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(54) Title: MODIFIED SOLUBLE FGF RECEPTOR FC FUSIONS WITH IMPROVED BIOLOGICAL ACTIVITY

(57) Abstract: The invention relates to modified soluble FGF receptor Fc fusions comprising a fusion of a soluble fragment or domain of the FGF receptor part (targeting or binding moiety) with an Fc region of an immunoglobulin part (effector function moiety), having improved biological activity including ADCC/CDC activities, compositions containing them, and method of producing such modified soluble FGF receptor Fc fusion molecules.

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MODIFIED SOLUBLE FGF RECEPTOR Fc FUSIONS WITH IMPROVED BIOLOGICAL ACTIVITY

Field of the Invention and Introduction

The invention generally relates to modified soluble FGF receptor Fc fusions comprising a fusion of a soluble fragment or domain of the FGF receptor with an Fc region of an immunoglobulin, having improved biological activity, and compositions containing them. Methods of producing such modified soluble FGF receptor Fc fusion molecules are also described. Particularly, the modified soluble FGF receptor Fc fusions have improved anti-angiogenic activity and anti-tumoral antibody-dependent cell mediated cytotoxicity activities, namely ADCC (antibody-dependent cellular cytotoxicity) and/or CDC (complement-dependent cytotoxicity), and are thus useful in the treatment of cancer, metastatic tumors and for reducing tumor growth in a subject. Further described are methods of inhibiting tumor growth and methods for the treatment or prevention of pathological situations including, but not limited to, breast cancer, melanoma, leukemia, brain metastases, renal cancer, primary melanoma, primary colon cancer, primary bladder cancer, infantile hemangioma, ovarian cancer, prostate cancer and lung cancer.

Background of and Relevance of the Invention

Angiogenesis, *i.e.*, the formation of new blood vessels from pre-existing ones, involves a complex coordination of endothelial cell proliferation, migration, basement membrane degradation and neovessel organization (Ji *et al.*, 1998, *FASEB J.* **12**:1731-1738). The local, uncontrolled release of angiogenic growth factors and/or alterations of the production of natural angiogenic inhibitors, with a consequent alteration of the angiogenic balance (Hanahan *et al*, 1996, *Cell.* **86**: 353-64) are responsible for the uncontrolled endothelial cell proliferation that takes place during tumor neovascularization and in angiogenesis-dependent diseases (Folkman, 1995, *Nat. Med.* **1**:27-31).

Numerous natural inducers of angiogenesis have been identified, including members of the vascular endothelial growth factor (VEGF) family, angiopoietins, transforming growth factor-α and -β (TGF-α and -β), platelet-derived growth factors (PDGF), tumor necrosis factor- α (TNF- α), interleukins, chemokines, and the members of the fibroblast growth factor (FGF) family. These potent angiogenic factors are often over-

expressed by tumor tissues (Presta, 2005, *Cytokine & Growth Factors Reviews*. **16**: 159-178; Grose, 2005, *Cytokine & Growth Factors Reviews*. **16**: 179-186). Indeed, FGFs, and more specially FGF2, are over-expressed in numerous human cancer including melanoma (Halaban, 1996, *Semin Oncol*. **23**:673-81; Hanada, 2001, *Cancer Res*. **61:** 5511-5516), leukemia (Krejci *et al*, 2001 *Leukemia*. **15**:228-37, Bieker *et al*, 2003, *Cancer Res*. **63**: 7241-7246) renal cancer (Hanada, 2001, *Cancer Res*. **61:** 5511-5516), colon cancer (Tassi, 2006, *Cancer Res*. **66**:1191-1198), ovarian cancer (Whitworth *et al*, 2005, *Clin Cancer Res*. **11**:4282-4288, Gan *et al*, 2006, *Pharm Res*. **23**:1324-31), prostate cancer (Aigner *et al*, 2002 *Oncogene*, **21**:5733-42; Kwabi-Addo *et al*, 2004, *Endocr Relat Cancer*. **11**:709-24) and lung cancer (Takanami *et al*, 1996, *Pathol Res Pract*. **192**:1113-20; Volm *et al*, 1997, *Anticancer Res*. **17**:99-103; Brattstrom *et al*, 1998, *Anticancer Res*. **18**: 1123-1127). In addition, FGF2 over-expression can be correlated with a chemoresistance in certains cancers including bladder, breast, head and neck cancers (Gan *et al*, 2006, *Pharm Res*. **23**:1324-31).

With respect to FGF family members, as FGFs secreted by tumor cells have affinities for the glycosaminoglycan side-chains of cell surface and matrix proteoglycans, these secreted FGFs are most likely sequestered nearby tumor cells forming FGF reservoirs. This particularity makes FGF addressing a good strategy to direct an active molecule that needs a target molecule stably expressed and easily accessible.

Various antibody-based products are currently used as therapeutic drugs and several monoclonal antibodies (mAbs) are now approved in various therapeutic areas such as oncology, inflammation, infectious disease and cardiovascular disease. These mAbs induce tumor cells killing by multiple mechanisms including recruitment of immune system (Harris, 2004, *Lancet Oncol*, **5**: 292-302). The Fc moiety of mAbs is responsible for these immune-mediated effector functions that include two major mechanisms: Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity (CDC). ADCC occurs when an mAb first binds via its antigen-binding site to its target on tumor cells, and then the Fc portion is recognized by specific Fc receptors (FcR) on effector cells (i.e. NK, neutrophiles, macrophages...) that attack the target cell. CDC is a process where a cascade of different complement proteins become activated when an mAb binds to C1q leading to formation of C3b on the surface of antibody-coated tumor cells near the site of complement activation. The presence of C3b controls formation of the C5–C9 membrane attack complex that can insert into the membrane to lyse tumor cells (Sharkey, 2007, *CA Cancer J Clin*, **56**: 226-243). The ability of mAbs to stimulate ADCC depends on their isotype. IgG1 and IgG3 antibodies bind highly to FcRs, while IgG4 and IgG2 antibodies bind weakly. CDC

capacitity of mAb also depends on mAb isotype. IgG3 and, to a lesser extent, IgG1 are the most effective isotypes for stimulating the classic complement cascade. IgG2 mAbs are less efficient in activating the complement cascade, whereas IgG4 is unable to do so (Strome, 2007, *The Oncologist*, **12**:1084-1095).

The use of a fusion protein that can have, as antibodies, a dual functionality with a binding part exhibiting a specific targeting and an effector part able to induce the lysis of target cells by recruitment of immune system, is one aspect of these therapeutic strategies. In addition, to be useful in therapy, this molecule would need to have advantageous pharmacokinetic properties PK. The Fc moiety can detectably increase the serum half life of the modified soluble FGF receptor Fc fusion, but there is still a need for fusion protein with a longer serum half life. Finally, if this fusion protein is to be used as a drug, it is necessary that it is produced reliably, efficiently and with appropriate productivity.

Thus there is a need for a fusion protein with ADCC and/or CDC activities targeting FGF for treatment of cancer, metastatic tumors and for reducing tumor growth in a subject, with improved PK features, and which can be produced efficiently.

It is an object of the present invention to provide a fusion protein which goes at least some way to addressing the above desiderata; and/or to at least provide the public with a useful choice.

The applicants have now discovered that soluble fusion proteins between soluble FGF receptor part (binding or targeting moiety) and Fc part (effector function moiety) (sFGFR-Fc) that are modified to have a particular glycan profile have in fact substantially improved biological activities, including ADCC and/or CDC activities, and may thus be used as efficacious anti-angiogenic and anti-tumoral drugs, for the treatment of uncontrolled cell growth or cancer. These modified soluble fusion proteins have advantageous PK properties due to their sialylation rate, and can be produced with appropriate productivity and minimal aggregation because of their glycosylation pattern.

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an

admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

In the description in this specification reference may be made to subject matter that is not within the scope of the claims of the current application. That subject matter should be readily identifiable by a person skilled in the art and may assist in putting into practice the invention as defined in the claims of this application.

Summary of the Invention

The present invention is thus directed to a modified soluble FGF receptor Fc fusion comprising a fusion of a soluble fragment or domain of a FGF receptor with an Fc region of an immunoglobulin, wherein at least the 5th N-glycosylation site of the FGF receptor moiety is occupied, and at most 45% of the N-glycans of the FGF receptor moiety have no sialyl group and wherein the average number of sialic acid per N-glycan of the FGF receptor moiety is 1.2 or above and wherein the FGF receptor is FGF receptor 2 (FGFR2). In addition, according to a further preferred embodiment of the invention, the 3rd, 4th, 6th and 7th N-glycosylation sites of the FGF receptor moiety are occupied. Preferably, all N-glycosylation sites are occupied. In a further preferred embodiment, the average number of sialic acid per N-glycan in the FGF receptor moiety of the fusions of the invention is at least 0.9; even more preferably, it is at least 1.2. Each N-glycan molecule of the modified soluble FGF receptor Fc fusion according to the present invention comprises 3 mannose residues, on average 1.5 to 3.0 galactose residues, 3.5 to 5 of N-acetylglucosamine, and 0.6 to 1 fucose residues.

The present invention is also directed to modified soluble FGF receptor Fc fusions comprising a fusion of a soluble fragment or domain of a FGF receptor with an Fc region of an immunoglobulin, wherein all N-glycosylation sites are occupied, and wherein at most 45% of the N-glycans of the FGF receptor moiety have no sialyl group and wherein the N-glycan of the Fc region is 60 to 100% fucosylated.

In one embodiment, the soluble fragment or domain of the FGF receptor is the soluble or extracellular domain of FGF receptor 1 (sFGFR1) or of FGF receptor 2 (sFGFR2).

In another embodiment, the soluble fragment or domain of the FGF receptor is the soluble or extracellular domain of FGF receptor 1 isotype or variant IIIc (sFGFR1(IIIc)) or of FGF receptor 2 isotype IIIc (sFGFR2(IIIc)).

Also described is a polynucleotide encoding the modified fusion FGF receptor of the invention.

According to a preferred embodiment, the modified soluble FGF receptor Fc fusion is encoded by a polynucleotide having the nucleotide sequence as set forth in the SEQ ID NO: 1, or a polynucleotide having at least 80% identity with the nucleotide sequence of SEQ ID NO: 1. In a further preferred embodiment, the modified soluble FGF receptor Fc fusion of the invention has the amino acid sequence as set forth in the SEQ ID NO : 2 or a sequence having at least 95%, 97%, 98%, or 99% identity with the sequence as set forth in SEQ ID NO: 2.

Also described is a vector comprising a polynucleotide of the invention. Also described is a cell comprising a vector of the invention.

The modified soluble FGF receptor Fc fusion of the invention has ADCC and/or CDC activity and is thus useful for the treament of diseases such as cancer.

The present invention also relates to pharmaceutical composition comprising a modified soluble FGF receptor Fc fusion of the invention.

Also described are combinations of the modified soluble FGF receptor Fc fusion with a chemotherapeutic agent or a biotherapeutic agent with anti-tumoral and/or anti-angiogenic properties.

The invention also relates to the modified soluble FGF receptor Fc fusion of the invention as a medicament.

The invention also relates to the use of a modified soluble FGF receptor Fc fusion of the invention for the preparation of a medicament for treating cancer.

The invention also relates to a method for treating cancer, said method comprising administering to a subject a modified soluble FGF receptor Fc fusion of the invention in a therapeutically effective amount.

Also described is a method of preventing or reducing tumor growth and volume and metastatic tumors comprising administering to a subject the modified soluble FGF receptor Fc fusion of the present invention in a therapeutically effective amount.

Brief Description of the Figures

Figure 1A and B show maps of the expression vectors for SIAT6 (ST3GAL3) and B4GT1(B4GALT1), respectively.

Figure 2 A and B show the nucleic acid (SEQ ID No. 9) and amino acid (SEQ ID No. 10) sequences of B4GT1(B4GALT1) for expression from pXL4551

Figure 3 A and B show the nucleic acid (SEQ ID No. 11) and amino acid (SEQ ID No. 12) sequences of SIAT6 (ST3GAL3) for expression from pXL4544

Figure 4 A corresponds to the nucleic acid sequence of sFGFR2-Fc for expression from pXL4410, pXL4429 or pXL4636 (SEQ ID N° 1), **Fig 4 B** to the amino acid sequence of sFGFR2-Fc (the N-glycosylation sites are indicated in bold type) encoded by pXL4410 pXL4429 or pXL4636 (SEQ ID N° 2), **Fig 4 C** to the amino acid sequence of sFGFR2 (SEQ ID N° 4), **Fig 4 D** to the amino acid sequence of Fc (SEQ ID N° 6), **Fig 4 E** to the amino acid sequence of the linker, and **Fig 4F** to the amino acid sequence of the signal peptide (SEQ ID N° 8). It is the signal peptide described for interleukin-2. It was observed that fusion of this peptide upstream of the sequence represented by SEQ ID No.2 leads to a secreted protein with a homogeneous N-terminal amino acid sequence.

Figure 5 is a schematic representation of the strategy used for constructing pXL4636, encoding sFGFR2-Fc and Glutamine Synthetase

Figure 6 A and B show maps of the expression vectors pXL4429 for sFGFR2-Fc and DHFR and pXL4417 encoding neomycin resistance gene

Figure 7 is a graph showing the correlation between the average number of sialic acid per sFGFR2 N-glycan and clearance of sFGFR2-Fc in blood - Optimal ratio > 1.2. preferred ratio > 0.9.

Figure 8 shows SDS-PAGE (non reducing conditions) of 1 μg of sFGFR2-Fc incubated in the absence (-) or in the presence (+) of PNGase F. M: molecular weight marker

Figure 9 shows the N-glycan position and numbering in sFGFR2-Fc dimer. The numbering starts from the N-terminal amino acid sequence of FGFR2

(FGFR2_HUMAN) such that position N1 correspond to Asn83, N2 to Asn123, N3 to Asn228, N4 to Asn241, N5 to Asn 265, N6 to Asn 297, N7 to Asn318, N8 to Asn331. Position N297 correspond to the Asn position on human Fc (IgG1).

Figure 10 is a graph showing the kinetics of disappearance of protein sFGFR2-Fc (squares) in blood over time up to 72 hours.

Figure 11 shows the amount of recoveries of protein sFGFR2-Fc in plasma and liver expressed in % of injected dose.

Figure 12 is a graph of the A549 tumor volume analysis after treatment up to day 40 (100 μg/mouse/admin: triangles; 300 μg/mouse/admin: squares; 500 μg/mouse/admin: closed circles; PBS control: open circles).

Figure 13 is a graph of the A549 tumor weight analysis after treatment at day 40.

Figure 14 is a graph of the H460 tumor volume analysis after treatment up to day 22 (treated group: closed circles; PBS control group: open circles).

Figure 15 is a graph of the H460 tumor weight analysis after treatment at day 22.

Figure 16 is a graph of the evaluation of the *in vitro* ADCC activity of sFGFR2-Fc on H460 and A549 tumor cells.

Figure 17 shows graphs of the A549 tumor volume analysis when implanted in three mouse strains, i.e. SCID (**Fig. 17A**), NOD/SCID (**Fig. 17B**), and SCID/bg (**Fig. 17C**), up to day 41 (sFGFR2-Fc 100 μg/mouse/admin: diamonds; PBS control: open circles).

Figure 18 shows graphs of the H460 tumor volume analysis when implanted in three mouse strains, i.e. SCID (**Fig. 18A**), NOD/SCID (**Fig. 18B**), and SCID/bg (**Fig. 18C**), up to day 22 (sFGFR2-Fc 100 μg/mouse/admin: diamonds; PBS control: open circles).

Figure 19A shows a map of the plasmid encoding sFGFR2-Fc (A265 in Fc), and **Fig. 19B** shows the protein sequence of sFGFR2-Fc (A265 in Fc) (SEQ ID No. 14).

Position 392 is the position of the mutation in the Fc domain (Asp265Ala) and is represented in bold type.

Figure 20 is a graph showing the H460 tumor volume analysis when implanted in nude mouse strains up to day 23 (open circles: PBS control; diamonds: sFGFR2-Fc 500 μg/mouse/admin; open square: sFGFR2-Fc (A265Fc) 500 μg/mouse/admin). **: p<0.01 vs control Anova & Newman-Keuls post test)

Detailed Description

The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

Throughout this disclosure, applicants refer to journal articles, patent documents, published references, web pages, sequence information available in databases, and other sources of information. One skilled in the art can use the entire contents of any of the cited sources of information to make and use aspects of this invention. Each and every cited source of information is specifically incorporated herein by reference in its entirety. Portions of these sources may be included in this document as allowed or required. However, the meaning of any term or phrase specifically defined or explained in this disclosure shall not be modified by the content of any of the sources. The description and examples that follow are merely exemplary of the scope of this invention and content of this disclosure. One skilled in the art can devise and construct numerous modifications to the examples listed below without departing from the scope of this invention.

The present invention relates to soluble protein fusions comprising domains of FGF receptors. The invention generally encompasses FGF receptor fragments, domains, and especially soluble or extracellular domains. The extracellular domain of the FGF receptor is linked to an appropriate fusion partner, such as an immunoglobulin Fc unit. Therefore, in the broadest sense, the modified soluble FGF receptor fusions of the invention can be FGF receptor (FGFR) proteins, fragments, domains, extracellular domains, soluble domains, and any of these linked to a fusion partner, especially an Fc region fusion partner.

This applicant has found that a modified soluble FGF receptor Fc fusion comprising a fusion of a soluble domain of a FGF receptor with an Fc region of an immunoglobulin wherein at most 45% of the N-glycans have no sialyl group have advantageous

properties. Advantages of the sialylation pattern of the modified soluble FGF receptor Fc fusion of the invention include a better pharmacokinetic profile and an improved resistance to cleavage in vivo.

Typically N-glycans are attached cotranslationally through specific asparagine (Asn) residues. The consensus sequence Asn-X-Ser/Thr (where X is any amino acid except Pro) is essential but not sufficient in that local secondary structure may determine addition (Jefferis *et al.*, 2006, *Nature Biotechnology* **24**:1241). Several factors are believed to account for unoccupied N-glycosylation sites (Jones *et al.*, 2005, *Biochimica et Biophysica Acta* **1726**: 121). Several N-glycosylation sites are present within the soluble FGF receptors of the invention. For example, there are 8 N-glycosylation sites in the extracellular domain of FGFR2IIIc (see Fig. 9). The N-glycans contain a conserved oligosaccharide core linked to Asn. This core is composed of three mannose (Man) and two N-acetylglucosamine (GlcNAcs) monosaccharide residues. Additional GlcNAcs are normally linked to β1,2-linked to the α6 Man or α3 Man, while the N-acetylneuraminic acid (NeuAcα2,6), galactose (Galβ1,4), fucose (Fucα1,6) and bisecting GlcNAc (β1,4) can be present or absent (Jefferis *et al.*, 2006, *Nature Biotechnology* **24**:1241).

The applicant has demonstrated that the presence of a N-glycan on the 5th N-glycosylation site from the N-terminus of the FGFR moiety confers advantageous properties for productivity and low aggregation, as shown in the Experimental Examples. In particular, in the absence of glycosylation on this particular site, productivity drops dramatically, while aggregation is increased. In addition, the presence of this N-glycan was found to be necessary for FGF binding.

The present invention is thus directed to a modified soluble FGF receptor Fc fusion comprising a fusion of a soluble domain of a FGF receptor with an Fc region of an immunoglobulin, wherein at least the 5th N-glycosylation site of the FGF receptor moiety is occupied, and at most 45% of the N-glycans of said FGF receptor moiety have no sialyl group.

According to a further embodiment of the invention, the 3rd, 4th, 6th and 7th N-glycosylation sites of the FGF receptor moiety are occupied. When at least 7 of the N-glycosylation sites of the FGF receptor moiety are glycosylated, the fusion of the invention has even better properties as regards productivity and low aggregation. Thus, in another aspect, the invention is directed to a fusion of a soluble fragment or domain

of a FGF receptor with an Fc region of an immunoglobulin, wherein at least 7 N-glycosylation sites are occupied and at most 45 % of the N-glycans of the FGF receptor Fc fusion have no sialyl group. In a specific embodiment of the invention, all the N-glycosylation sites are occupied.

In another aspect, the modified soluble FGF receptor Fc fusion of the invention has an average number of sialic acid per N-glycan of the FGF receptor moiety is at least 0.9, i.e. this number can be 0.9 or any value above 0.9. In a preferred embodiment, the modified soluble FGF receptor Fc fusion of the invention has an average number of sialic acid per N-glycan of at least 1.2. Such a ratio was found by the applicant to ensure a maximized concentration in the blood of the soluble FGF receptor fusion of the invention, that would be comparable to the optimal concentration in the blood found for Fc molecules.

The present invention is further directed to a modified soluble FGF receptor Fc fusion comprising a fusion of a soluble fragment or domain of a FGF receptor with an Fc region of an immunoglobulin, wherein all N-glycosylation sites are occupied, and wherein at most 45% of the N-glycans of the FGF receptor moiety have no sialyl group and wherein the N-glycans of the Fc region are not fucosylated. In another embodiment, the modified soluble FGF receptor Fc fusion of the invention is partially fucosylated, e.g., 0 to 60 % fucosylated. In yet another embodiment, the modified soluble FGF receptor Fc fusion of the invention is entirely fucosylated. In a preferred embodiment, the modified soluble FGF receptor Fc fusion of the invention is 60 to 100 % fucosylated. In a further preferred embodiment, each N-glycan molecule of the modified soluble FGF receptor Fc fusion according to the present invention further comprises 3 mannose residues, and a mean of 1.5 to 3.0 galactose residues, 3.5 to 5 of N-acetylglucosamine per molecule of glycan, and 0.6 to 1 fucose residues.

According to the invention, the modified soluble FGF receptor Fc fusion binds FGF ligand with high affinity. For example, said fusion binds FGF2 with a K_D value measured by Biacore™ comprised between 1 and 5 nM. In a preferred embodiment of the invention, the K_D value of said fusion for FGF2 measured by Biacore™ is around 1.5 nM.

Such modified fusions as described above are useful as potent and therapeutically effective inhibitors of tumor growth. Indeed the applicant has demonstrated that the modified soluble FGF receptor Fc fusions of the invention are capable of inhibiting tumor growth in vivo. Moreover, said modified soluble FGF receptor Fc fusions of the invention are capable of triggering ADCC and/or CDC responses both in vitro and in vivo. As effective ADCC and/or CDC mediating molecules, these compounds are especially useful to treat FGF-overexpressing cancerous tumors.

The FGFR sequences used for the FGFR compounds, full length or fragments of an FGFR, synthetic FGFR sequences, extracellular domains, soluble domains, or fusions of these, can be selected from any available or known sequences. FGFR belongs to the tyrosine kinase family of receptors and to the immunoglobulin (Ig) supergene family. In transmembrane forms of the receptor, the tyrosine kinase domain is intracellular and the Ig-like domains are extracellular. Both transmembrane and secreted forms bind FGF. There are at least four genes that encode FGFRs that have a common structure of two or three extracellular immunoglobulin (Ig)-like loops (IgI-IgIII) and one intracellular tyrosine kinase domain. Alternative splicing products are also known, from exons encoding the extracellular region, resulting in multiple receptor forms. The third Ig-like loop leads to at least three receptor variants and two membrane-spanning forms are produced by alternative splicing of two exons (IIIb and IIIc) encoding the second half of loop III. For example, a selective polyadenylation site preceding exons IIIb and IIIc is used to produce a soluble form of FGFR1 (IIIa). In humans and mice, the IgIIIa splice variant of FGFR1 encodes a protein that apparently has no hydrophobic membrane-spanning domain and may therefore be a secreted or soluble form of the receptor. The FGFR compounds may also utilize sequences from FGFR1 (Protein locus on NCBI, NP_075598); FGFR2 (Protein locus on NCBI, NP_000132); FGFR3 (Protein locus on NCBI, P22607); and FGFR4 (Protein locus on NCBI, NP_002002) (Kiefer *et al.*, 1991, *Growth Factors* **5**:115-127).

Soluble forms of FGF receptors, comprising the extracellular domains, have also been discussed in U.S. Pat. Nos. 5,288,855; 6,656,728; WO 91/00916; WO 92/00999; WO 00/46380; WO 2005/016966; WO 2005/113295; WO 2006/113277; WO 2007/014123; and European Patent 529 076. The FGFR fragments, domains, or soluble or extracellular domains as used in the invention may include the fragment of an FGFR that is extracellular in its native form or consists of all or part of the naturally secreted form. Furthermore, the FGFR sequences as used in this invention can be those specifically described or listed and sequences having about 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% amino acid sequence identity over the full length of the polypeptide sequence described or depicted, or having about 95%, 90%, 85%, 80%, or 75% nucleic acid sequence identity over the polypeptide encoding region for nucleic acids encoding the FGFR sequences of the invention. The fragment or domain could also include additional amino acids or other regions of the FGFR as long as these

additional amino acids or regions do not prevent or significantly reduce the ability of the FGFR compound to be used as described in this invention. A polypeptide or fusion protein consisting essentially of a FGFR domain or fragment may contain other amino acids as long as the ability to be expressed in a mammalian cell and bind to FGF are retained, and optionally, in addition, as long as the ability to reduce cell growth or reduce vascularization is retained.

In one embodiment, the soluble fragment or domain of the FGF receptor is the soluble or extracellular domain of FGF receptor 1 (sFGFR1) or FGF receptor 2 (sFGFR2). The fragments of FGFR1 and FGFR2 selected can have one or more of the following mutations, an N-terminal deletion; of 1-7 amino acids; or N-term substitution; a deletion of the loop1 sequence; a deletion of the acidic box sequence. Polynucleotides encoding amino acid sequence mutants can be prepared by a variety of methods known in the art, including, but not limited to, oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis and cassette mutagenesis of an earlier prepared mutant or a non-mutant version of the molecule of interest (see, e.g., Kunkel, 1985, *Proc Natl Acad Sci USA* **82**:488).

In another embodiment, the soluble fragment or domain of the FGF receptor is the soluble or extracellular domain of FGF receptor 1 isotype IIIc (sFGFR1(IIIc)) and FGF receptor 2 isotype IIIc (sFGFR2(IIIc)).

Preferred embodiments include soluble fragment or domain of the FGF receptor 2 isotype or variant IIIc encoded by a polynucleotide having the sequence of SEQ ID NO: 3, and/or having the amino acid sequence of SEQ ID NO: 4 or a sequence with at least 95%, 97%, 98%, or 99% identity with the SEQ ID NO: 4.

According to this latest embodiment, the modified soluble FGF receptor Fc fusion (sFGFR2-Fc) of the present invention advantageously has a high affinity for its natural ligand FGF2 or high K_D value of the order of the nanomolar, comprised between 1 and 5 nM and more precisely around 1.5 nM.

Specific examples of immunoglobulin domains include, but are not limited to, the Fc region of an immunoglobulin molecule; the hinge region of an immunoglobulin molecule; the CH_1 region of an immunoglobulin molecule; the CH_2 region of an immunoglobulin molecule; the CH_3 region of an immunoglobulin molecule; the CH_4 region of an immunoglobulin molecule; and the light chain of an immunoglobulin molecule, and humanized variants of any of these. The sequences for these regions are also available to one of skill in the art (*see, for example,* Huck *et al.*, 1986, *Nucleic Acids Res.* **14**:1779).

As used in the specification and claims, "immunoglobulin Fc region or Fc" means the carboxyl-terminal portion of an immunoglobulin heavy chain constant region. The Fc regions are particularly important in determining the biological functions of the immunoglobulin and these biological functions are termed effector functions. As known in the art, the heavy chains of the immunoglobulin subclasses comprise four or five domains: IgM and IgE have five heavy chain domains, and IgA, IgD and IgG have four heavy chain domains. The Fc region of IgA, IgD and IgG is a dimer of the hinge-CH_2--CH_3 domains, and in IgM and IgE it is a dimer of the hinge-CH_2--CH_3--CH_4 domains. Further the CH_3 domain of IgM and IgE is structurally equivalent to the CH_2 domain of IgG, and the CH_4 domain of IgM and IgE is the homolog of the CH_3 domain of IgG (see, W. E. Paul, ed., 1993, Fundamental Immunology, Raven Press, New York, New York). Any of the known Fc regions would be useful as the Fc region in the modified soluble FGF receptor Fc fusions of the invention.

