FGFR EXTRACELLULAR DOMAIN ACIDIC REGION MUTEINS

[001] This application claims a priority benefit under 35 U.S.C. § 119(e) from U.S. Provisional Application No. 61/086,121, filed August 4, 2008, which is incorporated by reference herein in its entirety.

TECHNICAL FIELD

[002] The present invention relates to fibroblast growth factor receptor (FGFR) extracellular domains (ECDs) that have been engineered to exhibit decreased tissue binding by increasing the number of acidic amino acid residues within the D1-D2 linker region. The invention further relates to polypeptide and polynucleotide sequences, vectors, host cells, compositions, and kits comprising or encoding such molecules. The invention also relates to methods of making and using FGFR ECD acidic region muteins to treat proliferative disorders, including cancer, disorders of angiogenesis, and macular degeneration.

BACKGROUND ART

[003] Fibroblast growth factors (FGFs) and their receptors (FGFRs) are a highly conserved group of proteins with instrumental roles in angiogenesis, vasculogenesis, and wound healing, as well as in tissue patterning and limb formation in embryonic development. FGFs and FGFRs affect cell migration, proliferation, and survival, providing wide-ranging impacts on health and disease.

[004] The FGFR family comprises four major types of receptors, FGFR1, FGFR2, FGFR3, and FGFR4. These receptors are transmembrane proteins having an extracellular domain (ECD), a transmembrane domain, and an intracytoplasmic tyrosine kinase domain. Each of the extracellular domains contains either two or three immunoglobulin (Ig) domains. When there are three Ig domains, they are referred to as D1, D2, and D3. Receptors having two Ig domains typically lack D1. An acidic motif, called the acid box, is located in the linker region between D1 and D2 in the FGFR extracellular domain. The D2 domain of FGFRs contains a heparin binding site. FGFR4 also contains a heparin binding site in D1. The acid box is believed to interact with the heparin binding site in the D2 domain. Furthermore, it has been shown that the FGFR1 and FGFR3 D1 domains are capable of interacting with the D2 and D3 domains. It has been hypothesized that the FGFR1 acid box-mediated interactions with the D2 domain, and the FGFR1 D1 domain-mediated interactions with the D2 and D3 domains play an

autoinhibitory role that prevents receptor oligomerization in the absence of FGF ligand. Finally, extracellular FGFR activation by FGF ligand binding to an FGFR initiates a cascade of signaling events inside the cell, beginning with oligomerization of the receptor and activation of receptor tyrosine kinase activity.

[005] To date, there are 22 known FGFs, each with the capacity to bind one or more FGFRs. *See, e.g.*, Zhang et al., *J. Biol. Chem.* 281:15, 694-15,700 (2006). Several FGFs can bind to and activate each of one or more FGFRs, often with large differences in their affinities for the different FGFRs. Heparin sulfate proteoglycan ("heparin") is required for the binding of FGFs to FGFRs under certain circumstances. *See, e.g.*, Ornitz et al., *Mol. Cell Biol.* 12:240 (1992). For example, the mitogenic response to FGF2 (also known as basic FGF (bFGF)) mediated by FGFR1 has been shown to depend on the presence of heparin. *See, e.g.*, Ornitz et al., *Mol. Cell Biol.* 12:240 (1992).

SUMMARY

[006] The FGFR4 extracellular domain ("ECD") binds with high affinity to FGF2 and FGF19 ligands, among others. By using the FGFR4 ECD as a 'ligand trap' to bind, for example, free FGF2 and FGF19, one may effectively treat proliferative disorders, including cancer, disorders of angiogenesis, and macular degeneration. Experiments using purified Fc fusions of the wild-type FGFR4 ECD ("FGFR4 ECD Fc fusions") showed that they exhibited poor bioavailability and a short serum half-life, which is at least partially due to excessive tissue binding, when administered to mice using intravenous (IV) methods. *See* **FIG. 4 and Table 5.** In contrast, Fc fusions of the FGFR1 ECD exhibited higher bioavailability and serum half-life. Furthermore, the FGFR4 ECD Fc fusions exhibited high levels of *in vitro* binding to extracellular matrix (ECM) components, whereas Fc fusions of the FGFR1, FGFR2, or FGFR3 ECD showed minimal or undetectable levels of ECM binding. *See* **FIG. 5.**

[007] As noted above, the FGFRs typically contain an acidic motif, called an acid box, between the D1 and D2 domains. The D1 domain and the D1-D2 linker region, which contains the acid box, are more divergent than the D2 and D3 domains between FGFR4 and the other three FGFRs. Further, the FGFR1, FGFR2, and FGFR3 acid boxes all contain a greater number of acidic amino acid residues than the FGFR4 acid box. *See* **FIGs. 11A, 11B, and 11C**, respectively. Based on experiments described herein, we hypothesize that the relatively "weak" FGFR4 acid box, which contains fewer acidic amino acid residues than the FGFR1, FGFR2, and FGFR3 acid boxes, may be less

effective at preventing tissue binding, and may therefore be responsible for the greater ECM binding of the FGFR4 ECD Fc fusions observed *in vitro*.

[008] We have engineered FGFR4 ECDs, called FGFR4 ECD acidic region muteins, that have an increase in the total number of acidic residues within the D1-D2 linker and thus a "stronger" acid box region to reduce ECM binding and potentially reduce tissue binding *in vivo* and to increase the bioavailability of FGFR4 ECD fusion proteins. We have discovered that FGFR4 ECD acidic region muteins that contain an increased number of acidic residues within the D1-D2 linker exhibit decreased ECM binding. We have also discovered that certain FGFR4 ECD acidic region muteins exhibit decreased tissue binding and increased bioavailability. Thus, by increasing the total number of acidic residues within the D1-D2 linker, thus "strengthening" the FGFR4 acid box, we have engineered FGFR4 ECD acidic region muteins with improved properties, including decreased ECM binding and decreased tissue binding, which can in turn lead to increased bioavailability of FGFR4 ECD fusion proteins.

[008a] In a first aspect, the present invention refers to a polypeptide comprising an FGFR4 extracellular domain (ECD) acidic region mutein, wherein the FGFR4 ECD acidic region mutein is selected from an FGFR4 ECD acidic region chimera and an FGFR4 long acid box variant, and wherein the FGFR4 ECD acidic region mutein has a greater number of acidic residues in the D1-D2 linker region than wild-type FGFR4 ECD.

[008b] In a second aspect, the present invention refers to an FGFR4 ECD fusion molecule comprising a polypeptide as defined above and a fusion partner.

[008c] In a third aspect, the present invention refers to a polynucleotide comprising a nucleic acid sequence that encodes the polypeptide as defined above or an FGFR4 ECD fusion molecule as defined above.

[008d] In a fourth aspect, the present invention refers to a pharmaceutical composition comprising the polypeptide or FGFR4 fusion molecule as defined above, and a pharmaceutically acceptable carrier.

[008e] In a fifth aspect, the present invention refers to the use of the pharmaceutical composition as defined above in the manufacture of a medicament for treating an angiogenic disorder in a patient.

[008f] In a sixth aspect, the present invention refers to a polypeptide comprising an FGFR2 ECD acidic region mutein, wherein the FGFR2 ECD acidic region mutein has a greater number of acidic residues in the D1-D2 linker region than wild-type FGFR2 ECD.

[008g] In a seventh aspect, the present invention refers to a polypeptide comprising an FGFR3 ECD acidic region mutein, wherein the FGFR3 ECD acidic region mutein has a greater number of acidic residues in the D1-D2 linker region than wild –type FGFR3 ECD.

[009] In one approach for generating "stronger" FGFR4 ECD acidic region muteins, we have replaced certain non-acidic amino acid residues with acidic amino acid residues, such that the total number of acidic residues within the FGFR4 ECD long acid box is increased relative to the wild-type FGFR4 ECD long acid box. This class of FGFR4 ECD acidic region muteins is referred to herein as "FGFR4 ECD long acid box variants." We discovered that FGFR4 ECD long acid box variants that comprise two more acidic amino acid residues than the wild-type FGFR4 ECD long acid box exhibited decreased ECM binding. *See* **FIG. 14**. FGFR4 ECD long acid box variants that contained four more acidic amino acid residues in the long acid box exhibited even further decreased ECM binding. *See id.*

[010] In another approach for generating "stronger" FGFR4 ECD acidic region muteins, we have replaced all or portions of the D1-D2 linker region of FGFR4 with all or portions of the D1-D2 linker region of FGFR1, FGFR2, or FGFR3 to generate polypeptides in a class of FGFR4 ECD acidic region muteins referred to herein as "FGFR4 ECD acidic region chimeras." FGFR4 ECD acidic region chimeras include FGFR4 ECD D1-D2 linker chimeras, FGFR4 ECD exon 4 chimeras, FGFR4 ECD acid box chimeras, FGFR4 ECD long acid box chimeras, and FGFR4 ECD short acid box chimeras. We found that at least certain FGFR4 ECD D1-D2 linker chimeras, FGFR4 ECD exon 4 chimeras, and FGFR4 ECD acid box chimeras, retained the ability to bind to

both FGF2 and FGF19, but showed decreased levels of ECM binding *in vitro* when compared to a parental FGFR4 ECD Fc fusion. *See* **Tables 3 and 4**, and **FIGs. 6 and 12**. Further, the FGFR4 ECD acidic region chimeras showed decreased binding to the surface of hepatocytes when compared to a parental FGFR4 ECD Fc fusion. *See* **FIG. 7**. *In vitro* ECM binding by an FGFR4 acidic region chimera was further reduced by introducing an N-glycan mutation either adjacent to the amino-terminus of the FGFR4 ECD D1-D2 linker or in the D2 heparin binding site. *See* **FIG. 15**.

[011] In *in vivo* studies, an FGFR4 ECD acidic region mutein exhibited substantially improved bioavailability and serum half-life relative to a parental FGFR4 ECD Fc fusion. *See* **FIG. 8**. Mice injected with the FGFR4 ECD acidic region mutein showed a statistically significant reduction in tumor burden in certain tumor models when compared to a control group, showing that the FGFR4 ECD acidic region muteins possess a similar anti-tumor activity as FGFR4 ECDs administered *in vivo*. *See* **FIG. 9**. The FGFR4 ECD acidic region muteins may therefore be used, e.g., to treat proliferative disorders, including cancer, disorders of angiogenesis, and macular degeneration.

[012] Both the FGFR2 ECD-Fc and FGFR3 ECD-Fc fusion proteins showed significantly lower levels of ECM binding *in vitro* than a parental FGFR4 ECD-Fc, however they showed slightly higher levels of ECM binding than an FGFR1 ECD-Fc fusion protein at higher protein concentrations. *See* **FIG. 5**. Although the FGFR2 and FGFR3 acid boxes contain a greater number of acidic amino acid residues than the FGFR4 acid box, they both contain fewer acidic amino acid residues than the FGFR1 acid box. *See* **FIGs. 11D** and **11E**, respectively. *In vitro* ECM binding experiments showed that FGFR2 and FGFR3 ECD acidic region muteins in which the total number of acidic residues within the FGFR2 and FGFR3 long acid boxes was increased exhibited decreased ECM binding relative to the parental FGFR2 and FGFR3 ECDs. *See* **FIGs. 17A and 17B**, respectively.

[013] In certain examples, FGFR1, FGFR2, and FGFR3 ECDs may be engineered to have a decrease in the total number of acidic residues within the D1-D2 linker, and thus a "weaker" acid box to increase tissue binding *in vivo* and to decrease the bioavailability of the FGFR1, FGFR2, and FGFR3 ECD fusion proteins. Such "weakened" FGFR1, FGFR2, and FGFR3 ECDs may be useful, for example, when delivered locally, to prevent toxicity and/or side effects that might occur with systemic administration.

[014] In certain examples, a polypeptide comprising an FGFR4 ECD acidic region mutein is provided. In certain examples, an isolated polypeptide comprising an FGFR4 ECD acidic region mutein is provided. In certain examples, an FGFR4 ECD acidic region mutein is an FGFR4 ECD D1-D2 linker chimera. In certain examples, the FGFR4 ECD D1-D2 linker chimera comprises a D1-D2 linker selected from an FGFR1 D1-D2 linker, an FGFR2 D1-D2 linker, and an FGFR3 D1-D2 linker, in place of the FGFR4 D1-D2 linker. In certain examples, the FGFR4 ECD D1-D2 linker chimera comprises an amino acid sequence selected from SEQ ID NOs: 22, 26, 28, and 32, in place of an FGFR4 D1-D2 linker selected from SEQ ID NOs: 16 and 17. In certain examples, the FGFR4 ECD D1-D2 linker chimera comprises an amino acid sequence selected from SEQ ID NOs: 35 to 38.

[015] In certain examples, an FGFR4 ECD acidic region mutein is an FGFR4 ECD exon 4 chimera. In certain examples, the FGFR4 ECD exon 4 chimera comprises an exon 4 selected from an FGFR1 exon 4, an FGFR2 exon 4, and an FGFR3 exon 4, in place of the FGFR4 exon 4. In certain examples, the FGFR4 ECD exon 4 chimera comprises an amino acid sequence selected from SEQ ID NOs: 23, 92, 29, and 33, in place of an FGFR4 D1-D2 linker selected from SEQ ID NOs: 18 and 19. In certain examples, the FGFR4 ECD exon 4 chimera comprises an amino acid sequence selected from SEQ ID NOs: 39 to 42.

[016] In certain examples, an FGFR4 ECD acidic region mutein is an FGFR4 ECD acid box chimera. In certain examples, the FGFR4 ECD acid box chimera comprises an acid box selected from the FGFR1 acid box, the FGFR2 acid box, and the FGFR3 acid box, in place of the FGFR4 acid box. In certain examples, the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 24, 30, and 34 in place of the FGFR4 acid box having an amino acid sequence of SEQ ID NO: 20. In certain examples, an FGFR4 ECD acid box chimera comprises an acid box region selected from an FGFR1 acid box region, an FGFR2 acid box region, and an FGFR3 acid box region, in place of the FGFR4 acid box. In certain examples, the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 56 to 65, in place of the FGFR4 acid box having an amino acid sequence of SEQ ID NO: 20. In certain examples, the FGFR4 ECD acid box chimera comprises an acid box region selected from an FGFR1 acid box region, an FGFR2 acid box region, and an FGFR3 acid box region, in place of an FGFR4 acid box region. In certain examples,

the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 56 to 65, in place of an FGFR4 acid box region having an amino acid sequence selected from SEQ ID NOs: 46 to 55. In certain examples, the FGFR4 ECD acid box chimera comprises an FGFR1 acid box region having the amino acid sequence of SEQ ID NO: 56 in place of an FGFR4 acid box region having the amino acid sequence of SEQ ID NO: 51. In certain examples, the FGFR4 ECD acid box chimera comprises an acid box selected from the FGFR1 acid box, the FGFR2 acid box, and the FGFR3 acid box, in place of an FGFR4 acid box region. In certain examples, the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 24, 30, and 34 in place of an FGFR4 acid box region having an amino acid sequence selected from SEQ ID NOs: 46 to 55. In certain examples, the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 43 to 45 and 157.

[017] In certain examples, an FGFR4 ECD acidic region chimera is an FGFR4 ECD long acid box chimera. In certain examples, the FGFR4 ECD long acid box chimera comprises a long acid box selected from an FGFR1 long acid box, an FGFR2 long acid box, and an FGFR3 long acid box, in place of the FGFR4 long acid box. In certain examples, the FGFR4 long acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 98 to 100, in place of an FGFR4 long acid box selected from SEQ ID NOs: 96 and 97. In certain examples, the FGFR4 long acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 105 to 107.

[018] In certain examples, an FGFR4 ECD acidic region mutein is an FGFR4 ECD short acid box chimera. In certain examples, the FGFR4 ECD short acid box chimera comprises a short acid box selected from an FGFR1 short acid box, an FGFR2 short acid box, and an FGFR3 short acid box, in place of the FGFR4 short acid box. In certain examples, the FGFR4 ECD short acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 102 to 104, in place of an FGFR4 short acid box having an amino acid sequence of SEQ ID NO: 101. In certain examples, the FGFR4 ECD short acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 108 to 110.

In certain examples, the FGFR4 ECD acidic region mutein is an FGFR4 ECD long acid box variant. In certain examples, the the FGFR4 ECD long acid box variant comprises a variant of the FGFR4 ECD that has an increased number of acidic amino acid residues in the long acid box relative to the FGFR4 wild-type long acid box. In certain

such examples, at least two, three, or four non-acidic residues within the long acid box of the FGFR4 ECD are each independently replaced with an acidic residue selected from Glu (E) and Asp (D). In certain such examples, at least one acidic residue is inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2. In certain examples, two acidic residues are inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2. In certain such examples, the number of acidic residues in the FGFR4 long acid box is at least seven. In certain such examples, FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 106 to 117 (SEQ ID NO: 149); FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 107 to 117 (SEQ ID NO: 150); FGFR4 ECD residues 104 to 110 (SEQ ID NO: 146) are replaced with FGFR1 ECD residues 105-113 (SEQ ID NO: 151); FGFR4 ECD residues 113 to 116 (SEQ ID NO: 147) are replaced with FGFR1 ECD residues 116-119 (SEQ ID NO: 152); or FGFR4 ECD residues 109 to 113 (SEQ ID NO: 148) are replaced with FGFR1 ECD residues 112-116 (SEQ ID NO: 153). In certain such examples, FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 106 to 117 (SEQ ID NO: 149); FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 107 to 117 (SEQ ID NO: 150); FGFR4 ECD residues 104 to 110 (SEQ ID NO: 146) are replaced with FGFR1 ECD residues 105-113 (SEQ ID NO: 151); FGFR4 ECD residues 113 to 116 (SEQ ID NO: 147) are replaced with FGFR1 ECD residues 116-119 (SEQ ID NO: 152); or FGFR4 ECD residues 109 to 113 (SEQ ID NO: 148) are replaced with FGFR1 ECD residues 112-116 (SEQ ID NO: 153).

[019] In certain examples, an FGFR4 ECD fusion molecule comprising an FGFR4 ECD acidic region mutein and a fusion partner is provided. In certain examples, an isolated FGFR4 ECD fusion molecule comprising an FGFR4 ECD acidic region mutein and a fusion partner is provided. In certain examples, an FGFR4 ECD fusion molecule comprising an amino acid sequence selected from SEQ ID NOs: 35 to 45, 105 to 121, and 157 is provided. In certain examples, an isolated FGFR4 ECD fusion molecule comprising an amino acid sequence selected from SEQ ID NOs: 35 to 45, 105 to 121, and 157 is provided. In certain examples, an FGFR4 ECD fusion molecule comprising the amino acid sequence of SEQ ID NO: 35, is provided. In certain examples, an isolated FGFR4 ECD fusion molecule comprising the amino acid sequence of SEQ ID NO: 35, is provided. In certain examples, the fusion partner is selected from Fc, albumin, and polyethylene glycol. In certain examples, the fusion

partner is Fc. In certain examples, an FGFR4 ECD fusion molecule comprising an amino acid sequence selected from SEQ ID NOs: 86 to 88, 124 to 140, 143, 144, and 158 is provided. In certain examples, an isolated FGFR4 ECD fusion molecule comprising an amino acid sequence selected from SEQ ID NOs: 86 to 88, 124 to 140, 143, 144, and 158 is provided. In certain examples, an FGFR4 ECD fusion molecule comprising the amino acid sequence of SEQ ID NO: 86 is provided. In certain examples, an isolated FGFR4 ECD fusion molecule comprising the amino acid sequence of SEQ ID NO: 86 is provided. In certain examples, an FGFR4 ECD fusion molecule consisting of the amino acid sequence of SEQ ID NO: 86 is provided. In certain examples, an isolated FGFR4 ECD fusion molecule consisting of the amino acid sequence of SEQ ID NO: 86 is provided.

[020] In certain examples, a pharmaceutical composition comprising an FGFR4 ECD acidic region mutein and a pharmaceutically acceptable carrier is provided. In certain examples, a polynucleotide comprising a nucleic acid sequence that encodes an FGFR4 ECD acidic region mutein is provided.

[021] In certain examples, a method of treating an angiogenic disorder in a patient comprising administering to the patient a pharmaceutical composition comprising an FGFR4 ECD acidic region mutein is provided. In certain examples, a method of treating cancer in a patient comprising administering to the patient a pharmaceutical composition comprising an FGFR4 ECD acidic region mutein is provided. In certain examples, the cancer is selected from colon, liver, lung, breast, and prostate cancers. In certain examples, a method of treating macular degeneration in a patient comprising administering to the patient a pharmaceutical composition comprising an FGFR4 ECD acidic region mutein is provided.

[022] In certain examples, the FGFR4 acidic region mutein comprises at least one point mutation that inhibits glycosylation. In certain examples, the at least one point mutation that inhibits glycosylation is selected from N91A, N156A, N237A, N269A, N290A, and N301A. In certain examples, the FGFR4 acidic region mutein comprises an amino acid sequence selected from SEQ ID NOs: 120, 121, and 168.

[023] In certain examples, an FGFR2 ECD acidic region mutein is provided. In certain examples, the FGFR2 ECD acidic region mutein is an FGFR2 ECD short acid box chimera. In certain examples, the FGFR2 ECD short acid box chimera comprises at least the FGFR1 short acid box in place of at least the FGFR2 short acid box.

In certain such examples, FGFR2 ECD residues 111 to 118 (SEQ ID NO: 155) are replaced with FGFR1 ECD residues 105 to 112 (SEQ ID NO: 154). In certain examples, the FGFR2 ECD short acid box chimera comprises the amino acid sequence of SEQ ID NO: 122.

[024] In certain examples, an FGFR3 ECD acidic region mutein is provided. In certain examples, the FGFR3 ECD acidic region mutein is an FGFR3 ECD short acid box chimera. In certain examples, the FGFR3 ECD short acid box chimera comprises at least the FGFR1 short acid box in place of at least the FGFR3 short acid box. In certain such examples, FGFR3 ECD residues 110 to 117 (SEQ ID NO: 156) are replaced with FGFR1 ECD residues 105 to 112 (SEQ ID NO: 154). In certain examples, the FGFR3 ECD short acid box chimera comprises the amino acid sequence of SEQ ID NO: 123.

BRIEF DESCRIPTION OF THE DRAWINGS

[025] **FIG. 1** shows the extracellular domain (ECD) amino acid sequence of FGFR4 with a 17 amino acid C-terminal deletion, which was fused to an Fc domain in the parental FGFR4 ECD-Fc (also referred to herein as "R4Mut4"). The amino acid sequence in Figure 1 includes the signal peptide, which is cleaved in the mature fusion protein. The numbers refer to the amino acid position, and certain domains within the ECD are illustrated in gray above the amino acid numbers. The amino acid positions within the signal peptide are given negative values because they are cleaved in the mature fusion protein. The first amino acid residue of the mature fusion protein is designated as amino acid position 1. The linker between the first and second Ig domains (referred to herein interchangeably as the "linker domain," "linker region," "D1-D2 linker," and "D1-D2 linker region") is illustrated in a darker gray.

[026] **FIG. 2** shows a sequence alignment of the linker domains from FGFR1 and FGFR4 and the boundaries and sequence of the swapped regions in the three variants, called FGFR4ECD(ABMut1: delta17)-Fc (ABMut1), FGFR4ECD(ABMut2: delta17)-Fc (ABMut2), and FGFR4ECD(ABMut3: delta17)-Fc (ABMut3). Acidic residues within the D1-D2 linker are indicated with underlining and bold font.

[027] **FIG. 3** shows the binding of R1Mut4, three different preparations of R4Mut4, R4Mut4 plus heparin, and an IgG control to hepatocytes, detected by flow

cytometry, as described in Example 5. The number of cells (counts) is shown on the Y-axis, and the X-axis shows the relative fluorescent signal, in log units.

[028] **FIG. 4** is a graphical representation of the plasma concentration (ng/ml) in mice of R4Mut4, administered with and without heparin, as described in Example 6. The plasma concentration is shown on the Y-axis, and was determined using an FGF2-binding ELISA. The X-axis shows the time following administration. The dashed line represents the lower limit of reproducible R4Mut4 detectability in the ELISA (approximately 156 ng/ml). Each data point represents the average from 5 animals, with the error bars representing the standard error of the mean.

[029] **FIG. 5** shows the binding of commercially-available FGFR1 ECD-Fc, FGFR2 ECD-Fc, FGFR3 ECD-Fc, and FGFR4 ECD-Fc to Matrigel plates, as described in Example 7. The X-axis shows the concentration of the Fc fusion proteins and the Y-axis shows the absorbance at 450 nm following incubation of the bound Fc fusion protein with OPD substrate. All binding reactions were carried out in triplicate and the data points represent the average values obtained.

[030] **FIG. 6** shows the binding of R1Mut4, R4Mut4, and FGFR4 ECD acidic region chimeras ABMut1, ABMut2, and ABMut3 to Matrigel plates, as described in Example 9. The X-axis shows the concentration of the Fc fusion proteins and the Y-axis shows the absorbance at 450 nm following incubation of the bound Fc fusion protein with OPD substrate. All binding reactions were carried out in triplicate and the data points represent the average value obtained.

[031] **FIG. 7** shows the binding of R1Mut4, R4Mut4, FGFR4 ECD acidic region chimeras ABMut1, ABMut2, and ABMut3, and an IgG control, to hepatocytes detected by flow cytometry, as described in Example 10. The number of cells (counts) is shown on the Y-axis, and the X-axis shows the relative fluorescent signal, in log units.

[032] **FIG. 8** is a graphical representation of the plasma concentrations (ng/ml) of R4Mut4 and ABMut1 following their administration to mice, as described in Example 11. The plasma concentrations are shown on the Y-axis, and were determined using an FGF2-binding ELISA. The X-axis shows the time following administration. The dashed line represents the lower limit of reproducible R4Mut4 detectability in the ELISA (approximately 8 ng/ml). Data from five animals for each time point are included in the figure.

[033] **FIG. 9** shows the results of the xenograft experiment described in Example 12. Mice were inoculated with tumor cells, and tumor growth was measured after administration of R4Mut4, ABMut1, or vehicle alone. The tumor size is shown on the Y-axis, and the number of days following tumor inoculation is shown on the X-axis. The dosing schedule for each treatment group is shown in Table 9, and the p-values of each treatment group at days 14 and 21 are shown in Table 10.

[034] **FIG. 10** shows the amino acid sequence of the FGFR4 ECD acidic region, along with the locations of certain regions within the FGFR4 ECD acidic region, as defined herein.

[035] **FIG. 11** shows amino acid sequence alignments between (A) the FGFR4 ECD acidic region and the FGFR1 ECD acidic region, (B) the FGFR4 ECD acidic region and the FGFR2 ECD acidic region, (C) the FGFR4 ECD acidic region and the FGFR3 ECD acidic region, (D) the FGFR1 ECD acidic region and the FGFR2 ECD acidic region, and (E) the FGFR1 ECD acidic region and the FGFR3 ECD acidic region.

