METHOD FOR TREATING A GD2 POSITIVE CANCER

The present invention relates to a method for treating a GD2 positive cancer by administering a preparation comprising an anti-GD2 antibody to a patient, wherein the preparation is administered in a dose sufficient to induce tumor cell lysis (cytolysis threshold dose), and wherein said cytolysis threshold dose is administered until the predetermined overall patient dose has been administered.
Method for treating a GD2 positive cancer

Field of the invention

The present invention relates to a method for treating a GD2 positive cancer by administering a preparation comprising an anti-GD2 antibody to a patient, wherein the preparation is administered in a minimal dose to induce tumor cell lysis (cytolysis threshold dose), and wherein said cytolysis threshold dose is administered continuously over 24 hours per day for a treatment period until the predetermined overall patient dose has been administered.

Background to the invention

Neuroblastoma, after brain cancer, is the most frequent solid cancer in children under five years of age. In high-risk neuroblastoma, more than half of the patients receiving standard therapy have a relapse and ultimately die from the disease. 90% of cases occur between ages zero to six. The worldwide incidence in industrialized countries is around 2000 cases per year.

Monoclonal antibodies against specific antigens are increasingly being used in oncology. The entirely different mode of action compared to cytotoxic therapies have made them a valuable asset as is shown by forerunners like trastuzumab, cetuximab, bevacizumab, rituximab and others. The disialoganglioside GD2 is a glycosphingolipid expressed primarily on the cell surface. GD2 expression in normal tissues is rare and primarily restricted to the central nervous system (CNS), peripheral nerves and melanocytes. In cancerous cells, GD2 is uniformly expressed in neuroblastomas and most melanomas and to a variable degree in bone and soft-tissue sarcomas, small cell lung cancer, renal cell carcinoma, and brain tumors (Navid et al., Curr Cancer Drug Targets 2010). Because of the relatively tumor-selective expression combined with its presence on the cell surface, GD2 represents a promising target for antibody-based cancer immunotherapy.

Accordingly, several anti-GD2 antibodies are subject to preclinical or clinical investigation in neuroblastoma, melanoma and other GD2-related cancers.

APN311 is the chimeric monoclonal anti-GD2 antibody ch14.18 produced in Chinese hamster ovary (CHO) cells, which is the standard mammalian cell line for production of commercially available antibodies. In a Phase I clinical study in relapsed/refractory neuroblastoma patients remissions were achieved with this antibody as single agent. A Phase III trial comprising treatment with APN311 was initiated in 2006 by the International Society of Paediatric Oncology European Neuroblastoma (SIOPEN) and is presently investigating the effects on event-free and overall survival related to treatment with APN311 together with isotretinoin, i.e. cis-retinoic acid (cis-RA), with or without s.c. IL-2. In a comparable US study using a treatment package of 4 drugs, namely a related antibody produced in SP2/0 murine hybridoma cells together with i.v.
Interleukin-2 (IL-2), Granulocyte-macrophage colony-stimulating factor (GM-CSF) and isotretinoin, interesting survival improvement was seen in children with neuroblastoma in complete remission following initial therapies and no evidence of disease.

APN301 is an immunocytokine comprising a humanized anti-GD2 antibody (hu4.18) and IL-2 as a fusion protein. The antibody portion specifically binds to the GD2 antigen that is strongly expressed on neuroblastoma and several other cancers. IL-2 is a cytokine that recruits multiple immune effector cell types. In neuroblastoma patients, APN301 is designed to localize GD2-positive tumor cells via the antibody component. The fused IL-2 then stimulates the patient's immune system against the tumor by activation of both, NK and T cells, whereas the Fc portion of the antibody is designed to trigger tumor cell killing by antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). The immunocytokine has shown activity in a Phase II clinical study in children with relapsed/refractory neuroblastoma (Shusterman et al.; JCO 2010) and was also tested in a Phase I/II study in late stage malignant melanoma, showing immune activation.

Other anti-GD2 antibodies in research or development are, for example, the monoclonal antibody 3F8 (chimeric in phase II, as well as humanized in phase I), and 886 (specific to O-acetylated GD2, preclinical). Furthermore, anti-idiotype antibodies such as e.g. 4B5, 1A7, and A1G4 have been under investigation as potential tumor vaccines, however, their development seems to be abandoned.

Another version of the 14.18 anti-GD2 antibody is hul4.18K322A as described in WO2005/070967, which has a point mutation in the Fc region in order to reduce CDC, but maintain ADCC, e.g. by expression in a cell line suitable for enhancing ADCC, such as YB2/0. The reduction in CDC is considered to result in reduced pain associated with the antibody treatment.

Anti-tumor activity of antibodies generally occurs via either complement dependent cytotoxicity (CDC or complement fixation) or through anti-body dependent cell-mediated cytotoxicity (ADCC). These two activities are known in the art as "effector functions" and are mediated by antibodies, particularly of the IgG class. All of the IgG subclasses (IgG1, IgG2, IgG3, IgG4) mediate ADCC and complement fixation to some extent, with IgG1 and IgG3 being most potent for both activities. ADCC is believed to occur when Fc receptors on natural killer (NK) cells bind to the Fc region of antibodies bound to antigen on a cell's surface. Fc receptor binding signals the NK cell to kill the target cell. CDC is believed to occur by multiple mechanisms; one mechanism is initiated when an antibody binds to an antigen on a cell's surface. Once the antigen-antibody complex is formed, the Clq molecule is believed to bind the antigen-antibody complex. Clq then cleaves itself to initiate a cascade of enzymatic activation and cleavage of other complement proteins, which then bind the target cell surface and facilitate its death through, for example, cell lysis and/or ingestion by a macrophage.
However, CDC is considered to cause the side effect of pain, especially for anti-GD2 antibodies. As described in WO2005/070967, neurons may be particularly sensitive to complement fixation because this process involves the creation of channels in a cell membrane, allowing an uncontrolled ion flux. In pain-sensing neurons, even a small amount of complement fixation may be significant to generate action potentials. Thus, any amount of CDC resulting from anti-GD2 antibody binding on neurons will result in pain.

