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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 December 2012 (06.12.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/163544 A1

(51) International Patent Classification:
A61P 35/00 (2006.01) *A61M 1/34* (2006.01)
A61K 38/19 (2006.01) *C07K 16/28* (2006.01)

(21) International Application Number:
PCT/EP2012/002340

(22) International Filing Date:
1 June 2012 (01.06.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/492,200 1 June 2011 (01.06.2011) US

(71) Applicant *(for all designated States except US)*: **BIOPHERESIS TECHNOLOGIES, INC.** [US/US]; 400 Galleria Parkway, Suite 1950, Atlanta, GA 30339 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HEINRICH, Günther** [DE/DE]; Klenzestrasse 11, 82319 Starnberg (DE). **TEN HAGEN, Timo, L.M.** [NL/NL]; P.O. Box 1738, NL-3000 Rotterdam (NL). **EGGERMONT, Alexander** [NL/FR]; 39bis, rue Camille Desmoulins, F-94800 Villejuilf (FR). **WIESNER, Peter** [DE/DE]; Am Zapfenberg 18, 69121 Heidelberg (DE).

(74) Agent: **LAHRTZ, Fritz**; Isenbruck Bösl Hörschler LLP, Patentanwälte, Prinzregentenstraße 68, 81675 München (DE).

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

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

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2012/163544 A1

(54) **Title**: REMOVAL OF SOLUBLE TUMOR NECROSIS FACTOR RECEPTOR 2 (STNFR2)

(57) **Abstract**: The present invention relates to a device having molecules binding to soluble tumor necrosis factor receptor 2 (sTNFR2, TNFRSFIB) immobilized on a solid support, wherein no molecules binding to soluble tumor necrosis factor receptor 1 (sTNFRl) are immobilized. Further, the present invention relates to a method for the removal of sTNFR2 from blood, plasma or a blood fraction. Moreover, the present invention relates to a molecule specifically binding to sTNFR2, but not binding to sTNFRl, for use in a method for treating the human or animal body and to blood, plasma or a blood fraction obtainable from the method of the present invention for use in a method for treating cancer.

Removal of soluble tumor necrosis factor receptor 2 (sTNFR2)

The present invention relates to a device having molecules binding to soluble tumor necrosis factor receptor 2 (sTNFR2, TNFRSF1B) immobilized on a solid support, wherein no molecules binding to soluble tumor necrosis factor receptor 1 (sTNFR1) are immobilized. Further, the present invention relates to a method for the removal of sTNFR2 from blood, plasma or a blood fraction. Moreover, the present invention relates to a molecule specifically binding to sTNFR2, but not binding to sTNFR1, for use in a method for treating the human or animal body and to blood, plasma or a blood fraction obtainable from the method of the present invention for use in a method for treating cancer.

In the developed countries, cancer is one of the major causes of death throughout the population. Therefore, various therapeutics have been developed since decades. However, so far, no therapeutic approach is fully satisfying. Today, cancer therapy is mainly based on chemotherapy, targeted small molecules, radiation therapy, surgery, immunotherapy, monoclonal antibody therapy and/or other methods. Conventional chemotherapeutic cancer therapy is based on the use of agents that have cytotoxic effects predominantly on neoplastic cells. The predominant killing of neoplastic cells occurs due to the fact that cancer cells replicate faster than most other cells of the adult human body. However, it is widely known that also other cell types replicate relatively fast, such as, e.g., hair follicle cells and mucosa cells. Therefore, these cells are also affected by cytotoxic agents and the patient is exposed to side effects, in many instances severe side effects, of the treatment. Adverse events related to the chemotherapeutic treatment can lead to the premature discontinuation of the therapy, in some instances chemotherapy can even cause the death of the patient.

Further, surgery and radiation are used to reduce tumor bulk. Success rates for both therapeutic approaches are limited due to inoperable organs or sites of the human body, insensitivity of organs to radiation, or acquired radioresistance of primary or metastatic tumor lesions. A surgical procedure may even require the amputation of a limb, a breast, a testicle, an inner organ, a part of the skin and/or parts of one or more of the aforementioned. Further, a surgical procedure may lead to an artificial anus and/or to a bladder catheter. Likewise, radiation may lead to severe symptoms of intoxication.

Other treatments have been tried in an attempt to improve mortality and morbidity. Today, some types of cancer can be treated by immunotherapy (e.g., therapeutic vaccines such as sipuleucel-T, or cytokine-based immunotherapy (e.g. Interleukin-2, Interferon-alpha)), monoclonal antibody therapy (e.g. bevacizumab), targeted small molecules (e.g., imatinib) and/or other methods, such as, e.g., cryoablation or isolated organ perfusion. These treatments may bear significant advantages in comparison to the conventional chemotherapeutic treatments described above. However, many of these treatments have limited efficacy, are associated with severe toxicities, and/or can only be used for one or few types of cancer. Therefore, there are numerous types of cancer which can, so far, only be treated with a conventional cytotoxic agents, or which cannot be treated effectively at all. Irrespective of recent advances there remains a high unmet medical need associated with common malignancies with high mortality, high morbidity, high rates of recurrence, short periods of disease- or progression-free survival, rapid tumor growth, and high risk of formation of (micro-) metastases.

The addition of cytokines, alone or in combination, such as, e.g., tumor necrosis factor alpha (TNF-alpha), interferon gamma (IFN-γ), and interleukin-2 (IL-2) have been used clinically, but have either demonstrated durable remissions only in small numbers of patients treated, or produced no significant clinical responses in overall target populations.

Further, it has been demonstrated that the removal of molecules based on the molecular weight by means of ultrapheresis (therapeutic apheresis, plasmapheresis), appeared to promote an immune attack on the tumors by the patient's own white blood cells (US 4,708,713). In particular, it has been demonstrated that the removal of components present in the blood having a molecular weight of 120,000 Da (dalton) or less can lead to an induction of an immune response against transformed, infected or diseased tissue (US 6,620,382). However, the unspecific removal of a large fraction of components having a molecular weight of 120,000 Da may lead to significant disadvantages as also molecules that have positive effects on the patient's vitality such as, e.g., TNF-α, IL-2, or IFN-γ are separated from the high-molecular weight components containing, e.g., antibodies.Further it has been demonstrated that the concomitant removal of soluble tumor necrosis factor receptor 1 (sTNFR1) and soluble tumor necrosis factor receptor 2 (sTNFR2) by means of, e.g., immobilized antibodies specific for both sTNFR1 and sTNFR2, may have a positive effect on cancer treatment (WO 01/37873).

Finally it has been demonstrated that the concomitant depletion of the sTNFRl, sTNFR2, and soluble interleukin-2 receptor (sIL-2R) plasma concentrations may lead to beneficial

clinical responses in patients suffering from different types of solid malignancies (Lentz and Kumar, 2008).

However, it has not been shown whether or not the removal of any one of sTNFRl, sTNFR2, or sIL-2R alone has a positive impact on cancer treatment.

The present invention relates to the removal of soluble tumor necrosis factor receptor 2 (sTNFR2), wherein molecules binding to sTNFR2, but not binding to soluble tumor necrosis factor receptor 1 (sTNFR1), are immobilized on a solid support and wherein sTNFR1 is not removed.

Surprisingly, it could be demonstrated that the selective removal of sTNFR2 alone has a positive impact on cancer therapy. Previously, only the concomitant removal of both sTNFRs, i.e., sTNFRl and sTNFR2, resulted in clinical effects.

In a first aspect, the present invention refers to a device having molecules binding to soluble tumor necrosis factor receptor 2 (sTNFR2, TNFRSF1B) immobilized on a solid support, wherein no molecules binding to soluble tumor necrosis factor receptor 1 (sTNFR1) are immobilized.

The term "device" may be understood in the broadest sense as any assembly known in the art to enable the purification and/or handling of liquid solutions, such as, e.g., any hollow-ware, a column, a column matrix, a filter, a membrane, in particular a dialysis membrane, a semi-permeable material, a bead, in particular a microbead or a nanobead or a tubing. In particular the device may be a therapeutic, diagnostic, medical, analytical, or laboratory device or an implant.

As used herein, the term "soluble tumor necrosis factor receptor 2 (sTNFR2)" refers to a polypeptide that has a sequence identity of at least at least 75%, even more preferably at least 80%, even more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95%, and most preferably 100% of the sequence of SEQ ID NO:1. In the art, several designations are common for sTNFR2, such as, e.g., "soluble tumor necrosis factor receptor 2", "soluble tumor necrosis factor receptor type II", "soluble tumor necrosis factor receptor 1B", "soluble tumor necrosis factor receptor superfamily member 1B", "soluble tumor necrosis factor receptor superfamily 1B", "soluble tumor necrosis factor-binding protein 2", "sTNF-alpha receptor", "TNFR1B", "sTNFR-II", "TNFRSF1B", "sTBP-2", "sTBP-II", "sTBP2", "sTBPII", "p75", "p80", "sTNF-R2" or "CD120b". It will be understood that these names may be understood interchangeably.

The sTNFR2 polypeptide may bear the property to bind to tumor necrosis factor (TNF). TNF may be, e.g., TNF-alpha. As used herein, the terms "TNF", "cachexin" or "cachectin", TNF-α and "tumor necrosis factor-alpha" may be understood interchangeably.

In nature, sTNFR2 may be generated by shedding, thus, by proteolytic cleavage of the full-length TNFR2 associated with the membrane. Typically, shedding will be executed by one or more matrix metalloprotease(s).

As used in the context of the present invention, the term "polypeptide" may be understood in the broadest sense as a linear or branched polymer mainly composed of amino acids. A polypeptide may be a protein. The polypeptide may further contain posttranslational modifications. Posttranslational modifications are well-known in the art and may comprise but may not be limited to lipidation, phosphorylation, sulfatation, glucosylation, truncation, cyclization of several amino acid moieties, cyclization of the polypeptide strand, oxidation, reduction, decarboxylation, acetylation, amidation, deamidation, nitration, nitrosylation, disulfide bond formation, pyroglutamate formation, amino acid addition, cofactor addition (e.g., biotinylation, heme addition) and complexation of metal ions, non-metal ions, peptides or small molecules and addition of iron-sulfide clusters. Further, co-factors, such as, e.g., ATP, ADP, NAD+, NADH+H^+, $NADP^+$, NADPH+H^+, metal ions, cations, anions, lipids may be bound to the polypeptide, irrespective on their biological impact. Moreover, the protein may be elongated by one or more amino acids. The sTNFR2 of the present invention may be any sTNFR2 found throughout the population, preferably throughout the population of the species of the patient that may be treated with the body-fluid from which sTNFR2 has been removed. sTNFR2 may be the form of sTNFR2 that is most common throughout the population or may be a mutated form of sTNFR2, such as, e.g., a single nucleotide polymorphism (SNP) of sTNFR2. In this context, the term "mutated" may include, e.g., single amino acid exchanges (point mutations), truncated and elongated sTNFR2 and/or sTNFR2 bearing posttranslational modifications. Further, sTNFR2 may or may not be fused to other molecule(s). Most preferably, sTNFR2 originates from the patient itself. Herein, the sTNFR2 may be expressed by or result from enzymatic shedding from any cell type. sTNFR2 may, to a large extent, be expressed by or released from malignant or benign neoplastic cells and/or by cells belonging to the immune system. Herein, the cancer cells may be cells of a malignant, a semi-malignant, a pre-malignant or a benign primary tumor, or cells of secondary tumors, e.g. lymph node and distant metastases.

As used herein, the term "soluble tumor necrosis factor receptor 1 (sTNFR1)" refers to a polypeptide that has a sequence identity of at least at least 75%, even more preferably at least 80%, even more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95%, and most preferably 100% of the sequence of SEQ ID NO:2. In the art, several designations are common for sTNFR1, such as, e.g., "soluble tumor necrosis factor receptor 1", "soluble tumor necrosis factor receptor type I", "soluble tumor necrosis factor receptor 1A", "soluble tumor necrosis factor receptor superfamily member 1A", "soluble tumor necrosis factor receptor superfamily 1A", " soluble tumor necrosis factor-binding protein 1" , "soluble TNF-alpha receptor", "TNFR1A", "sTNFR-I", "TNFRSF1A", "sTBP-1", "sTBP-I", "sTBP1", "sTBPI", "p55", "p60", "sTNF-R1", "sTNF-R55", or "CD120a". It will be understood that these names may be understood interchangeably.

In nature, sTNFR1 may be generated by shedding, thus, by proteolytic cleavage of the full length TNFR1 associated with the membrane. Typically, shedding will be executed by one or more matrix metalloprotease(s).

The device of the present invention may bear molecules binding to sTNFR2. As used herein, the term "binding to" refers to an intermolecular interaction of the sTNFR2 polypeptide with any molecule binding to sTNFR2. Herein, the molecule binding sTNFR2 may be any molecule binding to sTNFR2 with a dissociation constant (Kd) of less than 10 mM, less than 1 mM, less than 100 µM, less than 10 µM, less than 1 µM, less than 100 nM, or less than 10 nM. Preferably, the molecule binding sTNFR2 may bind to sTNFR2 with a Kd of less than 1 nM, more preferably less than 100 µM, even more preferably less than 10 µM, even more preferably less than 1 µM, in particular less than 100 nM. The molecule binding to sTNFR2 may be understood as any binding partner of sTNFR2 or any sTNFR2-binding molecule. Preferably, said molecule binding to sTNFR2 may have a higher binding affinity to sTNFR2 than to other proteins present in blood or plasma such as, e.g., albumins (e.g., serum albumin), globulins (e.g., immunoglobulins), other cytokines, interleukins, chemokines, fibrinogens, antitrypsins and/or complement factors. Further, said molecule binding to sTNFR2 may bear a significantly higher affinity to sTNFR2 than to sTNFR1. Therefore, said molecule binding to sTNFR2 may bear more than 5fold, preferably more than 10fold, more preferably more than 50fold, even more preferably more than 100fold higher binding affinity to sTNFR2 than to sTNFR1. The molecules binding to sTNFR2 may be of natural or of synthetical origin. Further, the molecules binding to sTNFR2 may be conjugated or bound to other molecules that may refer to but may not be limited to one or more protein tag(s) (e.g., dihydrofolate, polyhistidine tag(s) ((poly)His tag(s)), streptavidin), one or more binding moiety/moieties

comprising, e.g., biotin, methotrexate, glycocorticoid(s), one or more active ester(s) (e.g., N-hydroxysuccinimidyl (NHS) ester(s)), one or more isothiocyanate(s), one or more maleimide(s), one or more glutaraldehyde derivative(s), one or more carbodiimide derivative(s) or combinations thereof, one or more insoluble and/or soluble polymer(s) (e.g., polyethylene glycol (PEG), hydroxypropyl methacrylate (HPMA), polyethylene imine (PEI)), one or more antibody/antibodies or derivative(s) thereof, micro- or nanobeads (e.g., functionalized silica beads, polysaccharide-based beads), polymersomes, liposomes, one or more linker or spacer molecule(s) such as, e.g., peptide linker(a), a polyethylene glycol (PEG) linker(s), saccharide linker(s), fatty acid linker(s), alkyl linker(s), antibody linker(s), or a combination of two or more thereof. The molecule binding to sTNFR2 may be conjugated to one or more binding moieties and/or one or more linker molecules during synthesis (i.e., as fusion protein) or *ex post* synthesis or expression.

The device of the present invention may not comprise any molecules binding to sTNFR1 with an binding affinity of a Kd of less than 10 nM, more preferably less than 100 nM, even more preferably less than 250 nM, even more preferably less than 500 nM, even more preferably less than 750 nM, even more preferably less than 1 μM, even more preferably less than 2 μM, , even more preferably less than 5 μM, even more preferably less than 10 μM, even more preferably less than 50 μM, even more preferably less than 100 μM.

The molecule binding sTNFR2 may be immobilized to a solid support by any means known in the art. As used herein, the term "immobilized to a solid support" means that the molecule is bound to any solid material. Herein, the term "bound" means that the molecule is covalently or quasi-covalently bound. A quasi-covalent bond is a non-covalent bond with a binding affinity with a Kd of less than 50 nM, such as, e.g., the dihydrofolate-methotrexate interaction, or the streptavidin-biotin interaction. The molecule binding sTNFR2 may be immobilized directly or via linker. For instance a bispecific linker can bind to the surface of the solid support with one active group whereas it binds to the molecule binding sTNFR2 with another active group.

For instance, a glass surface of a solid support may be activated by silane conditioning as known in the art. Then, the surface of the solid support may further be conjugated with bispecific linker carrying, e.g., a maleimide or a succinimidyl ester (e.g., N-hydroxysuccinimidyl (NHS) esters) on the other end. A succinimidyl ester can bind to free amino groups of the molecule binding sTNFR2, a maleimide preferably binds to free thiol groups of the molecule binding sTNFR2. An active ester such as an acid halogenide (e.g., an acid chloride or an acid bromide) may bind to free amino groups or free hydroxyl

groups of the molecule binding to sTNFR2. Thereby, the molecule binding sTNFR2 is immobilized on the surface of the solid support. Alternatively, antibodies binding to the molecule binding to sTNFR2 can be immobilized on the surface of a solid support. Then, in a second step, a fluid containing the molecule binding to sTNFR2 is incubated with the antibody-coated surface. As used herein, the term "coated" may be understood in the broadest sense as the conjugation of any molecular structure(s) to the surface of the solid support.

