bar chart of the number of tender joints for patients from the dose group receiving 25 mg subcutaneous BT061. Figure 16B shows a bar chart of the number of swollen joints in patients from the same dose group. Six patients in each group received the antibody dose while two received a placebo.

Figure 17A and 17 B provide results from the clinical trial with rheumatoid arthritis patients as described in Example 9. The Figures show the changes of individual parameters (in %) for one responder (Figure 17A) and one non-responder (Figure 17B) from the 25 mg subcutaneous dose group. In the Figures "Pat's GA" and "Phy's GA" refer to the patient's

global assessment and physician's global assessment, respectively. The term "PA of pain" refers to the patient's assessment of pain.

Figure 18A and 18B provide further results from the clinical trial with rheumatoid arthritis patients as described in Example 9. The Figures show the number of tender joints in patients from the 1.25 mg subcutaneous dose group (Figure 18A) and from the 6.25 mg subcutaneous dose group (Figure 18B).

Figure 19A and 19B provide further results from the clinical trial with rheumatoid arthritis patients as described in Example 9. The Figures show the number of tender joints in patients from the 50 mg subcutaneous dose group (Figure 19A) and from the 6.25 mg intravenous dose group (Figure 19B).

Figure 20 shows the alignment of the polypeptide sequences of murine B-F5 V_K (SEQ ID No: 8), FK-001 (SEQ ID Nos: 9, 10, 11 and 12), L4L (SEQ ID No: 18), and L4M (SEQ ID No: 2) in the design of the humanised form of B-F5 (i.e. BT061).

Figure 21 shows the alignment of the polypeptide sequences of murine B-F5 V_H (SEQ ID No: 7), M26 (SEQ ID Nos: 13, 14, 15 and 16), H37L (SEQ ID No: 1), and H37V (SEQ ID No: 17) in the design of the humanised form of B-F5;

The invention will now be described in more detail.

The agents that are suitable for use in the present invention are those which are capable of activating CD4+CD25+ regulatory T cells. The agent may be a polypeptide, a protein or an antibody. Where the agent is an antibody it may be a monoclonal antibody. Preferably the antibody is a monoclonal anti-CD4 antibody. The antibody may also preferably be an IgG1 antibody and may be an unmodified IgG1 antibody.

In a preferred aspect of the invention the agent does not cause a substantial increase in the level of pro-inflammatory cytokines in the subject's blood plasma after administration as compared to anti-CD3 antibodies. In particular, the levels of IFN-γ, TNF-α, IL-6 and/or IL-2

after administration of the agent are not substantially raised compared to plasma levels measured in healthy subjects (see Table A1). Specifically, if the ULN for a specific cytokine given in Table A1 is taken as X then within 96 hours after administration of the agent of the invention there may be less than a 20 fold increase in X. Preferably there may be less than a 10 fold increase in X. More preferably these levels are during the period of 10 minutes after the start of administration to 96 hours after completion of administration.

It is possible that in autoimmune patients, cytokine levels prior to administration of the agent are already higher than those observed in healthy subjects (ULN given in Table A1) e.g. due to a modified activation status of immune cells compared to the activation status of the cells in healthy subjects. In those cases, the concentration for a specific cytokine directly prior to administration of the agent is taken as X and within 96 hours after administration of the agent of the invention there may be less than a 20 fold increase in X. Preferably there may be less than a 10 fold increase in X. More preferably these levels are during the period of 10 minutes after the start of administration to 96 hours after the completion of administration.

Cytokine	**ULN (pg/mL)**
Il-2	19.4
IL-6	4.4
TNF-alpha	2.8
IFN-gamma	3.8

Table A1: Cytokine levels measured in plasma of healthy volunteers. The ULN (upper limit of normal) is calculated based on mean values measured in 39 individual subjects + 2 x standard deviation.

In a further preferred aspect of the invention the agent does not cause a substantial long-lasting decrease in the cell count of CD4+ lymphocytes in the subject's blood plasma. Specifically, within the period of 72 to 96 hours after administration the cell count of CD4+ lymphocytes in the subject's blood plasma may be above 250 cells/µl (or at least 250 cells/µl).

Preferably the cytokine and CD4+ lymphocyte effects described above are seen in at least 80% of patients treated.

To prevent negative impact on the immune system, e.g. a decrease in the lymphocyte cell count or induction of cytokine release, it is known in the art to utilise antibodies (especially T cell interacting antibodies) of subclass IgG2, IgG3 or IgG4 because antibodies of the IgG1 subclass display higher Fc receptor interactions. It is also known in the art to modify antibodies (especially T cell interacting antibodies) by Fc mutation, deglycosylation, glycomodification or glycoengineering to reduce Fc receptor interactions.

The present inventors have found that the avoidance of antibodies of the IgG1 subclass and modifications are not necessary for the agent of the present invention. In particular, the agent of the present invention does not display substantial long-lasting CD4+ cell depletion or induce substantial cytokine release as compared to anti-CD3 antibodies.

Accordingly, in a preferred aspect of the invention the agent is an unmodified IgG1 antibody, i.e. an antibody which does not include an Fc mutation, and has not been subject to deglycosylation, glycomodification or glycoengineering to reduce Fc receptor interactions, or a fragment or a derivative thereof.

The antibodies which are most suitable for use in the present invention are humanized anti-CD4 antibodies, or fragments or derivatives thereof, which are capable of activating CD4+CD25+ regulatory T cells. Examples of antibodies which are capable of capable of activating CD4+CD25+ regulatory T cells are discussed in Becker *et al.*, (European Journal of Immunology (2007), Vol. 37: pages 1217-1223).

Generally, the antibody used in the invention further comprises a human constant region (Fc). This constant region can be selected among constant domains from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgGl, IgG2, IgG3 and IgG4. Preferred constant regions are selected among constant domains of IgG, in particular IgGl.

The present invention also includes any fragment of the antibody comprising the V regions thereof. This comprises in particular Fab, Fab', $F(ab)'_2$, Fv and scFv fragments.

In a particularly preferred aspect of the present invention the antibody is a humanized anti-CD4 antibody or fragment or derivative thereof derived from the mouse monoclonal anti-CD4 antibody B-F5. An example of such an antibody is the BT061 antibody.

BT061 antibody, fragments and derivatives thereof

The humanized antibody BT061 (hB-F5) is derived from mouse B-F5 mAb, and has V domains defined by the following polypeptide sequences:

- H chain V domain: EEQLVESGGGLVKPGGSLRLSCAASGFSFSDCRMYWLRQA PGKGLEWIGVISVKSENYGANYAESVRGRFTISRDDSKNTVYLQMNSLKTEDTAVY YCSAS YYRYDVGAWFAYWGQGTLVTVSS (SEQ ID NO: 1)
- L chain V domain:

DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYIYWYQQ KPGQPPKLLIYLASILESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSRELPWT FG QGTKVEIK (SEQ ID NO: 2).

Derivatives of this antibody are also suitable for use in the present invention. Derivatives include those with V domains defined by polypeptide sequences having at least 80%, preferably at least 90 %, most preferably at least 95% sequence identity with SEQ ID NO: 1 or SEQ ID No: 2.

Particularly preferred antibodies are those which comprise the complementarity-determining regions (CDRs) of the mouse B-F5 mAb, and retain the ability of hB-F5 to activate CD4+CD25+ regulatory T cells. The location of the CDRs within the V_H and V_K domains is shown in Figures 20 and 21. Such antibodies can optionally have variations in the sequence of the CDRs that do not substantially affect the specificity and/or affinity of binding.

Generally, the hB-F5 antibody used in the invention further comprises a human constant region (Fc). As indicated above, this constant region can be selected among constant domains from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype,

including IgGl, IgG2, IgG3 and IgG4. Preferred constant regions are selected among constant domains of IgG, in particular IgGl.

The present invention also includes any fragment of a BT061 antibody comprising the V regions thereof. This comprises in particular Fab, Fab', $F(ab)'_2$, Fv and scFv fragments.

A polynucleotide encoding the V domain of the H chain or of the L chain of a BT061 antibody may be fused with a polynucleotide coding for the constant region of a human H or L chain, for the purpose of expressing the complete H and L chains obtained in this way; a sequence coding a signal peptide allowing the secretion of the protein can also be added.

The invention also makes use of expression cassettes wherein a polynucleotide as described above is linked to appropriate control sequences allowing the regulation of its transcription and translation in a chosen host cell, and recombinant vectors comprising a polynucleotide or an expression cassette of the invention.

These recombinant DNA constructs can be obtained and introduced in host cells by the well-known techniques of recombinant DNA and genetic engineering.

The invention also makes use of a host cell, transformed by a polynucleotide of the invention. Useful host-cells within the framework of the present invention can be prokaryotic or eukaryotic cells. Among suitable eukaryotic cells, one will mention, by way of example, plant cells, cells of yeasts such as *Saccharomyces,* cells of insects such as *Drosophila,* or *Spodoptera,* and mammal cells such as HeLa, CHO, 3T3, C127, BHK, COS, etc.

The construction of expression vectors used in the invention, and the transformation of host-cells can be made by the standard techniques of molecular biology.

The BT061 (hB-F5) antibody used in the invention can be obtained by culturing a host cell containing an expression vector comprising a nucleic acid sequence encoding said antibody, under conditions suitable for the expression thereof, and recovering said antibody from the host cell culture.

Construction of humanized B-F5

Design of humanized B-F5 V_H and V_K regions

DNA sequences encoding mouse B-F5 V_H and V_K regions are respectively shown in Figure 3 and Figure 4 and under sequence identifiers SEQ ID NO:5 and SEQ ID NO:6. The human V_H and V_K on which the mouse CDRs are grafted were selected by searching databases for human V_H most like the original mouse B-F5 V_H and V_K. V_H region of a human antibody (M26; Accession Number A36006) had the highest homology with B-F5 V_H. VK region of another human antibody (FK-001; NAKATANI et *al., Biotechnology, 7* (1989), 805-810)) had the highest homology with B-F5 V_K.

Two types of V_K differing between them in that the 4th residue was Leucine or Methionine were constructed and designated as L4L and L4M. Two types of VH differing between them in that the 37th amino acid residue was Leucine or Valine, were constructed and designated as H37L and H37V. The alignment of the polypeptide sequences of B-F5, FK-001, L4L, and L4M is shown in Figure 20. The alignment of the polypeptide sequences of B-F5, M26, H37L, and H37V is shown in Figure 21. The FR residues previously reported to be important for the packing of CDRs (Chothia et al., Nature 342(1989), 877; Foote et al., J. Mol. Biol., 224(1992), 487) are boxed.

By combining these V_H and V_K, 4 versions of V regions were designed.

Expression of humanized B-F5

The subsequent steps for production of humanized B-F5 were the same as those disclosed in US Patent 5,886,152 for humanized B-B10.

Briefly, expression plasmids for the H chain (V_H humanized region fused to the constant region of a human y-1 chain (TAKAHASHI et *al., Cell,* 29 (1982), 671-679)) and the L chain (VK humanized region fused to the constant region of FK-001 K chain) of humanized B-F5 were constructed separately. In these plasmids, the expression of humanized B-F5 is driven by the promoter/enhancer of the gene of human monoclonal IgM, FK-001. Figure 5 and 6 respectively show the fragments of the plasmids encoding the VH and V_K regions of humanized BF-5. The sequences encoding the V region are underlined and the corresponding

polypeptide sequences are indicated underneath the nucleotide sequence. Both plasmids and pSV2neo were simultaneously introduced into mouse myeloma Sp2/0 (ATCC CRL-1581) using Lipofectin[ni]. Transfectomas producing human IgG were selected by ELISA, using an anti-human IgG (y chain) antibody and an anti-human Ig K chain antibody.