Accordingly, the prior art teaches that it is advantageous to reduce complement fixation so as to reduce the level of side effects in a patient and that the antitumor activity of anti-GD2 antibodies results primarily from ADCC, and not substantially from complement fixation (see e.g. WO2005/070967).

In contrast, a key aspect of the invention is that CDC function of an anti-GD2 antibody is essential for the anti-tumor effect of the anti-GD2 antibody. Another key aspect of the invention is that it is possible to reduce and manage the side effect of pain by determining the dose of the anti-GD2 antibody depending on the CDC and/or whole blood cytolytic activity. Another key finding of the invention is that the side effect of pain can be substantially reduced by administering the anti-GD2 antibody as a continuous infusion until the predetermined overall patient dose has been administered.

**Brief description of the invention**

The present invention relates to a method for treating a GD2 positive cancer by administration of a preparation comprising an anti-GD2 antibody to a patient, wherein the preparation is administered in a dose sufficient to induce tumor cell lysis (cytolysis threshold dose), and wherein said cytolysis threshold dose is administered for a treatment period until the predetermined overall patient dose has been administered. Said administration of the preparation comprising an anti-GD2 antibody can be done continuously for 24 h per day, e.g. by using a mini-pump, for the treatment period (i.e. the number of consecutive days) until the predetermined overall patient dose has been administered.

In related aspect the invention provides an anti-GD2 antibody for use in said treatment. In a further related aspect the invention provides the use of an anti-GD2 antibody in the preparation of a medicament for said treatment. The invention is further defined by the claims. All preferred embodiments of the invention as further described herein relate to all aspects of the invention equally.

**Brief description of the figures**

Figure 1 shows the results of a WBT (with whole blood) and a CDC assay (with serum) of two healthy donors in the presence of APN311. As can be seen, there is a substantial difference in WBT lysis between the two donors: 50% lysis is reached at APN311
concentrations of 2 versus 10 ng/mL whole blood. However, there is no difference in CDC: 50% lysis of both donors is reached at APN311 concentrations of 1000 ng/ml serum. In both assays (WBT and CDC assay), the same incubation time (20h) has been used, as well as the same final concentration of complement.

Figure 2 shows the results of a WBT (with whole blood) and a CDC assay (with serum) of one healthy donor in the presence of APN301 or APN311. There is a substantial difference in WBT lysis between the two preparations: 50% lysis is reached at an APN311 concentration of 21,27 ng/mL versus an APN301 concentration of 234,3 ng/mL. However, the difference in CDC is less substantial: 50% lysis is reached at an APN311 concentration of 470,4 ng/mL versus an APN301 concentration of 619,2 ng/mL.

Figure 3 shows the results of a WBT and CDC assay with whole blood or plasma of a healthy donor spiked with 5 μg/mL APN311 compared to the whole blood or plasma of a patient treated with APN311. The patient sample was collected on day 17 of the treatment cycle, i.e. at the end of the treatment period with APN311, which is from day 8 to 18 of the treatment cycle.

Figure 4 shows the results of the WBT as shown in Figure 3 compared to the same samples with the addition of a 5-fold excess of specific anti-iodiotype (anti-ID) antibody, which inhibits the target cell lysis.

Figure 5 shows the results of the CDC assay as shown in Figure 3 compared to the same samples with the addition of a 5-fold excess of specific anti-ID antibody, which inhibits the target cell lysis.

Figure 6 shows the pharmacokinetic of APN311 in serum of patients. The numbers above the mean serum levels at indicate the number of patients included in said mean at this day of sample collection. The treatment period with APN311 was from day 8 to 18, the two treatment periods with IL-2 were on days 1 to 5 and 8 to 12 of the treatment cycle. One patient received APN311 in a dose of 5 mg/m²/day, all others in a dose of 10 mg/m²/day.

Figure 7 shows the CDC assay results on day 1, 8, and 15 of the treatment cycle of patients treated with APN311, as measured by a calcein release CDC assay. The treatment period with APN311 was from day 8 to 18, the two treatment periods with IL-2 were on days 1 to 5 and 8 to 12. One patient received APN311 in a dose of 5 mg/m²/day, all others in a dose of 10 mg/m²/day.

Figures 8 and 9 show examples of schematic treatment schedules for the treatment with a preparation comprising an anti-GD2 antibody combined with other treatments.
Detailed description of the invention

It has surprisingly turned out that a treatment with a preparation comprising an anti-GD2 antibody in a dose determined by cytolysis capacity has a beneficial effect in cancer therapy, especially on side effects such as pain. If the preparation comprising an anti-GD2 antibody is administered in a dose as low as possible but sufficient to induce CDC and/or whole blood cytolysis, and is administered in said cytolysis threshold dose for a treatment period until the predetermined overall patient dose has been administered, pain can be substantially reduced and thus, the administration of morphine or other analgesics can be substantially reduced or even stopped.

In one aspect, the invention concerns a method for treating a GD2 positive cancer by administering a preparation comprising an anti-GD2 antibody to a patient, wherein the preparation is administered in a dose sufficient to induce tumor cell lysis (cytolysis threshold dose), and wherein said cytolysis threshold dose is administered until the predetermined overall patient dose has been administered.

In one embodiment, the minimal cytolysis threshold dose is determined individually for each patient. The term "predetermined overall patient dose" as used herein shall mean the overall patient dose per treatment cycle, as further specified below.