[036] **FIG. 12** shows the binding of high concentrations of R4Mut4 and ABMut1 fusion proteins expressed in CHO or 293-T cells to Matrigel plates, as described in Example 13. The X-axis shows the concentration of the Fc fusion proteins and the Y-axis shows the absorbance at 450 nm following incubation of the bound Fc fusion protein with OPD substrate.

[037] **FIG. 13** shows the binding of R1Mut4, R4Mut4, ABMut1, R4Mut4(N104D), R4Mut4(P109D), R4Mut4(R113E), and R4Mut4(S116E) fusion proteins to Matrigel plates, as described in Example 14. The X-axis shows the concentration of the Fc fusion proteins and the Y-axis shows the absorbance at 450 nm following incubation of the bound Fc fusion protein with OPD substrate.

[038] **FIG. 14** shows the binding of R1Mut4, R4Mut4, ABMut1, R4(104-114):R1(106-117), R4(104-114):R1(107-117), R4(104-110):R1(105-113), R4(113-116):R1(116-119), and R4(109-113):R1(112-116) fusion proteins to Matrigel plates, as described in Example 15. The X-axis shows the concentration of the Fc fusion proteins and the Y-axis shows the absorbance at 450 nm following incubation of the bound Fc fusion protein with OPD substrate.

[039] **FIG. 15** shows the binding of R4Mut4, ABMut1, ABMut1(N91A), and ABMut1(N159A) fusion proteins to Matrigel plates, as described in Example 16. The X-axis shows the concentration of the Fc fusion proteins and the Y-axis shows the

absorbance at 450 nm following incubation of the bound Fc fusion protein with OPD substrate.

[040] **FIG. 16** shows the binding of the R1Mut4, R4Mut4, ABMut1, R4(D1-D2):R2(D1-D2), and R4(D1-D2):R3(D1-D2) fusion proteins to Matrigel plates, as described in Example 17. The X-axis shows the concentration of the Fc fusion proteins and the Y-axis shows the absorbance at 450 nm following incubation of the bound Fc fusion protein with OPD substrate.

[041] **FIG. 17** shows the binding of FGFR2 ECD-Fc, FGFR3 ECD-Fc, R2(111-118):R1(105-112), and R3(110-117):R1(105-112) fusion proteins to Matrigel plates, as described in Example 18. The X-axis shows the concentration of the Fc fusion proteins and the Y-axis shows the absorbance at 450 nm following incubation of the bound Fc fusion protein with OPD substrate.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[042] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

[043] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[044] Certain techniques used in connection with recombinant DNA, oligonucleotide synthesis, tissue culture and transformation (e.g., electroporation, lipofection), enzymatic reactions, and purification techniques are known in the art. Many such techniques and procedures are described, e.g., in Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), among other places. In addition, certain techniques for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients are also known in the art.

[045] In this application, the use of "or" means "and/or" unless stated otherwise. In the context of a multiple dependent claim, the use of "or" refers back to more than one preceding independent or dependent claim in the alternative only. Also, terms such as "element" or "component" encompass both elements and components comprising one unit

and elements and components that comprise more than one subunit unless specifically stated otherwise.

[046] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[047] The terms "**nucleic acid molecule**" and "**polynucleotide**" may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA.

[048] The terms "**polypeptide**" and "**protein**" are used interchangeably, and refer to a polymer of amino acid residues. Such polymers of amino acid residues may contain natural and/or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. The terms "polypeptide" and "protein" include natural and non-natural amino acid sequences, and both full-length proteins and fragments thereof. Those terms also include post-translationally modified polypeptides and proteins, including, for example, glycosylated, sialylated, acetylated, and/or phosphorylated polypeptides and proteins.

[049] The terms "**acidic amino acid,**" "**acidic amino acid residue,**" and "**acidic residue**" are used interchangeably herein and refer to an amino acid residue that is negatively charged at physiological pH. Acidic amino acids include, but are not limited to, aspartic acid (Asp, D) and glutamic acid (Glu, E).

[050] The terms "**non-acidic amino acid,**" "**non-acidic amino acid residue,**" and "**non-acidic residue**" are used interchangeably and refer to an amino acid residue that is not negatively charged at physiological pH.

[051] The terms "**FGFR extracellular domain**" and "**FGFR ECD**" include FGFR1 ECDs, FGFR2 ECDs, FGFR3 ECDs, and FGFR4 ECDS, as defined herein.

[052] The terms "**FGFR1 extracellular domain**" and "**FGFR1 ECD**" include native FGFR1 ECDs, FGFR1 ECD fragments, and FGFR1 ECD variants. As used herein, the term "**native FGFR1 ECD**" refers to an FGFR1 ECD having an amino acid sequence selected from SEQ ID NOs: 21 and 25. As used herein, the term "**FGFR1 ECD fragment**" refers a polypeptide having an amino acid sequence selected from SEQ ID NOs: 21 and 25, but wherein amino acid residues have been deleted from the amino-terminus and/or carboxy-terminus, wherein the fragments are capable of binding to FGF2. As used herein, the term "**FGFR1 ECD variants**" refers to variants of the portion of the

FGFR1 polypeptide that extends into the extracellular space and variants of fragments thereof that comprise D1, D2, and D3, wherein the variants are capable of binding to FGF2. Such variants may contain amino acid additions, deletions, and substitutions, provided that the FGFR1 ECD variants remain capable of ligand binding.

[053] In certain embodiments, an FGFR1 ECD lacks a signal peptide. In certain embodiments, an FGFR1 ECD includes at least one signal peptide, which may be selected from a native FGFR1 signal peptide and/or a heterologous signal peptide.

[054] The terms "**FGFR2 extracellular domain**" and "**FGFR2 ECD**" include native FGFR2 ECDs, FGFR2 ECD fragments, and FGFR2 ECD variants. As used herein, the term "**native FGFR2 ECD**" refers to an FGFR2 ECD having an amino acid sequence of SEQ ID NO: 27. As used herein, the term "**FGFR2 ECD fragment**" refers a polypeptide having an amino acid sequence selected from SEQ ID NO: 27, but wherein amino acid residues have been deleted from the amino-terminus and/or carboxy-terminus, wherein the fragments are capable of binding to FGF2. A non-limiting exemplary FGFR2 ECD fragment has the amino acid sequence of SEQ ID NO: 160, which corresponds to the amino acid sequence of SEQ ID NO: 27, but with the last three carboxy-terminal amino acid residues, YLE, deleted. As used herein, the term "**FGFR2 ECD variants**" refers to variants of the portion of the FGFR2 polypeptide that extends into the extracellular space and variants of fragments thereof that comprise D1, D2, and D3, wherein the variants are capable of binding to FGF2. Such variants may contain amino acid additions, deletions, and substitutions, provided that the FGFR2 ECD variant remains capable of ligand binding. FGFR2 ECD variants may include amino acid substitutions within the FGFR2 ECD that inhibit N-glycosylation, referred to interchangeably herein as "**FGFR2 ECD glycosylation mutants**" and "**FGFR2 ECD N-glycan mutants**." In certain embodiments, at least one amino acid within the FGFR2 ECD is mutated to prevent glycosylation at that site in the polypeptide. Non-limiting exemplary FGFR2 ECD amino acids that may be glycosylated include N62, N102, N207, N220, N244, N276, N297, and N310 in SEQ ID NO: 27. Non-limiting exemplary amino acid mutations in FGFR4 ECD glycosylation mutants include N62A, N102A, N207A, N220A, N244A, N276A, N297A, and N310A in SEQ ID NO: 27.

[055] In certain embodiments, an FGFR2 ECD lacks a signal peptide. In certain embodiments, an FGFR2 ECD includes at least one signal peptide, which may be selected from a native FGFR2 signal peptide and/or a heterologous signal peptide.

[056] The terms "**FGFR3 extracellular domain**" and "**FGFR3 ECD**" include native FGFR3 ECDs, FGFR3 ECD fragments, and FGFR3 ECD variants. As used herein, the term "**native FGFR3 ECD**" refers to an FGFR3 ECD having an amino acid sequence of SEQ ID NO: 31. As used herein, the term "**FGFR3 ECD fragment**" refers a polypeptide having an amino acid sequence selected from SEQ ID NO: 31, but wherein amino acid residues have been deleted from the amino-terminus and/or carboxy-terminus, wherein the fragments are capable of binding to FGF2. A non-limiting exemplary FGFR3 ECD fragment has the amino acid sequence of SEQ ID NO: 161, which corresponds to the amino acid sequence of SEQ ID NO: 31, but with the last three carboxy-terminal amino acid residues, YAG, deleted. As used herein, the term "**FGFR3 ECD variants**" refers to variants of the portion of the FGFR3 polypeptide that extends into the extracellular space and variants of fragments thereof that comprise D1, D2, and D3, wherein the variants are capable of binding to FGF2. Such variants may contain amino acid additions, deletions, and substitutions, provided that the FGFR3 ECD variant remains capable of ligand binding. FGFR3 ECD variants may include amino acid substitutions within the FGFR3 ECD that inhibit N-glycosylation, referred to interchangeably herein as "**FGFR3 ECD glycosylation mutants**" and "**FGFR3 ECD N-glycan mutants.**" In certain embodiments, at least one amino acid within the FGFR3 ECD is mutated to prevent glycosylation at that site in the polypeptide. Non-limiting exemplary FGFR3 ECD amino acids that may be glycosylated include N76, N203, N240, N272, N293, and N306 in SEQ ID NO: 31. Non-limiting exemplary amino acid mutations in FGFR3 ECD glycosylation mutants include N76A, N203A, N240A, N272A, N293A, and N306A in SEQ ID NO: 31.

[057] In certain embodiments, an FGFR3 ECD lacks a signal peptide. In certain embodiments, an FGFR3 ECD includes at least one signal peptide, which may be selected from a native FGFR3 signal peptide and/or a heterologous signal peptide.

[058] The terms "**FGFR4 extracellular domain**" and "**FGFR4 ECD**" include native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants. As used herein, the term "**native FGFR4 ECD**" refers to an FGFR4 ECD having an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, and 93. As used herein, the term "**FGFR4 ECD fragment**" refers a polypeptide having an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, and 93, but wherein all or a portion of the sequence LEASEEVE (SEQ ID NO: 70) has been deleted from the amino terminus and/or all or a portion of the sequence LPEEDPTWTAAAPEARYTD (SEQ ID NO: 71) has been deleted from the carboxy

terminus of the polypeptide, wherein the fragments are capable of binding to FGF2 and/or FGF19. Non-limiting exemplary FGFR4 ECD fragments have the amino acid sequences shown in SEQ ID NOs: 6 to 10 and 76 to 81. As used herein, the term "**FGFR4 ECD variants**" refers to variants of the portion of the FGFR4 polypeptide that extends into the extracellular space and variants of fragments thereof that comprise D1, D2, and D3, wherein the variants are capable of binding to FGF2 and/or FGF19. Such variants may contain amino acid additions, deletions, and substitutions, provided that the FGFR4 ECD variant remains capable of binding to FGF2 and/or FGF19 (see below for a discussion of the structure/function relationship of FGFR extracellular domains).

[059] FGFR4 ECD variants may include amino acid substitutions within the FGFR4 ECD that inhibit N-glycosylation, referred to interchangeably herein as "**FGFR4 ECD glycosylation mutants**" and "**FGFR4 ECD N-glycan mutants.**" In certain embodiments, one or more amino acids within the FGFR4 ECD are mutated to prevent glycosylation at that site in the polypeptide. Non-limiting exemplary FGFR4 ECD amino acids that may be glycosylated include N91, N156, N237, N269, N290, and N301 in SEQ ID NOs: 1 and 2. Non-limiting exemplary amino acid mutations in FGFR4 ECD glycosylation mutants include N91A, N156A, N237A, N269A, N290A, and N301A in SEQ ID NOs: 1 and 2.

[060] In certain embodiments, an FGFR4 ECD lacks a signal peptide. In certain embodiments, an FGFR4 ECD includes at least one signal peptide, which may be selected from a native FGFR4 signal peptide and/or a heterologous signal peptide.

[061] The terms "**FGFR4 2Ig extracellular domain**" and "**FGFR4 2Ig ECD**" include FGFR4 2Ig ECDs, FGFR4 2Ig ECD fragments, and FGFR4 2Ig ECD variants. As used herein, the term "**FGFR4 2Ig ECD**" refers to a polypeptide comprising the acid box and domains D2 and D3 , wherein the polypeptide has an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, and 93, but with at least a portion of D1 deleted. Exemplary FGFR4 2Ig ECDs have the amino acid sequence of SEQ ID NO: 94. As used herein, the term "**FGFR4 2Ig ECD fragment**" refers to an FGFR4 2Ig ECD polypeptide wherein all or a portion of the sequence LPEEDPTWTAAAPEARYTD (SEQ ID NO: 71) has been deleted from the carboxy-terminus of the polypeptide. As used herein, the term "**FGFR4 2Ig ECD variants**" refers to variants of the FGFR4 2Ig ECDs and FGFR4 2Ig ECD fragments discussed above, wherein the variants are capable of binding to FGF2 and/or FGF19. Such variants may contain amino acid additions, deletions, and substitutions,

provided that the FGFR4 2 Ig ECD variant remains capable of binding to FGF2 and/or FGF19 (see below for a discussion of the structure/function relationship of FGFR extracellular domains).

[062] An "**FGFR4 ECD D1-D2 linker chimera**" refers to an FGFR4 ECD selected from native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants, in which the linker region between immunoglobulin-like domain I (D1) and immunoglobulin-like domain II (D2) (referred to herein interchangeably as the "linker domain," "linker region," "D1-D2 linker," and "D1-D2 linker region") has been replaced with the D1-D2 linker region from FGFR1, FGFR2, or FGFR3. The D1-D2 linker of the FGFR4 ECD has the sequence DSLTSSNDDEDPKSHRDPSNRHSYPQQ (SEQ ID NO: 16), which is amino acids 98 to 124, inclusive, of SEQ ID NO: 1; or has the sequence DSLTSSNDDEDPKSHRDLSNRHSYPQQ (SEQ ID NO: 17), which is amino acids 98 to 124, inclusive, of SEQ ID NO: 2. The D1-D2 linker of FGFR1 has the sequence DALPSSEDDDDDDDSSSEEKETDNTKPNPV (SEQ ID NO: 22), which is amino acids 99 to 128, inclusive, of SEQ ID NO: 21; or has the sequence DALPSSEDDDDDDDSSSEEKETDNTKPNRMPV (SEQ ID NO: 26), which is amino acids 99 to 130, inclusive, of SEQ ID NO: 25. The D1-D2 linker of FGFR2 has the sequence DAISSGDDEDDTDGAEDFVSENSNNKR (SEQ ID NO: 28), which is amino acids 105 to 131, inclusive, of SEQ ID NO: 27. The D1-D2 linker of FGFR3 has the sequence DAPSSGDDEDGEDEAEDTGVDTG (SEQ ID NO: 32), which is amino acids 105 to 127, inclusive, of SEQ ID NO: 31. Certain exemplary FGFR4 ECD D1-D2 linker chimeras include, but are not limited to, FGFR4 ECD D1-D2 linker chimeras having the amino acid sequences of SEQ ID NOs: 35 to 38.

[063] An "**FGFR4 2Ig ECD D1-D2 linker chimera**" refers to an FGFR4 2Ig ECD selected from FGFR4 2Ig ECDs, FGFR4 2Ig ECD fragments, and FGFR4 2Ig ECD variants, in which the linker region between immunoglobulin-like domain I (D1) and immunoglobulin-like domain II (D2) (referred to herein interchangeably as the "linker domain," "linker region," "D1-D2 linker," and "D1-D2 linker region") has been replaced with the D1-D2 linker region from FGFR1, FGFR2, or FGFR3, as described above for FGFR4 ECD D1-D2 linker chimeras.

[064] The terms "**corresponding amino acid residue**" and "**corresponding residue**" are used interchangeably herein to refer to an amino acid residue or a gap in the amino acid sequence (as indicated by "-") of a first FGFR ECD D1-D2 linker region that

lines up with an amino acid residue or a gap in the amino acid sequence (as indicated by "-") of a second FGFR ECD D1-D2 linker region shown in a sequence alignment. As defined herein, the corresponding amino acid residues between FGFR4 and FGFR1 are shown in **FIG. 11A**. As defined herein, the corresponding amino acid residues between FGFR4 and FGFR2 are shown in **FIG. 11B**. As defined herein, the corresponding amino acid residues between FGFR4 and FGFR3 are shown in **FIG. 11C**. As defined herein, the corresponding amino acid residues between FGFR1 and FGFR2 are shown in **FIG. 11D**. As defined herein, the corresponding amino acid residues between FGFR1 and FGFR3 are shown in **FIG. 11E**. In certain embodiments, the amino acid residue of a first FGFR ECD is replaced by the corresponding amino acid residue of a second FGFR ECD. In certain such embodiments, when the corresponding amino acid of the second FGFR ECD is a gap, the amino acid residue of the first FGFR ECD is deleted. In certain such embodiments, when the amino acid residue of the first FGFR ECD is a gap, the corresponding amino acid residue of the second FGFR ECD is inserted into the first FGFR ECD.

[065] The terms "**corresponding amino acid sequence**" and "**corresponding sequence**" are used interchangeably herein to refer to the sequence of amino acid residues within a particular region of an FGFR ECD.

[066] An "**FGFR4 ECD exon 4 chimera**" refers to an FGFR4 ECD selected from native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants, in which the amino acid sequence encoded by exon 4 (referred to herein interchangeably as "exon 4" or "exon 4 region") has been replaced with the amino acid sequence encoded by exon 4 from FGFR1, FGFR2, or FGFR3. Exon 4 of the FGFR4 ECD encodes the sequence DSLTSSNDDEDPKSHRDPSNRHSYPQ (SEQ ID NO: 18), which is amino acids 98 to 123, inclusive, of SEQ ID NO: 1; or encodes the sequence DSLTSSNDDEDPKSHRDLSNRHSYPQ (SEQ ID NO: 19), which is amino acids 98 to 123, inclusive, of SEQ ID NO: 2. Exon 4 of FGFR1 encodes the sequence DALPSSEDDDDDDDSSSEEKETDNTKPN (SEQ ID NO: 23), which is amino acids 99 to 126, inclusive, of SEQ ID NO: 21; or encodes the sequence DALPSSEDDDDDDDSSSEEKETDNTKPNRM (SEQ ID NO: 92), which is amino acids 99 to 128, inclusive, of SEQ ID NO: 25. Exon 4 of FGFR2 encodes the sequence DAISSGDDEDDTDGAEDFVSENSNNK (SEQ ID NO: 29), which is amino acids 105 to 130, inclusive, of SEQ ID NO: 27. Exon 4 of FGFR3 encodes the sequence DAPSSGDDEDGEDEAEDTGVDT (SEQ ID NO: 33), which is amino acids 105 to 126,

inclusive, of SEQ ID NO: 31. Certain exemplary FGFR4 ECD exon 4 chimeras include, but are not limited to, FGFR4 ECD exon 4 chimeras having the amino acid sequences of SEQ ID NOs: 39 to 42.

[067] An "**FGFR4 2Ig ECD exon 4 chimera**" refers to an FGFR4 2Ig ECD selected from FGFR4 2Ig ECDs, FGFR4 2Ig ECD fragments, and FGFR4 2Ig ECD variants, in which the amino acid sequence encoded by exon 4 (referred to herein interchangeably as "exon 4" or "exon 4 region") has been replaced with the amino acid sequence encoded by exon 4 from FGFR1, FGFR2, or FGFR3, as described above for FGFR4 ECD exon 4 chimeras.

[068] An "**FGFR4 ECD acid box chimera**" refers to an FGFR4 ECD selected from native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants, in which at least the acid box has been replaced with at least the acid box from FGFR1, FGFR2, or FGFR3. As defined herein, the acid box of FGFR4 has the sequence DDEDPKSHR (SEQ ID NO: 20). As defined herein, the acid box of FGFR1 has the sequence EDDDDDDDSS SE (SEQ ID NO: 24). As defined herein, the acid box of FGFR2 has the sequence DDEDDTD (SEQ ID NO: 30). As defined herein, the acid box of FGFR3 has the sequence DDEDGE (SEQ ID NO: 34).

[069] An "**FGFR4 2Ig ECD acid box chimera**" refers to an FGFR4 ECD selected from FGFR4 2Ig ECDs, FGFR4 2Ig ECD fragments, and FGFR4 2Ig ECD variants, in which at least the acid box has been replaced with at least the acid box from FGFR1, FGFR2, or FGFR3, as described above for FGFR4 ECD acid box chimeras.

[070] As used herein, the term "**acid box region**" means a region of an FGFR ECD that includes the acid box defined above, along with additional amino acids from the FGFR ECD sequence on either the amino-terminus, the carboxy-terminus, or both the amino-terminus and the carboxy-terminus of the acid box, up to and including all of the additional amino acids found in the D1-D2 linker of the FGFR ECD, as defined above. As defined herein, the term FGFR4 ECD acid box chimera includes polypeptides in which the acid box of FGFR4 is replaced with an acid box region from FGFR1, FGFR2, or FGFR3. The term FGFR4 ECD acid box chimera also includes polypeptides in which an acid box region of FGFR4 is replaced with an acid box region from FGFR1, FGFR2, or FGFR3. The term FGFR4 ECD acid box chimera also includes polypeptides in which an acid box region of FGFR4 is replaced with the acid box from FGFR1, FGFR2, or FGFR3. Certain

exemplary FGFR4 ECD acid box chimeras include, but are not limited to, FGFR4 ECD acid box chimeras having the amino acid sequences of SEQ ID NOs: 43 to 45 and 157.

[071] A "**long acid box**" refers to a region of an FGFR ECD that includes the acid box and certain additional amino acid residues on the amino-terminus and/or carboxy-terminus of the acid box. As defined herein, the long acid box of the FGFR4 ECD has the sequence NDDEDPKSHRDPSNR (SEQ ID NO: 96), which is amino acids 104 to 118, inclusive, of SEQ ID NO: 1; or has the sequence NDDEDPKSHRDLSNR (SEQ ID NO: 97), which is amino acids 104 to 118, inclusive, of SEQ ID NO: 2. As defined herein, the long acid box of the FGFR1 ECD has the sequence EDDDDDDDSSSEEKETD (SEQ ID NO: 98), which is amino acids 105 to 121, inclusive, of SEQ ID NOs: 21 and 25. As defined herein, the long acid box of the FGFR2 ECD has the sequence DDEDDTDGAEDFVSE (SEQ ID NO: 99), which is amino acids 111 to 125, inclusive, of SEQ ID NO: 27. As defined herein, the long acid box of the FGFR3 ECD has the sequence GDDEDGEDEAED (SEQ ID NO: 100), which is amino acids 110 to 121, inclusive, of SEQ ID NO: 31.

[072] The term "**FGFR4 ECD long acid box chimera**" refers to an FGFR4 ECD selected from native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants, in which at least the long acid box, but not more than the D1-D2 linker region, has been replaced with at least the long acid box, but not more than the D1-D2 linker region, from FGFR1, FGFR2, or FGFR3.

[073] The term "**FGFR4 2Ig ECD long acid box chimera**" refers to an FGFR4 ECD selected from native FGFR4 2Ig ECDs, FGFR4 2Ig ECD fragments, and FGFR4 2 Ig ECD variants, in which at least the long acid box, but not more than the D1-D2 linker region, has been replaced with at least the long acid box, but not more than the D1-D2 linker region, from FGFR1, FGFR2, or FGFR3.

[074] The term "**short acid box**" refers to a region of an FGFR ECD having a stretch of consecutive acidic amino acid residues within the acid box. As defined herein, the short acid box of the FGFR4 ECD has the sequence DDED (SEQ ID NO: 101), which is amino acids 105 to 108, inclusive, of SEQ ID NOs: 1 and 2. As defined herein, the short acid box of the FGFR1 ECD has the sequence EDDDDDDD (SEQ ID NO: 102), which is amino acids 105 to 112, inclusive, of SEQ ID NOs: 21 and 25. As defined herein, the short acid box of the FGFR2 ECD has the sequence DDEDD (SEQ ID NO: 103), which is amino acids 111 to 115, inclusive, of SEQ ID NO: 27. As defined herein,

the short acid box of the FGFR3 ECD has the sequence DDED (SEQ ID NO: 104), which is amino acids 111 to 114, inclusive, of SEQ ID NO: 31.

[075] The term "**FGFR4 ECD short acid box chimera**" refers to an FGFR4 ECD selected from native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants, in which at least the short acid box, but not more than the D1-D2 linker region, from FGFR4 has been replaced with at least the short acid box, but not more than the D1-D2 linker region, from FGFR1, FGFR2, or FGFR3.

[076] The term "**FGFR4 2Ig ECD short acid box chimera**" refers to an FGFR4 ECD selected from native FGFR4 2Ig ECDs, FGFR4 2Ig ECD fragments, and FGFR4 2Ig ECD variants, in which at least the short acid box, but not more than the D1-D2 linker region, from FGFR4 has been replaced with at least the short acid box, but not more than the D1-D2 linker region, from FGFR1, FGFR2, or FGFR3.

[077] The term "**FGFR2 ECD short acid box chimera**" refers to an FGFR2 ECD selected from native FGFR2 ECDs, FGFR2 ECD fragments, and FGFR2 ECD variants in which at least the short acid box, but not more than the D1-D2 linker region, from FGFR2 has been replaced with at least the short acid box, but not more than the D1-D2 linker region, from FGFR1. In certain embodiments of the FGFR2 ECD short acid box chimera, at least the FGFR2 acid box, but not more than the D1-D2 linker region, is replaced with the FGFR1 short acid box.

[078] The term "**FGFR3 ECD short acid box chimera**" refers to an FGFR3 ECD selected from native FGFR3 ECDs, FGFR3 ECD fragments, and FGFR3 ECD variants in which at least the short acid box, but not more than the D1-D2 linker region, from FGFR3 has been replaced with at least the short acid box, but not more than the D1-D2 linker region, from FGFR1. In certain embodiments of the FGFR3 ECD short acid box chimera, at least the FGFR3 acid box, but not more than the D1-D2 linker region, is replaced with the FGFR1 short acid box.

[079] The term "**FGFR4 ECD acidic region chimera**" is used herein for convenience to refer to the following five types of molecules: FGFR4 ECD D1-D2 linker chimeras, FGFR4 ECD exon 4 chimeras, FGFR4 ECD acid box chimeras, FGFR4 ECD long acid box chimeras, and FGFR4 ECD short acid box chimeras.