In one embodiment, the gene encoding the Fc region of human IgG (Fc_γ) is obtained by reverse transcription and PCR using RNA prepared from human leukocytes and appropriate 5' and 3' primers. The resulting DNA fragments contain complete sequences of the hinge, CH_2, and CH_3 domains of IgG and can be used as the template to generate variants in which certain amino acids are substituted, as known in the art. A primer encoding a peptide linker, including an optional restriction enzyme site, can be incorporated into the PCR process. The resulting DNA fragments are inserted into a holding vector and confirmed by DNA sequencing.

Preferably, the Fc region of immunoglobulin gamma-1 is used, which includes at least part of the hinge region, CH_1 region, CH_2 region, and CH_3 region. In addition, the Fc region of immunoglobulin gamma-1 can be a CH_1-deleted –Fc or a CH_2-deleted-Fc, and includes a part of a hinge region and a CH_3 region wherein the CH_1 and/or CH_2 region has been deleted. A CH_2-deleted-Fc has been described by Gillies *et al.*, (1990, *Hum. Antibod. Hybridomas*, 1:47).

Most preferably, the Fc region of IgG1 comprises the sequence encoded by a polynucleotide having the sequence of SEQ ID NO: 5, and/or the amino acid sequence as set forth in SEQ ID NO: 6 or a sequence with at least 95% identity with SEQ ID NO: 6. However, Fc regions from the other classes of immunoglobulins, IgA, IgD, IgE, and IgM, would also be useful as the Fc region.

Further, deletion constructs of these Fc regions, in which one or more of the constant domains are deleted, may be prepared. One of ordinary skill in the art could prepare such deletion constructs using well known molecular biology techniques. In addition, the Fc region used can be one that has about 99%, or about 98%, or about 95%, or about 90%, or about 85%, or about 80%, or about 75% amino acid identity to that shown in SEQ ID NO:6.

Specific mutations as compared to SEQ ID NO: 6 that can be selected from and used individually or in any combination are: a deletion or substitution of one of the Cys within the first 20 N-term amino acids; a deletion of Cys at position 5 in SEQ ID NO: 6; or a substitution of Cys at position 5. The Fc region sequence chosen can detectably increase the serum half life of the modified soluble FGF receptor Fc fusion.

The modified soluble FGF receptor Fc fusion of the invention may comprise a hinge or a spacer region can be used between the soluble receptor part and the Fc region, (Ashkenazi *et al.*, 1997, *Current Opinion in Immunology*, **9**:195-200). Examples include a flexible peptide linker of about 20 or fewer amino acids in length. More preferably, the peptide linker may be at least three amino acids in length, and/or a peptide linker comprising two or more of the following amino acids: glycine, serine, alanine, and threonine. In a preferred embodiment, the peptide linker does not include a protease cleavage site. Most preferred linker is SAL (Ser-Ala-Leu).

The present invention also provides for the construction of polynucleotides encoding the modified soluble FGF receptor Fc fusion according to the present invention as well as a vector capable of expressing the modified soluble FGF receptor Fc fusion when introduced into an appropriate host cell. According to the preferred embodiment, the polynucleotide encoding the modified soluble FGF receptor Fc fusion has the sequence of SEQ ID NO: 1, or a sequence sharing at least 80% identity with SEQ ID NO: 1. As used herein, "vector" is understood to mean any nucleic acid comprising a nucleotide sequence of interest and capable of being incorporated into a host cell, and optionally to express an encoded protein or polypeptide. Vectors include linear nucleic acids, plasmids, phagemids, cosmids and the like, all within the knowledge of a person skilled in the art. Polynucleotides encoding the FGFR or fusion compound of the invention, as well as vectors containing these nucleic acids and host cells wherein these vectors have been introduced, are also specifically incorporated into the scope of the invention.

Most preferably, the fusion molecules of the invention are encoded by DNA comprising an extracellular domain of an FGFR fused at the C terminus to the Fcγ1 region of the human immunoglobulin γ1 gene. The Fcγ1 region of the immunoglobulin γ1 gene includes at least a portion of the hinge domain and CH_3 domain, or at least a portion of the hinge domain, CH_2 domain and CH_3 domain. The DNA encoding the chimeric polypeptide molecules according to the present invention can be in its genomic configuration or its cDNA configuration. Signal peptides may be used to efficiently initiate the transport of a protein across the membrane of the endoplasmic reticulum. Signal sequences have been well characterized in the art and are known typically to contain 16 to 30 amino acid residues, although other sizes are possible. A detailed discussion of signal peptide sequences is provided by von Heijne (1986, *Nucleic Acids Res.*, **14**:4683). According to a preferred embodiment, the signal peptide is taken from Interleukin-2 signal peptide (SEQ ID No. 8) as known in the art. The applicant has observed that fusing this peptide to an extracellular domain of an FGFR leads to a secreted protein with a homogenous N-terminal amino acid sequence, which is not the case when the endogenous FGFR signal peptide is used.

An expression vector containing the coding sequences of the modified soluble FGF receptor Fc fusion of the invention placed under the control of appropriate transcriptional and translational regulatory sequences can be constructed by recombinant DNA technology as known in the art. Such expression vector is introduced into a host cell by any technique known to the person skilled in the art. The resulting vector-containing cell is then grown to produce a modified soluble FGF receptor Fc fusion or fragment thereof, using any technique known to the person skilled in the art.

According to the invention, a variety of expression systems may be used to express the modified soluble FGF receptor Fc fusion molecules. In one aspect, such expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transiently transfected with the appropriate nucleotide coding sequences, express a modified soluble FGF receptor Fc fusion molecule of the invention in situ. Mammalian cells are commonly used for the expression of a recombinant modified soluble FGF receptor Fc fusion molecule, especially for the expression of whole recombinant modified soluble FGF receptor Fc fusion molecule. For example, mammalian cells such as HEK293 or CHO cells, in conjunction with a vector, containing the expression signal such as one

carrying the major intermediate early gene promoter element from human cytomegalovirus, are an effective system for expressing the modified soluble FGF receptor Fc fusions of the invention (Foecking *et al.*, 1986, *Gene* **45**:101; Cockett *et al.*, 1990, *Bio/Technology* **8**:2).

In addition, a host cell is chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing of protein products may be important for the function of the protein. Different host cells have features and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems are chosen to ensure the correct modification and processing of the expressed modified soluble FGF receptor Fc fusion of interest. Hence, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, COS, HEK293, 3T3 or myeloma cells. The host cell may be co-transfected with two or more expression vectors, including the vector expressing the protein of the invention. For example, a host cell can be transfected with a first vector encoding a modified soluble FGF receptor Fc fusion polypeptide, as described above, and a second vector encoding a glycosyltransferase polypeptide. Alternatively, the second vector could express a small interfering RNA against a glycosyltransferase.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. In one embodiment of the invention, cell lines which stably express the modified soluble FGF receptor Fc fusion molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells are transformed with DNA under the control of the appropriate expression regulatory elements, including promoters, enhancers, transcription terminators, polyadenylation sites, and other appropriate sequences known to the person skilled in art, and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for one to two days in an enriched media, and then are moved to a selective media. The selectable marker on the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into a chromosome and be expanded into a cell line.

A number of selection systems may be used according to the invention, including but not limited to the Herpes simplex virus thymidine kinase (Wigler *et al.*, 1977, *Cell* **11**:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska *et al.*, 1992, Proc Natl Acad Sci USA 48:202), glutamate synthase selection in the presence of methionine sulfoximide (*Adv Drug Del Rev*, 2006, **58**: 671, and website or literature of Lonza Group Ltd.) and adenine phosphoribosyltransferase (Lowy *et al.*, 1980, *Cell* **22**:817) genes in tk, hgprt or aprt cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler *et al.*, 1980, *Proc Natl Acad Sci USA* **77**:357); gpt, which confers resistance to mycophenolic acid (Mulligan *et al.*, 1981, *Proc Natl Acad Sci USA* **78**:2072); neo, which confers resistance to the aminoglycoside, G-418 (Wu *et al.*, 1991, *Biotherapy* **3**:87); and hygro, which confers resistance to hygromycin (Santerre *et al.*, 1984, *Gene* **30**:147). Methods known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, John Wiley & Sons (1993). The expression levels of a modified soluble FGF receptor Fc fusion molecule can be increased by vector amplification. When a marker in the vector system expressing a modified soluble FGF receptor Fc fusion is amplifiable, an increase in the level of inhibitor present in the culture will increase the number of copies of the marker gene. Since the amplified region is associated with the gene encoding the modified soluble FGF receptor Fc fusion of the invention, production of said modified soluble FGF receptor Fc fusion will also increase (Crouse *et al.*, 1983, *Mol Cell Biol* **3**:257).

A number of factors are known to the person skilled in the art to influence the glycosylation level of a glycoprotein. For example, modified mammalian host cells can be used to alter the glycosylation profile of modified soluble FGF receptor Fc fusion by increasing or diminishing the expression of glycosyltransferase. Such modified mammalian host cells include, but are not limited to, CHO, COS, HEK293, PER.C6, 3T3, YB2/0 and myeloma cells (Stanley *et al.*, 1986, *Archives of Biochemistry and Biophysics*, **249**:533; Mori *et al.*, 2006, *Biotechnology and Bioengineering* **94**:68; Chitlaru *et al.*, 1998, *Biochem. J.* **336**:647; Umana *et al.*, 1999 *Nature Biotechnology* **17**:176). It is also known to the person skilled in the art that bioprocess factors affect

glycoprotein oligosaccharide biosynthesis (Goochee *et al.*, 1994, *Curr Opin Biotechnol.* 5:546). The effect of cell culture conditions, such as glucose or ammonium ions concentration, pH, serum, and the effects of other bioprocess factors, such as cell growth rate, cultivation time, influence N-linked glycosylation (*Biotechnol. Bioeng.* **39**:327 (1993); *Biotechnol. Bioeng.* **68**:370 (2000); *Bio/technology* **11**:720 (1993); *Cytotechnology* **17**:13 (1995); *Biochem J.* **272**:333 (1990)). It is also known that in addition to the host cells and the bioprocess factors, the oligosaccharide processing is influenced by the local environment at each N-glycosylation site pending on the local glycoprotein environment. Site-to-site differences may be extensive as was observed with t-PA or may involve more subtle differences in branching and terminal processing as observed for the three N-glycosylation sites of EPO (Goochee *et al*, 1991, *Bio/Technology* **9**: 1347).

After it has been produced, a modified soluble FGF receptor Fc fusion of the invention may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by Protein A affinity for Fc after and so on), centrifugation, differential solubility or by any other standard technique for the purification of proteins.

Quantitative sialic acid identification (N-acetylneuraminic acid residues), carbohydrate composition analysis and quantitative oligosaccharide mapping of N-glycans in the purified modified soluble FGF receptor Fc fusion proteins can be performed essentially as described previously (Saddic *et al.* Methods Mol. Biol. 194:23-36 (2002) and Anumula *et al.* Glycobiology 8:685-694 (1998)).

Fusion proteins incorporating soluble FGF receptor domains can be produced by methods familiar to those in the art for any other mammalian, expressable or biologically active fusion, *mutatis mutandis*. For example, methods reported to combine the Fc regions of IgG with the domains of cytokines and soluble receptors can be adopted to design and produce the FGFR compounds of the invention (*see, for example*, Capon *et al.*, Nature, 337:525-531 (1989); Chamow *et al.*, Trends Biotechnol., 14:52-60 (1996); U.S. 5,116,964, 5,349,053 and 5,541,087). Other examples of receptor-Ig fusion proteins that can be adopted include those of U.S. 5,726,044; 5,707,632; and 5,750,375. Because FGF receptor extracellular domains share a significant degree of homology to the immunoglobulin gene family and the FGFR extracellular domain contains Ig-like segments, the use of Fc regions is especially preferred. In one example, the fusion is a homodimeric protein linked through cysteine

residues in the hinge region of IgG Fc, resulting in a molecule with similar characteristics to an IgG molecule. One advantage to using an Fc region is extended circulating half-life. In addition, the glycosylation modifications of the modified soluble FGF receptor Fc fusion proteins of the invention lead to improved pharmacokinetic properties, as the fusions of the invention exhibit *in vivo* pharmacokinetic profiles comparable to human IgG of a similar isotype.

Also described is a method for making a modified soluble FGF receptor Fc fusion protein comprising a FGFR fragment or domain, a flexible peptide linker, and a human IgG Fc variant, which method comprises: (a) generating a CHO-derived cell line; (b) growing the cell line under conditions such that the recombinant fusion protein is expressed; and (c) purifying the expressed protein from step (b). In this case, preferably, the flexible peptide linker comprising at least about 3 amino acids between the soluble FGF receptor and the human IgG Fc variant comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine. In additional and related embodiments, the linker peptide is not present or is only one amino acid in length. In a preferred embodiment, the peptide linker does not include a protease cleavage site. The most preferred linker is SAL (Ser-Ala-Leu).

Preferably, the modified soluble FGF receptor fusion protein is produced in CHO cells in a suspension mode as described in the Examples herein.

As shown in the Examples herein, the modified soluble FGF receptor Fc fusions of the present invention have anti-tumoral activity, at least through induction of ADCC and/or CDC responses, and are thus useful in the treatment of metastatic tumors and diseases such as cancer. One aspect of the invention is thus directed to a modified soluble FGF receptor Fc fusion as described above with ADCC and/or CDC activities.

Of special interest are modified soluble FGF receptor Fc fusions with enhanced ability to mediate cellular cytotoxic effector functions such as ADCC. Such proteins may be obtained by making single or multiple substitutions in the Fc region of the molecule, thus altering its interaction with the Fc receptors. Methods for designing such mutants can be found for example in Lazar *et al.* (2006, *Proc. Natl. Acad. Sci. U.S.A.* **103**(11): 4005-4010) and Okazaki *et al.* (2004, *J. Mol. Biol.* **336**(5):1239-49). See also WO 03/074679, WO 2004/029207, WO 2004/099249, WO2006/047350. WO 2006/019447.

WO 2006/105338, WO 2007/041635. It is also possible to use cell lines specifically engineered for production of improved modified soluble FGF receptor Fc fusions. In particular, these lines have altered regulation of the glycosylation pathway, resulting in modified soluble FGF receptor Fc fusions which are poorly fucosylated or even totally defucosylated. Such cell lines and methods for engineering them are disclosed in e.g. Shinkawa *et al.* (2003, *J. Biol. Chem.* **278**(5): 3466-3473), Ferrara *et al.* (2006, *J. Biol. Chem.* **281**(8): 5032-5036; 2006, *Biotechnol. Bioeng.* **93**(5): 851-61), EP 1331266, EP 1498490, EP 1498491, EP 1676910, EP 1792987, and WO 99/54342.

Methods of inhibiting tumor growth in a subject, and methods for the treatment or prevention of metastasis in a subject, comprising administering an efficient amount of such modified soluble FGF receptor Fc fusions as described above, are described. The invention also relates to a method for treating cancer, said method comprising administering to a subject a modified soluble FGF receptor Fc fusion of the invention in a therapeutically effective amount. The invention thus also relates to the modified soluble FGF receptor Fc fusion as described above as a medicament. Also described is the use of the modified soluble FGF receptor Fc fusion as described above for the preparation of a medicament for treating or inhibiting tumor growth in a subject. The invention also relates to the use of a modified soluble FGF receptor Fc fusion of the invention for the preparation of a medicament for treating cancer.

Another aspect of the invention relates to pharmaceutical compositions of the modified soluble FGF receptor Fc fusion of the invention. The pharmaceutical compositions of the invention typically comprise the modified soluble FGF receptor Fc fusion of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, buffers, salt solutions, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The type of carrier can be selected based upon the intended route of administration. In various embodiments, the carrier is suitable for intravenous, intraperitoneal, subcutaneous, intramuscular, topical, transdermal or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of media and agents for pharmaceutically active substances is well known in the art. As detailed herebelow, additional active compounds can also be incorporated into the compositions, such as anti-cancer and/or anti-angiogenesis agents; in particular, the additional active

compound can be an anti-angiogenic agent, a chemotherapeutic agent, or a low-molecular weight agent. A typical pharmaceutical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 100 mg of the combination. Actual methods for preparing parenterally administrable compounds will be known or apparent to those skilled in the art and are described in more detail in for

example, Remington's Pharmaceutical Science, 17th ed., Mack Publishing Company, Easton, Pa. (1985), and the 18th and 19th editions thereof, which are incorporated herein by reference.

The modified soluble FGF receptor Fc fusion of the invention can also be prepared with carriers and controlled-release formulations, including implants and microencapsulated delivery systems. Biodegradable and biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are generally known to those skilled in the art.

The modified soluble FGF receptor Fc fusion in the composition preferably is formulated in an effective amount. An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired result, such as modulation of FGF and/or FGFR activities and induction of ADCC and/or CDC responses. A "therapeutically effective amount" means an amount sufficient to influence the therapeutic course of a particular disease state. A therapeutically effective amount is also one in which any toxic or detrimental effects of the agent are outweighed by the therapeutically beneficial effects.

For therapeutic applications, the modified soluble FGF receptor Fc fusions of the invention are administered to a mammal, preferably a human, in a pharmaceutically acceptable dosage form such as those discussed above, including those that may be administered to a human intravenously as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intraarticular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The modified soluble FGF receptor Fc fusions also are suitably administered by intratumoral, peritumoral, intralesional, or perilesional routes, to exert local as well as systemic therapeutic effects. The intraperitoneal route is expected to be particularly useful, for example, in the treatment of ovarian tumors.

Dosage regimens may be adjusted to provide the optimum response. For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased. The compositions of the invention can be administered to a subject to effect cell growth activity in a subject. As used herein, the term "subject" is intended to include living organisms in which an FGF-

dependent cell growth exists and specifically includes mammals, such as rabbits, dogs, cats, mice, rats, monkey transgenic species thereof, and humans.

The modified soluble FGF receptor Fc fusions and the pharmaceutical compositions of the invention are useful in the treatment or prevention of a variety of cancers, including (but not limited to) the following: carcinoma, including that of the bladder, breast, colon, head and neck, kidney, including renal cell carcinoma, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin; including squamous cell carcinoma ; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Burkitt's lymphoma ; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer and teratocarcinoma, and other cancers yet to be determined which are caused by FGF overexpression. In a preferred embodiment, the modified soluble FGF receptor Fc fusion of the invention is used to treat melanoma, leukemia, renal cancer, colon cancer, ovarian cancer, prostate cancer, lung cancer, bladder cancer, breast cancer, or head and neck cancer.

Also described is the use of the modified soluble FGF receptor Fc fusion described above for the preparation of a medicament for treating or inhibiting cancer-related diseases in a subject. Also described is a method of treating diseases and processes that result from cancer cell proliferation, and a composition for treating or repressing the growth of a cancer. Also descsribed are compositions and methods useful for gene therapy for the modulation of cancer. The method of the present invention may be used in particular for the treatment of melanoma, leukemia, renal cancer, colon cancer, ovarian cancer, prostate cancer, lung cancer, bladder cancer, breast cancer, or head and neck cancer.

The effectiveness of the modified soluble FGF receptor Fc fusion in preventing or treating disease may be improved by administering said fusion serially or in

combination with another agent that is effective for those purposes, such as tumor necrosis factor (TNF), an antagonist capable of inhibiting or neutralizing the angiogenic activity of acidic or basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), or hepatocyte growth factor (HGF), an antagonist capable of inhibiting or neutralizing the coagulant activities of tissue factor, protein C, or protein S (see WO 91/01753), an antagonist such as an antibody capable of binding to HER2 receptor (see US 5,772,997), or one or more conventional therapeutic agents such as, for example, alkylating agents, folic acid antagonists, anti-metabolites of nucleic acid metabolism, antibiotics, pyrimidine analogs, 5-fluorouracil, cisplatin, purine nucleosides, amines, amino acids, triazol nucleosides, or corticosteroids.

In another aspect of the invention, the administration is combined with an administration of therapeutically effective amount of chemotherapeutic agent, such as for example, taxol (paclitaxel) or taxotere (docetaxel).

Chemotherapeutic agents include without any limitations, anti-microtubule agents such as diterpenoids and vinca alkaloids; platinum coordination complexes; alkylating agents such as nitrogen mustards, oxazaphosphorines, alkylsulfonates, nitrosoureas, and triazenes; antibiotic agents such as anthracyclins, actinomycins and bleomycins; topoisomerase II inhibitors such as epipodophyllotoxins; antimetabolites such as purine and pyrimidine analogues and antifolate compounds; topoisomerase I inhibitors such as camptothecins; hormones and hormonal analogues; signal transduction pathway inhibitors; non-receptor tyrosine kinase angiogenesis inhibitors; immunotherapeutic agents; proapoptotic agents; and cell cycle signaling inhibitors. In addition, the methods of the invention can be combined with another anti-cancer treatment, anti-angiogenic agent, or chemotherapeutic agent or radiation therapy. A preferred example is docetaxel or taxotere. Other examples include, gemcitabine, cisplatin diterpenoids and vinca alkaloids, paclitaxel, vinblastine, vincristine, and vinorelbine, carboplatin, cyclophosphamide, melphalan, and chlorambucil, busulfan, carmustine, dacarbazine, cyclophosphamide, melphalan, chlorambucil, busulfan, carmustine, dacarbazine, anti-neoplastic agents including, but not limited to, actinomycins such as dactinomycin, anthrocyclins such as daunorubicin and doxorubicin, bleomycins, epipodophyllotoxins, etoposide and teniposide; antimetabolite neoplastic agents, 5-fluorouracil, methotrexate, cytarabine, mecaptopurine, thioguanine, camptothecins, irinotecan HCl, and topotecan HCl.

A variety of different chemotherapeutic agents or anti-cancer polypeptides can also be selected. Information sources such as www.clinicaltrials.gov, www.ncbi.nlm.nih, and www.drugs.com, include references to polypeptides and agents that can be selected.

Such other agents, *e.g.* anti-angiogenic agents or chemotherapeutic agents may be present in the composition being administered or may be administered separately. In one aspect of the invention, the administration is performed with the other active principle, either simultaneously, separately or sequentially over time. When the administration is performed simultaneously, the two active principles may be combined in a single pharmaceutical composition, comprising the two compositions, such as a tablet or a gel capsule. On the other hand, the two active principles may, whether or not they are administered simultaneously, be present in separate pharmaceutical compositions. To this end, the combination may be in the form of a kit comprising, on the one hand, the modified soluble FGF receptor Fc fusion as described above and, on the other hand, the second active principle, the modified soluble FGF receptor Fc fusion as described above and the second active principle being in separate compartments and being intended to be administered simultaneously, separately, or sequentially over time.

The combination useful according to the present invention can be administered especially for tumor therapy in combination with chemotherapy, protein therapy (i.e. using a therapeutic agent such as an antibody or recombinant protein), gene therapy, radiotherapy, immunotherapy, surgical intervention, or a combination of these. Long-term therapy is equally possible as is adjuvant therapy in the context of other treatment strategies, as described above.

The examples that follow are merely exemplary of the scope of this invention and content of this disclosure. One skilled in the art can devise and construct numerous modifications to the examples listed below without departing from the scope of this invention.

Examples

Example 1: Production in HEK293 of sFGFR2-Fc with a high sialic acid content and low blood clearance.

cDNAs encoding human α-1,4-galactosyltransferase (B4GT1) (SEQ ID No. 9) or human β-2,3-sialyltransferase (SIAT6) (SEQ ID No. 11) were retrieved from clone collection (Invitrogen) and cloned into the mammalian expression vector pXL4214 from which expression is driven from the CMV promoter. The same expression vector was also used to clone the protein fusion sFGFR2-Fc and generate pXL4410. Map of plasmids pXL4551 encoding B4GT1, pXL4544 encoding SIAT6 and pXL4410 encoding the modified soluble FGFR2IIIc-Fc fusion (herein designated as sFGFR2-Fc) are presented on Fig. 1 and 5 as well as the nucleic acid and corresponding amino acid sequence of B4GT1 (Fig. 2), SIAT6 (Fig. 3) and sFGFR2-Fc (Fig.4 A and B). sFGFR2-Fc was produced in adherent HEK293 EBNA cells (Invitrogen) by transient transfection of one to three expression plasmids encoding sFGFR2-Fc, B4GT1or SIAT6 complexed with JET PEI (Q-Biogen). Plasmid ratio was 90/5/5 for pXL4410/pXL4544/pXL4551. Plasmid ratio had to be optimized to ensure optimal productivity and quality of the sFGFR2-Fc polypeptide. Secreted proteins were harvested eight days post-transfection and centrifuged. Proteins were purified by affinity chromatography on Protein G Sepharose (Amersham Biosciences) after elution from the column with 100 mM glycine/HCl pH 2.7. The sFGFR2-Fc proteins were formulated in PBS and 0.22 µm filtered. Protein concentration was determined by the microBC Assay (Interchim).

Quantitative sialic acid identification, carbohydrate composition analysis and quantitative oligosaccharide mapping of N-glycans in the sFGFR2-Fc purified proteins were performed essentially as described previously (Saddic *et al.* 2002. Methods Mol. Biol. 194:23-36 and Anumula *et al.*1998. Glycobiology 8:685-694). First, sialic acid residues were released after mild hydrolysis of sFGFR2-Fc and fluorescently labeled with ortho-phenylenediamine and separated by reversed-phase HPLC. Individual peaks were detected by fluorescence detection (excitation, 230 nm; emission, 425 nm), identified and quantitated by comparison with N-acetylneuraminic and N-glycolylneuraminic acid standards. Second, the carbohydrate composition was determined after acid hydrolysis of sFGFR2-Fc samples to release the individual monosaccharides. After hydrolysis, the monosaccharides (neutral and amino sugars) were derivatized with anthranilic acid and then separated by reversed-phase HPLC and detected by fluorescence detection (excitation, 360 nm; emission, 425 nm). Individual

peaks were identified and quantitated by comparison with monosaccharide standards.Third, oligosaccharides were enzymatically released with PNGase F and fluorescently labeled with anthranilic acid before separation according to their number of sialic acid residues by normal phase-anion exchange HPLC on an Asahipak-NH2P (Phenomenex) column. Labeled glycans were detected and quantitated by fluorescence detection (excitation, 360 nm; emission, 425 nm). The average number of sialic acid per N-glycan in the FGFR2 domain was calculated based on the total amount of moles of N-glycan per mole of FGFR2-Fc and the moles of N-glycan per Fc mole obtained after release of the Fc by papaïn.

The purified sFGFR2-Fc proteins were injected in the tail of Swiss Nude mice (Charles River). A total of three mice were used per protein batch. Blood was collected 6-hour post injection of 500 µg of sFGFR2-Fc, plasma was obtained and FGFR2-Fc concentration was determined by ELISA utilizing the sandwich method with an anti-human FGFR2 monoclonal antibody (R&D system) and an anti-human IgG-HRP conjugate polyclonal antibody (Pierce) (2 analysis at 2 dilutions in triplicate). In the control experiments mice were pretreated with fetuin and asiolofetuin one hour prior to injection of sFGFR2-Fc.

Table 1 summarizes the condition of production of the different sFGFR2-Fc batches, the N-glycan profile and monosaccharide composition of each batch and the plasma concentration of sFGFR2-Fc 6-hour post intravenous injection in mice.

Table 1- N-Glycan content and pharmacokinetic of FGFR2-Fc produced in HEK293

Expressed protein in HEK293EBNA	sFGFR2-Fc	sFGFR2-Fc + SIAT6	sFGFR2-Fc + SIAT6 & B4GT1
% sFGFR2-Fc sialylated species:			
1-non sialylated	69 %	46 %	34 %
2-monosialylated	23 %	19 %	22 %
2-disialylated	6 %	26 %	35 %
3-trisialylated	1.5 %	10 %	10 %
Sialic acid content:			
1-pmol of N-acetylneuraminic acid per pmol of sFGFR2-Fc	3.4	6.4	6.8
2-Average number of sialic acid per sFGFR2 N-glycan	0.4		1
Monosaccharide composition of sFGFR2-Fc N-glycans for 3 mannoses			
1-Glucosamine (number per 3 mannoses)	4.3	4.1	4.1
2-Galactose (number per 3 mannoses)	1.5	1.4	1.6
Number of fucose in Fc N-glycans for 3 mannoses	0.73	0.61	0.63
Blood clearance			
[sFGFR2-Fc] in plasma 6-hour post i.v. injection (ng/mL)	258		20000
[sFGFR2-Fc] in plasma 6-hour post i.v. injection (ng/mL) when mice were pretreated with fetuin	229	Not done	Not done
[sFGFR2-Fc] in plasma 6-hour post i.v. injection (ng/mL) when mice were pretreated with asialofetuin	19276	Not done	Not done

Improved sialylation pattern of sFGFR2-Fc fusion proteins produced in HEK293EBNA has been demonstrated by transient co-expression of the fusion protein with human α-1,4-galactosyltransferase or human β-2,3-sialyltransferase. This large improvement of sialylation status was evidenced by a 2-fold reduction of the percentage of non-sialylated glycans, a 2-fold increase in the total sialic acid content per mol of protein and a 2.5-fold increase in the average number of sialic acid per N-glycan. Of note the monosaccharide content was not affected (see Table 1).