The cytolysis threshold dose may be determined by a complement dependent cytolysis (CDC) assay or a whole blood test (WBT). The WBT is an assay in which the target cells or target components (i.e. cells, liposomes or other cell-like compartments to be lysed) are contacted with whole blood from the patient. The CDC assay can be, for example, a standard CDC assay as known in the art (e.g. as described in Indusogie et al., J Immunol 2000, Zeng et al., Molecular Immunology 2005, or in WO2005/070967). The CDC assay and/or the WBT may be done with GD2 positive target cells, such as tumor cell lines of the GD2 positive cancer to be treated. For example, if the patient to be treated has neuroblastoma, the cell line may be a neuroblastoma cell line, such as e.g. LAN-1 cells. In another example, if the patient to be treated has melanoma, the cell line may be a melanoma cell line, such as e.g. M21 cells. In still another embodiment, the target cells of the CDC assay and/or the WBT are tumor cells obtained from the patient. In another embodiment, the target component of the CDC assay and/or WBT is a liposome displaying GD2 on the surface. The target cells or target components are labelled with a signalling component, e.g. with a radioactive component, such as $^{51}$Cr, or with a fluorescent component, such as Calcein. The signalling component is comprised by the target cell or target component, i.e. is inside of the target cell or target component (e.g. a liposome packed with the signalling component and displaying GD2 on the surface), and is released upon lysis of the
target cell or target component. Thus, the signalling component provides the assay readout. Target cell or target component lysis can be measured by release of said signalling component by a scintillation counter or spectrophotometry. For example, the target cell or target component lysis can be measured by determining the amount of $^{51}$Cr released into the supernatant by a scintillation counter. The percentage of lysis may be determined by the following equation: $rac{100 \times (\text{experimental release} - \text{spontaneous release})}{(\text{maximum release} - \text{spontaneous release})}.$

For the CDC assay, the cytolytic components (or effector components) are provided by serum or plasma obtained from the patient comprising the complement system components. For the WBT, the cytolytic components (or effector components) are provided by whole blood obtained from the patient comprising the complement system components as well as cellular components. For the CDC and/or WBT, the serum, plasma, or whole blood may be added to the target cells or target components in different dilutions. Furthermore, one or more samples of the CDC assay and/or WBT may be spiked with an anti-GD2 antibody in different dilutions, e.g. for generation of a standard curve.

In another embodiment, one or more anti-idiotypic anti-GD2 antibodies may be added to a sample to inhibit the target cell lysis, e.g. as a negative control.

If the cytolysis threshold dose is determined for a patient before the start of the treatment with the preparation comprising an anti-GD2 antibody, the anti-GD2 antibody or the preparation comprising the anti-GD2 antibody is added in different dilutions to the CDC assay and/or WBT samples (in addition to the patient serum, plasma, or blood), so that the cytolysis threshold dose can be determined.

If the cytolysis threshold dose is determined for a patient during the treatment with the preparation comprising an anti-GD2 antibody, the serum, plasma, or whole blood of the patient (which comprises the anti-GD2 antibody) is added in different dilutions to the CDC assay and/or WBT samples (without the addition of separate anti-GD2 antibody), so that the cytolysis threshold dose can be determined.

The dose sufficient to induce CDC and/or whole blood cytolysis may be defined as the dose that achieves at least 50% of the maximal possible target cell lysis in that respective assay (a specific CDC assay or WBT). In one embodiment, the dose is defined as the dose that achieves at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% of maximal possible cell lysis in the respective assay (a specific CDC assay or WBT).

The dose determined in such assay is a serum-, plasma-, and/or blood-level of anti-GD2 antibody. The dose of the preparation comprising the anti-GD2 antibody to be administered to patient to achieve such blood, plasma and/or serum antibody levels has then to
be determined accordingly based on pharmacokinetic data for said preparation. As shown in Figures 1 and 2, antibody levels as low as 470 to 1,000 ng/mL serum or plasma are sufficient to induce at least 50% tumor cell lysis in that CDC assay, e.g. 470.4 ng/mL (Fig. 2), or 1000 ng/mL (Fig. 1) of APN311, and 619.2 ng/mL of APN301 (Fig. 2). Accordingly, in one embodiment of the invention, the cytolysis threshold dose is 470 to 1000 ng/mL serum or plasma, or 470 to 10000 ng/mL serum or plasma. The dose of the preparation comprising the anti-GD2 antibody to be administered to the patient is determined accordingly, i.e. it is administered in a dose to achieve said serum or plasma levels within the first 1-3 days of treatment with the preparation comprising the anti-GD2 antibody (e.g. on day 1, 2, or 3 of the treatment period with the preparation comprising the anti-GD2 antibody), and said serum or plasma level is maintained over the entire treatment period with the preparation comprising the anti-GD2 antibody. As shown in Figures 1 and 2, antibody levels as low as 2 to 234.3 ng/mL whole blood are sufficient to induce at least 50% tumor cell lysis in that WBT, e.g. 2 ng/mL (Fig. 1), 10 ng/mL (Fig. 1), or 21,27 ng/mL (Fig. 2), of APN311 and 234,3 ng/mL of APN301 (Fig. 2). Accordingly, in one embodiment of the invention, the cytolysis threshold dose is 2 to 250 ng/mL whole blood, or 2 to 2500 ng/mL whole blood. The dose of the preparation comprising the anti-GD2 antibody to be administered to the patient is determined accordingly, i.e. it is administered in a dose to achieve said serum or plasma levels within the first 1-3 days of treatment with the preparation comprising the anti-GD2 antibody (e.g. on day 1, 2, or 3 of the treatment period with the preparation comprising the anti-GD2 antibody), and said serum or plasma level is maintained over the entire treatment period with the preparation comprising the anti-GD2 antibody. As can be seen in Figure 6, serum levels of 1000 ng/mL (or 1 μg/mL) can be achieved within the first one or two days of anti-GD2 antibody treatment, if the preparation comprising the anti-GD2 antibody is administered in a dose of 10 mg/m^2/day as a continuous intravenous (i.v.) infusion, i.e. for 24 h per day, using a mini-pump. Thus, in one embodiment, the preparation comprising the anti-GD2 antibody is administered in a dose of 5, 7, 10 or 15, especially 10 mg/m^2/day or any range in between these doses as a continuous intravenous infusion (24 h per day). In one embodiment, the cytolysis threshold dose is achieved within the first, second or third day of the treatment with the preparation comprising the anti-GD2 antibody. Figure 7 shows that 50% of cytolysis can be achieved within the first four days of the treatment with the preparation comprising the anti-GD2 antibody, if the preparation comprising the anti-GD2 antibody is administered in a dose of 10 mg/m^2/day as a continuous intravenous (i.v.) infusion, i.e. for 24 h per day, using a mini-pump.

