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(54) **Title**: ANTI-FGFR2/3 ANTIBODIES AND METHODS USING SAME

(57) **Abstract**: The invention provides dual specific anti-FGFR2 and FGFR3 (FGFR2/3) antibodies, and compositions comprising and methods of using these antibodies.

ANTI-FGFR2/3 ANTIBODIES AND METHODS USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority of provisional U.S. Application No. 62/075,740 filed November 5, 2014, which is herein incorporated by reference in its entirety.

SEQUENCE LISTING

None.

FIELD OF THE INVENTION

The present invention relates generally to dual specific anti-FGFR2/3 antibodies, and uses of same.

BACKGROUND OF THE INVENTION

Fibroblast growth factors (FGFs) and their receptors (FGFRs) play critical roles during embryonic development, tissue homeostasis and metabolism (Eswarakumar, V.P., Lax, I., and Schlessinger, J. 2005. Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 16:139-149; L'Hote, C.G., and Knowles, M.A. 2005. Cell responses to FGFR3 signalling: growth, differentiation and apoptosis. Exp Cell Res 304:417-431; Dailey, L., Ambrosetti, D., Mansukhani, A., and Basilico, C. 2005. Mechanisms underlying differential responses to FGF signaling. Cytokine Growth Factor Rev 16:233-247). In humans, there are 22 FGFs (FGF1-14, FGF16-23) and four FGF receptors with tyrosine kinase domain (FGFR1-4). FGFRs consist of an extracellular ligand binding region, with two or three immunoglobulin-like domains (IgD1-3), a single-pass transmembrane region, and a cytoplasmic, split tyrosine kinase domain. FGFR1, 2 and 3 each have two major alternatively spliced isoforms, designated IIIb and IIIc. These isoforms differ by about 50 amino acids in the second half of IgD3, and have distinct tissue distribution and ligand

specificity. In general, the IIIb isoform is found in epithelial cells, whereas IIIc is expressed in mesenchymal cells. Upon binding FGF in concert with heparan sulfate proteoglycans, FGFRs dimerize and become phosphorylated at specific tyrosine residues. This facilitates the recruitment of critical adaptor proteins, such as FGFR substrate 2 α (FRS2α), leading to activation of multiple signaling cascades, including the mitogen-activated protein kinase (MAPK) and PI3K-AKT pathways (Eswarakumar, V.P., Lax, I., and Schlessinger, J. 2005. Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 16:139-149; Dailey, L., Ambrosetti, D., Mansukhani, A., and Basilico, C. 2005. Mechanisms underlying differential responses to FGF signaling. Cytokine Growth Factor Rev 16:233-247; Mohammadi, M., Olsen, S.K., and Ibrahimi, O.A. 2005. Structural basis for fibroblast growth factor receptor activation. Cytokine Growth Factor Rev 16:107-137). Consequently, FGFs and their cognate receptors regulate a broad array of cellular processes, including proliferation, differentiation, migration and survival, in a context-dependent manner.

Aberrantly activated FGFRs have been implicated in specific human malignancies (Eswarakumar, V.P., Lax, I., and Schlessinger, J. 2005. Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 16:139-149; Grose, R., and Dickson, C. 2005. Fibroblast growth factor signaling in tumorigenesis. Cytokine Growth Factor Rev 16:179-186). In particular, the t(4;14) (p16.3;q32) chromosomal translocation occurs in about 15-20% of multiple myeloma patients, leading to overexpression of FGFR3 and correlates with shorter overall survival (Chang, H., Stewart, A.K., Qi, X.Y., Li, Z.H., Yi, Q.L., and Trudel, S. 2005. Immunohistochemistry accurately predicts FGFR3 aberrant expression and t(4;14) in multiple myeloma. Blood 106:353-355; Chesi, M., Nardini, E., Brents, L.A., Schrock, E., Ried, T., Kuehl, W.M., and Bergsagel, P.L. 1997. Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. Nat Genet 16:260-

264; Fonseca, R., Blood, E., Rue, M., Harrington, D., Oken, M.M., Kyle, R.A., Dewald, G.W., Van Ness, B., Van Wier, S.A., Henderson, K.J., et al. 2003. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood 101:4569-4575; Moreau, P., Facon, T., Leleu, X., Morineau, N., Huyghe, P., Harousseau, J.L., Bataille, R., and Avet-Loiseau, H. 2002. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. Blood 100:1579-1583). FGFR3 is implicated also in conferring chemoresistance to myeloma cell lines in culture (Pollett, J.B., Trudel, S., Stern, D., Li, Z.H., and Stewart, A.K. 2002. Overexpression of the myeloma-associated oncogene fibroblast growth factor receptor 3 confers dexamethasone resistance. Blood 100:3819-3821), consistent with the poor clinical response of t(4;14)+ patients to conventional chemotherapy (Fonseca, R., Blood, E., Rue, M., Harrington, D., Oken, M.M., Kyle, R.A., Dewald, G.W., Van Ness, B., Van Wier, S.A., Henderson, K.J., et al. 2003. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood 101:4569-4575). Overexpression of mutationally activated FGFR3 is sufficient to induce oncogenic transformation in hematopoietic cells and fibroblasts (Bernard-Pierrot, I., Brams, A., Dunois-Larde, C., Caillault, A., Diez de Medina, S.G., Cappellen, D., Graff, G., Thiery, J.P., Chopin, D., Ricol, D., et al. 2006. Oncogenic properties of the mutated forms of fibroblast growth factor receptor 3b. Carcinogenesis 27:740-747; Agazie, Y.M., Movilla, N., Ischenko, I., and Hayman, M.J. 2003. The phosphotyrosine phosphatase SHP2 is a critical mediator of transformation induced by the oncogenic fibroblast growth factor receptor 3. Oncogene 22:6909-6918; Ronchetti, D., Greco, A., Compasso, S., Colombo, G., Dell'Era, P., Otsuki, T., Lombardi, L., and Neri, A. 2001. Deregulated FGFR3 mutants in multiple myeloma cell lines with t(4;14): comparative analysis of Y373C, K650E and the novel G384D mutations. Oncogene 20:3553-3562; Chesi, M., Brents, L.A., Ely, S.A., Bais, C., Robbiani, D.F., Mesri, E.A., Kuehl, W.M., and Bergsagel, P.L. 2001. Activated fibroblast

growth factor receptor 3 is an oncogene that contributes to tumor progression in multiple myeloma. Blood 97:729-736; Plowright, E.E., Li, Z., Bergsagel, P.L., Chesi, M., Barber, D.L., Branch, D.R., Hawley, R.G., and Stewart, A.K. 2000. Ectopic expression of fibroblast growth factor receptor 3 promotes myeloma cell proliferation and prevents apoptosis. Blood 95:992-998), and murine bone marrow transplantation models (Chen, J., Williams, I.R., Lee, B.H., Duclos, N., Huntly, B.J., Donoghue, D.J., and Gilliland, D.G. 2005. Constitutively activated FGFR3 mutants signal through PLCgamma-dependent and -independent pathways for hematopoietic transformation. Blood 106:328-337; Li, Z., Zhu, Y.X., Plowright, E.E., Bergsagel, P.L., Chesi, M., Patterson, B., Hawley, T.S., Hawley, R.G., and Stewart, A.K. 2001. The myeloma-associated oncogene fibroblast growth factor receptor 3 is transforming in hematopoietic cells. Blood 97:2413-2419). Accordingly, FGFR3 has been proposed as a potential therapeutic target in multiple myeloma. Indeed, several small-molecule inhibitors targeting FGFRs, although not selective for FGFR3 and having cross-inhibitory activity toward certain other kinases, have demonstrated cytotoxicity against FGFR3-positive myeloma cells in culture and in mouse models (Trudel, S., Ely, S., Farooqi, Y., Affer, M., Robbiani, D.F., Chesi, M., and Bergsagel, P.L. 2004. Inhibition of fibroblast growth factor receptor 3 induces differentiation and apoptosis in t(4;14) myeloma. Blood 103:3521-3528; Trudel, S., Li, Z.H., Wei, E., Wiesmann, M., Chang, H., Chen, C., Reece, D., Heise, C., and Stewart, A.K. 2005. CHIR-258, a novel, multitargeted tyrosine kinase inhibitor for the potential treatment of t(4;14) multiple myeloma. Blood 105:2941-2948; Chen, J., Lee, B.H., Williams, I.R., Kutok, J.L., Mitsiades, C.S., Duclos, N., Cohen, S., Adelsperger, J., Okabe, R., Coburn, A., et al. 2005. FGFR3 as a therapeutic target of the small molecule inhibitor PKC412 in hematopoietic malignancies. Oncogene 24:8259-8267; Paterson, J.L., Li, Z., Wen, X.Y., Masih-Khan, E., Chang, H., Pollett, J.B., Trudel, S., and Stewart, A.K. 2004. Preclinical studies of fibroblast growth factor receptor 3 as a therapeutic target in multiple

myeloma. Br J Haematol 124:595-603; Grand, E.K., Chase, A.J., Heath, C., Rahemtulla, A., and Cross, N.C. 2004. Targeting FGFR3 in multiple myeloma: inhibition of t(4;14)-positive cells by SU5402 and PD173074. Leukemia 18:962-966).

FGFR3 overexpression has been documented also in a high fraction of bladder cancers (Gomez-Roman, J.J., Saenz, P., Molina, M., Cuevas Gonzalez, J., Escuredo, K., Santa Cruz, S., Junquera, C., Simon, L., Martinez, A., Gutierrez Banos, J.L., et al. 2005. Fibroblast growth factor receptor 3 is overexpressed in urinary tract carcinomas and modulates the neoplastic cell growth. Clin Cancer Res 11:459-465; Tomlinson, D.C., Baldo, O., Harnden, P., and Knowles, M.A. 2007. FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer. J Pathol 213:91-98). Furthermore, somatic activating mutations in FGFR3 have been identified in 60-70% of papillary and 16-20% of muscle-invasive bladder carcinomas (Tomlinson, D.C., Baldo, O., Harnden, P., and Knowles, M.A. 2007. FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer. J Pathol 213:91-98; van Rhijn, B.W., Montironi, R., Zwarthoff, E.C., Jobsis, A.C., and van der Kwast, T.H. 2002. Frequent FGFR3 mutations in urothelial papilloma. J Pathol 198:245-251). In cell culture experiments, RNA interference (Bernard-Pierrot, I., Brams, A., Dunois-Larde, C., Caillault, A., Diez de Medina, S.G., Cappellen, D., Graff, G., Thiery, J.P., Chopin, D., Ricol, D., et al. 2006. Oncogenic properties of the mutated forms of fibroblast growth factor receptor 3b. Carcinogenesis 27:740-747; Tomlinson, D.C., Hurst, C.D., and Knowles, M.A. 2007. Knockdown by shRNA identifies S249C mutant FGFR3 as a potential therapeutic target in bladder cancer. Oncogene 26:5889-5899) or an FGFR3 single-chain Fv antibody fragment inhibited bladder cancer cell proliferation (Martinez-Torrecuadrada, J., Cifuentes, G., Lopez-Serra, P., Saenz, P., Martinez, A., and Casal, J.I. 2005. Targeting the extracellular domain of fibroblast growth factor receptor 3 with human single-chain Fv antibodies inhibits bladder carcinoma cell line

proliferation. Clin Cancer Res 11:6280-6290). A recent study demonstrated that an FGFR3 antibody-toxin conjugate attenuates xenograft growth of a bladder cancer cell line through FGFR3-mediated toxin delivery into tumors (Martinez-Torrecuadrada, J.L., Cheung, L.H., Lopez-Serra, P., Barderas, R., Canamero, M., Ferreiro, S., Rosenblum, M.G., and Casal, J.I. 2008. Antitumor activity of fibroblast growth factor receptor 3-specific immunotoxins in a xenograft mouse model of bladder carcinoma is mediated by apoptosis. Mol Cancer Ther 7:862-873). However, it remains unclear whether FGFR3 signaling is indeed an oncogenic driver of in vivo growth of bladder tumors. Moreover, the therapeutic potential for targeting FGFR3 in bladder cancer has not been defined on the basis of in vivo models. Publications relating to FGFR3 and anti-FGFR3 antibodies include U.S. Patent Publication no. 2005/0147612; Rauchenberger et al, J Biol Chem 278 (40):38194-38205 (2003); WO2006/048877; Martinez-Torrecuadrada et al, (2008) Mol Cancer Ther 7(4): 862-873; WO2007/144893; Trudel et al. (2006) 107(10): 4039-4046; Martinez-Torrecuadrada et al (2005) Clin Cancer Res 11 (17): 6280-6290; Gomez-Roman et al (2005) Clin Cancer Res 11:459-465; Direnzo, R et al (2007) Proceedings of AACR Annual Meeting, Abstract No. 2080; WO2010/002862. Crystal structures of FGFR3:anti-FGFR3 antibody are disclosed in U.S. Pat. Pub. No. 20100291114.

While FGFR2 and FGFR3 can be inhibited without disrupting adult-tissue homeostasis, blocking the closely related FGFR1 and FGFR4, which regulate specific metabolic functions, carries a greater safety risk. An anti-FGFR3 antibody dislosed in U.S. patent publication no. 20100291114 was re-engineered here to create function-blocking antibodies that bind with dual specificity to FGFR3 and FGFR2 but spare FGFR1 and FGFR4. Thus a dual-specific antibody was desiged and made that blocks FGF binding to FGFR2 and FGFR3 (*i.e.*, FGFR2/3), thereby inhibiting downstream signaling, without blocking FGFR1 or FGFR4.

It is clear that there continues to be a need for agents that have clinical attributes that are optimal for development as therapeutic agents.

As described herein, an antibody that binds monospecifically to FGFR3, was redesigned for binding to other FGFR family members through multiple rounds of engineering, including recruiting binding to FGFR2 and removing binding to FGFR4. The first step of engineering was carried out to gain FGFR2 binding using phage display library. Each phage library constituted mutagenesis of one contacting CDR, and the range of mutagenesis covered as many residues in that CDR as allowed by library size. Choosing multiple consecutive positions for mutagenesis permitted significant freedom in the CDR backbones. Most of the resulting clones that were able to engage FGFR2 harbored all 5 mutations in CDR H2. The crystal structure demonstrated that the full range of mutagenesis was coupled with complete remodeling of the geometry of the CDR loop. The solutions to spatial reorganizations of a CDR are numerous, as evidenced by the identification of diverse H2 mutants that had gained binding to FGFR2. Such a large variety of solutions are not typically seen as outcomes from standard affinity maturation experiments, whereby the recovered sequences usually contain sparse positions on individual CDRs. Therefore, acquiring additional specificity for homologous antigens may require larger mutagenesis freedom than affinity maturation.

The second round of engineering was refinement of specificity to remove FGFR4 binding. Detailed structural analysis of contact residues between the antibody CDR loops and the antigen surface was used to guide the design of phage display libraries. Selected antibody variants showed reduction in FGFR4 binding with retention of binding to FGFR2/3. The sequence solutions to this specificity refinement step were more limited compared to the first round of engineering. The refinement step further demonstrated the ability to differentiate binding specificities among closely related antigens antibody re-engineering.

The dual-specific antibodies generated through the antibody engineering described herein bind to two closely related antigens, namely FGFR2 and FGFR3 (anti-FGFR2/3 antibodies). These anti-FGFR2/3 antibodies (2B.1.3 antibody variants) are regular IgG molecules in that they use identical heavy and light chains. Certain anti-FGFR2/3 antibodies of this invention can bind to two FGFR2 isoforms, two FGFR3 isoforms or one FGFR2 and one FGFR3 isoform in a bivalent or monovalent manner respectively. This contrasts to conventional bispecific IgG, which commonly use two different heavy/light-chain pairs to bind to two different antigens in a monovalent manner. The dual-specific antibodies described share some similarities with "two-in-one" antibodies (Grand, E.K., Chase, A.J., Heath, C., Rahemtulla, A., and Cross, N.C. 2004. Targeting FGFR3 in multiple myeloma: inhibition of t(4;14)-positive cells by SU5402 and PD173074. Leukemia 18:962-966). Bostrom *et al.* randomized all 3 light-chain CDRs of Herceptin and selected for a second specificity as well as the parental specificity. As expected, the second specificity comes from the dominant contributions of light-chain CDRs (Grand, E.K., Chase, A.J., Heath, C., Rahemtulla, A., and Cross, N.C. 2004. Targeting FGFR3 in multiple myeloma: inhibition of t(4;14)-positive cells by SU5402 and PD173074. Leukemia 18:962-966; Gomez-Roman, J.J., Saenz, P., Molina, M., Cuevas Gonzalez, J., Escuredo, K., Santa Cruz, S., Junquera, C., Simon, L., Martinez, A., Gutierrez Banos, J.L., et al. 2005. Fibroblast growth factor receptor 3 is overexpressed in urinary tract carcinomas and modulates the neoplastic cell growth. Clin Cancer Res 11:459-465). In one case, although EGFR and Her3 are homologous, the binding epitopes by an anti-EGFR/Her3 "two-in-one" antibody are different (Gomez-Roman, J.J., Saenz, P., Molina, M., Cuevas Gonzalez, J., Escuredo, K., Santa Cruz, S., Junquera, C., Simon, L., Martinez, A., Gutierrez Banos, J.L., et al. 2005. Fibroblast growth factor receptor 3 is overexpressed in urinary tract carcinomas and modulates the neoplastic cell growth. Clin Cancer Res 11:459-465). The approach described herein differs from "two-in-one" antibodies

in that it appreciates the sequence and structure similarities between the two homologous antigens, and focuses on a more limited set of mutagenesis so as to retain the parental epitope during engineering.

The antibody engineering presented here started from an existing and extensively characterized antibody anti-FGFR antibody that has potential utility for cancer therapy. Since introduction of the first therapeutic monoclonal antibody in the mid-1980s, there have been many clinically and commercially successful antibody drugs in different disease areas, including trastuzumab, cetuximab, adalimumab, bevacizumab, etc. These antibodies displayed exceptional activities in inhibiting their molecular targets. On the other hand, like the FGFR family, multiple homologous proteins are pursued as molecular targets for their various disease associations. Traditional discovery routes to obtain antibodies targeting a functional epitope, either animal immunization or other display-based library selections, are not guaranteed to be successful. Alternatively, as described herein, an antibody can be engineered to acquire specificity towards homologous targets, thereby providing an alternative route for antibody discovery. Moreover, this approach takes advantage of the favorable properties of previously developed antibodies by maintaining the functional epitopes and presumably the biological functions as well. As the clinical antibody repertoire expands, more antibodies could be engineered instead of being discovered *ab initio*. Potential applications may include protein families that comprise multiple members as disease targets, such as the EGFR family (Tomlinson, D.C., Baldo, O., Harnden, P., and Knowles, M.A. 2007. FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer. J Pathol 213:91-98), the TNFR family (van Rhijn, B.W., Montironi, R., Zwarthoff, E.C., Jobsis, A.C., and van der Kwast, T.H. 2002. Frequent FGFR3 mutations in urothelial papilloma. J Pathol 198:245-251), the TAM family (Tomlinson, D.C., Hurst, C.D., and Knowles, M.A. 2007. Knockdown by shRNA identifies S249C mutant

FGFR3 as a potential therapeutic target in bladder cancer. Oncogene 26:5889-5899; Martinez-Torrecuadrada, J., Cifuentes, G., Lopez-Serra, P., Saenz, P., Martinez, A., and Casal, J.I. 2005. Targeting the extracellular domain of fibroblast growth factor receptor 3 with human single-chain Fv antibodies inhibits bladder carcinoma cell line proliferation. Clin Cancer Res 11:6280-6290), the Ephrin family (Martinez-Torrecuadrada, J.L., Cheung, L.H., Lopez-Serra, P., Barderas, R., Canamero, M., Ferreiro, S., Rosenblum, M.G., and Casal, J.I. 2008. Antitumor activity of fibroblast growth factor receptor 3-specific immunotoxins in a xenograft mouse model of bladder carcinoma is mediated by apoptosis. Mol Cancer Ther 7:862-873). As in the traditional discovery processes, engineered antibodies towards homologs should be considered as new molecules, and still need full characterization of their biochemical, biophysical and biologic properties for any potential therapeutic applications.

All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

The invention is based in part on the identification of a variety of FGFR binding agents (such as antibodies, and fragments thereof) that bind FGFR2 and FGFR3 ("FGFR2/3"). FGFR3 presents an important and advantageous therapeutic target, and the invention provides compositions and methods based on binding of the agents to FGFR3, specifically agents that bind FGFR. Specifically, invention provides compositions and methods based on binding of the agents to FGFR2/3 (i.e., binding of the agents that have dual specificity for FGFR2 and FGFR3). FGFR2/3 binding agents of the invention, as described herein, provide important therapeutic and diagnostic agents for use in targeting pathological conditions associated with expression and/or activity of the FGFR3 and/or FGFR2 signaling pathways. Accordingly, the invention provides methods, compositions, kits, and articles of manufacture related to FGFR3 and FGFR2 binding.

The present invention provides antibodies that bind to FGFR2 and FGFR3 (anti-FGFR2/3 antibodies). In one aspect, the invention features an isolated antibody that binds an FGFR3. In some embodiments, the antibody binds a FGFR3 IIIb isoform and/or a FGFR3 IIIc isoform. In some embodiments, the antibody binds a mutated FGFR3 (e.g., one or more of FGFR3 IIIb R248C, S249C, G372C, Y375C, K652E, and/or one or more of FGFR3 IIIc R248C, S249C, G370C, Y373C, K650E). In some embodiments, the antibody binds monomeric FGFR3 (e.g., monomeric FGFR3 IIIb and/or IIIc isoforms). In some embodiments, the antibody promotes formation of monomeric FGFR3, such as by stabilizing the monomeric FGFR3 form relative to the dimeric FGFR3 form. In some embodiments, the antibody binds FGFR2 or a variant thereof. In some embodiments, the antibody binds FGFR2 and any one or more of the FGFR3 variants described herein.

In one aspect, the invention provides an isolated anti-FGFR2/3 antibody, wherein a full length IgG form of the antibody binds human FGFR3 with a Kd of 1 x 10^{-7} M or higher affinity. In one aspect, the invention provides an isolated anti-FGFR2/3 antibody, wherein a full length IgG form of the antibody binds human FGFR2 with a Kd of 1 x 10^{-7} M or higher affinity. As is well-established in the art, binding affinity of a ligand to its receptor can be determined using any of a variety of assays, and expressed in terms of a variety of quantitative values. Accordingly, in one embodiment, the binding affinity is expressed as Kd values and reflects intrinsic binding affinity (e.g., with minimized avidity effects). Generally and preferably, binding affinity is measured *in vitro*, whether in a cell-free or cell-associated setting. Any of a number of assays known in the art, including those described herein, can be used to obtain binding affinity measurements, including, for example, Biacore, radioimmunoassay (RIA), and ELISA. In some embodiments, the full length IgG form of the antibody binds human FGFR3 with a Kd of 1 x 10^{-8} M or higher affinity, with a Kd of 1 x 10^{-9} M or higher affinity, or with a Kd of 1 x 10^{-10} M or higher affinity. In some embodiments,

the full length IgG form of the antibody binds human FGFR2 with a Kd of 1 x 10^{-8} M or higher affinity, with a Kd of 1 x 10^{-9} M or higher affinity, or with a Kd of 1 x 10^{-10} M or higher affinity. In some embodiments, the full length IgG form of the antibody binds human FGFR2 and FGFR3 with Kds of 1 x 10^{-8} M or higher affinity, with Kds of 1 x 10^{-9} M or higher affinity, or with Kds of 1 x 10^{-10} M or higher affinity.

Generally, the anti-FGFR2/3 antibodies of the present invention are antagonist antibodies. Thus, in one aspect, the anti-FGFR2/3 antibodies inhibit FGFR3 activity (e.g., FGFR3-IIIb and/or FGFR3-IIIc activity). In some embodiments, the anti-FGFR2/3 antibody (generally in bivalent form) does not possess substantial FGFR3 agonist function. In some embodiments, the anti-FGFR2/3 antagonist antibody (generally in bivalent form) possesses little or no FGFR3 agonist function. In one embodiment, an antibody of the invention (generally in bivalent form) does not exhibit an FGFR3 agonist activity level that is above background level that is of statistical significance.

In one aspect, binding of the antibody to a FGFR3 may inhibit dimerization of the receptor with another unit of the receptor, whereby activation of the receptor is inhibited (due, at least in part, to a lack of receptor dimerization). Inhibition can be direct or indirect.

In one aspect, the invention provides anti-FGFR2/3 antibodies that do not possess substantial apoptotic activity (e.g., does not induce apoptosis of a cell, e.g., a transitional cell carcinoma cell or a multiple myeloma cell, such as a multiple myeloma cell comprising a FGFR3 translocation, such as a t(4;14) translocation). In some embodiments, the anti-FGFR2/3 antibody possesses little or no apoptotic function. In some embodiment, the FGFR2/3 antibodies do not exhibit apoptotic function that is above background level that is of statistical significance.

In one aspect, the invention provides anti-FGFR2/3 antibodies that do not induce substantial FGFR3 down-regulation. In some embodiments, the anti-FGFR2/3 antibody

induces little or no receptor down-regulation. In some embodiment, the FGFR2/3 antibodies do not induce receptor down-regulation that is above background level that is of statistical significance.

In one aspect, the invention provides anti-FGFR2/3 antibodies that possess effector function. In one embodiment, the effector function comprises antibody-dependent cell-mediated cytotoxicity (ADCC). In one embodiment, the anti-FGFR2/3 antibodies of this invention (in some embodiments, a naked anti-FGFR2/3 antibody) are capable of killing a cell, in some embodiments, a multiple myeloma cells (e.g., multiple myeloma cells comprising a translocation, e.g., a t(4;14) translocation). In some embodiments, the the anti-FGFR2/3 antibodies of this invention are capable of killing a cell that expresses about 10,000 FGFR3 molecules per cell or more (such as about 11,000, about 12,000, about 13,000, about 14,000, about 15,000, about 16,000, about 17,000, about 18,000 or more FGFR3 molecules per cell). In other embodiments, the cell expresses about 2000, about 3000, about 4000, about 5000, about 6000, about 7000, about 8000, or more FGFR3 molecules per cell. In some embodiments, the the anti-FGFR2/3 antibodies of this invention are capable of killing a cell that expresses about 10,000 FGFR2 molecules per cell or more (such as about 11,000, about 12,000, about 13,000, about 14,000, about 15,000, about 16,000, about 17,000, about 18,000 or more FGFR3 molecules per cell). In other embodiments, the cell expresses about 2000, about 3000, about 4000, about 5000, about 6000, about 7000, about 8000, or more FGFR2 molecules per cell.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit constitutive FGFR3 activity. In some embodiments, constitutive FGFR3 activity is ligand-dependent FGFR3 constitutive activity. In some embodiments, constitutive FGFR3 activity is ligand-independent constitutive FGFR3 activity. In one aspect, the anti-FGFR2/3 antibodies of the

invention inhibit constitutive FGFR2 activity. In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit constitutive FGFR2 and FGFR3 activity.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{R248C}. As used herein the term "comprising a mutation corresponding to FGFR3-IIIb^{R248C}" is understood to encompass FGFR3-IIIb^{R248C} and FGFR3-IIIc^{R248C}, as well as additional FGFR3 forms comprising an R to C mutation at a position corresponding to FGFR3-IIIb R248. One of ordinary skill in the art understands how to align FGFR3 sequences in order identify corresponding residues between respective FGFR3 sequences, e.g., aligning a FGFR3- IIIc sequence with a FGFR3-IIIb sequence to identify the position in FGFR3 corresponding R248 position in FGFR3-IIIb. In some embodiments, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3-IIIb^{R248C} and/or FGFR3-IIIc^{R248C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{K652E}. For convenience, the term "comprising a mutation corresponding to FGFR3-IIIb ^{K652E}" is understood to encompass FGFR3-IIIb ^{K652E} and FGFR3-IIIc ^{K650E}, as well as additional FGFR3 forms comprising a K to E mutation at a position corresponding to FGFR3-IIIb K652. One of ordinary skill in the art understands how to align FGFR3 sequences in order identify corresponding residues between respective FGFR3 sequences, e.g., aligning a FGFR3- IIIc sequence with a FGFR3-IIIb sequence to identify the position in FGFR3 corresponding K652 position in FGFR3-IIIb. In some embodiments, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3-IIIb ^{K652E} and/or FGFR3-IIIc ^{K650E}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{S249C}. For convenience, the term "comprising a mutation corresponding to FGFR3-IIIb^{S249C} " is understood to encompass

FGFR3-IIIb ^{S249C} and FGFR3-IIIc ^{S249C}, as well as additional FGFR3 forms comprising an S to C mutation at a position corresponding to FGFR3-IIIb S249. In some embodiments, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3-IIIb^{S249C} and/or FGFR3-IIIc ^{S249C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{G372C}. For convenience, the term "comprising a mutation corresponding to FGFR3-IIIb^{G372C} " is understood to encompass FGFR3-IIIb ^{G372C} and FGFR3-IIIc ^{G370C}, as well as additional FGFR3 forms comprising a G to C mutation at a position corresponding to FGFR3-IIIb G372. In some embodiments, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3-IIIb^{G372C} and/or FGFR3-IIIc ^{G370C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{Y375C}. For convenience, the term "comprising a mutation corresponding to FGFR3-IIIb^{Y375C} " is understood to encompass FGFR3-IIIb^{Y375C} and FGFR3-IIIc^{Y373C}, as well as additional FGFR3 forms comprising an S to C mutation at a position corresponding to FGFR3-IIIb S249. In some embodiments, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3-IIIb^{Y375C} and/or FGFR3-IIIc^{Y373C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit (a) FGFR3-IIIb^{K652E} and (b) one or more of FGFR3-IIIb^{R248C}, FGFR3-IIIb^{Y375C}, FGFR3 -IIIb^{S249C}, and FGFR3IIIb ^{G372C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit (a) FGFR3-IIIc^{K650E} and (b) one or more of FGFR3-IIIc^{R248C}, FGFR3-IIIc^{Y373C}, FGFR3 -IIIc^{S249C}, and FGFR3IIIc ^{G370C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit (a) FGFR3-IIIb^{R248C} and (b) one or more of FGFR3-IIIb^{K652E}, FGFR3-IIIb^{Y375C}, FGFR3-IIIb^{S249C}, and FGFR3-IIIb^{G372C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit (a) FGFR3-IIIc^{R248C} and (b) one or more of FGFR3-IIIc^{K650E}, FGFR3-IIIc^{Y373C}, FGFR3-IIIc^{S249C}, and FGFR3-IIIc^{G370C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit (a) FGFR3-IIIb^{G372C} and (b) one or more of FGFR3-IIIb^{K652E}, FGFR3-IIIb^{Y375C}, FGFR3-IIIb^{S249C}, and FGFR3-IIIb^{R248C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit (a) FGFR3-IIIc^{G370C} and (b) one or more of FGFR3-IIIc^{K650E}, FGFR3-IIIc^{Y373C}, FGFR3-IIIc^{S249C}, and FGFR3-IIIc^{R248C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3-IIIb^{R248C}, FGFR3-IIIb^{K652E}, FGFR3-IIIb^{Y375C}, FGFR3-IIIb ^{S249C}, and FGFR3-IIIb ^{G372C}.

In one aspect, the anti-FGFR2/3 antibodies of the invention inhibit FGFR3-IIIc^{R248C}, FGFR3-IIIc^{K650E}, FGFR3-IIIc^{Y373C}, FGFR3-IIIc ^{S249C}, and FGFR3-IIIc ^{G370C}.

In one aspect, the invention provides an isolated anti-FGFR2/3 antibody comprising at least one, two, three, four, or five hypervariable region (HVR) sequences selected from: SEQ ID NO 1: RASQDVDTSLA, SEQ ID NO 2: SASFLYS, SEQ ID NO 3: QQSTGHPQT, SEQ ID NO 4: GFPFTSQGIS, SEQ ID NO 5: RTHLGDGSTNYADSVKG, and SEQ ID NO 6: ARTYGIYDTYDKYTEYVMDY. In a specific embodiment, the invention provides the 2B.1.3.10 anti-FGFR2/3 antibody comprising HVR-L1: RASQDVDTSLA, HVR-L2: SASFLYS, HVR-L3: QQSTGHPQT, HVR-H1: GFPFTSQGIS, HVR-H2: RTHLGDGSTNYADSVKG, and HVR-H3: ARTYGIYDTYDKYTEYVMDY.

In one aspect, the invention provides an isolated anti-FGFR2/3 antibody comprising at least one, two, three, four, or five hypervariable region (HVR) sequences selected from: SEQ ID NO 7: RASQDVDTSLA, SEQ ID NO 8: SASFLYS, SEQ ID NO 9: QQSTGHPQT, SEQ ID NO 10: GFPFTSTGIS, SEQ ID NO 11: RTHLGDGSTNYADSVKG, and SEQ ID NO

12: ARTYGIYDTYDMYTEYVMDY. In a specific embodiment, the invention provides the 2B.1.3.12 anti-FGFR2/3 antibody comprising HVR-L1: RASQDVDTSLA, HVR-L2: SASFLYS, HVR-L3: QQSTGHPQT, HVR-H1: GFPFTSTGIS, HVR-H2: RTHLGDGSTNYADSVKG, and HVR-H3: ARTYGIYDTYDMYTEYVMDY.

In certain embodiments, the HVR-H1 of an anti-FGFR2/3 antibody described herein comprises the sequence FTS at positions 4-6 of SEQ ID NO:4.

In certain embodiments, at least one HVR of an anti-FGFR2/3 antibody described herein is a variant HVR, where the variant HVR sequence comprises modification of at least one residue (at least two residues, at least three or more residues) of the sequence depicted in SEQ ID NOs:1-6. The modification desirably is a substitution, insertion, or deletion. In some embodiments, a HVR-L1 variant comprises 1-6 (1, 2, 3, 4, 5, or 6) substitutions. In some embodiments, a HVR-L2 variant comprises 1-6 (1, 2, 3, 4, 5, or 6) substitutions. In some embodiments, a HVR-L3 variant comprises 1-6 (1, 2, 3, 4, 5, or 6) substitutions. In some embodiments, a HVR-H1 variant comprises 1-6 (1, 2, 3, 4, 5, or 6) substitutions. In some embodiments, a HVR-H2 variant comprises 1-6 (1, 2, 3, 4, 5, or 6) substitutions. In some embodiments, a HVR-H3 variant comprises 1-6 (1, 2, 3, 4, 5, or 6) substitutions.

In certain embodiments, the HVR-H1 of an anti-FGFR2/3 antibody described herein is a variant HVR-H1 wherein the variant HVR-H1 comprises substitutions at amino acids P3 and/or Q7 (SEQ ID NO:4). In specific embodiments, the variant HVR-H1 comprises a P3T substitution. In specific embodiments, the variant HVR-H1 comprises a Q7T or a Q7L substitution. In specific embodiments, the variant HVR-H1 comprises a P3T and a Q7L substitution. In specific embodiments, the variant HVR-H1 comprises a P3T and a Q7T substitution. In certain embodiments, the variant HVR-H1 comprises a sequence selected from the group listed in **Table 11**: TFTST, PFTSL, PFTSQ, and PFTST.

In certain embodiments, the HVR-H3 of an anti-FGFR2/3 antibody described herein is a variant HVR-H3 wherein the variant HVR-H3 comprises substitutions at amino acids T9, D11, and/or K12 (SEQ ID NO:6). In specific embodiments, the variant HVR-H3 comprises a T9I substitution. In specific embodiments, the variant HVR-H3 comprises a T9L substitution. In specific embodiments, the variant HVR-H3 comprises a D11V substitution. In specific embodiments, the variant HVR-H3 comprises a D11G substitution. In specific embodiments, the variant HVR-H3 comprises a D11E substitution. In specific embodiments, the variant HVR-H3 comprises a K12D substitution. In specific embodiments, the variant HVR-H3 comprises a K12N substitution. In specific embodiments, the variant HVR-H3 comprises a K12G substitution. In specific embodiments, the variant HVR-H3 comprises a K12E substitution. In specific embodiments, the variant HVR-H3 comprises a K12M substitution. In specific embodiments, the variant HVR-H3 comprises a T9L, a D11V, and a K12D substitution. In specific embodiments, the variant HVR-H3 comprises only a K12D substitution. In specific embodiments, the variant HVR-H3 comprises a T9I, a D11G, and a K12G substitution. In specific embodiments, the variant HVR-H3 comprises only a K12E substitution. In specific embodiments, the variant HVR-H3 comprises a T9I and a D11E substitution. In specific embodiments, the variant HVR-H3 comprises only a K12M substitution. In certain embodiments, the variant HVR-H3 comprises a sequence selected from the group listed in **Table 11**: LYVD, TYDN, IYGG, TYDE, IKEK, TYDK, and TYDM.

In certain embodiemnts, the HVR-H1 of an anti-FGFR2/3 antibody described herein is a variant HVR-H1 wherein the variant HVR-H1 comprises substitutions at amino acids P3 and/or Q7 (SEQ ID NO:4) and the HVR-H3 of an anti-FGFR2/3 antibody described herein is a variant HVR-H3 wherein the variant HVR-H3 comprises substitutions at amino acids T9, D11, and/or K12 (SEQ ID NO:6). In certain embodiments, the variant HVR-H1 and HVR-

H3 of an anti-FGFR2/3 antibody of this invention comprise sequences selected from the group listed in **Table 11**: TFTST (HVR-H1) and LYVD (HVR-H3), TFTST (HVR-H1) and TYDN (HVR-H3), TFTST (HVR-H1) and IYGG (HVR-H3), TFTST (HVR-H1) and TYDE (HVR-H3), PFTSL (HVR-H1) and IYEK (HVR-H3), PFTSQ (HVR-H1) and TYDK (HVR-H3), PFTST (HVR-H1) and TYDM (HVR-H3).

In certain embodiments, the anti-FGFR2/3 antibody of this invention comprises a HVR-H2 sequence selected from the group consisting of the sequences recited in SEQ ID NOs:13-44. In certain embodiments, the anti-FGFR2/3 antibody of this invention comprises a HVR-H2 sequence selected from the group consisting of the sequences recited in SEQ ID NOs:45-50.

In specific embodiments, the anti-FGFR2/3 antibodies of this invention bind to FGFR2-IIIb (SEQ ID NOs:51 and 52), FGFR2-IIIc (SEQ ID NOs:53 and 54), FGFR3-IIIb (SEQ ID NOs:55 and 56), and/or FGFR3-IIIc (SEQ ID NOs:57 and 58). In certain embodiments, the anti-FGFR2/3 antibodies of this invention bind to FGFR2-IIIb, FGFR2-IIIc, FGFR3-IIIb, and FGFR3-IIIc. In specific embodiments, the anti-FGFR2/3 antibodies of this invention bind to an FGFR selected from the group consisting of FGFR2-IIIb, FGFR2-IIIc, FGFR3-IIIb, and FGFR3-IIIc. In specific embodiments, the anti-FGFR2/3 antibodies of this invention bind to two FGFRs selected from the group consisting of FGFR2-IIIb, FGFR2-IIIc, FGFR3-IIIb, and FGFR3-IIIc. In specific embodiments, the anti-FGFR2/3 antibodies of this invention bind to three FGFRs selected from the group consisting of FGFR2-IIIb, FGFR2-IIIc, FGFR3-IIIb, and FGFR3-IIIc.

Antibodies of the invention can comprise any suitable framework variable domain sequence, provided binding activity to FGFR3 and FGFR2 are substantially retained. For example, in some embodiments, antibodies of the invention comprise a human subgroup III heavy chain framework consensus sequence. In one embodiment of these antibodies, the

framework consensus sequence comprises a substitution at position 71, 73, and/or 78. In some embodiments of these antibodies, position 71 is A, 73 is T and/or 78 is A. In one embodiment, these antibodies comprise heavy chain variable domain framework sequences of huMAb4D5-8 (HERCEPTIN®, Genentech, Inc., South San Francisco, CA, USA) (also referred to in U.S. Patent Nos. 6,407,213 & 5,821,337, and Lee et al., J. Mol. Biol. (2004), 340(5):1073-1093). In one embodiment, these antibodies further comprise a human κI light chain framework consensus sequence. In a particular embodiment, these antibodies comprise light chain HVR sequences of huMAb4D5-8 as described in U.S. Patent Nos. 6,407,213 & 5,821,337.) In one embodiment, these antibodies comprise light chain variable domain sequences of huMAb4D5-8 (HERCEPTIN®, Genentech, Inc., South San Francisco, CA, USA) (also referred to in U.S. Patent Nos. 6,407,213 & 5,821,337, and Lee et al., J. Mol. Biol. (2004), 340(5):1073-1093).

In one embodiment, the amino acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:59.

In one embodiment, the amino acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:60.

In one embodiment, the amino acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:61.

In one embodiment, the amino acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:62.

In one embodiment, the amino acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:63.

In one embodiment, the amino acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:64.

In one embodiment, the amino acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:65.

In one embodiment, the amino acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:66.

In one embodiment, the amino acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:75.

In one embodiment, the amino acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:76.

In one embodiment, the amino acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:77.

In one embodiment, the amino acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:78.

In one embodiment, the amino acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:79.

In one embodiment, the amino acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:80.

In one embodiment, the amino acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:81.

In one embodiment, the amino acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:82.

In specific embodiments, the antibody of this invention comprises a light chain comprising amino acid SEQ ID NO:59 and a heavy chain amino acid sequence comprising SEQ ID NO:75. In specific embodiments, the antibody of this invention comprises a light chain amino acid sequence comprising SEQ ID NO:60 and a heavy chain amino acid sequence comprising SEQ ID NO:76. In specific embodiments, the antibody of this

invention comprises a light chain amino acid sequence comprising SEQ ID NO:61 and a heavy chain amino acid sequence comprising SEQ ID NO:77. In specific embodiments, the antibody of this invention comprises a light chain amino acid sequence comprising SEQ ID NO:62 and a heavy chain amino acid sequence comprising SEQ ID NO:78. In specific embodiments, the antibody of this invention comprises a light chain amino acid sequence comprising SEQ ID NO:63 and a heavy chain amino acid sequence comprising SEQ ID NO:79. In specific embodiments, the antibody of this invention comprises a light chain amino acid sequence comprising SEQ ID NO:64 and a heavy chain amino acid sequence comprising SEQ ID NO:60. In specific embodiments, the antibody of this invention comprises a light chain amino acid sequence comprising SEQ ID NO:65 and a heavy chain amino acid sequence comprising SEQ ID NO:81. In specific embodiments, the antibody of this invention comprises a light chain amino acid sequence comprising SEQ ID NO:66 and a heavy chain amino acid sequence comprising SEQ ID NO:82.

In one embodiment, the nucleic acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:67.

In one embodiment, the nucleic acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:68.

In one embodiment, the nucleic acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:69.

In one embodiment, the nucleic acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:70.

In one embodiment, the nucleic acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:71.

In one embodiment, the nucleic acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:72.

In one embodiment, the nucleic acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:73.

In one embodiment, the nucleic acid sequence of the light chain of an antibody of this invention comprises SEQ ID NO:74.

In one embodiment, the nucleic acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:83.

In one embodiment, the nucleic acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:84.

In one embodiment, the nucleic acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:85.

In one embodiment, the nucleic acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:86.

In one embodiment, the nucleic acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:87.

In one embodiment, the nucleic acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:88.

In one embodiment, the nucleic acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:89.

In one embodiment, the nucleic acid sequence of the heavy chain of an antibody of this invention comprises SEQ ID NO:90.

In specific embodiments, the antibody of this invention comprises a light chain comprising nucleic acid SEQ ID NO:67 and a heavy chain nucleic acid sequence comprising SEQ ID NO:83. In specific embodiments, the antibody of this invention comprises a light chain nucleic acid sequence comprising SEQ ID NO:68 and a heavy chain nucleic acid sequence comprising SEQ ID NO:84. In specific embodiments, the antibody of this

invention comprises a light chain nucleic acid sequence comprising SEQ ID NO:69 and a heavy chain nucleic acid sequence comprising SEQ ID NO:85. In specific embodiments, the antibody of this invention comprises a light chain nucleic acid sequence comprising SEQ ID NO:70 and a heavy chain nucleic acid sequence comprising SEQ ID NO:86. In specific embodiments, the antibody of this invention comprises a light chain nucleic acid sequence comprising SEQ ID NO:71 and a heavy chain nucleic acid sequence comprising SEQ ID NO:87. In specific embodiments, the antibody of this invention comprises a light chain nucleic acid sequence comprising SEQ ID NO:72 and a heavy chain nucleic acid sequence comprising SEQ ID NO:88. In specific embodiments, the antibody of this invention comprises a light chain nucleic acid sequence comprising SEQ ID NO:73 and a heavy chain nucleic acid sequence comprising SEQ ID NO:89. In specific embodiments, the antibody of this invention comprises a light chain nucleic acid sequence comprising SEQ ID NO:74 and a heavy chain nucleic acid sequence comprising SEQ ID NO:90.

In certain embodiments, the anti-FGFR2/3 antibody comprises a light chain amino acid sequence comprising SEQ ID NO:65 and a heavy chain nucleic acid sequence comprising SEQ ID NO:81. In specific embodiments, the anti-FGFR2/3 antibody has the following CDRs:

HVR-L1: RASQDVDTSLA (SEQ ID NO:1)

HVR-L2: SASFLYS (SEQ ID NO:2)

HVR-L3: QQSTGHPQT (SEQ ID NO:3)

HVR-H1: GFPFTSQGIS (SEQ ID NO:4)

HVR-H2: RTHLGDGSTNYADSVKG (SEQ ID NO:5)

HVR-H3: ARTYGIYDTYDKYTEYVMDY (SEQ ID NO:6)

In certain embodiments, the anti-FGFR2/3 antibody comprises a light chain amino acid sequence comprising SEQ ID NO:66 and a heavy chain nucleic acid sequence

comprising SEQ ID NO:82. In certain embodiments, the anti-FGFR2/3 antibody has the following CDRs:

HVR-L1: RASQDVDTSLA (SEQ ID NO:7)

HVR-L2: SASFLYS (SEQ ID NO:8)

HVR-L3: QQSTGHPQT (SEQ ID NO:9)

HVR-H1: GFPFTSTGIS (SEQ ID NO:10)

HVR-H2: RTHLGDGSTNYADSVKG (SEQ ID NO:11)

HVR-H3: ARTYGIYDTYDMYTEYVMDY (SEQ ID NO:12)

In certain embodiments the anti-FGFR2/3 antibody binds to a region within amino acids 153-251 of a FGFR2 (SEQ ID NOs:52 and 54):

APYWTNTEKMEKRLHAVPAANTVKFRCPAGGNPMPTMRWLKNGKEFKQEHRIGGYKVRNQHWSLIMESVVPSDKGNYTCVVENEYGSINHTYHLDVVER.

In certain embodiments the anti-FGFR2/3 antibody binds to a region within amino acids 150-248 of a FGFR3 (SEQ ID NOs:56 and 58):

APYWTRPERMDKKLLAVPAANTVRFRCPAAGNPTPSISWLKNGREFRGEHRIGGIKLRHQQWSLVMESVVPSDRGNYTCVVENKFGSIRQTYTLDVLER.

In a preferred embodiment the anti-FGFR2/3 antibody binds to a region within amino acids 153-251 of a FGFR2 (SEQ ID NOs:52 and 54) and to a region within amino acids 150-248 of a FGFR3 (SEQ ID NOs:56 and 58).

In certain embodiments the anti-FGFR2/3 antibody binds to a region within amino acids 157-181 (TNTEKMEKRLHAVPAANTVKFRCPA) of a FGFR2 (SEQ ID NOs:52 and 54) (**Figure 9**). In certain embodiments the anti-FGFR2/3 antibody binds to a region within amino acids 207-220 (YKVRNQHWSLIMES) of a FGFR2 (SEQ ID NOs:52 and 54) (**Figure 9**). In specific embodiments, the anti-FGFR2/3 antibody binds to a region of

FGFR2-IIIb that aligns with SEQ ID NO:52. In specific embodiments, the anti-FGFR2/3 antibody binds to a region of FGFR2-IIIc that aligns with SEQ ID NO:54.

In certain embodiments, the anti-FGFR2/3 antibody binds to amino acids 157-181 (TNTEKMEKRLHAVPAANTVKFRCPA) of FGFR2-IIIb (SEQ ID NO:52). In certain embodiments, the anti-FGFR2/3 antibody binds to amino acids 157-181 of FGFR2-IIIc (SEQ ID NO:54). In certain embodiments, the anti-FGFR2/3 antibody binds to amino acids 207-220 (YKVRNQHWSLIMES) of a FGFR2 (SEQ ID NOs:52 and 54) (**Figure 9**). In certain embodiments, the anti-FGFR2/3 antibody binds to amino acids 207-220 of FGFR2-IIIb (SEQ ID NO:52). In certain embodiments, the anti-FGFR2/3 antibody binds to amino acids 207-220 of FGFR2-IIIc (SEQ ID NO:54).

In a specific embodiment, the anti-FGFR2/3 antibody binds to a region within amino acids 157-181(TNTEKMEKRLHAVPAANTVKFRCPA) of a FGFR2 (SEQ ID NOs:52 and 54) and to a region within amino acids 207-220 (YKVRNQHWSLIMES) of FGFR2-IIIb (SEQ ID NOs:52 and 54). In a specific embodiment, the anti-FGFR2/3 antibody binds to amino acids 157-181(TNTEKMEKRLHAVPAANTVKFRCPA) of a FGFR2 (SEQ ID NOs:52 and 54) and to amino acids 207-220 (YKVRNQHWSLIMES) of FGFR2-IIIb (SEQ ID NOs:52 and 54).

In certain embodiments the anti-FGFR2/3 antibody binds to a region within amino acids 154-178 (TRPERMDKKLLAVPAANTVRFRCPA) of FGFR3-IIIb (SEQ ID NO:56). In certain embodiments the anti-FGFR2/3 antibody binds to a region within amino acids 154-178 (TRPERMDKKLLAVPAANTVRFRCPA) of FGFR3-IIIc (SEQ ID NO:58). In certain embodiments the anti-FGFR2/3 antibody binds to a region within amino acids 204-217 (IKLRHQQWSLVMES) of FGFR3-IIIb (SEQ ID NO:56). In certain embodiments the anti-FGFR2/3 antibody binds to a region within amino acids 204-217 (IKLRHQQWSLVMES) of FGFR3-IIIc (SEQ ID NO:58). In specific embodiments, the anti-FGFR2/3 antibody binds to

a region of FGFR3-IIIb that aligns with SEQ ID NO:56. In specific embodiments, the anti-FGFR2/3 antibody binds to a region of FGFR3-IIIb that aligns with SEQ ID NO:58.

In specific embodiments, the anti-FGFR2/3 antibody binds to amino acids 154-178 (TRPERMDKKLLAVPAANTVRFRCPA) of FGFR3-IIIb (SEQ ID NO:56). In specific embodiments, the anti-FGFR2/3 antibody binds to amino acids 154-178 (TRPERMDKKLLAVPAANTVRFRCPA) of FGFR3-IIIc (SEQ ID NO:58). In specific embodiments, the anti-FGFR2/3 antibody binds to amino acids 204-217 (IKLRHQQWSLVMES) of FGFR3-IIIb (SEQ ID NO:56). In specific embodiments, the anti-FGFR2/3 antibody binds to amino acids 204-217 (IKLRHQQWSLVMES) of FGFR3-IIIc (SEQ ID NO:58).

In a preferred embodiment, the anti-FGFR2/3 antibody binds to the following epitopes of an FGFR2: TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO:91) and YKVRNQHWSLIMES (SEQ ID NO:92). In a preferred embodiment, the anti-FGFR2/3 antibody binds to the following epitopes of an FGFR3: TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO:93) and IKLRHQQWSLVMES (SEQ ID NO:94). In preferred embodiments, the anti-FGFR2/3 antibody binds to following epitopes:

FGFR2: TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO:91) and YKVRNQHWSLIMES (SEQ ID NO:92), and

FGFR3: TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO:93) and IKLRHQQWSLVMES (SEQ ID NO:94).

In certain embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:91-94. In certain embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:91 and 92. In certain embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:91-93. In certain embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:91, 93, and 94. In certain

embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:91 and 94. In certain embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:92-94. In certain embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:92 and 93. In certain embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:92 and 94. In certain embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:93 and 91. In certain embodiments, the anti-FGFR2/3 antibody binds to SEQ ID NOs:91, 92, and 94. In certain embodiments, the anti-FGFR2/3 antibody binds to a combination of any two or more epitopes provided in SEQ ID NOs:91-94.

In certain embodiments, the anti-FGFR2/3 antibody binds to a region within amino acids 153-251 of FGFR2-IIIb (SEQ ID NO:52). In certain embodiments, the anti-FGFR2/3 antibody binds to a region within amino acids 153-251 of FGFR2-IIIc (SEQ ID NO:54). In preferred embodiments, the anti-FGFR2/3 antibody binds to a region within amino acids 153-251 of FGFR2-IIIb (SEQ ID NO:52) and FGFR2-IIIc (SEQ ID NO:54). In certain embodiments, the anti-FGFR2/3 antibody binds to a region within amino acids 150-248 of FGFR3-IIIb (SEQ ID NO:56). In certain embodiments, the anti-FGFR2/3 antibody binds to a region within amino acids 150-248 of FGFR3-IIIb (SEQ ID NO:58). In preferred embodiments, the anti-FGFR2/3 antibody binds to a region within amino acids 150-248 of FGFR3-IIIb (SEQ ID NO:56) and FGFR3-IIIc (SEQ ID NO:58).

In a preferred embodiment, the anti-FGFR2/3 antibody binds to a region within amino acids 153-251 of FGFR2-IIIb (SEQ ID NO:52) and/or FGFR2-IIIc (SEQ ID NO:54) and to a region within amino acids 150-248 of FGFR3-IIIb (SEQ ID NO:56) and/or FGFR3-IIIc (SEQ ID NO:58).

In some embodiments, the anti-FGFR2/3 antibody binds to an epitope comprising one or more amino acids selected from T157, N158, T159, E160, K161, M162, E163, K164, R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178,

C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody binds to an epitope comprising amino acids T157, N158, T159, E160, K161, M162, E163, K164, R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178, C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 4 angstroms or less from one or more amino acids T157, N158, T159, E160, K161, M162, E163, K164, R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178, C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 4 angstroms or less from amino acids T157, N158, T159, E160, K161, M162, E163, K164, R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178, C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 3.5 angstroms or less from one or more amino acids T157, N158, T159, E160, K161, M162, E163, K164, R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178, C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 3.5 angstroms or less from amino acids T157, N158, T159, E160, K161, M162, E163, K164, R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178, C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 3.0 angstroms or less from one or more amino acids T157, N158, T159, E160, K161, M162, E163, K164, R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178, C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 3.0 angstroms or less from amino acids T157, N158, T159, E160, K161, M162, E163, K164,

R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178, C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 4.0, 3.75, 3.5, 3.25, or 3.0 angstroms or less from one or more amino acids T157, N158, T159, E160, K161, M162, E163, K164, R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178, C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 4.0, 3.75, 3.5, 3.25, or 3.0 angstroms or less from amino acids T157, N158, T159, E160, K161, M162, E163, K164, R165, L166, H167, A168, V169, P170, A171, A172, N173, T174, V175, K176, F177, R178, C179, P180, and A181 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the one or more amino acids and/or the one or more amino acid residues is about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 amino acids and/or amino acid residues. In some embodiments, the epitope is determined by crystallography (e.g., crystallography methods described in the Examples). In preferred embodiments, the anti-FGFR2/3 antibody binds to human FGFR2 (hFGFR2) (e.g., SEQ ID NOs:52 and 54).

In some embodiments, the anti-FGFR2/3 antibody binds to an epitope comprising one or more amino acids selected from Y207, K208, V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody binds to an epitope comprising amino acids Y207, K208, V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 4 angstroms or less from one or more amino acids Y207, K208, V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 4 angstroms or less from amino acids Y207, K208,

V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 3.5 angstroms or less from one or more amino acids Y207, K208, V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 3.5 angstroms or less from amino acids Y207, K208, V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 3.0 angstroms or less from one or more amino acids Y207, K208, V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 3.0 angstroms or less from amino acids Y207, K208, V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 4.0, 3.75, 3.5, 3.25, or 3.0 angstroms or less from one or more amino acids Y207, K208, V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR2 is positioned 4.0, 3.75, 3.5, 3.25, or 3.0 angstroms or less from amino acids Y207, K208, V209, R210, N211, Q212, H213, W214, S215, L216, I217, M218, E219, and S220 of FGFR2 (e.g., SEQ ID NOs:52 and 54). In some embodiments, the one or more amino acids and/or the one or more amino acid residues is about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 amino acids and/or amino acid residues. In some embodiments, the epitope is determined by crystallography (e.g., crystallography methods described in the Examples).). In preferred embodiments, the anti-FGFR2/3 antibody binds to human FGFR2 (hFGFR2) (e.g., SEQ ID NOs:52 and 54).

In some embodiments, the anti-FGFR2/3 antibody binds to an epitope comprising one or more amino acids selected from T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170, T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody binds to an epitope comprising amino acids T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170, T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 4 angstroms or less from one or more amino acids T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170, T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 4 angstroms or less from amino acids T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170, T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 3.5 angstroms or less from one or more amino acids T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170, T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 3.5 angstroms or less from amino acids T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170, T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 3.0 angstroms or less from one or more amino acids T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170,

T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 3.0 angstroms or less from amino acids T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170, T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). . In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 4.0, 3.75, 3.5, 3.25, or 3.0 angstroms or less from one or more amino acids T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170, T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 4.0, 3.75, 3.5, 3.25, or 3.0 angstroms or less from amino acids T154, R155, P156, E157, R158, M159, D160, K161, K162, L163, L164, A165, V166, P167, A168, A169, N170, T171, V172, R173, F174, R175, C176, P177, and A178 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the one or more amino acids and/or the one or more amino acid residues is about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 amino acids and/or amino acid residues. In some embodiments, the epitope is determined by crystallography (e.g., crystallography methods described in the Examples). In preferred embodiments, the anti-FGFR2/3 antibody binds to human FGFR3 (hFGFR3) (e.g., SEQ ID NOs:56 and 58).

In some embodiments, the anti-FGFR2/3 antibody binds to an epitope comprising one or more amino acids selected from I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody binds to an epitope comprising amino acids I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 4 angstroms or less from one or more amino

acids I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 4 angstroms or less from amino acids I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 3.5 angstroms or less from one or more amino acids I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 3.5 angstroms or less from amino acids I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 3.0 angstroms or less from one or more amino acids I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 3.0 angstroms or less from amino acids I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 4.0, 3.75, 3.5, 3.25, or 3.0 angstroms or less from one or more amino acids I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the anti-FGFR2/3 antibody when bound to FGFR3 is positioned 4.0, 3.75, 3.5, 3.25, or 3.0 angstroms or less from amino acids I204, K205, L206, R207, H208, Q209, Q210, W211, S212, L213, V214, M215, E216, and S217 of FGFR3 (e.g., SEQ ID NOs:56 and 58). In some embodiments, the one or more amino acids and/or the one or more amino acid residues is about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 amino acids and/or amino acid

residues. In some embodiments, the epitope is determined by crystallography (e.g., crystallography methods described in the Examples). In preferred embodiments, the anti-FGFR2/3 antibody binds to human FGFR3 (hFGFR3) (e.g., SEQ ID NOs:56 and 58).

In specific embodiments, the anti-FGFR2/3 antibody binds to one epitope on FGFR2 selected from SEQ ID NOs: 91 and 92 and one epitope on FGFR3 selected from SEQ ID NOs: 93 and 94. In certain embodiments, the anti-FGFR2/3 antibody binds to two epitopes on FGFR2 comprising SEQ ID NOs: 91 and 92 and one epitope on FGFR3 selected from SEQ ID NOs: 93 and 94. In certain embodiments, the anti-FGFR2/3 antibody binds to one epitope on FGFR2 selected from SEQ ID NOs: 91 and 92 and two epitopes on FGFR3 comprising SEQ ID NOs: 93 and 94. In a preferred embodiment, the anti-FGFR2/3 antibody binds to two epitopes on FGFR2 comprising SEQ ID NOs: 91 and 92 and two epitopes on FGFR3 comprising SEQ ID NOs: 93 and 94 (**Figure 9**).

In one aspect, the invention provides an anti-FGFR2/3 antibody that binds a polypeptide comprising, consisting essentially of or consisting of the following amino acid sequence: TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO:91) and/or YKVRNQHWSLIMES (SEQ ID NO:92).

In one aspect, the invention provides an anti-FGFR2/3 antibody that binds a polypeptide comprising, consisting essentially of or consisting of the following amino acid sequence: TRRERMDKKLLAVPAANTVRFRCPA (SEQ ID NO:93) and/or IKLRHQQWSLVMES (SEQ ID NO:94).

In one aspect, the invention provides an anti-FGFR2/3 antibody that binds a polypeptide comprising, consisting essentially of or consisting of the following amino acid sequence: TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO:91) or YKVRNQHWSLIMES (SEQ ID NO:92) and TRRERMDKKLLAVPAANTVRFRCPA (SEQ ID NO:93) or IKLRHQQWSLVMES (SEQ ID NO:94).

In one embodiment, an anti-FGFR2/3 antibody of the invention specifically binds an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with the sequence TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO:91) and/or YKVRNQHWSLIMES (SEQ ID NO:92). In one embodiment, an anti-FGFR2/3 antibody of the invention specifically binds an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with the sequence TRRERMDKKLLAVPAANTVRFRCPA (SEQ ID NO:93) and/or IKLRHQQWSLVMES (SEQ ID NO:94).

In one embodiment, an anti-FGFR2/3 antibody of the invention specifically binds an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with the sequence TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO:91) or YKVRNQHWSLIMES (SEQ ID NO:92) and an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with the sequence TRRERMDKKLLAVPAANTVRFRCPA (SEQ ID NO:93) or IKLRHQQWSLVMES (SEQ ID NO:94).

One of ordinary skill in the art understands how to align FGFR3 sequences in order identify corresponding residues between respective FGFR3 sequences. Similarly, one of ordinary skill in the art understands how to align FGFR2 sequences in order identify corresponding residues between respective FGFR2 sequences.

In one aspect, the invention provides an anti-FGFR2/3 antibody that competes with any of the above-mentioned antibodies for binding to FGFR3 and/or FGFR2. In one aspect, the invention provides an anti- FGFR2/3 antibody that binds to the same or a similar epitope on FGFR3 and/or FGFR2 as any of the above-mentioned antibodies.

As is known in the art, and as described in greater detail herein, the amino acid position/boundary delineating a hypervariable region of an antibody can vary, depending on the context and the various definitions known in the art (as described below). Some positions within a variable domain may be viewed as hybrid hypervariable positions in that these positions can be deemed to be within a hypervariable region under one set of criteria while being deemed to be outside a hypervariable region under a different set of criteria. One or more of these positions can also be found in extended hypervariable regions (as further defined below).

In some embodiments, the antibody is a monoclonal antibody. In other embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is selected from the group consisting of a chimeric antibody, an affinity matured antibody, a humanized antibody, and a human antibody. In certain embodiments, the antibody is an antibody fragment. In some embodiments, the antibody is a Fab, Fab', Fab'-SH, $F(ab')_2$, or scFv.

In some embodiment, the FGFR2/3 antibody is a one-armed antibody (i.e., the heavy chain variable domain and the light chain variable domain form a single antigen binding arm) comprising an Fc region, wherein the Fc region comprises a first and a second Fc polypeptide, wherein the first and second Fc polypeptides are present in a complex and form a Fc region that increases stability of said antibody fragment compared to a Fab molecule comprising said antigen binding arm. See, e.g., WO2006/015371.

In one embodiment, the antibody is a chimeric antibody, for example, an antibody comprising antigen binding sequences from a non-human donor grafted to a heterologous non-human, human, or humanized sequence (e.g., framework and/or constant domain sequences). In one embodiment, the non-human donor is a mouse. In a further embodiment, an antigen binding sequence is synthetic, e.g., obtained by mutagenesis (e.g., phage display screening, etc.). In a particular embodiment, a chimeric antibody of the invention has murine

V regions and a human C region. In one embodiment, the murine light chain V region is fused to a human kappa light chain. In another embodiment, the murine heavy chain V region is fused to a human IgG1 C region.

Humanized antibodies of the invention include those that have amino acid substitutions in the framework region (FR) and affinity maturation variants with changes in the grafted CDRs. The substituted amino acids in the CDR or FR are not limited to those present in the donor or recipient antibody. In other embodiments, the antibodies of the invention further comprise changes in amino acid residues in the Fc region that lead to improved effector function including enhanced CDC and/or ADCC function and B-cell killing. Other antibodies of the invention include those having specific changes that improve stability. In other embodiments, the antibodies of the invention comprise changes in amino acid residues in the Fc region that lead to decreased effector function, e.g., decreased CDC and/or ADCC function and/or decreased B-cell killing. In some embodiments, the antibodies of the invention are characterized by decreased binding (such as absence of binding) to human complement factor C1q and/or human Fc receptor on natural killer (NK) cells. In some embodiments, the antibodies of the invention are characterized by decreased binding (such as the absence of binding) to human FcγRI, FcγRIIA, and/or FcγRIIIA. In some embodiments, the antibodies of the invention are of the IgG class (e.g., IgG1 or IgG4) and comprise at least one mutation in E233, L234, G236, D265, D270, N297, E318, K320, K322, A327, A330, P331, and/or P329 (numbering according to the EU index). In some embodiments, the antibodies comprise the mutations L234A/L235A or D265A/N297A.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108. See also US 2004/0093621. Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in

the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878 and US Patent No. 6,602,684. Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087. See also, WO 1998/58964 and WO 1999/22764 concerning antibodies with altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 on antigen-binding molecules with modified glycosylation.In one aspect, the invention provides FGFR3 binding polypeptides comprising any of the antigen binding sequences provided herein, wherein the FGFR3 binding polypeptides specifically bind to a FGFR3, e.g., a human and/or cyno and/or mouse FGFR3.

The antibodies of the invention bind (such as specifically bind) FGFR3 (e.g. FGFR3-IIIb and/or FGFR3-IIIc) and FGFR2 (e.g. FGFR2-IIIb and/or FGFR2-IIIc), and in some embodiments, may modulate (e.g. inhibit) one or more aspects of FGFR3 and/or FGFR2 signaling (such as FGFR3 phosphorylation) and/or disruption of any biologically relevant FGFR3 and/or FGFR3 ligand biological pathway and/or disruption of any biologically relevant FGFR2 and/or FGFR2 ligand biological pathway, and/or treatment and/or prevention of a tumor, cell proliferative disorder or a cancer; and/or treatment or prevention of a disorder associated with FGFR3 and/or FGFR2 expression and/or activity (such as increased FGFR3 and/or FGFR2 expression and/or activity). In some embodiments, the FGFR2/3 antibody specifically binds to a polypeptide consisting of or consisting essentially of a FGFR3 (e.g., a human or mouse FGFR3) and/or a FGFR2 (e.g., a human or mouse FGFR3). In some embodiments, the antibody specifically binds FGFR3 with a Kd of 1 x 10^{-7} M or higher affinity. In some embodiments, the antibody specifically binds FGFR2 with a Kd of 1 x 10^{-7} M or higher affinity. In some embodiments, the antibody specifically binds FGFR3 and FGF2 with Kds of 1 x 10^{-7} M or higher affinity.