This 2.5-fold increase in sialylated N-glycan is directed correlated with the significantly improved pharmacokinetic parameters of sFGFR2-Fc. In particular a 100-fold increase of the sFGFR2-Fc presence in the plasma has been measured 6-hour post iv injection in mice.

The pharmacokinetic was also improved by 100-fold when mice were pretreated with asialofetuin compared to the pretreatment with fetuin. Asialofetuin, but not fetuin, is known to bind to the hepatic asialoglycoprotein receptor (ASGPR) (Webster *et al.*, 2003, *Xenobiotica* **33**:945)

Taken together, these results indicate that specific binding to asialoglycoprotein receptor via exposed terminal galactose residues from N-glycan are responsible for clearance of sFGFR2-Fc whereas the presence of one sialic acid per N-glycan on sFGFR2-Fc decreased significantly this clearance.

Example 2: Screening of CHO stable clones expressing sFGFR2-Fc protein with an average number of sialic acid residues per FGFR2 N-glycan greater than 1.2 for optimal pharmacokinetics

Mammalian expression plasmid pXL4636 for stable expression of sFGFR2-Fc in CHO cells was generated from plasmid pEE14.4 encoding glutamine synthetase selection marker (Lonza) and plasmid pXL4410 containing the cDNA sequence encoding sFGFR2-Fc, Fig 5. Plasmid pXL4636 was introduced into CHO K1 cells by nucleofection utilizing AMAXA cell line Nucleofactor kit as recommended by the supplier. Transfected cells were transferred into selective medium and after cell amplification the seven best producer CHO/GS semi-clones (SC# 9, 11, 26, 58, 112, 118, 170) were screened for the sialic acid content of the purified sFGFR2-Fc molecules. The two semi-clones with the highest sialic acid content (SC # 11and 118) were selected for cloning and up-scale production; in particular clone from SC# 118 was further described as GC111.

Although sFGFR2-Fc molecules produced from all the semi-clones had a high sialic acid content, they did not lead to the same pharmacokinetics. Interestingly, it was observed that from two semi-clones (SC#11 and 118), sFGFR2-Fc with the highest sialic acid content led to the highest sFGFR2-Fc concentration in the blood 6-hour post iv injection in mice, as described on Table 2. And from the two clones (SC# 9 and 170) sFGFR2-Fc with the lowest sialic acid content had the lowest sFGFR2-Fc concentration in the blood 6-hour post iv injection in mice.

Table 2- CHO stable clones expressing sFGFR2-Fc protein with a ratio of sialic acid residues per sFGFR2 N-glycan greater than 1.2 for optimal pharmacokinetics

Purified sFGFR2-Fc from CHO-GS semi-clone SC #	SC# 9	SC# 11	SC# 26	SC# 58	SC# 112	SC# 118	SC# 170
% sFGFR2-Fc sialylated species							
1-non sialylated	50	42	48	48	45	43	53
2-monosialylated	28	30	30	30	30	30	28
3-disialylated	14	18	14	15	16	17	13
4-trisialylated	8	10	7	8	9	10	6
Average number of sialic acid per sFGFR2 N-glycan	1.07	1.45				1.29	0.98
[sFGFR2-Fc]/ [sFGFR2-Fc]max [sFGFR2-Fc] found in plasma 6-hour post i.v. injection [sFGFR2-Fc]max found for SC#11	55%	100 %				81 %	45 %

The screening of clones based on high sialylated N-glycan of sFGFR2-Fc is predictive of optimal pharmacokinetics parameters of sFGFR2-Fc.

Example 3: Correlation between the average number of sialic acid per sFGFR2-Fc N-glycan and the clearance of sFGFR2-Fc in blood

In other experiment similar to the experiment described in Example 2, stable CHO/DHFR clones expressing sFGFR2-Fc were generated using the DHFR selection and amplification system with the appropriate mammalian expression plasmids pXL4429 and plasmid pXL4417 (Fig. 6). These CHO-DHFR clones have also been screened for the content of sialic acid per sFGFR2-Fc molecule and the clearance of sFGFR2-Fc produced by these clones was also assayed.

The average number of sialic acid per N-glycan in the FGFR2 domain was calculated based on the total amount of moles of N-glycan per mole of FGFR2-Fc and the moles of N-glycan per Fc mole obtained after release of the Fc by papaïn. The results presented on Fig. 7 showed that a ratio of sialic acid residues per FGFR2 N-glycan greater than 1.2 ensured optimal sFGFR2-Fc concentration in the blood as compared to the optimal concentration in the blood found for Fc molecules. Only the selected clones reached this optimal ratio.
Therefore screening clones by the ratio of sialic acid residues per FGFR2 N-glycan allows the skilled person to predict the low clearance of sFGFR2-Fc in blood.

Example 4: Amino acid sequence of the sFGFR2-Fc fusion protein

The protein sFGFR2-Fc encoded by plasmid pXL4410 or pXL4636 or pXL4429 (Figure 5 and 6) is a fusion protein of the soluble FGFR2 human sequence with the Fc fragment derived from the human IgG1 sequence.

The sequence of the polynucleotide encoding sFGFR2-Fc is set forth in SEQ ID NO: 1 and in Figure 4 A. Likewise, the full amino acid sequence of the sFGFR2-Fc protein is set forth in SEQ ID NO: 2 in Figure 4B. Amino acids from positions 1 to 350 correspond to the FGFR2IIIc isotype (see Fig 4 C SEQ ID; NO: 4) and are the amino acids from position 27 to 376 described in SwissProt (FGFR2_HUMAN). Amino acids from positions 354 to 584 are amino acids of IgG1 from position 99 to 329 as described in SwissProt (IGHG1_HUMAN); see Fig 4 D and SEQ ID NO: 6. Amino acids from positions 351 to 353 are amino acids of a synthetic linker: SAL (Ser Ala Leu) see Fig 4 E.

Example 5: Defined N-glycan content of sFGFR2-Fc produced in CHO stable clone GC111

Conditions have been optimized to produce sFGFR2-Fc such that the ratio of sialic acid residues per sFGFR2 N-glycan would be higher than 1.2. This example provides conditions to reach this condition.

A 5-L Celligen bioreactor (New Brunswick) filled with 4.4 L of CD-CHO protein-free media, supplemented with 100 µM MSX and 1X GS supplements was seeded at an initial cell density of 3.5 x 10^5 cells/mL of clone GC111 and cultured at 37°C. Sparger aeration was employed using a mixture of oxygen, nitrogen, air and carbon dioxide or pur oxygen, and dissolved oxygen was maintained at 30 % of air saturation. The pH

was maintained at 7.2 by addition of carbon dioxide and injection of 1 M sodium bicarbonate in the culture medium. The agitation rate used was 110 rpm using a Cell Lift Impeller. The feed solution (450 g/L glucose) was continuously fed to maintain the glucose at a target level of 2-3 g/L. Feeding was started at day 5 when residual glucose concentration reached 2.5 g/L. Continuous nutrient feeding was based on the predicted cell growth and glucose consumption with a nutrient feeding rate equal to the glucose consumption rate. The glutamate concentration was maintained at 1-3 mmol/L by pulse addition following daily off-line control. The cell culture was monitored for cell count, viability and metabolites (glucose, lactate, glutamine, ammonia and glutamate) and for product concentration during the production phase. The culture was stopped when cell viability dropped down to 55%, and the culture harvest was collected.
The cell culture harvest was clarified and sFGFR2-Fc was purified by affinity chromatography (ProsepvA, Millipore) and two ion-exchange chromatography steps then filtered sterile and stored in phosphate buffer saline before further analysis and *in vivo* testing.

The apparent molecular mass of sFGFR2-Fc obtained from SDS-PAGE analysis under non-reducing conditions was 180 kDa. This was in contrast with the theoretical molecular mass of 130 kDa calculated based on the amino acid sequence of the sFGFR2-Fc homodimer. This large difference between apparent and calculated molecular masses was attributed to the additional presence of about 30 % of N-glycans, see Table 3. Indeed upon digestion of the sFGFR2-Fc by Peptide-N-glycosidase F (PNGase F, Roche) followed by analysis by SDS-PAGE under non-reducing conditions, the molecular mass of deglycosylated sFGFR2-Fc was around 160 kDa (Fig. 7).
The carbohydrate composition and the N-glycan profile of sFGFR2-Fc was analyzed as described on Example 1 and reported on Table 3.

Table 3 Carbohydrate composition and N-glycan profile of sFGFR2-Fc produced in optimal conditions

Expressed protein from stable CHO-GS clone GC111	sFGFR2-Fc
% sFGFR2-Fc sialylated species:	
1-non sialylated	30 %
2-Monosialylated	34 %
3-Disialylated	23 %
4-trisialylated	13 %
Average number of sialic acid per sFGFR2 N-glycan	1.34

Monosaccharide composition of sFGFR2-Fc N-glycans per 3 mannoses	
1-Glucosamine (number per 3 mannoses)	4.9
2-Galactose (number per 3 mannoses)	2.7
3-Fucose (number per 3 mannoses)	0.96
Fucose composition of Fc N-glycan per 3 mannoses	1.1

Compared to the results obtained on Table 2, sFGFR2-Fc, produced from clone GC111 in the optimal conditions, had more sialylated N-glycan than sFGFR2-Fc produced from the semi-clone SC#118, parent of clone GC111 produced in standard conditions. For example the % of non-sialylated species decreased from 43% to 30%.

Based on the total amount of moles of N-glycan per mole of FGFR2-Fc and the moles of N-glycan per Fc mole obtained after release of the Fc by papaïn, it was measured that there were seven N-glycans per FGFR2. Therefore most the sites of the FGFR2 domain are almost all fully occupied.

Example 6: N-glycosylation sites important for FGF affinity and productivity

The sFGFR2-Fc fusion protein has eight N-glycosylation sites in the FGFR2 domain and one in the Fc domain, see Fig 8 and Table 5 for definition of N-glycan positions N1 to N8. Proteins 4493 and 4565, two variants of sFGFR2-Fc no longer having Ig-like domain 1, were produced. 4493 exhibited characteristics similar to wild type FGFR2-Fc derived from pXL4636 in terms of productivity and binding to FGF-2 or heparin. In contrast, 4565 in which all glycosylation sites in the sFGFR2 domain were mutated (N- to Q substitution) could not be characterized due to the more than 50-fold reduction in productivity of the mutant, see Table 4.

Table 4. Physico-chemical characteristics of sFGFR2-Fc variants

Protein ID	N-Glycosylation sites in FGFR2 domain	Amino acid residue at N-glycosylation site	Production (mg/L)	FGF-2 binding **	Heparin binding

				K_D (10^{-9} M)	[NaCl] for elution from heparin (mM)
4410	8 (N1 to N8)	N	75	1.39	370
4493	6 (N3 to N8)	N	67	1.19	442
4565	6 (N3 to N8)	Q	< 1	ND	ND

** Binding to FGF-2 was determined on a BIAcore™ instrument utilizing a "two-state reaction with conformation change" model as described by Gamsjaeger *et al.*, Biochem. J. 7 Apr. 2005 / BJ20050156. In brief, integration was performed on the entire sensogram except in the "bulk effect" areas. Concentration range was selected between 1 to 8 nM. The simultaneous ka/kd kinetics method allows the measurements of two ka and two kd assuming the following equations:

ka1 and kd1 for $A+B \rightleftharpoons AB$

ka2 and kd2 for $AB \rightleftharpoons AB^*$ that may represent dimerization of (FGF - FGFR2-Fc) complexes

The dissociation constant K_D was calculated from the following formula:

$$\frac{1}{(ka_1 / kd_1) \times (1 + ka_2 / kd_2)}$$

According to the two competing models reported (the symetric two-end model from Mohammadi and the asymmetric model from Pellegrini), sites N3 to N7 could potentially interact with amino acid residues of either FGF1 or FGFR2c and/or interact with heparin, whereas site N8 is unlikely to be involved in interactions in all crystal structures (Pellegrini *et al.* 2000. *Nature* **407**: 1029; Ibrahimi *et al.* 2005 *Mol. Cell. Biol.* **25** : 671).

Based on the above information, it was relevant to study the N-glycosylation sites at positions N3 to N7 by substitution of the corresponding Asn to Gln and by keeping position N8 unchanged to allow for significant productivity. The positional influence of N-glycans on physico-chemical characteristics of sFGFR2-Fc was evaluated

statistically with a two-level fractional factorial experiment. Five variables (glycosylation site occupancy at positions N3 to N7) were selected and 16 independent constructs were studied. The experimental 2^{5-1} fractional design and data analysis were performed as described (Statistics for Experimenters, G. Box Ed., Willey, 1978).

Fractional Factorial Design

Site-specific N-glycosylation variants were designed based on 4493 and the factorial design at two levels with five variables (N3 to N7) that could either be at a plus level (N) or a minus level (Q). The design was fractional with 16 constructs (2^{5-1}, i.e. resolution V design), allowing the identification of the main effects and of 2-factor interactions but confounding 2-factor with 3-factor interactions.

The 16 plasmid constructs were obtained by sequencial PCR and cloning to generate the N to Q substitution at position N3, N4, N5, N6 or N7 but keeping position N8 and the N-glycosylation site of the Fc domain unchanged. These protein variants only differed from 4493 by the 2- or 4-point mutations listed on Table 5.

Table 5. Design matrix

		N-glycosylation position status						
Asn postion on Figure 9	Construct	N3	N4	N5	N6	N7	N8	N 297 (Fc)
Asn position on FGFR2_HUMAN		228	241	265	297	318	331	
	4572	-	-	-	-	+	+	+
	4570	+	-	-	-	-	+	+

4577	-	+	-	-	-	+	+
4571	+	+	-	-	+	+	+
4587	-	-	+	-	-	+	+
4585	+	-	+	-	+	+	+
4586	-	+	+	-	+	+	+
4573	+	+	+	-	-	+	+
4574	-	-	-	+	-	+	+
4575	+	-	-	+	+	+	+
4588	-	+	-	+	+	+	+
4576	+	+	-	+	-	+	+
4579	-	-	+	+	+	+	+
4569	+	-	+	+	-	+	+
4578	-	+	+	+	-	+	+
4493	+	+	+	+	+	+	+

The 16 designed constructs were tested for production at small scale. Two variants (4572 and 4574) were very low producers (around 2 mg/L). The remaining 14 constructs were produced at liter-scale and purified in parallel under standard conditions with reasonable product recovery. They were then analyzed by SDS-PAGE, gel filtration, BIAcore™. Results were analyzed with the statistical fractional factorial resolution- 2^{5-1} DOE.

Productivity

N-glycosylation at N3, N4, N5, N6, N7 had a positive contribution on productivity. There was a similar quantitative effect for all the positions studied (i.e. N3, N4, N5, N6 and N7) and no significant effect of two-factor interactions.

Table 6. Productivity

Construct	Titer (mg/L) from production at 1-L
4572	0
4570	13
4577	9
4571	34

Response (mg/L)	Variable
28	Mean
17	N3
14	N4
20	N5

4587	5
4585	40
4586	42
4573	32
4574	0
4575	30
4588	37
4576	27
4579	30
4569	54
4578	37
4493	67

13	N6
13	N7
-8	N3-N4
3	N3-N5
1	N3-N6
-2	N3-N7
-2	N4-N5
-1	N4-N6
6	N4-N7
4	N5-N6
0	N5-N7
-1	N6-N7

Aggregation

Purified proteins were analyzed by gel filtration (Superdex 2000) to quantitate the percentage of high molecular weight species (HMW; %) in purified preparations. The highest value (worst-case) of 80.2 % HMW was obtained for 4587 and was used in the DOE analysis for the two constructs (4572 and 4574) that could not be produced (the mean percentage of HMW value obtained from the 14 constructs was also used for comparison, giving a similar conclusion). N-glycosylation at position N5 and to a lesser extent at position N6 disfavored the appearance of HMW species. The N5-N6 interaction exhibited a similar effect on aggregation.

Table 7. Aggregate formation

Construct	HMW (%)
4572	80
4570	73.0
4577	71.6
4571	72.1
4587	80.2

Response(%)	Variable
57	Mean
-12	N3
-5	N4
-33	N5
-18	N6

4585	47.5
4586	47.7
4573	57.5
4574	80
4575	69.6
4588	71.8
4576	72.9
4579	35.2
4569	12.0
4578	39.4
4493	3.6

-7	N7
6	N3-N4
-8	N3-N5
-5	N3-N6
2	N3-N7
-2	N4-N5
3	N4-N6
-4	N4-N7
-18	N5-N6
-6	N5-N7
1	N6-N7

Binding to FGF-2

The binding affinity to FGF-2 was determined with BIAcore™ for each construct. Dissociation constant (K_D) values between 0.49 and 2.31 nM were obtained for constructs showing measurable affinity. For constructs that did not bind to FGF-2 or could not be produced, a value of 10 nM was used for the DOE analysis. N-glycosylation at positions N5 and to a lower extent at positions N6 and N3 had a positive effect on binding to FGF-2. Interactions N3-N5 & N5-N6 had a significant positive effect on binding whereas interactions N3-N6 and N4-N7 had a negative effect on binding.

Table 8. Binding to FGF-2

Construct	K_D (nM)
4572	10
4570	10
4577	10
4571	10
4587	10

Response(nM)	Variable
6.8	Mean
-2.2	N3
-0.5	N4
-6.5	N5
-2.3	N6

4585	2.7
4586	10
4573	0.49
4574	10
4575	10
4588	10
4576	10
4579	2.31
4569	0.99
4578	0.66
4493	1.19

0.5	N7
0.0	N3-N4
-2.2	N3-N5
2.0	N3-N6
0.1	N3-N7
-0.5	N4-N5
0.1	N4-N6
2.0	N4-N7
-2.3	N5-N6
0.5	N5-N7
0.0	N6-N7

This resolution-V 2^{5-1} D.O.E. revealed that N-glycosylation had a positive contribution on productivity, at positions N3, N4, N5, N6, N7; had a positive impact on binding to FGF-2, at positions N5 >> N6 and N3; and disfavored the appearance of high molecular-weight molecules at all positions, especially N5 > N6.

Therefore N-glycan occupancy is mandatory at position N5, and recommended at positions N3, N4, N6 and N7 respectively (position 265, 228, 241, 297and 318 respectively on FGFR2_HUMAN (Swissprot).

Example 7: Pharmacokinetics of sFGFR2-Fc fusion protein

In example 7, sFGFR2-Fc is the fusion protein corresponding to amino acid sequence SEQ ID N° 2 with an average number of sialic acid per sFGFR2 N-glycan of 1.3. Three mice per time point were injected with 500 µg of the fusion protein in the tail vein. At various time points after injection of the product, blood was collected, Figure 10 shows protein concentration in plasma and liver. Figure 11 shows the amount of protein recovery at early time points in plasma and liver expressed in percentage of injected dose.

Pharmacokinetic parameters were calculated using non-compartmental analysis. Elimination half-life was calculated with the last 6 data points that provided the best fit of the log-linear terminal phase (Table 9).

Table 9

Parameter	Units	sFGFR2-Fc
t½	hr	70.1
Tlast	hr	72.0
Clast	µg/mL	27.6
AUClast	hr*µg/mL	3138
Clobs	mL/hr/kg	4.2
Vss	mL/kg	406

AUClast	Area under the curve from the time of dosing to the last measurable concentration.
Cl obs	Total body clearance for extravascular administration
Clast	Concentration corresponding to Tlast
t1/2	Terminal half-life
Tlast	Time of last measurable (non-zero) concentration.
Vss	an estimate of the volume of distribution at steady state

Following intravenous administration, sFGFR2-Fc showed a favorable pharmacokinetic profile with a long elimination half-life (almost 3 days) and a reduced total body clearance. The volume of distribution was limited and lower than total body water volume, suggesting limited tissue distribution. At 72 h, sFGFR2-Fc plasma concentration stays at a very high concentration and clearance is also good. Pharmacokinetic parameters of sFGFR2-Fc are very compatible with the use of this fusion protein as a therapeutic. In effect as shown in the Examples, plasma concentration after intravenous injection in mice is substantially high, and clearance stays very low at 72 h after injection. Importantly, the kinetics of sFGFR2-Fc cleavage in vivo is also very low, since the sFGFR2-Fc was only partially cleaved (40% after 18h) and the full-length molecule concentration remained unchanged until 72 h. Therefore, the fusion molecule, as listed in SEQ ID NO: 2 and an average number of sialic acid per sFGFR2 N-glycan of 1.3, showed a favorable pharmacokinetic profile with a long elimination half-life (almost 3 days) and a satisfactory total body clearance.

Example 8: Efficacy of the sFGFR2-Fc fusion protein in A549 subcutaneous tumor model

In this example, sFGFR2-Fc is the fusion protein corresponding to amino acid sequence SEQ ID N° 2 with an average number of sialic acid per sFGFR2 N-glycan of 1.3.

A549 subcutaneous tumor model

A549 cells line had been identified as a tumor cell line to assess the efficacy of sFGFR2-Fc. It has been demonstrated in vitro by the Applicant that this cell line expresses FGF2 and also that sFGFR2-Fc can abrogate autocrine proliferation of these cells. A subcutaneous A549 tumor model was set up by the Applicant in Balb/c nude mice. Moreover the tumor in vivo expresses FGF2.
In this experiment, the efficacy of sFGFR2-Fc molecule was assessed on A549 tumor growth model. Products were injected subcutaneously twice a week. Different doses, i.e. 25, 15, and 5 mg/kg of the sFGFR2-Fc were assessed.
After overall analysis of tumor volume evolution of the three treated group with sFGFR2-Fc were statically different to the tumor evolution of the group treated with PBS. sFGFR2-Fc is efficient on tumor growth in this model at the dose of 5 mg/kg twice a week.

Experimental Design

5.10^6 A549 cells in 200 µl were injected subcutaneous in Balb/c nude mice at day 0. After cell injection the same day, mice were randomized per block of 4 in four groups based on body weight. The treatments started after randomization the day after cell injection. Each group received subcutaneously 500, 300 or 100 µg/mouse/administration corresponding respectively to 25, 15 and 5 mg/kg, two times per week: Monday and Friday, during 39 days.

Results

Tumor volume analysis

As shown in Figure 12, the volume of the tumor was analyzed up to day 40. The groups 100 µg (triangle), 300 µg (squares) and 500 µg/mouse/administration (closed circles) were statistically different from group PBS (open circles). We concluded that sFGFR2-Fc decreased tumor growth at the dose 25, 15 and 5 mg/kg.

Tumor weight at day 40

As shown in Figure 13, the tumors were harvested and weighed at the end of the experiment. The groups 100 µg, 300 µg and 500 µg/mouse/administration were

statically different to group PBS. We concluded that sFGFR2-Fc according to the present invention decreased tumor weight at the dose 25, 15 and 5 mg/kg.

Evolution of tumor growth of sFGFR2-Fc_100, sFGFR2-Fc _300 and sFGFR2-Fc _500 groups were statistically different to the evolution of the group treated with PBS. The sFGFR2-Fc fusion protein according to the present invention (amino acid sequence SEQ ID N° 2 with an average number of sialic acid per sFGFR2 N-glycan of 1.3, is able to substantially decrease tumor growth at the dose of 5 mg/kg.

Example 9: Efficacy of sFGFR2-Fc fusion protein in H460 subcutaneous tumor model

In example 9, sFGFR2-Fc is the fusion protein corresponding to amino acid sequence SEQ ID N° 2 with an average number of sialic acid per sFGFR2 N-glycan of 1.6.

H460 subcutaneous tumor model

H460 cells line had been identified as a tumor cell line to assess the efficacy of sFGFR2-Fc. It has been demonstrated in vitro that this cell line expresses FGF2. In this experiment the efficacy of sFGFR2-Fc was assessed on tumor growth. Products were injected subcutaneously twice a week at the dose of 25 mg/kg. After overall analysis of tumor volume evolution, sFGFR2-Fc decreased tumor growth.

Experimental Design

5.10^6 H460 cells in 200 µl were injected subcutaneous in right flank of Balb/c nude mice at day 0. After cells injection, on the day of cell inoculation (Day 0) mice were randomized on the body weight measured and will be allocated to the treatments groups.

Treatments were administered twice a week by subcutaneously injections (200 µl) for 3 consecutive weeks (Monday and Friday). The first administration was performed on Day 1 after cell inoculation to maximize the exposure of the cells to the treatment.

Results

Tumor volume analysis

Figure 14 shows the tumor volume analysis up to day 22. We concluded that sFGFR2-Fc substantially decreased the tumor growth.

Tumor weight analysis

Figure 15 shows the tumor weight analysis at day 22. We concluded that sFGFR2-Fc substantially decreased the tumor weight.

Evolution of tumor growth of sFGFR2-Fc group was statically different of evolution of group treated with PBS. We clearly concluded that the fusion protein according to the present invention (amino acid sequence SEQ ID N° 2 with an average number of sialic acid per sFGFR2 N-glycan of 1.6 is able to substantially decrease H460 tumor growth at the dose of 25 mg/kg.

Example 10: Evaluation of in vitro ADCC activity of sFGFR2-Fc fusion protein on A549 and H460 cell lines

In this example sFGFR2-Fc is the fusion protein corresponding to amino acid sequence SEQ ID N° 2 with an average number of sialic acid per sFGFR2 N-glycan greater than 1.6. The capacity of sFGFR2-Fc to mediate an *in vitro* ADCC activity has been evaluated in both selected models H460 and A549 tumor cells (see examples 8 and 9).

Experimental Design

Tumor cells (A549 and H460) in PBS 2% BSA (1 million/mL) have been incubated 30 min at 4°C with 500 ng/mL of FGF2 (R&DSystems) and 2 µg/mL of sFGFR2-Fc or control human IgG1 (Sigma). Tumor cells have been diluted in RPMI 1% FBS and incubated in 96-well plate at 5000 cells per well. Purified NK have been added in ratio NK/tumor cells 20/1 and 6/1. Plates have been incubated 4 hours at 37°C, then centrifuged and lactate dehydrogenase has been titrated in the supernatant (kit ROCHE). 100% lysis was obtained using triton X100 0.2%. Specific sFGFR2-Fc induced ADCC was calculated as required by the manufacturer.

Results

The results illustrated on Figure 16, show a tumor cell lysis mediated by natural killer cells (NK) in the presence of sFGFR2-Fc on both A549 and H460 tumor cells close to 25 % in the 20/1 (NK/tumor cell) conditions, indicating that sFGFR2-Fc is able to mediate ADCC effect on these tumor cells.

Example 11: Evaluation of in vivo ADCC and CDC activities of sFGFR2-Fc fusion protein in A549 subcutaneous tumor model

In this example, sFGFR2-Fc is the fusion protein corresponding to amino acid sequence SEQ ID N° 2 with an average number of sialic acid per sFGFR2 N-glycan greater than 1.6.

A549 subcutaneous tumor model

A549 cell line has been identified as a tumor cell line to assess the efficacy of sFGFR2-Fc, since these cells expressed high level of FGF2 in vivo and were sensitive to *in vitro* sFGFR2-Fc-mediated-ADCC.

Mouse strains

Three different mouse strains (SCID, NOD/SCID and SCID/bg mice) were selected to evaluate the capacity of sFGFR2-Fc to mediate *in vivo* ADCC and/or CDC activities. SCID mice kept NK and complement functions and are able to develop ADCC and CDC responses and were selected as positive control. NOD/SCID mice have neither NK function nor the ability to stimulate complement activity and were unable to develop neither ADCC nor CDC activities. SCID/bg mice have no NK function and were unable to develop ADCC activity.
In this experiment, the efficacy of sFGFR2-Fc molecule line was assessed on A549 tumor subcutaneously implanted in three different mouse strains. sFGFR2-Fc was injected subcutaneously twice a week at the dose of 5 mg/kg during the entire study course.

Experimental Design

A549 was subcutaneously implanted in SCID, NOD/SCID and SCID/bg mice as previously described (see example 8). The treatments have been administered twice a

week by subcutaneously injections for 6 consecutive weeks. During the study course, the body weight and the tumor volume have been measured twice a week.