The preparation may be administered to a subject in need thereof. In one embodiment, the subject is a GD2 positive cancer patient. A GD2 positive cancer is a type of cancer, in which GD2 is
expressed on tumor cells and comprises, for example, neuroblastoma, glioblastoma, medulloblastoma, astrocytoma, melanoma, small-cell lung cancer, desmoplastic small round cell tumor, osteosarcoma, rhabdomyosarcoma, and other soft tissue sarcomas. In one embodiment, the patient suffers from primary refractory or relapsed high risk-neuroblastoma, or from minimal residual disease in high-risk neuroblastoma. The patient may have previously been treated or is simultaneously treated with another therapy, such as e.g. surgery, chemotherapy, radiation, stem cell transplantation, cytokine treatment (e.g. with IL-2 and/or GM-CSF), and/or retinoid treatment (e.g. with isotretinoin).

The antibody can be selected from the group of recombinant or artificial, including single chain antibodies, mammalian antibodies, human or humanized antibodies. It may comprise or be selected from constant and/or variable portions of an antibody in particular selected from Fc, Fc-like, Fv, Fab, F(ab)2, Fab', F(ab')2, scFv, scfc, VHH. Preferably the antibody comprises a light and heavy chain of an antibody. The antibody may comprise one or two antigen binding regions, which may bind the same or different antigen, e.g. GD2, that may be bound specifically. The inventive antibodies can be directed - e.g. generated by immunization against - the antigens as defined above. The anti-GD2 antibody may be a humanized or chimeric GD2 antibody, e.g. a humanized or chimeric 14.18, 3F8 or 8B6 antibody, or an antigen-binding fragment thereof. The anti-GD2 antibody may have one or more amino acid modifications, such as e.g. a modified Fc region. In one embodiment, the anti-GD2 antibody is hul4.18K322A. In another embodiment, the anti-GD2 antibody is a chimeric 14.18 antibody. In one embodiment, the anti-GD2 antibody has the light chain nucleotide sequence of SEQ ID NO:1 (see also Example 1) and the heavy chain nucleotide sequence of SEQ ID NO:2 (see also Example 1). In one embodiment, the anti-GD2 antibody has the light chain amino acid sequence of SEQ ID NO:3 (see also Example 1) and the heavy chain amino acid sequence of SEQ ID NO:4 (see also Example 1). The relative molecular mass of the antibody comprising of two light and two heavy chains may be approximately 150,000 Dalton. In one embodiment, the anti-GD2 antibody is APN311. The anti-GD2 antibody may be expressed in CHO cells, in SP2/0 cells, or in other suitable cell lines, such as e.g. HEK-293, MRC-5, Vero, PerC6, or NS0. In one embodiment, the anti-GD2 antibody is a chimeric 14.18 antibody expressed in SP2/0 cells.

The anti-GD2 antibody may also be an immunocytokine comprising a fusion protein of an anti-GD2 antibody (or an antigen-binding fragment thereof) and a cytokine. The antibody part of the immunocytokine may be a humanized or chimeric GD2 antibody, e.g. a humanized or chimeric 14.18, 3F8 or 8B6 antibody. The antibody part of the immunocytokine protein may have one or more amino acid modifications, such as e.g. a modified Fc region. In one embodiment, the antibody part of the immunocytokine is hul4.18K322A. In another embodiment, the antibody part of the immunocytokine is a humanized
14.18 antibody. The cytokine part of the anti-GD2 antibody-cytokine fusion protein may be, for example, IL-2 or Interleukin-12 (IL-12). The antibody and the cytokine are fused together and may comprise a linker sequence. In one embodiment, the immunocytokine has the light chain nucleotide sequence of SEQ ID NO:5 (see also Example 1) and the heavy chain nucleotide sequence of SEQ ID NO:6 (see also Example 1). In one embodiment, the immunocytokine has the light chain amino acid sequence of SEQ ID NO:7 (see also Example 1) and the heavy chain amino acid sequence of SEQ ID NO:8 (see also Example 1). In one embodiment, the immunocytokine is APN301. The immunocytokine may be expressed in NSO cells, or in other suitable cell lines, such as e.g. CHO, HEK-293, MRC-5, Vero, or PerC6.

The preparation comprising an anti-GD2 antibody may further comprise salts and WFI. In one embodiment, the preparation comprising an anti-GD2 antibody may further comprise a buffer, e.g. phosphate buffered saline, comprising said salts and WFI.

The preparation comprising an anti-GD2 antibody may further comprise stabilising agents, preservatives and other carriers or excipients. The preparation comprising an anti-GD2 antibody may be freeze-dried. In one embodiment, the preparation comprising an anti-GD2 antibody comprises an anti-GD2 antibody-cytokine fusion (e.g. hul4.18-IL-2) and further comprises sucrose, L-arginine, citric acid monohydrate, polysorbate 20, and hydrochloric acid. In an embodiment, the anti-GD2 antibody is hul4.18-IL-2 and the preparation comprises 4 mg/mL immunocytokine, 20 mg/mL sucrose, 13.9 mg/mL L-arginine, 2 mg/mL polysorbate 20, and 2.1 mg/mL citric acid monohydrate. In an embodiment, said preparation comprising an immunocytokine and other excipients is freeze-dried, can be reconstituted in 4 mL of 0.9% sodium chloride, and the resulting solution has a pH of 5.5. In one embodiment, the preparation comprising an anti-GD2 antibody does not comprise stabilising agents, preservatives and other excipients.

The anti-GD2 antibody may be administered in daily doses of 1 to 50 mg/m², e.g. 1, 2, 3, 4, 5, 6, 7, 7.5, 8, 9, 10, 12, 15, 20, 25, 30, 32, 40, 45, or 50 mg/m² or any range in between these periods. For example, a daily dose of 10 mg/m² means that the patient receives 10 mg anti-GD2 antibody per m² of body surface per day. The anti-GD2 antibody may be administered in a dose of 100, 150 or 210 mg/m²/cycle or any range in between these doses. The total dose per patient per treatment cycle may be defined as the predetermined overall patient dose.

