CRYSTAL STRUCTURES AND METHODS USING SAME

Inventor: Christian Wiesmann, Bottmingen (CH)

Assignee: Genentech, Inc., South San Francisco, CA (US)

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Related U.S. Application Data
Continuation of application No. 12/661,852, filed on Mar. 24, 2010, now abandoned.

Publication Classification
Int. Cl.
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C12N 9/12  (2006.01)
G06F 19/16  (2011.01)
C12N 9/96  (2006.01)

U.S. Cl. 424/94.3; 435/188; 435/194; 703/11

ABSTRACT
The present invention relates generally to the fields of molecular biology and growth factor regulation. More specifically, the invention concerns modulators of FGFR3 function, and the identification and uses of said modulators.
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GVPSSFSSTGDDTFTLTISSLQPEDFATYQOOQSGSTGQPTFGQGTVKVEIR (SEQ ID NO: 133)

Clone 184.6.58

VH
EVQLVESGGGLVQPGSRSLRLSCAASGFTFTSPGISWVRQAPGKGLEWV
ARIFYTSGTYNSGTVKGRFTISADTSKNTAYLQMNLRAEFTAVYCAR
ARTYGIYDLYVQDYTEYVMVQGDYGTLV (SEQ ID NO: 134)

VL
DIQMTQSPSSLSASVGDRVITCRAASQDVSDSLAWYQQKPGKAPLLIYSASFLYS
GVPSSFSSTGDDTFTLTISSLQPEDFATYQOOQSGSTGQPTFGQGTVKVEIR (SEQ ID NO: 135)

Clone 184.6.62

VH
EVQLVESGGGLVQPGSRSLRLSCAASGFTFTSPGISWVRQAPGKGLEWV
ARIFYTSGTYNSGTVKGRFTISADTSKNTAYLQMNLRAEFTAVYCAR
ARTYGIYDLYVQDYTEYVMVQGDYGTLV (SEQ ID NO: 136)

VL
DIQMTQSPSSLSASVGDRVITCRAASQDVSDSTAVWYQQKPGKAPLLIYSASFLYS
GVPSSFSSTGDDTFTLTISSLQPEDFATYQOOQSGSTGQPTFGQGTVKVEIR (SEQ ID NO: 137)

Clone 184.6.1.NS D30E

VH
EVQLVESGGGLVQPGSRSLRLSCAASGFTFTSPGISWVRQAPGKGLEWV
GRIFYTSGTYNSGTVKGRFTISADTSKNTAYLQMNLRAEFTAVYCAR
ARTYGIYDLYVQDYTEYVMVQGDYGTLV (SEQ ID NO: 138)

VL
DIQMTQSPSSLSASVGDRVITCRAASQDVTSLAWYQQKPGKAPLLIYSASFLYS
GVPSSFSSTGDDTFTLTISSLQPEDFATYQOOQSGSTGQPTFGQGTVKVEIR (SEQ ID NO: 139)

FIG. 2A
Clone 1G6

HVR-H1: GYSFTDYNMY (SEQ ID NO:164)
HVR-H2: WIGYIEPYNGGTSYNQKFKG (SEQ ID NO:167)
HVR-H3: ASPNYDSSPFAY (SEQ ID NO:170)
HVR-L1: SASSSVSYMH (SEQ ID NO:155)
HVR-L2: TWIYDTSILAS (SEQ ID NO:158)
HVR-L3: QQWTSNPLT (SEQ ID NO:161)

Clone 6G1

HVR-H1: GYVFTHYNMY (SEQ ID NO:165)
HVR-H2: WIGYIEPYNGGTSYNQKFKG (SEQ ID NO:168)
HVR-H3: ARGGPGFDV (SEQ ID NO:171)
HVR-L1: SASSSVSYMH (SEQ ID NO:156)
HVR-L2: RWIYDTSKLAS (SEQ ID NO:159)
HVR-L3: QQWSSYPPT (SEQ ID NO:162)

Clone 15B2

HVR-H1: GYAFTSYNY (SEQ ID NO:166)
HVR-H2: WIGYIDPYIGGTSYNQKFKG (SEQ ID NO:169)
HVR-H3: ARWGIDVGYGMDY (SEQ ID NO:172)
HVR-L1: LASQTIGTWLA (SEQ ID NO:157)
HVR-L2: LLIYAATSLAD (SEQ ID NO:160)
HVR-L3: QQLYSPPW (SEQ ID NO:163)

FIG. 2B
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<td>QVQLVQQGAEVKPGASVKSCKACGYYTPT</td>
<td>-H1-</td>
<td>WVRQAPGQQLRWS</td>
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<td>WVRQAPGQQLRWS</td>
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<td>II</td>
<td>QVQLVQQGAEVKPGASVKSCKACGYYTPT</td>
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<td>WVRQAPGQQLRWS</td>
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<td>WVRQAPGQQLRWS</td>
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<td>III</td>
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**Acceptor**

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**Second Acceptor**

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**FIG. 3A**
Framework Sequences of huMAb4D5-8 Light Chain

LC-FR1 1Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys2 (SEQ ID NO: 42)

LC-FR2 35Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr49 (SEQ ID NO: 43)

LC-FR3 57Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys86 (SEQ ID NO: 44)

LC-FR4 98Phe Gly Gln Gly Thr Lys Val Glu Ile Lys107 (SEQ ID NO: 45)

Framework Sequences of huMAb4D5-8 Heavy Chain

HC-FR1 1Glu Val Gln Leu Val Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser25 (SEQ ID NO: 46)

HC-FR2 36Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val46 (SEQ ID NO: 47)

HC-FR3 66Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn83 Ser83a Leu83b Arg83c Ala Glu Asp Thr Ala Val Tyr Tyr Cys92 (SEQ ID NO: 175)

HC-FR4 103Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser113 (SEQ ID NO: 176)

FIG. 5

Framework Sequences of huMAb4D5-8 Light Chain Modified at Position 66 (Underlined)

LC-FR1 1Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys23 (SEQ ID NO: 42)

LC-FR2 35Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr49 (SEQ ID NO: 43)

LC-FR3 57Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys86 (SEQ ID NO: 177)

LC-FR4 98Phe Gly Gln Gly Thr Lys Val Glu Ile Lys107 (SEQ ID NO: 45)

Framework Sequences of huMAb4D5-8 Heavy Chain Modified at Positions 71, 73 and 78 (Underlined)

HC-FR1 1Glu Val Gln Leu Val Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser25 (SEQ ID NO: 46)

HC-FR2 36Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val46 (SEQ ID NO: 47)

HC-FR3 66Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn83 Ser83a Leu83b Arg83c Ala Glu Asp Thr Ala Val Tyr Tyr Cys92 (SEQ ID NO: 178)

HC-FR4 103Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser113 (SEQ ID NO: 176)

FIG. 6
**FIG. 8A**

**FIG. 8B**

**FIG. 8C**

**FIG. 8D**

**FIG. 8E**
**FIG. 9A**

Graph showing cell viability as a percentage of FGF1 with various antibody concentrations.

**FIG. 9B**

Western blot analysis showing the effects of Mock, Ctrl, and R3Mab on FGF1, pFGFR3, Total FGFR3, pMAPK, and Total MAPK.

**FIG. 9C**

Diagram of protein domains: IgD1, IgD2, IgD3, TM, TK1, and TK2 with their corresponding mutations and percentages: R248C 9.7%, S249C 66.6%, G372C 4.3%, Y375C 15.1%, K652M 1.0%, K652Q 0.1%, K652E 1.4%.
FIG. 9D

FIG. 9E

FIG. 9F

FIG. 9G

FIG. 9H
**FIG. 10A**

<table>
<thead>
<tr>
<th>R3Mab (µg/ml)</th>
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<tr>
<td>0</td>
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</tr>
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<td>100</td>
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**FIG. 10B**

164  178
FGFR3 LAVPAANTVRFRCPA Peptide 3
FGFR1 HAVPAAKTVFKCPGS

269  283
FGFR3 SDVEFHCKVYSDAQPS Peptide 11
FGFR1 SNVEFMCKVYSDPQP
**FIG. 11A**

![Graph showing [3H] Thymidine Incorporation (Fold Change vs. Untreated).](image)

**FIG. 11B**

![Western blot images of FGF1, pFGFR3, Total FGFR3, pFRS2Y196, Total FRS2, pAktS473, Total Akt, pMAPK, Total MAPK.](image)

**FIG. 11C**

![Colony Count image.](image)

**FIG. 11D**

![Bar graph showing Colony Count / Well.](image)

**FIG. 11E**

![Western blot images of FGF1, pFGFR3, Total FGFR3.](image)
**FIG. 12A**

**FIG. 12B**

**FIG. 12C**
**FIG. 13D**

Mean Tumor Volume (mm³)

- Vehicle
- Control Ab
- R3Mab.
- R3Mab. 5 mg/kg
- R3Mab. 50 mg/kg

**FIG. 13E**

<table>
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</table>

- FGFR3 Dimer
- FGFR3 Monomer
- FGFR3
- β-actin
- pMAPK
- Total MAPK
**FIG. 14A**

Mean Tumor Volume (mm$^3$)

**FIG. 14B**

Mean Tumor Volume (mm$^3$)

**FIG. 14C**

% of Maximum Lysis vs. Antibody (ng/ml)

**FIG. 14D**

% of Maximum Lysis vs. Antibody (ng/ml)
**FIG. 15A**

RT112 (WT FGFR3)

![Graph showing relative CPM (Fold to Mock) for RT112 (WT FGFR3) with bars indicating different conditions.]

**FIG. 15B**

SW780 (WT FGFR3)

![Graph showing relative CPM (Fold to Mock) for SW780 (WT FGFR3) with bars indicating different conditions.]