[080] The term "**FGFR4 2Ig ECD acidic region chimera**" is used herein for convenience to refer to the following five types of molecules: FGFR4 2Ig ECD D1-D2

linker chimeras, FGFR4 2Ig ECD exon 4 chimeras, FGFR4 2Ig ECD acid box chimeras, FGFR4 2Ig ECD long acid box chimeras, and FGFR4 2Ig ECD short acid box chimeras.

[081] The term "**FGFR4 ECD long acid box variant**" refers to variants of the FGFR4 ECD selected from native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants, that have increased acidity in the long acid box relative to the FGFR4 wild-type long acid box. In certain embodiments of FGFR4 ECD long acid box variants, at least two non-acidic residues within the long acid box of the FGFR4 ECD are each independently replaced with an acidic residue. In certain embodiments of FGFR4 ECD long acid box variants, at least one residue within the long acid box of the FGFR4 ECD is replaced with the corresponding amino acid residue from FGFR1, FGFR2, or FGFR3. In certain embodiments of FGFR4 ECD long acid box variants, at least one acidic residue within the long acid box of the FGFR4 ECD is replaced with a different acidic residue. In certain embodiments of FGFR4 ECD long acid box variants, one or two acidic residues are inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2. In certain embodiments of FGFR4 ECD long acid box variants, up to three non-acidic residues are deleted from the long acid box of the FGFR4 ECD. In certain embodiments of FGFR4 ECD long acid box variants, the total number of acidic residues within the long acid box of an FGFR4 ECD acidic region variant, including any acidic residues inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2, is at least seven.

[082] The term "**FGFR4 2Ig ECD long acid box variant**" refers to variants of the FGFR4 ECD selected from native FGFR4 2Ig ECDs, FGFR4 2Ig ECD fragments, and FGFR4 2Ig ECD variants, that have increased acidity in the long acid box relative to the FGFR4 wild-type long acid box. In certain embodiments of FGFR4 2Ig ECD long acid box variants, at least two non-acidic residues within the long acid box of the FGFR4 2Ig ECD are replaced with an acidic residue. In certain embodiments of FGFR4 2Ig ECD long acid box variants, at least one residue within the long acid box of the FGFR4 2Ig ECD is replaced with the corresponding amino acid residue from FGFR1, FGFR2, or FGFR3. In certain embodiments of FGFR4 2Ig ECD long acid box variants, at least one acidic residue within the long acid box of the FGFR4 2Ig ECD is replaced with a different acidic residue. In certain embodiments of FGFR4 2Ig ECD long acid box variants, one or two acidic residues are inserted between amino acids 15 and 16 of SEQ ID NO: 94, which is an exemplary FGFR4 2Ig ECD. In certain embodiments of FGFR4 2Ig ECD long acid box variants, up to three non-acidic residues are deleted from the long acid box of the

FGFR4 ECD. In certain embodiments of FGFR4 2Ig ECD long acid box variants, the total number of acidic residues within the long acid box of an FGFR4 2Ig ECD acidic region variant, including any acidic residues inserted between amino acids 15 and 16 of SEQ ID NO: 94, is at least seven.

[083] An "**FGFR4 ECD acidic region mutein**" is an FGFR4 ECD selected from native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants having a greater number of acidic residues in the D1-D2 linker region than the wild-type FGFR4 ECD. The term FGFR4 ECD acidic region mutein is used herein to refer to the following types of molecules: FGFR4 ECD acidic region chimeras, including FGFR4 ECD D1-D2 linker chimeras, FGFR4 ECD exon 4 chimeras, FGFR4 ECD acid box chimeras, FGFR4 ECD long acid box chimeras, and FGFR4 ECD short acid box chimeras; and FGFR4 ECD long acid box variants.

[084] An "**FGFR4 2Ig ECD acidic region mutein**" is an FGFR4 2Ig ECD selected from native FGFR4 2Ig ECDs, FGFR4 2Ig ECD fragments, and FGFR4 2Ig ECD variants having a greater number of acidic residues in the D1-D2 linker region than the wild-type FGFR4 2Ig ECD. The term FGFR4 2Ig ECD acidic region mutein is used herein to refer to the following types of molecules: FGFR4 2Ig ECD acidic region chimeras, including FGFR4 2Ig ECD D1-D2 linker chimeras, FGFR4 2Ig ECD exon 4 chimeras, FGFR4 2Ig ECD acid box chimeras, FGFR4 2Ig ECD long acid box chimeras, and FGFR4 2Ig ECD short acid box chimeras; and FGFR4 2Ig ECD long acid box variants.

[085] An "**FGFR2 ECD acidic region mutein**" is an FGFR2 ECD selected from native FGFR2 ECDs, FGFR2 ECD fragments, and FGFR2 ECD variants having a greater number of acidic residues in the D1-D2 linker region than the wild-type FGFR2 ECD.

[086] An "**FGFR3 ECD acidic region mutein**" is an FGFR3 ECD selected from native FGFR3 ECDs, FGFR3 ECD fragments, and FGFR3 ECD variants having a greater number of acidic residues in the D1-D2 linker region than the wild-type FGFR3 ECD.

[087] The term "**FGFR4 ECD fusion molecule**" refers to a molecule comprising a polypeptide selected from an FGFR4 ECD and an FGFR4 ECD acidic region mutein, and a fusion partner. The term "**FGFR4 2Ig ECD fusion molecule**" refers to a molecule comprising a polypeptide selected from an FGFR4 2Ig ECD and an FGFR4 2Ig ECD acidic region mutein, and a fusion partner. The term "**FGFR2 ECD fusion molecule**" refers to a molecule comprising a polypeptide selected from an FGFR2 ECD and an

FGFR2 ECD acidic region mutein, and a fusion partner. In certain embodiments, an FGFR2 ECD fusion molecule contains a "GS" linker between the FGFR2 ECD or the FGFR2 ECD acidic region mutein and the fusion partner. The term "**FGFR3 ECD fusion molecule**" refers to a molecule comprising a polypeptide selected from an FGFR3 ECD and an FGFR3 ECD acidic region mutein, and a fusion partner. In certain embodiments, an FGFR3 ECD fusion molecule contains a "GS" linker between the FGFR3 ECD or the FGFR3 ECD acidic region mutein and the fusion partner. The fusion partner may be linked to either the amino-terminus or the carboxy-terminus of the polypeptide. In certain embodiments, the polypeptide and the fusion partner are covalently linked. If the fusion partner is also a polypeptide ("the fusion partner polypeptide"), the polypeptide and the fusion partner polypeptide may be part of a continuous amino acid sequence. In such cases, the polypeptide and the fusion partner polypeptide may be translated as a single polypeptide from a coding sequence that encodes both the polypeptide and the fusion partner polypeptide. In certain embodiments, the polypeptide and the fusion partner are covalently linked through other means, such as, for example, a chemical linkage other than a peptide bond. Many methods of covalently linking polypeptides to other molecules (for example, fusion partners) are known in the art. One skilled in the art can select a suitable method of covalent linkage based on the particular polypeptide and fusion partner to be covalently linked.

[088] In certain embodiments, the polypeptide and the fusion partner are noncovalently linked. In certain such embodiments, they may be linked, for example, using binding pairs. Exemplary binding pairs include, but are not limited to, biotin and avidin or streptavidin, an antibody and its antigen, etc.

[089] Certain exemplary fusion partners include, but are not limited to, an immunoglobulin Fc domain, albumin, and polyethylene glycol. The amino acid sequences of certain exemplary Fc domains are shown in SEQ ID NOs: 72 to 74.

[090] The term "**signal peptide**" refers to a sequence of amino acid residues that facilitates secretion of a polypeptide from a mammalian cell. A signal peptide is typically cleaved upon export of the polypeptide from the mammalian cell. Certain exemplary signal peptides include, but are not limited to, the signal peptides of FGFR1, FGFR2, FGFR3, and FGFR4, such as, for example, the amino acid sequences of SEQ ID NOs: 66 to 69, and 75. Certain exemplary signal peptides also include signal peptides from

heterologous proteins. A "signal sequence" refers to a polynucleotide sequence that encodes a signal peptide.

[091] A "**vector**" refers to a polynucleotide that is used to express a polypeptide of interest in a host cell. A vector may include one or more of the following elements: an origin of replication, one or more regulatory sequences (such as, for example, promoters and/or enhancers) that regulate the expression of the polypeptide of interest, and/or one or more selectable marker genes (such as, for example, antibiotic resistance genes and genes that can be used in colorimetric assays, e.g., β-galactosidase). One skilled in the art can select suitable vector elements for the particular host cell and application at hand.

[092] A "**host cell**" refers to a cell that can be or has been a recipient of a vector or isolated polynucleotide. Host cells may be prokaryotic cells or eukaryotic cells. Exemplary eukaryotic cells include mammalian cells, such as primate or non-primate animal cells; fungal cells; plant cells; and insect cells. Certain exemplary mammalian cells include, but are not limited to, 293 and CHO cells.

[093] The term "**isolated**" as used herein refers to a molecule that has been separated from at least some of the components with which it is typically found in nature. For example, a polypeptide is referred to as "isolated" when it is separated from at least some of the components of the cell in which it was produced. Where a polypeptide is secreted by a cell after expression, physically separating the supernatant containing the polypeptide from the cell that produced it is considered to be "isolating" the polypeptide. Similarly, a polynucleotide is referred to as "isolated" when it is not part of the larger polynucleotide (such as, for example, genomic DNA or mitochondrial DNA, in the case of a DNA polynucleotide) in which it is typically found in nature, or is separated from at least some of the components of the cell in which it was produced, e.g., in the case of an RNA polynucleotide. Thus, a DNA polynucleotide that is contained in a vector inside a host cell may be referred to as "isolated" so long as that polynucleotide is not found in that vector in nature.

[094] The terms "**subject**" and "**patient**" are used interchangeably herein to refer to mammals, including, but not limited to, rodents, simians, humans, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets.

[095] The term "**angiogenesis**" refers to the development of new blood vessels, including capillary vessels. It can take place in healthy tissue or diseased tissue, such as,

for example, cancer and macular degeneration. The term includes neovascularization, revascularization, angiopoiesis, and vasculogenesis. New blood vessel growth typically results from stimulation of endothelial cells by angiogenic factors which may be active in proliferative conditions, such as in cancer or macular degeneration. An "angiogenic factor" is one that promotes angiogenesis.

[096] The term "**angiogenic disorder**" refers to a condition in which there is inappropriate development of new blood vessels.

[097] "**Treatment**," as used herein, covers any administration or application of a therapeutic for disease in a mammal, including a human, and includes inhibiting the disease, arresting its development, or relieving the disease, for example, by causing regression, or restoring or repairing a lost, missing, or defective function; or stimulating an inefficient process. Treatment may achieved with surgery, radiation, and/or administration of one or more molecules, including, but not limited to, small molecules and polymers, such as polypeptides.

[098] A "**pharmaceutically acceptable carrier**" refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed. For example, if the therapeutic agent is to be administered orally, the carrier may be a gel capsule. If the therapeutic agent is to be administered subcutaneously, the carrier ideally is not irritable to the skin and does not cause injection site reaction.

FGFR4 Extracellular Domains

[099] Certain exemplary FGFR4 ECDs include native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants. As noted above, an FGFR4 ECD fragment may have all or a portion of the sequence LEASEEVE (SEQ ID NO: 70) deleted from the amino terminus and/or all or a portion of the sequence LPEEDPTWTAAAPEARYTD (SEQ ID NO: 71) deleted from the carboxy terminus of the polypeptide. Exemplary FGFR4 ECDs include, but are not limited to, FGFR4 ECDs having amino acid sequences selected from SEQ ID NOs: 1, 2, 3, 93, 6 to 10, and 76 to 81.

[0100] One skilled in the art can create FGFR4 ECD variants that are capable of binding to FGF2 and/or FGF19 based on the extensive data available on the structure/function relationship for FGFRs.

[0101] In certain embodiments, an FGFR4 ECD is isolated.

FGFR4 ECD Acidic Region Chimeras

[0102] An FGFR4 ECD acidic region chimera is an FGFR4 ECD selected from native FGFR4 ECDs, FGFR4 ECD fragments, and FGFR4 ECD variants having a greater number of acidic residues in the D1-D2 linker region than the wild-type FGFR4 ECD. FGFR4 ECD acidic region chimeras include FGFR4 ECD D1-D2 linker chimeras, FGFR4 ECD exon 4 chimeras, FGFR4 ECD acid box chimeras, FGFR4 ECD long acid box chimeras, and FGFR4 ECD short acid box chimeras. In certain embodiments, an FGFR4 ECD acidic region chimera is isolated.

[0103] Exemplary FGFR4 ECD D1-D2 linker chimeras include, but are not limited to, FGFR4 ECDs in which the D1-D2 linker of FGFR4, DSLTSSNDDEDPKSHRDPSNRHSYPQQ or DSLTSSNDDEDPKSHRDLSNRHSYPQQ (SEQ ID NO: 16 or 17, respectively) has been replaced with the D1-D2 linker of FGFR1, DALPSSEDDDDDDDSSSEEKETDNTKPNPV or DALPSSEDDDDDDDSSSEEKETDNTKPNRMPV (SEQ ID NO: 22 or 26, respectively (collectively referred to as "FGFR4 ECD R1 D1-D2 linker chimeras" and "FGFR4 ECD R1 RM D1-D2 linker chimeras," respectively), the D1-D2 linker of FGFR2, DAISSGDDEDDTDGAEDFVSENSNNKR (SEQ ID NO: 28) (collectively referred to as "FGFR4 ECD R2 D1-D2 linker chimeras"), or the D1-D2 linker of FGFR3, DAPSSGDDEDGEDEAEDTGVDTG (SEQ ID NO: 32) (collectively referred to as "FGFR4 ECD R3 D1-D2 linker chimeras").

[0104] As discussed above for FGFR4 ECDs, FGFR4 ECD D1-D2 linker chimeras may include or lack a signal peptide. FGFR4 ECD D1-D2 linker chimeras may have all or a portion of the sequence LEASEEVE (SEQ ID NO: 70) deleted from the amino terminus and/or all or a portion of the sequence LPEEDPTWTAAAPEARYTD (SEQ ID NO: 71) deleted from the carboxy terminus of the polypeptide. Exemplary FGFR4 ECD D1-D2 linker chimeras include, but are not limited to, the FGFR4 ECD D1-D2 linker chimeras having amino acid sequences selected from SEQ ID NOs: 35 to 38. In certain embodiments, FGFR4 ECD D1-D2 linker chimeras comprise at least one FGFR4 ECD

glycosylation mutation. Exemplary FGFR4 ECD D1-D2 linker chimeras comprising glycosylation mutations include, but are not limited to, the FGFR4 ECD D1-D2 linker chimera of SEQ ID NO: 35 with the N91A glycosylation mutation, the N159A glycosylation mutation, or both the N91A and N159A glycosylation mutations. In certain embodiments, an FGFR4 ECD D1-D2 linker chimera glycosylation mutants comprise an amino acid sequence selected from SEQ ID NOs: 120, 121, and 168.

[0105] Exemplary FGFR4 ECD exon 4 chimeras include, but are not limited to, FGFR4 ECDs in which the FGFR4 exon 4 amino acid sequence, DSLTSSNDDEDPKSHRDPSNRHSYPQ or DSLTSSNDDEDPKSHRDLSNRHSYPQ (SEQ ID NOs: 18 and 19, respectively), has been replaced with the FGFR1 exon 4 amino acid sequence, DALPSSEDDDDDDDSSSEEKETDNTKPN (SEQ ID NO: 23) or DALPSSEDDDDDDDSSSEEKETDNTKPNRM (SEQ ID NO: 92) (collectively referred to as "FGFR4 ECD R1 exon 4 chimeras"), the FGFR2 exon 4 amino acid sequence, DAISSGDDEDDTDGAEDFVSENSNNK (SEQ ID NO: 29) (collectively referred to as "FGFR4 ECD R2 exon 4 chimeras"), or the FGFR3 exon 4 amino acid sequence, DAPSSGDDEDGEDEAEDTGVDT (SEQ ID NO: 33) (collectively referred to as "FGFR4 ECD R3 exon 4 chimeras").

[0106] As discussed above for FGFR4 ECDs, FGFR4 ECD exon 4 chimeras may include or lack a signal peptide. FGFR4 ECD exon 4 chimeras may have all or a portion of the sequence LEASEEVE (SEQ ID NO: 70) deleted from the amino terminus and/or all or a portion of the sequence LPEEDPTWTAAAPEARYTD (SEQ ID NO: 71) deleted from the carboxy terminus of the polypeptide. Exemplary FGFR4 ECD exon 4 chimeras include, but are not limited to, the FGFR4 ECD exon 4 linker chimeras having amino acid sequences selected from SEQ ID NOs: 39-42. In certain embodiments, an FGFR4 ECD exon 4 chimera comprises the amino acid sequence of SEQ ID NO: 39. In certain embodiments, FGFR4 ECD exon 4 chimeras comprise at least one FGFR4 ECD glycosylation mutation.

[0107] Exemplary FGFR4 ECD acid box chimeras include, but are not limited to, FGFR4 ECDs in which at least the FGFR4 acid box, defined herein as DDEDPKSHR (SEQ ID NO: 20) has been replaced with at least the FGFR1 acid box, defined herein as EDDDDDDDSSSE (SEQ ID NO: 24) (collectively referred to as "FGFR4 ECD R1 acid box chimeras"), at least the FGFR2 acid box, defined herein as DDEDDTD (SEQ ID NO: 30) (collectively referred to as "FGFR4 ECD R2 acid box chimeras"), or at least the

FGFR3 acid box, defined herein as DDEDGE (SEQ ID NO: 34) (collectively referred to as "FGFR4 ECD R3 acid box chimeras").

[0108] In certain embodiments, additional amino acids flanking the acid box sequences noted above are also replaced in the FGFR4 ECD and/or are also inserted from the FGFR1, FGFR2, or FGFR3 ECD. Such acid boxes including additional amino acids are called "acid box regions." In certain embodiments, additional amino acids from the FGFR1, FGFR2, or FGFR3 acid box region are included up to, and including, the next acidic amino acid. Thus, for example, an FGFR4 acid box or FGFR4 acid box region (e.g., an amino acid sequence selected from SEQ ID NOs: 20 and 46 to 55) may be replaced with an FGFR1 acid box or FGFR1 acid box region having an amino acid sequence selected from EDDDDDDDSSSE (SEQ ID NO: 24), EDDDDDDDSSSEE (SEQ ID NO: 56), EDDDDDDDSSSEEKE (SEQ ID NO: 57), and EDDDDDDDSSSEEKETD (SEQ ID NO: 58). Similarly, an FGFR4 acid box or FGFR4 acid box region may replaced with an FGFR2 acid box or FGFR2 acid box region having an amino acid sequence selected from DDEDDTD (SEQ ID NO: 30), DDEDDTDGAE (SEQ ID NO: 59), DDEDDTDGAED (SEQ ID NO: 60), and DDEDDTDGAEDFVSE (SEQ ID NO: 61). Finally, an FGFR4 acid box or FGFR4 acid box region may replaced with an FGFR3 acid box or FGFR3 acid box region having an amino acid sequence selected from DDEDGE (SEQ ID NO: 34), DDEDGED (SEQ ID NO: 62), DDEDGEDE (SEQ ID NO: 63), DDEDGEDEAE (SEQ ID NO: 64), and DDEDGEDEAED (SEQ ID NO: 65). In certain embodiments, additional amino acids may also be replaced, up to and including all of the amino acids in the D1-D2 linker, for example, in order to retain a particular spacing or structure in the acid box region.

[0109] As non-limiting examples, an FGFR4 acid box having the amino acid sequence of SEQ ID NO: 21 or an FGFR4 acid box region having an amino acid sequence selected from SEQ ID NOs: 46 to 55 may be replaced with (1) an FGFR1 acid box having the amino acid sequence of SEQ ID NO: 24 or an FGFR1 acid box region having an amino acid sequence selected from SEQ ID NOs: 56 to 58; (2) an FGFR2 acid box having the amino acid sequence of SEQ ID NO: 30 or an FGFR2 acid box region having an amino acid sequence selected from SEQ ID NOs: 59 to 61; or (3) an FGFR3 acid box having the amino acid sequence of SEQ ID NO: 34 or an FGFR3 acid box region having an amino acid sequence selected from SEQ ID NOs: 62 to 65. In certain embodiments,

the FGFR4 acid box region of SEQ ID NO: 51 is replaced with the FGFR1 acid box region of SEQ ID NO: 56.

[0110] As discussed above for FGFR4 ECDs, FGFR4 ECD acid box chimeras may include or lack a signal peptide. FGFR4 ECD acid box chimeras may have all or a portion of the sequence LEASEEVE (SEQ ID NO: 70) deleted from the amino terminus and/or all or a portion of the sequence LPEEDPTWTAAAPEARYTD (SEQ ID NO: 71) deleted from the carboxy terminus of the polypeptide. Exemplary FGFR4 ECD acid box chimeras include, but are not limited to, FGFR4 ECD acid box chimeras having amino acid sequences selected from SEQ ID NOs: 44 to 45 and 157. In certain embodiments, FGFR4 ECD acid box chimeras comprise at least one FGFR4 ECD glycosylation mutation.

[0111] Exemplary FGFR4 ECD long acid box chimeras include, but are not limited to, FGFR4 ECDs in which at least the long acid box of FGFR4, NDDEDPKSHRDPSNR or NDDEDPKSHRDLSNR (SEQ ID NO: 96 or 97, respectively), but not more than the D1-D2 linker region, has been replaced with at least the long acid box of FGFR1, EDDDDDDDSSSEEKETD (SEQ ID NO: 98), but not more than the D1-D2 linker region (collectively referred to as "FGFR4 ECD R1 long acid box chimeras"); at least the long acid box of FGFR2, DDEDDTDGAEDFVSE (SEQ ID NO: 99), but not more than the D1-D2 linker region (collectively referred to as "FGFR4 ECD R2 long acid box chimeras"); or at least the long acid box of FGFR3, GDDEDGEDEAED (SEQ ID NO: 100), but not more than the D1-D2 linker region (collectively referred to as "FGFR4 ECD R3 long acid box chimeras").

[0112] As discussed above for FGFR4 ECDs, FGFR4 ECD long acid box chimeras may include or lack a signal peptide. FGFR4 ECD long acid box chimeras may have all or a portion of the sequence LEASEEVE (SEQ ID NO: 70) deleted from the amino terminus and/or all or a portion of the sequence LPEEDPTWTAAAPEARYTD (SEQ ID NO: 71) deleted from the carboxy terminus of the polypeptide. Exemplary FGFR4 ECD long acid box chimeras include, but are not limited to, the FGFR4 ECD long acid box chimeras having amino acid sequences selected from SEQ ID NOs: 105-107. In certain embodiments, FGFR4 ECD long acid box chimeras comprise at least one FGFR4 ECD glycosylation mutation.

[0113] Exemplary FGFR4 ECD short acid box chimeras include, but are not limited to, FGFR4 ECDs in which at least the short acid box of FGFR4, DDED (SEQ ID NO: 101), but not more than the D1-D2 linker region, has been replaced with at least the

short acid box of FGFR1, EDDDDDDD (SEQ ID NO: 102), but not more than the D1-D2 linker region (collectively referred to as "FGFR4 ECD R1 short acid box chimeras"); at least the short acid box of FGFR2, DDEDD (SEQ ID NO: 103), but not more than the D1-D2 linker region (collectively referred to as "FGFR4 ECD R2 short acid box chimeras"); or at least the short acid box of FGFR3, DDED (SEQ ID NO: 104), but not more than the D1-D2 linker region (collectively referred to as "FGFR4 ECD R3 short acid box chimeras").

[0114] As discussed above for FGFR4 ECDs, FGFR4 ECD short acid box chimeras may include or lack a signal peptide. FGFR4 ECD short acid box chimeras may have all or a portion of the sequence LEASEEVE (SEQ ID NO: 70) deleted from the amino terminus and/or all or a portion of the sequence LPEEDPTWTAAAPEARYTD (SEQ ID NO: 71) deleted from the carboxy terminus of the polypeptide. Exemplary FGFR4 ECD short acid box chimeras include, but are not limited to, the FGFR4 ECD short acid box chimeras having amino acid sequences selected from SEQ ID NOs: 108-110. In certain embodiments, FGFR4 ECD short acid box chimeras comprise at least one FGFR4 ECD glycosylation mutation.

FGFR4 ECD Long Acid Box Variants

[0115] FGFR4 ECD long acid box variants include variants of the FGFR4 ECD that have an increased number of acidic amino acid residues in the long acid box relative to the FGFR4 wild-type long acid box. Exemplary FGFR4 ECD long acid variants include, but are not limited to, variants of the FGFR4 ECD in which at least two non-acidic residues within the long acid box of the FGFR4 ECD are each replaced with acidic residues; variants of the FGFR4 ECD in which at least one residue within the long acid box of the FGFR4 ECD is replaced with the corresponding amino acid residue from FGFR1, FGFR2, or FGFR3; variants of the FGFR4 ECD in which at least one acidic residue within the FGFR4 ECD long acid box is replaced with another acidic residue; variants of the FGFR4 ECD in which up to three non-acidic residues are deleted from the long acid box of the FGFR4 ECD; and variants of the FGFR4 long acid box in which the total number of acidic residues within the FGFR4 ECD long acid box, including any acidic residues inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2, is at least seven.

[0116] As non-limiting examples of FGFR4 ECD long acid box variants, FGFR4 ECD residue N104 is replaced with the corresponding D107 residue of FGFR1; FGFR4

ECD residue P109 is replaced with the corresponding D112 residue of FGFR1; FGFR4 ECD residue R113 is replaced with the corresponding E116 residue of FGFR1; and FGFR4 ECD residue S116 is replaced with the corresponding E119 residue of FGFR1. As non-limiting examples of FGFR4 ECD long acid box variants, FGFR4 ECD residues 104 to 114, NDDEDPKSHRD (SEQ ID NO: 145), are replaced with FGFR1 ECD residues 106 to 117, DDDDDDDSSSEE (SEQ ID NO: 149); FGFR4 ECD residues 104 to 114, NDDEDPKSHRD (SEQ ID NO: 145), are replaced with FGFR1 ECD residues 107 to 117, DDDDDDSSSEE (SEQ ID NO: 150); FGFR4 ECD residues 104 to 110, NDDEDPK (SEQ ID NO: 146), are replaced with FGFR1 ECD residues 105-113, EDDDDDDDS (SEQ ID NO: 151); FGFR4 ECD residues 113 to 116, RDPS (SEQ ID NO: 147), are replaced with FGFR1 ECD residues 116-119, EEKE (SEQ ID NO: 152); and FGFR4 ECD residues 109 to 113, PKSHR (SEQ ID NO: 148), are replaced with FGFR1 ECD residues 112-116, DSSSE (SEQ ID NO: 153).