Characterisation of the different versions of humanized B-F5

Estimation of CD4 binding activity

Culture supernatants of transfectomas producing the four versions of hB-F5 were collected, and concentrated. The different antibodies were purified from culture supernatants by affinity chromatography using protein A Sepharose and assessed for their CD4 binding activity by measuring, by means of competitive ELISA, their inhibitory activities against the binding of biotinylated mB-F5 to soluble CD4 coated on microtiter plates. Incubation, time is 2 hours for 37°C and overnight for 4°C.

The relative binding activities of hB-F5s (binding activity of mB-F5 was taken as 100%) are shown in Table A below:

Table A

Antibody	Temp (°C)	Relative binding activity (% of mB-F5)
H37L/L4L	4	80
	37	30
H37L/L4M	4	80
	37	30
H37V/L4L	4	10-20
	37	10
H37V/L4M	4	10-20
	37	10

From the results shown in Table A, it appears that the 37th residue of Leucine, is critical to maintain CD4 binding activity of hB-F5 because the CD4 binding activity *is* several-fold reduced by conversion of ^{37}Leu to ^{37}Val. On the contrary, the 4th residue of V_K is found to be not so important for the CD4 binding activity. As the structural difference between ^{37}Leu and ^{37}Val of V_H is not clearly demonstrated by molecular modeling, the superiority of H37L to H37V in CD4 binding activity was unexpected.

H37L/L4L and H37L/L4M were chosen for evaluation.

Investigation of the in vitro biological activities of humanized B-F5

The *in vitro* biological activities of mouse B-F5 and humanized B-F5s (H37L/L4M IgG1 and H37L/L4L IgG 1) were evaluated. Humanized B-F5s of IgG2 type (H37L/L4M IgG2 and H37L/L4L IgG2) were also tested.

The *in vitro* biological activities of mB-F5 and the four types of hB-F5s were evaluated using peripheral blood mononuclear cells (PBMCs) from healthy donors. PBMCs were activated by ConA (2.5 pg/ml, 3 days) or PPD (10 pg/ml, 4 days) in the presence of murine or hB-F5s, and were monitored for their proliferative responses by 3H-thymidine incorporation.

Murine and hB-F5s could moderately inhibit ConA-induced proliferation, but the activities varied from antibody to antibody and/or from donor to donor. Also, murine and hB-F5s were able to inhibit Ag-specific PBMC proliferation induced by PPD.

IgG1 type of hB-F5 inhibited PPD-induced proliferation more effectively (as high as 70% inhibition) than mB-F5. IgG1 type seemed to be more effective than IgG2 type of which inhibitory activity was almost the same as mB-F5. For IgGl type, H37L/L4M was more effective than H37L/L4L. IgG2 type of H37L/L4M and H37L/L4L had almost the same inhibitory activities. In short, the inhibitory activities of B-F5s against PPD-induced PBMC proliferation were as follows: H37L/L4M IgG1 > H37L/L4L IgG1 > H37L/L4M IgG2 = H37L/L4L IgG2 = mB-F5.

Considering the efficacy of the *in vitro* biological activity and the smaller number of mouse amino acids, H37L/L4M IgG1 was chosen for further evaluation, and it is this antibody which is named BT061 and is employed to demonstrate the present invention in the Examples provided in this application.

Compositions and uses

As has been mentioned, the pharmaceutical composition and medicaments used in the present invention, are preferably capable of treating an autoimmune disease in patients benefiting from longer term therapies.

In some embodiments (higher doses) the preferred doses of the agent are those from 10 mg to 100 mg, 10 mg to 80 mg, 15 mg to 80 mg, 20 mg to 75 mg, preferably from 20 mg to 60 mg and most preferably from 25 mg to 60 mg.

Alternatively, the dosage may be defined based on the cumulative dose over plurality of doses. In particular, for a plurality of doses over a period of 10 days it is preferred that the cumulative dose is between greater than 25 mg but less than or equal to 200 mg, more preferably between 28 mg and 100 mg, and most preferably between 30 mg and 100 mg. Further, the cumulative dose over a period of 5 days are preferably greater than 15 mg but less than or equal to 100 mg, more preferably between 18 mg and 100 mg and most preferably between 20 mg and 100 mg. In this aspect of the invention it is particularly preferred that the dosages are administered subcutaneously.

In an alternative embodiment (lower doses) the preferred doses are those of 0.2 mg to 30 mg, 0.2 to 20 mg per dose, 0.3 mg to 7.5 mg, 0.3 to 5 mg per dose or preferably 0.3 to 1 mg per dose.

Alternatively, the dosage may be defined based on the cumulative dose over plurality of doses. In particular, for a plurality of doses over a period of 10 days it is preferred that the cumulative dose is between 0.2 to less than 25 mg, more preferably between 0.2 and 20 mg and most preferably between 0.2 to less than 10 mg. Further, the cumulative dosage over a period of 5 days should be between 0.2 to less than 15 mg, preferably between 0.2 and 12 mg, and more preferably between 0.2 to less than 5 mg.

In this aspect of the invention it is preferred that, when the dose is to be administered intravenously, the dosages over a period of 10 days are between 0.2 to less than 10 mg, most

preferably between 0.2 and 7.5 mg. Alternatively, where the doses are to be administered subcutaneously or intramuscularly, it is preferred that the dosages over a period of 10 days are between 1 mg to 30 mg, more preferably between 5 mg and 30 mg.

The dose can also be calculated on the basis of the body weight of the subject or the body surface area (BSA) of the subject. Body surface area (BSA) can be calculated according to any known method. Examples of BSA calculation methods are as follows:

Mosteller formula: $(BSA\ (m^2) = ([Height(cm) \times Weight(kg)]/ 3600)^{1/2}$

(Mosteller RD: Simplified Calculation of Body Surface Area. *N Engl J Med* 1987 Oct 22;317(17):1098)

DuBois and DuBois formula: $BSA\ (m^2) = 0.20247 \times Height(m)^{0.725} \times Weight(kg)^{0.425}$

(DuBois D; DuBois EF: A formula to estimate the approximate surface area if height and weight be known. *Arch Int Med* 1916 17:863-71.

Haycock formula: $BSA\ (m^2) = 0.024265 \times Height(cm)^{0.3964} \times Weight(kg)^{0.5378}$

(Haycock G.B., Schwartz G.J.,Wisotsky D.H. Geometric method for measuring body surface area: A height weight formula validated in infants, children and adults. *The Journal of Pediatrics* 1978 93:1:62-66)

Gehan and George formula: $BSA\ (m^2) = 0.0235 \times Height(cm)^{0.42246} \times Weight(kg)^{0.51456}$

(Gehan EA, George SL, Estimation of human body surface area from height and weight. *Cancer Chemother Rep* 1970 54:225-35)

Boyd formula: $BSA\ (m^2) = 0.0003207 \times Height(cm)^{0.3} \times Weight(grams)^{(0.7285 - (0.0188 \times LOG(grams)))}$

According to the higher dose embodiments of the invention the dose of the agent to the subject in terms of body weight is from 0.1 to 2 mg/kg, preferably 0.15 to 1.5 mg/kg, and most preferably 0.2 to 1 mg/kg. In terms of BSA the dose of the agent to the subject is from 5 to 60 mg/m^2 BSA, preferably from 6 to 50 mg/m^2, and most preferably 8 to 40 mg/m^2.

In these aspects of the invention, where the dose is based on the body surface area or the body weight of the subject it is preferred that the doses over a period of 10 days are between 10 mg/m^2 and 120 mg/m^2, more preferably between 16 mg/m^2 and 120 mg/m^2, or between 0.2 mg/kg and 4 mg/kg, more preferably between 0.4 mg/kg and 4 mg.kg. It is particularly preferred that the dosages are administered subcutaneously.

According to the lower dose embodiments of the invention the dose of the agent to the subject in terms of body weight is from 1 to 500 μg/kg, preferably 2 to 400 μg/kg, more preferably 2 to 250 μg/kg and most preferably 2.5 to 20 μg/kg. In terms of BSA the dose of the agent to the subject is from 0.12 to 15 mg/m^2, more preferably 0.20 to 10 mg/m^2 and most preferably 0.30 to 0.50 mg/m^2.

In these aspect of the invention where the dose is based on the body surface area or the body weight of the subject it is preferred that, when the dose is to be administered intravenously, the dosages over a period of 10 days are between 0.20 to 10 mg/m^2, more preferably between 0.20 to 4 mg/m^2, or between 2 to 250 μg/kg, more preferably between 2 to 100 μg/kg. Alternatively, where the doses are to be administered subcutaneously or intramuscularly, it is preferred that the dosages over a period of 10 days are between 0.30 to 20 mg/m^2, more preferably between 0.5 to 20 mg/m^2, or between 2.5 to 500 μg/kg more preferably between 20 to 500 μg/kg.

The frequency of administration is not especially limited, provided that it affords the advantages of the log term treatment. In the invention, it is preferred that the plurality of doses are administered on at least the following bases: 3-daily, weekly, every four weeks, every 6 weeks, every 12 weeks, every 24 weeks, every calendar month, every 3 calendar months, every six calendar months or yearly. Thus, the doses may be separated by at least three days, or alternatively by at least one week, by at least 10 days, by at least one month, by at least 3 months, by at least 6 months, or by at least one year (meaning that the doses are taken at least every 3 days, every week, every 10 days, every month, every 3 months, every 6 months, or every year). In a further alternative the plurality of doses are taken from every 3 to 31 days, every 3-10 days, every 3-6 days or from every 1-12 months.

The invention also provides a kit for a use as defined above, wherein the kit comprises a plurality of medicament dosages as defined above for simultaneous, sequential or separate administration to a subject.

It also provides a method of treatment of an autoimmune disease, which method comprises administering a medicament as defined above, in a manner as defined above, to a subject.

As has been mentioned, the pharmaceutical composition and medicaments used in the present invention, are preferably capable of treating an autoimmune disease in patients benefiting from longer term therapies. Such patients include but are not limited to severe cases with a long history of the disease.

Generally the pharmaceutical composition and medicaments used according to the present invention are for treating an autoimmune disease. Preferably the autoimmune disease is selected from psoriasis, rheumatoid arthritis, multiple sclerosis, type-1 diabetes, inflammatory bowel diseases, Crohn's disease, Hashimoto's thyreoiditis, autoimmune thyreoiditis, autoimmune myasthenia gravis, systemic lupus erythematosus, ulcerative colitis, atopic dermatitis, myocarditis and transplantation-related diseases such as graft-versus-host or host-versus graft reactions, or general organ tolerance issues.

In a particularly preferred aspect of the invention the pharmaceutical compositions are for treating the autoimmune disease psoriasis. In particular, such pharmaceutical compositions are to be administered intravenously or subcutaneously in the dosages specified herein.

Psoriasis is a disorder which causes psoriatic lesions or plaques on the sufferer's skin.

The Psoriasis Area and Severity Index (PASI) score is commonly used to evaluate and record the level of psoriasis exhibited by sufferers. PASI scoring involves the assessment of erythema (E), infiltration (I), and desquamation (D), and body surface area involvement (A) over 4 body regions (head (h), trunk (t), upper (u) and lower (l) extremities). Table B below shows how the scoring system works.