In a preferred embodiment, the device is an extracorporeal device through which a patient's blood, plasma or blood fraction can be circulated prior to being returned into the patient.

As used herein, the term "extracorporeal device" may be understood in the broadest sense as any device that is used *ex vivo*, thus, outside of the living body of the patient. The extracorporeal device may be a column, optionally coated with a molecule binding to sTNFR2 and/or filled with a column matrix coated with a molecule binding to sTNFR2, a filter system coated with a molecule binding to sTNFR2, a membrane, in particular a dialysis membrane, coated with a molecule binding to sTNFR2, a tubing coated with a molecule binding to sTNFR2, or any other device coated having a molecule binding to sTNFR2 immobilized on a solid support.

The extracorporeal device may get in contact with the body fluid containing sTNFR2 directly. Alternatively, the body fluid containing sTNFR2 may first be subjected to a previous purification step and may subsequently get in contact with the body fluid containing sTNFR2. For instance, the suspended or emulsified cells of a body fluid such as, e.g., blood cells, may first be removed from the body fluid. Then, blood plasma is generated. The blood plasma may get in contact with the extracorporeal device that enables the removal of sTNFR2. Subsequently, the cells may optionally be remixed with the plasma or returned into the patient separately. The separation of cells and body fluid may be used to protect the column(s), the filter or membrane device from being clogged. Likewise, a size exclusion filter may be suited upstream the extracorporeal device that enables the removal of sTNFR2. This filter may be used to protect a column, filter or membrane device from being clogged.

As used herein, the term "get in contact" may be understood in the broadest sense as exposing the molecules binding to sTNFR2 to the body fluid, enabling the sTNFR2 to bind to the molecules binding to sTNFR2.

Alternatively or additionally, one or more type(s) of molecule(s) or one or more type(s) of cell(s) may be removed by other means such as, e.g., affinity chromatography, anion exchange chromatography, cation exchange chromatography, hydrophobicity chromatography, or electrophoresis. This process may be conducted upstream and/or downstream to the removal of sTNFR2 or may be conducted on the same solid support as the removal of sTNFR2.

The extracorporeal device may be used offline, online or a combination thereof. As used herein, the term "offline" may be understood in the broadest sense as a system that is used batch-wise. Therefore, a certain volume of the body fluid extracted from a patient, is exposed to the device of the present invention and sTNFR2 is removed. Subsequently, the body fluid, from which sTNFR2 has been removed, is further analyzed and/or is optionally returned to the patient from whom the fluid has been obtained or to one or more other patient(s), in particular other patient(s) of the same patient as the graft patient the body fluid is obtained from. As used herein, the term "online" may be understood in the broadest sense as a system that is used in a continuous flow and/or is connected with the patient's blood or lymphatic vessel(s). Highly preferably, the body fluid extracted from a patient, is directly obtained from said patient, conducted through the extracorporeal device and, thereby, exposed to the molecules binding to sTNFR2 and is finally led back to said patient. This process may preferably be controlled manually and/or computer-assisted. In the device, the body fluid may be brought to or kept at any temperature sufficient for treating a body fluid, preferably the body fluid may be cooled to a temperature in the range of between 2°C and 5°C, between 5°C and 10°C, between 10°C and 15°C, between 15°C and 25°C, between 25°C and 37°C, or may be kept at body temperature, preferably at approximately 37°C. More preferably, the body fluid is cooled to a temperature in the range of between 2°C and 5°C or kept at body temperature, preferably at approximately 37°C, in particular kept at approximately 37°C. When returned into the patient, the body fluid may preferably bear body temperature, thus a temperature of approximately 37°C. The extracorporeal device may also serve as a combination of an offline and an online method. Then, e.g., the body fluid may be extracted from a patient in a pulsed mode, sTNFR2 may be removed in the extracorporeal device in a pulsed mode and/or the body fluid may be led back to the patient in a pulsed mode. Herein, the term "pulsed mode" means that there is no continuous flow, but the sample is rather treated batch-wise every few seconds or every few minutes, whereas the process comprising extracting the body fluid and removing sTNFR2 is conducted automatically.

As used herein, the term "blood" may be understood in the broadest sense as blood of a human or an animal. Preferably, the blood is the blood of the patient who receives the blood, plasma or blood fraction of which sTNFR2 has been removed.

As used herein, the term "plasma" may be understood in the broadest sense as blood from which the cells (e.g., red blood cells (RBCs) and leucocytes) as well as the platelets have been removed. Preferably, the plasma is obtained from the blood of the patient who receives the plasma, blood or blood fraction of which sTNFR2 has been removed.

As used herein, the term "blood fraction" may be understood in the broadest sense as any fraction of blood from which one or more factors have been removed. Exemplarily, the RBCs, some types of or all leucocytes, platelets, and/or one or more proteins may be removed. Preferably, the term "blood fraction" refers to (blood) serum, thus, plasma of which further one or more coagulation factor(s) has/have been removed. Preferably, in serum, at least fibrin and/or fibrinogen has been removed. A blood fraction may also be plasma or serum of which further proteins of a certain molecular weight range have been removed. This can be proteins of a molecular weight above a certain threshold or below a certain threshold, preferably proteins above a certain threshold are removed, more preferably proteins of more than 50,000 Da, more than 100,000 Da, more than 120,000 Da, more than 150,000 Da or more than 200,000 Da. Even more preferably, proteins of a molecular weight of more than 120,000 Da may be removed from the blood fraction. Alternatively, sTNFR2 may also be removed from any other body fluid such as, e.g., lymph (endolymph and/or perilymph), cerebrospinal fluid (*liquor cerebrospinalis*).

Preferably, the blood, plasma, blood fraction or body fluid is obtained from an organism directly before or during the removal of sTNFR2. More preferably, the blood, plasma, blood fraction or body fluid is obtained from a patient. Alternatively, the blood, plasma, blood fraction or body fluid may have been obtained previously. If the body fluid was obtained previously, it may be stored by any means known in the art, e.g., the body fluid may be stored at room temperature, at 4°C, at -20°C, at -80°C, or in liquid nitrogen. It may be stored for minutes, hours, days, months, or years. For storage, the body fluid may also be dried (e.g., by evaporation, vacuum evaporation, or freeze drying). Before the removal of sTNFR2, the residua, thus, dry foam or powder, may be dissolved in water or a respective aqueous buffer as known in the art.

As used throughout the invention, the term "patient" may refer to every living subject that may be treated. The patient may be an animal, in particular a mammal including humans. Most particularly, the patient is a human. The patient may be a subject that has an

increased sTNFR2 level. Said increased sTNFR2 level may be due to the presence of malignant or benignant neoplastic cells and/or by cancer cells in the patient.

The term "obtained from" as used herein may be understood in the broadest sense as the gaining of the body fluid by any means known in the art. Exemplarily, blood, plasma, serum, cerebrospinal fluid and/or lymph may be obtained by taking a sample by means of an acus, a needle, a spicule or the like. Further, many devices for obtaining blood are well-known in the context of dialyzers and kidney machines (e.g., syringes, venipuncture devices, vacutainer) and can be used without particular expert skills.

The body fluid obtained from a patient may be an analytical sample of a volume of less than 10 ml, less than 1 ml, less than 100 µl, less than 10 µl, or even less than 1 µl. An analytical sample may the consumed during the analytical procedure and/or may be discarded after the analytical procedure has been conducted.

Preferably, the obtained body fluid from which the sTNFR2 has been removed may be injected or recirculated into the patient the sample has been extracted from or into another patient, more preferably into the patient the sample has been extracted from.

In a preferred embodiment, the molecules binding to sTNFR2 are

(i) antibodies, preferably monoclonal antibodies, antibody fragments or antibody mimetics;
(ii) cofactors,
(iii) cytokines, in particular tumor necrosis factor alpha (sTNFSF2) or sTNFSF2 mutein;
(iv) synthetic polypeptides, in particular *Staphylococcus aureus* Protein A, human LDL receptor, lipocalin or fibronectin; and/or
(v) small molecules, in particular ensemblins, macrocyclic scaffolds, Aptamers and/or peptide staples;

in particular wherein the molecules binding to sTNFR2 are monoclonal antibodies, antibody fragments or single chain antibodies.

An antibody may be a monoclonal or a polyclonal antibody of any species or origin. It may bind to any epitope(s) comprised in the sTNFR2 polypeptide (e.g., linear epitope(s), structural epitope(s), primary epitope(s), secondary epitope(s)), including its posttranslational modifications. The epitope may be accessible by the antibody in the natural configuration of the sTNFR2 or may be a hidden epitope. Preferably, the epitope is accessible in the natural configuration of the sTNFR2. The antibodies may be of natural

origin, of gene technologic origin and/or of synthetical origin. Optionally, the antibody may also be a CovX antibody. Optionally, the antibody may also be a cameloid species antibody.

As used herein, the term "antibody fragments" may be understood in the broadest sense as any fragment of an antibody that still bears binding affinity to its target. Exemplarily, the antibody fragment may be a fragment antigen binding (Fab fragment), a truncated antibody comprising one or both complementarity determining region(s) (CDR(s)) or the variable fragment (Fv) of an antibody. The antibody fragments may be of natural origin, of gene technologic origin and/or of synthetical origin.

As used herein, the term "antibody mimetic" may be understood in the broadest sense as organic compounds that, like antibodies, can specifically bind antigens and that typically have a molecular mass in a range of from approximately 3 kDa to approximately 25 kDa. Antibody mimetics may be, e.g., Affibody molecules (Affibodies), Affilins, Affitins, Anticalins, Avimers, DARPins, Fynomers, Kunitz domain peptides, single-domain antibodies (e.g., V_HH antibodies or V_{NAR} antibodies) Monobodies, Diabodies, Triabodies, flexibodies and tandabs. The antibody mimetics may be of natural origin, of gene technologic origin and/or of synthetical origin.

The term "cofactor" may be understood in the broadest sense as a molecule binding to sTNFR2 in the natural environment to facilitate or enhance binding affinity or any other function thereof.

As used herein, the term "cytokine" may be understood in the broadest as any small protein, peptide, or glycoprotein secreted in the body to signal in intercellular communication, that may bind to sTNFR2, such as, e.g., tumor necrosis factor alpha (sTNFSF2).

As used throughout the invention, the term "mutein" refers to any mutated protein. The amino acid sequence of the mutein may comprise alterations or substitutions in the primary amino acid residues, may be truncated and/or may be elongated compared to the corresponding wildtype protein. The mutein may have more than 50%, preferably more than 60%, more preferably more than 70%, even more preferably more than 80%, even more preferably more than 90%, most preferably more than 95% sequence homology to the corresponding wildtype protein.

As used herein, the term "synthetic polypeptide" refers to any polypeptide that binds to sTNFR2. A synthetic polypeptide may be obtained from chemical synthesis or from gene technological processes. A synthetic polypeptide may be, e.g., *Staphylococcus aureus* Protein A, low-density lipoprotein (LDL) receptor, lipocalin or fibronectin.

As used herein, the term "small molecule" refers to any molecule that binds to sTNFR2 that has a molecular mass of less than 5000 Da, preferably less than 2000 Da, more preferably less than 1500 Da, even more preferably less than 1000 Da, even more preferably less than 800 Da. The small molecule may be, e.g., an ensemblin, a macrocyclic scaffold (e.g., an ensemblin), an Aptamer and/or a peptide staple. Further, the small molecule may also be any other small molecule. It may be obtained from a natural source and be identified in a screening process. It will be understood by a person skilled in the art, that the small molecule may also be conjugated to a polymeric scaffold, such as, e.g., hydroxypropyl methacrylamide (HPMA), polyethylene imine (PEI), carboxymethyl cellulose (CMC), polyethylene glycol (PEG), collagen fiber, a silk fiber, an agarose fiber, or a micro- or nanobead.

Most preferably, the device of the present invention may comprise monoclonal antibodies targeted against sTNFR2.

Further, the molecules binding to sTNFR2 may be selected from the group consisting of

(i) antibodies, preferably monoclonal antibodies, antibody fragments or antibody mimetics;
(ii) cofactors,
(iii) cytokines, in particular tumor necrosis factor alpha (TNFSF2) or TNFSF2 mutein;
(iv) synthetic polypeptides, in particular *Staphylococcus aureus* Protein A, human LDL receptor, lipocalin or fibronectin; and
(v) small molecules, in particular ensemblins, macrocyclic scaffolds, Aptamers and/or peptide staples;

in particular wherein the molecules binding to sTNFR2 are monoclonal antibodies, antibody fragments or single chain antibodies.

In a further preferred embodiment, the molecules binding to sTNFR2 are immobilized on

(i) a column, in particular an adsorbent column;
(ii) a filter, in particular a capillary membrane filter with a pore size of between about 0.04 and 0.05 μm or a parallel plate filter with a pore size of between about 0.04 and 0.08 μm;

(iii) semi-permeable material, preferably a membrane, in particular a dialysis membrane;

(iv) a bead, in particular a microbead or a nanobead; and/or

(v) the surface of said device, in particular the surface of a tubing of said device.

As used herein, the term "column" refers to any hollow chromatography device that is itself coated with a molecule binding to sTNFR2 and/or filled with a column matrix bearing a molecule binding to sTNFR2. As used herein, the term "column matrix" refers to any chromatographic material and may preferably be a bead material. The beads may be spherical or may bear any other shape known in the art to conduct chromatographic material. The beads may be of any material known in the art to be useful for preparing chromatographic material such as, e.g., silica, sugar-based bead material (e.g., agarose, sepharose), plastic bead material (e.g., polystyrene). The beads may bear a neutral, a positive or a negative zeta potential. The column may be a flow-through device or may be used batch-wise. When using a flow-through method, the column may be, e.g, an ultrapheresis column, a high performance liquid chromatography (HPLC) column, a fast protein liquid chromatography (FPLC) column, a flash chromatography (flash) column, a Rapid Refluid Liquid Chromatography (RRLC) column, a Rapid Separation Liquid Chromatography (RSLC) column, an Ultra Fast Liquid Chromatography (UFLC) column, an Ultra Performance Liquid Chromatography (UPLC) column or any other chromatography column known by those skilled in the art. Chromatography is widely used in protein purification. When the molecule binding to sTNFR2 is immobilized on the surface of the column device and/or on the column matrix an affinity chromatographic device is obtained. However, this device may concomitantly separate molecules by size exclusion chromatography, hydrophobicity chromatography, etc. The chromatographic methods may be combined with one or more filter(s), membrane(s) and/or semi-permeable material(s).

The term "filter" as used herein may refer to any device bearing pores. The pores may have an average pore size of an average pore size in the range of few nanometers or even less for separating high-molecular weight molecules, such as proteins, of different molecular weights from another or the pores may have an average pore size of few micrometers for separating solid particles such as cells (e.g., red blood cells (RBCs), leucocytes, tumor cells), from another and/or from the liquid compartments of a body fluid (e.g., blood or lymph). The filter may have a molecular exclusion size that excludes molecules of less than 1,000 Da, less than 10,000 Da, less than 25,000 Da, less than 50,000 Da, less than 75,000 Da, less than 100,000 Da less than 125,000 Da, less than 150,000 Da, less than

175,000 Da, less than 200,000 Da, less than 250,000 Da, or less than 300,000 Da. The filter may preferably be a capillary membrane filter with a pore size of between 0.001 and 10 μm, more preferably of between 0.01 and 1 μm, even more preferably of between 0.01 and 0.1 μm, even more preferably of between 0.01 and 0.09 μm, μm, even more preferably of between 0.01 and 0.08 μm, even more preferably of between 0.01 and 0.07, even more preferably of between 0.02 and 0.07 μm, even more preferably of between 0.02 and 0.06, even more preferably of between 0.03 and 0.06 μm, even more preferably of between 0.04 and 0.06 μm, and most preferably of between 0.04 and 0.05 μm. Alternatively, the a preferred filter may be a parallel plate filter with a pore size of between 0.001 and 10 μm, more preferably of between 0.01 and 1 μm, even more preferably of between 0.01 and 0.1 μm, even more preferably of between 0.01 and 0.09 μm, even more preferably of between 0.01 and 0.08 μm, even more preferably of between 0.01 and 0.07 μm, even more preferably of between 0.02 and 0.1 μm, even more preferably of between 0.02 and 0.09 μm, even more preferably of between 0.04 and 0.08 μm, even more preferably of between 0.04 and 0.07 μm, and most preferably of between 0.04 and 0.06 μm. Several filters with the same, similar or different pore size(s) may be combined with another. The filter may be any kind of filter. The filter may be of any material, such as, e.g., plastic (e.g., nylon, polysterene), metal, alloy, glass, ceramics, cellophane, cellulose, or composite material. The filter may be hydrophobic or hydrophilic. The surface of the filter may be neutral or positively charged or negatively charged. The filter may be a part of a plasmapheresis and/or an ultrapheresis device as known in the art (see, e.g., WO 99/61085 and WO 01/37873). The filter may be a dead-end filter. Alternatively, the filter may also be a cross-flow filter. As used herein, the terms "cross-flow filter", "crossflow filter", "tangential flow filter" may be understood interchangeably. Filtration may be conducted batch-wise of in a continuous flow method. Preferably, the filtration is conducted in a continuous flow method. The filter may be combined with one or more column(s), membrane(s) and/or semi-permeable material(s).