[Images showing Western blot analysis for FGFR3 and Tubulin expression across different conditions.]
FIG. 16A

FGFR3 shRNA2-4

No Dox
G1: 57.9%
S: 27.7%
G2: 11.1%

+ Dox
G1: 72.9%
S: 17.8%
G2: 7.87%

FGFR3 shRNA6-16

No Dox
G1: 58.3%
S: 25.8%
G2: 9.09%

+ Dox
G1: 71.3%
S: 20.5%
G2: 5.14%
FIG. 16B
FIG. 17

Cell Viability (% of with FGF1) vs. Ab (μg/ml) for different mutant FGFR3 proteins (Ba/F3-FGFR3, Ba/F3-FGFR3K652E, Ba/F3-FGFR3R248C, Ba/F3-FGFR3S249C, Ba/F3-FGFR3I37G370C, Ba/F3-FGFR3Y375C) with and without FGF1 in the presence of Ab (g/ml). The graphs illustrate the impact of different Ab concentrations on cell viability under varying conditions.
FIG. 21
FIG. 22A

Relative CPM (Fold to No FGF9)

FIG. 22B

Relative CPM (Fold to Untreated)

FIG. 22C

Relative CPM (Fold to Untreated)
**FIG. 23B**

![Graphs showing binding data for FGFR3 receptors in different cell lines.](image)

**KMS11**
- Bound/FREE: 0.077, 0.070, 0.063, 0.056, 0.049, 0.042, 0.035, 0.028, 0.021, 0.014, 0.007, 0.000
- Bound (Picomolar): 14, 28, 42, 56
- FGFR3 (Receptors/Cell) Kd (nM): 13.550, 0.35

**OPM2**
- Bound/FREE: 0.051, 0.034, 0.017, 0.000
- Bound (Picomolar): 16, 32, 48
- FGFR3 (Receptors/Cell) Kd (nM): 15.682, 0.64

**RT112**
- Bound/FREE: 0.028, 0.024, 0.020, 0.016, 0.012, 0.008, 0.004, 0.000
- Bound (Picomolar): 2.3, 3.3, 4.3, 5.3, 6.3, 7.3
- FGFR3 (Receptors/Cell) Kd (nM): 2720, 0.16

**UMUC-14**
- Bound/FREE: 0.018, 0.016, 0.014, 0.012, 0.010, 0.008, 0.006, 0.004, 0.002, 0.000
- Bound (Picomolar): 0, 1, 2, 3, 4, 5, 6
- FGFR3 (Receptors/Cell) Kd (nM): 2642, 0.34
**FIG. 24A**

**FIG. 24B**
FIELD OF THE INVENTION

[0003] The present invention relates generally to the field of molecular biology. More specifically, the invention concerns anti-FGFR3 antibodies, and uses of same.

BACKGROUND OF THE INVENTION

[0004] Fibroblast growth factors (FGFs) and their receptors (FGFRs) play critical roles during embryonic development, tissue homeostasis and metabolism (1-3). In humans, there are 22 FGFs (FGF1-14, FGF16-23) and four FGFR 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 IIb and Mc. 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 Mb isoform is found in epithelial cells, whereas IIb 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 FGF substrate 2 α (FRS2α), leading to activation of multiple signaling cascades, including the mitogen-activated protein kinase (MAPK) and PI3K-AKT pathways (1, 3, 4). Consequently, FGFs and their cognate receptors regulate a broad array of cellular processes, including proliferation, differentiation, migration and survival, in a context-dependent manner.

[0005] Aberrantly activated FGFRs have been implicated in specific human malignancies (1, 5). 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.


SUMMARY OF THE INVENTION

[0007] It is clear that there continues to be a need for agents that have clinical attributes that are optimal for development as therapeutic agents. The invention described herein meets this need and provides other benefits.

[0008] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.
In one aspect, the invention provides a heavy atom derivative of a crystal of the invention. In one aspect, the invention provides a composition comprising a crystal of the invention.

In one aspect, the invention provides a computer-implemented method, a computer system and machine-readable data storage medium comprising a data storage material encoded with machine-readable instructions for causing a display of a graphical three-dimensional representation of a structure of a portion of a crystal of anti-FGFR3 antibody (or structural homolog thereof) in complex with FGFR3 (or a structural homolog and/or portion thereof). In some embodiments, the computer is programmed with instructions for transforming the structure coordinates into the graphical three-dimensional representation of the structure and/or displaying the graphical three-dimensional representation. In some embodiments, the structure coordinates include the coordinates of the backbone atoms of the portion of the crystal and/or one or more of the contact residues between the anti-FGFR3 antibody and the FGFR3 in the complex (e.g., some or all of the coordinates shown in Table 6).

In one aspect, the amino acid residues that form a binding site for an inhibitor binding site on FGFR3 are identified and are useful, for example, in methods to model the structure of an FGFR3 binding site and to identify agents that can bind or fit into the binding site. This use includes the rational design of modulators of FGFR3 activity. For example, these modulators include ligands that interact with FGFR3 and modulate FGFR/FGF activities.

In some embodiments, the crystals are formed from an FGFR3 sequence comprising sequence

In some embodiments, the anti-FGFR3 antibody comprises:

(a) at least one, two, three, four, or five hypervariable region (HVR) sequences selected from:

(i) HVR-L1 comprising sequence A1-A11, wherein A1-A11 is RASQDVTSLTA (SEQ ID NO: 87);

(ii) HVR-L2 comprising sequence B1-B7, wherein B1-B7 is SASFLYYS (SEQ ID NO: 88);

(iii) HVR-L3 comprising sequence C1-C9, wherein C1-C9 is QQSTGHPQTVT (SEQ ID NO: 89);

(iv) HVR-H1 comprising sequence D1-D10, wherein D1-D10 is GFTTFSTGIS (SEQ ID NO: 84);

(v) HVR-H2 comprising sequence E1-E18, wherein E1-E18 is GRYPTSGTNYDVSQGK (SEQ ID NO: 85);

and

HVR-H3 comprising sequence F1-F20, wherein F1-F20 is ARTYG_1YDVYDYVMDY (SEQ ID NO: 86).

(b) at least one 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 NO: 18, 48-131 and 140-145. The modification desirably is a substitution, insertion, or deletion.

In some embodiments, an HVR-L1 variant comprises 1-6, 1, 2, 3, 4, 5, or 6 substitutions in any combination of the following positions: A5 (V or D), A6 (V or I), A7 (D, E or S), A8 (T or I), A9 (A or S) and A10 (V or L). In some embodiments, an HVR-L2 variant comprises 1-6, 1, 2, 3, 4, 5, or 6 substitutions in any combination of the following positions: B1 (S or G), B4 (F or S or T) and B6 (A or Y). In some embodiments, a HVR-L3 variant comprises 1-6, 1, 2, 3, 4, 5, or 6 substitutions in any combination of the following positions: C3 (G or S or T), C4 (T or Y or A), C5 (G or S or T or A), C6 (A or H or D or T or N), C7 (Q or P or S), and C8 (S or Y or L or P or Q). In some embodiment, a HVR-H1 variant comprises 1-3, 1, 2, or 3 substitutions in any combination of the following positions: D3 (S or T), D5 (W or Y or S or T), D6 (S or G or T). In some embodiment, a HVR-H2 variant comprises 1-6, 1, 2, 3, 4, 5, or 6 substitutions in any combination of the following positions: E2 (R or S), E6 (Y or A or L or S or T), E7 (A or R or D or G or Y or S or N or F), E8 (A or D or G), E9 (T or S), E10 (K or F or T or S), E11 (Y or H or N or I).

In some embodiments, the anti-FGFR3 antibody comprises:

(a) at least one, two, three, four, or five hypervariable region (HVR) sequences selected from:

(i) HVR-L1 comprising sequence RASQDVTSLTA (SEQ ID NO: 274);

(ii) HVR-L2 comprising sequence SASFLYYS (SEQ ID NO: 275);

and

a heavy chain variable region comprising sequence

(iii) HVR-H1 comprising sequence GFTTFSTGIS (SEQ ID NO: 276);

(iv) HVR-H2 comprising sequence GRYPTSGTNYDVSQGK (SEQ ID NO: 277);

and

(iv) HVR-H3 comprising sequence ARTYG_1YDVYDYVMDY (SEQ ID NO: 278).
X₁ is D, E or S, X₂ is T or I, X₃ is A or S, and X₄ is V or L (SEQ ID NO:146),

[0030] (ii) HVR-L2 comprising sequence X₁ASFLX₃S wherein X₁ is S or G and X₃ is A or Y (SEQ ID NO:147),

[0031] (iii) HVR-L3 comprising sequence QXX₃X₃X₄X₅X₆T wherein X₁ is G, S or T, X₂ is Y or A, X₃ is G, S, T or A, X₄ is A, H, D, T or N, X₅ is Q, P or S, X₆ is S, Y, L, P or Q (SEQ ID NO:148),

[0032] (iv) HVR-H1 comprising sequence GFX₃F₄X₅TGIS wherein X₁ is S or T, X₂ is W, Y, S or T, X₃ is S, G, or T (SEQ ID NO:149),

[0033] (v) HVR-H2 comprising sequence GRIYPX₃X₄X₅X₆YADSVKG wherein X₁ is Y, A, L, 5, or T, X₂ is A, Q, D, G, Y, 5, N or F, X₃ is A, D, or G, X₄ is T or 5, X₅ is K, F, T, or S, X₆ is Y, H, N or I (SEQ ID NO:150), and

[0034] (vi) HVR-H3 comprising sequence ARTYGYDLYVDTYEYMID (SEQ ID NO:151).

[0035] In some embodiments, HVR-L1 comprises sequence QASQX₃VXX₄X₅VA wherein X₁ is V or D, X₂ is D, or S, X₃ is T or I, X₄ is A or S (SEQ ID NO:152). In some embodiments, HVR-L3 comprises sequence QXX₃X₄X₅X₆T wherein X₁ is S, G, or T, X₂ is Y, T, or A, X₃ is T or G, X₄ is T, H or N, X₅ is P or S, X₆ is P, Q, Y, or L (SEQ ID NO:153). In some embodiments, HVR-H2 comprises sequence GRIYPX₃X₄GSTX₅YADSVKG wherein X₁ is T or L, X₂ is N, Y, S, G, A, or Q; X₃ is N or H (SEQ ID NO:154).

[0036] In one aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, wherein each, in order, comprises SEQ ID NO:1, 2, 3, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 4, 5, 6.

[0037] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, wherein each, in order, comprises SEQ ID NO:7, 8, 9, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 10, 11, 12.

[0038] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, wherein each, in order, comprises SEQ ID NO:13, 14, 15, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO:16, 17, 18.

[0039] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, wherein each, in order, comprises SEQ ID NO: 48, 49, 50, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 51, 52, 53.

[0040] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, wherein each, in order, comprises SEQ ID NO: 54, 55, 56, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 57, 58, 59.

[0041] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, wherein each, in order, comprises SEQ ID NO:90, 61, 62, 63, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 63, 64, 65.