TABLE B – PASI scoring system

Degree of severity (per body region)	Value given
No symptoms	0
Slight	1
Moderate	2
Marked	3
Very marked	4

Surface involved (per body region)	Value given
< 10%	1
10-29%	2
30-49%	3
50-69%	4
70-89%	5
90-100%	6

Because the head, upper extremities, trunk, and lower extremities correspond to approximately 10, 20, 30, and 40% of body surface area, respectively, the PASI score is calculated by the formula:

$$PASI = 0.1(E_h + I_h + D_h)A_h + 0.2(E_u + I_u + D_u)A_u + 0.3(E_t + I_t + D_t)A_t + 0.4\,(E_l + I_l + D_l)A_l$$

PASI score ranges from 0-72. A score of 0 means no psoriasis, while a score of 72 represents the most severe psoriasis.

In a preferred embodiment of this aspect the pharmaceutical composition of the present invention is capable of treating psoriasis by providing at least a 40 %, and preferably at least a 50 %, improvement in the PASI score of the patient. Preferably the subject has a PASI score of at least 10 prior to treatment. These effects may be seen at least 56 days after administration of a single dose of the antibody, more preferably at least 75 days after administration of a single dose of the antibody. In particular, these effects may be seen in at least 80% of patients.

In a further aspect of the present invention the pharmaceutical compositions are for treating rheumatoid arthritis.

Rheumatoid arthritis is an autoimmune disease which causes chronic inflammation of joints and surrounding tissues, and can also affect other tissues and body organs.

Improvement in rheumatoid arthritis exhibited by a treated patient is commonly assessed using the American College of Rheumatology (ACR) core set of parameters (Felson et al., Arthritis & Rheumatism, 1995, 38(6), 727-735). This system defines a value of ACR 20 as a 20% improvement in tender and swollen joint counts and 20% improvement in 3 of the 5 remaining ACR core set measures: patient and physician global assessments, pain, disability, and an acute phase reactant, such as C-reactive protein (CRP).

In particular, the pharmaceutical compositions for treating rheumatoid arthritis are preferably to be administered intramuscularly or subcutaneously in the dosages specified herein.

Present treatment of arthritis includes first line drugs for controlling pain and inflammation classified as non-steroidal anti-inflammatory drugs (NSAIDs), e.g., aspirin, ibuprofen, naproxen, etc. Secondary treatment of arthritis includes corticosteroids (e.g. prednisone and dexamethasone), slow acting antirheumatic drugs (SAARDs) or disease-modifying anti-rheumatic drugs (DMARDs), e.g., methotrexate, penicillinamine, cyclophosphamide, gold salts, azothipoprine, leflunomide, etc.

Corticosteroids, the synthetic versions of the body's cortisone hormone, are used to inhibit RA progression (e.g. prednisone and dexamethasone).

Another group of drugs called biological-response modifiers (BRMs) has also been developed for treatment of RA including antagonists to TNF-alpha (adalimumab, infliximab, etanercept) which work through binding to its receptor or directly binding to the TNF-alpha protein.

In one embodiment of this aspect of the invention the compositions are to be administered in combination with drugs currently used to treat rheumatoid arthritis. In particular, the compositions are to be administered with one of the drugs mentioned above, preferably methotrexate.

Known drugs, such as methotrexate, and the pharmaceutical composition of the present invention can be administered simultaneously, sequentially or separately.

The invention will now be described further in relation to the following specific embodiments.

EXAMPLES

EXAMPLE 1 - *Investigation of the immuno-modulating capacities of BT061 regarding T cell proliferation.*

Method

Whole-blood cultures were performed with freshly drawn peripheral blood. Briefly, using 19G needles blood from three healthy volunteers was collected into heparinised syringes. The blood was seeded into 96-well culture plates not later than 60 min after donation.

The antibody used in the invention (BT061, lot 40588, or lot 70A0013B) was added to the cultures before stimulation of the leukocytes at 5 different concentrations (see "Test substances" below) The cells were allowed to interact with the antibody for 90 min at 37°C, 5 % CO_2 in humidified atmosphere, then four different stimulants were added to separate cultures:

(a) anti-CD3 antibodies (R&D Systems; 50 ng/ml)

(b) phytohaemagglutinin (PHA, Biochrom KG; 3 pa/ml) together with anti-CD28 antibodies (Becton-Dickinson; 1 μg/ml);

(c) lipopolysaccharide (LPS, subtype 055:B15 from Sigma Aldrich; 1 μg/ml);

(d) SE-B (Bernhard-Nocht-Institut; 25 ng/ml) together with anti-CD28 antibodies (Becton-Dickinson; 1 μg/ml).

All whole-blood cultures were incubated for 24 hrs at 37 °C, 5% CO_2 (humidified atmosphere). Then the culture supernatants were collected for the determination the cytokine

endpoints, except for PHA/antiCD28 stimulated cultures, which were incubated for 48 hrs in order to obtain a sufficient stimulation of Th2 cells.

The results are set out in Figure 1.

Results

BT061 displayed no significant effect on the major activities of monocytes/macrophages and on Th1 as well as Th2 activities in whole blood cultures from healthy volunteers. There was a concentration-dependent effect on Treg cells (demonstrated as an increase in TGF-beta release).

In particular, the results confirm that there is:

- No modulation of the inflammatory cytokine IL-2 at concentrations of up to 50 µg/ml corresponding to high dose application of up to 150mg in patients
- No induction of the inflammatory cytokine IFN-gamma at concentrations of up to 50 µg/ml corresponding to high dose application of up to 150mg in patients
- No modulation of the Th1/Th2 cytokines at concentrations of up to 50 µg/ml corresponding to high dose application in patients
- Only very sporadic up-regulation of IL-6 by a marginal increment occurred, the implications of which are discussed controversial
- Increase in TGF-beta release (Treg cells)
- No significant effect on major regulatory activities of monocytes/macrophages and on Th1 and Th2 activities.

EXAMPLE 2 - *Testing of BT061 for its influence on cultivated human PBMCs primed for an anti-tetanus toxoid response and cytokine assay*

Method

Proliferation assay

Freshly isolated PBMC were cultivated in 96 well flat bottom microtiter-plates in a volume of 200 µl/well (4 x 10^5 cells/well). The test-item (Anti CD4 mAb BT061) was used in the concentrations of 20 µg/ml, 4 µg/ml and 0.8 µg/ml (additionally 40 µg/ml within the pre

test); Tetanus-Toxoid was used in concentrations of 25 µg/ml, 5 µg/ml and 1 µg/ml. For the negative control cell culture medium was taken. All cultures were set up as triplicates.

For the ConA-stimulation a concentration of 2.5 µg/ml and a volume of 200 µl/well was used. The PBMC was adjusted to a density of 1 x 10^6/ml and dispensed in a volume of 100 µl per well.

At the end of the culture period, cell proliferation was detected by adding 0.4 µCi of ^{3}H-thymidine per well for sixteen hours. At the end of the culture period the cells were detached from the surface using EDTA solution and harvested on glass fibre filters using a scatron cell harvester. The amount of radioactivity incorporated into DNA in each well was measured in a scintillation counter and is proportional to the number of proliferating cells, which in turn is a function of the number of leukocytes that were stimulated to enter the S-phase of the cell cycle. The readout parameter was counts per minute (cpm) and the stimulation index (SI) for each concentration defined as $cpm_{compound}$ / cpm_{blank}.

Cytokine assays

All cytokines were quantified in the culture supernatants using commercial ELISA kits, according to the respective manufacturers instructions. The reagents employed are set out in Table 1 below:

Table 1

substance	source	code	lot
Human IFN-γ OptEIA Set	BD Biosciences	555142	MF31099
Human IL-1 ELISA Set	Bender	BMS243/2MST	n.a.
Human IL-4 OptEIA Set	BD Biosciences	555194	MF10142
Human IL-5 OptEIA Set	BD Biosciences	555202	MF31662
Human IL-6 OptEIA Set	BD Biosciences	555220	MF25815
Human IL-10 OptEIA Set	BD Biosciences	555157	MF24968
Human TGF-β OptEIA Set	BD Biosciences	559119	47704
Human TNF-α OptEIA Set	BD Biosciences	555212	MF31447

* additional material, required for the Interleukin-1 ELISA

Interleukin (IL)-1 levels in culture supernatants were determined using the human IL-1 ELISA Set A (Bender) test kit according to the manufacturers instructions. The range of the test, predetermined by the standard delivered with the kit, was specified at 1.3 to 130 pg/ml for undiluted samples.

IL-4, 5, 6 and 10 levels in culture supernatants as well as transforming growth factor (TGF) β1 and tumour necrosis factor (TNF) α were determined using the OptEIA (BD biosciences) test kit according to the manufacturers instructions. The range of the tests, predetermined by the standards delivered with the kits was specified at 3.8 to 330 pg/ml for IFN-γ, 6,3 to 616 pg/ml (for IL-4, 5, 10 and TNF), measuring undiluted samples and 7.6 to 660 pg/ml for IL-6 using twofold dilutions of the samples.

Two (for monocyte cultures) or eight (for PBMC cultures) cytokine determinations by ELISA, each from independent micro-cultures were included in the calculation of mean and standard deviation. Titres above the upper range of the test (e.g. 616 pg/ml in the case of TNF) were set to this value for calculation. The lower range of the test was subtracted from each mean value prior calculation.

Results

BT061 (also termed humanised B-F5, or simply hB-F5) is able to suppress dose-dependently the tetanus-toxoid specific T cell proliferation; there has been no effect on the total number of T cells. General suppression of cytokine release has been demonstrated.

Table 2 below shows the influence of the anti CD4 mAb BT061 on a tetanus-toxoid-specific T-cell proliferation assay, measured in triplicates.

Displayed are the means and SD of the ^{3}H-Tdr-incorporation, measured in triplicates as well as the stimulation index (SI, defined as $cpm_{compound}$ / cpm_{nil}) for each concentration and the level of significance in the unpaired, two sided t-test against the medium control (n.s.: not significant; *: $p< 0,05$; **: $p< 0,01$; ***: $p< 0,001$).

Table 2

anti CD4 (BT061)	Tetanus Toxoid			
20 µg/ml	25 µg/ml	5 µg/ml	1 µg/ml	nil
Mean (cpm)	5123	2426	2589	456
SD (cpm)	597	989	1069	363
Mean (SI)	11,23	5,32	5,67	1,00
SD (SI)	10,24	6,40	6,85	1,59
t-test (compound vs. nil)				
p=	0,000	0,032	0,031	
	***	*	*	
4 µg/ml	25 µg/ml	5 µg/ml	1 µg/ml	nil
Mean (cpm)	6591	5005	2957	358
SD (cpm)	468	518	1333	57
Mean (SI)	18,39	13,97	8,25	1,00
SD (SI)	4,21	3,65	5,02	0,32
t-test (compound vs. nil)				
p=	0,000	0,000	0,028	
	***	***	*	
0,8 µg/ml	25 µg/ml	5 µg/ml	1 µg/ml	nil
Mean (cpm)	8787	6748	3902	414
SD (cpm)	1886	763	218	12
Mean (SI)	21,24	16,31	9,43	1,00
SD (SI)	5,18	2,32	0,80	0,06
t-test (compound vs. nil)				
p=	0,002	0,000	0,000	
nil	25 µg/ml	5 µg/ml	1 µg/ml	nil
Mean (cpm)	13783	10244	6970	650
SD (cpm)	1826	758	1351	95
Mean (SI)	21,22	15,77	10,73	1,00
SD (SI)	5,91	3,47	3,65	0,29
t-test (compound vs. nil)				
p=	0,000	0,000	0,001	
	***	***	**	
Viab-ctr	ConA	nil		
Mean (cpm)	94473	404		
SD (cpm)	4200	239		
Mean (SI)	233,94	1,00		
SD (SI)	148,86	1,18		
t-test (compound vs. nil)				
p=	0,000			
	***			

Table 3

This table shows the influence of the anti CD4 mAb BT061 on the cytokine production of tetanus- toxoid- stimulated PBMC cultures. Mean and SD of eight individual micro cultures are given.