Results

As shown in Figure 17, the volume of the tumor was analyzed up to day 41 in the 3 experiments. In SCID mice, the group treated by sFGFR2-Fc at 5 mg/kg (diamonds) was statistically different from group control (open circles) and showed a tumor inhibition (calculated as 100 - (Treated group volume / Control group volume x 100)) of 39%. In the NOD/SCID mice, sFGFR2-Fc at 5 mg/kg exhibited no activity. In the SCID/bg mice, sFGFR2-Fc at 5 mg/kg recovered partially its activity (tumor inhibition = 18%). According to these results, CDC and ADCC mechanisms were involved in sFGFR2-Fc efficacy.

Example 12: Evaluation of in vivo ADCC and CDC activities of sFGFR2-Fc fusion protein in H460 subcutaneous tumor model

In this example sFGFR2-Fc is the fusion protein corresponding to amino acid sequence SEQ ID N° 2 with an average number of sialic acid per sFGFR2 N-glycan greater than 1.6.

H460 subcutaneous tumor model

H460 cell line has been identified as a tumor cell line to assess the efficacy of sFGFR2-Fc, these cells expressed high level of FGF2 in vivo and were sensitive to *in vitro* sFGFR2-Fc mediated-ADCC.

Mouse strains

As previously described in example 11, three mouse strains with different immune functions (SCID, NOD/SCID and SCID/bg mice) were selected to evaluate the capacity of sFGFR2-Fc to mediate in vivo ADCC and/or CDC activities in H460.

In this experiment, the efficacy of sFGFR2-Fc molecule was assessed on H460 tumor subcutaneously implanted in the three different mouse strains. sFGFR2-Fc was injected subcutaneously twice a week at the dose of 25 mg/kg during the entire study course.

Experimental Design

H460 was subcutaneously implanted in SCID, NOD/SCID and SCID/bg mice as previously described (see example 9). The treatments have been administered twice a week by subcutaneously injections for 3 consecutive weeks. During the study course, the body weight and the tumor volume have been measured twice a week.

Results

As shown in Figure 18, the volume of the tumor was analyzed up to day 22 in the 3 experiments. In SCID mice, the group treated with sFGFR2-Fc at 25 mg/kg (diamonds) was statistically different from group control (open circles) and showed a tumor inhibition of 29%. In the NOD/SCID mice, sFGFR2-Fc at 5 mg/kg exhibited a lost of activity with a tumor inhibition of 14%. In the SCID/bg mice, sFGFR2-Fc at 5 mg/kg recovered all its activity (tumor inhibition = 32%). In these studies, as observed in the A549 tumor model (see example 11), CDC and ADCC mechanisms were involved in sFGFR2-Fc efficacy.

Example 13 Evaluation of in vivo efficacy of sFGFR2-Fc compared to sFGFR2-Fc (A265 Fc) in H460 subcutaneous tumor model

Shields at al. described a point mutation Asp265Ala in the Fc domain of human IgG1 named (A265 Fc) that conferred reduced binding to all FcγR receptors and very low antibody-dependent cell cytotoxicity (2001 J. Biol. Chem 276:6591). This point mutation was introduced into the Fc domain of sFGFR2-Fc coding DNA sequence of plasmid pXL4547 (Fig. 19), resulting in the polynucleotide sequence represented by SEQ ID No. 13. Stable CHO/DHFR clones expressing sFGFR2-Fc (A265 Fc) (SEQ ID NO. 14) were generated using the DHFR selection and amplification system with the appropriate mammalian expression plasmids pXL4547 and plasmid pXL4417 as described in Example 3. The protein sFGFR2-Fc (A265 Fc) was then produced and purified for *in vivo* studies. It was verified that its glycan content was similar to the glycan content found for sFGFR2-Fc produced in Examples 3 or 5.

Expressed protein from stable CHO-DHFR	sFGFR2-Fc (A265 Fc)
% sFGFR2-Fc sialylated species:	
1-non sialylated	40 %
2-Monosialylated	28 %

3-Disialylated	22 %
4-trisialylated	10 %
Monosaccharide composition of sFGFR2-Fc N-glycans per 3 mannoses	
1-Glucosamine (number per 3 mannoses)	4.35
2-Galactose (number per 3 mannoses)	2.74
3-Fucose (number per 3 mannoses)	0.89

460 subcutaneous tumor model

H460 cell line has been identified as a tumor cell line to assess the efficacy of sFGFR2-Fc; these cells expressed high level of FGF2 in vivo and were sensitive to in vitro sFGFR2-Fc-mediated-ADCC.

In this experiment, the efficacy of sFGFR2-Fc molecule and the modified sFGFR2-Fc (A265 Fc) molecule was assessed on H460 tumor subcutaneously implanted in nude mice.

Experimental Design

H460 was subcutaneously implanted in nude Balb/C mice as previously described (see example 9). The treatments have been administered twice a week during the entire study course. During the study course, the body weight and the tumor volume have been measured twice a week.

Results

As shown in Figure 20, the volume of the tumor was analyzed up to day 23. The group treated by sFGFR2-Fc at 25 mg/kg (diamonds) was statistically different from the control group (open circles) and showed a tumor inhibition of 50%. The modified sFGFR2-Fc (A265 Fc) at 25 mg/kg exhibited a lost of activity with only a tumor inhibition of 25% (NS). The mutation within the Fc of sFGFR2-Fc (A265 Fc) induced a decrease of the activity.

Since this modification was shown to decrease antibody-dependent cell cytotoxicity, example 13 provides an indirect evidence that sFGFR2-Fc acted *in vivo* by a mechanism involving ADCC.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A modified soluble FGF receptor Fc fusion comprising a fusion of a soluble fragment or domain of a FGF receptor with an Fc region of an immunoglobulin, wherein at least the 5th N-glycosylation site of the FGF receptor moiety is occupied, and at most 45% of the N-glycans of said FGF receptor moiety have no sialyl group, and wherein the average number of sialic acid per N-glycan of the FGF receptor moiety is 1.2 or above, and the FGF receptor is FGF receptor 2 (FGFR2).

2. The modified soluble FGF receptor Fc fusion of claim 1 , wherein, in addition, the 3rd, 4th, 6th and 7th N-glycosylation sites of the FGF receptor moiety are occupied.

3. The modified soluble FGF receptor Fc fusion of claim 2, wherein at least 7 N-glycosylation sites of the FGF receptor moiety are occupied.

4. The modified soluble FGF receptor Fc fusion of claim 3, wherein all N-glycosylation sites of the FGF receptor moiety are occupied.

5. The modified soluble FGF receptor Fc fusion of any one of the previous claims, wherein the K_D value of said fusion for FGF2 measured by Biacore™ is comprised between 1 and 5 nM.

6. The modified soluble FGF receptor Fc fusion of claim 5, wherein the K_D value of said fusion for FGF2 measured by Biacore™ is around 1.5 nM.

7. The modified soluble FGF receptor Fc fusion of any one of the previous claims wherein said fusion possesses ADCC and/or CDC activities.

8. The modified soluble FGF receptor Fc fusion of any one of the previous claims, wherein said modified soluble FGF receptor Fc fusion comprises 3 mannose residues, a mean of 1.5 to 3.0 galactose residues, a mean of 3.5 to 5 of N- acetylglucosamine residues, and a mean of 0.6 to 1 fucose residues per molecule of glycan.

9. The modified soluble FGF receptor Fc fusion of any one of the previous claims, wherein the FGF receptor is FGF receptor 2 isotype IIIc.

10. The modified soluble FGF receptor Fc fusion of any one of the previous claims, wherein the FGF receptor soluble domain has a sequence as set forth in SEQ ID NO: 4, or a sequence having an identity of at least 95% with the SEQ ID NO: 4.

11. The modified soluble FGF receptor Fc fusion of any one of the previous claims, wherein the Fc portion has a sequence as set forth in SEQ ID NO: 6, or a sequence having an identity of at least 95% with the SEQ ID NO: 6.

12. The modified soluble FGF receptor Fc fusion of any one of the previous claims, wherein said modified soluble FGF receptor Fc fusion further comprises a linker sequence of at least 3 amino acid residues.

13. The modified soluble FGF receptor Fc fusion of claim 12, wherein the linker sequence is SAL (Ser-Ala-Leu).

14. The modified soluble FGF receptor Fc fusion of any one of the previous claims, wherein modified soluble FGF receptor Fc fusion has a polypeptide sequence as set forth in SEQ ID NO: 2, or a sequence having an identity of at least 95% with the SEQ ID NO: 2.

15. The modified soluble FGF receptor Fc fusion of any one of the previous claims, wherein modified soluble FGF receptor Fc fusion further comprises the signal peptide of SEQ ID NO: 8.

16. The modified soluble FGF receptor Fc fusion of any one of claims 1 to 15 as a medicament.

17. A pharmaceutical composition comprising a modified fusion FGF receptor of any one of claims 1 to 15.

18. The pharmaceutical composition of claim 17, wherein said composition contains a further therapeutic agent.

19. The use of a modified soluble FGF receptor Fc fusion of any one of claims 1 to 15 for the preparation of a medicament for treating cancer.

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20. The use of claim 19, comprising the use of a further therapeutic agent in the manufacture of the same or different medicament.

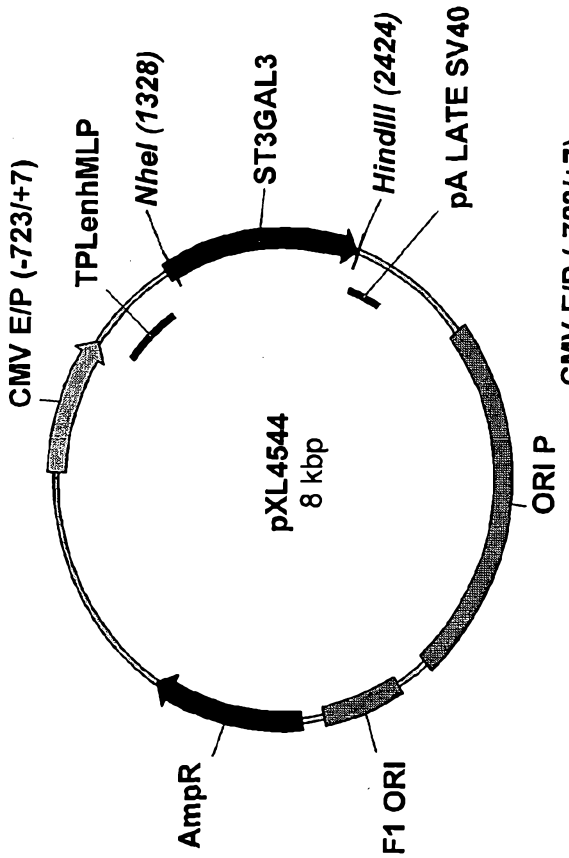
21. The use of claim 20, or the pharmaceutical composition of claim 18, wherein said further therapeutic agent is an anti-angiogenic agent or a chemotherapeutic agent.

22. The use or the pharmaceutical composition of claim 21, wherein said anti-angiogenic agent is a tumor necrosis factor, or an antagonist of an acidic or basic fibroblast growth factor (FGF), hepatocyte growth factor (HGF), tissue factor (TF), protein C, protein S, platelet-derived growth factor (PDGF), or HER2 receptor, or wherein said chemotherapeutic agent is selected from the group: anti-microtubule agents; platinum coordination complexes; alkylating agents; antibiotic agents; topoisomerase II inhibitors; antimetabolites; topoisomerase I inhibitors; hormones and hormone analogues; signal transduction pathway inhibitors; non-receptor tyrosine kinase angiogenesis inhibitors; immunotherapeutic agents; pro-apoptotic agents; and cell cycle signaling inhibitors.

23. The use or the pharmaceutical composition of claim 21, wherein said chemotherapeutic agent is selected from the group of taxol and taxotere.

24. The use of claim 19, wherein said cancer is selected from the group of carcinoma, including that of the bladder, breast, colon, head and neck, kidney, including renal cell carcinoma, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin; including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Burkitt's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscarama, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer, prostate cancer, and teratocarcinoma.

25. A method for treating cancer, said method comprising administering to a subject a modified soluble FGF receptor Fc fusion of any one of claims 1 to 15 in a therapeutically effective amount.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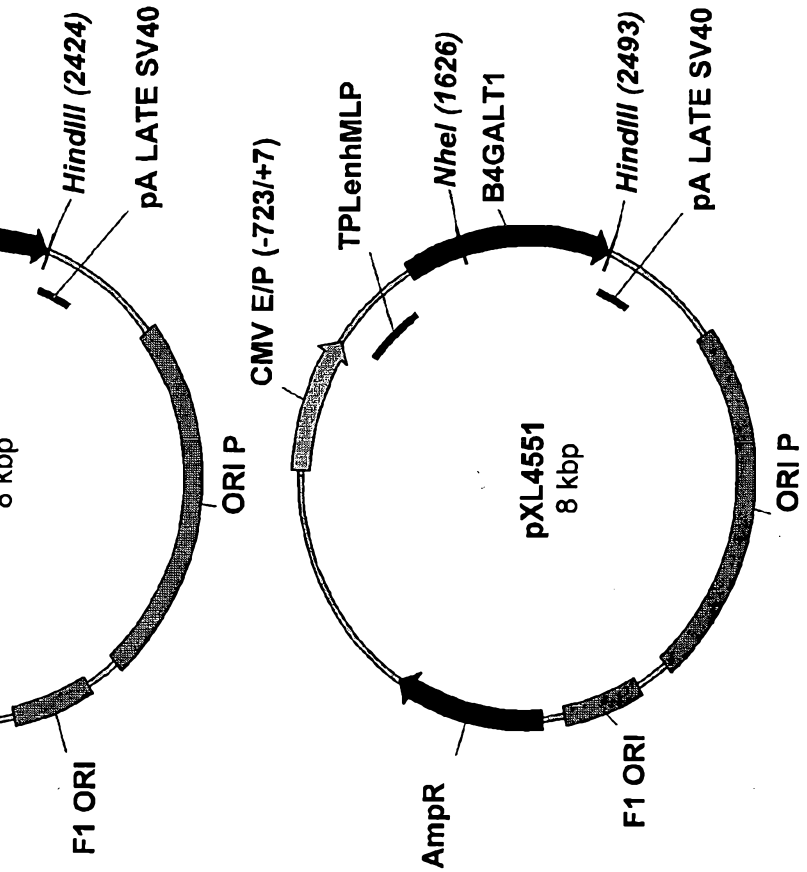
TPLenhMLP
NheI (1328)
ST3GAL3
HindIII (2424)
pA LATE SV40
ORI P
F1 ORI
AmpR
pXL4544
8 kbp

Fig. 1A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TPLenhMLP
NheI (1626)
B4GALT1
HindIII (2493)
pA LATE SV40
ORI P
F1 ORI
AmpR
pXL4551
8 kbp

Fig. 1B

Fig. 2A

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1281                ATGAGG CTTCGGGAGC CGCTCCTGAG CGGCAGCGCC GCGATGCCAG GCGCGTCCCT ACAGCGGGCC
                    TACTCC GAAGCCCTCG GCGAGGACTC GCCGTCGCGG CGCTACGGTC CGCGCAGGGA TGTCGCCCGG
1361    TGCCGCCTGC TCGTGGCCGT CTGCGCTCTG CACCTTGGCG TCACCCTCGT TTACTACCTG GCTGGCCGCG ACCTGAGCCG
        ACGGCGGACG AGCACCGGCA GACGCGAGAC GTGGAACCGC AGTGGGAGCA AATGATGGAC CGACCGGCGC TGGACTCGGC
1441    CCTGCCCCAA CTGGTCGGAG TCTCCACACC GCTGCAGGGC GGCTCGAACA GTGCCGCCGC CATCGGGCAG TCCTCCGGGG
        GGACGGGGTT GACCAGCCTC AGAGGTGTGG CGACGTCCCG CCGAGCTTGT CACGGCGGCG GTAGCCCGTC AGGAGGCCCC
1521    AGCTCCGGAC CGGAGGGGCC CGGCCGCCGC CTCCTCTAGG CGCCTCCTCC CAGCCGCGCC CGGGTGGCGA CTCCAGCCCA
        TCGAGGCCTG GCCTCCCCGG GCCGGCGGCG GAGGAGATCC GCGGAGGAGG GTCGGCGCGG GCCCACCGCT GAGGTCGGGT
1601    GTCGTGGATT CTGGCCCTGG CCCCGCTAGC AACTTGACCT CGGTCCCAGT GCCCCACACC ACCGCACTGT CGCTGCCCGC
        CAGCACCTAA GACCGGGACC GGGGCGATCG TTGAACTGGA GCCAGGGTCA CGGGGTGTGG TGGCGTGACA GCGACGGGCG
1681    CTGCCCTGAG GAGTCCCCGC TGCTTGTGGG CCCCATGCTG ATTGAGTTTA ACATGCCTGT GGACCTGGAG CTCGTGGCAA
        GACGGGACTC CTCAGGGGCG ACGAACACCC GGGGTACGAC TAACTCAAAT TGTACGGACA CCTGGACCTC GAGCACCGTT
1761    AGCAGAACCC AAATGTGAAG ATGGGCGGCC GCTATGCCCC CAGGGACTGC GTCTCTCCTC ACAAGGTGGC CATCATCATT
        TCGTCTTGGG TTTACACTTC TACCCGCCGG CGATACGGGG GTCCCTGACG CAGAGAGGAG TGTTCCACCG GTAGTAGTAA
1841    CCATTCCGCA ACCGGCAGGA GCACCTCAAG TACTGGCTAT ATTATTTGCA YCCAGTCCTG CAGCGCCAGC AGCTGGACTA
        GGTAAGGCGT TGGCCGTCCT CGTGGAGTTC ATGACCGATA TAATAAACGT RGGTCAGGAC GTCGCGGTCG TCGACCTGAT
1921    TGGCATCTAT GTTATCAACC AGGCGGGAGA CACTATATTC AATCGTGCTA AGCTCCTCAA TGTTGGCTTT CAAGAAGCCT
        ACCGTAGATA CAATAGTTGG TCCGCCCTCT GTGATATAAG TTAGCACGAT TCGAGGAGTT ACAACCGAAA GTTCTTCGGA
2001    TGAAGGACTA TGACTACACC TGCTTTGTGT TTAGTGACGT GGACCTCATT CCAATGAATG ACCATAATGC GTACAGGTGT
        ACTTCCTGAT ACTGATGTGG ACGAAACACA AATCACTGCA CCTGGAGTAA GGTTACTTAC TGGTATTACG CATGTCCACA
2081    TTTTCACAGC CACGGCACAT TTCCGTTGCA ATGGATAAGT TTGGATTCAG CCTACCTTAT GTTCAGTATT TTGGAGGTGT
        AAAAGTGTCG GTGCCGTGTA AAGGCAACGT TACCTATTCA AACCTAAGTC GGATGGAATA CAAGTCATAA AACCTCCACA
2161    CTCTGCTCTA AGTAAACAAC AGTTTCTAAC CATCAATGGA TTTCCTAATA ATTATTGGGG CTGGGGAGGA GAAGATGATG
        GAGACGAGAT TCATTTGTTG TCAAAGATTG GTAGTTACCT AAAGGATTAT TAATAACCCC GACCCCTCCT CTTCTACTAC
2241    ACATTTTTAA CAGATTAGTT TTTAGAGGCA TGTCTATATC TCGCCCAAAT GCTGTGGTCG GGAGGTGTCG CATGATCCGC
        TGTAAAAATT GTCTAATCAA AAATCTCCGT ACAGATATAG AGCGGGTTTA CGACACCAGC CCTCCACAGC GTACTAGGCG
2321    CACTCAAGAG ACAAGAAAAA TGAACCCAAT CCTCAGAGGT TTGACCGAAT TGCACACACA AAGGAGACAA TGCTCTCTGA
        GTGAGTTCTC TGTTCTTTTT ACTTGGGTTA GGAGTCTCCA AACTGGCTTA ACGTGTGTGT TTCCTCTGTT ACGAGAGACT
2401    TGGTTTGAAC TCACTCACCT ACCAGGTGCT GGATGTACAG AGATACCCAT TGTATACCCA AATCACAGTG GACATCGGGA
        ACCAAACTTG AGTGAGTGGA TGGTCCACGA CCTACATGTC TCTATGGGTA ACATATGGGT TTAGTGTCAC CTGTAGCCCT
2481    CACCGAGCTA G
        GTGGCTCGAT C
```

Fig. 2B

```
1     MRLREPLLSG SAAMPGASLQ RACRLLVAVC ALHLGVTLVY YLAGRDLSRL
51    PQLVGVSTPL QGGSNSAAAI GQSSGELRTG GARPPPPLGA SSQPRPGGDS
101   SPVVDSGPGP ASNLTSVPVP HTTALSLPAC PEESPLLVGP MLIEFNMPVD
151   LELVAKQNPN VKMGGRYAPR DCVSPHKVAI IIPFRNRQEH LKYWLYYLHP
201   VLQRQQLDYG IYVINQAGDT IFNRAKLLNV GFQEALKDYD YTCFVFSDVD
251   LIPMNDHNAY RCFSQPRHIS VAMDKFGFSL PYVQYFGGVS ALSKQQFLTI
301   NGFPNNYWGW GGEDDDIFNR LVFRGMSISR PNAVVGRCRM IRHSRDKKNE
351   PNPQRFDRIA HTKETMLSDG LNSLTYQVLD VQRYPLYTQI TVDIGTPS
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Fig. 3A

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1281                    ATGGGA CTCTTGGTAT TTGTGCGCAA TCTGCTGCTA GCCCTCTGCC TCTTTCTGGT ACTGGGATTT
                        TACCCT GAGAACCATA AACACGCGTT AGACGACGAT CGGGAGACGG AGAAAGACCA TGACCCTAAA
1361            TTGTATTATT CTGCGTGGAA GCTACACTTA CTCCAGTGGG AGGAGGACTC CAATTCAGTG GTTCTTTCCT TTGACTCCGC
                AACATAATAA GACGCACCTT CGATGTGAAT GAGGTCACCC TCCTCCTGAG GTTAAGTCAC CAAGAAAGGA AACTGAGGCG
1441            TGGACAAACA CTAGGCTCAG AGTATGATCG GTTGGGCTTC CTCCTGAATC TGGACTCTAA ACTGCCTGCT GAATTAGCCA
                ACCTGTTTGT GATCCGAGTC TCATACTAGC CAACCCGAAG GAGGACTTAG ACCTGAGATT TGACGGACGA CTTAATCGGT
1521            CCAAGTACGC AAACTTTTCA GAGGGAGCTT GCAAGCCTGG CTATGCTTCA GCCTTGATGA CGGCCATCTT CCCCCGGTTC
                GGTTCATGCG TTTGAAAAGT CTCCCTCGAA CGTTCGGACC GATACGAAGT CGGAACTACT GCCGGTAGAA GGGGGCCAAG
1601            TCCAAGCCAG CACCCATGTT CCTGGATGAC TCCTTTCGCA AGTGGGCTAG AATCCGGGAG TTCGTGCCGC CTTTTGGGAT
                AGGTTCGGTC GTGGGTACAA GGACCTACTG AGGAAAGCGT TCACCCGATC TTAGGCCCTC AAGCACGGCG GAAAACCCTA
1681            CAAAGGTCAA GACAATCTGA TCAAAGCCAT CTTGTCAGTC ACCAAAGAGT ACCGCCTGAC CCCTGCCTTG GACAGCCTCC
                GTTTCCAGTT CTGTTAGACT AGTTTCGGTA GAACAGTCAG TGGTTTCTCA TGGCGGACTG GGGACGGAAC CTGTCGGAGG
1761            GCTGCCGCCG CTGCATCATC GTGGGCAATG GAGGCGTTCT TGCCAACAAG TCTCTGGGGT CACGAATTGA CGACTATGAC
                CGACGGCGGC GACGTAGTAG CACCCGTTAC CTCCGCAAGA ACGGTTGTTC AGAGACCCCA GTGCTTAACT GCTGATACTG
1841            ATTGTGGTGA GACTGAATTC AGCACCAGTG AAAGGCTTTG AGAAGGACGT GGGCAGCAAA ACGACACTGC GCATCACCTA
                TAACACCACT CTGACTTAAG TCGTGGTCAC TTTCCGAAAC TCTTCCTGCA CCCGTCGTTT TGCTGTGACG CGTAGTGGAT
1921            CCCCGAGGGC GCCATGCAGC GGCCTGAGCA GTACGAGCGC GATTCTCTCT TTGTCCTCGC CGGCTTCAAG TGGCAGGACT
                GGGGCTCCCG CGGTACGTCG CCGGACTCGT CATGCTCGCG CTAAGAGAGA AACAGGAGCG GCCGAAGTTC ACCGTCCTGA
2001            TTAAGTGGTT GAAATACATC GTCTACAAGG AGAGAGTGAG TGCATCGGAT GGCTTCTGGA AATCTGTGGC CACTCGAGTG
                AATTCACCAA CTTTATGTAG CAGATGTTCC TCTCTCACTC ACGTAGCCTA CCGAAGACCT TTAGACACCG GTGAGCTCAC
2081            CCCAAGGAGC CCCCTGAGAT TCGAATCCTC AACCCATATT TCATCCAGGA GGCCGCCTTC ACCCTCATTG GCCTGCCCTT
                GGGTTCCTCG GGGGACTCTA AGCTTAGGAG TTGGGTATAA AGTAGGTCCT CCGGCGGAAG TGGGAGTAAC CGGACGGGAA
2161            CAACAATGGC CTCATGGGCC GGGGGAACAT CCCTACCCTT GGCAGTGTGG CAGTGACCAT GGCACTACAC GGCTGTGACG
                GTTGTTACCG GAGTACCCGG CCCCCTTGTA GGGATGGGAA CCGTCACACC GTCACTGGTA CCGTGATGTG CCGACACTGC
2241            AGGTGGCAGT CGCAGGATTT GGCTATGACA TGAGCACACC CAACGCACCC CTGCACTACT ATGAGACCGT TCGCATGGCA
                TCCACCGTCA GCGTCCTAAA CCGATACTGT ACTCGTGTGG GTTGCGTGGG GACGTGATGA TACTCTGGCA AGCGTACCGT
2321            GCCATCAAAG AGTCCTGGAC GCACAATATC CAGCGAGAGA AAGAGTTTCT GCGGAAGCTG GTGAAAGCTC GCGTCATCAC
                CGGTAGTTTC TCAGGACCTG CGTGTTATAG GTCGCTCTCT TTCTCAAAGA CGCCTTCGAC CACTTTCGAG CGCAGTAGTG
2401            TGATCTAAGC AGTGGCATCT GA
                ACTAGATTCG TCACCGTAGA CT
```

Fig. 3B

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  1     MGLLVFVRNL LLALCLFLVL GFLYYSAWKL HLLQWEEDSN SVVLSFDSAG
 51     QTLGSEYDRL GFLLNLDSKL PAELATKYAN FSEGACKPGY ASALMTAIFP
101     RFSKPAPMFL DDSFRKWARI REFVPPFGIK GQDNLIKAIL SVTKEYRLTP
151     ALDSLRCRRC IIVGNGGVLA NKSLGSRIDD YDIVVRLNSA PVKGFEKDVG
201     SKTTLRITYP EGAMQRPEQY ERDSLFVLAG FKWQDFKWLK YIVYKERVSA
251     SDGFWKSVAT RVPKEPPEIR ILNPYFIQEA AFTLIGLPFN NGLMGRGNIP
301     TLGSVAVTMA LHGCDEVAVA GFGYDMSTPN APLHYYETVR MAAIKESWTH
351     NIQREKEFLR KLVKARVITD LSSGI
```