[0042] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO:66, 67, 68, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 69, 70, 71.

[0043] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO:72, 73, 74, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 75, 76, 77.

[0044] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO:78, 79, 80, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 81, 82, 83.

[0045] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO: 84, 85, 86, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 87, 88, 89.

[0046] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO: 90, 91, 92, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 93, 94, 95.

[0047] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO: 96, 97, 98, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 99, 100, 101.

[0048] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO: 102, 103, 104, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 105, 106, 107.

[0049] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO: 108, 109, 110, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 111, 112, 113.

[0050] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO: 114, 115, 116, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 117, 118, 119.

[0051] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO: 120, 121, 122, and/or a light chain variable region comprising HVR-L₁, HVR-L₂, and HVR-L₃, where each, in order, comprises SEQ ID NO: 123, 124, 125.

[0052] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H₁, HVR-H₂, HVR-H₃, where each, in order, comprises SEQ ID NO:126, 127, 128, and/or a light chain variable region com-
prising HVR-L1, HVR-L2, and HVR-L3, where each, in order, comprises SEQ ID NO:129, 130, 131.

[0053] In another aspect, an anti-FGFR3 antibody comprises a heavy chain variable region comprising HVR-H1, HVR-H2, HVR-H3, where each, in order, comprises SEQ ID NO:143, 144, 145, and/or a light chain variable region comprising HVR-L1, HVR-L2, and HVR-L3, where each, in order, comprises SEQ ID NO:140, 141, 142.

[0054] The amino acid sequences of SEQ ID NOs:1-18, 48-131 and 140-145 are numbered with respect to individual HVRs (i.e., H1, H2 or H3) as indicated in FIG. 1, the numbering being consistent with the Kabat numbering system as described below.

[0055] In some embodiments, the anti-FGFR3 antibody comprises a heavy chain variable region comprising SEQ ID NO:132 and a light chain variable region.

[0056] In some embodiments, the anti-FGFR3 antibody comprises a light chain variable region comprising SEQ ID NO:133, and a heavy chain variable region.

[0057] In some embodiments, the anti-FGFR3 antibody comprises a heavy chain variable region comprising SEQ ID NO:132 and a light chain variable region comprising SEQ ID NO:133.

[0058] In some embodiments, the anti-FGFR3 antibody comprises a heavy chain variable region comprising SEQ ID NO:134 and a light chain variable region.

[0059] In some embodiments, the anti-FGFR3 antibody comprises a light chain variable region comprising SEQ ID NO:135, and a heavy chain variable region.

[0060] In some embodiments, the anti-FGFR3 antibody comprises a light chain variable region comprising SEQ ID NO:134 and a light chain variable region comprising SEQ ID NO:135.

[0061] In some embodiments, the anti-FGFR3 antibody comprises a heavy chain variable region comprising SEQ ID NO:136 and a light chain variable region.

[0062] In some embodiments, the anti-FGFR3 antibody comprises a light chain variable region comprising SEQ ID NO:137, and a heavy chain variable region.

[0063] In some embodiments, the anti-FGFR3 antibody comprises a heavy chain variable region comprising SEQ ID NO:136 and a light chain variable region comprising SEQ ID NO:137.

[0064] In some embodiments, the anti-FGFR3 antibody comprises a heavy chain variable region comprising SEQ ID NO:138 and a light chain variable region.

[0065] In some embodiments, the anti-FGFR3 antibody comprises a light chain variable region comprising SEQ ID NO:139, and a heavy chain variable region.

[0066] In some embodiments, the anti-FGFR3 antibody comprises a light chain variable region comprising SEQ ID NO:138 and a light chain variable region comprising SEQ ID NO:139.

[0067] In some embodiments, the anti-FGFR3 antibody comprises a heavy chain variable region comprising SEQ ID NO:136 and a light chain variable region comprising SEQ ID NO:137.

[0068] In some embodiments, the anti-FGFR3 antibody comprises: at least one, two, three, four, five, and/or six hyper-variable region (HVR) sequences selected from the group consisting of:

- [0069] (a) HVR-L1 comprising sequence SASSSVSYMH (SEQ ID NO:155), SASSSVSYMH (SEQ ID NO:156) or LASSQITGWLA (SEQ ID NO:157),
- [0070] (b) HVR-L2 comprising sequence TWIYDTSILAS (SEQ ID NO:158), RWIYDTSKLAS (SEQ ID NO:159), or LLIYAATSLAD (SEQ ID NO:160),
- [0071] (c) HVR-L3 comprising sequence QQWTSNPLT (SEQ ID NO:161), QQWSSYPPT (SEQ ID NO:162), or QQLYSPPW (SEQ ID NO:163),
- [0072] (d) HVR-H1 comprising sequence GYSFTDYNM (SEQ ID NO:164), GYVFHTHYNM (SEQ ID NO:165), or GYAFISYNMY (SEQ ID NO:166),
- [0073] (e) HVR-H2 comprising sequence WIGYIEPYNGGTSYNKFKG (SEQ ID NO:167), WIGYIEPYNGGTSYNKFKG (SEQ ID NO:168), or WIGYDTPYGGTSTYNKFKG (SEQ ID NO:169), and
- [0074] (f) HVR-H3 comprising sequence ASPNYYDDSP-FAY (SEQ ID NO:170), ARGGQGPFDV (SEQ ID NO:171), or ARWGDYDVGAMDY (SEQ ID NO:172).

[0075] In some embodiments, the anti-FGFR3 antibody comprises: at least one, two, three, four, five, and/or six hyper-variable region (HVR) sequences selected from the group consisting of:

- [0076] (a) HVR-L1 comprising sequence SASSSVSYMH (SEQ ID NO:155),
- [0077] (b) HVR-L2 comprising sequence TWIYDTSILAS (SEQ ID NO:158),
- [0078] (c) HVR-L3 comprising sequence QQWTSNPLT (SEQ ID NO:161),
- [0079] (d) HVR-H1 comprising sequence GYSFT-DYNM (SEQ ID NO:164),
- [0080] (e) HVR-H2 comprising sequence WIGYIEPYNGGTSYNKFKG (SEQ ID NO:167), and
- [0081] (f) HVR-H3 comprising sequence ASPNYYDDSP-FAY (SEQ ID NO:170).

[0082] In some embodiments, the anti-FGFR3 antibody comprises: at least one, two, three, four, five, and/or six hyper-variable region (HVR) sequences selected from the group consisting of:

- [0083] (a) HVR-L1 comprising sequence SASSSVSYMH (SEQ ID NO:156),
- [0084] (b) HVR-L2 comprising sequence RWIY-DTSKLAS (SEQ ID NO:159),
- [0085] (c) HVR-L3 comprising sequence QQWSSYPPT (SEQ ID NO:162),
- [0086] (d) HVR-H1 comprising sequence GYVFHTHYNM (SEQ ID NO:165),
- [0087] (e) HVR-H2 comprising sequence WIGYDTPYGGTSTYNKFKG (SEQ ID NO:168), and
- [0088] (f) HVR-H3 comprising sequence ARGGQGPFDV (SEQ ID NO:171).

[0089] In some embodiments, the anti-FGFR3 antibody comprises: at least one, two, three, four, five, and/or six hyper-variable region (HVR) sequences selected from the group consisting of:

- [0090] (a) HVR-L1 comprising sequence LASQITGWLA (SEQ ID NO:157),
- [0091] (b) HVR-L2 comprising sequence LLIYAATSLAD (SEQ ID NO:160),
- [0092] (c) HVR-L3 comprising sequence QQQLYSPPW (SEQ ID NO:163),
- [0093] (d) HVR-H1 comprising sequence GYAFTSYNMY (SEQ ID NO:166),
- [0094] (e) HVR-H2 comprising sequence WIGYIDPYGGTSTYNKFKG (SEQ ID NO:169), and
- [0095] (f) HVR-H3 comprising sequence ARGWGDYDVGAMDY (SEQ ID NO:172).
In some embodiments, the anti-FGFR3 antibody comprises (a) a light chain comprising (i) HVR-L1 comprising sequence SASSSSVSYMH (SEQ ID NO:155); (ii) HVR-L2 comprising sequence TWIYDTSILAS (SEQ ID NO:158); and (iii) HVR-L3 comprising sequence QQWTSNPLT (SEQ ID NO:161); and/or (b) a heavy chain comprising (i) HVR-H1 comprising sequence GYSFDTYNMY (SEQ ID NO:164); (ii) HVR-H2 comprising sequence WIGYIEPYNGTYSYQFKFG (SEQ ID NO:167); and (iii) HVR-H3 comprising sequence ASPNYDSSSPFAY (SEQ ID NO:170).

In some embodiments, the anti-FGFR3 antibody comprises (a) a light chain comprising (i) HVR-L1 comprising sequence SASSSSVSYMH (SEQ ID NO:156); (ii) HVR-L2 comprising sequence RWIYDTSKLAS (SEQ ID NO:159); and (iii) HVR-L3 comprising sequence QQWSSYPPT (SEQ ID NO:162); and/or (b) a heavy chain comprising (i) HVR-H1 comprising sequence GYVFTHNYM (SEQ ID NO:165); (ii) HVR-H2 comprising sequence WIGYIEPYNGTYSYQFKFG (SEQ ID NO:168); and (iii) HVR-H3 comprising sequence ARQGYDPFDYW (SEQ ID NO:171).

In some embodiments, the anti-FGFR3 antibody comprises (a) a light chain comprising (i) HVR-L1 comprising sequence LASQHTWLA (SEQ ID NO:157); (ii) HVR-L2 comprising sequence LLIYAASTSAL (SEQ ID NO:160); and (iii) HVR-L3 comprising sequence QQLYSSPWWT (SEQ ID NO:163); and/or (b) a heavy chain comprising (i) HVR-H1 comprising sequence GYAFSTSYMN (SEQ ID NO:166); (ii) HVR-H2 comprising sequence WIGYIDPYIIGTYSQFKFG (SEQ ID NO:169); and (iii) HVR-H3 comprising sequence ARWQGDYDVAGMDY (SEQ ID NO:172).