The preparation comprising an anti-GD2 antibody may be administered as intravenous infusion. The preparation comprising an anti-GD2 antibody may be administered for 10, 14, 15, or 21 consecutive days or any range in between these periods. The preparation comprising an anti-GD2 antibody may be administered as a continuous intravenous infusion over a time period of 24 hours per day. For such continuous infusion, an osmotic mini-pump may be used. In one embodiment, the preparation comprising an anti-GD2 antibody
is administered as continuous intravenous infusion for 24 hours per day for 10, 14, 15 or 21 consecutive days or any range in between these periods, in daily doses as specified above (e.g. 7, 10, or 15 mg/m²/day), e.g. 10 mg/m²/day for 10 days, 15 mg/m²/day for 10 days, 7 mg/m²/day for 14 days, 15 mg/m²/day for 14 days, 10 mg/m²/day for 15 days, 7 mg/m²/day for 21 days, or 10 mg/m²/day for 21 days or any range in between these doses.

In one embodiment, the retinoid is a retinoic acid, e.g. isotretinoin.

The immunocytokine may be administered in daily doses of 0.8 to 50 mg/m², e.g. 0.8, 1.6, 2, 3.2, 4, 4.8, 5, 6, 7, 7.5, 8, 9, 10, 12, 14.4, 15, 20, 25, 30, 32, 40, 45, or 50 mg/m² or any range in between these doses. For example, a daily dose of 10 mg/m² means that the patient receives 10 mg immunocytokine per m² of body surface per day.

In one embodiment, one milligram of fusion protein contains approximately 0.8 mg of huIL-4.18 antibody and approximately $3 \times 10^6$ U of IL-2. The preparation comprising an immunocytokine may be administered subcutaneously or as intravenous infusion, e.g. once a day. The preparation comprising an immunocytokine may be administered i.v. over a time period of 1 to 24 hours per day, e.g. 1, 1.5, 2, 4, 5, 8, 10, 12, 20, 23, or 24 hours per day or any range in between these periods. The preparation comprising an immunocytokine may be administered for 2, 3, 4, 5, 10, 14, 15, or 21 consecutive days or any range in between these periods. In one embodiment, the preparation comprising an immunocytokine is administered once a day as intravenous infusion for 4 hours per day for 3 consecutive days. In another embodiment, the preparation comprising an immunocytokine is administered once a day as intravenous infusion (e.g. for 4 hours per day) or as subcutaneous injection on 5 consecutive days. In another embodiment, the preparation comprising an immunocytokine is administered as continuous intravenous infusion for 24 hours per day for 10, 14, 15 or 21 consecutive days. For such continuous infusion, an osmotic mini-pump may be used.

The treatment period with the preparation comprising an anti-GD2 antibody may be preceded and/or accompanied by one or more treatment periods with a cytokine. In one embodiment, the cytokine is Granulocyte colony-stimulating factor (G-CSF), GM-CSF, IL-2, and/or IL-12. The cytokine may be administered subcutaneously (e.g. once a day), or as intravenous infusion. In one embodiment, the cytokine is IL-2 and is administered subcutaneously once a day in a dose of $6 \times 10^6$ IU/m²/day, e.g. on days 1 and 2 and on days 8 to 14 of the treatment cycle. In one embodiment, the overall patient dose of IL-2 is $60 \times 10^6$ IU/m²/cycle. In another embodiment, the cytokine is GM-CSF and is administered intravenously once a day over 2 hours in a dose of 250 micrograms/m²/day.

The treatment period with the preparation comprising an anti-GD2 antibody may be followed by one or more treatment periods with a retinoid. In one embodiment, the retinoid is a retinoic acid, e.g. isotretinoin.
Any such treatment period may be repeated. Any such treatment period may be followed by an interval of no treatment. In one embodiment, the interval may be an interval free of any treatment. In another embodiment, the interval is free of administration of the same preparation or treatment, however, other preparations or treatments may be administered during the interval.

Furthermore, the method according to the present invention may be accompanied by a treatment with analgesics. In one embodiment, the analgesic is an opioid, e.g. morphine. In one embodiment, the patient that is treated with the method according to the invention is also treated with GM-CSF, IL-2, and/or isotretinoin, and optionally morphine.

The treatment period with the preparation comprising an anti-GD2 antibody may be combined with one or more treatment periods with a cytokine, one or more treatment periods with a retinoid, and/or one or more treatment periods with an analgesic. In one embodiment, the treatment period with the preparation comprising an anti-GD2 antibody combined with one or more of any such other treatment periods represent one treatment cycle.

In one embodiment, the treatment period with the preparation comprising an anti-GD2 antibody is preceded by a treatment period with the cytokine. In one embodiment, the treatment period with the preparation comprising an anti-GD2 antibody is accompanied by a treatment period with the cytokine. In one embodiment, the treatment period with the preparation comprising an anti-GD2 antibody is preceded by a treatment period with the cytokine and accompanied by another treatment period with the cytokine.

A "treatment period" with a specific preparation or treatment as used herein means the period of time in which said specific preparation or treatment is administered to the patient, i.e. the time period of subsequent treatment days. For example, if the preparation comprising a cytokine is administered for 5 consecutive days, followed by one or more days of no administration of the preparation comprising a cytokine, then the treatment period with the preparation comprising a cytokine comprises 5 days. In another example, if the preparation comprising the anti-GD2 antibody is administered continuously over 24 h for 10 consecutive days, followed by one or more days of no administration of the preparation comprising the anti-GD2 antibody, then the treatment period with the preparation comprising the anti-GD2 antibody comprises 10 days. In another example, if isotretinoin is administered twice a day for 14 days, followed by one or more days of no isotretinoin administration, then the treatment period with isotretinoin comprises 14 days. Any such treatment periods may be repeated and/or overlap. For example, the treatment schedules as depicted in Figures 8 and 9 comprise two 5-day treatment periods with IL-2, the second of which is overlapping with the 10-day (or 14-, 15-, or 21-day) treatment period with chl4.18, followed by a 14-day treatment period with isotretinoin.
The terms "combined" or "combination" as used herein in relation to treatment periods shall mean that two or more treatment periods are comprised in one treatment cycle. Said two or more treatment periods may partially or entirely overlap, or may not overlap and may be separated by an interval of no treatment.

The term "treatment cycle" as used herein means a course of one or more treatments or treatment periods that is repeated on a regular schedule with periods of rest in between. For example, a treatment given for one week followed by three weeks of rest is one treatment cycle. In one embodiment, one treatment cycle comprises one treatment period with the preparation comprising an anti-GD2 antibody. The treatment cycle may optionally further comprise one or more treatment periods with a cytokine, one or more treatment periods with a retinoid, and/or one or more treatment periods with an analgesic.

