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(54) Title: ANTI-CANCER THERAPY USING A LEPTIN ANTAGONIST AND AN iNKT-CELL ACTIVATOR

(57) Abstract: The present invention relates to the treatment of cancer. More specifically the invention shows that the anti-cancer activity in mammals can be augmented by administering to the mammalian host a combination of a synergistically effective amount of a leptin antagonist and an invariant natural killer T (iNKT) cell activator.

Anti-cancer therapy using a leptin antagonist and an iNKT-cell activator

Field of the invention

The present invention relates to the treatment of cancer. More specifically the invention shows that the anti-cancer activity in mammals can be augmented by administering to the mammalian host a combination of a synergistically effective amount of a leptin antagonist and an invariant natural killer T (iNKT) cell activator.

Background

It would be advantageous to treat malignant tumours in humans using two or more anti-cancer drugs. This so called combination therapy is currently in use in research and in the clinic. The anti-cancer drugs may be anti-metabolites, alkylating agents, antibiotics, antibodies, immune-stimulants, cytokines and the like. Combinations of said drugs are administered in an attempt to obtain a synergistic, cytotoxic effect on most cancers, e.g., carcinomas, myelomas, melanomas, lymphomas and sarcomas, and to reduce or eliminate emergence of drug-resistant cells and to reduce side effects of each drug.

In multiple myeloma (MM), a fatal plasma cell neoplastic cancer, a complex crosstalk of different cell types within the bone marrow (BM) confers survival and growth to MM cells and induces angiogenesis, bone destruction, drug resistance and immune escape (Lemaire M, Deleu S, De Bruyne E, et al., Adv Cancer Res. 2011;110:19–42). Fatty deposits can occupy up to 70% of the BM cavity with aging, yet little attention has been given to the role of adipocytes and adipokines in MM development. Adipocytes may contribute to MM by promoting migration and proliferation through secretion of adipokines such as leptin and are known to be negative regulators of the hematopoietic environment (Naveiras O, Nardi V, Wenzel PL, Fahey F, Daley GQ, Nature. 2009;460(7252):259-263). Recently, it became clear that adipose tissue contains a wide range of immune cells. Reciprocal interactions of immune mediators with adipocytes have been described giving rise to the emerging field of the immunometabolism (Mathis D, Shoelson S., Nat. Rev. Immunol. 2011;11(2):81 doi). Invariant natural killer T (iNKT) cells, a prototypic T cell subset with important roles in anti-tumour immunity, have been shown to closely interact with adipocytes in a CD1d dependent manner (Huh JY, Kim I, Park J, et al., Mol. Cell. Biol. 2013;33(2):328–339). In MM, defective IFN-γ production by iNKT cells and reduced iNKT cell frequencies are observed in patients and different animal models (Dhodapkar M V, Geller MD, Chang DH, et al., J. Exp. Med. 2003;197(12):1667–76). Moreover, CD1d expression levels on antigen presenting cells are progressively downregulated upon MM progression (Spanoudakis E, Hu M, Naresh K, et al., Blood. 2009;113(11):2498–507). We therefore hypothesized that a crosstalk between adipose tissue and iNKT cell function plays a crucial role in MM development through release of the adipokine leptin.

iNKT cells have been shown to be involved in various immune responses, both in mice and in humans, ranging from self-tolerance to development of autoimmunity and responses to pathogens and tumours (Taniguchi, M. et al., Annu Rev Immunol. 2003;21:483).

α-galactosylceramides (α -GalCer) have originally been isolated from a marine sponge Agelas mauritianus and it was found that these compounds exhibit anti-tumour and immuno-stimulating activity in pre-clinical animal models (see for example patent EP0609437B1). The use of α-GalCer and analogous CD1d ligands has allowed to define iNKT cells as potent immunoregulatory cells that bridge the innate and adaptive response.

The design of various analogs of α-GalCer and its non-glycosidic analogues is extensively described in literature (Kerzerho, J. et al., J Immunol. 2012 Mar 1; 188(5): 2254–2265, Wojino, J. et al., ACS Chem Biol. 2012 May 18;7(5):847-55 or Guillaume, J., Bioorg Med Chem. 2015 Jul 1;23(13):3175-82).

In an effort to selectively elicit either a Th1 or Th2 biased response upon antigen challenge, structural analogs of α-GalCer have been developed. Modifications include either the lipid tails responsible for CD1d binding or the sugar moiety that directly interacts with the TCR.

Structural analogs that alter the polar head that is directly recognized by the TCR have also been synthesized. These ligands do not alter intracellular processing as they share the lipid chain with α-GalCer or previously synthesized analogs, thus their influence on iNKT activation would only derive from the stability of the binding to CD1d and the direct interaction with the semi-invariant TCR.

KRN7000 is a synthetic α -GalCer that has been most frequently used in experimental studies. Clinical studies using KRN7000 have been disappointing since no clinical anti-tumour effects were recorded (Giaccone G et al (2002) Clin Cancer Res 8: 3702). Furthermore, it was observed that the use of sequential doses of α -GalCer can lead to an anergic state of T-cells (Parekh VV et al (2005) J Clin. Invest. 115(9):2572-83).

A hallmark of BM changes occurring during aging is the increase in adipocyte numbers. However, its impact on development of hematological malignancies, particularly MM is unresolved. Leptin, an adipokine released by adipocytes crucial in energy homeostasis, also displays immune modulatory properties but its role in anti-tumour immunity is unclear.

In a novel approach, we investigated the crosstalk between leptin-leptin receptor, MM and iNKT cell mediated anti-tumour immunity. A marked and progressive increase in serum leptin serum levels and upregulation of the leptin receptor expression on iNKT cells during MM progression was observed. In vitro functional analysis highlighted leptin inhibits iNKT cell function. In a pilot study, we evaluated the in vivo effect of leptin receptor antagonism in the presence of iNKT cell activation with the prototypic glycolipid, α-galactosylceramide (α -GalCer), in the 5T33 myeloma model. Surprisingly, we observed a complete protection in the combined regimen (iNKT activation and leptin receptor blockade), which was found to be linked to alleviating iNKT cell anergy. These findings indicate leptin has a crucial immune suppressive role in myeloma development.

Based on our study unveiling a previously unknown link between leptin and iNKT cells in MM development, we demonstrate that increased leptin and iNKT leptin receptor (LR) levels are linked to disease progression. Leptin

controls iNKT cells by inhibiting their function, preventing long term anti-tumour effects in MM. Conversely, LR signaling on activated iNKT cells markedly promotes tumour protection by alleviating iNKT cell anergy.

In summary, overall our data reveal leptin-leptin receptor as a novel check point inhibitor strategy to target MM. In the present invention we show that blocking leptin receptor signalling on activated iNKT cells protects against multiple myeloma by modulating iNKT anergy. Specifically, we found that the use of a therapeutic amount of a leptin antagonist in combination with an iNKT-cell activator provides a surprising synergism in treating various forms of cancer.

Summary

It is an object of the invention to provide pharmaceutical compositions which comprise a leptin antagonist and an iNKT-cell activator.

In one aspect, said iNKT-cell activator is α-galactosylceramide or a functional derivative thereof. A functional derivative retains the capacity to activate invariant natural killer cells.

Accordingly, in one aspect, the invention relates to a pharmaceutical composition comprising a leptin antagonist and α-galactosylceramide. In another aspect, said pharmaceutical composition comprises a leptin antagonist and a functional derivative of α-galactosylceramide capable of activating invariant natural killer T cells.

In one aspect, said leptin antagonist is a leptin receptor antagonist.

Accordingly, in another aspect, the invention provides a pharmaceutical composition which comprises an antagonist of the leptin receptor and an iNKT-cell activator.

In one aspect, said antagonist of the leptin receptor is an antibody with a specificity for the leptin receptor.

In other aspects, said pharmaceutical composition comprising a leptin antagonist and an iNKT-cell activator further comprises a chemotherapeutic agent.

This invention also relates to a pharmaceutical composition, comprising the particular components as described above, and one or more physiologically acceptable excipients.

The pharmaceutical composition according to the invention is particularly useful in treating cancer.

More specifically, the pharmaceutical composition can be used to treat multiple myeloma.

In another aspect, the pharmaceutical composition is useful in the treatment of melanoma. In yet another aspect, the pharmaceutical composition according to the invention can be used to treat breast cancer.

In an aspect according to the invention, the leptin antagonist and the iNKT-cell activator are simultaneously administered. In another aspect according to the invention, administration of the leptin antagonist and the iNKT-cell

activator takes place successively. In yet another aspect according to the invention, the leptin antagonist and the iNKT-cell activator are sequentially administered.

According to specific aspects of the invention, simultaneous, successive and sequential administration of the leptin antagonist and the iNKT-cell activator can be combined. According to other specific aspects of the invention, the leptin antagonist and the iNKT-cell activator can alternatingly be administered simultaneously, successively and sequentially.

Brief description of the figures

Figure 1: The effect of leptin and its receptor on iNKT cells in MM.