The term "membrane" as used in the context of the solid support may be understood in the broadest sense as any thin, flexible solid material. The membrane may contain pores and serve as filter. Then at least part of the fluid flow goes through the membrane. Alternatively, the membrane may also be impermeable. Then, the fluid flow passes by the membrane on which molecules binding to sTNFR2 are immobilized. Several or even numerous membranes may be used in one device and form a functional array of membranes. The membrane may be a dialysis membrane. Then the membrane is permeable for some molecules (in particular small molecules) (semi-permeable). Dialysis is based on diffusion and osmosis, respectively, and is well known in the art. Dialysis is widely used in protein purification and is also used to provide an artificial replacement for lost kidney

function in people with renal failure. The membrane may be of any material, such as, e.g., plastic (e.g., nylon, polysterene), metal, alloy, glass, ceramics, cellophane, cellulose, or composite material. The membrane may be hydrophobic or hydrophilic. The surface of the membrane may be neutral or positively charged or negatively charged. The membrane may be combined with one or more column(s), filter(s) and/or semi-permeable material(s).

The term "semi-permeable material" may refer to any material that allows the passage of some molecules whereas other molecules can not or nearly not pass. The semi-permeability can base, e.g., on size exclusion and/or on the charge of the semi-permeable material. The semi-permeable material may be a solid material. The semi-permeable material may be a membrane or a thick material and may be of any material, such as, e.g., plastic (e.g., nylon, polysterene), metal, alloy, glass, ceramics, cellophane, cellulose, or composite material. The semi-permeable material may be hydrophobic or hydrophilic. The surface of the semi-permeable material may be neutral or positively charged or negatively charged. The semi-permeable material may have a smooth or porous surface structure. The semi-permeable material may be combined with one or more column(s), filter(s) and/or membrane(s).

The term "bead" may refer to any small spherical particle. In particular a bead may be a microbead or a nanobead. A microbead typically bears an average diameter in the range of approximately from 1 μm and 1000 μm. A nanobead typically bears an average diameter in the range of approximately from 1 nm and 1000 nm. The bead may be of any material such as, e.g., of silica, of metal- or alloy-based material, of sugar-based material (e.g., agarose, sepharose), of plastic (e.g., polystyrene) or may be a quantumdot. The bead may be hydrophobic or hydrophilic. The surface of the bead may have a neutral, a positive or a negative zeta potential. The beads may be compact or may be porous. A bead may be used in a column or may be used in fluid. The beads may be combined with one or more column(s), filter(s), semi-permeable material(s) and/or membrane(s).

The term "tubing" may be understood as any hollow solid material through which a fluid may flow. It may be flexible or inflexible. A tubing may be column filed with a matrix, but may also be not filled with a matrix. Optionally, the tubing may be the tubing of a capillary electrophoresis (CE). The tubing may also be any kind of semi-permeable material. The tubing may be of any material, such as, e.g., plastic (e.g., nylon, polysterene), metal, alloy, glass, ceramics, cellophane, cellulose, or composite material and may be hydrophobic or hydrophilic. The surface of the tubing may be neutral, positively charged or negatively charged. A tubing may be combined with one or more column(s), filter(s), semi-permeable material(s) and/or membrane(s).

The term "surface of a device" may refer to any kind of device. In particular, the device may be a therapeutic, a medical, or a laboratory device. The surface of the device may be of any material, such as, e.g., plastic (e.g., nylon, polysterene), metal, alloy, glass, ceramics, cellophane, cellulose, or composite material. The surface of the device may be hydrophobic or hydrophilic. The surface of the device may be neutral or positively charged or negatively charged.

Further, the molecules binding to sTNFR2 may be immobilized on a solid support selected from the group consisting of

(i) a column, in particular an adsorbent column;
(ii) a filter, in particular a capillary membrane filter with a pore size of between about 0.04 and 0.05 μm or a parallel plate filter with a pore size of between about 0.04 and 0.08 μm;
(iii) semi-permeable material, preferably a membrane, in particular a dialysis membrane;
(iv) a bead, in particular a microbead or a nanobead; and
(v) the surface of said device, in particular the surface of a tubing of said device.

In a preferred embodiment, the device further comprises

(i) a primary filter to separate ultrafiltrate or plasma from the patient's blood;
(ii) a centrifuge to separate ultrafiltrate or plasma from the patient's blood;
(iii) a filter which removes components of a molecular weight of 120,000 Da or less from the blood, plasma or blood fraction;
(iv) a centrifuge which removes components of a molecular weight of 120,000 Da or less from the blood, plasma or blood fraction;
(v) means for administering radiation to patent's tissue; and/or
(vi) one or more cannula(s) and tubing(s) suitable for connecting the device to the patient.

As used herein, the term "primary filter" may be understood as a filter that is connected upstream to the solid support on which the sTNFR2-binding molecules are immobilized. The primary filter may protect said solid support from cells. When a primary filter is used, preferably only the flow-through of the primary filter, thus the ultrafiltrate, gets in contact with said solid support.

As used herein, the term "ultrafiltrate" may be understood in the broadest sense as a solution, emulsion or suspension from which parts of the high-molecular weight components have been removed. Preferably, the ultrafiltrate is a solution from which at least the blood cells (e.g., red blood cells (RBCs) and lymphocytes) have been removed. Even more preferably, also the cellular fragments such as e.g., platelets have been removed. When cells and cellular fragments have been removed from blood, the person skilled in the art will recognize plasma. Optionally, further one or more coagulation factors may be removed. Then, the person skilled in the art will recognize serum. Optionally, in the ultrafiltrate, also protein fraction(s) above a given size exclusion level may be removed. The size exclusion level may be more than 1,000 Da, more than 10,000 Da, more than 25,000 Da, more than 50,000 Da, more than 75,000 Da, more than 100,000 Da more than 125,000 Da, more than 150,000 Da, more than 175,000 Da, more than 200,000 Da, more than 250,000 Da, or more than 300,000 Da. Preferably, molecules of a molecular weight of more than 120,000 Da may be removed from the ultrafiltrate.

The ultrafiltrate may be generated by any means known in the art. Exemplarily, it may be generated by means of a filter or by centrifugation. Centrifugation may be any centrifugation of liquids known in the art. Centrifugation may be conducted batch-wise of in a continuous flow method. Preferably, the centrifugation is conducted in a continuous flow method. Filtration and centrifugation may be combined with another, in particular in a continuous flow method, or may be combined with any other method known in the art. Optionally, centrifugation may be gradient centrifugation (e.g., sucrose gradient centrifugation, equilibrium centrifugation).
Before the body fluid is recirculated into the patient, the cells and/or protein faction(s) may optionally be reunited with the ultrafiltrate from which the sTNFR2 has been removed. Preferably, at least most of the cells are added to the purified ultrafiltrate prior to being reinjected into the patient.

The term "administering radiation to patent's tissue" may comprise, but may not be limited to expose the patient, a patient's limb, a patient's organ, or a patient's tissue to any kind of radiation used for therapeutic purposes, such as, e.g., x-ray radiation, ultraviolet (UV) radiation (e.g., UV-A, UV-B, and/or UV-C radiation), alpha radiation, beta radiation, gamma radiation, or cosmic radiation. Radiation therapy may also include, but may not be limited to Intensity-Modulated Radiation Therapy (IMRT), 3-Dimensional Conformal Radiotherapy (3DCRT), Stereotactic body radiation therapy (SBRT), Stereotactic radiosurgery (SRS), image-guided radiation therapy (IGRT), Particle Therapy (e.g, proton therapy), Brachytherapy, Radioisotope Therapy (RIT) (e.g., with iodine-131, lutetium-177,

strontium-89 and samarium (^{153}Sm) lexidronam and/or yttrium-90). Preferably, a tumor and/or metastases of the patient are subjected to radiation.

Further, the device may further comprise a technical unit selected from the group consisting of

(i) a primary filter to separate ultrafiltrate or plasma from the patient's blood;
(ii) a centrifuge to separate ultrafiltrate or plasma from the patient's blood;
(iii) a filter which removes components of a molecular weight of 120,000 Da or less from the blood, plasma or blood fraction;
(iv) a centrifuge which removes components of a molecular weight of 120,000 Da or less from the blood, plasma or blood fraction;
(v) means for administering radiation to patent's tissue; and
(vi) one or more cannula(s) and tubing(s) suitable for connecting the device to the patient.

In a further preferred embodiment of the present invention, the device further has one or more molecules binding to other plasma protein(s) immobilized on a solid support, preferably wherein said molecules are molecules binding to cytokines, chemokines, soluble cytokine receptors, soluble chemokine receptors, other soluble decoy receptors, angiogenic factors, growth factors, and bone morphogenic factors more preferably molecules binding to soluble interleukin receptors (sILRs), in particular molecules binding to soluble interleukin-2 receptor alpha (sIL-2R).

As used herein, the terms "soluble interleukin-2 receptor alpha", "soluble IL-2 receptor alpha", "sIL-2R", "sIL2aR", "sIL2αR", "sIL2-R", "sIL2-RA" and "CD25 alpha" may be understood interchangeably.

As used herein, the term "other plasma protein" may be understood in the broadest sense as any protein found in blood plasma that may modulate an immune response and/or may control cell growth, angiogenesis and/or apoptosis, except for sTNFR1. Exemplarily, said plasma protein may be a cytokine. Exemplarily, a cytokine may be an interleukin (IL) (e.g., IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9 IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, macrophage-inhibitory factor-1), a lymphokine (e.g., granulocyte-macrophage colony-stimulating factor (GM-CSF), an interferon (e.g., IFN-α, IFN-β, IFN-γ), a chemokine (e.g., a CCL, a CXCL, an XCL, growth-related oncogene-alpha, CX3CL or a monocyte chemoattractant protein (MCP)). An "other plasma protein" may also be a soluble cytokine receptor (e.g., sIL-2R, or the soluble interferon-gamma receptor), a colony stimulating

factor (e.g., M-CSF, G-CSF), an angiogenic factor, a growth factor (e.g., TGF-beta 1, TGF-beta 3, HGF, VEGF, basic FGF, IGF-1, IGF-2, PDGFR, EGF, CTGF), a bone morphogenic factor (e.g., osteoprotegerin, osteopontin, macrophage inhibitory cytokine-1 other soluble decoy receptors (e.g., soluble VCAM-1, soluble E-selectin, soluble P-selectin, soluble E-cadherin, soluble ILT-3, soluble MICA, soluble MICB, sULBP2, sICAM-1, soluble Fas) or one or more mutein(s) of the aforementioned. In particular, the plasma protein may be a molecule binding to sIL-2R.

In a preferred embodiment, the device has been sterilized and/or endotoxin has been removed from the device.

Sterilization may lead to a decreased concentration of pathogens and/or microorganisms such as, e.g., bacteria, viruses, fungals, moulds, bacterial spores, fungal spores, mould spores, worms, worm eggs, or eukaryotic protocoa. Sterilization may be accomplished by any means known in the art such as, e.g., employment of radiation (e.g., UV radiation, gamma radiation) or one or more antimicrobic agent(s) known in the art.

An endotoxin may be any endotoxin known in the art, such as e.g., lipopolysaccharide (LPS) or delta endotoxin. Typically, an endotoxin originates from the cell wall of a gram-negative bacterium.

In a second aspect, the present invention refers to a method for the removal of sTNFR2 from blood, plasma or a blood fraction, wherein molecules binding to sTNFR2, but not binding to sTNFR1, are immobilized on a solid support and brought into contact with the blood, plasma or a blood fraction and wherein sTNFR1 is not removed.

As used herein, the term "removal" may be understood in the broadest sense as the depletion or decrease of the concentration of sTNFR2. After removal, the concentration of the sTNFR2 may be decreased to less than 50%, less than 25%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, or less than 0.1% of the initial concentration. The initial concentration may be the concentration found in any fluid, in particular the concentration naturally found in a body fluid.

Most preferably, the molecule binding to sTNFR2 may be a monoclonal antibody targeted against sTNFR2.

In the context of the present invention, the molecule binding to sTNFR2 is not binding to sTNFR1. As used herein, the term "not binding to" refers to a binding affinity of the sTNFR2-binding molecule to sTNFR1 that is more than 5fold, more than 10fold, more than 50fold, more than 100fold, more than 500fold, more than 1000fold, or more than 10000fold weaker than to sTNFR2.

The solid support may not comprise any molecules binding to sTNFR1 with an binding affinity of a Kd of less than 10 nM, more preferably less than 100 nM, even more preferably less than 250 nM, even more preferably less than 500 nM, even more preferably less than 750 nM, even more preferably less than 1 μM, even more preferably less than 2 μM, even more preferably less than 5 μM, even more preferably less than 10 μM, even more preferably less than 50 μM, even more preferably less than 100 μM.

However, the solid support that bears the sTNFR2-binding molecules may further bear molecules binding to other plasma proteins except molecules binding to sTNFR1.

In a preferred embodiment, the method is conducted by means of a device of the present invention.

In a further preferred embodiment, sTNFR2 is removed from a patient's blood, plasma or blood fraction *ex vivo* in an extracorporeal device.

In a further preferred embodiment, the patient is suffering from cancer.

As used herein, the term "suffering from" may be understood in the broadest sense as having a certain disease. The patient may or may not bear any symptoms of said disease. The disease may be any disease, in particular a tumorous disease, cancer. The patient may or may not show any further symptoms caused by increased sTNFR2 level such as faster tumor growth, depleted immune response against tumorous or neoplastic tissue.

As used herein, the term "cancer" as used herein may be understood in the broadest sense as any type of a cancerous, neoplastic and/or tumorous disease. It may refer to the formation of malignant, semi-malignant, pre-malignant or benignant neoplastic tissue, herein designated as "tumor" or may lead to the formation of a malignant cell in suspension. A tumor may be any kind of tumor, such as a solid tumor or a metastasis. The tumor may be a primary tumor or a secondary tumor. As used herein, the terms "tumor", "cancerous swelling", "neoplasia", "lesion" and "carcinoma *in situ*" may be understood interchangeably. The tumor may be a benign, pre-malignant or malignant tumor. The

tumor may origin from any tissue that can develop a tumor, such as, e.g., sarcoma (e.g., myxosarcoma, histiocytoma, liposarcoma, chondrosarcoma, osteosarcoma, angiosarcoma, leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma, synovialcarcinoma, Ewing sarcoma, hemangiosarcoma, lymphangiosarcoma, adenosarcoma, carciosarcoma), bronchiocarcinoma, prostate carcinoma, cervix carcinoma, ovarian carcinoma, mamma carcinoma, bronchial carcinoma, melanoma, esophagus carcinoma, rectal carcinoma, pancreas carcinoma, bladder carcinoma, kidney carcinoma, blastoma (e.g., hepatoblastoma, retinoblastoma, nephroblastoma, neuroblastoma, medulloblastoma, glioblastoma), head and neck tumor, cerebral tumor, lymphoma (e.g., Hodgkin lymphoma, Non-Hodgkin lymphoma, multiple lymphoma, malignant lymphoma), fibroma (e.g., adenofibroma, neurofibroma), leukemia (e.g., acute myeloic leukemia, chronic myeloic leukemia), endothelioma (e.g., hemangioendothelioma), adenoma (e.g., fibroadenoma), semioma, dysgerminoma, luteoma, mesenchymal tumors, granulose cell tumor, ymphangioma, meningeoma, mesothelioma, schwannoma, chorionepitheliom, hepatoma, leukemia, teratoma, basaliom, neurodoctrine tumors, insulinoma, pheochromocytoma, melanoma, astrocytoma, anaplastic meningeoma, carcinoides, and/or (aggressive) fibromatosa.

The cancer may be located in any organ, such as, e.g., brain, eye, skin, lung, liver, oral cavity, pharynx, stomach, small intestine, duodenum, colon, urinary bladder, kidney, gall bladder, pancreas, vaginal tract, testicles, breast, a muscle, blood, lymph, a lymph node, esophagus, heart, a bone, bone marrow, spleen, a blood vessel, a lymphatic vessel, cervix, uterus, spinal cord, or an ovary or may be located in connective tissue, mucosa tissue, or nerve tissue.

Alternatively, the patient who is treated is suffering from another disease associated with an increased sTNFR2 level. As used in the context of the present invention, the term "increased sTNFR2 level" may be understood in the broadest sense as an elevated concentration of sTNFR2 in a body fluid when compared to the sTNFR2 level found throughout the population. The increased sTNFR2 level may be a sTNFR2 level that is more than 1.5fold, more than 2fold, more than 5fold, more than 10fold, more than 50fold, or even more than 100fold higher than the sTNFR2 level found throughout the population.

In a preferred embodiment, the method is combined with the removal of one or more other plasma protein(s), preferably cytokines, chemokines, soluble cytokine receptors, other soluble decoy receptors, angiogenic factors, growth factors, and bone morphogenic factors more preferably molecules binding to sILRs, in particular molecules binding to sIL-2R.

A further aspect of the present invention refers to a molecule specifically binding to sTNFR2, but not binding to sTNFR1, for use in a method for treating the human or animal body.

Most preferably, this molecule may be a monoclonal antibody targeted against sTNFR2.

In a preferred embodiment, the treating is treating cancer.

In a further preferred embodiment, the molecule is used in a method of the present invention.