In one embodiment, one treatment cycle comprises 28 to 49 days, e.g. 28, 35, 42, or 49 days or any range in between these periods. The treatment cycle starts with the day when the patient first receives any of the treatments comprised in said cycle (day 1), e.g. the administration of an preparation comprising an anti-GD2 antibody, and/or the cytokine, and/or any other preparation or treatment.

The treatment period with the anti-GD2 antibody and/or with a cytokine may be followed by a treatment period with a retinoid (e.g. isotretinoin), either directly or with an interval of one or more days of no treatment, e.g. 1, 2, 3, 4, or 5 days of no treatment. In one embodiment, isotretinoin is administered orally twice a day in a dose of 160 mg/m²/day for 14 days, e.g. from day 19 to day 32 of the treatment cycle. The treatment period with isotretinoin may be followed by an interval of one or more days of no treatment, e.g. 1, 2, 3, 4, or 5 days of no treatment.

In one embodiment, the treatment cycle comprises two 5-day treatment periods with the cytokine, e.g. on days 1 to 5 and 8 to 12 of the treatment cycle, one 10-day treatment period with the anti-GD2 antibody (e.g. with 10 or 15 mg/m²/day to administer a dose of 100 or 150 mg/m²/cycle), e.g. on days 8 to 17 of the treatment cycle, and one 14-day treatment period with isotretinoin, e.g. on days 19 to 32 of the treatment cycle, followed by 3 days of no treatment, before the next cycle begins on day 36, which is then day 1 of the second treatment cycle.

In one embodiment, the treatment cycle comprises two 5-day treatment periods with the cytokine, e.g. on days 1 to 5 and 8 to 12 of the treatment cycle, one 14-day treatment period with the anti-GD2 antibody (e.g. with 7 or 15 mg/m²/day to administer a dose of 100 or 210 mg/m²/cycle), e.g. on days 8 to 21 of the treatment cycle, and one 14-day treatment period with isotretinoin, e.g. on days 26 to 39 of the treatment cycle, followed by 3 days of no treatment, before the next cycle begins on day 43, which is then day 1 of the second treatment cycle.
In one embodiment, the treatment cycle comprises two 5-day treatment periods with the cytokine, e.g. on days 1 to 5 and 8 to 12 of the treatment cycle, one 15-day treatment period with the anti-GD2 antibody (e.g. with 10 mg/m²/day to administer a dose of 150 mg/m²/cycle), e.g. on days 8 to 22 of the treatment cycle, and one 14-day treatment period with isotretinoin, e.g. on days 26 to 39 of the treatment cycle, followed by 3 days of no treatment, before the next cycle begins on day 43, which is then day 1 of the second treatment cycle.

In one embodiment, the treatment cycle comprises two 5-day treatment periods with the cytokine, e.g. on days 1 to 5 and 8 to 12 of the treatment cycle, one 21-day treatment period with the anti-GD2 antibody (e.g. with 7 or 10 mg/m²/day to administer a dose of 150 or 210 mg/m²/cycle), e.g. on days 8 to 28 of the treatment cycle, and one 14-day treatment period with isotretinoin, e.g. on days 33 to 46 of the treatment cycle, followed by 3 days of no treatment, before the next cycle begins on day 50, which is then day 1 of the second treatment cycle.

The treatment cycle may be repeated, either identical or in an amended form, e.g. with a different dose or schedule, or with different additional treatments (e.g. with one or more other cytokines). Thus, a treatment (i.e. the overall continuous treatment period) may comprise 2 or more cycles, or up to 10 cycles. In one embodiment, the treatment comprises 3, 4, 5, 6, 7, 8, 9, or 10 cycles.

In one embodiment, the 35, 42 or 49 day treatment cycle as specified above is repeated 4 or 5 times, so that the overall continuous treatment period comprises 5 or 6 treatment cycles.

Examples

Example 1: APN311 and APN301 sequences and related data

APN311 Sequence Data

1. Molecular Weight (MW) and pI (calculated)

<table>
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<th></th>
<th>pI 1)</th>
<th>MW [D] 1)</th>
<th>No. of AS</th>
<th>Conditions</th>
<th>2D-DIGE 2)</th>
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<td>144701.10</td>
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1) Calculated via http://web.expasy.org/compute_pi/
2) Due to the molecular weight of the dyes, shifts to slightly higher molecular weights are to be expected for 2D-DIGE

2. NUCLEOTIDE SEQUENCE (cDNA, incl. leader)
"TAG" works as a "stop codon" and therefore is not translated into the peptide sequence.

**Light Chain (SEQ ID NO:1):**

1. ATT - GTA - GGG - CCA - GGC - AGC - CAG - TCT - CCA - GCA - ACC - ACT - AGA
2. CTG - TCC - TCG - AGA - AGT - CTT - CTA - TTT - CCA - GCT - GAT - ACC - ACT - AGA

**Heavy Chain (SEQ ID NO:2):**

2. GTC - CAA - CAG - TCT - GCA - GCT - AGG - CAG - ACC - ATT - TTA - CTT - ACT - TCT - TCA
5. ATG - GTA - TGT - TGC - AGA - TAT - TTA - TTA - GCA - TCA - TCA - TCT - CTG - CCA - ACC
9. GCT - GCT - CGG - GCG - GCG - TGG - GAT - TGG - TGG - TGC - TGG - GAT - TGG - TGG - TGG
13. GGT - AAA - AGA

**nucleotide 1 to 60 (striked out): leader sequence**

**last nucleotide (striked out): stop codon**

3. Peptide Sequence (incl. signal peptide)
The signal peptide is split off during post translational processing and is not part of the final recombinant protein anymore.