A) Total blood was collected and leptin serum levels were measured in C57BL/KaLwRij mice at week 1, 2 and 3 after inoculation with 5T33MM cells (n=4). Levels were compared to naïve C57BL/KaLwRij mice (n=4). B) Mean Fluorescence Intensity (MFI) of the leptin receptor expression on iNKT cells in the liver, bone marrow, spleen and blood at week 1, 2 and 3 after inoculation with 5T33MM cells. The MFI is compared to leptin receptor levels in naïve C57BL/KaLwRij mice (n=3). C) Plasma leptin levels of 6 MM patients are compared with healthy controls D) Leptin receptor expression levels on the total T cell population in the peripheral blood and bone marrow of healthy controls and MM patients are represented in percentage and by mean fluorescence intensity (MFI). E) Mean IFNγ levels of 3 separate co-culture experiments are illustrated. Respective conditions are MM cells + iNKT cells; iNKT cells + α-GalCer- loaded Dc's; iNKT cells + α-GalCer- loaded Dc's + MM cells; iNKT cells + α-GalCer- loaded Dc's + MM cells + leptin receptor antagonist; iNKT cells + α-GalCer- loaded Dc's + leptin receptor antagonist; iNKT cells + α-GalCer-loaded Dc's + leptin; iNKT cells + α-GalCer- loaded Dc's + leptin + MM. *Natural killer T cells (NKT); Dendritic cells (DC); Alpha- Galactosylceramide (α-G); Leptin Receptor antagonist (2.17-mAlb); Leptin (L). * p<0.05, ** p<0.01, *** p<0.001*

Figure 2: The combination effects of blocking the leptin receptor and iNKT cell activation in MM in vivo.

A) Timeline of the experiment. C57BL/KaLwRij mice were inoculated together with 2μg α-GalCer at day 0. α-GalCer was re-injected weekly at day 6 and day 13. Injections with the leptin antagonist 2.17-mAlb (200μg/mice) were started at day 4 and repeated during 10 days. Treatment groups were naïve (n=5), MM + CTR nb (n=8), MM + 2.17-mAlb (n=10), MM + α-GalCer (n=10), MM + α-GalCer + 2.17-mAlb (n=10). B) Mice were weighed daily. Weight curves from one mouse representative for each group are illustrated. C) Differences in end weight compared to the initial weight of mice are represented. D) Fat pad weight at the end of the experiment are shown. E) IFNγ levels in the serum 16h after the first stimulation and second stimulation (one week later) with α-GalCer are represented. F) Total blood was collected and the serum M-spike was measured using ELISA. G) Serum TNFα and IL-6 levels were determined by ELISA at the end of the experiment for each group. *Alpha- Galactosylceramide (α-G); Leptin Receptor antagonist (2.17-mAlb); CTR nb (Control nanobody, BcII10-mAlb nanobody) Tumour necrosis factor alpha (TNFα); Interleukine 6 (IL-6). * p<0.05, ** p<0.01, *** p<0.001*

Detailed description

The present invention will be described with respect to particular embodiments and with reference to certain drawings but the invention is not limited thereto but only by the claims. Any reference signs in the claims shall not be construed as limiting the scope. The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes. Where the term "comprising" is used in the present description and claims, it does not exclude other elements or steps. Where an indefinite or definite article is used when referring to a singular noun e.g. "a" or "an", "the", this includes a plural of that noun unless something else is specifically stated.

Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

The following terms or definitions are provided solely to aid in the understanding of the invention. Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook et al., Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Press, Plainsview, New York (2012); and Ausubel et al., Current Protocols in Molecular Biology (Supplement 114), John Wiley & Sons, New York (2016), for definitions and terms of the art. The definitions provided herein should not be construed to have a scope less than understood by a person of ordinary skill in the art.

The present invention relates to a combination of a leptin antagonist and an iNKT-cell activator and the use of said combination as an anti-tumour therapeutic agent.

Accordingly, the present invention provides a pharmaceutical composition comprising a leptin antagonist and an iNKT-cell activator.

In a particular embodiment said pharmaceutical composition comprises synergistically effective amounts of the leptin antagonist and the iNKT-cell activator.

In another particular embodiment said leptin antagonist is from mammalian species, preferably human.

In a particular embodiment said leptin antagonist is recombinant.

In another particular embodiment said leptin antagonist is a leptin receptor antagonist.

In another particular embodiment said leptin receptor antagonist is an antibody which provides a specificity for the leptin receptor.

In another embodiment said iNKT-cell activator is α-galactosylceramide or a functional derivative thereof capable of activating invariant natural killer T cells.

In yet another embodiment the pharmaceutical composition comprising a leptin receptor antagonist and an iNKT-cell activator further comprises a chemotherapeutic agent.

In yet another embodiment the invention provides the use of a pharmaceutical composition comprising a leptin receptor antagonist and an iNKT-cell activator for the treatment of cancer.

In a particular embodiment the invention provides the use of a pharmaceutical composition comprising a leptin receptor antagonist and an iNKT-cell activator for the therapeutic treatment of MM.

In another particular embodiment the invention provides the use of a pharmaceutical composition comprising a leptin receptor antagonist and an iNKT-cell activator for the therapeutic treatment of melanoma.

In a particular embodiment the invention provides the use of a pharmaceutical composition comprising a leptin receptor antagonist and an iNKT-cell activator for the therapeutic treatment of breast cancer.

In yet another embodiment the invention provides the use of a pharmaceutical composition comprising a leptin receptor antagonist and an iNKT-cell activator for the treatment of metastasis.

In another embodiment said leptin receptor antagonist and said iNKT-cell activator are administered simultaneously.

In a particular embodiment said leptin receptor antagonist and said iNKT-cell activator are administered successively.

In a preferred embodiment the administration of the leptin receptor antagonist precedes the administration of the iNKT-cell activator.

In yet another preferred embodiment the administration of the iNKT-cell activator precedes the administration of the leptin receptor antagonist.

In a particular embodiment said leptin receptor antagonist and said iNKT-cell activator are administered sequentially.

In yet another preferred embodiment the administration of the leptin receptor antagonist precedes the administration of the iNKT-cell activator wherein said leptin receptor antagonist is administered sequentially and the first dose is administered at least one hour before the administration of the iNKT-cell activator. In a particular embodiment said first dose of the leptin receptor antagonist is administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours before the administration of the iNKT-cell activator. In yet another particular embodiment said first dose of the leptin receptor antagonist is administered at least one day before the administration of the iNKT-cell activator. In another particular embodiment said first dose of the leptin receptor antagonist is administered at least two days before the administration of the iNKT-cell activator.

Another preferred embodiment the leptin receptor antagonist is administered daily for at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9 or at least 10 consecutive days.

According to the invention, the leptin receptor antagonist can also be administered over a long period, for example over weeks, months or even years. This can be useful to suppress tumour development and tumour growth.

In yet another preferred embodiment the administration with the iNKT-cell activator is weekly for at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9 or at least 10 consecutive weeks.

According to the invention, the iNKT-cell activator can also be administered over a long period, for example over months or even years. This can be useful to suppress tumour development and tumour growth.

It should be clear to the skilled practitioner that the dose and dosage regimen will depend mainly on whether the leptin receptor antagonist and iNKT-cell activator are being administered separately or as a mixture, the type of cancer, the patient, and the patient's history. The amount must be effective to achieve a tumour reduction that is synergistic. If multiple doses are employed (such as preferred with the leptin receptor antagonist) the frequency of administration will depend, for example, on the type of host and type of cancer, dosage amounts, etc. For some types of cancers, daily administration will be effective, whereas for others, administration every other day or every third day will be effective, but daily administration will be ineffective. The practitioner will be able to ascertain upon routine experimentation which route of administration and frequency of administration are most effective in any particular case.

By a “leptin receptor antagonist” are meant the various forms of leptin receptor antagonists as described in the non-limiting examples below:

Leptin shows three binding sites for the interaction with the leptin receptor, sites I-III. Preferably, the leptin receptor antagonist binds to the leptin receptor on binding site II, but fails to induce receptor clustering and thus signaling. Both mutations in leptin site III (leptin S120A/T121A; Peelman, F. et al., J Biol Chem. 2004; 279(39):41038-41046) and leptin site I (leptin L39A/D40A/F41A; Niv-Spector, L. et al., Biochem J. 2005;391(Pt 2):221-230) result in potent leptin receptor antagonists both *in vitro* and *in vivo*. More preferably, residue D23 of mouse leptin receptor antagonist (L39A/D40A/F41) is replaced with a non-negatively charged amino acid (most specifically with Leu) to develop superactive mouse, human, ovine and rat leptin receptor antagonists (D23L/L39A/D40A/F41A). Appropriate methods are disclosed in the art (Gertler, A. and Elinav, E., Curr Pharm Des. 2014;20(4):659-65).

The leptin receptor antagonist can also be synthesized based on the wild-type sequence of leptin binding sites I and III. The preparation of a leptin receptor antagonist based on site I is disclosed in Catalano, S. et al., J Cell Mol Med. 2015 May;19(5):1122-32, and based on site III in Otvos, L. et al., Biopolymers. 2011;96(2):117-125.