[0117] As discussed above for FGFR4 ECDs, FGFR4 ECD long acid box variants may include or lack a signal peptide. FGFR4 ECD long acid box variants may have all or a portion of the sequence LEASEEVE (SEQ ID NO: 70) deleted from the amino terminus and/or all or a portion of the sequence LPEEDPTWTAAAPEARYTD (SEQ ID NO: 71) deleted from the carboxy terminus of the polypeptide. Exemplary FGFR4 ECD long acid box muteins include, but are not limited to, the FGFR4 ECD long acid box variants having amino acid sequences selected from SEQ ID NOs: 111 to 119. In certain embodiments, FGFR4 ECD long acid box variants comprise at least one FGFR4 ECD glycosylation mutation.

FGFR2 ECD Short Acid Box Chimeras

[0118] Exemplary FGFR2 ECD short acid box chimeras include, but are not limited to, FGFR2 ECDs in which at least the FGFR2 acid box is replaced with the FGFR1 short acid box. As a non-limiting example of a short acid box chimera, FGFR2 ECD residues 111 to 118, DDEDDTDG (SEQ ID NO: 155), are replaced with FGFR1 ECD residues 105 to 112, EDDDDDDD (SEQ ID NO: 154).

[0119] As discussed above for FGFR4 ECDs, FGFR2 ECD short acid box chimeras may include or lack a signal peptide. Further, FGFR2 ECD short acid box chimeras include FGFR2 ECD short acid box chimeras in which one or more amino acid residues have been deleted from the amino-terminus and/or the carboxy-terminus of the ECD, and wherein the FGFR2 ECD short acid box chimeras are capable of binding to

FGF2. Non-limiting exemplary FGFR2 ECD short acid box chimeras include, but are not limited to, FGFR2 ECD short acid box chimeras having amino acid sequences selected from SEQ ID NOs: 122 and 164.

FGFR3 ECD Short Acid Box Chimeras

[0120] Exemplary FGFR3 ECD short acid box chimeras include, but are not limited to, FGFR3 ECDs in which at least the FGFR3 acid box is replaced with the FGFR1 short acid box. As a non-limiting example of a short acid box chimera, FGFR3 ECD residues 110 to 117, GDDEDGED (SEQ ID NO: 156), are replaced with FGFR1 ECD residues 105 to 112, EDDDDDDD (SEQ ID NO: 154)

[0121] As discussed above for FGFR4 ECDs, FGFR3 ECD short acid box chimeras may include or lack a signal peptide. Further, FGFR3 ECD short acid box chimeras include FGFR3 ECD short acid box chimeras in which one or more amino acid residues have been deleted from the amino-terminus and/or the carboxy-terminus of the ECD, and wherein the FGFR3 ECD short acid box chimeras are capable of binding to FGF2. Non-limiting exemplary FGFR3 ECD short acid box chimeras include, but are not limited to, the FGFR3 ECD short acid box chimera having the amino acid sequences selected from SEQ ID NOs: 123 and 165.

FGFR ECD Fusion Molecules

[0122] FGFR ECD fusion molecules comprising an FGFR ECD and a fusion partner are provided. In certain embodiments, an FGFR ECD fusion molecule is isolated.

Fusion Partners and Conjugates

[0123] In certain embodiments, a fusion partner is selected that imparts favorable pharmacokinetics and/or pharmacodynamics on the FGFR ECD fusion molecule. For example, in certain embodiments, a fusion partner is selected that increases the half-life of the FGFR ECD fusion molecule relative to the corresponding FGFR ECD without the fusion partner. By increasing the half-life of the molecule, a lower dose and/or less-frequent dosing regimen may be required in therapeutic treatment. Further, the resulting decreased fluctuation in FGFR ECD serum levels may improve the safety and tolerability of the FGFR ECD-based therapeutics.

[0124] Many different types of fusion partners are known in the art. One skilled in the art can select a suitable fusion partner according to the intended use. Non-limiting exemplary fusion partners include polymers, polypeptides, lipophilic moieties, and succinyl groups. Exemplary polypeptide fusion partners include serum albumin and an

antibody Fc domain. Exemplary polymer fusion partners include, but are not limited to, polyethylene glycol, including polyethylene glycols having branched and/or linear chains.

Oligomerization Domain Fusion Partners

[0125] In various embodiments, oligomerization offers certain functional advantages to a fusion protein, including, but not limited to, multivalency, increased binding strength, and the combined function of different domains. Accordingly, in certain embodiments, a fusion partner comprises an oligomerization domain, for example, a dimerization domain. Exemplary oligomerization domains include, but are not limited to, coiled-coil domains, including alpha-helical coiled-coil domains; collagen domains; collagen-like domains, and certain immunoglobulin domains. Certain exemplary coiled-coil polypeptide fusion partners include the tetranectin coiled-coil domain; the coiled-coil domain of cartilage oligomeric matrix protein; angiopoietin coiled-coil domains; and leucine zipper domains. Certain exemplary collagen or collagen-like oligomerization domains include, but are not limited to, those found in collagens, mannose binding lectin, lung surfactant proteins A and D, adiponectin, ficolin, conglutinin, macrophage scavenger receptor, and emilin.

Antibody Fc Immunoglobulin Domain Fusion Partners

[0126] Many Fc domains that could be used as fusion partners are known in the art. One skilled in the art can select an appropriate Fc domain fusion partner according to the intended use. In certain embodiments, a fusion partner is an Fc immunoglobulin domain. An Fc fusion partner may be a wild-type Fc found in a naturally occurring antibody, a variant thereof, or a fragment thereof. Non-limiting exemplary Fc fusion partners include Fcs comprising a hinge and the CH2 and CH3 constant domains of a human IgG, for example, human IgG1, IgG2, IgG3, or IgG4. Certain additional Fc fusion partners include, but are not limited to, human IgA and IgM. In certain embodiments, an Fc fusion partner comprises a C237S mutation. In certain embodiments, an Fc fusion partner comprises a hinge, CH2, and CH3 domains of human IgG2 with a P331S mutation, as described in U.S. Patent No. 6,900,292. Certain exemplary Fc domain fusion partners are shown in SEQ ID NOs: 72 to 74, 170, and 171.

[0127] Certain exemplary FGFR4 ECD fusion molecules comprising an FGFR4 ECD include, but are not limited to, polypeptides having the amino acid sequences of SEQ ID NOs: 5 and 11 to 15. Certain exemplary FGFR4 ECD fusion molecules comprising an FGFR4 ECD acidic region chimera include, but are not limited to, polypeptides having the

amino acid sequences of SEQ ID NOs: 86 to 88 and 158. In certain embodiments, an FGFR4 ECD fusion molecule comprises the amino acid sequence of SEQ ID NO: 86. In certain embodiments, an FGFR4 ECD fusion molecule consists of the amino acid sequence of SEQ ID NO: 86.

Albumin Fusion Partners and Albumin-binding Molecule Fusion Partners

[0128] In certain embodiments, a fusion partner is an albumin. Certain exemplary albumins include, but are not limited to, human serum album (HSA) and fragments of HSA that are capable of increasing the serum half-life and/or bioavailability of the polypeptide to which they are fused. In certain embodiments, a fusion partner is an albumin-binding molecule, such as, for example, a peptide that binds albumin or a molecule that conjugates with a lipid or other molecule that binds albumin. In certain embodiments, a fusion molecule comprising HSA is prepared as described, e.g., in U.S. Patent No. 6,686,179.

Polymer Fusion Partners

[0129] In certain embodiments, a fusion partner is a polymer, for example, polyethylene glycol (PEG). PEG may comprise branched and/or linear chains. In certain embodiments, a fusion partner comprises a chemically-derivatized polypeptide having at least one PEG moiety attached. Pegylation of a polypeptide may be carried out by any method known in the art. One skilled in the art can select an appropriate method of pegylating a particular polypeptide, taking into consideration the intended use of the polypeptide. Certain exemplary PEG attachment methods include, for example, EP 0 401 384; Malik et al., *Exp. Hematol.*, 20:1028-1035 (1992); Francis, *Focus on Growth Factors*, 3:4-10 (1992); EP 0 154 316; EP 0 401 384; WO 92/16221; and WO 95/34326. As non-limiting examples, pegylation may be performed via an acylation reaction or an alkylation reaction, resulting in attachment of one or more PEG moieties via acyl or alkyl groups. In certain embodiments, PEG moieties are attached to a polypeptide through the α- or ε-amino group of one or more amino acids, although any other points of attachment known in the art are also contemplated.

[0130] Pegylation by acylation typically involves reacting an activated ester derivative of a PEG moiety with a polypeptide. A non-limiting exemplary activated PEG ester is PEG esterified to N-hydroxysuccinimide (NHS). As used herein, acylation is contemplated to include, without limitation, the following types of linkages between a polypeptide and PEG: amide, carbamate, and urethane. See, e.g., Chamow, *Bioconjugate*

Chem., 5:133-140 (1994). Pegylation by alkylation typically involves reacting a terminal aldehyde derivative of a PEG moiety with a polypeptide in the presence of a reducing agent. Non-limiting exemplary reactive PEG aldehydes include PEG propionaldehyde, which is water stable, and mono C1-C10 alkoxy or aryloxy derivatives thereof. See, e.g., U.S. Patent No. 5,252,714.

[0131] In certain embodiments, a pegylation reaction results in poly-pegylated polypeptides. In certain embodiments, a pegylation reaction results in mono-, di-, and/or tri-pegylated polypeptides. One skilled in the art can select appropriate pegylation chemistry and reaction conditions to achieve the desired level of pegylation. Further, desired pegylated species may be separated from a mixture containing other pegylated species and/or unreacted starting materials using various purification techniques known in the art, including among others, dialysis, salting-out, ultrafiltration, ion-exchange chromatography, gel filtration chromatography, and electrophoresis.

Exemplary Attachment of Fusion Partners

[0132] The fusion partner may be attached, either covalently or non-covalently, to the amino-terminus or the carboxy-terminus of the FGFR ECD. The attachment may also occur at a location within the FGFR ECD other than the amino-terminus or the carboxy-terminus, for example, through an amino acid side chain (such as, for example, the side chain of cysteine, lysine, histidine, serine, or threonine).

[0133] In either covalent or non-covalent attachment embodiments, a linker may be included between the fusion partner and the FGFR ECD. Such linkers may be comprised of amino acids and/or chemical moieties. One skilled in the art can select a suitable linker depending on the attachment method used, the intended use of the FGFR ECD fusion molecule, and the desired spacing between the FGFR ECD and the fusion partner.

[0134] Exemplary methods of covalently attaching a fusion partner to an FGFR ECD include, but are not limited to, translation of the fusion partner and the FGFR ECD as a single amino acid sequence and chemical attachment of the fusion partner to the FGFR ECD. When the fusion partner and the FGFR ECD are translated as single amino acid sequence, additional amino acids may be included between the fusion partner and the FGFR ECD as a linker. In certain embodiments, the linker is glycine-serine ("GS"). In certain embodiments, the linker is selected based on the polynucleotide sequence that encodes it, to facilitate cloning the fusion partner and/or FGFR ECD into a single

expression construct (for example, a polynucleotide containing a particular restriction site may be placed between the polynucleotide encoding the fusion partner and the polynucleotide encoding the FGFR ECD, wherein the polynucleotide containing the restriction site encodes a short amino acid linker sequence).

[0135] When the fusion partner and the FGFR ECD are covalently coupled by chemical means, linkers of various sizes can typically be included during the coupling reaction. One skilled in the art can select a suitable method of covalently attaching a fusion partner to an FGFR ECD depending, for example, on the identity of the fusion partner and the particular use intended for the FGFR ECD fusion molecule. One skilled in the art can also select a suitable linker type and length, if one is desired.

[0136] Exemplary methods of non-covalently attaching a fusion partner to an FGFR ECD include, but are not limited to, attachment through a binding pair. Exemplary binding pairs include, but are not limited to, biotin and avidin or streptavidin, an antibody and its antigen, etc.. Again, one skilled in the art can select a suitable method of non-covalently attaching a fusion partner to an FGFR ECD depending, for example, on the identity of the fusion partner and the particular use intended for the FGFR ECD fusion molecule. The selected non-covalent attachment method should be suitable for the conditions under which the FGFR ECD fusion molecule will be used, taking into account, for example, the pH, salt concentrations, and temperature.

Nucleic Acid Molecules Encoding the Polypeptides of the Invention

[0137] Nucleic acid molecules comprising polynucleotides that encode the polypeptides of the invention are provided. Nucleic acid molecules comprising polynucleotides that encode FGFR ECD fusion molecules in which the FGFR ECD and the fusion partner are translated as a single polypeptide, are also provided. Such nucleic acid molecules can be constructed by one skilled in the art using recombinant DNA techniques conventional in the art.

[0138] In certain embodiments, a polynucleotide encoding a polypeptide of the invention comprises a nucleotide sequence that encodes a signal peptide, which, when translated, will be fused to the amino-terminus of the FGFR polypeptide of the invention. As discussed above, the signal peptide may be the native signal peptide, the signal peptide of FGFR1, FGFR2, FGFR3, or FGFR4, or may be another heterologous signal peptide. The amino acid sequences for certain exemplary FGFR signal peptides are shown, e.g., in SEQ ID NOs: 66 to 69 and 75. Certain exemplary signal peptides are known in the art,

and are described, e.g., in the online Signal Peptide Database maintained by the Department of Biochemistry, National University of Singapore, http://proline.bic.nus.edu.sg/spdb/index.html (*see also* Choo et al., *BMC Bioinformatics*, 6: 249 (2005)); and in PCT Publication No. WO 2006/081430.

[0139] In certain embodiments, the nucleic acid molecule comprising the polynucleotide encoding the gene of interest is an expression vector that is suitable for expression in a selected host cell.

Expression and Production of the Proteins of the Invention

Vectors

[0140] Vectors comprising polynucleotides that encode the polypeptides of the invention are provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, etc. One skilled in the art can select a suitable vector depending on the polypeptide to be expressed and the host cell chosen for expression.

[0141] In certain embodiments, a vector is selected that is optimized for expression of polypeptides in CHO-S or CHO-S-derived cells. Exemplary such vectors are described, e.g., in Running Deer et al., *Biotechnol. Prog.* 20:880-889 (2004).

[0142] In certain embodiments, a vector is chosen for *in vivo* expression of the polypeptides of the invention in animals, including humans. In certain such embodiments, expression of the polypeptide is under the control of a promoter that functions in a tissue-specific manner. For example, liver-specific promoters are described, e.g., in PCT Publication No. WO 2006/076288.

Host Cells

[0143] The polypeptides of the invention can be expressed, in various embodiments, in prokaryotic cells, such as bacterial cells; or eukaryotic cells, such as fungal cells, plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Certain exemplary eukaryotic cells that can be used to express polypeptides include, but are not limited to, Cos cells, including Cos 7 cells; 293 cells, including 293-6E and 293-T cells; CHO cells, including CHO-S and DG44 cells; and NS0 cells. One skilled in the art can select a suitable host cell depending on the polypeptide to be expressed, the desired use of that polypeptide, and the scale of the production (e.g., a small amount for laboratory use, or a larger amount for pharmaceutical use). In certain embodiments, a particular eukaryotic

host cell is selected based on its ability to make certain desired post-translational modifications of the polypeptide of the invention. For example, in certain embodiments, CHO cells produce FGFR4 ECD acidic region muteins and/or FGFR4 ECD fusion molecules that have a higher level of glycosylation and/or sialylation than the same polypeptide produced in 293 cells.

[0144] Introduction of a nucleic acid into a desired host cell can be accomplished by any method known in the art, including, but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, etc. Certain exemplary methods are described, e.g., in Sambrook et al., *Molecular Cloning, A Laboratory Manual,* 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to methods known in the art.

[0145] In certain embodiments, a polypeptide can be produced *in vivo* in an animal that has been engineered or transfected with a nucleic acid molecule encoding the polypeptide, according to methods known in the art.

Purification of FGFR ECD Polypeptides

[0146] The polypeptides of the invention can be purified by various methods known in the art. Such methods include, but are not limited to, the use of affinity matrices, ion exchange chromatography, and/or hydrophobic interaction chromatography. Suitable affinity ligands include any ligands of the FGFR ECD or of the fusion partner, or antibodies thereto. For example, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind to an Fc fusion partner to purify a polypeptide of the invention. Antibodies to the polypeptides of the invention may also be used to purify the polypeptides of the invention. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may also suitable for purifying certain polypeptides. Many methods of purifying polypeptides are known in the art. One skilled in the art can select a suitable method depending on the identity of the polypeptide or molecule to be purified and on the scale of the purification (i.e., the quantity of polypeptide or molecule produced).

Therapeutic Compositions

Routes of Administration and Carriers

[0147] In various embodiments, the polypeptides of the invention can be administered *in vivo* by various routes known in the art, including, but not limited to,

intravenous, intra-arterial, subcutaneous, parenteral, intranasal, intramuscular, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. The subject compositions can be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. Nucleic acid molecules encoding the polypeptides of the invention can be coated onto gold microparticles and delivered intradermally by a particle bombardment device, or "gene gun," as described in the literature (see, e.g., Tang et al., *Nature* 356:152-154 (1992)). One skilled in the art can select the appropriate formulation and route of administration according to the intended application.

[0148] In various embodiments, compositions comprising the polypeptides of the invention are provided in formulation with pharmaceutically acceptable carriers, a wide variety of which are known in the art (see, e.g., Gennaro, *Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus*, 20th ed. (2003); Ansel et al., *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 7th ed., Lippencott Williams and Wilkins (2004); Kibbe et al., *Handbook of Pharmaceutical Excipients*, 3rd ed., Pharmaceutical Press (2000)). Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, carriers, and diluents, are available to the public. Moreover, various pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are also available to the public. Certain non-limiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. One skilled in the art can select a suitable carrier according to the intended use.

[0149] In various embodiments, compositions comprising polypeptides of the invention can be formulated for injection by dissolving, suspending, or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids, or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. In various embodiments, the compositions may be formulated for inhalation, for example, using pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen, and the like. The compositions may also be formulated, in various embodiments, into sustained release

microcapsules, such as with biodegradable or non-biodegradable polymers. A non-limiting exemplary biodegradable formulation includes poly lactic acid-glycolic acid polymer. A non-limiting exemplary non-biodegradable formulation includes a polyglycerin fatty acid ester. Certain methods of making such formulations are described, for example, in EP 1 125 584 A1. One skilled in the art can select a suitable formulation depending on the intended route of administration, using techniques and components known in the art.

[0150] Pharmaceutical packs and kits comprising one or more containers, each containing one or more doses of the polypeptides of the invention are also provided. In certain embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising a polypeptide of the invention, with or without one or more additional agents. In certain embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In various embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective pH range. Alternatively, in certain embodiments, the composition may be provided as a lyophilized powder that can be reconstituted upon addition of an appropriate liquid, for example, sterile water. In certain embodiments, the composition comprises one or more substances that inhibit protein aggregation, including, but not limited to, sucrose and arginine. In certain embodiments, a composition of the invention comprises heparin and/or a proteoglycan.

[0151] Pharmaceutical compositions are administered in an amount effective for treatment and/or prophylaxis of the specific indication. The effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, and/or the age of the subject being treated. In general, the polypeptides of the invention are to be administered in an amount in the range of about 50 ug/kg body weight to about 30 mg/kg body weight per dose. Optionally, the polypeptides of the invention can be administered in an amount in the range of about 100 ug/kg body weight to about 20 mg/kg body weight per dose. Further optionally, the polypeptides of the invention can be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose.

[0152] The compositions comprising the polypeptides of the invention can be administered as needed to subjects. Determination of the frequency of administration can

be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like. In certain embodiments, an effective dose of the polypeptide of the invention is administered to a subject one or more times. In various embodiments, an effective dose of the polypeptide of the invention is administered to the subject at least twice a month, once a week, twice a week, or three times a week. In various embodiments, an effective dose of the polypeptide of the invention is administered to the subject for at least a week, at least a month, at least three months, at least six months, or at least a year.

Combination Therapy

[0153] Polypeptides of the invention may be administered alone or with other modes of treatment. They may be provided before, substantially contemporaneous with, or after other modes of treatment, for example, surgery, chemotherapy, radiation therapy, or the administration of a biologic, such as a therapeutic antibody.

Methods of Treating Diseases Using FGFR ECD Polypeptides

[0154] Results from the experiments described herein show that FGFR4 ECD acidic region muteins retain FGFR4's ability to bind FGF2 and/or FGF19. See, *e.g.*, **Example 8**, including **Tables 3 and 4**. Thus, those chimeras can be used in a variety of treatment methods in a similar manner to native FGFR4 ECD (*see, e.g.*, U.S. Publication No. US 2008/0171689).

[0155] For example, polypeptides of the invention may be used as ligand traps *in vivo* to treat diseases associated with one or more ligands of the FGFR family, such as FGF2 and/or FGF19. The FGFR ECD polypeptide ligand traps may be used, for example, to treat a range of cancers and/or angiogenic disorders. In certain embodiments, the FGFR ECD polypeptide ligand traps comprise a fusion partner such as an Fc, albumin, or polyethylene glycol (discussed above).

[0156] In certain embodiments, FGFR4 ECD acidic region muteins may be used to treat colon cancer. Expression of both FGFR4 and FGF19 has been detected in primary colon tumors and several colon tumor cell lines (see, *e.g.*, Desnoyers, Oncogene, 27:85-97 (2008); U.S. Patent Application No. 20070248604). Administration of a monoclonal antibody to FGF19 significantly reduced tumor growth in two human colon cancer cell line xenograft models (HCT116 and Colo201; *see* Desnoyers). Experiments herein demonstrate that the FGFR4 ECD acidic region chimera ABMut1 reduced tumor growth

in an HCT116 xenograft model. Reduced tumor growth was also seen in a Colo201 model following administration of ABMut1 (data not shown). Thus, in certain embodiments, the FGFR4 ECD acidic region muteins of the invention may be administered, e.g., as described above, to patients who have colon cancer. In certain embodiments, the FGFR4 ECD acidic region muteins may be administered to colon cancer patients along with at least one other therapeutic regimen and/or agent.

[0157] FGFR4 and FGF19 expression have also been detected in liver and lung tumors (see, *e.g.*, Desnoyers, Oncogene, 27:85-97 (2008); U.S. Patent Publication No. 2007/0248604). A monoclonal antibody against FGF19 reduced tumor burden in an FGF19-transgenic mouse hepatocellular carcinoma model (*see* Desnoyers). FGFR4 expression has also been implicated in breast cancer (see, *e.g.*, U.S. Patent No. 7,297,774). High FGFR4 mRNA levels in estrogen receptor-positive breast carcinomas correlated with poor clinical benefit in patients on tamoxifen as a first-line treatment (Meijer et al., Endocr. Relat. Cancer., 15(1):101-11 (2008)). Thus, FGFR4 ECD acidic region muteins may also be used to treat liver cancers, breast cancers, including infiltrating ductal carcinoma and adenocarcinoma, and lung carcinomas, including small cell lung carcinomas and non-small cell lung carcinomas.

[0158] FGFR1 and FGFR4 overexpression have also been detected in prostate cancer, with a greater frequency of high levels of protein expression in grade 5 cancers than in grades 1-3 (Sahadevan et al., J. Pathol., 213(1):82-90 (2007)). Suppression of FGFR4 by RNA interference blocked prostate cancer cell proliferation *in vitro* (see *id.*). FGFR4 ECD acidic region muteins may therefore also be used to treat prostate cancers.

[0159] FGFR ligands, such as FGF2, are known stimulators of angiogenesis. Thus, FGFR ECD polypeptides may be administered to patients with angiogenic disorders such as cancer and/or macular degeneration in order to inhibit angiogenesis. Additional cancers that may be treated with the FGFR ECD polypeptides of the invention include, for example, sarcomas and carcinomas including, but not limited to fibrosarcomas, myxosarcomas, liposarcomas, chondrosarcomas, osteogenic sarcomas, chordomas, angiosarcomas, endotheliosarcomas, lymphangiosarcomas, lymphangioendotheliosarcomas, synoviomas, mesotheliomas, Ewing's tumors, leiomyosarcomas, rhabdomyosarcomas, gastic cancers, pancreatic cancers, ovarian cancers, prostate cancers, squamous cell carcinomas, basal cell carcinomas, adenocarcinomas, sweat gland carcinomas, sebaceous gland carcinomas, papillary

carcinomas, papillary adenocarcinomas, cystadenocarcinomas, medullary carcinomas, bronchogenic carcinomas, renal cell carcinomas, hepatomas, liver metastases, bile duct carcinomas, choriocarcinomas, seminomas, embryonal carcinomas, thyroid carcinomas such as anaplastic thyroid cancers, Wilms' tumors, cervical cancers, testicular tumors, bladder carcinomas, epithelial carcinomas, gliomas, astrocytomas, medulloblastomas, craniopharyngiomas, ependymomas, pinealomas, hemangioblastomas, acoustic neuromas, oligodendrogliomas, meningiomas, melanomas, neuroblastomas, glioblastomas, and retinoblastomas. Also among the cancers within the scope of the invention are hematologic malignancies; prostate cancer; bladder cancer; pancreatic cancer; ovarian cancer, salivary cancer; pituitary cancer; renal cell carcinoma; melanoma; glioblastoma; and retinoblastoma.

[0160] Tumors comprising dysproliferative changes, such as hyperplasias, metaplasias, and dysplasias, may be treated, modulated, or prevented with FGFR ECD polypeptides as well, such as those found in epithelial tissues, including the cervix, esophagus, and lung, for example. Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. By way of example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is a disorderly form of non-neoplastic cell growth, involving losses in individual cell uniformity and in the cell's architectural orientation. Dysplasia characteristically occurs where there exists chronic irritation or inflammation and is often found in the cervix, respiratory passages, oral cavity, and gall bladder. Other examples of benign tumors which can be treated, modulated, or prevented in accordance with the present invention include arteriovenous (AV) malformations, particularly in intracranial sites and myoleomas.

[0161] Since FGFs contribute to normal bone formation and are expressed locally in the bone stromal environment, they may play a role in seeding, growth, and survival of bone metastases. FGFs have been implicated in bone formation, affecting osteoprogenitor cell replication, osteoblast differentiation, and apoptosis. Thus, agents that block FGF/FGFR interactions, including FGFR ECD polypeptides, can be used to treat bone

metastases in cancers such as prostate cancer and breast cancer. Such agents will not only inhibit local osteoblastic conversion events, but also inhibit initial seeding, growth, and survival of the cancer bone metastases.

EXAMPLES

[0162] The examples discussed below are intended to be purely exemplary of the invention and should not be considered to limit the invention in any way. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Construction of Certain FGFR4 ECD-Fc Fusion Molecules.