Fig. 4A

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6241                                                ATGTACA GGATGCAACT CCTGTCTTGC ATTGCACTAA GTCTTGCACT
                                                    TACATGT CCTACGTTGA GGACAGAACG TAACGTGATT CAGAACGTGA
6321        TGTCACGAAT TCATTAGTTG AGGATACCAC ATTAGAGCCA GAAGAGCCAC CAACTAAATA CCAAATCTCT CAACCAGAAG
            ACAGTGCTTA AGTAATCAAC TCCTATGGTG TAATCTCGGT CTTCTCGGTG GTTGATTTAT GGTTTAGAGA GTTGGTCTTC
6401        TGTACGTGGC TGCACCAGGG GAGTCGCTAG AGGTGCGCTG CCTGTTGAAA GATGCCGCCG TGATCAGTTG GACTAAGGAT
            ACATGCACCG ACGTGGTCCC CTCAGCGATC TCCACGCGAC GGACAACTTT CTACGGCGGC ACTAGTCAAC CTGATTCCTA
6481        GGGGTGCACT TGGGGCCCAA CAATAGGACA GTGCTTATTG GGGAGTACTT GCAGATAAAG GGCGCCACGC CTAGAGACTC
            CCCCACGTGA ACCCCGGGTT GTTATCCTGT CACGAATAAC CCCTCATGAA CGTCTATTTC CCGCGGTGCG GATCTCTGAG
6561        CGGCCTCTAT GCTTGTACTG CCAGTAGGAC TGTAGACAGT GAAACTTGGT ACTTCATGGT GAATGTCACA GATGCCATCT
            GCCGGAGATA CGAACATGAC GGTCATCCTG ACATCTGTCA CTTTGAACCA TGAAGTACCA CTTACAGTGT CTACGGTAGA
6641        CATCCGGAGA TGATGAGGAT GACACCGATG GTGCGGAAGA TTTTGTCAGT GAGAACAGTA ACAACAAGAG AGCACCATAC
            GTAGGCCTCT ACTACTCCTA CTGTGGCTAC CACGCCTTCT AAAACAGTCA CTCTTGTCAT TGTTGTTCTC TCGTGGTATG
6721        TGGACCAACA CAGAAAAGAT GGAAAAGCGG CTCCATGCTG TGCCTGCGGC CAACACTGTC AAGTTTCGCT GCCCAGCCGG
            ACCTGGTTGT GTCTTTTCTA CCTTTTCGCC GAGGTACGAC ACGGACGCCG GTTGTGACAG TTCAAAGCGA CGGGTCGGCC
6801        GGGGAACCCA ATGCCAACCA TGCGGTGGCT GAAAAACGGG AAGGAGTTTA AGCAGGAGCA TCGCATTGGA GGCTACAAGG
            CCCCTTGGGT TACGGTTGGT ACGCCACCGA CTTTTTGCCC TTCCTCAAAT TCGTCCTCGT AGCGTAACCT CCGATGTTCC
6881        TACGAAACCA GCACTGGAGC CTCATTATGG AAAGTGTGGT CCCATCTGAC AAGGGAAATT ATACCTGTGT GGTGGAGAAT
            ATGCTTTGGT CGTGACCTCG GAGTAATACC TTTCACACCA GGGTAGACTG TTCCCTTTAA TATGGACACA CCACCTCTTA
6961        GAATACGGGT CCATCAATCA CACGTACCAC CTGGATGTTG TGGAGCGATC GCCTCACCGG CCCATCCTCC AAGCCGGACT
            CTTATGCCCA GGTAGTTAGT GTGCATGGTG GACCTACAAC ACCTCGCTAG CGGAGTGGCC GGGTAGGAGG TTCGGCCTGA
7041        GCCGGCAAAT GCCTCCACAG TGGTCGGAGG AGACGTAGAG TTTGTCTGCA AGGTTTACAG TGATGCCCAG CCCCACATCC
            CGGCCGTTTA CGGAGGTGTC ACCAGCCTCC TCTGCATCTC AAACAGACGT TCCAAATGTC ACTACGGGTC GGGGTGTAGG
7121        AGTGGATCAA GCACGTGGAA AAGAACGGCA GTAAATACGG GCCCGACGGG CTGCCCTACC TCAAGGTTCT CAAGGCCGCC
            TCACCTAGTT CGTGCACCTT TTCTTGCCGT CATTTATGCC CGGGCTGCCC GACGGGATGG AGTTCCAAGA GTTCCGGCGG
7201        GGTGTTAACA CCACGGACAA AGAGATTGAG GTTCTCTATA TTCGGAATGT AACTTTTGAG GACGCTGGGG AATATACGTG
            CCACAATTGT GGTGCCTGTT TCTCTAACTC CAAGAGATAT AAGCCTTACA TTGAAAACTC CTGCGACCCC TTATATGCAC
7281        CTTGGCGGGT AATTCTATTG GGATATCCTT TCACTCTGCA TGGTTGACAG TTCTGCCAGC GCCTGGAAGA GAAAAGGAGA
            GAACCGCCCA TTAAGATAAC CCTATAGGAA AGTGAGACGT ACCAACTGTC AAGACGGTCG CGGACCTTCT CTTTTCCTCT
7361        TTACAGCTTC CCCAGACTAC CTGTCAGCGC TAGAGCCCAA ATCTTGTGAC AAAACTCACA CATGCCCACC GTGCCCAGCA
            AATGTCGAAG GGGTCTGATG GACAGTCGCG ATCTCGGGTT TAGAACACTG TTTTGAGTGT GTACGGGTGG CACGGGTCGT
7441        CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA
            GGACTTGAGG ACCCCCCTGG CAGTCAGAAG GAGAAGGGGG GTTTTGGGTT CCTGTGGGAG TACTAGAGGG CCTGGGGACT
7521        GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC
            CCAGTGTACG CACCACCACC TGCACTCGGT GCTTCTGGGA CTCCAGTTCA AGTTGACCAT GCACCTGCCG CACCTCCACG
7601        ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG
            TATTACGGTT CTGTTTCGGC GCCCTCCTCG TCATGTTGTC GTGCATGGCA CACCAGTCGC AGGAGTGGCA GGACGTGGTC
7681        GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA
            CTGACCGACT TACCGTTCCT CATGTTCACG TTCCAGAGGT TGTTTCGGGA GGGTCGGGGG TAGCTCTTTT GGTAGAGGTT
7761        AGCCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGATGAGCT GACCAAGAAC CAGGTCAGCC
            TCGGTTTCCC GTCGGGGCTC TTGGTGTCCA CATGTGGGAC GGGGGTAGGG CCCTACTCGA CTGGTTCTTG GTCCAGTCGG
7841        TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC
            ACTGGACGGA CCAGTTTCCG AAGATAGGGT CGCTGTAGCG GCACCTCACC CTCTCGTTAC CCGTCGGCCT CTTGTTGATG
7921        AAGACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA
            TTCTGGTGCG GAGGGCACGA CCTGAGGCTG CCGAGGAAGA AGGAGATGTC GTTCGAGTGG CACCTGTTCT CGTCCACCGT
8001        GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC
            CGTCCCCTTG CAGAAGAGTA CGAGGCACTA CGTACTCCGA GACGTGTTGG TGATGTGCGT CTTCTCGGAG AGGGACAGAG
8081        CGGGTTGA
            GCCCAACT
```

Fig. 4B

```
  1         LVEDTTLEPE EPPTKYQISQ PEVYVAAPGE SLEVRCLLKD AAVISWTKDG
 51         VHLGPNNRTV LIGEYLQIKG ATPRDSGLYA CTASRTVDSE TWYFMVNVTD
101         AISSGDDEDD TDGAEDFVSE NSNNKRAPYW TNTEKMEKRL HAVPAANTVK
151         FRCPAGGNPM PTMRWLKNGK EFKQEHRIGG YKVRNQHWSL IMESVVPSDK
201         GNYTCVVENE YGSINHTYHL DVVERSPHRP ILQAGLPANA STVVGGDVEF
251         VCKVYSDAQP HIQWIKHVEK NGSKYGPDGL PYLKVLKAAG VNTTDKEIEV
301         LYIRNVTFED AGEYTCLAGN SIGISFHSAW LTVLPAPGRE KEITASPDYL
351         SALEPKSCDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
401         VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
451         WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ
501         VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV
551         DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPG
```

Fig. 4C

```
  1         LVEDTTLEPE EPPTKYQISQ PEVYVAAPGE SLEVRCLLKD AAVISWTKDG
 51         VHLGPNNRTV LIGEYLQIKG ATPRDSGLYA CTASRTVDSE TWYFMVNVTD
101         AISSGDDEDD TDGAEDFVSE NSNNKRAPYW TNTEKMEKRL HAVPAANTVK
151         FRCPAGGNPM PTMRWLKNGK EFKQEHRIGG YKVRNQHWSL IMESVVPSDK
201         GNYTCVVENE YGSINHTYHL DVVERSPHRP ILQAGLPANA STVVGGDVEF
251         VCKVYSDAQP HIQWIKHVEK NGSKYGPDGL PYLKVLKAAG VNTTDKEIEV
301         LYIRNVTFED AGEYTCLAGN SIGISFHSAW LTVLPAPGRE KEITASPDYL
```

Fig. 4D

```
  1         EPKSCDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 51         VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
101         GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL
151         TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS
201         RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G
```

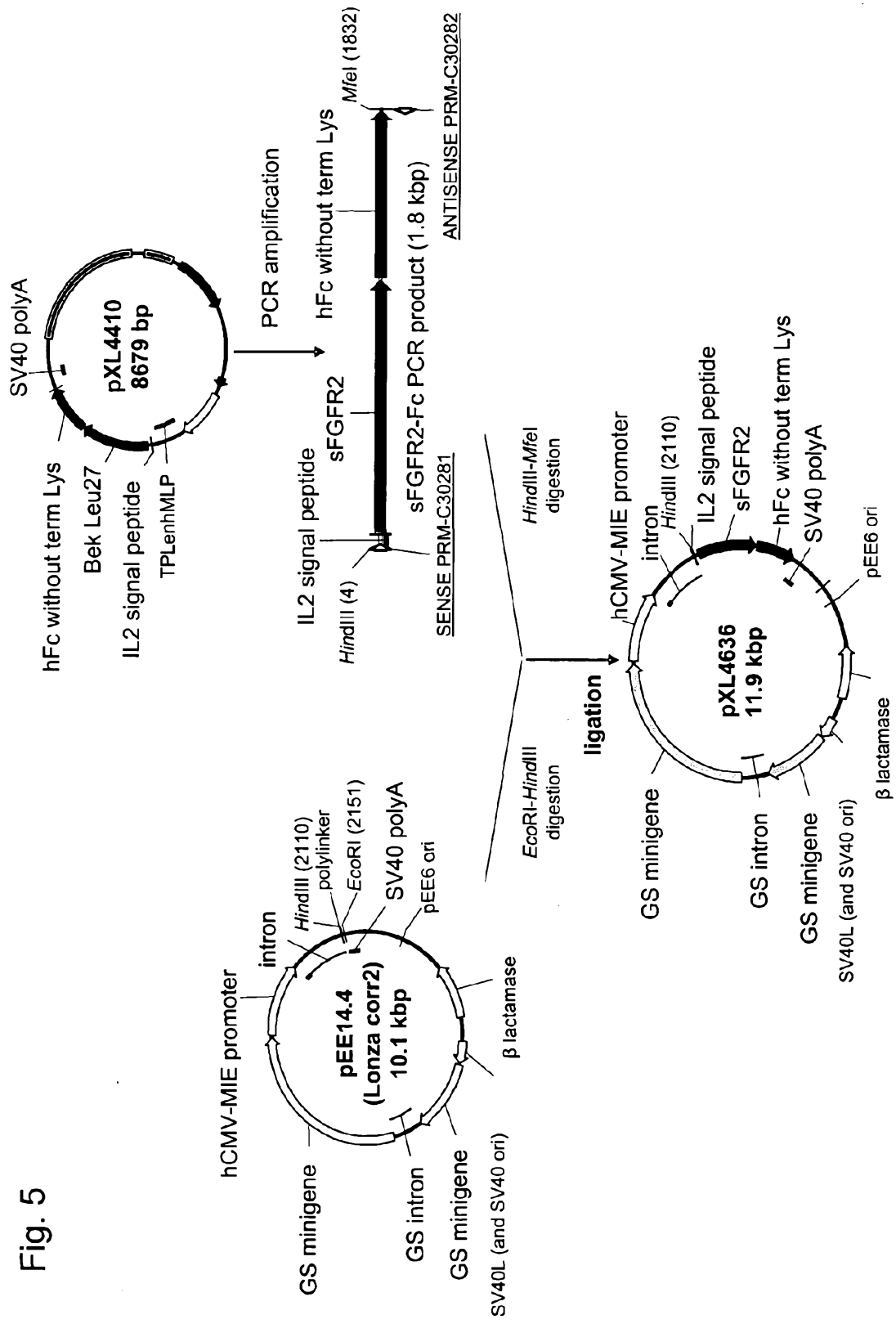
Fig. 4E

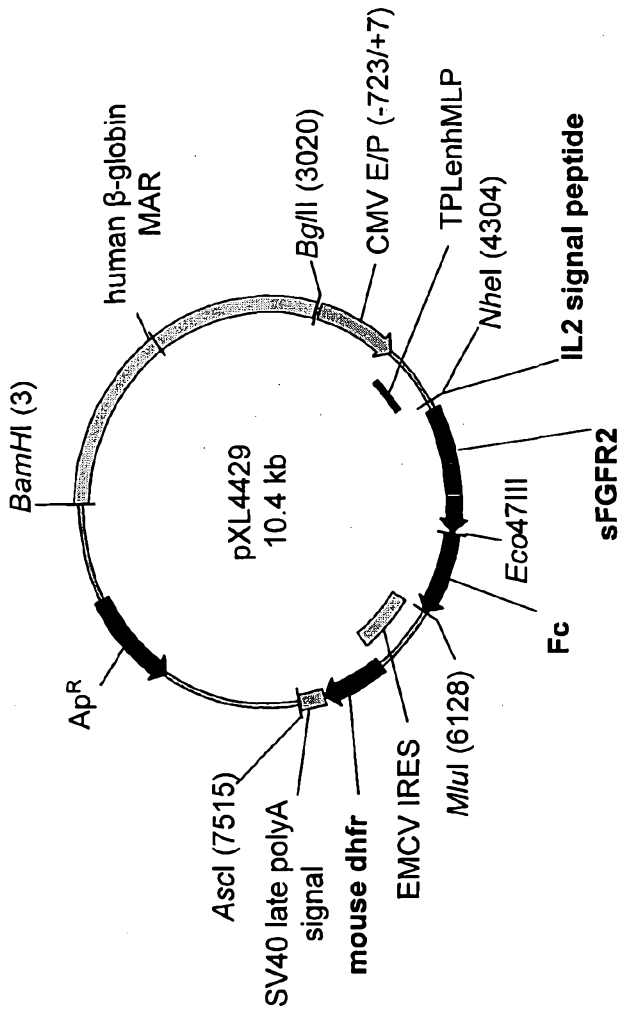
```
  1         SAL
```

Fig. 4F