In a preferred embodiment, a nanobody-based approach for the design of a leptin receptor antagonist is applied. Methods for this approach can be found in Zabeau, L. et al., Biochem J. 2012 Jan 1;441(1):425-34.

The skilled person knows how to design appropriate leptin receptor antagonists based on the information spread in this field.

In another preferred embodiment, leptin receptor antagonists are modified with polyethylene glycol (PEG) and the like as known in the art.

In yet another embodiment, the leptin receptor antagonist comprises an antibody binding to albumin.

The skilled person is able to apply suitable methods with the aim to extend the half-life of the leptin receptor antagonist.

The preferred range of the leptin receptor antagonist used in the pharmaceutical composition of the present invention is between 1-100 mg/kg.

Any compound activating iNKT cell function can be used in the scope of the present invention. In view of the person skilled in the art, those compounds can be easily selected or designed. A broad range of these compounds is commonly available as well.

Compounds activating iNKT cell function are disclosed in US Patent Application 2012093875.

As used herein, the term "iNKT-cell activator" has its general meaning in the art and refers to any derivative or analogue derived from a lipid, that is typically presented in a CD1d context by antigen presentating cells (APCs) and that can activate iNKT cells, i.e. promote, in a specific manner, cytokine production by iNKT cells. “α-galactosylceramide or “α -GalCer” can be seen as an example of a compound activating iNKT cell function. As used herein, the term "α-galactosylceramide compound" or "α-GalCer compound" has its general meaning in the art and refers to any functional derivative or analogue derived from a glycosphingolipid that contains a galactose carbohydrate attached by an a-linkage to a ceramide lipid that has an acyl and sphingosine chains of variable lengths (Van Kaer L. a - Galactosylceramide therapy for autoimmune diseases: Prospects and obstacles. Nat. Rev. Immunol. 2005; 5: 31-42). A functional derivative retains the capacity to activate iNKT cells.

Various publications have described α-GalCer compounds and their synthesis. An exemplary, but by no means exhaustive, list of such references includes Morita, et al., J. Med. Chem., 25 38:2176 (1995); Sakai, et al., J. Med. Chem., 38: 1836 (1995); Morita, et al., Bioorg. Med. Chem. Lett., 5:699 (1995); Takakawa, et al., Tetrahedron, 54:3150 (1998); Sakai, et al., Org. Lett., 1 :359 (1998); Figueroa-Perez, et al., Carbohydr. Res., 328:95 (2000); Plettenburg, et al., J. Org. Chem., 67:4559 (2002); Yang, et al., Angew. Chem., 116:3906 (2004); Yang, et al., Angew. Chem. Int. Ed., 43:3818 (2004); Yu, et al., Proc. Natl. Acad. Sci. USA, 102(9):3383-3388 (2005); Trappeniers M, et al., J Am Chem Soc., 130(49) (2008); Leung L, et al., ChemMedChem., 4(3):329-34 (2009); Trappeniers M, et al., ChemMedChem., 3(7):1061-70 (2008); Pauwels N, et al., Bioorg Med Chem., 20(24):7149-54, (2012); Guillaume J, Bioorg Med Chem., 23(13):3175-82 (2015); Aspeslagh S, et al., J Immunol.,191(6):2916-25 (2013); Pauwels N, et al., Org Biomol Chem., 9(24):8413-21 (2011); Aspeslagh S, EMBO J., 30(11):2294-305 (2011), and Trappeniers M, et al., Org Lett.; 12(13):2928-31 (2010).

Examples of patents and patent applications describing instances of α -GalCer compounds include U.S. Pat. No.

5,936,076; U.S. Pat. No. 6,531,453 U.S. Pat. No. 5,S53,737, U.S. Pat. No. 8,022,043, US Patent Application 2003030611, US Patent Application 20030157135, US Patent Application 20040242499, US Patent Application 20040127429, US Patent Application 20100104590, EP0609437, EP2958595 and International patent application W02006026389.

A typical -GalCer compound is KRN7000 ((2S 3S, 4R)-1-0-(alfaD-galactopyranosyl)-N - hexacosanoyl-2-amino-1,3,4-octadecanetriol)) (KRN7000, a novel immunomodulator, and its antitumour activities. Kobayashi E, Motoki K, Uchida T, Fukushima H, Koezuka Y. Oncol Res. 1995;7(10-l l):529-34).

Other examples include:

(2S,3R)-1-(α-D-galactopyranosyloxy)-2-tetracosanoylamino-3-octadecanol,

(2S, 3R)- 2- docosanoylamina -1-(α -D- galactopyranosyloxy)-3-octadecanol,

(2S ,3R) -1- (α -D- galactopyranosyloxy) -2-icosanoylamino-3-octadecanol,

(2S ,3R) -1- (α -D- galactopyranosyloxy) -2-octadecanoylamino-3-octadecanol,

(2S ,3R) -1- (α -D- galactopyranosyloxy) -2-tetradecanoylamino-3-octadecanol,

(2S,3R)- 2 - decanoylamino-l-(α-D-40galactopyranosyloxy)-3-octadecanol,

(2S ,3R) -l-(α -D- galactopyranosyloxy) -2-tetracosanoylamino-3-tetradecanol,

(2S,3R)-l-(α -D-galactopyranosyloxy) -2-tetradecanoylamino-3-hexadecanol,

(2R,3S) -l-(α -D- galactopyranosyloxy) -2-tetradecanoylamino-3-hexadecanol,

(2S, 3S) -l-(α -D- galactopyranosyloxy)- 2-tetradecanoylamino-3-hexadecanol,

(2S,3R)-l-(α -D-galactopyranosyloxy)-2[(R)-2-hydroxytetracosanoylamino]-3-octadecanol,

(2S,3R,4E)-l-(α -D -galactopyranosyloxy)-2-octadecanoy lamino-4-octadecen-3-ol,

(2S,3R,4E)-l-(α -D-galactopyranosyloxy)-2- tetradecanoylamino-4-octadecen-3-ol,

(2S,3S,4R)-l-(α -D -galactopyranosyloxy)-2-tetracosanoylamino-3,4-octadecanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-tetracosanoylamino-3,4-heptadecanediol,

(2S,3S,4R)-l-(α -D -galactopyranosyloxy)-2-tetracosanoylamino-3,4-pentadecanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-tetracosanoylamino-3,4-undecanediol,

(2S,3S,4R)-l-(α -D -galactopyranosyloxy)-2-hexacosanoylamino-3,4-heptadecanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-[(R)-2-hydroxytetracosanoylamino]-3,4 octadecanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-[(R)-2-hydroxytetracosanoylamino]-3,4 heptadecanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-[(R)-2-hydroxytetracosanoylamino]-3,4 pentadecanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-[(R)-2-hydroxytetracosanoylamino]-3,4 undecanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-[(R)-2-hydroxyhexacosanoylamino]-3,4 octadecanediol, (2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-[(R)-2-hydroxyhexacosanoylamino]-3,4- nonadecanediol,

(2S,3S,4R)-l-(-D-galactopyranosyloxy)-2-[(R)-2-hydroxyhexacosanoylamina]-3,4- icosanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-[(S)-2-hydroxytetracosanoylamino]-3,4- heptadecanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-[(R)-2-hydroxytetracosanoylamino]-3,4- hexadecanediol,

(2S,3S,4R)-l-(α -D-galactopyranosyloxy)-2-[(S)-2-hydroxytetracosanoylamino] -16-methyl- 3 ,4-heptadecanediol,