1 Asp Ile Gin Met Thr Gin Ser Pro Ser Ser Leu Ser 
Ala Ser Val Gly Arg Val Thr Ile Thr Cys Asx 
Ala Ser Gin Asx Val Aan Thr Ala Val Ala Trp Tyr 
Gln Gin Lys Pro Gly Lys Ala Pro Lys Leu Ile 
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser 
Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr 
Leu Thr Ile Ser Ser Leu Gin Pro Glu Asp Phe Ala 
Thr Tyr Tyr Cys Gin Gin His Tyr Thr Thr Pro 
The Phe Gin Gly Thr Lys Val Glu Ile Lys 107

(Substituted residues with respect to huMAb4D5-8 are indicated in bold/italics.

Antibodies of the invention can comprise any suitable framework variable domain sequence, provided binding activity to FGFR3 is 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, Calif., USA) (also referred to in U.S. Pat. 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 κ light chain framework consensus sequence. In particular, these antibodies comprise light chain HVR sequences of huMAb4D5-8 as described in U.S. Pat. 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, Calif., USA) (also referred to in U.S. Pat. Nos. 6,407,213 & 5,821,337, and Lee et al., J. Mol. Biol. (2004), 340(5):1073-1093).

In one embodiment, an antibody of the invention comprises a heavy chain variable domain, wherein the framework sequence comprises the sequence of SEQ ID NOS:19 and 203-215, 20 and 206-208, 21 and 209-211, 22 and 212-214, 23 and 215-217, 24 and 218-220, 25 and 221-223, 26 and 224-226, 27 and 227-229, 28 and 230-232, 29 and 233-235, 30 and 236-238, 31 and 239-241, 32 and 242-244, 33 and 245-247, 34 and 248-250, 35 and 251-253, 36 and 254-256, and/or 37 and 257-259, and HVR H1, H2, and H3 sequences are SEQ ID NOS:13, 14 and/or 15, respectively. In another embodiment, the framework sequence comprises the sequence of SEQ ID NOS:19 and 203-205, 20 and 206-208, 21 and 209-211, 22 and 212-214, 23 and 215-217, 24 and 218-220, 25 and 221-223, 26 and 224-226, 27 and 227-229, 28 and 230-232, 29 and 233-235, 30 and 236-238, 31 and 239-241, 32 and 242-244, 33 and 245-247, 34 and 248-250, 35 and 251-253, 36 and 254-256, and/or 37 and 257-259, and HVR H1, H2, and H3 sequences are SEQ ID NOS:48, 49.
and/or 50, respectively. In yet another embodiment, the framework sequence comprises the sequence of SEQ ID NOS: 19 and 203-205, 20 and 206-208, 21 and 209-211, 22 and 212-214, 23 and 215-217, 24 and 218-220, 25 and 221-223, 26 and 224-226, 27 and 227-229, 28 and 230-232, 29 and 233-235, 30 and 236-238, 31 and 239-241, 32 and 242-244, 33 and 245-247, 34 and 248-250, 35 and 251-253, 36 and 254-256, and/or 37 and 257-259, and HVR H1, H2, and H3 sequences are SEQ ID NOS: 84, 85, and/or 86, respectively. In a further embodiment, the framework sequence comprises the sequence of SEQ ID NOS: 19 and 203-205, 20 and 206-208, 21 and 209-211, 22 and 212-214, 23 and 215-217, 24 and 218-220, 25 and 221-223, 26 and 224-226, 27 and 227-229, 28 and 230-232, 29 and 233-235, 30 and 236-238, 31 and 239-241, 32 and 242-244, 33 and 245-247, 34 and 248-250, 35 and 251-253, 36 and 254-256, and/or 37 and 257-259, and HVR H1, H2, and H3 sequences are SEQ ID NOS: 108, 109, and/or 110, respectively.

[0102] In a particular embodiment, an antibody of the invention comprises a light chain variable domain, wherein the framework sequence comprises the sequence of SEQ ID NOS: 38 and 260-262, 39 and 263-265, 40 and 266-268, and/or 41 and 269-271, and HVR L1, L2, and L3 sequences are SEQ ID NOS: 16, 17, and/or 18, respectively. In another embodiment, an antibody of the invention comprises a light chain variable domain, wherein the framework sequence comprises the sequence of SEQ ID NOS: 38 and 260-262, 39 and 263-265, 40 and 266-268, and/or 41 and 269-271, and HVR L1, L2, and L3 sequences are SEQ ID NOS: 51, 52 and/or 53, respectively. In an additional embodiment, an antibody of the invention comprises a light chain variable domain, wherein the framework sequence comprises the sequence of SEQ ID NOS: 38 and 260-262, 39 and 263-265, 40 and 266-268, and/or 41 and 269-271, and HVR L1, L2, and L3 sequences are SEQ ID NOS: 87, 88 and/or 89, respectively. In yet another embodiment, an antibody of the invention comprises a light chain variable domain, wherein the framework sequence comprises the sequence of SEQ ID NOS: 38 and 260-262, 39 and 263-265, 40 and 266-268, and/or 41 and 269-271, and HVR L1, L2, and L3 sequences are SEQ ID NOS: 111, 112, and/or 113, respectively.

[0103] In another aspect, an antibody of the invention comprises a heavy chain variable domain comprising the sequence of SEQ ID NO: 132 and/or a light chain variable domain comprising the sequence of SEQ ID NO: 133. In another aspect, an antibody of the invention comprises a heavy chain variable domain comprising the sequence of SEQ ID NO: 134 and/or a light chain variable domain comprising the sequence of SEQ ID NO: 135. In another aspect, an antibody of the invention comprises a heavy chain variable domain comprising the sequence of SEQ ID NO: 136 and/or a light chain variable domain comprising the sequence of SEQ ID NO: 137. In another aspect, an antibody of the invention comprises a heavy chain variable domain comprising the sequence of SEQ ID NO: 138 and/or a light chain variable domain comprising the sequence of SEQ ID NO: 139.

[0104] In one aspect, the invention provides an anti-FGFR3 antibody that binds a polypeptide comprising, consisting essentially of or consisting of the following amino acid sequence: LAVPAANTVVRCPA (SEQ ID NO: 179) and/or SDVEFHCKVYSDAQHP (SEQ ID NO: 180).

[0105] In some embodiments, the antibody binds a polypeptide comprising, consisting essentially of or consisting of amino acid numbers 164-178 and/or 269-283 of human FGFR3.

[0106] In one embodiment, an anti-FGFR3 antibody of the invention specifically binds an amino acid sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, 98% sequence identity or similarity with the sequence LAVPAANTVVRCPA (SEQ ID NO: 179) and/or SDVEFHCKVYSDAQHP (SEQ ID NO: 180).

[0107] In one aspect, the anti-FGFR3 antibody of the present invention binds to at least one, two, three, four, or any number up to all of residues 154, 155, 158, 159, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 202, 205, 207, 210, 212, 214, 216, 217, 241, 246, 247, 248, 278, 279, 280, 281, 282, 283, 314, 315, 316, 317 and/or 318 of FGFR3-IIIb polypeptide, or equivalent residues of FGFR3-IIIc polypeptide. One of ordinary skill in the art understands how to align FGFR3 sequences in order identify corresponding residues between respective FGFR3 sequences. Combinations of two or more residues can include any of residues 154, 155, 158, 159, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 202, 205, 207, 210, 212, 214, 216, 217, 241, 246, 247, 248, 278, 279, 280, 281, 282, 283, 314, 315, 316, 317 and/or 318, or mixtures thereof, of FGFR3-IIIb polypeptide, or equivalent residues of FGFR3-IIIc polypeptide. In some embodiments, the anti-FGFR3 antibody binds to at least one, two, three, four, or any number up to all of residues 158, 159, 169, 170, 171, 173, 175, 205, 207, and/or 315, or mixtures thereof, of FGFR3-IIIb polypeptide, or equivalent residues of FGFR3-IIIc polypeptide. In some embodiments, the anti-FGFR3 antibody binds to at least one, two, three, four, or any number up to all of residues 158, 159, 169, 170, 171, 173, 175, 205, 207, and/or 315, or mixtures thereof, of FGFR3-IIIb polypeptide, or equivalent residues of FGFR3-IIIc polypeptide.

[0108] In one aspect, the invention provides methods of screening for a candidate inhibitor substance that inhibits FGFR3 activity, said method comprising: detecting binding, if any, of the candidate substance to a FGFR3 binding site, wherein the candidate substance is identified by a method comprising comparing amount of FGFR3 activity in a sample with amount of FGFR3 activity in a reference sample comprising similar amounts of FGFR3 as the first sample but that has not been contacted with said candidate substance, whereby a decrease in amount of FGFR3 activity in the first sample compared to the reference sample indicates that the candidate substance is capable of inhibiting FGFR3 activity.

[0109] In another aspect, the invention provides methods of screening for a candidate inhibitory substance that inhibits FGFR3 activation, the method comprising screening for a candidate inhibitory substance that binds FGFR3 binding site and inhibits FGFR3 activity.

[0110] In some embodiments, the methods comprising selecting for a substance that binds to at least one, two, three, four, or any number up to all of residues 154, 155, 158, 159, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 202, 205, 207, 210, 212, 214, 216, 217, 241, 246, 247, 248, 278, 279, 280, 281, 282, 283, 314, 315, 316, 317 and/or 318.
the sequence of FGFR3-IIIb polypeptide, or equivalent residues of FGFR3-IIIc polypeptide.

[0111] In some embodiments, the sample comprises FGFR3 and FGFR3 ligand (such as FGF1 or FGF9). In some embodiments, the sample comprises a mammalian cell expressing FGFR3. In some embodiments, the FGFR3 is transgenically expressed. In some embodiments, the sample comprises a B/FC cell expressing FGFR3.

[0112] In some embodiments, FGFR3 activity comprises FGF (such as FGF1 and/or FGF9) binding, FGFR3 downstream signaling, FGFR3 phosphorylation, FGFR3 binding to a ligand (e.g., FGF1, FGF9), FGFR3 dimerization, promotion of formation of 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, decrease in amount of FGFR3 activity is reduction or blocking of FGF (such as FGF1 and/or FGF9) binding to FGFR3, reduction or blocking of FGFR3 activation, reduction or blocking of FGFR3 downstream signaling), reduction or blocking of FGFR3 dimerization 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).