In a preferred embodiment, the molecule is part of a device of the present invention or is immobilized on a bead or an implant, preferably wherein said bead or implant is in contact with the blood stream, the lymph or the extracellular matrix, in particular in contact with the blood stream.

The term "blood stream" may be understood in the broadest sense as circulating blood. The blood stream may be in a vessel of a patient or may be in a tube, a filter, a column or any other device outside of the body.

In a preferred embodiment, the method further comprises the removal of one or more other plasma protein(s), preferably cytokines, chemokines, soluble cytokine receptors, other soluble decoy receptors, angiogenic factors, growth factors, and bone morphogenic factors more preferably molecules binding to sILRs, in particular molecules binding to soluble interleukin-2 receptor alpha (sIL-2R).

In a further aspect, the present invention refers to blood, plasma or a blood fraction obtainable from a method of the present invention for use in a method for treating cancer.

The blood, plasma or a blood fraction of which sTNFR2 has been removed may be injected or recirculated into the patient by any means known in the art. For instance, the sample may be injected by means of a tubing and/or an acus, a needle, a spicule or the like. These means are well-known in the context of dialyzers and kidney machines and can be used without particular expert skills.

As the method of the present invention removes sTNFR2 from a blood, plasma or a blood fraction, the method may have an impact on the whole organism systemically, independent on where the tumor is located. Therefore, the method is independent on barriers, such as

the blood-brain barrier, the blood-testis barrier, the blood-milk barrier or the mother-embryo/fetus barrier. Therefore, the method may also enable the systemic treatment of target tissue beyond the barrier. A patient suffering from a brain disease such as, e.g., e brain tumor (e.g., an astrocytome, or a glioblastome) may be treated by systemic treatment without targeting the brain directly. A fetus or embryo may be treated indirectly by treating the pregnant mother systemically. As used herein, the terms "systemic treatment" or "treating systemically" may be understood in the broadest sense as the treatment of a subject in total. Exemplarily, a body fluid such as, e.g., blood, may be extracted somewhere from the patient's body (e.g., from the basilica vein), conducted through the extracorporeal device of the present invention and, subsequently, being injected or recirculated somewhere in the patient's body (e.g., recirculated in the same or the other basilica vein), whereas the tissue to be treated (e.g., cancer tissue) may be located somewhere else in the patient's body (e.g., somewhere in the inner organs). Nevertheless, the treatment may be successful as a body fluid such as blood is circulating through the entire body of the patient. A treatment may be successful when the local concentration, but also when the overall concentration of sTNFR2 in the patient is depleted.

The treatment as described herein may be the sole treatment of the patient or may be combined with one or more other treatment(s) of the patient. Additional treatment may be any treatment known in the art, such as, e.g., therapy with one or more chemotherapeutic(s), other anti cancer drug(s) and/or agent(s) (e.g., alkylating agents (e.g., mechlorethamine, cyclophosphamide, chlorambucil, Ifosfamide), anti-metabolites (e.g., 5-fluorouracil, azathioprine, 6-mercaptopurine, mercaptopurine, pyrimidines, thioguanine, fludarabine, floxuridine, cytosine arabinoside (cytarabine), pemetrexed, raltitrexed, pralatrexate, methotrexate), plant alkaloids and terpenoids (e.g., vinca alkaloids (vincristine, vinblastine, vinorelbine, vindesine), taxanes (e.g., paclitaxel), cytoxan), topoisomerase inhibitors (e.g., camptothecins: irinotecan, topotecan, etoposide, etoposide phosphate, teniposide), melphalan, antineoplastica (e.g., doxorubicin (adriamycin), doxorubicin lipo, epirubicin, bleomycin)), actinomycin D, aminoglutethimide, amsacrine, anastrozole, antagonists of purine and pyrimidine bases, anthracyclines, aromatase inhibitors, asparaginase, antiestrogens, bexarotene, buserelin, busulfan, camptothecin derivatives, capecitabine, carmustine, a platin (e.g., cisplatin, carboplatin, oxaliplatin), cladribine, cytarabine, cytosine arabinoside, alkylating cytostatics, dacarbazine, daunorubicin, docetaxel, epirubicin, estramustine, etoposide, exemestane, fludarabine, fluorouracil, folic acid antagonists, formestane, gemcitabine, glucocorticoids, goserelin, hormones and hormone antagonists, hycamtin, hydroxyurea, idarubicin, irinotecan, letrozole, leuprorelin, lomustine, mercaptopurine, miltefosine, mitomycins, mitosis inhibitors, mitoxantrone, nimustine, procarbazine, tamoxifen, temozolomide, teniposide,

testolactone, thiotepa, topoisomerase inhibitors, treosulfan, tretinoin, triptorelin, trofosfamide, cytostatically active antibiotics, everolimus, pimecrolimus, tacrolimus, azithromycin, spiramycin, sirolimus (rapamycin), roxithromycin, ascomycin, bafilomycin, erythromycin, midecamycin, josamycin, concancamycin, clarithromycin, troleandomycin, folimycin, tobramycin, mutamycin, dactinomycin, dactinomycin, rebeccamycin, a statin (e.g., cerivastatin, simvastatin, lovastatin, somatostatin, fluvastatin, nystatin, rosuvastatin, atorvastatin, pravastatin, pitavastatin, pentostatin,), 4-hydroxyoxycyclophosphamide, bendamustine, thymosin α-1, aclarubicin, fludarabine-5'-dihydrogen phosphate, hydroxycarbamide, aldesleukin, pegaspargase, cepharanthine, epothilone A and B, azathioprine, mycophenolate mofetil, c-myc antisense, b-myc antisense, betulinic acid, camptothecin, melanocyte stimulating hormone (α-MSH), activated protein C, IL-1β inhibitor, fumaric acid and esters thereof, dermicidin, calcipotriol, taclacitol, lapachol, β-lapachone, podophyllotoxin, betulin, podophyllic acid 2-ethyl hydrazide, sagramostim, (rhuGM-CSF), peginterferon α-2b, lenograstim (r-HuG-CSF), filgrastim, macrogol, cephalomannine, selectin (cytokine antagonist), CETP inhibitor, cadherins, cytokinin inhibitors, COX inhibitor (COX-2 or COX-3 inhibitor), angiopeptin, ciprofloxacin, fluroblastin, bFGF antagonists, probucol, prostaglandins, 1,11-dimethoxyeanthin-6-one, 1-hydroxy-11-methoxycanthin-6-one, scopoletin, colchicine, NO donors, pentaerythrityl tetranitrate, sydnonimines, S-nitroso derivatives, staurosporine, β-estradiol, α-estradiol, estriol, estrone, ethinyl estradiol, fosfestrol, medroxyprogesterone, estradiol cypionates, estradiot benzoates, tranilast, kamebakaurin, verapamil, ciclosporin A, paclitaxel and derivatives thereof such as 6-α-hydroxy paclitaxel, baccatin, taxotere, mofebutazone, acemetacin, diclofenac, lonazolac, dapsone, o-carbamoyl-phenoxy-acetic acid, lidocaine, ketoprofen, mefenamic acid, piroxicam, meloxicam, chloroquine phosphate, penicillamine, hydroxychloroquine, auranofin, sodium aurothiomalate, oxaceprol, celecoxib, β-sitosterol, ademetionine, myrtecaine, polidocanol, nonivamide, levomenthol, benzocaine, aescin, elipticine, D-24851 (Calbiochem), colcemid, cytochalasin A-E, indanocine, nocodazole, bacitracin, vitronectin receptor antagonists, azelastine, free nucleic acids, nucleic acids incorporated into virus transmitters, DNA and RNA fragments, plasminogen activator inhibitor-1, plasminogen activator inhibitor-2, antisense oligonucleotide, VEGF inhibitors, IGF-1, active agents from the group of antibiotics such as cefadroxil, cefazolin, cefaclor, cefoxitin, gentamicin, penicillins, dicloxacillin, oxacillin, sulfonamides, metronidazole, antithrombotics, argatroban, aspirin, abciximab, synthetic antithrombin, bivalirudin, coumadin, enoxaparin, GpIIb/IIIa platelet membrane receptor, antibodies to factor X_a inhibitor, heparin, hirudin, r-hirudin, PPACK, protamine, prourokinase, streptokinase, warfarin, urokinase, vasodilators, dipyramidole, trapidil, nitroprussides, PDGF antagonists, triazolopyrimidine, seramin, ACE inhibitors, captopril, cilazapril, lisinopril, enalapril, losartan, thioprotease inhibitors, prostacyclin,. vapiprost, interferon α, β and γ, histamine

antagonists, serotonin blockers, apoptosis inhibitors, apoptosis regulators, NF-kB or Bcl-xL antisense oligonucleotides, halofuginone, nifedipine, tocopherol, molsidomine, tea polyphenols, epicatechin gallate, epigallocatechin gallate, boswellic acids and derivatives thereof, leflunomide, anakinra, etanercept, sulfasalazine, tetracycline, triamcinolone, procainimide, retinoic acid, quinidine, disopyramide, flecainide, propafenone, sotalol, amiodarone, natural and synthetically obtained steroids such as bryophyllin A, inotodiol, maquiroside A, mansonine, strebloside, hydrocortisone, betamethasone, dexamethasone, fenoprofen, ibuprofen, indomethacin, naproxen, phenylbutazone, acyclovir, ganciclovir, zidovudine, antimycotics, clotrimazole, flucytosine, griseofulvin, ketoconazole, miconazole, terbinafine, chloroquine, mefloquine, quinine, natural terpenoids, hippocaesculin, barringtogenol-C21-angelate 14-dehydroagrostistachin, agroskerin, agrostistachin, 17-hydroxyagrostistachin, ovatodiolids, 4,7-oxycycloanisomelic acid, baccharinoids B1, B2, B3 and B7, tubeimoside, bruceanol A, B and C, bruceantinoside C, yadanziosides N and P, isodeoxyelephantopin, tomenphantopin A and B, coronarin A, B, C and D, ursolic acid, hyptatic acid A, zeorin, iso-iridogermanal, maytenfoliol, effusantin A, excisanin A and B, longikaurin B, sculponeatin C, kamebaunin, leukamenin A and B, 13,18-dehydro-6-alpha-senecioyloxychaparrine, taxamairin A and B, regenilol, triptolide, cymarin, apocymarin, aristolochic acid, anopterin, hydroxyanopterin, anemonin, protoanemonin, berberine, cheliburin chloride, cicutoxin, sinococuline, combrestatin A and B, cudraisoflavone A, curcumin, dihydronitidine, nitidine chloride, 12-beta-hydroxypregnadiene-3,20-dione bilobol, ginkgol, ginkgolic acid, helenalin, indicine, indicine-N-oxide, lasiocarpine, inotodiol, glycoside 1a, justicidin A and B, larreatin, malloterin, mallotochromanol, isobutyrylmallotochromanol, marchantin A, maytansine, lycoridicin, margetine, pancratistatin, liriodenine, bisparthenolidine, oxoushinsunine, aristolactam-AII, periplocoside A, ghalakinoside, deoxypsorospermin, psychorubin, ricin A, sanguinarine, manwu wheat acid, methylsorbifolin, chromones of spathelia, stizophyllin, akagerine, dihydrousambaraensine, hydroxyusambarine, strychnopentamine, strychnophylline, usambarine, usambarensine, daphnoretin, lariciresinol, methoxylariciresinol, syringaresinol, umbelliferone, afromoson, acetylvismione B, desacetylvismione A, vismione A and B), radiation therapy (e.g, Intensity-Modulated Radiation Therapy (IMRT), 3-Dimensional Conformal Radiotherapy (3DCRT), Stereotactic body radiation therapy (SBRT), Stereotactic radiosurgery (SRS), image-guided radiation therapy (IGRT), Particle Therapy (e.g, proton therapy), Brachytherapy, Radioisotope Therapy (RIT) (e.g., with iodine-131, lutetium-177, strontium-89 and samarium (^{153}Sm) lexidronam and/or yttrium-90)), antiangiogenic therapy (e.g., carboxyamidotriazole, TNP-470, CM101, , Suramin, SU5416, Thrombospondin, VEGFR antagonists, angiostatic steroids + heparin, Cartilage-Derived Angiogenesis Inhibitory Factor, matrix metalloproteinase inhibitors, 2-methoxyestradiol, Tecogalan,

tetrathiomolybdate, thalidomide, thrombospondin, soluble VEGFR-1 and NRP-1, Angiopoietin 2, angiostatin (e.g., TSP-1 and TSP-2 angiostatin), endostatin, vasostatin, canstatin, calreticulin, platelet factor-4, TIMP and CDAI, Meth-1 and Meth-2, CXCL10prothrombin (kringle domain-2), antithrombin III fragment prolactin, VEGI, SPARC, osteopontin, maspin, proliferin-related protein, restin), kinase inhibitors (e.g., imatinib, imatinib mesylate, gefitinib, erlotinib, pazopanib, apatinib), polyclonal or monoclonal antibodies (e.g., rituximab, trastuzumab, cetuximab, bevacizumab, basiliximab, daclizumab), proteasome inhibitors (e.g., bortezomib), cytokine or hormone therapy (e.g., selective estrogen receptor modulator tamoxifen, or with IFN-α, IFN-β, IFN-γ, IL-4, IL-12, IL-18, platelet factor-4)), PARP inhibitors (e.g., iniparib, olaparib) and/or combinations of two or more thereof.

The therapy may also include the oral intake of an agent or a pharmaceutically acceptable composition thereof, the injections of an agent or a pharmaceutically acceptable composition thereof, the perfusion of an agent or a pharmaceutically acceptable composition thereof. Further, the removal of sTNFR2 may be combined with any surgical procedure known in the art such as, e.g., the section or resection of the tumor, the amputation of a limb, a breast, a testicle, or a certain tissue or parts thereof. Moreover, the removal of sTNFR2 may be combined with radiation therapy.

All embodiments described above also apply to the following aspects of the present invention, because also the following aspects relate to the removal of sTNFR2 from the blood of tumor patients. Also for the following aspects, it is preferred that sTNFR2 is removed specifically from the blood of the patient, as discussed in detail above. However, it is equally possible to apply methods like ultrapheresis which unspecifically remove sTNFR2 from the blood.

Furthermore, it is also included with the following aspects of the invention that in addition to sTNFR2, also other soluble molecules including but not limited to soluble TNF receptor 1 (sTNFR1) or soluble IL-2 receptor (sIL-2R) are removed from the blood from the patient. Such methods are e.g. described in WO2005/107802.

In a preferred embodiment of the following aspects, sTNFR1 and sTNFR2 are removed from the blood of the patients. In a further preferred embodiment, sTNFR1, sTNFR2 and sIL-2R are removed from the blood of the patients.

In a further aspect, the present invention also relates to a method for sensitizing a tumor patient to a treatment with a chemotherapeutic agent, comprising the step of removing soluble TNF receptor 2 (sTNFR2) from the blood of said patient.

As described in Example 2, the present inventors have found that by removing soluble TNF receptor 2 from the blood, the treated subject is sensitized for the treatment with a chemotherapeutic agent. Consequently, by removing sTNFR2 from the blood from the patient, it is possible to reduce the dose of the chemotherapeutic agent, which reflects an important improvement in tumor treatment.

Any of numerous chemotherapeutic drugs can be used in the methods or uses of the invention. These compounds fall into several different categories, including, for example, alkylating agents, antineoplastic antibiotics, antimetabolites, and natural source derivatives.

Examples of alkylating agents that can be used in the invention include busulfan, caroplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide (i.e., cytoxan), dacarbazine, ifosfamide, lomustine, mecholarethamine, melphalan, procarbazine, streptozocin, and thiotepa.

Examples of antineoplastic antibiotics include bleomycin, dactinomycin, daunorubicin, doxorubicin, idarubicin, mitomycin (e.g., mitomycin C), mitoxantrone, pentostatin, and plicamycin.

Examples of antimetabolites include fluorodeoxyuridine, cladribine, cytarabine, floxuridine, fludarabine, flurouracil (e.g., 5-fluorouracil (5FU)), gemcitabine, hydroxyurea, mercaptopurine, methotrexate, and thioguanine.

Examples of natural source derivatives include docetaxel, etoposide, irinotecan, taxanes (e.g. paclitaxel), teniposide, topotecan, vinblastine, vincristine, vinorelbine, prednisone, and tamoxifen.

Additional examples of chemotherapeutic agents that can be used in the invention include asparaginase and mitotane.

Furthermore, also C2 ceramide can be used.

In an especially preferred embodiment, the chemotherapeutic drug is selected from the group consisting of actinomycin-D, mitomycin C, cisplatin, doxorubicin, etoposide, verapamil, podophyllotoxin, 5-FU, taxans such as paclitaxel, and carboplatin.

sTNFR2 can be removed by any means known to the person skilled in the art. Preferably, sTNFR2 is removed with the help of a molecule binding to sTNFR2. Such molecules are discussed in detail above. Furthermore, such molecules also include molecules that do not bind specifically to sTNFR2, as e.g. polyclonal antibodies recognizing soluble TNF receptor 2 and soluble TNF receptor 1.

In a preferred embodiment, said molecule is an antibody against sTNFR2, preferably a monoclonal antibody against sTNFR2. Such antibodies have also been described above.

In a preferred embodiment of the present invention, the sTNFR2 is removed with the help of a device of the invention.