(2S,3S,4R)-l-(α-D-galactopyranosyloxy)-16-methyl-2-tetracosanoylamino-3,4- heptadecanediol,
(2S,3S,4R)-l-(α-D-galactopyranosyloxy)-2-[(R)-2-hydroxytricosanoylamino] -16- methyl- 3, 4-heptadecanediol,
(2S,3S,4R)-l-(α-D-galactopyranosyloxy)-2-[(R)-2-hydroxypentacosanoylamino]-16- methyl-3 ,4-octadecanediol,
(2S,3R)-l-(α-D -galactopyranosyloxy) -2-oleoylamino-3-octadecanol,
(2S ,3S,4R)-l-(α -D-galactopyranosyloxy)-2-hexacosanoylamino-3,4-octadecanediol;
(2S ,3S,4R)-l-(α-D-galactopyranosyloxy)-2-octacosanoylamino-3,4-heptadecanediol
(2R,3R)-l-(-D -galactopyranosyloxy)-2-tetradecanoylamino-3-hexadecanol
(2S,3R,4S,5R)-2-((2S,3S,4R)-2-(4-hexyl-lH-l,2,3-triazol-l-yl)-3,4-dihydroxyoctadecyloxy)- 6-(hydroxymethyl)-tetrahydro-28-pyrane-3,4,5-triol;
(2S,3R,4S,5R)-2-((2S,3S,4R)-2-(4-heptyl-lH-l,2,3-triazol-l-yl)-3,4-dihydroxyoctadecyloxy)-6-(hydroxymethyl)-tetrahydro-28-pyrane-3,4,5-triol;
(2S,3R,4S,5R)-2-((2S,3S,4R)-2-(4-hexadecyl-lH -1 ,2,3-triazol-l-yl)-3,4- dihydroxyoctadecyloxy)-6-(hydroxymethyl)-tetrahydro-28-pyrane-3,4,5-triol;
(2S,3R,4S,5R)-2-((2S,3S,4R)-3,4-dihydroxy-2-(4-tricosyl-l H -1 ,2,3-triazol-l- yl)octadecyloxy)-6-(hydroxymethyl)-tetrahydro-28-pyrane-3,4,5-triol;
(2S,3R,4S,5R)-2-((2S,3S,4R)-3,4-dihydroxy-2-(4-tetracosyl-l H -1 ,2,3-triazol-l- yl)octadecyloxy)-6-(hydroxymethyl)-tetrahydro-2H-pyrane-3,4,5-triol; (2S,3R,4S,5R)-2-((2S,3S,4R)-3,4-dihydroxy-2-(4-pentacosyl-lH -1 ,2,3-triazol-l-yl)octadecyloxy)-6-(hydroxymethyl)-tetrahydro-28-pyrane-3,4,5-triol;
(2S,3R,4S,5R)-2-((2S,3S,4R)-3,4-dihydroxy-2-(4-(6-phenylhexyl)-lH -1 ,2,3-triazol-l- yl)octadecyloxy)-6-(hydroxymethyl)-tetrahydro-28-pyrane-3,4,5-triol;
(2S,3R,4S,5R)-2-((2S,3S,4R)-3,4-dihydroxy-2-(4-(7 -phenylheptyl)-lH -1 ,2,3-triazol-l- yl)octadecyloxy)-6-(hydroxymethyl)-tetrahydro-28-pyrane-3,4,5-triol;
(2S,3R,4S,5R)-2-((2S,3S,4R)-3,4-dihydroxy-2-(4-(8-phenyloctyl)-l H -1 ,2,3-triazol-l- yl)octadecyloxy)-6-(hydroxymethyl)-tetrahydro-28-pyrane-3,4,5-triol;
11- amino-N-((2S,3S,4R)-3,4-dihydroxy-l-((2S,3R,4S,5R)-3,4,5-trihydroxy-6- (hydroxymethyl)-tetrahydro-28-pyran-2-yloxy)octadecan-2-yl)undecanamide;
12- amino-N-((2S,3S,4R)-3,4-dihydroxy-l-((2S,3R,4S,5R)-3,4,5-trihydroxy-6- (hydroxymethyl)-tetrahydro-2H-pyran-2-oxy)octadecan-2-yl)dodecanamide;
N-((2S,3S,4R)-3,4-dihydroxy-l-((2S,3R,4S,5R)-3,4,5-trihydroxy-6-(hydroxymethyl)- tetrahydro-2Hpyran-2-yloxy)octadecan-2-yl)- 11 -hydroxyundecanamide;
N-((2S,3S,4R)-3,4-dihydroxy-l-((2S,3R,4S,5R)-3,4,5-trihydroxy-6-(hydroxymethyl)- tetrahydro-2Hpyran-2-yloxy)octadecan-2-yl)-12-hydroxydodecanamide;
8-(diheptylamino)-N-((2S,3S,4R)-3,4-dihydroxy-l-((2S,3R,4S,5R)-3,4,5-trihydroxy-6- (hydroxymethyl)-tetrahydro-2H-pyran-2-yloxy)octadecan-2-yl)octanamide;
N-((2S,3S,4R)-3,4-dihydroxy-l-((2S,3R,4S,5R)-3,4,5-trihydroxy-6-(hydroxymethyl)- tetrahydro-2Hpyran-2-yloxy)octadecan-2-yl)-l l-(dipentylamino)undecanamide;
l l-(diheptylamino)-N-((2S,3S,4R)-3,4-dihydroxy-l-((2S,3R,4S,5R)-3,4,5-trihydroxy-6- (hydroxymethyl)-tetrahydro-

2H-pyran-2-yloxy)octadecan-2-yl)undecanamide;
N-((2S,3S,4R)-3,4-dihydroxy-l-((2S,3R,4S,5R)-3,4,5-trihydroxy-6-(hydroxymethyl)- tetranydro-2Hpyran-2-y loxy)octadecan-2-yl)-11-mercaptoundecanamide;
N-((2S,3S,4R)-3,4-dihydroxy-l-((2S,3R,4S,5R)-3,4,5-dihydroxy-6-(hydroxymethyl)- tetrahydro-2Hpyran-2-yloxy)octadecan-2-yl)-12-mercaptododecanamide.

In some embodiments α -GalCer compounds are modified with polyethylene glycol (PEG) and the like as known in the art. The skilled person is able to apply suitable methods with the aim to extend the half-life of the iNKT-cell activator.

As used herein, the term "pegylated" refers to the conjugation of a compound moiety (i.e. α -GalCer compound) with conjugate moiety(ies) containing at least one polyalkylene unit. In particular, the term pegylated refers to the conjugation of the compound moiety (i.e. α -GalCer compound) with a conjugate moiety having at least one polyethylene glycol unit.

Derivatives of α -GalCer also include functional derivatives of α -GalCer which have been modified for chemical coupling (conjugation) to another molecule.

The phrase "activate iNKT cells" refers for instance to the observed induction of cytokine production, such as IFN-γ in iNKT cells by an iNKT-cell activator.

In one specific embodiment, the particulate entity according to the invention comprises (2S,3S,4R)-l-O-(alpha-D-galactosyl)-N-hexacosanoyl-2-amino-l,3,4-octadecanetriol or its functional derivative.

The listing and above given examples of iNKT-cell activators are non-limiting. The skilled person has access to suitable iNKT-cell activators as their design and optimization is extensively spread in both scientific and patent literature. Any compound activating iNKT-cell function is envisaged by the present invention.

The preferred range of α -GalCer used in the pharmaceutical composition of the present invention is between 20-200 µg/kg.

Thus, the combination of the iNKT-cell activator and the leptin receptor antagonist is found to provide a surprising synergism in treating various forms of cancer such as myeloma, melanoma and breast cancer.

As used herein, the term "therapeutic" treatment refers to administration to the host of the leptin receptor antagonist and the iNKT-cell activator after or before the host has contracted cancer, as determined by any means. The treatment is not considered therapeutic if an existing tumour burden is not decreased or more preferentially eliminated. The treatment is also not considered therapeutic if the development of a tumour burden is not decreased or more preferentially prevented.

As used herein, the term "cancer" refers to any neoplastic disorder, including such cellular disorders as, for example, renal cell cancer, Kaposi's sarcoma, chronic leukemia, breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, myeloma, melanoma, colon cancer, bladder cancer, mastocytoma, lung cancer, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, and gastrointestinal or stomach cancer. Metastasis of said various forms of cancer are also envisaged by the term “cancer”. Preferably, the cancer is myeloma, melanoma and breast cancer.

As used herein, the term "synergistically effective amount" as applied to the leptin receptor antagonist and the iNKT-cell activator refers to the amount of each component of the pharmaceutical composition which is effective for a decrease of tumour cells and which produces an effect which does not intersect, in a dose-response plot of the dose of the leptin receptor antagonist versus a dose of the iNKT-cell activator versus decrease of tumour cells, either the dose leptin receptor antagonist axis or the dose iNKT-cell activator axis. The dose response curve used to determine synergy in the art is fully described in A. Goodman *et al*, ed. 12, the Pharmacological Basis of Therapeutics, MacMillan Publishing Co., Inc., New York (2011). The optimum synergistic amounts can be determined, using a 95% confidence limit, by varying factors such as dose level, schedule and response, and using a computer-generated model that generates isobolograms from the dose response curves for various combinations of the iNKT-cell activator and the leptin receptor antagonist. The highest decrease in tumour cells on the dose response curve correlates with the optimum dosage levels.

As used herein, the term "recombinant" refers to a leptin receptor antagonist produced by recombinant DNA techniques wherein generally the gene coding for the leptin receptor antagonist is cloned by known recombinant DNA technology. The recombinant host may be eucaryotic or procaryotic host.

As used herein, the term "pharmaceutically acceptable" refers to a carrier medium that does not interfere with the effectiveness of the biological activity of the active ingredients and that is not toxic to the hosts to which it is administered.

The administration of the pharmaceutical composition of the invention may take place by any suitable technique, including parenteral administration. Examples of parenteral administration include subcutaneous, intravenous, intra-arterial, intramuscular, and intraperitoneal administration(s).

The dosage amount which appears to be most effective herein is one which results in tumour regression or complete regression and is not toxic to the host. This optimum level will depend on many factors, for example, on the type of host and type of cancer, route, schedule of administration, existing tumour burden, the type of iNKT-cell activator and the leptin receptor antagonist, and the definition of toxicity. Toxicity to the host may be defined by the extent and type of side effects or by the amount of body weight loss or by death after a certain period of time. If body weight loss is the criterion for toxicity, typically a loss of 10-20% by weight will be tolerated, with greater than 20% loss being considered toxic.