In some embodiments, the anti-FGFR2/3 antibody of the invention is not an anti-FGFR3 antibody described in U.S. Patent Publication no. 2005/0147612 (e.g., antibody MSPRO2, MSPRO12, MSPRO59, MSPRO11, MSPRO21, MSPRO24, MSPRO26, MSPRO28, MSPRO29, MSPRO43, MSPRO55), antibody described in Rauchenberger et al, J Biol Chem 278 (40):38194-38205 (2003); an antibody described in PCT Publication No. WO2006/048877 (e.g., antibody PRO-001), an antibody described in Martinez-Torrecuadrada et al, Mol Cancer Ther (2008) 7(4): 862-873 (e.g., scFvαFGFR3 3C), an antibody described in Direnzo, R et al (2007) Proceedings of AACR Annual Meeting, Abstract No. 2080 (e.g., D11), or an antibody described in WO 2010/002862 (e.g., antibodies 15D8, 27H2, 4E7, 2G4, 20B4).

In one aspect, the invention provides compositions comprising one or more antibodies of the invention and a carrier. In one embodiment, the carrier is pharmaceutically acceptable.

In another aspect, the invention provides nucleic acids encoding a FGFR2/3 antibody of the invention.

In yet another aspect, the invention provides vectors comprising a nucleic acid of the invention.

In a further aspect, the invention provides compositions comprising one or more nucleic acids of the invention and a carrier. In one embodiment, the carrier is pharmaceutically acceptable.

In one aspect, the invention provides host cells comprising a nucleic acid or a vector of the invention. A vector can be of any type, for example, a recombinant vector such as an expression vector. Any of a variety of host cells can be used. In one embodiment, a host cell is a prokaryotic cell, for example, *E. coli*. In another embodiment, a host cell is a eukaryotic cell, for example a mammalian cell such as Chinese Hamster Ovary (CHO) cell.

In a further aspect, the invention provides methods of making an antibody of the invention. For example, the invention provides methods of making an anti-FGFR2/3 antibody (which, as defined herein includes full length antibody and fragments thereof), said method comprising expressing in a suitable host cell a recombinant vector of the invention encoding the antibody, and recovering the antibody. In some embodiments, the method comprises culturing a host cell comprising nucleic acid encoding the antibody so that the nucleic acid is expressed. In some embodiments, the method further comprises recovering the antibody from the host cell culture. In some embodiments, the antibody is recovered from the host cell culture medium. In some embodiments, the method further comprises combining the recovered antibody with a pharmaceutically acceptable carrier, excipient, or carrier to prepare a pharmaceutical formulation comprising the humanized antibody.

In one aspect, the invention provides an article of manufacture comprising a container; and a composition contained within the container, wherein the composition comprises one or more FGFR2/3 antibodies of the invention. In one embodiment, the composition comprises a nucleic acid of the invention. In another embodiment, a composition comprising an antibody further comprises a carrier, which in some embodiments is pharmaceutically acceptable. In one embodiment, an article of manufacture of the invention further comprises instructions for administering the composition (e.g., the antibody) to an individual (such as instructions for any of the methods described herein).

In another aspect, the invention provides a kit comprising a first container comprising a composition comprising one or more anti-FGFR2/3 antibodies of the invention; and a second container comprising a buffer. In one embodiment, the buffer is pharmaceutically acceptable. In one embodiment, a composition comprising an antibody further comprises a carrier, which in some embodiments is pharmaceutically acceptable. In another embodiment,

a kit further comprises instructions for administering the composition (e.g., the antibody) to an individual.

In a further aspect, the invention provides an anti-FGFR2/3 antibody of the invention for use as a medicament.

In a further aspect, the invention provides an anti-FGFR2/3 antibody of the invention for use in treating or preventing a disorder, such as a pathological condition associated with FGFR3 activation and/or expression (in some embodiments, over-expression). In a further aspect, the invention provides an anti-FGFR2/3 antibody of the invention for use in treating or preventing a disorder, such as a pathological condition associated with FGFR2 activation and/or expression (in some embodiments, over-expression). In a further aspect, the invention provides an anti-FGFR2/3 antibody of the invention for use in treating or preventing a disorder, such as a pathological condition associated with FGFR2 and FGFR3 activation and/or expression (in some embodiments, over-expression). In some embodiments, the disorder is a cancer, a tumor, and/or a cell proliferative disorder. In some embodiments, the cancer, a tumor, and/or a cell proliferative disorder is multiple myeloma or bladder cancer (e.g., transitional cell carcinoma), breast cancer or liver cancer.

In a further aspect, the invention provides an anti-FGFR2/3 antibody of the invention for use in treating or preventing a disorder such as a skeletal disorder. In some embodiments, the disorder is achondroplasia, hypochondroplasia, dwarfism, thantophoric dysplasia (TD; clinical forms TD1 and TDII), or craniosynostosis syndrome.

In a further aspect, the invention provides an anti-FGFR2/3 antibody of the invention for use in reducing cell proliferation.

In a further aspect, the invention provides an anti-FGFR2/3 antibody of the invention for use in killing a cell. In some embodiments, the cell is a multiple myeloma cell. In some embodiments, the cell is killed by ADCC. In some embodiments, the antibody is a naked

antibody. In some embodiments, the cell over-expresses FGFR3. In some embodiments, the cell over-expresses FGFR2. In some embodiments, the cell over-expresses FGFR2 and FGFR3.

In a further aspect, the invention provides an anti-FGFR2/3 antibody of the invention for use in depleting cells, such as multiple myeloma cells. In some embodiments, the cell is killed by ADCC. In some embodiments, the antibody is a naked antibody. In some embodiments, the cell over-expresses FGFR3.

In a further aspect, the invention provides use of an anti-FGFR2/3 antibody of the invention in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disorder, such as a pathological condition associated with FGFR3, FGFR2, or FGFR2 and FGFR3 activation and/or expression (in some embodiments, over-expression). In some embodiments, the disorder is a cancer, a tumor, and/or a cell proliferative disorder. In some embodiments, the cancer, a tumor, and/or a cell proliferative disorder is multiple myeloma or bladder cancer (e.g., transitional cell carcinoma), breast cancer or liver cancer. In some embodiments, the disorder is a skeletal disorder, e.g., achondroplasia, hypochondroplasia, dwarfism, thantophoric dysplasia (TD; clinical forms TD1 and TDII), or craniosynostosis syndrome.

In one aspect, the invention provides use of a nucleic acid of the invention in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disorder, such as a pathological condition associated with FGFR3, FGFR2, or FGFR2 and FGFR3 activation and/or expression (in some embodiments, over-expression). In some embodiments, the disorder is a cancer, a tumor, and/or a cell proliferative disorder. In some embodiments, the cancer, a tumor, and/or a cell proliferative disorder is multiple myeloma or bladder cancer (e.g., transitional cell carcinoma), breast cancer or liver cancer. In some embodiments, the disorder is a skeletal disorder, e.g., achondroplasia, hypochondroplasia,

dwarfism, thantophoric dysplasia (TD; clinical forms TD1 and TDII), or craniosynostosis syndrome.

In another aspect, the invention provides use of an expression vector of the invention in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disorder, such as a pathological condition associated with FGFR3, FGFR2, or FGFR2 and FGFR3 activation and/or expression (in some embodiments, over-expression). In some embodiments, the disorder is a cancer, a tumor, and/or a cell proliferative disorder. In some embodiments, the cancer, a tumor, and/or a cell proliferative disorder is multiple myeloma or bladder cancer (e.g., transitional cell carcinoma), breast cancer or liver cancer. In some embodiments, the disorder is a skeletal disorder, e.g., achondroplasia, hypochondroplasia, dwarfism, thantophoric dysplasia (TD; clinical forms TD1 and TDII), or craniosynostosis syndrome.

In yet another aspect, the invention provides use of a host cell of the invention in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disorder, such as a pathological condition associated with FGFR3, FGFR2, or FGFR2 and FGFR3 activation and/or expression (in some embodiments, over-expression). In some embodiments, the disorder is a cancer, a tumor, and/or a cell proliferative disorder. In some embodiments, the cancer, a tumor, and/or a cell proliferative disorder is multiple myeloma or bladder cancer (e.g., transitional cell carcinoma), breast cancer or liver cancer. In some embodiments, the disorder is a skeletal disorder, e.g., achondroplasia, hypochondroplasia, dwarfism, thantophoric dysplasia (TD; clinical forms TD1 and TDII), or craniosynostosis syndrome.

In a further aspect, the invention provides use of an article of manufacture of the invention in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disorder, such as a pathological condition associated with FGFR3, FGFR2, or

FGFR2 and FGFR3 activation and/or expression (in some embodiments, over-expression). In some embodiments, the disorder is a cancer, a tumor, and/or a cell proliferative disorder. In some embodiments, the cancer, a tumor, and/or a cell proliferative disorder is multiple myeloma or bladder cancer (e.g., transitional cell carcinoma), breast cancer or liver cancer. In some embodiments, the disorder is a skeletal disorder, e.g., achondroplasia, hypochondroplasia, dwarfism, thantophoric dysplasia (TD; clinical forms TD1 and TDII), or craniosynostosis syndrome.

In one aspect, the invention also provides use of a kit of the invention in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disorder, such as a pathological condition associated with FGFR3, FGFR2, or FGFR2 and FGFR3 activation and/or expression (in some embodiments, over-expression). In some embodiments, the disorder is a cancer, a tumor, and/or a cell proliferative disorder. In some embodiments, the cancer, a tumor, and/or a cell proliferative disorder is multiple myeloma or bladder cancer (e.g., transitional cell carcinoma) , breast cancer or liver cancer. In some embodiments, the disorder is a skeletal disorder, e.g., achondroplasia, hypochondroplasia, dwarfism, thantophoric dysplasia (TD; clinical forms TD1 and TDII), or craniosynostosis syndrome.

In a further aspect, the invention provides use of an anti-FGFR2/3 antibody of the invention in the preparation of a medicament for inhibition of cell proliferation.In a further aspect, the invention provides use of an anti-FGFR2/3 antibody of the invention in the preparation of a medicament for cell killing. In some embodiments, the cell is a multiple myeloma cell. In some embodiments, the cell is killed by ADCC. In some embodiments, the antibody is a naked antibody. In some embodiments, the cell over-expresses FGFR3. In some embodiments, the cell over-expresses FGFR2. In some embodiments, the cell over-expresses FGFR3 and FGFR2.

In a further aspect, the invention provides use of an anti-FGFR2/3 antibody of the invention in the preparation of a medicament for depleting cells, such as multiple myeloma cells. In some embodiments, the cell is killed by ADCC. In some embodiments, the antibody is a naked antibody. In some embodiments, the cell over-expresses FGFR3. In some embodiments, the cell over-expresses FGFR2. In some embodiments, the cell over-expresses FGFR3 and FGFR2.

The invention provides methods and compositions useful for modulating disorders associated with expression and/or signaling of FGFR3, such as increased expression and/or signaling or undesired expression and/or signaling. The invention provides methods and compositions useful for modulating disorders associated with expression and/or signaling of FGFR2, such as increased expression and/or signaling or undesired expression and/or signaling. The invention provides methods and compositions useful for modulating disorders associated with expression and/or signaling of FGFR3 and FGFR2, such as increased expression and/or signaling or undesired expression and/or signaling.

Methods of the invention can be used to affect any suitable pathological state. Exemplary disorders are described herein, and include a cancer selected from the group consisting of non-small cell lung cancer, ovarian cancer, thyroid cancer, testicular cancer, endometrial cancer, head and neck cancer, brain cancer (e.g., neuroblastoma or meningioma), skin cancer (e.g., melanoma, basal cell carcinoma, or squamous cell carcinoma), bladder cancer (e.g., transitional cell carcinoma), breast carcinoma, gastric cancer, colorectal cancer (CRC), hepatocellular carcinoma, cervical cancer, lung cancer, pancreatic cancer, prostate cancer, and hematologic malignancies (e.g., T-cell acute lymphoblastic leukemia (T-ALL), B-cell acute lymphoblastic leukemia (B-ALL), acute myelogenous leukemia (AML), B-cell malignancies, Hodgkin lymphoma, and multiple myeloma). In some embodiments, the disorder is invasive transitional cell carcinoma. In some embodiments, the disorder is

multiple myeloma. Additional exemplary disorders include skeletal disorders, such as achondroplasia, hypochondroplasia, dwarfism, thantophoric dysplasia (TD; clinical forms TD1 and TDII), or craniosynostosis syndrome.

In certain embodiments, the cancer expresses FGFR3, amplified FGFR3, translocated FGFR3, and/or mutated FGFR3. In certain embodiments, the cancer expresses activated FGFR3. In certain embodiments, the cancer expresses translocated FGFR3 (e.g., a t(4;14) translocation). In certain embodiments, the cancer expresses constitutive FGFR3. In some embodiments, the constitutive FGFR3 comprises a mutation in the tyrosine kinase domain and/or the juxtamembrane domain and/or a ligand-binding domain. In certain embodiments, the cancer expresses ligand-independent FGFR3. In some embodiments, the cancer expresses ligand-dependent FGFR3.

In some embodiments, the cancer expresses FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{S248C}. In some embodiments, the cancer expressed FGFR3-IIIb ^{S248C} and/or FGFR3-IIIc^{S248C}.

In some embodiments, the cancer expresses FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{K652E}. In some embodiments, the cancer expressed FGFR3-IIIb ^{K652E} and/or FGFR3-IIIc ^{K650E}.

FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{S249C}. In some embodiments, the cancer expresses FGFR3-IIIb^{S249C} and/or FGFR3-IIIc ^{S249C}.

In one aspect, the cancer expresses FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{G372C}. In some embodiments, the cancer expresses FGFR3-IIIb^{G372C} and/or FGFR3-IIIc ^{G370C}.

In one aspect, the cancer expresses FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{Y375C}. In some embodiments, the cancer expresses FGFR3-IIIb^{Y375C} and/or FGFR3-IIIc^{Y373C}.

In some embodiments, the cancer expresses (a) FGFR3-IIIb^{K652E} and (b) one or more of FGFR3-IIIb^{R248C}, FGFR3-IIIb^{Y375C}, FGFR3 -IIIb^{S249C}, and FGFR3IIIb ^{G372C}.

In some embodiments, the cancer expresses (a) FGFR3-IIIb^{R248C} and (b) one or more of FGFR3-IIIb^{K652E}, FGFR3-IIIb^{Y375C}, FGFR3-IIIb^{S249C}, and FGFR3-IIIb^{G372C}.

In some embodiments, the cancer expresses (a) FGFR3-IIIb^{G372C} and (b) one or more of FGFR3-IIIb^{K652E}, FGFR3-IIIb^{Y375C}, FGFR3-IIIb^{S249C}, and FGFR3-IIIb^{R248C}.

In some embodiments, the cancer expresses FGFR3-IIIb^{R248C}, FGFR3-IIIb^{K652E}, FGFR3-IIIb^{Y375C}, FGFR3-IIIb ^{S249C}, and FGFR3-IIIb ^{G372C}.

In certain embodiments, the cancer expresses increased levels of phospho-FGFR3, phospho-FRS2 and/or phospho-MAPK relative to a control sample (e.g., a sample of normal tissue) or level.

In certain embodiments, the cancer expresses FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2. In certain embodiments, the cancer expresses activated FGFR2. In certain embodiments, the cancer expresses translocated FGFR2. In certain embodiments, the cancer expresses constitutive FGFR2. In certain embodiments, the cancer expresses ligand-independent FGFR2. In some embodiments, the cancer expresses ligand-dependent FGFR2.

In some embodiments, the cancer expresses FGFR2 comprising a mutation.

In certain embodiments, the cancer expresses: 1) FGFR3, amplified FGFR3, translocated FGFR3, and/or mutated FGFR3 and 2) FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2. In certain embodiments, the cancer expresses activated FGFR3 and a FGFR2 as described above. In certain embodiments, the cancer expresses translocated FGFR3 (e.g., a t(4;14) translocation) and a FGFR2 as described above. In certain embodiments, the cancer expresses constitutive FGFR3 and a FGFR2 as described above. In some embodiments, the constitutive FGFR3 comprises a mutation in the tyrosine

kinase domain and/or the juxtamembrane domain and/or a ligand-binding domain. In certain embodiments, the cancer expresses ligand-independent FGFR3 and a FGFR2 as described above. In some embodiments, the cancer expresses ligand-dependent FGFR3 and a FGFR2 as described above.

In some embodiments, the cancer expresses FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{S248C} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2). In some embodiments, the cancer expresses FGFR3-IIIb ^{S248C} and/or FGFR3-IIIc^{S248C} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

In some embodiments, the cancer expresses FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{K652E} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2). In some embodiments, the cancer expresses FGFR3-IIIb ^{K652E} and/or FGFR3-IIIc ^{K650E} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{S249C} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2). In some embodiments, the cancer expresses FGFR3-IIIb^{S249C} and/or FGFR3-IIIc ^{S249C} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

In one aspect, the cancer expresses FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{G372C} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2). In some embodiments, the cancer expresses FGFR3-IIIb^{G372C} and/or FGFR3-IIIc ^{G370C} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

In one aspect, the cancer expresses FGFR3 comprising a mutation corresponding to FGFR3-IIIb^{Y375C} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2). In some embodiments, the cancer expresses FGFR3-IIIb^{Y375C} and/or FGFR3-IIIc^{Y373C} and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

In some embodiments, the cancer expresses (a) FGFR3-IIIb^{K652E} and (b) one or more of FGFR3-IIIb^{R248C}, FGFR3-IIIb^{Y375C}, FGFR3 -IIIb^{S249C}, and FGFR3IIIb ^{G372C} and (c) a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

In some embodiments, the cancer expresses (a) FGFR3-IIIb^{R248C} and (b) one or more of FGFR3-IIIb^{K652E}, FGFR3-IIIb^{Y375C}, FGFR3-IIIb^{S249C}, and FGFR3-IIIb^{G372C} and (c) a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

In some embodiments, the cancer expresses (a) FGFR3-IIIb^{G372C} and (b) one or more of FGFR3-IIIb^{K652E}, FGFR3-IIIb^{Y375C}, FGFR3-IIIb^{S249C}, and FGFR3-IIIb^{R248C} and (c) a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

In some embodiments, the cancer expresses (a) FGFR3-IIIb^{R248C}, FGFR3-IIIb^{K652E}, FGFR3-IIIb^{Y375C}, FGFR3-IIIb ^{S249C}, and FGFR3-IIIb ^{G372C}, and (b) a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

In certain embodiments, the cancer expresses increased levels of phospho-FGFR3, phospho-FRS2 and/or phospho-MAPK relative to a control sample (e.g., a sample of normal tissue) or level and a FGFR2 as described above (e.g. FGFR2, amplified FGFR2, translocated FGFR2, and/or mutated FGFR2).

In some embodiments, the cancer expresses (e.g., on the cell surface) about 10,000 FGFR3 molecules per cell or more (such as 11,000, 12,000, 13,000, 14,000, 15,000, 16,000, 17,000, 18,000 or more FGFR3 receptors). In some embodiments, the cancer expresses about 13000 FGFR3 molecules. In other embodiments, the cancer expresses about 5000, 6000, 7000, 8000, or more FGFR3 molecules. In some embodiments, the cancer expresses less than about 4000, 3000, 2000, 1000, or fewer FGFR3 molecules. In some embodiments, the cancer expresses less than about 1000 FGFR3 molecules. In some embodiments, the cancer expresses (e.g., on the cell surface) about 10,000 FGFR2 molecules per cell or more (such as 11,000, 12,000, 13,000, 14,000, 15,000, 16,000, 17,000, 18,000 or more FGFR2 receptors). In some embodiments, the cancer expresses about 13000 FGFR2 molecules. In other embodiments, the cancer expresses about 5000, 6000, 7000, 8000, or more FGFR2 molecules. In some embodiments, the cancer expresses less than about 4000, 3000, 2000, 1000, or fewer FGFR2 molecules. In some embodiments, the cancer expresses less than about 1000 FGFR2 molecules. In some embodiments, the cancer expresses (e.g., on the cell surface) about 10,000 FGFR3 and 10,000 FGFR2 molecules per cell or more (such as 11,000, 12,000, 13,000, 14,000, 15,000, 16,000, 17,000, 18,000 or more FGFR3 receptors and 11,000, 12,000, 13,000, 14,000, 15,000, 16,000, 17,000, 18,000 or more FGFR2 receptors). In some embodiments, the cancer expresses about 13000 FGFR3 molecules and 13000 FGFR2 molecules. In other embodiments, the cancer expresses about 5000, 6000, 7000, 8000, or more FGFR3 molecules and about 5000, 6000, 7000, 8000, or more FGFR2 molecules. In some embodiments, the cancer expresses less than about 4000, 3000, 2000, 1000, or fewer FGFR3 molecules and less than about 4000, 3000, 2000, 1000, or fewer FGFR2 molecules. In some embodiments, the cancer expresses less than about 1000 FGFR3 molecules and less than about 1000 FGFR2 molecules.

In one embodiment, a cell that is targeted in a method of the invention is a cancer cell. For example, a cancer cell can be one selected from the group consisting of a breast cancer cell, a colorectal cancer cell, a lung cancer cell (e.g., a non-small cell lung cancer cell), a thyroid cancer cell, a multiple myeloma cell, a testicular cancer cell, a papillary carcinoma cell, a colon cancer cell, a pancreatic cancer cell, an ovarian cancer cell, a cervical cancer cell, a central nervous system cancer cell, an osteogenic sarcoma cell, a renal carcinoma cell, a hepatocellular carcinoma cell, a bladder cancer cell (e.g., a transitional cell carcinoma cell), a gastric carcinoma cell, a head and neck squamous carcinoma cell, a melanoma cell, a leukemia cell, a multiple myeloma cell (e.g. a multiple myeloma cell comprising a t(4:14) FGFR3 translocation)and a colon adenoma cell. In one embodiment, a cell that is targeted in a method of the invention is a hyperproliferative and/or hyperplastic cell. In another embodiment, a cell that is targeted in a method of the invention is a dysplastic cell. In yet another embodiment, a cell that is targeted in a method of the invention is a metastatic cell.

In one aspect, the invention provides methods for inhibiting cell proliferation in a subject, the method comprising administering to the subject an effective amount of an anti-FGFR2/3 antibody to reduce cell proliferation.

In one aspect, the invention provides methods for killing a cell in a subject, the method comprising administering to the subject an effective amount of an anti-FGFR2/3 antibody to kill a cell. In some embodiments, the cell is a multiple myeloma cell. In some embodiments, the cell is killed by ADCC. In some embodiments, the antibody is a naked antibody. In some embodiments, the cell over-expresses FGFR3. In some embodiments, the cell over-expresses FGFR2. In some embodiments, the cell over-expresses FGFR3 and FGFR2.

In one aspect, the invention provides methods for depleting cells (such as multiple myeloma cells) in a subject, the method comprising administering to the subject an effective

amount of an anti-FGFR2/3 antibody to kill a cell. In some embodiments, the cell is killed by ADCC. In some embodiments, the antibody is a naked antibody. In some embodiments, the cell over-expresses FGFR2/3.

In one aspect, the invention provides methods for treating or preventing a skeletal disorder. In some embodiments, the disorder is achondroplasia, hypochondroplasia, dwarfism, thantophoric dysplasia (TD; clinical forms TD1 and TDII), or craniosynostosis syndrome.

Methods of the invention can further comprise additional treatment steps. For example, in one embodiment, a method further comprises a step wherein a targeted cell and/or tissue (e.g., a cancer cell) is exposed to radiation treatment or a chemotherapeutic agent.

In one aspect, the invention provides methods comprising administration of an effective amount of an anti-FGFR2/3 antibody in combination with an effective amount of another therapeutic agent (such as an anti-angiogenesis agent, another antibody, a chemotherapeutic agent, a cytotoxic agent, an immunosuppressive agent, a prodrug, a cytokine, cytotoxic radiotherapy, a corticosteroid, an anti-emetic, a cancer vaccine, an analgesic, or a growth inhibitory agent). For example, anti-FGFR2/3 antibodies are used in combinations with an anti-cancer agent or an anti-angiogenic agent to treat various neoplastic or non-neoplastic conditions. In particular examples, the anti-FGFR2/3 antibodies are used in combination with velcade, revlimid, tamoxifen, letrozole, exemestane, anastrozole, irinotecan, cetuximab, fulvestrant, vinorelbine, bevacizumab, vincristine, cisplatin, gemcitabine, methotrexate, vinblastine, carboplatin, paclitaxel, docetaxel, pemetrexed, 5-fluorouracil, doxorubicin, bortezomib, lenalidomide, dexamethasone, melphalin, prednisone, vincristine, and/or thalidomide.

Depending on the specific cancer indication to be treated, the combination therapy of the invention can be combined with additional therapeutic agents, such as chemotherapeutic agents, or additional therapies such as radiotherapy or surgery. Many known chemotherapeutic agents can be used in the combination therapy of the invention. Preferably those chemotherapeutic agents that are standard for the treatment of the specific indications will be used. Dosage or frequency of each therapeutic agent to be used in the combination is preferably the same as, or less than, the dosage or frequency of the corresponding agent when used without the other agent(s).

In another aspect, the invention provides any of the anti-FGFR2/3 antibodies described herein, wherein the anti-FGFR2/3 antibody comprises a detectable label.

In another aspect, the invention provides a complex of any of the anti-FGFR2/3 antibodies described herein and FGFR2/3. In some embodiments, the complex is *in vivo* or *in vitro*. In some embodiments, the complex comprises a cancer cell. In some embodiments, the anti-FGFR2/3 antibody is detectably labeled.

The present disclosure also provides antibodies that bind to beta-Klotho (KLB) and bispecific antibodies that bind to both KLB and FGFR2 and/or FGFR3 (the "FGFR2/3 + KLB bispecific antibody"), and methods of using the same. In specific embodiments, the FGFR2/3 + KLB bispecific antibody can be used to treat metabolic diseases and disorders including weight loss and improvement in glucose and lipid metabolism. In certain embodiments, the FGFR2/3 + KLB bispecific antibody can be used to treat metabolic disorders or diseases without a significant impact on the liver and without a significant loss in bone mass. In preferred embodiments, the FGFR2/3 + KLB bispecific antibody is used to treat non-alcoholic steatohepatitis (NASH).

In certain embodiments, the bispecific antibody is an isolated antibody. In certain embodiments, the bispecific antibody can bind to both KLB and FGFR2, KLB and FGFR3,

or all three of KLB, FGFR2, and FGFR3, wherein the antibody binds to the C-terminal domain of KLB. In certain embodiments, the bispecific antibody binds to a fragment of KLB including the amino acid sequence SSPTRLAVIPWGVRKLLRWVRRNYGDMDIYITAS (SEQ ID NO: 103).

In certain embodiments, the bispecific antibody that binds KLB also binds to an epitope within a fragment of FGFR2 including the amino acid sequence TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) or YKVRNQHWSLIMES (SEQ ID NO:92). In certain embodiments, the bispecific antibody that binds KLB also binds to an epitope within a fragment of FGFR3 including the amino acid sequence TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO:94). In certain embodiments, the bispecific antibody that binds KLB also binds to an epitope within a fragment of FGFR2 including the amino acid sequence TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) or YKVRNQHWSLIMES (SEQ ID NO:92) and binds to an epitope within a fragment of FGFR3 including the amino acid sequence TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO:94).

In certain embodiments, the bispecific antibody that binds KLB also binds to an epitope within a fragment of FGFR2 having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with amino acid sequence TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and/or YKVRNQHWSLIMES (SEQ ID NO:92). In certain embodiments, the bispecific antibody that binds KLB also binds to an epitope within a fragment of FGFR3 having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with amino acid sequence TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO:94). In certain embodiments, the bispecific antibody that binds KLB also binds

to an epitope within a fragment of FGFR2 having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with amino acid sequence TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and/or YKVRNQHWSLIMES (SEQ ID NO:92) and also binds to an epitope within a fragment of FGFR3 having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with amino acid sequence TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO:94).

In certain embodiments, the bispecific antibody that binds KLB also binds FGFR2 within the amino acid sequence range of 157 to 181 of SEQ ID NOs: 52 or 54. In certain embodiments, the bispecific antibody that binds KLB also binds FGFR2 within the amino acid sequence range of 207 to 220 of SEQ ID NOs: 52 or 54. In certain embodiments, the bispecific antibody that binds KLB also binds FGFR2 within the amino acid sequence range of 157 to 181 and 207 to 220 of SEQ ID NOs: 52 or 54.

In certain embodiments, the bispecific antibody that binds KLB and FGFR2/3 inhibits constitutive FGFR2 and/or FGFR3 activity. In certain embodiments, the constitutive FGFR2/3 activity is ligand-dependent constitutive FGFR2/3 activity. In certain embodiments, the constitutive FGFR2/3 activity is ligand-independent constitutive FGFR2/3 activity. In certain embodiments, the constitutive FGFR2/3 activity is FGFR2 and FGFR3 activity.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure reduces blood glucose levels *in vivo*. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure does not significantly affect bone density. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure does not have a significant impact on the liver. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure induces ERK and MEK phosphorylation in the

liver at significantly lower levels than FGF21 induces. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure binds to KLB with a K_d from 10^{-8} M to 10^{-13} M. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure can bind to a FGFR2 and/or FGFR3 protein with a K_d from 10^{-8} M to 10^{-13} M. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure can bind to FGFR2 and /or FGFR3 with a K_d from 10^{-8} M to 10^{-13} M.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure binds to an epitope present on KLB. For example, and not by way of limitation, the present disclosure provides an FGFR2/3 + KLB bispecific antibody can bind the same epitope on KLB as an antibody shown in **Figures 11A and 11B**. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure can bind the same epitope as the 12A11 or the 8C5 antibody. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure can bind to an epitope within the C-terminal domain of KLB. In certain embodiments, the an FGFR2/3 + KLB bispecific antibody of the present disclosure can bind to a fragment of KLB consisting of the amino acid sequence SSPTRLAVIPWGVRKLLRWVRRNYGDMDIYITAS (SEQ ID NO: 103).

In certain embodiments, the KLB arm of any of the FGFR2/3 + KLB bispecific antibodies of the present disclosure is an arm of any KLB antibody described in US20150218276 which is incorporated herein in its entirety.

In certain embodiments, the FGFR2/3 arm of any of the FGFR2/3 + KLB bispecific antibodies of the present disclosure is an arm of any FGFR2/3 antibodies described herein.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first antibody, or antigen binding portion thereof, that includes a heavy chain variable region and a light chain variable region, where the heavy chain variable region

includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 104, and the light chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 105. In certain embodiments, the second antibody, or antigen binding portion thereof, includes a heavy chain variable region and a light chain variable region, where the heavy chain variable region includes amino acids having a sequence that is at least 95% identical to a sequence set forth in column 2 of **Table 1**, and the light chain variable region includes amino acids having a sequence that is at least 95% identical to a sequence set forth in column 3 of **Table 1**.

Table 1. HC and LC sequences of exemplary FGFR2/3 antibodies

Antibody	HC SEQ ID NO:	LC SEQ ID NO:
2B.1.3	75	59
2B.1.95	76	60
2B.1.73	77	61
2B.1.32	78	62
2B.1.88	79	63
2B.1.1	80	64
2B.1.3.10	81	65
2B.1.3.12	82	66

In certain embodiments, an anti-KLB/anti-FGFR1 bispecific antibody of the present disclosure includes a first antibody, or antigen binding portion thereof, which includes a heavy chain region and a light chain region, where the heavy chain region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 106, and the light chain region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 107. In certain embodiments, the second antibody, or antigen binding portion thereof, includes a heavy chain region and a light chain region, where the heavy chain region includes amino acids having a sequence that is at least 95% identical to a sequence set forth in column 2 of **Table 1**, and the light chain region

includes amino acids having a sequence that is at least 95% identical to a sequence set forth in column 3 of **Table 1**.

In preferred embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, that includes a heavy chain variable region and a light chain variable region, where the heavy chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 104, and the light chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 105; and the second anti-FGFR2/3 antibody, or antigen binding portion thereof, includes a heavy chain variable region and a light chain variable region, where the heavy chain variable region includes amino acids having a sequence that is at least 95% identical to a sequence set SEQ ID NO: 66, and the light chain variable region includes amino acids having a sequence that is at least 95% identical to a sequence set forth in SEQ ID NO: 82.

In preferred embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, that includes a heavy chain variable region and a light chain variable region, where the heavy chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 106, and the light chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 107; and the second anti-FGFR2/3 antibody, or antigen binding portion thereof, includes a heavy chain variable region and a light chain variable region, where the heavy chain variable region includes amino acids having a sequence that is at least 95% identical to a sequence set SEQ ID NO: 82, and the light chain variable region includes amino acids having a sequence that is at least 95% identical to a sequence set forth in SEQ ID NO: 66.

In preferred embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, that includes a heavy chain variable region and a light chain variable region, where the heavy chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 106, and the light chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 107; and the second anti-FGFR2/3 antibody, or antigen binding portion thereof, includes a heavy chain and a light chain, where the heavy chain includes amino acids having a sequence that is at least 95% identical to a sequence set SEQ ID NO: 282, and the light chain includes amino acids having a sequence that is at least 95% identical to a sequence set forth in SEQ ID NO: 283.

In preferred embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, that includes a heavy chain variable region and a light chain variable region, where the heavy chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 106, and the light chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 107; and the second anti-FGFR2/3 antibody, or antigen binding portion thereof, wherein the CDRs on the light chain, comprise amino acids having a sequence that are at least 90%, 91%, 92%, 93%, 94%, 95%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a SEQ ID NOs: 7-9 (CDRL1, CDRL2, and CDRL3), and wherein the CDRs on the heavy chain, comprise amino acids having a sequence that are at least 90%, 91%, 92%, 93%, 94%, 95%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10-12 (CDRH1, CDRH2, and CDRH3).

In preferred embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, that

includes a heavy chain variable region and a light chain variable region, where the heavy chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 106, and the light chain variable region includes amino acids having a sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 107; and the second anti-FGFR2/3 antibody, or antigen binding portion thereof, wherein the CDRs on the light chain, comprise amino acids having a sequence that are at least 90%, 91%, 92%, 93%, 94%, 95%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a SEQ ID NOs: 276-278 (CDRL1, CDRL2, and CDRL3), and wherein the CDRs on the heavy chain, comprise amino acids having a sequence that are at least 90%, 91%, 92%, 93%, 94%, 95%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 279-281 (CDRH1, CDRH2, and CDRH3).

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, comprising: (a) HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 230-232 and 236-247, (b) HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-137, and (c) HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 142 and 248-262 .

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, comprising (a) HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 230-232 and 236-247, (b) HVR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 142 and 248-262 , and (c) HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 263-278.

In certain embodiments, an FGFR2/3 + KLB bispecific bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof,

comprising (a) HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 279-293, (b) HVR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 294-309, and (c) HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 310-324.

In certain embodiments, an FGFR2/3 + KLB bispecific bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 119, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 150, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 166, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 181, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 197, and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 212.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 122, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 153, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 169, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 184, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 200, and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 215.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes one arm from an anti-KLB antibody, or antigen binding portion thereof, selected from any of the anti-KLB antibodies disclosed herein or in US20150218276 which is incorporated herein in its entirety and one arm of an FGFR2/3 antibody disclosed herein. In specific embodiments the arms of the FGFR2/3 + KLB bispecific are selected from the following combinations:

a) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NO: 82 (heavy chain) and SEQ ID NO: 66 (light chain));

b) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NO: 82 (heavy chain) and SEQ ID NO: 66 (light chain));

c) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 7-9 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 10-12 (CDRH1, CDRH2, and CDRH3));

d) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 7-9 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 10-12 (CDRH1, CDRH2, and CDRH3));

e) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NO: 282 (heavy chain) and SEQ ID NO: 283 (light chain));

f) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NO: 282 (heavy chain) and SEQ ID NO: 283 (light chain));

g) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 276-278 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 279-281 (CDRH1, CDRH2, and CDRH3)); and

h) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 276-278 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 279-281 (CDRH1, CDRH2, and CDRH3)).

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, comprising (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104 and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, comprising (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 106 and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 107.

In another aspect, an FGFR2/3 + KLB bispecific antibody of the present disclosure includes a first anti-KLB antibody, or antigen binding portion thereof, comprising (a) a heavy chain variable region having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 104; (b) a light chain variable region having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 105; and (c) a heavy chain variable region as in (a) and a light chain variable region as in (b).

In certain embodiments, FGFR2/3 + KLB bispecific antibody of the present disclosure is a monoclonal antibody. In certain embodiments, the antibody is a human, humanized, or chimeric antibody. In certain embodiments, the antibody has reduced effector function.

In another aspect, the present disclosure provides an isolated nucleic acid encoding an FGFR2/3 + KLB bispecific antibody of the present disclosure. In certain embodiments, the present disclosure provides a host cell comprising a nucleic acid encoding an FGFR2/3 + KLB bispecific antibody of the present disclosure. In certain embodiments, the present disclosure provides a method of producing an FGFR2/3 + KLB bispecific antibody comprising culturing a host cell of the present disclosure so that the antibody is produced. In certain embodiments, this method further comprises recovering the FGFR2/3 + KLB bispecific antibody from the host cell.

The present disclosure further provides a pharmaceutical formulation that includes one or more antibodies of the invention and a pharmaceutically acceptable carrier. Specifically, the present disclosure provides a pharmaceutical formulation that includes an FGFR2/3 + KLB bispecific antibody described herein. In certain embodiments, the pharmaceutical formulation comprises an additional therapeutic agent.

In another aspect, the present disclosure provides an FGFR2/3 + KLB bispecific antibody of the invention for use as a medicament. In certain embodiments, the an anti-KLB/anti-FGFR1 bispecific antibody is for use in treating metabolic disorders, *e.g.*, polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), obesity, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, hypertension, type 2 diabetes, non-type 2 diabetes, type 1 diabetes, latent autoimmune diabetes (LAD), and maturity onset diabetes of the young (MODY). In certain embodiments, an FGFR2/3 + KLB bispecific antibody is for use in treating type 2 diabetes. In certain

embodiments, an FGFR2/3 + KLB bispecific antibody is for use in treating obesity. In certain embodiments, the present disclosure provides an an FGFR2/3 + KLB bispecific antibody for use in treating Bardet-Biedl syndrome, Prader-Willi syndrome, Alstrom syndrome, Cohen syndrome, Albright's hereditary osteodystrophy (pseudohypoparathyroidism), Carpenter syndrome, MOMO syndrome, Rubinstein-Taybi syndrome, fragile X syndrome and Börjeson-Forssman-Lehman syndrome. In certain embodiments, the an FGFR2/3 + KLB bispecific antibody is for use in treating NASH.

In another aspect, the present disclosure provides the use of an FGFR2/3 + KLB bispecific antibody, disclosed herein, in the manufacture of a medicament for treatment of metabolic disorders, *e.g.*, polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), obesity, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, hypertension, type 2 diabetes, non-type 2 diabetes, type 1 diabetes, latent autoimmune diabetes (LAD), and maturity onset diabetes of the young (MODY), and aging and related diseases such as Alzheimer's disease, Parkinson's disease and ALS. In certain embodiments, the metabolic disorder is type 2 diabetes. In certain embodiments, the metabolic disorder is NASH.

In another aspect, the present disclosure provides a method of treating an individual having a disease selected from the group consisting of polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), obesity, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, hypertension, type 2 diabetes, non-type 2 diabetes, type 1 diabetes, latent autoimmune diabetes (LAD), and maturity onset diabetes of the young (MODY), and aging and related diseases such as Alzheimer's disease, Parkinson's disease and ALS, the method comprising administering to the individual an effective amount of one or more FGFR2/3 + KLB bispecific antibodies of the present disclosure. In certain embodiments, the disease is diabetes, *e.g.*, type 2 diabetes. In certain embodiments, the

disease is obesity. In certain embodiments, the present disclosure provides a method of treating an individual having a disease and/or disorder selected from the group consisting of Bardet-Biedl syndrome, Prader-Willi syndrome, Alstrom syndrome, Cohen syndrome, Albright's hereditary osteodystrophy (pseudohypoparathyroidism), Carpenter syndrome, MOMO syndrome, Rubinstein-Taybi syndrome, fragile X syndrome and Börjeson-Forssman-Lehman syndrome, the method comprising administering to the individual an effective amount of one or more FGFR2/3 + KLB bispecific antibodies of the present disclosure. In certain embodiments, the method further includes administering an additional therapeutic agent to the individual. In certain embodiments, a method using one or more FGFR2/3 + KLB bispecific antibodies of the present disclosure does not affect liver function in an individual. In certain embodiments, the present disclosure provides a method for inducing weight loss comprising administering to an individual an effective amount of one or more antibodies of the present disclosure.

In another embodiment, an FGFR2/3 + KLB bispecific antibody of the present disclosure can be used as a medicament and includes one arm from an anti-KLB antibody, or antigen binding portion thereof, selected from any of the anti-KLB antibodies disclosed herein or in US20150218276 which is incorporated herein in its entirety and one arm of an FGFR2/3 antibody disclosed herein. In specific embodiments the arms of the FGFR2/3 + KLB bispecific antibody that can be used as a medicament are selected from the following combinations:

a) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NO: 82 (heavy chain) and SEQ ID NO: 66 (light chain));

b) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NO: 82 (heavy chain) and SEQ ID NO: 66 (light chain));

c) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 7-9 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 10-12 (CDRH1, CDRH2, and CDRH3));

d) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 7-9 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 10-12 (CDRH1, CDRH2, and CDRH3));

e) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NO: 282 (heavy chain) and SEQ ID NO: 283 (light chain));

f) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NO: 282 (heavy chain) and SEQ ID NO: 283 (light chain));

g) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 276-278 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 279-281 (CDRH1, CDRH2, and CDRH3)); and

h) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 276-278 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 279-281 (CDRH1, CDRH2, and CDRH3)).

In another embodiment, an FGFR2/3 + KLB bispecific antibody of the present disclosure can be used to treat a metabolic disease (e.g., NASH or a related disease) and includes one arm from an anti-KLB antibody, or antigen binding portion thereof, selected from any of the anti-KLB antibodies disclosed herein or in US20150218276 which is incorporated herein in its entirety and one arm of an FGFR2/3 antibody disclosed herein. In specific embodiments the arms of the FGFR2/3 + KLB bispecific antibody that can be used to treat a metabolic disease (e.g., NASH or a related disease) are selected from the following combinations:

a) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NO: 82 (heavy chain) and SEQ ID NO: 66 (light chain));

b) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NO: 82 (heavy chain) and SEQ ID NO: 66 (light chain));

c) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.3.12

anti-FGFR2/3 antibody (comprising SEQ ID NOs: 7-9 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 10-12 (CDRH1, CDRH2, and CDRH3));

d) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.3.12 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 7-9 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 10-12 (CDRH1, CDRH2, and CDRH3));

e) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NO: 282 (heavy chain) and SEQ ID NO: 283 (light chain));

f) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NO: 282 (heavy chain) and SEQ ID NO: 283 (light chain));

g) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 104 (HCVR) and SEQ ID NO: 105 (LCVR)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 276-278 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 279-281 (CDRH1, CDRH2, and CDRH3)); and

h) One arm from the 8C5.K4.M4L.H3.KNV anti-KLB antibody (comprising SEQ ID NO: 106 (heavy chain) and SEQ ID NO: 107 (light chain)) and one arm from the 2B.1.1.6 anti-FGFR2/3 antibody (comprising SEQ ID NOs: 276-278 (CDRL1, CDRL2, and CDRL3) and SEQ ID NO: 279-281 (CDRH1, CDRH2, and CDRH3)).

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the inhibitory effects of engineered 2B.1 antibodies for FGF7-stimulated MCF-7 cell proliferation. Error bars represent SEM.

FIGURES 2A-2C show the crystal structure of the complex between FGFR2 D2 domain and the Fab fragment of Mab 2B.1.3. Figure 2A shows the overall structure of the complex. FGFR2-D2 was colored as magenta, the heavy chain of the Fab 2B.1.3 green and the light chain blue. Figure 2B shows the overlay of the structures of FGFR2-D2:2B.1.3 and FGFR3-D2D3:R3Mab. The former complex was colored the same as in Figure 2A. FGFR3-D2D3 was colored in yellow, and R3Mab gray. Figure 2C shows the zoom-in representation of the boxed area in Figure 2B showing the structural differences between the two complexes. Color scheme is the same as in Figure 2B.

FIGURES 3A-3D show the differential blocking of FGF ligands by R3Mab variants. Figure 3A shows the blocking of FGF-7 binding to human FGFR2-IIIb. Figure 3B shows the blocking of FGF-1 binding to human FGFR2-IIIc. Figure 3C shows the blocking of FGF-1 binding to human FGFR3-IIIb. Figure 3D shows the blocking of FGF-1 binding to human FGFR3-IIIc. Figure 3E shows the blocking of FGF-19 binding to human FGFR4.

FIGURES 4A-4C show that the 2B.1 variants inhibit FGFR2 signaling *in vitro* and suppress *in vivo* xenograft growth. Figure 4A shows the blocking of FGF7-stimulated FGFR2 signaling by 2B.1 variants in gastric cancer cell line SNU-16. Figure 4B shows the effect of 2B.1.3.10 and 2B.1.3.12 on the growth of FGFR2-dependent SNU-16 xenografts compared to control antibody. Figure 4C shows the effects of 2B.1.3.10 and 2B.1.3.12 on the growth of FGFR3-dependent RT112 bladder cancer xenografts.

FIGURE 5 shows the surface areas on FGFR3-IIIb contacted by R3Mab (PDB 3GRW). The surface of the D2 and D3 domains of FGFR3-IIIb is shown in gray. The contact

areas by individual CDR loops of R3Mab are colored. The contact areas by each CDR and their percentages of overall contact areas are labeled as numbers in parentheses.

FIGURE 6 shows the sequence logo of CDR H2 from phage libraries selected for binding to FGFR2-IIIb prepared using Weblogo 3 (Crooks, G. E., G. Hon, J. M. Chandonia and S. E. Brenner (2004). "WebLogo: a sequence logo generator." Genome Res 14(6): 1188-1190).

FIGURE 7 shows the overall structural alignment of the complexes of FGFR2-D2:2B.1.3 and FGFR3-D2D3:R3Mab.

FIGURE 8A show blocking of FGF7-stimulated FGFR2 signaling by 2B.1 variants in breast cancer cell line MFM-223x2.2. FIGURE 8B shows the effects of 2B.1.3.10 and 2B.1.3.12 on the growth of FGFR2-dependent MFM-223x2.2 breast cancer xenografts. Mice under experiment showed estrogen toxicity. n = 10 per group; error bars represent SEM.

FIGURES 9A-9D shows the epitopes of the 2B.1.3.10 (*i.e.*, 1.3.10) and 2B.1.3.12 (*i.e.*, 1.3.12) anti-FGFR2/3 antibodies. Figure 9A shows the FGFR2-IIIb sequence and the epitopes of the anti-FGFR2/3 1.3.10 and 1.3.12 antibodies are underlined and in bold. Figure 9B shows the FGFR2-IIIc sequence and the epitopes of the anti-FGFR2/3 1.3.10 and 1.3.12 antibodies are underlined and in bold. Figure 9C shows the FGFR3-IIIb sequence and the epitopes of the anti-FGFR2/3 1.3.10 and 1.3.12 antibodies are underlined and in bold. Figure 9D shows the FGFR3-IIIc sequence and the epitopes of the anti-FGFR2/3 1.3.10 and 1.3.12 antibodies are underlined and in bold. Antibody 2B.1.3.10 binds to epitopes on FGFR2 that are composed of two beta-strands with residue numbers of 157-181 and 207-220 according to SEQ ID NOs: 52 and 54 (see also SEQ ID NOs: 91 and 92 for epitope sequences). Antibody 2B.1.3.10 also binds to epitopes on FGFR3 that are composed of two beta-strands with residue numbers of 154-178 and 204-217 according to SEQ ID NOs: 56 and 58 (see also SEQ ID NOs: 93 and 94 for epitope sequences). 2B.1.3.12 binds to the same epitopes as 2B.1.3.10.

In particular, 2B.1.3.12 binds to the epitope on FGFR2 that is composed of two beta-strands with residue numbers of 157-181 and 207-220. 2B.1.3.12 also binds to an epitope on FGFR3 that is composed of two beta-strands with residue numbers of 154-178 and 204-217.

FIGURE 10 shows a chart of the nucleic acid and amino acid SEQ ID NOs corresponding to anti-FGFR2/3 antibodies 1.3, 1.95, 1.73, 1.32, 1.88, 1.1, 1.3.10, and 1.3.12.

Figure 11A depicts the light chain variable region sequences for 17 anti-KLB antibodies. The CDR L1 sequences are, in order, SEQ ID NOs: 279-293; the CDR L2 sequences are, in order, SEQ ID NOs: 294-309; and the CDH L3 sequences are, in order, SEQ ID NOs: 123-137. The light chain variable region sequences are, in order, SEQ ID NOs: 111-127. Figure 11B depicts the heavy chain variable region sequences for 17 anti-KLB antibodies. The CDR H1 sequences for the antibodies are, in order (11F1-8C5), SEQ ID NOs: 230-232 and 236-247; the CDR H2 sequences are, in order, SEQ ID NOs: 142 and 248-262 ; the CDR H3 sequences are, in order, SEQ ID NOs: 263-278. The heavy chain variable region sequences for the antibodies are, in order, SEQ ID NOs: 216-232.

Figure 12 depicts the median shift observed in the FACS plot at 0.8 μg/ml measuring binding of various anti-KLB antibodies to 293 cells expressing hKLB.

Figure 13 depicts the relative binding of various anti-KLB antibodies to hKLB-ECD-HIS protein.

Figure 14A shows the N-terminal amino acid sequence of mouse KLB protein (SEQ ID NO: 165), and the corresponding amino acid sequence encoded by the *Klb* allele in the KO mice (SEQ ID NO: 166) are shown. A missense mutation in *Klb* gene results in a frame-shift after the second amino acid in the KO allele, as shown with red letters. Figure 14B shows KLB protein expression in epididymal white adipose tissue in wildtype (+/+) and *KLB* knockout (-/-) mice. Figure 14C shows that KLB is important for BsAb20 to affect glucose metabolism. Glucose tolerance test (GTT) in DIO mice that received four weekly injections

of BsAb20 or control IgG at 3 mpk. GTT was conducted on day 23, three days after the last injection. The mice were on HFD for 20 weeks prior to GTT. *p<0.05.

Figures 15A and 15B show detection of human FGFR2 (Figure 15A) and FGFR3 (Figure 15B) in SNU-16 xenograft tumors. Tumor samples were lysed and subjected to Western blot analysis for human FGFR2 and FGFR3 proteins. Tumors collected from the current study *showed* signal for FGFR3 (Figure 15B, Lane 5-24). In addition, tumors collected from a previous SNU-16 study (Figure 15B, Lane 3) and the *in vitro*-cultured SNU-16 cells (Figure B, Lane 4) also showed detectable but weaker FGFR3 expression.

Figures 16A-16C shows seven 2B1.1 variants that were expressed and tested for agonist activity and FGFR2, FGFR3, and FGFR4 binding. Figure 16A shows a chart detailing the anti-FGFR2/3 antibody variant, the sequence of the CDR H1-H3 of each variant, and the FGFR3 affinity measured by Biacor assays and ELISA. Figure 16B shows binding affinity for FGFR3 of the variants as measured by ELISA. Figure 16C shows binding affinity for FGFR4 of the variants as measured by ELISA.

Figures 17A and 17B show a comparison of anti-FGFR2/3 antibody variant activity against the FGFRs using a luciferase assay. Figure 17A shows FGFR3 and FGFR4 activity. Figure 17B shows FGFR2 and FGFR1 activity.

Figure 18 shows the anti-FGFR2/3 antibody variant decision matrix used for selecting which anti-FGFR2/3 antibody should be used for the FGFGR2/3 + KLB bispecific antibody.

Figures 19A-19C show FGFR activity of selected anti-FGFR2/3 antibody variants. Figure 19A shows FGFR3 activity. Figure 19B shows FGFR2 activity. Figure 19C shows FGFR4 activity.

DETAILED DESCRIPTION OF THE INVENTION

Fibroblast growth factors (FGFs) and their tyrosine kinase receptors (FGFRs) play key roles in regulating specific pathways during embryonic development, as well as

homeostasis of diverse tissues, wound healing processes and certain metabolic functions in the adult animal. In humans there are 4 highly homologous FGFRs (FGFR1-4) and 22 FGFs (FGF1-14 and FGF16-23) (Goetz R & Mohammadi M (2013) Exploring mechanisms of FGF signalling through the lens of structural biology. *Nat Rev Mol Cell Biol* 14(3):166-180; Turner N & Grose R (2010) Fibroblast growth factor signalling: from development to cancer. *Nat Rev Cancer* 10(2):116-129; Beenken A & Mohammadi M (2009) The FGF family: biology, pathophysiology and therapy. *Nat Rev Drug Discov* 8(3):235-253; Wesche J, Haglund K, & Haugsten EM (2011) Fibroblast growth factors and their receptors in cancer. *Biochem J* 437(2):199-213). The FGFRs comprise an extracellular region with 3 immunoglobulin domains (D1, D2 and D3), a single-pass transmembrane region and a split cytoplasmic kinase moiety (Goetz R & Mohammadi M (2013) Exploring mechanisms of FGF signalling through the lens of structural biology. *Nat Rev Mol Cell Biol* 14(3):166-180; Mohammadi M, Olsen SK, & Ibrahimi OA (2005) Structural basis for fibroblast growth factor receptor activation. *Cytokine Growth Factor Rev* 16(2):107-137). Alternative splicing gives rise to two major variants of FGFRs 1-3, termed isoforms IIIb and IIIc, which differ in the second half of D3 and consequently in ligand-binding specificity (Chang, H., Stewart, A.K., Qi, X.Y., Li, Z.H., Yi, Q.L., and Trudel, S. 2005. Immunohistochemistry accurately predicts FGFR3 aberrant expression and t(4;14) in multiple myeloma. Blood 106:353-355).

Dysregulated signaling by FGFRs 1-4 is associated with pathogenesis in several cancer types (L'Hote, C.G., and Knowles, M.A. 2005. Cell responses to FGFR3 signalling: growth, differentiation and apoptosis. Exp Cell Res 304:417-431; Dailey, L., Ambrosetti, D., Mansukhani, A., and Basilico, C. 2005. Mechanisms underlying differential responses to FGF signaling. Cytokine Growth Factor Rev 16:233-247). Genomic FGFR alterations, which include gene amplification, chromosomal translocation and activating mutations, can drive aberrant activation of the FGF pathway and promote neoplastic transformation of normal

cells. FGFR2 gene amplification occurs in ~10% of gastric and ~4% of triple-negative breast cancers (Chesi, M., Nardini, E., Brents, L.A., Schrock, E., Ried, T., Kuehl, W.M., and Bergsagel, P.L. 1997. Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. Nat Genet 16:260-264; Fonseca, R., Blood, E., Rue, M., Harrington, D., Oken, M.M., Kyle, R.A., Dewald, G.W., Van Ness, B., Van Wier, S.A., Henderson, K.J., et al. 2003. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood 101:4569-4575; Moreau, P., Facon, T., Leleu, X., Morineau, N., Huyghe, P., Harousseau, J.L., Bataille, R., and Avet-Loiseau, H. 2002. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. Blood 100:1579-1583), while FGFR3 amplification is associated with specific subsets of bladder cancer (Moreau, P., Facon, T., Leleu, X., Morineau, N., Huyghe, P., Harousseau, J.L., Bataille, R., and Avet-Loiseau, H. 2002. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. Blood 100:1579-1583; Pollett, J.B., Trudel, S., Stern, D., Li, Z.H., and Stewart, A.K. 2002. Overexpression of the myeloma-associated oncogene fibroblast growth factor receptor 3 confers dexamethasone resistance. Blood 100:3819-3821). Missense FGFR mutations are also found in multiple types of cancer (L'Hote, C.G., and Knowles, M.A. 2005. Cell responses to FGFR3 signalling: growth, differentiation and apoptosis. Exp Cell Res 304:417-431; Agazie, Y.M., Movilla, N., Ischenko, I., and Hayman, M.J. 2003. The phosphotyrosine phosphatase SHP2 is a critical mediator of transformation induced by the oncogenic fibroblast growth factor receptor 3. Oncogene 22:6909-6918). Specifically, amino-acid substitutions in the linker region between D2 and D3, e.g. S252W in FGFR2 and S249C in FGFR3, augment FGF-driven signaling and tumor-cell proliferation and represent hot spots for somatic mutation (Agazie, Y.M., Movilla, N., Ischenko, I., and Hayman, M.J. 2003. The

phosphotyrosine phosphatase SHP2 is a critical mediator of transformation induced by the oncogenic fibroblast growth factor receptor 3. Oncogene 22:6909-6918; Ronchetti, D., Greco, A., Compasso, S., Colombo, G., Dell'Era, P., Otsuki, T., Lombardi, L., and Neri, A. 2001. Deregulated FGFR3 mutants in multiple myeloma cell lines with t(4;14): comparative analysis of Y373C, K650E and the novel G384D mutations. Oncogene 20:3553-3562). Activating mutations also occur in the tyrosine kinase region of FGFRs (Chesi, M., Brents, L.A., Ely, S.A., Bais, C., Robbiani, D.F., Mesri, E.A., Kuehl, W.M., and Bergsagel, P.L. 2001. Activated fibroblast growth factor receptor 3 is an oncogene that contributes to tumor progression in multiple myeloma. Blood 97:729-736).

Targeting the FGF-FGFR pathway has been a major area of focus for cancer drug development. This effort has included small-molecule tyrosine kinase inhibitors (TKIs), blocking antibodies, as well as ligand traps (Moreau, P., Facon, T., Leleu, X., Morineau, N., Huyghe, P., Harousseau, J.L., Bataille, R., and Avet-Loiseau, H. 2002. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. Blood 100:1579-1583). Current high-potency FGFR TKIs have limited selectivity for different FGFRs (Moreau, P., Facon, T., Leleu, X., Morineau, N., Huyghe, P., Harousseau, J.L., Bataille, R., and Avet-Loiseau, H. 2002. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. Blood 100:1579-1583), which may impact their therapeutic window. For example, disruption of FGF23 signaling through hetero-complexes of FGFR1 and the co-receptor Klothoβ can lead to hyperphosphatemia and tissue calcification in patients (Plowright, E.E., Li, Z., Bergsagel, P.L., Chesi, M., Barber, D.L., Branch, D.R., Hawley, R.G., and Stewart, A.K. 2000. Ectopic expression of fibroblast growth factor receptor 3 promotes myeloma cell proliferation and prevents apoptosis. Blood 95:992-998; Chen, J., Williams, I.R., Lee, B.H., Duclos, N., Huntly, B.J., Donoghue, D.J., and Gilliland, D.G.

2005. Constitutively activated FGFR3 mutants signal through PLCgamma-dependent and -independent pathways for hematopoietic transformation. Blood 106:328-337), whereas blockade of FGF19 signaling through FGFR4 hetero-complexes with Klothoβ can disrupt bile acid metabolism (Li, Z., Zhu, Y.X., Plowright, E.E., Bergsagel, P.L., Chesi, M., Patterson, B., Hawley, T.S., Hawley, R.G., and Stewart, A.K. 2001. The myeloma-associated oncogene fibroblast growth factor receptor 3 is transforming in hematopoietic cells. Blood 97:2413-2419). More selective antibodies have been developed to antagonize ligand signaling through individual FGFRs, including FGFR1 (Trudel, S., Ely, S., Farooqi, Y., Affer, M., Robbiani, D.F., Chesi, M., and Bergsagel, P.L. 2004. Inhibition of fibroblast growth factor receptor 3 induces differentiation and apoptosis in t(4;14) myeloma. Blood 103:3521-3528), FGFR2 (Trudel, S., Li, Z.H., Wei, E., Wiesmann, M., Chang, H., Chen, C., Reece, D., Heise, C., and Stewart, A.K. 2005. CHIR-258, a novel, multitargeted tyrosine kinase inhibitor for the potential treatment of t(4;14) multiple myeloma. Blood 105:2941-2948) and FGFR3 (Chen, J., Lee, B.H., Williams, I.R., Kutok, J.L., Mitsiades, C.S., Duclos, N., Cohen, S., Adelsperger, J., Okabe, R., Coburn, A., et al. 2005. FGFR3 as a therapeutic target of the small molecule inhibitor PKC412 in hematopoietic malignancies. Oncogene 24:8259-8267). However, antibodies recognizing more than one FGFR have not yet been reported.

The previously described monospecific anti-FGFR3 antibody R3Mab effectively blocks binding of FGF1 and FGF9 to both the IIIb and IIIc isoforms of wild-type FGFR3, as well as to certain cancer-associated mutant forms of FGFR3 (Chen, J., Lee, B.H., Williams, I.R., Kutok, J.L., Mitsiades, C.S., Duclos, N., Cohen, S., Adelsperger, J., Okabe, R., Coburn, A., et al. 2005. FGFR3 as a therapeutic target of the small molecule inhibitor PKC412 in hematopoietic malignancies. Oncogene 24:8259-8267; Paterson, J.L., Li, Z., Wen, X.Y., Masih-Khan, E., Chang, H., Pollett, J.B., Trudel, S., and Stewart, A.K. 2004. Preclinical

studies of fibroblast growth factor receptor 3 as a therapeutic target in multiple myeloma. Br J Haematol 124:595-603). X-ray structural analysis revealed that R3Mab binds to a specific epitope on FGFR3 that is required for ligand binding. R3Mab displayed potent antitumor activity in mice against human bladder cancer and multiple myeloma tumor xenografts. In the present study, structure-guided phage display was used iteratively to re-engineer R3Mab into derivative antibodies that carry dual specificity for FGFR3 and FGFR2 while sparing FGFR1 and FGFR4. The practical aim of this study was to broaden the potential therapeutic scope beyond that of the parent molecule while avoiding added safety risks. The re-engineered antibodies displayed inhibition of FGF-stimulated tumor-cell growth *in vitro* and significant efficacy against human cancer xenografts overexpressing FGFR2 or FGFR3 *in vivo*.

The invention herein provides anti-FGFR2/3 antibodies that are useful for, e.g., treatment or prevention of disease states associated with expression and/or activity of FGFR2 and/or FGFR3, such as increased expression and/or activity or undesired expression and/or activity. In specific embodiments, the invention herein provides anti-FGFR2/3 antibodies that are useful for, e.g., treatment or prevention of disease states associated with expression and/or activity of FGFR2 and FGFR3, such as increased expression and/or activity or undesired expression and/or activity.In some embodiments, the antibodies of the invention are used to treat a tumor, a cancer, and/or a cell proliferative disorder.

In another aspect, the anti-FGFR2/3 antibodies of the invention find utility as reagents for detection and/or isolation of FGFR2 and/or FGFR3, such as detection of FGFR3 in various tissues and cell type. In a specific embodiment, the anti-FGFR2/3 antibodies of the invention find utility as reagents for detection and/or isolation of FGFR2 and FGFR3, such as detection of FGFR2 and FGFR3 in various tissues and cell type.

The invention further provides methods of making and using anti-FGFR2/3 antibodies, and polynucleotides encoding anti-FGFR2/3 antibodies.

General techniques

The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, et al. eds., (2003)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R. I. Freshney, ed. (1987)).

Definitions

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

An "antibody that competes for binding" with a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is described in "Antibodies," Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY).

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (*e.g.*, methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

An "effective amount" of an agent, *e.g.*, a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. For example, and not by way of limitation, an "effective

amount" can refer to an amount of an antibody, disclosed herein, that is able to alleviate, minimize and/or prevent the symptoms of the disease and/or disorder, prolong survival and/or prolong the period until relapse of the disease and/or disorder.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The terms "host cell," "host cell line," and "host cell culture" as used interchangeably herein, refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

An "individual" or "subject," as used interchangeably herein, is a mammal. Mammals include, but are not limited to, domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats). In certain embodiments, the individual or subject is a human.

The term "monoclonal antibody," as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, *e.g.*, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a

monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the presently disclosed subject matter may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (*e.g.*, a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "package insert," as used herein, refers to instructions customarily included in commercial packages of therapeutic products, that contain information about the

indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the nucleic acid (for example, an antibody encoding nucleic acid) where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

"Isolated nucleic acid encoding an antibody" (including references to a specific antibody, *e.g.*, an anti-KLB antibody) refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991). Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and

82c, etc according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The phrase "substantially similar," or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values (generally one associated with an antibody of the invention and the other associated with a reference/comparator antibody) such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values). The difference between said two values is preferably less than about 50%, preferably less than about 40%, preferably less than about 30%, preferably less than about 20%, preferably less than about 10% as a function of the value for the reference/comparator antibody.

"Binding affinity" generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Desirably the Kd is 1×10^{-7}, 1×10^{-8}, 5×10^{-8}, 1×10^{-9}, 3×10^{-9}, 5×10^{-9}, or even 1×10^{-10} or higher affinity. Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative embodiments are described in the following.

In one embodiment, the "Kd" or "Kd value" according to this invention is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay that measures solution binding affinity of Fabs for antigen by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (Chen, et al., (1999) J. Mol. Biol. 293:865-881). To establish conditions for the assay, microtiter plates (Dynex) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbant plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of an anti-VEGF antibody, Fab-12, in Presta et al., (1997) Cancer Res. 57:4593-4599). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, 65 hours) to insure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% Tween-20 in PBS. When the plates have dried, 150 μl/well of scintillant (MicroScint-20; Packard) is added, and the plates are counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays. According to another embodiment the Kd or Kd value is measured by using surface plasmon resonance assays using a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS)

according to the supplier's instructions. Antigen is diluted with 10mM sodium acetate, pH 4.8, into 5μg/ml (~0.2μM) before injection at a flow rate of 5μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% Tween 20 (PBST) at 25°C at a flow rate of approximately 25μl/min. In some embodiments, the following modifications are used for the surface Plasmon resonance assay method: antibody is immobilized to CM5 biosensor chips to achieve approximately 400 RU, and for kinetic measurements, two-fold serial dilutions of target protein (e.g., FGFR3-IIIb or –IIIc) (starting from 67 nM) are injected in PBST buffer at 25°C with a flow rate of about 30 ul/minute. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2) by simultaneous fitting the association and dissociation sensorgram. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen, Y., et al., (1999) J. Mol. Biol. 293:865-881. If the on-rate exceeds 10^6 M^{-1} S^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-Aminco spectrophotometer (ThermoSpectronic) with a stir red cuvette.