**Light Chain (SEQ ID NO:3):**

Heavy Chain (SEQ ID NO: 4):

```
1  M-G-W-T-W--F--i--i--s-V-T-G-v-s-s-
241 C-P-P-C-P-A-P-E-L-L-G-G-P-S-V-F-L-P-P
261 K-P-K-D-T-L-M-I-S-R-T-P-E-V-T-C-V-V-V-D
361 P-Q-V-Y-T-L-P-P-S-R-E-E-M-T-K-N-Q-V-S-L
401 Q-P-E-N-N-Y-K-T-T-P-P-V-L-D-S-D-G-S-F-F
421 L-Y-S-K-L-T-V-D-K-S-R-W-Q-Q-G-N-V-F-S-C
461 G-K
```

amino acid 1 to 20 (striked out): leader sequence

---

**APN301 Sequence Data**

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1) Calculated via http://web.expasy.org/compute_pi/
2) Due to the molecular weight of the dyes, shifts to slightly higher molecular weights are to be expected for 2D-DIGE
3) IL-2 should not be cleaved off the immunocytokine under reducing condition, as it is bound covalently and therefore the heavy chain, antibody (1/2) and the antibody should not be present on a 2D-DIGE

2. NUCLEOTIDE SEQUENCE (cDNA, incl. leader).
"TAG" and "TGA" work as "stop codons" and therefore are not translated into the peptide sequence.

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Heavy Chain (incl. IL-2; SEQ ID NO:6):

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<td>CAC</td>
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</table>

nucleotide 1 to 57 (striked out): leader sequence
nucleotide 1387 to 1385: IL-2 sequence
last nucleotide (striked out): stop codon

3. PEPTIDE SEQUENCE (incl. signal peptide):
The signal peptide is split off during post translational processing and is not part of the final recombinant protein anymore.
Example 2: CDC assay method

Principle for CDC (complement dependent cytotoxicity)
Induction of tumor cell cytotoxicity of normal human serum or plasma in the presence of APN301 or APN311, or of patients' serum or plasma after infusion of one of these antibodies, to the GD2 antigen
positive LAN-1 neuroblastoma cancer cell line (target cells) was determined in a $^{51}$Chromium release assay. The target cells were incubated with Na$_2$$^{51}$Cr (VI)$_6$, which permeates the cell membrane and binds to cytoplasmatic proteins in the Cr-III-valent form. When these cells are lysed after incubation with serum or plasma and antibodies or patients' serum or plasma, radioactivity is released into the supernatant dependent on the lytic capacity in the tested samples.

Spontaneous background lysis and total (maximally achievable) lysis by a surfactant were determined in each individual experiment. After subtracting spontaneous lysis, the lysis induced by the tested samples was calculated as % of total lysis.

**Serum or plasma sampling:**
Whole blood from normal human donors or from patients was sampled using heparinized vacutainer vials for plasma or serum clotting vials for serum. Vials were centrifuged at 2000 g for 20 minutes. The supernatant plasma or serum could be used immediately for the assay or stored at -20°C (no thawing and re-freezing allowed).

**Labelling of target cells with $^{51}$Cr:**
LAN-1 cells were cultivated in RPMI 1640 with 10% heat inactivated FCS. The day preceding the assay they were transferred into fresh flasks and fresh medium.

The assay was carried out in a 96-well flat bottom cell culture plate, using $4\times10^4$ labelled cells per well with an activity of 800nCi $^{51}$Cr per well.

The needed amount of cells was harvested from the culture flasks, the suspension centrifuged and resuspended in 1ml of PBS def. with 0.1% EDTA and 1% FCS. The calculated volume of the $^{51}$Cr solution was added, cells were incubated for 90 minutes at 37°C and 5% CO2 under gentle rotation of the tube.

Then the cell suspension was washed twice with cell culture medium to remove radioactivity from outside the cells. This medium contained additionally 100 U/ml penicillin G and 100 µg/ml streptomycin sulphate. The pellet of labelled cells after the washing steps was resuspended to the wanted concentration of $4\times10^5$ cells per ml.

**Assay procedures:**
For the assessment of cytolytic capacity of antibodies, the following was pipetted:
- 50 µl of the samples (antibody dilutions)
- 100 µl 1:4 pre-diluted normal human serum or plasma
- 100 µl $^{51}$Cr labeled cell suspension ($4\times10^5$ per ml)

For the assessment of cytolytic capacity of patients' whole blood, the following was pipetted:
50 µl medium
100 µl 1:4 pre-diluted patients' plasma or serum
100 µl \(^{51}\)Cr labeled cell suspension (4x10^5 per mL)

Assay plates were incubated in a CO₂ incubator at 37°C, 5% CO₂,
for 4 hours, or when compared directly to a WBT, for 20 hours.

The above described CDC assay method has been used for the
results as shown in Figures 1, 2, 3, and 5.

A similar CDC assay method has been used for the results as
shown in Figure 7, however, calcein has been used as label for the
LAN-1 cells instead of chromium.

Example 3: WBT method

Principle for WBT (whole blood test):

Induction of tumor cell cytotoxicity of normal human whole
blood in the presence of APN301 or APN311, or of patients' whole
blood after infusion of one of these antibodies, to the GD2 antigen
positive LAN-1 neuroblastoma cancer cell line (target cells) was
determined in a \(^{51}\)Chromium release assay.

The target cells were incubated with Na\(_2^{51}\)Cr(VI)O\(_4\), which
permeates the cell membrane and binds to cytoplasmatic proteins in
the Cr-III-valent form. When these cells are lysed after incubation
with whole blood and antibodies or patients' whole blood,
radioactivity is released into the supernatant dependent on the
lytic capacity in the tested samples.

Spontaneous background lysis and total (maximally achievable)
lysis by a surfactant were determined in each individual experiment.
After subtracting spontaneous lysis, the lysis induced by the tested
samples was calculated as % of total lysis.

Blood sampling:
Whole blood from normal human donors or from patients was
sampled using heparinized vacutainer vials.

Labelling of target cells with \(^{51}\)Cr:
LAN-1 cells were cultivated in RPMI 1640 with 10% heat
inactivated FCS. The day preceding the assay they were transferred
into fresh flasks and fresh medium.

The assay was carried out in a 96-well flat bottom cell culture
plate, using 4x10^4 labelled cells per well with an activity of 800nCi
\(^{51}\)Cr per well.