For parenteral administration the pharmaceutical composition will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion), preferably in a pharmaceutically acceptable carrier medium that is inherently non-toxic and non-therapeutic. Examples of such vehicles include saline, Ringer's solution, dextrose solution, mannitol and normal serum albumin. Non-aqueous vehicles such as fixed oils and ethyl oleate may also be used. The carrier medium may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The leptin receptor antagonist will typically be formulated in such carriers at a concentration of about 0.01 mg/ml to 20 mg/ml.

Alternatively, the pharmaceutical composition may be made into a sterile, stable lyophilized formulation in which the purified compounds (i.e. leptin receptor antagonist and iNKT-cell activator) can be mixed with a water-soluble carrier such as mannitol, which provides bulk, and a sufficient amount of a surfactant (such as for example sodium dodecyl sulfate) to ensure the solubility of the iNKT-cell activator in water. The formulation is suitable for reconstitution in aqueous injections for parenteral administration and it is stable and well-tolerated in human patients.

It should be clear that the pharmaceutical composition and its uses can also be applied for the treatment of veterinary animals. For such applications the leptin receptor antagonist may be prepared from tissue cultures or by recombinant techniques, and from any mammalian source, such as, e.g. rabbit, primate, pig, cow, cat and dog.

The various aspects of the invention are further described by the following examples, which are not intended to limit the invention in any manner.

It is to be understood that although particular embodiments, specific configurations as well as materials and/or molecules, have been discussed herein for cells and methods according to the present invention, various changes or modifications in form and detail may be made without departing from the scope and spirit of this invention. The following examples are provided to better illustrate particular embodiments, and they should not be considered limiting the application. The application is limited only by the claims.

Examples

Materials and methods

All patients gave informed consent, and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Animal experiments were performed in C57BL/KaLwRij mice or in Balb/c mice and were approved by the Ethical Committee of the Vrije Universiteit Brussels. Liver and BM cells were isolated as previously described (Jacques P, Venken K, Beneden K Van, et al., 2010;62(4):988–999; Vanderkerken K, Croucher P., *Immunol. Rev.* 2003;194:196–206). Mice lymphocytes were stained with CD1d/α-GalCer tetramer, mouse anti-leptin receptor biotinylated antibody (R&D Systems), TCRβ, CD3, NK1.1, anti-APC HRP conjugated antibody (all eBioscience), CD4 and 7-AAD (Both BD Biosciences) and acquired on a FACSCanto II (BD Biosciences). Human mononuclear cells were stained with CD3, CD4, CD8 (eBioscience), anti-human leptin receptor (R&D Systems) and acquired on a FACSLSRII (BD Biosciences).

Analyses were performed using FlowJo software (Tree Star Inc.). Isolation of 5T33MM cells and dendritic cells (DCs) were performed as reported (Jacques P, Venken K, Beneden K Van, et al., 2010;62(4):988–999; Lutz MB, Kukutsch N, Ogilvie ALJ, et al., 1999;77–92). Isolation and expansion of iNKT cells was based on our optimized protocol (Govindarajan, Srinath Elewaut D, Drennan M., *J Vis Exp.* 2015;105:doi: 10.3791/53256).

$5x10^4$ 5T33MMvv cells, $5x10^4$ iNKT cells, 10^5 α-GalCer loaded or vehicle loaded DCs were co-cultured in supplemented RPMI-1640 medium. Leptin (0,25 µg/ml) and/or bispecific nanobody 2.17m-Alb (50µg/ml) were added. Construction, production, and purification of the nanobody 2.17m-Alb was described elsewhere (Zabeau L, Verhee A, Catteeuw D, et al., *Biochem. J.* 2011;441:425–434). Supernatants of co-cultures were collected after 72 h for IFN-γ measurements by ELISA (eBioscience), following manufacturer's instructions.

C57BL/KaLwRij mice were inoculated as described previously and weekly i.p. injected with 2µg α-GalCer (Vanderkerken K, Croucher P., *Immunol. Rev.* 2003;194:196–206; Radl J, Glopper E De, Schuit HRE, Zucher C., *J. Immunol.* 1979;122(2):609–613). Serum IFN-γ levels were determined by ELISA. Mice were treated during 10 days with 2.17m-Alb or control BclL10-mAlb (i.p., 200 µg $mouse^{-1}$ day^{-1}) and weighed daily. At early established disease, organs were dissected and blood was collected to evaluate the TNFα, IL-6 and serum M–spike by ELISA.

The melanin-positive melanoma B16Bl6 cells (Hart *et al.*, 1979) are cultured in RPMI 1640 (Invitrogen) supplemented with 10% FCS, 50 IU/ml penicillin G, 50µg/ml streptomycin sulphate, 2 mM L-glutamine and 0,4 mM Na-pyruvate. For tumour inoculation cells are detached from the culture flask by a short EDTA treatment, washed three times in endotoxin-free sterile PBS (Sigma), and resuspended in PBS at $12x10^6$ cells/ml.

On day 0 C57Bl/6J mice are inoculated with $6x10^5$ cells (in 50µl PBS) subcutaneously in the back just in front of the hind limb. Treatment is started when the tumour size index (TSI), i.e. the product of the largest perpendicular diameters in mm, reached 10-50 (day 7-12).

2.17m-Alb or control BclI10-mAlb (i.p., 200 µg mouse−1 day−1) is given daily for 10 consecutive days via paralesional injection (subcutaneous injection right next to the tumour but outside the nodule). Mice are weekly i.p. injected with 2µg α-GalCer (Vanderkerken K, Croucher P., Immunol. Rev. 2003;194:196–206; Radl J, Glopper E De, Schuit HRE, Zucher C., J. Immunol. 1979;122(2):609–613). All agents are diluted in PBS to a final volume of 100µl/injection. Control mice are injected with 100µl PBS. TSI and body weight are measured every day prior to injection and every two or three days when treatment is finished.

4T1, a mammary carcinoma cell line (Aslakson, C.J. and Miller, F.R., *Cancer Res* 52: 1399–1405 (1992)) was cultured as previously described (Li, Q. et al., *Cancer Res* 64: 1130–1139 (2004); Pulaski, B.A. et al., *Cancer Res* 60: 2710–2715 (2000)).

On day 0 Balb/c mice are inoculated with $1x10^5$ cells (in 50µl PBS) subcutaneously in the back just in front of the hind limb. Treatment is started when the tumour size index (TSI), i.e. the product of the largest perpendicular

diameters in mm, reached 10-50 (day 8-14). As a treatment, mice receive α-GalCer, 2.17m-Alb or control BcII10-mAlb (i.p., 200 µg mouse−1 day−1) according to the treatment scheme above.

RESULTS Example 1

To investigate the role of leptin signaling in MM, serum leptin levels were assessed in the 5T33MM model during disease development. Levels increased significantly starting from week 2 (Figure 1A). In addition, leptin receptor (LR) expression levels on iNKT cells progressively increased from week 1 in liver, bone marrow and blood (Figure 1B). In MM patients a significant increase of in plasma leptin levels were seen relative to healthy controls (Figure 1C). A significantly higher percentage of T cells from MM patients expressed LR (Figure 1D). We next assessed the effect of leptin on iNKT cell function within an MM context using co-culture experiments (Figure 1E). IFN-γ levels dropped when iNKT cells stimulated with α-GalCer loaded dendritic cells (DCα-G) were co-cultured with MM cells or leptin. Remarkably, stimulated iNKT cells co-cultured with both MM cells and leptin completely lost capacity to secrete IFN-γ. 5T33MM cells known to express the LR do not secrete leptin (Caers, J. et al., Leukemia. 2007;21(7):1580–1584). These data suggest that leptin acts directly as a suppressor on iNKT cells but possibly also indirectly through potentiating an immunosuppressive effect of MM cells.

We further explored if LR could be a potential target in MM by using a 2.17-mAlb nanobody which inhibits binding of leptin to the leptin receptor (Zabeau, L. et al., Biochem. J. 2011;441:425-434). α-GalCer stimulated iNKT cells significantly decreased BM tumour load and serum M-spike in MM diseased mice (Nur, H. et al., PLoS One. 2013;8(5):e65075; Mattarollo, S.R. et al., Blood. 2012;120(15):3019-3029). We hypothesized that LR signaling blockade combined with iNKT cell stimulation could lead to synergistic anti-myeloma effects *in vivo* (Figure 2A). Body weight was measured daily (Figure 2B): weight gain could be observed in groups who received LR antagonists consistent with earlier reports, while it remained stable in control treated mice (Figure 2B and 2C). Masses of fat pads significantly increased upon treatment with 2.17-mAlb (Figure 2D). Spleen and liver weights remained unchanged (data not shown). Serum IFN-γ levels significantly increased in both groups receiving their first α-GalCer stimulation (Figure 2E). However, anergy, which occurs when iNKT cells are activated with α-GalCer, is a crucial problem in NKT based therapies. Indeed, this hypoactive state was observed in the control group after a second restimulation, which did not induce an IFN-γ response. In contrast, mice receiving the combined regimen with LR antagonist displayed intact IFN-γ levels after a second restimulation (Figure 2E). Thus, combining α-GalCer with the LR antagonist amplified and prolonged iNKT cell activation and prevented iNKT anergy. LR antagonists therefore bypass hypo-activation of iNKT cells (Figure 2E). This maintained iNKT activity had effects on tumour burden, as measured by the serum M spike. The α-GalCer treated group showed significant decreases in M-spike compared to untreated mice (Figure 2F), in line with earlier reports (Nur, H. et al., PLoS One. 2013;8(5):e65075). Despite this reduction, a substantial fraction of mice did not respond sufficiently. Similar to the IFN-γ levels, marked synergy was observed when combining α-GalCer with 2.17-mAlb, with low measurable serum M spike levels as a result as opposed to single treatments (Figure 2F). Surprisingly, also the group treated with LR antagonist alone had significantly lower M- spike levels, even though increased IFN-γ levels were not observed during treatment, indicating that the LR antagonism also induces anti-myeloma effects independently of iNKT cell-agonists (Figure 2E and 2F). These effects were also

seen at the level of TNFα and IL-6 and confirmed the remarkable impact blocking of leptin signaling has on MM (Figure2G). Thus, leptin impacts MM development in a dual way, by direct modulation of MM cells but also indirectly by promoting anti-tumour immunity, i.e. controlling iNKT cell function.