An "on-rate" or "rate of association" or "association rate" or "k_{on}" according to this invention can also be determined with the same surface plasmon resonance technique described above using a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly,

carboxymethylated dextran biosensor chips (CM5, BIAcore Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10mM sodium acetate, pH 4.8, into 5μg/ml (~0.2uM) before injection at a flow rate of 5μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% Tween 20 (PBST) at 25°C at a flow rate of approximately 25μl/min. In some embodiments, the following modifications are used for the surface Plasmon resonance assay method: antibody is immobilized to CM5 biosensor chips to achieve approximately 400 RU, and for kinetic measurements, two-fold serial dilutions of target protein (e.g., FGFR3-IIIb or –IIIc) (starting from 67 nM) are injected in PBST buffer at 25°C with a flow rate of about 30 ul/minute. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2) by simultaneous fitting the association and dissociation sensorgram. The equilibrium dissociation constant (Kd) was calculated as the ratio k_{off}/k_{on}. See, e.g., Chen, Y., et al., (1999) J. Mol. Biol. 293:865-881. However, if the on-rate exceeds 10^6 M^{-1} S^{-1} by the surface plasmon resonance assay above, then the on-rate is preferably determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-Aminco spectrophotometer (ThermoSpectronic) with a stir red cuvette.

The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector

is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "recombinant vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector.

"Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase, or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after synthesis, such as by conjugation with a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with

charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, alpha-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and a basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR_2 ("amidate"), P(O)R, P(O)OR', CO or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

"Oligonucleotide," as used herein, generally refers to short, generally single stranded, generally synthetic polynucleotides that are generally, but not necessarily, less than about 200 nucleotides in length. The terms "oligonucleotide" and "polynucleotide" are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides.

"Percent (%) amino acid sequence identity" with respect to a peptide or polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in the chart below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in, e.g., WO2007/001851. The ALIGN-2 program should be compiled for use on a

UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

In some embodiments, two or more amino acid sequences are at least 50%, 60%, 70%, 80%, or 90% identical. In some embodiments, two or more amino acid sequences are at least 95%, 97%, 98%, 99%, or even 100% identical. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "FGFR3," as used herein, refers, unless specifically or contextually indicated otherwise, to any native or variant (whether native or synthetic) FGFR3 polypeptide (e.g., FGFR3-IIIb isoform or FGFR3-IIIc isoform). The term "native sequence" specifically encompasses naturally occurring truncated forms (e.g., an extracellular domain sequence or a transmembrane subunit sequence), naturally occurring variant forms (e.g., alternatively

spliced forms) and naturally-occurring allelic variants. The term "wild-type FGFR3" generally refers to a polypeptide comprising an amino acid sequence of a naturally occurring FGFR3 protein. The term "wild type FGFR3 sequence" generally refers to an amino acid sequence found in a naturally occurring FGFR3.

The term "FGFR3 ligand," (interchangeably termed "FGF") as used herein, refers, unless specifically or contextually indicated otherwise, to any native or variant (whether native or synthetic) FGFR3 ligand (for example, FGF1, FGF2, FGF4, FGF8, FGF9, FGF17, FGF18, FGF23) polypeptide. The term "native sequence" specifically encompasses naturally occurring truncated forms (e.g., an extracellular domain sequence or a transmembrane subunit sequence), naturally occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants. The term "wild-type FGFR3 ligand" generally refers to a polypeptide comprising an amino acid sequence of a naturally occurring FGFR3 ligand protein. The term "wild type FGFR3 ligand sequence" generally refers to an amino acid sequence found in a naturally occurring FGFR3 ligand.

The term "FGFR3 activation" refers to activation, or phosphorylation, of the FGFR3 receptor. Generally, FGFR3 activation results in signal transduction (*e.g.* that caused by an intracellular kinase domain of a FGFR3 receptor phosphorylating tyrosine residues in FGFR3 or a substrate polypeptide). FGFR3 activation may be mediated by FGFR ligand binding to a FGFR3 receptor of interest. FGFR3 ligand (e.g., such as FGF1 or FGF9) binding to FGFR3 may activate a kinase domain of FGFR3 and thereby result in phosphorylation of tyrosine residues in the FGFR3 and/or phosphorylation of tyrosine residues in additional substrate polypeptides(s).

The term "FGFR2," as used herein, refers, unless specifically or contextually indicated otherwise, to any native or variant (whether native or synthetic) FGFR2 polypeptide (e.g., FGFR2-IIIb isoform or FGFR2-IIIc isoform). The term "native sequence" specifically

encompasses naturally occurring truncated forms (e.g., an extracellular domain sequence or a transmembrane subunit sequence), naturally occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants. The term "wild-type FGFR2" generally refers to a polypeptide comprising an amino acid sequence of a naturally occurring FGFR2 protein. The term "wild type FGFR2 sequence" generally refers to an amino acid sequence found in a naturally occurring FGFR2.

The term "FGFR2 ligand," (interchangeably termed "FGF2") as used herein, refers, unless specifically or contextually indicated otherwise, to any native or variant (whether native or synthetic) FGFR2 ligand. The term "native sequence" specifically encompasses naturally occurring truncated forms (e.g., an extracellular domain sequence or a transmembrane subunit sequence), naturally occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants. The term "wild-type FGFR2 ligand" generally refers to a polypeptide comprising an amino acid sequence of a naturally occurring FGFR2 ligand protein. The term "wild type FGFR2 ligand sequence" generally refers to an amino acid sequence found in a naturally occurring FGFR2 ligand.

The term "FGFR2 activation" refers to activation, or phosphorylation, of the FGFR2 receptor. FGFR2 activation may be mediated by FGFR ligand binding to a FGFR2 receptor of interest. FGFR2 ligand binding to FGFR2 may activate a kinase domain of FGFR2 and thereby result in phosphorylation of tyrosine residues in the FGFR2 and/or phosphorylation of tyrosine residues in additional substrate polypeptides(s).

The term "FGFR2/3 antibody" refers to dual-specific antibodies that bind to FGFR2 and FGFR3. Non-limiting examples of FGFR2/3 antibodies include the dual specific monoclonoal antibodies 2B.1.3.10 and 2B.1.3.12 as described herein. The terms FGFR2/3 and "FGFR 2 and FGFR3" and "FGFR3 and FGFR2" are used interchangeably herein

The term "constitutive" as used herein, as for example applied to receptor kinase activity, refers to continuous signaling activity of a receptor that is not dependent on the presence of a ligand or other activating molecules. Depending on the nature of the receptor, all of the activity may be constitutive or the activity of the receptor may be further activated by the binding of other molecules (e. g. ligands). Cellular events that lead to activation of receptors are well known among those of ordinary skill in the art. For example, activation may include oligomerization, e.g., dimerization, trimerization, etc., into higher order receptor complexes. Complexes may comprise a single species of protein, i.e., a homomeric complex. Alternatively, complexes may comprise at least two different protein species, i.e., a heteromeric complex. Complex formation may be caused by, for example, overexpression of normal or mutant forms of receptor on the surface of a cell. Complex formation may also be caused by a specific mutation or mutations in a receptor.

The term "ligand-independent" as used herein, as for example applied to receptor signaling activity, refers to signaling activity that is not dependent on the presence of a ligand. A receptor having ligand-independent kinase activity will not necessarily preclude the binding of ligand to that receptor to produce additional activation of the kinase activity.

The term "ligand-dependent" as used herein, as for example applied to receptor signaling activity, refers to signaling activity that is dependent on the presence of a ligand.

The phrase "gene amplification" refers to a process by which multiple copies of a gene or gene fragment are formed in a particular cell or cell line. The duplicated region (a stretch of amplified DNA) is often referred to as "amplicon." Usually, the amount of the messenger RNA (mRNA) produced, i.e., the level of gene expression, also increases in the proportion of the number of copies made of the particular gene expressed.

A "tyrosine kinase inhibitor" is a molecule which inhibits to some extent tyrosine kinase activity of a tyrosine kinase such as FGFR2 and FGFR3 receptors.

A cancer or biological sample which "displays FGFR3 expression, amplification, or activation" is one which, in a diagnostic test, expresses (including overexpresses) FGFR3, has amplified FGFR3 gene, and/or otherwise demonstrates activation or phosphorylation of a FGFR3. A cancer or biological sample which "displays FGFR2 expression, amplification, or activation" is one which, in a diagnostic test, expresses (including overexpresses) FGFR2, has amplified FGFR2 gene, and/or otherwise demonstrates activation or phosphorylation of a FGFR2. A cancer or biological sample which "displays FGFR2/3 expression, amplification, or activation" or "displays FGFR2 and FGFR3 expression, amplicification, or activation" is one which, in a diagnostic test, expresses (including overexpresses) FGFR2 and FGFR3, has amplified FGFR2 and FGFR3 genes, and/or otherwise demonstrates activation or phosphorylation of a FGFR2 and a FGFR3.

"Klotho-beta," "KLB" and "beta-Klotho," as used herein, refers to any native beta-Klotho from any vertebrate source, including mammals such as primates (*e.g.*, humans) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed KLB as well as any form of KLB that results from processing in the cell. The term also encompasses naturally occurring variants of KLB, *e.g.*, splice variants or allelic variants. A non-limiting example of a human KLB amino acid sequence targeted by an antibody of the present disclosure, excluding the signal sequence, is as follows:

FSGDGRAIWSKNPNFTPVNESQLFLYDTFPKNFFWGIGTGALQVEGSWKKDGKG
PSIWDHFIHTHLKNVSSTNGSSDSYIFLEKDLSALDFIGVSFYQFSISWPRLFPDGIV
TVANAKGLQYYSTLLDALVLRNIEPIVTLYHWDLPLALQEKYGGWKNDTIIDIFN
DYATYCFQMFGDRVKYWITIHNPYLVAWHGYGTGMHAPGEKGNLAAVYTVGH
NLIKAHSKVWHNYNTHFRPHQKGWLSITLGSHWIEPNRSENTMDIFKCQQSMVS
VLGWFANPIHGDGDYPEGMRKKLFSVLPIFSEAEKHEMRGTADFFAFSFGPNNFK
PLNTMAKMGQNVSLNLREALNWIKLEYNNPRILIAENGWFTDSRVKTEDTTAIY

MMKNFLSQVLQAIRLDEIRVFGYTAWSLLDGFEWQDAYTIRRGLFYVDFNSKQK
ERKPKSSAHYYKQIIRENGFSLKESTPDVQGQFPCDFSWGVTESVLKPESVASSPQ
FSDPHLYVWNATGNRLLHRVEGVRLKTRPAQCTDFVNIKKQLEMLARMKVTHY
RFALDWASVLPTGNLSAVNRQALRYYRCVVSEGLKLGISAMVTLYYPTHAHLGL
PEPLLHADGWLNPSTAEAFQAYAGLCFQELGDLVKLWITINEPNRLSDIYNRSGN
DTYGAAHNLLVAHALAWRLYDRQFRPSQRGAVSLSLHADWAEPANPYADSHW
RAAERFLQFEIAWFAEPLFKTGDYPAAMREYIASKHRRGLSSSALPRLTEAERRL
LKGTVDFCALNHFTTRFVMHEQLAGSRYDSDRDIQFLQDITRLSSPTRLAVIPWG
VRKLLRWVRRNYGDMDIYITASGIDDQALEDDRLRKYYLGKYLQEVLKAYLIDK
VRIKGYYAFKLAEEKSKPRFGFFTSDFKAKSSIQFYNKVISSRGFPFENSSSRCSQT
QENTECTVCLFLVQKKPLIFLGCCFFSTLVLLLSIAIFQRQKRRKFWKAKNLQHIPL
KKGKRVVS (SEQ ID NO: 233).

In certain embodiments, a KLB protein can include a N-terminal signal sequence having the amino acid sequence

MKPGCAAGSPGNEWIFFSTDEITTRYRNTMSNGGLQRSVILSALILLRAVTG (SEQ ID NO: 234).

The term "C-terminal domain of KLB" refers to the carboxy-terminal glycosidase-like domain of KLB. For example, the C-terminal domain of the exemplary KLB protein shown in SEQ ID NO: 233 comprises the following amino acid sequence:

FPCDFSWGVTESVLKPESVASSPQFSDPHLYVWNATGNRLLHRVEGVRLKTRPAQC
TDFVNIKKQLEMLARMKVTHYRFALDWASVLPTGNLSAVNRQALRYYRCVVSEGL
KLGISAMVTLYYPTHAHLGLPEPLLHADGWLNPSTAEAFQAYAGLCFQELGDLVKL
WITINEPNRLSDIYNRSGNDTYGAAHNLLVAHALAWRLYDRQFRPSQRGAVSLSLH
ADWAEPANPYADSHWRAAERFLQFEIAWFAEPLFKTGDYPAAMREYIASKHRRGLS
SSALPRLTEAERRLLKGTVDFCALNHFTTRFVMHEQLAGSRYDSDRDIQFLQDITRLS

SPTRLAVIPWGVRKLLRWVRRNYGDMDIYITASGIDDQALEDDRLRKYYLGKYLQE VLKAYLIDKVRIKGYYAFKLAEEKSKPRFGFFTSDFKAKSSIQFYNKVISSRGFPFENS SSR (SEQ ID NO: 235).

The terms "anti-KLB antibody" and "an antibody that binds to KLB" refer to an antibody that is capable of binding KLB with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting KLB. In one embodiment, the extent of binding of an anti-KLB antibody to an unrelated, non-KLB protein is less than about 10% of the binding of the antibody to KLB as measured, *e.g.*, by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to KLB has a dissociation constant (K_d) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (*e.g.*, 10^{-8} M or less, *e.g.*, from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-KLB antibody binds to an epitope of KLB that is conserved among KLB from different species. In certain embodiments, an anti-KLB antibody binds to an epitope on KLB that is in the C-terminal part of the protein.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier," as used herein, refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the

individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In certain embodiments, antibodies of the present disclosure can be used to delay development of a disease or to slow the progression of a disease. "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. As it relates to the FGFR2/3 antibody, those in need of treatment include those already having a benign, pre-cancerous, or non-metastatic tumor as well as those in which the occurrence or recurrence of cancer is to be prevented.

A cancer or biological sample which "displays FGFR3 activation" is one which, in a diagnostic test, demonstrates activation or phosphorylation of FGFR3. Such activation can be determined directly (*e.g.* by measuring FGFR3 phosphorylation by ELISA) or indirectly. A cancer or biological sample which "displays FGFR2 activation" is one which, in a diagnostic test, demonstrates activation or phosphorylation of FGFR2. Such activation can be determined directly or indirectly. A cancer or biological sample which "displays FGFR2 and FGFR3 activation" is one which, in a diagnostic test, demonstrates activation or phosphorylation of FGFR2 and FGFR3. Such activation can be determined directly or indirectly.

A cancer or biological sample which "displays constitutive FGFR3 activation" is one which, in a diagnostic test, demonstrates constitutive activation or phosphorylation of a FGFR3. Such activation can be determined directly (*e.g.* by measuring c-FGFR3 phosphorylation by ELISA) or indirectly. A cancer or biological sample which "displays constitutive FGFR2 activation" is one which, in a diagnostic test, demonstrates constitutive

activation or phosphorylation of a FGFR2. Such activation can be determined directly or indirectly. A cancer or biological sample which "displays constitutive FGFR2 and FGFR3 activation" is one which, in a diagnostic test, demonstrates constitutive activation or phosphorylation of a FGFR2 and a FGFR3. Such activation can be determined directly or indirectly.

A cancer or biological sample which "displays FGFR3 amplification" is one which, in a diagnostic test, has amplified FGFR3 gene. A cancer or biological sample which "displays FGFR2 amplification" is one which, in a diagnostic test, has amplified FGFR2 gene. A cancer or biological sample which "displays FGFR2 and FGFR3 amplification" is one which, in a diagnostic test, has amplified FGFR2 and FGFR3 genes.

A cancer or biological sample which "displays FGFR3 translocation" is one which, in a diagnostic test, has translocated FGFR3 gene. An example of a FGFR3 translocation is the t(4;14) translocation, which occurs in some multiple myeloma tumors. A cancer or biological sample which "displays FGFR2 translocation" is one which, in a diagnostic test, has translocated FGFR2 gene. A cancer or biological sample which "displays FGFR2 and FGFR3 translocation" is one which, in a diagnostic test, has translocated FGFR2 and FGFR3 genes.

A "phospho-ELISA assay" herein is an assay in which phosphorylation of one or more FGFR (e.g. FGFR2 and FGFR3), substrate or downstream signaling molecules is evaluated in an enzyme-linked immunosorbent assay (ELISA) using a reagent, usually an antibody, to detect a phosphorylated FGFR (e.g. FGFR2 and FGFR3), substrate, or downstream signaling molecule. In some embodiments, an antibody which detects phosphorylated FGFR2, FGFR3, or pMAPK is used. In a specific embodiment, an antibody which detects phosphorylated FGFR2 and FGFR3 is used. The assay may be performed on cell lysates, preferably from fresh or frozen biological samples.

A cancer or biological sample which "displays ligand-independent FGFR3 activation" is one which, in a diagnostic test, demonstrates ligand-independent activation or phosphorylation of a FGFR3. Such activation can be determined directly (*e.g.* by measuring FGFR3 phosphorylation by ELISA) or indirectly. A cancer or biological sample which "displays ligand-independent FGFR2 activation" is one which, in a diagnostic test, demonstrates ligand-independent activation or phosphorylation of a FGFR2. Such activation can be determined directly or indirectly. A cancer or biological sample which "displays ligand-independent FGFR2/3 activation" is one which, in a diagnostic test, demonstrates ligand-independent activation or phosphorylation of a FGFR2 and FGFR3. Such activation can be determined directly or indirectly.

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demonstrates ligand-independent activation or phosphorylation of a FGFR2. Such activation can be determined directly or indirectly. A cancer or biological sample which "displays ligand-independent FGFR2/3 activation" is one which, in a diagnostic test, demonstrates ligand-independent activation or phosphorylation of a FGFR2/3. Such activation can be determined directly or indirectly.

A cancer cell with "FGFR3 overexpression or amplification" is one which has significantly higher levels of a FGFR3 protein or gene compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. FGFR3 overexpression or amplification may be determined in a diagnostic or prognostic assay by evaluating increased levels of the FGFR3 protein present on the surface of a cell (*e.g.* via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of FGFR3 -encoding nucleic acid in the cell, *e.g.* via fluorescent *in situ* hybridization (FISH; see WO98/45479 published October, 1998), southern blotting, or polymerase chain reaction (PCR) techniques, such as quantitative real time PCR (qRT-PCR). Aside from the above assays, various *in vivo* assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.* a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.* by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

A cancer cell with "FGFR2 overexpression or amplification" is one which has significantly higher levels of a FGFR2 protein or gene compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. FGFR2 overexpression or amplification may be determined in a diagnostic or prognostic assay by evaluating increased levels of the FGFR2

protein present on the surface of a cell (*e.g.* via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of FGFR2 -encoding nucleic acid in the cell, *e.g.* via fluorescent *in situ* hybridization (FISH; see WO98/45479 published October, 1998), southern blotting, or polymerase chain reaction (PCR) techniques, such as quantitative real time PCR (qRT-PCR). Aside from the above assays, various *in vivo* assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.* a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.* by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

A cancer cell with "FGFR2/3 overexpression or amplification" is one which has significantly higher levels of FGFR2 and FGFR3 proteins or genes compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. FGFR2 and FGFR3 overexpression or amplification may be determined in a diagnostic or prognostic assay by evaluating increased levels of the FGFR2 and FGFR3 proteins present on the surface of a cell (*e.g.* via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of FGFR2 and FGFR3 -encoding nucleic acid in the cell, *e.g.* via fluorescent *in situ* hybridization (FISH; see WO98/45479 published October, 1998), southern blotting, or polymerase chain reaction (PCR) techniques, such as quantitative real time PCR (qRT-PCR). Aside from the above assays, various *in vivo* assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.* a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.* by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

The term “mutation”, as used herein, means a difference in the amino acid or nucleic acid sequence of a particular protein or nucleic acid (gene, RNA) relative to the wild-type protein or nucleic acid, respectively. A mutated protein or nucleic acid can be expressed from or found on one allele (heterozygous) or both alleles (homozygous) of a gene, and may be somatic or germ line. In the instant invention, mutations are generally somatic. Mutations include sequence rearrangements such as insertions, deletions, and point mutations (including single nucleotide/amino acid polymorphisms).

To "inhibit" is to decrease or reduce an activity, function, and/or amount as compared to a reference.

An agent possesses “agonist activity or function” when an agent mimics at least one of the functional activities of a polypeptide of interest (e.g., FGFR ligand, such as FGF1 or FGF9).

An “agonist antibody”, as used herein, is an antibody which mimics at least one of the functional activities of a polypeptide of interest (e.g., FGFR ligand, such as FGF1 or FGF9).

Protein “expression” refers to conversion of the information encoded in a gene into messenger RNA (mRNA) and then to the protein.

Herein, a sample or cell that “expresses” a protein of interest (such as a FGF receptor or FGF receptor ligand) is one in which mRNA encoding the protein, or the protein, including fragments thereof, is determined to be present in the sample or cell.

An “ immunoconjugate” (interchangeably referred to as “antibody-drug conjugate,” or “ADC”) means an antibody conjugated to one or more cytotoxic agents, such as a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., a protein toxin, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

The term "Fc region", as used herein, generally refers to a dimer complex comprising the C-terminal polypeptide sequences of an immunoglobulin heavy chain, wherein a C-terminal polypeptide sequence is that which is obtainable by papain digestion of an intact antibody. The Fc region may comprise native or variant Fc sequences. Although the boundaries of the Fc sequence of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc sequence is usually defined to stretch from an amino acid residue at about position Cys226, or from about position Pro230, to the carboxyl terminus of the Fc sequence. The Fc sequence of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during purification of the antibody or by recombinant engineering of the nucleic acid encoding the antibody. Accordingly, a composition comprising an antibody having an Fc region according to this invention can comprise an antibody with K447, with all K447 removed, or a mixture of antibodies with and without the K447 residue.

By "Fc polypeptide" herein is meant one of the polypeptides that make up an Fc region. An Fc polypeptide may be obtained from any suitable immunoglobulin, such as IgG_1, IgG_2, IgG_3, or IgG_4 subtypes, IgA, IgE, IgD or IgM. In some embodiments, an Fc polypeptide comprises part or all of a wild type hinge sequence (generally at its N terminus). In some embodiments, an Fc polypeptide does not comprise a functional or wild type hinge sequence.

A "blocking" antibody or an antibody "antagonist" is one which inhibits or reduces biological activity of the antigen it binds. Preferred blocking antibodies or antagonist antibodies completely inhibit the biological activity of the antigen.

A "naked antibody" is an antibody that is not conjugated to a heterologous molecule, such as a cytotoxic moiety or radiolabel.

An antibody having a "biological characteristic" of a designated antibody is one which possesses one or more of the biological characteristics of that antibody which distinguish it from other antibodies that bind to the same antigen.

In order to screen for antibodies which bind to an epitope on an antigen bound by an antibody of interest, a routine cross-blocking assay such as that described in Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed.

To increase the half-life of the antibodies or polypeptide containing the amino acid sequences of this invention, one can attach a salvage receptor binding epitope to the antibody (especially an antibody fragment), as described, e.g., in US Patent 5,739,277. For example, a nucleic acid molecule encoding the salvage receptor binding epitope can be linked in frame to a nucleic acid encoding a polypeptide sequence of this invention so that the fusion protein expressed by the engineered nucleic acid molecule comprises the salvage receptor binding epitope and a polypeptide sequence of this invention. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.,* IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule (e.g., Ghetie et al., *Ann. Rev. Immunol.* 18:739-766 (2000), Table 1). Antibodies with substitutions in an Fc region thereof and increased serum half-lives are also described in WO00/42072, WO 02/060919; Shields et al., *J. Biol. Chem.* 276:6591-6604 (2001); Hinton, *J. Biol. Chem.* 279:6213-6216 (2004)). In another embodiment, the serum half-life can also be increased, for example, by attaching other polypeptide sequences. For example, antibodies or other polypeptides useful in the methods of the invention can be attached to serum albumin or a portion of serum albumin that binds to the FcRn receptor or a serum albumin binding peptide so that serum albumin binds to the antibody or polypeptide, e.g., such polypeptide sequences are disclosed in WO01/45746. In one preferred embodiment, the

serum albumin peptide to be attached comprises an amino acid sequence of DICLPRWGCLW (SEQ ID NO:183). In another embodiment, the half-life of a Fab is increased by these methods. *See also*, Dennis et al. *J. Biol. Chem.* 277:35035-35043 (2002) for serum albumin binding peptide sequences.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule that contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, or more nucleotides or 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 190, 200 amino acids or more.

The phrase "little to no agonist function" with respect to an antibody of the invention, as used herein, means the antibody does not elicit a biologically meaningful amount of agonist activity, e.g., upon administration to a subject. As would be understood in the art, amount of an activity may be determined quantitatively or qualitatively, so long as a comparison between an antibody of the invention and a reference counterpart can be done. The activity can be measured or detected according to any assay or technique known in the art, including, e.g., those described herein. The amount of activity for an antibody of the invention and its reference counterpart can be determined in parallel or in separate runs. In some embodiments, a bivalent antibody of the invention does not possess substantial agonist function.

The terms "apoptosis" and "apoptotic activity" are used in a broad sense and refer to the orderly or controlled form of cell death in mammals that is typically accompanied by one or more characteristic cell changes, including condensation of cytoplasm, loss of plasma membrane microvilli, segmentation of the nucleus, degradation of chromosomal DNA or loss of mitochondrial function. This activity can be determined and measured using techniques

known in the art, for instance, by cell viability assays, FACS analysis or DNA electrophoresis, and more specifically by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmatic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies).

The terms "antibody" and "immunoglobulin" are used interchangeably in the broadest sense and include monoclonal antibodies (e.g., full length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies so long as they exhibit the desired biological activity) and may also include certain antibody fragments (as described in greater detail herein). An antibody can be human, humanized, and/or affinity matured.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. "Antibody fragments" comprise only a portion of an intact antibody, wherein the portion preferably retains at least one, preferably most or all, of the functions normally associated with that portion when present in an intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. In one embodiment, an antibody fragment comprises an antigen binding site of the intact antibody and thus retains the ability to bind antigen. In another embodiment, an antibody fragment, for example one that comprises the Fc region, retains at least one of the biological functions normally associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an in vivo half life substantially similar to an intact antibody. For e.g., such an antibody fragment may comprise on antigen binding arm linked to an Fc sequence capable of conferring in vivo stability to the fragment.

The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity

Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B
	(Kabat Numbering)			
H1	H31-H35	H26-H35	H26-H32	H30-H35
	(Chothia Numbering)			
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102 or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat *et al.*, *supra* for each of these definitions.

"Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also the following review articles and references cited therein: Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994).

"Chimeric" antibodies (immunoglobulins) have a portion of the heavy and/or light chain identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder

of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Humanized antibody as used herein is a subset of chimeric antibodies.

"Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv see Pluckthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

An "antigen" is a predetermined antigen to which an antibody can selectively bind. The target antigen may be polypeptide, carbohydrate, nucleic acid, lipid, hapten or other naturally occurring or synthetic compound. Preferably, the target antigen is a polypeptide.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH - VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human

antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

An "affinity matured" antibody is one with one or more alterations in one or more CDRs thereof which result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. Bio/Technology 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et al., Proc Nat. Acad. Sci, USA 91:3809-3813 (1994); Schier et al., Gene 169:147-155 (1995); Yelton et al., J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al., J. Mol. Biol. 226:889-896 (1992).

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.,* B cell receptor); and B cell activation.

"Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on

hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 or Presta U.S. Patent No. 6,737,056 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al., *PNAS (USA)* 95:652-656 (1998).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source, *e.g.*, from blood.

"Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see review M. in Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al., Immunomethods* 4:25-34 (1994); and de Haas et al., *J. Lab. Clin. Med.* 126:330-41 (1995).

Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al., J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)) and regulates homeostasis of immunoglobulins. WO 00/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. The content of that patent publication is specifically incorporated herein by reference. See, also, Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).

Methods of measuring binding to FcRn are known (see, e.g., Ghetie 1997, Hinton 2004). Binding to human FcRn in vivo and serum half life of human FcRn high affinity binding polypeptides can be assayed, e.g, in transgenic mice or transfected human cell lines expressing human FcRn, or in primates administered with the Fc variant polypeptides.

"Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.,* as described in Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996), may be performed.

Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US patent No. 6,194,551B1 and WO 99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie et al., *J. Immunol.* 164:4178-4184 (2000).

The term "Fc region-comprising polypeptide" refers to a polypeptide, such as an antibody or immunoadhesin, which comprises an Fc region. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during purification of the polypeptide or by recombinant engineering the nucleic acid

encoding the polypeptide. Accordingly, a composition comprising a polypeptide having an Fc region according to this invention can comprise polypeptides with K447, with all K447 removed, or a mixture of polypeptides with and without the K447 residue.

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework, or from a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or human consensus framework may comprise the same amino acid sequence thereof, or may contain pre-existing amino acid sequence changes. Where pre-existing amino acid changes are present, preferably no more than 5 and preferably 4 or less, or 3 or less, pre-existing amino acid changes are present. Where pre-existing amino acid changes are present in a VH, preferably those changes are only at three, two, or one of positions 71H, 73H, and 78H; for instance, the amino acid residues at those positions may be 71A, 73T, and/or 78A. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residue in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al.

A "VH subgroup III consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable heavy subgroup III of Kabat et al. In one embodiment, the VH subgroup III consensus framework amino acid sequence comprises at

least a portion or all of each of the following sequences: EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:95)-H1-WVRQAPGKGLEWV (SEQ ID NO:96)-H2-RFTISRDNSKNTLYLQMNSLRAEDTAVYYC (SEQ ID NO:97)-H3-WGQGTLVTVSS (SEQ ID NO:98).

A "VL subgroup I consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable light kappa subgroup I of Kabat et al. In one embodiment, the VH subgroup I consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences: DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:99)-L1-WYQQKPGKAPKLLIY (SEQ ID NO:100)-L2-GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO:101)-L3-FGQGTKVEIK (SEQ ID NO:102).

As used herein, "antibody mutant" or "antibody variant" refers to an amino acid sequence variant of an antibody wherein one or more of the amino acid residues of the species-dependent antibody have been modified. Such mutants necessarily have less than 100% sequence identity or similarity with the species-dependent antibody. In one embodiment, the antibody mutant will have an amino acid sequence having at least 75% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the species-dependent antibody, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%. Identity or similarity with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical (i.e same residue) or similar (i.e. amino acid residue from the same group based on common side-chain properties, see below) with the species-dependent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody

sequence outside of the variable domain shall be construed as affecting sequence identity or similarity

A "disorder" or "disease" is any condition that would benefit from treatment with a substance/molecule or method of the invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include malignant and benign tumors; carcinoma, blastoma, and sarcoma.

The term "therapeutically effective amount" refers to an amount of a therapeutic agent to treat or prevent a disease or disorder in a mammal. In the case of cancers, the therapeutically effective amount of the therapeutic agent may reduce the number of cancer cells; reduce the primary tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy in vivo can, for example, be measured by assessing the duration of survival, time to disease progression (TTP), the response rates (RR), duration of response, and/or quality of life.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Included in this definition are benign and malignant cancers. By "early stage cancer" or "early stage tumor" is meant a cancer that is not invasive or metastatic or is classified as a Stage 0, I, or II cancer. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma (including medulloblastoma and retinoblastoma), sarcoma (including liposarcoma and synovial cell sarcoma), neuroendocrine tumors (including carcinoid tumors, gastrinoma, and

islet cell cancer), mesothelioma, schwannoma (including acoustic neuroma), meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (*e.g.* epithelial squamous cell cancer), lung cancer including small-cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer (including metastatic breast cancer), colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophagael cancer, tumors of the biliary tract, as well as head and neck cancer and multiple myeloma.

The term "pre-cancerous" refers to a condition or a growth that typically precedes or develops into a cancer. A "pre-cancerous" growth will have cells that are characterized by abnormal cell cycle regulation, proliferation, or differentiation, which can be determined by markers of cell cycle regulation, cellular proliferation, or differentiation.

By "dysplasia" is meant any abnormal growth or development of tissue, organ, or cells. Preferably, the dysplasia is high grade or precancerous.

By "metastasis" is meant the spread of cancer from its primary site to other places in the body. Cancer cells can break away from a primary tumor, penetrate into lymphatic and blood vessels, circulate through the bloodstream, and grow in a distant focus (metastasize) in normal tissues elsewhere in the body. Metastasis can be local or distant. Metastasis is a sequential process, contingent on tumor cells breaking off from the primary tumor, traveling through the bloodstream, and stopping at a distant site. At the new site, the cells establish a blood supply and can grow to form a life-threatening mass.

Both stimulatory and inhibitory molecular pathways within the tumor cell regulate this behavior, and interactions between the tumor cell and host cells in the distant site are also significant.

By "non-metastatic" is meant a cancer that is benign or that remains at the primary site and has not penetrated into the lymphatic or blood vessel system or to tissues other than the primary site. Generally, a non-metastatic cancer is any cancer that is a Stage 0, I, or II cancer, and occasionally a Stage III cancer.

By "primary tumor" or "primary cancer" is meant the original cancer and not a metastatic lesion located in another tissue, organ, or location in the subject's body.

By "benign tumor" or "benign cancer" is meant a tumor that remains localized at the site of origin and does not have the capacity to infiltrate, invade, or metastasize to a distant site.

By "tumor burden" is meant the number of cancer cells, the size of a tumor, or the amount of cancer in the body. Tumor burden is also referred to as tumor load.

By "tumor number" is meant the number of tumors.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. Preferably, the subject is a human.

The term "anti-cancer therapy" refers to a therapy useful in treating cancer. Examples of anti-cancer therapeutic agents include, but are limited to, e.g., chemotherapeutic agents, growth inhibitory agents, cytotoxic agents, agents used in radiation therapy, anti-angiogenesis agents, apoptotic agents, anti-tubulin agents, and other agents to treat cancer , anti-CD20 antibodies, platelet derived growth factor inhibitors (e.g., Gleevec™ (Imatinib Mesylate)), a COX-2 inhibitor (e.g., celecoxib), interferons, cytokines, antagonists (e.g., neutralizing antibodies) that bind to one or more of the following targets ErbB2, ErbB3, ErbB4, PDGFR-

beta, BlyS, APRIL, BCMA or VEGF receptor(s), TRAIL/Apo2, and other bioactive and organic chemical agents, etc. Combinations thereof are also included in the invention.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (see, e.g., Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin

(including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2- ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol- Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine;

mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; VELCADE bortezomib; REVLIMID lenalidomide; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); inhibitors of PKC-alpha, Raf, H-Ras, EGFR (e.g., erlotinib (Tarceva^{TM})) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON· toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Raf and H-Ras; ribozymes such as a VEGF expression inhibitor (e.g., ANGIOZYME® ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN®

topoisomerase 1 inhibitor; ABARELIX® rmRH; Vinorelbine and Esperamicins (see U.S. Pat. No. 4,675,187), and pharmaceutically acceptable salts, acids or derivatives of any of the above.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, e.g., Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions*, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella et al., "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt et al., (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

By "radiation therapy" is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell altogether. It will be appreciated that there will be many ways known in the art to determine the dosage and duration of treatment. Typical treatments are given as a one time administration and typical dosages range from 10 to 200 units (Grays) per day.

A "biological sample" (interchangeably termed "sample" or "tissue or cell sample") encompasses a variety of sample types obtained from an individual and can be used in a

diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom, and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides, or embedding in a semi-solid or solid matrix for sectioning purposes. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples. The source of the biological sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood constituents; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the individual. In some embodiments, the biological sample is obtained from a primary or metastatic tumor. The biological sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like.

For the purposes herein a "section" of a tissue sample is meant a single part or piece of a tissue sample, e.g., a thin slice of tissue or cells cut from a tissue sample. It is understood that multiple sections of tissue samples may be taken and subjected to analysis according to the present invention. In some embodiments, the same section of tissue sample is analyzed at both morphological and molecular levels, or is analyzed with respect to both protein and nucleic acid.

The word "label" when used herein refers to a compound or composition which is conjugated or fused directly or indirectly to a reagent such as a nucleic acid probe or an antibody and facilitates detection of the reagent to which it is conjugated or fused. The label may itself be detectable (e.g., radioisotope labels or fluorescent labels) or, in the case of an

enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

Anti-FGFR2/3 Antibody Compositions and Methods of Using anti-FGFR2/3 Antibodies

This invention encompasses compositions, including pharmaceutical compositions, comprising an anti-FGFR2/3 antibody; and polynucleotides comprising sequences encoding an anti-FGFR2/3 antibody. As used herein, compositions comprise one or more antibodies that bind to FGFR2 and FGFR3, and/or one or more polynucleotides comprising sequences encoding one or more antibodies that bind to FGFR2 and FGFR3. These compositions may further comprise suitable carriers, such as pharmaceutically acceptable excipients including buffers, which are well known in the art.

The invention also encompasses isolated antibody and polynucleotide embodiments. The invention also encompasses substantially pure antibody and polynucleotide embodiments.

The invention also encompasses method of treating a disorder, e.g. multiple myeloma or transitional stage carcinoma (e.g., invasive transitional stage carcinoma) using an anti-FGFR2/3 antibody (as described herein or as known in the art).

Anti-FGFR2/3 Antibody Compositions

The anti-FGFR2/3 antibodies of the invention are preferably monoclonal. Also encompassed within the scope of the invention are Fab, Fab', Fab'-SH and $F(ab')_2$ fragments of the anti-FGFR2/3 antibodies provided herein. These antibody fragments can be created by traditional means, such as enzymatic digestion, or may be generated by recombinant techniques. Such antibody fragments may be chimeric or humanized. These fragments are useful for the diagnostic and therapeutic purposes set forth below.

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

The anti-FGFR2/3 monoclonal antibodies of the invention can be made using the hybridoma method first described by Kohler *et al., Nature,* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Antibodies to FGFR2/3 may be raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of FGFR2/3 and an adjuvant. FGFR2/3 may be prepared using methods well-known in the art, some of which are further described herein. For example, recombinant production of human and mouse FGFR2/3 is described below. In one embodiment, animals are immunized with a FGFR2/3 fused to the Fc portion of an immunoglobulin heavy chain. In a preferred embodiment, animals are immunized with a FGFR2/3-IgG1 fusion protein. Animals ordinarily are immunized against immunogenic conjugates or derivatives of FGFR2/3 with monophosphoryl lipid A (MPL)/trehalose dicrynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, MT) and the solution is injected intradermally at multiple sites. Two weeks later the animals are boosted. 7 to 14 days later animals are bled and the serum is assayed for anti-FGFR2/3 titer. Animals are boosted until titer plateaus.

Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a

hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against FGFR2/3. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoadsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The anti-FGFR2/3 antibodies of the invention can be made by using combinatorial libraries to screen for synthetic antibody clones with the desired activity or activities. In principle, synthetic antibody clones are selected by screening phage libraries containing phage that display various fragments of antibody variable region (Fv) fused to phage coat protein. Such phage libraries are panned by affinity chromatography against the desired antigen. Clones expressing Fv fragments capable of binding to the desired antigen are adsorbed to the antigen and thus separated from the non-binding clones in the library. The binding clones are then eluted from the antigen, and can be further enriched by additional cycles of antigen adsorption/elution. Any of the anti-FGFR3 antibodies of the invention can be obtained by designing a suitable antigen screening procedure to select for the phage clone of interest followed by construction of a full length anti-FGFR2/3 antibody clone using the Fv sequences from the phage clone of interest and suitable constant region (Fc) sequences described in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3.

The antigen-binding domain of an antibody is formed from two variable (V) regions of about 110 amino acids, one each from the light (VL) and heavy (VH) chains, that both present three hypervariable loops or complementarity-determining regions (CDRs). Variable domains can be displayed functionally on phage, either as single-chain Fv (scFv) fragments, in which VH and VL are covalently linked through a short, flexible peptide, or as Fab fragments, in which they are each fused to a constant domain and interact non-covalently, as described in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994). As used herein, scFv encoding phage clones and Fab encoding phage clones are collectively referred to as "Fv phage clones" or "Fv clones".

Repertoires of VH and VL genes can be separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be searched for antigen-binding clones as described in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994). Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned to provide a single source of human antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths *et al.*, *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning the unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992).

Filamentous phage is used to display antibody fragments by fusion to the minor coat protein pIII. The antibody fragments can be displayed as single chain Fv fragments, in which VH and VL domains are connected on the same polypeptide chain by a flexible polypeptide spacer, e.g., as described by Marks *et al.*, *J. Mol. Biol.*, 222: 581-597 (1991), or as Fab fragments, in which one chain is fused to pIII and the other is secreted into the bacterial host

cell periplasm where assembly of a Fab-coat protein structure which becomes displayed on the phage surface by displacing some of the wild type coat proteins, e.g., as described in Hoogenboom *et al.*, *Nucl. Acids Res.*, 19: 4133-4137 (1991).

In general, nucleic acids encoding antibody gene fragments are obtained from immune cells harvested from humans or animals. If a library biased in favor of anti-FGFR2/3 clones is desired, the individual is immunized with FGFR2/3 to generate an antibody response, and spleen cells and/or circulating B cells other peripheral blood lymphocytes (PBLs) are recovered for library construction. In a preferred embodiment, a human antibody gene fragment library biased in favor of anti-FGFR2/3 clones is obtained by generating an anti-FGFR2/3 antibody response in transgenic mice carrying a functional human immunoglobulin gene array (and lacking a functional endogenous antibody production system) such that FGFR2/3 immunization gives rise to B cells producing human antibodies against FGFR2/3. The generation of human antibody-producing transgenic mice is described below.

Additional enrichment for anti-FGFR2/3 reactive cell populations can be obtained by using a suitable screening procedure to isolate B cells expressing FGFR2/3-specific membrane bound antibody, e.g., by cell separation with FGFR2/3 affinity chromatography or adsorption of cells to fluorochrome-labeled FGFR2/3 followed by flow-activated cell sorting (FACS).

Alternatively, the use of spleen cells and/or B cells or other PBLs from an unimmunized donor provides a better representation of the possible antibody repertoire, and also permits the construction of an antibody library using any animal (human or non-human) species in which FGFR2/3 is not antigenic. For libraries incorporating in vitro antibody gene construction, stem cells are harvested from the individual to provide nucleic acids encoding unrearranged antibody gene segments. The immune cells of interest can be obtained from a

variety of animal species, such as human, mouse, rat, lagomorpha, luprine, canine, feline, porcine, bovine, equine, and avian species, etc.

Nucleic acid encoding antibody variable gene segments (including VH and VL segments) are recovered from the cells of interest and amplified. In the case of rearranged VH and VL gene libraries, the desired DNA can be obtained by isolating genomic DNA or mRNA from lymphocytes followed by polymerase chain reaction (PCR) with primers matching the 5' and 3' ends of rearranged VH and VL genes as described in Orlandi *et al.*, *Proc. Natl. Acad. Sci. (USA),* 86: 3833-3837 (1989), thereby making diverse V gene repertoires for expression. The V genes can be amplified from cDNA and genomic DNA, with back primers at the 5' end of the exon encoding the mature V-domain and forward primers based within the J-segment as described in Orlandi *et al.* (1989) and in Ward *et al., Nature*, 341: 544-546 (1989). However, for amplifying from cDNA, back primers can also be based in the leader exon as described in Jones *et al.*, *Biotechnol.*, 9: 88-89 (1991), and forward primers within the constant region as described in Sastry *et al.*, *Proc. Natl. Acad. Sci. (USA),* 86: 5728-5732 (1989). To maximize complementarity, degeneracy can be incorporated in the primers as described in Orlandi *et al.* (1989) or Sastry *et al.* (1989). Preferably, the library diversity is maximized by using PCR primers targeted to each V-gene family in order to amplify all available VH and VL arrangements present in the immune cell nucleic acid sample, e.g. as described in the method of Marks *et al.*, *J. Mol. Biol.*, 222: 581-597 (1991) or as described in the method of Orum *et al.*, *Nucleic Acids Res.*, 21: 4491-4498 (1993). For cloning of the amplified DNA into expression vectors, rare restriction sites can be introduced within the PCR primer as a tag at one end as described in Orlandi *et al.* (1989), or by further PCR amplification with a tagged primer as described in Clackson *et al.*, *Nature*, 352: 624-628 (1991).

Repertoires of synthetically rearranged V genes can be derived in vitro from V gene segments. Most of the human VH-gene segments have been cloned and sequenced (reported in Tomlinson *et al.*, *J. Mol. Biol.,* 227: 776-798 (1992)), and mapped (reported in Matsuda *et al.*, *Nature Genet.,* 3: 88-94 (1993); these cloned segments (including all the major conformations of the H1 and H2 loop) can be used to generate diverse VH gene repertoires with PCR primers encoding H3 loops of diverse sequence and length as described in Hoogenboom and Winter, *J. Mol. Biol.,* 227: 381-388 (1992). VH repertoires can also be made with all the sequence diversity focused in a long H3 loop of a single length as described in Barbas *et al.*, *Proc. Natl. Acad. Sci. USA*, 89: 4457-4461 (1992). Human Vκ and Vλ segments have been cloned and sequenced (reported in Williams and Winter, *Eur. J. Immunol.*, 23: 1456-1461 (1993)) and can be used to make synthetic light chain repertoires. Synthetic V gene repertoires, based on a range of VH and VL folds, and L3 and H3 lengths, will encode antibodies of considerable structural diversity. Following amplification of V-gene encoding DNAs, germline V-gene segments can be rearranged in vitro according to the methods of Hoogenboom and Winter, *J. Mol. Biol.,* 227: 381-388 (1992).

Repertoires of antibody fragments can be constructed by combining VH and VL gene repertoires together in several ways. Each repertoire can be created in different vectors, and the vectors recombined in vitro, e.g., as described in Hogrefe *et al.*, *Gene*, 128:119-126 (1993), or in vivo by combinatorial infection, e.g., the loxP system described in Waterhouse *et al.*, *Nucl. Acids Res.,* 21:2265-2266 (1993). The *in vivo* recombination approach exploits the two-chain nature of Fab fragments to overcome the limit on library size imposed by *E. coli* transformation efficiency. Naive VH and VL repertoires are cloned separately, one into a phagemid and the other into a phage vector. The two libraries are then combined by phage infection of phagemid-containing bacteria so that each cell contains a different combination and the library size is limited only by the number of cells present (about 10^{12} clones). Both

vectors contain in vivo recombination signals so that the VH and VL genes are recombined onto a single replicon and are co-packaged into phage virions. These huge libraries provide large numbers of diverse antibodies of good affinity (K_d^{-1} of about 10^{-8} M).

Alternatively, the repertoires may be cloned sequentially into the same vector, e.g., as described in Barbas *et al.*, *Proc. Natl. Acad. Sci. USA,* 88:7978-7982 (1991), or assembled together by PCR and then cloned, e.g. as described in Clackson *et al.*, *Nature*, 352: 624-628 (1991). PCR assembly can also be used to join VH and VL DNAs with DNA encoding a flexible peptide spacer to form single chain Fv (scFv) repertoires. In yet another technique, "in cell PCR assembly" is used to combine VH and VL genes within lymphocytes by PCR and then clone repertoires of linked genes as described in Embleton *et al.*, *Nucl. Acids Res.*, 20:3831-3837 (1992).

The antibodies produced by naive libraries (either natural or synthetic) can be of moderate affinity (K_d^{-1} of about 10^6 to 10^7 M^{-1}), but affinity maturation can also be mimicked *in vitro* by constructing and reselecting from secondary libraries as described in Winter *et al.* (1994), *supra*. For example, mutations can be introduced at random in vitro by using error-prone polymerase (reported in Leung *et al.*, *Technique,* 1:1230-232 and 236-247 (1989)) in the method of Hawkins *et al.*, *J. Mol. Biol.,* 226: 889-896 (1992) or in the method of Gram *et al.*, *Proc. Natl. Acad. Sci USA*, 89: 3576-3580 (1992). Additionally, affinity maturation can be performed by randomly mutating one or more CDRs, e.g. using PCR with primers carrying random sequence spanning the CDR of interest, in selected individual Fv clones and screening for higher affinity clones. WO 96/07754 (published 14 March 1996) described a method for inducing mutagenesis in a complementarity determining region of an immunoglobulin light chain to create a library of light chain genes. Another effective approach is to recombine the VH or VL domains selected by phage display with repertoires of naturally occurring V domain variants obtained from unimmunized donors and screen for

higher affinity in several rounds of chain reshuffling as described in Marks *et al.*, *Biotechnol.*, 10:779-783 (1992). This technique allows the production of antibodies and antibody fragments with affinities in the 10^{-9} M range.

FGFR2 and FGFR3 nucleic acid and amino acid sequences are known in the art. Nucleic acid sequence encoding the FGFR2 and FGFR3 can be designed using the amino acid sequence of the desired region of FGFR2 and FGFR3. For example, the FGFR3 can be designed using the amino acid sequence of R3Mab As is well-known in the art, there are two major splice isoforms of FGFR3, FGFR3 IIIb and FGFR3 IIIc. FGFR3 sequences are well-known in the art and may include the sequence of UniProKB/Swiss-Prot accession number P22607 (FGFR3 IIIc) or P22607_2 (FGFR3 IIIb). FGFR2 and FGFR3 mutations have been identified and are well-known in the art and include the following mutations (with reference to the sequences shown in UniProKB/Swiss-Prot accession number P22607 (FGFR3 IIIc) or P22607_2 (FGFR3 IIIb):

FGFR3-IIIb	FGFR3 IIIc
R248C	R248C
S249C	S249C
G372C	G370C
Y375C	Y373C
G382R	G380R
K652E	K650E

Nucleic acids encoding FGFR2 and/or FGFR3 can be prepared by a variety of methods known in the art. These methods include, but are not limited to, chemical synthesis by any of the methods described in Engels *et al.*, *Agnew. Chem. Int. Ed. Engl.*, 28: 716-734 (1989), such as the triester, phosphite, phosphoramidite and H-phosphonate methods. In one

embodiment, codons preferred by the expression host cell are used in the design of the FGFR2 and/or FGFR3 encoding DNA. Alternatively, DNA encoding FGFR2 and/or FGFR3 can be isolated from a genomic or cDNA library.

Following construction of the DNA molecule encoding the FGFR2 and/or FGFR3, the DNA molecule is operably linked to an expression control sequence in an expression vector, such as a plasmid, wherein the control sequence is recognized by a host cell transformed with the vector. In general, plasmid vectors contain replication and control sequences which are derived from species compatible with the host cell. The vector ordinarily carries a replication site, as well as sequences which encode proteins that are capable of providing phenotypic selection in transformed cells. Suitable vectors for expression in prokaryotic and eukaryotic host cells are known in the art and some are further described herein. Eukaryotic organisms, such as yeasts, or cells derived from multicellular organisms, such as mammals, may be used.

Optionally, the DNA encoding the FGFR2 and/or FGFR3 is operably linked to a secretory leader sequence resulting in secretion of the expression product by the host cell into the culture medium. Examples of secretory leader sequences include stII, ecotin, lamB, herpes GD, lpp, alkaline phosphatase, invertase, and alpha factor. Also suitable for use herein is the 36 amino acid leader sequence of protein A (Abrahmsen *et al.*, *EMBO J.*, 4: 3901 (1985)).

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ precipitation and electroporation.

Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell. Methods for transfection are well known in the art, and some are further described herein.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. Methods for transformation are well known in the art, and some are further described herein.

Prokaryotic host cells used to produce the FGFR2 and/or FGFR3 can be cultured as described generally in Sambrook *et al.*, *supra*.

The mammalian host cells used to produce the FGFR2 and/or FGFR3 can be cultured in a variety of media, which is well known in the art and some of which is described herein.

The host cells referred to in this disclosure encompass cells in *in vitro* culture as well as cells that are within a host animal.

Purification of FGFR2 and/or FGFR3 may be accomplished using art-recognized methods, some of which are described herein.

The purified FGFR2 and/or FGFR3 can be attached to a suitable matrix such as agarose beads, acrylamide beads, glass beads, cellulose, various acrylic copolymers, hydroxyl methacrylate gels, polyacrylic and polymethacrylic copolymers, nylon, neutral and ionic carriers, and the like, for use in the affinity chromatographic separation of phage display clones. Attachment of the FGFR2 and/or FGFR3 protein to the matrix can be accomplished by the methods described in *Methods in Enzymology*, vol. 44 (1976). A commonly employed technique for attaching protein ligands to polysaccharide matrices, e.g. agarose, dextran or cellulose, involves activation of the carrier with cyanogen halides and subsequent coupling of the peptide ligand's primary aliphatic or aromatic amines to the activated matrix.

Alternatively, FGFR2 and/or FGFR3 can be used to coat the wells of adsorption plates, expressed on host cells affixed to adsorption plates or used in cell sorting, or conjugated to biotin for capture with streptavidin-coated beads, or used in any other art-known method for panning phage display libraries.

The phage library samples are contacted with immobilized FGFR2 and/or FGFR3 under conditions suitable for binding of at least a portion of the phage particles with the adsorbent. Normally, the conditions, including pH, ionic strength, temperature and the like are selected to mimic physiological conditions. The phages bound to the solid phase are washed and then eluted by acid, e.g. as described in Barbas *et al.*, *Proc. Natl. Acad. Sci USA*, 88: 7978-7982 (1991), or by alkali, e.g. as described in Marks *et al.*, *J. Mol. Biol.,* 222: 581-597 (1991), or by FGFR3 antigen competition, e.g. in a procedure similar to the antigen competition method of Clackson *et al.*, *Nature*, 352: 624-628 (1991). Phages can be enriched 20-1,000-fold in a single round of selection. Moreover, the enriched phages can be grown in bacterial culture and subjected to further rounds of selection.

The efficiency of selection depends on many factors, including the kinetics of dissociation during washing, and whether multiple antibody fragments on a single phage can simultaneously engage with antigen. Antibodies with fast dissociation kinetics (and weak binding affinities) can be retained by use of short washes, multivalent phage display and high coating density of antigen in solid phase. The high density not only stabilizes the phage through multivalent interactions, but favors rebinding of phage that has dissociated. The selection of antibodies with slow dissociation kinetics (and good binding affinities) can be promoted by use of long washes and monovalent phage display as described in Bass *et al.*, *Proteins*, 8: 309-314 (1990) and in WO 92/09690, and a low coating density of antigen as described in Marks *et al.*, *Biotechnol.,* 10: 779-783 (1992).

It is possible to select between phage antibodies of different affinities, even with affinities that differ slightly, for FGFR2 and/or FGFR3. However, random mutation of a selected antibody (e.g. as performed in some of the affinity maturation techniques described above) is likely to give rise to many mutants, most binding to antigen, and a few with higher affinity. With limiting FGFR2 and/or FGFR3, rare high affinity phage could be competed out. To retain all the higher affinity mutants, phages can be incubated with excess biotinylated FGFR2 and/or FGFR3, but with the biotinylated FGFR2 and/or FGFR3 at a concentration of lower molarity than the target molar affinity constant for FGFR2 and/or FGFR3. The high affinity-binding phages can then be captured by streptavidin-coated paramagnetic beads. Such "equilibrium capture" allows the antibodies to be selected according to their affinities of binding, with sensitivity that permits isolation of mutant clones with as little as two-fold higher affinity from a great excess of phages with lower affinity. Conditions used in washing phages bound to a solid phase can also be manipulated to discriminate on the basis of dissociation kinetics.

FGFR2/3 clones may be activity selected. In one embodiment, the invention provides FGFR2/3 antibodies that block the binding between a FGFR3 receptor and its ligand (such as FGF1 and/or FGF9) and FGFR2 and its ligand. Fv clones corresponding to such FGFR2/3 antibodies can be selected by (1) isolating FGFR2/3 clones from a phage library as described above, and optionally amplifying the isolated population of phage clones by growing up the population in a suitable bacterial host; (2) selecting FGFR2/3 and a second protein against which blocking and non-blocking activity, respectively, is desired; (3) adsorbing the anti-FGFR2/3 phage clones to immobilized FGFR2/3; (4) using an excess of the second protein to elute any undesired clones that recognize FGFR2/3-binding determinants which overlap or are shared with the binding determinants of the second protein; and (5) eluting the clones which remain adsorbed following step (4). Optionally, clones with the desired blocking/non-

blocking properties can be further enriched by repeating the selection procedures described herein one or more times.

DNA encoding the hybridoma-derived monoclonal antibodies or phage display Fv clones of the invention is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide primers designed to specifically amplify the heavy and light chain coding regions of interest from hybridoma or phage DNA template). Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of the desired monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of antibody-encoding DNA include Skerra *et al.*, *Curr. Opinion in Immunol.*, 5: 256 (1993) and Pluckthun, *Immunol. Revs*, 130:151 (1992).

DNA encoding the Fv clones of the invention can be combined with known DNA sequences encoding heavy chain and/or light chain constant regions (e.g., the appropriate DNA sequences can be obtained from Kabat *et al.*, supra) to form clones encoding full or partial length heavy and/or light chains. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. A Fv clone derived from the variable domain DNA of one animal (such as human) species and then fused to constant region DNA of another animal species to form coding sequence(s) for "hybrid," full length heavy chain and/or light chain is included in the definition of "chimeric" and "hybrid" antibody as used herein. In a preferred embodiment, a Fv clone derived from human variable DNA is fused to human constant region DNA to form coding sequence(s) for all human, full or partial length heavy and/or light chains.

DNA encoding anti-FGFR2/3 antibody derived from a hybridoma of the invention can also be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of homologous murine sequences derived from the hybridoma clone (e.g., as in the method of Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). DNA encoding a hybridoma or Fv clone-derived antibody or fragment can be further modified by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In this manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of the Fv clone or hybridoma clone-derived antibodies of the invention.

Bispecific Antibodies

In one aspect, the invention is based, in part, on the discovery of bispecific antibodies that bind to both KLB and FGFR2/3 ("FGFR2/3 + KLB bispecific antibodies"). In certain aspects, the FGFR2/3 + KLB bispecific antibodies can be used in the treatment of metabolic diseases and disdorders, such treatment resulting in weight loss and/or improvement in glucose and lipid metabolism without a significant impact on the liver and without significant loss in bone mass. In certain aspects, the FGFR2/3 + KLB bispecific antibodies can be used in the treatment of NASH.

In certain embodiments, the FGFR2/3 + KLB bispecific antibodies disclosed herein comprise a first arm of any of the anti-FGFR2/3 antibodies disclosed herein and a second arm of any anti-KLB antibody disclosed herein or disclosed in US20150218276 which is incorporated herein in its entirity.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure does not have a significant impact on the liver, *e.g.*, liver function.. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure does not

modulate the activity of an FGFR/KLB receptor complex in the liver as compared to the modulation of an FGFR/KLB receptor complex in the liver by an FGF21 protein. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure does not result in the inhibition of the FGFR4/KLB complex and/or does not result in the elevation of liver enzymes such as, but not limited to, ALT, AST, ALP and GLDH. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure does not function as an agonist of the FGFR2c/KLB complex and/or the FGFR3c/KLB complex in the liver, which can lead to activated MAPK signaling and/or altered expression of Spry4 and Dusp6 in the liver. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure does not result in the activation of MAPK signaling in the liver as compared to the activation of MAPK signaling by an FGF21 protein. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure does not function as an agonist of the FGFR4/KLB complex in the liver.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure can be humanized. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure can be a monoclonal antibody, including a chimeric, humanized or human antibody. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure can be an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In certain embodiments, the FGFR2/3 + KLB bispecific antibody is a full length antibody, *e.g.*, an intact IgG1 antibody, or other antibody class or isotype as defined herein. In a certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure can incorporate any of the features, singly or in combination, as described in detailed below.

FGFR2/3 + KLB bispecific antibodies of the present disclosure are useful, *e.g.*, for the diagnosis or treatment of metabolic disorders. Non-limiting examples of metabolic disorders include polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), obesity, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, hypertension, type 2 diabetes, non-type 2 diabetes, type 1 diabetes, latent autoimmune diabetes (LAD), maturity onset diabetes of the young (MODY), and aging and related diseases such as Alzheimer's disease, Parkinson's disease and ALS. In preferred aspects, the metabolic disease is NASH.

In certain embodiments, the FGFR2/3 + KLB bispecific antibodies of the present disclosure are can be used, *e.g.*, for the diagnosis or treatment of metabolic disorders. Non-limiting examples of metabolic disorders include polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), obesity, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, hypertension, type 2 diabetes, non-type 2 diabetes, type 1 diabetes, latent autoimmune diabetes (LAD), maturity onset diabetes of the young (MODY), and aging and related diseases such as Alzheimer's disease, Parkinson's disease and ALS. In preferred aspects, the metabolic disease is NASH.

Exemplary Anti-KLB Antibodies

In one aspect, the present disclosure provides isolated antibodies that bind to a KLB protein. In certain embodiments, an anti-KLB antibody of the present disclosure binds to the C-terminal domain of KLB. In certain embodiments, an anti-KLB antibody of the present disclosure binds to a fragment of KLB that comprises the amino acid sequence SSPTRLAVIPWGVRKLLRWVRRNYGDMDIYITAS (SEQ ID NO: 103). In certain embodiments, the antibody binds to the same epitope as an anti-KLB antibody, *e.g.*, 8C5, described herein.

In certain embodiments, an anti-KLB antibody of the present disclosure comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising an amino acid sequence of any one of SEQ ID NOs: 230-232 and 236-247, *e.g.*, 244 or 247; (b) HVR-H2 comprising an amino acid sequence of any one of SEQ ID NOs: 142 and 248-262 , *e.g.*, 259 or 262; (c) HVR-H3 comprising an amino acid sequence of any one of SEQ ID NOs: 263-278, *e.g.*, 166 or 169; (d) HVR-L1 comprising an amino acid sequence of any one of SEQ ID NOs: 279-293, *e.g.*, 171 or 184; (e) HVR-L2 comprising an amino acid sequence of any one of SEQ ID NOs: 294-309, *e.g.*, 197 or 200; and (f) HVR-L3 comprising an amino acid sequence of any one of SEQ ID NOs: 310-324, *e.g.*, 212 or 215.

In certain embodiments, the present disclosure provides an anti-KLB antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising SEQ ID NO: 119; (b) HVR-H2 comprising SEQ ID NO: 150; (c) HVR-H3 comprising SEQ ID NO: 166; (d) HVR-L1 comprising SEQ ID NO: 171; (e) HVR-L2 comprising SEQ ID NO: 197; and (f) HVR-L3 comprising SEQ ID NO: 212. In certain embodiments, the present disclosure provides an anti-KLB antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising SEQ ID NO: 122; (b) HVR-H2 comprising SEQ ID NO 153; (c) HVR-H3 comprising SEQ ID NO: 169; (d) HVR-L1 comprising SEQ ID NO 184; (e) HVR-L2 comprising SEQ ID NO: 200; and (f) HVR-L3 comprising SEQ ID NO: 215.

The present disclosure further provides an anti-KLB antibody that comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 104. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions as disclosed below), insertions, or deletions relative to the reference sequence, but an anti-KLB

antibody comprising that sequence retains the ability to bind to KLB. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 104. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Alternatively or additionally, the anti-KLB antibody comprises the VH sequence in SEQ ID NO: 104, including post-translational modifications of that sequence as disclosed below. In certain embodiments, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 122, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 153, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 169.

In another aspect, the present disclosure provides an anti-KLB antibody, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 105. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-KLB antibody comprising that sequence retains the ability to bind to KLB. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 105. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Alternatively or additionally, the anti-KLB antibody comprises the VL sequence in SEQ ID NO: 105, including post-translational modifications of that sequence. In certain embodiments, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 184; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 200; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 215.

The present disclosure further provides an anti-KLB antibody, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In certain embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 104 and SEQ ID NO: 105, respectively, including post-translational modifications of those sequences.

In certain embodiments, an anti-KLB antibody binds to a fragment of KLB consisting of the amino acid sequence SSPTRLAVIPWGVRKLLRWVRRNYGDMDIYITAS (SEQ ID NO: 103).