[0163] The cloning, expression and purification of the R1Mut4 fusion protein used in these examples has been previously described (WO 2007/014123). The cloning of the parental FGFR4 ECD-Fc fusion protein used in these examples has also been described (WO 2007/014123, called "R4Mut4"). For transient expression in 293-6E cells, R4Mut4 was cloned into and expressed from vector pTT5 (Biotechnology Research Institute, Montreal, Canada). Chimeras of the R4Mut4 fusion protein were constructed using PCR and conventional mutagenesis techniques. The R4Mut4 chimeras were originally cloned into the mammalian expression vector pcDNA3.1 (Invitrogen) for transient expression in 293-6E cells.

[0164] The primary sequence and domain structure of the FGFR4 moiety in the parental R4Mut4 construct is shown in **FIG. 1**. The stretch of amino acids between the first and second immunoglobulin (Ig) domains (amino acids 98 to 124) is denoted interchangeably herein as the "linker domain" "linker region," "D1-D2 linker," and "D1-D2 linker region" (see **FIG. 1**). Within the linker domain is a smaller region called the "acid box" ("AB") found within the FGFR family. Three chimeras of the parental R4Mut4 were constructed, in which regions within the R4Mut4 linker domain were replaced with the corresponding sequences from the FGFR1 linker domain. **FIG. 2** shows a sequence alignment of the linker domains from FGFR1 and FGFR4 and the boundaries and sequence of the swapped regions in the three variants, called ABMut1, ABMut2, and

ABMut3 (see **Table 1**), that were constructed. **Table 1** lists the various FGFR-Fc fusion proteins used in these examples with names and brief descriptions.

Table 1. FGFR-Fc Fusion Proteins

Protein Name	SEQ ID #	Brief Description	Short name
FGFR4ECD(delta17)-Fc	14	Parental FGFR4ECD-Fc, which has a 17 amino acid carboxy-terminal deletion from the FGFR4 ECD.	R4Mut4
FGFR4ECD(ABMut1: delta17)-Fc	86	The D1-D2 linker from FGFR1 is swapped into R4Mut4.	ABMut1
FGFR4ECD(ABMut2: delta17)-Fc	87	Exon 4 from FGFR1 is swapped into R4Mut4.	ABMut2
FGFR4ECD(ABMut3: delta17)-Fc	158	An acid box region from FGFR1 is swapped into R4Mut4.	ABMut3
FGFR4ECD(2Ig+Linker)-GS linker-Fc	89	The first Ig domain of R4Mut4 is deleted, but the D1-D2 linker is retained with a GS linker.	R4(2Ig+L)
FGFR4ECD(2Ig-Linker)-GS linker-Fc	90	Both the first Ig domain and the D1-D2 linker are deleted from R4Mut4 with a GS linker.	R4(2Ig-L)
FGFR1ECD(delta14)-Fc	91	FGFR1 ECD-Fc fusion protein with 14 amino acid C-terminal deletion.	R1Mut4
FGFR1ECD-Fc	N/A	Commercially-available FGFR1 ECD-Fc fusion protein	R1Fc
FGFR2ECD-Fc	N/A	Commercially-available FGFR2 ECD-Fc fusion protein	R2Fc
FGFR3ECD-Fc	N/A	Commercially-available FGFR3 ECD-Fc fusion protein	R3Fc
FGFR4ECD-Fc	N/A	Commercially-available FGFR4 ECD-Fc fusion protein	R4Fc
FGFR4ECD(R4Mut4(N104D): delta17)-Fc	130	R4Mut4 with N104D point mutation.	R4Mut4(N104D)
FGFR4ECD(R4Mut4(P109D): delta17)-Fc	131	R4Mut4 with P109D point mutation.	R4Mut4(P109D)
FGFR4ECD(R4Mut4(R113E): delta17)-Fc	132	R4Mut4 with R113E point mutation.	R4Mut4(R113E)
FGFR4ECD(R4Mut4(S116E): delta17)-Fc	133	R4Mut4 with S116E point mutation.	R4Mut4(S116E)
FGFR4ECD(R4Mut4(104-114):FGFR1(106-117); delta 17)-Fc	134	Residues 106 to 117 from FGFR1 swapped into R4Mut4.	R4(104-114):R1(106-117)

FGFR4ECD(R4Mut4(104-114):FGFR1(107-117): delta 17)-Fc	135	Residues 107 to 117 from FGFR1 swapped into R4Mut4.	R4(104-114):R1(107-117)
FGFR4ECD(R4Mut4(104-110):FGFR1(105-113): delta 17)-Fc	136	Residues 105 to 113 from FGFR1 swapped into R4Mut4.	R4(104-110):R1(105-113)
FGFR4ECD(R4Mut4(113-116):FGFR1(116-119): delta 17)-Fc	137	Residues 116 to 119 from FGFR1 swapped into R4Mut4.	R4(113-116):R1(116-119)
FGFR4ECD(R4Mut4(109-113):FGFR1(112-116): delta 17)-Fc	138	Residues 112 to 116 from FGFR1 swapped into R4Mut4.	R4(109-113):R1(112-116)
FGFR4ECD(ABMut1(N91A): delta 17)-Fc	139	ABMut1 with N91A point mutation.	ABMut1(N91A)
FGFR4ECD(ABMut1(N159A): delta 17)-Fc	140	ABMut1 with N159A point mutation.	ABMut1(N159A)
FGFR4ECD(R4Mut4(D1-D2):FGFR2(D1-D2): delta 17)-Fc	143	D1-D2 linker from FGFR2 swapped into R4Mut4.	R4(D1-D2):R2(D1-D2)
FGFR4ECD(R4Mut4(D1-D2):FGFR3(D1-D2): delta 17)-Fc	144	D1-D2 linker from FGFR3 swapped into R4Mut4.	R4(D1-D2):R3(D1-D2)
FGFR2ECD(delta3)-GS linker-Fc	162	Parental FGFR2ECD-Fc, which has a 3 amino acid carboxy-terminal deletion from the FGFR2 ECD and a GS linker.	FGFR2-Fc
FGFR3ECD(delta3)-GS linker-Fc	163	Parental FGFR3ECD-Fc, which has a 3 amino acid carboxy-terminal deletion from the FGFR3 ECD and a GS linker.	FGFR3-Fc
FGFR2ECD(FGFR2(111-118):FGFR1(105-112): delta3)-GS linker-Fc	166	Residues 105-112 from FGFR1 swapped into FGFR2-Fc.	R2(111-118):R1(105-112)
FGFR3ECD(FGFR3(110-117):FGFR1(105-112): delta3)-GS linker-Fc	167	Residues 105-112 from FGFR1 swapped into FGFR3-Fc.	R3(110-117):R1(105-112)

[0165] CHO-S host cells can give higher yields and/or different glycosylation patterns for recombinant proteins when compared to 293-6E host cells. For expression of the fusion proteins in CHO-S host cells, we used the pTT5 and pDEF38 (ICOS Corporation, Bothell, WA) vectors. R4Mut4 and the FGFR4 ECD-Fc acidic region muteins were subcloned into the pTT5 and pDEF38 vectors using PCR and conventional subcloning techniques.

[0166] DG44 (Invitrogen, Carlsbad, CA) is a derivative cell line of the CHO-S cell line that we have found can give higher yields of recombinant proteins. For expression of the fusion proteins in DG44 host cells, we used the vector pDEF38.

Example 2: Transient Expression of Fusion Proteins in 293-6E and CHO-S Host Cells.

[0167] In certain Examples herein, fusion protein was transiently expressed in 293-6E cells. The R4Mut4/pTT5 expression vector described in Example 1 was designed to provide transient expression in 293-6E host cells. The 293-6E host cells used for expression were previously adapted to serum-free suspension culture in Free-Style medium (Invitrogen). The cells were transfected with the expression vector while in logarithmic growth phase (log phase growth) at a cell density of between 9 x 10^5/ml and 1.2 x 10^6/ml.

[0168] In order to transfect 500 ml of 293-6E cell suspension, a transfection mixture was made by mixing 500 micrograms (ug) of the expression vector DNA in 25 ml of sterile phosphate buffered saline (PBS) with 1 mg of polyethylenimine (from a 1 mg/ml solution in sterile water) in 25 ml of sterile PBS. This transfection mixture was incubated for 15 min at room temperature. Following incubation, the transfection mixture was added to the 293-6E cells in log phase growth for transfection. The cells and the transfection mixture were then incubated at 37°C in 5% CO_2 for 24 hours. Following incubation, Trypton-N1 (Organotechnie S.A., La Courneuve, France; 20% solution in sterile FreeStyle medium) was added to a final concentration of 0.5% (v/v). The mixture was maintained at 37°C and 5% CO_2 for about 6 – 8 days until the cells reached a density of about 3 – 4 x 10^6 cells/ml and showed a viability of >80%. To harvest the fusion protein from the cell culture medium, cells were pelleted at 400 x g for 15 min at 4°C and the supernatant was decanted. The supernatant was cleared of cell debris by centrifugation at 3,315 x g for 15 min at 4°C. The cleared supernatant containing the fusion protein was then submitted for purification.

[0169] To provide small batches (1 – 2 mg) of R4Mut4 for *in vivo* study in a short period of time, transient production from suspension CHO-S host cells was carried out using the plasmid construct R4Mut4/pDEF38. Briefly, suspension CHO-S cells (Invitrogen) were cultured in Freestyle CHO expression medium supplemented with L-Glutamine (Invitrogen). The day before transfection, the CHO-S cells were seeded into a shaker flask at a density of about 5x10^5/ml, which then reached a density of about 1x10^6/ml on the day of transfection. In order to transfect 125 ml of cell suspension,

156.25 ug of the expression vector DNA was mixed with 2.5 ml of OptiPro serum free medium. 156.25 ul of FreestyleMax transfection reagent (Invitrogen) was separately mixed with 2.5 ml of OptiPro serum free medium. The transfection mixture was made by combining the DNA/OptiPro medium mixture and the FreestyleMax/Optipro medium mixture for 10 min at room temperature. Following incubation, the transfection mixture was added to the CHO-S cells. The cells and the transfection mixture were then incubated at 37°C in 5% CO_2 for 6 days. Following incubation, the cell density was about 3.3-3.7 $x10^6$/ml with a viability of about 82-88%. The supernatant from the culture was separated from the cells by centrifugation and collected for purification. Using this method, 1 mg of R4Mut4 can be produced from 400 ml of transiently transfected cell culture in about 1 week.

[0170] When indicated below, the R4Mut4 variants ABMut1, ABMut2, and ABMut3 were similarly produced by transient expression in CHO-S cells using the pDEF38 expression vectors described in **Example** 1.

Example 3: Purification of Expressed Proteins.

[0171] FGFR ECD-Fc fusion proteins expressed from recombinant host cells were purified from the cell culture supernatant using a first purification step of Protein-A affinity chromatography, followed by a second purification step of butyl hydrophobic interaction chromatography. For the Protein-A affinity chromatography step, the components of the media were separated on a Mabselect Protein-A Sepharose column (GE Healthcare Bio-Sciences, Piscataway, NJ), which will bind to the Fc region of the fusion molecule. The column was equilibrated with ten column volumes of a sterile buffer of 10 mM Tris, 100 mM NaCl, pH 8.0; then the cell culture supernatant was applied to the column. The column was washed with eight column volumes of sterile 10 mM Tris, 100 mM NaCl buffer, pH 8.0. The bound material, including R4Mut4, was then eluted at a rate of 10 ml/min with a one step elution using seven column volumes of elution buffer (100 mM glycine, 100 mM NaCl, pH 2.7). Ten ml fractions were collected in tubes containing one ml 1 M Tris pH 8.0 (Ambion, Austin, TX) to neutralize the eluate. Fractions comprising R4Mut4 were identified by gel electrophoresis and pooled.

[0172] For the second purification step of butyl hydrophobic interaction chromatography, pooled Protein-A column eluates were further purification on a butyl Sepharose column using a GE Healthcare Akta Purifier 100 (GE Healthcare Bio-Sciences, Piscataway, NJ). The column was first equilibrated with five column volumes of sterile

10 mM Tris, 1 M ammonium sulfate, pH 8.0. A half volume of 3 M ammonium sulfate was then added to the eluate, which was then applied to the equilibrated butyl Sepharose column. The column was washed with four column volumes of the equilibration buffer and the bound material was eluted at a rate of five ml/min with a linear gradient starting at 50% equilibration buffer / 50% elution buffer (10 mM Tris pH 8.0) and ending at 90% elution buffer / 10% equilibration buffer over a total volume of 20 column volumes. Finally, an additional two column volumes of 100% elution buffer was used. Fourteen ml fractions were collected. R4Mut4 was eluted with approximately 40-60% elution buffer. The fractions containing the bulk of the R4Mut4 were identified by gel electrophoresis and pooled.

[0173] After purification, endotoxin levels were checked by the limulus amoebocyte lysate (LAL) assay (Cambrex, Walkersville, MD). Endotoxin levels were confirmed to be less than or equal to 1 endotoxin unit (EU) per mg of R4Mut4.

Example 4: Stable Production in DG44 Cells.

[0174] The expression vector R4Mut4/pDEF38, described in Example 1, was used to transfect DG44 host cells for stable production of R4Mut4. The untransfected DHFR-negative CHO cell line, DG44, was cultured in CHO-CD serum free medium (Irvine Scientific, Irvine, CA) supplemented with 8 mM L-Glutamine, 1x Hypoxanthine/Thymidine (HT; Invitrogen), and 18 ml/L of Pluronic-68 (Invitrogen). About 50 ug of R4Mut4/pDEF38 plasmid DNA was linearized by digestion with restriction enzyme PvuI, then precipitated by addition of ethanol, briefly air-dried, and then resuspended in 400 ul of sterile, distilled water. The DG44 cells were seeded into a shaker flask at a density of about $4x10^5$/ml the day before transfection, and reached a density of about $0.8x10^6$/ml on the day of transfection. The cells were harvested by centrifugation and about $1x10^7$ cells were used per transfection.

[0175] For transfection, each cell pellet was resuspended in 0.1 ml of Nucleofector V solution and transferred to an Amaxa Nucleofector cuvette (Amaxa, Cologne, Germany). About 5 ug of the resuspended linearized plasmid DNA was added and mixed with the suspended DG44 cells in the cuvette. Cells were then electroporated with an Amaxa Nucleofector Device II using program U-024. Electroporated cells were cultured in CHO-CD medium for two days and then transferred into selective medium (CHO-CD serum free medium supplemented with 8 mM L-Glutamine and 18 ml/L Pluronic-68). The selective medium was changed once every week. After about 12 days, 1 ug/ml R3

Long IGF I growth factor (Sigma, St. Louis, MO) was added to the medium and the culture was continued for another week until confluent. The supernatants from pools of stably transfected cell lines were assayed by a sandwich R4Mut4 ELISA to determine the product titer. This transfection method generated an expression level of about 30 ug/ml of R4Mut4 from the pools of stably transfected cells.

Example 5. Binding to Hepatocytes *In Vitro*.

[0176] Preliminary experiments demonstrated that R4Mut4 had antitumor properties in a xenograft model yet exhibited a very fast initial serum concentration decline when injected into the tail vein of the mouse. (Data not shown.) Follow-up experiments demonstrated that, unlike the FGFR1 ECD fusion protein R1Mut4, R4Mut4 bound in a concentration-dependent manner to the extracellular matrix Matrigel *in vitro*. *See, e.g.,* **FIG. 6** and **Example 9**. To ascertain whether the R4Mut4 was binding to liver *in vivo*, further *in vitro* binding experiments were conducted to determine whether R4Mut4 and R1Mut4 bound to hepatocytes and also to determine the heparin-sensitivity of that binding.

[0177] R4Mut4 and R1Mut4 were expressed and purified from CHO-S cells as described in **Examples 2 and 3**. For this experiment, three different batches of R4Mut4 were tested; each had been expressed and purified independently. Human IgG1 control protein was obtained from Caltag (now part of Invitrogen).

[0178] Hepatocytes were isolated from adult rats. Rats were anesthetized with isoflurane and the animals were kept as close to 37°C as possible with a heating element. A midline incision was made and the organs were removed from the cavity to access the portal vein. The portal vein was cannulated with a butterfly catheter secured with a bulldog clamp. The pump was started at 8 ml/min with Hanks Balanced Salt Solution without Ca^{2+} or Mg^{2+}, with 10 mM Hepes, 0.5 mM EGTA, 50 ug/ml gentamicin, pH 7.38. The inferior vena cava (IVC) was then cut about 2 cm below the liver and an exit point was created by cutting the heart. The flow was adjusted to 40 ml/min and once clear signs of perfusion were observed, the IVC was clamped between the liver and the posterior cut of the IVC. After 4 minutes, Liver Digest Media (Invitrogen) was added to the perfusion line. Both solutions were perfused together for 30 seconds, and then the Hanks Balanced Salt Solution was stopped. The flow rate of the Liver Digest Media was then decreased to 20 ml/min and the perfusion continued for approximately 10 minutes. The liver was excised and small slits in the capsule of each lobe were made. The liver was placed on

sterile gauze affixed to the top of a beaker and gently rolled around while continuously rinsing the hepatocytes into the beaker using Liver Digest Media.

[0179] The media containing the hepatocytes was poured into 50 ml conical tubes and centrifuged for approximately 2 minutes at 400 rpm. The media was aspirated off and 25 ml of Culture Media (Williams Medium E (Sigma), 100 IU/ml of penicillin (Cellgro), 100 ug/ml of streptomycin (Cellgro), 1X ITS (Insulin, Transferrin and Sodium Selenite, from Sigma) and 10% FBS (Cellgro)) was added to half of the tubes and the cells were resuspended. The resuspended cells were then decanted into the remaining tubes and the volume of Culture Media was brought to 50 ml in each tube. The tubes were centrifuged again as above and the decanting step was repeated. The cells were then resuspended in ice-cold staining buffer (Ca^{2+} and Mg^{2+} free PBS (Invitrogen) supplemented with 1% bovine serum albumin (BSA; w/v) and 0.1% NaN_3 (w/v), both from Sigma) at a concentration of 500,000 cells per ml. The hepatocytes were then incubated with 5 ug/ml of R1Mut4, R4Mut4, or control human IgG1 for 30 minutes. In samples where heparin was present, the R1Mut4, R4Mut4, or control human IgG1 was pre-incubated with a 10-fold molar excess of sodium heparin (from porcine intestinal mucosa, Catalog # 086K2231, Sigma) before mixing with the hepatocytes.

[0180] Following incubation with the fusion proteins or control IgG1, with or without heparin, the cells were washed in staining buffer and incubated with 5 ug/ml biotinylated goat anti- human Fc (Becton Dickinson) on ice for 30 min. The cells were then washed in staining buffer and incubated with 1 ug/ml streptavidin-APC conjugate (Becton Dickinson) on ice for 30 min. The cells were again washed with staining buffer and then stained with 0.5 ug/ml propidium iodide (Sigma). Non-viable cells that had absorbed the propidium iodide were gated out such that only viable cells were included in the flow cytometry analysis. The viable cells were quantitated for APC fluorescence. As shown in **FIG. 3**, R4Mut4 showed 10-fold more binding to rat hepatocytes than either control IgG1 or R1Mut4 in that experiment. In addition, all three batches of R4Mut4 bound the rat hepatocytes with similar affinity, indicating that the high affinity of R4Mut4 for rat hepatocytes was not due to unusual expression or purification conditions. Finally, the addition of heparin prevented R4Mut4 binding to hepatocytes in that experiment.

Example 6. Heparin Increases the Cmax of R4Mut4 in Plasma *In Vivo*.

[0181] The experiment discussed in Example 5 demonstrated that R4Mut4 bound to hepatocytes and that heparin could interfere with that binding. To test whether heparin

could increase in the amount of R4Mut4 present in the serum *in vivo*, R4Mut4 was administered to mice with and without exogenous heparin, and the amount of R4Mut4 in the serum was analyzed by ELISA at different time points following injection. The R4Mut4 injected in the experiment was expressed and purified from CHO-S cells as described in Examples 2 and 3.

[0182] Thirty male Balb/C mice (Jackson Laboratories, Bar Harbor, ME) were weighed and sorted into two groups of 15 mice each based on a random distribution of body weight. Mice in the first group were given an intravenous injection (via tail vein) of 3 mg/kg R4Mut4 in a total volume of 0.5 ml PBS (Cellgro, Herndon, VA). Mice in the second group were given an intravenous injection (via tail vein) of 3 mg/kg R4Mut4 that had been premixed with 5 mg/kg sodium heparin (from porcine intestinal mucosa, Sigma) for 30 minutes-2 hours before IV dosing.

[0183] Blood from the mice was collected at 6 different time points after IV administration, at approximately 2 minutes, 2 hours, 8 hours, 1 day, 4 days, and 6 days. Blood was collected from each mouse only twice, the first by a retro-orbital bleed and the second terminally by cardiac puncture. The 15 mice from each IV group were further divided into three subgroups of 5 mice. The first subgroup was used to collect blood at 2 minutes and 2 hours. The second subgroup was used to collect blood at 24 hours and 4 days. The third subgroup was used to collect blood at 8 hours and 6 days.

[0184] The retro-orbital bleeds were collected through heparin-coated capillary tubes (Fisher Scientific, Pittsburgh, PA) into K_2-EDTA coated tubes (BD Biosciences; San Jose, CA), placed on wet ice for approximately 30 minutes and then spun at 10,621 x g (10,000 rpm) in a microfuge for 8 minutes. The second, terminal bleed, was collected through uncoated syringes into K_2-EDTA coated tubes (BD Biosciences) and then processed as above. Following centrifugation, the plasma was removed and frozen at -80°C until analyzed by ELISA.

[0185] For detection of R4Mut4 in plasma samples, a direct ELISA for FGF2 binding activity was used. Briefly, Maxisorp 96-well plates (Nunc, Rochester, NY) were coated with recombinant human FGF2 (PeproTech, Rocky Hill, NJ) in PBS (Mediatech, Herndon, VA) at 1 ug/ml overnight at 4°C. The plates were then blocked with blocking buffer (1% BSA (Sigma) in PBS) for 2-3 hours at room temperature. The plates were washed 6 times with wash buffer (0.05% Tween-20 (v/v; Sigma) in PBS). Various dilutions of the test samples and R4Mut4 standards were made with a constant final

concentration of 5% plasma in each sample. 100 ul of test sample was added to each well and then incubated for approximately 90 minutes at room temperature. The plates were washed 6 times with wash buffer, and then a peroxidase-conjugated AffiPure goat anti-human IgG-Fc antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted at 1:60,000 in assay diluent (1% BSA and 0.05% Tween-20 in PBS) was added to each well and incubated for approximately 1 hour at room temperature. The plates were washed 6 times with wash buffer. 100 ul per well of TMB substrate (Pierce Biotechnology, Chicago, IL) was then added and incubated for 10 minutes. The reactions were quenched with 50 ul stop solution (2 N H_2SO_4). The absorbance at 450 nm was then read on a SPECTRAmax PLUS microplate reader (Molecular Devices, Sunnyvale, CA).

[0186] Results for all six time points from that experiment are shown in **Table 2** and the first 4 time points are shown graphically in **FIG. 4**.

Table 2. R4Mut4 Serum Levels

	Average R4Mut4 serum plasma concentration +/- standard deviation (ng/ml)	
Time point	No Heparin	With Heparin
2 minutes	5247 +/- 1367	44404 +/-7362
2 hours	1329 +/- 236	6516 +/- 1293
8 hours	628 +/- 138	592 +/- 109
1 day	204 +/- 45	273 +/- 27
4 day	Below limit of detection (156 ng/ml)	Below limit of detection (156 ng/ml)
6 day	Below limit of detection (156 ng/ml)	Below limit of detection (156 ng/ml)

[0187] Those results demonstrate that co-administration of heparin with R4Mut4 increased the maximum R4Mut4 concentration in the plasma (C_{max}) by approximately 9-fold over R4Mut4 administered alone at the 2 min time point and approximately 7-fold at the 2 hour time point in that experiment. The two curves merge at the 8 hour time point. These data show that heparin increases the amount of R4Mut4 that is free in the serum but that the protection was lost by 8 hours in that experiment. Previous reports have shown that heparin has a short half-life *in vivo* (~45-60 minutes; see Bjornsson and Levy, J. Pharmacol. Exp. Ther. (1979) *210*:243-246) and this may play a role in the duration of the protective effect of heparin in that experiment.

Example 7. FGFR ECD-Fc Binding to Extracellular Matrix Components *In Vitro*.

[0188] *In vitro* binding studies were conducted to characterize the ability of each of the four FGFR ECD-Fc fusion proteins to bind to extracellular matrix components (ECM). For this study, wild-type FGFR1 ECD-Fc, FGFR2 ECD-Fc, FGFR3 ECD-Fc and FGFR4 ECD-Fc fusion proteins were purchased from R&D Systems (Minneapolis, MN).

[0189] 96-well Matrigel plates (Becton-Dickinson) were blocked with a 1% (w/v) solution of BSA (Sigma). The FGFR ECD-Fc fusion proteins were serially diluted, transferred to the Matrigel plates, and allowed to bind for 1 hour at room temperature. Unbound fusion proteins were removed by washing three times with PBS (EMD Biosciences; La Jolla, CA) and 0.5% Tween (v/v; Sigma). Bound fusion proteins were detected using a peroxidase-conjugated anti-human Fc antibody (Bethel Laboratories, Montgomery, TX) and OPD (o-phenylenediamine dihydrochloride) substrate (Sigma), according to the manufacturer's instructions. FGFR4 ECD-Fc bound tightly to the Matrigel plates with an EC_{50} of approximately 300 ng/mL in that experiment. *See* **FIG. 5**. FGFR1 ECD-Fc, FGFR2 ECD-Fc, and FGFR3 ECD-Fc all showed significantly weaker binding to the Matrigel plates than the FGFR4 ECD-Fc, with half-maximal binding not observed in that experiment even at concentrations of 10,000 ng/ml. *See* **FIG. 5**.

Example 8. FGFR4 ECD Acidic Region Chimera-Fc Fusion Proteins Bind to FGF2 and FGF19.

[0190] In order to reduce tissue binding and improve the pharmacokinetic profile, three FGFR4 ECD acidic region chimeras were fused to Fc, expressed in 293-6E cells, and purified. In addition, two FGFR4 2Ig ECD-Fc fusion proteins in which D1 or D1 + the acid box region were deleted, were constructed, expressed, and purified.