```
  1    MYRMQLLSCI ALSLALVTNS
```

Fig. 5


SV40 polyA
pXL4410
8679 bp
hFc without term Lys
Bek Leu27
IL2 signal peptide
TPLenhMLP
PCR amplification
hFc without term Lys
MfeI (1832)
IL2 signal peptide
sFGFR2
HindIII (4)
sFGFR2-Fc PCR product (1.8 kbp)
SENSE PRM-C30281
ANTISENSE PRM-C30282
HindIII-MfeI digestion
EcoRI-HindIII digestion
ligation
hCMV-MIE promoter
intron
HindIII (2110)
polylinker
EcoRI (2151)
SV40 polyA
pEE6 ori
pEE14.4
(Lonza corr2)
10.1 kbp
β lactamase
GS minigene
GS intron
GS minigene
SV40L (and SV40 ori)
hCMV-MIE promoter
intron
HindIII (2110)
IL2 signal peptide
sFGFR2
hFc without term Lys
SV40 polyA
pEE6 ori
pXL4636
11.9 kbp
β lactamase
GS minigene
GS intron
GS minigene
SV40L (and SV40 ori)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10.4 kb
BamHI (3)
human β-globin MAR
BglII (3020)
CMV E/P (-723/+7)
TPLenhMLP
NheI (4304)
IL2 signal peptide
sFGFR2
Eco47III
Fc
MluI (6128)
EMCV IRES
mouse dhfr
SV40 late polyA signal
AscI (7515)
ApR

Fig. 6A

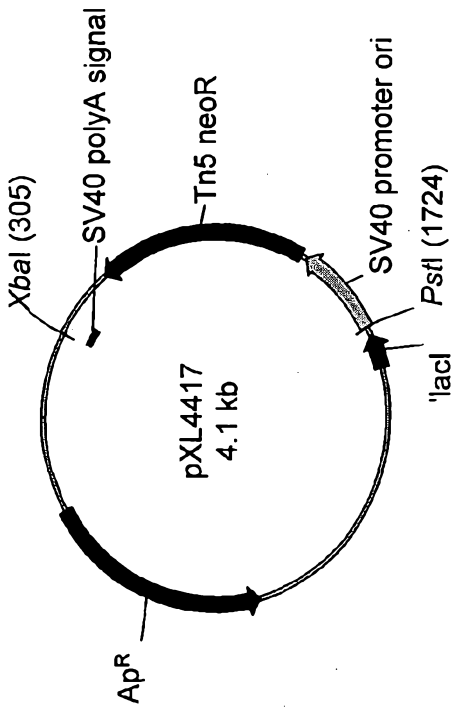
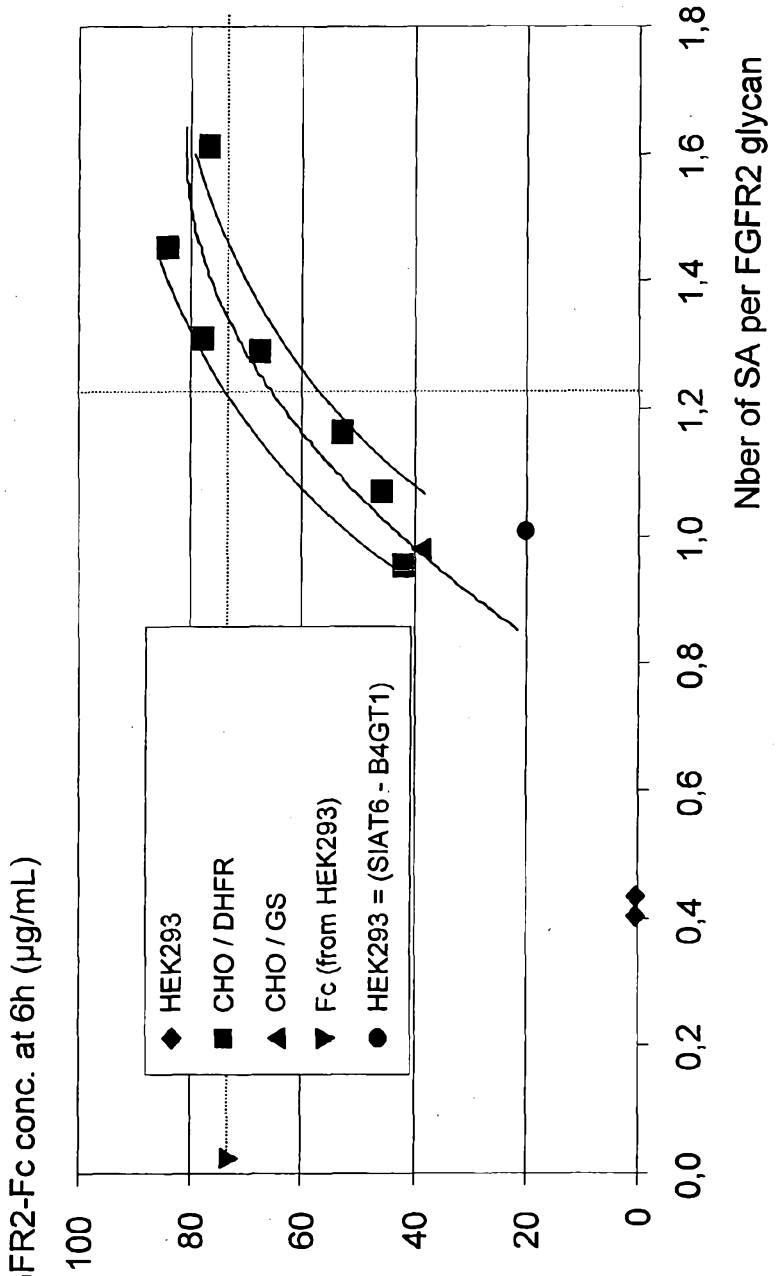

pXL4417
4.1 kb
XbaI (305)
SV40 polyA signal
Tn5 neoR
SV40 promoter ori
PstI (1724)
'lacI
ApR

Fig. 6B

Fig. 7


FGFR2-Fc conc. at 6h (µg/mL)
100
80
60
40
20
0
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
1,6
1,8
Nber of SA per FGFR2 glycan
HEK293
CHO / DHFR
CHO / GS
Fc (from HEK293)
HEK293 = (SIAT6 - B4GT1)

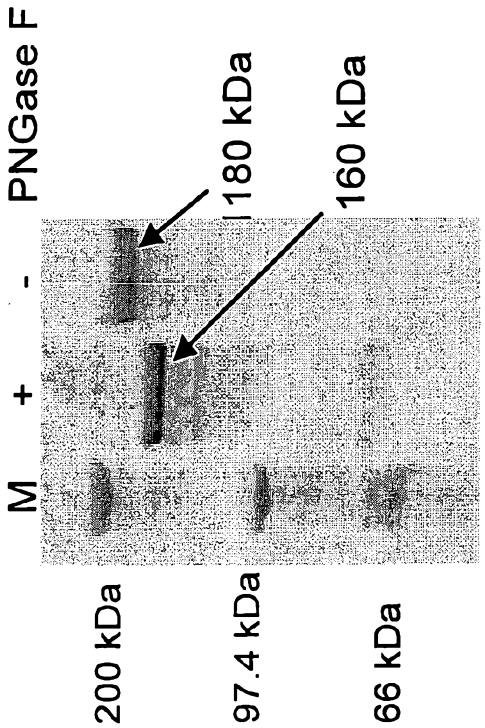


Fig. 8

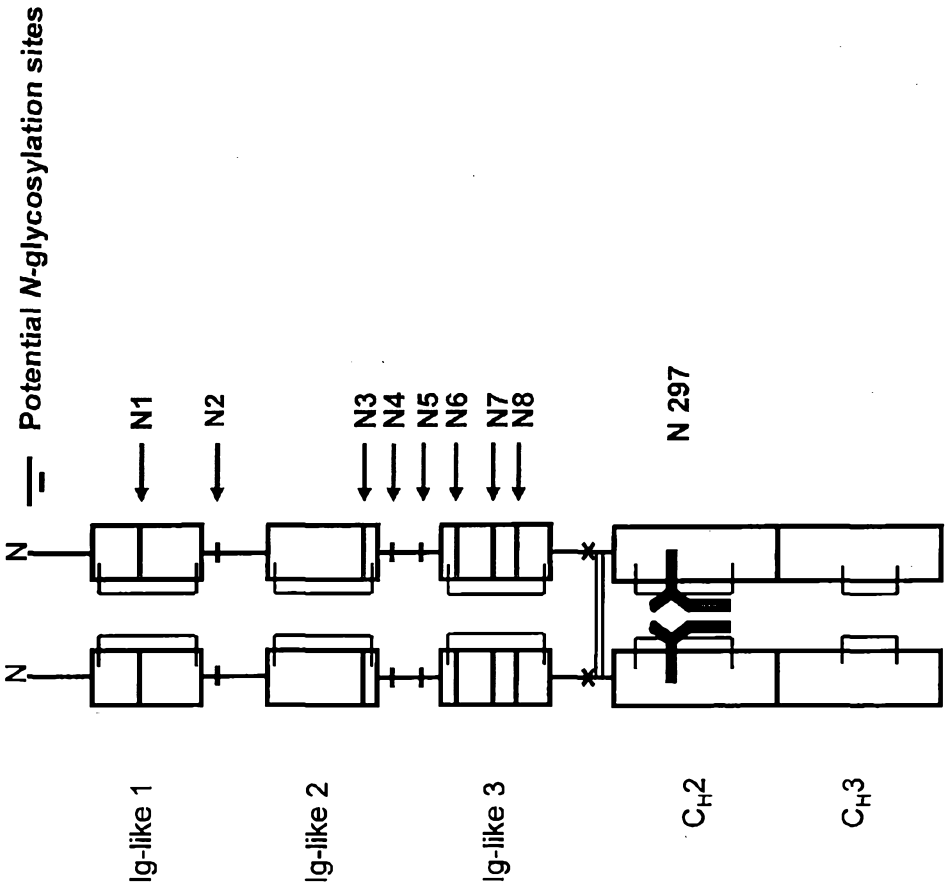
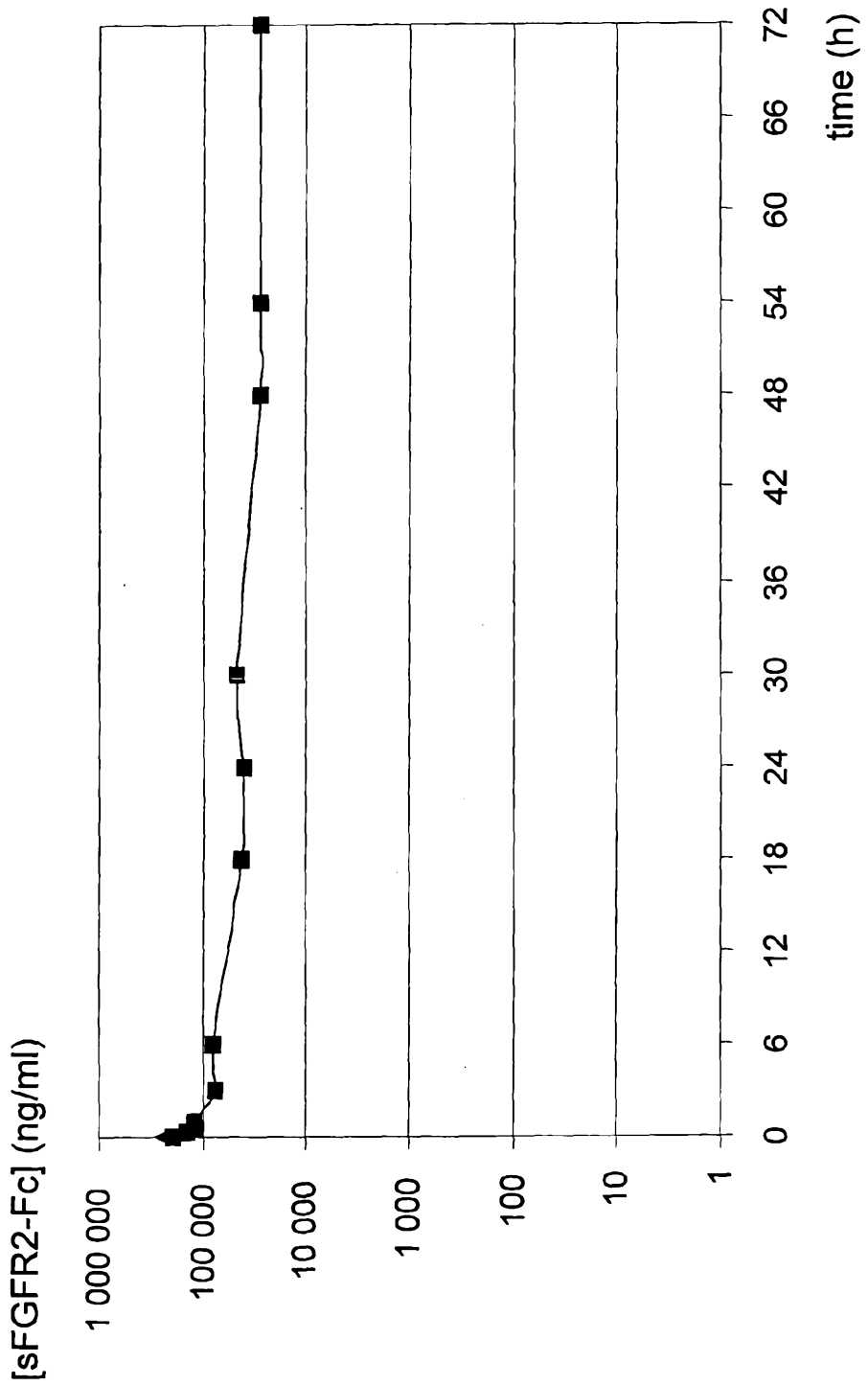

Potential N-glycosylation sites
N1
N2
N3
N4
N5
N6
N7
N8
N 297
N
N
Ig-like 1
Ig-like 2
Ig-like 3
C_H2
C_H3

Fig. 9

Fig. 10


[sFGFR2-Fc] (ng/ml)
1 000 000
100 000
10 000
1 000
100
10
1
0
6
12
18
24
30
36
42
48
54
60
66
72
time (h)

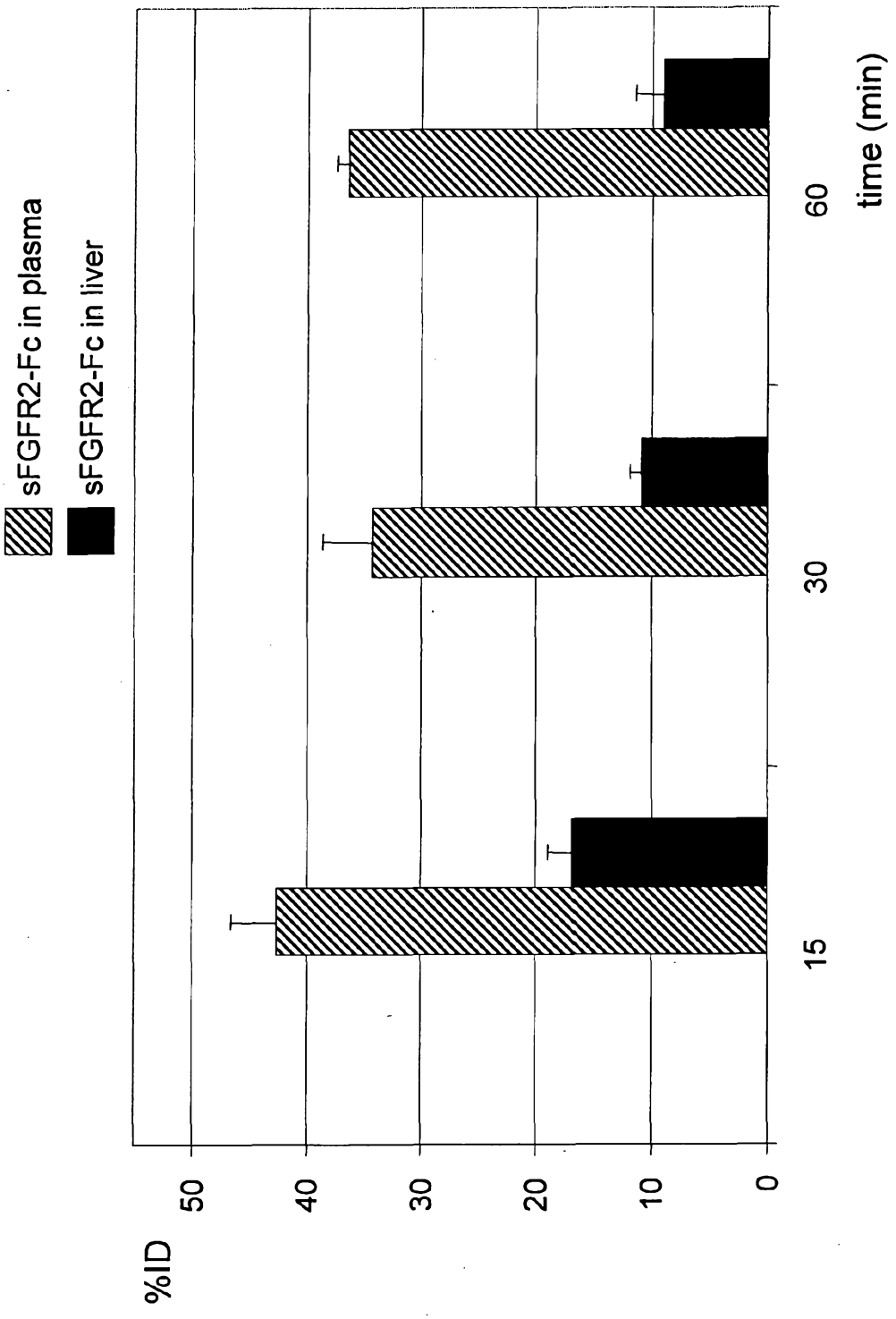

sFGFR2-Fc in plasma
sFGFR2-Fc in liver
%ID
0
10
20
30
40
50
15
30
60
time (min)

Fig. 11

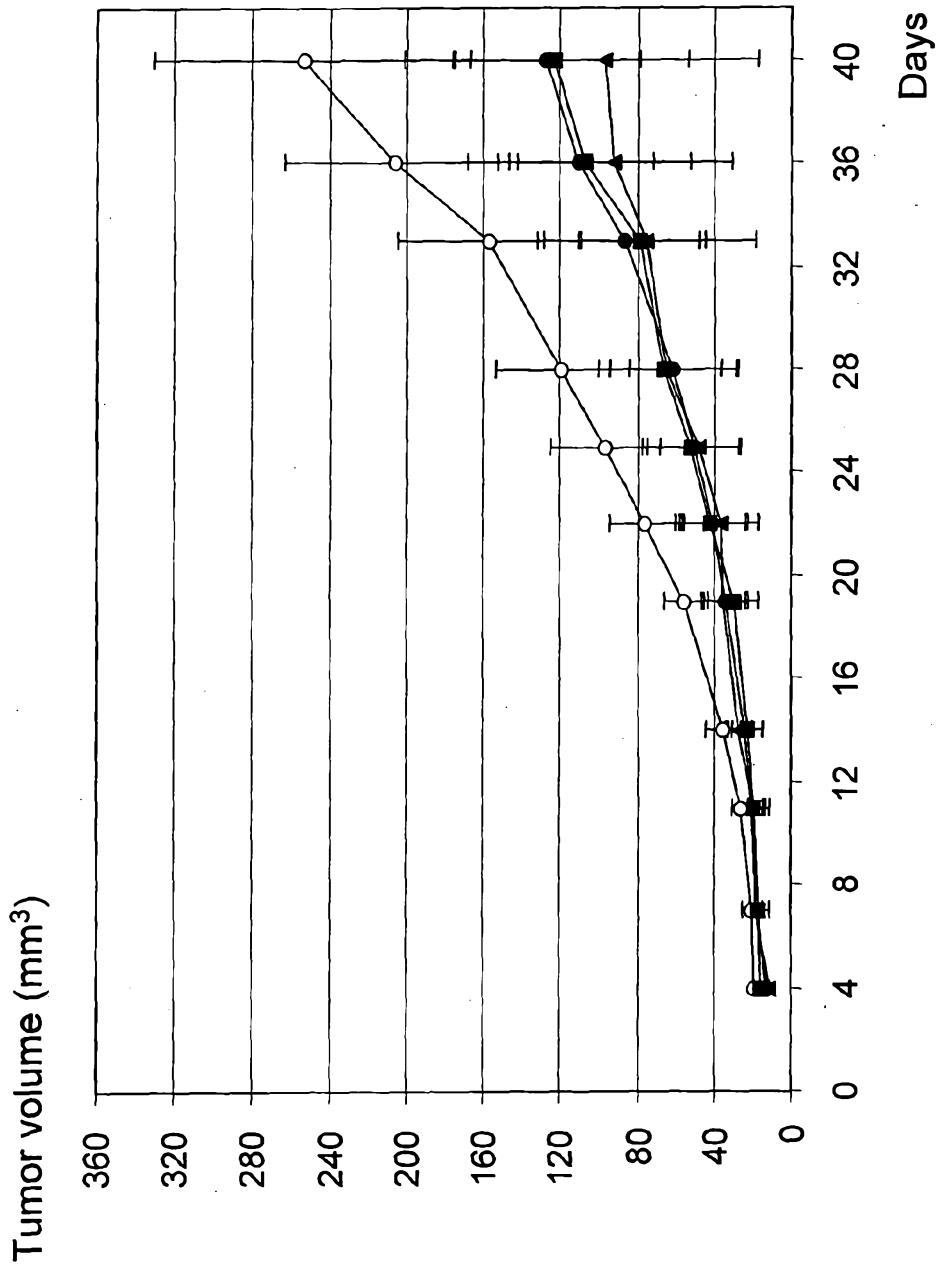

Tumor volume (mm3)
360
320
280
240
200
160
120
80
40
0
0
4
8
12
16
20
24
28
32
36
40
Days

Fig. 12

Fig. 13

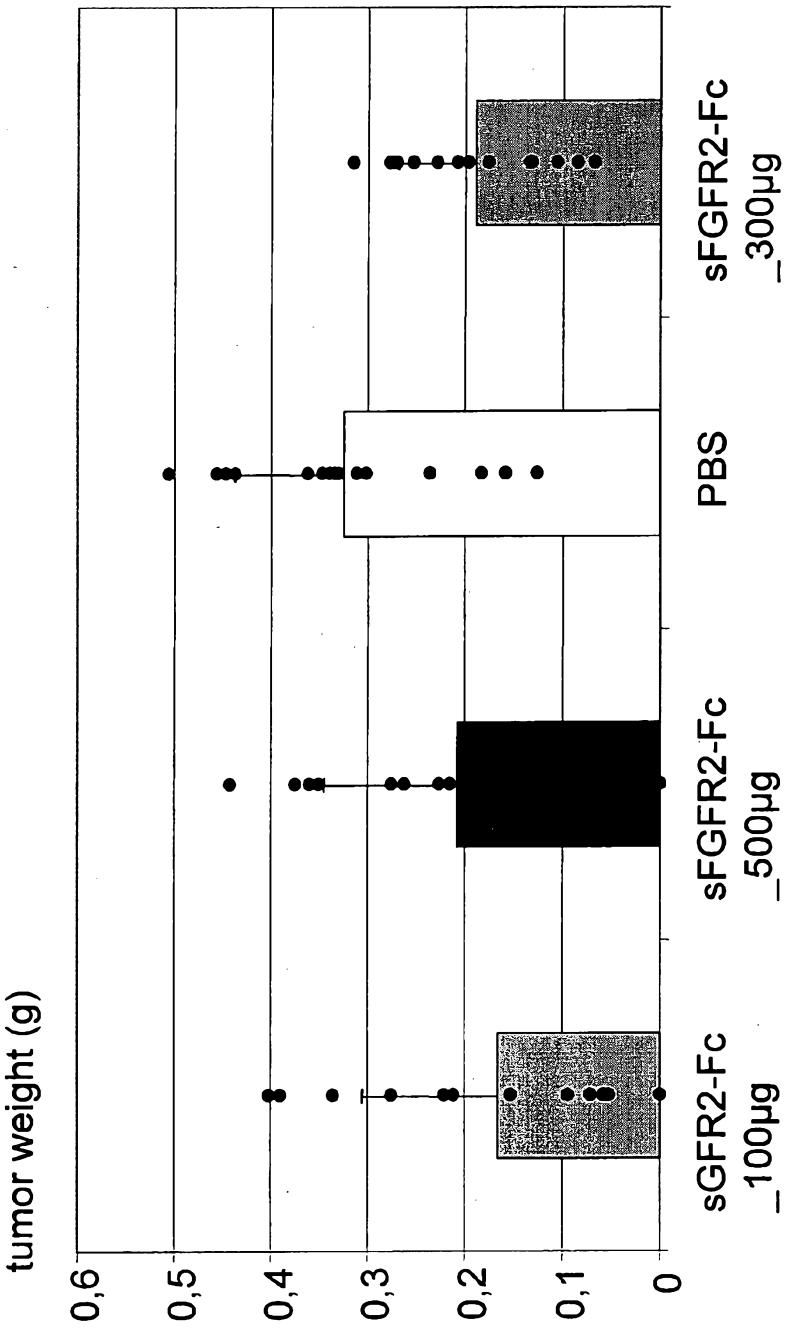

tumor weight (g)
0,6
0,5
0,4
0,3
0,2
0,1
0
sGFR2-Fc _100µg
sFGFR2-Fc _500µg
PBS
sFGFR2-Fc _300µg

Fig. 14

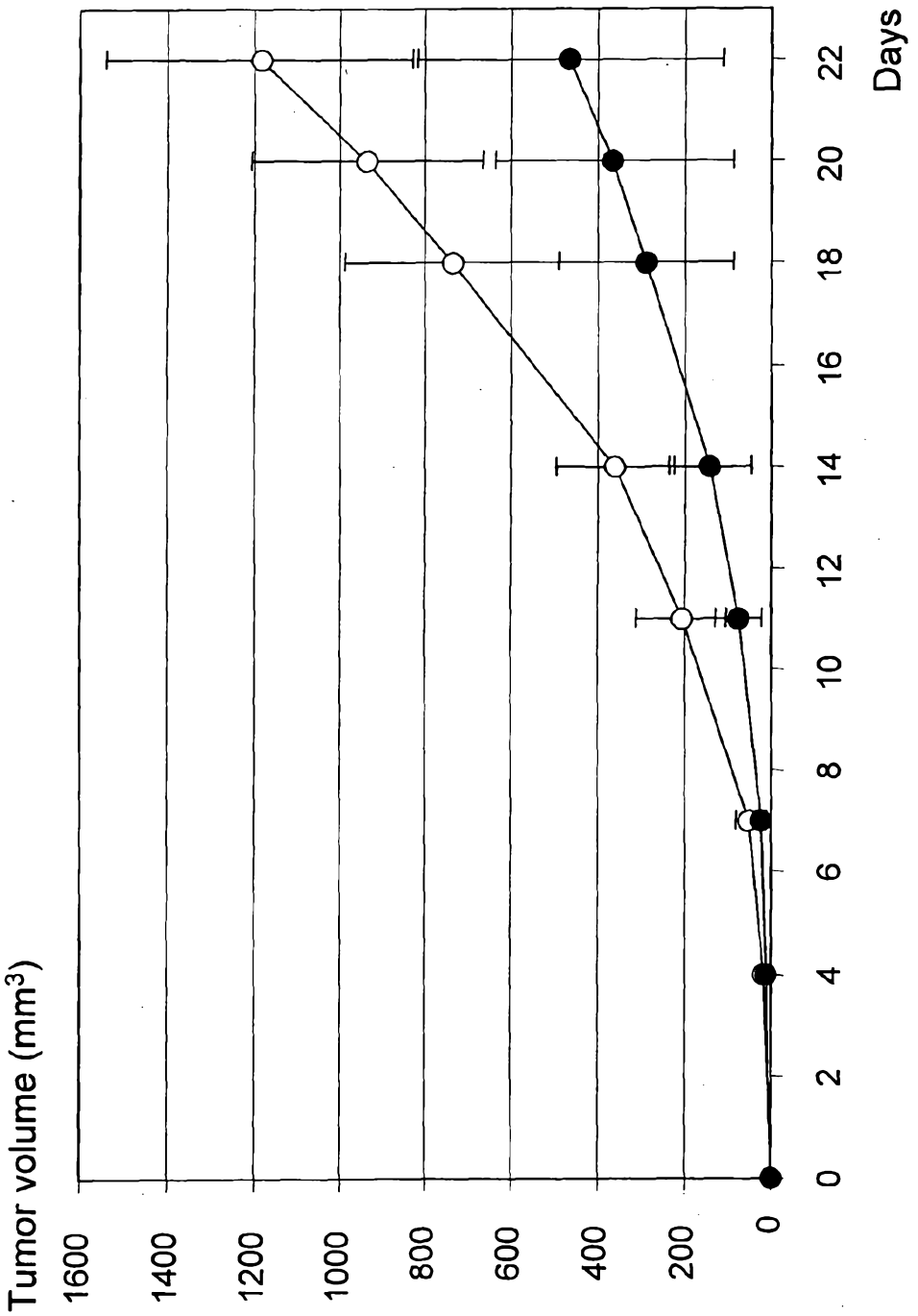
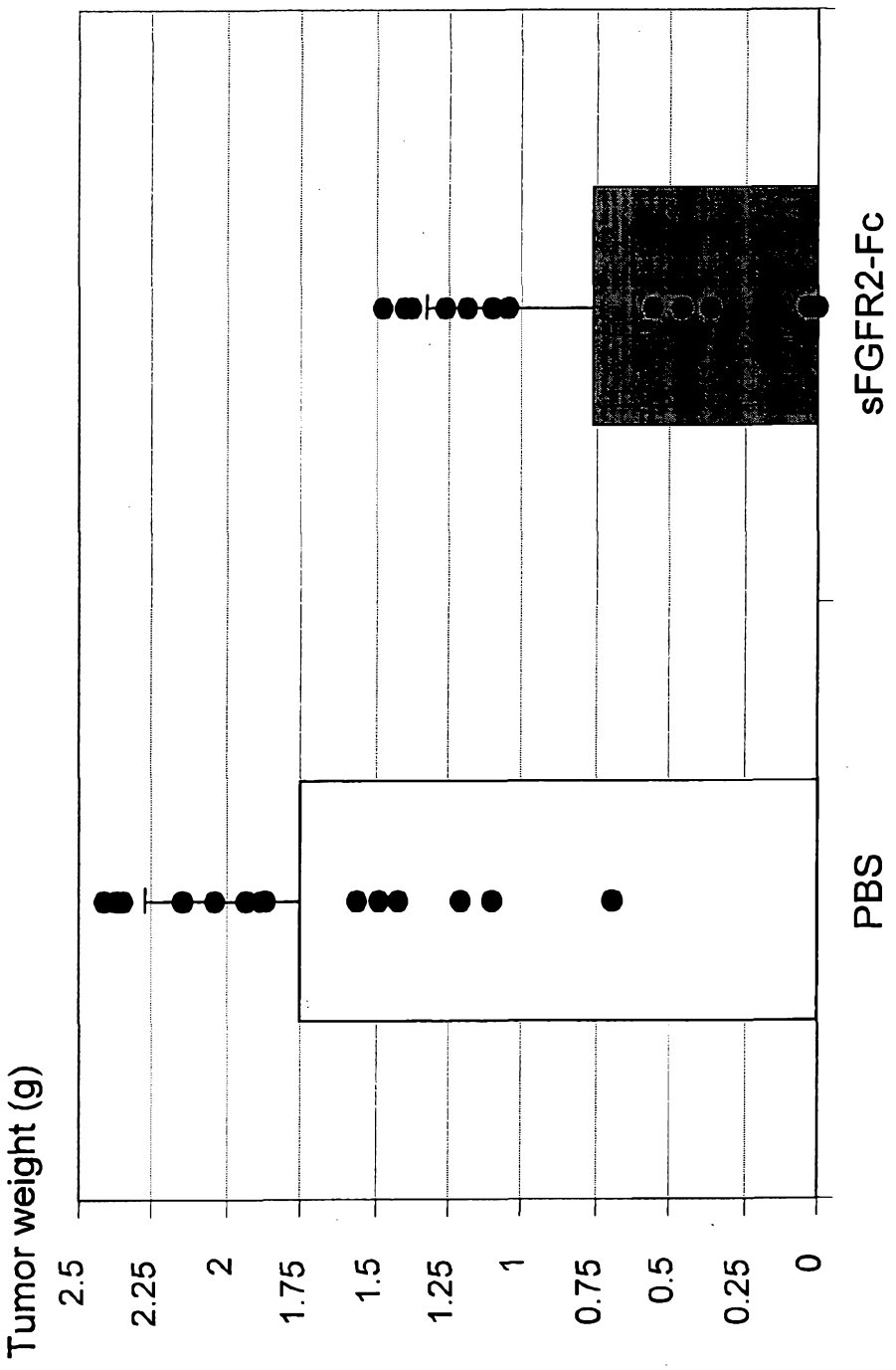

Tumor volume (mm3)
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
18
20
22
Days

Fig. 15



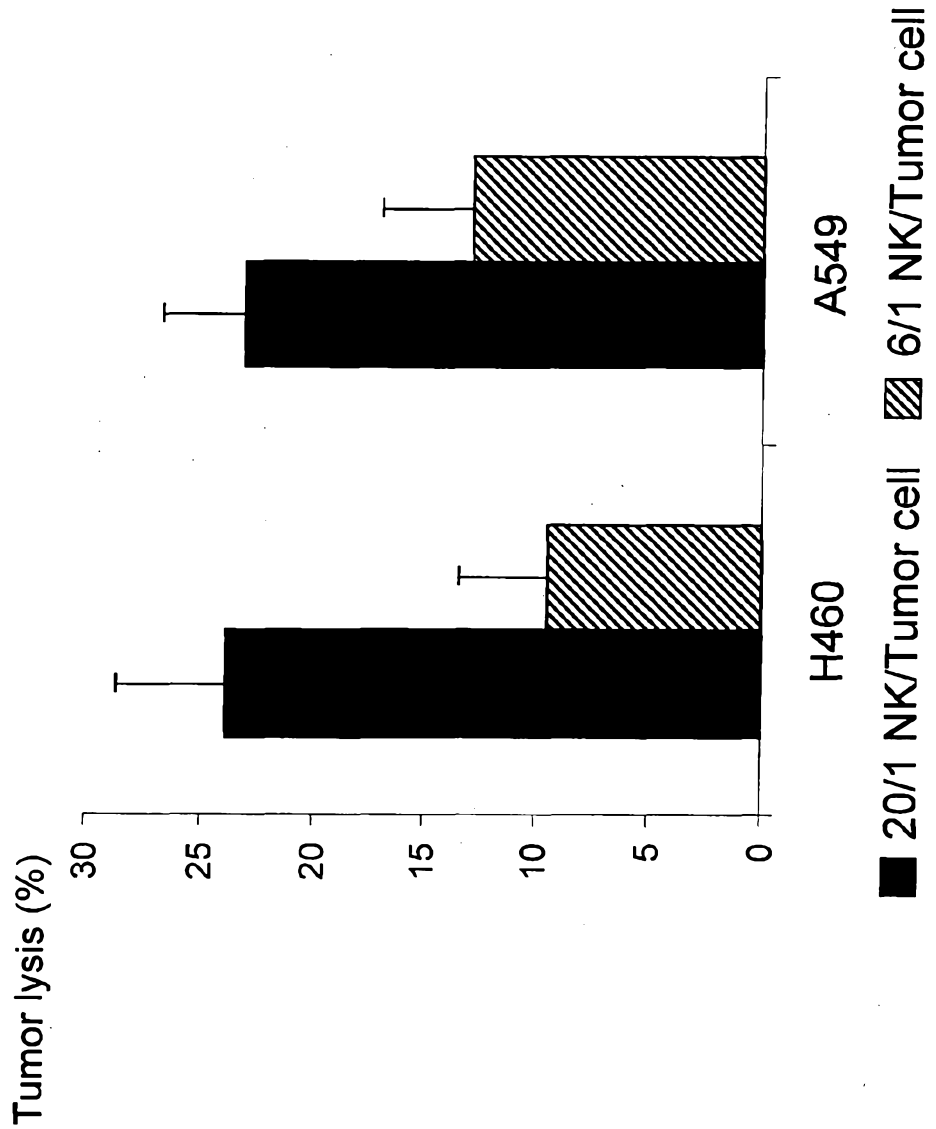

Tumor lysis (%)
30
25
20
15
10
5
0
H460
A549
20/1 NK/Tumor cell
6/1 NK/Tumor cell

Fig. 16

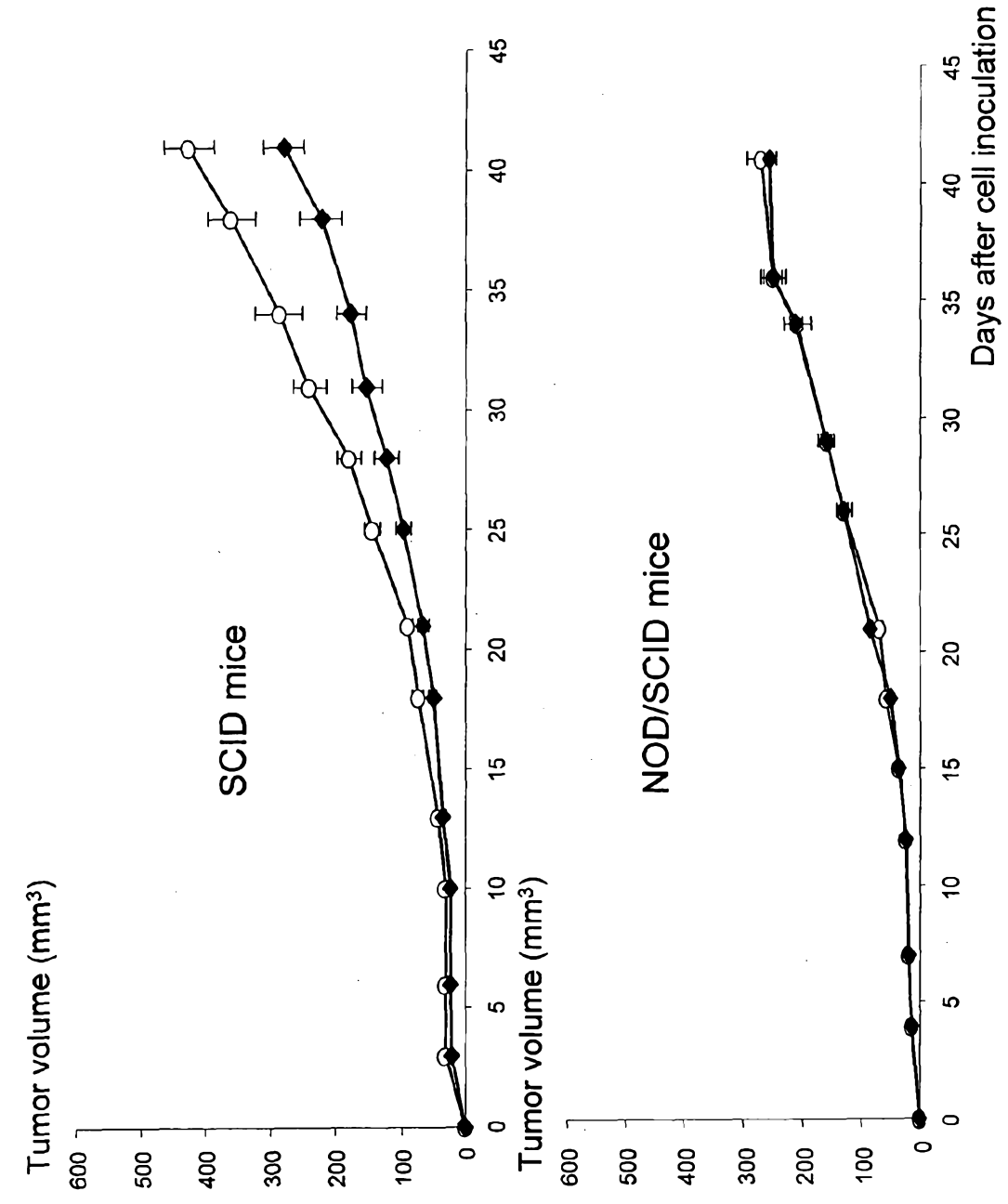


Fig. 17A

Fig. 17B

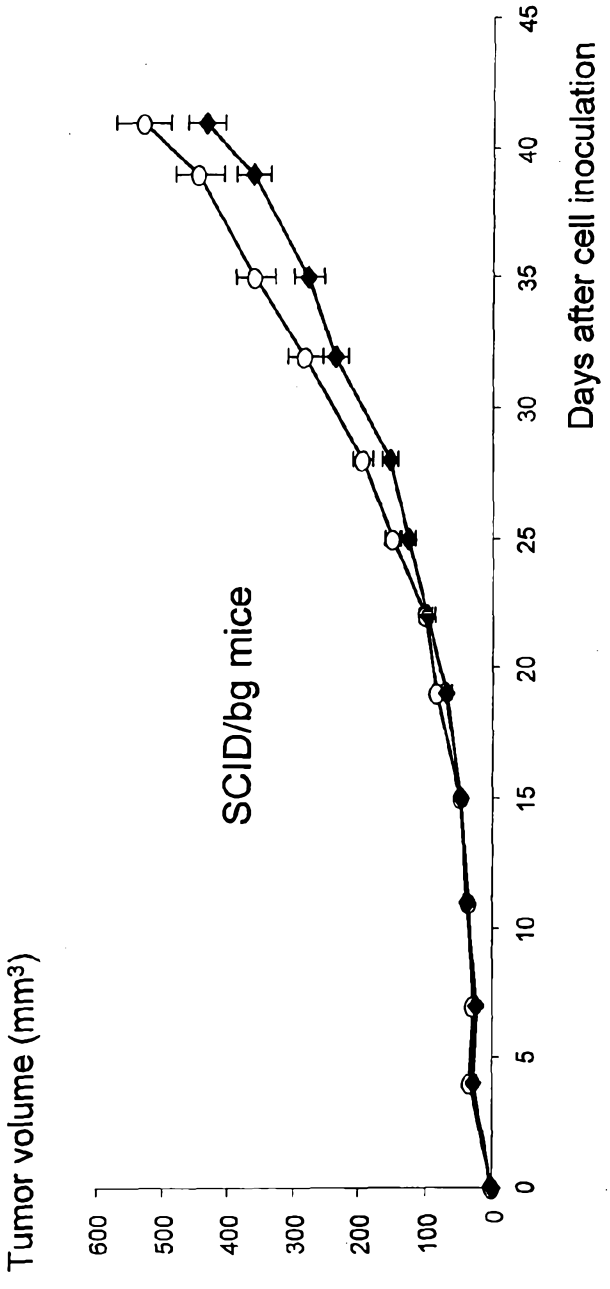

Tumor volume (mm³)
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
45
Days after cell inoculation
SCID/bg mice

Fig. 17C

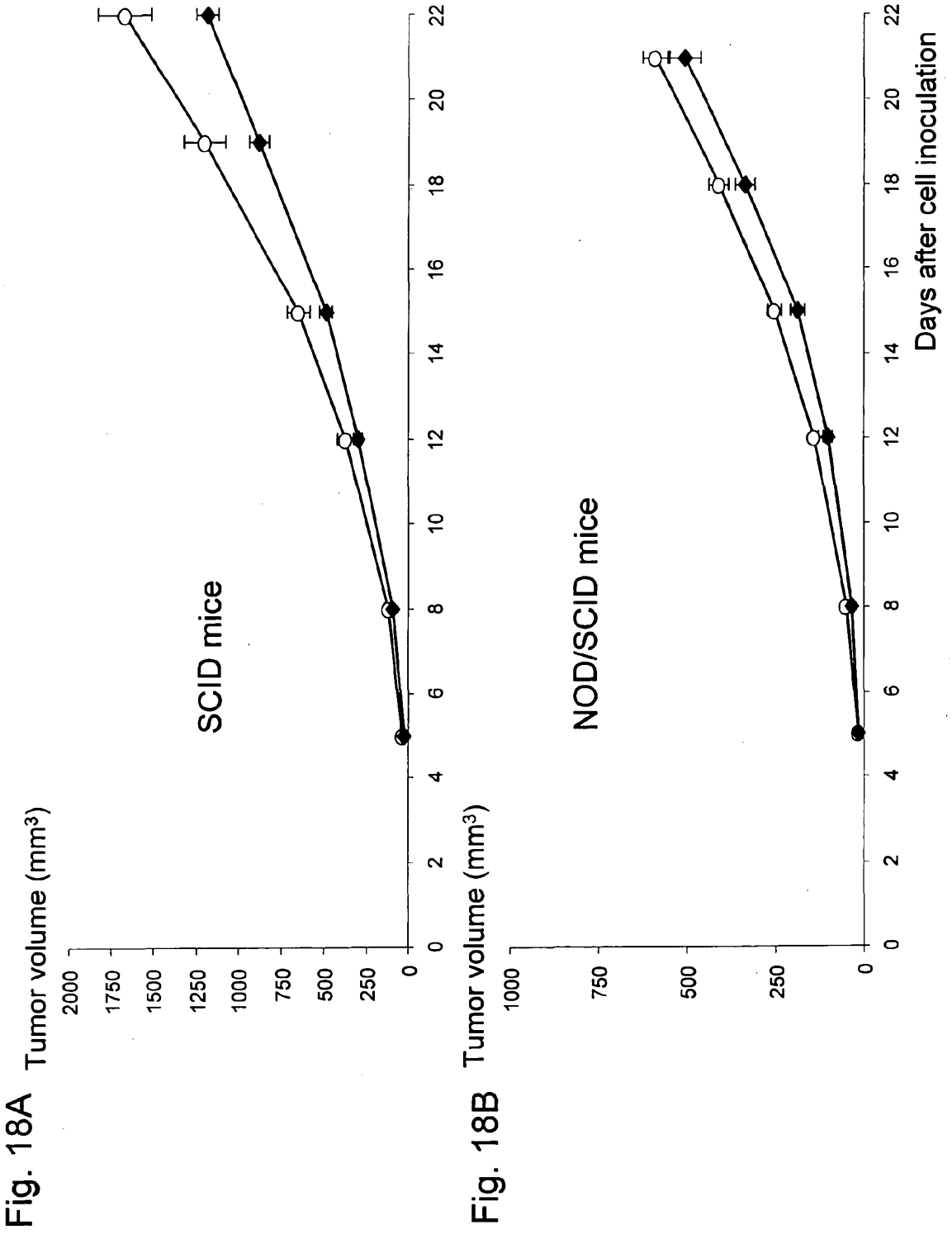


Fig. 18A

Fig. 18B

Fig. 18C

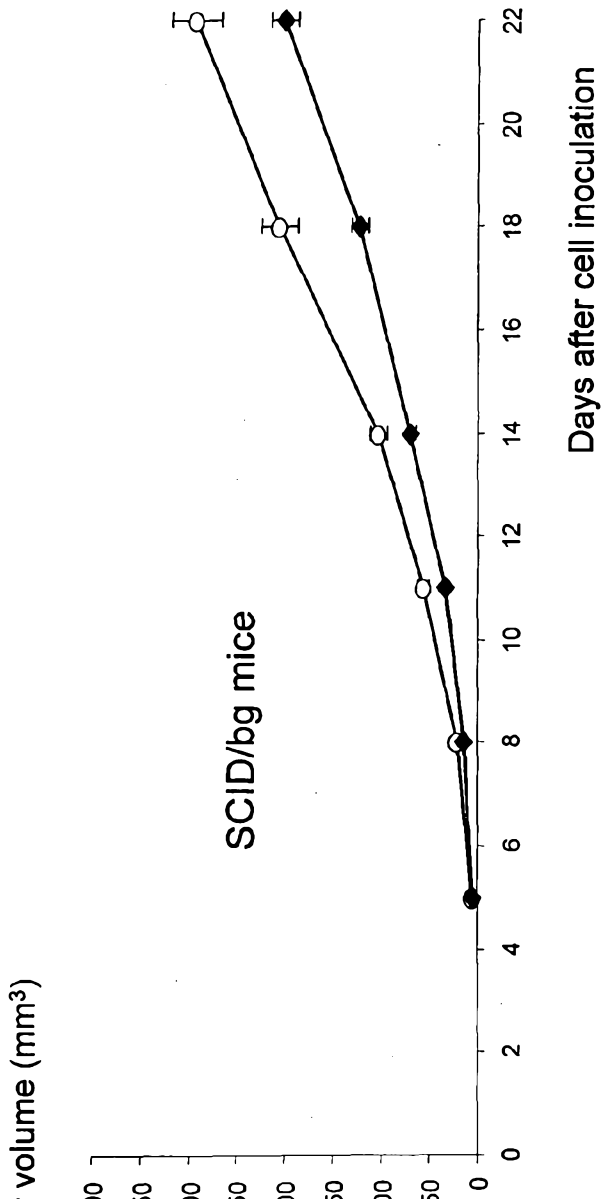

Tumor volume (mm3)
2000
1750
1500
1250
1000
750
500
250
0
SCID/bg mice
0
2
4
6
8
10
12
14
16
18
20
22
Days after cell inoculation

Fig. 19A

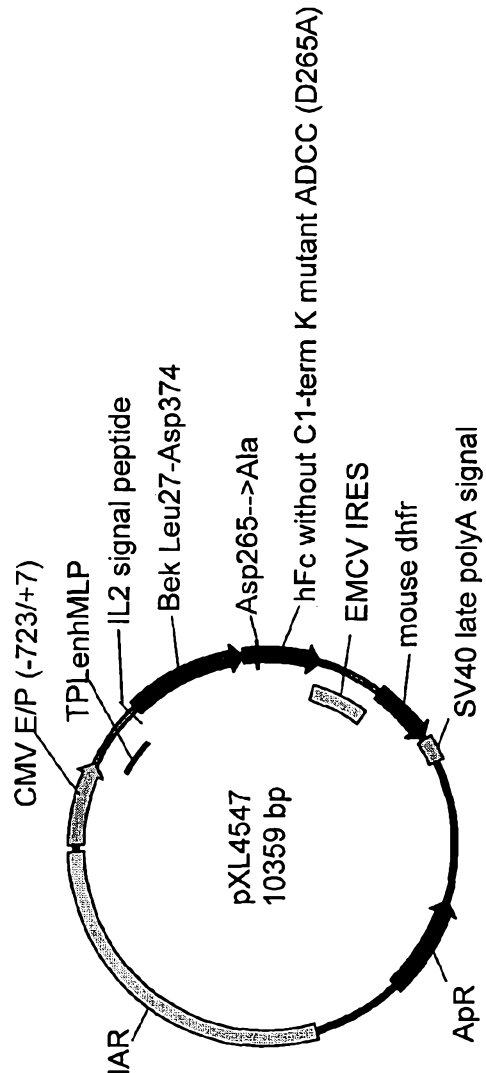


Fig. 19B