In summary, our study unveils a previously unknown link between leptin and iNKT cells in MM development. We demonstrate that increased leptin and iNKT LR levels are linked to disease progression. Leptin controls iNKT cells by inhibiting their function, thereby preventing long term anti-tumour effects in MM. Conversely, LR signaling on activated iNKT cells markedly promotes tumour protection by alleviating iNKT cell anergy. Overall, a pharmacological targeting of leptin signaling represents a novel strategy for immunotherapy in MM.

RESULTS Example 2

Synergism between an iNKT-cell activator and a leptin receptor antagonist in the B16 melanoma model

To determine the anti-tumour potential of activation of iNKT cells in combination with a leptin receptor antagonist, C57BL/6 mice bearing a subcutaneously growing B16Bl6 tumour are treated with a combination of the specific iNKT cell agonist alfa-GalCer and a leptin receptor antagonist.

Treatment with either α-GalCer or a leptin receptor antagonist alone has no significant effect on tumour growth, while the combination of α-GalCer and the leptin receptor antagonist results in reduced tumour growth.

RESULTS Example 3

Synergism between an iNKT-cell activator and a leptin receptor antagonist in the 4T1 breast cancer model

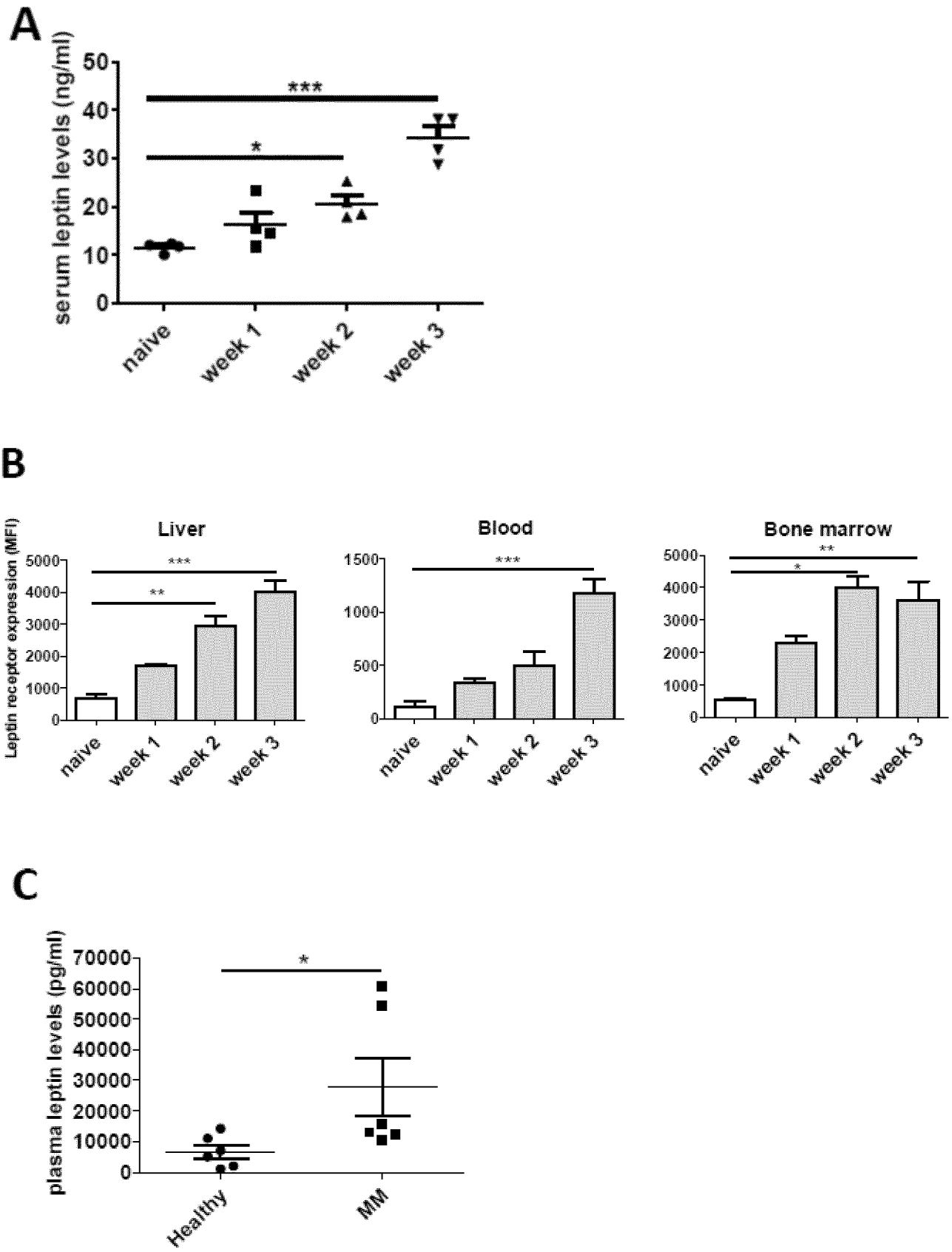
To determine the anti-tumour potential of activation of iNKT cells in combination with a leptin receptor antagonist, Balb/c mice bearing a subcutaneously growing 4T1 tumour are treated with a combination of the specific iNKT cell agonist alfa-GalCer and a leptin receptor antagonist.

Treatment with either α-GalCer or a leptin receptor antagonist alone has no significant effect on tumour growth, while the combination of α-GalCer and the leptin receptor antagonist results in reduced tumour growth.

Claims

1. A pharmaceutical composition comprising a leptin antagonist and an iNKT-cell activator.
2. A pharmaceutical composition according to claim 1 wherein the leptin antagonist is a leptin receptor antagonist.
3. A pharmaceutical composition according to any of claims 1 or 2 wherein said leptin antagonist is an antibody with a specificity for the leptin receptor.
4. A pharmaceutical composition according to claim 1 wherein the iNKT-cell activator is α-galactosylceramide or a functional derivative thereof capable of activating invariant natural killer T cells.
5. A pharmaceutical composition according to any of claims 1-4 further comprising a chemotherapeutic agent.
6. A pharmaceutical composition according to any of claims 1-5 for use to treat cancer.
7. A pharmaceutical composition according to any of claims 1-5 for use to treat multiple myeloma.
8. A pharmaceutical composition according to any of claims 1-5 for use to treat melanoma.
9. A pharmaceutical composition according to any of claims 1-5 for use to treat breast cancer.
10. A pharmaceutical composition according to claim 6 for use to treat cancer wherein the leptin antagonist and the iNKT-cell activator are administered simultaneously.
11. A pharmaceutical composition according to claim 6 for use to treat cancer wherein the leptin antagonist and the iNKT-cell activator are administered successively.
12. A pharmaceutical composition according to claim 6 for use to treat cancer wherein the leptin antagonist and the iNKT-cell activator are administered sequentially.