[0191] The FGF2 and FGF19 ligand binding affinity and kinetics of the parental R4Mut4 and the five different FGFR4 ECD-Fc fusion proteins (collectively "the R4 proteins") were determined using Biacore® X surface plasmon resonance (SPR) technology (Uppsala, Sweden). FGF2 was selected because it is broadly expressed in adult tissue and has been implicated in cancer progression and angiogenesis. FGF19 was selected because, in the absence of other protein cofactors, it binds specifically to FGFR4. Briefly, Protein-A was covalently linked to a CM5 chip, according to manufacturer's instructions. The R4 proteins were produced in 293-6E host cells as described in Example 2, purified as described in Example 3, and then bound to the chip by interaction of the Fc domain with Protein-A. The R4 proteins were captured onto flow channels 2-4, while

channel 1 served as a reference. FGF2 was purchased from Peprotech (Rocky Hill, NJ) and FGF19 was purchased from R&D Systems. Each FGF ligand was injected at 5 concentrations (100 nM, 25 nM, 6.25 nM, 1.56 nM, and 0 nM) for 2 minutes and dissociation was monitored for 4 minutes. 50 uM Heparin was included in the running buffer. The association constant, dissociation constant, affinity, and binding capacity of each of the R4 proteins for FGF2 and FGF19 was calculated using the Biacore T100 Evaluation software package using the 1:1 binding model.

[0192] The results of that experiment are shown in **Tables 3 and 4.**

Table 3. FGF2 Ligand Binding

Protein Name	**k_a (1/M·ms)**	**k_d (1/s)·1000**	**K_D (nM)**	**R_{max} (RU)**
R4Mut4 (experiment 1)	59	0.23	3.90	46
R4Mut4 (experiment 2)	45	0.27	5.88	53
ABMut1	160	0.27	1.70	56
ABMut2	114	0.26	2.26	57
ABMut3	242	0.35	1.44	58
R4(2Ig+L)	313	0.79	2.54	62
R4(2Ig-L)	306	0.73	2.40	51

Table 4. FGF19 Ligand Binding

Protein Name	**k_a (1/M·ms)**	**k_d (1/s)·1000**	**K_D (nM)**	**R_{max} (RU)**
R4Mut4 (experiment 1)	176	0.63	3.60	55
R4Mut4 (experiment 2)	184	0.61	3.32	50
ABMut1	213	0.68	3.18	45
ABMut2	250	0.64	2.58	44
ABMut3	211	0.74	3.50	40
R4(2Ig+L)	80	2.76	34.31	26
R4(2Ig-L)	118	2.14	18.15	18

[0193] As shown in **Tables 3 and 4,** the three chimeras ABMut1, ABMut2, and ABMut3, had an affinity equal to or greater than the parental R4Mut4 for both FGF2 and FGF19 in that experiment, as measured by the equilibrium dissociation constant (K_D).

[0194] In addition, FGFR4 ECD-Fc fusion proteins in which D1 was deleted, in either the presence (R4(2Ig+L)) or absence (R4(2Ig-L)) of the D1-D2 linker region, bound

FGF2 with an affinity equal to or greater than the parental R4Mut4 in that experiment, as measured by the equilibrium dissociation constant (K_D). Deletion of D1 reduced binding to FGF19 by approximately ten-fold in the presence of the D1-D2 linker region (R4(2Ig+L)), and by approximately five-fold in the absence of the D1-D2 linker region (R4(2Ig-L)) in that experiment.

[0195] Those results show that all of the R4 proteins tested retained the ability to bind to FGF2 and/or FGF19, although the D1 deletion proteins exhibited weaker binding to FGF19 than the parental or the acidic region chimeras in that experiment.

Example 9. FGFR4 ECD Acidic Region Chimera-Fc Fusion Protein Binding to Extracellular Matrix (ECM) Components *In Vitro*.

[0196] *In vitro* binding studies were conducted to characterize the ability of the FGFR4 ECD acidic region chimeras to bind to ECM. FGFR4-Fc acidic region chimera-Fc fusion proteins were expressed in 293-6E cells, as described in Example 2, and all were purified as described in Example 3. The parental R4Mut4 was expressed in CHO cells as described in Example 2 and also purified as described in Example 3.

[0197] Binding experiments were performed as described in Example 7, and a graphical representation of the results is shown in **FIG. 6.** In this experiment, the parental R4Mut4 bound to Matrigel plates with an EC_{50} of approximately 100 ng/ml. All three FGFR ECD acidic region chimera-Fc fusion proteins showed minimal binding to Matrigel plates up to a concentration of 10,000 ng/ml in that experiment. Therefore, the substitution of the FGFR1 D1-D2 linker, FGFR1 exon 4, or FGFR1 acid box region for the corresponding region of the FGFR4 ECD abrogated the *in vitro* extracellular matrix binding of the FGFR4 ECD in that experiment.

Example 10. FGFR4 ECD Acidic Region Chimera-Fc Fusion Protein Binding to Hepatocytes *In Vitro*.

[0198] FGFR4 ECD acidic region chimera-Fc fusion proteins ABMut1, ABMut2 and ABMut3 were tested for their ability to bind hepatocytes. The fusion proteins were expressed in 293-6E cells, as described in Example 2, and purified as described in Example 3. The R1Mut4 and R4Mut4 proteins were expressed in CHO-S cells, as described in Example 2, and purified as described in Example 3. Human IgG1 control protein was obtained from Caltag.

[0199] The hepatocyte binding experiments were performed as described in Example 5 and a graphical representation of the results is shown in **FIG. 7.** In that experiment, all three FGFR4 ECD acidic region chimera-Fc fusion proteins showed

hepatocyte binding that was equivalent to R1Mut4, and was greatly reduced compared to the parental R4Mut4.

Example 11. Pharmacokinetics of an FGFR4 ECD Acidic Region Chimera-Fc Fusion Protein in Mice.

[0200] The pharmacokinetic properties of ABMut1 were compared to the parental R4Mut4 *in vivo*. Both proteins were expressed in CHO-S cells as described in Example 2 and purified as described in Example 3.

[0201] Forty female Balb/C mice (Charles River Laboratories, Wilmington, MA) were weighed and sorted into two groups of 20 mice based on a random distribution of body weight. Mice in the first group received R4Mut4 and mice in the second group received ABMut1. Each mouse received 5 mg protein per kilogram body weight via intravenous injection through the tail vein in a total volume of 0.2 ml PBS (Cellgro).

[0202] Blood plasma was collected at 9 different time points following IV administration, at approximately 2 minutes, 30 minutes, 2 hours, 7 hours, 1 day, 2 days, 3 days, 5 days, and 7 days. Blood was collected from each mouse two or three times, the first one or two collections by a retro-orbital bleed and the last terminally by cardiac puncture. The 20 mice from each treatment group were further divided into four subgroups of 5 mice. Each group was bled at the times shown in **Table 5**.

Table 5. Sample Collection Times and Methods

	Time points of blood collection (following IV administration)	
Group (n = 5)	Retro-orbital bleed	Terminal cardiac puncture
Group 1	2 and 5 minutes	7 days
Group 2	30 minutes	7 hours
Group 3	2 hours	24 hours
Group 4	2 days	3 days

[0203] Plasma serum was collected and the R4Mut4 and ABMut1 protein levels determined by a direct FGF2 binding ELISA as described in Example 7. The results are shown in **FIG. 8** and certain pharmacokinetic parameters are given in **Table 6**. The results show that the replacement of the D1-D2 linker of the FGFR4 ECD with the FGFR1 D1-D2 linker increased the half-life ($t_{1/2}$) of the administered protein by 126%, increased the maximum observed plasma concentration (C_{max}) by 183%, and increased the clearance time (CL) by 302%. The improved pharmacokinetic profile of ABMut1 shows that it is

present in the blood, at a therapeutic concentration, longer than the parental R4Mut4 molecule.

Table 6. Pharmacokinetic Parameters of R4Mut4 and ABMut1

Protein	$t_{1/2}$ (in hours)	C_{max} (in ug/ml)	CL (in ml/hr/kg)
R4Mut4	15.7	18.7	23.6
ABMut1	35.5	52.9	94.8
Change (%)	126%	183%	302%

Example 12. Activity in HCT116sc Cancer Xenograft Model.

The anti-cancer activities of the parental R4Mut4 and ABMut1 were tested in a xenograft colon cancer model using human colon carcinoma HCT116sc cells. The HCT116sc cell line is a sub-line of the HCT116 colon carcinoma cell line (ATCC, Manassas, VA) isolated from a subcutaneous HCT116 tumor and selected for more consistent *in vivo* growth using standard cell culture and xenograft techniques. To prepare the HCT116sc cells for the xenograft experiment, the cells were cultured for five passages in RPMI 1640 media supplemented with 10% FBS (vol/vol), 2 mM L-Glutamine, 100 IU/ml of penicillin and 100 ug/ml of streptomycin (all from Cellgro) at 37°C in a humidified atmosphere with 5% CO_2. Semi-confluent cells (~80%) were re-suspended in PBS without calcium and magnesium (Cellgro) at a concentration of $1x10^8$ cells per ml. Matrigel basement membrane matrix (BD Biosciences) was added to 50% (vol/vol) to give a final concentration of $5x10^7$ cells per ml and the mixture stored on ice until implantation into mice.

[0204] For the xenograft experiments, sixty CB17 SCID mice (Charles River Laboratories) were used. On day 1, the body weight of each mouse was measured. The mice were randomly distributed into 6 groups of 10 mice based on their body weight. Once assigned to a treatment group, the mice were shaved on the right hind flank and then inoculated subcutaneously with 5×10^6 (100 ul) of the HCT116sc cells prepared as described above.

[0205] On the next day, animals were dosed with the test articles according to the dosing scheme shown in **Table 7**. R4Mut4 and ABMut1 were expressed in CHO-S cells and purified as described in **Examples 2 and 3**, respectively.

Table 7. HCT116sc Xenograft Dosing Groups

Group	Number of Animals	Test Article and Dose (mg test article per weight mouse)	Dosing Route and Schedule
1	10	Vehicle	Intravenous, 2X/week
2	10	R4Mut4, 20 mg/kg	Intraperitoneal, daily
3	10	R4Mut4, 10 mg/kg	Intravenous, 2X/week
4	10	R4Mut4, 20 mg/kg	Intravenous, 2X/week
5	10	ABMut1, 10 mg/kg	Intravenous, 2X/week
6	10	ABMut1, 20 mg/kg	Intravenous, 2X/week

[0206] Tumor sizes were measured in each mouse on days 7, 14, and 21 following the day of tumor cell inoculation. The length and width of each tumor was measured using calipers and the tumor size calculated according to the formula:

$$\text{Tumor size } (mm^3) = \text{width}^2 \text{ (mm)} \times \text{length (mm)} \times (\pi/6)$$

[0207] **FIG. 9** shows the results of that experiment. All groups of mice that received R4Mut4 or ABMut1 showed a diminution of tumor growth compared to vehicle-treated animals. Table 8 shows the average percent inhibition of tumor growth for each treatment group at days 14 and 21 compared to the vehicle treated group, and the corresponding p-values. P-values were calculated using an ANOVA analysis followed by the Bonferonni t-test. *See, e.g.*, Mathematical Statistics and Data Analysis, 1988, Wadsworth & Brooks, Pacific Grove, CA. This analysis demonstrated that ABMut1 reduced tumor growth to a similar or greater extent than the parental R4Mut4 in that experiment.

Table 8. HCT116sc Xenograft Results

Group	Day 14: Percent inhibition; p-value	Day 21: Percent inhibition; p-value
R4Mut4, 20 mg/kg, IP	43%, p-value = 0.003	33%, p-value = 0.014
R4Mut4, 10 mg/kg, IV	42%; p-value = 0.003	27%; p-value = 0.068
R4Mut4, 20 mg/kg, IV	37%; p-value = 0.011	21%; p-value = 0.083

ABMut1, 10 mg/kg, IV	42%; p-value = 0.003	29%; p-value = 0.015
ABMut1, 20 mg/kg, IV	52%; p-value = 0.002	40%; p-value = 0.003

Example 13. Increasing the Concentration of an FGFR4 ECD Acidic Region Chimera Leads to Detectable ECM Binding *In Vitro*.

[0208] As described in **Example 9** and shown in **FIG. 6**, early *in vitro* binding experiments showed minimal binding of three FGFR4 ECD acidic region chimera-Fc fusion proteins (ABMut1, ABMut2, and ABMut3) to ECM components when up to 10,000 ng/ml of purified protein was incubated with Matrigel plates. Experiments were carried out to determine whether higher concentrations (up to 1 mg/ml) of the ABMut1 fusion protein could exhibit increased levels of ECM binding in the same *in vitro* ECM binding assay.

[0209] In these experiments, the R1Mut4, R4Mut4, and ABMut1 fusion proteins were used. The R1Mut4 fusion protein served as a negative control for ECM binding (data not shown), and the R4Mut4 fusion protein served as a positive control for ECM binding. All three fusion proteins were expressed in CHO cells as described in **Example 2** and purified as described in **Example 3**. The R4Mut4 and ABMut1 fusion proteins were also transiently expressed in 293-T cells. For transient expression in 293-T cells, 0.5-0.65 x 10^6 cells were plated in each well of a 6-well plate (with or without poly-lysine coating) in 2 ml DMEM supplemented with 10% FBS. A Fugene™ (Roche) stock was made by combining 93.5 ul Optimem with 6.5 ul Fugene™, followed by a 5 min incubation. A DNA stock was made by combining 1.3 ug DNA with Optimem to a final volume of 100 ul. The Fugene™ stock (100 ul) was added to the DNA stock (100 ul), and the combined solution (200 ul) was added to one well of the 6-well plate. The solution was gently swirled and allowed to incubate with the cells for 30 min at room temperature. The cells were incubated in a humidified incubator with 5% CO_2. After 40 hours, the medium was removed, the cells were washed, and 1.5 ml Optimem was added to each well. Forty-nine hours after the medium was changed, the supernatant was collected, spun at 1,400 rpm for 10 min, and transferred to a fresh tube. The fusion proteins were purified from the culture medium as described in **Example 3**, except that only the first purification step of Protein-A affinity chromatography was used. Protein levels were determined using AlphaScreen (hu IgG AlphaLISA; Perkin-Elmer #AL205C). ECM binding experiments were carried out as described in **Example 7**, except that up to 1 mg/ml of each FGFR ECD-Fc fusion protein was used in the *in vitro* ECM binding assay. A graphical representation of the

results is shown in **FIG. 12**. As shown in **FIG. 12**, detectable levels of ECM binding were observed for the ABMut1 fusion protein at higher concentrations, although ECM binding by the ABMut1 fusion protein was still much lower than that of the R4Mut4 fusion protein.

Example 14. Replacement of Certain Individual Non-Acidic Residues in the FGFR4 ECD Long Acid Box with the Corresponding Acidic Residues from FGFR1 is Not Sufficient to Inhibit ECM Binding *In Vitro*.

[0210] Experiments were carried out to determine whether the replacement of individual non-acidic residues in the FGFR4 ECD long acid box with the corresponding acidic residues from FGFR1 could inhibit ECM binding *in vitro*. This experiment used four FGFR4 ECD long acid box variants in which a single non-acidic residue from the FGFR4 ECD of R4Mut4 was replaced with the corresponding acidic residue from FGFR1. Conventional cloning and site-directed mutagenesis methods were employed to generate clones in the pTT5 vector encoding the R4Mut4(N104D), R4Mut4(P109D), R4Mut4(R113E), and R4Mut4(S116E) fusion proteins. The R4Mut4(N104D), R4Mut4(P109D), R4Mut4(R113E), and R4Mut4(S116E) long acid box variants correspond to SEQ ID NOs: 130, 131, 132, and 133, respectively. The R4Mut4(N104D), R4Mut4(P109D), R4Mut4(R113E), and R4Mut4(S116E) variants each contained a single amino acid change at amino acids 104, 109, 113, and 116, respectively, in SEQ ID NOs: 1 and 2. *In vitro* ECM binding of the four FGFR4 ECD long acid box variants with single amino acid substitutions was compared to the R1Mut4, R4Mut4, and ABMut1 fusion proteins.

[0211] All of the fusion proteins, including R1Mut4, R4Mut4, ABMut1, R4Mut4(N104D), R4Mut4(P109D), R4Mut4(R113E), and R4Mut4(S116E) were transiently expressed in 293-T cells and purified as described in **Example 1**. Protein levels were determined using AlphaScreen (hu IgG AlphaLISA; Perkin-Elmer #AL205C). The concentrations of purified R4Mut4(N104D) and R4Mut4(S116E) were too low to reliably determine ECM binding. The concentrations of the purified R4Mut4(P109D) and R4Mut4(R113E) fusion proteins were lower than the concentrations of the purified R1Mut4, R4Mut4, and ABMut1 fusion proteins, and did not permit an analysis of their ECM binding at the highest concentrations. ECM binding experiments were carried out as described in **Example 7**, except that higher protein levels were used for most of the fusion proteins tested in the *in vitro* ECM binding assay. A graphical representation of the results is shown in **FIG. 13**. As shown in **FIG. 13**, the R4Mut4 variants with single amino acid

substitutions that were expressed at sufficient levels to determine ECM binding (i.e., the R4Mut4(P109D) and R4Mut4(R113E)) did not exhibit decreased ECM binding relative to R4Mut4.

Example 15. FGFR4 ECD Long Acid Box Variants that Contain at Least Two Additional Acidic Residues Exhibit Decreased ECM Binding.

[0212] Experiments described in **Example 14** showed that increasing the total number of acidic amino acid residues in the long acid box of an FGFR4 ECD acidic region mutein by one was not sufficient to inhibit ECM binding *in vitro*. Thus, experiments were carried out to determine whether a further increase in the number of acidic amino acid residues in the long acid box of an FGFR4 ECD acidic region mutein, including any acidic amino acid residues inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2, could inhibit ECM binding *in vitro*.

[0213] Five FGFR4 ECD long acid box variant fusion molecules, called R4(104-114):R1(106-117), R4(104-114):R1(107-117), R4(104-110):R1(105-113), R4(113-116):R1(116-119), and R4(109-113):R1(112-116), corresponding to SEQ. ID. NOs: 134, 135, 136, 137, and 138, respectively, were used in these experiments. In the R4(104-114):R1(106-117) FGFR4 ECD long acid box variant, amino acids 106 to 117 of the FGFR1 ECD replace amino acids 104 to 114 of the FGFR4 ECD. In the R4(104-114):R1(107-117) FGFR4 ECD long acid box variant, amino acids 107 to 117 of the FGFR1 ECD replace amino acids 104 to 114 of the FGFR4 ECD. In the R4(104-110):R1(105-113) FGFR4 ECD long acid box variant, amino acids 105 to 113 of the FGFR1 ECD replace amino acids 104 to 110 of the FGFR4 ECD. In the R4(113-116):R1(116-119) FGFR4 ECD long acid box variant, amino acids 116 to 119 of the FGFR1 ECD replace amino acids 113 to 116 of the FGFR4 ECD. In the R4(109-113):R1(112-116) FGFR4 ECD long acid box variant, amino acids 112 to 116 of the FGFR1 ECD replace amino acids 109 to 113 of the FGFR4 ECD. Conventional cloning and site-directed mutagenesis were employed to generate clones in the pTT5 vector encoding the R4(104-114):R1(106-117), R4(104-114):R1(107-117), R4(104-110):R1(105-113), R4(113-116):R1(116-119), and R4(109-113):R1(112-116) fusion proteins using the R4Mut4 parental clone as a template. *In vitro* ECM binding of the four FGFR4 ECD long acid box variants was compared to the R1Mut4, R4Mut4, and ABMut1 fusion proteins.

[0214] All of the fusion proteins, including R1Mut4, R4Mut4, ABMut1, R4(104-114):R1(106-117), R4(104-114):R1(107-117), R4(104-110):R1(105-113), R4(113-116):R1(116-119), and R4(109-113):R1(112-116) were transiently expressed in 293-T

cells and purified as described in **Example 11**. Protein levels were determined using AlphaScreen (hu IgG AlphaLISA; Perkin-Elmer #AL205C). ECM binding experiments were carried out as described in **Example 7**, except that up to 1 mg/ml of the FGFR ECD-Fc fusion proteins were used in the *in vitro* ECM binding assay. A graphical representation of the results is shown in **FIG. 14**. As shown in **FIG. 14**, at least the R4(104-114):R1(106-117) and R4(104-110):R1(105-113) FGFR4 ECD long acid box variants exhibited ECM binding levels that were intermediate between the R4Mut4 and ABMut1 fusion proteins.

Example 16. FGFR4 ECD Acidic Region Chimeras Lacking Individual N-Glycan Sites Exhibit Decreased ECM Binding.

[0215] The FGFR4 ECD contains five N-glycan sites as determined by mass spectrometry. (Data not shown.) The FGFR4 ECD N-glycan sites at amino acids N91 and N156 of SEQ. ID. NOs: 1 and 2 are located adjacent to the amino-terminus of the FGFR4 D1-D2 linker and in the D2 heparin binding domain, respectively. In the ABMut1 FGFR4 ECD D1-D2 linker chimera of SEQ ID NO: 25, those N-glycan sites are located at amino acids N91 and N159. Experiments were carried out to determine whether the introduction of either the N91A or the N159A N-glycan mutation could further reduce the *in vitro* ECM binding of the ABMut1 fusion protein. Conventional cloning and site-directed mutagenesis methods were employed to generate clones in the pTT5 vector encoding the ABMut1 fusion protein with the N91A or the N159A N-glycan mutation, referred to herein as ABMut1(N91A) and ABMut1(N159A), respectively. ABMut1(N91A) and ABMut1(N159A) fusion proteins correspond to SEQ ID NOs: 139 and 140, respectively.

[0216] The R4Mut4, ABMut1, ABMut1(N91A), and ABMut1(N159A) fusion proteins were used in these experiments. All four fusion proteins were transiently expressed in CHO-S cells. Briefly, a 500 ml culture of CHO-S cells (Invitrogen) was established by inoculating 0.5 x 10^6 cells/ml in fresh 37°C Freestyle CHO medium containing 8 mM L-Glutamine (Invitrogen). The cells were grown in a 2 l plastic flask and were derived from a seed strain that was continuously maintained up to passage 20. The following day, the cells were counted and diluted, if necessary, to 1 x 10^6 cells/ml in 37°C Freestyle CHO medium (Invitrogen) with a cell viability greater than 95%. The cells were transfected by transferring 10 ml of 37°C OptiPRO SFM medium containing 8 mM L-Glutamine (dilution media) into two 50 ml tubes. To the first tube (A), 625 ul of FreestyleMax transfection reagent (Invitrogen) were added. To the second tube (B), 625 ug of DNA were added. Both tubes were gently mixed by inverting, and the contents of

tube A were immediately added to tube B, followed by gentle mixing by inversion. The mixture was incubated at room temperature for between 10 to 20 min, and was then delivered drop-wise into the 500 ml cell culture in the 2 l culture flask while slowly swirling the flask. The culture was then transferred to an incubator at 37°C, 5% CO_2, 125 rpm. After six days, the cell viability was greater than 80%, and the culture supernatant was collected into a centrifuge bottle. The supernatant was centrifuged at 1,000 xg for 10 min, transferred to a new centrifuge bottle, and centrifuged at 4,000 xg for 10 min. The supernatant was collected into a new bottle and filtered through a 0.2 um filter. The supernatant was stored at 37°C prior to the purification step. The fusion proteins were purified from the culture supernatant as described in **Example 3**, except that Q Sepharose anion exchange chromatography was used as the second purification step. Protein-A eluates were applied to a Q Sepharose HP column (GE Healthcare 17-1014-01) equilibrated with five column volumes of sterile buffer (10 mM Tris, 50 mM NaCl, pH 8.0). The column was washed with five column volumes of the same buffer and the bound material was eluted at a rate of five ml/min with a linear gradient of 15 column volumes of elution buffer (10 mM Tris, 2 M NaCl, pH 8.0), followed by five column volumes with 100% elution buffer. Fourteen ml fractions were collected and the fractions comprising the FGFR ECD-Fc were identified by gel electrophoresis and pooled. FGFR ECD-Fc fusion proteins eluted with approximately 10-25% elution buffer.

[0217] Protein levels were determined based on absorbance measurements at 280 nm. ECM binding experiments were carried out as described in **Example 7**, except that up to 1 mg/ml of the fusion proteins was used in the *in vitro* ECM binding assay. A graphical representation of the results is shown in **FIG. 15**. As shown in **FIG. 15**, the ABMut1 fusion protein with either the N91A or the N159A N-glycan mutation exhibited a further decrease in *in vitro* ECM binding, which would also predict a further increase in both C_{max} and bioavailability.

[0218] An FGF2 competition ELISA assay was carried out to determine whether the ABMut1(N91A) and ABMut1(N159A) fusion proteins could inhibit the binding of FGF2 or FGF19 to surface-bound FGFR4 ECD-Fc (R4Mut4). In these assays, ABMut1 was the reference standard, and ABMut1(N91A) and ABMut1(N159A) were the test samples. Purified ABMut1, ABMut1(N91A), and ABMut1(N159A) were serially diluted in sample diluent (PBS containing 1% BSA (fraction V; Sigma #A3059), 0.05% Tween-20, 200 ng/ml FGF2 (PreproTech #100-18B) or 50 ng/ml FGF19 (PeproTech #100-32),

and 20 ug/ml heparin (Sigma #H3149)) to concentrations ranging from 1.5 ng/ml to 90,000 ng/ml. The protein mixtures were incubated for 60 min. A 96-well plate was incubated with 100 ul of 5 ug/ml R4Mut4 overnight at 4°C, washed three times, blocked in blocking buffer (PBS containing 1% BSA) for between one and two hours at room temperature, and washed three times. The protein mixtures (100 ul) were then transferred to the wells of the 96-well plate and incubated for one hour at room temperature with shaking.

[0219] In this assay, FGF2 or FGF19 that was not bound to the test samples or the reference standard during the initial incubation step would be free to bind to the surface-bound R4Mut4. The wells were washed three times using a plate washer, followed by detection using biotinylated anti-FGF2 antibody (R&D Systems #BAM233) or biotinylated anti-FGF19 antibody (R&D Systems #BAF969) with the VECTASTAIN ABC Kit (Vector Laboratories #PK-4000). Biotinylated anti-FGF2 antibody or biotinylated anti-FGF19 was diluted to 1 ug/ml in assay diluent (PBS containing 1% BSA and 0.05% Tween-20), and 100 ul was added to each well, followed by a one hour incubation at room temperature with shaking. The ABC solution was reconstituted by mixing three drops of solution A with three drops of solution B in 15 ml PBS, and the solution was allowed to stand for 30 min at room temperature. The plates were washed six times using a plate washer and 100 ul of the freshly reconstituted ABC solution were added to each well, followed by a 45 min to one hour incubation at room temperature. TMB substrate (100 ul) was added to each well, followed by incubation for 6 to 8 min at room temperature in the dark with gentle shaking. One hundred microliters of stop solution were added to each well, and the plates were mixed by tapping. The plate optical density (OD) was read at 450 nm with 570 nm subtraction.