```
   1  LVEDTTLEPE EPPTKYQISQ PEVYVAAPGE SLEVRCLLKD AAVISWTKDG
  51  VHLGPNNRTV LIGEYLQIKG ATPRDSGLYA CTASRTVDSE TWYFMVNVTD
 101  AISSGDDEDD TDGAEDFVSE NSNNKRAPYW TNTEKMEKRL HAVPAANTVK
 151  FRCPAGGNPM PTMRWLKNGK EFKQEHRIGG YKVRNQHWSL IMESVVPSDK
 201  GNYTCVVENE YGSINHTYHL DVVERSPHRP ILQAGLPANA STVVGGDVEF
 251  VCKVYSDAQP HIQWIKHVEK NGSKYGPDGL PYLKVLKAAG VNTTDKEIEV
 301  LYIRNVTFED AGEYTCLAGN SIGISFHSAW LTVLPAPGRE KEITASPDKT
 351  HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VAVSHEDPEV
 401  KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV
 451  SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY
 501  PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF
 551  SCSVMHEALH NHYTQKSLSL SPG
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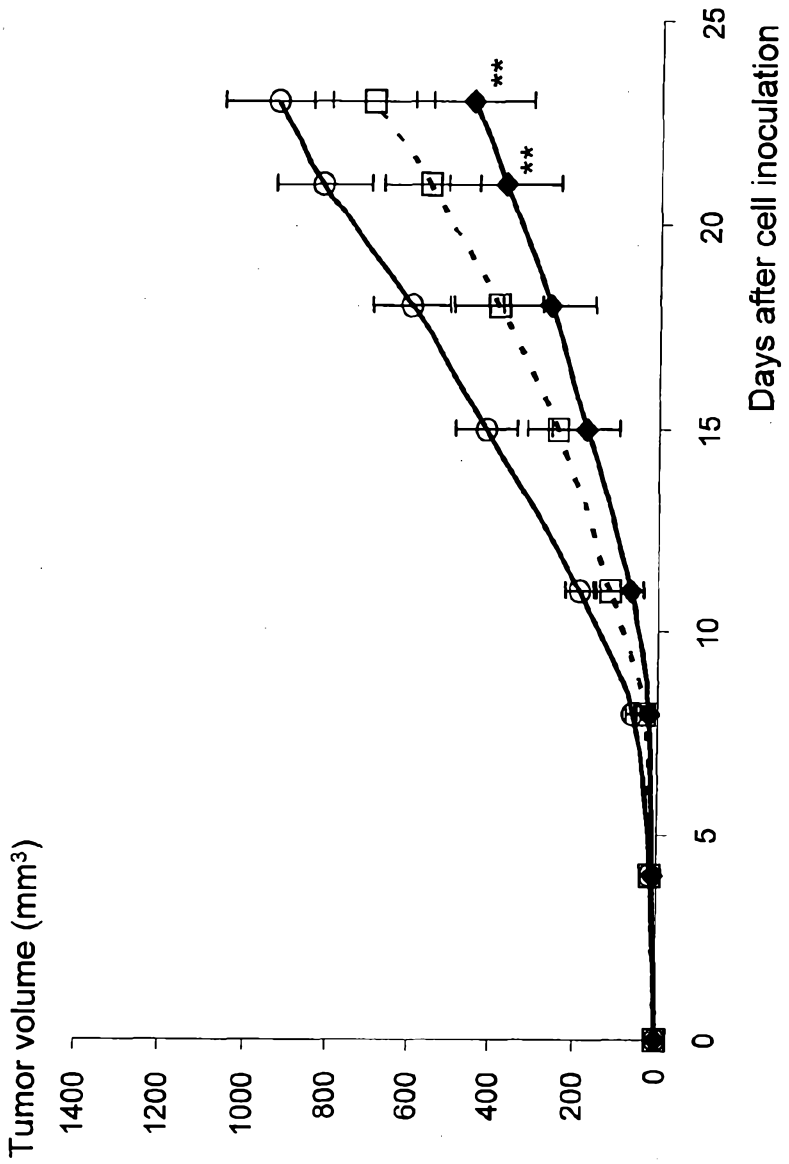
Fig. 20


Tumor volume (mm³)
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
Days after cell inoculation
**
**

SEQUENCE LISTING

<110> CENTELION

<120> MODIFIED SOLUBLE FGF RECEPTOR Fc FUSIONS WITH IMPROVED BIOLOGICAL ACTIVITY

<130> FR2006-084 PCT

<150> EP 06291824.8
<151> 2006-11-28

<150> EP 07290042.6
<151> 2007-01-11

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<170> PatentIn version 3.3

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caa atc tct caa cca gaa gtg tac gtg gct gca cca ggg gag tcg cta       96
Gln Ile Ser Gln Pro Glu Val Tyr Val Ala Ala Pro Gly Glu Ser Leu
            20                  25                  30

gag gtg cgc tgc ctg ttg aaa gat gcc gcc gtg atc agt tgg act aag      144
Glu Val Arg Cys Leu Leu Lys Asp Ala Ala Val Ile Ser Trp Thr Lys
        35                  40                  45

gat ggg gtg cac ttg ggg ccc aac aat agg aca gtg ctt att ggg gag      192
Asp Gly Val His Leu Gly Pro Asn Asn Arg Thr Val Leu Ile Gly Glu
    50                  55                  60

tac ttg cag ata aag ggc gcc acg cct aga gac tcc ggc ctc tat gct      240
Tyr Leu Gln Ile Lys Gly Ala Thr Pro Arg Asp Ser Gly Leu Tyr Ala
65                  70                  75                  80

tgt act gcc agt agg act gta gac agt gaa act tgg tac ttc atg gtg      288
Cys Thr Ala Ser Arg Thr Val Asp Ser Glu Thr Trp Tyr Phe Met Val
                85                  90                  95

aat gtc aca gat gcc atc tca tcc gga gat gat gag gat gac acc gat      336
Asn Val Thr Asp Ala Ile Ser Ser Gly Asp Asp Glu Asp Asp Thr Asp
            100                 105                 110

ggt gcg gaa gat ttt gtc agt gag aac agt aac aac aag aga gca cca      384
Gly Ala Glu Asp Phe Val Ser Glu Asn Ser Asn Asn Lys Arg Ala Pro
        115                 120                 125

tac tgg acc aac aca gaa aag atg gaa aag cgg ctc cat gct gtg cct      432
Tyr Trp Thr Asn Thr Glu Lys Met Glu Lys Arg Leu His Ala Val Pro
    130                 135                 140

gcg gcc aac act gtc aag ttt cgc tgc cca gcc ggg ggg aac cca atg      480
Ala Ala Asn Thr Val Lys Phe Arg Cys Pro Ala Gly Gly Asn Pro Met
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145 150 155 160

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Pro Thr Met Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Gln Glu His
165 170 175

cgc att gga ggc tac aag gta cga aac cag cac tgg agc ctc att atg 576
Arg Ile Gly Gly Tyr Lys Val Arg Asn Gln His Trp Ser Leu Ile Met
180 185 190

gaa agt gtg gtc cca tct gac aag gga aat tat acc tgt gtg gtg gag 624
Glu Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Val Val Glu
195 200 205

aat gaa tac ggg tcc atc aat cac acg tac cac ctg gat gtt gtg gag 672
Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr His Leu Asp Val Val Glu
210 215 220

cga tcg cct cac cgg ccc atc ctc caa gcc gga ctg ccg gca aat gcc 720
Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Ala
225 230 235 240

tcc aca gtg gtc gga gga gac gta gag ttt gtc tgc aag gtt tac agt 768
Ser Thr Val Val Gly Gly Asp Val Glu Phe Val Cys Lys Val Tyr Ser
245 250 255

gat gcc cag ccc cac atc cag tgg atc aag cac gtg gaa aag aac ggc 816
Asp Ala Gln Pro His Ile Gln Trp Ile Lys His Val Glu Lys Asn Gly
260 265 270

agt aaa tac ggg ccc gac ggg ctg ccc tac ctc aag gtt ctc aag gcc 864
Ser Lys Tyr Gly Pro Asp Gly Leu Pro Tyr Leu Lys Val Leu Lys Ala
275 280 285

gcc ggt gtt aac acc acg gac aaa gag att gag gtt ctc tat att cgg 912
Ala Gly Val Asn Thr Thr Asp Lys Glu Ile Glu Val Leu Tyr Ile Arg
290 295 300

aat gta act ttt gag gac gct ggg gaa tat acg tgc ttg gcg ggt aat 960
Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn
305 310 315 320

tct att ggg ata tcc ttt cac tct gca tgg ttg aca gtt ctg cca gcg 1008
Ser Ile Gly Ile Ser Phe His Ser Ala Trp Leu Thr Val Leu Pro Ala
325 330 335

cct gga aga gaa aag gag att aca gct tcc cca gac tac ctg tca gcg 1056
Pro Gly Arg Glu Lys Glu Ile Thr Ala Ser Pro Asp Tyr Leu Ser Ala
340 345 350

cta gag ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca 1104
Leu Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
355 360 365

gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa 1152
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
370 375 380

ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg 1200
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
385 390 395 400

gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac 1248
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
405 410 415

gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag 1296
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu

```
            420                 425                 430

cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac     1344
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
        435                 440                 445

cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa     1392
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
    450                 455                 460

gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag     1440
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
465                 470                 475                 480

ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg     1488
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
                485                 490                 495

acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc     1536
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
            500                 505                 510

agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac     1584
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
        515                 520                 525

tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc     1632
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
    530                 535                 540

tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc     1680
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
545                 550                 555                 560

ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag     1728
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
                565                 570                 575

aag agc ctc tcc ctg tct ccg ggt tga                                 1755
Lys Ser Leu Ser Leu Ser Pro Gly
            580


<210>  2
<211>  584
<212>  PRT
<213>  Homo sapiens

<400>  2

Leu Val Glu Asp Thr Thr Leu Glu Pro Glu Glu Pro Pro Thr Lys Tyr
1               5                   10                  15


Gln Ile Ser Gln Pro Glu Val Tyr Val Ala Ala Pro Gly Glu Ser Leu
            20                  25                  30


Glu Val Arg Cys Leu Leu Lys Asp Ala Ala Val Ile Ser Trp Thr Lys
        35                  40                  45


Asp Gly Val His Leu Gly Pro Asn Asn Arg Thr Val Leu Ile Gly Glu
    50                  55                  60


Tyr Leu Gln Ile Lys Gly Ala Thr Pro Arg Asp Ser Gly Leu Tyr Ala
65                  70                  75                  80
```

Cys Thr Ala Ser Arg Thr Val Asp Ser Glu Thr Trp Tyr Phe Met Val
85 90 95

Asn Val Thr Asp Ala Ile Ser Ser Gly Asp Asp Glu Asp Asp Thr Asp
100 105 110

Gly Ala Glu Asp Phe Val Ser Glu Asn Ser Asn Asn Lys Arg Ala Pro
115 120 125

Tyr Trp Thr Asn Thr Glu Lys Met Glu Lys Arg Leu His Ala Val Pro
130 135 140

Ala Ala Asn Thr Val Lys Phe Arg Cys Pro Ala Gly Gly Asn Pro Met
145 150 155 160

Pro Thr Met Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Gln Glu His
165 170 175

Arg Ile Gly Gly Tyr Lys Val Arg Asn Gln His Trp Ser Leu Ile Met
180 185 190

Glu Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Val Val Glu
195 200 205

Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr His Leu Asp Val Val Glu
210 215 220

Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Ala
225 230 235 240

Ser Thr Val Val Gly Gly Asp Val Glu Phe Val Cys Lys Val Tyr Ser
245 250 255

Asp Ala Gln Pro His Ile Gln Trp Ile Lys His Val Glu Lys Asn Gly
260 265 270

Ser Lys Tyr Gly Pro Asp Gly Leu Pro Tyr Leu Lys Val Leu Lys Ala
275 280 285

Ala Gly Val Asn Thr Thr Asp Lys Glu Ile Glu Val Leu Tyr Ile Arg
290 295 300

Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn
305 310 315 320

Ser Ile Gly Ile Ser Phe His Ser Ala Trp Leu Thr Val Leu Pro Ala
325 330 335

Pro Gly Arg Glu Lys Glu Ile Thr Ala Ser Pro Asp Tyr Leu Ser Ala
340 345 350

Leu Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
355 360 365

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
370 375 380

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
385 390 395 400

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
405 410 415

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
420 425 430

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
435 440 445

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
450 455 460

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
465 470 475 480

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
485 490 495

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
500 505 510

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
515 520 525

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
530 535 540

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
545 550 555 560

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
565 570 575

Lys Ser Leu Ser Leu Ser Pro Gly
580

<210> 3
<211> 1050
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1050)

<400> 3

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tta gtt gag gat acc aca tta gag cca gaa gag cca cca act aaa tac        48
Leu Val Glu Asp Thr Thr Leu Glu Pro Glu Glu Pro Pro Thr Lys Tyr
1               5                  10                  15

caa atc tct caa cca gaa gtg tac gtg gct gca cca ggg gag tcg cta        96
Gln Ile Ser Gln Pro Glu Val Tyr Val Ala Ala Pro Gly Glu Ser Leu
            20                  25                  30

gag gtg cgc tgc ctg ttg aaa gat gcc gcc gtg atc agt tgg act aag       144
Glu Val Arg Cys Leu Leu Lys Asp Ala Ala Val Ile Ser Trp Thr Lys
        35                  40                  45

gat ggg gtg cac ttg ggg ccc aac aat agg aca gtg ctt att ggg gag       192
Asp Gly Val His Leu Gly Pro Asn Asn Arg Thr Val Leu Ile Gly Glu
    50                  55                  60

tac ttg cag ata aag ggc gcc acg cct aga gac tcc ggc ctc tat gct       240
Tyr Leu Gln Ile Lys Gly Ala Thr Pro Arg Asp Ser Gly Leu Tyr Ala
65                  70                  75                  80

tgt act gcc agt agg act gta gac agt gaa act tgg tac ttc atg gtg       288
Cys Thr Ala Ser Arg Thr Val Asp Ser Glu Thr Trp Tyr Phe Met Val
                85                  90                  95

aat gtc aca gat gcc atc tca tcc gga gat gat gag gat gac acc gat       336
Asn Val Thr Asp Ala Ile Ser Ser Gly Asp Asp Glu Asp Asp Thr Asp
            100                 105                 110

ggt gcg gaa gat ttt gtc agt gag aac agt aac aac aag aga gca cca       384
Gly Ala Glu Asp Phe Val Ser Glu Asn Ser Asn Asn Lys Arg Ala Pro
        115                 120                 125

tac tgg acc aac aca gaa aag atg gaa aag cgg ctc cat gct gtg cct       432
Tyr Trp Thr Asn Thr Glu Lys Met Glu Lys Arg Leu His Ala Val Pro
    130                 135                 140

gcg gcc aac act gtc aag ttt cgc tgc cca gcc ggg ggg aac cca atg       480
Ala Ala Asn Thr Val Lys Phe Arg Cys Pro Ala Gly Gly Asn Pro Met
145                 150                 155                 160

cca acc atg cgg tgg ctg aaa aac ggg aag gag ttt aag cag gag cat       528
Pro Thr Met Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Gln Glu His
                165                 170                 175

cgc att gga ggc tac aag gta cga aac cag cac tgg agc ctc att atg       576
Arg Ile Gly Gly Tyr Lys Val Arg Asn Gln His Trp Ser Leu Ile Met
            180                 185                 190

gaa agt gtg gtc cca tct gac aag gga aat tat acc tgt gtg gtg gag       624
Glu Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Val Val Glu
        195                 200                 205

aat gaa tac ggg tcc atc aat cac acg tac cac ctg gat gtt gtg gag       672
Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr His Leu Asp Val Val Glu
    210                 215                 220

cga tcg cct cac cgg ccc atc ctc caa gcc gga ctg ccg gca aat gcc       720
Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Ala
225                 230                 235                 240

tcc aca gtg gtc gga gga gac gta gag ttt gtc tgc aag gtt tac agt       768
Ser Thr Val Val Gly Gly Asp Val Glu Phe Val Cys Lys Val Tyr Ser
                245                 250                 255
```

```
gat gcc cag ccc cac atc cag tgg atc aag cac gtg gaa aag aac ggc      816
Asp Ala Gln Pro His Ile Gln Trp Ile Lys His Val Glu Lys Asn Gly
            260                 265                 270

agt aaa tac ggg ccc gac ggg ctg ccc tac ctc aag gtt ctc aag gcc      864
Ser Lys Tyr Gly Pro Asp Gly Leu Pro Tyr Leu Lys Val Leu Lys Ala
        275                 280                 285

gcc ggt gtt aac acc acg gac aaa gag att gag gtt ctc tat att cgg      912
Ala Gly Val Asn Thr Thr Asp Lys Glu Ile Glu Val Leu Tyr Ile Arg
    290                 295                 300

aat gta act ttt gag gac gct ggg gaa tat acg tgc ttg gcg ggt aat      960
Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn
305                 310                 315                 320

tct att ggg ata tcc ttt cac tct gca tgg ttg aca gtt ctg cca gcg     1008
Ser Ile Gly Ile Ser Phe His Ser Ala Trp Leu Thr Val Leu Pro Ala
                325                 330                 335

cct gga aga gaa aag gag att aca gct tcc cca gac tac ctg             1050
Pro Gly Arg Glu Lys Glu Ile Thr Ala Ser Pro Asp Tyr Leu
            340                 345                 350


<210>  4
<211>  350
<212>  PRT
<213>  Homo sapiens

<400>  4

Leu Val Glu Asp Thr Thr Leu Glu Pro Glu Glu Pro Pro Thr Lys Tyr
1               5                   10                  15

Gln Ile Ser Gln Pro Glu Val Tyr Val Ala Ala Pro Gly Glu Ser Leu
            20                  25                  30

Glu Val Arg Cys Leu Leu Lys Asp Ala Ala Val Ile Ser Trp Thr Lys
        35                  40                  45

Asp Gly Val His Leu Gly Pro Asn Asn Arg Thr Val Leu Ile Gly Glu
    50                  55                  60

Tyr Leu Gln Ile Lys Gly Ala Thr Pro Arg Asp Ser Gly Leu Tyr Ala
65                  70                  75                  80

Cys Thr Ala Ser Arg Thr Val Asp Ser Glu Thr Trp Tyr Phe Met Val
                85                  90                  95

Asn Val Thr Asp Ala Ile Ser Ser Gly Asp Asp Glu Asp Asp Thr Asp
            100                 105                 110

Gly Ala Glu Asp Phe Val Ser Glu Asn Ser Asn Asn Lys Arg Ala Pro
        115                 120                 125

Tyr Trp Thr Asn Thr Glu Lys Met Glu Lys Arg Leu His Ala Val Pro
    130                 135                 140
```

Ala Ala Asn Thr Val Lys Phe Arg Cys Pro Ala Gly Gly Asn Pro Met
145 150 155 160

Pro Thr Met Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Gln Glu His
165 170 175

Arg Ile Gly Gly Tyr Lys Val Arg Asn Gln His Trp Ser Leu Ile Met
180 185 190

Glu Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Val Val Glu
195 200 205

Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr His Leu Asp Val Val Glu
210 215 220

Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Ala
225 230 235 240

Ser Thr Val Val Gly Gly Asp Val Glu Phe Val Cys Lys Val Tyr Ser
245 250 255

Asp Ala Gln Pro His Ile Gln Trp Ile Lys His Val Glu Lys Asn Gly
260 265 270

Ser Lys Tyr Gly Pro Asp Gly Leu Pro Tyr Leu Lys Val Leu Lys Ala
275 280 285

Ala Gly Val Asn Thr Thr Asp Lys Glu Ile Glu Val Leu Tyr Ile Arg
290 295 300

Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn
305 310 315 320

Ser Ile Gly Ile Ser Phe His Ser Ala Trp Leu Thr Val Leu Pro Ala
325 330 335

Pro Gly Arg Glu Lys Glu Ile Thr Ala Ser Pro Asp Tyr Leu
340 345 350

<210> 5
<211> 696
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(696)

<400> 5
gag ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca 48
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