BT061 µg/ml	Tet.Tox. µg/ml	IL-1pg/ml mean	IL-1pg/ml SD	IL-4pg/ml mean	IL-4pg/ml SD	IL-5pg/ml mean	IL-5pg/ml SD	IL-6pg/ml mean	IL-6pg/ml SD	IL-10pg/ml mean	IL-10pg/ml SD	IFN-γ pg/ml mean	IFN-γ pg/ml SD	TGF-β1 pg/ml mean	TGF-β1 pg/ml SD	TNF α pg/ml mean	TNF α pg/ml SD
20	25	0,0	0,0	0,0	0,0	26,3	10,1	141,8	16,9	22,9	4,5	0,9	1,6	35,5	46,1	2,2	3,1
4	25	0,0	0,0	0,0	0,0	53,0	20,4	143,3	40,3	20,9	5,3	3,3	3,6	15,9	23,8	2,1	2,2
0,8	25	0,0	0,0	0,0	0,0	83,7	41,8	144,2	15,2	21,4	4,1	17,9	24,1	14,3	27,1	5,5	4,0
0	25	0,1	0,2	0,2	0,6	150,1	59,5	133,3	13,5	27,2	5,9	41,0	34,8	12,4	27,6	8,5	5,5
25	0	0,5	0,0	0,0	0,0	0,0	0,0	124,8	16,9	8,7	2,5	0,0	0,0	8,8	18,4	0,0	0,0
0	0	0,0	0,0	0,0	0,0	0,0	0,0	123,7	14,5	6,7	1,9	0,0	0,0	5,4	13,1	0,0	0,0

Table 4

This table shows the influence of the anti CD4 mAb BT061 on the production of inflammatory cytokines LPS- stimulated monocyte cultures. Mean and SD of two individual micro cultures are given.

	IL-1 pg/ml				IL-6 pg/ml				TNF α pg/ml			
					LPS 1				LPS 1			
BT061	LPS 1 µg/ml		nil		µg/ml		nil		µg/ml		nil	
µg/ml	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
20,0	40	14,7	0	0	253	2,4	121	12,2	609	0,0	0,3	0,4
4,0	39	8,1	0	0	256	14,9	135	8,9	597	12,7	0,0	0
0,8	34	14,8	0,2	0,3	257	16,6	128	5,9	605	5,7	0,0	0
0,0	56	2,2	0	0	233	16,6	117	14,1	600	3,2	0,6	0,8

The data in Tables 2-4 demonstrate the following:

- A dose dependent suppression of tetanus toxoid-induced T cell proliferation (recall response) has been demonstrated even at high doses of BT061, showing that BT061 does not abrogate all immune responses. Doses used apply to a corresponding high dose application of up to 60 mg in patients
- General suppression of cytokine release (dose-dependent reduction of IFN-gamma, IL-5 and TNF-alpha, with no change in IL-1, IL-4, IL-6, IL-10) and without affecting Th1/Th2 balance.
- An increase in TGF-beta release

EXAMPLE 3 - *A flow cytometric test for BT061 (anti-CD4 mAb) induced ADCC (Antibody-dependent, cell-mediated cytotoxicity)*

HuT 78 target cells were labelled with BT061 (hB-F5) and incubated with PBMC cells as effectors. Dead cells could be detected due to the uptake of the DNA dye propidium iodine after an incubation time of 30 minutes. Results are shown in Table 5.

Table 5

Concentration [µg/ml]	Tecelac /ATG) (positive control) mean fluorescence intensity	Negative control mean fluorescence intensity	Specific cell death (ADCC) [%]
75	31,78	11,96	19,82
37,05	25,09	12,70	12,39
18,75	22,25	10,59	11,66
9,38	14,95	10,63	4,32

Concentration [µg/ml]	hB-F5 mean fluorescence intensity	Negative control mean fluorescence intensity	Specific cell death (ADCC) [%]
70,85	10,96	13,41	-2,45
35,43	11,06	14,05	-2,99
17,71	11,68	12,16	-0,48
8,86	10,54	10,23	0,31

The data in the table demonstrate that there was no induction of ADCC by BT061 (hB-F5) even at high concentrations

EXAMPLE 4 – *Apoptosis*

In a flow cytometric test for *BT061 (anti-CD4 mAb)* induced apoptosis, PBMCs from full blood were incubated with BT061 or the positive control.

After an incubation time of 7 days, the detection of apoptotic cells was performed by staining of apoptotic cells with Annexin-V-Fluoresceine. The results are shown in Table 6.

Table 6

7 d stimulation experiments	Stimulus	
	Positive control (ATG/Tecelac)	*BT061*
Antibody concentration [µg/ml]	Increase of the ratio of apoptotic cells [%]	
400	0,8	-6,9
200	13,6	-6,4
100	19,5	-0,7
50	17,3	-0,4
25	18,3	-0,5
12,50	8,2	-0,1

The data demonstrate that there was no induction of apoptosis even at high concentrations of BT061.

EXAMPLE 5 - *Complement binding*

In a flow cytometric test for binding of the complement factor C1qPBMCs have been isolated and incubated with *BT061 (anti-CD4 mAb)*, followed by an incubation with purified recombinant C1q.

ATG (Tecelac) served as the positive control.

Detection was performed with a FITC labelled detection antibody against C1q. Results are shown in Table 7 below.

Table 7

antibody concentration [µg/ml]	anti-CD4 mAb BT061	Positive control
	Mean fluorescence intensity	
150	199	782
75,0	200	766
37,5	205	732
18,8	217	672
9,4	210	541
4,7	198	491
2,3	199	371
1,2	193	302

The data show that no complement binding can be seen, even at high concentrations.

EXAMPLE 6 - *CD4-activated CD25+ Tregs suppress cytokine synthesis and CD25 re-expression of CD8+ T cells*

Figure 7 shows that CD4-activated CD25+ Tregs suppress cytokine synthesis and CD25 re-expression of CD8+ T cells. Top: CD4-activated CD25+ Tregs suppress cytokine synthesis of co-activated CD8+ T cells.

Method

CD25+ Tregs were stimulated with/without anti-CD3 mAb or anti-CD4mAb (B-F5, 1 µg/ml each) for 48 h. Thereafter, cells were co-cultured with irradiated T cell-depleted syngeneic PBMC and allogeneic CD8+ T cells (ratio 1:1). Production of cytokines was analysed on day 7. Alloreactive CD8+ T cells were re-stimulated with PHA/PMA for 5h in presence of monensin, fixed and stained with anti-CD8 and cytokine-specific mAbs according to the respective manufacturer instruction (all BD PharMingen). Cytokine synthesis was analysed by flow cytometry drawing a gate on CD8+ lymphocytes. CD25 re-expression by CD8+ T cells was measured according to following procedure: CD25+ Tregs were stimulated

with/without anti-CD3 mAb or anti-CD4mAb (B-F5, 1 μg/ml each) for 48 h. Thereafter, cells were co-cultured with T cell-depleted syngeneic PBMC and allogeneic CD8+ T cells. On day 10, CD8+ T cells were re-stimulated with allogeneic CD3-depleted PBMC from the same donor as used for primary stimulation (ratio 1:1) and CD25 re-expression of CD8+ T cells was analyzed 24 h after re-stimulation flow cytometry.

Results

Activated CD25+ Tregs do not only suppress the proliferation of conventional T cells but also inhibit the production of cytokines and the ability of CD8+T cells to express the a-chain of the IL-2 receptor, CD25 . As shown in Figure 7, CD4-activated CD25+ Tregs effectively inhibited IL-2 and IFN-γ production of alloreactive CD8+ T cells, similar to anti-CD3-stimulated CD25+ Tregs. Simultaneously, they rendered suppressed CD8+ T cells unable to express CD25 upon re-stimulation (Figure 7). Taken together, these data demonstrate that CD4 stimulation fully activates the suppressive properties of CD25+ Tregs.

EXAMPLE 7 – *Safety and tolerability of escalating doses of BT061*

A study was conducted to monitor the safety and tolerability of BT061 using escalating doses of the antibody in healthy male and female volunteers between the ages of ≥18 to ≤ 75 years.

Thirty volunteers received BT061 by intravenous administration in 10 dosage groups, with 3 volunteers per group. Further, 15 volunteers received BT061 by subcutaneous administration in 5 dosage groups also with 3 volunteers per group. The administration of BT061 intravenously is illustrated Table 8 below:

TABLE 8 – Intravenous dose of BT061

Administration of BT061			
Total dose of BT061 mab	**Volume of BT061-12.5 mg**	**Volume of BT061-25 mg**	**Volume of BT061-50 mg**
3.5 µg	0.28 µl	—	—
20 µg	1.6 µl	—	—
100 µg	8 µl	—	—
500 µg	40 µl	—	—
2.5 mg	0.2 ml	—	—
5 mg	0.4 ml	—	—
10 mg	0.8 ml	—	—
20 mg	—	0.8 ml	—
40 mg	—	—	0.8 ml
60 mg	0.8 ml	—	1 ml

Each dose is diluted with 0.9% sodium chloride injection up to a total volume of 20 ml. The dose is administered as a single continuous intravenous infusion over 2 hours.

The administration of BT061 subcutaneously is illustrated in Table 9 below:

TABLE 9 – Subcutaneous dose of BT061

Administration of BT061			
Total dose of BT061 mab	**Volume of BT061-12.5 mg**	**Volume of BT061-25 mg**	**Volume of BT061-50 mg**
5 mg	0.4 ml	—	—
10 mg	0.8 ml	—	—
20 mg	—	0.8 ml	—
40 mg	—	—	0.8 ml
60 mg	—	—	1 ml + 0.2 ml

Each dose is injected as a single bolus injection.

The volunteers were assessed over a period of 3 months after the injection.

For subcutaneous application plasma samples were taken before administration and at 3, 6, 12, 24, 36, 48, 56, 72, 88, 96, 120, 144 and 168 hours after administration and on day 75.

For intravenous application, plasma samples were taken before administration and at 30 minutes, 1, 2, 3, 6, 12, 24, 36, 48, 72, 96, 120, 144 and 168 hours after administration.

The plasma samples were analyzed using standard ELISA methodology to establish cytokine levels. The relevant cytokines analyzed included: IFN-γ, TNF-α, IL-6, and IL-2.

The plasma samples were also analyzed using standard methods of flow cytometry to measure the number of CD4+ lymphocytes.

Results

It was found that intravenous and subcutaneous doses up to 60 mg were generally well tolerated.

Cytokine levels

Induction of cytokine release is a common immediate complication occurring with the use of T cell interacting therapeutic antibodies, such as ATG, OKT3, CAMPATH-1H and humanized anti-CD3 mAbs (TRX4, Visilizumab and Teplizumab). The symptoms mainly include moderate fever, headaches and self-limiting gastrointestinal manifestations. Side effects correlated with cytokine induction after antibody administration require the application of additional drugs such as the antihistamine diphenhydramine hydrochloride and/or the anti-inflammatory agent ibuprofen.

With the use of OKT3 (muromonab-CD3), a murine CD3 specific therapeutic monoclonal antibody, there have even been deaths reported, and severe side effects limit the clinical use of this antibody mainly to immunosuppressed patients.