The invention further relates to a molecule binding to sTNR2 for use in a method for sensitizing a tumor patient to a treatment with a chemotherapeutic agent. Such molecules have been described in detail above, and all these embodiments also apply to this aspect of the invention.

In a preferred embodiment, the molecule is an antibody against sTNFR2, preferably a monoclonal antibody against sTNFR2.

The invention further relates to a method for treating a tumor in a patient, comprising administering to a patient a chemotherapeutic drug in an amount sufficient for the treatment of the tumor, wherein from the blood of said patient sTNFR2 has been removed.

The treatment of tumors with chemotherapeutic agents is a standard procedure known to the person skilled in the art. All embodiments defined above also apply to this aspect of the invention. Examples of chemotherapeutic agents which can be used in the context of the present invention are given above.

Furthermore, the present invention also relates to a chemotherapeutic agent for use in a method for treating a tumor in a patient, wherein from the blood of said patient sTNFR2 has been removed. All embodiments discussed above also apply to this aspect of the invention.

The invention is further explained by the following example and figures, which are intended to illustrate, but not limit the scope of the present invention.

Figures

Figure 1 shows the treatment scheme.

Figure 2 shows the apheresis equipment. In the first part of the system (single arrow, ⟶), blood was pumped from the animal (*A. carotis*) via a plasma separator and retraced to the animal by a venous catheter. The separated plasma entered the second circuit (double-lined arrow, ⟹) and passed the adsorber before it was returned to the rat (*V. jugularis*).

Figure 3 shows the mean tumor diameter in tumor-bearing Brown Norway rats treated with Sham apheresis (dashed line, open circles) or sTNFR2 apheresis (solid line, filled squares), respectively, and 0.25 mg/kg melphalan. The results of two independent study parts, study part I (3A) and study part II (3B), are shown.

Figure 4 shows the change of mean tumor diameter during course of treatment of tumor-bearing Wag/Rij rats treated with **Sham** apheresis and 0.25 mg/kg **low** dose melphalan (**Sham low,** solid line, diamonds) or **sTNFR2** apheresis (**sTNFR2 low**, dotted line, squares), respectively Sham apheresis or sTNFR2 apheresis at **high** melphalan concentrations of 0,50 mg/kg (**Sham high** dotted line, triangles, respectively **sTNFR2 high**, solid line, circles)

Figure 5 shows the body weight of Wag/Rij rats during the course of treatment with **low** doses of melphalan (0,25 mg/kg), **Sham** apheresis (**Sham low**, solid line, diamonds), **sTNFR2** apheresis (**sTNFR2 low**, dotted line, squares) and with high doses of melphalan (0,50 mg/kg) and Sham apheresis (**Sham high**, dotted line, triangles), respectively sTNFR2 apheresis /**sTNFR2 high**, solid line, circles).

Examples

Example 1

Example 1 shows the influence of the removal of sTNFR2 in Brown Norway rats bearing a non-immunogenic sarcoma BN 175 on tumor growth in the presence of systemically administered melphalan.

1. Aim

The project aimed to reveal the influence of sTNFR2 on the promotion of tumor growth by removing sTNFR2 using plasmapheresis under the presence of melphalan treatment (0.25 mg/kg twice per week). Melphalan is a chemotherapeutic drug that belongs to the class of nitrogen mustard alkylating agents. The effect of the adsorption of the following factors on tumor growth was investigated in the presence of melphalan:

1. Sham adsorption (no antibodies were coupled to the column, mock column); and
2. adsorption of TNF receptor 2 (sTNFR2) alone.

2. Material and Methods

2.1. Animals

Brown Norway rats were purchased from Charles River Laboratories with a mean body weight of 200 g (N = 10 for study part I and N=14 for study part II). The animals were about 10 weeks old. As soon as the animals arrived in the test facility, they were housed individually in separated cages under following conditions:

- Temperature of 21+2°C
- Relative humidity (45-65%)
- 12-h/12-h dark/light cycle (lights on at 06:00 AM)
- *Ad libitum* access to tap-water and commercial pelleted laboratory chow (Art. No. V1536; Ssniff® Spezialdiäten GmbH, D-59494 Soest, Germany)

During the study strict hygiene conditions were maintained. One to two weeks of acclimatization were allowed. During this period, the rats were periodically handled in order to limit animal stress during the study phase.

2.2. Preparation of Columns

NHS activated 1 ml Sepharose columns was purchased from GE Healthcare (Order No. 17-0716.01). Based on the amount of antibodies used for human treatment using the OncoSorb® column, 0.12 mg of affinity purified goat anti-mouse sTNFR2 antibodies (purchased from R&D Systems Order No. AF-426-PB) were coupled according to the

manufacturer's procedure to NHS activated Sepharose. The antibodies were able to remove secreted rat sTNFR2 molecules out of rat plasma as shown by affinity purification of the receptor from rat plasma.

2.3. Design

The study was performed in two parts:
Part I: October - November 2010 and part II: November - December 2010):

Tumor tissue transfer and catheter insertion:

On the day of tumor tissue implantation (day -x), tumor material from a single sarcoma-bearing donor rat was taken and washed in RPMI 1640 culture medium. Then, the non-immunogenic Brown Norway 175 sarcoma tissue pieces (approx. 1 mm^3) were implanted under the skin of the Brown Norway rats to induce cancer growth. The method described by Prof. ten Hagen from Rotterdam was employed). The day of tumor tissue transfer was October 20, 2010, for study part I and November 22, 2010, for study part II. Together with tumor tissue implantation or 1-2 days thereafter, chronical arterial and venous catheters were inserted under general anesthesia (i.p. injection of 0.8 mg Rompun®, Bayer and 4 mg Ketamin®, Sanofi, Germany) into the *Arteria carotis* and the *Vena jugularis*. Catheters were flushed three times per week with heparinized saline during the study.

Follow-up monitoring and treatment

After nearly one week recovery (in part I 9.4 ± 1.3 days and in part II 7.9 ± 0.7 days after tumor tissue transfer) when the tumor had reached an average size of 11 mm +/- 1 mm in diameter, the first apheresis treatment was commenced to treat the growing tumor (day 0). This time point for the first apheresis was chosen based on the knowledge that tumors of this size - >11 ± 1 mm in diameter - exhibit a very fast growth rate doubling their volume every two to three days. Each animal was treated up to 4 times by plasmapheresis, The time between two plasmapheresis treatments was 2-5 days. During each plasmapheresis treatment, plasma was pumped through the adsorber for about one hour. The adsorbers were columns containing affinity purified goat anti-mouse sTNFR2 antibodies. The adsorbers for the plasmapheresis procedure were obtained from BioPheresis GmbH (Heidelberg, Germany) and had a volume of 1 ml.

Part I of the Study

In part I of the study, catheters and sarcoma tissue were implanted in rats randomized into two groups consisting of two animals each. Animals of each group were treated with 0.25 mg/kg melphalan i.v. twice per week. In addition, the animals were treated with the following plasmapheresis procedures:

Group 1 (**Sham**, N=2):	Sham plasmapheresis (without anti-mouse-TNFR2 antibodies)
Group 2 (**sTNFR2**, N=2):	Plasmapheresis using a 1 ml column containing 120 µg affinity-purified goat anti-mouse-TNFR2 antibodies

Part II of the Study

Under consideration of the results obtained in part I of the study, part II of the study was performed, using Brown Norway rats bearing identical sarcomas (BN-175). Before the first apheresis treatment began, rats which had tumors of 11 ± 2 mm in diameter) and which had functional catheters were divided into the following two groups:

Group 1 (**Sham**, N=3):	Sham plasmapheresis (without anti-mouse-TNFR2 antibodies)
Group 2 (**sTNFR2,** N=2):	Plasmapheresis using a 1 ml column containing 120 µg affinity-purified goat anti-mouse-TNFR2 antibodies.

The study was performed as shown in the scheme of Figure 1.

During the entire study the following parameters were monitored:

- Body weight (3 times per week)
- Health status (3 times per week)
- Tumor growth (diameters were measured in two different special directions (A and B)), measurement of tumor size with a caliper daily before first plasmapheresis and in between plasmapheresis treatments.

Apheresis equipment

Before each apheresis treatment, a heparin bolus was given (90 IU/100 g body weight (b.w.)). The extracorporeal system was fully filled with heparinized saline and thereafter, the catheter endings were connected with the extracorporeal system as shown in Figure 2.

In the first part of the system, blood was pumped (multi-channel pump, Petro Gas Ausrüstungen, Berlin, Germany) from the animal (*A. carotis*) via a plasma separator (Saxonia medical and Alpha Plan, Radeberg, Germany) and retraced to the animal by a venous catheter. The separated plasma entered the second circuit and passed the adsorber before it was returned to the rat (*V. jugularis*).

The following parameters will be or were measured before and after each apheresis:

1. Bound sTNFR2 will be determined by elution of the respective (bound) protein from the column after each apheresis by analysis of the eluate.
2. The following parameters were determined before and after each plasmapheresis:
   a.) Hematocrit
   b.) White blood cell counts
   c.) Body temperature (one hour after the end of apheresis)
   d.) Body weight
   e.) Plasma was sampled (300 µl blood) for later determination of concentrations of TNF-α and IL-2.

The rats were sacrificed 21 days after first apheresis treatment. 50 % of the tumor (maximally a piece of 2 x 2 cm) was fixed in formalin to determine necrotic tissue and a comparable piece was shock-frozen and stored at -70°C for later histological investigation.

Preparation of the columns before and after apheresis:

Before each plasmapheresis each column was flushed with at least 6 ml 0.9% saline. After the apheresis treatment, each column was treated in the following way:

1. It was rinsed with 12 ml 0.9% saline.
2. It was washed with 3 ml glycine buffer (pH 2.8) to elute the bound proteins/factors.
3. It was washed with 6 ml 0.9% saline.
4. Column was kept in PBS-azide (for storage).

The eluate from each column was frozen for further analyses.

Chemotherapeutic drug application:

• The start of chemotherapeutic treatment with melphalan was combined with the apheresis treatment.

• Melphalan was given systemically twice per week, on day 0 (directly after the first apheresis), on day 3.8±0.9 (study part I) or 2.3±0.5 (study part II), directly after the second apheresis and thereafter on days 7 and 11.

• Melphalan was given by intravenous injection (via the venous catheter) in a dose of 0.25 mg/kg. After each injection, the catheter was flushed with 500 µl saline.

3. Ethics

The study was be performed under the animal license of the Institut für Diabetes "Gerhard Katsch" GmbH , Karlsburg, Germany. Procedures related to this study have been reviewed and approved for the Institut für Diabetes "Gerhard Katsch" GmbH, Karlsburg, Germany

by the Animal Ethics Committee of the Country Mecklenburg-Vorpommern, Schwerin, Germany (file number: LALLF M-V/TSD/7221.3-1.1-032/08).

4. Data handling and mathematical analysis

All data were documented for statistical evaluations with Excel 2003. For calculation of the tumor size, the mean tumor diameter was calculated each day for each animal. Results were expressed as the means ± SD for each experimental group. By separate analysis the tumor diameter for each study part and the comparable tendency concerning the tumor growth depression was shown. The body weight and the tumor diameter were analyzed in dependence of the days after the first apheresis.

5. Results

5.1. Follow-up parameter

Body weight

At the time of the tumor tissue transfer and the catheter insertion the rats had a mean body weight of 265 ± 11 g (part I) and 253 ±16 g (part II). The body weight was comparable between the investigated groups. The procedure of catheter insertion was followed by a decline of body weight during the following days. The body weight at the beginning was reached after 4 to 7 days. The start of apheresis treatments 9.4 ± 1.3 (part I) and 7.9 ± 0.7 days after tumor tissue transfer was followed by a slight decline in body weight, more prominently expressed in part II of the study.

Tumor growth

During the first days after the tumor tissue transfer, the tumors were growing 1.22 ± 0.13 mm per day in part I and slightly faster in part II (1.51 ± 0.16 mm per day). The limit of 11 ± 1 mm mean diameter was reached after 9.4 ± 1.3 (part I) and 7.9 ± 0.7 (part II) days. Study part I demonstrates that, from day 18 after the first apheresis onwards, there is a tendency of tumor size reduction in the group treated with sTNFR2 compared to the group subjected to Sham apheresis. This tendency is verified by the results found in a further study part. Study part II clearly demonstrates that the mean tumor diameter in the group treated with sTNFR2 is smaller compared to the group subjected to Sham apheresis from day 10 after the first apheresis onwards. In particular, the results of study part II demonstrate the inhibitory effect of sTNFR2 apheresis on tumor growth between days 11 and 15 following the first apheresis and from day 18 after the first apheresis onwards.

Consequently, sTNFR2 apheresis, has a positive impact on reducing the tumor size. Therefore, sTNFR2 apheresis as described herein is usable in a method for treating and

preventing a tumorous disease in a patient in need thereof. When apheresis and melphalan treatment were discontinued, the tumors began to grow again, as had been expected on the basis of previous experiments using identical tumor tissue in the same rat strain, Brown Norway, with isolated limb perfusion with TNF-α and melphalan. These prior experiments had been conducted by A. M. M. Eggermont and Timo L. M. ten Hagen. The described treatment effects are documented by the mean diameters (Figures 3A and 3B).

White blood cell counts during follow-up

The animals from group 1 undergoing Sham apheresis treatment had no changes in white blood cell counts during the observation period.

5.2. Parameter directly before and after apheresis

In the following table, the time point of each apheresis treatment (days after first treatment) is shown for each included animal. The animal with the number 371 received only 3 plasmapheresis treatments due to a loss of catheter function.

Table 1

Days of apheresis treatment in tumor-bearing Brown Norway rats treated with Sham apheresis or sTNFR2 apheresis, respectively, with concomitant treatment with 0.25 mg/kg melphalan. Data for each animal and means for the different groups are shown.

Sham

Animal No.	1. apheresis	2. apheresis	3. apheresis	4. apheresis
S 436	0	4		
S 442	0	4	7	10
370	0	3	8	10
375	0	3	8	10
377	0	2	6	8
mean	**0.0**	**3.2**	**7.3**	**9.5**
SD	0.0	0.8	1.0	1.0

sTNFR2

Animal No.	1. apheresis	2. apheresis.	3. apheresis.	4. apheresis
S 440	0	4	6	8
S 432	0	4	6	9
371	0	2	6	
369	0	3	7	9
mean	**0.0**	**3.3**	**6.3**	**8.7**
SD	0.0	1.0	0.5	0.6

The follow-up parameters are documented independent of the number of apheresis treatments.

Body weight: There are nearly no changes in body weight during apheresis.

Hematocrit: Apheresis induced a comparable decrease of hematocrit in both groups.

White blood cell counts: Apheresis induced a comparable decrease of white blood cell counts in both groups.

Body temperature: Body temperature before and 1 h after the plasmapheresis treatment was comparable in both groups.

Filtered plasma volume: *(Extracorporeal filtered plasma volume)* During the apheresis procedures plasma volumes in the range between 0.9 ml and 14.29 ml were passed through the adsorbers.

Table 2

Extracorporeal adsorbed plasma volumes during each apheresis in tumor-bearing Brown Norway rats treated with Sham apheresis or sTNFR2 apheresis, respectively, are shown. Data for each animal and each apheresis and means are given.

Extracorporeal (filtered) plasma volumes during apheresis (in ml)

Animal No.	1st apheresis	2nd apheresis	3rd apheresis	4th apheresis
S 436	4.57	7.29		
S 442	6.85	9.16	1.05	2.13
370	5.83	5.36	8.81	8.62
375	8.33	0.9	4.89	5.68
377	9.04	9.7	6.2	9.11
Mean	**6.9**	**6.5**	**5.2**	**6.4**
SD	1.8	3.6	3.2	3.2

sTNFR2

Animal No.	1st apheresis	2nd apheresis	3rd apheresis	4th apheresis
S 440	5.7	4.92	7.13	10.57
S 432	9.16	9.16	6.44	8
371	7.65	7.91	6.34	
369	6.93	8.86	9.34	8.56
Mean	**7.4**	**7.7**	**7.3**	**9.0**
SD	1.4	1.9	1.4	1.4

Parameters when animals were sacrificed

Twenty-one days after the first apheresis treatment, all animals were sacrificed and plasma was separated and stored at -20 °C for later analysis. At this point in time, the animals from both groups had comparable body weight and hematocrit.

Table 3

Date of sacrificing, body weight (b.w.) and hematocrit (HK) at termination are given for tumor-bearing Brown Norway rats treated with Sham apheresis or sTNFR2 apheresis, respectively. Data for each animal and means are shown below.

Sham				Na.-Hep.-plasma
Animal No.	day	b.w. (g)	HK (%)	500µl/vial
S 436	Nov 19, 2010	275	46	4 x
S 442	Nov 19, 2010	302	44	4 x
370	Dec 20, 2010	262	41	5 x
375	Dec 21, 2010	281	43	5 x
377	Dec 21, 2010	259	47	6 x
mean		**276**	**44**	
SD		17	2	
Student's T-test		1,000	1,000	

sTNFR2				Na.-Hep.-plasma
Animal No.	day	b.w. (g)	HK (%)	500µl/vial
S 440	Nov 18, .2010	305	43	5 x
S 432	Nov 19, 2010	268	42	5 x
371	Dec 21, 2010	297	48	5 x
369	Dec 21, 2010	272	42	6 x
mean		**286**	**44**	
SD		18	3	
Student's T-test		0,440	0,804	

6. Conclusions

- An animal model for plasmapheresis treatments of sarcomas was established successfully.
- Plasmapheresis was well tolerated in all animals.
- Sham plasmapheresis showed a tumor growth inhibiting effect. The data indicate that plasmapheresis leads to a better uptake of the cytotoxic drug in the tumor.
- Removal of sTNFR2 by plasmapheresis in the presence of melphalan (0.25 mg/ kg rat body weight twice per week) has a tumor growth inhibiting effect.