[0113] The invention also provides an antagonist molecule that inhibits FGFR3, wherein the molecule binds to at least one, two, three, four, or any number up to all of residues 158, 170, 171, 173, 175, and/or 315 of the sequence of FGFR3-IIIb polypeptide, or equivalent residues of FGFR3-IIIc polypeptide. In some embodiments, the antagonist molecule binds to at least one, two, three, four, or any number up to all of residues 154, 155, 158, 159, 161, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 202, 205, 207, 210, 212, 214, 216, 217, 241, 246, 247, 248, 278, 279, 280, 281, 282, 283, 314, 315, 316, 317, 318 of the sequence of FGFR3-IIIb polypeptide, or equivalent residues of FGFR3-IIIc polypeptide. In some embodiments, the antagonist molecule comprises an antibody.

[0114] The invention also provides methods using the antagonist molecules, including treatment and diagnostic methods.

[0115] In another aspect, the disclosure includes FGFR3 polypeptides and polynucleotides encoding the polypeptides. The disclosure includes a polynucleotide encoding a polypeptide and/or a polypeptide having at least 90% sequence identity to the polypeptide comprising sequence of amino acids 154-318 of human FGFR3, not including the polypeptide having the amino acid sequence of human FGFR3. In some embodiments, the disclosure includes a polynucleotide encoding a polypeptide and/or a polypeptide having at least 90% sequence identity to the polypeptide comprising any of amino acid residue 154 to amino acid residue 177, amino acid residue 202 to amino acid residue 217, amino acid residue 241 to amino acid residue 248, amino acid residue 278 to amino acid residue 283 and/or amino acid residue 314 to amino acid residue 318 FGFR3, not including the polypeptide comprising the amino acid sequence of FGFR3. In some embodiments, the polypeptide binds a human FGFR3 ligand (such as FGF1 or FGF9).
NO:116; HVR-L1 is SEQ ID NO:117; HVR-L2 is SEQ ID NO:118; HVR-L3 is SEQ ID NO:119)

[0132] Clone 184.6.58.N54A (HVR-H1 is SEQ ID NO:120; HVR-H2 is SEQ ID NO:121; HVR-H3 is SEQ ID NO:122; HVR-L1 is SEQ ID NO:123; HVR-L2 is SEQ ID NO:124; HVR-L3 is SEQ ID NO:125)

[0133] Clone 184.6.58.N54Q (HVR-H1 is SEQ ID NO:126; HVR-H2 is SEQ ID NO:127; HVR-H3 is SEQ ID NO:128; HVR-L1 is SEQ ID NO:129; HVR-L2 is SEQ ID NO:130; HVR-L3 is SEQ ID NO:131)

[0134] Clone 184.6.1 NS D3OE (HVR-H1 is SEQ ID NO:143; HVR-H2 is SEQ ID NO:144; HVR-H3 is SEQ ID NO:145; HVR-L1 is SEQ ID NO:140; HVR-L2 is SEQ ID NO:141; HVR-L3 is SEQ ID NO:142)

[0135] Amino acid positions are numbered according to the Kabat numbering system as described below.

[0136] FIGS. 2A and 2B: depict (A) the amino acid sequences of the heavy chain variable regions and light chain variable regions of anti-FGFR3 antibodies 184.6.1.N54S, 184.6.58, and 184.6.62; and (B) the hypervariable regions of anti-FGFR3 antibodies 1G6, GG1, and 15B2.

[0137] FIGS. 3A, 3B, and 4: depict exemplary human constant framework sequences for use in practicing the instant invention with sequence identifiers as follows:

Variable Heavy (VH) Consensus Frameworks (FIG. 3A, 3B)

[0138] human VH subgroup I consensus framework minus Kabat CDRs (SEQ ID NOS:19 and 203-205)
human VH subgroup I consensus framework minus extended hypervariable regions (SEQ ID NOS:20 and 206-208, 21 and 209-211, 22 and 212-214)
human VH subgroup II consensus framework minus Kabat CDRs (SEQ ID NOS:23 and 215-217)
human VH subgroup II consensus framework minus extended hypervariable regions (SEQ ID NOS:24 and 218-220, 25 and 221-223, 26 and 224-226)
human VH subgroup II consensus framework minus extended Kabat CDRs (SEQ ID NOS:27 and 227-229)
human VH subgroup III consensus framework minus extended hypervariable regions (SEQ ID NOS:28 and 230-232, 29 and 233-235, 30 and 236-238)
human VH acceptor framework minus Kabat CDRs (SEQ ID NOS:31 and 239-241)
human VH acceptor framework minus extended hypervariable regions (SEQ ID NOS:32 and 242-244, 33 and 224-247)
human VH acceptor 2 framework minus Kabat CDRs (SEQ ID NOS:34 and 248-250)
human VH acceptor 2 framework minus extended hypervariable regions (SEQ ID NOS:35 and 251-253, 36 and 254-256, 37 and 257-259)

Variable Light (VL) Consensus Frameworks (FIG. 4)

[0139] human VL kappa subgroup I consensus framework (SEQ ID NO:38 and 260-262)
human VL kappa subgroup II consensus framework (SEQ ID NO:39 and 263-265)
human VL kappa subgroup III consensus framework (SEQ ID NO:40 and 265-268)
human VL kappa subgroup IV consensus framework (SEQ ID NO:41 and 269-271)

[0140] FIG. 5: depicts framework region sequences of huMAb4D5-8 light (SEQ ID NOS:42-45) and heavy chains (SEQ ID NOS:46, 47, 175, 176). Numbers in superscript/bold indicate amino acid positions according to Kabat.

[0141] FIG. 6: depicts modified/variant framework region sequences of huMAb4D5-8 light (SEQ ID NOS:42, 43, 177, 45) and heavy chains (SEQ ID NOS:46, 47, 178, and 176). Numbers in superscript/bold indicate amino acid positions according to Kabat.

[0142] FIG. 7: FGFR3 knockdown in bladder cancer cell RT112 inhibits proliferation and induces G1 cell cycle arrest in vitro, and suppresses tumor growth in vivo. Three different FGFR3 shRNAs were cloned into a Tet-inducible expression vector. RT112 cells stably expressing FGFR3 shRNAs or a control shRNA were established with puromycin selection. (A) Representative blots showing FGFR3 expression in selected clones treated with or without doxycycline (Dox, 0, 0.1 and 1 µg/ml, left to right). (B) [3H]-Thymidine incorporation by RT112 stable cells. RT112 stable clones were cultured with or without 1 µg/ml doxycycline for 3 days prior to 16 hour-incubation with [3H]-thymidine (1 µCi per well). Counts of incorporated [3H]-thymidine were normalized to that from cells without doxycycline induction. Error bars represent SEM. (C) DNA fluorescence flow cytometry histograms of RT112 stable cells. RT112 clones expressing control shRNA or FGFR3 shRNA4 were cultured with or without 1 µg/ml doxycycline for 72 hours, and the nuclei were stained with propidium iodide (PI). Similar results were obtained for FGFR3 shRNA2 and 6 (FIG. 16). (D) The growth of RT112 cells expressing control shRNA (n=9 per treatment group) or FGFR3 shRNA4 (n=11 per treatment group) in mice. Mice were given 5% sucrose alone or supplemented with 1 mg/ml doxycycline, and tumor size was measured twice a week. Error bars represent SEM. Similar results were obtained for FGFR3 shRNA2 and 6 (FIG. 16). Lower panel: Expression of FGFR3 protein in tumor lysates extracted from control shRNA or FGFR3 shRNA4 stable cell xenograft tissues.

[0143] FIG. 8: R3Mab blocks FGF/FGFR3 interaction. (A) Selective binding of human FGFR3 by R3Mab. Human FGFR1-4 Fe chimeric proteins were immobilized and incubated with increasing amount of R3Mab. Specific binding was detected using an anti-human Fab antibody. (B-E) Blocking of FGFI binding to human FGFR3-IIIb (B) or IIc (C) by R3Mab. Specific binding was detected by using a biotinylated FGFI-specific polyclonal antibody. (D-E) Blocking of FGFI binding to human FGFR3-IIIb (D) or Mc (E) by R3Mab. Specific binding was detected by using a biotinylated FGFI9-specific polyclonal antibody. Error bars represent standard error of the mean (SEM) and are sometimes smaller than symbols.

[0144] FIG. 9: R3Mab inhibits Ba/F3 cell proliferation driven by wild type and mutated FGFR3. (A) Inhibitory effect of R3Mab on the viability of Ba/F3 cells expressing wild type human FGFR3-IIIb. Cells were cultured in medium without FGFI (no FGFI), or in the presence of 10 ng/ml FGFI plus 10 µg/ml heparin alone (FGFI), or in combination with a control antibody (Control) or R3Mab. Cell viability was assessed with CellTiter-Glo (Promega) after 72 hr incubation with antibodies. (B) Inhibition of FGFR3 and MAPK phosphorylation by R3Mab in Ba/F3 FGFR3-IIIb stable cells. Cells were treated with 15 ng/ml FGFI and 10 µg/ml heparin (+) or heparin alone (−) for 10 minutes, following pre-incubation with a Control Ab (Ctrl), decreasing amount of R3Mab (1, 0.2, 0.04 µg/ml respectively) in PBS, or PBS alone (Mock) for
3 hours. Lysates were immunoblotted to assess phosphorylation of FGFR3 and p44/42 MAPK with antibodies to pFGFR3 and pMAPK, respectively. (C) Schematic representation of FGFR3 mutant hot spots and frequency in bladder cancer (sequence numbering depicted is based on the FGFR3-IIIb isoform amino acid sequence) based on published data (32). TM, transmembrane domain; TK1 and TK2, tyrosine kinase domain 1 and 2. (D) Inhibitory effect of R3Mab on the viability of BaF3 cells expressing cancer-associated FGFR3 mutants. G372C is derived from Mc isoform, and the rest are derived from Mb isoform. Sequence numbering for all mutants is based on the FGFR3-IIIb isoform amino acid sequence (including the G372C mutant, which would be numbered G370C based on the FGFR3-IIIc isoform amino acid sequence). Cell viability was assessed after 72 hour incubation with antibodies as described in (A). Error bars represent SEM.