Although humanized FcR-non-binding CD3-specific monoclonal antibodies that are presently used in the clinic for the treatment of autoimmune disease (Teplizumab and TRX4) exhibit reduced side effects induced by T-cell activation and/or by activation of Fc receptor

expressing cells after the first dose, as compared with FcR-binding CD3-specific antibodies such as OKT3, some degree of T-cell activation and activation of Fc receptor expressing cells is still observed that leads to cytokine release generally connected to cytokine dependent side effects.

In the present study it was surprisingly found that cytokine induction observed in healthy volunteers after intravenous or subcutaneous application of BT061 was low and transient as compared to anti-CD3 antibodies. Cytokine induction generally increased with increasing dosage. However, even at the highest doses of 40 to 60 mg cytokine induction is much lower than that seen with other T cell interacting monoclonal antibodies (Figure 8A and B).

The median peak concentrations for the cytokines observed at any time point within 96 hours after administration using the highest doses (40 mg to 60 mg of BT061) are shown in Figures 8 and 9.

The median peak concentration for each cytokine is calculated as follows: The median of the highest cytokine concentrations observed after administration of the antibody.

Figure 8A and B show the TNFα and IL-6 release observed in healthy volunteers after intravenous or subcutaneous administration of BT061 in comparison to those released after administration of anti-CD3 monoclonal antibodies, Teplizumab and TRX4. The normal values of these cytokines were taken from Straub et al., (2007, Arthr. & Rheumat.). Figure 9 shows the IL-2 and IFN-γ plasma levels after administration of intravenous or subcutaneous BT061. The median peak levels were calculated from the 40 and 60 mg dose group measured within 4 days after antibody injection. The upper limit of normal (ULN) was calculated based on cytokine levels measured in 39 healthy subjects, where ULN = mean value + 2 x standard deviation.

In comparison to Teplizumab and TRX4 (results taken from Herold et al., 2002, New Engl. J. Med, and Keymeulen et al., 2005 New Engl. J. Med, respectively) BT061 induced only marginal and transient cytokine release. TNF-α and IL-6 levels were slightly increased. Figure 8 shows that the median peak values of IL-6 and TNFα cytokine levels detected in

plasma after application of BT061 (40 and 60 mg) are lower than those seen after treatment with the CD3 specific therapeutic antibodies Teplizumab and TRX4.

Further, in contrast to the anti-CD3 mAbs, BT061 did not lead to substantially increased levels of IFN-γ and IL-2 (Figure 9) as was reported for the application of TRX4 (Keymeulen et al. 2005).

CD4+ lymphocytes

In addition, the trial also included a study of the numbers of CD4-positive lymphocytes in plasma samples collected.

The results of the intravenous administration are shown below in Tables 10, 11 and 12. Table 13 shows the results of the trial with subcutaneous administration. The results are shown graphically in Figures 10 and 11.

TABLE 10 CD4+ cell counts in individual healthy volunteers after 3.5 µg to 2.5 mg intravenous administration of BT061

TIME	DOSE													
	3.5 µg	3.5 µg	3.5 µg	20 µg	20 µg	20 µg	100 µg	100 µg	100 µg	500 µg	500 µg	500 µg	2.5 mg	2.5 mg
Predose	998	878	1025	955	1209	666	1164	800	543	759	493	1240	891	782
3h	1098	746	1020	708	1121	642	452	791	319	777	566	1058	392	461
6h	922	710	1063	746	1091	590	353	627	314	805	381	1115	487	512
12h	898	1183	942	1016	1667	1055	465	1065	477	955	643	1505	669	881
24h	868	825	769	699	1043	517	399	606	347	687	439	1017	521	701
36h	948	1035	1148	861	1549	836	559	1016	485	869	688	1542	976	1207
48h	798	542	834	566	1119	711	384	644	413	730	509	1108	574	815
72h	798	626	766	622	942	575	400	749	503	696	484	969	537	689
96h	469	715	838	583	978	531	475	698	400	800	390	1026	499	785
120h	736	643	843	604	942	472	401	650	511	830	488	1168	501	782
144h	663	593	766	635	1200	510	372	573	432	705	394	953	518	745
168h	753	576	695	616	1012	505	348	646	426	740	475	1073	568	773
day 14	716	615	625	696	867	645	400	771	451	713	600	1398	663	883
day 21	941	378	707	637	971	633	391	805	404	651	533	1241	530	758
day 28	821	569	840	652	863	649	497	775	503	752	445	1109	590	914
day 42	922	442	784	676	1044	700	569	758	455	700	617	1120	770	901
day 56	573	597	828	839	871	553	489	607	441	674	616	1311	634	1017
day 75	1821	512	692	659	1058	553	466	735	459	645	522	1026	731	1032
day 90														
minimum cell count (72 h to day 75)	469	378	625	583	863	472	348	573	400	645	390	953	499	689
Maximum Reduction of CD4+ cells (%)	53.0	56.9	39.0	39.0	28.6	29.1	70.1	28.4	26.3	15.0	20.9	23.1	44.0	11.9

TABLE 11 CD4+ cell counts in individual healthy volunteers after 2.5 mg to 20 mg intravenous administration of BT061

TIME	DOSE							
	2.5 mg	5 mg	5 mg	5 mg	10 mg	10 mg	10 mg	20 mg
Predose	1080	1116	623	1160	840	835	1281	700
3h	488	313	108	111	104	143	132	63
6h	514	445	164	246	122	82	120	110
12h	699	763	346	573	297	285	366	199
24h	726	617	282	539	470	496	772	351
36h	985	1102	505	721	414	985	1417	677
48h	738	807	390	687	388	794	942	493
72h	711	736	419	700	440	830	919	504
96h	680	791	395	806	516	641	918	538
120h	649	669	438	750	543	674	1002	558
144h	662	723	407	676	448	549	942	579
168h	579	777	309	652	473	525	876	510
day 14	726	692	354	475	357	701	908	484
day 21	601	811	480	514	481	654	978	618
day 28	874	681	339	602	414	755	887	496
day 42	923	843	300	694	507	614	889	639
day 56	847		450	571	551	805	1006	420
day 75	1239		365	627	685	853	1080	537
day 90		721						
minimum cell count (72 h to day 75)	579	669	300	475	357	525	876	420
Maximum Reduction of CD4+ cells (%)	46.4	40.1	51.8	59.1	57.5	37.1	31.6	40.0

In particular, Figure 10 shows the CD4 cell counts (cells per ml plasma) in volunteers treated with the single intravenous dose of BT061. The data points represent the mean values of the 3 patients in each dose group. Dotted lines indicate the upper limit of normal (ULN) and the lower limit of normal (LLN). The ULN and the LLN (mean value + (or -) the standard deviation) were calculated, based on cell counts from healthy volunteers, as 443 CD4 cells per µl (lower limit of normal; LLN) and 1324 CD4 cells per µl (upper limit of normal; ULN).

Figure 11 shows the CD4 cell counts (cells per ml plasma) in volunteers treated with the single subcutaneous dose of BT061. As with Figure 10, the data points represent the mean values of the 3 patients in each dose group. Dotted lines indicate the upper limit of normal (ULN) and the lower limit of normal (LLN).

TABLE 12 CD4+ cell counts in individual healthy volunteers after 20 mg to 60 mg intravenous administration of BT061

TIME	DOSE							
	20 mg	20 mg	40 mg	40 mg	40 mg	60 mg	60 mg	60 mg
Predose	843	1233	1152	789	976	900	989	2539
3h	69	186	72	137	48	63	55	71
6h	83	245	87	147	69	81	78	109
12h	214	469	262	221	212	360	276	182
24h	266	490	208	222	292	315	285	313
36h	562	1019	489	475		707	561	569
48h	359	792	460	455	703	413	500	565
72h	392	909	591	545	625	709	688	806
96h	468	755	567	545	733	717	737	774
120h	391	795	578	517	636	718	685	646
144h	347	897	548	523	760	714	732	606
168h	331	853	630	577	720	656	822	662
day 14	334	899	683	495	675	851	546	423
day 21	396	1077	744	487	711	627	639	867
day 28	579	1030	637	458	582	466	757	1376
day 42	346	847	439	472	619	1179	814	1607
day 56	337	947	556	557	570	686	686	1298
day 75	597	824	986	440	813	648	748	1199
day 90								
minimum cell count (72 h to day 75)	331	755	439	440	570	466	546	423
Maximum Reduction of CD4+ cells (%)	60.7	38.8	61.9	44.2	41.6	48.2	44.8	83.3

TABLE 13 CD4+ cell counts in individual healthy volunteers after subcutaneous application of BT061

TIME	DOSAGE														
	5 mg	5 mg	5 mg	10 mg	10 mg	10 mg	20 mg	20 mg	20 mg	40 mg	40 mg	40 mg	60 mg	60 mg	60 mg
Predose	1053	553	663	962	697	891	863	692	621	689	946	840	1114	719	1345
24h	858	402	707	640	623	615	537	535	500	177	326	379	580	470	333
36h	1131	916	946	1232	955	1019	762	666	944	268	463	870	503	527	487
48h	625	526	550	610	757	537	628	599	587	354	586	686	739	735	551
72h	814	661	580	589	709	610	700	587	596	299	719	734	860	813	539
96h	799	582	694	666	732	579	643	665	589	283	605	653	852	743	608
120h	823	566	712	653	673	659	642	528	633	323	835	629	873	639	555
144h	890	543	809	744	767	784	603	620	627	239	626	568	801	924	591
168h	894	450	437	686	655	516	620	550	581	262	515	569	1010	843	820
day 10	951	606	690	1213	724	928	683	616	778	354	715	809	757	1123	836
day 14	719	647	948	969	724	859	843	642	1033	307	705	541	1198	1359	527
day 21	859	552	750	655	708	685	904	605	752	293	1097	630	1074	901	750
day 28	894	546	758	778	653	766	757	680	768	298	861	667		880	578
day 42	854	461	1009	1235	805	785	775	469	634	308	678	709	568	1149	854
day 56	806	560	958	977	665	753	801	589	531	342	541	948	1126	945	505
day 75	852	592	772	1268	679	906	714	688	551	340	551	659			
minimum cell count (72 h to day 75)	719	450	437	589	653	516	603	469	531	239	515	541	568	639	505
Maximum Reduction of CD4+ cells (%)	31.7	18.6	34.1	38.8	6.3	42.1	30.1	32.2	14.5	65.3	45.6	35.6	49.0	11.1	62.5

Many CD4 specific monoclonal antibodies known in the art (such as those reviewed in Strand et al., 2007) achieve immuno-suppression via CD4-positive lymphocyte depletion. The drawback of these antibodies is that treated individuals become immuno-compromised, and are susceptible to other infections.

In contrast this study showed that BT061 induced no massive long-lasting depletion of CD4-positive cells. However, a transient decline of CD4-positive lymphocytes was observed with a recovery to norm values in the peripheral blood within 72 h after administration of the antibody.

At the 72 h time point after application of BT061, CD4 cell counts in four volunteers of the intravenous dose groups showed CD4 levels that were below these norm values as follows: 1 volunteer of the 100 µg intravenous dose: 400 CD4 cells per µl; 1 volunteer of the 5 mg group: 419 CD4 cells per µl; 1 volunteer of the 10 mg group: 440 CD4 cells per µl; and 1 volunteer of the 20 mg group: 392 CD4 cells per µl.

However, these values were only slightly below norm values. CD4 cell counts in the remaining 26 volunteers of the intravenous dose groups were within the norm values 72 hours after administration of BT061.

In the subcutaneous dose groups, after 72 h, only one out of 15 volunteer showed CD4 cell counts below norm values.