The results of this pilot study look promising. Potential for further improvements may exist with regard to the dose of melphalan. Other agents and additional controls should be taken into consideration for corroborating further evidence to support the initial findings.

Table 4

Body weight (g) in dependence from the day of the first apheresis (day 1)

Sham	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 436	255		250	247	253	243	250			256	258	258	256	258			270	272	270	271		
S 442	288	276		283	285	280	280	276			284	278	279	278	279			293	290	295	299	
370	272	243	222	218	225	235		252	254	252	254	248	248		246	245	238	251	252	256		262
375	256	246	248	230	232		239	240	236	236	241	232		257	252	243	248	262	261		269	281
377	234	227	230	215	218		222	212	218	217	220	225		235	233	235	250	244	247		252	259
mean	**261**	**248**	**238**	**239**	**243**	**253**	**248**	**245**	**236**	**240**	**251**	**248**	**261**	**257**	**253**	**241**	**252**	**264**	**264**	**274**	**273**	**267**
SD	20	20	14	28	27	24	24	27	18	18	23	21	16	18	19	5	13	19	17	20	24	12

sTNFR2	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 440	269	257	263		269	257	269	269	272			281	280	281	281	285			295	298	302	305
S 432	245		253	253	259	245	251			258	253	256	246	247			255	254	262	265		
371	285	274	275	260	266		274	269	268	270	263	262		263	263	265	279	286	285		295	297
369	256	248	242	231	218		209	223	216	222	220	232		241	245	246	255	248	250		263	272
mean	**264**	**260**	**258**	**248**	**253**	**251**	**251**	**254**	**252**	**250**	**245**	**258**	**263**	**258**	**263**	**265**	**263**	**263**	**273**	**282**	**287**	**291**
SD	17	13	14	15	24	8	30	27	31	25	23	20	24	18	18	20	14	20	21	23	21	17
Student's T test	0.835	0.433	0.079	0.616	0.565	0.934	0.881	0.687	0.485	0.569	0.732	0.514	0.916	0.939	0.498	0.105	0.318	0.908	0.495	0.721	0.505	0.118

Table 5

Mean tumor diameters (mm)

Sham	day after first apheresis																								
Animal No.	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 436	0.00	4.93	4.78	9.32	12.08		9.61	8.60	9.54	10.88	11.28			5.81	7.48	8.03		7.68			8.31	9.29	6.16	6.93	6.58	
S 442		0.00	5.37	7.10	12.52	10.84		13.52	15.46	11.01	8.81	8.59			9.70	10.57	7.46	6.92	8.62			8.88	12.86	13.08	15.57	
370		8.57	8.85		11.93	12.00	11.71	12.77	12.12	12.88		12.05	12.30	14.73	14.84	10.38	9.41		11.56	10.66	9.18	11.34	11.45	16.10		16.17
375	5.00	7.95		10.93	11.50	12.37	13.45	12.80	13.67		12.20	11.10	11.60	11.41	9.00	8.93		7.54	11.83	10.83	12.16	14.00	16.08		22.72	23.97
377	7.42	8.53		10.43	13.54	12.73	13.98	13.77	17.91		15.96	15.18	15.34	15.14	13.79	12.60		14.10	13.96	13.78	15.00	13.40	18.54		20.85	24.68
mean	**4.14**	**5.99**	**6.33**	**9.44**	**12.31**	**11.98**	**12.19**	**12.29**	**13.74**	**11.59**	**12.06**	**11.73**	**13.08**	**11.77**	**10.96**	**10.10**	**8.44**	**9.06**	**11.49**	**11.75**	**11.16**	**11.38**	**13.02**	**12.03**	**16.43**	**21.60**
SD	3.78	3.67	2.20	1.70	0.78	0.82	1.97	2.11	3.19	1.12	2.96	2.72	1.99	4.31	3.18	1.75	1.38	3.38	2.20	1.75	3.04	2.32	4.73	4.67	7.23	4.72

sTNFR2	day after first apheresis																								
Animal No.	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 440			6.74	7.24	10.62	8.06	9.81		11.12	9.67	9.17	9.23	10.60			8.35	10.59	10.79	7.51	9.70			7.84	10.49	12.30	
S 432	6.12	6.80	8.26	8.47	10.52		9.52	10.10	8.49	10.82	11.70			7.68	6.35	6.39	6.13	6.36			6.69	7.67	4.64	7.14	6.56	
371	7.46	6.97		10.63	12.23	12.44	15.64	16.19	17.29		14.83	15.83	16.55	17.34	12.59	10.58		11.64	8.79	13.08	16.15	15.59	18.76		21.57	23.39
369	7.70	8.33		9.68	10.45	11.38	10.73	12.35	13.76		10.33	9.67	11.09	9.75	8.88	8.57		6.22	6.30	8.76	8.00	10.31	12.02		12.60	14.89
mean	**7.09**	**7.37**	**7.50**	**9.00**	**10.96**	**10.63**	**11.42**	**12.88**	**12.66**	**10.24**	**11.51**	**11.58**	**12.75**	**11.59**	**9.27**	**8.47**	**8.36**	**8.75**	**7.53**	**10.51**	**10.28**	**11.19**	**10.81**	**8.82**	**13.26**	**19.14**
SD	0.85	0.84	1.08	1.47	0.85	2.29	2.86	3.08	3.76	0.82	2.44	3.69	3.30	5.08	3.14	1.72	3.15	2.86	1.25	2.27	5.13	4.03	6.10	2.37	6.20	6.01
Student's T-test	0.258	0.559		0.709	0.041	0.312	0.676	0.757	0.656		0.783	0.952	0.888	0.962	0.494	0.204		0.893	0.040	0.495	0.785	0.933	0.559		0.530	0.639

References

US 4,708,713
US 6,620,382
WO 01/37873
WO 05/107802

Brunstein F, Hoving S, Seynhaeve AL, van Tiel ST, Guetens G, de Bruijn EA, Eggermont AM, ten Hagen TL; Synergistic antitumor activity of histamine plus melphalan in isolated limb perfusion: preclinical studies; J Natl Cancer Inst. 2004 Nov 3;96(21):1603-10.

Manusama ER, Nooijen PT, Stavast J, Durante NM, Marquet RL, Eggermont AM; Synergistic antitumour effect of recombinant human tumour necrosis factor alpha with melphalan in isolated limb perfusion in the rat; Br J Surg. 1996 Apr;83(4):551-5.

Hoving S, Brunstein F, aan de Wiel-Ambagtsheer G, van Tiel ST, de Boeck G, de Bruijn EA, Eggermont AM, ten Hagen TL; Synergistic antitumor response of interleukin 2 with melphalan in isolated limb perfusion in soft tissue sarcoma-bearing rats; Cancer Res. 2005 May 15;65(10):4300-8.

Lentz M, Kumar K; Reduction of plasma levels of soluble tumor necrosis factor and interleukin-2 receptors by means of a novel immunoadsorption column, Ther Apher Dial. 2008 Dec;12(6):491-9.

Example 2

Example 2 shows the influence of the removal of sTNFR2 in Wag/Rij rats bearing the immunogenic ROS-1 osteosarcoma on tumor growth and animal weight in the presence of systemically administered melphalan.

Aim

The project aimed to confirm the efficacy of the combination of Melphalan chemotherapy and the removal of sTNFR2 by apheresis as described in example 1 in a second tumor model using Wag/Rij rats bearing the immunogenic ROS-1 osteosarcoma. In addition, the experiments also aimed to find the optimal dosage of highly toxic Melphalan, combined with apheresis treatment.

1 Material and Methods

1.1. Animals

WAG/Rij rats with a mean body weight of 200 g (N = 20), were purchased from Harlan Laboratories (Niederlande) on 5th of April 2012. They were housed individually in separated cages under identical conditions as described for example 1. The rats were periodically handled in order to limit animal stress during the study phase.

2.2. Preparation of Adsorber Columns

The preparation of columns was done by Biopheresis GmbH in Heidelberg. The procedure, reagents and consumables were identical as described for example 2, antibodies were from the same batch to ensure optimal comparability.

2.3. Tumor tissue handling and transfer:

Frozen ROS-1 tumor cells, delivered from Prof. T. Ten Hagen in March 2012 and stored at -70°C up to the culturing, were thawed on 3rd of March and transferred in Minimum Essential Medium Eagle (SIGMA-ALDRICH, M4655) containing 10% fetal calf serum (FCS) 2012. After cultivation in a culture flask with DMEM and 10% FCS during 8 days, the cells were adhered by trypsination and counted. 1.5 Mill. cells per animal were injected subcutaneously in tested Wag/rij rats on 11th of April.

Under light inhalation anaesthesia (Sevorane®), ROS-1 tumor cells (1.500.000 in 500 µl per animal) were injected under the skin of the left flank region of the WAG rat to induce cancer growth (the method was detailed by Prof. ten Hagen; Rotterdam).

Rats with palpable tumors were instrumented with a chronic arterial and venous catheter. The catheters were inserted under general anaesthesia (i.p. injection of 0.8 mg Rompun®, Bayer and 4 mg Ketamin®, Sanofi, Germany) into the *Arteria carotis* and the *Vena jugularis*. Catheters were flushed with heparinized saline three times per week before the study.

When the tumor was grown to a mean diameter of >11 ± 3 mm, first chemotherapy and apheresis therapy was initiated on the following day.

2.4. Apheresis equipment

Apheresis equipment and procedure of apheresis treatment (including handling / rinsing of Adsorber Columns before and after apheresis) was identical as for example 1.

2.5. Chemotherapy and Apheresis:

The apheresis treatment was combined with intra-venous chemotherapy directly after the apheresis treatment . All animals were repeatedly administered a chemotherapeutic drug (Melphalan: Alkeran® , GlaxoSmithKline).

Each animal was treated with up to 6 apheresis cycles in intervals of 2-3 days (2-3 cycles per week). The duration of each plasmapheresis was one hour.

The adsorbers for the plasmapheresis procedure with a volume of 1 ml were provided by BioPheresis GmbH.

2.6. Treatment scheme

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Melphalan-application	x		x					x		x				
Apheresis	x		x		x			x		x		x		

Table 6: Treatment scheme

The first apheresis therapy was initiated directly when the tumor was grown to a mean diameter of 11±3 mm, followed by application of melphalan chemotherapy (day 0). On day 2, the second apheresis was performed and also the second Melphalan injection was administered. Melphalan was administered two times per week, for two weeks (four injections). Apheresis treatments were performed three times per week, for two weeks.

The animals were monitored without further treatment up to day 21.

Groups:

Sham: Sarcoma wearing rats with Sham apheresis (using the "empty HiTrap column") and 0.25 mg/kg **low** dose Melphalan (n=2) respectively 0.50 mg/kg (**high** dose) Melphalan (n=2)

sTNFR2 Sarcoma wearing rats with TNFR2 apheresis and **low** dose (0.25 mg/kg) Melphalan (n=2) respectively 0.50 mg/kg (**high** dose) Melphalan (n=2)

2.7. Monitored parameters:

Parameters monitored daily

- Body weight daily
- Health status
- Tumor growth, daily measurement of tumor size with a calliper

Parameters monitored before and after apheresis:

- Hematocrit
- Body temperature

At the end of the study, 21 days after tumor tissue transplantation, the tumor tissue was taken out, weighted and conserved for optional later histological investigation.

3. Ethics

The study was be performed under the animal license of the Institut für Diabetes "Gerhard Katsch" GmbH , Karlsburg, Germany. Procedures related to this study have been reviewed and approved for the Institut für Diabetes "Gerhard Katsch" GmbH, Karlsburg, Germany by the Animal Ethics Committee of the Country Mecklenburg-Vorpommern, Schwerin,

4. Data handling and mathematical analysis

Data handling and mathematical analysis was identical as described for example 1.

5. Results

5.1. Follow-up parameter

Tumour growth (delta mean tumour diameter)

The animals met inclusion criteria (mean diameter 11 ± 3 mm) 26 to 29 days after tumor cell injection. In the group of Sham apheresis / melphalan treated rats tumour size increased continuously during the twenty-one days of monitoring. In the sTNFR2 / melphalan group a tendency to a reduced tumour growth was shown from day 5 on.

The described effects are summarized in Table 7 and shown in figure 4 as the change of tumor size compared to initial size (delta mean diameter).

Sham low and **Sham high** are control data where animals have been treated with "empty HiTrap columns" (containing 1 ml NHS activated Sepharose, that have undergone the same blocking procedure as antibody loaded columns), and repetitive administration of 0,25 mg/kg (low) respectively 0,50 mg/ml (high). **sTNFR2 high** and **low**, respectively, are data where animals have been treated with apheresis using specifically loaded apheresis columns that can specifically bind sNTNFR2.

The data demonstrate that removal of sTNFR2 by apheresis had a significant impact to reduce tumor growth even in the animals that were treated with the lower dose of 0,25 mg/kg Melphalan. The reduction of tumor growth was comparable to that observed with control animals that were treated with a higher dose of 0,50 mg/kg melphalan and that had been treated with a Sham apheresis that does not remove sTNFR2.
It appears that removal of sTNFR2 is sensitizing the tumor for melphalan treatment, which would allow a reduction of melphalan level in the plasma, which is beneficial for better toleration of the cytotoxic substance melphalan.

Sham low	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 47	0,00	1,04	1,67	1,73	1,17	2,23	2,87	2,33	2,11	2,56	3,74	3,43	1,90	6,39	6,67	6,80	7,93	7,05	8,57	8,99	8,87	14,21
S 30	0,00	-0,62	0,39	0,30	1,28	0,74	2,01	1,98	3,40	3,47	2,83	4,93	4,57	5,82	5,22	7,20	7,12	7,44	9,68	7,55	8,66	
mean	**0,00**	**0,21**	**1,03**	**1,01**	**1,23**	**1,49**	**2,44**	**2,15**	**2,75**	**3,01**	**3,29**	**4,18**	**3,23**	**6,10**	**5,94**	**7,00**	**7,52**	**7,24**	**9,12**	**8,27**	**8,77**	
SD	0,00	1,17	0,91	1,01	0,08	1,05	0,61	0,25	0,91	0,65	0,64	1,06	1,88	0,41	1,03	0,28	0,57	0,27	0,78	1,02	0,15	

sTNFR2 low	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 40	0,00	1,34	2,53	0,98	0,21	2,48	1,14	2,52	1,10	3,44	3,50	4,16	3,62	4,05	4,99	5,32	6,14	6,24	7,09	6,34	8,85	8,42
S 48	0,00	-0,55	0,27	-1,00	0,58	0,73	0,92	1,11	0,78	1,60	-2,07	1,81	2,16	3,50	3,34	3,25	2,34	3,80	4,26	5,55	4,95	
mean	**0,00**	**0,39**	**1,40**	**-0,01**	**0,40**	**1,61**	**1,03**	**1,82**	**0,94**	**2,52**	**0,72**	**2,98**	**2,89**	**3,78**	**4,17**	**4,28**	**4,24**	**5,02**	**5,67**	**5,95**	**6,90**	
SD	0,00	1,33	1,60	1,41	0,26	1,24	0,16	1,00	0,23	1,30	3,94	1,66	1,03	0,39	1,17	1,47	2,69	1,73	2,01	0,56	2,76	

Sham high	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 39	0,00	0,10	1,48	0,33	-0,26	0,61	1,88	2,12	1,60	2,67	1,82	4,50	3,74	4,47	6,62	4,79	5,75	6,63	6,26	5,82	7,84	7,14
S 42	0,00	-1,55	-1,60	-0,20	2,04	0,66	1,25	0,38	-0,33	0,51	1,73	2,77	0,09	1,50	1,47	1,64	2,57	1,94	0,42	5,24	3,01	
mean	**0,00**	**-0,72**	**-0,06**	**0,06**	**0,89**	**0,64**	**1,56**	**1,25**	**0,63**	**1,59**	**1,78**	**3,63**	**1,92**	**2,98**	**4,04**	**3,21**	**4,16**	**4,28**	**3,34**	**5,53**	**5,42**	
SD	0,00	1,16	2,17	0,38	1,62	0,03	0,44	1,23	1,36	1,52	0,06	1,23	2,58	2,10	3,64	2,22	2,25	3,32	4,13	0,41	3,41	

sTNFR2 high	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 21	0,00	-0,36	0,26	-0,23	0,72	0,85	-0,58	1,68	1,89	1,37	1,68	2,91	3,12	3,76	3,33	3,20	4,70	4,69	4,91	5,64	6,79	6,97
S 45	0,00	0,68	-0,67	0,38	0,78	1,29	0,94	0,48	1,94	3,79	2,72	2,40	3,44	2,75	2,77	3,46	3,16	3,30	6,29	5,91		
mean	**0,00**	**0,16**	**-0,21**	**0,08**	**0,75**	**1,07**	**0,18**	**1,08**	**1,92**	**2,58**	**2,20**	**2,66**	**3,28**	**3,25**	**3,05**	**3,33**	**3,93**	**3,99**	**5,60**	**5,78**		
SD	0,00	0,74	0,66	0,43	0,04	0,31	1,08	0,85	0,04	1,71	0,73	0,36	0,22	0,71	0,39	0,19	1,09	0,98	0,97	0,19		

Table 7

Delta mean tumor diameter, the difference to tumor diameter measured at each animal at the days of the first apheresis, in mm. Sham low and Sham high are control data where animals have been treated with "empty HiTrap columns" (containing 1 ml NHS activated Sepharose, that have undergone the same blocking procedure as antibody loaded columns), and repetitive administration of 0,25 mg/kg (low) respectively 0,50 mg/ml (high). sTNFR2 high and low, respectively, are data where animals have been treated with apheresis using specifically loaded apheresis columns that can specifically bind sNTNFR2.