Bispecific Anti-FGFR2/3 Antibody

The present disclosure further provides bispecific antibodies that bind to both KLB and FGFR2/3 (*i.e.*, FGFR2/3 + KLB bispecific antibodies). A bispecific antibody has two different binding specificities, *see, e.g.*, U.S. Patent Nos. 5,922,845 and 5,837,243; Zeilder (1999) J. Immunol. 163:1246-1252; Somasundaram (1999) Hum. *Antibodies* 9:47-54; Keler (1997) *Cancer Res.* 57:4008-4014. For example, and not by way of limitation, the presently disclosed subject matter provides bispecific antibodies having one binding site (*e.g.*, antigen binding site) for a first epitope present on KLB and a second binding site for a second epitope present on FGFR2/3. For example, and not by way of limitation, the present disclosure provides an antibody where one arm binds KLB and comprises any of the anti-KLB antibody sequences described herein and the second arm binds to FGFR2/3 and comprises any of the anti-FGFR2/3 antibody sequences described herein. In certain embodiments, an FGFR2/3 + KLB bispecific antibody of the present disclosure has one binding site for a first epitope present on KLB and a second binding site for a second epitope present on FGFR2/3.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody, or an antigen-binding portion thereof, includes a heavy chain and a light chain region. In certain

embodiments, the full length heavy chain includes amino acids having a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 106. In certain embodiments, the full length light chain includes amino acids having a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 107. In certain embodiments, the full length heavy chain includes amino acids having the sequence set forth in SEQ ID NO: 106. In certain embodiments, the full length light chain includes amino acids having the sequence set forth in SEQ ID NO: 107.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody, or an antigen-binding portion thereof, includes a heavy chain variable region and a light chain variable region. In certain embodiments, the heavy chain variable region includes amino acids having a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 104. In certain embodiments, the light chain variable region includes amino acids having a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 105. In certain embodiments, the heavy chain variable region includes amino acids having the sequence set forth in SEQ ID NO: 104. In certain embodiments, the light chain variable region includes amino acids having the sequence set forth in SEQ ID NO: 105.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising an amino acid sequence of any one of SEQ ID NOs: 230-232 and 236-247, *e.g.*, 244 or 247; (b) HVR-H2 comprising an amino acid sequence of any one of SEQ ID NOs: 142 and 248-262 , *e.g.*, 259 or 262; (c) HVR-H3 comprising an amino acid sequence of any one of SEQ ID NOs: 263-278, *e.g.*, 275 or 278; (d) HVR-L1 comprising an amino acid sequence of any one of SEQ ID NOs: 279-293, *e.g.*, 280 or 293; (e) HVR-L2 comprising an amino acid sequence of any one

of SEQ ID NOs: 294-309, *e.g.*, 306 or 309; and (f) HVR-L3 comprising an amino acid sequence of any one of SEQ ID NOs: 310-324, *e.g.*, 321 or 324.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody, comprises at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising SEQ ID NO: 119; (b) HVR-H2 comprising SEQ ID NO: 150; (c) HVR-H3 comprising SEQ ID NO: 166; (d) HVR-L1 comprising SEQ ID NO: 171; (e) HVR-L2 comprising SEQ ID NO: 197; and (f) HVR-L3 comprising SEQ ID NO: 212. In certain embodiments, the present disclosure provides an anti-KLB antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising SEQ ID NO: 122; (b) HVR-H2 comprising SEQ ID NO: 153; (c) HVR-H3 comprising SEQ ID NO: 169; (d) HVR-L1 comprising SEQ ID NO: 184; (e) HVR-L2 comprising SEQ ID NO: 200; and (f) HVR-L3 comprising SEQ ID NO: 215.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody includes a heavy chain variable region that comprises CDR1, CDR2, and CDR3 domains, and a light chain variable region that comprises CDR1, CDR2, and CDR3 domains. In certain embodiments, the heavy chain variable region CDR1 domain includes an amino acid sequence having a sequence set forth in SEQ ID NO: 230-232 and 236-247. In certain embodiments, the heavy chain variable region CDR2 domain includes an amino acid sequence a sequence set forth in SEQ ID NO: 142 and 248-262 . In certain embodiments, the heavy chain variable region CDR3 domain includes an amino acid sequence having a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 263-278. In certain embodiments, the light chain variable region CDR1 domain includes an amino acid sequence having a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 279-293. In certain embodiments, the light chain variable region CDR2 domain includes an amino acid sequence having a sequence that

is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 294-309. In certain embodiments, the light chain variable region CDR3 domain includes an amino acid sequence having a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 310-324.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody, includes a heavy chain variable region that comprises CDR1, CDR2, and CDR3 domains, and a light chain variable region that comprises CDR1, CDR2, and CDR3 domains. In certain embodiments, the heavy chain variable region CDR1 domain includes an amino acid sequence having a sequence set forth in SEQ ID NO: 230-232 and 236-247. In certain embodiments, the heavy chain variable region CDR2 domain includes an amino acid sequence having a sequence set forth in SEQ ID NO: 103 and 248-262 . In certain embodiments, the heavy chain variable region CDR3 domain includes an amino acid sequence having a sequence set forth in SEQ ID NO: 263-278. In certain embodiments, the light chain variable region CDR1 domain includes an amino acid sequence having a sequence set forth in SEQ ID NO: 279-293. In certain embodiments, the light chain variable region CDR2 domain includes an amino acid sequence having a sequence set forth in SEQ ID NO: 294-309. In certain embodiments, the light chain variable region CDR3 domain includes an amino acid sequence having a sequence set forth in SEQ ID NO: 310-324.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody, includes a heavy chain variable region CDR1 having the sequence set forth in SEQ ID NO: 122; a heavy chain variable region CDR2 having the sequence set forth in SEQ ID NO: 153; a heavy chain variable region CDR3 having the sequence set forth in SEQ ID NO: 169; a light chain variable region CDR1 having the sequence set forth in SEQ ID NO: 184; a light chain variable region CDR2 having the sequence set forth in SEQ ID NO: 200; and a light chain variable region CDR3 having the sequence set forth in SEQ ID NO: 215.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody includes a first antibody, or antigen binding portion thereof, and includes a second antibody, or antigen binding portion thereof, where the first antibody, or antigen binding portion thereof, binds to an epitope present on KLB, and the second antibody, or antigen binding portion thereof, bind to an epitope present on FGFR2/3. For example, and not by way of limitation, the first antibody, or antigen binding portion thereof, can include a heavy chain variable region and a light chain variable region; and the second antibody, or antigen binding portion thereof, can include a heavy chain variable region and a light chain variable region. In certain embodiments, the heavy chain variable region of the first antibody, or antigen binding portion thereof, includes amino acids having a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 104. In certain embodiments, the light chain variable region of the first antibody, or antigen binding portions thereof, includes amino acids having a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 105. In certain embodiments, the heavy chain of the second antibody (anti-FGFR2/3 antibody) or antigen binding portion thereof includes amino acids having a sequence that is at least 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 282. In certain embodiments, the light chain of the second antibody (anti-FGFR2/3 antibody), or antigen binding portions thereof, includes amino acids having a sequence that is at least 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 283.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody that binds to the same epitope as an anti-KLB antibody is provided herein. For example, in certain embodiments, an FGFR2/3 + KLB bispecific antibody is provided that binds to the same

epitope as an anti-KLB antibody comprising the VH sequence of SEQ ID NO: 104 and a VL sequence of SEQ ID NO: 105. In certain embodiments, an FGFR2/3 + KLB bispecific antibody is provided that binds to a fragment of KLB consisting of the amino acid sequence SSPTRLAVIPWGVRKLLRWVRRNYGDMDIYITAS (SEQ ID NO: 103).

In certain embodiments, an FGFR2/3 + KLB bispecific antibody is provided that binds to a fragment of KLB having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 103.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody binds to the same epitope as an anti-KLB antibody is provided herein. For example, in certain embodiments, an FGFR2/3 + KLB bispecific antibody is provided that binds to the same epitope as an anti-KLB antibody comprising the full length heavy chain sequence of SEQ ID NO: 106 and a full length light chain sequence of SEQ ID NO: 107.

In certain embodiments, the present disclosure provides an FGFR2/3 + KLB bispecific antibody that binds to the same epitope as an anti-FGFR2/3 antibody provided herein. For example, in certain embodiments, an FGFR2/3 + KLB bispecific antibody is provided that binds to the same epitope as an anti-FGFR2/3 antibody comprising the VH sequence of SEQ ID NO: 82 and a VL sequence of SEQ ID NO: 66. In certain embodiments, an FGFR2/3 + KLB bispecific antibody is provided that binds to an epitope on FGFR2 comprising amino acid sequence TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and/or YKVRNQHWSLIMES (SEQ ID NO: 92) and/or also binds to an epitope on FGFR3 comprising amino acid sequence TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and/or IKLRHQQWSLVMES (SEQ ID NO: 94).

In certain embodiments, the present disclosure provides an FGFR2/3 + KLB bispecific antibody that binds to the same epitope as an anti-FGFR2/3 antibody provided herein. For example, in certain embodiments, an FGFR2/3 + KLB bispecific antibody is provided that binds to the same epitope as the 2B.1.3.12, 2B.1.3.10, or the 2B.1.1.6 anti-FGFR2/3 antibodies disclosed herein. In certain embodiments, an FGFR2/3 + KLB bispecific antibody is provided that binds to the same epitopes as the anti-FGFR2/3 antibodies 2B.1.3.10 and 2B.1.3.12 (*i.e.*, the FGFR2/3 + KLB bispecific antibody binds to the same epitope(s) on FGFR2 comprising amino acid sequence TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and/or YKVRNQHWSLIMES (SEQ ID NO: 92) and/or also binds to the same epitope(s) on FGFR3 comprising amino acid sequence TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and/or IKLRHQQWSLVMES (SEQ ID NO: 94) as the 2B.1.3.10 and 2B.1.3.12 do).

In certain embodiments, the present disclosure provides an FGFR2/3 + KLB bispecific antibody that competes for binding to FGFR2/3 with the 2B.1.3.10 and 2B.1.3.12 antibodies provided herein.

In certain embodiments, an FGFR2/3 + KLB bispecific antibody is provided that binds to a fragment of KLB having an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 103, and binds to or competes for binding to the FGFR2 epitopes selected from TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and YKVRNQHWSLIMES (SEQ ID NO:92) and binds to or competes for biding to the FGFR3 epitopes selected from TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO: 94).

In certain embodiments, an anti-KLB/anti-FGFR1 bispecific antibody is provided that binds to a fragment of KLB having the amino acid sequence set forth in SEQ ID NO: 103 and

binds to or competes for binding to the FGFR2 epitopes provided in SEQ ID NOs: 91 and 92 and binds to or competes for binding to the FGFR3 epitopes provided in SEQ ID NOs: 93 and 94.

Antibody Fragments

The present invention encompasses antibody fragments. In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased in vivo half-life comprising a salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv) (see, e.g., WO 93/16185; U.S. Pat. Nos. 5,571,894 and 5,587,458). Fv and sFv are the only species with intact combining sites that are devoid of constant regions; thus,

they are suitable for reduced nonspecific binding during in vivo use. sFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an sFv. See Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment may also be a "linear antibody," e.g., as described, for example, in U.S. Pat. No. 5,641,870. Such linear antibody fragments may be monospecific or bispecific.

Humanized Antibodies

The present invention encompasses humanized antibodies. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.* (1986) *Nature* 321:522-525; Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeyen *et al.* (1988) *Science* 239:1534-1536), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the

entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework for the humanized antibody (Sims *et al.* (1993) *J. Immunol.* 151:2296; Chothia *et al.* (1987) *J. Mol. Biol.* 196:901. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; Presta *et al.* (1993) *J. Immunol.*, 151:2623.

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Human antibodies

Human anti-FGFR2/3 antibodies of the invention can be constructed by combining Fv clone variable domain sequence(s) selected from human-derived phage display libraries with known human constant domain sequences(s) as described above. Alternatively, human

monoclonal anti-FGFR2/3 antibodies of the invention can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner *et al.*, *J. Immunol.*, 147:86 (1991).

It is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits *et al.*, *Proc. Natl. Acad. Sci USA,* 90: 2551 (1993); Jakobovits *et al.*, *Nature,* 362: 255 (1993); Bruggermann *et al.*, *Year in Immunol.,* 7:33 (1993).

Gene shuffling can also be used to derive human antibodies from non-human, e.g., rodent, antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called "epitope imprinting," either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described above is replaced with a repertoire of human V domain genes, creating a population of non-human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the corresponding non-human chain in the primary phage display clone, i.e. the epitope governs (imprints) the choice of the human chain partner. When the process is

repeated in order to replace the remaining non-human chain, a human antibody is obtained (see PCT WO 93/06213 published April 1, 1993). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin.

Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for FGFR3 and the other is for FGFR2. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express FGFR3, FGFR2, or FGFR2/3. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g., $F(ab')_2$ bispecific antibodies).

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305: 537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 published May 13, 1993, and in Traunecker *et al.*, *EMBO J.,* 10: 3655 (1991).

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2,

and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1), containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh *et al., Methods in Enzymology*, 121:210 (1986).

According to another approach, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side

chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.,* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/00373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al., Science*, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli,* which can be chemically coupled to form bispecific antibodies. Shalaby *et al., J. Exp. Med.*,

175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al., J. Immunol.*, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al. J. Immunol.* 147: 60 (1991).

Multivalent Antibodies

A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fe region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)n -VD2-(X2)n -Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

Antibody Variants

In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding

affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed immunoglobulins are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include

the fusion to the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 (Presta, L.). See also US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878, Jean-Mairet *et al.* and US Patent No. 6,602,684, Umana *et al.* Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087, Patel *et al.* See, also, WO 1998/58964 (Raju, S.) and WO 1999/22764 (Raju, S.) concerning antibodies with

altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 (Umana *et al.*) on antigen-binding molecules with modified glycosylation.

The preferred glycosylation variant herein comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,*knockout CHO cells (Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004)).

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid (at leat two, at leat three, at least 4 or more) residue in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in the chart below, under the heading of "preferred substitutions." If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in the chart below, or as further

described below in reference to amino acid classes, may be introduced and the products screened.

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: asp, glu;

(4) basic: his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g., 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibodies thus generated are displayed from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein

and antibodies with superior properties in one or more relevant assays may be selected for further development.

Nucleic acid molecules encoding amino acid sequence variants of the antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody.

It may be desirable to introduce one or more amino acid modifications in an Fc region of the immunoglobulin polypeptides of the invention, thereby generating a Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions including that of a hinge cysteine.

In accordance with this description and the teachings of the art, it is contemplated that in some embodiments, an antibody used in methods of the invention may comprise one or more alterations as compared to the wild type counterpart antibody, e.g., in the Fc region. These antibodies would nonetheless retain substantially the same characteristics required for therapeutic utility as compared to their wild type counterpart. For example, it is thought that certain alterations can be made in the Fc region that would result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in WO99/51642. See also Duncan & Winter *Nature* 322:738-40 (1988); US Patent No. 5,648,260; US Patent No. 5,624,821; and WO94/29351 concerning other examples of Fc region variants. WO00/42072 (Presta) and WO 2004/056312 (Lowman) describe antibody variants with improved or diminished binding to FcRs. The content of these patent publications are specifically incorporated herein by reference. See, also, Shields

et al. J. Biol. Chem. 9(2): 6591-6604 (2001). Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). These antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US patent No. 6,194,551B1, WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie *et al., J. Immunol.* 164: 4178-4184 (2000).

Antibody Derivatives

The antibodies of the present invention can be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. Preferably, the moieties suitable for derivatization of the antibody are water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymers are attached, they can be the same or different molecules. In general, the number and/or type of polymers

used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

Screening for antibodies with desired properties

The antibodies of the present invention can be characterized for their physical/chemical properties and biological functions by various assays known in the art (some of which are disclosed herein). In some embodiments, antibodies are characterized for any one or more of reduction or blocking of FGF (such as FGF1 and/or FGF9) binding, reduction or blocking of FGFR3 activation, reduction or blocking of FGFR3 downstream molecular signaling, disruption or blocking of FGFR3 binding to a ligand (e.g., FGF1, FGF9), reduction or blocking of FGFR3 dimerization, promotion of formation of monomeric FGFR3, binding to monomeric FGFR3, and/or treatment and/or prevention of a tumor, cell proliferative disorder or a cancer; and/or treatment or prevention of a disorder associated with FGFR3 expression and/or activity (such as increased FGFR3 expression and/or activity). In some embodiments, the antibodies are screened for increased FGFR3 activation, increased FGFR3 downstream molecule signaling, apoptotic activity, FGFR3 down-regulation, and effector function (e.g., ADCC activity). In certain embodiments, antibodies are characterized for any one or more of reduction or blocking of FGFR2 activation, reduction or blocking of FGFR2 downstream molecular signaling, disruption or blocking of FGFR2 binding to a ligand, reduction or blocking of FGFR2 dimerization, promotion of formation of monomeric FGFR2, binding to monomeric FGFR2, and/or treatment and/or prevention of a tumor, cell proliferative disorder or a cancer; and/or treatment or prevention of a disorder associated with FGFR2 expression and/or activity (such as increased FGFR2 expression and/or activity). In some embodiments, the antibodies are screened for increased FGFR2 activation, increased FGFR2 downstream molecule signaling, FGFR2 down-regulation, and effector function (e.g.,

ADCC activity). In certain embodiments, antibodies are characterized for any one or more of reduction or blocking of FGFR2 and FGFR3 activation, reduction or blocking of FGFR2 and FGFR3 downstream molecular signaling, disruption or blocking of FGFR2 and FGFR3 binding to a ligand (e.g., FGF1, FGF9), reduction or blocking of FGFR2 and FGFR3 dimerization, promotion of formation of monomeric FGFR2 and FGFR3, binding to monomeric FGFR2 and monomeric FGFR3, and/or treatment and/or prevention of a tumor, cell proliferative disorder or a cancer; and/or treatment or prevention of a disorder associated with FGFR2 and FGFR3 expression and/or activity (such as increased FGFR2 and/or FGFR3 expression and/or activity). In some embodiments, the antibodies are screened for increased FGFR2 and FGFR3 activation, increased FGFR2 and FGFR3 downstream molecule signaling, apoptotic activity, FGFR2 and FGFR3 down-regulation, and effector function (e.g., ADCC activity).

The purified antibodies can be further characterized by a series of assays including, but not limited to, N-terminal sequencing, amino acid analysis, non-denaturing size exclusion high pressure liquid chromatography (HPLC), mass spectrometry, ion exchange chromatography and papain digestion.

In certain embodiments of the invention, the antibodies produced herein are analyzed for their biological activity. In some embodiments, the antibodies of the present invention are tested for their antigen binding activity. The antigen binding assays that are known in the art and can be used herein include without limitation any direct or competitive binding assays using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, and protein A immunoassays. Illustrative antigen binding and other assay are provided below in the Examples section.

If an anti-FGFR2/3 antibody that inhibits cell growth is desired, the candidate antibody can be tested in *in vitro* and/or *in vivo* assays that measure inhibition of cell growth. If an anti-FGFR2/3 antibody that does or does not promote apoptosis is desired, the candidate antibody can be tested in assays that measure apoptosis. Methods for examining growth and/or proliferation of a cancer cell, or determining apoptosis of a cancer cell are well known in the art and some are described and exemplified herein. Exemplary methods for determining cell growth and/or proliferation and/or apoptosis include, for example, BrdU incorporation assay, MTT, [3H]-thymidine incorporation (e.g., TopCount assay (PerkinElmer)), cell viability assays (e.g., CellTiter-Glo (Promega)), DNA fragmentation assays, caspase activation assays, tryptan blue exclusion, chromatin morphology assays and the like.

In one embodiment, the present invention contemplates an antibody that possesses effector functions. In certain embodiments, the Fc activities of the antibody are measured. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991). An example of an in vitro assay to assess ADCC activity of a molecule of interest is described in US Patent No. 5,500,362 or 5,821,337. An assay to detect ADCC activity is also exemplified herein. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA)

95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed. FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art, e.g., those described in the Examples section.

If an anti-FGFR2/3 antibody that binds monomeric FGFR2 and/or FGFR3 is desired, the candidate antibody can be tested in assays (such as in vitro assays) that measure binding to monomeric FGFR2 and/or FGFR3 and promotion of the formation of monomeric FGFR2 and/or FGFR3. Such assays are known in the art and some assays are described and exemplified herein.

If an anti-FGFR2/3 antibody that inhibits FGFR2 and/or FGFR3 dimerization is desired, the candidate antibody can be tested in dimerization assays, e.g., as described herein.

In some embodiments, the FGFR2 and/or FGFR3 agonist function of the candidate antibody is determined. Methods for assessing agonist function or activity of FGFR2 and/or FGFR3 antibodies are known in the art and some are also described herein.

In some embodiments, ability of an FGFR2/3 antibody to promote FGFR2 and/or FGFR3 receptor down-regulation is determined, e.g., using methods described and exemplified herein. In one embodiment, a FGFR2/3 antibody is incubated with suitable test cells, e.g., bladder cancer cell lines (e.g., RT112), and after a suitable period of time, cell lysates are harvested and examined for total FGFR2 and FGFR3 levels. FACS analysis may also be used to examine surface FGFR2 and FGFR3 receptor levels following incubation with candidate FGFR2/3 antibodies

Vectors, Host Cells, and Recombinant Methods

For recombinant production of an antibody of the invention, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, preferred host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species.

a. Generating antibodies using prokaryotic host cells:

i. Vector Construction

Polynucleotide sequences encoding polypeptide components of the antibody of the invention can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the purpose of the present invention. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to: an origin of replication, a selection marker gene, a

promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species. pBR322 contains genes encoding ampicillin (Amp) and tetracycline (Tet) resistance and thus provides easy means for identifying transformed cells. pBR322, its derivatives, or other microbial plasmids or bacteriophage may also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of endogenous proteins. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter et al., U.S. Patent No. 5,648,237.

In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as λGEM.TM.-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as *E. coli* LE392.

The expression vector of the invention may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, e.g., the presence or absence of a nutrient or a change in temperature.

A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the light or heavy chain by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the invention. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the β-galactamase and lactose promoter systems, a tryptophan (trp) promoter system and hybrid promoters such as the tac or the trc promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding the target light and heavy chains (Siebenlist et al., (1980) Cell 20: 269) using linkers or adaptors to supply any required restriction sites.

In one aspect of the invention, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of this invention should be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA, and MBP. In one embodiment of

the invention, the signal sequences used in both cistrons of the expression system are STII signal sequences or variants thereof.

In another aspect, the production of the immunoglobulins according to the invention can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. In that regard, immunoglobulin light and heavy chains are expressed, folded and assembled to form functional immunoglobulins within the cytoplasm. Certain host strains (e.g., the *E. coli* trxB- strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits. Proba and Pluckthun Gene, 159:203 (1995).

Prokaryotic host cells suitable for expressing antibodies of the invention include Archaebacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include Escherichia (e.g., *E. coli*), Bacilli (e.g., *B. subtilis*), Enterobacteria, Pseudomonas species (e.g., *P. aeruginosa*), *Salmonella typhimurium*, *Serratia marcescans*, Klebsiella, Proteus, Shigella, Rhizobia, Vitreoscilla, or Paracoccus. In one embodiment, gram-negative cells are used. In one embodiment, E. coli cells are used as hosts for the invention. Examples of *E. coli* strains include strain W3110 (Bachmann, Cellular and Molecular Biology, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 ΔfhuA (ΔtonA) ptr3 lac Iq lacL8 ΔompTΔ(nmpc-fepE) degP41 kanR (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as *E. coli* 294 (ATCC 31,446), E. coli B, E. coliλ 1776 (ATCC 31,537) and *E. coli* RV308(ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass et al., Proteins, 8:309-314 (1990). It is generally necessary to select the appropriate bacteria taking into consideration replicability of

the replicon in the cells of a bacterium. For example, *E. coli*, Serratia, or Salmonella species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

ii. Antibody Production

Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

Prokaryotic cells used to produce the polypeptides of the invention are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include Luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the

culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol.

The prokaryotic host cells are cultured at suitable temperatures. For *E. coli* growth, for example, the preferred temperature ranges from about 20°C to about 39°C, more preferably from about 25°C to about 37°C, even more preferably at about 30°C. The pH of the medium may be any pH ranging from about 5 to about 9, depending mainly on the host organism. For *E. coli*, the pH is preferably from about 6.8 to about 7.4, and more preferably about 7.0.

If an inducible promoter is used in the expression vector of the invention, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the invention, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. Preferably, the phosphate-limiting medium is the C.R.A.P medium (see, e.g., Simmons et al., J. Immunol. Methods (2002), 263:133-147). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

In one embodiment, the expressed polypeptides of the present invention are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

In one aspect of the invention, antibody production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1000 liters of capacity, preferably about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose (the preferred carbon/energy source). Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, e.g., an OD550 of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

To improve the production yield and quality of the polypeptides of the invention, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of the secreted antibody polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD, and/or DsbG) or FkpA (a peptidylprolyl cis,trans-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen et al., (1999) J. Biol. Chem. 274:19601-19605; Georgiou et al., U.S. Patent No. 6,083,715; Georgiou et al., U.S. Patent No. 6,027,888; Bothmann and Pluckthun (2000) J. Biol. Chem. 275:17100-17105; Ramm and Pluckthun, (2000) J. Biol. Chem. 275:17106-17113; Arie et al., (2001) Mol. Microbiol. 39:199-210.

To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present invention. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI, and combinations thereof. Some *E. coli* protease-deficient strains are available and described in, for example, Joly et al., (1998), supra; Georgiou et al., U.S. Patent No. 5,264,365; Georgiou et al., U.S. Patent No. 5,508,192; Hara et al., Microbial Drug Resistance, 2:63-72 (1996).

In one embodiment, *E. coli* strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins are used as host cells in the expression system of the invention.

iii. Antibody Purification

Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75.

In one aspect, Protein A immobilized on a solid phase is used for immunoaffinity purification of the full length antibody products of the invention. Protein A is a 41kD cell wall protein from *Staphylococcus aureas* which binds with a high affinity to the Fc region of antibodies. Lindmark et al., (1983) J. Immunol. Meth. 62:1-13. The solid phase to which Protein A is immobilized is preferably a column comprising a glass or silica surface, more preferably a controlled pore glass column or a silicic acid column. In some applications, the

column has been coated with a reagent, such as glycerol, in an attempt to prevent nonspecific adherence of contaminants.

As the first step of purification, the preparation derived from the cell culture as described above is applied onto the Protein A immobilized solid phase to allow specific binding of the antibody of interest to Protein A. The solid phase is then washed to remove contaminants non-specifically bound to the solid phase. Finally the antibody of interest is recovered from the solid phase by elution.

b. Generating antibodies using eukaryotic host cells:

The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(i) Signal sequence component

A vector for use in a eukaryotic host cell may also contain a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide of interest. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

The DNA for such precursor region is ligated in reading frame to DNA encoding the antibody.

(ii) Origin of replication

Generally, an origin of replication component is not needed for mammalian expression vectors. For example, the SV40 origin may typically be used only because it contains the early promoter.

(iii) Selection gene component

Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, where relevant, or (c) supply critical nutrients not available from complex media.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL-9096).

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

(iv) Promoter component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the antibody polypeptide nucleic acid. Promoter sequences are known for eukaryotes. Virtually alleukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

Antibody polypeptide transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus, and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446. A modification of this system is described in U.S. Patent No. 4,601,978. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

(v) Enhancer element component

Transcription of DNA encoding the antibody polypeptide of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody polypeptide-encoding sequence, but is preferably located at a site 5' from the promoter.

(vi) Transcription termination component

Expression vectors used in eukaryotic host cells will typically also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding an antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

(vii) Selection and transformation of host cells

Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in

suspension culture, Graham et al., J. Gen. Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)) ; mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

(viii) Culturing the host cells

The host cells used to produce an antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem.102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as

HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

(ix) Purification of antibody

When using recombinant techniques, the antibody can be produced intracellularly, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most

often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX™resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

Immunoconjugates

The invention also provides immunoconjugates (interchangeably termed "antibody-drug conjugates" or "ADC"), comprising any of the anti-FGFR2/3 antibodies described herein conjugated to a cytotoxic agent such as a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e., drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drg. Del. Rev. 26:151-172; U.S. Patent No. 4,975,278) allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal

cells as well as the tumor cells sought to be eliminated (Baldwin et al., (1986) Lancet pp. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera et al. (ed.s), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland et al., (1986) Cancer Immunol. Immunother., 21:183-87). Drugs used in these methods include daunomycin, doxorubicin, methotrexate, and vindesine (Rowland et al., (1986) supra). Toxins used in antibody-toxin conjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et al (2000) Jour. of the Nat. Cancer Inst. 92(19):1573-1581; Mandler et al., (2000) Bioorganic & Med. Chem. Letters 10:1025-1028; Mandler et al., (2002) Bioconjugate Chem. 13:786-791), maytansinoids (EP 1391213; Liu et al., (1996) Proc. Natl. Acad. Sci. USA 93:8618-8623), and calicheamicin (Lode et al., (1998) Cancer Res. 58:2928; Hinman et al., (1993) Cancer Res. 53:3336-3342). The toxins may effect their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition. Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) is an antibody-radioisotope conjugate composed of a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and ^{111}In or ^{90}Y radioisotope bound by a thiourea linker-chelator (Wiseman et al., (2000) Eur. Jour. Nucl. Med. 27(7):766-77; Wiseman et al., (2002) Blood 99(12):4336-42; Witzig et al., (2002) J. Clin. Oncol. 20(10):2453-63; Witzig et al., (2002) J. Clin. Oncol. 20(15):3262-69). Although ZEVALIN has activity against B-cell non-Hodgkin's Lymphoma (NHL), administration results in severe and prolonged cytopenias in most patients. MYLOTARG™ (gemtuzumab

ozogamicin, Wyeth Pharmaceuticals), an antibody drug conjugate composed of a hu CD33 antibody linked to calicheamicin, was approved in 2000 for the treatment of acute myeloid leukemia by injection (Drugs of the Future (2000) 25(7):686; US Patent Nos. 4,970,198; 5,079,233; 5,585,089; 5,606,040; 5,6937,62; 5,739,116; 5,767,285; 5,773,001). Cantuzumab mertansine (Immunogen, Inc.), an antibody drug conjugate composed of the huC242 antibody linked via the disulfide linker SPP to the maytansinoid drug moiety, DM1, is advancing into Phase II trials for the treatment of cancers that express CanAg, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), an antibody drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, is under development for the potential treatment of prostate tumors. The auristatin peptides, auristatin E (AE) and monomethylauristatin (MMAE), synthetic analogs of dolastatin, were conjugated to chimeric monoclonal antibodies cBR96 (specific to Lewis Y on carcinomas) and cAC10 (specific to CD30 on hematological malignancies) (Doronina et al., (2003) Nature Biotechnology 21(7):778-784) and are under therapeutic development.

Chemotherapeutic agents useful in the generation of immunoconjugates are described herein (e.g., above). Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. See, e.g., WO 93/21232 published October 28, 1993. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re. Conjugates of the antibody and cytotoxic agent are

made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, aurostatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein.

i. Maytansine and maytansinoids

In some embodiments, the immunoconjugate comprises an antibody (full length or fragments) of the invention conjugated to one or more maytansinoid molecules.

Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Patent No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification, derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through the non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

Immunoconjugates containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. Liu et al., Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described immunoconjugates comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an in vivo tumor growth assay. Chari et al., Cancer Research 52:127-131 (1992) describe immunoconjugates in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/neu oncogene. The cytotoxicity of the TA.1-maytansinoid conjugate was tested in vitro on the human breast cancer cell line SK-BR-3, which expresses 3 x 10^5 HER-2 surface antigens per cell. The drug conjugate achieved a degree of cytotoxicity similar to the free maytansinoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

Antibody-maytansinoid conjugates are prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. See, e.g., U.S. Patent No. 5,208,020 (the disclosure of which is hereby expressly incorporated by reference). An average of 3-4

maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody, although even one molecule of toxin/antibody would be expected to enhance cytotoxicity over the use of naked antibody. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed, for example, in U.S. Patent No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytansinoids are maytansinol and maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

There are many linking groups known in the art for making antibody-maytansinoid conjugates, including, for example, those disclosed in U.S. Patent No. 5,208,020 or EP Patent 0 425 235 B1, Chari et al., Cancer Research 52:127-131 (1992), and U.S. Patent Application No. 10/960,602, filed Oct. 8, 2004, the disclosures of which are hereby expressly incorporated by reference. Antibody-maytansinoid conjugates comprising the linker component SMCC may be prepared as disclosed in U.S. Patent Application No. 10/960,602, filed Oct. 8, 2004. The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents, disulfide and thioether groups being preferred. Additional linking groups are described and exemplified herein.

Conjugates of the antibody and maytansinoid may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives

(such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particularly preferred coupling agents include N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (Carlsson et al., Biochem. J. 173:723-737 (1978)) and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage.

The linker may be attached to the maytansinoid molecule at various positions, depending on the type of the link. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. The reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In a preferred embodiment, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

ii. Auristatins and dolastatins

In some embodiments, the immunoconjugate comprises an antibody of the invention conjugated to dolastatins or dolostatin peptidic analogs and derivatives, the auristatins (U.S. Patent Nos. 5,635,483 and 5,780,588). Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob. Agents and Chemother. 45(12):3580-3584) and have anticancer (U.S. Patent No. 5,663,149) and antifungal activity (Pettit et al., (1998) Antimicrob. Agents Chemother. 42:2961-2965). The dolastatin or auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172).

Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in "Monomethylvaline

Compounds Capable of Conjugation to Ligands," U.S. Ser. No. 10/983,340, filed Nov. 5, 2004, the disclosure of which is expressly incorporated by reference in its entirety.

Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (see E. Schröder and K. Lübke, "The Peptides," volume 1, pp. 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry. The auristatin/dolastatin drug moieties may be prepared according to the methods of: U.S. Patent Nos. 5,635,483 and 5,780,588; Pettit et al., (1989) J. Am. Chem. Soc. 111:5463-5465; Pettit et al., (1998) Anti-Cancer Drug Design 13:243-277; Pettit, G.R., et al., Synthesis, 1996, 719-725; and Pettit et al., (1996) J. Chem. Soc. Perkin Trans. 1 5:859-863. See also Doronina (2003) Nat. Biotechnol. 21(7):778-784; "Monomethylvaline Compounds Capable of Conjugation to Ligands," US Ser. No. 10/983,340, filed Nov. 5, 2004, hereby incorporated by reference in its entirety (disclosing, e.g., linkers and methods of preparing monomethylvaline compounds such as MMAE and MMAF conjugated to linkers).

iii. Calicheamicin

In other embodiments, the immunoconjugate comprises an antibody of the invention conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. For the preparation of conjugates of the calicheamicin family, see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296 (all to American Cyanamid Company). Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman et al., Cancer Research 53:3336-3342 (1993), Lode et al., Cancer Research 58:2925-2928 (1998) and the aforementioned U.S. patents to American Cyanamid). Another anti-tumor drug that the

antibody can be conjugated is QFA which is an antifolate. Both calicheamicin and QFA have intracellular sites of action and do not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody mediated internalization greatly enhances their cytotoxic effects.

iv. Other cytotoxic agents

Other antitumor agents that can be conjugated to the antibodies of the invention include BCNU, streptozoicin, vincristine and 5-fluorouracil, the family of agents known collectively LL-E33288 complex described in U.S. Patent Nos. 5,053,394 and 5,770,710, as well as esperamicins (U.S. Patent No. 5,877,296).

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

The present invention further contemplates an immunoconjugate formed between an antibody and a compound with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

For selective destruction of the tumor, the antibody may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the conjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc^{99m} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such

as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

The radio- or other labels may be incorporated in the conjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as tc^{99m} or I^{123}, Re^{186}, Re^{188} and In^{111} can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker et al (1978) Biochem. Biophys. Res. Commun. 80: 49-57 can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A). See pages 467-498, 2003-2004 Applications Handbook and Catalog.

v. Preparation of antibody drug conjugates

In the antibody drug conjugates (ADC) of the invention, an antibody (Ab) is conjugated to one or more drug moieties (D), e.g. about 1 to about 20 drug moieties per antibody, through a linker (L). The ADC of Formula I may be prepared by several routes, employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent, to form Ab-L, via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with the nucleophilic group of an antibody. Additional methods for preparing ADC are described herein.

$$Ab–(L–D)_p \qquad I$$

The linker may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl ("PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC'), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"). Additional linker components are known in the art and some are described herein. See also "Monomethylvaline Compounds Capable of

Conjugation to Ligands," U.S. Ser. No. 10/983,340, filed Nov. 5, 2004, the contents of which are hereby incorporated by reference in its entirety.

In some embodiments, the linker may comprise amino acid residues. Exemplary amino acid linker components include a dipeptide, a tripeptide, a tetrapeptide or a pentapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe). Exemplary tripeptides include: glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). Amino acid residues which comprise an amino acid linker component include those occurring naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. Amino acid linker components can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzymes, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol). Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through the reaction of lysines with 2-iminothiolane (Traut's reagent) resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into the antibody by introducing one,

two, three, four, or more cysteine residues (e.g., preparing mutant antibodies comprising one or more non-native cysteine amino acid residues).

Antibody drug conjugates of the invention may also be produced by modification of the antibody to introduce electrophilic moieties, which can react with nucleophilic substituents on the linker reagent or drug. The sugars of glycosylated antibodies may be oxidized, e.g., with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g., by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either glactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the protein that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, proteins containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; U.S. Patent No. 5,362,852). Such aldehyde can be reacted with a drug moiety or linker nucleophile.

Likewise, nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

Alternatively, a fusion protein comprising the antibody and cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. The length of DNA may comprise respective regions encoding the two portions of the conjugate either adjacent one

another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the individual, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a radionucleotide).

Methods using anti-FGFR2/3 antibodies

The present invention features the use of an FGFR2/3 antibody as part of a specific treatment regimen intended to provide a beneficial effect from the activity of this therapeutic agent. The present invention is particularly useful in treating cancers of various types at various stages.

The term cancer embraces a collection of proliferative disorders, including but not limited to pre-cancerous growths, benign tumors, and malignant tumors. Benign tumors remain localized at the site of origin and do not have the capacity to infiltrate, invade, or metastasize to distant sites. Malignant tumors will invade and damage other tissues around them. They can also gain the ability to break off from the original site and spread to other parts of the body (metastasize), usually through the bloodstream or through the lymphatic system where the lymph nodes are located. Primary tumors are classified by the type of tissue from which they arise; metastatic tumors are classified by the tissue type from which the cancer cells are derived. Over time, the cells of a malignant tumor become more abnormal and appear less like normal cells. This change in the appearance of cancer cells is called the tumor grade, and cancer cells are described as being well-differentiated (low grade), moderately-differentiated, poorly-differentiated, or undifferentiated (high grade). Well-differentiated cells are quite normal appearing and resemble the normal cells from

which they originated. Undifferentiated cells are cells that have become so abnormal that it is no longer possible to determine the origin of the cells.

Cancer staging systems describe how far the cancer has spread anatomically and attempt to put patients with similar prognosis and treatment in the same staging group. Several tests may be performed to help stage cancer including biopsy and certain imaging tests such as a chest x-ray, mammogram, bone scan, CT scan, and MRI scan. Blood tests and a clinical evaluation are also used to evaluate a patient's overall health and detect whether the cancer has spread to certain organs.

To stage cancer, the American Joint Committee on Cancer first places the cancer, particularly solid tumors, in a letter category using the TNM classification system. Cancers are designated the letter T (tumor size), N (palpable nodes), and/or M (metastases). T1, T2, T3, and T4 describe the increasing size of the primary lesion; N0, N1, N2, N3 indicates progressively advancing node involvement; and M0 and M1 reflect the absence or presence of distant metastases.

In the second staging method, also known as the Overall Stage Grouping or Roman Numeral Staging, cancers are divided into stages 0 to IV, incorporating the size of primary lesions as well as the presence of nodal spread and of distant metastases. In this system, cases are grouped into four stages denoted by Roman numerals I through IV, or are classified as "recurrent." For some cancers, stage 0 is referred to as "in situ" or "Tis," such as ductal carcinoma in situ or lobular carcinoma in situ for breast cancers. High grade adenomas can also be classified as stage 0. In general, stage I cancers are small localized cancers that are usually curable, while stage IV usually represents inoperable or metastatic cancer. Stage II and III cancers are usually locally advanced and/or exhibit involvement of local lymph nodes. In general, the higher stage numbers indicate more extensive disease, including greater tumor size and/or spread of the cancer to nearby lymph nodes and/or organs adjacent to the primary

tumor. These stages are defined precisely, but the definition is different for each kind of cancer and is known to the skilled artisan.

Many cancer registries, such as the NCI's Surveillance, Epidemiology, and End Results Program (SEER), use summary staging. This system is used for all types of cancer. It groups cancer cases into five main categories:

In situ is early cancer that is present only in the layer of cells in which it began.

Localized is cancer that is limited to the organ in which it began, without evidence of spread.

Regional is cancer that has spread beyond the original (primary) site to nearby lymph nodes or organs and tissues.

Distant is cancer that has spread from the primary site to distant organs or distant lymph nodes.

Unknown is used to describe cases for which there is not enough information to indicate a stage.

In addition, it is common for cancer to return months or years after the primary tumor has been removed. Cancer that recurs after all visible tumor has been eradicated, is called recurrent disease. Disease that recurs in the area of the primary tumor is locally recurrent, and disease that recurs as metastases is referred to as a distant recurrence.

The tumor can be a solid tumor or a non-solid or soft tissue tumor. Examples of soft tissue tumors include leukemia (e.g., chronic myelogenous leukemia, acute myelogenous leukemia, adult acute lymphoblastic leukemia, acute myelogenous leukemia, mature B-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia, polymphocytic leukemia, or hairy cell leukemia) or lymphoma (e.g., non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, or Hodgkin's disease). A solid tumor includes any cancer of body tissues other than blood, bone marrow, or the lymphatic system. Solid tumors can be further divided into

those of epithelial cell origin and those of non-epithelial cell origin. Examples of epithelial cell solid tumors include tumors of the gastrointestinal tract, colon, breast, prostate, lung, kidney, liver, pancreas, ovary, head and neck, oral cavity, stomach, duodenum, small intestine, large intestine, anus, gall bladder, labium, nasopharynx, skin, uterus, male genital organ, urinary organs, bladder, and skin. Solid tumors of non-epithelial origin include sarcomas, brain tumors, and bone tumors. Other examples of tumors are described in the Definitions section.

In some embodiments, the patient herein is subjected to a diagnostic test e.g., prior to and/or during and/or after therapy. Generally, if a diagnostic test is performed, a sample may be obtained from a patient in need of therapy. Where the subject has cancer, the sample may be a tumor sample, or other biological sample, such as a biological fluid, including, without limitation, blood, urine, saliva, ascites fluid, or derivatives such as blood serum and blood plasma, and the like.

The biological sample herein may be a fixed sample, *e.g.* a formalin fixed, paraffin-embedded (FFPE) sample, or a frozen sample.

Various methods for determining expression of mRNA or protein include, but are not limited to, gene expression profiling, polymerase chain reaction (PCR) including quantitative real time PCR (qRT-PCR), microarray analysis, serial analysis of gene expression (SAGE), MassARRAY, Gene Expression Analysis by Massively Parallel Signature Sequencing (MPSS), proteomics, immunohistochemistry (IHC), etc. Preferably mRNA is quantified. Such mRNA analysis is preferably performed using the technique of polymerase chain reaction (PCR), or by microarray analysis. Where PCR is employed, a preferred form of PCR is quantitative real time PCR (qRT-PCR). In one embodiment, expression of one or more of the above noted genes is deemed positive expression if it is at the median or above, *e.g.* compared to other samples of the same tumor-type. The median expression level can be

determined essentially contemporaneously with measuring gene expression, or may have been determined previously.

The steps of a representative protocol for profiling gene expression using fixed, paraffin-embedded tissues as the RNA source, including mRNA isolation, purification, primer extension and amplification are given in various published journal articles (for example: Godfrey *et al. J. Molec. Diagnostics* 2: 84-91 (2000); Specht *et al., Am. J. Pathol.* 158: 419-29 (2001)). Briefly, a representative process starts with cutting about 10 microgram thick sections of paraffin-embedded tumor tissue samples. The RNA is then extracted, and protein and DNA are removed. After analysis of the RNA concentration, RNA repair and/or amplification steps may be included, if necessary, and RNA is reverse transcribed using gene specific promoters followed by PCR. Finally, the data are analyzed to identify the best treatment option(s) available to the patient on the basis of the characteristic gene expression pattern identified in the tumor sample examined.

Detection of gene or protein expression may be determined directly or indirectly.

One may determine expression or translocation or amplification of FGFR2 and/or FGFR3 in the cancer (directly or indirectly). Various diagnostic/prognostic assays are available for this. In one embodiment, FGFR3 overexpression may be analyzed by IHC. Parafin embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a FGFR2 and/or FGFR3 protein staining intensity criteria as follows:

Score 0 no staining is observed or membrane staining is observed in less than 10% of tumor cells.

Score 1+ a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.

Score 2+ a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

Score 3+ a moderate to strong complete membrane staining is observed in more than 10% of the tumor cells.

In some embodiments, those tumors with 0 or 1+ scores for each of FGFR2 and FGFR3 overexpression assessment may be characterized as not overexpressing FGFR2 and FGFR3, whereas those tumors with 2+ or 3+ scores may be characterized as overexpressing each of FGFR2 and FGFR3.

In some embodiments, tumors overexpressing each of FGFR2 and FGFR3 may be rated by immunohistochemical scores corresponding to the number of copies of each of FGFR2 and FGFR3 molecules expressed per cell, and can been determined biochemically:

0 = 0-90 copies/cell,

1+ = at least about 100 copies/cell,

2+ = at least about 1000 copies/cell,

3+ = at least about 10,000 copies/cell.

Alternatively, or additionally, FISH assays may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the presence or and/or extent (if any) of FGFR2 and/or FGFR3 amplification or translocation in the tumor.

FGFR2 and FGFR3 activation may be determined directly (e.g., by phospho-ELISA testing, or other means of detecting phosphorylated receptor) or indirectly (e.g., by detection of activated downstream signaling pathway components, detection of receptor dimers (e.g., homodimers, heterodimers), detection of gene expression profiles and the like.

Similarly, constitutive FGFR2 and FGFR3 and/or ligand-independent or ligand-dependent FGFR2 and FGFR3 may be detected directly or indirectly (e.g., by detection of receptor mutations correlated with constitutive activity, by detection of receptor amplification correlated with constitutive activity and the like).

Methods for detection of nucleic acid mutations are well known in the art. Often, though not necessarily, a target nucleic acid in a sample is amplified to provide the desired amount of material for determination of whether a mutation is present. Amplification techniques are well known in the art. For example, the amplified product may or may not encompass all of the nucleic acid sequence encoding the protein of interest, so long as the amplified product comprises the particular amino acid/nucleic acid sequence position where the mutation is suspected to be.

In one example, presence of a mutation can be determined by contacting nucleic acid from a sample with a nucleic acid probe that is capable of specifically hybridizing to nucleic acid encoding a mutated nucleic acid, and detecting said hybridization. In one embodiment, the probe is detectably labeled, for example with a radioisotope (^{3}H, ^{32}P, ^{33}P etc), a fluorescent agent (rhodamine, fluorescene etc.) or a chromogenic agent. In some embodiments, the probe is an antisense oligomer, for example PNA, morpholino-phosphoramidates, LNA or 2'-alkoxyalkoxy. The probe may be from about 8 nucleotides to about 100 nucleotides, or about 10 to about 75, or about 15 to about 50, or about 20 to about 30. In another aspect, nucleic acid probes of the invention are provided in a kit for identifying FGFR2 and/or FGFR3 mutations in a sample, said kit comprising an oligonucleotide that specifically hybridizes to or adjacent to a site of mutation in the nucleic acid encoding FGFR2 and/or FGFR3. The kit may further comprise instructions for treating patients having tumors that contain FGFR2 and/or FGFR3 mutations with a FGFR2 and/or FGFR3 antagonist based on the result of a hybridization test using the kit.

Mutations can also be detected by comparing the electrophoretic mobility of an amplified nucleic acid to the electrophoretic mobility of corresponding nucleic acid encoding wild-type FGFR2 and/or FGFR3. A difference in the mobility indicates the presence of a

mutation in the amplified nucleic acid sequence. Electrophoretic mobility may be determined by any appropriate molecular separation technique, for example on a polyacrylamide gel.

Nucleic acids may also be analyzed for detection of mutations using Enzymatic Mutation Detection (EMD) (Del Tito et al, Clinical Chemistry 44:731-739, 1998). EMD uses the bacteriophage resolvase T_4 endonuclease VII, which scans along double-stranded DNA until it detects and cleaves structural distortions caused by base pair mismatches resulting from nucleic acid alterations such as point mutations, insertions and deletions. Detection of two short fragments formed by resolvase cleavage, for example by gel eletrophoresis, indicates the presence of a mutation. Benefits of the EMD method are a single protocol to identify point mutations, deletions, and insertions assayed directly from amplification reactions, eliminating the need for sample purification, shortening the hybridization time, and increasing the signal-to-noise ratio. Mixed samples containing up to a 20-fold excess of normal nucleic acids and fragments up to 4 kb in size can been assayed. However, EMD scanning does not identify particular base changes that occur in mutation positive samples, therefore often requiring additional sequencing procedures to identify the specific mutation if necessary. CEL I enzyme can be used similarly to resolvase T_4 endonuclease VII, as demonstrated in US Pat. No. 5,869,245.

Another simple kit for detecting mutations is a reverse hybridization test strip similar to Haemochromatosis StripAssay™ (Viennalabs http://www.bamburghmarrsh.com/pdf/4220.pdf) for detection of multiple mutations in HFE, TFR2 and FPN1 genes causing Haemochromatosis. Such an assay is based on sequence specific hybridization following amplification by PCR. For single mutation assays, a microplate-based detection system may be applied, whereas for multi-mutation assays, test strips may be used as "macro-arrays". Kits may include ready-to use reagents for sample prep, amplification and mutation detection. Multiplex amplification protocols provide

convenience and allow testing of samples with very limited volumes. Using the straightforward StripAssay format, testing for twenty and more mutations may be completed in less than five hours without costly equipment. DNA is isolated from a sample and the target nucleic acid is amplified *in vitro* (e.g., by PCR) and biotin-labelled, generally in a single ("multiplex") amplification reaction. The amplification products are then selectively hybridized to oligonucleotide probes (wild-type and mutant specific) immobilized on a solid support such as a test strip in which the probes are immobilized as parallel lines or bands. Bound biotinylated amplicons are detected using streptavidin-alkaline phosphatase and color substrates. Such an assay can detect all or any subset of the mutations of the invention. With respect to a particular mutant probe band, one of three signaling patterns are possible: (i) a band only for wild-type probe which indicates normal nucleic acid sequence, (ii) bands for both wild-type and a mutant probe which indicates heterozygous genotype, and (iii) band only for the mutant probe which indicates homozygous mutant genotype. Accordingly, in one aspect, the invention provides a method of detecting mutations of the invention comprising isolating and/or amplifying a target FGFR2 and/or FGFR3 nucleic acid sequence from a sample, such that the amplification product comprises a ligand, contacting the amplification product with a probe which comprises a detectable binding partner to the ligand and the probe is capable of specifically hydribizing to a mutation of the invention, and then detecting the hybridization of said probe to said amplification product. In one embodiment, the ligand is biotin and the binding partner comprises avidin or streptavidin. In one embodiment, the binding partner comprises steptavidin-alkaline which is detectable with color substrates. In one embodiment, the probes are immobilized for example on a test strip wherein probes complementary to different mutations are separated from one another. Alternatively, the amplified nucleic acid is labelled with a radioisotope in which case the probe need not comprise a detectable label.

Alterations of a wild-type gene encompass all forms of mutations such as insertions, inversions, deletions, and/or point mutations. In one embodiment, the mutations are somatic. Somatic mutations are those which occur only in certain tissues, e.g., in the tumor tissue, and are not inherited in the germ line. Germ line mutations can be found in any of a body's tissues.

A sample comprising a target nucleic acid can be obtained by methods well known in the art, and that are appropriate for the particular type and location of the tumor. Tissue biopsy is often used to obtain a representative piece of tumor tissue. Alternatively, tumor cells can be obtained indirectly in the form of tissues/fluids that are known or thought to contain the tumor cells of interest. For instance, samples of lung cancer lesions may be obtained by resection, bronchoscopy, fine needle aspiration, bronchial brushings, or from sputum, pleural fluid or blood. Mutant genes or gene products can be detected from tumor or from other body samples such as urine, sputum or serum. The same techniques discussed above for detection of mutant target genes or gene products in tumor samples can be applied to other body samples. Cancer cells are sloughed off from tumors and appear in such body samples. By screening such body samples, a simple early diagnosis can be achieved for diseases such as cancer. In addition, the progress of therapy can be monitored more easily by testing such body samples for mutant target genes or gene products.

Means for enriching a tissue preparation for tumor cells are known in the art. For example, the tissue may be isolated from paraffin or cryostat sections. Cancer cells may also be separated from normal cells by flow cytometry or laser capture microdissection. These, as well as other techniques for separating tumor from normal cells, are well known in the art. If the tumor tissue is highly contaminated with normal cells, detection of mutations may be more difficult, although techniques for minimizing contamination and/or false positive/negative results are known, some of which are described hereinbelow. For example,

a sample may also be assessed for the presence of a biomarker (including a mutation) known to be associated with a tumor cell of interest but not a corresponding normal cell, or vice versa.

Detection of point mutations in target nucleic acids may be accomplished by molecular cloning of the target nucleic acids and sequencing the nucleic acids using techniques well known in the art. Alternatively, amplification techniques such as the polymerase chain reaction (PCR) can be used to amplify target nucleic acid sequences directly from a genomic DNA preparation from the tumor tissue. The nucleic acid sequence of the amplified sequences can then be determined and mutations identified therefrom. Amplification techniques are well known in the art, e.g., polymerase chain reaction as described in Saiki et al., Science 239:487, 1988; U.S. Pat. Nos. 4,683,203 and 4,683,195.

It should be noted that design and selection of appropriate primers are well established techniques in the art.

The ligase chain reaction, which is known in the art, can also be used to amplify target nucleic acid sequences. See, e.g., Wu et al., Genomics, Vol. 4, pp. 560-569 (1989). In addition, a technique known as allele specific PCR can also be used. See, e.g., Ruano and Kidd, Nucleic Acids Research, Vol. 17, p. 8392, 1989. According to this technique, primers are used which hybridize at their 3'ends to a particular target nucleic acid mutation. If the particular mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435, and in Newton et al., Nucleic Acids Research, Vol. 17, p.7, 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Single stranded conformation polymorphism (SSCP) analysis

can also be used to detect base change variants of an allele. See, e.g. Orita et al., Proc. Natl. Acad. Sci. USA Vol. 86, pp. 2766-2770, 1989, and Genomics, Vol. 5, pp. 874-879, 1989. Other techniques for detecting insertions and deletions as known in the art can also be used.

Alteration of wild-type genes can also be detected on the basis of the alteration of a wild-type expression product of the gene. Such expression products include both mRNA as well as the protein product. Point mutations may be detected by amplifying and sequencing the mRNA or via molecular cloning of cDNA made from the mRNA. The sequence of the cloned cDNA can be determined using DNA sequencing techniques which are well known in the art. The cDNA can also be sequenced via the polymerase chain reaction (PCR).

Mismatches are hybridized nucleic acid duplexes which are not 100% complementary. The lack of total complementarity may be due to deletions, insertions, inversions, substitutions or frameshift mutations. Mismatch detection can be used to detect point mutations in a target nucleic acid. While these techniques can be less sensitive than sequencing, they are simpler to perform on a large number of tissue samples. An example of a mismatch cleavage technique is the RNase protection method, which is described in detail in Winter et al., Proc. Natl. Acad. Sci. USA, Vol. 82, p. 7575, 1985, and Meyers et al., Science, Vol. 230, p. 1242, 1985. For example, a method of the invention may involve the use of a labeled riboprobe which is complementary to the human wild-type target nucleic acid. The riboprobe and target nucleic acid derived from the tissue sample are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full-length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the target nucleic acid

mRNA or gene, but can a portion of the target nucleic acid, provided it encompasses the position suspected of being mutated. If the riboprobe comprises only a segment of the target nucleic acid mRNA or gene, it may be desirable to use a number of these probes to screen the whole target nucleic acid sequence for mismatches if desired.

In a similar manner, DNA probes can be used to detect mismatches, for example through enzymatic or chemical cleavage. See, e.g., Cotton et al., Proc. Natl. Acad. Sci. USA, Vol. 85, 4397, 1988; and Shenk et al., Proc. Natl. Acad. Sci. USA, Vol. 72, p. 989, 1975. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, Human Genetics, Vol. 42, p. 726, 1988. With either riboprobes or DNA probes, the target nucleic acid mRNA or DNA which might contain a mutation can be amplified before hybridization. Changes in target nucleic acid DNA can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

Target nucleic acid DNA sequences which have been amplified may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the target nucleic acid gene harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the target gene sequence. By use of a battery of such allele-specific probes, target nucleic acid amplification products can be screened to identify the presence of a previously identified mutation in the target gene. Hybridization of allele-specific probes with amplified target nucleic acid sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under stringent hybridization conditions indicates the presence of the same mutation in the tumor tissue as in the allele-specific probe.

Alteration of wild-type target genes can also be detected by screening for alteration of the corresponding wild-type protein. For example, monoclonal antibodies immunoreactive

with a target gene product can be used to screen a tissue, for example an antibody that is known to bind to a particular mutated position of the gene product (protein). For example, an antibody that is used may be one that binds to a deleted exon or that binds to a conformational epitope comprising a deleted portion of the target protein. Lack of cognate antigen would indicate a mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant gene product. Antibodies may be identified from phage display libraries. Such immunological assays can be done in any convenient format known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered protein can be used to detect alteration of wild-type target genes.

Primer pairs are useful for determination of the nucleotide sequence of a target nucleic acid using nucleic acid amplification techniques such as the polymerase chain reaction. The pairs of single stranded DNA primers can be annealed to sequences within or surrounding the target nucleic acid sequence in order to prime amplification of the target sequence. Allele-specific primers can also be used. Such primers anneal only to particular mutant target sequence, and thus will only amplify a product in the presence of the mutant target sequence as a template. In order to facilitate subsequent cloning of amplified sequences, primers may have restriction enzyme site sequences appended to their ends. Such enzymes and sites are well known in the art. The primers themselves can be synthesized using techniques which are well known in the art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available. Design of particular primers is well within the skill of the art.

Nucleic acid probes are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA and in the RNase protection method for detecting point mutations already discussed above. The probes can be used to detect target nucleic acid amplification products. They may also be used to detect mismatches with the wild type gene

or mRNA using other techniques. Mismatches can be detected using either enzymes (e.g., S1 nuclease), chemicals (e.g., hydroxylamine or osmium tetroxide and piperidine), or changes in electrophoretic mobility of mismatched hybrids as compared to totally matched hybrids. These techniques are known in the art. See Novack et al., Proc. Natl. Acad. Sci. USA, Vol. 83, p. 586, 1986. Generally, the probes are complementary to sequences outside of the kinase domain. An entire battery of nucleic acid probes may be used to compose a kit for detecting mutations in target nucleic acids. The kit allows for hybridization to a large region of a target sequence of interest. The probes may overlap with each other or be contiguous.

If a riboprobe is used to detect mismatches with mRNA, it is generally complementary to the mRNA of the target gene. The riboprobe thus is an antisense probe in that it does not code for the corresponding gene product because it is complementary to the sense strand. The riboprobe generally will be labeled with a radioactive, colorimetric, or fluorometric material, which can be accomplished by any means known in the art. If the riboprobe is used to detect mismatches with DNA it can be of either polarity, sense or anti-sense. Similarly, DNA probes also may be used to detect mismatches.

In some instances, the cancer does or does not overexpress FGFR2 and/or FGFR3. Receptor overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the receptor protein present on the surface of a cell (e.g. via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of receptor-encoding nucleic acid in the cell, e.g. via fluorescent in situ hybridization (FISH; see WO98/45479 published October, 1998), southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). Aside from the above assays, various in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, e.g. a radioactive isotope, and binding of the antibody to cells in the patient

can be evaluated, e.g. by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

Chemotherapeutic Agents

The combination therapy of the invention can further comprise one or more chemotherapeutic agent(s). The combined administration includes coadministration or concurrent administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

The chemotherapeutic agent, if administered, is usually administered at dosages known therefor, or optionally lowered due to combined action of the drugs or negative side effects attributable to administration of the antimetabolite chemotherapeutic agent. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner.

Various chemotherapeutic agents that can be combined are disclosed herein.

In some embodiments, chemotherapeutic agents to be combined are selected from the group consisting of lenalidomide (REVLIMID), proteosome inhibitors (such as bortezomib (VELCADE) and PS342), bora taxoid (including docetaxel and paclitaxel), vinca (such as vinorelbine or vinblastine), platinum compound (such as carboplatin or cisplatin), aromatase inhibitor (such as letrozole, anastrazole, or exemestane), anti-estrogen (e.g. fulvestrant or tamoxifen), etoposide, thiotepa, cyclophosphamide, pemetrexed, methotrexate, liposomal doxorubicin, pegylated liposomal doxorubicin, capecitabine, gemcitabine, melthalin, doxorubicin, vincristine, COX-2 inhibitor (for instance, celecoxib), or steroid (e.g., dexamethasone and prednisone). In some embodiments (e.g., embodiments involving treatment of t(4;14)+ multiple myeloma, dexamethasone and lenalidomide, or dexamethasone, or bortezomib, or vincristine, doxorubicin and dexamethason, or thalidomide

and dexamethasone, or liposomal doxorubicin, vincristine and dexamethasone, or lenalidomide and dexamethasone, or bortezomib and dexamethasone, or bortezomib, doxorubicin, and dexamethasone are combined. In some embodiments (e.g., embodiments involving bladder cancer), gemcitabine and cisplatin, or a taxane (e.g., paclitaxel, docetaxel), or pemetrexed, or methotrexate, vinblastine, doxorubicin and cisplatin, or carboplatin, or mitomycin C in combination with 5-Fluorouracil, or cisplatin, or cisplatin and 5-Fluorouracil are combined.

Formulations, Dosages and Administrations

The therapeutic agents used in the invention will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular subject being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, the drug-drug interaction of the agents to be combined, and other factors known to medical practitioners.

Therapeutic formulations are prepared using standard methods known in the art by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences (20^{th} edition), ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, PA). Acceptable carriers, include saline, or buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagines, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such

as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, PLURONICS™, or PEG.

Optionally, but preferably, the formulation contains a pharmaceutically acceptable salt, preferably sodium chloride, and preferably at about physiological concentrations. Optionally, the formulations of the invention can contain a pharmaceutically accepTable preservative. In some embodiments the preservative concentration ranges from 0.1 to 2.0%, typically v/v. Suitable preservatives include those known in the pharmaceutical arts. Benzyl alcohol, phenol, m-cresol, methylparaben, and propylparaben are preferred preservatives. Optionally, the formulations of the invention can include a pharmaceutically acceptable surfactant at a concentration of 0.005 to 0.02%.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, *supra*.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat.

No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

The therapeutic agents of the invention are administered to a human patient, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. An *ex vivo* strategy can also be used for therapeutic applications. Ex vivo strategies involve transfecting or transducing cells obtained from the subject with a polynucleotide encoding a FGFR2, FGFR3, or FGFR2/3 antagonist. The transfected or transduced cells are then returned to the subject. The cells can be any of a wide range of types including, without limitation, hemopoietic cells (e.g., bone marrow cells, macrophages, monocytes, dendritic cells, T cells, or B cells), fibroblasts, epithelial cells, endothelial cells, keratinocytes, or muscle cells.

For example, if the FGFR2/3 antagonist is an antibody, the antibody is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antibody is suitably administered by pulse infusion, particularly with declining doses of the antibody. Preferably the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

In another example, the FGFR2/3 antagonist compound is administered locally, e.g., by direct injections, when the disorder or location of the tumor permits, and the injections can be repeated periodically. The FGFR2/3 antagonist can also be delivered systemically to the subject or directly to the tumor cells, e.g., to a tumor or a tumor bed following surgical excision of the tumor, in order to prevent or reduce local recurrence or metastasis.

Administration of the therapeutic agents in combination typically is carried out over a defined time period (usually minutes, hours, days or weeks depending upon the combination selected). Combination therapy is intended to embrace administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner.

The therapeutic agent can be administered by the same route or by different routes. For example, the anti-FGFR2/3 antibody in the combination may be administered by intravenous injection while a chemotherapeutic agent in the combination may be administered orally. Alternatively, for example, both of the therapeutic agents may be administered orally, or both therapeutic agents may be administered by intravenous injection,

depending on the specific therapeutic agents. The sequence in which the therapeutic agents are administered also varies depending on the specific agents.

Depending on the type and severity of the disease, about 1 μg/kg to 100 mg/kg of each therapeutic agent is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to about 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until the cancer is treated, as measured by the methods described above. However, other dosage regimens may be useful.

The present application contemplates administration of the FGFR2/3 antibody by gene therapy. See, for example, WO96/07321 published March 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or when combined with another composition(s) effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice, such as cancer. Moreover, the article of manufacture may comprise (a) a first container with a composition contained

therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the first and second antibody compositions can be used to treat a particular condition, e.g., cancer. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

The following are examples of the methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

EXAMPLES

Example 1. Broadening the specificity of anti-FGFR3 antibodies. Experiments were performed to broaden the binding specificity of an anti-FGFR2/3 antibody. Specifically, experiments were performed to develop antibodies for cancer therapy with dual specificity for FGFR3 and FGFR2 that do not bind the highly related receptors FGFR1 and FGFR4. The starting point was the monospecific antibody R3Mab, which binds to the FGFR3 IIIb and IIIc isoforms with sub-nanomolar affinities (Qing, J., X. Du, Y. Chen, P. Chan, H. Li, P. Wu, S. Marsters, S. Stawicki, J. Tien, K. Totpal, S. Ross, S. Stinson, D. Dornan, D. French, Q. R. Wang, J. P. Stephan, Y. Wu, C. Wiesmann and A. Ashkenazi (2009). "Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice." The Journal of clinical investigation **119**(5): 1216-1229). R3Mab shows robust inhibition of FGFR3 signaling and tumor growth *in vivo* (Qing, J., X. Du, Y. Chen, P. Chan, H. Li, P. Wu, S. Marsters, S. Stawicki, J. Tien, K. Totpal, S. Ross, S. Stinson,

D. Dornan, D. French, Q. R. Wang, J. P. Stephan, Y. Wu, C. Wiesmann and A. Ashkenazi (2009). "Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice." The Journal of clinical investigation 119(5): 1216-1229) and has been studied in phase I clinical trials.

The antibody re-design strategy was guided by the previously determined crystallographic structure of an R3Mab Fab fragment in complex with FGFR3-IIIb (PDB 3GRW) (Qing, J., X. Du, Y. Chen, P. Chan, H. Li, P. Wu, S. Marsters, S. Stawicki, J. Tien, K. Totpal, S. Ross, S. Stinson, D. Dornan, D. French, Q. R. Wang, J. P. Stephan, Y. Wu, C. Wiesmann and A. Ashkenazi (2009). "Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice." The Journal of clinical investigation 119(5): 1216-1229). This structure indicates that R3Mab interacts with both the D2 and D3 domains of FGFR3-IIIb. Although D2 was subsequently found here to be sufficient for R3Mab binding, initial analyses were based on the contacts on this original structure. Most of the contact surface on the FGFR3-IIIb antigen was contributed by the antibody complementarity-determining regions (CDRs) H3 (46%), H1 (23%) and L2 (22%), with small contributions from CDR H2 and framework region (FR) residues (Qing, J., X. Du, Y. Chen, P. Chan, H. Li, P. Wu, S. Marsters, S. Stawicki, J. Tien, K. Totpal, S. Ross, S. Stinson, D. Dornan, D. French, Q. R. Wang, J. P. Stephan, Y. Wu, C. Wiesmann and A. Ashkenazi (2009). "Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice." The Journal of clinical investigation 119(5): 1216-1229.) (**Figure 5**). The similarity between FGFR3-IIIb and the intended additional FGFR2-IIIb antigen were compared. The D2D3 regions of these two homologs share 68% protein-sequence identity, while their D2 domains share 76% identity (**Table 2**). **Table 2** shows the percentage identities between the two isoforms of the same FGFR (Bold), the complete sequences of the D2D3 domains including the isoform-dependent regions in the D3 (Underline), and the D2D3 domains lacking the isoform-dependent regions (Bold and Underline). Since D3 of the R3Mab-bound FGFR3-IIIb had a different geometry as compared to all other FGFR

structures (Qing, J., X. Du, Y. Chen, P. Chan, H. Li, P. Wu, S. Marsters, S. Stawicki, J. Tien, K. Totpal, S. Ross, S. Stinson, D. Dornan, D. French, Q. R. Wang, J. P. Stephan, Y. Wu, C. Wiesmann and A. Ashkenazi (2009). "Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice." The Journal of clinical investigation 119(5): 1216-1229), the structures of FGFR2-IIIb and FGFR3-IIIb were superimposed on their D2 regions, which yielded a calculated root mean squared deviation (RMSD) of α-carbons of 0.78 Å. Based on this analysis, experiments were designed to re-engineer R3Mab to bind and inhibit FGFR2 as well.

Table 2. Sequence identities between FGFR proteins

Identity of FGFR (%)

	FGFR1-IIIb	FGFR1-IIIc	FGFR2-IIIb	FGFR2-IIIc	FGFG3-IIIb	FGFR3-IIIc	FGFR4
FGFR1-IIIb	100		**77.6**		**71.4**		**67.1**
FGFR1-IIIc	**88.0**	100					
FGFR2-IIIb	79.1	71.6	100		**75.8**		**71.4**
FGFR2-IIIc	70.2	79.3	**88.9**	100			
FGFR3-IIIb	64.7	63.5	68.1	65.9	100		**78.9**
FGFR3-IIIc	65.9	74.5	70.2	76.9	**85.1**	100	
FGFR4	64.1	68.8	68.9	71.2	70.5	75.0	100

To construct a phage display library, mutations were designed that cover most residues in each of the individual heavy-chain CDRs and a selection of the contact residues on all CDRs (**Table 3**). In **Table 3**, N = G, A, T or C; K = G or T. R3Mab variants displayed as Fab fragments on phage particles were selected for binding to FGFR2-IIIb. We did not perform selection on FGFR3 at this stage as we wanted to keep the selection stringency low when bringing in the new FGFR2 specificity. After the first round of panning, the phage outputs from the individual libraries were combined and subjected to 3 further rounds of

selection. 95 clones, designated as 2B.1 series, were screened by ELISA. Among these, 81 clones, representing 32 unique sequences, bound to FGFR2-IIIb. All binding clones were apparently derived from the H2 library, because they contained mutations in CDR H2 but not elsewhere (**Table 4**). **Table 4** identifies residues the same as those in R3Mab with underlining. **Table 4** identifies residues differing from those in R3Mab with italics. As used in **Table 4**, "ND" refers to not detectable and "NA" refers to not available due to protein aggregation. All selected antibodies showed substantially improved binding to FGFR2-IIIb relative to R3Mab, with K_D values ranging from 0.3 to 17 nM (**Table 4**). Remarkably, the mutated H2 sequences contained significant variation, lacking clear consensus and differing from R3Mab at 4 or 5 positions (**Table 4**, **Fig. 6**). Thus, there are multiple possible solutions to conferring high-affinity binding of FGFR2-IIIb onto R3Mab.