In conclusion, in contrast to depleting CD4 specific mAbs, even at high doses BT061 only induced a transient decline of CD4-positive cells followed by a general recovery. From the transient decline and rapid general recovery it is concluded that a transient redistribution of the CD4-positive cells has taken place, rather than depletion of these cells.

Thus the data supports the use of BT061 as an agent to be administered in a plurality of doses over a long time period.

EXAMPLE 8 – *Clinical trial of BT061 in patients with moderate to severe chronic psoriasis*

The ability of hB-F5 BT061 to treat an autoimmune disease is being tested on 56 patients suffering from moderate to severe chronic psoriasis. The trial comprises a single dose escalation study to assess the safety and efficacy of hB-F5.

The conditions of the trial are as follows:

The 56 patients are divided into seven dose groups, each group comprising eight individuals. Five dose groups (dose groups I to V) are to receive the antibody or placebo by intravenous administration and two dose groups (dose groups VI and VII) are to receive the antibody or placebo via subcutaneous administration. Two patients in each dose group receive a placebo, while the remaining six patients in each dose group receive a dose of BT061. In dose group I the six patients receive 0.5 mg of intravenous BT061. In dose groups II to V the six patients receive 2.5 mg, 5 mg, 10 mg, or 20 mg of BT061, respectively. In dose groups VI and VII where the administration is subcutaneous, the six patients receive 12.5 mg or 25 mg of BT061, respectively.

For intravenous administration the antibody/placebo is to be infused in the forearm vein according to medically accepted procedures. In the present case the total volume is administered as a single continuous intravenous infusion over a period of 2 hours via a perfusor (Fresenius Pilot C, Fresenius AG, Germany). Each dose of the antibody is diluted with a 0.9% sodium chloride injection (B. Braun Melsungen AG, Germany) up to a total volume of 20 ml.

For subcutaneous administration the antibody is to be administered as a single subcutaneous injection. The same procedure applies for the placebo.

The level of psoriasis exhibited by each patient is recorded using the Psoriasis Area and Severity Index (PASI) score. As described above higher PASI scores corresponds to a higher level of psoriasis. Patients enrolled onto the trial have a moderate to severe chronic psoriasis, i.e. a PASI score of 10 or above.

The patient's PASI score is assessed before the trial to provide a "baseline" value at day 0, and repeatedly during the trial at days 5, 7, 14, 21, 28, 42, 56 and 75.

Dose Group I

Six patients from dose group I received a single intravenous application of 0.5 mg of BT061, while two patients from dose group 1 received the placebo. The dose per weight and the dose per body surface area (BSA) for each patient are shown in Table 14. Body surface area was calculated according to the Mosteller formula described herein.

The PASI scores for the patients in dose group I are shown in Table 14 together with the percentage improvement in the PASI score from the baseline.

Dose Group II

Six patients from dose group II received a single intravenous injection of 2.5mg of BT061 while two patients from dose group 2 received the placebo. The dose per weight and the dose per body surface area (BSA) for each patient is shown in Table 15.

The PASI scores for the patients in dose group II are shown in Table 15 together with the percentage improvement in the PASI score from the baseline.

Dose Group III

Six patients from dose group III received a single intravenous injection of 5.0 mg of BT061 while two patients from dose group III received the placebo. The dose per weight and the dose per body surface area (BSA) for each patient are shown in Table 15B.

The PASI scores for the patients in dose group III are shown in Table 15B together with the percentage improvement in the PASI score from the baseline.

Dose Group IV

Six patients from dose group IV are receiving a single intravenous injection of 10.0 mg of BT061 while two patients from dose group IV received the placebo. The dose per weight and the dose per body surface area (BSA) for the patients is shown in Table 15C.

The PASI scores for the patients in dose group IV are shown in Table 15C together with the percentage improvement in the PASI score from the baseline.

TABLE 14 – PASI scores for the patients in dose group I (0.5 mg intravenous dose) over the course of the trial

Patient	1	2	3	4	5	6	7	8
Rel. Dose [µg/kg]/ [mg/m^2]	5.2 / 0.23	5.9 / 0.25	4.6 / 0.22	5.3 / 0.24	4.8 / 0.22	8.5 / 0.31	4.7 / 0.21	7.0 / 0.28
PASI Score (relative change / improvement to baseline)								
Baseline	12.2	13.5	12.1	14.0	12.1	12.6	12.1	15.2
Day 5	12.0 (2%)	12.1 (10%)	9.1 (25%)	12.2 (13%)	10.9 (10%)	11.8 (6%)	9.4 (22%)	10.2 (33%)
Day 7	12.0 (0%)	10.7 (21%)	7.7 (36%)	11.6 (17%)	11.2 (7%)	10.7 (15%)	9.3 (23%)	9.0 (41%)
Day 14	11.8 (3%)	9.5 (30%)	5.7 (53%)	9.6 (31%)	8.7 (28%)	10.4 (17%)	7.8 (36%)	7.2 (53%)
Day 21	11.6 (5%)	10.0 (26%)	5.1 (58%)	7.2 (49%)	8.0 (34%)	8.0 (37%)	6.0 (50%)	6.5 (57%)
Day 28	-	10.4 (23%)	4.8 (60%)	8.4 (40%)	8.2 (32%)	6.5 (48%)	6.4 (47%)	6.4 (58%)
Day 42	11.4 (7%)	11.2 (17%)	5.1 (58%)	8.4 (40%)	7.9 (35%)	6.1 (52%)	5.5 (55%)	5.5 (55%)
Day 56	11.4 (7%)	10.1 (25%)	4.2 (65%)	9.2 (34%)	7.9 (35%)	5.7 (55%)	5.3 (56%)	5.3 (56%)
Day 75	11.6 (5%)	7.7 (43%)	5.1 (58%)	9.8 (30%)	9.6 (21%)	5.1 (60%)	5.4 (55%)	7.8 (49%)

TABLE 15A - PASI scores for the patients in dose group II (2.5 mg intravenous dose) over the course of the trial

Patient	1	2	3	4	5	6	7	8
Rel. Dose [µg/kg]/ [mg/m^2]	27.8 / 1.20	31.6 / 1.30	31.8 / 1.28	23.6 / 1.08	28.4 / 1.19	39.4 / 1.49	26.5 / 1.17	28.4 / 1.19
PASI Score (relative change / improvement to baseline)								
Baseline	17.1	13.8	10.9	18.6	15.0	12.6	13.9	13.5
Day 5	14.5 (15%)	15.5 (+12%)	6.8 (38%)	17.6 (5)	12.1 (19%)	12.6 (0%)	19.5 (+40%)	15.6 (+16%)
Day 7	12.3 (28%)	13.6 (1%)	5.2 (52%)	19.7 (+6%)	14.0 (7%)	13.0 (+3%)	17.0 (+22%)	14.8 (+10%)
Day 14	10.8 (37%)	15.5 (+12%)	3.9 (64%)	11.1 (40%)	11.8 (21%)	16.6 (+32%)	11.4 (18%)	8.0 (41%)
Day 21	9.7 (43%)	12.2 (12%)	3.9 (64%)	13.7 (26%)	12.4 (17%)	11.4 (10%)	7.9 (43%)	7.1 (47%)
Day 28	8.6 (50%)	9.4 (32%)	1.5 (86%)	7.6 (59%)	12.2 (19%)	12.8 (+2%)	8.0 (41%)	6.5 (52%)
Day 42	8.8 (49%)	8.5 (38%)	1.3 (88%)	10.2 (45%)	12.2 (19%)	11.0 (13%)	7.8 (44%)	9.0 (33%)
Day 56	6.3 (63%)	8.3 (40%)	1.3 (88%)	-	14.2 (5%)	9.7 (23%)	8.2 (41%)	7.8 (42%)
Day 75	6.0 (65%)	5.6 (59%)	1.9 (83%)	9.2 (51%)	14.7 (2%)	10.4 (17%)	10.2 (27%)	8.1 (40%)

TABLE 15B - PASI scores for the patients in dose group III (5.0 mg intravenous dose) over course of trial

Patient	1	2	3	4	5	6	7	8
Rel. Dose [µg/kg]/ [mg/m^2]	70.4 / 2.73	57.5 / 2.40	43.9 /	59.5 /	52.4 /	56.8 /	71.4 /	66.2 /
	PASI Score (relative change / improvement to baseline)							
Baseline	15.8	14.1	17.4	12.4	17.3	12.4	13.5	10.4
Day 5	13.0 (18%)	27.2 (+93%)	17.7 (+2%)	12.0 (3%)	16.8 (3%)	10.6 (15%)	-	-
Day 7	14.3 (9%)	18.9 (+34%)	15.6 (10%)	11.1 (10%)	17.6 (+2%)	11.4 (8%)	11.0 (19%)	8.2 (21%)
Day 14	13.5 (15%)	30.3 (+115%)	14.0 (20%)	9.3 (25%)	14.4 (17%)	13.0 (+5%)	9.4 (30%)	7.6 (27%)
Day 21	10.1 (36%)	23.1 (+64%)	14.4 (17%)	9.2 (26%)	14.7 (15%)	11.6 (6%)	9.4 (30%)	-
Day 28	9.6 (39%)	23.1 (+64%)	13.4 (23%)	10.2 (18%)	13.8 (20%)	11.2 (10%)	8.3 (39%)	8.6 (17%)
Day 42	9.2 (42%)	20.1 (+43%)	14.4 (17%)	10.2 (18%)	13.2 (24%)	12.6 (+2%)	8.3 (39%)	-
Day 56	10.0 (37%)	20.1 (+43%)	15.8 (9%)	-	13.2 (24%)	10.6 (15%)	9.6 (20%)	-
Day 75	12.8 (19%)	22.5 (+60%)	16.0 (8%)	9.0 (27%)	13.2 (24%)	13.2 (+6%)	13.4 (1%)	9.6 (8%)

TABLE 15C - PASI scores for the patients in dose group IV (10.0 mg intravenous dose) over course of trial

Patient	1	2	3	4	5	6	7	8
Rel. Dose [µg/kg]/ [mg/m^2]	173.0 /	142.9 /	102.8 /	115.6 /	119.6/	108.8/	75.9/	106.6/
	PASI Score (relative change / improvement to baseline)							
Baseline	14.6	11.0	21.6	22.0	19.0	11.6	14.0	12.4
Day 5	12.8 (12%)	11.0 (0%)	21.6 (0%)	16.8 (24%)	18.8 (1%)	11.2 (3%)	14.2 (+1%)	11.4 (8%)
Day 7	12.8 (12%)	11.0 (0%)	21.6 (0%)	16.8 (24%)	18.2 (4%)	11.2 (3%)	14.2 (+1%)	8.4 (32%)
Day 14	11.4 (22%)	11.0 (0%)	21.6 (0%)	18.1 (18%)	16.7 (12%)			
Day 21	11.4 (22%)	11.0 (0%)	22.5 (+4%)	19.0 (14%)	17.3 (9%)			
Day 28	11.4 (22%)	8.9 (19%)	22.0 (7%)	17.7 (20%)				
Day 42	11.0 (25%)	9.4 (15%)	22.6 (+5%)	18.8 (15%)				
Day 56	11.4 (22%)	9.8 (11%)						
Day 75								

Further, the PASI scores against time for individual patients are shown in graph form in Figures 12A to 12H and in Figures 13A to 13H. The graphs shown in Figures 12A to 12H represent PASI scores for patients from dose group I, while the graphs shown in Figures 13A to 13H represent PASI scores for patients from dose group II.

As can be seen from the results shown in Tables 14 and 15, 75% of all the patients from dose group I and dose group II show a clear improvement in their PASI scores, i.e. at least a 40% improvement over the baseline value, after a single dose. It should be noted that 25% of the patients in dose group I and dose group II received a placebo.