Body weight

Two days after tumour tissue transfer, the rats had a body weight of 242 ± 8 g. The procedure of catheter insertion was followed by a decline of body weight during the next days and the initial body weight was reached again after 4 to 7 days. The start of apheresis treatments and Melphalan applications was followed by a decline in body weight gain surprisingly only in the Sham apheresis control group. The interruption of Melphalan treatment from day 2 to 7 followed by a slight reincrease of body weight in the both groups and the treatment with chemotherapeutic on day 7 induced a slight reduction again.

In the groups treated with TNFR-2 apheresis no prominent decline of body weight was induced by the Melphalan treatment.

It appears that removal of TNFR-2 by apheresis has a similar effect in a rat model as the administration of TNF in limb perfusion experiments, as published by Prof. Timo ten Hagen. He has shown that the Melphalan uptake in tumor tissue is enhanced after addition of TNF. This could lead to a reduction of melphalan level in the plasma, which is beneficial for better toleration of the cytotoxic substance Melphalan.

Body weight development of WG rats injected with sarcoma cells and treated with apheresis and Melphalan from day 0 on are summarized in Table 8 and shown in figure 5, where means of two animals per group are shown.

Sham low and **Sham high** are control data where animals have been treated with "empty HiTrap columns" (containing 1 ml NHS activated Sepharose, that have undergone the same blocking procedure as antibody loaded columns), and repetitive administration of 0,25 mg/kg (**low**) respectively 0,50 mg/ml (**high**). **sTNFR2 high** and **low**, respectively, are data where animals have been treated with apheresis using specifically loaded apheresis columns that can specifically bind sNTNFR2.

Sham low	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 47	269	266	272	264	265	269	272	276	269	275	268	279	268	273	274	279	284	282	285	290	293	292
S 30	264	256	256	263	264	264	262	268	265	269	257	266	263	268	271	275	277	282	283	280		
mean	**267**	**261**	**264**	**264**	**265**	**267**	**267**	**272**	**267**	**272**	**263**	**273**	**266**	**271**	**273**	**277**	**281**	**282**	**284**	**285**		
SD	4	7	11	1	1	4	7	6	3	4	8	9	4	4	2	3	5	0	1	7		
sTNFR2 low	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 40	283	278	282	278	284	275	282	283	281	288	280	286	282	286	290	294	295	301	304	306	308	
S 48	266	263	270	267	274	273	276	282	280	286	282	287	278	288	290	292	295	299	301	303		
mean	**275**	**271**	**276**	**273**	**279**	**274**	**279**	**283**	**281**	**287**	**281**	**287**	**280**	**287**	**290**	**293**	**295**	**300**	**303**	**305**		
SD	12	11	8	8	7	1	4	1	1	1	1	1	3	1	0	1	0	1	2	2		
Sham high	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 39	275	266	269	261	268	268	272		270	276	268	266	267	269	275	280	280	282	283	287	287	
S 42	266	252	243	243	245	250	249	254	251	255	252	259	254	263	268	269	272	278	283	281		
mean	**271**	**259**	**256**	**252**	**257**	**259**	**261**	**254**	**261**	**266**	**260**	**263**	**261**	**266**	**272**	**275**	**276**	**280**	**283**	**284**		
SD	6	10	18	13	16	13	16	n.a.	13	15	11	5	9	4	5	8	6	3	0	4		
sTNFR2 high	day																					
Animal-No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
S 21	265	272	278	275	276	263	269	275	266	265	262	266	268	275	278	280	284	287	294	295	290	
S 45	300	293	294	289	290	289	295	299	298	298	290	300	298	300	295	302	305	309	307			
mean	**283**	**283**	**286**	**282**	**283**	**276**	**282**	**287**	**282**	**282**	**276**	**283**	**283**	**288**	**287**	**291**	**295**	**298**	**301**			
SD	25	15	11	10	10	18	18	17	23	23	20	24	21	18	12	16	15	16	9			

Table 8

Body weight (g) during the course of treatment. The first apheresis started on day 0. Sham low and Sham high are control data where animals have been treated with "empty HiTrap columns" (containing 1 ml NHS activated Sepharose, that have undergone the same blocking procedure as antibody loaded columns), and repetitive administration of 0,25 mg/kg (low) respectively 0,50 mg/ml (high). sTNFR2 high and low, respectively, are data where animals have been treated with apheresis using specifically loaded apheresis columns that can specifically bind sNTNFR2.

Hematocrit, Body temperature

No significant difference between the different groups has been observed during the observation period.

6. Conclusions

- The tumor growth inhibiting effect of removal of sTNFR2 by plasmapheresis demonstrated in example 1 could be verified with a second tumor model.
- Plasmapheresis was well tolerated in all animals.
- Removal of sTNFR2 by plasmapheresis in the presence of melphalan (0.25 mg/ kg rat body weight twice per week) has a tumor growth inhibiting effect.
- The data indicate that the tumor is sensitized for melphalan by removal of sTNFR2 with apheresis

Claims

1. A device having molecules binding to soluble tumor necrosis factor receptor 2 (sTNFR2, TNFRSF1B) immobilized on a solid support, wherein no molecules binding to soluble tumor necrosis factor receptor 1 (sTNFR1) are immobilized.

2. The device of claim 1, wherein said device is an extracorporeal device through which a patient's blood, plasma or blood fraction can be circulated prior to being returned into the patient.

3. The device of claim 1 or 2, wherein the molecules binding to sTNFR2 are
   (i) antibodies, preferably monoclonal antibodies, antibody fragments or antibody mimetics;
   (ii) cofactors,
   (iii) cytokines, in particular tumor necrosis factor alpha (TNFSF2) or TNFSF2 mutein;
   (iv) synthetic polypeptides, in particular *Staphylococcus aureus* Protein A, human LDL receptor, lipocalin or fibronectin; and/or
   (v) small molecules, in particular ensemblins, macrocyclic scaffolds, Aptamers and/or peptide staples;

   in particular wherein the molecules binding to sTNFR2 are monoclonal antibodies, antibody fragments or single chain antibodies.

4. The device of at least one of claims 1 to 3, wherein the molecules binding to sTNFR2 are immobilized on
   (i) a column, in particular an adsorbent column;
   (ii) a filter, in particular a capillary membrane filter with a pore size of between about 0.04 and 0.05 μm or a parallel plate filter with a pore size of between about 0.04 and 0.08 μm;
   (iii) semi-permeable material, preferably a membrane, in particular a dialysis membrane;
   (iv) a bead, in particular a microbead or a nanobead; and/or
   (v) the surface of said device, in particular the surface of a tubing of said device.

5. The device of at least one of claims 1 to 4, wherein said device further comprises
   (i) a primary filter to separate ultrafiltrate or plasma from the patient's blood;
   (ii) a centrifuge to separate ultrafiltrate or plasma from the patient's blood;
   (iii) a filter which removes components of a molecular weight of 120,000 Da or less from the blood, plasma or blood fraction;
   (iv) a centrifuge which removes components of a molecular weight of 120,000 Da or less from the blood, plasma or blood fraction;
   (v) means for administering radiation to patent's tissue; and/or
   (vi) one or more cannula(s) and tubing(s) suitable for connecting the device to the patient;

6. The device of at least one of claims 1 to 5, wherein said device further has one or more molecules binding to other plasma protein(s) immobilized on a solid support, preferably wherein said molecules are molecules binding to cytokines, chemokines, soluble cytokine receptors, other soluble decoy receptors, angiogenic factors, growth factors, and bone morphogenic factors more preferably molecules binding to soluble interleukin receptors (sILRs), in particular molecules binding to soluble interleukin-2 receptor alpha (sIL-2R).

7. The device of at least one of claims 1 to 6, wherein the device has been sterilized and/or endotoxin has been removed from the device.

8. A method for the removal of sTNFR2 from blood, plasma or a blood fraction, wherein molecules binding to sTNFR2, but not binding to sTNFR1, are immobilized on a solid support and brought into contact with the blood, plasma or a blood fraction and wherein sTNFR1 is not removed.

9. The method of claims 8, wherein said method is conducted by means of a device of at least one of claims 1 to 7.

10. The method of claim 8 or 9, wherein sTNFR2 is removed from a patient's blood, plasma or blood fraction *ex vivo* in an extracorporeal device.

11. The method of claim 10, wherein the patient is suffering from cancer.

12. The method of at least one of claims 8 to 11, wherein said method is combined with the removal of one or more other plasma protein(s), preferably cytokines, chemokines, soluble cytokine receptors, other soluble decoy receptors, angiogenic

factors, growth factors, and bone morphogenic factors more preferably molecules binding to sILRs, in particular molecules binding to soluble sIL-2R.

13. A molecule specifically binding to sTNFR2, but not binding to sTNFR1, for use in a method for treating the human or animal body.

14. The molecule of claim 13, wherein the treating is treating cancer.

15. The molecule of claim 13 or 14, wherein said molecule is used in a method of at least one of claims 8 to 12.

16. The molecule of claim 13 or 14, wherein said molecule is part of a device of at least one of claims 1 to 7 or is immobilized on a bead or an implant, preferably wherein said bead or implant is in contact with the blood stream, the lymph or the extracellular matrix, in particular in contact with the blood stream.

17. The molecule of at least one of claims 13 to 16, wherein said method further comprises the removal of one or more other plasma protein(s), preferably cytokines, chemokines, soluble cytokine receptors, other soluble decoy receptors, angiogenic factors, growth factors, and bone morphogenic factors more preferably molecules binding to sILRs, in particular molecules binding to soluble interleukin-2 receptor alpha (sIL-2R).

18. Blood, plasma or a blood fraction obtainable from a method of at least one of claims 8 to 12 for use in a method for treating cancer.

19. A method for sensitizing a tumor patient to a treatment with a chemotherapeutic agent, comprising the step of removing soluble TNF receptor 2 (sTNFR2) from the blood of said patient.

20. The method of claim 19, wherein the sTNFR2 is removed with the help of a molecule binding to sTNFR2.

21. The method of claim 20, wherein said molecule is an antibody against sTNFR2, preferably a monoclonal antibody against sTNFR2.

22. The method of any of claims 19 to 21, wherein the sTNFR2 is removed with the help of a device according to any of claims 1 to 7.

23. A method for treating a tumor in a patient, comprising administering to a patient a chemotherapeutic drug in an amount sufficient for the treatment of the tumor, wherein from the blood of said patient sTNFR2 has been removed.

24. The method of claim 23, with the features as defined in any of claims 20 to 22.

25. A molecule binding to sTNR2 for use in a method for sensitizing a tumor patient to a treatment with a chemotherapeutic agent

26. The molecule for use according to claim 23, wherein the molecule has the features as defined in any of claims 13 to 17.

27. The molecule for use according to any of claims 25 or 26, wherein the molecule is an antibody against sTNFR2, preferably a monoclonal antibody against sTNFR2.

28. A chemotherapeutic agent for use in a method for treating a tumor in a patient, wherein from the blood of said patient sTNFR2 has been removed.

29. The chemotherapeutic agent for use according to claim 28, with the features as defined in any of claims 26 or 27.

Fig. 1

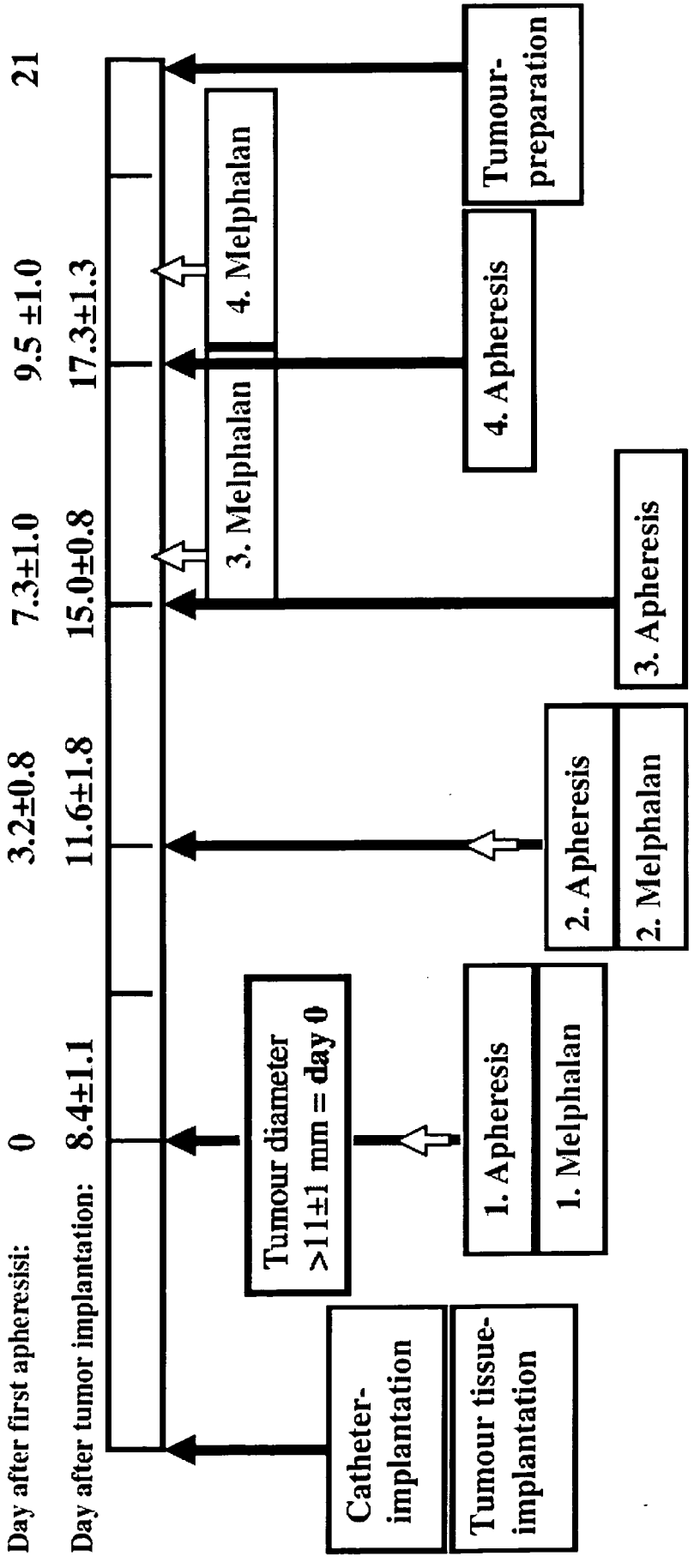

Day after first apheresisi: 0 3.2±0.8 7.3±1.0 9.5 ±1.0 21
Day after tumor implantation: 8.4±1.1 11.6±1.8 15.0±0.8 17.3±1.3
Catheter-implantation
Tumour tissue-implantation
Tumour diameter >11±1 mm = day 0
1. Apheresis
1. Melphalan
2. Apheresis
2. Melphalan
3. Melphalan
3. Apheresis
4. Apheresis
4. Melphalan
Tumour-preparation