[0220] The OD values were then plotted versus the protein concentration on a log scale to generate standard curves. The OD value for each well was directly proportional to the amount of bound FGF2 or FGF19, and was inversely proportional to the amount of active FGFR4 ECD-Fc fusion protein in the test solution. The concentration profiles for the test samples and the reference standards were fit using a 4-parameter logistic. The relative binding activity (% bioactivity) of each test sample was calculated by dividing the IC_{50} value for the standard reference by the IC_{50} value for the test sample, which was then multiplied by 100%. The relative FGF2 binding activities of ABMut1(N91A) and ABMut1(N159A) in this assay were 44% and 42%, respectively. The relative FGF19

binding activities of ABMut1(N91A) and ABMut1(N159A) in this assay were 51% and 56%, respectively.

Example 17. FGFR4 ECD D1-D2 Linker Chimeras with the FGFR2 or FGFR3 D1-D2 Linker Exhibit Decreased ECM Binding *In Vitro*.

[0221] Experiments were carried out to determine whether FGFR4 ECD D1-D2 linker chimeras in which the FGFR4 D1-D2 linker was replaced with either the FGFR2 D1-D2 linker ("R4(D1-D2):R2(D1-D2)") or the FGFR3 D1-D2 linker ("R4(D1-D2):R3(D1-D2)") exhibited decreased binding to ECM components *in vitro*. Both the FGFR2 D1-D2 linker and the FGFR3 D1-D2 linker contain more acidic residues than the FGFR4 D1-D2 linker. Conventional cloning techniques were employed to generate clones in the pTT5 vector encoding the R4(D1-D2):R2(D1-D2) and R4(D1-D2):R3(D1-D2) fusion proteins. R4(D1-D2):R2(D1-D2) and R4(D1-D2):R3(D1-D2) correspond to SEQ ID NOs: 143 and 144, respectively.

[0222] The R1Mut4, R4Mut4, ABMut1, R4(D1-D2):R2(D1-D2), and R4(D1-D2):R3(D1-D2) fusion proteins were used in these experiments. All of the fusion proteins were expressed in 293-T cells as described in **Example 11**. Protein levels were determined using AlphaScreen (hu IgG AlphaLISA; Perkin-Elmer #AL205C). The concentration of the purified R4(D1-D2):R3(D1-D2) fusion protein was lower than the concentrations of the purified R1Mut4, R4Mut4, ABMut1, and R4(D1-D2):R2(D1-D2) fusion proteins, and did not permit an analysis of its ECM binding at higher concentrations. ECM binding experiments were carried out as described in **Example 7**, except that up to nearly 1 mg/ml of the FGFR ECD-Fc fusion proteins were used in the *in vitro* ECM binding assay. A graphical representation of the results is shown in **FIG. 16.** As shown in **FIG. 16**, both the R4(D1-D2):R2(D1-D2) and R4(D1-D2):R3(D1-D2) fusion proteins exhibited ECM binding levels similar to that of the ABMut1 fusion protein.

Example 18. FGFR2 and FGFR3 Short Acid Box Chimeras with the FGFR1 Short Acid Box Exhibit Decreased ECM Binding *In Vitro*.

[0223] Experiments were carried out to determine whether an increase in the total number of acidic residues within the long acid box of FGFR2 and FGFR3 could further decrease their ECM binding *in vitro*. FGFR2 and FGFR3 short acid box chimeras were generated in which the amino acid residues of the short acid box of FGFR1 replaced the corresponding amino acid residues within the FGFR2 and FGFR3 long acid box, referred to as R2(111-118):R1(105-112) and R3(110-117):R1(105-112), respectively. Conventional cloning techniques were employed to generate clones in the pTT5 vector

encoding the R2(111-118):R1(105-112) and R3(110-117):R1(105-112) fusion proteins. R2(111-118):R1(105-112) and R3(110-117):R1(105-112) correspond to SEQ ID NOs: 166 and 167, respectively.

[0224] The FGFR2 ECD-Fc, FGFR3 ECD-Fc, R2(111-118):R1(105-112), and R3(110-117):R1(105-112) fusion proteins were used in these experiments. The FGFR2 ECD-Fc, FGFR3 ECD-Fc fusion proteins, R2(111-118):R1(105-112), and R3(110-117):R1(105-112) fusion proteins were transiently expressed in CHO-S cells and purified as described in **Example 16.** Protein levels were determined using absorbance measurements at 280 nm. ECM binding experiments were carried out as described in **Example 7,** except that up to 1 mg/ml of each fusion protein was used in the *in vitro* ECM binding assays. Graphical representations of the results are shown in **FIGs. 17A-B.** As shown in **FIG. 17A** and **FIG. 17B,** the R2(111-118):R1(105-112) and R3(110-117):R1(105-112) fusion proteins exhibited slightly less ECM binding *in vitro* relative to the FGFR2 ECD-Fc and FGFR3 ECD-Fc fusion proteins, respectively.

INDUSTRIAL APPLICABILITY

[0225] The FGFR ECD acidic region muteins and the FGFR ECD fusion molecules described herein are useful in treating proliferative diseases and diseases involving angiogenesis, including cancer and macular degeneration. They can be used to diagnose, prevent, and treat these diseases.

TABLE OF SEQUENCES

[0226] Table 11 provides certain sequences discussed herein. All FGFR sequences are shown without the signal peptide unless otherwise indicated.

Table 11: Sequences and Descriptions

SEQ. ID. NO.	Description	Sequence
1	FGFR4 ECD	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
2	FGFR4 ECD P115L	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE

		RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDLSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA *VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY* VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
3	FGFR4 ECD D276V	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGAVGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
93	FGFR4 ECD T158A	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPAPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
4	FGFR4 ECD + linker + Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTDGS EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
5	FGFR4 ECD + Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTDEP KSSDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

6	FGFR4 ECD Δ5	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APE
7	FGFR4 ECD Δ10	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTW
8	FGFR4 ECD Δ15	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEE
9	FGFR4 ECD Δ17	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
10	FGFR4 ECD Δ18	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL
11	FGFR4 ECD Δ5 + Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVINGS SFGADGFPYV QVLKTADINS SEVEVLYLRN VSAEDAGEYT CLAGNSIGLS YQSAWLTVLP EEDPTWTAAA PEEPKSSDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV

		KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
12	FGFR4 ECD Δ10 + Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTEP KSSDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
13	FGFR4 ECD Δ15 + Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEEPKSSDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
14	FGFR4 ECD Δ17 + Fc (also called FGFR4ECD (delta17)-Fc and R4Mut4)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEPKSSDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
15	FGFR4 ECD Δ18 + Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA

		VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
16	FGFR4 D1-D2 linker	DSLTSSNDDED PKSHRDPSNR HSYPQQ
17	FGFR4 P115L D1-D2 linker	DSLTSSNDDED PKSHRDLSNR HSYPQQ
18	FGFR4 exon 4	DSLTSSNDDE DPKSHRDPSN RHSYPQ
19	FGFR4 P115L exon 4	DSLTSSNDDE DPKSHRDLSN RHSYPQ
20	FGFR4 acid box	DDEDPKSHR
21	FGFR1 ECD	RPSPTLPEQ AQPWGAPVEV ESFLVHPGDL LQLRCRLRDD VQSINWLRDG VQLAESNRTR ITGEEVEVQD SVPADSGLYA CVTSSPSGSD TTYFSVNVSD ALPSSEDDDD DDDSSSEEKE TDNTKPNPVA PYWTSPEKME KKLHAVPAAK TVKFKCPSSG TPNPTLRWLK NGKEFKPDHR IGGYKVRYAT WSIIMDSVVP SDKGNYTCIV ENEYGSINHT YQLDVVERSP HRPILQAGLP ANKTVALGSN VEFMCKVYSD PQPHIQWLKH IEVNGSKIGP DNLPYVQILK TAGVNTTDKE MEVLHLRNVS FEDAGEYTCL AGNSIGLSHH SAWLTVLEAL EERPAVMTSP LYLE
22	FGFR1 D1-D2 linker	DALPSSEDDDD DDDSSSEEKE TDNTKPNPV
23	FGFR1 exon 4	DALPSSEDDD DDDDSSSEEK ETDNTKPN
24	FGFR1 acid box	EDDDDDDDSS SE
25	FGFR1 RM ECD	RPSPTLPEQ AQPWGAPVEV ESFLVHPGDL LQLRCRLRDD VQSINWLRDG VQLAESNRTR ITGEEVEVQD SVPADSGLYA CVTSSPSGSD TTYFSVNVSD ALPSSEDDDD DDDSSSEEKE TDNTKPNRMP VAPYWTSPEK MEKKLHAVPA AKTVKFKCPS SGTPNPTLRW LKNGKEFKPD HRIGGYKVRY ATWSIIMDSV VPSDKGNYTC IVENEYGSIN HTYQLDVVER SPHRPILQAG LPANKTVALG SNVEFMCKVY SDPQPHIQWL KHIEVNGSKI GPDNLPYVQI LKTAGVNTTD KEMEVLHLRN VSFEDAGEYT CLAGNSIGLS HHSAWLTVLE ALEERPAVMT SPLYLE
26	FGFR1 RM D1-D2 linker	DALPSSEDDDD DDDSSSEEKE TDNTKPNRMP V
92	FGFR1 RM exon 4	DALPSSEDDD DDDDSSSEEK ETDNTKPNRM
27	FGFR2 ECD	RPSPSLVED TTLEPEEPPT KYQISQPEVY VAAPGESLEV RCLLKDAAVI SWTKDGVHLG PNNRTVLIGE YLQIKGATPR DSGLYACTAS RTVDSETWYF MVNVTDAISS GDDEDDTDGA EDFVSENSNN KRAPYWTNTE KMEKRLHAVP AANTVKFRCP AGGNPMPTMR WLKNGKEFKQ EHRIGGYKVR NQHWSLIMES VVPSDKGNYT CVVENEYGSI NHTYHLDVVE RSPHRPILQA GLPANASTVV GGDVEFVCKV YSDAQPHIQW IKHVEKNGSK YGPDGLPYLK VLKAAGVNTT DKEIEVLYIR NVTFEDAGEY

		TCLAGNSIGI SFHSAWLTVL PAPGREKEIT ASPDYLE
28	FGFR2 D1-D2 linker	DAISSGDDED DTDGAEDFVS ENSNNKR
29	FGFR2 exon 4	DAISSGDDED DTDGAEDFVS ENSNNK
30	FGFR2 acid box	DDEDDTD
31	FGFR3 ECD	ESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS HEDSGAYSCR QRLTQRVLCH FSVRVTDAPS SGDDEDGEDE AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP DGTPYVTVLK TAGANTTDKE LEVLSLHNVT FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE EELVEADEAG SVYAG
32	FGFR3 D1-D2 linker	DAPSSGDDEDG EDEAEDTGVD TG
33	FGFR3 exon 4	DAPSSGDDED GEDEAEDTGV DT
34	FGFR3 acid box	DDEDGE
35	FGFR4 ECD Δ17 R1 D1-D2 linker chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNPVAPY WTHPQRMEKK LHAVPAGNTV KFRCPAAGNP TPTIRWLKDG QAFHGENRIG GIRLRHQHWS LVMESVVPSD RGTYTCLVEN AVGSIRYNYL LDVLERSPHR PILQAGLPAN TTAVVGSDVE LLCKVYSDAQ PHIQWLKHIV INGSSFGADG FPYVQVLKTA DINSSEVEVL YLRNVSAEDA GEYTCLAGNS IGLSYQSAWL TVLP
36	FGFR4 ECD Δ17 R1 RM D1-D2 linker chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNRMPVA PYWTHPQRME KKLHAVPAGN TV KFRCPAAGNP TPTIRWLKDG QAFHGENRIG GIRLRHQHWS LVMESVVPSD RGTYTCLVEN AVGSIRYNYL LDVLERSPHR PILQAGLPAN TTAVVGSDVE LLCKVYSDAQ PHIQWLKHIV INGSSFGADG FPYVQVLKTA DINSSEVEVL YLRNVSAEDA GEYTCLAGNS IGLSYQSAWL TVLP
37	FGFR4 ECD Δ17 R2 D1-D2 linker chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAI SSGDDEDDTD GAEDFVSENS NNKRAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY

		VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
38	FGFR4 ECD Δ17 R3 D1-D2 linker chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAP SSGDDEDGED EAEDTGVDTG APYWTHPQRM EKKLHAVPAG NTVKFRCPAA GNPTPTIRWL KDGQAFHGEN RIGGIRLRHQ HWSLVMESVV PSDRGTYTCL VENAVGSIRY NYLLDVLERS PHRPILQAGL PANTTAVVGS DVELLCKVYS DAQPHIQWLK HIVINGSSFG ADGFPYVQVL KTADINSSEV EVLYLRNVSA EDAGEYTCLA GNSIGLSYQS AWLTVLP
39	FGFR4 ECD Δ17 R1 exon 4 chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNQAPYW THPQRMEKKL HAVPAGNTVK FR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
40	FGFR4 ECD Δ17 R1 RM exon 4 chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNRMQAPYW THPQRMEKKL HAVPAGNTVK FR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
41	FGFR4 ECD Δ17 R2 exon 4 chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAISSGDDED DTDGAEDFVS ENSNNKQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
42	FGFR4 ECD Δ17 R3 exon 4 chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAPSSGDDED GEDEAEDTGV DTQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
43	FGFR4 ECD Δ17 R1 acid box chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNEDDDDDDDSS SEDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING

		SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
44	FGFR4 ECD Δ17 R2 acid box chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDDT DDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
45	FGFR4 ECD Δ17 R3 acid box chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDGE DPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
46	FGFR4 acid box region 1	DDEDPKSHRD
47	FGFR4 acid box region 2	DDEDPKSHRD P
48	FGFR4 acid box region 3	DDEDPKSHRD PS
49	FGFR4 acid box region 4	DDEDPKSHRD PSN
50	FGFR4 acid box region 5	DDEDPKSHRD PSNR
51	FGFR4 acid box region 6	NDDEDPKSHR D
52	FGFR4 acid box region 7	NDDEDPKSHR DP
53	FGFR4 acid box region 8	NDDEDPKSHR DPS
54	FGFR4 acid box region 9	NDDEDPKSHR DPSN
55	FGFR4 acid box region 10	NDDEDPKSHR DPSNR
56	FGFR1 acid box region 1	EDDDDDDDSS SEE
57	FGFR1 acid box region 2	EDDDDDDDSS SEEKE
58	FGFR1 acid box region 3	EDDDDDDDSS SEEKETD

59	FGFR2 acid box region 1	DDEDDTDGAE
60	FGFR2 acid box region 2	DDEDDTDGAED
61	FGFR2 acid box region 3	DDEDDTDGAEDFVSE
62	FGFR3 acid box region 1	DDEDGED
63	FGFR3 acid box region 2	DDEDGEDE
64	FGFR3 acid box region 3	DDEDGEDEAE
65	FGFR3 acid box region 4	DDEDGEDEAED
66	FGFR1 signal peptide	MWSWKCLLFWAVLVTATLCTA
67	FGFR2 signal peptide	MVSWGRFICLVVVTMATLSLA
68	FGFR3 signal peptide	MGAPACALALCVAVAIVAGASS
69	FGFR4 signal peptide	MRLLLALLGI LLSVPGPPVL S
70	FGFR4 N-terminal sequence	LEASEEVE
71	FGFR4 C-terminal sequence	LPEEDPTWTAA APEARYTD
72	Fc C237S	EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
73	Fc	ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
74	Fc	ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
75	FGFR4 V10I signal peptide	MRLLLALLGI LLSVPGPPVL S
76	FGFR4 ECD NΔ2	ASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE

		RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
77	FGFR4 ECD NΔ3	SEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
78	FGFR4 ECD NΔ5	EVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
79	FGFR4 ECD NΔ7	ELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
80	FGFR4 ECD NΔ8	LE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
81	FGFR4 ECD NΔ8 Δ17	LE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
82	FGFR4 ECD w/ signal	MRLLLALLGI LLSVPGPPVL SLEASEEVELE PCLAPSLEQQ

	peptide	EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEEDPTWTAA APEARYTD
83	FGFR1 RM ECD w/ signal peptide	MWSWKCLLFW AVLVTATLCT ARPSPTLPEQ AQPWGAPVEV ESFLVHPGDL LQLRCRLRDD VQSINWLRDG VQLAESNRTR ITGEEVEVQD SVPADSGLYA CVTSSPSGSD TTYFSVNVSD ALPSSEDDDD DDDSSSEEKE TDNTKPNRMP VAPYWTSPEK MEKKLHAVPA AKTVKFKCPS SGTPNPTLRW LKNGKEFKPD HRIGGYKVRY ATWSIIMDSV VPSDKGNYTC IVENEYGSIN HTYQLDVVER SPHRPILQAG LPANKTVALG SNVEFMCKVY SDPQPHIQWL KHIEVNGSKI GPDNLPYVQI LKTAGVNTTD KEMEVLHLRN VSFEDAGEYT CLAGNSIGLS HHSAWLTVLE ALEERPAVMT SPLYLE
84	FGFR2 ECD w/ signal peptide	MVSWGRFICL VVVTMATLSL ARPSFSLVED TTLEPEEPPT KYQISQPEVY VAAPGESLEV RCLLKDAAVI SWTKDGVHLG PNNRTVLIGE YLQIKGATPR DSGLYACTAS RTVDSETWYF MVNVTDAISS GDDEDDTDGA EDFVSENSNN KRAPYWTNTE KMEKRLHAVP AANTVKFRCP AGGNPMPTMR WLKNGKEFKQ EHRIGGYKVR NQHWSLIMES VVPSDKGNYT CVVENEYGSI NHTYHLDVVE RSPHRPILQA GLPANASTVV GGDVEFVCKV YSDAQPHIQW IKHVEKNGSK YGPDGLPYLK VLKAAGVNTT DKEIEVLYIR NVTFEDAGEY TCLAGNSIGI SFHSAWLTVL PAPGREKEIT ASPDYLE
85	FGFR3 ECD w/ signal peptide	MGAPACALAL CVAVAIVAGA SSESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS HEDSGAYSCR QRLTQRVLCH FSVRVTDAPS SGDDEDGEDE AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP DGTPYVTVLK TAGANTTDKE LEVLSLHNVT FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE EELVEADEAG SVYAG
86	FGFR4 ECD Δ17 R1 D1-D2 linker chimera + Fc (also called FGFR4ECD (ABMut1: delta 17)-Fc and ABMut1)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNPVAPY WTHPQRMEKK LHAVPAGNTV KFRCPAAGNP TPTIRWLKDG QAFHGENRIG GIRLRHQHWS LVMESVVPSD RGTYTCLVEN AVGSIRYNYL LDVLERSPHR PILQAGLPAN TTAVVGSDVE LLCKVYSDAQ PHIQWLKHIV INGSSFGADG FPYVQVLKTA DINSSEVEVL YLRNVSAEDA GEYTCLAGNS IGLSYQSAWL TVLPEPKSSD KTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
87	FGFR4 ECD Δ17 R1	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE

	exon 4 chimera + Fc (also called FGFR4ECD (ABMut2: delta17)-Fc and ABMut2)	RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNQAPYW THPQRMEKKL HAVPAGNTVK FR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEPKSSD KTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
88	FGFR4 ECD Δ17 R1 acid box chimera + Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNEDDDDDDDSS SEDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL PEPKSSD KTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK
89	FGFR4 ECD 2Ig + D1-D2 linker + GS linker + Fc (also called FGFR4ECD(2Ig+Linker)-Fc and R4(2Ig+L))	LEASEEVELED SLTSSNDDED PKSHRDPSNR HSYPQQAPYW THPQRMEKKL HAVPAGNTVK FRCPAAGNPT PTIRWLKDGQ AFHGENRIGG IRLRHQHWSL VMESVVPSDR GTYTCLVENA VGSIRYNYLL DVLERSPHRP ILQAGLPANT TAVVGSDVEL LCKVYSDAQP HIQWLKHIVI NGSSFGADGF PYVQVLKTAD INSSEVEVLY LRNVSAEDAG EYTCLAGNSI GLSYQSAWLT VLPEEDPTWT AAAPEARYTD GSEPKSSDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK
90	FGFR4 ECD 2Ig - D1-D2 linker + GS linker + Fc (also called FGFR4ECD(2Ig-Linker)-Fc and R4(2Ig-L))	LEASEEVELEA PYWTHPQRME KKLHAVPAGN TVKFRCPAAG NPTPTIRWLK DGQAFHGENR IGGIRLRHQH WSLVMESVVP SDRGTYTCLV ENAVGSIRYN YLLDVLERSP HRPILQAGLP ANTTAVVGSD VELLCKVYSD AQPHIQWLKH IVINGSSFGA DGFPYVQVLK TADINSSEVE VLYLRNVSAE DAGEYTCLAG NSIGLSYQSA WLTVLPEEDP TWTAAAPEAR YTDGSEPKSS DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK
91	FGFR1 ECD Δ14 + Fc	RPSPTLPEQA QPWGAPVEVE SFLVHPGDLL QLRCRLRDDV QSINWLRDGV QLAESNRTRI TGEEVEVQDS VPADSGLYAC

	(also called FGFR1ECD(delta14)-Fc and R1Mut4)	VTSSPSGSDT TYFSVNVSDA LPSSEDDDDD DDSSSEEKET DNTKPNPVAP YWTSPEKMEK KLHAVPAAKT VKFKCPSSGT PNPTLRWLKN GKEFKPDHRI GGYKVRYATW SIIMDSVVPS DKGNYTCIVE NEYGSINHTY QLDVVERSPH RPILQAGLPA NKTVALGSNV EFMCKVYSDP QPHIQWLKHI EVNGSKIGPD NLPYVQILKT AGVNTTDKEM EVLHLRNVSF EDAGEYTCLA GNSIGLSHHS AWLTVLEALE PKSSDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
94	FGFR4 ECD 2Ig + D1-D2 linker	LEASEEVELED SLTSSNDDED PKSHRDPSNR HSYPQQAPYW THPQRMEKKL HAVPAGNTVK FRCPAAGNPT PTIRWLKDGQ AFHGENRIGG IRLRHQHWSL VMESVVPSDR GTYTCLVENA VGSIRYNYLL DVLERSPHRP ILQAGLPANT TAVVGSDVEL LCKVYSDAQP HIQWLKHIVI NGSSFGADGF PYVQVLKTAD INSSEVEVLY LRNVSAEDAG EYTCLAGNSI GLSYQSAWLT VLPEEDPTWT AAAPEARYTD
95	FGFR4 ECD 2Ig - D1-D2 linker	LEASEEVELEA PYWTHPQRME KKLHAVPAGN TVKFRCPAAG NPTPTIRWLK DGQAFHGENR IGGIRLRHQH WSLVMESVVP SDRGTYTCLV ENAVGSIRYN YLLDVLERSP HRPILQAGLP ANTTAVVGSD VELLCKVYSD AQPHIQWLKH IVINGSSFGA DGFPYVQVLK TADINSSEVE VLYLRNVSAE DAGEYTCLAG NSIGLSYQSA WLTVLPEEDP TWTAAAPEAR YTD
96	FGFR4 long acid box	NDDEDPKSHR DPSNR
97	FGFR4 P115L long acid box	NDDEDPKSHR DLSNR
98	FGFR1 long acid box	EDDDDDDDSS SEEKETD
99	FGFR2 long acid box	DDEDDTDGAE DFVSE
100	FGFR3 long acid box	GDDEDGEDEA ED
101	FGFR4 short acid box	DDED
102	FGFR1 short acid box	EDDDDDDD
103	FGFR2 short acid box	DDEDD
104	FGFR3 short acid box	DDED
105	FGFR4 ECD Δ17 R1 long acid box chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS EDDDDDDDSSSEEKETD HS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
106	FGFR4 ECD Δ17 R2	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC

	long acid box chimera	LARGSMIVLQ NLTLITGDSL TSS DDEDDTDGAEDFVSE HS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
107	FGFR4 ECD Δ17 R3 long acid box chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS GDDEDGEDEAED HS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
108	FGFR4 ECD Δ17 R1 short acid box chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSN EDDDDDDD PK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
109	FGFR4 ECD Δ17 R2 short acid box chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSN DDEDD PK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
110	FGFR4 ECD Δ17 R3 short acid box chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSN DDED PK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
111	FGFR4 ECD Δ17 N104D	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSDDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
112	FGFR4 ECD Δ17 P109D	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC

		LARGSMIVLQ NLTLITGDSL TSSNDDEDDK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
113	FGFR4 ECD Δ17 R113E	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHEDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
114	FGFR4 ECD Δ17 S116E	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPENRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
115	FGFR4 ECD Δ17 R4(104-114):R1(106-117)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS DDDDDDDSSSEE PSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
116	FGFR4 ECD Δ17 R4(104-114):R1(107-117)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS DDDDDDSSSEE PSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
117	FGFR4 ECD Δ17 R4(104-110):R1 (105-113)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS EDDDDDDDS SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
118	FGFR4 ECD Δ17 R4(113-116):R1(116-	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC

	119)	LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SH EEKE NRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
119	FGFR4 ECD Δ17 R4(109-113):R1(112-116)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDED DSSSE DPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
120	FGFR4 ECD Δ17 R1 D1-D2 linker chimera (N91A)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ ALTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNPVAPY WTHPQRMEKK LHAVPAGNTV KFRCPAAGNP TPTIRWLKDG QAFHGENRIG GIRLRHQHWS LVMESVVPSD RGTYTCLVEN AVGSIRYNYL LDVLERSPHR PILQAGLPAN TTAVVGSDVE LLCKVYSDAQ PHIQWLKHIV INGSSFGADG FPYVQVLKTA DINSSEVEVL YLRNVSAEDA GEYTCLAGNS IGLSYQSAWL TVLP
121	FGFR4 ECD Δ17 R1 D1-D2 linker chimera (N159A)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNPVAPY WTHPQRMEKK LHAVPAGNTV KFRCPAAGAP TPTIRWLKDG QAFHGENRIG GIRLRHQHWS LVMESVVPSD RGTYTCLVEN AVGSIRYNYL LDVLERSPHR PILQAGLPAN TTAVVGSDVE LLCKVYSDAQ PHIQWLKHIV INGSSFGADG FPYVQVLKTA DINSSEVEVL YLRNVSAEDA GEYTCLAGNS IGLSYQSAWL TVLP
122	FGFR2 ECD R2(111-118):R1(105-112)	RPSFSLVED TTLEPEEPPT KYQISQPEVY VAAPGESLEV RCLLKDAAVI SWTKDGVHLG PNNRTVLIGE YLQIKGATPR DSGLYACTAS RTVDSETWYF MVNVTDAISS G EDDDDDDD A EDFVSENSNN KRAPYWTNTE KMEKRLHAVP AANTVKFRCP AGGNPMPTMR WLKNGKEFKQ EHRIGGYKVR NQHWSLIMES VVPSDKGNYT CVVENEYGSI NHTYHLDVVE RSPHRPILQA GLPANASTVV GGDVEFVCKV YSDAQPHIQW IKHVEKNGSK YGPDGLPYLK VLKAAGVNTT DKEIEVLYIR NVTFEDAGEY TCLAGNSIGI SFHSAWLTVL PAPGREKEIT ASPDYLE
123	FGFR3 ECD R3(110-117):R1(105-112)	ESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS HEDSGAYSCR QRLTQRVLCH FSVRVTDAPS S EDDDDDDD E AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP DGTPYVTVLK TAGANTTDKE LEVLSLHNVT FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE EELVEADEAG SVYAG