Table 3. Library design for recruiting FGFR2 binding specificity

CDR H1

Residues	25	26	27	28	29	30	31	32	33
Codons	TCT	GGC	TTC	ACC	TTC	ACT	AGT	ACT	GGG
Amino acids	S	G	F	T	F	T	S	T	G
H1 Lib.	NNK	NNK		NNK		NNK	NNK	NNK	NNK
H2 Lib.									
H3 Lib.									
Combined Lib.							NNT	NNK	NNC

CDR H2

Residues	51	52	52a	53	54
Codons	ATT	TAT	CCT	ACT	AAC
Amino acids	I	Y	P	T	N
H1 Lib.					
H2 Lib.	NNK	NNK	NNK	NNK	NNK
H3 Lib.					
Combined Lib.		NNK			

CDR H3

Residues	96	97	98	99	100	100a
Codons	TAC	GGC	ATC	TAC	GAC	CTG
Amino acids	Y	G	I	Y	D	L
H1 Lib.						
H2 Lib.						
H3 Lib.	NNK	NNK	NNK	NNC	NNK	NNK
Combined Lib.				NNC	NNK	NNK

CDR L2

Residues	52	53	54
Codons	TCC	TTC	CTC
Amino acids	S	F	L

H1 Lib.			
H2 Lib.			
H3 Lib.			
Combined Lib.		NNC	

Table 4. Residues differing from those in R3Mab.

Variant ID	CDR-H2 sequence	Times found (n)	FGFR2-IIIb K_D (nM)
R3Mab	IYPTN	0	ND
2B.1.1	*YWAWD*	3	0.29
2B.1.88	I*WMFT*	4	0.64
2B.1.38	*FWAYD*	1	1.1
2B.1.20	*LDVFW*	1	1.2
2B.1.32	*WVGFT*	9	1.2
2B.1.49	*LSFFS*	1	1.3
2B.1.86	*LSFWT*	1	1.3
2B.1.9	*YHPYL*	8	1.4
2B.1.73	*MIFYN*	1	1.4
2B.1.74	*YHPFR*	1	1.4
2B.1.14	*LWYFD*	1	1.6
2B.1.71	*VWMFD*	1	1.6
2B.1.28	*FWAWS*	2	1.8
2B.1.95	*LIFFT*	2	1.8
2B.1.50	*LNFYS*	2	2.0
2B.1.81	*VNNFY*	1	2.1
2B.1.25	*WHPWM*	1	2.3
2B.1.3	*THLGD*	1	2.6
2B.1.65	*YNAYT*	1	2.7
2B.1.94	*LVFFS*	3	3.1
2B.1.78	*LSFYS*	4	3.2
2B.1.72	*VHPFE*	1	3.5
2B.1.44	*WWSWG*	1	3.6
2B.1.52	*FSLGD*	1	3.9
2B.1.30	*VSFFS*	1	4.1
2B.1.82	I*NFFS*	1	4.9
2B.1.93	I*DNYW*	13	5.1
2B.1.55	*VDVFW*	3	5.9
2B.1.35	*WHPFR*	5	9.4
2B.1.33	*YHPFH*	2	15
2B.1.80	*YWAFS*	2	17
2B.1.92	*WVAFS*	2	NA

Next six variants were selected for measurements of binding to FGFR3 based on their affinities (< 3 nM) for FGFR2 and sequence diversity. All the variants showed improved affinities for FGFR3-IIIb (**Table 5**). To further assess their ability to inhibit receptor-dependent cell growth,

proliferation of MCF7 breast carcinoma cells was assayed either with or without FGF7–a specific ligand for FGFR2-IIIb (Goetz, R. and M. Mohammadi (2013). "Exploring mechanisms of FGF signalling through the lens of structural biology." Nat Rev Mol Cell Biol 14(3): 166-180;Bai, A., K. Meetze, N. Y. Vo, S. Kollipara, E. K. Mazsa, W. M. Winston, S. Weiler, L. L. Poling, T. Chen, N. S. Ismail, J. Jiang, L. Lerner, J. Gyuris and Z. Weng (2010). "GP369, an FGFR2-IIIb-specific antibody, exhibits potent antitumor activity against human cancers driven by activated FGFR2 signaling." Cancer research 70(19): 7630-7639). Variant 2B.1.3 exhibited the greatest antagonist activity, as compared to other variants, which showed less or no inhibition, or even displayed stimulatory effect (**Figure 2**). Hence, 2B.1.3 was carried over as a functional antibody for further characterizations.

Table5. Binding affinities of selected 2B.1.3 variants for FGFR2-IIIb and FGFR3-IIIb.

Clone	CDR H2	FGFR2-IIIb K_D (nM)	FGFR3-IIIb K_D (nM)
R3Mab	IYPTN	ND	0.24
2B.1.3	THLGD	2.6	0.09
2B.1.95	LIFFT	1.8	0.19
2B.1.73	MIFYN	1.4	0.09
2B.1.32	WVGFT	1.2	0.06
2B.1.88	IWMFT	0.64	0.05
2B.1.1	YWAWD	0.29	0.09

*Residues the same as those in R3Mab are underlined.

Since all FGFR homologs share nearly 70% sequence identity between each other (**Table 2**), binding of re-engineered variant 2B.1.3 to other FGFRs was evaluated. Mab 2B.1.3 bound FGFR2-IIIc with similar affinity as FGFR2-IIIb (**Table 6**). Mab 2B.1.3 also showed several-fold higher affinity for FGFR3-IIIb and FGFR3–IIIc than did R3Mab, even though the selection strategy used was based on binding to FGFR2-IIIb. The increased

affinity for FGFR3 was consistently exhibited by all the other selected variants tested (data now shown). Moreover, Mab 2B.1.3 also bound to FGFR4, with a K_D value of 32 nM, yet showed no detectable binding to FGFR1 (**Table 6**). Therefore, variant 2B.1.3 is trispecific, binding to FGFR2, FGFR3 and FGFR4, but not FGFR1.

Table 6. Binding affinities of R3Mab and its variants to all FGFR homologs

K_D (nM)

	FGFR1-IIIb	FGFR1-IIIc	FGFR2-IIIb	FGFR2-IIIc	FGFR3-IIIb	FGFR3-IIIc	FGFR4
R3Mab	ND*	ND	ND	ND	0.24	0.61	ND
2B.1.3	ND	ND	2.6	2.0	0.09	0.07	32
2B.1.3.10	ND	ND	2.9	1.1	0.11	0.25	ND
2B.1.3.12	ND	ND	3.0	6.1	0.50	0.72	ND

*ND: not detecTable at 500 nM.

Example 2. The structure Mab2B.1 and FGFR2-IIIb complex was determined. Specifically, to obtain direct insight into how the re-engineered variant 2B.1.3 acquired specificity for FGFR2, the crystal structure of its complex with FGFR2 was determined (**Fig. 2A**, **Table 7**). FGFR2-IIIb D2D3 was first generated by expression in *E. coli* and refolding from inclusion bodies and judged to be intact by SDS-PAGE and mass spectrometry. However, in crystals this protein contained only the isoform-independent D2 domain, suggesting proteolysis between D2 and D3 during the crystallization process. The previously determined FGFR3-IIIb:R3Mab complex structure contained both the D2 and D3 domains of FGFR3-IIIb. The whole complex of FGFR2-D2:Mab 2B.1.3 was superimposed closely onto the FGFR3-IIIb:R3Mab structure (**Fig. 7**), with an overall α-carbon RMSD of 1.4 Å, indicating that the re-engineering retained the same binding geometry as the original antibody R3Mab. The FGFR3:R3Mab crystal structure suggests considerable interactions between

FGFR3 D3 and the CDR H1 loop. Therefore, to investigate the involvement of D3 in binding, proteins of the D2 domains of FGFR2 and FGFR3 were prepared and their binding affinity to R3Mab and Mab 2B.1.3 measured. Only very minor differences in binding affinity between D2 alone and the D2D3 domains were observed for both receptors (**Table 8**). Thus, D2 is primarily responsible for binding of R3Mab and its derivatives, whereas D3 plays a minimal role.

Table 7. Data collection and refinement statistics of the affinity between 2B.1.3 and FGFR2-D2

Data collection	
Space group	C2
Cell dimensions	
a, *b*, *c* (Å)	76.09, 181.24, 94.43
a, b, g (°)	90.0, 113.7, 90.0
Resolution (Å)	50.0-2.36 (2.47-2.36) *
R_{sym}	0.094 (0.489)
$I / \sigma I$	14.4(1.9)
Completeness (%)	98.3(99.3)
Redundancy	2.5(2.5)
Refinement	
Resolution (Å)	50.0-2.36
No. reflections	46,583
R_{work} / R_{free}	0.198/0.243
No. atoms	
Protein	8298
Water	152
B-factors	
Protein	33.6
Water	25.1
r.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.2

*Values in parentheses are for the highest-resolution shell.

Table 8. Comparison of the binding affinities of D2 alone and D2D3 domains of FGFR2 and FGFR3 to R3Mab or Mab 2B.1.3.

K_D (nM)

	FGFR2-D2	FGFR2-IIIb (D2D3)	FGFR3-D2	FGFR3-IIIb (D2D3)
R3Mab	ND*	ND*	0.26	0.24
2B.1.3	1.0	0.71	<0.1**	0.09

* ND: not detectable at 200 nM; ** reached the fitting limit of Biacore.

The CDR H2 sequence in Mab 2B.1.3, THLGD, is completely different from the parental H2 sequence in R3Mab, IYPTN. As expected, the conformations of the CDR H2 loops in the two Mabs differ substantially (**Fig. 2C**). Upon aligning the variable domains of Mab 2B.1.3 onto those of R3Mab (**Fig. 2B**), the H3 loop also appears twisted by a few degrees, resulting in a distance of 2.6 Å between the Cα atoms of the H3 tip residue Y100b in both structures (**Fig. 2C**). Accordingly, the position of the FGFR2 D2 domain overall is shifted by ~3 Å from that of the FGFR3 D2 domain. Comparison of the interface between the variants and the FGFR antigens revealed that such reorganizations of the H2 and H3 CDR loops in Mab 2B.1.3 significantly improved packing against the FGFR2 surface. In the parental structure, the shape complementarity (sc) score between R3Mab and FGFR3-D2 is 0.731. If the D2 domain of FGFR2 is aligned onto and replaces FGFR3 D2, the sc between R3Mab and FGFR2 D2 drops to 0.685. This may explain the lack of R3Mab binding to FGFR2 (**Table 6**). However, in the new crystal structure, the sc score between 2B.1.3 and FGFR2-D2 dramatically increased to 0.768, which is consistent with the gain of high-affinity binding to FGFR2 through re-engineering of R3Mab.

Due to the remarkable similarity among FGFRs, 2B.1.3 cross-reacts with multiple homologs in the family. Although FGFR1 binding was not acquired along with FGFR2 binding, FGFR4 interaction was. Considering that FGFR4 inhibition carries an increased risk of toxicity (Pai, R., D. French, N. Ma, K. Hotzel, E. Plise, L. Salphati, K. D. Setchell, J. Ware, V. Lauriault, L. Schutt, D.

Hartley and D. Dambach (2012). "Antibody-mediated inhibition of fibroblast growth factor 19 results in increased bile acids synthesis and ileal malabsorption of bile acids in cynomolgus monkeys." Toxicol Sci **126**(2): 446-456), a second round of re-engineering was undertaken to eliminate FGFR4 binding.

Example 3. Further Re-engineering of the FGFR3 antibody was performed to remove FGFR4 Binding.

To generate a Mab 2B.1.3 derivative that binds FGFR2 and FGFR3 but not FGFR4, it antigen residues were identified that likely interacted with the antibody but differ between the various FGFRs (**Table 9**), assuming that Mab 2B.1.3 recognizes all FGFRs in an analogous mode to its interaction with FGFR2. Three phage display libraries were constructed based on the 2B.1.3 template, with random mutagenesis at selected positions on the contacted CDRs H1, H3 and L2 (**Table 10**). During engineering, we tried to focus on binding to FGFR2 instead of maintaining both FGFR2 and FGFR3, as we did in the previous engineering. Therefore, selection was undertaken with immobilized FGFR2-IIIb alone during panning. To counter-select FGFR4 binders, phage particles were incubated with excessive amount of soluble FGFR4-Fc proteins. The concentrations of FGFR4-Fc were increased up to 0.46 μM for successive rounds of selection (see Methods). Individual clones from round 4 (n = 96) were assayed by ELISA with FGFR2-IIIb and FGFR4, and ranked by the ratio of FGFR2 to FGFR4 binding-ELISA values. Six clones with the highest FGFR2/FGFR4 binding ratios were sequenced, expressed as IgG and characterized for binding to FGFR2-IIIb and FGFR4 (**Table 11**). Characterized clones from the H3/L2 libraries 2B.1.3.2, 2B.1.3.4 and 2B.1.3.6 contained mutations only in CDR H3, not CDR L2, whereas characterized clones from the H1/H3 library 2B.1.3.8, 2B.1.3.10 and 2B.1.3.12 contained mutations in both CDR H1 and H3. Although the 4 residues in H3 from L100a to D100d were fully randomized, Y100b remained unchanged, suggesting that the interaction of Y100b with FGFR2 is crucial for

binding. In addition, L100a was conservatively mutated to Thr or Ile, and V100c mostly to Asp. The H1/H3 mutants containing an additional H1 mutation of T28P displayed slightly higher affinities for FGFR2. These antibodies bind FGFR2 with K_D values of 1.4 to 6.6 nM, but showed minimal binding to FGFR4 when using concentrations as high as 1 μM for measurements, except that clone 2B.1.3.8 still retained detectable yet weak affinity for FGFR4 (**Table 11**). Residues that are the same as those in R3Mab are underlined and those residues that differ from those in R3Mab are in bold (**Table 11**). The convergence in both sequences and affinities of the 2B.1.3 variants indicated that the last rounds of phage selection had reached the limit of enrichment for binders with desired functions, i.e., diminished FGFR4 binding and retention of tight FGFR2 binding.

Table 9. Residue variations between FGFR2 and FGFR4 at the positions that make potential contacts to 2B.1.3.

Residues	155	158	162	169	205	214
FGFR2	N	K	R	A	K	I
FGFR3	R	R	K	A	K	V
FGFR4	H	R	K	G	R	V
CDR Contacts*	H3 Y100b	H3 Y100b	H3 L100a	H1 T32	L2 Y49, F53	L2 F53

*Cut-off distance for contacts is 4.5 Å.

Table 10. Library design for removing FGFR4 binding specificity from the engineered antibody 2B.1.3

CDR H1

Residue	28	29	30	31	32
Codons	ACC	TTC	ACT	AGT	ACT
Amino acid	T	F	T	S	T
Lib.H1+H3	NNK				NNK
Lib.H1+L2	NNK				NNK
Lib.H3+L2					

CDR H2

Residue	100a	100b	100c	100d
Codons	CTG	TAC	GTG	GAC
Amino acid	L	Y	V	D
Lib.H1+H3	NNK	NNK	NNK	NNK
Lib.H1+L2				
Lib.H3+L2	NNK	NNK	NNK	NNK

CDR H1

Residue	49	50	51	52	53	54	55	56
Codons	TAC	TCG	GCA	TCC	TTC	CTC	TAC	TCT
Amino acid	Y	S	A	S	F	L	Y	S
Lib.H1+H3								
Lib.H1+L2	NNK				NNK	NNK		NNK
Lib.H3+L2	NNK				NNK	NNK		NNK

N = G, A, T or C; K = G or T

Table 11. 2B.1.3 variants with minimal FGFR4 binding and maintained FGFR2 binding

Clone	CDR H1	CDR H3	FGFR2-IIIb K_D (nM)	FGFR4 K_D (nM)
2B.1.3	TFTST	LYVD	2.6	32
2B.1.3.2	TFTST	**TYDN**	6.6	>1,000
2B.1.3.4	TFTST	**IYGG**	5.8	>1,000

2B.1.3.6	TFTST	**TYDE**	5.9	>1,000
2B.1.3.8	**P**FTS**L**	**I**Y**EK**	1.4	~300
2B.1.3.10	**P**FTS**Q**	**TYDK**	2.9	>1,000
2B.1.3.12	**P**FTST	**TYDM**	3.0	>1,000

Considering that greater differential in binding to FGFR2 and FGFR4 as well as fewer mutations are preferable, Mab 2B.1.3.10 and 2B.1.3.12 were selected for further characterization. Both antibodies showed no binding to FGFR1 and retained strong binding to FGFR3 with affinities slightly weaker than 2B.1.3 (**Table 6**). Therefore, after the second-step engineering, the 2B.1.3 derivatives Mab 2B.1.3.10 and 2B.1.3.12 cross-react with FGFR2 and FGFR3, but do not recognize FGFR4.

We next checked the abilities of the R3Mab variants to block FGF ligand binding to the specific FGFRs. R3Mab blocks FGF ligand binding to both the FGFR3-IIIb and -IIIc isoforms. Owing to their different specificities for different FGFRs, the blocking spectrum of each of the new antibodies varied (**Fig. 3**). All the engineered antibodies showed blocking activities for both FGFR2 and FGFR3, while R3Mab did not inhibit FGF7 binding to FGFR2-IIIb or FGF1 binding to FGFR2-IIIc. Whereas 2B.1.3 strongly inhibited FGF19 binding to FGFR4, 2B.1.3.10 and 2B.1.3.12 did not block the latter interaction, due to substantially diminished FGFR4 affinity.

Example 4. Re-engineered Mab variants inhibit FGFR2- or FGFR3-dependent tumor-cell growth.

The newly engineered variants 2B.1.3.10 and 2B.1.3.12 display dual specificity for FGFR2 and FGFR3. To investigate their biological activities, we examined their effects on receptor-dependent signaling and proliferation in different types of tumor cells. First the new variants were assessed for inhibition of growth of FGFR2-overexpressing tumor cells *in vitro*. Both the SNU-16 gastric carcinoma and MFM-223x2.2 triple-negative breast carcinoma cell lines have amplification

of *FGFR2*, evident by increased *FGFR2* gene-copy numbers and protein over-expression (Kunii, K., L. Davis, J. Gorenstein, H. Hatch, M. Yashiro, A. Di Bacco, C. Elbi and B. Lutterbach (2008). "FGFR2-amplified gastric cancer cell lines require FGFR2 and Erbb3 signaling for growth and survival." Cancer research 68(7): 2340-2348.). In SNU-16 cells, 2B.1.3.10 and 2B.1.3.12 substantially suppressed FGF7-induced FGFR2 phosphorylation. In addition, the two 2B.1.3 variants markedly reduced phosphorylation of the downstream signaling molecules FRS2α, MAPK, PLCγ1 and AKT (**Fig. 4A**). Similarly, both variants diminished phosphorylation of FGFR2, FRS2α, MAPK and Her3 in FGF7-treated MFM-223x2.2 cells (**Fig. 8**).

Next, the ability of the dual-specific Mab 2B.1.3.10 and 2B.1.3.12 to inhibit *in vivo* FGFR2-dependent or FGFR3-dependent growth of tumor xenografts was investigated. For FGFR2-specific treatment, mice injected with the human gastric cancer cells SNU-16 were dosed with non-specific IgG control antibody and the dual-specific Mabs, 2B.1.3.10 and 2B.1.3.12. Compared with the control antibody, the dual-specific antibodies displayed about 67% and 57% of tumor growth inhibition (**Fig. 4B**). In another experiment, 2B.1.3.10 and 2B.1.3.12 also retarded the growth of MFM-223x2.2 tumor xenografts in mice (**Fig. 8**). Therefore, these two engineered antibodies showed potency in inhibiting FGFR2-dependent tumor growth. Since they retain the parental specificity for FGFR3 after engineering, inhibition of FGFR3-dependent tumor growth was investigated. As anticipated, both Mab 2B.1.3.10 and 2B.1.3.12 suppressed the growth of RT112 tumor xenografts (**Fig. 4C**). Collectively, the engineered antibodies can serve as dual agents to effectively inhibit both FGFR2- and FGFR3-dependent cancer cell growth. However, the potencies of the engineered variants in the RT112 model were reduced compared to the parental R3Mab, possibly due to modified pharmacokinetics.

The RT112 cell line expresses FGFR3 but not FGFR2. As anticipated, both Mab 2B.1.3.10 and 2B.1.3.12, which retained the parental specificity for FGFR3 after engineering,

as well as the parental antibody R3Mab, suppressed the growth of FGFR3-overexpressing RT112 tumor xenografts (**Fig. 4B**). The engineered variants 2B.1.3.10 and 2B.1.3.12 in the study, with tumor growth inhibition (TGI) values of 48% and 64%, displayed weaker potency than the parental R3Mab (TGI 82%), which could be possibly due to modified pharmacokinetics. For FGFR2-based efficacy, we turned to the SNU-16 cell line, which expresses readily detectable FGFR2 along with very low FGFR3 levels. Mice bearing SNU-16 xenografts were dosed with non-specific IgG control antibody, the parental R3Mab, or the engineered variants 2B.1.3.10 or 2B.1.3.12. The engineered variants displayed similar TGI values of 63% and 61%, respectively (**Fig. 4C**). Surprisingly, R3Mab, although not binding to FGFR2, also showed a measurable TGI of 44%. The tumor samples were then collected and analyzed for FGFR2 and FGFR3 expression (**Fig. 15**). FGFR3 was upregulated in the SNU-16 tumor xenografts *in vivo*, which may explain the observed inhibitory effect of R3Mab in this model. Regardless, the engineered variants showed significantly stronger activity as compared to R3Mab ($p<0.001$, day 31). In another experiment, 2B.1.3.10 and 2B.1.3.12 also retarded the growth of MFM-223x2.2 tumor xenografts in mice (**Fig. 8A and Fig. 8B**). Collectively, the engineered antibodies can serve as dual agents to effectively inhibit both FGFR2- and FGFR3-dependent cancer cell growth.

Example 5. FGFR2-binding R3Mab variants were generated by phage library selection.

Phagemid displaying R3Mab Fab fragment have been previously described (Qing, J., X. Du, Y. Chen, P. Chan, H. Li, P. Wu, S. Marsters, S. Stawicki, J. Tien, K. Totpal, S. Ross, S. Stinson, D. Dornan, D. French, Q. R. Wang, J. P. Stephan, Y. Wu, C. Wiesmann and A. Ashkenazi (2009). "Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice." The Journal of clinical investigation 119(5): 1216-1229.). Three consecutive stop codons were introduced to replace 3 residues in each of the

H1, H2, H3 or L2 CDR loops of R3Mab, which served as the template for constructing phage display library. Random mutations were then incorporated into each of the above CDR loops (**Table 3**) using the method of Kunkel et al. (Kunkel TA, Bebenek K, & McClary J (1991) Efficient site-directed mutagenesis using uracil-containing DNA. *Methods Enzymol* 204:125-139.). Purified library DNA was then transformed into SS320 competent cells by electroporation (BTX ECM 630) (Clackson T & Lowman HB (2004) *Phage display: A practical approach* (Oxford University Press). The transformed library cells were grown overnight in 2YT medium at 37°C to allow the propagation of phage particles (Clackson T & Lowman HB (2004) *Phage display: A practical approach* (Oxford University Press). To sort for FGFR2 binders, 2 μg/mL of His-tagged FGFR2-IIIb, was coated on the 96-well MaxiSorp plates. 1 OD of purified phage suspensions from each library was incubated separately with the immobilized antigen for the first round of panning. After brief washing with phosphate buffer saline plus 0.05% Tween 20 (PBST), bound phage particles were eluted with low pH. Collected phage from individual libraries were pooled together and propagated in XL1-Blue cells for subsequent rounds of panning. For the fourth round of panning, the washing step was extended to three 15-time washings with intervals of 30-min PBST incubations so as to enrich the tight binders. XL1-Blue cells were infected with the recovered phage particles from round 4 and plated on 2YT agar. 96 randomly picked colonies were cultured individually for phage production. The supernatants were assayed to verify FGFR2-IIIb binding by phage ELISA. Meanwhile, phagemid DNA was extracted from each clone and sequenced.

Example 6. A library was construction and FGFR2 binders that did not bind FGFR4 were selected.

The phage display libraries were constructed based on the phagemid displaying the Fab fragment of antibody 2B.1.3. Stop templates for Kunkel mutagenesis included 3 stop

codons in either the CDR H1 or H3 loops or both. Selected positions in CDR H1, H3 or L2 loops were subject to random mutagenesis (**Table 10**). Library preparation procedures were the same as described above. For selection of clones that have reduced FGFR4 binding while retaining FGFR2 specificity, in the first round, 1.5 OD of phage library was mixed with 0.5 nM FGFR4-Fc proteins. The mixture was incubated overnight at 4°C in a MaxiSorp plate that was pre-coated with 2 μg/mL FGFR2-IIIb. Bound phage particles were washed briefly, eluted and propagated for the next round of selection. In the second round, 1.5 OD of phage preparations were mixed with 10 nM FGFR4-Fc and incubated at 4°C overnight. For the third and fourth rounds, 0.5 OD of phage preparations were mixed with 460 nM FGFR4-Fc proteins, and shaken at room temperature (RT) for 20 min before being incubated with coated FGFR2-IIIb. After being incubated with FGFR2-IIIb for 30 min at RT, the MaxiSorp plates were washed 3 times with 10-min intervals of PBST incubations. Eluted phage particles were used to infect XL1-Blue cells and plated on 2YT agar. Randomly picked clones were cultured for phage ELISA assays and DNA sequencing as described above.

Example 7. Phage ELISA binding assays were performed. A 384-well MaxiSorp plate was coated overnight at 4°C with 30 μL 1 μg/mL E25 (control antibody), FGFR2-IIIb-His, FGFR2-IIIc-His or FGFR4-His in each quadrant. After blocking with 2% BSA in PBS for 1 h at RT, 30 μL of 10-fold diluted phage supernatant was added into quadrant. The plate was shaken at RT for 2 h. To detect the bound phage particles, HRP-conjugated anti-M13 monoclonal antibody (GE Healthcare) was 1:3000 diluted and incubated in the plate for 15 min. TMB peroxidase substrate was added into each well to allow color development. The reaction was stopped by the addition of 100 μL 1M phosphoric acid before the plate was read at the absorbance of 450 nM.

Example 8. Surface Plasmon Resonance (SPR) assays were performed.

The binding affinities of R3Mab variants for FGFR antigens were determined using a Biacore T100 (GE Healthcare). A saturated amount of anti-human Fc monoclonal antibody was immobilized onto a CM5 biosensor chip by following the product instructions. About 500 resonance units of R3Mab-derived antibody molecules were captured in each flow cell. FGFR antigens of various concentrations were injected at a flow rate 30 μL/min. After each binding cycle, flow cells were regenerated using 3M $MgCl_2$. Kinetic analyses were performed using the T100 evaluation software to obtain the kinetic and affinity constants.

Example 9. Protein expression, purification and structure determination.

The human FGFR2-IIIb ECD (residue 140-369) was amplified by PCR and subcloned into pET-21b(+) vector (Novagen). The protein was expressed as inclusion bodies in *E. coli* BL21(DE3)pLysS cells. The inclusion bodies were washed with 20 mM Tris pH7.5, 5% Glycerol, 1 mM EDTA and 2% Triton X-100, before being dissolved in 6 M Guanidine-HCl, 20 mM Tris pH8, 10 mM TCEP. For in vitro folding, inclusion body was rapidly diluted to 50 mg/L into the refolding buffer containing 100 mM Tris pH 8.0, 0.4 M L-arginine HCl, 2 mM EDTA, 3.7 mM cystamine and 6.6 mM cysteamine. After 72 h at 4°C, the folding mixture was concentrated and passed through a 5 mL Heparin HP column (GE Healthcare). The sample was further purified with a MonoS column and a Superdex 200 column. The 2B.1.3 Fab was expressed and purified as described (Qing, J., X. Du, Y. Chen, P. Chan, H. Li, P. Wu, S. Marsters, S. Stawicki, J. Tien, K. Totpal, S. Ross, S. Stinson, D. Dornan, D. French, Q. R. Wang, J. P. Stephan, Y. Wu, C. Wiesmann and A. Ashkenazi (2009). "Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice." The Journal of clinical investigation 119(5): 1216-1229). The FGFR2 and Fab proteins were separately dialyzed against 10 mM Tris pH 7.0, 5 mM NaCl before being mixed together at a molar ratio of 1:1. The protein mixture was diluted to 2 mg/mL for

crystallization. Crystals were grown at 20 % (w/v) PEG3350, 0.1 M sodium citrate pH 5.5, 0.2 M ammonium sulfate using vapor diffusion method. As the crystals were sensitive to cryoprotection solutions and cracked once being transferred out of the mother liquor, a diffractable crystal was eventually harvested from a tray that were left untouched for four months with the concentration of PEG3350 high enough to serve cryoprotection. Thus the crystals were directly taken out of the drop and flash frozen in liquid nitrogen. Diffraction data was collected with a beam wavelength of 1 Å at the Advanced Light Source of the Lawrence Berkeley National Laboratory. Data processing was carried out using HKL2000 and Scalepack (Otwinowski Z & Minor W (1997) Processing of X-ray diffraction data collected in oscillation mode. Methods in enzymology 276:307-326.). The structure was solved with molecular replacement using the program Phaser in the CCP4 suite (McCoy AJ, et al. (2007) Phaser crystallographic software. J Appl Crystallogr 40(Pt 4):658-674. Winn MD, et al. (2011) Overview of the CCP4 suite and current developments. Acta Crystallogr D Biol Crystallogr 67(Pt 4):235-242). The search models for Fab and FGFR2-D2 were PDB 3GRW and 3CU1, respectively. Two complexes were found in an asymmetric unit cell. Rigid body and simulated annealing refinements were conducted using Phenix (Adams PD, *et al.* (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* 66(Pt 2):213-221). Manual model building was performed with the program Coot (Emsley P, Lohkamp B, Scott WG, & Cowtan K (2010) Features and development of Coot. *Acta Crystallogr D Biol Crystallogr* 66(Pt 4):486-501). Subsequent refinements of positional and atomic displacement parameters were carried out using Phenix. Water molecules were added with a distance cutoff of 3.4 Å. The final model was validated by the program MolProbity (Chen VB, et al. (2010) MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr 66(Pt 1):12-21). Ramachandran outliers were not detected.

Example 10. FGF Ligand-Blocking ELISA.

A 96-well MaxiSorp plate was coated overnight at 4°C with 1.5 μg/mL anti-human Fc antibody (Jackson ImmunoResearch Lab). After blocking with 2% BSA in PBS for 1 h at RT, 0.25 μg/mL FGFR-Fc fusion proteins were incubated at RT for 2 h. The plate was washed 5 times before being added with the antibody and FGF ligand mixtures, which was prepared as 49 μL 100 ng/mL FGF ligand, 1 μL 25 mg/mL Heparin (Sigma-Aldrich) and 50 μL antibody dilutions. After shaking at RT for 2 h, the plate was washed 5 times. Bound ligand was detected by subsequent incubations at RT with 0.5 μg/mL biotinylated anti-FGF antibodies (R&D Biosystems) for 0.5 h, 1:2,500-diluted Streptavidin-HRP (Invitrogen) for 0.5 h and the TMB substrate until enough color development.

Example 11. Cell lines.

SNU16 and MFM-223x2.2 cell lines were obtained from an internal cell bank. The cell line RT112 was obtained from ATCC. The cells were cultured in RPMI medium supplemented with 10% FBS. All cell lines are tested for mycoplasma, cross contamination and genetically fingerprinted when new stocks are generated to ensure quality and confirm ancestry. *Cell line fingerprinting: SNP fingerprinting.* SNP genotypes are performed each time new stocks are expanded for cryopreservation. Cell line identity is verified by high-throughput SNP genotyping using Fluidigm multiplexed assays. SNPs were selected based on minor allele frequency and presence on commercial genotyping platforms. SNP profiles are compared to SNP calls from available internal and external data (when available) to determine or confirm ancestry. In cases where data is unavailable or cell line ancestry is questionable, DNA or cell lines are re-purchased to perform profiling to confirm cell line ancestry. *SNPs.* rs11746396, rs16928965, rs2172614, rs10050093, rs10828176, rs16888998, rs16999576, rs1912640, rs2355988, rs3125842, rs10018359, rs10410468, rs10834627, rs11083145, rs11100847, rs11638893, rs12537, rs1956898, rs2069492, rs10740186,

rs12486048, rs13032222, rs1635191, rs17174920, rs2590442, rs2714679, rs2928432, rs2999156, rs10461909, rs11180435, rs1784232, rs3783412, rs10885378, rs1726254, rs2391691, rs3739422, rs10108245, rs1425916, rs1325922, rs1709795, rs1934395, rs2280916, rs2563263, rs10755578, rs1529192, rs2927899, rs2848745, rs10977980. *Short Tandem Repeat (STR) Profiling.* STR profiles are determined for each line using the Promega PowerPlex 16 System. This is performed once and compared to external STR profiles of cell lines (when available) to determine cell line ancestry. *Loci analyzed.* Detection of sixteen loci (15 STR loci and Amelogenin for gender identification), including D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, AMEL, vWA, D8S1179 and TPOX.

Example 12. Immunoblotting.

Cells were seeded on tissue culture plates for 24 hours, pre-treated with 10 μg/ml FGFR blocking or control anti-gD antibody, then stimulated with 25 ng/ml FGF-7 (R&D Systems) in the presence of 20 μg/ml heparin (Sigma) for 15 minutes. Cells were placed on ice and protein immediately harvested with IP lysis buffer (Thermo Scientific). Protein lysates were passed through a syringe, cleared by centrifugation, then quantified using BCA protein assay (Thermo Scientific). Protein was separated on 4-12% Bis-Tris gels (Life Technologies), transferred to nitrocellulose membranes, blocked with 5% BSA or milk in TBST for 30 minutes, then blotted with primary antibody overnight at 4C. Antibodies used: phospho-FGFR (Y653/654), phospho-FRS2 (Y196), phospho-ERK1/2 (T202/Y204), ERK1/2, phospho-AKT (S473), AKT, phospho-HER3 (Y1289), HER3, phospho-PLCgamma1 (Y783), PLCgamma1 (Cell Signaling); FGFR2, FRS2 (Santa Cruz Biotechnology); beta-actin (Sigma). Membranes were washed and incubated with appropriate HRP conjugated secondary antibodies for 1 hour, then washed and detected with SuperSignal

West Femto Chemiluminescent Substrate (Thermo Scientific). Luminescence signal was acquired with FluorChem Q (Alpha Innotech).

Example 13. Xenograft Experiments.

All procedures were approved by and conformed to the guidelines and principles set by the Institutional Animal Care and Use Committee of Genentech and were carried out in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility. 0.36 mg estrogen pellets were implanted subcutaneously (s.c.) 1 day prior to cell inoculation. 10 million MFM-223 x2.2 breast cancer cells suspended in HBSS with matrigel were inoculated in the mammary fat pad #4 of 6-8-week-old female NCR nude mice (Taconic™). SNU-16 tumor fragments of about 15-30 mm^3 were implanted s.c. into right flanks of 6-8-week-old female Balb/c nude mice (Shanghai Laboratory Animal). 7 million RT-112 bladder carcinoma cells suspended in HBSS with matrigel were inoculated s.c. in the 6-8-week-old female C.B-17 SCID mice (Charles River Lab). When the mean tumor volume reached 100-200 mm^3 (day 0), mice were randomized into groups of 6 (SNU-16, RT112) or 7 (MFM-223 x2.2) and were treated starting on day 1 with twice weekly intraperitoneal (i.p.) injections of 2B1.3.10 or 2B1.3.12 (10, 30 or 50 mg/kg). Control groups were treated with a control human IgG1 antibody diluted in PBS (30 mg/kg). Tumor volumes were measured in two dimensions (length and width) using Ultra Cal IV calipers (Model 54 10 111, Fred V. Fowler Company). The tumor volume was calculated using the following formula: Tumor volume (mm^3) = (length × $width^2$) × 0.5. Animal body weights were measured using an Adventurer Pro AV812 scale (Ohaus). Percent body weight change was calculated using the following formula: Body weight change (%) = [(WeightDay new - WeightDay 0)/WeightDay 0] x 100%. Percent body weight was tracked for each animal during the study and percent body weight change for each group was calculated and plotted.

Example 14. Identification FGFR2/3 + KLB bispecific antibodies

Along with the anti-tumor activity of the anti-FGFR2/3 antibodies described here, bispecific antibodies directed to FGFR2/3 and KLB ("FGFR2/3 + KLB bispecific antibodies") can be made for use in treating proliferative disorders and diseases associated with FGFR2 and/or FGFR3 expression and more specifically for metabolic diseases. Metabolic diseases that may be treated by FGFR2/3 + KLB bispecific antibodies include but are not limited to: polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), obesity, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, hypertension, type 2 diabetes, non-type 2 diabetes, type 1 diabetes, latent autoimmune diabetes (LAD), maturity onset diabetes of the young (MODY), type 2 diabetes, obesity, Bardet-Biedl syndrome, Prader-Willi syndrome, Alstrom syndrome, Cohen syndrome, Albright's hereditary osteodystrophy (pseudohypoparathyroidism), Carpenter syndrome, MOMO syndrome, Rubinstein-Taybi syndrome, fragile X syndrome and Börjeson-Forssman-Lehman syndrome. More specifically, the FGFR2/3 + KLB bispecific antibodies may be used for the treatment of NASH.

Initially, experiments were performed to compare the activity of the anti-FGFR2/3 antibody variants (**Figs. 17A** and **17B**). Specifically, 239T FGFR1 deletion cells were seeded at a density of 0.9 x 10^6 in 96 well plates on Day 1. On Day 2, the cells were transfected with constructs including FGFR, FF Luciferase, Renilla Luciferase (transfection efficiency control), and Elk1. On Day 3, the cells were stimulated in serum-free media with anti-FGFR2/3 antibody variants 2B1.3, 2B1.3.12, 2B1.1.2, 2B1.1.4, 2B1.1.6, 2B1.1.8, 2B1.1.10, 2B1.1, and 2B1.1.12. Initial concentrations were 10μg/mL and a series of dilutions were performed 1/5. Reactions were carried out for 7.5 hrs and were stopped by removing media from the plates and addint 1x Passive Lysis buffer. The plates were then analyzed using a

Wallace Envision plate reader after adding Luciferase substrate and normalized to Renilla expression.

Based in part on the Luciferase assay in addition to other assays performed but not described herein, an anti-FGFR2/3 antibody variant decision matrix was assembled (**Fig. 18**). 2B1.3 was shown to block growth in MCF-7/FGF7 assay and showed FGF19 blocking. 2B1.3.12 blocked tumor progression.

Based on the decision matrix, variants 2B1.3.12, 2B1.1.6, and 2B1.1 were further examined (**Figs. 19A-19C**) and the activity of each was tested. Furthermore, FGFR binding was examined for 2B1.1, 2B1.3, and 2B1.3.12 (**Table 12**). In **Table 12**, NB refers to no binding and ND refers to not determined. 2B.1.1 for FGFR4 was measured by capturing IgG and flowing the FGFR4-6xHis (experiment identified with a * in **Table 12**). Later, the KD for the 2B.1.3 variants were determined by capturing FGFR4-Fc and flowing the antibody Fab fragments (**Table 12**).

Table 12: Binding affinities of R3Mab variants for human FGFR.

Clone	FGFR1-IIIb, IIIc	FGFR2-IIIb	FGFR2-IIIc	FGFR3-IIIb	FGFR3-IIIc	FGFR4
R3Mab	NB	NB	NB	0.24	0.61	NB
2B.1.1	NB	0.29	2.8	ND	ND	2.8*
2B.1.3	NB	2.6	2.0	0.09	0.07	32
2B.1.3.12	NB	3.0	6.1	0.50	0.72	>1,000

Thereafter, seven 2B1.1 variants were expressed and agonist activity for FGFR2, FGFR3, and FGFR4 binding was tested (**Figs.16A-16C**). All 2B1.1 variants showed sub-nM to low-pM affinity ranges to FGFR3 using the Biacore assay. Due to FGFR4 protein stickiness, the binding affinity is best determined by Biacore with Fabs as the analyte. Most of the variants showed weak binding to FGFR4 by ELISA except for 2B1.1 and 2B1.1.4.

Based on the experiments described in this Example 14, variants 2B1.3.12 and 2B1.1.6 were selected for bispecific assembly with an anti-KLB antibody.

Example 14. Generation of FGFR2/3 + KLB bispecific antibodies

FGFR2/3 + KLB bispecific antibodies can be made using any bispecific antibody production method. In specific exmples, FGFR2/3 + KLB bispecific antibodies of this invention can be made using the the knob and hole technique.

HEK293 cells can be co-transfected with a mixture of four expression vectors encoding the heavy and light chains of anti-FGFR2/3 antibody variant 2B1.3.12 or 2B1.1.6 and the heavy and light chains of one of the anti-KLB antibodies described herein (*see e.g.,* **Tables 13** and **14**).

Table 13. CDR H sequences for murine anti-KLB monoclonal antibodies.

Antibody	CDR H1	CDR H2	CDR H3
11F1	SYGIS (SEQ ID NO: 108)	TVSSGGRYTYYPDSVKG (SEQ ID NO: 138)	GGDGYALDY (SEQ ID NO: 154)
6D12	DYYMN (SEQ ID NO: 109)	WIDPENDDTIYDPKFQG (SEQ ID NO: 139)	FTTVFAY (SEQ ID NO: 155)
11D4	NYGVS (SEQ ID NO: 110)	VIWGDGSINYHSALIS (SEQ ID NO: 140)	THDWFDY (SEQ ID NO: 156)
8E1	DTYMN (SEQ ID NO: 111)	RIDPSNGNAKYDPKFQG (SEQ ID NO: 141)	RALGNGYALGY (SEQ ID NO: 157)
46C3	DTYIH (SEQ ID NO: 112)	RIDPANGNTKYDPKFQD (SEQ ID NO: 142)	GTSYSWFAY (SEQ ID NO: 158)
8H7	SYWIH (SEQ ID NO: 113)	EIDPSVSNSNYNQKFKG (SEQ ID NO: 143)	LGVMVYGSSPFWFAY (SEQ ID NO: 159)
21H3	SYWIH (SEQ ID NO: 113)	EIDPSVSNSNYNQKFKG (SEQ ID NO: 143)	LGVMVYGSSPFWFAY (SEQ ID NO: 159)
25F7	DTFTH (SEQ ID NO: 114)	RIDPSNGNTKYDPKFQG (SEQ ID NO: 144)	RALGNGYAMDY (SEQ ID NO: 160)
14E6	EYTMN (SEQ ID NO: 115)	GINPNNGETSYNQKFKG (SEQ ID NO: 145)	KTTNY (SEQ ID NO: 161)
14C6	SYWIE (SEQ ID NO: 116)	EIFPGGGSTIYNENFRD (SEQ ID NO: 146)	RGYYDAAWFDY (SEQ ID NO: 162)
24A1	DYEMH (SEQ ID NO: 117)	AIWPENADSVYNQKFKG (SEQ ID NO: 147)	EGGNY (SEQ ID NO: 163)
5F8	DTYIH (SEQ ID NO: 118)	RIDPANGNTKYDPKFQG (SEQ ID NO: 148)	SGNYGAMDY (SEQ ID NO: 164)
6C1	SYWIE (SEQ ID NO: 116)	EILPGSDSTKYVEKFKV (SEQ ID NO: 149)	GGYHYPGWLVY (SEQ ID NO: 165)
12A11	RYWMS (SEQ ID NO: 119)	EISPDSSTINYTPSLKD (SEQ ID NO: 150)	PSPALDY (SEQ ID NO: 166)

12B8	NYGMN (SEQ ID NO: 120)	WIDTDTGEATYTDDFKG (SEQ ID NO: 151)	EEYGLFGFPY (SEQ ID NO: 167)
14C10	TSAMGIG (SEQ ID NO: 121)	HIWWDDDKRYNPALKS (SEQ ID NO: 152)	IDGIYDGSFYAMDY (SEQ ID NO: 168)
8C5	TYGVH (SEQ ID NO: 122)	VIWSGGSTDYNAAFIS (SEQ ID NO: 153)	DYGSTYVDAIDY (SEQ ID NO: 169)

Table 14. CDR L sequences for murine anti-KLB monoclonal antibodies.

Antibody	CDR L1	CDR L2	CDR L3
11F1	SASQVISNYLN (SEQ ID NO: 170)	FTSSLRS (SEQ ID NO: 185)	QQYSKLPWT (SEQ ID NO: 201)
6D12	SASSSGRYTF (SEQ ID NO: 171)	DTSKLAS (SEQ ID NO: 186)	FQGTGYPLT (SEQ ID NO: 202)
11D4	RASQDISNYFN (SEQ ID NO: 172)	YTSRLQS (SEQ ID NO: 187)	HQVRTLPWT (SEQ ID NO: 203)
8E1	KASDHINNWLA (SEQ ID NO: 173)	GTTNLET (SEQ ID NO: 188)	QQYWNTPFT (SEQ ID NO: 204)
46C3	RSSQNIVHSDGNTYLE (SEQ ID NO: 174)	KVSNRFS (SEQ ID NO: 189)	FQGSHVLT (SEQ ID NO: 205)
8H7	KASQFVSDAVA (SEQ ID NO: 175)	SASYRYT (SEQ ID NO: 190)	QQHYIVPYT (SEQ ID NO: 206)
21H3	KASQFVSDAVA (SEQ ID NO: 175)	SASYRYT (SEQ ID NO: 190)	QQHYIVPYT (SEQ ID NO: 206)
25F7	KASDHINNWLA (SEQ ID NO: 173)	GASNLET (SEQ ID NO: 191)	QQYWNTPFT (SEQ ID NO: 204)
14E6	RASQEISGYLS (SEQ ID NO: 176)	AASTLDS (SEQ ID NO: 192)	LQYGSYPWT (SEQ ID NO: 207)
14C6	SASSSLSSSYLY (SEQ ID NO: 177)	GASNLAS (SEQ ID NO: 193)	HQWSSYPLT (SEQ ID NO: 208)
24A1	KSSQSLLNSGNQKNSLA (SEQ ID NO: 178)	LASTRES (SEQ ID NO: 194)	QQHHSTPYT (SEQ ID NO: 209)
5F8	RASSSVNHMY (SEQ ID NO: 179)	YTSTLAP (SEQ ID NO: 195)	QQFTISPSMYT (SEQ ID NO: 210)
6C1	KASQNVDSYVA (SEQ ID NO: 180)	SASYRFS (SEQ ID NO: 196)	QQYNISPYT (SEQ ID NO: 211)
12A11	RASQSISDYVY (SEQ ID NO: 181)	YASQSIS (SEQ ID NO: 197)	QNGHNFPYT (SEQ ID NO: 212)
12B8	KASEDIYNRLA (SEQ ID NO: 182)	AATSLET (SEQ ID NO: 198)	QQYWSNPLT (SEQ ID NO: 213)
14C10	RASESVDSYGNSFMH (SEQ ID NO: 183)	RASNLES (SEQ ID NO: 199)	QQSNEDYT (SEQ ID NO: 214)
8C5	RASESVESYGNRYMT (SEQ ID NO: 184)	RAANLQS (SEQ ID NO: 200)	QQSNEDPWT (SEQ ID NO: 215)

The heavy chain of anti-FGFR2/3 and anti-KLB can be respectively tagged with the Flag peptide and Oct-Histidine so that heterodimeric IgG can be purified by sequential affinity purification from conditioned medium. Partially purified heterodimeric IgG can then be analyzed in a GAL-ELK1 based luciferase assay to identify KLB-dependent agonists. To

minimize mispairing of heavy and light chains, anti-FGFR2/3 can be expressed with human Fab constant region, and anti-KLB can be expressed with mouse Fab constant region. The tagged-bispecific IgGs can then be initially tested in a crude form using combinations of one arm from either FGFR2/3 antibody variant 2B1.3.12 or 2B1.1.6 and one arm from any of the KLB antibodies described herein. Specifically, the the anti-KLB antibody from which the KLB arm originates may comprise:

8C5.K4.M4L.H3.KNV Heavy Chain Variable Region

EVQLVESGGGLVQPGGSLRLSCAASDFSLTTYGVHWVRQAPGKGLEWLGVI WSGGSTDYNAAFISRLTISKDNSKNTVYLQMNSLRAEDTAVYYCARDYGSTYVDAI DYWGQGTLVTVSS (SEQ ID NO: 104)

8C5.K4.M4L.H3.KNV Full Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASDFSLTTYGVHWVRQAPGKGLEWLGVI WSGGSTDYNAAFISRLTISKDNSKNTVYLQMNSLRAEDTAVYYCARDYGSTYVDAI DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK (SEQ ID NO: 106)

8C5.K4.M4L.H3.KNV Light Chain Variable Region

DIVLTQSPDSLAVSLGERATINCRASESVESYGNRYMTWYQQKPGQPPKLLIY RAANLQSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSNEDPWTFGQGTKVEI K (SEQ ID NO: 105)

8C5.K4.M4L.H3.KNV Full Light Chain

DIVLTQSPDSLAVSLGERATINCRASESVESYGNRYMTWYQQKPGQPPKLLIY RAANLQSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSNEDPWTFGQGTKVEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 107)

Furthermore, bispecific antibodies can be produced with human IgG1 constant region (wild-type, with effector function) and with human IgG1 constant region with N297G

mutation to eliminate the effector function, or mouse constant region with dual [D265G/N297G] mutations (DANG) to eliminate effector function.

Example 15: Testing of Bispecific antibodies.

Various bispecific antibody combinations of 8C5.K4H3.M4L.KNV (*see* Example 14 above) and different anti-FGFR2/3 arms can be made and tested in the GAL-ELK1-based luciferase assay in HEK293 cells with or without KLB. Each bispecific antibody combination can induced luciferase activity in a dose-dependent manner in cells expressing recombinant FGFR2 or 3 and KLB, but not in cells without KLB expression. This data can confirm that the FGFR2/3 +KLB bispecifics retain the advantages of the parent antibodies, *e.g.*, 2B1.3.12 or 2B1.1.6. Furthermore, the binding affinity of an FGFR2/3 +KLB bispecific antibody that has a humanized 8C5 arm (8C5.K4.M4L.H3.KNV) and an arm of either the 2B1.3.12 or 2B1.1.6 variant can be determined for KLB binding, FGFR2 binding, and FGFR3 binding.

In addition to the various embodiments depicted and claimed, the disclosed subject matter is also directed to other embodiments having other combinations of the features disclosed and claimed herein. As such, the particular features presented herein can be combined with each other in other manners within the scope of the disclosed subject matter such that the disclosed subject matter includes any suitable combination of the features disclosed herein. The foregoing description of specific embodiments of the disclosed subject matter has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the disclosed subject matter to those embodiments disclosed.

It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions and methods of the disclosed subject matter without departing from the spirit or scope of the disclosed subject matter. Thus, it is intended that the

disclosed subject matter include modifications and variations that are within the scope of the appended claims and their equivalents.

Various publications, patents and patent applications are cited herein, the contents of which are hereby incorporated by reference in their entireties.

SEQUENCES

SEQ ID NO:1
2B.1.3.10 HVR-L1
RASQDVDTSLA

SEQ ID NO:2
2B.1.3.10 HVR-L2
SASFLYS

SEQ ID NO:3
2B.1.3.10 HVR-L3
QQSTGHPQT

SEQ ID NO:4
2B.1.3.10 HVR-H1
GFPFTSQGIS

SEQ ID NO:5
2B.1.3.10 HVR-H2
RTHLGDGSTNYADSVKG

SEQ ID NO:6
2B.1.3.10 HVR-H3
ARTYGIYDTYDKYTEYVMDY

SEQ ID NO:7
2B.1.3.12 HVR-L1
RASQDVDTSLA

SEQ ID NO:8
2B.1.3.12 HVR-L2
SASFLYS

SEQ ID NO:9
2B.1.3.12 HVR-L3
QQSTGHPQT

SEQ ID NO:10

2B.1.3.12 HVR-H1
GFPFTSTGIS

SEQ ID NO:11
2B.1.3.12 HVR-H2
RTHLGDGSTNYADSVKG

SEQ ID NO:12
2B.1.3.12 HVR-H3
ARTYGIYDTYDMYTEYVMDY

SEQ ID NO:13
2B.1.1 HVR-H2
YWAWD

SEQ ID NO:14
2B.1.88 HVR-H2
IWMFT

SEQ ID NO:15
2B.1.38 HVR-H2
FWAYD

SEQ ID NO:16
2B.1.20 HVR-H2
LDVFW

SEQ ID NO:17
2B.1.32 HVR-H2
WVGFT

SEQ ID NO:18
2B.1.49 HVR-H2
LSFFS

SEQ ID NO:19
2B.1.86 HVR-H2
LSFWT

SEQ ID NO:20
2B.1.9 HVR-H2
YHPYL

SEQ ID NO:21
2B.1.73 HVR-H2
MIFYN

SEQ ID NO:22
2B.1.74 HVR-H2
YHPFR

SEQ ID NO:23
2B.1.14 HVR-H2
LWYFD

SEQ ID NO:24
2B.1.71 HVR-H2
VWMFD

SEQ ID NO:25
2B.1.28 HVR-H2
FWAWS

SEQ ID NO:26
2B.1.95 HVR-H2
LIFFT

SEQ ID NO:27
2B.1.50 HVR-H2
LNFYS

SEQ ID NO:28
2B.1.81 HVR-H2
VNNFY

SEQ ID NO:29
2B.1.25 HVR-H2
WHPWM

SEQ ID NO:30
2B.1.3 HVR-H2
THLGD

SEQ ID NO:31
2B.1.65 HVR-H2
YNAYT

SEQ ID NO:32
2B.1.94 HVR-H2
LVFFS

SEQ ID NO:33
2B.1.78 HVR-H2
LSFYS

SEQ ID NO:34
2B.1.72 HVR-H2
VHPFE

SEQ ID NO:35
2B.1.44 HVR-H2
WWSWG

SEQ ID NO:36
2B.1.52 HVR-H2
FSLGD

SEQ ID NO:37
2B.1.30 HVR-H2
VSFFS

SEQ ID NO:38
2B.1.82 HVR-H2
INFFS

SEQ ID NO:39
2B.1.93 HVR-H2
IDNYW

SEQ ID NO:40
2B.1.55 HVR-H2
VDVFW

SEQ ID NO:41
2B.1.35 HVR-H2
WHPFR

SEQ ID NO:42
2B.1.33 HVR-H2
YHPFH

SEQ ID NO:43
2B.1.80 HVR-H2
YWAFS

SEQ ID NO:44
2B.1.92 HVR-H2
WVAFS

SEQ ID NO:45
2B.1.3 HVR-H2
THLGD

SEQ ID NO:46
2B.1.95 HVR-H2
LIFFT

SEQ ID NO:47
2B.1.73 HVR-H2

MIFYN

SEQ ID NO:48
2B.1.32 HVR-H2
WVGFT

SEQ ID NO:49
2B.1.88 HVR-H2
IWMFT

SEQ ID NO:50
2B.1.1 HVR-H2
YWAWD

SEQ ID NO:51
FGFR2-IIIb nucleic acid sequence
ATGGTCAGCTGGGGTCGTTTCATCTGCCTGGTCGTGGTCACCATGGCAACCTTGT
CCCTGGCCCGGCCCTCCTTCAGTTTAGTTGAGGATACCACATTAGAGCCAGAAGA
GCCACCAACCAAATACCAAATCTCTCAACCAGAAGTGTACGTGGCTGCGCCAGG
GGAGTCGCTAGAGGTGCGCTGCCTGTTGAAAGATGCCGCCGTGATCAGTTGGACT
AAGGATGGGGTGCACTTGGGGCCCAACAATAGGACAGTGCTTATTGGGGAGTAC
TTGCAGATAAAGGGCGCCACGCCTAGAGACTCCGGCCTCTATGCTTGTACTGCCA
GTAGGACTGTAGACAGTGAAACTTGGTACTTCATGGTGAATGTCACAGATGCCAT
CTCATCCGGAGATGATGAGGATGACACCGATGGTGCGGAAGATTTTGTCAGTGA
GAACAGTAACAACAAGAGAGCACCATACTGGACCAACACAGAAAAGATGGAAA
AGCGGCTCCATGCTGTGCCTGCGGCCAACACTGTCAAGTTTCGCTGCCCAGCCGG
GGGGAACCCAATGCCAACCATGCGGTGGCTGAAAAACGGGAAGGAGTTTAAGCA
GGAGCATCGCATTGGAGGCTACAAGGTACGAAACCAGCACTGGAGCCTCATTAT
GGAAAGTGTGGTCCCATCTGACAAGGGAAATTATACCTGTGTAGTGGAGAATGA
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CGGCCCATCCTCCAAGCCGGACTGCCGGCAAATGCCTCCACAGTGGTCGGAGGA
GACGTAGAGTTTGTCTGCAAGGTTTACAGTGATGCCCAGCCCCACATCCAGTGGA
TCAAGCACGTGGAAAAGAACGGCAGTAAATACGGGCCCGACGGGCTGCCCTACC
TCAAGGTTCTCAAGCACTCGGGGATAAATAGTTCCAATGCAGAAGTGCTGGCTCT
GTTCAATGTGACCGAGGCGGATGCTGGGGAATATATATGTAAGGTCTCCAATTAT
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CCTGGAAGAGAAAAGGAGATTACAGCTTCCCCAGACTACCTGGAGATAGCCATT
TACTGCATAGGGGTCTTCTTAATCGCCTGTATGGTGGTAACAGTCATCCTGTGCC
GAATGAAGAACACGACCAAGAAGCCAGACTTCAGCAGCCAGCCGGCTGTGCACA
AGCTGACCAAACGTATCCCCCTGCGGAGACAGGTAACAGTTTCGGCTGAGTCCA
GCTCCTCCATGAACTCCAACACCCCGCTGGTGAGGATAACAACACGCCTCTCTTC
AACGGCAGACACCCCCATGCTGGCAGGGGTCTCCGAGTATGAACTTCCAGAGGA
CCCAAAATGGGAGTTTCCAAGAGATAAGCTGACACTGGGCAAGCCCCTGGGAGA
AGGTTGCTTTGGGCAAGTGGTCATGGCGGAAGCAGTGGGAATTGACAAAGACAA
GCCCAAGGAGGCGGTCACCGTGGCCGTGAAGATGTTGAAAGATGATGCCACAGA
GAAAGACCTTTCTGATCTGGTGTCAGAGATGGAGATGATGAAGATGATTGGGAA
ACACAAGAATATCATAAATCTTCTTGGAGCCTGCACACAGGATGGGCCTCTCTAT
GTCATAGTTGAGTATGCCTCTAAAGGCAACCTCCGAGAATACCTCCGAGCCCGGA
GGCCACCCGGGATGGAGTACTCCTATGACATTAACCGTGTTCCTGAGGAGCAGAT
GACCTTCAAGGACTTGGTGTCATGCACCTACCAGCTGGCCAGAGGCATGGAGTA

CTTGGCTTCCCAAAAATGTATTCATCGAGATTTAGCAGCCAGAAATGTTTTGGTA
ACAGAAAACAATGTGATGAAAATAGCAGACTTTGGACTCGCCAGAGATATCAAC
AATATAGACTATTACAAAAAGACCACCAATGGGCGGCTTCCAGTCAAGTGGATG
GCTCCAGAAGCCCTGTTTGATAGAGTATACACTCATCAGAGTGATGTCTGGTCCT
TCGGGGTGTTAATGTGGGAGATCTTCACTTTAGGGGGCTCGCCCTACCCAGGGAT
TCCCGTGGAGGAACTTTTTAAGCTGCTGAAGGAAGGACACAGAATGGATAAGCC
AGCCAACTGCACCAACGAACTGTACATGATGATGAGGGACTGTTGGCATGCAGT
GCCCTCCCAGAGACCAACGTTCAAGCAGTTGGTAGAAGACTTGGATCGAATTCTC
ACTCTCACAACCAATGAGGAATACTTGGACCTCAGCCAACCTCTCGAACAGTATT
CACCTAGTTACCCTGACACAAGAAGTTCTTGTTCTTCAGGAGATGATTCTGTTTTT
TCTCCAGACCCCATGCCTTACGAACCATGCCTTCCTCAGTATCCACACATAAACG
GCAGTGTTAAAACATGA

SEQ ID NO:52
FGFR2-IIIb amino acid sequence
MVSWGRFICLVVVTMATLSLARPSFSLVEDTTLEPEEPPTKYQISQPEVYVAAPGESL
EVRCLLKDAAVISWTKDGVHLGPNNRTVLIGEYLQIKGATPRDSGLYACTASRTVDS
ETWYFMVNVTDAISSGDDEDDTDGAEDFVSENSNNKRAPYWTNTEKMEKRLHAVP
AANTVKFRCPAGGNPMPTMRWLKNGKEFKQEHRIGGYKVRNQHWSLIMESVVPSD
KGNYTCVVENEYGSINHTYHLDVVERSPHRPILQAGLPANASTVVGGDVEFVCKVY
SDAQPHIQWIKHVEKNGSKYGPDGLPYLKVLKHSGINSSNAEVLALFNVTEADAGEY
ICKVSNYIGQANQSAWLTVLPKQQAPGREKEITASPDYLEIAIYCIGVFLIACMVVTVI
LCRMKNTTKKPDFSSQPAVHKLTKRIPLRRQVTVSAESSSSMNSNTPLVRITTRLSST
ADTPMLAGVSEYELPEDPKWEFPRDKLTLGKPLGEGCFGQVVMAEAVGIDKDKPKE
AVTVAVKMLKDDATEKDLSDLVSEMEMMKMIGKHKNIINLLGACTQDGPLYVIVE
YASKGNLREYLRARRPPGMEYSYDINRVPEEQMTFKDLVSCTYQLARGMEYLASQK
CIHRDLAARNVLVTENNVMKIADFGLARDINNIDYYKKTTNGRLPVKWMAPEALFD
RVYTHQSDVWSFGVLMWEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTNELY
MMMRDCWHAVPSQRPTFKQLVEDLDRILTLTTNEEYLDLSQPLEQYSPSYPDTRSSC
SSGDDSVFSPDPMPYEPCLPQYPHINGSVKT

SEQ ID NO:53
FGFR2-IIIc nucleic acid sequence
ATGGTCAGCTGGGGTCGTTTCATCTGCCTGGTCGTGGTCACCATGGCAACCTTGT
CCCTGGCCCGGCCCTCCTTCAGTTTAGTTGAGGATACCACATTAGAGCCAGAAGA
GCCACCAACCAAATACCAAATCTCTCAACCAGAAGTGTACGTGGCTGCGCCAGG
GGAGTCGCTAGAGGTGCGCTGCCTGTTGAAAGATGCCGCCGTGATCAGTTGGACT
AAGGATGGGGTGCACTTGGGGCCCAACAATAGGACAGTGCTTATTGGGGAGTAC
TTGCAGATAAAGGGCGCCACGCCTAGAGACTCCGGCCTCTATGCTTGTACTGCCA
GTAGGACTGTAGACAGTGAAACTTGGTACTTCATGGTGAATGTCACAGATGCCAT
CTCATCCGGAGATGATGAGGATGACACCGATGGTGCGGAAGATTTTGTCAGTGA
GAACAGTAACAACAAGAGAGCACCATACTGGACCAACACAGAAAAGATGGAAA
AGCGGCTCCATGCTGTGCCTGCGGCCAACACTGTCAAGTTTCGCTGCCCAGCCGG
GGGGAACCCAATGCCAACCATGCGGTGGCTGAAAAACGGGAAGGAGTTTAAGCA
GGAGCATCGCATTGGAGGCTACAAGGTACGAAACCAGCACTGGAGCCTCATTAT
GGAAAGTGTGGTCCCATCTGACAAGGGAAATTATACCTGTGTAGTGGAGAATGA
ATACGGGTCCATCAATCACACGTACCACCTGGATGTTGTGGAGCGATCGCCTCAC
CGGCCCATCCTCCAAGCCGGACTGCCGGCAAATGCCTCCACAGTGGTCGGAGGA
GACGTAGAGTTTGTCTGCAAGGTTTACAGTGATGCCCAGCCCCACATCCAGTGGA
TCAAGCACGTGGAAAAGAACGGCAGTAAATACGGGCCCGACGGGCTGCCCTACC

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TCTATATTCGGAATGTAACTTTTGAGGACGCTGGGGAATATACGTGCTTGGCGGG
TAATTCTATTGGGATATCCTTTCACTCTGCATGGTTGACAGTTCTGCCAGCGCCTG
GAAGAGAAAAGGAGATTACAGCTTCCCCAGACTACCTGGAGATAGCCATTTACT
GCATAGGGGTCTTCTTAATCGCCTGTATGGTGGTAACAGTCATCCTGTGCCGAAT
GAAGAACACGACCAAGAAGCCAGACTTCAGCAGCCAGCCGGCTGTGCACAAGCT
GACCAAACGTATCCCCCTGCGGAGACAGGTAACAGTTTCGGCTGAGTCCAGCTC
CTCCATGAACTCCAACACCCCGCTGGTGAGGATAACAACACGCCTCTCTTCAACG
GCAGACACCCCCATGCTGGCAGGGGTCTCCGAGTATGAACTTCCAGAGGACCCA
AAATGGGAGTTTCCAAGAGATAAGCTGACACTGGGCAAGCCCCTGGGAGAAGGT
TGCTTTGGGCAAGTGGTCATGGCGGAAGCAGTGGGAATTGACAAAGACAAGCCC
AAGGAGGCGGTCACCGTGGCCGTGAAGATGTTGAAAGATGATGCCACAGAGAAA
GACCTTTCTGATCTGGTGTCAGAGATGGAGATGATGAAGATGATTGGGAAACAC
AAGAATATCATAAATCTTCTTGGAGCCTGCACACAGGATGGGCCTCTCTATGTCA
TAGTTGAGTATGCCTCTAAAGGCAACCTCCGAGAATACCTCCGAGCCCGGAGGC
CACCCGGGATGGAGTACTCCTATGACATTAACCGTGTTCCTGAGGAGCAGATGAC
CTTCAAGGACTTGGTGTCATGCACCTACCAGCTGGCCAGAGGCATGGAGTACTTG
GCTTCCCAAAAATGTATTCATCGAGATTTAGCAGCCAGAAATGTTTTGGTAACAG
AAAACAATGTGATGAAAATAGCAGACTTTGGACTCGCCAGAGATATCAACAATA
TAGACTATTACAAAAAGACCACCAATGGGCGGCTTCCAGTCAAGTGGATGGCTC
CAGAAGCCCTGTTTGATAGAGTATACACTCATCAGAGTGATGTCTGGTCCTTCGG
GGTGTTAATGTGGGAGATCTTCACTTTAGGGGGCTCGCCCTACCCAGGGATTCCC
GTGGAGGAACTTTTTAAGCTGCTGAAGGAAGGACACAGAATGGATAAGCCAGCC
AACTGCACCAACGAACTGTACATGATGATGAGGGACTGTTGGCATGCAGTGCCC
TCCCAGAGACCAACGTTCAAGCAGTTGGTAGAAGACTTGGATCGAATTCTCACTC
TCACAACCAATGAGGAATACTTGGACCTCAGCCAACCTCTCGAACAGTATTCACC
TAGTTACCCTGACACAAGAAGTTCTTGTTCTTCAGGAGATGATTCTGTTTTTTCTC
CAGACCCCATGCCTTACGAACCATGCCTTCCTCAGTATCCACACATAAACGGCAG
TGTTAAAACATGA