Fig. 2

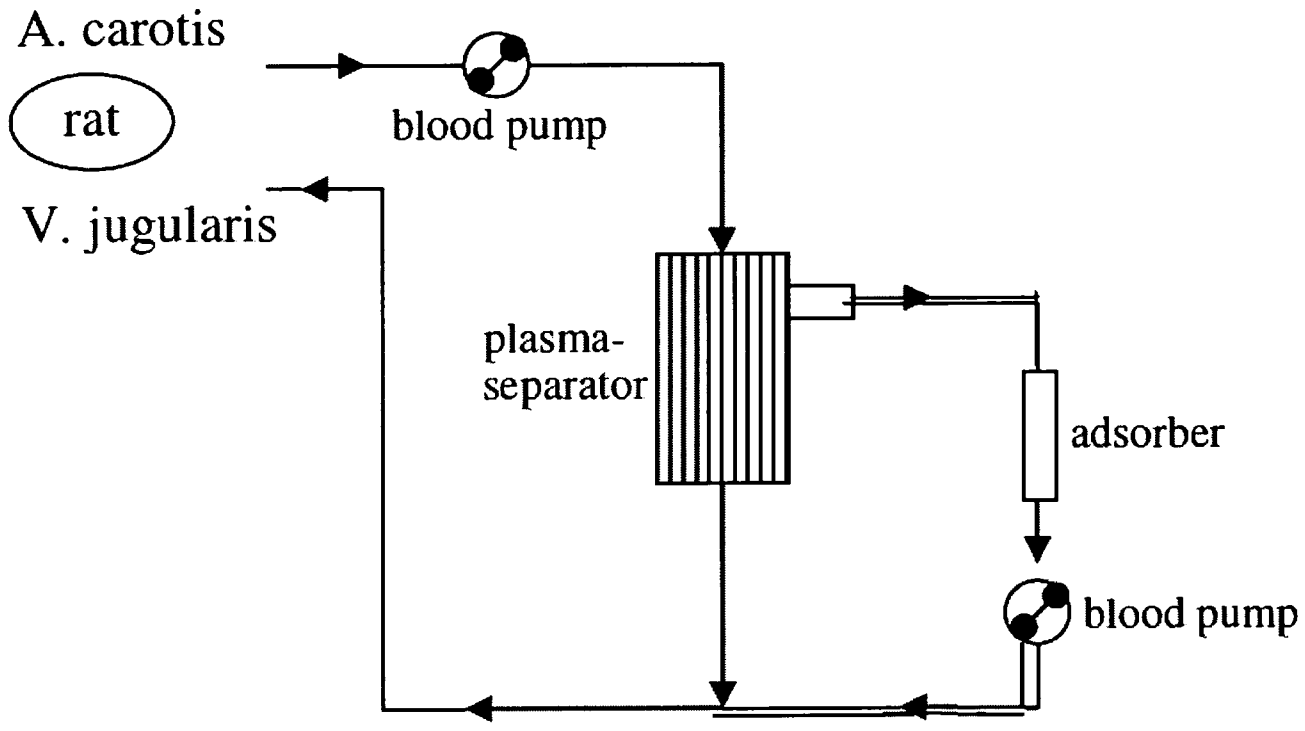

A. carotis
rat
V. jugularis
blood pump
plasma-
separator
adsorber
blood pump

Fig. 3A

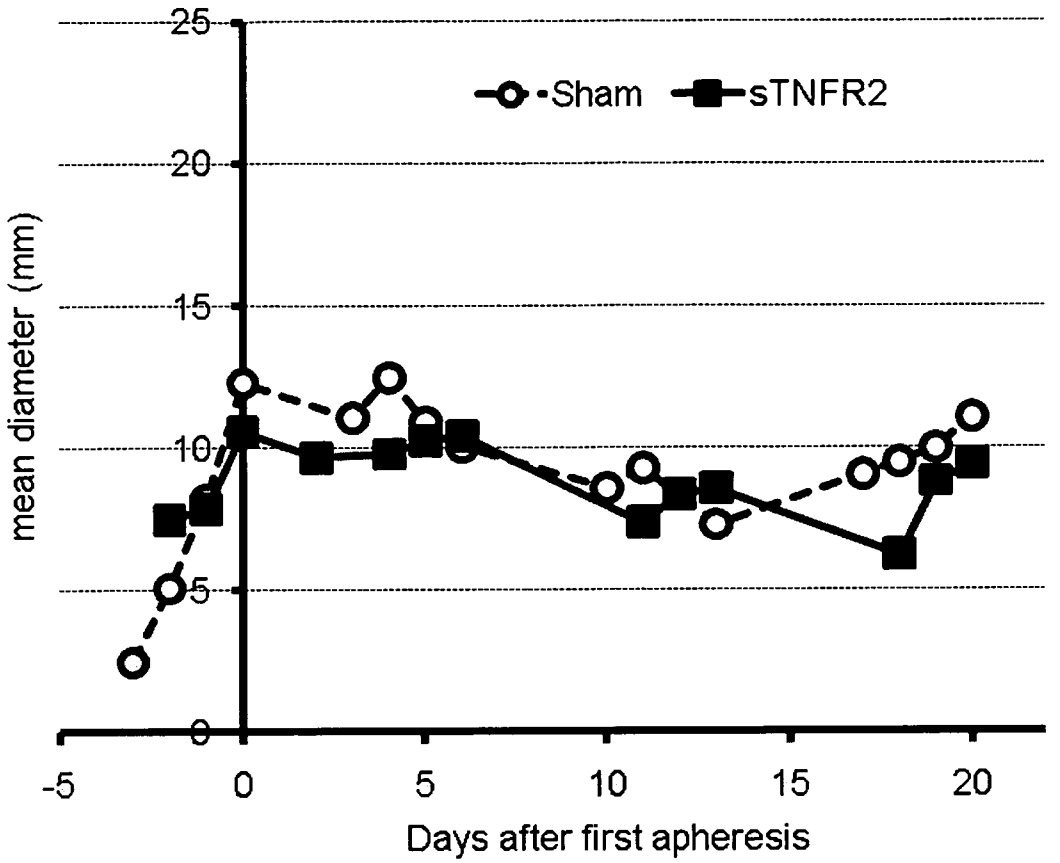

25
20
15
10
5
0
Sham
sTNFR2
mean diameter (mm)
-5
0
5
10
15
20
Days after first apheresis

Fig. 3B

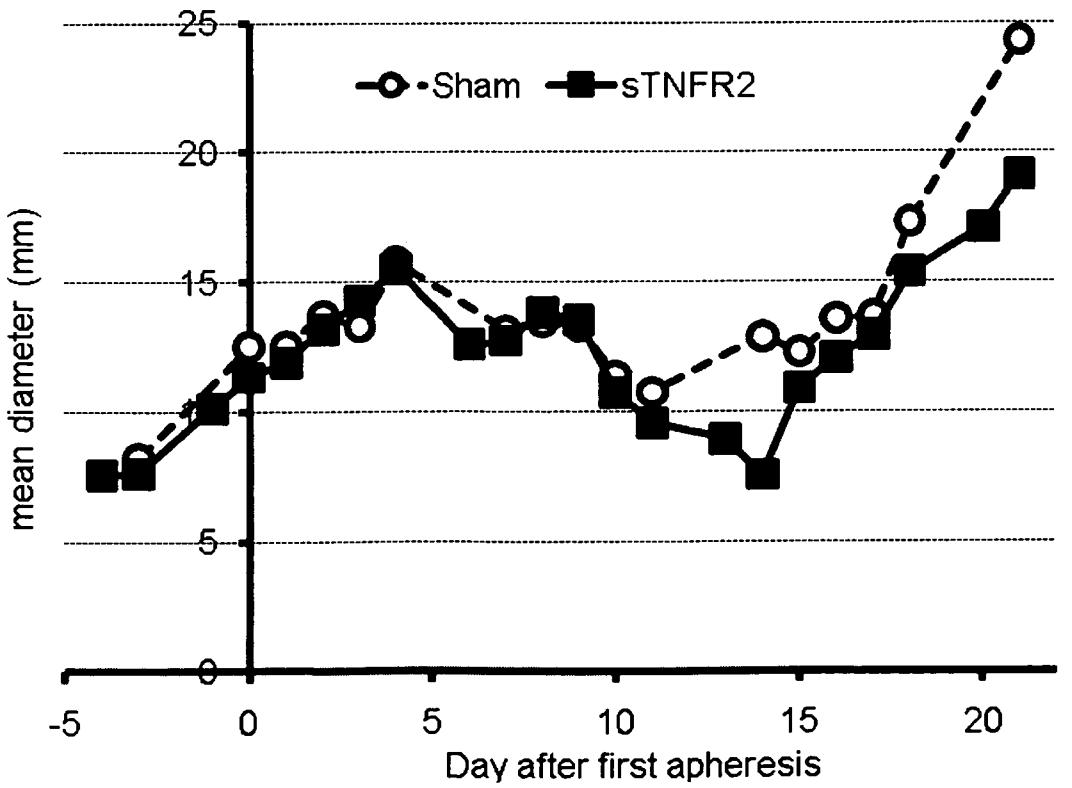

25
20
15
10
5
0
Sham
sTNFR2
mean diameter (mm)
-5
0
5
10
15
20
Day after first apheresis

Figure 4

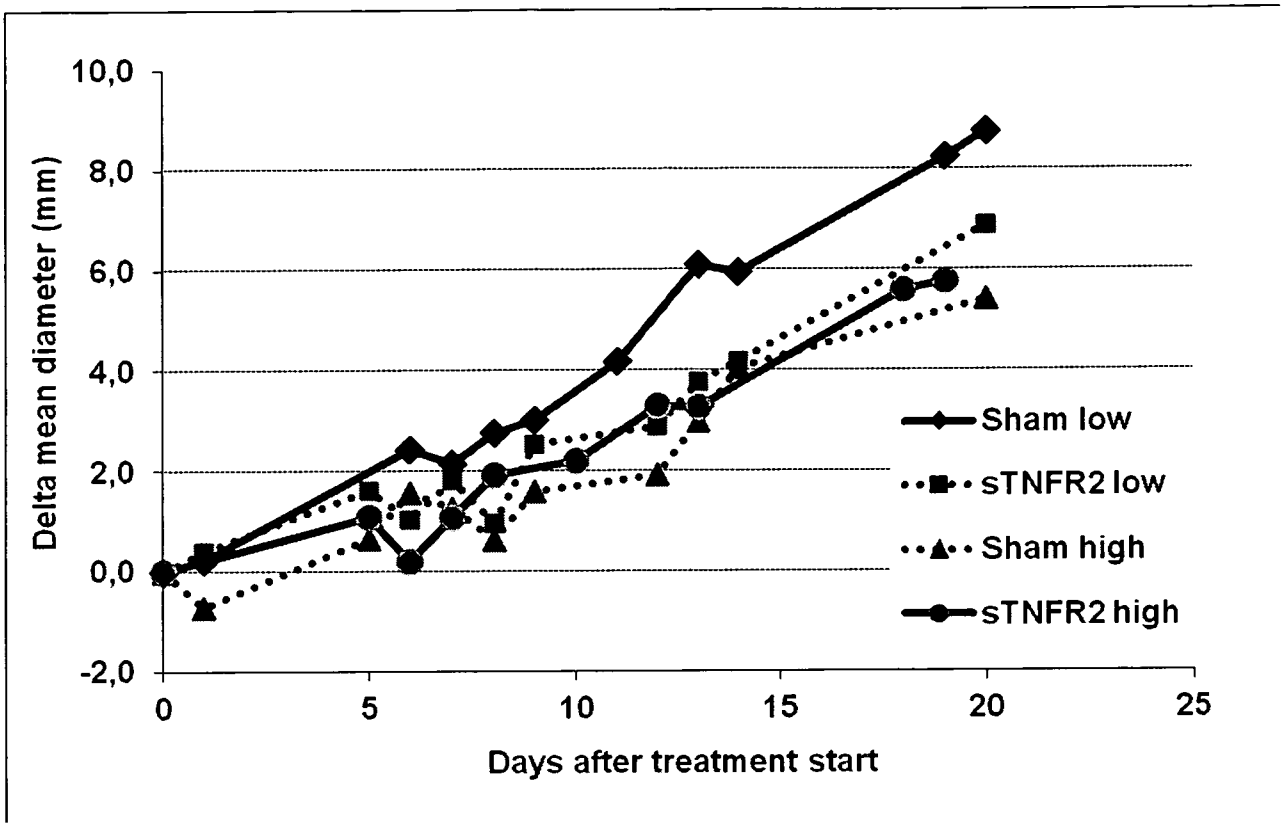
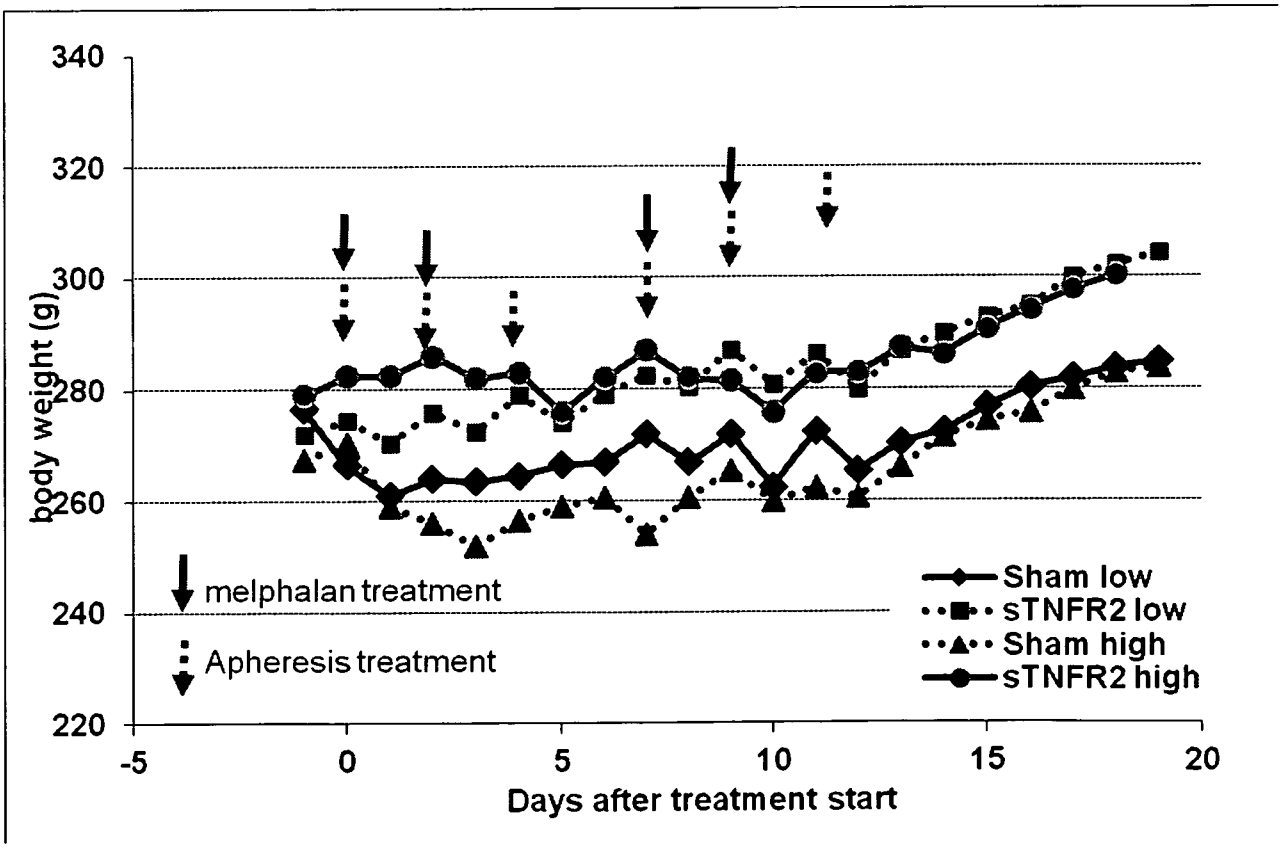


Figure 5



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/002340

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P35/00 A61K38/19 A61M1/34 C07K16/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61M G01N A61P

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

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/127585 A2 (VIRXSYS CORP [US]; SLEPUSHKIN VLADIMIR [US]; HUMEAU LAURENT [US]) 30 November 2006 (2006-11-30) column 66 - column 68	1,3,4,6
X	US 5 817 528 A (BOEHM WOLFGANG [DE] ET AL) 6 October 1998 (1998-10-06) claims	1-7

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 October 2012	09/10/2012

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/002340

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MEREDITH LENTZ ET AL: "Reduction of Plasma Levels of Soluble Tumor Necrosis Factor and Interleukin-2 Receptors by Means of a Novel Immunoadsorption Column", THERAPEUTIC APHERESIS AND DIALYSIS, vol. 12, no. 6, 1 December 2008 (2008-12-01), pages 491-499, XP55001372, ISSN: 1744-9979, DOI: 10.1111/j.1744-9987.2008.00640.x page 491	8-29
X	WO 01/37873 A2 (LENTZ M RIGDON [US]) 31 May 2001 (2001-05-31) cited in the application page 3 - page 19	8-29

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/002340

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006127585 A2	30-11-2006	AU 2006251621 A1	30-11-2006
		CA 2606928 A1	30-11-2006
		CN 101238218 A	06-08-2008
		EP 1888758 A2	20-02-2008
		JP 2008539796 A	20-11-2008
		US 2008274091 A1	06-11-2008
		WO 2006127585 A2	30-11-2006
US 5817528 A	06-10-1998	AT 294959 T	15-05-2005
		AU 691297 B2	14-05-1998
		AU 2594495 A	05-12-1995
		CA 2190268 A1	23-11-1995
		DE 69534187 D1	09-06-2005
		DE 69534187 T2	17-11-2005
		EP 0804734 A1	05-11-1997
		ES 2242189 T3	01-11-2005
		JP 3161604 B2	25-04-2001
		JP H09508976 A	09-09-1997
		US 5817528 A	06-10-1998
		WO 9531727 A1	23-11-1995
WO 0137873 A2	31-05-2001	AT 445414 T	15-10-2009
		AU 783238 B2	06-10-2005
		AU 4505601 A	04-06-2001
		CA 2390095 A1	31-05-2001
		DK 1227843 T3	15-02-2010
		EP 1227843 A2	07-08-2002
		EP 2087907 A2	12-08-2009
		ES 2356369 T3	07-04-2011
		JP 3852755 B2	06-12-2006
		JP 2003514622 A	22-04-2003
		JP 2006249112 A	21-09-2006
		WO 0137873 A2	31-05-2001