Figure 1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40
30
20
10
0
***
*
naive
week 1
week 2
week 3
B
Liver
Blood
Bone marrow
Leptin receptor expression (MFI)
5000
4000
3000
2000
1000
0
1500
1000
500
***
**
*
naive
week 1
week 2
week 3
C
plasma leptin levels (pg/ml)
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50000
40000
30000
20000
10000
0
*
Healthy
MM

Figure 1 continued

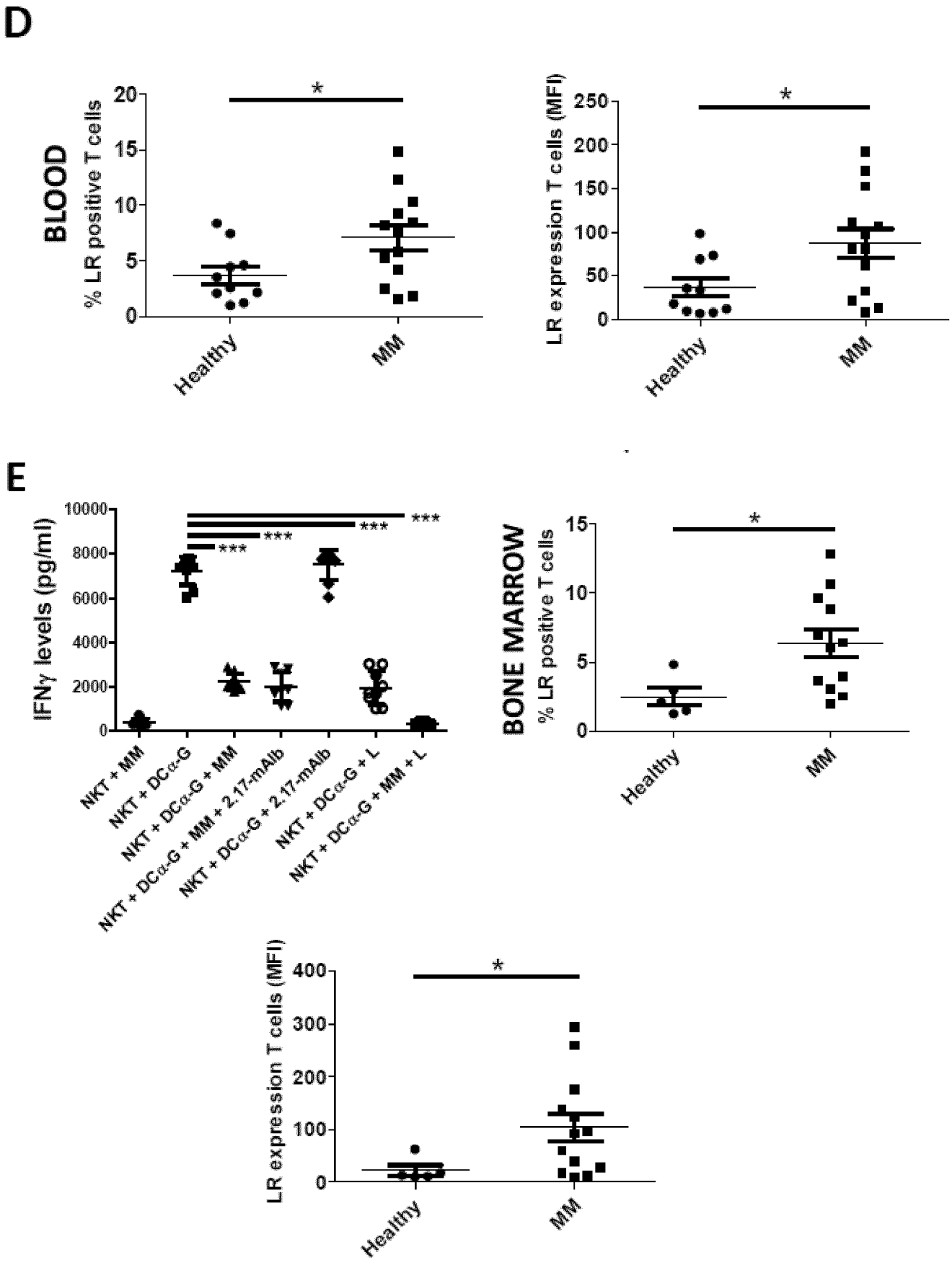
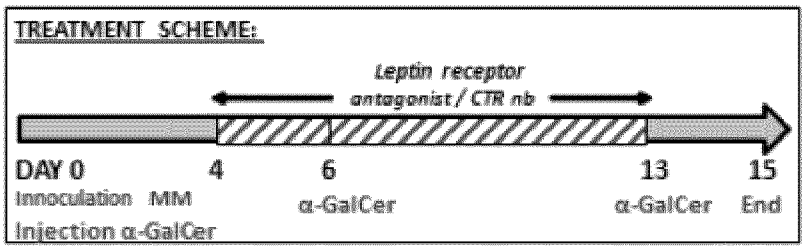


Figure 2

A


TREATMENT SCHEME:
Leptin receptor
antagonist / CTR nb
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Injection α-GalCer
4
6
α-GalCer
13
α-GalCer
15
End

B

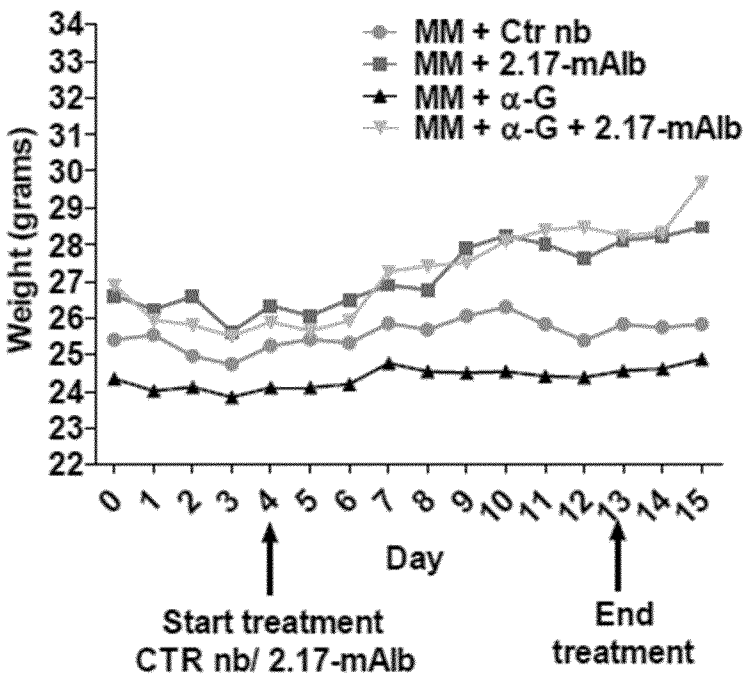

MM + Ctr nb
MM + 2.17-mAlb
MM + α-G
MM + α-G + 2.17-mAlb
Weight (grams)
34
33
32
31
30
29
28
27
26
25
24
23
22
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Day
Start treatment
CTR nb/ 2.17-mAlb
End
treatment

Figure 2 continued

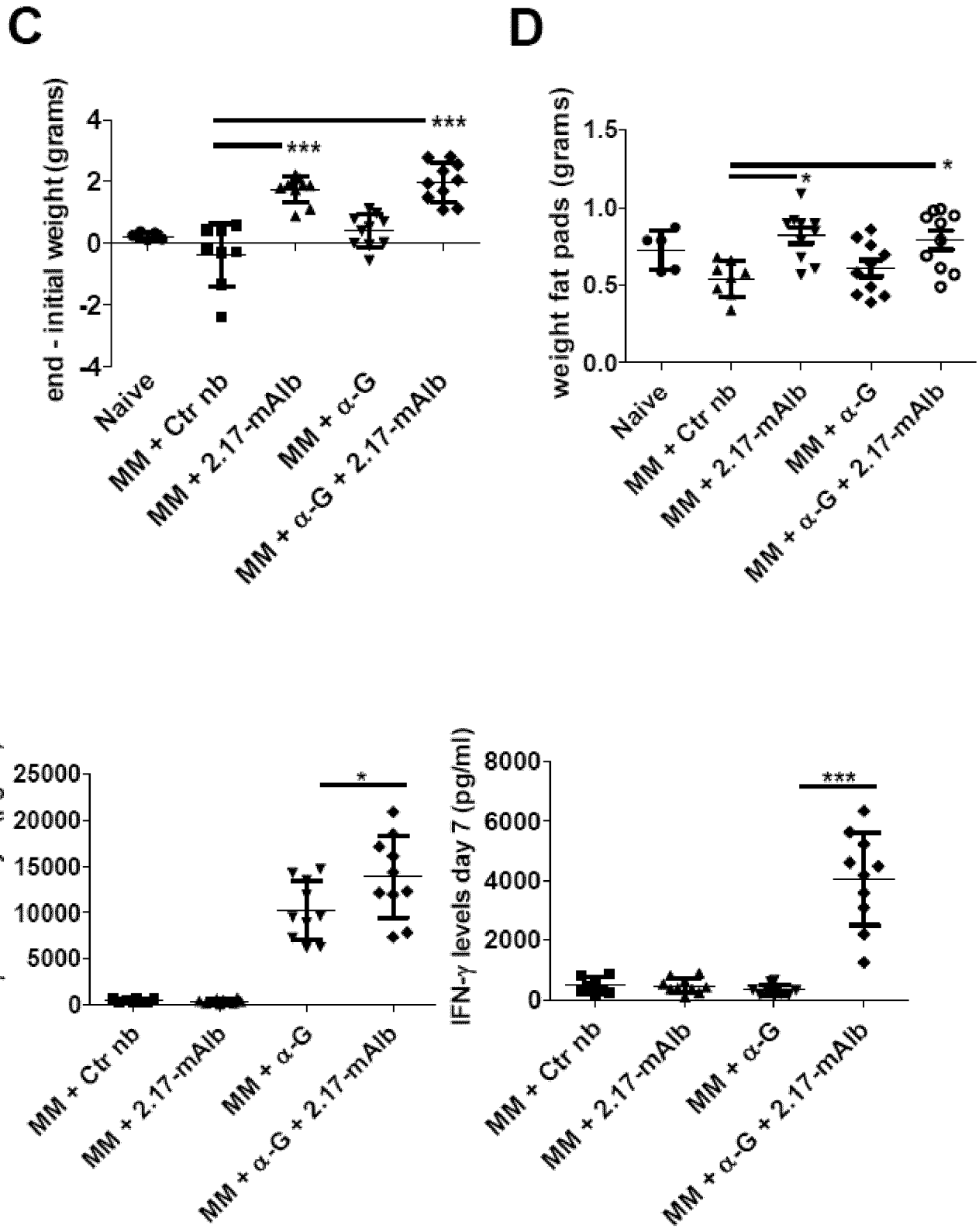
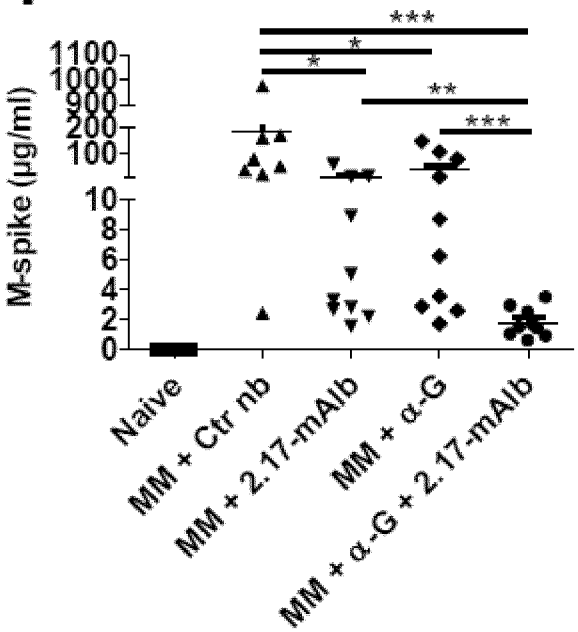