SEQ ID NO:54
FGFR2-IIIc amino acid sequence
MVSWGRFICLVVVTMATLSLARPSFSLVEDTTLEPEEPPTKYQISQPEVYVAAPGESL
EVRCLLKDAAVISWTKDGVHLGPNNRTVLIGEYLQIKGATPRDSGLYACTASRTVDS
ETWYFMVNVTDAISSGDDEDDTDGAEDFVSENSNNKRAPYWTNTEKMEKRLHAVP
AANTVKFRCPAGGNPMPTMRWLKNGKEFKQEHRIGGYKVRNQHWSLIMESVVPSD
KGNYTCVVENEYGSINHTYHLDVVERSPHRPILQAGLPANASTVVGGDVEFVCKVY
SDAQPHIQWIKHVEKNGSKYGPDGLPYLKVLKAAGVNTTDKEIEVLYIRNVTFEDAG
EYTCLAGNSIGISFHSAWLTVLPAPGREKEITASPDYLEIAIYCIGVFLIACMVVTVILC
RMKNTTKKPDFSSQPAVHKLTKRIPLRRQVTVSAESSSSMNSNTPLVRITTRLSSTAD
TPMLAGVSEYELPEDPKWEFPRDKLTLGKPLGEGCFGQVVMAEAVGIDKDKPKEAV
TVAVKMLKDDATEKDLSDLVSEMEMMKMIGKHKNIINLLGACTQDGPLYVIVEYAS
KGNLREYLRARRPPGMEYSYDINRVPEEQMTFKDLVSCTYQLARGMEYLASQKCIH
RDLAARNVLVTENNVMKIADFGLARDINNIDYYKKTTNGRLPVKWMAPEALFDRV
YTHQSDVWSFGVLMWEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTNELYMM
MRDCWHAVPSQRPTFKQLVEDLDRILTLTTNEEYLDLSQPLEQYSPSYPDTRSSCSSG
DDSVFSPDPMPYEPCLPQYPHINGSVKT

SEQ ID NO:55
FGFR3-IIIb nucleic acid sequence

ATGGGCGCCCCTGCCTGCGCCCTCGCGCTCTGCGTGGCCGTGGCCATCGTGGCCG
GCGCCTCCTCGGAGTCCTTGGGGACGGAGCAGCGCGTCGTGGGGCGAGCGGCAG
AAGTCCCGGGCCCAGAGCCCGGCCAGCAGGAGCAGTTGGTCTTCGGCAGCGGGG
ATGCTGTGGAGCTGAGCTGTCCCCCGCCCGGGGGTGGTCCCATGGGGCCCACTGT
CTGGGTCAAGGATGGCACAGGGCTGGTGCCCTCGGAGCGTGTCCTGGTGGGGCC
CCAGCGGCTGCAGGTGCTGAATGCCTCCCACGAGGACTCCGGGGCCTACAGCTG
CCGGCAGCGGCTCACGCAGCGCGTACTGTGCCACTTCAGTGTGCGGGTGACAGA
CGCTCCATCCTCGGGAGATGACGAAGACGGGGAGGACGAGGCTGAGGACACAG
GTGTGGACACAGGGGCCCCTTACTGGACACGGCCCGAGCGGATGGACAAGAAGC
TGCTGGCCGTGCCGGCCGCCAACACCGTCCGCTTCCGCTGCCCAGCCGCTGGCAA
CCCCACTCCCTCCATCTCCTGGCTGAAGAACGGCAGGGAGTTCCGCGGCGAGCA
CCGCATTGGAGGCATCAAGCTGCGGCATCAGCAGTGGAGCCTGGTCATGGAAAG
CGTGGTGCCCTCGGACCGCGGCAACTACACCTGCGTCGTGGAGAACAAGTTTGG
CAGCATCCGGCAGACGTACACGCTGGACGTGCTGGAGCGCTCCCCGCACCGGCC
CATCCTGCAGGCGGGGCTGCCGGCCAACCAGACGGCGGTGCTGGGCAGCGACGT
GGAGTTCCACTGCAAGGTGTACAGTGACGCACAGCCCCACATCCAGTGGCTCAA
GCACGTGGAGGTGAATGGCAGCAAGGTGGGCCCGGACGGCACACCCTACGTTAC
CGTGCTCAAGTCCTGGATCAGTGAGAGTGTGGAGGCCGACGTGCGCCTCCGCCTG
GCCAATGTGTCGGAGCGGGACGGGGGCGAGTACCTCTGTCGAGCCACCAATTTC
ATAGGCGTGGCCGAGAAGGCCTTTTGGCTGAGCGTTCACGGGCCCCGAGCAGCC
GAGGAGGAGCTGGTGGAGGCTGACGAGGCGGGCAGTGTGTATGCAGGCATCCTC
AGCTACGGGGTGGGCTTCTTCCTGTTCATCCTGGTGGTGGCGGCTGTGACGCTCT
GCCGCCTGCGCAGCCCCCCCAAGAAAGGCCTGGGCTCCCCCACCGTGCACAAGA
TCTCCCGCTTCCCGCTCAAGCGACAGGTGTCCCTGGAGTCCAACGCGTCCATGAG
CTCCAACACACCACTGGTGCGCATCGCAAGGCTGTCCTCAGGGGAGGGCCCCAC
GCTGGCCAATGTCTCCGAGCTCGAGCTGCCTGCCGACCCCAAATGGGAGCTGTCT
CGGGCCCGGCTGACCCTGGGCAAGCCCCTTGGGGAGGGCTGCTTCGGCCAGGTG
GTCATGGCGGAGGCCATCGGCATTGACAAGGACCGGGCCGCCAAGCCTGTCACC
GTAGCCGTGAAGATGCTGAAAGACGATGCCACTGACAAGGACCTGTCGGACCTG
GTGTCTGAGATGGAGATGATGAAGATGATCGGGAAACACAAAAACATCATCAAC
CTGCTGGGCGCCTGCACGCAGGGCGGGCCCCTGTACGTGCTGGTGGAGTACGCG
GCCAAGGGTAACCTGCGGGAGTTTCTGCGGGCGCGGCGGCCCCCGGGCCTGGAC
TACTCCTTCGACACCTGCAAGCCGCCCGAGGAGCAGCTCACCTTCAAGGACCTGG
TGTCCTGTGCCTACCAGGTGGCCCGGGGCATGGAGTACTTGGCCTCCCAGAAGTG
CATCCACAGGGACCTGGCTGCCCGCAATGTGCTGGTGACCGAGGACAACGTGAT
GAAGATCGCAGACTTCGGGCTGGCCCGGGACGTGCACAACCTCGACTACTACAA
GAAGACAACCAACGGCCGGCTGCCCGTGAAGTGGATGGCGCCTGAGGCCTTGTT
TGACCGAGTCTACACTCACCAGAGTGACGTCTGGTCCTTTGGGGTCCTGCTCTGG
GAGATCTTCACGCTGGGGGGCTCCCCGTACCCCGGCATCCCTGTGGAGGAGCTCT
TCAAGCTGCTGAAGGAGGGCCACCGCATGGACAAGCCCGCCAACTGCACACACG
ACCTGTACATGATCATGCGGGAGTGCTGGCATGCCGCGCCCTCCCAGAGGCCCA
CCTTCAAGCAGCTGGTGGAGGACCTGGACCGTGTCCTTACCGTGACGTCCACCGA
CGAGTACCTGGACCTGTCGGCGCCTTTCGAGCAGTACTCCCCGGGTGGCCAGGAC
ACCCCCAGCTCCAGCTCCTCAGGGGACGACTCCGTGTTTGCCCACGACCTGCTGC
CCCCGGCCCCACCCAGCAGTGGGGGCTCGCGGACGTGA

SEQ ID NO:56
FGFR3-IIIb amino acid sequence
MGAPACALALCVAVAIVAGASSESLGTEQRVVGRAAEVPGPEPGQQEQLVFGSGDA
VELSCPPPGGGPMGPTVWVKDGTGLVPSERVLVGPQRLQVLNASHEDSGAYSCRQR

LTQRVLCHFSVRVTDAPSSGDDEDGEDEAEDTGVDTGAPYWTRPERMDKKLLAVP
AANTVRFRCPAAGNPTPSISWLKNGREFRGEHRIGGIKLRHQQWSLVMESVVPSDRG
NYTCVVENKFGSIRQTYTLDVLERSPHRPILQAGLPANQTAVLGSDVEFHCKVYSDA
QPHIQWLKHVEVNGSKVGPDGTPYVTVLKSWISESVEADVRLRLANVSERDGGEYL
CRATNFIGVAEKAFWLSVHGPRAAEEELVEADEAGSVYAGILSYGVGFFLFILVVAA
VTLCRLRSPPKKGLGSPTVHKISRFPLKRQVSLESNASMSSNTPLVRIARLSSGEGPTL
ANVSELELPADPKWELSRARLTLGKPLGEGCFGQVVMAEAIGIDKDRAAKPVTVAV
KMLKDDATDKDLSDLVSEMEMMKMIGKHKNIINLLGACTQGGPLYVLVEYAAKGN
LREFLRARRPPGLDYSFDTCKPPEEQLTFKDLVSCAYQVARGMEYLASQKCIHRDLA
ARNVLVTEDNVMKIADFGLARDVHNLDYYKKTTNGRLPVKWMAPEALFDRVYTH
QSDVWSFGVLLWEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTHDLYMIMREC
WHAAPSQRPTFKQLVEDLDRVLTVTSTDEYLDLSAPFEQYSPGGQDTPSSSSSGDDS
VFAHDLLPPAPPSSGGSRT

SEQ ID NO:57
FGFR3-IIIc nucleic acid sequence
ATGGGCGCCCCTGCCTGCGCCCTCGCGCTCTGCGTGGCCGTGGCCATCGTGGCCG
GCGCCTCCTCGGAGTCCTTGGGGACGGAGCAGCGCGTCGTGGGGCGAGCGGCAG
AAGTCCCGGGCCCAGAGCCCGGCCAGCAGGAGCAGTTGGTCTTCGGCAGCGGGG
ATGCTGTGGAGCTGAGCTGTCCCCCGCCCGGGGGTGGTCCCATGGGGCCCACTGT
CTGGGTCAAGGATGGCACAGGGCTGGTGCCCTCGGAGCGTGTCCTGGTGGGGCC
CCAGCGGCTGCAGGTGCTGAATGCCTCCCACGAGGACTCCGGGGCCTACAGCTG
CCGGCAGCGGCTCACGCAGCGCGTACTGTGCCACTTCAGTGTGCGGGTGACAGA
CGCTCCATCCTCGGGAGATGACGAAGACGGGGAGGACGAGGCTGAGGACACAG
GTGTGGACACAGGGGCCCCTTACTGGACACGGCCCGAGCGGATGGACAAGAAGC
TGCTGGCCGTGCCGGCCGCCAACACCGTCCGCTTCCGCTGCCCAGCCGCTGGCAA
CCCCACTCCCTCCATCTCCTGGCTGAAGAACGGCAGGGAGTTCCGCGGCGAGCA
CCGCATTGGAGGCATCAAGCTGCGGCATCAGCAGTGGAGCCTGGTCATGGAAAG
CGTGGTGCCCTCGGACCGCGGCAACTACACCTGCGTCGTGGAGAACAAGTTTGG
CAGCATCCGGCAGACGTACACGCTGGACGTGCTGGAGCGCTCCCCGCACCGGCC
CATCCTGCAGGCGGGGCTGCCGGCCAACCAGACGGCGGTGCTGGGCAGCGACGT
GGAGTTCCACTGCAAGGTGTACAGTGACGCACAGCCCCACATCCAGTGGCTCAA
GCACGTGGAGGTGAATGGCAGCAAGGTGGGCCCGGACGGCACACCCTACGTTAC
CGTGCTCAAGACGGCGGGCGCTAACACCACCGACAAGGAGCTAGAGGTTCTCTC
CTTGCACAACGTCACCTTTGAGGACGCCGGGGAGTACACCTGCCTGGCGGGCAA
TTCTATTGGGTTTTCTCATCACTCTGCGTGGCTGGTGGTGCTGCCAGCCGAGGAG
GAGCTGGTGGAGGCTGACGAGGCGGGCAGTGTGTATGCAGGCATCCTCAGCTAC
GGGGTGGGCTTCTTCCTGTTCATCCTGGTGGTGGCGGCTGTGACGCTCTGCCGCC
TGCGCAGCCCCCCCAAGAAAGGCCTGGGCTCCCCCACCGTGCACAAGATCTCCC
GCTTCCCGCTCAAGCGACAGGTGTCCCTGGAGTCCAACGCGTCCATGAGCTCCAA
CACACCACTGGTGCGCATCGCAAGGCTGTCCTCAGGGGAGGGCCCCACGCTGGC
CAATGTCTCCGAGCTCGAGCTGCCTGCCGACCCCAAATGGGAGCTGTCTCGGGCC
CGGCTGACCCTGGGCAAGCCCCTTGGGGAGGGCTGCTTCGGCCAGGTGGTCATG
GCGGAGGCCATCGGCATTGACAAGGACCGGGCCGCCAAGCCTGTCACCGTAGCC
GTGAAGATGCTGAAAGACGATGCCACTGACAAGGACCTGTCGGACCTGGTGTCT
GAGATGGAGATGATGAAGATGATCGGGAAACACAAAAACATCATCAACCTGCTG
GGCGCCTGCACGCAGGGCGGGCCCCTGTACGTGCTGGTGGAGTACGCGGCCAAG
GGTAACCTGCGGGAGTTTCTGCGGGCGCGGCGGCCCCCGGGCCTGGACTACTCCT
TCGACACCTGCAAGCCGCCCGAGGAGCAGCTCACCTTCAAGGACCTGGTGTCCT
GTGCCTACCAGGTGGCCCGGGGCATGGAGTACTTGGCCTCCCAGAAGTGCATCC

ACAGGGACCTGGCTGCCCGCAATGTGCTGGTGACCGAGGACAACGTGATGAAGA
TCGCAGACTTCGGGCTGGCCCGGGACGTGCACAACCTCGACTACTACAAGAAGA
CAACCAACGGCCGGCTGCCCGTGAAGTGGATGGCGCCTGAGGCCTTGTTTGACC
GAGTCTACACTCACCAGAGTGACGTCTGGTCCTTTGGGGTCCTGCTCTGGGAGAT
CTTCACGCTGGGGGGCTCCCCGTACCCCGGCATCCCTGTGGAGGAGCTCTTCAAG
CTGCTGAAGGAGGGCCACCGCATGGACAAGCCCGCCAACTGCACACACGACCTG
TACATGATCATGCGGGAGTGCTGGCATGCCGCGCCCTCCCAGAGGCCCACCTTCA
AGCAGCTGGTGGAGGACCTGGACCGTGTCCTTACCGTGACGTCCACCGACGAGT
ACCTGGACCTGTCGGCGCCTTTCGAGCAGTACTCCCCGGGTGGCCAGGACACCCC
CAGCTCCAGCTCCTCAGGGGACGACTCCGTGTTTGCCCACGACCTGCTGCCCCCG
GCCCCACCCAGCAGTGGGGGCTCGCGGACGTGA

SEQ ID NO:58
FGFR3-IIIc amino acid sequence
MGAPACALALCVAVAIVAGASSESLGTEQRVVGRAAEVPGPEPGQQEQLVFGSGDA
VELSCPPPGGGPMGPTVWVKDGTGLVPSERVLVGPQRLQVLNASHEDSGAYSCRQR
LTQRVLCHFSVRVTDAPSSGDDEDGEDEAEDTGVDTGAPYWTRPERMDKKLLAVP
AANTVRFRCPAAGNPTPSISWLKNGREFRGEHRIGGIKLRHQQWSLVMESVVPSDRG
NYTCVVENKFGSIRQTYTLDVLERSPHRPILQAGLPANQTAVLGSDVEFHCKVYSDA
QPHIQWLKHVEVNGSKVGPDGTPYVTVLKTAGANTTDKELEVLSLHNVTFEDAGEY
TCLAGNSIGFSHHSAWLVVLPAEEELVEADEAGSVYAGILSYGVGFFLFILVVAAVTL
CRLRSPPKKGLGSPTVHKISRFPLKRQVSLESNASMSSNTPLVRIARLSSGEGPTLANV
SELELPADPKWELSRARLTLGKPLGEGCFGQVVMAEAIGIDKDRAAKPVTVAVKML
KDDATDKDLSDLVSEMEMMKMIGKHKNIINLLGACTQGGPLYVLVEYAAKGNLRE
FLRARRPPGLDYSFDTCKPPEEQLTFKDLVSCAYQVARGMEYLASQKCIHRDLAARN
VLVTEDNVMKIADFGLARDVHNLDYYKKTTNGRLPVKWMAPEALFDRVYTHQSD
VWSFGVLLWEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTHDLYMIMRECWH
AAPSQRPTFKQLVEDLDRVLTVTSTDEYLDLSAPFEQYSPGGQDTPSSSSSGDDSVFA
HDLLPPAPPSSGGSRT

SEQ ID NO:59
2B.1.3 light chain, amino acid
DIQMTQSPSSLSASVGDRVTITCRASQDVDTSLAWYKQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGHPQTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:60
2B.1.95 light chain, amino acid
DIQMTQSPSSLSASVGDRVTITCRASQDVDTSLAWYKQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGHPQTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:61
2B.1.73 light chain, amino acid
DIQMTQSPSSLSASVGDRVTITCRASQDVDTSLAWYKQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGHPQTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:62
2B.1.32 light chain, amino acid
DIQMTQSPSSLSASVGDRVTITCRASQDVDTSLAWYKQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGHPQTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:63
2B.1.88 light chain, amino acid
DIQMTQSPSSLSASVGDRVTITCRASQDVDTSLAWYKQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGHPQTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:64
2B.1.1 light chain, amino acid
DIQMTQSPSSLSASVGDRVTITCRASQDVDTSLAWYKQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGHPQTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:65
2B.1.3.10 light chain, amino acid
DIQMTQSPSSLSASVGDRVTITCRASQDVDTSLAWYKQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGHPQTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:66
2B.1.3.12 light chain, amino acid
DIQMTQSPSSLSASVGDRVTITCRASQDVDTSLAWYKQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGHPQTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:67
2B.1.3 light chain, nucleic acid
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGG
TCACCATCACCTGCCGTGCCAGTCAGGATGTTGATACTTCTCTGGCCTGGTATAA
ACAGAAACCAGGAAAAGCTCCGAAGCTTCTGATTTACTCGGCATCCTTCCTCTAC
TCTGGAGTCCCTTCTCGCTTCTCTGGTAGCGGTTCCGGGACGGATTTCACTCTGAC
CATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAATCTACC
GGTCATCCTCAGACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGAACTGTG
GCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
CTGCTTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:68
2B.1.95 light chain, nucleic acid
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGG
TCACCATCACCTGCCGTGCCAGTCAGGATGTTGATACTTCTCTGGCCTGGTATAA
ACAGAAACCAGGAAAAGCTCCGAAGCTTCTGATTTACTCGGCATCCTTCCTCTAC
TCTGGAGTCCCTTCTCGCTTCTCTGGTAGCGGTTCCGGGACGGATTTCACTCTGAC
CATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAATCTACC
GGTCATCCTCAGACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGAACTGTG
GCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
CTGCTTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:69
2B.1.73 light chain, nucleic acid
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGG
TCACCATCACCTGCCGTGCCAGTCAGGATGTTGATACTTCTCTGGCCTGGTATAA
ACAGAAACCAGGAAAAGCTCCGAAGCTTCTGATTTACTCGGCATCCTTCCTCTAC
TCTGGAGTCCCTTCTCGCTTCTCTGGTAGCGGTTCCGGGACGGATTTCACTCTGAC
CATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAATCTACC
GGTCATCCTCAGACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGAACTGTG
GCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
CTGCTTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:70
2B.1.32 light chain, nucleic acid
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGG
TCACCATCACCTGCCGTGCCAGTCAGGATGTTGATACTTCTCTGGCCTGGTATAA
ACAGAAACCAGGAAAAGCTCCGAAGCTTCTGATTTACTCGGCATCCTTCCTCTAC
TCTGGAGTCCCTTCTCGCTTCTCTGGTAGCGGTTCCGGGACGGATTTCACTCTGAC
CATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAATCTACC
GGTCATCCTCAGACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGAACTGTG
GCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
CTGCTTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:71
2B.1.88 light chain, nucleic acid
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGG
TCACCATCACCTGCCGTGCCAGTCAGGATGTTGATACTTCTCTGGCCTGGTATAA

ACAGAAACCAGGAAAAGCTCCGAAGCTTCTGATTTACTCGGCATCCTTCCTCTAC
TCTGGAGTCCCTTCTCGCTTCTCTGGTAGCGGTTCCGGGACGGATTTCACTCTGAC
CATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAATCTACC
GGTCATCCTCAGACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGAACTGTG
GCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
CTGCTTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:72
2B.1.1 light chain, nucleic acid
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGG
TCACCATCACCTGCCGTGCCAGTCAGGATGTTGATACTTCTCTGGCCTGGTATAA
ACAGAAACCAGGAAAAGCTCCGAAGCTTCTGATTTACTCGGCATCCTTCCTCTAC
TCTGGAGTCCCTTCTCGCTTCTCTGGTAGCGGTTCCGGGACGGATTTCACTCTGAC
CATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAATCTACC
GGTCATCCTCAGACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGAACTGTG
GCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
CTGCTTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:73
2B.1.3.10 light chain, nucleic acid
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGG
TCACCATCACCTGCCGTGCCAGTCAGGATGTTGATACTTCTCTGGCCTGGTATAA
ACAGAAACCAGGAAAAGCTCCGAAGCTTCTGATTTACTCGGCATCCTTCCTCTAC
TCTGGAGTCCCTTCTCGCTTCTCTGGTAGCGGTTCCGGGACGGATTTCACTCTGAC
CATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAATCTACC
GGTCATCCTCAGACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGAACTGTG
GCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
CTGCTTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:74
2B.1.3.12 light chain, nucleic acid
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGG
TCACCATCACCTGCCGTGCCAGTCAGGATGTTGATACTTCTCTGGCCTGGTATAA
ACAGAAACCAGGAAAAGCTCCGAAGCTTCTGATTTACTCGGCATCCTTCCTCTAC
TCTGGAGTCCCTTCTCGCTTCTCTGGTAGCGGTTCCGGGACGGATTTCACTCTGAC
CATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAATCTACC
GGTCATCCTCAGACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGAACTGTG
GCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA

CTGCTTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:75
2B.1.3 heavy chain, amino acid
EVQLVESGGGLVQPGGSLRLSCAASGFTFTSTGISWVRQAPGKGLEWVGRTHLGDG
STNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGIYDLYVDYTEY
VMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

SEQ ID NO:76
2B.1.95 heavy chain, amino acid
EVQLVESGGGLVQPGGSLRLSCAASGFTFTSTGISWVRQAPGKGLEWVGRLIFFTGS
TNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGIYDLYVDYTEYV
MDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

SEQ ID NO:77
2B.1.73 heavy chain, amino acid
EVQLVESGGGLVQPGGSLRLSCAASGFTFTSTGISWVRQAPGKGLEWVGRMIFYNGS
TNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGIYDLYVDYTEYV
MDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

SEQ ID NO:78
2B.1.32 heavy chain, amino acid
EVQLVESGGGLVQPGGSLRLSCAASGFTFTSTGISWVRQAPGKGLEWVGRWVGFTG
STNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGIYDLYVDYTEY
VMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA

LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

SEQ ID NO:79
2B.1.88 heavy chain, amino acid
EVQLVESGGGLVQPGGSLRLSCAASGFTFTSTGISWVRQAPGKGLEWVGRIWMFTG
STNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGIYDLYVDYTEY
VMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

SEQ ID NO:80
2B.1.1 heavy chain, amino acid
EVQLVESGGGLVQPGGSLRLSCAASGFTFTSTGISWVRQAPGKGLEWVGRYWAWD
GSTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGIYDLYVDYTE
YVMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPGK

SEQ ID NO:81
2B.1.3.10 heavy chain, amino acid
EVQLVESGGGLVQPGGSLRLSCAASGFPFTSQGISWVRQAPGKGLEWVGRTHLGDG
STNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGIYDTYDKYTEY
VMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

SEQ ID NO:82
2B.1.3.12 heavy chain, amino acid
EVQLVESGGGLVQPGGSLRLSCAASGFPFTSTGISWVRQAPGKGLEWVGRTHLGDG
STNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGIYDTYDMYTEY
VMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG

QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

SEQ ID NO:83
2B.1.3 heavy chain, nucleic acid
GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTC
CGTTTGTCCTGTGCAGCTTCTGGCTTCACCTTCACTAGTACTGGGATTAGCTGGGT
GCGTCAGGCCCCGGGTAAGGGCCTGGAATGGGTTGGTAGGACGCATTTGGGTGA
TGGTTCTACTAACTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGAC
ACATCCAAAAACACAGCCTACCTACAAATGAACAGCTTAAGAGCTGAGGACACT
GCCGTCTATTATTGTGCTCGTACCTACGGCATCTACGACCTGTACGTGGACTACA
CGGAGTACGTTATGGACTACTGGGGTCAAGGAACCCTGGTCACCGTCTCCTCGGC
CTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCT
GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT
GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCCCTCTA
GCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA
CCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCC
CACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
CGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGAAGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGA
ACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTA
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
TCTCCGGGTAAA

SEQ ID NO:84
2B.1.95 heavy chain, nucleic acid
GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTC
CGTTTGTCCTGTGCAGCTTCTGGCTTCACCTTCACTAGTACTGGGATTAGCTGGGT
GCGTCAGGCCCCGGGTAAGGGCCTGGAATGGGTTGGTAGGTTAATTTTTTTTACA
GGTTCTACTAACTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGACA
CATCCAAAAACACAGCCTACCTACAAATGAACAGCTTAAGAGCTGAGGACACTG
CCGTCTATTATTGTGCTCGTACCTACGGCATCTACGACCTGTACGTGGACTACAC
GGAGTACGTTATGGACTACTGGGTCAAGGAACCCTGGTCACCGTCTCCTCGGCCT
CCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC
GGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTC
CTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCCCTCTAGCA
GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCA
AGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCAC
CGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAA
ACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTG
GACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG

GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTA
CCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCAT
CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATC
CCGGGAAGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA
CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC
AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCC
GTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTC
CGGGTAAA

SEQ ID NO:85
2B.1.73 heavy chain, nucleic acid
GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTC
CGTTTGTCCTGTGCAGCTTCTGGCTTCACCTTCACTAGTACTGGGATTAGCTGGGT
GCGTCAGGCCCCGGGTAAGGGCCTGGAATGGGTTGGTAGGATGATTTTTTATAAT
GGTTCTACTAACTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGACA
CATCCAAAAACACAGCCTACCTACAAATGAACAGCTTAAGAGCTGAGGACACTG
CCGTCTATTATTGTGCTCGTACCTACGGCATCTACGACCTGTACGTGGACTACAC
GGAGTACGTTATGGACTACTGGGGTCAAGGAACCCTGGTCACCGTCTCCTCGGCC
TCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTG
GGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGA
CGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGT
CCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCCCTCTAGC
AGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
AAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCA
CCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAA
AACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGT
ACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG
AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCA
TCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
CCCGGGAAGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCT
TCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA
ACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAG
CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT
CCGGGTAAA

SEQ ID NO:86
2B.1.32 heavy chain, nucleic acid
GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTC
CGTTTGTCCTGTGCAGCTTCTGGCTTCACCTTCACTAGTACTGGGATTAGCTGGGT
GCGTCAGGCCCCGGGTAAGGGCCTGGAATGGGTTGGTAGGTGGGTCGGATTTAC
AGGTTCTACTAACTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGAC
ACATCCAAAAACACAGCCTACCTACAAATGAACAGCTTAAGAGCTGAGGACACT
GCCGTCTATTATTGTGCTCGTACCTACGGCATCTACGACCTGTACGTGGACTACA
CGGAGTACGTTATGGACTACTGGGGTCAAGGAACCCTGGTCACCGTCTCCTCGGC
CTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCT

GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT
GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCCCTCTA
GCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA
CCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCC
CACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
CGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGAAGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGA
ACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTA
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
TCTCCGGGTAAA

SEQ ID NO:87
2B.1.88 heavy chain, nucleic acid
GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTC
CGTTTGTCCTGTGCAGCTTCTGGCTTCACCTTCACTAGTACTGGGATTAGCTGGGT
GCGTCAGGCCCCGGGTAAGGGCCTGGAATGGGTTGGTAGGATTTGGATGTTTAC
AGGTTCTACTAACTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGAC
ACATCCAAAAACACAGCCTACCTACAAATGAACAGCTTAAGAGCTGAGGACACT
GCCGTCTATTATTGTGCTCGTACCTACGGCATCTACGACCTGTACGTGGACTACA
CGGAGTACGTTATGGACTACTGGGGTCAAGGAACCCTGGTCACCGTCTCCTCGGC
CTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCT
GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT
GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCCCTCTA
GCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA
CCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCC
CACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
CGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGAAGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGA
ACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTA
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
TCTCCGGGTAAA

SEQ ID NO:88
2B.1.1 heavy chain, nucleic acid
GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTC
CGTTTGTCCTGTGCAGCTTCTGGCTTCCCGTTCACTAGTCAGGGGATTAGCTGGGT
GCGTCAGGCCCCGGGTAAGGGCCTGGAATGGGTTGGTAGGACGCATTTGGGTGA
TGGTTCTACTAACTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGAC
ACATCCAAAAACACAGCCTACCTACAAATGAACAGCTTAAGAGCTGAGGACACT
GCCGTCTATTATTGTGCTCGTACCTACGGCATCTACGACACGTATGATAAGTACA
CGGAGTACGTTATGGACTACTGGGGTCAAGGAACCCTGGTCACCGTCTCCTCGGC
CTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCT
GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT
GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCCCTCTA
GCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA
CCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCC
CACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
CGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGAAGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGA
ACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTA
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
TCTCCGGGTAAA

SEQ ID NO:89
2B.1.3.10 heavy chain, nucleic acid
GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTC
CGTTTGTCCTGTGCAGCTTCTGGCTTCCCGTTCACTAGTCAGGGGATTAGCTGGGT
GCGTCAGGCCCCGGGTAAGGGCCTGGAATGGGTTGGTAGGACGCATTTGGGTGA
TGGTTCTACTAACTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGAC
ACATCCAAAAACACAGCCTACCTACAAATGAACAGCTTAAGAGCTGAGGACACT
GCCGTCTATTATTGTGCTCGTACCTACGGCATCTACGACACGTATGATAAGTACA
CGGAGTACGTTATGGACTACTGGGGTCAAGGAACCCTGGTCACCGTCTCCTCGGC
CTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCT
GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT
GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCCCTCTA
GCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA
CCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCC
CACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
CGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA

CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGAAGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGA
ACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTA
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
TCTCCGGGTAAA

SEQ ID NO:90
2B.1.3.12 heavy chain, nucleic acid
GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTC
CGTTTGTCCTGTGCAGCTTCTGGCTTCCCGTTCACTAGTACGGGGATTAGCTGGGT
GCGTCAGGCCCCGGGTAAGGGCCTGGAATGGGTTGGTAGGACGCATTTGGGTGA
TGGTTCTACTAACTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGAC
ACATCCAAAAACACAGCCTACCTACAAATGAACAGCTTAAGAGCTGAGGACACT
GCCGTCTATTATTGTGCTCGTACCTACGGCATCTACGACACGTATGATATGTACA
CGGAGTACGTTATGGACTACTGGGGTCAAGGAACCCTGGTCACCGTCTCCTCGGC
CTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCT
GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT
GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCCCTCTA
GCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA
CCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCC
CACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
CGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGAAGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGA
ACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTA
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
TCTCCGGGTAAA

SEQ ID NO:91
2B.1.3.10 FGFR2-IIIb and FGFR2-IIIc epitope 1
TNTEKMEKRLHAVPAANTVKFRCPA

SEQ ID NO:92
2B.1.3.10 FGFR2-IIIb and FGFR2-IIIc epitope 2
YKVRNQHWSLIMES

SEQ ID NO:93
2B.1.3.10 FGFR3-IIIb and FGFR3-IIIc epitope 1
TRPERMDKKLLAVPAANTVRFRCPA

SEQ ID NO:94
2B.1.3.10 FGFR3-IIIb and FGFR3-IIIc epitope 2
IKLRHQQWSLVMES

SEQ ID NO:95
VH subgroup III consensus framework
EVQLVESGGGLVQPGGSLRLSCAAS

SEQ ID NO:96
VH subgroup III consensus framework
WVRQAPGKGLEWV

SEQ ID NO:97
VH subgroup III consensus framework
RFTISRDNSKNTLYLQMNSLRAEDTAVYYC

SEQ ID NO:98
VH subgroup III consensus framework
WGQGTLVTVSS

SEQ ID NO:99
VL subgroup I consensus framework
DIQMTQSPSSLSASVGDRVTITC

SEQ ID NO:100
VL subgroup I consensus framework
WYQQKPGKAPKLLIY

SEQ ID NO:101
VL subgroup I consensus framework
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC

SEQ ID NO:102
VL subgroup I consensus framework
FGQGTKVEIK

SEQ ID NO:103
Ser Ser Pro Thr Arg Leu Ala Val Ile Pro Trp Gly Val Arg Lys Leu Leu Arg Trp Val Arg Arg Asn Tyr Gly Asp Met Asp Ile Tyr Ile Thr Ala Ser

SEQ ID NO:104
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Asp Phe Ser Leu Thr Thr Tyr Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Ser Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Ile Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Gly Ser Thr Tyr Val Asp Ala Ile Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

SEQ ID NO:105
Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Glu Ser Tyr Gly Asn Arg Tyr Met Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Arg Ala Ala Asn Leu Gln Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn Glu Asp Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys

SEQ ID NO:106
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Asp Phe Ser Leu Thr Thr Tyr Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Ser Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Ile Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Gly Ser Thr Tyr Val Asp Ala Ile Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Gly Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

SEQ ID NO:107
Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Glu Ser Tyr Gly Asn Arg Tyr Met Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Arg Ala Ala Asn Leu Gln Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Ser Asn Glu Asp Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys

SEQ ID NO:108
Ser Tyr Gly Ile Ser

SEQ ID NO:109
Asp Tyr Tyr Met Asn

SEQ ID NO:110
Asn Tyr Gly Val Ser

SEQ ID NO:111
Asp Thr Tyr Met Asn

SEQ ID NO:112
Asp Thr Tyr Ile His

SEQ ID NO:113
Ser Tyr Trp Ile His
SEQ ID NO:114
Asp Thr Phe Thr His

SEQ ID NO:115
Glu Tyr Thr Met Asn

SEQ ID NO:116
Ser Tyr Trp Ile Glu

SEQ ID NO:117
Asp Tyr Glu Met His

SEQ ID NO:118
Asp Thr Tyr Ile His

SEQ ID NO:119
Arg Tyr Trp Met Ser

SEQ ID NO:120
Asn Tyr Gly Met Asn

SEQ ID NO:121
Thr Ser Ala Met Gly Ile Gly

SEQ ID NO:122
Thr Tyr Gly Val His

SEQ ID NO:123
Gln Gln Tyr Ser Lys Leu Pro Trp Thr

SEQ ID NO:124
Phe Gln Gly Thr Gly Tyr Pro Leu Thr

SEQ ID NO:125
His Gln Val Arg Thr Leu Pro Trp Thr

SEQ ID NO:126
Gln Gln Tyr Trp Asn Thr Pro Phe Thr

SEQ ID NO:127
Phe Gln Gly Ser His Val Leu Thr

SEQ ID NO:128
Gln Gln His Tyr Ile Val Pro Tyr Thr

SEQ ID NO:129
Leu Gln Tyr Gly Ser Tyr Pro Trp Thr

SEQ ID NO:130
His Gln Trp Ser Ser Tyr Pro Leu Thr

SEQ ID NO:131
Gln Gln His His Ser Thr Pro Tyr Thr

SEQ ID NO:132
Gln Gln Phe Thr Ile Ser Pro Ser Met Tyr Thr

SEQ ID NO:133
Gln Gln Tyr Asn Ile Ser Pro Tyr Thr

SEQ ID NO:134
Gln Asn Gly His Asn Phe Pro Tyr Thr

SEQ ID NO:135
Gln Gln Tyr Trp Ser Asn Pro Leu Thr

SEQ ID NO:136
Gln Gln Ser Asn Glu Asp Tyr Thr

SEQ ID NO:137
Gln Gln Ser Asn Glu Asp Pro Trp Thr

SEQ ID NO:138
Thr Val Ser Ser Gly Gly Arg Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly

SEQ ID NO:139
Trp Ile Asp Pro Glu Asn Asp Asp Thr Ile Tyr Asp Pro Lys Phe Gln Gly

SEQ ID NO:140
Val Ile Trp Gly Asp Gly Ser Ile Asn Tyr His Ser Ala Leu Ile Ser

SEQ ID NO:141
Arg Ile Asp Pro Ser Asn Gly Asn Ala Lys Tyr Asp Pro Lys Phe Gln Gly

SEQ ID NO:142
Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe Gln Asp

SEQ ID NO:143
Glu Ile Asp Pro Ser Val Ser Asn Ser Asn Tyr Asn Gln Lys Phe Lys Gly

SEQ ID NO:144
Arg Ile Asp Pro Ser Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe Gln Gly

SEQ ID NO:145
Gly Ile Asn Pro Asn Asn Gly Glu Thr Ser Tyr Asn Gln Lys Phe Lys Gly

SEQ ID NO:146
Glu Ile Phe Pro Gly Gly Gly Ser Thr Ile Tyr Asn Glu Asn Phe Arg Asp

SEQ ID NO:147
Ala Ile Trp Pro Glu Asn Ala Asp Ser Val Tyr Asn Gln Lys Phe Lys Gly

SEQ ID NO:148
Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe Gln Gly

SEQ ID NO:149
Glu Ile Leu Pro Gly Ser Asp Ser Thr Lys Tyr Val Glu Lys Phe Lys Val

SEQ ID NO:150
Glu Ile Ser Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu Lys Asp

SEQ ID NO:151
Trp Ile Asp Thr Asp Thr Gly Glu Ala Thr Tyr Thr Asp Asp Phe Lys Gly

SEQ ID NO:152

SEQ ID NO:152
His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala Leu Lys Ser

SEQ ID NO:153
Val Ile Trp Ser Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Ile Ser

SEQ ID NO:154
Gly Gly Asp Gly Tyr Ala Leu Asp Tyr

SEQ ID NO:155
Phe Thr Thr Val Phe Ala Tyr

SEQ ID NO:156
Thr His Asp Trp Phe Asp Tyr

SEQ ID NO:157
Arg Ala Leu Gly Asn Gly Tyr Ala Leu Gly Tyr

SEQ ID NO:158
Gly Thr Ser Tyr Ser Trp Phe Ala Tyr

SEQ ID NO:159
Leu Gly Val Met Val Tyr Gly Ser Ser Pro Phe Trp Phe Ala Tyr

SEQ ID NO:160
Arg Ala Leu Gly Asn Gly Tyr Ala Met Asp Tyr

SEQ ID NO:161
Lys Thr Thr Asn Tyr

SEQ ID NO:162
Arg Gly Tyr Tyr Asp Ala Ala Trp Phe Asp Tyr

SEQ ID NO:163
Glu Gly Gly Asn Tyr

SEQ ID NO:164
Ser Gly Asn Tyr Gly Ala Met Asp Tyr

SEQ ID NO:165
Gly Gly Tyr His Tyr Pro Gly Trp Leu Val Tyr

SEQ ID NO:166
Pro Ser Pro Ala Leu Asp Tyr

SEQ ID NO:167
Glu Glu Tyr Gly Leu Phe Gly Phe Pro Tyr

SEQ ID NO:168
Ile Asp Gly Ile Tyr Asp Gly Ser Phe Tyr Ala Met Asp Tyr

SEQ ID NO:169
Asp Tyr Gly Ser Thr Tyr Val Asp Ala Ile Asp Tyr

SEQ ID NO:170
Ser Ala Ser Gln Val Ile Ser Asn Tyr Leu Asn

SEQ ID NO:171
Ser Ala Ser Ser Ser Gly Arg Tyr Thr Phe

SEQ ID NO:172
Arg Ala Ser Gln Asp Ile Ser Asn Tyr Phe Asn

SEQ ID NO:173
Lys Ala Ser Asp His Ile Asn Asn Trp Leu Ala

SEQ ID NO:174
Arg Ser Ser Gln Asn Ile Val His Ser Asp Gly Asn Thr Tyr Leu Glu

SEQ ID NO:175
Lys Ala Ser Gln Phe Val Ser Asp Ala Val Ala

SEQ ID NO:176
Arg Ala Ser Gln Glu Ile Ser Gly Tyr Leu Ser

SEQ ID NO:177
Ser Ala Ser Ser Ser Leu Ser Ser Ser Tyr Leu Tyr

SEQ ID NO:178
Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln Lys Asn Ser Leu Ala

SEQ ID NO:179
Arg Ala Ser Ser Ser Val Asn His Met Tyr

SEQ ID NO:180
Lys Ala Ser Gln Asn Val Asp Ser Tyr Val Ala

SEQ ID NO:181
Arg Ala Ser Gln Ser Ile Ser Asp Tyr Val Tyr

SEQ ID NO:182
Lys Ala Ser Glu Asp Ile Tyr Asn Arg Leu Ala

SEQ ID NO:183
Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His

SEQ ID NO:184
Arg Ala Ser Glu Ser Val Glu Ser Tyr Gly Asn Arg Tyr Met Thr

SEQ ID NO:185
Phe Thr Ser Ser Leu Arg Ser

SEQ ID NO:186
Asp Thr Ser Lys Leu Ala Ser

SEQ ID NO:187
Tyr Thr Ser Arg Leu Gln Ser

SEQ ID NO:188
Gly Thr Thr Asn Leu Glu Thr

SEQ ID NO:189
Lys Val Ser Asn Arg Phe Ser

SEQ ID NO:190
Ser Ala Ser Tyr Arg Tyr Thr

SEQ ID NO:191
Gly Ala Ser Asn Leu Glu Thr

SEQ ID NO:192
Ala Ala Ser Thr Leu Asp Ser

SEQ ID NO:193
Gly Ala Ser Asn Leu Ala Ser

SEQ ID NO:194
Leu Ala Ser Thr Arg Glu Ser

SEQ ID NO:195
Tyr Thr Ser Thr Leu Ala Pro

SEQ ID NO:196
Ser Ala Ser Tyr Arg Phe Ser

SEQ ID NO:197
Tyr Ala Ser Gln Ser Ile Ser

SEQ ID NO:198
Ala Ala Thr Ser Leu Glu Thr

SEQ ID NO:199
Arg Ala Ser Asn Leu Glu Ser

SEQ ID NO:200
Arg Ala Ala Asn Leu Gln Ser

SEQ ID NO:201
Gln Gln Tyr Ser Lys Leu Pro Trp Thr

SEQ ID NO:202
Phe Gln Gly Thr Gly Tyr Pro Leu Thr

SEQ ID NO:203
His Gln Val Arg Thr Leu Pro Trp Thr

SEQ ID NO:204
Gln Gln Tyr Trp Asn Thr Pro Phe Thr

SEQ ID NO:205
Phe Gln Gly Ser His Val Leu Thr

SEQ ID NO:206
Gln Gln His Tyr Ile Val Pro Tyr Thr

SEQ ID NO:207
Leu Gln Tyr Gly Ser Tyr Pro Trp Thr

SEQ ID NO:208
His Gln Trp Ser Ser Tyr Pro Leu Thr

SEQ ID NO:209
Gln Gln His His Ser Thr Pro Tyr Thr

SEQ ID NO:210
Gln Gln Phe Thr Ile Ser Pro Ser Met Tyr Thr

SEQ ID NO:211
Gln Gln Tyr Asn Ile Ser Pro Tyr Thr

SEQ ID NO:212
Gln Asn Gly His Asn Phe Pro Tyr Thr

SEQ ID NO:213
Gln Gln Tyr Trp Ser Asn Pro Leu Thr

SEQ ID NO:214
Gln Gln Ser Asn Glu Asp Tyr Thr

SEQ ID NO:215
Gln Gln Ser Asn Glu Asp Pro Trp Thr

SEQ ID NO:216
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Pro Ser Gly Phe Thr Phe Ser Ser Tyr Gly Ile Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Val Ser Ser Gly Gly Arg Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Glu Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Thr Arg Gly Gly Asp Gly Tyr Ala Leu Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser

SEQ ID NO:217
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Leu Val Asn Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Met Asn Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Thr Gly Trp Ile Asp Pro Glu Asn Asp Asp Thr Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Val Tyr Leu Gln Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Phe Thr Thr Val Phe Ala Tyr Trp Gly His Gln Thr Met Val Thr Val Ser Ala

SEQ ID NO:218
Gln Val Gln Val Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Asp Gly Ser Ile Asn Tyr His Ser Ala Leu Ile Ser Arg Leu Thr Ile Thr Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Leu Asn Ser Leu Glu Ala Asp Asp Thr AlaThr Tyr Tyr Cys Ala Lys Thr His Asp Trp Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala

SEQ ID NO:219
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ala Asp Phe Asn Ile Lys Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ser Asn Gly Asn Ala Lys Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Ser Ser Ser Asn Thr Ala Tyr Leu His Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr CysAla Ser Arg Ala Leu Gly Asn Gly Tyr Ala Leu Gly Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser

SEQ ID NO:220
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Asp Phe Asn Ile Ile Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe Gln Asp Lys Ala Ala Leu Thr Ser Asp Thr Asp Ser Asn Thr Ala Tyr Leu Leu Phe Asn Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Thr Ser Tyr Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Ser Val Ser Ala

SEQ ID NO:221
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Ile Val Lys Pro Gly Ala Ser Val Arg Leu Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr Trp Ile His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Glu Ile Asp Pro Ser Val Ser Asn Ser Asn Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Val Arg Leu Gly Val Met Val Tyr Gly Ser Ser Pro Phe Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala

SEQ ID NO:222
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Ile Val Lys Pro Gly Ala Ser Val Arg Leu Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr Trp Ile His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Glu Ile Asp Pro Ser Val Ser Asn Ser Asn Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe CysVal Arg Leu Gly Val Met Val Tyr Gly Ser Ser Pro Phe Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala

SEQ ID NO:223
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Leu Lys Pro Gly Ala Ser Val Arg Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Gln Asp Thr Phe Thr His Trp Val Arg Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ser Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe Gln Gly Lys Ala Lys Ile Leu Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ile Gly Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ser Arg Ala Leu Gly Asn Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser

SEQ ID NO:224
Glu Val Pro Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Pro Ser Gly Asp Thr Phe Thr Glu Tyr Thr Met Asn Trp Val Arg Gln Ser His Gly Lys Ser Leu Glu Trp Ile Gly Gly Ile Asn Pro Asn Asn Gly Glu Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Phe Met Asp Leu Arg Ile Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Lys Thr Thr Asn Tyr Trp Gly Gln Gly Thr Thr Leu Ile Val Ser Ser

SEQ ID NO:225
Gln Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala Ser Val Arg Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ser Tyr Trp Ile Glu Trp Val Lys Gln Arg Ser Gly His Gly Leu Glu Trp Ile Gly Glu Ile Phe Pro Gly Gly Gly Ser Thr Ile Tyr Asn Glu Asn Phe Arg Asp Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Tyr Tyr Asp Ala Ala Trp Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala

SEQ ID NO:226
Gln Val Gln Leu Lys Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Glu Met His Trp Met Lys Gln Thr Pro Val Tyr Gly Leu Glu Trp Ile Gly Ala Ile Trp Pro Glu Asn Ala Asp Ser Val Tyr Asn Gln Lys Phe Lys Gly Lys Val Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Asp Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Thr Arg Glu Gly Gly Asn Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser

SEQ ID NO:227
Glu Val Gln Leu Gln Gln Ser Gly Thr Glu Leu Val Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ser Ser Asp Phe Asn Ile Lys Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Asp Trp Leu Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ala Met Thr Ser Asp Thr Ser Ser Asn Thr Ala Tyr Leu Arg Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ser Ser Gly Asn Tyr Gly Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser

SEQ ID NO:228
Gln Val Gln Leu Gln Gln Ser Gly Asp Glu Leu Met Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Val Thr Gly Asn Thr Phe Ser Ser Tyr Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Glu Ile Leu Pro Gly Ser Asp Ser Thr Lys Tyr Val Glu Lys Phe Lys Val Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr CysAla Arg Gly Gly Tyr His Tyr Pro Gly Trp Leu Val Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala

SEQ ID NO:229
Glu Val Lys Phe Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Phe Ser Arg Tyr Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Glu Ile Ser Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu Lys Asp Lys Phe Val Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Ser Lys Val Arg Ser Ala Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Pro Ser Pro Ala Leu Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala

SEQ ID NO:230
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu Thr Ala Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Asp Leu Lys Trp Met Gly Trp Ile Asp Thr Asp Thr Gly Glu Ala Thr Tyr Thr Asp Asp Phe Lys Gly Arg Phe Val Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Met Ala Thr Tyr Phe Cys Ala Arg Glu Glu Tyr Gly Leu Phe Gly Phe Pro Tyr Trp Gly His Gly Thr Leu Val Thr Val Ser Ala

SEQ ID NO:231
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser Ala Met Gly Ile Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Arg Tyr Asn Pro Ala Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val Phe Leu Lys Ile Ala Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Phe Cys Ala Arg Ile Asp Gly Ile Tyr Asp Gly Ser Phe Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser

SEQ ID NO:232
Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln Ser Leu Ser Val Ala Cys Thr Val Ser Asp Phe Ser Leu Thr Thr Tyr Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Ser Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Ile Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Phe Lys Met Asn Ser Leu Gln Thr Thr Asp Thr Ala Ile Tyr Tyr Cys Ala Arg Asp Tyr Gly Ser Thr Tyr Val Asp Ala Ile Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser

SEQ ID NO:233
Phe Ser Gly Asp Gly Arg Ala Ile Trp Ser Lys Asn Pro Asn Phe Thr Pro Val Asn Glu Ser Gln Leu Phe Leu Tyr Asp Thr Phe Pro Lys Asn Phe Phe Trp Gly Ile Gly Thr Gly Ala Leu Gln Val Glu Gly Ser Trp Lys Lys Asp Gly Lys Gly Pro Ser Ile Trp Asp His Phe Ile His Thr His Leu Lys Asn Val Ser Ser Thr Asn Gly Ser Ser Asp Ser Tyr Ile Phe Leu Glu Lys Asp Leu Ser Ala Leu Asp Phe Ile Gly Val Ser Phe Tyr Gln Phe Ser Ile Ser Trp Pro Arg Leu Phe Pro Asp Gly Ile Val Thr Val Ala Asn Ala Lys Gly Leu Gln Tyr Tyr Ser Thr Leu Leu Asp Ala Leu Val Leu Arg Asn Ile Glu Pro Ile Val Thr Leu Tyr His Trp Asp Leu Pro Leu Ala Leu Gln Glu Lys Tyr Gly Gly Trp Lys Asn Asp Thr Ile Ile Asp Ile Phe Asn Asp Tyr Ala Thr Tyr Cys Phe Gln Met Phe Gly Asp Arg Val Lys Tyr Trp Ile Thr Ile His Asn Pro Tyr Leu Val Ala Trp His Gly Tyr Gly Thr Gly Met His Ala Pro Gly Glu Lys Gly Asn Leu Ala Ala Val Tyr Thr Val Gly His Asn Leu Ile Lys Ala His Ser Lys Val Trp His Asn Tyr Asn Thr His Phe Arg Pro His Gln Lys Gly Trp Leu Ser Ile Thr Leu Gly Ser His Trp Ile Glu Pro Asn Arg Ser Glu Asn Thr Met Asp Ile Phe Lys Cys Gln Gln Ser Met Val Ser Val Leu Gly Trp Phe Ala Asn Pro Ile His Gly Asp Gly Asp Tyr Pro Glu Gly Met Arg Lys Lys Leu Phe Ser Val Leu Pro Ile Phe Ser Glu Ala Glu Lys His Glu Met Arg Gly Thr Ala Asp Phe Phe Ala Phe Ser Phe Gly Pro Asn Asn Phe Lys Pro Leu Asn Thr Met Ala Lys Met Gly Gln Asn Val Ser Leu Asn Leu Arg Glu Ala Leu Asn Trp Ile Lys Leu Glu Tyr Asn Asn Pro Arg Ile Leu Ile Ala Glu Asn Gly Trp Phe Thr Asp Ser Arg Val Lys Thr Glu Asp Thr Thr Ala Ile Tyr Met Met Lys Asn Phe Leu Ser Gln Val Leu Gln Ala Ile Arg Leu Asp Glu Ile Arg Val Phe Gly Tyr Thr Ala Trp Ser Leu Leu Asp Gly Phe Glu Trp Gln Asp Ala Tyr Thr Ile Arg Arg Gly Leu Phe Tyr Val Asp Phe Asn Ser Lys Gln Lys Glu Arg Lys Pro Lys Ser Ser Ala His Tyr Tyr Lys Gln Ile Ile Arg Glu Asn Gly Phe Ser Leu Lys Glu Ser Thr Pro Asp Val Gln Gly Gln Phe Pro Cys Asp Phe Ser Trp Gly Val Thr Glu Ser Val Leu Lys Pro Glu Ser Val Ala Ser Ser Pro Gln Phe Ser Asp Pro His Leu Tyr Val Trp Asn Ala Thr Gly Asn Arg Leu Leu His Arg Val Glu Gly Val Arg Leu Lys Thr Arg Pro Ala Gln Cys Thr Asp Phe Val Asn Ile Lys Lys Gln Leu Glu Met Leu Ala Arg Met Lys Val Thr His Tyr Arg Phe Ala Leu Asp Trp Ala Ser Val Leu Pro Thr Gly Asn Leu Ser Ala Val Asn Arg Gln Ala Leu Arg Tyr Tyr Arg Cys Val Val Ser Glu Gly Leu Lys Leu Gly Ile Ser Ala Met Val Thr Leu Tyr Tyr Pro Thr His Ala His Leu Gly Leu Pro Glu Pro Leu Leu His Ala Asp Gly Trp Leu Asn Pro Ser Thr Ala Glu Ala Phe Gln Ala Tyr Ala Gly Leu Cys Phe Gln Glu Leu Gly Asp Leu Val Lys Leu Trp Ile Thr Ile Asn Glu Pro Asn Arg Leu Ser Asp Ile Tyr Asn Arg Ser Gly Asn Asp Thr Tyr Gly Ala Ala His Asn Leu Leu Val Ala His Ala Leu Ala Trp Arg Leu Tyr Asp Arg Gln Phe Arg Pro Ser Gln Arg Gly Ala Val Ser Leu Ser Leu His Ala Asp Trp Ala Glu Pro Ala Asn Pro Tyr Ala Asp Ser His Trp Arg Ala Ala Glu Arg Phe Leu Gln Phe Glu Ile Ala Trp Phe Ala Glu Pro Leu Phe Lys Thr Gly Asp Tyr Pro Ala Ala Met Arg Glu Tyr Ile Ala Ser Lys His Arg Arg Gly Leu Ser Ser Ser Ala Leu Pro Arg Leu Thr Glu Ala Glu Arg Arg Leu Leu Lys Gly Thr Val Asp Phe Cys Ala Leu Asn His Phe Thr Thr Arg Phe Val Met His Glu Gln Leu Ala Gly Ser Arg Tyr Asp Ser Asp Arg Asp Ile Gln Phe Leu Gln Asp Ile Thr Arg Leu Ser Ser Pro Thr Arg Leu Ala Val Ile Pro Trp Gly Val Arg Lys Leu Leu Arg Trp Val Arg Arg Asn Tyr Gly Asp Met Asp Ile Tyr Ile Thr Ala Ser Gly Ile Asp Asp Gln Ala Leu Glu Asp Asp Arg Leu Arg Lys Tyr Tyr Leu Gly Lys Tyr Leu Gln Glu Val Leu Lys Ala Tyr Leu Ile Asp Lys Val Arg Ile Lys Gly Tyr Tyr Ala Phe Lys Leu Ala Glu Glu Lys

Ser Lys Pro Arg Phe Gly Phe Phe Thr Ser Asp Phe Lys Ala Lys Ser Ser Ile Gln Phe Tyr Asn Lys Val Ile Ser Ser Arg Gly Phe Pro Phe Glu Asn Ser Ser Ser Arg Cys Ser Gln Thr Gln Glu Asn Thr Glu Cys Thr Val Cys Leu Phe Leu Val Gln Lys Lys Pro Leu Ile Phe Leu Gly Cys Cys Phe Phe Ser Thr Leu Val Leu Leu Leu Ser Ile Ala Ile Phe Gln Arg Gln Lys Arg Arg Lys Phe Trp Lys Ala Lys Asn Leu Gln His Ile Pro Leu Lys Lys Gly Lys Arg Val Val Ser

SEQ ID NO:234
Met Lys Pro Gly Cys Ala Ala Gly Ser Pro Gly Asn Glu Trp Ile Phe Phe Ser Thr Asp Glu Ile Thr Thr Arg Tyr Arg Asn Thr Met Ser Asn Gly Gly Leu Gln Arg Ser Val Ile Leu Ser Ala Leu Ile Leu Leu Arg Ala Val Thr Gly

SEQ ID NO:235
Phe Pro Cys Asp Phe Ser Trp Gly Val Thr Glu Ser Val Leu Lys Pro Glu Ser Val Ala Ser Ser Pro Gln Phe Ser Asp Pro His Leu Tyr Val Trp Asn Ala Thr Gly Asn Arg Leu Leu His Arg Val Glu Gly Val Arg Leu Lys Thr Arg Pro Ala Gln Cys Thr Asp Phe Val Asn Ile Lys Lys Gln Leu Glu Met Leu Ala Arg Met Lys Val Thr His Tyr Arg Phe Ala Leu Asp Trp Ala Ser Val Leu Pro Thr Gly Asn Leu Ser Ala Val Asn Arg Gln Ala Leu Arg Tyr Tyr Arg Cys Val Val Ser Glu Gly Leu Lys Leu Gly Ile Ser Ala Met Val Thr Leu Tyr Tyr Pro Thr His Ala His Leu Gly Leu Pro Glu Pro Leu Leu His Ala Asp Gly Trp Leu Asn Pro Ser Thr Ala Glu Ala Phe Gln Ala Tyr Ala Gly Leu Cys Phe Gln Glu Leu Gly Asp Leu Val Lys Leu Trp Ile Thr Ile Asn Glu Pro Asn Arg Leu Ser Asp Ile Tyr Asn Arg Ser Gly Asn Asp Thr Tyr Gly Ala Ala His Asn Leu Leu Val Ala His Ala Leu Ala Trp Arg Leu Tyr Asp Arg Gln Phe Arg Pro Ser Gln Arg Gly Ala Val Ser Leu Ser Leu His Ala Asp Trp Ala Glu Pro Ala Asn Pro Tyr Ala Asp Ser His Trp Arg Ala Ala Glu Arg Phe Leu Gln Phe Glu Ile Ala Trp Phe Ala Glu Pro Leu Phe Lys Thr Gly Asp Tyr Pro Ala Ala Met Arg Glu Tyr Ile Ala Ser Lys His Arg Arg Gly Leu Ser Ser Ser Ala Leu Pro Arg Leu Thr Glu Ala Glu Arg Arg Leu Leu Lys Gly Thr Val Asp Phe Cys Ala Leu Asn His Phe Thr Thr Arg Phe Val Met His Glu Gln Leu Ala Gly Ser Arg Tyr Asp Ser Asp Arg Asp Ile Gln Phe Leu Gln Asp Ile Thr Arg Leu Ser Ser Pro Thr Arg Leu Ala Val Ile Pro Trp Gly Val Arg Lys Leu Leu Arg Trp Val Arg Arg Asn Tyr Gly Asp Met Asp Ile Tyr Ile Thr Ala Ser Gly Ile Asp Asp Gln Ala Leu Glu Asp Asp Arg Leu Arg Lys Tyr Tyr Leu Gly Lys Tyr Leu Gln Glu Val Leu Lys Ala Tyr Leu Ile Asp Lys Val Arg Ile Lys Gly Tyr Tyr Ala Phe Lys Leu Ala Glu Glu Lys Ser Lys Pro Arg Phe Gly Phe Phe Thr Ser Asp Phe Lys Ala Lys Ser Ser Ile Gln Phe Tyr Asn Lys Val Ile Ser Ser Arg Gly Phe Pro Phe Glu Asn Ser Ser Ser Arg

SEQ ID NO:236
Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ile Cys Ser Ala Ser Gln Val Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile Tyr Phe Thr Ser Ser Leu Arg Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Pro Glu Asp Val Ala Thr Tyr Phe Cys Gln Gln Tyr Ser Lys Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys

SEQ ID NO:237
Glu Asn Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro GlyGlu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Gly Arg Tyr Thr Phe Trp Tyr Gln Gln Lys Ser Asn Thr Ala Pro Lys Leu Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Gly Arg Phe Ser Gly Ser Gly Ser Gly Asn Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Val Ala Thr Tyr Tyr Cys Phe Gln Gly Thr Gly Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys

SEQ ID NO:238
Asp Ile Gln Met Thr Gln Thr Pro Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Asn Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr Phe Asn Trp Tyr Gln Gln Lys Pro Asn Gly Thr Ile Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln Glu Asp Lys Ala Thr Tyr Phe Cys His Gln Val Arg Thr Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys

SEQ ID NO:239
Asp Ile Gln Met Thr Gln Ser Ser Ser Tyr Leu Ser Val Ser Leu Gly Gly Ser Val Thr Ile Thr Cys Lys Ala Ser Asp His Ile Asn Asn Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Asn Ala Pro Arg Leu Leu Ile Tyr Gly Thr Thr Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Arg Asp Tyr Ile Leu Ser Ile Thr Ser Leu Gln Ser Glu Asp Val Ala Ser Tyr Tyr Cys Gln Gln Tyr Trp Asn Thr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys

SEQ ID NO:240
Ala Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser Asp Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Arg Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Gly Asp Leu Gly Val Tyr Tyr Cys Phe Gln GlySer His Val Leu Thr Phe Gly Ala Gly Thr Arg Leu Glu Leu Lys

SEQ ID NO:241
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Phe Val Ser Asp Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Cys Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Arg Thr Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Ile Val Pro Tyr Thr Phe Gly Gly Gly Thr Thr Leu Glu Ile Glu

SEQ ID NO:242
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Phe Val Ser Asp Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Cys Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Arg Thr Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Ile Val Pro Tyr Thr Phe Gly Gly Gly Thr Thr Leu Glu Ile Glu

SEQ ID NO:243
Asp Ile Gln Met Thr Gln Ser Ser Ser Tyr Leu Ser Val Ser Leu Gly Gly Arg Val Thr Ile Thr Cys Lys Ala Ser Asp His Ile Asn Asn Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Asn Ala Pro Arg Leu Leu Ile Ser Gly Ala Ser Asn Leu Glu Thr Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Lys Asp Tyr Thr Leu Thr Ile Thr Ser Leu Gln Thr Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Tyr Trp Asn Thr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys

SEQ ID NO:244
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln Glu Ile Ser Gly Tyr Leu Ser Trp Leu Gln Gln Lys Pro Asp Gly Thr Ile Lys Arg Leu Ile Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val Pro Arg Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Ser Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln Tyr Gly Ser Tyr Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys

SEQ ID NO:245
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr Leu Thr Cys Ser Ala Ser Ser Ser Leu Ser Ser Ser Tyr Leu Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp Ile Tyr Gly Ala Ser Asn Leu Ala Ser Gly Val Pro Gly Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Ser Tyr Phe Cys His Gln Trp Ser Ser Tyr Pro Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu Leu Lys

SEQ ID NO:246
Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Pro Met Ser Val Gly Gln Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln Lys Asn Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Val Tyr Leu Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Ile Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln His His Ser Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys

SEQ ID NO:247
Glu Ser Val Leu Thr Gln Ser Pro Ala Leu Met Ser Ala Ser Leu Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Asn His Met Tyr Trp Tyr Gln Gln Lys Ser Asp Ala Ser Pro Lys Leu Trp Ile Tyr Tyr Thr Ser Thr Leu Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Asn Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Gly Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Phe Thr Ile Ser Pro Ser Met Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys

SEQ ID NO:248
Gly Thr Asp Val Met Asp Tyr

SEQ ID NO:249
Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala

SEQ ID NO:250
Ser Ala Ser Phe Leu Tyr Ser

SEQ ID NO:251
Gln Gln Ser Tyr Thr Thr Pro Pro Thr

SEQ ID NO:252
Lys Leu His Ala Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro

SEQ ID NO:253
Phe Lys Pro Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr

SEQ ID NO:254
Phe Ser Gly Asp Gly Arg Ala Ile Trp Ser Lys Asn Pro Asn Phe Thr Pro Val Asn Glu Ser Gln Leu Phe Leu Tyr Asp Thr Phe Pro Lys Asn Phe Phe Trp Gly Ile Gly Thr Gly Ala Leu Gln Val Glu Gly Ser Trp Lys Lys Asp Gly Lys Gly Pro Ser Ile Trp Asp His Phe Ile His Thr His Leu Lys Asn Val Ser Ser Thr Asn Gly Ser Ser Asp Ser Tyr Ile Phe Leu Glu Lys Asp Leu Ser Ala Leu Asp Phe Ile Gly Val Ser Phe Tyr Gln Phe Ser Ile Ser Trp Pro Arg Leu Phe Pro Asp Gly Ile Val Thr Val Ala Asn Ala Lys Gly Leu Gln Tyr Tyr Ser Thr Leu Leu Asp Ala Leu Val Leu Arg Asn Ile Glu Pro Ile Val Thr Leu Tyr His Trp Asp Leu Pro Leu Ala Leu Gln Glu Lys Tyr Gly Gly Trp Lys Asn Asp Thr Ile Ile Asp Ile Phe Asn Asp Tyr Ala Thr Tyr Cys Phe Gln Met Phe Gly Asp Arg Val Lys Tyr Trp Ile Thr Ile His Asn Pro Tyr Leu Val Ala Trp His Gly