[0145] FIG. 10: Epitope mapping for R3Mab and crystal structure of the complex between R3Mab Fab fragment and IgD2-D3 of human FGFR3-IIIb. (A) Epitope determined by the binding of 13 peptides spanning IgD2-D3 of human FGFR3 to R3Mab. Each biotinylated peptide was captured onto streptavidin-coated microtiter well and incubated with R3Mab. Specifically bound R3Mab was detected using a goat anti-human IgG antibody. (B) Sequence alignment of human FGFR3 peptides 3 (LAVPAANTVRKFCPA (SEQ ID NO:179)) and 11 (SDVFEHKCVYSDAQ (SEQ ID NO:180)) with extracellular segments of human FGFR1 (peptide 3: HAVPAAXTXF3KCC (SEQ ID NO:181); peptide 11: SNVFMCKVYSDQQ (SEQ ID NO:182)). FGFR1 residues engaged in the primary FGFR2-FGFR1 interaction, heparin binding, and receptor-receptor association are shown in bold, italics, and underlined font, respectively. Functional assignment of FGFR1 residues is based on Plotnikov et al (34). (C) Structure of R3Mab Fab (shown in ribbon-helix, light chain grey, heavy chain black) in complex with human FGFR IgD2-D3 (shown in molecular surface, white). Receptor residues involved in ligand binding and dimerization are colored in grey/crosshatched and dark grey respectively based on Plotnikov et al (34). (D) The close-up of the crystal structure shows that CDR H3 and H4 from the Fab constitute the major interaction sites with IgD2 and IgD3 of FGFR3. (E) Superposition of FGFR3-IIIc-FGFI complex (PDB code 1RY7) with FGFR3-IIIb-Fab complex. FGFR3-IIIc and FGFI are colored in grey and dark grey respectively. FGFR3-IIIb is shown in white and the Fab is shown in light grey for light chain, dark grey for heavy chain. IgD2 was used as the anchor for superposition. Note the well-supersuperposed IgD2 from both structures and the new conformation adopted by IgD3 of FGFR3-IIIb when bound by R3Mab. (F) Another representation of the superposition of FGFR3-IIIc-FGFI complex (PDB code 1RY7) with FGFR3-IIIb-Fab complex. FGFR3-IIIc and FGFI are shown as molecular surfaces that are grey/mesh texture and dark grey/dotted texture, respectively. FGFR3-IIIb is shown in white and the Fab is shown in grey for light chain, black for heavy chain. IgD2 was used as the anchor for superposition. Note the well-supersuperposed IgD2 from both structures and the new conformation adopted by IgD3 of FGFR3-IIIb when bound by R3Mab.

[0146] FIG. 11: R3Mab inhibits proliferation, clonal growth and FGFR3 signaling in bladder cancer cells expressing wild type or mutated FGFR3. (A) Inhibition of [3H]-thymidine incorporation by R3Mab in bladder cancer cell line RT112. Error bars represent SEM. (B) Blocking of FGFI-activated FGFR3 signaling by R3Mab (15 μg/ml) in bladder cancer cell line RT112 as compared to treatment medium alone (Mock) or a control antibody (Ctrl). Cell lysates were immunoprecipitated with anti-FGFR3 antibody and assessed for FGFR3 phosphorylation with an anti-phospho-tyrosine antibody (4G10). Lysates were immunoblotted to detect phosphorylation of AKT (pAKT1-3,Tyr473) and p44/42 MAPK (pMAPKThr202/Tyr204). (C) Inhibition of clonal growth by R3Mab (10 μg/ml) in bladder cancer cell line UMUC-14 (harboring FGFR3G372C) as compared to treatment medium alone (Mock) or a control antibody (Ctrl). (D) Quantitation of the study in (C) reporting the number of colonies larger than 120 μm in diameter per well from a replicate of 12 wells. Error bars represent SEM. P<3.4x10^-5 versus Mock or Ctrl. (E) Inhibition of FGFR3 phosphorylation in UMUC-14 cells by R3Mab (15 μg/ml). FGFR3 phosphorylation was analyzed as in (B). Note constitutive phosphorylation of FGFR3 in this cell line.

[0147] FIG. 12: R3Mab decreases steady-state level of disulfide-linked FGFR3G372C dimer by driving the dimer-monomer equilibrium toward monomeric state. (A) Effect of R3Mab on FGFR3G372C dimer in UMUC-14 cells. Cells were incubated with R3Mab (15 μg/ml) or a control antibody (Ctrl) for 3 hours, and whole cell lysates were analyzed by immunoblot under non-reducing and reducing conditions. (B) Effect of free-sulfhydryl blocker DTNB on FGFR3G372C dimer-monomer equilibrium in UMUC-14 cells. UMUC-14 cells were treated with increasing concentration of DTNB for 3 hours, and cell lysates were analyzed as in (A). (C) Effect of R3Mab on purified recombinant FGFR3G372C dimer in vitro. FGFR3G372C dimer composed of IgD2-D3 was purified through size-exclusion column, and incubated with PBS (Mock), a control antibody (Ctrl), or R3Mab at 37°C. Samples were collected at indicated time for immunoblot analysis under non-reducing conditions. FGFR3 dimer-monomer was detected using anti-FGFR3 hybridoma antibody 6G1 (A-C).

[0148] FIG. 13: R3Mab inhibits xenograft growth of bladder cancer cells and allograft growth of Baf/E3-FGFR3G372C (A) Effect of R3Mab on the growth of pre-established RT112 bladder cancer xenografts compared with vehicle control, n=10 per group. (B) Inhibition of FGFR3 signaling in RT112 tumor tissues by R3Mab. In a separate experiment, RT112 xenograft tumors that were treated with 15 mg/kg of a control antibody (Ctrl) or R3Mab for 48 hours or 72 hours were collected (n=3 per group), homogenized and analyzed for FR2/2a and MAPK activation by immunoblot. (C) Effect of R3Mab on the growth of pre-established UMUC-14 bladder cancer xenografts, n=10 per group. (D) Effect of R3Mab on the growth of pre-established UMUC-14 bladder cancer xenografts, n=10 per group. (E) Effect of R3Mab on FGFR3G372C dimer and signaling in UMUC-14 tumor tissues. UMUC-14 xenograft tumors that were treated with 30 mg/kg of a control antibody (Ctrl) or R3Mab for 24 hours or 72 hours were collected (n=3 per group), homogenized, and analyzed for FGFR3G372C dimer-monomer as well as MAPK activation by immunoblot. FGFR3 dimer-monomer was detected using an anti-FGFR3 rabbit polyclonal antibody sc9007 to avoid interference from mouse IgG in tumor lysates. Error bars represent SEM.

[0149] FIG. 14: ADCC contributes to the anti-tumor efficacy of R3Mab in (4:4) positive multiple myeloma models. (A-B) Effect of R3Mab on the growth of pre-established OPM2 (A) and RMM11 (B) myeloma xenografts.
group. (C-F) Cytolysis of myeloma cell lines OPM2 (C) and KMS11 (D), or bladder cancer cell lines RT112 (E) and UMUC-14 (F) induced by R3Mab in cell culture. Myeloma or bladder cancer cells were incubated with freshly isolated human PBMC in the presence of R3Mab or a control antibody. Cytotoxicity was determined by measuring LDH released in the supernatant. (G-H) Effect of R3Mab or its DNAA mutant on the growth of pre-established OPM2 (G) and KMS11 (H) myeloma xenografts. n=10 per group. Error bars represent SEM and are sometimes smaller than symbols.

**Figure 15:** Knockdown of FGFR3 with siRNA inhibits cell proliferation of bladder cancer cell lines. Six to seven different FGFR3 siRNAs and three non-specific control siRNAs were designed and synthesized in Genentech. Bladder cancer cell lines RT112 (A), SW780 (B), RT4 (C) and UMUC-14 (D) were plated into 96-well plate (3000 cells per well) and allowed to attach overnight, and transiently transfected with 25 nM siRNA in complex with RNAiMax (Invitrogen). 72 hr post-transfection, [3H]-thymidine (1 μCi per well) was added to the culture (A, C, and D) for another 16 hour incubation. Incorporated [3H]-thymidine was quantitated with TopCount. Data were normalized to that from cells transfected with RNAiMax alone (Mock). Error bars represent SEM. Lower panel: Representative blots showing FGFR3 expression in siRNA transfected cells. (B) Cell viability was measured with CellTiter-Glo (Promega) 96 hours after transfection. Error bars represent SEM.

**Figure 16:** FGFR3 knockdown in bladder cancer cell line RT112 induces G1 cell cycle arrest in vitro, and suppresses tumor growth in vivo. Three different FGFR3 siRNAs were designed and cloned into a Tet-inducible shRNA expression retrovector. RT112 stable clones expressing FGFR3 shRNAs or control shRNA were established with puromycin selection. (A) DNA fluorescence flow cytometry histograms of propidium iodide (PI)-stained nuclei obtained from RT112 stable cells expressing FGFR3 shRNA2 or shRNA6 following treatment with or without 1 μg/ml doxycycline for 72 hours. (B) The growth of RT112 stable cells expressing FGFR3 shRNA2-4 (n=11 per treatment group) or FGFR3shRNA6-16 (n=10 per treatment group) in mm/m mice. Tumor bearing mice received 5% sucrose only (solid circle) or 5% sucrose plus 1 mg/ml doxycycline (solid square), and tumors were measured with calipers twice a week. Error bars represent SEM.

**Figure 17:** Effect of anti-FGFR3 hyperboid antibodies 16G, 6G1 and 15B2 on Ba/F3 cell proliferation driven by wild type and mutated FGFR3. Anti-FGFR3 hyperboid antibodies were generated by immunizing BALB/c mice with human FGFR3-IIIb/Fc or human FGFR3-IIIc/Fc chimera. Fused hyperboid cells were selected using hypoxanthin-aminopterin-thymidine selection in Medium D from the ClonaCell® hyperboid selection kit (StemCell Technologies, Inc., Vancouver, BC, Canada). Hyperboid antibodies were sequentially screened for their ability to bind to FGFR3-IIIb and FGFR3-IIIc by ELISA and to recognize cell surface FGFR3 by FACS. Selected hyperboids were then cloned by limiting dilution. 16G, 6G1 and 15B2 are clones used to assess the effect on the proliferation of Ba/F3 cells expressing wild type or mutated FGFR3 similarly as described in Fig. 9A. Error bars represent SEM.

**Figure 18:** Comparison of R3Mab epitopes determined by peptide mapping and crystal structure analysis. (A) Epitope revealed by the structure of the R3Mab Fab fragment in complex with the extracellular IgD2-D3 segment of human FGFR3. FGFR3 residues contacted by Fab heavy chain and light chain are colored in black and grey, respectively. (B) Location of peptides 3 and 11 on FGFR3.