The needed amount of cells was harvested from the culture
flasks, the suspension centrifuged and resuspended in 1ml of PBS
def. with 0,1% EDTA and 1% FCS. The calculated volume of the \(^{51}\)Cr
solution was added, cells were incubated for 90 minutes at 37°C and
5% CO₂ under gentle rotation of the tube.
Then the cell suspension was washed twice with cell culture medium to remove radioactivity from outside the cells. This medium contained additionally 100 U/ml penicillin G and 100 µg/ml streptomycin sulfate. The pellet of labelled cells after the washing steps was resuspended to the wanted concentration of 4x10^5 cells per ml.

**Assay procedures:**

For the assessment of cytolytic capacity of antibodies pipette:
- 50 µl of the samples (antibody dilutions)
- 100 µl 1:2 pre-diluted normal human whole blood
- 100 µl ^51^Cr labeled cell suspension (4x10^5 per ml)

For the assessment of cytolytic capacity of patients' whole blood pipette:
- 50µl medium
- 100µl 1:2 pre-diluted patient's blood
- 100µl ^51^Cr labeled cell suspension (4x10^5 per ml)

Assay plates are incubated in a CO_2 incubator at 37°C, 5% CO_2, for 20 hours.

The above described WBT method has been used for the results as shown in Figures 1, 2, 3, and 4.
Claims

1. A method for treating a GD2 positive cancer by administering preparation comprising an anti-GD2 antibody to a patient, wherein the preparation is administered in a dose sufficient to induce tumor cell lysis (cytolysis threshold dose), and wherein said cytolysis threshold dose is administered until the predetermined overall patient dose has been administered.

2. A method according to claim 1, wherein the minimal cytolysis threshold dose is determined individually for each patient.

3. A method according to claim 1, wherein the minimal cytolysis threshold dose is determined by a complement dependent cytolysis assay.

4. A method according to claim 1, wherein the minimal cytolysis threshold dose is determined by a whole blood test.

5. A method according to any one of claims 1 to 3, wherein the target cell of the CDC assay and/or WBT is a tumor cell line of a GD2 positive cancer.

6. A method according to any one of claims 1 to 3, wherein the target component of the CDC assay and/or WBT is a liposome packed with a signalling component and displaying GD2 on the surface.

7. A method according to any one of claims 1 to 6, wherein the preparation comprising an anti-GD2 antibody is administered intravenously for 24 hours per day by using a minipump.

8. A method according to any one of claims 1 to 8, wherein the anti-GD2 antibody is a chimeric or humanized antibody.

9. A method according to any one of claims 1 to 9, wherein the anti-GD2 antibody is APN311.

10. A method according to any one of claims 1 to 9, wherein the preparation comprising an anti-GD2 antibody is administered intravenously over 24 h per day in a dose of 10 mg/m²/day for 10 consecutive days.

11. A method according to any one of claims 1 to 10, wherein the administration of the preparation comprising an anti-GD2 antibody is preceded and/or accompanied by the administration of IL-2 and/or GM-CSF.

12. A method according to any one of claims 1 to 11, wherein the administration period of the preparation comprising an anti-GD2 antibody may be followed by an administration period of isotretinoin.

13. An anti-GD2 antibody for use in a treatment according to any one of claims 1 to 12.

14. Use of an anti-GD2 antibody in the preparation of a medicament for the treatment according to any one of claims 1 to 12.
Whole blood cytotoxicity against and complement lysis of LAN1 neuroblastoma cells by APN311

2 donors

Fig. 1
WBT or CDC in whole blood or plasma of a normal donor spiked with 5μg/ml APN311 or a study patient

![Bar chart showing lysis percentages for different dilutions and conditions.]

Fig. 3
Whole blood of a normal donor spiked with 5μg/ml APN311 or of a study patient +/-5-fold excess of specific anti-ID antibody - WBT

Fig. 4
Plasma from a normal donor spiked with 5μg/ml APN311 or a study patient +/- 5-fold excess of specific anti-ID antibody - CDC

Fig. 5
Treatment schedule

ch14.18/CHO
100 mg/m²/cycle, 10 day: continuous infusion i.v.

aldesleukin (IL2)
60 x 10^6 IU/m²/cycle, 10 days: two 5 day periods, 6 x 10^6 IU/m²/day s.c.

13-cis retinoid acid
2240 mg/m²/cycle, 14 days: 14 x 160 mg/m²/day p.o.

5 cycles; 36 day intervals

↑ = Aldesleukin (IL-2) 6 x 10^6 IU/m²/day s.c.
↑ = ch14.18/CHO 10 mg/m²/day continuous i.v. infusion
↑ = Isotretinoin (13-cis-RA) 160mg/m²/day b.i.d.
↓ = Time points for pharmacokinetics and immune phenotype / effector function
+ = Sample also used for human anti chimeric response determination

Fig. 8
Treatment Schedule

- Cycles always start on Mondays (respectively the next Monday after the last 13-cis RA dose) with Aldesleukin (IL2) s.c.
- Check time points (sampling days, start/end of ch14.18/CHO infusion as well as start/end of 13-cis RA p.o. medication) for the assigned ch14.18/CHO ctnt infusion duration
- The actual dose/day (7-10-15mg/m2/day ch14.18/CHO) will be assigned by the study centre on the bases of the respective open dose level
- The dose and schedule of a dose of Aldesleukin [6 x 10^8 IU/m2/day in two 5 day blocks (days 1-5 and 8-12)] and 13-cis-RA will be constant

![Diagram showing treatment schedule]

= Aldesleukin (IL-2) 6 x 10^8 IU/m2/day s.c.
= ch14.18/CHO 10 mg/m2/day continuous i.v. infusion: start always on Mondays for advised number of days
= Isotretinoin (13-cis-RA) 160mg/m2/day b.i.d.: start always on Fridays for 14 days
= Time points for pharmacokinetics and immune phenotype / effector function
= Sample also used for human anti chimeric response determination
= Samples for ADCC (1 Serum, 1 EDTA Plasma; day 1 (baseline), day 15)

Fig. 9
A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/395
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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[X] Further documents are listed in the continuation of Box C. See patent family annex.

- Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified).
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed
  - "Y" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  - "v" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  - "u" document member of the same patent family

Date of the actual completion of the international search: 11 December 2012

Date of mailing of the international search report: 07/01/2013

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

Authorized officer:
Merlos, Ana Maria
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