Tyr Gly Thr Gly Met His Ala Pro Gly Glu Lys Gly Asn Leu Ala Ala Val Tyr Thr Val Gly
His Asn Leu Ile Lys Ala His Ser Lys Val Trp His Asn Tyr Asn Thr His Phe Arg Pro His Gln
Lys Gly Trp Leu Ser Ile Thr Leu Gly Ser His Trp Ile Glu Pro Asn Arg Ser Glu Asn Thr Met
Asp Ile Phe Lys Cys Gln Gln Ser Met Val Ser Val Leu Gly Trp Phe Ala Asn Pro Ile His Gly
Asp Gly Asp Tyr Pro Glu Gly Met Arg Lys Lys Leu Phe Ser Val Leu Pro Ile Phe Ser Glu Ala
Glu Lys His Glu Met Arg Gly Thr Ala Asp Phe Phe Ala Phe Ser Phe Gly Pro Asn Asn Phe
Lys Pro Leu Asn Thr Met Ala Lys Met Gly Gln Asn Val Ser Leu Asn Leu Arg Glu Ala Leu
Asn Trp Ile Lys Leu Glu Tyr Asn Asn Pro Arg Ile Leu Ile Ala Glu Asn Gly Trp Phe Thr Asp
Ser Arg Val Lys Thr Glu Asp Thr Thr Ala Ile Tyr Met Met Lys Asn Phe Leu Ser Gln Val
Leu Gln Ala Ile Arg Leu Asp Glu Ile Arg Val Phe Gly Tyr Thr Ala Trp Ser Leu Leu Asp Gly
Phe Glu Trp Gln Asp Ala Tyr Thr Ile Arg Arg Gly Leu Phe Tyr Val Asp Phe Asn Ser Lys
Gln Lys Glu Arg Lys Pro Lys Ser Ser Ala His Tyr Tyr Lys Gln Ile Ile Arg Glu Asn Gly Phe
Ser Leu Lys Glu Ser Thr Pro Asp Val Gln Gly Gln Phe Pro Cys Asp Phe Ser Trp Gly Val Thr
Glu Ser Val Leu Lys Pro Glu Ser Val Ala Ser Ser Pro Gln Phe Ser Asp Pro His Leu Tyr Val
Trp Asn Ala Thr Gly Asn Arg Leu Leu His Arg Val Glu Gly Val Arg Leu Lys Thr Arg Pro
Ala Gln Cys Thr Asp Phe Val Asn Ile Lys Lys Gln Leu Glu Met Leu Ala Arg Met Lys Val
Thr His Tyr Arg Phe Ala Leu Asp Trp Ala Ser Val Leu Pro Thr Gly Asn Leu Ser Ala Val
Asn Arg Gln Ala Leu Arg Tyr Tyr Arg Cys Val Val Ser Glu Gly Leu Lys Leu Gly Ile Ser
Ala Met Val Thr Leu Tyr Tyr Pro Thr His Ala His Leu Gly Leu Pro Glu Pro Leu Leu His Ala
Asp Gly Trp Leu Asn Pro Ser Thr Ala Glu Ala Phe Gln Ala Tyr Ala Gly Leu Cys Phe Gln
Glu Leu Gly Asp Leu Val Lys Leu Trp Ile Thr Ile Asn Glu Pro Asn Arg Leu Ser Asp Ile Tyr
Asn Arg Ser Gly Asn Asp Thr Tyr Gly Ala Ala His Asn Leu Leu Val Ala His Ala Leu Ala
Trp Arg Leu Tyr Asp Arg Gln Phe Arg Pro Ser Gln Arg Gly Ala Val Ser Leu Ser Leu His
Ala Asp Trp Ala Glu Pro Ala Asn Pro Tyr Ala Asp Ser His Trp Arg Ala Ala Glu Arg Phe
Leu Gln Phe Glu Ile Ala Trp Phe Ala Glu Pro Leu Phe Lys Thr Gly Asp Tyr Pro Ala Ala Met
Arg Glu Tyr Ile Ala Ser Lys His Arg Arg Gly Leu Ser Ser Ser Ala Leu Pro Arg Leu Thr Glu
Ala Glu Arg Arg Leu Leu Lys Gly Thr Val Asp Phe Cys Ala Leu Asn His Phe Thr Thr Arg
Phe Val Met His Glu Gln Leu Ala Gly Ser Arg Tyr Asp Ser Asp Arg Asp Ile Gln Phe Leu
Gln Asp Ile Thr Arg Leu Ser Ser Pro Thr Arg Leu Ala Val Ile Pro Trp Gly Val Arg Lys Leu
Leu Arg Trp Val Arg Arg Asn Tyr Gly Asp Met Asp Ile Tyr Ile Thr Ala Ser Gly Ile Asp Asp
Gln Ala Leu Glu Asp Asp Arg Leu Arg Lys Tyr Tyr Leu Gly Lys Tyr Leu Gln Glu Val Leu
Lys Ala Tyr Leu Ile Asp Lys Val Arg Ile Lys Gly Tyr Tyr Ala Phe Lys Leu Ala Glu Glu Lys
Ser Lys Pro Arg Phe Gly Phe Phe Thr Ser Asp Phe Lys Ala Lys Ser Ser Ile Gln Phe Tyr Asn
Lys Val Ile Ser Ser Arg Gly Phe Pro Phe Glu Asn Ser Ser Ser Arg Cys Ser Gln Thr Gln Glu
Asn Thr Glu Cys Thr Val Cys Leu Phe Leu Val Gln Lys Lys Pro Leu Ile Phe Leu Gly Cys
Cys Phe Phe Ser Thr Leu Val Leu Leu Leu Ser Ile Ala Ile Phe Gln Arg Gln Lys Arg Arg Lys
Phe Trp Lys Ala Lys Asn Leu Gln His Ile Pro Leu Lys Lys Gly Lys Arg Val Val Ser

SEQ ID NO:255

Met Trp Ser Trp Lys Cys Leu Leu Phe Trp Ala Val Leu Val Thr Ala Thr Leu Cys Thr Ala
Arg Pro Ser Pro Thr Leu Pro Glu Gln Ala Gln Pro Trp Gly Ala Pro Val Glu Val Glu Ser Phe
Leu Val His Pro Gly Asp Leu Leu Gln Leu Arg Cys Arg Leu Arg Asp Asp Val Gln Ser Ile
Asn Trp Leu Arg Asp Gly Val Gln Leu Ala Glu Ser Asn Arg Thr Arg Ile Thr Gly Glu Glu
Val Glu Val Gln Asp Ser Val Pro Ala Asp Ser Gly Leu Tyr Ala Cys Val Thr Ser Ser Pro Ser
Gly Ser Asp Thr Thr Tyr Phe Ser Val Asn Val Ser Asp Ala Leu Pro Ser Ser Glu Asp Asp
Asp Asp Asp Asp Asp Asp Ser Ser Ser Glu Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val
Ala Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala Val Pro Ala Ala Lys Thr
Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly
Lys Glu Phe Lys Pro Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile
Met Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val Glu Asn Glu Tyr Gly Ser

Ile Asn His Thr Tyr Gln Leu Asp Val Val Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His Leu Arg Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu Glu Ile Ile Ile Tyr Cys Thr Gly Ala Phe Leu Ile Ser Cys Met Val Gly Ser Val Ile Val Tyr Lys Met Lys Ser Gly Thr Lys Lys Ser Asp Phe His Ser Gln Met Ala Val His Lys Leu Ala Lys Ser Ile Pro Leu Arg Arg Gln Val Thr Val Ser Ala Asp Ser Ser Ala Ser Met Asn Ser Gly Val Leu Leu Val Arg Pro Ser Arg Leu Ser Ser Ser Gly Thr Pro Met Leu Ala Gly Val Ser Glu Tyr Glu Leu Pro Glu Asp Pro Arg Trp Glu Leu Pro Arg Asp Arg Leu Val Leu Gly Lys Pro Leu Gly Glu Gly Cys Phe Gly Gln Val Val Leu Ala Glu Ala Ile Gly Leu Asp Lys Asp Lys Pro Asn Arg Val Thr Lys Val Ala Val Lys Met Leu Lys Ser Asp Ala Thr Glu Lys Asp Leu Ser Asp Leu Ile Ser Glu Met Glu Met Met Lys Met Ile Gly Lys His Lys Asn Ile Ile Asn Leu Leu Gly Ala Cys Thr Gln Asp Gly Pro Leu Tyr Val Ile Val Glu Tyr Ala Ser Lys Gly Asn Leu Arg Glu Tyr Leu Gln Ala Arg Arg Pro Pro Gly Leu Glu Tyr Cys Tyr Asn Pro Ser His Asn Pro Glu Glu Gln Leu Ser Ser Lys Asp Leu Val Ser Cys Ala Tyr Gln Val Ala Arg Gly Met Glu Tyr Leu Ala Ser Lys Lys Cys Ile His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr Glu Asp Asn Val Met Lys Ile Ala Asp Phe Gly Leu Ala Arg Asp Ile His His Ile Asp Tyr Tyr Lys Lys Thr Thr Asn Gly Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ala Leu Phe Asp Arg Ile Tyr Thr His Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu Trp Glu Ile Phe Thr Leu Gly Gly Ser Pro Tyr Pro Gly Val Pro Val Glu Glu Leu Phe Lys Leu Leu Lys Glu Gly His Arg Met Asp Lys Pro Ser Asn Cys Thr Asn Glu Leu Tyr Met Met Met Arg Asp Cys Trp His Ala Val Pro Ser Gln Arg Pro Thr Phe Lys Gln Leu Val Glu Asp Leu Asp Arg Ile Val Ala Leu Thr Ser Asn Gln Glu Tyr Leu Asp Leu Ser Met Pro Leu Asp Gln Tyr Ser Pro Ser Phe Pro Asp Thr Arg Ser Ser Thr Cys Ser Ser Gly Glu Asp Ser Val Phe Ser His Glu Pro Leu Pro Glu Glu Pro Cys Leu Pro Arg His Pro Ala Gln Leu Ala Asn Gly Gly Leu Lys Arg Arg

SEQ ID NO:256
Asp Tyr Lys Asp Asp Asp Asp Lys Leu Glu Phe Ser Gly Asp Gly Lys Ala Ile Trp Asp Lys Lys Gln Tyr Val Ser Pro Val Asn Pro Gly Gln Leu Phe Leu Tyr Asp Thr Phe Pro Lys Asn Phe Ser Trp Gly Val Gly Thr Gly Ala Phe Gln Val Glu Gly Ser Trp Lys Ala Asp Gly Arg Gly Pro Ser Ile Trp Asp Arg Tyr Val Asp Ser His Leu Arg Gly Val Asn Ser Thr Asp Arg Ser Thr Asp Ser Tyr Val Phe Leu Glu Lys Asp Leu Leu Ala Leu Asp Phe Leu Gly Val Ser Phe Tyr Gln Phe Ser Ile Ser Trp Pro Arg Leu Phe Pro Asn Gly Thr Val Ala Ala Val Asn Ala Lys Gly Leu Gln Tyr Tyr Arg Ala Leu Leu Asp Ser Leu Val Leu Arg Asn Ile Glu Pro Ile Val Thr Leu Tyr His Trp Asp Leu Pro Leu Thr Leu Gln Glu Glu Tyr Gly Gly Trp Lys Asn Ala Thr Met Ile Asp Leu Phe Asn Asp Tyr Ala Thr Tyr Cys Phe Gln Thr Phe Gly Asp Arg Val Lys Tyr Trp Ile Thr Ile His Asn Pro Tyr Leu Val Ala Trp His Gly Phe Gly Thr Gly Met His Ala Pro Gly Glu Lys Gly Asn Leu Thr Ala Val Tyr Thr Val Gly His Asn Leu Ile Lys Ala His Ser Lys Val Trp His Asn Tyr Asp Lys Asn Phe Arg Pro His Gln Lys Gly Trp Leu Ser Ile Thr Leu Gly Ser His Trp Ile Glu Pro Asn Arg Thr Glu Asn Met Glu Asp Val Ile Asn Cys Gln His Ser Met Ser Ser Val Leu Gly Trp Phe Ala Asn Pro Ile His Gly Asp Gly Asp Tyr Pro Glu Phe Met Lys Thr Ser Ser Val Ile Pro Glu Phe Ser Glu Ala Glu Lys Glu Glu Val Arg Gly Thr Ala Asp Phe Phe Ala Phe Ser Phe Gly Pro Asn Asn Phe Arg Pro Ser Asn Thr Val Val Lys Met Gly Gln Asn Val Ser Leu Asn Leu Arg Gln Val Leu Asn Trp Ile Lys Leu Glu Tyr Asp Asn Pro Arg Ile Leu Ile Ser Glu Asn Gly Trp Phe Thr Asp Ser Tyr Ile Lys Thr Glu Asp Thr Thr Ala Ile Tyr Met Met Lys Asn Phe Leu Asn Gln Val Leu Gln Ala Ile Lys Phe Asp Glu Ile Gln Val Phe Gly Tyr Thr Ala Trp Thr Leu Leu Asp Gly Phe Glu Trp Gln Asp Ala Tyr Thr Thr Arg

Arg Gly Leu Phe Tyr Val Asp Phe Asn Ser Glu Gln Lys Glu Arg Lys Pro Lys Ser Ser Ala His Tyr Tyr Lys Gln Ile Ile Gln Asp Asn Gly Phe Pro Leu Gln Glu Ser Thr Pro Asp Met Lys Gly Gln Phe Pro Cys Asp Phe Ser Trp Gly Val Thr Glu Ser Val Leu Lys Pro Glu Phe Thr Val Ser Ser Pro Gln Phe Thr Asp Pro His Leu Tyr Val Trp Asn Val Thr Gly Asn Arg Leu Leu Tyr Arg Val Glu Gly Val Arg Leu Lys Thr Arg Pro Ser Gln Cys Thr Asp Tyr Val Ser Ile Lys Lys Arg Val Glu Met Leu Ala Lys Met Lys Val Thr His Tyr Gln Phe Ala Leu Asp Trp Thr Ser Ile Leu Pro Thr Gly Asn Leu Ser Lys Ile Asn Arg Gln Val Leu Arg Tyr Tyr Arg Cys Val Val Ser Glu Gly Leu Lys Leu Gly Ile Ser Pro Met Val Thr Leu Tyr His Pro Thr His Ser His Leu Gly Leu Pro Met Pro Leu Leu Ser Ser Gly Gly Trp Leu Asn Thr Asn Thr Ala Lys Ala Phe Gln Asp Tyr Ala Gly Leu Cys Phe Lys Glu Leu Gly Asp Leu Val Lys Leu Trp Ile Thr Ile Asn Glu Pro Asn Arg Leu Ser Asp Met Tyr Asn Arg Thr Ser Asn Asp Thr Tyr Arg Ala Ala His Asn Leu Met Ile Ala His Ala Gln Val Trp His Leu Tyr Asp Arg Gln Tyr Arg Pro Val Gln His Gly Ala Val Ser Leu Ser Leu His Ser Asp Trp Ala Glu Pro Ala Asn Pro Tyr Val Glu Ser His Trp Lys Ala Ala Glu Arg Phe Leu Gln Phe Glu Ile Ala Trp Phe Ala Asp Pro Leu Phe Lys Thr Gly Asp Tyr Pro Leu Ala Met Lys Glu Tyr Ile Ala Ser Lys Lys Gln Arg Gly Leu Ser Ser Ser Val Leu Pro Arg Phe Thr Leu Lys Glu Ser Arg Leu Val Lys Gly Thr Ile Asp Phe Tyr Ala Leu Asn His Phe Thr Thr Arg Phe Val Ile His Lys Gln Leu Asn Thr Asn Cys Ser Val Ala Asp Arg Asp Val Gln Phe Leu Gln Asp Ile Thr Arg Leu Ser Ser Pro Ser Arg Leu Ala Val Thr Pro Trp Gly Met Arg Lys Leu Leu Gly Trp Ile Arg Arg Asn Tyr Arg Asp Met Asp Ile Tyr Val Thr Ala Asn Gly Ile Asp Asp Leu Ala Leu Glu Asp Asp Gln Ile Arg Lys Tyr Tyr Leu Glu Lys Tyr Val Gln Glu Ala Leu Lys Ala Tyr Leu Ile Asp Lys Val Lys Ile Lys Gly Tyr Tyr Ala Phe Lys Leu Thr Glu Glu Lys Ser Lys Pro Arg Phe Gly Phe Phe Thr Ser Asp Phe Lys Ala Lys Ser Ser Val Gln Phe Tyr Ser Lys Leu Ile Ser Ser Ser Gly Phe Ser Ser Glu Asn Arg Ser Pro Ala Cys Gly Gln Pro Pro Glu Asp Thr Glu Cys Ala Ile Cys Ser Phe Leu Thr

SEQ ID NO:257

Asp Tyr Lys Asp Asp Asp Asp Lys Leu Asp Phe Pro Gly Asp Gly Arg Ala Val Trp Ser Gln Asn Pro Asn Leu Ser Pro Val Asn Glu Ser Gln Leu Phe Leu Tyr Asp Thr Phe Pro Lys Asn Phe Phe Trp Gly Val Gly Thr Gly Ala Phe Gln Val Glu Gly Ser Trp Lys Lys Asp Gly Lys Gly Leu Ser Val Trp Asp His Phe Ile Ala Thr His Leu Asn Val Ser Ser Arg Asp Gly Ser Ser Asp Ser Tyr Ile Phe Leu Glu Lys Asp Leu Ser Ala Leu Asp Phe Leu Gly Val Ser Phe Tyr Gln Phe Ser Ile Ser Trp Pro Arg Leu Phe Pro Asp Gly Thr Val Ala Val Ala Asn Ala Lys Gly Leu Gln Tyr Tyr Asn Arg Leu Leu Asp Ser Leu Leu Leu Arg Asn Ile Glu Pro Val Val Thr Leu Tyr His Trp Asp Leu Pro Trp Ala Leu Gln Glu Lys Tyr Gly Gly Trp Lys Asn Glu Thr Leu Ile Asp Leu Phe Asn Asp Tyr Ala Thr Tyr Cys Phe Gln Thr Phe Gly Asp Arg Val Lys Tyr Trp Ile Thr Ile His Asn Pro Tyr Leu Val Ala Trp His Gly Tyr Gly Thr Gly Leu His Ala Pro Gly Glu Lys Gly Asn Val Ala Ala Val Tyr Thr Val Gly His Asn Leu Leu Lys Ala His Ser Lys Val Trp His Asn Tyr Asn Arg Asn Phe Arg Pro His Gln Lys Gly Trp Leu Ser Ile Thr Leu Gly Ser His Trp Ile Glu Pro Asn Arg Ala Glu Ser Ile Val Asp Ile Leu Lys Cys Gln Gln Ser Met Val Ser Val Leu Gly Trp Phe Ala Asn Pro Ile His Gly Asp Gly Asp Tyr Pro Glu Val Met Thr Lys Lys Leu Leu Ser Val Leu Pro Ala Phe Ser Glu Ala Glu Lys Asn Glu Val Arg Gly Thr Ala Asp Phe Phe Ala Phe Ser Phe Gly Pro Asn Asn Phe Lys Pro Leu Asn Thr Met Ala Lys Met Gly Gln Asn Val Ser Leu Asn Leu Arg Gln Val Leu Asn Trp Ile Lys Leu Glu Tyr Gly Asn Pro Arg Ile Leu Ile Ala Glu Asn Gly Trp Phe Thr Asp Ser Tyr Val Gln Thr Glu Asp Thr Thr Ala Ile Tyr Met Met Lys Asn Phe Leu Asn Gln Val Leu Gln Ala Ile Arg Leu Asp Gly Val Arg Val Phe Gly Tyr Thr Ala Trp Ser Leu Leu Asp Gly Phe Glu Trp Gln Asp Ala Tyr Asn Thr Arg Arg Gly Leu Phe Tyr Val Asp Phe Asn Ser Glu Gln Arg Glu Arg Arg Pro Lys Ser Ser Ala His Tyr Tyr Lys Gln Val Ile Gly Glu Asn Gly Phe Thr Leu Arg Glu Ala Thr Pro Asp Leu Gln Gly Gln Phe Pro Cys Asp Phe Ser Trp Gly Val Thr Glu Ser Val Leu

Lys Pro Glu Ser Val Ala Ser Ser Pro Gln Phe Ser Asp Pro His Leu Tyr Val Trp Asn Ala Thr Gly Asn Arg Met Leu His Arg Val Glu Gly Val Arg Leu Lys Thr Arg Pro Ala Gln Cys Thr Asp Phe Ile Thr Ile Lys Lys Gln Leu Glu Met Leu Ala Arg Met Lys Val Thr His Phe Arg Phe Ala Leu Asp Trp Ala Ser Val Leu Pro Thr Gly Asn Leu Ser Glu Val Asn Arg Gln Ala Leu Arg Tyr Tyr Arg Cys Val Val Thr Glu Gly Leu Lys Leu Asn Ile Ser Pro Met Val Thr Leu Tyr Tyr Pro Thr His Ala His Leu Gly Leu Pro Ala Pro Leu Leu His Ser Gly Gly Trp Leu Asp Pro Ser Thr Ala Lys Ala Phe Arg Asp Tyr Ala Gly Leu Cys Phe Arg Glu Leu Gly Asp Leu Val Lys Leu Trp Ile Thr Ile Asn Glu Pro Asn Arg Leu Ser Asp Val Tyr Asn Arg Thr Ser Asn Asp Thr Tyr Gln Ala Ala His Asn Leu Leu Ile Ala His Ala Ile Val Trp His Leu Tyr Asp Arg Gln Tyr Arg Pro Ser Gln Arg Gly Ala Leu Ser Leu Ser Leu His Ser Asp Trp Ala Glu Pro Ala Asn Pro Tyr Val Ala Ser His Trp Gln Ala Ala Glu Arg Phe Leu Gln Phe Glu Ile Ala Trp Phe Ala Glu Pro Leu Phe Lys Thr Gly Asp Tyr Pro Val Ala Met Arg Glu Tyr Ile Ala Ser Lys Thr Arg Arg Gly Leu Ser Ser Ser Val Leu Pro Arg Phe Ser Asp Ala Glu Arg Arg Leu Val Lys Gly Ala Ala Asp Phe Tyr Ala Leu Asn His Phe Thr Thr Arg Phe Val Met His Glu Gln Gln Asn Gly Ser Arg Tyr Asp Ser Asp Arg Asp Val Gln Phe Leu Gln Asp Ile Thr Arg Leu Ala Ser Pro Ser Arg Leu Ala Val Met Pro Trp Gly Glu Gly Lys Leu Leu Arg Trp Met Arg Asn Asn Tyr Gly Asp Leu Asp Val Tyr Ile Thr Ala Asn Gly Ile Asp Asp Gln Ala Leu Gln Asn Asp Gln Leu Arg Gln Tyr Tyr Leu Glu Lys Tyr Val Gln Glu Ala Leu Lys Ala Tyr Leu Ile Asp Lys Ile Lys Ile Lys Gly Tyr Tyr Ala Phe Lys Leu Thr Glu Glu Lys Ser Lys Pro Arg Phe Gly Phe Phe Thr Ser Asp Phe Lys Ala Lys Ser Ser Ile Gln Phe Tyr Asn Lys Leu Ile Thr Ser Asn Gly Phe Pro Ser Glu Asn Gly Gly Pro Arg Cys Asn Gln Thr Gln Gly Asn Pro Glu Cys Thr Val Cys Leu Leu Leu Leu

SEQ ID NO:258

Asp Tyr Lys Asp Asp Asp Asp Lys Leu Glu Phe Ser Gly Asp Gly Arg Ala Val Trp Ser Lys Asn Pro Asn Phe Thr Pro Val Asn Glu Ser Gln Leu Phe Leu Tyr Asp Thr Phe Pro Lys Asn Phe Phe Trp Gly Val Gly Thr Gly Ala Leu Gln Val Glu Gly Ser Trp Lys Lys Asp Gly Lys Gly Pro Ser Ile Trp Asp His Phe Val His Thr His Leu Lys Asn Val Ser Ser Thr Asn Gly Ser Ser Asp Ser Tyr Ile Phe Leu Glu Lys Asp Leu Ser Ala Leu Asp Phe Ile Gly Val Ser Phe Tyr Gln Phe Ser Ile Ser Trp Pro Arg Leu Phe Pro Asp Gly Ile Val Thr Val Ala Asn Ala Lys Gly Leu Gln Tyr Tyr Asn Thr Leu Leu Asp Ser Leu Val Leu Arg Asn
Ile Glu Pro Ile Val Thr Leu Tyr His Trp Asp Leu Pro Leu Ala Leu Gln Glu Lys Tyr Gly Gly Trp Lys Asn Asp Thr Ile Ile Asp Ile Phe Asn Asp Tyr Ala Thr Tyr Cys Phe Gln Thr Phe Gly Asp Arg Val Lys Tyr Trp Ile Thr Ile His Asn Pro Tyr Leu Val Ala Trp His Gly Tyr Gly Thr Gly Met His Ala Pro Gly Glu Lys Gly Asn Leu Ala Ala Val Tyr Thr Val Gly His Asn Leu Ile Lys Ala His Ser Lys Val Trp His Asn Tyr Asn Thr His Phe Arg Pro His Gln Lys Gly Trp Leu Ser Ile Thr Leu Gly Ser His Trp Ile Glu Pro Asn Arg Ser Glu Asn Thr Met Asp Ile Leu Lys Cys Gln Gln Ser Met Val Ser Val Leu Gly Trp Phe Ala Ser Pro Ile His Gly Asp Gly Asp Tyr Pro Glu Gly Met Lys Lys Lys Leu Leu Ser Ile Leu Pro Leu Phe Ser Glu Ala Glu Lys Asn Glu Val Arg Gly Thr Ala Asp Phe Phe Ala Phe Ser Phe Gly Pro Asn Asn Phe Lys Pro Leu Asn Thr Met Ala Lys Met Gly Gln Asn Val Ser Leu Asn Leu Arg Glu Ala Leu Asn Trp Ile Lys Leu Glu Tyr Asn Asn Pro Arg Ile Leu Ile Ala Glu Asn Gly Trp Phe Thr Asp Ser His Val Lys Thr Glu Asp Thr Thr Ala Ile Tyr Met Met Lys Asn Phe Leu Ser Gln Val Leu Gln Ala Ile Arg Leu Asp Glu Ile Arg Val Phe Gly Tyr Thr Ala Trp Ser Leu Leu Asp Gly Phe Glu Trp Gln Asp Ala Tyr Thr Ile Arg Arg Gly Leu Phe Tyr Val Asp Phe Asn Ser Lys Gln Lys Glu Arg Lys Pro Lys Ser Ser Ala His Tyr Tyr Lys Gln Ile Ile Arg Glu Asn Gly Phe Ser Leu Lys Glu Ala Thr Pro Asp Val Gln Gly Gln Phe Pro Cys Asp Phe Ser Trp Gly Val Thr Glu Ser Val Leu Lys Pro Glu Ser Val Ala Ser Ser Pro Gln Phe Ser Asp Pro Tyr Leu Tyr Val Trp Asn Ala Thr Gly Asn Arg Leu Leu His Arg Val Glu Gly Val Arg Leu Lys Thr Arg Pro Ala Gln Cys Thr Asp Phe Val Asn Ile Lys Lys Gln Leu Glu Met Leu Ala Arg Met Lys Val Thr His Tyr

Arg Phe Ala Leu Asp Trp Ala Ser Val Leu Pro Thr Gly Asn Leu Ser Ala Val Asn Arg Gln Ala Leu Arg Tyr Tyr Arg Cys Val Val Ser Glu Gly Leu Lys Leu Gly Ile Ser Ala Met Val Thr Leu Tyr Tyr Pro Thr His Ala His Leu Gly Leu Pro Glu Pro Leu Leu His Ala Gly Gly Trp Leu Asn Pro Ser Thr Val Glu Ala Phe Gln Ala Tyr Ala Gly Leu Cys Phe Gln Glu Leu Gly Asp Leu Val Lys Leu Trp Ile Thr Ile Asn Glu Pro Asn Arg Leu Ser Asp Ile Tyr Asn Arg Ser Gly Asn Asp Thr Tyr Gly Ala Ala His Asn Leu Leu Val Ala His Ala Leu Ala Trp Arg Leu Tyr Asp Arg Gln Phe Arg Pro Ser Gln Arg Gly Ala Val Ser Leu Ser Leu His Ala Asp Trp Ala Glu Pro Ala Asn Pro Tyr Ala Asp Ser His Trp Arg Ala Ala Glu Arg Phe Leu Gln Phe Glu Ile Ala Trp Phe Ala Glu Pro Leu Phe Lys Thr Gly Asp Tyr Pro Ala Ala Met Arg Glu Tyr Ile Ala Ser Lys His Arg Arg Gly Leu Ser Ser Ser Ala Leu Pro Arg Leu Thr Glu Ala Glu Arg Arg Leu Leu Lys Gly Thr Val Asp Phe Cys Ala Leu Asn His Phe Thr Thr Arg Phe Val Met His Glu Gln Leu Ala Gly Ser Arg Tyr Asp Ser Asp Arg Asp Ile Gln Phe Leu Gln Asp Ile Thr Arg Leu Ser Ser Pro Thr Arg Leu Ala Val Ile Pro Trp Gly Val Arg Lys Leu Leu Arg Trp Val Arg Arg Asn Tyr Gly Asp Met Asp Ile Tyr Ile Thr Ala Ser Gly Ile Asp Asp Gln Ala Leu Glu Asp Asp Arg Leu Arg Lys Tyr Tyr Leu Glu Lys Tyr Leu Gln Glu Val Leu Lys Ala Tyr Leu Ile Asp Lys Val Arg Ile Lys Gly Tyr Tyr Ala Phe Lys Leu Ala Glu Glu Lys Ser Lys Pro Arg Phe Gly Phe Phe Thr Ser Asp Phe Lys Ala Lys Ser Ser Ile Gln Phe Tyr Asn Lys Met Ile Ser Ser Ser Gly Phe Pro Ser Glu Asn Ser Ser Ser Arg Cys Ser Gln Thr Gln Lys Asn Thr Glu Cys Thr Val Cys Leu Phe Leu Ala

SEQ ID NO:259

Asp Tyr Lys Asp Asp Asp Asp Lys Leu Glu Phe Ser Gly Asp Gly Arg Ala Val Trp Ser Lys Asn Pro Asn Phe Thr Pro Val Asn Glu Ser Gln Leu Phe Leu Tyr Asp Thr Phe Pro Lys Asn Phe Phe Trp Gly Val Gly Thr Gly Ala Leu Gln Val Glu Gly Ser Trp Lys Lys Asp Gly Lys Gly Pro Ser Ile Trp Asp His Phe Val His Thr His Leu Lys Asn Val Ser Ser Thr Asn Gly Ser Ser Asp Ser Tyr Ile Phe Leu Glu Lys Asp Leu Ser Ala Leu Asp Phe Ile Gly Val Ser Phe Tyr Gln Phe Ser Ile Ser Trp Pro Arg Leu Phe Pro Asp Gly Ile Val Thr Val Ala Asn Ala Lys Gly Leu Gln Tyr Tyr Asn Ala Leu Leu Asp Ser Leu Val Leu Arg Asn Ile Glu Pro Ile Val Thr Leu Tyr His Trp Asp Leu Pro Leu Ala Leu Gln Glu Lys Tyr Gly Gly Trp Lys Asn Asp Thr Ile Ile Asp Ile Phe Asn Asp Tyr Ala Thr Tyr Cys Phe Gln Thr Phe Gly Asp Arg Val Lys Tyr Trp Ile Thr Ile His Asn Pro Tyr Leu Val Ala Trp His Gly Tyr Gly Thr Gly Met His Ala Pro Gly Glu Lys Gly Asn Leu Ala Ala Val Tyr Thr Val Gly His Asn Leu Ile Lys Ala His Ser Lys Val Trp His Asn Tyr Asn Thr His Phe Arg Pro His Gln Lys Gly Trp Leu Ser Ile Thr Leu Gly Ser His Trp Ile Glu Pro Asn Arg Ser Glu Asn Thr Met Asp Ile Leu Lys Cys Gln Gln Ser Met Val Ser Val Leu Gly Trp Phe Ala Asn Pro Ile His Gly Asp Gly Asp Tyr Pro Glu Gly Met Lys Lys Lys Leu Leu Ser Ile Leu Pro Leu Phe Ser Glu Ala Glu Lys Asn Glu Val Arg Gly Thr Ala Asp Phe Phe Ala Phe Ser Phe Gly Pro Asn Asn Phe Lys Pro Leu Asn Thr Met Ala Lys Met Gly Gln Asn Val Ser Leu Asn Leu Arg Glu Ala Leu Asn Trp Ile Lys Leu Glu Tyr Asn Asn Pro Gln Ile Leu Ile Ala Glu Asn Gly Trp Phe Thr Asp Ser His Val Lys Thr Glu Asp Thr Thr Ala Ile Tyr Met Met Lys Asn Phe Leu Ser Gln Val Leu Gln Ala Ile Arg Leu Asp Glu Ile Arg Val Phe Gly Tyr Thr Ala Trp Ser Leu Leu Asp Gly Phe Glu Trp Gln Asp Ala Tyr Thr Ile Arg Arg Gly Leu Phe Tyr Val Asp Phe Asn Ser Lys Gln Lys Glu Arg Lys Pro Lys Ser Ser Ala His Tyr Tyr Lys Gln Ile Ile Arg Glu Asn Gly Phe Ser Leu Lys Glu Ala Thr Pro Asp Val Gln Gly Gln Phe Pro Cys Asp Phe Ser Trp Gly Val Thr Glu Ser Val Leu Lys Pro Glu Ser Val Ala Ser Ser Pro Gln Phe Ser Asp Pro Tyr Leu Tyr Val Trp Asn Ala Thr Gly Asn Arg Leu Leu His Arg Val Glu Gly Val Arg Leu Lys Thr Arg Pro Ala Gln Cys Thr Asp Phe Val Asn Ile Lys Lys Gln Leu Glu Met Leu Ala Arg Met Lys Val Thr His Tyr Arg Phe Ala Leu Asp Trp Ala Ser Val Leu Pro Thr Gly Asn Leu Ser Ala Val Asn Arg Gln Ala Leu Arg Tyr Tyr Arg Cys Val Val Ser Glu Gly Leu Lys Leu Gly Ile Ser Ala Met Val Thr Leu Tyr Tyr Pro Thr His Ala His Leu Gly Leu Pro Glu Pro Leu Leu His Ala Gly Gly Trp Leu Asn Pro Ser Thr Val

Glu Ala Phe Gln Ala Tyr Ala Gly Leu Cys Phe Gln Glu Leu Gly Asp Leu Val Lys Leu Trp Ile Thr Ile Asn Glu Pro Asn Arg Leu Ser Asp Ile Tyr Asn Arg Ser Gly Asn Asp Thr Tyr Gly Ala Ala His Asn Leu Leu Val Ala His Ala Leu Ala Trp Arg Leu Tyr Asp Arg Gln Phe Arg Pro Ser Gln Arg Gly Ala Val Ser Leu Ser Leu His Ala Asp Trp Ala Glu Pro Ala Asn Pro Tyr Ala Asp Ser His Trp Arg Ala Ala Glu Arg Phe Leu Gln Phe Glu Ile Ala Trp Phe Ala Glu Pro Leu Phe Lys Thr Gly Asp Tyr Pro Ala Ala Met Arg Glu Tyr Ile Ala Ser Lys His Arg Arg Gly Leu Ser Ser Ser Ala Leu Pro Arg Leu Thr Glu Ala Glu Arg Arg Leu Leu Lys Gly Thr Val Asp Phe Cys Ala Leu Asn His Phe Thr Thr Arg Phe Val Met His Glu Gln Leu Ala Gly Ser Arg Tyr Asp Ser Asp Arg Asp Ile Gln Phe Leu Gln Asp Ile Thr Arg Leu Ser Ser Pro Thr Arg Leu Ala Val Ile Pro Trp Gly Val Arg Lys Leu Leu Arg Trp Val Arg Arg Asn Tyr Gly Asp Met Asp Ile Tyr Ile Thr Ala Ser Gly Ile Asp Asp Gln Ala Leu Glu Asp Asp Arg Leu Arg Lys Tyr Tyr Leu Glu Lys Tyr Leu Gln Glu Val Leu Lys Ala Tyr Leu Ile Asp Lys Val Arg Ile Lys Gly Tyr Tyr Ala Phe Lys Leu Ala Glu Glu Lys Ser Lys Pro Arg Phe Gly Phe Phe Thr Ser Asp Phe Lys Ala Lys Ser Ser Ile Gln Phe Tyr Asn Lys Met Ile Ser Ser Ser Gly Phe Pro Ser Glu Asn Ser Ser Ser Arg Cys Ser Gln Thr Gln Lys Asn Thr Glu Cys Thr Val Cys Leu Phe Leu Val

SEQ ID NO:260

Glu Pro Gly Asp Gly Ala Gln Thr Trp Ala Arg Phe Ser Arg Pro Pro Ala Pro Glu Ala Ala Gly Leu Phe Gln Gly Thr Phe Pro Asp Gly Phe Leu Trp Ala Val Gly Ser Ala Ala Tyr Gln Thr Glu Gly Gly Trp Gln Gln His Gly Lys Gly Ala Ser Ile Trp Asp Thr Phe Thr His His Pro Leu Ala Pro Pro Gly Asp Ser Arg Asn Ala Ser Leu Pro Leu Gly Ala Pro Ser Pro Leu Gln Pro Ala Thr Gly Asp Val Ala Ser Asp Ser Tyr Asn Asn Val Phe Arg Asp Thr Glu Ala Leu Arg Glu Leu Gly Val Thr His Tyr Arg Phe Ser Ile Ser Trp Ala Arg Val Leu Pro Asn Gly Ser Ala Gly Val Pro Asn Arg Glu Gly Leu Arg Tyr Tyr Arg Arg Leu Leu Glu Arg Leu Arg Glu Leu Gly Val Gln Pro Val Val Thr Leu Tyr His Trp Asp Leu Pro Gln Arg Leu Gln Asp Ala Tyr Gly Gly Trp Ala Asn Arg Ala Leu Ala Asp His Phe Arg Asp Tyr Ala Glu Leu Cys Phe Arg His Phe Gly Gly Gln Val Lys Tyr Trp Ile Thr Ile Asp Asn Pro Tyr Val Val Ala Trp His Gly Tyr Ala Thr Gly Arg Leu Ala Pro Gly Ile Arg Gly Ser Pro Arg Leu Gly Tyr Leu Val Ala His Asn Leu Leu Leu Ala His Ala Lys Val Trp His Leu Tyr Asn Thr Ser Phe Arg Pro Thr Gln Gly Gly Gln Val Ser Ile Ala Leu Ser Ser His Trp Ile Asn Pro Arg Arg Met Thr Asp His Ser Ile Lys Glu Cys Gln Lys Ser Leu Asp Phe Val Leu Gly Trp Phe Ala Lys Pro Val Phe Ile Asp Gly Asp Tyr Pro Glu Ser Met Lys Asn Asn Leu Ser Ser Ile Leu Pro Asp Phe Thr Glu Ser Glu Lys Lys Phe Ile Lys Gly Thr Ala Asp Phe Phe Ala Leu Cys Phe Gly Pro Thr Leu Ser Phe Gln Leu Leu Asp Pro His Met Lys Phe Arg Gln Leu Glu Ser Pro Asn Leu Arg Gln Leu Leu Ser Trp Ile Asp Leu Glu Phe Asn His Pro Gln Ile Phe Ile Val Glu Asn Gly Trp Phe Val Ser Gly Thr Thr Lys Arg Asp Asp Ala Lys Tyr Met Tyr Tyr Leu Lys Lys Phe Ile Met Glu Thr Leu Lys Ala Ile Lys Leu Asp Gly Val Asp Val Ile Gly Tyr Thr Ala Trp Ser Leu Met Asp Gly Phe Glu Trp His Arg Gly Tyr Ser Ile Arg Arg Gly Leu Phe Tyr Val Asp Phe Leu Ser Gln Asp Lys Met Leu Leu Pro Lys Ser Ser Ala Leu Phe Tyr Gln Lys Leu Ile Glu Lys Asn Gly Phe Pro Pro Leu Pro Glu Asn Gln Pro Leu Glu Gly Thr Phe Pro Cys Asp Phe Ala Trp Gly Val Val Asp Asn Tyr Ile Gln Val Asp Thr Thr Leu Ser Gln Phe Thr Asp Leu Asn Val Tyr Leu Trp Asp Val His His Ser Lys Arg Leu Ile Lys Val Asp Gly Val Val Thr Lys Lys Arg Lys Ser Tyr Cys Val Asp Phe Ala Ala Ile Gln Pro Gln Ile Ala Leu Leu Gln Glu Met His Val Thr His Phe Arg Phe Ser Leu Asp Trp Ala Leu Ile Leu Pro Leu Gly Asn Gln Ser Gln Val Asn His Thr Ile Leu Gln Tyr Tyr Arg Cys Met Ala Ser Glu Leu Val Arg Val Asn Ile Thr Pro Val Val Ala Leu Trp Gln Pro Met Ala Pro Asn Gln Gly Leu Pro Arg Leu Leu Ala Arg Gln Gly Ala Trp Glu Asn Pro Tyr Thr Ala Leu Ala Phe Ala Glu Tyr Ala Arg Leu Cys Phe Gln Glu Leu Gly His His Val Lys Leu Trp Ile Thr Met Asn Glu Pro Tyr Thr Arg Asn Met Thr Tyr Ser Ala Gly His Asn Leu Leu Lys Ala His Ala Leu Ala Trp His Val Tyr Asn Glu Lys Phe Arg His Ala Gln

Asn Gly Lys Ile Ser Ile Ala Leu Gln Ala Asp Trp Ile Glu Pro Ala Cys Pro Phe Ser Gln Lys Asp Lys Glu Val Ala Glu Arg Val Leu Glu Phe Asp Ile Gly Trp Leu Ala Glu Pro Ile Phe Gly Ser Gly Asp Tyr Pro Trp Val Met Arg Asp Trp Leu Asn Gln Arg Asn Asn Phe Leu Leu Pro Tyr Phe Thr Glu Asp Glu Lys Lys Leu Ile Gln Gly Thr Phe Asp Phe Leu Ala Leu Ser His Tyr Thr Thr Ile Leu Val Asp Ser Glu Lys Glu Asp Pro Ile Lys Tyr Asn Asp Tyr Leu Glu Val Gln Glu Met Thr Asp Ile Thr Trp Leu Asn Ser Pro Ser Gln Val Ala Val Val Pro Trp Gly Leu Arg Lys Val Leu Asn Trp Leu Lys Phe Lys Tyr Gly Asp Leu Pro Met Tyr Ile Ile Ser Asn Gly Ile Asp Asp Gly Leu His Ala Glu Asp Asp Gln Leu Arg Val Tyr Tyr Met Gln Asn Tyr Ile Asn Glu Ala Leu Lys Ala His Ile Leu Asp Gly Ile Asn Leu Cys Gly Tyr Phe Ala Tyr Ser Phe Asn Asp Arg Thr Ala Pro Arg Phe Gly Leu Tyr Arg Tyr Ala Ala Asp Gln Phe Glu Pro Lys Ala Ser Met Lys His Tyr Arg Lys Ile Ile Asp Ser Asn Gly Phe Pro Gly Pro Glu Thr Leu Glu Arg Phe Cys Pro Glu Glu Phe Thr Val Cys Thr Glu Cys Ser Phe Phe His Thr Arg Lys Ser Leu Leu Ala Phe Ile Ala Phe Leu Phe Phe Ala Ser Ile Ile Ser Leu Ser Leu Ile Phe Tyr Tyr Ser Lys Lys Gly Arg Arg Ser Tyr Lys Leu Glu Asp Tyr Lys Asp Asp Asp Asp Lys

SEQ ID NO:261
Ser Thr Tyr Ile Ser

SEQ ID NO:262
Glu Ile Asp Pro Tyr Asp Gly Ala Thr Asp Tyr Ala Asp Ser Val Lys Gly

SEQ ID NO:263
Glu His Phe Asp Ala Trp Val His Tyr Tyr Val Met Asp Tyr

SEQ ID NO:264
Phe Pro Cys Asp Phe Ser Trp Gly Val Thr Glu Ser Val Leu Lys Pro Glu Ser Val Ala Ser Ser Pro Gln Phe Ser Asp Pro His Leu Tyr Val Trp Asn Ala Thr Gly Asn Arg Leu Leu His Arg Val Glu Gly Val Arg Leu Lys Thr Arg Pro Ala Gln Cys Thr Asp Phe Val Asn Ile Lys Lys Gln Leu Glu Met Leu Ala Arg Met Lys Val Thr His Tyr Arg Phe Ala Leu Asp Trp Ala Ser Val Leu Pro Thr Gly Asn Leu Ser Ala Val Asn Arg Gln Ala Leu Arg Tyr Tyr Arg Cys Val Val Ser Glu Gly Leu Lys Leu Gly Ile Ser Ala Met Val Thr Leu Tyr Tyr Pro Thr His Ala His Leu Gly Leu Pro Glu Pro Leu Leu His Ala Asp Gly Trp Leu Asn Pro Ser Thr Ala Glu Ala Phe Gln Ala Tyr Ala Gly Leu Cys Phe Gln Glu Leu Gly Asp Leu Val Lys Leu Trp Ile Thr Ile Asn Glu Pro Asn Arg Leu Ser Asp Ile Tyr Asn Arg Ser Gly Asn Asp Thr Tyr Gly Ala Ala His Asn Leu Leu Val Ala His Ala Leu Ala Trp Arg Leu Tyr Asp Arg Gln Phe Arg Pro Ser Gln Arg Gly Ala Val Ser Leu Ser Leu His Ala Asp Trp Ala Glu Pro Ala Asn Pro Tyr Ala Asp Ser His Trp Arg Ala Ala Glu Arg Phe Leu Gln Phe Glu Ile Ala Trp Phe Ala Glu Pro Leu Phe Lys Thr Gly Asp Tyr Pro Ala Ala Met Arg Glu Tyr Ile Ala Ser Lys His Arg Arg Gly Leu Ser Ser Ser Ala Leu Pro Arg Leu Thr Glu Ala Glu Arg Arg Leu Leu Lys Gly Thr Val Asp Phe Cys Ala Leu Asn His Phe Thr Thr Arg Phe Val Met His Glu Gln Leu Ala Gly Ser Arg Tyr Asp Ser Asp Arg Asp Ile Gln Phe Leu Gln Asp Ile Thr Arg Leu Ser Ser Pro Thr Arg Leu Ala Val Ile Pro Trp Gly Val Arg Lys Leu Leu Arg Trp Val Arg Arg Asn Tyr Gly Asp Met Asp Ile Tyr Ile Thr Ala Ser Gly Ile Asp Asp Gln Ala Leu Glu Asp Asp Arg Leu Arg Lys Tyr Tyr Leu Gly Lys Tyr Leu Gln Glu Val Leu Lys Ala Tyr Leu Ile Asp Lys Val Arg Ile Lys Gly Tyr Tyr Ala Phe Lys Leu Ala Glu Glu Lys Ser Lys Pro Arg Phe Gly Phe Phe Thr Ser Asp Phe Lys Ala Lys Ser Ser Ile Gln Phe Tyr Asn Lys Val Ile Ser Ser Arg Gly Phe Pro Phe Glu Asn Ser Ser Ser Arg

SEQ ID NO:265
gttaccggct tctccggaga cgggaaagca atatgg

SEQ ID NO:266
Met Lys Pro Gly Cys Ala Ala Gly Ser Pro Gly Asn Glu Trp Ile Phe Phe Ser Thr Asp Glu Ile Thr Thr Arg Tyr Arg Asn Thr Met Ser Asn Gly Gly Leu Gln Arg Ser Val Ile Leu Ser Ala Leu Ile Leu Leu Arg Ala Val Thr Gly

SEQ ID NO:267
Phe Ser Gly Asp Gly Lys Ala Ile Trp Asp Lys Lys Gln Tyr Val Ser Pro Val Asn Pro Ser Gln Leu Phe Leu Tyr Asp Thr Phe Pro Lys Asn Phe Ser Trp Gly Val Gly Thr Gly Ala Phe Gln Val Glu Gly Ser Trp Lys Thr Asp Gly Arg Gly Pro Ser Ile Trp Asp Arg Tyr Val Tyr Ser His Leu Arg Gly Val Asn Gly Thr Asp Arg Ser Thr Asp Ser Tyr Ile Phe Leu Glu Lys Asp Leu Leu Ala Leu Asp Phe Leu Gly Val Ser Phe Tyr Gln Phe Ser Ile Ser Trp Pro Arg Leu Phe Pro Asn Gly Thr Val Ala Ala Val Asn Ala Gln Gly Leu Arg Tyr Tyr Arg Ala Leu Leu Asp Ser Leu Val Leu Arg Asn Ile Glu Pro Ile Val Thr Leu Tyr His Trp Asp Leu Pro Leu Thr Leu Gln Glu Glu Tyr Gly Gly Trp Lys Asn Ala Thr Met Ile Asp Leu Phe Asn Asp Tyr Ala Thr Tyr Cys Phe Gln Thr Phe Gly Asp Arg Val Lys Tyr Trp Ile Thr Ile His Asn Pro Tyr Leu Val Ala Trp His Gly Phe Gly Thr Gly Met His Ala Pro Gly Glu Lys Gly Asn Leu Thr Ala Val Tyr Thr Val Gly His Asn Leu Ile Lys Ala His Ser Lys Val Trp His Asn Tyr Asp Lys Asn Phe Arg Pro His Gln Lys Gly Trp Leu Ser Ile Thr Leu Gly Ser His Trp Ile Glu Pro Asn Arg Thr Asp Asn Met Glu Asp Val Ile Asn Cys Gln His Ser Met Ser Ser Val Leu Gly Trp Phe Ala Asn Pro Ile His Gly Asp Gly Asp Tyr Pro Glu Phe Met Lys Thr Gly Ala Met Ile Pro Glu Phe Ser Glu Ala Glu Lys Glu Glu Val Arg Gly Thr Ala Asp Phe Phe Ala Phe Ser Phe Gly Pro Asn Asn Phe Arg Pro Ser Asn Thr Val Val Lys Met Gly Gln Asn Val Ser Leu Asn Leu Arg Gln Val Leu Asn Trp Ile Lys Leu Glu Tyr Asp Asp Pro Gln Ile Leu Ile Ser Glu Asn Gly Trp Phe Thr Asp Ser Tyr Ile Lys Thr Glu Asp Thr Thr Ala Ile Tyr Met Met Lys Asn Phe Leu Asn Gln Val Leu Gln Ala Ile Lys Phe Asp Glu Ile Arg Val Phe Gly Tyr Thr Ala Trp Thr Leu Leu Asp Gly Phe Glu Trp Gln Asp Ala Tyr Thr Thr Arg Arg Gly Leu Phe Tyr Val Asp Phe Asn Ser Glu Gln Lys Glu Arg Lys Pro Lys Ser Ser Ala His Tyr Tyr Lys Gln Ile Ile Gln Asp Asn Gly Phe Pro Leu Lys Glu Ser Thr Pro Asp Met Lys Gly Arg Phe Pro Cys Asp Phe Ser Trp Gly Val Thr Glu Ser Val Leu Lys Pro Glu Phe Thr Val Ser Ser Pro Gln Phe Thr Asp Pro His Leu Tyr Val Trp Asn Val Thr Gly Asn Arg Leu Leu Tyr Arg Val Glu Gly Val Arg Leu Lys Thr Arg Pro Ser Gln Cys Thr Asp Tyr Val Ser Ile Lys Lys Arg Val Glu Met Leu Ala Lys Met Lys Val Thr His Tyr Gln Phe Ala Leu Asp Trp Thr Ser Ile Leu Pro Thr Gly Asn Leu Ser Lys Val Asn Arg Gln Val Leu Arg Tyr Tyr Arg Cys Val Val Ser Glu Gly Leu Lys Leu Gly Val Phe Pro Met Val Thr Leu Tyr His Pro Thr His Ser His Leu Gly Leu Pro Leu Pro Leu Leu Ser Ser Gly Gly Trp Leu Asn Met Asn Thr Ala Lys Ala Phe Gln Asp Tyr Ala Glu Leu Cys Phe Arg Glu Leu Gly Asp Leu Val Lys Leu Trp Ile Thr Ile Asn Glu Pro Asn Arg Leu Ser Asp Met Tyr Asn Arg Thr Ser Asn Asp Thr Tyr Arg Ala Ala His Asn Leu Met Ile Ala His Ala Gln Val Trp His Leu Tyr Asp Arg Gln Tyr Arg Pro Val Gln His Gly Ala Val Ser Leu Ser Leu His Cys Asp Trp Ala Glu Pro Ala Asn Pro Phe Val Asp Ser His Trp Lys Ala Ala Glu Arg Phe Leu Gln Phe Glu Ile Ala Trp Phe Ala Asp Pro Leu Phe Lys Thr Gly Asp Tyr Pro Ser Val Met Lys Glu Tyr Ile Ala Ser Lys Asn Gln Arg Gly Leu Ser Ser Ser Val Leu Pro Arg Phe Thr Ala Lys Glu Ser Arg Leu Val Lys Gly Thr Val Asp Phe Tyr Ala Leu Asn His Phe Thr Thr Arg Phe Val Ile His Lys Gln Leu Asn Thr Asn Arg Ser Val Ala Asp Arg Asp Val Gln Phe Leu Gln Asp Ile Thr Arg Leu Ser Ser Pro Ser Arg Leu Ala Val Thr Pro Trp Gly Val Arg Lys Leu Leu Ala Trp Ile Arg Arg Asn Tyr Arg Asp Arg Asp Ile Tyr Ile Thr Ala Asn Gly Ile Asp Asp Leu Ala Leu Glu Asp Asp Gln Ile Arg Lys Tyr Tyr Leu Glu Lys Tyr Val Gln Glu Ala Leu Lys Ala Tyr Leu Ile Asp Lys Val Lys Ile Lys Gly Tyr Tyr Ala Phe Lys Leu Thr Glu Glu Lys Ser Lys Pro Arg Phe Gly Phe Phe Thr Ser Asp Phe Arg Ala Lys Ser Ser Val Gln Phe Tyr

Ser Lys Leu Ile Ser Ser Ser Gly Leu Pro Ala Glu Asn Arg Ser Pro Ala Cys Gly Gln Pro Ala Glu Asp Thr Asp Cys Thr Ile Cys Ser Phe Leu Val

SEQ ID NO:268
Met Glu Lys Lys Leu His Ala Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp

SEQ ID NO:269
Ser Ser Pro Thr Arg Leu Ala Val Ile Pro Trp Gly Val Arg Lys Leu Leu Arg Trp Val Arg Arg Asn Tyr Gly Asp Met Asp Ile Tyr Ile Thr Ala Ser

SEQ ID NO:270
Ser Ser Pro Thr Arg Leu Ala Val Ile Pro Trp Gly Val Arg Lys Leu Leu Arg Trp Val Arg Arg Asn Tyr Gly Asp Met Asp Ile Tyr Ile Thr Ala Ser

SEQ ID NO:271
Ser Ser Pro Ser Arg Leu Ala Val Thr Pro Trp Gly Met Arg Lys Leu Leu Gly Trp Ile Arg Arg Asn Tyr Arg Asp Met Asp Ile Tyr Val Thr Ala Asn

SEQ ID NO:272
Ser Ser Pro Ser Arg Leu Ala Val Thr Pro Trp Gly Val Arg Lys Leu Leu Ala Trp Ile Arg Arg Asn Tyr Arg Asp Arg Asp Ile Tyr Ile Thr Ala Asn

SEQ ID NO:273
Ala Ser Pro Ser Arg Leu Ala Val Met Pro Trp Gly Glu Gly Lys Leu Leu Arg Trp Met Arg Asn Asn Tyr Gly Asp Leu Asp Val Tyr Ile Thr Ala Asn

SEQ ID NO:274
Phe Ser Gly Asp Gly Lys Ala Ile Trp Asp Lys Lys Gln Tyr Val Ser Pro

SEQ ID NO:275
Phe Ser Glu Thr Gly Lys Gln Tyr Gly Ile Lys Asn Ser Thr

SEQ ID NO:276
2B.1.1.6 HVR-L1
RASQDVDTSLA

SEQ ID NO:277
2B.1.1.6 HVR-L2
SASFLYS

SEQ ID NO:278
2B.1.1.6 HVR-L3
QQSTGHPQT

SEQ ID NO:279
2B.1.1.6 HVR-H1
GFTFTSTGIS

SEQ ID NO:280
2B.1.1.6 HVR-H2
RYWAWDGSTNYADSVKG

SEQ ID NO:281
2B.1.1.6 HVR-H3
ARTYGIYDTYDEYTEYVMDY

SEQ ID NO:282
2B.1.1.6 HC
EVQLVESGGGLVQPGGSLRLSCAASGFTFTSTGISWVRQAPGKGLEWVGRYWAWD
GSTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTYGIYDTYDEYTE
YVMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPGK

SEQ ID NO:283
2B.1.1.6 LC
DIQMTQSPSSLSASVGDRVTITCRASQDVDTSLAWYKQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGHPQTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

WHAT IS CLAIMED IS:

1. An isolated antibody that binds to FGFR2 and FGFR3, wherein the binding of FGFR1 and FGFR4 is not detected by surface plasmon resonance.

2. An isolated antibody, wherein the antibody binds FGFR2 and/or FGFR3.

3. The isolated antibody of claim 1 or 2, wherein the antibody binds FGFR2-IIIb, FGFR2-IIIc, FGFR3-IIIb, and/or FGFR3-IIIc.

4. The isolated antibody of any of claims 1-3, wherein the antibody binds human FGFR2 and/or FGFR3.

5. The isolated antibody of any of claims 1-4, wherein wherein the antibody possesses little or no agonist function.

6. The isolated antibody of any of claims 1-5, wherein the antibody possesses effector function.

7. The isolated antibody of claim 6, wherein the effector function comprises antibody-dependent cell-mediated cytotoxicity.

8. The isolated antibody of any of claims 1-7, wherein the antibody is a cytotoxic agent that inhibits or prevents the function of cells and/or causes destruction of cells.

9. The isolated antibody of claim 8, wherein the cells are cancer cells.

10. The isolated antibody of claim 9, wherein the cancer cells are multiple myeloma cells comprising a t(4;14) translocation, breast cancer cells, triple negative breast cancer cells, myeloma cells, and bladder cancer cells.

11. The isolated antibody of any of claims 1-10 wherein the antibody binds to an epitope, wherein the epitope includes at one the amino acid sequence selected from TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and YKVRNQHWSLIMES (SEQ ID NO:92).

12. The isolated antibody of any of claims 1-11 wherein the antibody binds to an epitope, wherein the epitope includes at least one amino acid sequence selected from TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO:94).

13. The isolated antibody of any of claims 1-12 wherein the antibody binds to an epitope, wherein the epitope includes the amino acid sequences of SEQ ID NOs: 91 and 92.

14. The isolated antibody of any of claims 1-13 wherein the antibody binds to an epitope, wherein the epitope includes the amino acid sequences of SEQ ID NOs: 93 and 94.

15. The isolated antibody of any of claims 1-12 wherein the antibody binds to a first and second epitope, wherein the first epitope includes the amino acid sequences of SEQ ID NOs: 91 and 92, and binds to a second epitope wherein the epitope includes the amino acid sequences of SEQ ID NOs: 93 and 94.

16. The isolated antibody of any of claims 1-15 wherein the antibody binds to an epitope wherein the epitope includes an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with amino acid sequence TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and/or YKVRNQHWSLIMES (SEQ ID NO:92).

17. The isolated antibody of any of claims 1-16 wherein the antibody binds to an epitope wherein the epitope includes an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with amino acid sequence TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO:94).

18. The isolated antibody of any of claims 1-17 wherein the antibody binds an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with amino acid sequence (a)

TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and/or YKVRNQHWSLIMES (SEQ ID NO:92), and (b) TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO:94).

19. The isolated antibody of any of claims 1-18 wherein the antibody binds to an epitope wherein the epitope includes an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity or similarity with amino acid sequences of SEQ ID NOs: 91, 92, 93, and 94.

20. The isolated antibody of any of claims 1-19 wherein the antibody binds within the amino acid sequence range of 157 to 181 of SEQ ID NOs:52 and/or 54.

21. The isolated antibody of any of claims 1-20 wherein the antibody binds within the amino acid sequence range of 207 to 220 of SEQ ID NOs:52 and/or 54.

22. The isolated antibody of any of claims 1-21 wherein the antibody binds within the amino acid sequence ranges of 157 to 181 and 207 to 220 of SEQ ID NOs:52 and 54.

23. The isolated antibody of any of claims 1-22, wherein the antibody inhibits constitutive FGFR2 and/or FGFR3 activity.

24. The isolated antibody of claim 23, wherein constitutive FGFR2 and/or FGFR3 activity is ligand-dependent constitutive FGFR2 and/or FGFR3 activity.

25. The isolated antibody of claim 23, wherein constitutive FGFR2 and/or FGFR3 activity is ligand-independent constitutive FGFR2 and/or FGFR3 activity.

26. The isolated antibody of any of claims 23-25, wherein the constitutive activity is FGFR2 and FGFR3 activity.

27. The isolated antibody of any of claims 1-26, wherein the antibody inhibits FGFR3 comprising a mutation corresponding to any one of the mutations selected from FGFR3-IIIb^{R248C}, FGFR3-IIIb^{K652E}, FGFR3-IIIb^{S249C}, FGFR3-IIIb^{G372C}, and FGFR3-IIIb^{Y375C}.

28. The isolated antibody of any of claims 1-27, wherein the antibody inhibits FGFR3 and FGFR2 activity.

29. The isolated antibody of any of claims 1-28, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, and 16.

30. The isolated antibody of any of claims 1-29, wherein the antibody comprises a light chain comprising an amino acid sequence selected from SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, and 15.

31. The isolated antibody of any of claims 1-30, wherein the antibody comprises a heavy chain and light chain selected from the group comprising:

a. a light chain of SEQ ID NO:1 and a heavy chain of SEQ ID NO:2,
b. a light chain of SEQ ID NO:3 and a heavy chain of SEQ ID NO:4,
c. a light chain of SEQ ID NO:5 and a heavy chain of SEQ ID NO:6,
d. a light chain of SEQ ID NO:7 and a heavy chain of SEQ ID NO:8,
e. a light chain of SEQ ID NO:9 and a heavy chain of SEQ ID NO:10,
f. a light chain of SEQ ID NO:11 and a heavy chain of SEQ ID NO:12,
g. a light chain of SEQ ID NO:13 and a heavy chain of SEQ ID NO:14, and
h. a light chain of SEQ ID NO:15 and a heavy chain of SEQ ID NO:16.

32. The isolated antibody of claim 31, wherein the antibody comprises a light chain of SEQ ID NO:13 and a heavy chain of SEQ ID NO:14.

33. The isolated antibody of claim 31, wherein the antibody comprises a light chain of SEQ ID NO:15 and a heavy chain of SEQ ID NO:16.

34. The isolated antibody of any of claims 1-33, wherein the antibody comprises three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:1,

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:2,

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:3,

(d) HVR-H1 comprises the amino acid sequence of SEQ ID NO:4,

(e) HVR-H2 comprises the amino acid sequence of SEQ ID NO:5, and

(f) HVR-H3 comprises the amino acid sequence of SEQ ID NO:6.

35. The isolated antibody of any of claims 1-33, wherein the antibody comprises three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3) and three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:7,

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:8,

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:9,

(d) HVR-H1 comprises the amino acid sequence of SEQ ID NO:10,

(e) HVR-H2 comprises the amino acid sequence of SEQ ID NO:11, and

(f) HVR-H3 comprises the amino acid sequence of SEQ ID NO:12.

36. The isolated monoclonal antibody of any of claims 1-35, wherein the antibody is a monoclonal antibody.

37. The isolated antibody of any of claims 1-36, wherein the antibody is selected from the group consisting of a chimeric antibody, a humanized antibody, an affinity matured antibody, a human antibody, and a bispecific antibody.

38. The isolated monoclonal antibody of claim 1-37, wherein the antibody is an antibody fragment.

39. A polynucleotide encoding an antibody of any of claims 1-38.

40. A vector comprising the polynucleotide of claim 39.

41. The vector of claim 40, wherein the vector is an expression vector.

42. A host cell comprising a vector of claim 40 or 41.

43. The host cell of claim 42, wherein the host cell is prokaryotic.

44. The host cell of claim 42, wherein the host cell is eukaryotic.

45. The host cell of claim 42, wherein the host cell is mammalian.

46. A method for making an anti-FGFR2/3 antibody, said method comprising culturing a host cell comprising polynucleotide encoding the antibody of any of claims 1-38 so that the polynucleotide is expressed, and optionally, recovering the antibody from the culture.

47. The method of claim 46, wherein the host cell is prokaryotic

48. The method of claim 46, wherein the host cell is eukaryotic.

49. A method for treating a tumor, a cancer, or a cell proliferative disorder, the method comprising administering an effective amount of an anti-FGFR3 antagonist antibody of any of claims 1-38 to a subject having a tumor, a cancer, or a cell proliferative disorder, whereby the tumor, cancer or cell proliferative disorder is treated.

50. The method of claim 49, wherein the cancer, tumor or cell proliferative disorder is multiple myeloma, bladder carcinoma, non-small cell lung cancer, ovarian cancer, thyroid cancer, head and neck cancer, liver cancer, breast carcinoma, gastric cancer, or colorectal cancer.

51. The method of claim 49, wherein the cancer, tumor or cell proliferative disorder is transitional cell carcinoma.

52. The method of claim 49, wherein the cancer, tumor or cell proliferative disorder is invasive transitional cell carcinoma.

53. The method of claim 49, wherein the cancer, tumor or cell proliferative disorder is multiple myeloma.

54. The method of any of claims 49-53, wherein the cancer, tumor or cell proliferative disorder expresses a FGFR3 translocation.

55. The method of any of claims 49-54, wherein the cancer, tumor or cell proliferative disorder expresses mutated FGFR3.

56. The method of any of claims 49-55, wherein the cancer, tumor or cell proliferative disorder expresses mutated FGFR2.

57. The method of any of claims 49-56, wherein the cancer, tumor or cell proliferative disorder wherein the tumor or cell overexpresses FGFR2 and/or FGFR3.

58. The method of claim 55 or 56, wherein the mutation is a constitutive mutation.

59. The method of claim 50, wherein the breast carcinoma is triple negative breast cancer.

60. The method of claim 50, wherein the cancer is gastric cancer.

61. The method of any of claims 49-60, further comprising administering to the subject an effective amount of another therapeutic agent.

62. A method for inhibiting cell proliferation, the method comprising administering an effective amount of an anti-FGFR2/3 antibody of any of claims 1-38 to a subject, whereby the cell proliferation is inhibited.

63. A method for depleting multiple myeloma cells, the method comprising administering an effective amount of an anti-FGFR2/3 antibody any of claims 1-38 to a subject, whereby the multiple myeloma cells are depleted.