**Figure 19:** R3Mab inhibits proliferation and FGFR3 signaling in bladder cancer cells containing wild type or mutated FGFR3. (A) Inhibition of cell viability by R3Mab in bladder cancer cell line RT4. Cell viability was assessed with CellTiter-Glo (Promega) after 96 hr incubation with the antibody. Error bars represent SEM. (B) Blocking of FGFI-activated FGFR3 signaling by R3Mab (15 μg/ml) in bladder cancer cell line RT4. (C) Inhibition of [3H]-thymidine incorporation by R3Mab in bladder cancer cell line RCC-97-7 (containing FGFR3<sup>3242</sup>C). Error bars represent SEM. (D) Inhibition of FGFR3 phosphorylation in TCC-97-7 cells by R3Mab (15 μg/ml). (E) Decrease of FGFR3<sup>3242</sup>C dimer in TCC-97-7 cells after 3 hours incubation with R3Mab (15 μg/ml) compared with a control antibody (Ctrl).

**Figure 20:** Effect of endocytosis inhibitors on the internalization of R3Mab and FGFR3<sup>3242</sup>C dimer in UMUC-14 cells. (A) Effect of endocytosis inhibitors on the internalization of R3Mab. UMUC-14 cells, pre-treated with various endocytosis inhibitor or DMISO for 1 hour at 37°C, were incubated with R3Mab (15 μg/ml) for 3 hours at 37°C to allow internalization. A low pH wash was used to remove cell surface R3Mab to visualize internalized antibody. Cells were fixed and stained with Alexa 488-labeled anti-human IgG. Image was taken using confocal microscopy. (B) Effect of endocytosis inhibitors on FGFR3<sup>3242</sup>C dimer in UMUC-14 cells treated with R3Mab. UMUC-14 cells, pre-treated with various endocytosis inhibitor or DMISO for 1 hour at 37°C, were incubated with mock (Lane 1), a control antibody (Lane 2), or R3Mab (15 μg/ml) for 3 hours at 37°C. Cell lysates were analyzed for FGFR3 protein under non-reducing or reducing conditions by immunoblot. Note that chlorpromazine (inhibitor of clathrin-mediated endocytosis) and genistein (pan-inhibitor of endocytosis) blocked R3Mab internalization, but had no effect on R3Mab-induced decrease of FGFR3<sup>3242</sup>C dimer.

**Figure 21:** Detection sensitivity of different anti-FGFR3 antibodies toward monomeric and dimeric FGFR3<sup>3242</sup>C dimer under reducing conditions. UMUC-14 cells were lysed after treatment with R3Mab (Lane 1), a control IgG1 (Lane 2), or PBS (Lane 3) for 3 hours, and cell lysates were subject to immunoblot analyses under reducing or non-reducing conditions. Note that IgG1 (murine hyperboid antibody generated at Genentech) detected both FGFR3<sup>3242</sup>C dimer and monomer, whereas s9007 (rabbit polyclonal antibody, Santa Cruz Biotechnology) or sc13121 (murine hyperboid antibody, Santa Cruz Biotechnology) preferentially detected the dimeric FGFR3<sup>3242</sup>C.

**Figure 22:** Effect of R3Mab on the proliferation of (t4; 14)+ multiple myeloma cells. (A) Inhibitory effect of R3Mab on [3H]-thymidine incorporation by UTMC-2 cells. UTMC-2 cells were grown in medium containing R3Mab or a control antibody in the presence of 25 ng/ml FG9 and 5 μg/ml heparin or heparin alone (No FG9). After 6 days incubation, [3H]-thymidine was added for 16 hr incubation. Data were normalized to that from cells grown in the absence of FG9 and antibody. (B-C) Effect of R3Mab on [3H]-thymidine incorporation by OPM2 (B) and KMS11 (C) cells. Cells grown in 1.5% FBS medium were treated with R3Mab or a control antibody for 6 days. Data were normalized to that from untreated cells. Error bars represent SEM.
FIG. 23: Cell surface expression levels of FGFR3 in myeloma and bladder cancer cells. (A) Cell surface FGFR3 expression in myeloma cells and bladder cancer cells assessed by FACS analysis. Cells were stained with phycoerythrin-conjugated mouse mAb against human FGFR3 (Fab766P, R&D Systems) or phycoerythrin-conjugated isotype control mouse IgG1 (BD Pharmingen). (B) Scatchard analysis of FGFR3 density in myeloma cells and bladder cancer cells. R3Mab was radiolabeled and incubated with cells in suspension with excess unlabeled antibody. After incubation at RT for 2 hours, cells were pelleted by centrifugation and washed twice. Specifically bound was determined. Receptor density and binding affinity (Kd) represent the mean from two binding experiments.

FIG. 24: Effect of R3Mab or its DNA mutant on xenograft growth of bladder carcinoma cells. (A) Effect of R3Mab and its DNA mutant (50 mg/kg each) on the growth of pre-established RT112 tumors. (B) Effect of R3Mab and its DNA mutant (50 mg/kg each) on the growth of pre-established UMUC-14 tumors. Error bars represent SEM.

DETAILED DESCRIPTION OF THE INVENTION

General Techniques


DEFINITIONS

The term “binding site,” as used herein, refers to a region of a molecule or molecular complex that, as a result of its shape, distribution of electrostatic charge, presentation of hydrogen-bond acceptors or hydrogen-bond donors, and/or distribution of nonpolar regions, favors associates with a ligand. Thus, a binding site may include or consist of features such as cavities, surfaces, or interfaces between domains. Ligands that may associate with a binding site include, but are not limited to, cofactors, substrates, receptors, agonists, and antagonists. The term binding site includes a functional binding site and/or a structural binding site. A structural binding site can include “in contact” amino acid residues as determined from examination of a three-dimensional structure. “Contact” can be determined using Van der Waals radii of atoms or by proximity sufficient to exclude solvent, typically water, from the space between the ligand and the molecule or molecular complex. In some embodiments, the FGFR3 residue in contact with an anti-FGFR3 antibody (e.g., YW184.6) or other substrate or inhibitor is a residue that has one atom within about 5 Å of an anti-FGFR3 antibody residue. Alternatively, “in contact” residue may be those that have a loss of solvent accessible surface area of at least about 10 Å and, more preferably at least about 50 Å to about 300 Å. Loss of solvent accessible surface can be determined by the method of Lee & Richards (J Mol Biol. 1971 Feb. 14; 55(3):379-400) and similar algorithms known to those skilled in the art, for instance as found in the SOLV module from C. Broger of F. Hoffmann-La Roche in Basel Switzerland.

Some of the “in contact” amino acid residues, if substituted with another amino acid type, may not cause any change in a biochemical assay, a cell-based assay, or an in vivo assay used to define a functional binding site but may contribute to the formation of a three-dimensional structure. A functional binding site includes amino acid residues that are identified as binding site residues based upon loss or gain of function, for example, loss of binding to ligand upon mutation of the residue. In some embodiments, the amino acid residues of a functional binding site are a subset of the amino acid residues of the structural binding site.

The term “FGFR3 binding site” refers to a region of FGFR3 that can favorably associate with a ligand. In some embodiments, the FGFR3 binding site may comprise, consist essentially of, or consist of one or more of the amino acid residues 154, 155, 158, 159, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 192, 205, 207, 210, 212, 214, 216, 217, 241, 246, 247, 248, 278, 279, 280, 281, 282, 283, 314, 315, 316, 317 and/or 318 of FGFR3-IIIb polypeptide (e.g., human FGFR3-IIIb disclosed in UniProtKB/Swiss-Prot accession number P22607...), and mixtures thereof, or equivalent residues of FGFR3-IIIc polypeptide (e.g., human FGFR3-IIIc disclosed in UniProtKB/Swiss-Prot accession number P22607...). In some embodiments, the FGFR3 binding site may comprise, consist essentially of, or consist of one or more of the amino acid residues 158, 159, 169, 170, 171, 173, 175, 205, 207, and/or 315 of FGFR3-IIIb polypeptide, and mixtures thereof, or equivalent residues of FGFR3-IIIc polypeptide. In some embodiments, the FGFR3 binding site may comprise, consist essentially of, or consist of one or more of the amino acid residues 158, 170, 171, 173, 175, and/or 315, or mixtures thereof, of FGFR3-IIIb polypeptide, or equivalent residues of FGFR3-IIIc polypeptide. A structurally equivalent ligand binding site is defined by a root mean square deviation from the structure coordinates of the backbone atoms of the amino acids that make up binding sites in FGFR3 for anti-FGFR3 antibody YW184.6 of at most about 0.70 Å, preferably about 5 Å.

“Crystal” as used herein, refers to one form of a solid state of matter in which atoms are arranged in a pattern that repeats periodically in three-dimensions, typically forming a lattice.

“Complementary or complement” as used herein, means the fit or relationship between two molecules that permits interaction, including for example, space, charge, three-dimensional configuration, and the like.

The term “corresponding” or “corresponds” refers to an amino acid residue or amino acid sequence that is found at the same position or positions in a sequence when the amino acid position or sequences are aligned with a reference sequence. In some embodiments, the reference sequence is a human FGFR3-IIIb disclosed in UniProtKB/Swiss-Prot accession number P22607... or a human FGFR3-IIIc disclosed in UniProtKB/Swiss-Prot accession number P22607... It will be appreciated that when the amino acid position or sequence is aligned with the reference sequence the numbering of the amino acids may differ from that of the reference sequence.
“Heavy atom derivative”, as used herein, means a derivative produced by chemically modifying a crystal with a heavy atom such as Hg, Au, or a halogen.

“Structural homolog” of FGFR3 as used herein refers to a protein that contains one or more amino acid substitutions, deletions, additions, or rearrangements with respect to the amino acid sequence of FGFR3, but that, when folded into its native conformation, exhibits or is reasonably expected to exhibit at least a portion of the tertiary (three-dimensional) structure of the FGFR3. In some embodiments, a portion of the three-dimensional structure refers to structural domains of the FGFR3, including the 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. Homolog tertiary structure can be probed, measured, or confirmed by known analytic or diagnostic methods, for example, X-ray, NMR, circular dichroism, a panel of monoclonal antibodies that recognize native FGFR3, and like techniques. For example, structurally homologous molecules can have substitutions, deletions or additions of one or more contiguous or noncontiguous amino acids, such as a loop or a domain. Structurally homologous molecules also include “modified” FGFR3 molecules that have been chemically or enzymatically derivatized at one or more constituent amino acid, including side chain modifications, backbone modifications, and N- and C-terminal modifications including acetylation, hydroxylation, methylation, amidation, and the attachment of carbohydrate or lipid moieties, cofactors, or like modifications.