```
cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc        96
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg       144
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg       192
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60

gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag       240
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80

tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag       288
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc       336
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc       384
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125

cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc       432
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140

aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc       480
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160

gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac       528
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac       576
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc       624
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag       672
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

agc ctc tcc ctg tct ccg ggt tga                                      696
Ser Leu Ser Leu Ser Pro Gly
225                 230
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<210> 6
<211> 231
<212> PRT
<213> Homo sapiens

<400> 6

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Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                   10                  15
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Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly
225 230

<210> 7
<211> 60
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(60)

<400> 7
atg tac agg atg caa ctc ctg tct tgc att gca cta agt ctt gca ctt 48

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Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1               5                   10                  15

gtc acg aat tca                                                    60
Val Thr Asn Ser
            20


<210>  8
<211>  20
<212>  PRT
<213>  Homo sapiens

<400>  8

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1               5                   10                  15


Val Thr Asn Ser
            20


<210>  9
<211>  1197
<212>  DNA
<213>  Homo sapiens


<220>
<221>  CDS
<222>  (1)..(1197)

<400>  9
atg agg ctt cgg gag ccg ctc ctg agc ggc agc gcc gcg atg cca ggc     48
Met Arg Leu Arg Glu Pro Leu Leu Ser Gly Ser Ala Ala Met Pro Gly
1               5                   10                  15

gcg tcc cta cag cgg gcc tgc cgc ctg ctc gtg gcc gtc tgc gct ctg     96
Ala Ser Leu Gln Arg Ala Cys Arg Leu Leu Val Ala Val Cys Ala Leu
            20                  25                  30

cac ctt ggc gtc acc ctc gtt tac tac ctg gct ggc cgc gac ctg agc    144
His Leu Gly Val Thr Leu Val Tyr Tyr Leu Ala Gly Arg Asp Leu Ser
        35                  40                  45

cgc ctg ccc caa ctg gtc gga gtc tcc aca ccg ctg cag ggc ggc tcg    192
Arg Leu Pro Gln Leu Val Gly Val Ser Thr Pro Leu Gln Gly Gly Ser
    50                  55                  60

aac agt gcc gcc gcc atc ggg cag tcc tcc ggg gag ctc cgg acc gga    240
Asn Ser Ala Ala Ala Ile Gly Gln Ser Ser Gly Glu Leu Arg Thr Gly
65                  70                  75                  80

ggg gcc cgg ccg ccg cct cct cta ggc gcc tcc tcc cag ccg cgc ccg    288
Gly Ala Arg Pro Pro Pro Pro Leu Gly Ala Ser Ser Gln Pro Arg Pro
                85                  90                  95

ggt ggc gac tcc agc cca gtc gtg gat tct ggc cct ggc ccc gct agc    336
Gly Gly Asp Ser Ser Pro Val Val Asp Ser Gly Pro Gly Pro Ala Ser
            100                 105                 110

aac ttg acc tcg gtc cca gtg ccc cac acc acc gca ctg tcg ctg ccc    384
Asn Leu Thr Ser Val Pro Val Pro His Thr Thr Ala Leu Ser Leu Pro
        115                 120                 125

gcc tgc cct gag gag tcc ccg ctg ctt gtg ggc ccc atg ctg att gag    432
Ala Cys Pro Glu Glu Ser Pro Leu Leu Val Gly Pro Met Leu Ile Glu
```

```
    130                 135                 140

ttt aac atg cct gtg gac ctg gag ctc gtg gca aag cag aac cca aat      480
Phe Asn Met Pro Val Asp Leu Glu Leu Val Ala Lys Gln Asn Pro Asn
145                 150                 155                 160

gtg aag atg ggc ggc cgc tat gcc ccc agg gac tgc gtc tct cct cac      528
Val Lys Met Gly Gly Arg Tyr Ala Pro Arg Asp Cys Val Ser Pro His
                165                 170                 175

aag gtg gcc atc atc att cca ttc cgc aac cgg cag gag cac ctc aag      576
Lys Val Ala Ile Ile Ile Pro Phe Arg Asn Arg Gln Glu His Leu Lys
            180                 185                 190

tac tgg cta tat tat ttg cat cca gtc ctg cag cgc cag cag ctg gac      624
Tyr Trp Leu Tyr Tyr Leu His Pro Val Leu Gln Arg Gln Gln Leu Asp
        195                 200                 205

tat ggc atc tat gtt atc aac cag gcg gga gac act ata ttc aat cgt      672
Tyr Gly Ile Tyr Val Ile Asn Gln Ala Gly Asp Thr Ile Phe Asn Arg
    210                 215                 220

gct aag ctc ctc aat gtt ggc ttt caa gaa gcc ttg aag gac tat gac      720
Ala Lys Leu Leu Asn Val Gly Phe Gln Glu Ala Leu Lys Asp Tyr Asp
225                 230                 235                 240

tac acc tgc ttt gtg ttt agt gac gtg gac ctc att cca atg aat gac      768
Tyr Thr Cys Phe Val Phe Ser Asp Val Asp Leu Ile Pro Met Asn Asp
                245                 250                 255

cat aat gcg tac agg tgt ttt tca cag cca cgg cac att tcc gtt gca      816
His Asn Ala Tyr Arg Cys Phe Ser Gln Pro Arg His Ile Ser Val Ala
            260                 265                 270

atg gat aag ttt gga ttc agc cta cct tat gtt cag tat ttt gga ggt      864
Met Asp Lys Phe Gly Phe Ser Leu Pro Tyr Val Gln Tyr Phe Gly Gly
        275                 280                 285

gtc tct gct cta agt aaa caa cag ttt cta acc atc aat gga ttt cct      912
Val Ser Ala Leu Ser Lys Gln Gln Phe Leu Thr Ile Asn Gly Phe Pro
    290                 295                 300

aat aat tat tgg ggc tgg gga gga gaa gat gat gac att ttt aac aga      960
Asn Asn Tyr Trp Gly Trp Gly Gly Glu Asp Asp Asp Ile Phe Asn Arg
305                 310                 315                 320

tta gtt ttt aga ggc atg tct ata tct cgc cca aat gct gtg gtc ggg     1008
Leu Val Phe Arg Gly Met Ser Ile Ser Arg Pro Asn Ala Val Val Gly
                325                 330                 335

agg tgt cgc atg atc cgc cac tca aga gac aag aaa aat gaa ccc aat     1056
Arg Cys Arg Met Ile Arg His Ser Arg Asp Lys Lys Asn Glu Pro Asn
            340                 345                 350

cct cag agg ttt gac cga att gca cac aca aag gag aca atg ctc tct     1104
Pro Gln Arg Phe Asp Arg Ile Ala His Thr Lys Glu Thr Met Leu Ser
        355                 360                 365

gat ggt ttg aac tca ctc acc tac cag gtg ctg gat gta cag aga tac     1152
Asp Gly Leu Asn Ser Leu Thr Tyr Gln Val Leu Asp Val Gln Arg Tyr
    370                 375                 380

cca ttg tat acc caa atc aca gtg gac atc ggg aca ccg agc tag         1197
Pro Leu Tyr Thr Gln Ile Thr Val Asp Ile Gly Thr Pro Ser
385                 390                 395
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<210> 10

<211> 398
<212> PRT
<213> Homo sapiens

<400> 10

Met Arg Leu Arg Glu Pro Leu Leu Ser Gly Ser Ala Ala Met Pro Gly
1 5 10 15

Ala Ser Leu Gln Arg Ala Cys Arg Leu Leu Val Ala Val Cys Ala Leu
20 25 30

His Leu Gly Val Thr Leu Val Tyr Tyr Leu Ala Gly Arg Asp Leu Ser
35 40 45

Arg Leu Pro Gln Leu Val Gly Val Ser Thr Pro Leu Gln Gly Gly Ser
50 55 60

Asn Ser Ala Ala Ala Ile Gly Gln Ser Ser Gly Glu Leu Arg Thr Gly
65 70 75 80

Gly Ala Arg Pro Pro Pro Pro Leu Gly Ala Ser Ser Gln Pro Arg Pro
85 90 95

Gly Gly Asp Ser Ser Pro Val Val Asp Ser Gly Pro Gly Pro Ala Ser
100 105 110

Asn Leu Thr Ser Val Pro Val Pro His Thr Thr Ala Leu Ser Leu Pro
115 120 125

Ala Cys Pro Glu Glu Ser Pro Leu Leu Val Gly Pro Met Leu Ile Glu
130 135 140

Phe Asn Met Pro Val Asp Leu Glu Leu Val Ala Lys Gln Asn Pro Asn
145 150 155 160

Val Lys Met Gly Gly Arg Tyr Ala Pro Arg Asp Cys Val Ser Pro His
165 170 175

Lys Val Ala Ile Ile Ile Pro Phe Arg Asn Arg Gln Glu His Leu Lys
180 185 190

Tyr Trp Leu Tyr Tyr Leu His Pro Val Leu Gln Arg Gln Gln Leu Asp
195 200 205

Tyr Gly Ile Tyr Val Ile Asn Gln Ala Gly Asp Thr Ile Phe Asn Arg
210 215 220

Ala Lys Leu Leu Asn Val Gly Phe Gln Glu Ala Leu Lys Asp Tyr Asp
225 230 235 240

Tyr Thr Cys Phe Val Phe Ser Asp Val Asp Leu Ile Pro Met Asn Asp
245 250 255

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His Asn Ala Tyr Arg Cys Phe Ser Gln Pro Arg His Ile Ser Val Ala
            260                 265                 270

Met Asp Lys Phe Gly Phe Ser Leu Pro Tyr Val Gln Tyr Phe Gly Gly
        275                 280                 285

Val Ser Ala Leu Ser Lys Gln Gln Phe Leu Thr Ile Asn Gly Phe Pro
    290                 295                 300

Asn Asn Tyr Trp Gly Trp Gly Gly Glu Asp Asp Asp Ile Phe Asn Arg
305                 310                 315                 320

Leu Val Phe Arg Gly Met Ser Ile Ser Arg Pro Asn Ala Val Val Gly
                325                 330                 335

Arg Cys Arg Met Ile Arg His Ser Arg Asp Lys Lys Asn Glu Pro Asn
            340                 345                 350

Pro Gln Arg Phe Asp Arg Ile Ala His Thr Lys Glu Thr Met Leu Ser
        355                 360                 365

Asp Gly Leu Asn Ser Leu Thr Tyr Gln Val Leu Asp Val Gln Arg Tyr
    370                 375                 380

Pro Leu Tyr Thr Gln Ile Thr Val Asp Ile Gly Thr Pro Ser
385                 390                 395


<210>  11
<211>  1128
<212>  DNA
<213>  Homo sapiens


<220>
<221>  CDS
<222>  (1)..(1128)

<400>  11
atg gga ctc ttg gta ttt gtg cgc aat ctg ctg cta gcc ctc tgc ctc       48
Met Gly Leu Leu Val Phe Val Arg Asn Leu Leu Leu Ala Leu Cys Leu
1               5                   10                  15

ttt ctg gta ctg gga ttt ttg tat tat tct gcg tgg aag cta cac tta       96
Phe Leu Val Leu Gly Phe Leu Tyr Tyr Ser Ala Trp Lys Leu His Leu
            20                  25                  30

ctc cag tgg gag gag gac tcc aat tca gtg gtt ctt tcc ttt gac tcc      144
Leu Gln Trp Glu Glu Asp Ser Asn Ser Val Val Leu Ser Phe Asp Ser
        35                  40                  45

gct gga caa aca cta ggc tca gag tat gat cgg ttg ggc ttc ctc ctg      192
Ala Gly Gln Thr Leu Gly Ser Glu Tyr Asp Arg Leu Gly Phe Leu Leu
    50                  55                  60

aat ctg gac tct aaa ctg cct gct gaa tta gcc acc aag tac gca aac      240
Asn Leu Asp Ser Lys Leu Pro Ala Glu Leu Ala Thr Lys Tyr Ala Asn
65                  70                  75                  80
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ttt tca gag gga gct tgc aag cct ggc tat gct tca gcc ttg atg acg      288
Phe Ser Glu Gly Ala Cys Lys Pro Gly Tyr Ala Ser Ala Leu Met Thr
                85                  90                  95

gcc atc ttc ccc cgg ttc tcc aag cca gca ccc atg ttc ctg gat gac      336
Ala Ile Phe Pro Arg Phe Ser Lys Pro Ala Pro Met Phe Leu Asp Asp
            100                 105                 110

tcc ttt cgc aag tgg gct aga atc cgg gag ttc gtg ccg cct ttt ggg      384
Ser Phe Arg Lys Trp Ala Arg Ile Arg Glu Phe Val Pro Pro Phe Gly
        115                 120                 125

atc aaa ggt caa gac aat ctg atc aaa gcc atc ttg tca gtc acc aaa      432
Ile Lys Gly Gln Asp Asn Leu Ile Lys Ala Ile Leu Ser Val Thr Lys
    130                 135                 140

gag tac cgc ctg acc cct gcc ttg gac agc ctc cgc tgc cgc cgc tgc      480
Glu Tyr Arg Leu Thr Pro Ala Leu Asp Ser Leu Arg Cys Arg Arg Cys
145                 150                 155                 160

atc atc gtg ggc aat gga ggc gtt ctt gcc aac aag tct ctg ggg tca      528
Ile Ile Val Gly Asn Gly Gly Val Leu Ala Asn Lys Ser Leu Gly Ser
                165                 170                 175

cga att gac gac tat gac att gtg gtg aga ctg aat tca gca cca gtg      576
Arg Ile Asp Asp Tyr Asp Ile Val Val Arg Leu Asn Ser Ala Pro Val
            180                 185                 190

aaa ggc ttt gag aag gac gtg ggc agc aaa acg aca ctg cgc atc acc      624
Lys Gly Phe Glu Lys Asp Val Gly Ser Lys Thr Thr Leu Arg Ile Thr
        195                 200                 205

tac ccc gag ggc gcc atg cag cgg cct gag cag tac gag cgc gat tct      672
Tyr Pro Glu Gly Ala Met Gln Arg Pro Glu Gln Tyr Glu Arg Asp Ser
    210                 215                 220

ctc ttt gtc ctc gcc ggc ttc aag tgg cag gac ttt aag tgg ttg aaa      720
Leu Phe Val Leu Ala Gly Phe Lys Trp Gln Asp Phe Lys Trp Leu Lys
225                 230                 235                 240

tac atc gtc tac aag gag aga gtg agt gca tcg gat ggc ttc tgg aaa      768
Tyr Ile Val Tyr Lys Glu Arg Val Ser Ala Ser Asp Gly Phe Trp Lys
                245                 250                 255

tct gtg gcc act cga gtg ccc aag gag ccc cct gag att cga atc ctc      816
Ser Val Ala Thr Arg Val Pro Lys Glu Pro Pro Glu Ile Arg Ile Leu
            260                 265                 270

aac cca tat ttc atc cag gag gcc gcc ttc acc ctc att ggc ctg ccc      864
Asn Pro Tyr Phe Ile Gln Glu Ala Ala Phe Thr Leu Ile Gly Leu Pro
        275                 280                 285

ttc aac aat ggc ctc atg ggc cgg ggg aac atc cct acc ctt ggc agt      912
Phe Asn Asn Gly Leu Met Gly Arg Gly Asn Ile Pro Thr Leu Gly Ser
    290                 295                 300

gtg gca gtg acc atg gca cta cac ggc tgt gac gag gtg gca gtc gca      960
Val Ala Val Thr Met Ala Leu His Gly Cys Asp Glu Val Ala Val Ala
305                 310                 315                 320

gga ttt ggc tat gac atg agc aca ccc aac gca ccc ctg cac tac tat     1008
Gly Phe Gly Tyr Asp Met Ser Thr Pro Asn Ala Pro Leu His Tyr Tyr
                325                 330                 335

gag acc gtt cgc atg gca gcc atc aaa gag tcc tgg acg cac aat atc     1056
Glu Thr Val Arg Met Ala Ala Ile Lys Glu Ser Trp Thr His Asn Ile
            340                 345                 350
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cag cga gag aaa gag ttt ctg cgg aag ctg gtg aaa gct cgc gtc atc     1104
Gln Arg Glu Lys Glu Phe Leu Arg Lys Leu Val Lys Ala Arg Val Ile
        355                 360                 365

act gat cta agc agt ggc atc tga                                   1128
Thr Asp Leu Ser Ser Gly Ile
    370                 375
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<210> 12
<211> 375
<212> PRT
<213> Homo sapiens

<400> 12

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Met Gly Leu Leu Val Phe Val Arg Asn Leu Leu Leu Ala Leu Cys Leu
1               5                   10                  15

Phe Leu Val Leu Gly Phe Leu Tyr Tyr Ser Ala Trp Lys Leu His Leu
            20                  25                  30

Leu Gln Trp Glu Glu Asp Ser Asn Ser Val Val Leu Ser Phe Asp Ser
        35                  40                  45

Ala Gly Gln Thr Leu Gly Ser Glu Tyr Asp Arg Leu Gly Phe Leu Leu
    50                  55                  60

Asn Leu Asp Ser Lys Leu Pro Ala Glu Leu Ala Thr Lys Tyr Ala Asn
65                  70                  75                  80

Phe Ser Glu Gly Ala Cys Lys Pro Gly Tyr Ala Ser Ala Leu Met Thr
                85                  90                  95

Ala Ile Phe Pro Arg Phe Ser Lys Pro Ala Pro Met Phe Leu Asp Asp
            100                 105                 110

Ser Phe Arg Lys Trp Ala Arg Ile Arg Glu Phe Val Pro Pro Phe Gly
        115                 120                 125

Ile Lys Gly Gln Asp Asn Leu Ile Lys Ala Ile Leu Ser Val Thr Lys
    130                 135                 140

Glu Tyr Arg Leu Thr Pro Ala Leu Asp Ser Leu Arg Cys Arg Arg Cys
145                 150                 155                 160

Ile Ile Val Gly Asn Gly Gly Val Leu Ala Asn Lys Ser Leu Gly Ser
                165                 170                 175

Arg Ile Asp Asp Tyr Asp Ile Val Val Arg Leu Asn Ser Ala Pro Val
            180                 185                 190

Lys Gly Phe Glu Lys Asp Val Gly Ser Lys Thr Thr Leu Arg Ile Thr
        195                 200                 205
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Tyr Pro Glu Gly Ala Met Gln Arg Pro Glu Gln Tyr Glu Arg Asp Ser
210 215 220

Leu Phe Val Leu Ala Gly Phe Lys Trp Gln Asp Phe Lys Trp Leu Lys
225 230 235 240

Tyr Ile Val Tyr Lys Glu Arg Val Ser Ala Ser Asp Gly Phe Trp Lys
245 250 255

Ser Val Ala Thr Arg Val Pro Lys Glu Pro Pro Glu Ile Arg Ile Leu
260 265 270

Asn Pro Tyr Phe Ile Gln Glu Ala Ala Phe Thr Leu Ile Gly Leu Pro
275 280 285

Phe Asn Asn Gly Leu Met Gly Arg Gly Asn Ile Pro Thr Leu Gly Ser
290 295 300

Val Ala Val Thr Met Ala Leu His Gly Cys Asp Glu Val Ala Val Ala
305 310 315 320

Gly Phe Gly Tyr Asp Met Ser Thr Pro Asn Ala Pro Leu His Tyr Tyr
325 330 335

Glu Thr Val Arg Met Ala Ala Ile Lys Glu Ser Trp Thr His Asn Ile
340 345 350

Gln Arg Glu Lys Glu Phe Leu Arg Lys Leu Val Lys Ala Arg Val Ile
355 360 365

Thr Asp Leu Ser Ser Gly Ile
370 375

<210> 13
<211> 1722
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1722)

<400> 13
tta gtt gag gat acc aca tta gag cca gaa gag cca cca act aaa tac 48
Leu Val Glu Asp Thr Thr Leu Glu Pro Glu Glu Pro Pro Thr Lys Tyr
1 5 10 15

caa atc tct caa cca gaa gtg tac gtg gct gca cca ggg gag tcg cta 96
Gln Ile Ser Gln Pro Glu Val Tyr Val Ala Ala Pro Gly Glu Ser Leu
20 25 30

gag gtg cgc tgc ctg ttg aaa gat gcc gcc gtg atc agt tgg act aag 144
Glu Val Arg Cys Leu Leu Lys Asp Ala Ala Val Ile Ser Trp Thr Lys
35 40 45

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gat ggg gtg cac ttg ggg ccc aac aat agg aca gtg ctt att ggg gag      192
Asp Gly Val His Leu Gly Pro Asn Asn Arg Thr Val Leu Ile Gly Glu
    50                  55                  60

tac ttg cag ata aag ggc gcc acg cct aga gac tcc ggc ctc tat gct      240
Tyr Leu Gln Ile Lys Gly Ala Thr Pro Arg Asp Ser Gly Leu Tyr Ala
65                  70                  75                  80

tgt act gcc agt agg act gta gac agt gaa act tgg tac ttc atg gtg      288
Cys Thr Ala Ser Arg Thr Val Asp Ser Glu Thr Trp Tyr Phe Met Val
                85                  90                  95

aat gtc aca gat gcc atc tca tcc gga gat gat gag gat gac acc gat      336
Asn Val Thr Asp Ala Ile Ser Ser Gly Asp Asp Glu Asp Asp Thr Asp
            100                 105                 110

ggt gcg gaa gat ttt gtc agt gag aac agt aac aac aag aga gca cca      384
Gly Ala Glu Asp Phe Val Ser Glu Asn Ser Asn Asn Lys Arg Ala Pro
        115                 120                 125

tac tgg acc aac aca gaa aag atg gaa aag cgg ctc cat gct gtg cct      432
Tyr Trp Thr Asn Thr Glu Lys Met Glu Lys Arg Leu His Ala Val Pro
    130                 135                 140

gcg gcc aac act gtc aag ttt cgc tgc cca gcc ggg ggg aac cca atg      480
Ala Ala Asn Thr Val Lys Phe Arg Cys Pro Ala Gly Gly Asn Pro Met
145                 150                 155                 160

cca acc atg cgg tgg ctg aaa aac ggg aag gag ttt aag cag gag cat      528
Pro Thr Met Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Gln Glu His
                165                 170                 175

cgc att gga ggc tac aag gta cga aac cag cac tgg agc ctc att atg      576
Arg Ile Gly Gly Tyr Lys Val Arg Asn Gln His Trp Ser Leu Ile Met
            180                 185                 190

gaa agt gtg gtc cca tct gac aag gga aat tat acc tgt gtg gtg gag      624
Glu Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Val Val Glu
        195                 200                 205

aat gaa tac ggg tcc atc aat cac acg tac cac ctg gat gtt gtg gag      672
Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr His Leu Asp Val Val Glu
    210                 215                 220

cga tcg cct cac cgg ccc atc ctc caa gcc gga ctg ccg gca aat gcc      720
Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Ala
225                 230                 235                 240

tcc aca gtg gtc gga gga gac gta gag ttt gtc tgc aag gtt tac agt      768
Ser Thr Val Val Gly Gly Asp Val Glu Phe Val Cys Lys Val Tyr Ser
                245                 250                 255

gat gcc cag ccc cac atc cag tgg atc aag cac gtg gaa aag aac ggc      816
Asp Ala Gln Pro His Ile Gln Trp Ile Lys His Val Glu Lys Asn Gly
            260                 265                 270

agt aaa tac ggg ccc gac ggg ctg ccc tac ctc aag gtt ctc aag gcc      864
Ser Lys Tyr Gly Pro Asp Gly Leu Pro Tyr Leu Lys Val Leu Lys Ala
        275                 280                 285

gcc ggt gtt aac acc acg gac aaa gag att gag gtt ctc tat att cgg      912
Ala Gly Val Asn Thr Thr Asp Lys Glu Ile Glu Val Leu Tyr Ile Arg
    290                 295                 300

aat gta act ttt gag gac gct ggg gaa tat acg tgc ttg gcg ggt aat      960
Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn
305                 310                 315                 320
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tct att ggg ata tcc ttt cac tct gca tgg ttg aca gtt ctg cca gcg     1008
Ser Ile Gly Ile Ser Phe His Ser Ala Trp Leu Thr Val Leu Pro Ala
                325                 330                 335

cct gga aga gaa aag gag att aca gct tcc cca gac aaa act cac aca     1056
Pro Gly Arg Glu Lys Glu Ile Thr Ala Ser Pro Asp Lys Thr His Thr
            340                 345                 350

tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc     1104
Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
        355                 360                 365

ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct     1152
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
    370                 375                 380

gag gtc aca tgc gtg gtg gtg gcc gtg agc cac gaa gac cct gag gtc     1200
Glu Val Thr Cys Val Val Val Ala Val Ser His Glu Asp Pro Glu Val
385                 390                 395                 400

aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca     1248
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
                405                 410                 415

aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc     1296
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
            420                 425                 430

ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc     1344
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
        435                 440                 445

aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc     1392
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
    450                 455                 460

aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca     1440
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
465                 470                 475                 480

tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc     1488
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
                485                 490                 495

aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg     1536
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
            500                 505                 510

cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac     1584
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
        515                 520                 525

ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg     1632
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
    530                 535                 540

cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac     1680
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
545                 550                 555                 560

aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt tga             1722
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
                565                 570
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<210> 14
<211> 573
<212> PRT

<213> Homo sapiens

<400> 14

Leu Val Glu Asp Thr Thr Leu Glu Pro Glu Glu Pro Pro Thr Lys Tyr
1 5 10 15

Gln Ile Ser Gln Pro Glu Val Tyr Val Ala Ala Pro Gly Glu Ser Leu
20 25 30

Glu Val Arg Cys Leu Leu Lys Asp Ala Ala Val Ile Ser Trp Thr Lys
35 40 45

Asp Gly Val His Leu Gly Pro Asn Asn Arg Thr Val Leu Ile Gly Glu
50 55 60

Tyr Leu Gln Ile Lys Gly Ala Thr Pro Arg Asp Ser Gly Leu Tyr Ala
65 70 75 80

Cys Thr Ala Ser Arg Thr Val Asp Ser Glu Thr Trp Tyr Phe Met Val
85 90 95

Asn Val Thr Asp Ala Ile Ser Ser Gly Asp Asp Glu Asp Asp Thr Asp
100 105 110

Gly Ala Glu Asp Phe Val Ser Glu Asn Ser Asn Asn Lys Arg Ala Pro
115 120 125

Tyr Trp Thr Asn Thr Glu Lys Met Glu Lys Arg Leu His Ala Val Pro
130 135 140

Ala Ala Asn Thr Val Lys Phe Arg Cys Pro Ala Gly Gly Asn Pro Met
145 150 155 160

Pro Thr Met Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Gln Glu His
165 170 175

Arg Ile Gly Gly Tyr Lys Val Arg Asn Gln His Trp Ser Leu Ile Met
180 185 190

Glu Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Val Val Glu
195 200 205

Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr His Leu Asp Val Val Glu
210 215 220

Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Ala
225 230 235 240

Ser Thr Val Val Gly Gly Asp Val Glu Phe Val Cys Lys Val Tyr Ser
245 250 255

Asp Ala Gln Pro His Ile Gln Trp Ile Lys His Val Glu Lys Asn Gly
260 265 270

Ser Lys Tyr Gly Pro Asp Gly Leu Pro Tyr Leu Lys Val Leu Lys Ala
275 280 285

Ala Gly Val Asn Thr Thr Asp Lys Glu Ile Glu Val Leu Tyr Ile Arg
290 295 300

Asn Val Thr Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn
305 310 315 320

Ser Ile Gly Ile Ser Phe His Ser Ala Trp Leu Thr Val Leu Pro Ala
325 330 335

Pro Gly Arg Glu Lys Glu Ile Thr Ala Ser Pro Asp Lys Thr His Thr
340 345 350

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
355 360 365

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
370 375 380

Glu Val Thr Cys Val Val Val Ala Val Ser His Glu Asp Pro Glu Val
385 390 395 400

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
405 410 415

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
420 425 430

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
435 440 445

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
450 455 460

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
465 470 475 480

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
485 490 495

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
500 505 510

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
515 520 525

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Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
    530                 535                 540

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
545                 550                 555                 560

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
                565                 570
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