In fact, in both dose groups 50% of the patients showed at least 50% improvement in their PASI scores, with one patient in dose group II showing an 88% improvement in the PASI score at day 56, (i.e. patient 3 in Table 15). Furthermore, the therapeutic effect is long-lasting even at these low doses, with the improvements still being seen in many patients at the end of the trial, 75 days after administration.

Patients in dose group III also show an improvement in their PASI score, with six out of eight patients showing a greater than 20% improvement and two of those six showing a greater than 30% improvement after treatment. However, the improvement was not as significant as that seen in patients from dose group I and dose group II which received a lower dose of the antibody. Some efficacy is also seen in the patients of dose group IV. In particular patients 1, 4, 5 and 8 in this dose group (as shown in Table 15C) show a clear improvement in their PASI scores, although this is limited in comparison to the patients of dose groups I to III.

The number of patients showing at least 40%, 50%, 60% and 75% improvement in PASI score is shown in Table 16.

TABLE 16 – Summary of results from Dose Groups I to III

	Dose group I* 0.5 mg BT-061	Dose group II* 2.5 mg BT-061	Dose Group III* 5.0 mg BT061
Improvement ≥ 40%	6/8 patients	6/8 patients	1/8 patients
Improvement ≥ 50%	4/8 patients	4/8 patients	0/8 patients
Improvement ≥ 60%	1/8 patients	2/8 patients	0/8 patients
Improvement ≥ 75%	0/8 patients	1/8 patients	0.8 patients

* per dose group: 75% of patients received BT061, 25% of patients received placebo

Figures 14 A and 14 B provide photographic evidence of the improvement in the level of psoriasis before and after treatment. Figure 14 A shows an area of psoriasis on the skin of a patient in dose group II prior to administration. Figure 14 B shows the same area of psoriasis 28 days after administration. The areas of improvement are marked on Figure 14 B with black boxes.

From these results it can clearly be seen that BT061 provides effective treatment of moderate and severe chronic psoriasis.

The results of this study, in combination with those described above in Example 7 which show that even high doses of the antibody of the invention are generally well-tolerated in humans, demonstrate the ability of the pharmaceutical compositions of the invention to provide effective long term treatment of autoimmune diseases with the dosage regimens described herein.

EXAMPLE 9 - *Clinical trial of BT061 in patients with rheumatoid arthritis*

The ability of BT061 to treat rheumatoid arthritis is being tested on patients suffering from this disease. The trial comprises a multiple dose study involving 96 patients, divided into 12 groups. In each group two patients receive a placebo while 6 patients receive BT061. Patients are dosed once a week over a period of 6 weeks.

Patients are divided into those receiving the antibody subcutaneously and those receiving the antibody intravenously. The subcutaneous dose groups are: 1.25 mg, 6.25 mg, 12. 5 mg, 25 mg, 50 mg, 75 mg and 100 mg. The intravenous dose groups are: 0.5 mg, 2 mg, 6.25 mg, 12.5 mg and 25 mg.

In the 1.25 mg subcutaneous dose group the patients are numbered 101, 102, 103, 104, 105, 106, 107 and 108. In the 6.25 mg subcutaneous dose group the patients are numbered 201-208. In the 12.5 mg subcutaneous dose group the patients are numbered 301-308. In the 25 mg subcutaneous dose group the patients are numbered 401-408. In the 50 mg subcutaneous dose group the patients are numbered 501-508. In the 6.25 mg intravenous dose group the patients are numbered 601-608.

The intravenous and subcutaneous administration procedure was the same as that described in Example 8 for the psoriasis trial.

The level of rheumatoid arthritis is recorded weekly by assessing the ACR parameters and in particular studying the number of tender and swollen joints and following the levels of C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR). These parameters are assessed before the trial to provide a "baseline" value at day 0, and repeatedly during the trial period and thereafter at 8, 22 and 43 days after the administration period is finished (i.e. follow up (FU) day 8, FU day 22 and FU day 43).

The Tables below provide the data obtained from the trial. Specifically Tables 17 to 22 provide the number of tender and swollen joints over the course of the trial.

Table 17 – Tender and swollen joint counts from the 1.25 mg subcutaneous dose group.

		Visits									
Patients – 1.25 mg SC dose group	**Joints (no.)**	**Screen Visit**	**Day 1 Week 1**	**Day 8 Week 2**	**Day 15 Week 3**	**Day 22 Week 4**	**Day 29 Week 5**	**Day 36 Week 6**	**Follow-up Day 43 Week 7**	**Follow-up Day 57 Week 9**	**Follow-up Day 78 Week 12**
101	tender	34	34	32	-	-	-	-	-	-	0
	swollen	10	10	18	-	-	-	-	-	-	0
102	tender	25	26	22	16	16	24	24	30	28	29
	swollen	12	13	9	10	9	15	12	9	18	15
103	tender	11	12	12	9	8	7	7	30	28	3
	swollen	7	8	8	6	6	6	6	9	18	2
104	tender	17	10	4	3	20	17	9	5	5	0
	swollen	8	6	0	0	0	8	2	0	0	0
105	tender	24	23	22	23	35	32	35	32	34	33
	swollen	14	14	14	17	18	18	19	19	20	20
106	tender	20	21	20	13	8	9	13	12	11	11
	swollen	9	12	9	10	5	5	6	5	5	7
107	tender	14	14	11	10	16	14	14	14	11	11
	swollen	8	9	8	8	5	5	5	6	6	6
108	tender	11	12	10	10	7	4	8	8	2	13
	swollen	10	11	7	11	10	9	11	7	6	8

Table 18 - Tender and swollen joint counts from the 6.25 mg subcutaneous dose group.

		Visits									
Patients – 6.25 mg SC dose group	**Joints (no.)**	**Screen Visit**	**Day 1 Week 1**	**Day 8 Week 2**	**Day 15 Week 3**	**Day 22 Week 4**	**Day 29 Week 5**	**Day 36 Week 6**	**Follow-up Day43 Week 7**	**Follow-up Day 57 Week 9**	**Follow-up Day 78 Week 12**
201	tender	16	17	15	14	12	15	13	11	9	9
	swollen	9	10	7	6	6	5	6	5	6	6
202	tender	14	10	10	10	8	8	8	8	9	13
	swollen	10	8	6	6	4	4	4	4	4	6
203	tender	15	14	10	8	10	10	8	7	5	5
	swollen	11	11	10	9	9	10	7	7	6	7
204	tender	19	22	16	0	0	10	2	10	11	0
	swollen	10	10	5	0	0	4	0	0	10	0
205	tender	21	21	0	0	14	30	10	37	-	12
	swollen	9	9	0	0	14	16	6	25	-	8
206	tender	16	16	15	13	13	17	19	-	-	5
	swollen	10	12	12	10	14	11	11	-	-	4
207	tender	17	28	28	11	9	15	17	14	18	22
	swollen	11	12	13	7	10	11	8	10	11	10
208	tender	13	12	9	8	9	11	10	-	-	12
	swollen	10	10	9	9	10	10	9	-	-	8

Table 19 – Tender and swollen joint counts from the 12.5 mg subcutaneous dose group.

		Visits									
Patients – 12..5 mg SC dose group	**Joints (no.)**	**Screen Visit**	**Day 1 Week 1**	**Day 8 Week 2**	**Day 15 Week 3**	**Day 22 Week 4**	**Day 29 Week 5**	**Day 36 Week 6**	**Follow-up Day 43 Week 7**	**Follow-up Day 57 Week 9**	**Follow-up Day 78 Week 12**
301	tender	18	18	16	16	16	16	16	20	14	14
	swollen	8	8	8	8	8	8	8	6	6	6
302	tender	36	36	34	35	31	-	-	-	-	30
	swollen	20	20	19	19	17	-	-	-	-	18
303	tender	20	19	19	16	15	14	16	18	-	19
	swollen	10	11	12	13	13	14	13	14	-	14
304	tender	10	10	10	10	10	10	10	10	10	8
	swollen	6	6	6	6	6	6	6	6	6	4
305	tender	16	16	14	14	13	13	13	12	10	10
	swollen	8	8	8	8	6	6	6	6	4	4
306	tender	27	27	18	18	12	23	28	-	-	29
	swollen	14	14	20	11	16	13	17	-	-	24
307	tender	25	23	23	17	17	17	17	15	13	11
	swollen	8	8	8	8	8	8	8	6	6	4
308	tender	20	20	18	8	8	8	8	8	8	4
	swollen	12	12	8	6	6	5	6	6	6	4

Table 20 - Tender and swollen joint counts from the 25 mg subcutaneous dose group.

		Visits									
Patients - 25 mg SC dose group	**Joints (no.)**	**Screen Visit**	**Day 1 Week 1**	**Day 8 Week 2**	**Day 15 Week 3**	**Day 22 Week 4**	**Day 29 Week 5**	**Day 36 Week 6**	**Follow-up Day43 Week 7**	**Follow-up Day 57 Week 9**	**Follow-up Day 78 Week 12**
401	tender	16	17	19	22	13	13	12	11	9	6
	swollen	10	11	8	9	12	11	8	5	8	5
402	tender	23	21	10	10	10	9	8	7	6	7
	swollen	8	11	5	6	6	5	4	3	3	3
403	tender	10	10	10	8	8	10	10	7	6	8
	swollen	8	8	8	6	5	5	5	5	5	5
404	tender	17	16	15	15	13	14	14	16		
	swollen	9	11	10	6	7	7	7	7		
405	tender	10	10	8	8	8	8	8	10	-	10
	swollen	6	6	4	4	4	4	4	6	-	6
406	tender	11	11	11	11	11	12	8	8	6	8
	swollen	6	6	6	6	5	5	3	3	2	4
407	tender	13	20	16	18	4	2	0	4	14	
	swollen	7	10	6	8	0	0	0	0	8	
408	tender	11	11	8	8	7	5	4	4	-	8
	swollen	9	9	5	5	4	6	6	3	-	6

Table 21 – Tender and swollen joint counts from the 50 mg subcutaneous dose group.

		Visits									
Patients - 50 mg SC dose group	**Joints (no.)**	**Screen Visit**	**Day 1 Week 1**	**Day 8 Week 2**	**Day 15 Week 3**	**Day 22 Week 4**	**Day 29 Week 5**	**Day 36 Week 6**	**Follow-up Day 43 Week 7**	**Follow-up Day 57 Week 9**	**Follow-up Day 78 Week 12**
501	tender	10	10	12	15	12	15	16	-	-	15
	swollen	10	10	10	10	10	13	13	-	-	13
502	tender	14	15	11	16	12	7	8			
	swollen	5	9	10	10	7	4	5			
503	tender	13	13	11	7	7	6	6	5	5	
	swollen	8	8	8	6	6	3	3	2	3	
504	tender	11	12	10	8	7	11	11	13		
	swollen	8	8	8	6	7	7	7	8		
505	tender	12	13	8	2	1					
	swollen	7	7	5	0	0					
506	tender	36	48	32							
	swollen	8	8	4							
507	tender										
	swollen										
508	tender										
	swollen										

Table 22 - Tender and swollen joint counts from the 6.25 mg intravenous dose group.