64. A composition comprising:

(a) an isolated bispecific antibody, or an antigen-binding portion thereof, that binds to beta-Klotho (KLB), FGFR2, and FGFR3;

(b) a nucleic acid encoding a bispecific antibody, or an antigen-binding portion thereof, that binds to KLB, FGFR2, and FGFR3;

(c) a host cell comprising a nucleic acid encoding a bispecific antibody, or an antigen-binding portion thereof, that binds to KLB, FGFR2, and FGFR3;

(d) a pharmaceutical formulation comprising an isolated bispecific antibody, or an antigen-binding portion thereof, that binds to KLB, FGFR2, and FGFR3.

65. The composition of claim 64, wherein the bispecific antibody, or an antigen-binding portion thereof, binds to an FGFR2/3 epitope and/or competes for binding to an FGFR2/3 epitope.

66. The composition of claim 65, wherein the FGFR2/3 epitope is an FGFR2 epitope selected from TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and YKVRNQHWSLIMES (SEQ ID NO:92).

67. The composition of claim 65, wherein the FGFR2/3 epitope is an FGFR2 epitope comprising both TNTEKMEKRLHAVPAANTVKFRCPA (SEQ ID NO: 91) and YKVRNQHWSLIMES (SEQ ID NO:92).

68. The composition of claim 65, wherein the FGFR2/3 epitope is an FGFR3 epitope selected from TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO:94).

69. The composition of claim 65, wherein the FGFR2/3 epitope is an FGFR2 epitope comprising both TRPERMDKKLLAVPAANTVRFRCPA (SEQ ID NO: 93) and IKLRHQQWSLVMES (SEQ ID NO:94).

70. The composition of any of claims 64-69, wherein the bispecific antibody, or an antigen-binding portion thereof, binds to a KLB epitope within a fragment of KLB consisting of the amino acid sequence SSPTRLAVIPWGVRKLLRWVRRNYGDMDIYITAS (SEQ ID NO: 103).

71. The composition of any of claims 64-70, wherein the anti-KLB antibody, or an antigen-binding portion thereof, comprises:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 108-122, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 138-153, and conservative substitutions thereof; and

(c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 154-169, and conservative substitutions thereof.

72. The composition of any of claims 64-71, wherein the anti-KLB antibody, or an antigen-binding portion thereof, comprises:

(a) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 170-184, and conservative substitutions thereof;

(b) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 185-200, and conservative substitutions thereof; and

(c) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 201-215, and conservative substitutions thereof.

73. The composition of any of claims 64-72, wherein the anti-FGFR2/3 antibody, or an antigen-binding portion thereof, comprises three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3) wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:7,

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:8, and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:9.

74. The composition of any of claims 64-73, wherein the anti-FGFR2/3 antibody, or an antigen-binding portion thereof, comprises three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) wherein:.

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:10,

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:11, and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:12.

75. The composition of any of claims 64-72, wherein the anti-FGFR2/3 antibody, or an antigen-binding portion thereof, comprises three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3) wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:276,

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:277, and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:278.

76. The composition of any of claims 64-72 and 75, wherein the anti-FGFR2/3 antibody, or an antigen-binding portion thereof, comprises three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) wherein:.

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:279,

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:280, and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:281.

77. The composition of any of claims 64-76 wherein the bispecific antibody, or an antigen-binding portion thereof, further binds to Fibroblast Growth Factor Receptor 4 (FGFR4).

78. The composition of any of claims 64-77, wherein the composition further comprises a pharmaceutically acceptable carrier.

79. A method of treating an individual having a disease selected from the group consisting of polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), obesity, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, hypertension, type 2 diabetes, non-type 2 diabetes, type 1 diabetes, latent autoimmune diabetes (LAD), maturity onset diabetes of the young (MODY), and aging and related diseases such as Alzheimer's disease, Parkinson's disease and ALS, Bardet-Biedl syndrome, Prader-Willi syndrome, Alstrom syndrome, Cohen syndrome, Albright's hereditary osteodystrophy (pseudohypoparathyroidism), Carpenter syndrome, MOMO syndrome, Rubinstein-Taybi syndrome, fragile X syndrome and Börjeson-Forssman-Lehman syndrome, comprising administering to the individual an effective amount a bispecific antibody, or an antigen-binding portion thereof, of any of claims 64-78.

80. The method of claim 79, wherein the disease is NASH.

81. The method of claim 79, wherein the bispecific antibody reduces blood glucose levels in vivo.

82. A method of producing a bispecific antibody of any of claims 64-78 comprising culturing one or more cells that comprises one or more nucleic acids encoding the bispecific antibody, or an antigen-binding portion thereof, that binds to KLB, FGFR2, and FGFR3.

83. The use of any of the bispecific antibodies of any of claims 64-78 for use in a medicament to treat a metabolic disease, wherein the disease is selected from the group consisting of polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), obesity, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, hypertension, type 2 diabetes, non-type 2 diabetes, type 1 diabetes, latent autoimmune diabetes (LAD), maturity onset diabetes of the young (MODY), and aging and related diseases such as Alzheimer's disease, Parkinson's disease and ALS, Bardet-Biedl

syndrome, Prader-Willi syndrome, Alstrom syndrome, Cohen syndrome, Albright's hereditary osteodystrophy (pseudohypoparathyroidism), Carpenter syndrome, MOMO syndrome, Rubinstein-Taybi syndrome, fragile X syndrome and Börjeson-Forssman-Lehman syndrome.

84. The use of any of the bispecific antibodies of any of claims 64-78 for use in a medicament to treat a metabolic disease, wherein the metabolic disease is NASH.

FIGURE 1

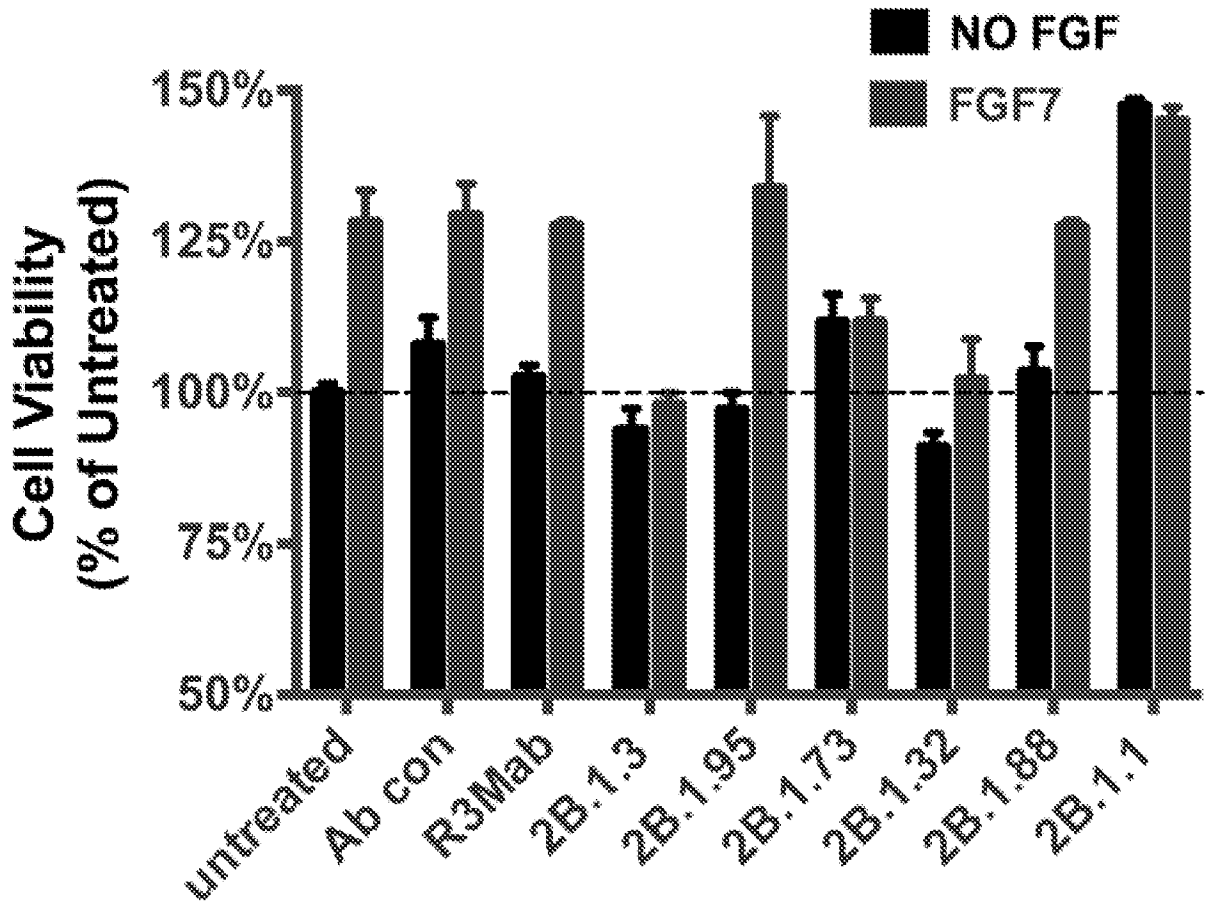
NO FGF
FGF7
Cell Viability
(% of Untreated)
150%
125%
100%
75%
50%
untreated
Ab con
R3Mab
2B.1.3
2B.1.95
2B.1.73
2B.1.32
2B.1.88
2B.1.1

FIGURE 2A

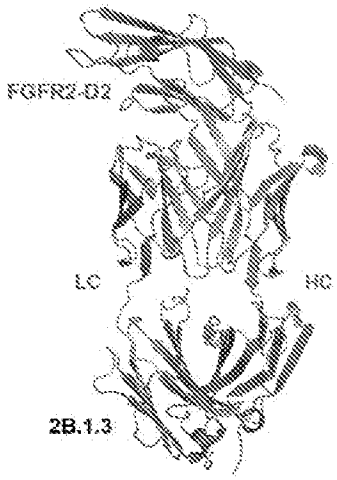

FIGURE 2B

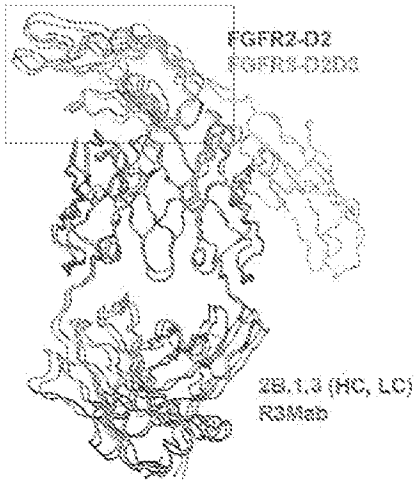

FIGURE 2C

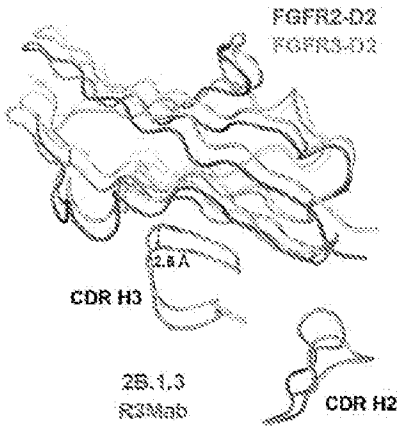

FIGURE 3A

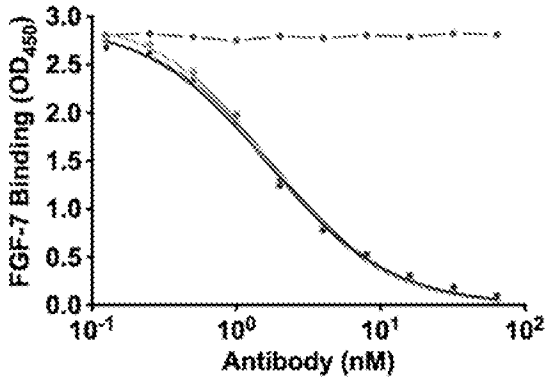

FIGURE 3B

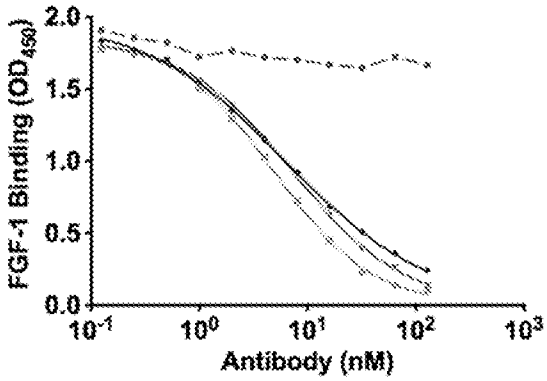

FIGURE 3C

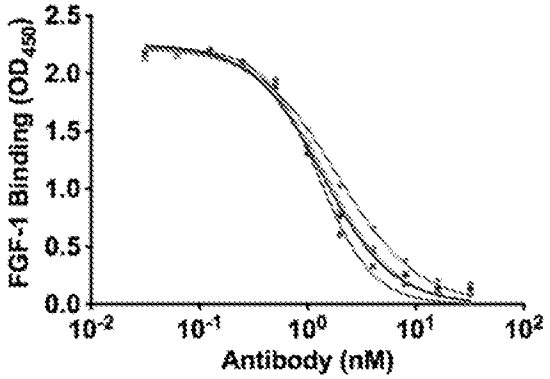

FIGURE 3D

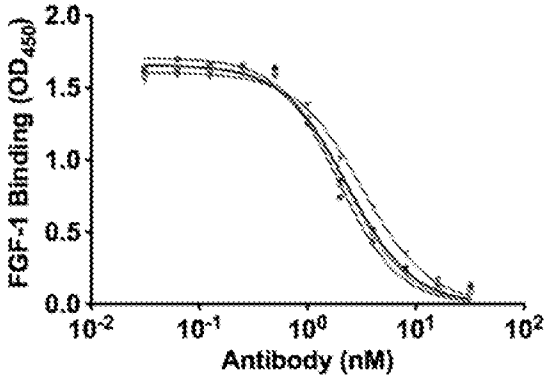

FIGURE 3E

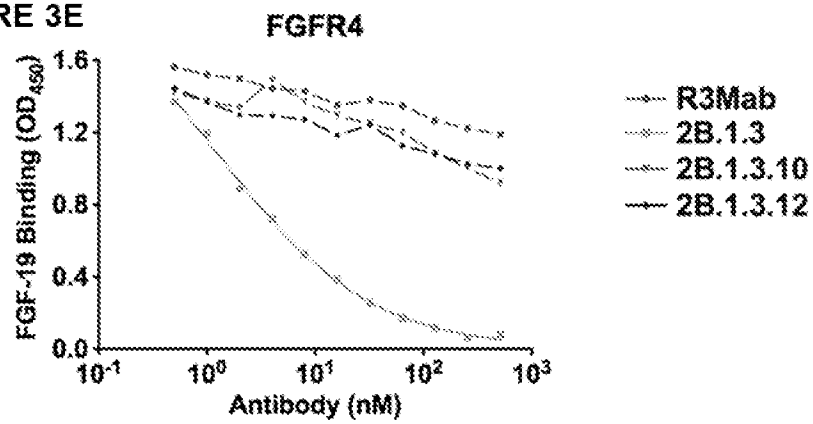

FIGURE 4A

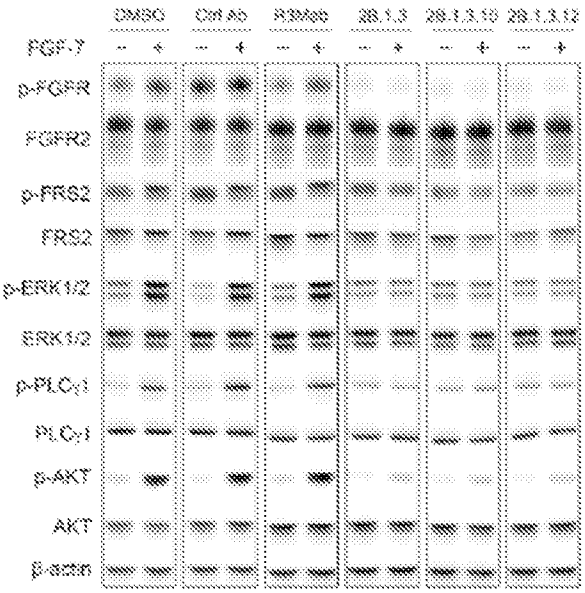

FIGURE 4B

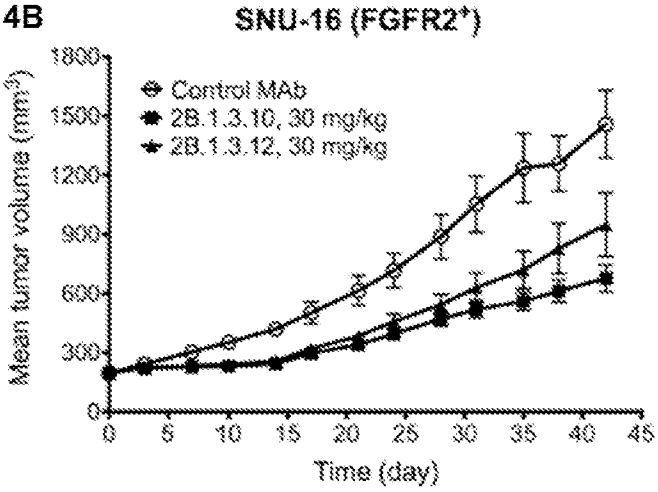

FIGURE 4C

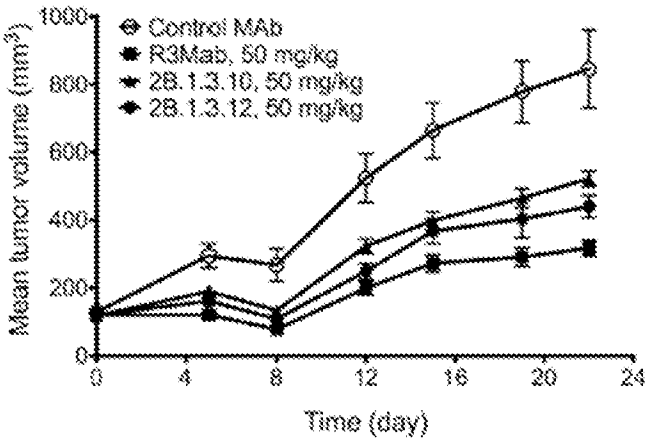

FIGURE 5

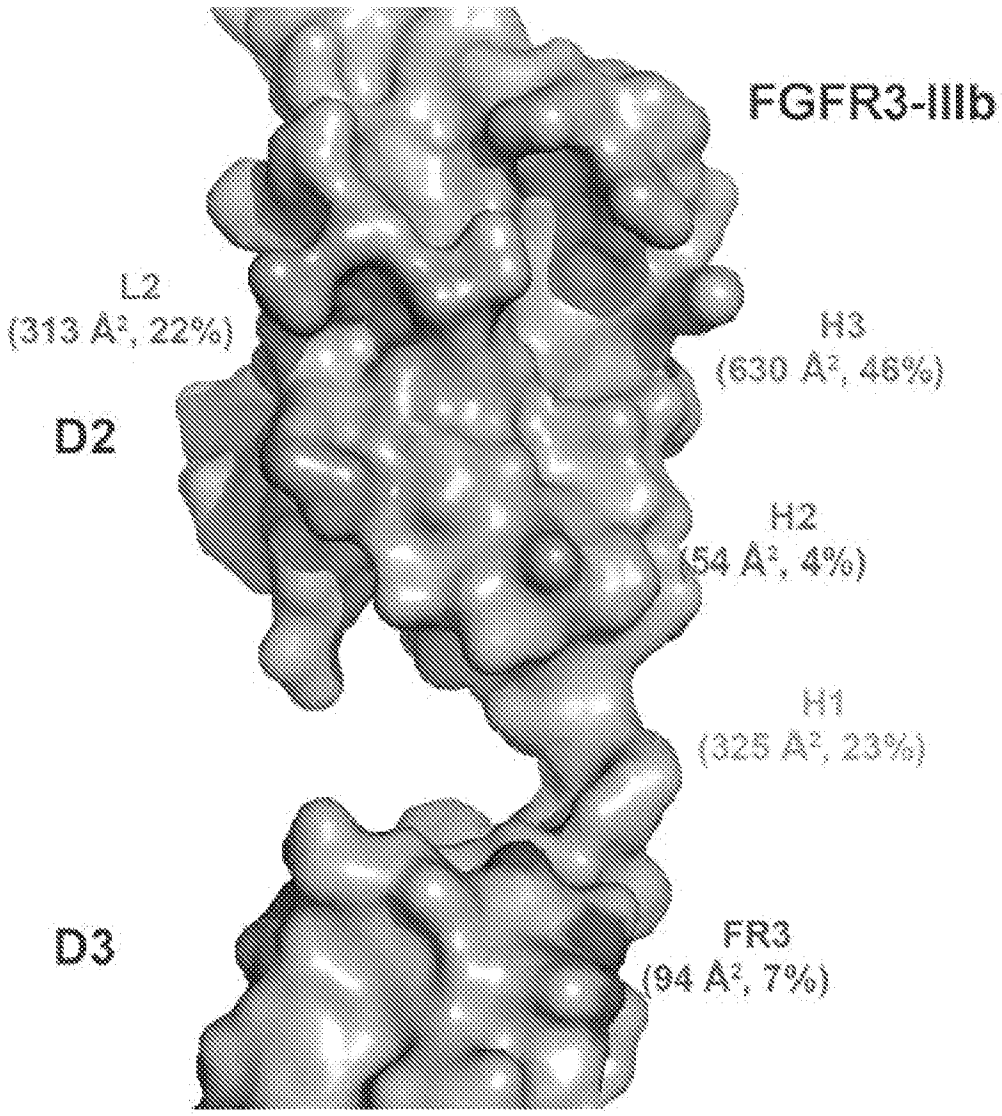
FGFR3-IIIb
L2
(313 Å², 22%)
H3
(630 Å², 46%)
D2
H2
(54 Å², 4%)
H1
(325 Å², 23%)
D3
FR3
(94 Å², 7%)

FIGURE 6

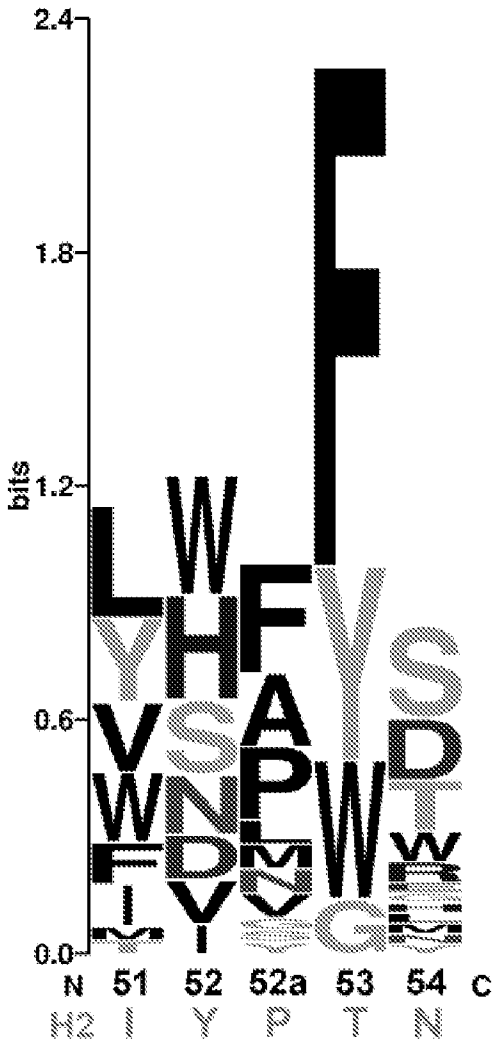

FIGURE 7

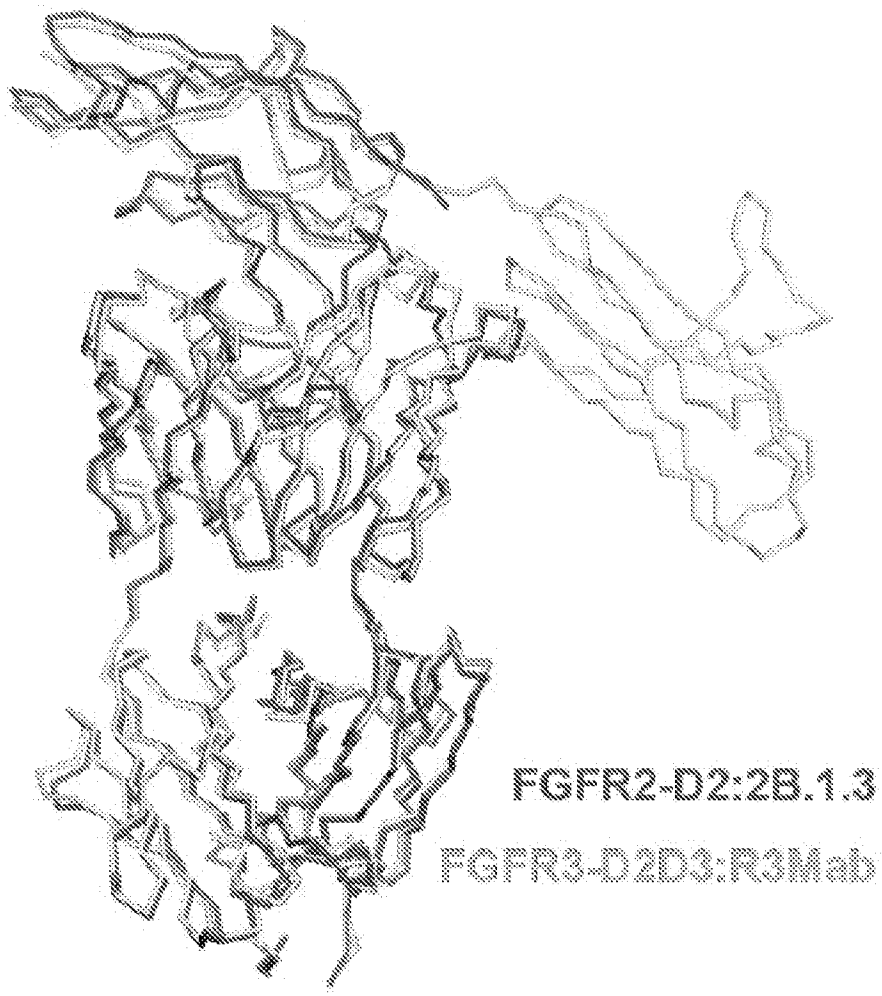

FIGURE 8A

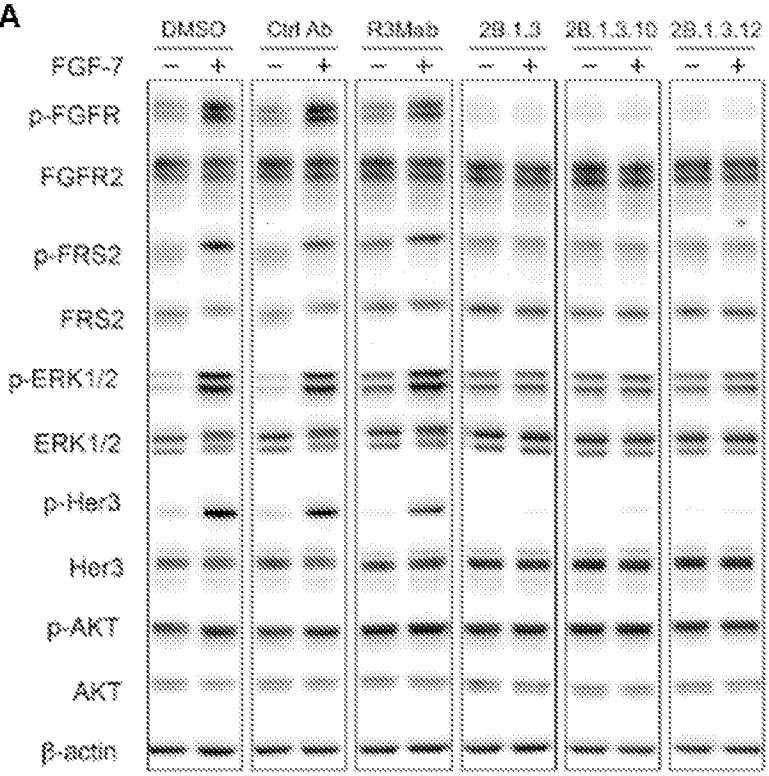

FIGURE 8B

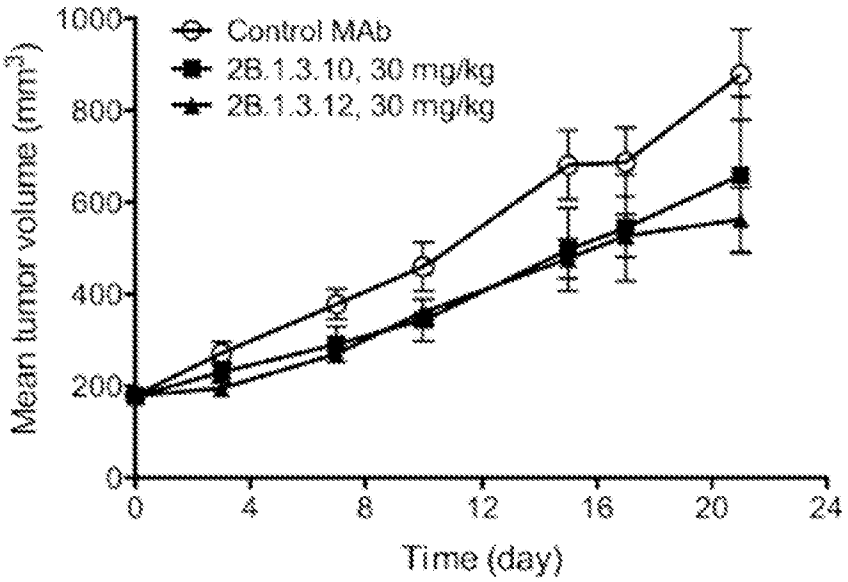

FIGURE 9A

FGFR2-IIIb

SEQ ID NO: 52

MVSWGRFICLVVVTMATLSLARPSFSLVEDTTLEPEEPPTKYQISQPEVYVAAPGESLEVRCLLKDAA

VISWTKDGVHLGPNNRTVLIGEYLQIKGATPRDSGLYACTASRTVDSETWYFMVNVTDAISSGDDEDD

TDGAEDFVSENSNNKRAPYW**TNTEKMEKRLHAVPAANTVKFRCPA**GGNPMPTMRWLKNGKEFKQEHRI

SEQ ID NO:91

GG**YKVRNQHWSLIMES**VVPSDKGNYTCVVENEYGSINHTYHLDVVERSPHRPILQAGLPANASTVVGG

SEQ ID NO:92

DVEFVCKVYSDAQPHIQWIKHVEKNGSKYGPDGLPYLKVLKHSGINSSNAEVLALFNVTEADAGEYIC

KVSNYIGQANQSAWLTVLPKQQAPGREKEITASPDYLEIAIYCIGVFLIACMVVTVILCRMKNTTKKP

DFSSQPAVHKLTKRIPLRRQVTVSAESSSSMNSNTPLVRITTRLSSTADTPMLAGVSEYELPEDPKWE

FPRDKLTLGKPLGEGCFGQVVMAEAVGIDKDKPKEAVTVAVKMLKDDATEKDLSDLVSEMEMMKMIGK

HKNIINLLGACTQDGPLYVIVEYASKGNLREYLRARRPPGMEYSYDINRVPEEQMTFKDLVSCTYQLA

RGMEYLASQKCIHRDLAARNVLVTENNVMKIADFGLARDINNIDYYKKTTNGRLPVKWMAPEALFDRV

YTHQSDVWSFGVLMWEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTNELYMMMRDCWHAVPSQR

PTFKQLVEDLDRILTLTTNEEYLDLSQPLEQYSPSYPDTRSSCSSGDDSVFSPDPMPYEPCLPQYPHI

NGSVKT

FIGURE 9B

FGFR2-IIIc

SEQ ID NO: 54

MVSWGRFICLVVVTMATLSLARPSFSLVEDTTLEPEEPPTKYQISQPEVYVAAPGESLEVRCLLKDAA

VISWTKDGVHLGPNNRTVLIGEYLQIKGATPRDSGLYACTASRTVDSETWYFMVNVTDAISSGDDEDD

TDGAEDFVSENSNNKRAPYW**TNTEKMEKRLHAVPAANTVKFRCPA**GGNPMPTMRWLKNGKEFKQEHRI

SEQ ID NO:91

GGY**KVRNQHWSLIMES**VVPSDKGNYTCVVENEYGSINHTYHLDVVERSPHRPILQAGLPANASTVVGG

SEQ ID NO:92

DVEFVCKVYSDAQPHIQWIKHVEKNGSKYGPDGLPYLKVLKAAGVNTTDKEIEVLYIRNVTFEDAGEY

TCLAGNSIGISFHSAWLTVLPAPGREKEITASPDYLEIAIYCIGVFLIACMVVTVILCRMKNTTKKPD

FSSQPAVHKLTKRIPLRRQVTVSAESSSSMNSNTPLVRITTRLSSTADTPMLAGVSEYELPEDPKWEF

PRDKLTLGKPLGEGCFGQVVMAEAVGIDKDKPKEAVTVAVKMLKDDATEKDLSDLVSEMEMMKMIGKH

KNIINLLGACTQDGPLYVIVEYASKGNLREYLRARRPPGMEYSYDINRVPEEQMTFKDLVSCTYQLAR

GMEYLASQKCIHRDLAARNVLVTENNVMKIADFGLARDINNIDYYKKTTNGRLPVKWMAPEALFDRVY

THQSDVWSFGVLMWEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTNELYMMMRDCWHAVPSQRP

TFKQLVEDLDRILTLTTNEEYLDLSQPLEQYSPSYPDTRSSCSSGDDSVFSPDPMPYEPCLPQYPHIN

GSVKT

FIGURE 9C

FGFR3-IIIb

SEQ ID NO: 56

MGAPACALALCVAVAIVAGASSESLGTEQRVVGRAAEVPGPEPGQQEQLVFGSGDAVELSCPPPGGGPMGPTVWV

KDGTGLVPSERVLVGPQRLQVLNASHEDSGAYSCRQRLTQRVLCHFSVRVTDAPSSGDDEDGEDEAEDTGVDTGA

PYW**TRPERMDKKLLAVPAANTVRFRCPA**AGNPTPSISWLKNGREFRGEHRIGG**IKLRHQQWSLVMES**VVPSDRGN

SEQ ID NO:93 SEQ ID NO:94

YTCVVENKFGSIRQTYTLDVLERSPHRPILQAGLPANQTAVLGSDVEFHCKVYSDAQPHIQWLKHVEVNGSKVGP

DGTPYVTVLKSWISESVEADVRLRLANVSERDGGEYLCRATNFIGVAEKAFWLSVHGPRAAEEELVEADEAGSVY

AGILSYGVGFFLFILVVAAVTLCRLRSPPKKGLGSPTVHKISRFPLKRQVSLESNASMSSNTPLVRIARLSSGEG

PTLANVSELELPADPKWELSRARLTLGKPLGEGCFGQVVMAEAIGIDKDRAAKPVTVAVKMLKDDATDKDLSDLV

SEMEMMKMIGKHKNIINLLGACTQGGPLYVLVEYAAKGNLREFLRARRPPGLDYSFDTCKPPEEQLTFKDLVSCA

YQVARGMEYLASQKCIHRDLAARNVLVTEDNVMKIADFGLARDVHNLDYYKKTTNGRLPVKWMAPEALFDRVYTH

QSDVWSFGVLLWEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTHDLYMIMRECWHAAPSQRPTFKQLVEDL

DRVLTVTSTDEYLDLSAPFEQYSPGGQDTPSSSSSGDDSVFAHDLLPPAPPSSGGSRT

FIGURE 9D

FGFR3-IIIc

SEQ ID NO: 56

MGAPACALALCVAVAIVAGASSESLGTEQRVVGRAAEVPGPEPGQQEQLVFGSGDAVELSCPPPGGGPMGPTVWV

KDGTGLVPSERVLVGPQRLQVLNASHEDSGAYSCRQRLTQRVLCHFSVRVTDAPSSGDDEDGEDEAEDTGVDTGA

PYW**TRPERMDKKLLAVPAANTVRFRCPA**AGNPTPSISWLKNGREFRGEHRIGG**IKLRHQQWSLVMES**VVPSDRGN

SEQ ID NO:93 SEQ ID NO:94

YTCVVENKFGSIRQTYTLDVLERSPHRPILQAGLPANQTAVLGSDVEFHCKVYSDAQPHIQWLKHVEVNGSKVGP

DGTPYVTVLKTAGANTTDKELEVLSLHNVTFEDAGEYTCLAGNSIGFSHHSAWLVVLPAEEELVEADEAGSVYAG

ILSYGVGFFLFILVVAAVTLCRLRSPPKKGLGSPTVHKISRFPLKRQVSLESNASMSSNTPLVRIARLSSGEGPT

LANVSELELPADPKWELSRARLTLGKPLGEGCFGQVVMAEAIGIDKDRAAKPVTVAVKMLKDDATDKDLSDLVSE

MEMMKMIGKHKNIINLLGACTQGGPLYVLVEYAAKGNLREFLRARRPPGLDYSFDTCKPPEEQLTFKDLVSCAYQ

VARGMEYLASQKCIHRDLAARNVLVTEDNVMKIADFGLARDVHNLDYYKKTTNGRLPVKWMAPEALFDRVYTHQS

DVWSFGVLLWEIFTLGGSPYPGIPVEELFKLLKEGHRMDKPANCTHDLYMIMRECWHAAPSQRPTFKQLVEDLDR

VLTVTSTDEYLDLSAPFEQYSPGGQDTPSSSSSGDDSVFAHDLLPPAPPSSGGSRT

FIGURE 10

	SEQ ID NO.			
Antibody	**Nucleic Acid (light chain)**	**Nucleic Acid (heavy chain)**	**Amino Acid (light chain)**	**Amino Acid (heavy chain)**
1.3	67	83	59	75
1.95	68	84	60	76
1.73	69	85	61	77
1.32	70	86	62	78
1.88	71	87	63	79
1.1	72	88	64	80
1.3.10	73	89	65	81
1.3.12	74	90	66	82

FIGURE 11A

CDR sequences according to Kabat definition are underlined

Light chain variable region

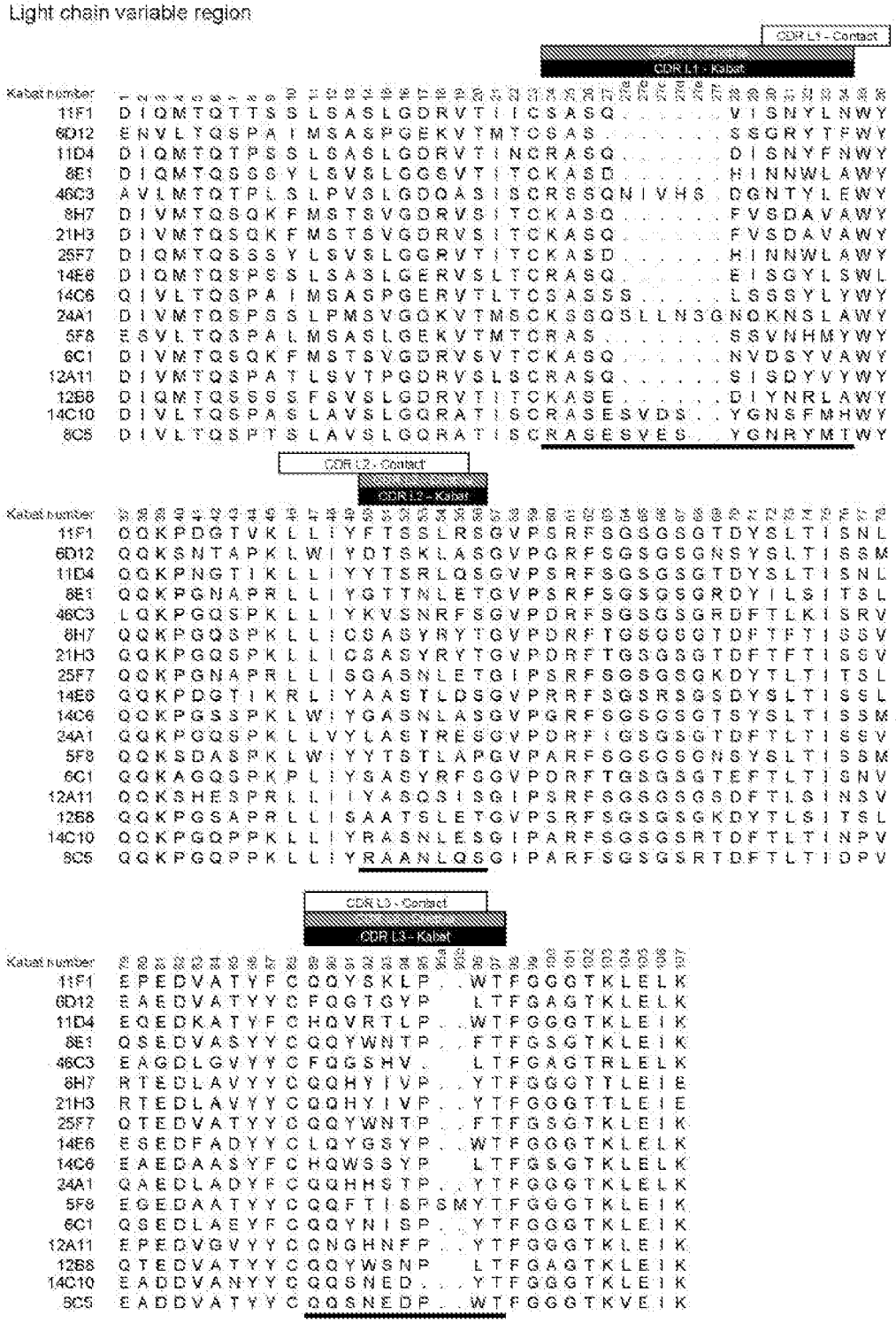

FIGURE 11B

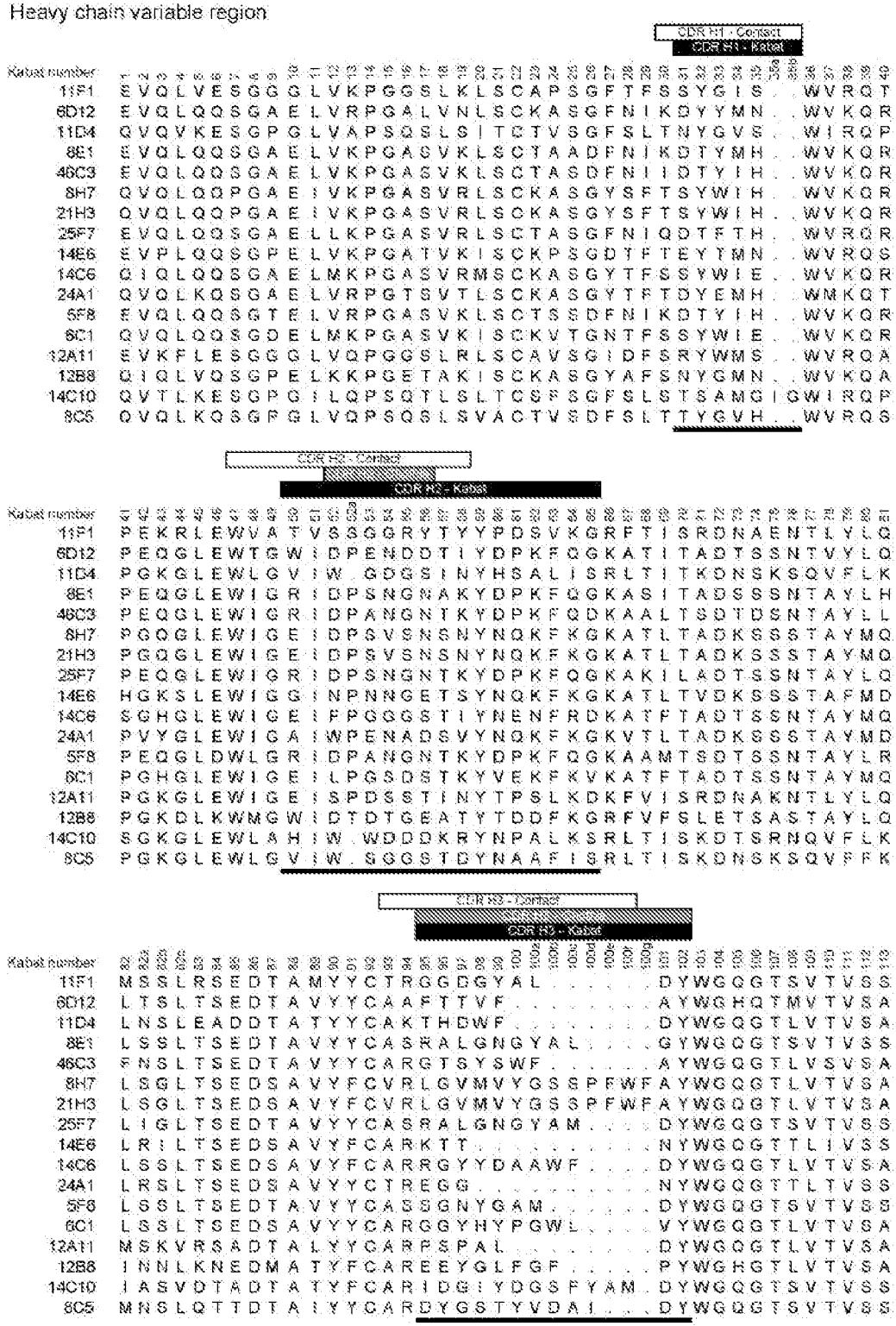
Heavy chain variable region
CDR H1 - Contact
CDR H1 - Kabat
CDR H2 - Contact
CDR H2 - Kabat
CDR H3 - Contact
CDR H3 - Kabat

FIGURE 12

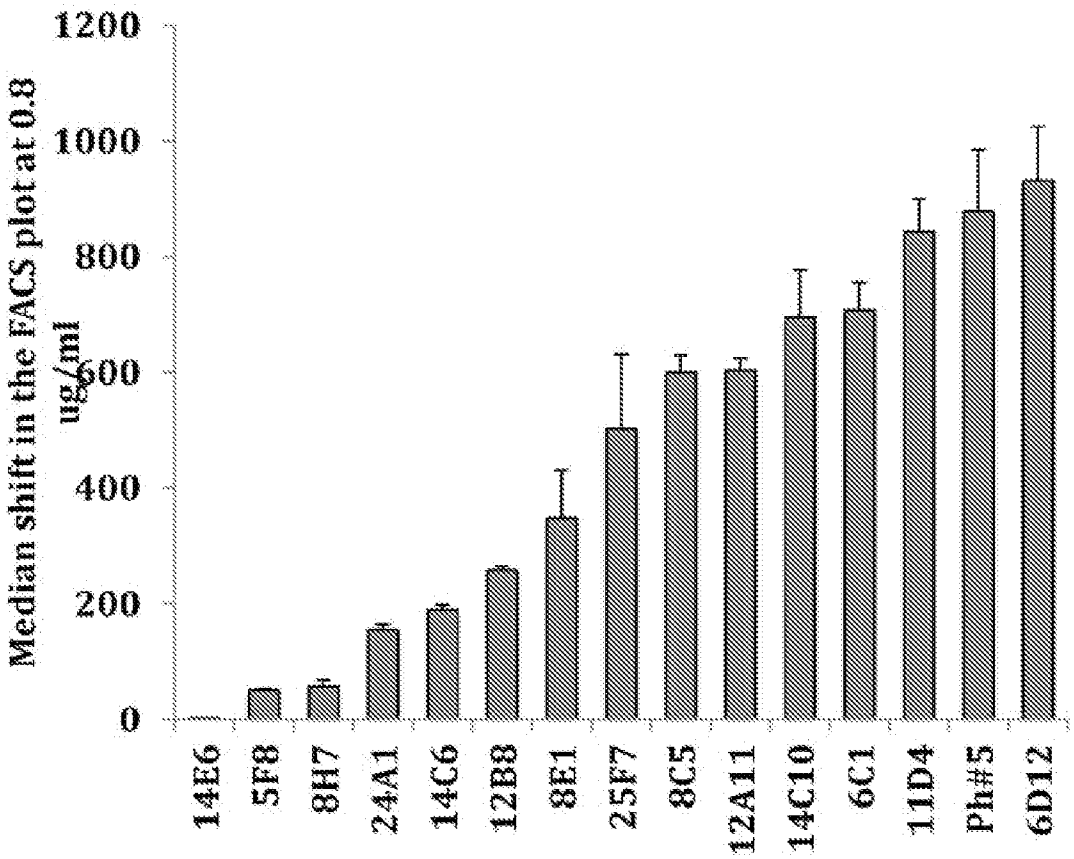
Median shift in the FACS plot at 0.8 ug/ml
1200
1000
800
600
400
200
0
14E6
5F8
8H7
24A1
14C6
12B8
8E1
25F7
8C5
12A11
14C10
6C1
11D4
Ph#5
6D12

FIGURE 13

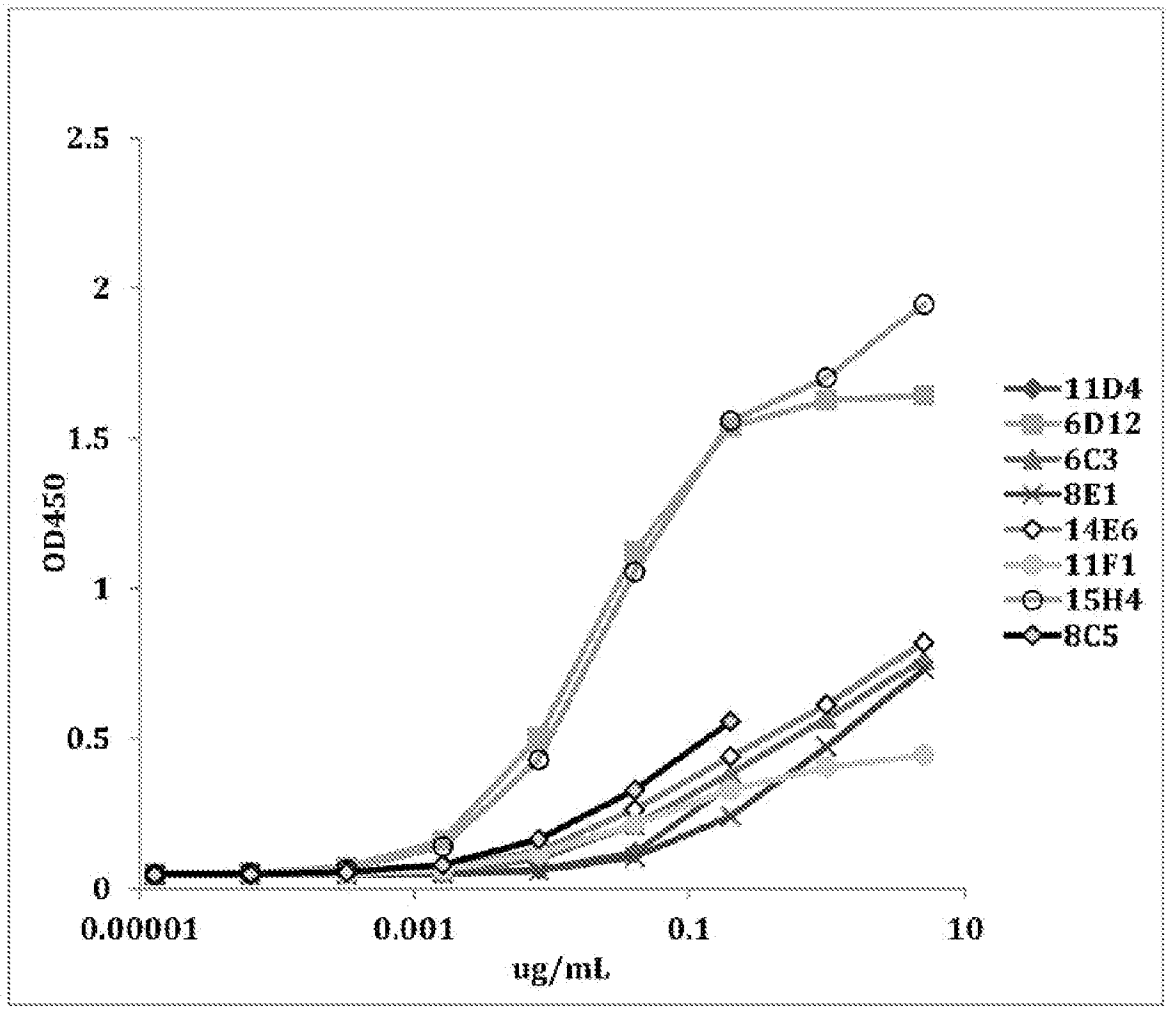
2.5
2
1.5
1
0.5
0
OD450
0.00001
0.001
0.1
10
ug/mL
11D4
6D12
6C3
8E1
14E6
11F1
15H4
8C5

FIGURE 14A

wt: FSGDGKAIMDKKQIVSP...

ko: FSETGKQIYGIKNST*

FIGURE 14B

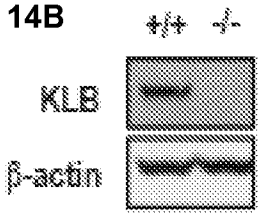

FIGURE 14C

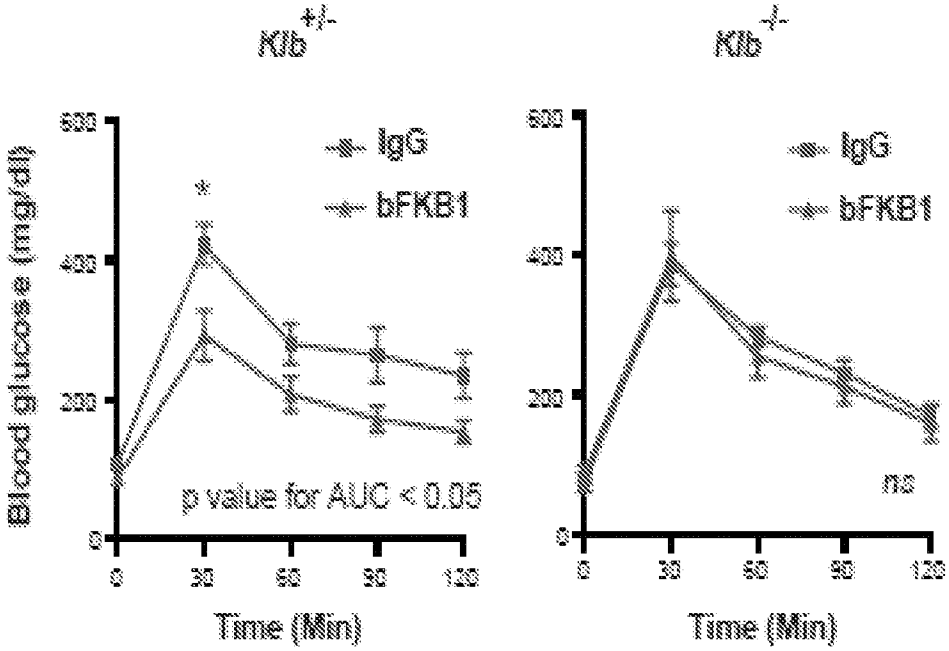

FIGURE 14D

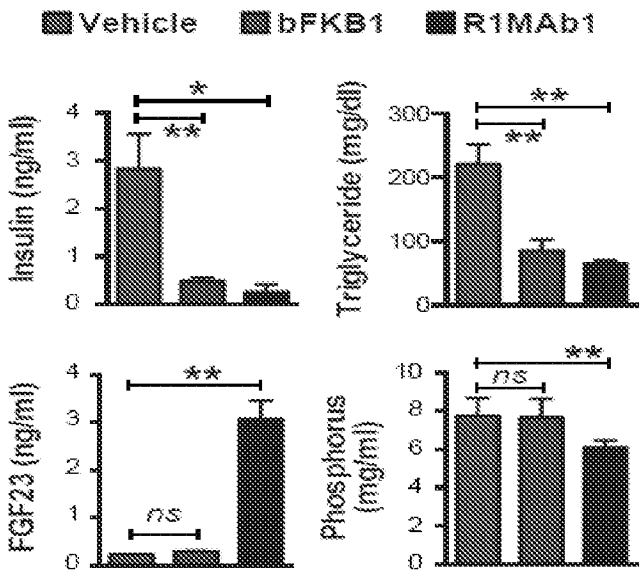

Figure 15A

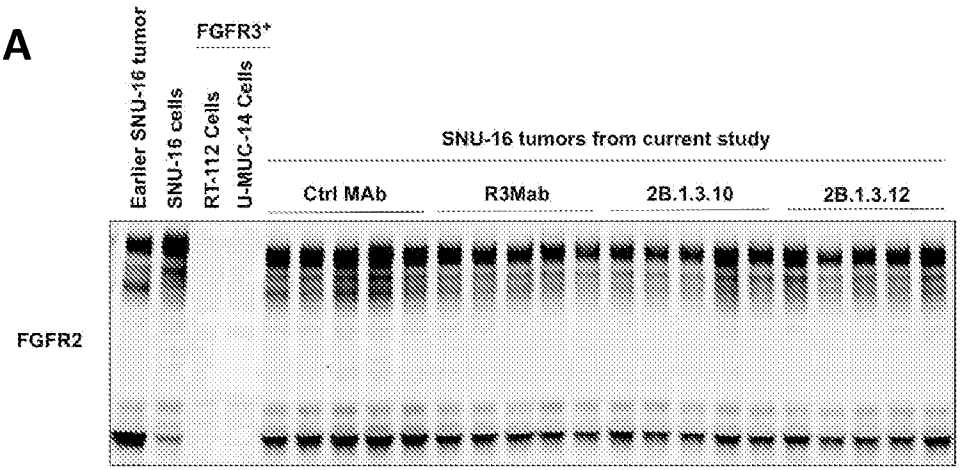

Figure 15B

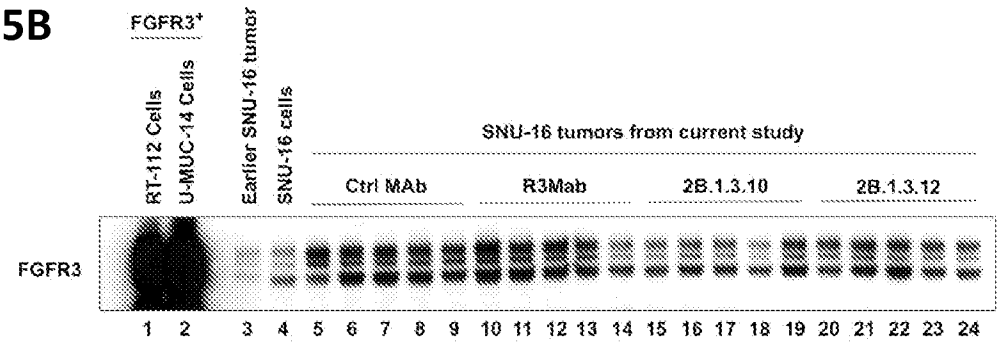

Figure 16A

Clone	CDR H1	CDR H2	CDR H3	FGFR3.IIIc KD (M)	BV ELISA
2B.1.1	TFTST	YWANO	LYYD	6.63E-11	0.22
2B.1.1.2	TFTST	YWANO	TYDN	9.08E-11	0.13
2B.1.1.4	TFTST	YWANO	IYGG	1.53E-10	0.23
2B.1.1.6	TFTST	YWANO	TYDE	7.96E-11	0.14
2B.1.1.8	PFTSL	YWANO	IYEK	1.12E-10	0.16
2B.1.1.10	PFTSQ	YWANO	TYDK	1.98E-10	0.12
2B.1.1.12	PFTST	YWANO	TYDM	1.42E-10	0.20
2B.1.3	TFTST	THLGD	LYYD	8.34E-11	0.15
2B.1.3.12	PFTST	THLGD	TYDM	2.80E-10	0.14

Figure 16B

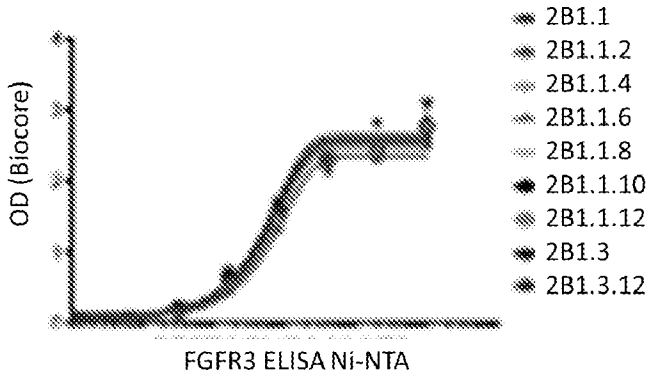

Figure 16C

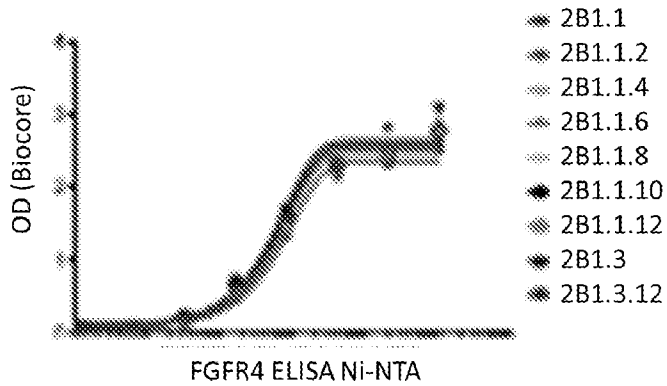

FIGURE 17A

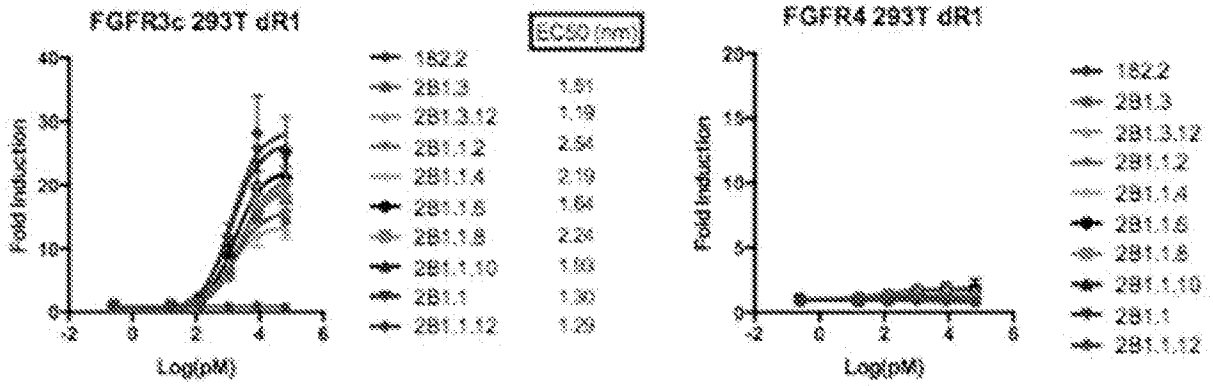

FIGURE 17B

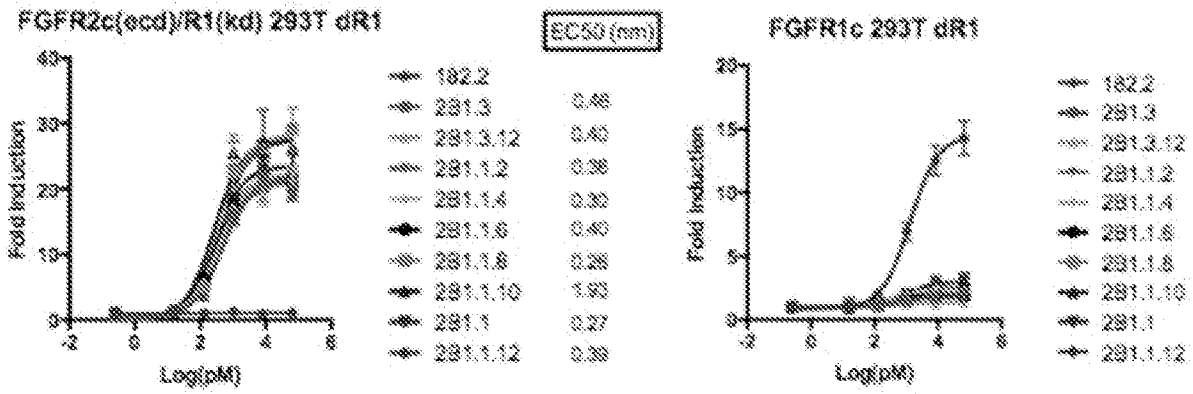

FIGURE 18

Variant	FGFR1c	FGFR2(ECD)/R1(KD)	FGFR3c	FGFR4	FGFR4 Binding affinity(nM)	Notes:
YW182.2	14.2	1	1	1		R1c Specific Ab
YW756.1	ND	24.7	1	1		Raised against R2
2B1.3	2.1	26	12.1	1	32	Blocks growth in MCF-7/FGF7 assay.FGF19 Blocking
2B1.3.12.3	1.3	27.7	11.2	1	<1000	Blocks Tumor Progression
2B1.1.2	1.6	35.3	17.5	1.2		
2B1.1.4	2.3	33.1	16.3	1.1		
2B1.1.6	1.8	42.3	19.3	1.5		
2B1.1.8	2.0	40.8	16.1	1.3		
2B1.1.10	2.3	38.7	18.9	1		
2B1.1	3	31.6	22.1	1.5	2.8	Growth agonist in MCF-7/FGF7 assay
2B1.1.12	2.6	30.3	24.9	1.5		?

FIGURE 19A

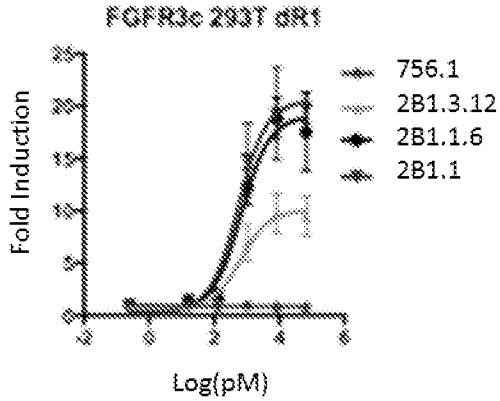

FIGURE 19B

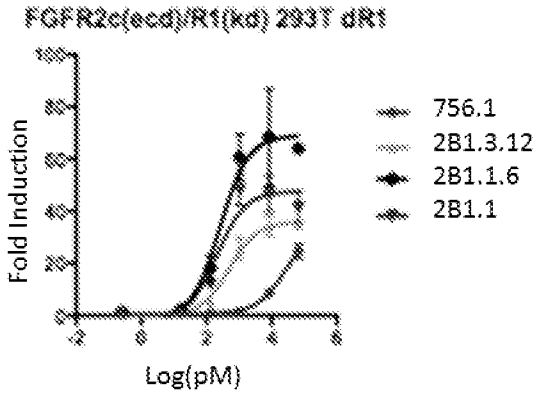

FIGURE 19C