Figure 2 continued

F



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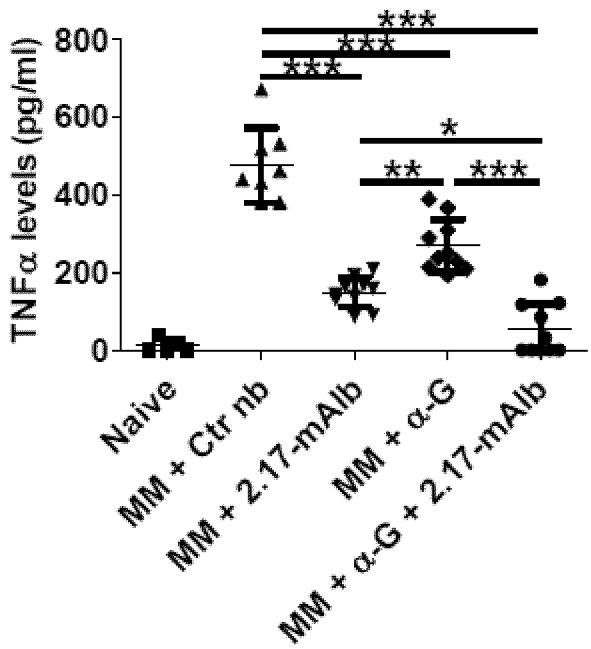

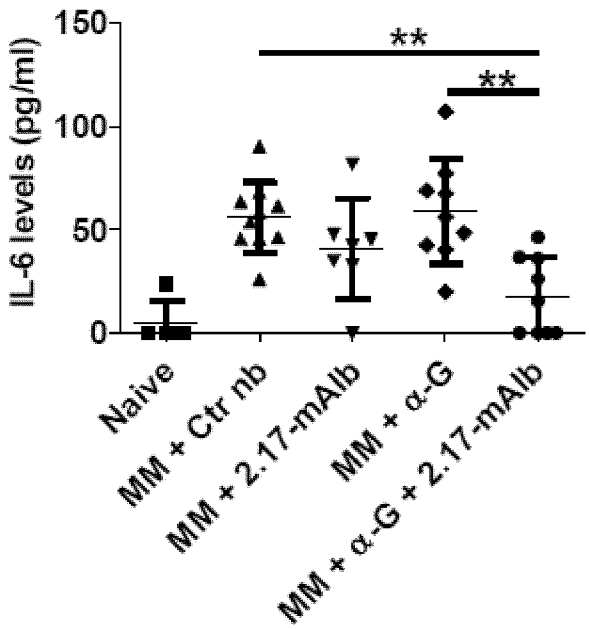

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/062322

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/26 A61K39/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KOEN VENKEN ET AL: "A bidirectional crosstalk between iNKT cells and adipocytes mediated by leptin modulates susceptibility for T cell mediated hepatitis", JOURNAL OF HEPATOLOGY, vol. 60, no. 1, 1 January 2014 (2014-01-01), pages 175-182, XP055332217, AMSTERDAM, NL ISSN: 0168-8278, DOI: 10.1016/j.jhep.2013.08.008 the whole document	1-12

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

[X] See patent family annex.

* Special categories of cited documents :

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

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 February 2017	14/02/2017

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	HANEEN NUR ET AL: "Stimulation of invariant natural killer T cells by [alpha]-Galactosylceramide activates the JAK-STAT pathway in endothelial cells and reduces angiogenesis in the 5T33 multiple myeloma model", BRITISH JOURNAL OF HAEMATOLOGY, vol. 167, no. 5, 21 August 2014 (2014-08-21), pages 651-663, XP055332220, GB ISSN: 0007-1048, DOI: 10.1111/bjh.13092 the whole document	1-12
Y	HANEEN NUR ET AL: "Preclinical Evaluation of Invariant Natural Killer T Cells in the 5T33 Multiple Myeloma Model", PLOS ONE, vol. 8, no. 5, 31 May 2013 (2013-05-31), page e65075, XP055332193, DOI: 10.1371/journal.pone.0065075 cited in the application the whole document	1-12
Y	W. SONG ET AL: "Generation of Antitumor Invariant Natural Killer T Cell Lines in Multiple Myeloma and Promotion of Their Functions via Lenalidomide: A Strategy for Immunotherapy", CLINICAL CANCER RESEARCH, vol. 14, no. 21, 1 November 2008 (2008-11-01), pages 6955-6962, XP055332222, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-07-5290 the whole document	1-12

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NOHA E. ESHEBA ET AL: "Assessment of leptin and resistin levels in non-obese multiple myeloma patients and their relation with Ig level and disease stage", EGYPTIAN NATIONAL CANCER INSTITUTE. JOURNAL, vol. 26, no. 2, 1 June 2014 (2014-06-01), pages 61-66, XP055332174, EG ISSN: 1110-0362, DOI: 10.1016/j.jnci.2014.02.002 the whole document -----	1-12
Y	J CAERS ET AL: "Neighboring adipocytes participate in the bone marrow microenvironment of multiple myeloma cells", LEUKEMIA., vol. 21, no. 7, 22 March 2007 (2007-03-22) , pages 1580-1584, XP055332796, US ISSN: 0887-6924, DOI: 10.1038/sj.leu.2404658 cited in the application the whole document -----	1-12
Y	LENNART ZABEAU ET AL: "Selection of non-competitive leptin antagonists using a random nanobody-based approach", BIOCHEMICAL JOURNAL, vol. 16, no. 1, 1 January 2012 (2012-01-01), pages 240-434, XP055332187, GB ISSN: 0264-6021, DOI: 10.1002/glia.20239 cited in the application the whole document -----	1-12
Y	LASZLO OTVOS ET AL: "Peptide-based leptin receptor antagonists for cancer treatment and appetite regulation", BIOPOLYMERS, vol. 96, no. 2, 23 March 2011 (2011-03-23) , pages 117-125, XP055332186, US ISSN: 0006-3525, DOI: 10.1002/bip.21377 cited in the application the whole document -----	1-12

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GERTLER ARIEH ET AL: "Novel superactive leptin antagonists and their potential therapeutic applications", CURRENT PHARMACEUTICAL DESIGN, BENTHAM SCIENCE PUBLISHERS LTD, NL, vol. 20, no. 4, 1 January 2014 (2014-01-01), pages 659-665, XP008182740, ISSN: 1873-4286 cited in the application the whole document	1-12
A	PEELMAN F ET AL: "Mapping of the leptin binding sites and design of a leptin antagonist", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 279, no. 39, 24 September 2004 (2004-09-24), pages 41038-41046, XP002379524, ISSN: 0021-9258, DOI: 10.1074/JBC.M404962200 the whole document	1-12
A	US 2011/256123 A1 (ILAN YARON [IL] ET AL) 20 October 2011 (2011-10-20) the whole document	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/062322

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2011256123 A1	20-10-2011	US 2011256123 A1	20-10-2011
		US 2015132287 A1	14-05-2015
		US 2016319015 A1	03-11-2016