124	FGFR4 ECD Δ17 R1 long acid box chimera-Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS EDDDDDDDSSSEEKETD HS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
125	FGFR4 ECD Δ17 R2 long acid box chimera-Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS DDEDDTDGAEDFVSE HS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
126	FGFR4 ECD Δ17 R3 long acid box chimera-Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS GDDEDGEDEAED HS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
127	FGFR4 ECD Δ17 R1 short acid box chimera-Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSN EDDDDDDD PK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
128	FGFR4 ECD Δ17 R2 short acid box chimera-	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC

	Fc	LARGSMIVLQ NLTLITGDSL TSSN DDEDD PK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
129	FGFR4 ECD Δ17 R3 short acid box chimera-Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSN DDED PK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
130	FGFR4 ECD Δ17 N104D + Fc (also called FGFR4ECD(R4Mut4(N104D): delta17)-Fc and R4Mut4(N104D))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSDDDEDPK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
131	FGFR4 ECD Δ17 P109D + Fc (also called FGFR4ECD(R4Mut4(P109D): delta17)-Fc and R4Mut4(P109D))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDDK SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS

		VMHEALHNHYTQKSLSLSPGK
132	FGFR4 ECD Δ17 R113E + Fc (also called FGFR4ECD(R4Mut4(R113E): delta17)-Fc and R4Mut4(R113E))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHEDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
133	FGFR4 ECD Δ17 S116E + Fc (also called FGFR4ECD(R4Mut4(S116E): delta17)-Fc and R4Mut4(S116E))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SHRDPENRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
134	FGFR4 ECD Δ17 R4(104-114):R1(106-117) + Fc (also called FGFR4ECD(R4Mut4(104-114):FGFR1(106-117): delta 17)-Fc and R4(104-114):R1(106-117))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS DDDDDDSSSEE PSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
135	FGFR4 ECD Δ17 R4(104-114):R1(107-117) + Fc (also called FGFR4ECD(R4Mut4(104-114):FGFR1(107-117): delta 17)-Fc and R4(104-114):R1(107-117))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS DDDDDDSSSEE PSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF

		NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
136	FGFR4 ECD Δ17 R4(104-110):R1(105-113) + Fc (also called FGFR4ECD(R4Mut4(104-110):FGFR1(105-113): delta 17)-Fc and R4(104-110):R1(105-113))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS EDDDDDDDS SHRDPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
137	FGFR4 ECD Δ17 R4(113-116):R1(116-119) + Fc (also called FGFR4ECD(R4Mut4(113-116):FGFR1(116-119): delta 17)-Fc and R4(113-116):R1(116-119))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDEDPK SH EEKE NRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
138	FGFR4 ECD Δ17 R4(109-113):R1(112-116) + Fc (also called FGFR4ECD(R4Mut4(109-113):FGFR1(112-116): delta 17)-Fc and R4(109-113):R1(112-116))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSSNDDED DSSSE DPSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
139	FGFR4 ECD Δ17 R1 D1-D2 linker chimera (N91A) + Fc (also called FGFR4ECD(ABMut1(N91A): delta 17)-Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ ALTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNPVAPY WTHPQRMEKK LHAVPAGNTV KFRCPAAGNP TPTIRWLKDG QAFHGENRIG GIRLRHQHWS LVMESVVPSD RGTYTCLVEN AVGSIRYNYL LDVLERSPHR PILQAGLPAN

	and ABMut1(N91A))	TTAVVGSDVE LLCKVYSDAQ PHIQWLKHIV INGSSFGADG FPYVQVLKTA DINSSEVEVL YLRNVSAEDA GEYTCLAGNS IGLSYQSAWL TVLP EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
140	FGFR4 ECD Δ17 R1 D1-D2 linker chimera (N159A) + Fc (also called FGFR4ECD(ABMut1(N159A): delta 17)-Fc and ABMut1(N159A))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNPVAPY WTHPQRMEKK LHAVPAGNTV KFRCPAAGAP TPTIRWLKDG QAFHGENRIG GIRLRHQHWS LVMESVVPSD RGTYTCLVEN AVGSIRYNYL LDVLERSPHR PILQAGLPAN TTAVVGSDVE LLCKVYSDAQ PHIQWLKHIV INGSSFGADG FPYVQVLKTA DINSSEVEVL YLRNVSAEDA GEYTCLAGNS IGLSYQSAWL TVLP EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
141	FGFR2 ECD R2(111-118):R1(105-112) + Fc	RPSFSLVED TTLEPEEPPT KYQISQPEVY VAAPGESLEV RCLLKDAAVI SWTKDGVHLG PNNRTVLIGE YLQIKGATPR DSGLYACTAS RTVDSETWYF MVNVTDAISS G EDDDDDDD A EDFVSENSNN KRAPYWTNTE KMEKRLHAVP AANTVKFRCP AGGNPMPTMR WLKNGKEFKQ EHRIGGYKVR NQHWSLIMES VVPSDKGNYT CVVENEYGSI NHTYHLDVVE RSPHRPILQA GLPANASTVV GGDVEFVCKV YSDAQPHIQW IKHVEKNGSK YGPDGLPYLK VLKAAGVNTT DKEIEVLYIR NVTFEDAGEY TCLAGNSIGI SFHSAWLTVL PAPGREKEIT ASPDYLE EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
142	FGFR3 ECD R3(110-117):R1(105-112) + Fc	ESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS HEDSGAYSCR QRLTQRVLCH FSVRVTDAPS S EDDDDDDD E AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP DGTPYVTVLK TAGANTTDKE LEVLSLHNVT FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE EELVEADEAG SVYAG EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
143	FGFR4 ECD Δ17 R2 D1-D2 linker chimera +	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC

	Fc (also called FGFR4ECD(R4Mut4(D1-D2):FGFR2(D1-D2): delta 17)-Fc and R4(D1-D2):R2(D1-D2))	LARGSMIVLQ NLTLITGDAI SSGDDEDDTD GAEDFVSENS NNKRAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
144	FGFR4 ECD Δ17 R3 D1-D2 linker chimera + Fc (also called FGFR4ECD(R4Mut4(D1-D2):FGFR3(D1-D2): delta 17)-Fc and R4(D1-D2):R3(D1-D2))	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDAP SSGDDEDGED EAEDTGVDTG APYWTHPQRM EKKLHAVPAG NTVKFRCPAA GNPTPTIRWL KDGQAFHGEN RIGGIRLRHQ HWSLVMESVV PSDRGTYTCL VENAVGSIRY NYLLDVLERS PHRPILQAGL PANTTAVVGS DVELLCKVYS DAQPHIQWLK HIVINGSSFG ADGFPYVQVL KTADINSSEV EVLYLRNVSA EDAGEYTCLA GNSIGLSYQS AWLTVLP EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
145	R4(104-114)	NDDEDPKSHR D
146	R4(104-110)	NDDEDPK
147	R4(113-116)	RDPS
148	R4(109-113)	PKSHR
149	R1(106-117)	DDDDDDDSSS EE
150	R1(107-117)	DDDDDDSSSE E
151	R1(105-113)	EDDDDDDDS
152	R1(116-119)	EEKE
153	R1(112-116)	DSSSE
154	R1(105-112)	EDDDDDDD
155	R2(111-118)	DDEDDTDG
156	R3(110-117)	GDDEDGED
157	FGFR4 ECD Δ17 R4(104-114):R1(105-117) acid box chimera	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS EDDDDDDDSSSEE PSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL

		QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P
158	FGFR4 ECD Δ17 R4(104-114):R1(105-117) acid box chimera + Fc (also called FGF4ECD(ABMut3: delta17)-Fc or ABMut3)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ NLTLITGDSL TSS EDDDDDDDSSSEE PSNRHS YPQQAPYWTH PQRMEKKLHA VPAGNTVKFR CPAAGNPTPT IRWLKDGQAF HGENRIGGIR LRHQHWSLVM ESVVPSDRGT YTCLVENAVG SIRYNYLLDV LERSPHRPIL QAGLPANTTA VVGSDVELLC KVYSDAQPHI QWLKHIVING SSFGADGFPY VQVLKTADIN SSEVEVLYLR NVSAEDAGEY TCLAGNSIGL SYQSAWLTVL P EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
159	R1(105-117)	EDDDDDDDSS SEE
160	FGFR2 ECD Δ3	RPSFSLVED TTLEPEEPPT KYQISQPEVY VAAPGESLEV RCLLKDAAVI SWTKDGVHLG PNNRTVLIGE YLQIKGATPR DSGLYACTAS RTVDSETWYF MVNVTDAISS GDDEDDTDGA EDFVSENSNN KRAPYWTNTE KMEKRLHAVP AANTVKFRCP AGGNPMPTMR WLKNGKEFKQ EHRIGGYKVR NQHWSLIMES VVPSDKGNYT CVVENEYGSI NHTYHLDVVE RSPHRPILQA GLPANASTVV GGDVEFVCKV YSDAQPHIQW IKHVEKNGSK YGPDGLPYLK VLKAAGVNTT DKEIEVLYIR NVTFEDAGEY TCLAGNSIGI SFHSAWLTVL PAPGREKEIT ASPD
161	FGFR3 ECD Δ3	ESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS HEDSGAYSCR QRLTQRVLCH FSVRVTDAPS SGDDEDGEDE AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP DGTPYVTVLK TAGANTTDKE LEVLSLHNVT FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE EELVEADEAG SV
162	FGFR2 ECD Δ3 + GS linker + Fc (also called FGFR2ECD(delta3)-GS linker-Fc and FGFR2-Fc)	RPSFSLVED TTLEPEEPPT KYQISQPEVY VAAPGESLEV RCLLKDAAVI SWTKDGVHLG PNNRTVLIGE YLQIKGATPR DSGLYACTAS RTVDSETWYF MVNVTDAISS GDDEDDTDGA EDFVSENSNN KRAPYWTNTE KMEKRLHAVP AANTVKFRCP AGGNPMPTMR WLKNGKEFKQ EHRIGGYKVR NQHWSLIMES VVPSDKGNYT CVVENEYGSI NHTYHLDVVE RSPHRPILQA GLPANASTVV GGDVEFVCKV YSDAQPHIQW IKHVEKNGSK YGPDGLPYLK VLKAAGVNTT DKEIEVLYIR NVTFEDAGEY TCLAGNSIGI SFHSAWLTVL PAPGREKEIT ASPD GS EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
163	FGFR3 ECD Δ3 + GS linker + Fc	ESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS

	(also called FGFR3ECD(delta3)-GS linker-Fc and FGFR3-Fc)	HEDSGAYSCR QRLTQRVLCH FSVRVTDAPS SGDDEDGEDE AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP DGTPYVTVLK TAGANTTDKE LEVLSLHNVT FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE EELVEADEAG SV GS EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
164	FGFR2 ECD Δ3 R2(111-118):R1(105-112)	RPSFSLVED TTLEPEEPPT KYQISQPEVY VAAPGESLEV RCLLKDAAVI SWTKDGVHLG PNNRTVLIGE YLQIKGATPR DSGLYACTAS RTVDSETWYF MVNVTDAISS G EDDDDDDD A EDFVSENSNN KRAPYWTNTE KMEKRLHAVP AANTVKFRCP AGGNPMPTMR WLKNGKEFKQ EHRIGGYKVR NQHWSLIMES VVPSDKGNYT CVVENEYGSI NHTYHLDVVE RSPHRPILQA GLPANASTVV GGDVEFVCKV YSDAQPHIQW IKHVEKNGSK YGPDGLPYLK VLKAAGVNTT DKEIEVLYIR NVTFEDAGEY TCLAGNSIGI SFHSAWLTVL PAPGREKEIT ASPD
165	FGFR3 ECD Δ3 R3(110-117):R1(105-112)	ESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS HEDSGAYSCR QRLTQRVLCH FSVRVTDAPS S EDDDDDDD E AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP DGTPYVTVLK TAGANTTDKE LEVLSLHNVT FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE EELVEADEAG SV
166	FGFR2 ECD Δ3 R2(111-118):R1(105-112) + GS linker + Fc (also called FGFR2ECD(FGFR2(111-118):FGFR1(105-112): delta3)-GS linker-Fc and R2(111-118):R1(105-112))	RPSFSLVED TTLEPEEPPT KYQISQPEVY VAAPGESLEV RCLLKDAAVI SWTKDGVHLG PNNRTVLIGE YLQIKGATPR DSGLYACTAS RTVDSETWYF MVNVTDAISS G EDDDDDDD A EDFVSENSNN KRAPYWTNTE KMEKRLHAVP AANTVKFRCP AGGNPMPTMR WLKNGKEFKQ EHRIGGYKVR NQHWSLIMES VVPSDKGNYT CVVENEYGSI NHTYHLDVVE RSPHRPILQA GLPANASTVV GGDVEFVCKV YSDAQPHIQW IKHVEKNGSK YGPDGLPYLK VLKAAGVNTT DKEIEVLYIR NVTFEDAGEY TCLAGNSIGI SFHSAWLTVL PAPGREKEIT ASPD GS EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
167	FGFR3 ECD Δ3 R3(110-117):R1(105-112) + GS linker + Fc (also called FGFR3ECD(FGFR3(110-117):FGFR1(105-112): delta3)-GS linker-Fc and R3(110-	ESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS HEDSGAYSCR QRLTQRVLCH FSVRVTDAPS S EDDDDDDD E AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP DGTPYVTVLK TAGANTTDKE LEVLSLHNVT

	117):R1(105-112))	FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE EELVEADEAG SV GS EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
168	FGFR4 ECD Δ17 R1 D1-D2 linker chimera (N91A, N159)	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ ALTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNPVAPY WTHPQRMEKK LHAVPAGNTV KFRCPAAGAP TPTIRWLKDG QAFHGENRIG GIRLRHQHWS LVMESVVPSD RGTYTCLVEN AVGSIRYNYL LDVLERSPHR PILQAGLPAN TTAVVGSDVE LLCKVYSDAQ PHIQWLKHIV INGSSFGADG FPYVQVLKTA DINSSEVEVL YLRNVSAEDA GEYTCLAGNS IGLSYQSAWL TVLP
169	FGFR4 ECD Δ17 R1 D1-D2 linker chimera (N91A, N159) + Fc	LEASEEVELE PCLAPSLEQQ EQELTVALGQ PVRLCCGRAE RGGHWYKEGS RLAPAGRVRG WRGRLEIASF LPEDAGRYLC LARGSMIVLQ ALTLITGDAL PSSEDDDDDD DSSSEEKETD NTKPNPVAPY WTHPQRMEKK LHAVPAGNTV KFRCPAAGAP TPTIRWLKDG QAFHGENRIG GIRLRHQHWS LVMESVVPSD RGTYTCLVEN AVGSIRYNYL LDVLERSPHR PILQAGLPAN TTAVVGSDVE LLCKVYSDAQ PHIQWLKHIV INGSSFGADG FPYVQVLKTA DINSSEVEVL YLRNVSAEDA GEYTCLAGNS IGLSYQSAWL TVLP EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
170	Fc	ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
171	Fc	ERKSSVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
172	FGFR1 ECD Δ14	RPSPTLPEQ AQPWGAPVEV ESFLVHPGDL LQLRCRLRDD VQSINWLRDG VQLAESNRTR ITGEEVEVQD SVPADSGLYA CVTSSPSGSD TTYFSVNVSD ALPSSEDDDD DDDSSSEEKE TDNTKPNPVA PYWTSPEKME KKLHAVPAAK TVKFKCPSSG TPNPTLRWLK NGKEFKPDHR IGGYKVRYAT WSIIMDSVVP SDKGNYTCIV ENEYGSINHT YQLDVVERSP HRPILQAGLP ANKTVALGSN VEFMCKVYSD PQPHIQWLKH IEVNGSKIGP DNLPYVQILK TAGVNTTDKE MEVLHLRNVS FEDAGEYTCL AGNSIGLSHH SAWLTVLEAL
173	FGFR1 ECD w/ signal peptide	MWSWKCLLFW AVLVTATLCT A RPSPTLPEQ AQPWGAPVEV ESFLVHPGDL LQLRCRLRDD VQSINWLRDG VQLAESNRTR

		ITGEEVEVQD SVPADSGLYA CVTSSPSGSD TTYFSVNVSD ALPSSEDDDD DDDSSSEEKE TDNTKPNPVA PYWTSPEKME KKLHAVPAAK TVKFKCPSSG TPNPTLRWLK NGKEFKPDHR IGGYKVRYAT WSIIMDSVVP SDKGNYTCIV ENEYGSINHT YQLDVVERSP HRPILQAGLP ANKTVALGSN VEFMCKVYSD PQPHIQWLKH IEVNGSKIGP DNLPYVQILK TAGVNTTDKE MEVLHLRNVS FEDAGEYTCL AGNSIGLSHH SAWLTVLEAL EERPAVMTSP LYLE

CLAIMS

1. A polypeptide comprising an FGFR4 extracellular domain (ECD) acidic region mutein, wherein the FGFR4 ECD acidic region mutein is selected from an FGFR4 ECD acidic region chimera and an FGFR4 long acid box variant, and wherein the FGFR4 ECD acidic region mutein has a greater number of acidic residues in the D1-D2 linker region than wild-type FGFR4 ECD.

2. The polypeptide of claim 1, wherein the FGFR4 ECD acidic region chimera is selected from an FGFR4 ECD D1-D2 linker chimera, an FGFR4 ECD exon 4 chimera, an FGFR4 ECD acid box chimera, an FGFR4 ECD long acid box chimera, and an FGFR4 ECD short acid box chimera.

3. The polypeptide of claim 2, wherein:

a) the FGFR4 ECD D1-D2 linker chimera comprises a D1-D2 linker selected from an FGFR1 D1-D2 linker, an FGFR2 D1-D2 linker, and an FGFR3 D1-D2 linker, in place of the FGFR4 D1-D2 linker;

b) the FGFR4 ECD exon 4 chimera comprises an exon 4 selected from an FGFR1 exon 4, an FGFR2 exon 4, and an FGFR3 exon 4, in place of the FGFR4 exon 4;

c) the FGFR4 ECD acid box chimera comprises:

i) an acid box selected from the FGFR1 acid box, the FGFR2 acid box, and the FGFR3 acid box, in place of the FGFR4 acid box;

ii) an acid box region selected from an FGFR1 acid box region, an FGFR2 acid box region, and an FGFR3 acid box region, in place of the FGFR4 acid box;

iii) an acid box region selected from an FGFR1 acid box region, an FGFR2 acid box region, and an FGFR3 acid box region, in place of an FGFR4 acid box region;

iv) an acid box selected from the FGFR1 acid box, the FGFR2 acid box, and the FGFR3 acid box, in place of an FGFR4 acid box region;

d) the FGFR4 ECD long acid box chimera comprises a long acid box selected from an FGFR1 long acid box, an FGFR2 long acid box, and an FGFR3 long acid box, in place of the FGFR4 long acid box; and

e) the FGFR4 ECD short acid box chimera comprises a short acid box selected from an FGFR1 short acid box, an FGFR2 short acid box, and an FGFR3 short acid box, in place of the FGFR4 short acid box.

4. The polypeptide of claim 3, wherein:

a) the FGFR4 ECD D1-D2 linker chimera comprises:

i) an amino acid sequence selected from SEQ ID NOs: 22, 26, 28, and 32, in place of an FGFR4 D1-D2 linker selected from SEQ ID NOs: 16 and 17; or

ii) an amino acid sequence selected from SEQ ID NOs: 35 to 38;

b) the FGFR4 ECD exon 4 chimera comprises:

i) an amino acid sequence selected from SEQ ID NOs: 23, 92, 29, and 33, in place of an FGFR4 exon 4 selected from SEQ ID NOs: 18 and 19; or

ii) an amino acid sequence selected from SEQ ID NOs: 39 to 42;

c) the FGFR4 ECD acid box chimera comprises:

i) an amino acid sequence selected from SEQ ID NOs: 24, 30, and 34 in place of the FGFR4 acid box having an amino acid sequence of SEQ ID NO: 20;

ii) an amino acid sequence selected from SEQ ID NOs: 56 to 65, in place of the FGFR4 acid box having an amino acid sequence of SEQ ID NO: 20; or

iii) an amino acid sequence selected from SEQ ID NOs: 56 to 65, in place of an FGFR4 acid box region having an amino acid sequence selected from SEQ ID NOs: 46 to 55;

iv) an amino acid sequence selected from SEQ ID NOs: 24, 30, and 34 in place of an FGFR4 acid box region having an amino acid sequence selected from SEQ ID NOs: 46 to 55; or

v) an amino acid sequence selected from SEQ ID NOs: 43 to 45 and 157;

d) the FGFR4 ECD long acid box chimera comprises:

i) an amino acid sequence selected from SEQ ID NOs: 98 to 100, in place of an FGFR4 long acid box selected from SEQ ID NOs: 96 and 97; or

ii) an amino acid sequence selected from SEQ ID NOs: 105 to 107; and

e) the FGFR4 ECD short acid box chimera comprises:

i) an amino acid sequence selected from SEQ ID NOs: 102 to 104, in place of an FGFR4 short acid box having an amino acid sequence of SEQ ID NO: 101; or

ii) an amino acid sequence selected from SEQ ID NOs: 108 to 110.

5. The polypeptide of claim 1, wherein the FGFR4 ECD long acid box variant comprises a variant of the FGFR4 ECD that has an increased number of acidic amino acid residues in the long acid box relative to the FGFR4 wild-type long acid box.

6. The polypeptide of claim 5, wherein:

a) at least two non-acidic residues within the long acid box of the FGFR4 ECD are each independently replaced with an acidic residue selected from Glu (E) and Asp (D);

b) at least three non-acidic residues within the long acid box of the FGFR4 ECD are each independently replaced with an acidic residue selected from Glu (E) and Asp (D); or

c) at least four non-acidic residues within the long acid box of the FGFR4 ECD are each independently replaced with an acidic residue selected from Glu (E) and Asp (D).

7. The polypeptide of claim 5, wherein:

a) at least one acidic residue is inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2; or

b) two acidic residues are inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2.

8. The polypeptide of claim 5, wherein the number of acidic residues in the FGFR4 long acid box is at least seven.

9. The polypeptide of claim 5, wherein:

a) FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 106 to 117 (SEQ ID NO: 149);

b) FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 107 to 117 (SEQ ID NO: 150);

c) FGFR4 ECD residues 104 to 110 (SEQ ID NO: 146) are replaced with FGFR1 ECD residues 105-113 (SEQ ID NO: 151);

d) FGFR4 ECD residues 113 to 116 (SEQ ID NO: 147) are replaced with FGFR1 ECD residues 116-119 (SEQ ID NO: 152);

e) FGFR4 ECD residues 109 to 113 (SEQ ID NO: 148) are replaced with FGFR1 ECD residues 112-116 (SEQ ID NO: 153); or

f) the FGFR4 ECD long acid box variant comprises an amino acid sequence selected from SEQ ID NOs: 111 to 119.

10. The polypeptide of claim 1, wherein the FGFR4 acidic region mutein comprises at least one point mutation that inhibits glycosylation.

11. The polypeptide of claim 10, wherein the at least one point mutation that inhibits glycosylation is selected from from N91A, N156A, N237A, N269A, N290A, and N301A.

12. The polypeptide of claim 10, wherein the FGFR4 acidic region mutein comprises an amino acid sequence selected from SEQ ID NOs: 120, 121, and 168.

13. An FGFR4 ECD fusion molecule comprising a polypeptide of any one of claims 1 to 12 and a fusion partner.

14. The FGFR4 ECD fusion molecule of claim 13, wherein the fusion partner is selected from Fc, albumin, and polyethylene glycol.

15. The FGFR4 ECD fusion molecule of claim 14, wherein the fusion partner is Fc.

16. The FGFR4 ECD fusion molecule of claim 15, wherein the FGFR4 fusion molecule comprises an amino acid sequence selected from SEQ ID NOs: 86 to 88, 124 to 140, 143, 144, and 158.

17. The FGFR4 ECD fusion molecule of claim 15 wherein the FGFR4 ECD fusion molecule has an amino acid sequence of SEQ ID NO: 86.

18. A polynucleotide comprising a nucleic acid sequence that encodes the polypeptide of any one of claims 1 to 12 or an FGFR4 ECD fusion molecule of any one of claims 13 to 17.

19. A pharmaceutical composition comprising the polypeptide or FGFR4 fusion molecule of any one of claims 1 to 17, and a pharmaceutically acceptable carrier.

20. Use of the pharmaceutical composition of claim 19 in the manufacture of a medicament for treating an angiogenic disorder in a patient .

21. The use of claim 20, wherein the angiogenic disorder is selected from cancer and macular degeneration.

22. The use of claim 21, wherein the cancer is selected from colon, liver, lung, breast, and prostate cancers.

23. A polypeptide comprising an FGFR2 ECD acidic region mutein, wherein the FGFR2 ECD acidic region mutein has a greater number of acidic residues in the D1-D2 linker region than wild-type FGFR2 ECD.

24. The polypeptide of claim 23, wherein the FGFR2 ECD acidic region mutein is an FGFR2 ECD short acid box chimera.

25. The polypeptide of claim 24, wherein the FGFR2 ECD short acid box chimera comprises at least the FGFR1 short acid box in place of at least the FGFR2 short acid box.

26. The polypeptide of claim 24, wherein:

a) FGFR2 ECD residues 111 to 118 (SEQ ID NO: 155) are replaced with FGFR1 ECD residues 105 to 112 (SEQ ID NO: 154); or

b) the FGFR2 ECD short acid box chimera comprises the amino acid sequence of SEQ ID NO: 122.

27. A polypeptide comprising an FGFR3 ECD acidic region mutein, wherein the FGFR3 ECD acidic region mutein has a greater number of acidic residues in the D1-D2 linker region than wild-type FGFR3 ECD.

28. The polypeptide of claim 27, wherein the FGFR3 ECD acidic region mutein is an FGFR3 ECD short acid box chimera.

29. The polypeptide of claim 28, wherein the FGFR3 ECD short acid box chimera comprises at least the FGFR1 short acid box in place of at least the FGFR3 short acid box.

30. The polypeptide of claim 28, wherein:

a) FGFR3 ECD residues 110 to 117 (SEQ ID NO: 156) are replaced with FGFR1 ECD residues 105 to 112 (SEQ ID NO: 154); or

b) the FGFR3 ECD short acid box chimera comprises the amino acid sequence of SEQ ID NO: 123.