		Visits									
Patients – 6.25 mg IV dose group	**Joints (no.)**	**Screen Visit**	**Day 1 Week 1**	**Day 8 Week 2**	**Day 15 Week 3**	**Day 22 Week 4**	**Day 29 Week 5**	**Day 36 Week 6**	**Follow-up Day43 Week 7**	**Follow-up Day 57 Week 9**	**Follow-up Day 78 Week 12**
601	tender	23	20	24	39	33	26	31			
	swollen	13	15	15	25	26	20	21			
602	tender	41	43	33	15	12	30	17	19	14	
	swollen	13	12	8	10	10	6	5	5	7	
603	tender	26	22	28	22	24	24				
	swollen	10	4	8	4	4	6				
604	tender	28	26	31	27	14	10	11	7	9	
	swollen	8	8	8	10	6	2	2	3	1	
605	tender	34	38	38	36						
	swollen	22	18	22	20						
606	tender	12	14								
	swollen	8	9								
607	tender	27	27								
	swollen	17	19								
608	tender										
	swollen										

Figure 15 shows the percentage of patients from the dose groups receiving 1.25 mg. 6.25 mg, 12.5 mg and 25 mg subcutaneous BT061 achieving at least a 20% improvement of relevant ACR parameters over the course of the trial, and the percentage of patients achieving at least an ACR20 response at week 7.

In particular, it can be seen that 50% of patients in the 25 mg subcutaneous dose group (i.e. 4 out of the 8 patients where 2 of the patients are receiving a placebo) achieved at least a 20% improvement of relevant ACR parameters at week 6. This figures increased to 5 out of the 8 patients at week 7, i.e. 5 out of the 8 patients achieved at least ACR20. One patient in this dose group achieved a more than 50% improvement of relevant ACR parameters at weeks 5 and 6 (full set of data not shown).

Positive results were also obtained by patients in other dose groups. One patients in the 6.25 mg subcutaneous dose group achieved at least a 50% improvement of relevant ACR parameters at week 4 while another achieved at least a 70% improvement of relevant ACR parameters at week 3 (full set of data not shown).

Figure 16 A and 16 B show results for the number of tender and swollen joints exhibited by patients from the 25 mg subcutaneous BT061 dose group over a six week period. Several patients exhibit a reduction in the number of tender and swollen joints over a period of the treatment. The results for one responder patient and one non-responder patient from this dose group are shown in Figures 17A and 17B, respectively. The responder shows a significant improvement in the number of tender and swollen joints and in pain levels.

A reduction in the numbers of tender and swollen joints is also seen in patients from the other dose groups. Figures 18A, 18B, 19A and 19B show the number of tender joints in the 1.25 mg subcutaneous, 6.25 mg subcutaneous, 50 mg subcutaneous and 6.25 mg intravenous dose groups respectively, over the course of the trial and in the weeks thereafter.

These results demonstrate the efficacy of the agent of the present invention in the treatment of rheumatoid arthritis within the dose ranges described herein.

CLAIMS:

1. A pharmaceutical composition for treating an autoimmune disease comprising a pharmaceutically acceptable carrier and an agent capable of activating CD4+CD25+ regulatory T cells, wherein the composition is to be administered to a subject at most every 3 days.

2. A pharmaceutical composition according to claim 1 wherein the period between administrations is at least four weeks.

3. A pharmaceutical composition according to claim 1 wherein the period between administrations is at least twelve weeks.

4. A pharmaceutical composition according to claim 1 wherein the period between administrations is at least twenty-four weeks.

5. A pharmaceutical composition according to claim 1, wherein the period between administrations is at least 6 calendar months.

6. A pharmaceutical composition according to claim 1, wherein the period between administrations is at least 1 year.

7. A pharmaceutical composition according to claim 1 wherein the composition is to be administered to a subject yearly over a period of 1 to 5 years.

8. A pharmaceutical composition according to any preceding claim, wherein the composition is to be administered to a subject in a dose of the agent from 0.2 mg to 200 mg per dose.

9. A pharmaceutical composition according any one of claims 1 to 7, wherein the composition is to be administered to a subject in a dose of the agent from 0.1 to 60 mg/m^2 body surface area of the subject.

10. A pharmaceutical composition according to any one of claims 1 to 7 wherein the composition is to be administered to a subject in a dose of the agent from 1 μg/kg to 2 mg/kg.

11. A pharmaceutical composition according to any one of claims 1 to 7 wherein the agent is present in a concentration of from10 μg/ml to 150 mg/ml.

12. A pharmaceutical composition according to any preceding claim wherein the autoimmune disease is selected from psoriasis, rheumatoid arthritis, multiple sclerosis, type-1 diabetes, inflammatory bowel diseases, Crohn's disease, Hashimoto's thyreoiditis, autoimmune thyreoiditis, autoimmune myasthenia gravis, systemic lupus erythematosus and transplantation-related diseases such as graft-versus-host or general organ tolerance issues.

13. A pharmaceutical composition according to any preceding claim wherein the autoimmune disease is psoriasis.

14. A pharmaceutical composition according to any preceding claim wherein the disease is rheumatoid arthritis.

15. A pharmaceutical composition according to any preceding claim wherein the composition is for parenteral administration.

16. A pharmaceutical composition according to claim 15, wherein the composition is for intramuscular administration, intravenous administration or subcutaneous administration.

17. A pharmaceutical composition according to claim 16 wherein the disease is psoriasis and the composition is for intravenous or subcutaneous administration.

18. A pharmaceutical composition according to claim 16 the composition is for intravenous administration and the composition is to be administered in a dose of 0.3 mg to 25 mg.

19. A pharmaceutical composition according to claim 16 wherein the disease is rheumatoid arthritis and the composition is for intravenous or subcutaneous administration.

20. A pharmaceutical composition according to any preceding claim wherein within the period of 10 minutes after the beginning of administration to 96 hours after completion of the administration the level of a cytokine in the subject's blood plasma is less than a 20 fold increase of the level immediately prior to administration, and wherein the cytokine is selected from IFN-γ, TNF-α, IL-6 and IL-2.

21. A pharmaceutical composition according to any one of claims 1 to 19 wherein within the period of 10 minutes after the beginning of administration to 96 hours after completion of the administration the level of a cytokine in the subject's blood plasma is less than a 20 fold increase of the upper limit of normal (ULN) value, wherein the cytokine is selected from IFN-γ, TNF-α, IL-6 and IL-2, and wherein the ULN value is 3.8, 2.8, 4.4, and 19.4 pg/ml respectively.

22. A pharmaceutical composition according to any preceding claim wherein within the period of 72 to 96 hours after administration the cell count of CD4+ lymphocytes in the subject's blood plasma is at least 200 cells/μl.

23. A pharmaceutical composition according to claim 22 wherein the cell count of CD4+ lymphocytes in the subject's blood plasma is at least 250 cells/μl.

24. A pharmaceutical composition according to any preceding claim wherein the agent is a humanized monoclonal antibody or fragment or derivative thereof.

25. A pharmaceutical composition according to claim 24 wherein the agent is an anti-CD4 antibody or fragment or derivative thereof.

26. A pharmaceutical composition according to any preceding claim wherein the agent is a humanized anti-CD4 antibody, or fragment or derivative thereof, having V domains defined by the following polypeptide sequences:

- H chain V domain:

EEQLVESGGGLVKPGGSLRLSCAASGFSFSDCRMYWLRQAPGKGLEWIGVISVKSEN YGANYAESVRGRFTISRDDSKNTVYLQMNSLKTEDTAVYYCSASYYRYDVGAWFA YWGQGTLVTVSS (SEQ ID NO: 1)

- L chain V domain:

DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYIYWYQQKPGQPPKLLIYLASILE SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSRELPWTFGQGTKVEIK (SEQ ID NO: 2).

or V domains comprising polypeptide sequences having at least 80% sequence identity with SEQ ID NO: 1 and SEQ ID NO: 2.

27. A pharmaceutical composition according to any preceding claim wherein the agent is a humanized anti-CD4 antibody derived from the mouse monoclonal anti-CD4 antibody B-F5.

28. A method of treatment of an autoimmune disease which method comprises administering a medicament to a subject, wherein the medicament comprises an agent capable of activating CD4+CD25+ regulatory T cells, and wherein the medicament is administered to the subject in a plurality of doses.

29. A method of treatment according to claim 28 wherein the medicament is administered to the subject according to any one of claims 2 to 7.

30. A method of treatment according to claim 28 or claim 29 wherein the medicament is administered to the subject in the dose specified in any one of claims 8 to 10.

31. A method of treatment according to any one of claims 28 to 30 wherein the autoimmune disease is as defined in any one of claims 12 to 14.

32. A method of treatment according to any one of claims 28 to 31 wherein the agent is a humanized anti-CD4 antibody or fragment or derivative thereof derived from the mouse monoclonal anti-CD4 antibody B-F5.

33. A method of treatment according to any one of claim 28 to 31 wherein the agent is a humanized anti-CD4 antibody or fragment or derivative thereof having V domains defined by the following polypeptide sequences:

- H chain V domain:

EEQLVESGGGLVKPGGSLRLSCAASGFSFSDCRMYWLRQAPGKGLEWIGVISVKSEN YGANYAESVRGRFTISRDDSKNTVYLQMNSLKTEDTAVYYCSASYYRYDVGAWFA YWGQGTLVTVSS (SEQ ID NO: 1)

- L chain V domain:

DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYIYWYQQKPGQPPKLLIYLASILE SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSRELPWTFGQGTKVEIK (SEQ ID NO: 2).

or V domains comprising polypeptide sequences having at least 80% sequence identity with SEQ ID NO: 1 and SEQ ID NO: 2.

34. A pharmaceutical composition according to any preceding claim wherein within the period of 3 to 6 hours after administration the cell count of CD4+ lymphocytes in the subject's blood plasma is below 250 cells/µl.

35. A pharmaceutical composition according to any one of claims 1 to 34 wherein within the period of 72 to 96 hours after administration the cell count of CD4+ lymphocytes in the subject's blood plasma is equal or higher than 50% of the cell count of the subject immediately prior to administration.

36. A pharmaceutical composition according to claim 13 wherein the composition is capable of treating psoriasis by providing at least a 40% improvement in the Psoriasis Area and Severity Index (PASI) score of the patient.

37. A pharmaceutical composition according to claim 13 wherein the composition is capable of treating psoriasis by providing at least a 50% improvement in the Psoriasis Area and Severity Index (PASI) score of the patient.

38. A pharmaceutical composition according to claim 36 or claim 37 wherein the improvement is seen at 56 days after administration of a single dose of the composition.

39. A pharmaceutical composition according to claim 36 or 37 wherein the improvement is seen at 75 days after administration of a single dose of the composition.

40. A pharmaceutical composition according to any one of claims 36 to 39 wherein the dose of the agent is 0.5 mg.

41. A pharmaceutical composition according to any one of claims 36 to 39 wherein the dose of the agent is 2.5 mg.

SEQUENCE LISTING

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<130> 314790.WO/JND/CJS

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<170> PatentIn version 3.3

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35 40 45

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Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
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Val Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
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35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Ile Leu Glu Ser Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
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Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
65                  70                  75                  80

Val Tyr Leu Gln Met Ser Arg Leu Arg Glu Glu Asp Thr Ala Thr Tyr
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Gly Tyr Ser Tyr Ile Tyr Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
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Lys Leu Leu Ile Tyr Leu Ala Ser Ile Leu Glu Ser Gly Val Pro Gly
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65                  70                  75                  80

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20 25 30

Arg Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Val Ile Ser Val Lys Ser Glu Asn Tyr Gly Ala Asn Tyr Ala Glu
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Ser Val Arg Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80

Val Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
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Tyr Cys Ser Ala Ser Tyr Tyr Arg Tyr Asp Val Gly Ala Trp Phe Ala
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Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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20 25 30

Gly Tyr Ser Tyr Ile Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Ile Leu Glu Ser Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln His Ser Arg
85 90 95

Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110