“Molecular complex”, as used herein, refers to a combination of bound substrate or ligand with polypeptide, such as an antibody bound to FGFR3, or a ligand bound to FGFR3.

“Machine-readable data storage medium”, as used herein, means a data storage material encoded with machine-readable data, wherein a machine programmed with instructions for using such data and is capable of displaying data in the desired format, for example, a graphical three-dimensional representation of molecules or molecular complexes.

“Scalable”, as used herein, means the increasing or decreasing of distances between coordinates (configuration of points) by a scalar factor while keeping the angles essentially the same.

“Space group symmetry”, as used herein, means the whole symmetry of the crystal that combines the translational symmetry of a crystalline lattice with the point group symmetry. A space group is designated by a capital letter identifying the lattice type (P, A, F, etc.) followed by the point group symbol in which the rotation and reflection elements are extended to include screw axes and glide planes. Note that the point group symmetry for a given space group can be determined by removing the cell centering symbol of the space group and replacing all screw axes by similar rotation axes and replacing all glide planes with mirror planes. The point group symmetry for a space group describes the true symmetry of its reciprocal lattice.

“Unit cell”, as used herein, means the atoms in a crystal that are arranged in a regular repeating pattern, in which the smallest repeating unit is called the unit cell. The entire structure can be reconstructed from knowledge of the unit cell, which is characterized by three lengths (a, b, and c) and three angles (α, β, and γ). The quantities a and b are the lengths of the sides of the base of the cell and γ is the angle between these two sides. The quantity c is the height of the unit cell. The angles α and β describe the angles between the base and the vertical sides of the unit cell.

“X-ray diffraction pattern” means the pattern obtained from X-ray scattering of the periodic assembly of molecules or atoms in a crystal. X-ray crystallography is a technique that exploits the fact that X-rays are diffracted by crystals. X-rays have the proper wavelength (in the Angstrom (Å) range, approximately 10-8 cm) to be scattered by the electron cloud of an atom of comparable size. Based on the diffraction pattern obtained from X-ray scattering of the periodic assembly of molecules or atoms in the crystal, the electron density can be reconstructed. Additional phase information can be extracted either from the diffraction data or from supplementing diffraction experiments to complete the reconstruction (the phase problem in crystallography). A model is then progressively built into the experimental electron density, refined against the data to produce an accurate molecular structure.

“X-ray structure coordinates” define a unique configuration of points in space. Those of skill in the art understand that a set of structure coordinates for a protein or a protein/ligand complex, or a portion thereof, define a relative set of points that, in turn, define a configuration in three dimensions. A similar or identical configuration can be defined by an entirely different set of coordinates, provided the distances and angles of coordinates remain essentially the same. In addition, a configuration of points can be defined by increasing or decreasing the distances between coordinates by a scalar factor, while keeping the angles essentially the same.


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.

[0179] “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 stronger. 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.

[0180] 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 to two or five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 μM 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 ensure 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 washed plate 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™-3000 or a BIACore™-2000 (BIACore, Inc., Piscataway, N.J.) at 25°C. With immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACore IIIc.) 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 10 mM sodium acetate, pH 4.8, into 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/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 kinetic 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 25 μl/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 10⁷
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 20 nM 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 spectrophotometer (Aviv Instruments) or a 8000-series SL-M-Amino spectrophotometer (ThermoSpectronic) with a stir red cuvette.

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, phosphoetriesters, phosphoamidites, carbamates, etc.) and charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nuclease, toxins, antibodies, signal peptides, glycosylated, etc.), and 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 polynucleotides. Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphate 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 arabino-, xylose or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and a basic nucleo-side 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(0)S (“thioate”), P(0)S(2) (“dithioate”), O(NR)2 (“amidate”), P(O)OR, P(O)OR2, CO or CH2 (“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 arylid. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

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 Table A 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. TXUS510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, Calif. 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:

$$\text{100 times the fraction } \frac{X}{Y}$$

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.

“Ligand,” as used herein, refers to an agent or compound that associates with a binding site on a molecule, for example, FGFR3 binding sites, and may be an antagonist or agonist of FGFR3 activity. Ligands include molecules that mimic anti-FGFR3 antibody (e.g., R3Mab) binding to FGFR3. A ligand may be 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.

“Compound” refers to molecule that associates with FGFR3 or a pharmaceutically acceptable salt, ester, amide, prodrug, isomer, or metabolite, thereof. “Pharmaceutically acceptable salt” refers to a formula of a compound that does not compromise the biological activity and properties of the compound. Pharmaceutically acceptable salts can be obtained by reacting a binding-active compound of the disclosure with inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

“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 polypeptide(s).

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, dimerization, trimerization, etc., into higher order receptor complexes. Complexes may comprise a single species of protein, i.e., a homeric complex. Alternatively, complexes may comprise at least two different protein species, i.e., a heteromic 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 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., FGFR3 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., FGFR3 ligand, such as FGF1 or FGF9).

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 IgG1, IgG2, IgG3, or IgG4 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 U.S. Pat. No. 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., IgG1, IgG2, IgG3, or IgG4) 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 halflives are also described in WO00/42072, WO 02/06919; 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 DILCPRWGCLW (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 “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 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.

**[0211]** 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.

**[0212]** The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (\(\kappa\)) and lambda (\(\lambda\)), based on the amino acid sequences of their constant domains.

**[0213]** 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., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called \(\alpha\), \(\delta\), \(\epsilon\), \(\gamma\), and \(\mu\), 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.

**[0214]** 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.

<table>
<thead>
<tr>
<th>Loop</th>
<th>Kabat</th>
<th>AbM</th>
<th>Chothia</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>H31-H35</td>
<td>H26-H35</td>
<td>H26-H32</td>
<td>H30-H35B</td>
</tr>
<tr>
<td>(Kabat Numbering)</td>
<td>(AbM Numbering)</td>
<td>(Chothia Numbering)</td>
<td>(Contact Numbering)</td>
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</tr>
<tr>
<td>L1</td>
<td>L24-L34</td>
<td>L24-L34</td>
<td>L26-L32</td>
<td>L30-L36</td>
</tr>
<tr>
<td>L2</td>
<td>L50-L56</td>
<td>L50-L56</td>
<td>L50-L52</td>
<td>L46-L55</td>
</tr>
<tr>
<td>L3</td>
<td>L89-L97</td>
<td>L89-L97</td>
<td>L91-L96</td>
<td>L89-L96</td>
</tr>
<tr>
<td>H1</td>
<td>H31-H35</td>
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<td>H26-H32</td>
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<td>H50-H58</td>
<td>H50-H58</td>
<td>H53-H55</td>
<td>H47-H58</td>
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<tr>
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<td>H95-H102</td>
<td>H95-H102</td>
<td>H96-H101</td>
<td>H93-H101</td>
</tr>
</tbody>
</table>

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.

**[0215]** "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

**[0216]** "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 312:522-525 (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 1-105-115 (1998); Harris, Biochem. Soc. Trans. 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biochem. 5:428-433 (1994).

**[0217]** "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. Pat. 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. [0218] “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, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0219] 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.

[0220] The term “diabodies” refers to small antibody fragments with two antigen-binding sites, which sites 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 404,087; WO 92/11611; and Holliger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

[0221] 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.


[0223] Antibody “effector functions” refer to those biological activities attributable to the Fe region (a native sequence Fe region or amino acid sequence variant Fe region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fe receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor); and B cell activation.

[0224] “Antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a form of cytotoxicity in which secreted IgG bound onto Fe receptors (FeRs) 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 FcγRII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FeR 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 U.S. Pat. No. 5,500,362 or 5,821,337 or Presta U.S. Pat. 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).

[0225] “Human effector cells” are leukocytes which express one or more FeRs and perform effector functions. Preferably, the cells express at least FcγRII 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.

[0226] “Fc receptor” or “FcR” describes a receptor that binds to the Fe region of an antibody. The preferred FcR is a native sequence human FeR. Moreover, a preferred FeR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII sub-classes, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIA (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 motifs (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motifs (ITIM) in its cytoplasmic domain. (see review M. in Duéron, 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, FeRn, which 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 FeRs. 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).

[0227] Methods of measuring binding to FeRn are known (see, e.g., Ghetti 1997, Hinton 2004). Binding to human FeRn in vivo and serum half life of human FeRn high affinity binding polypeptides can be assayed, e.g., in transgenic mice or transplanted human cell lines expressing human FeRn, or in primates administered with the Fe variant polypeptides.

[0228] “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 ini-
tiated 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.

[0229] Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in U.S. Pat. No. 6,194,551 B1 and WO 99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Ido et al., *J. Immunol.* 164:4178-4184 (2000).

[0230] The term “Fc-region comprising polypeptide” refers to a polypeptide, such as an antibody or immunoglobulin, 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, all K447 removed, or a mixture of polypeptides with and without the K447 residue.

[0231] An “acceptor human framework” for the purposes herein is the framework comprising the amino acid sequence of a VH or Fc region 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 at most three, two or one of positions 71H, 73H, and 78H; for instance, the amino acid residues at these positions may be 71A, 73I, 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.

[0232] A “human consensus framework” is a framework which represents the most commonly occurring amino acid residue in a selection of human immunoglobulin VI or VH framework sequences. Generally, the selection of human immunoglobulin VI 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.

[0233] 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:

- (SEQ ID NO: 184) EVQLVESGGGLVQPSGSLRLSCAAS-H1-
- (SEQ ID NO: 185) WMQRQAPGKGLEWVLHQ-
- (SEQ ID NO: 186) RFTISRDNSKNTLYLOMNLRGQDSFDNYCNW-
- (SEQ ID NO: 187) WQQGLTVVSS.

[0235] 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:

- (SEQ ID NO: 188) DIQMRTQPSLSSALGSVGRVTITC-L1-
- (SEQ ID NO: 189) WYQKQAPGKGLEWVLH2-
- (SEQ ID NO: 190) GVPFERGBGSSQGTDFTITSSLQPEDDTYC-L3-
- (SEQ ID NO: 191) FQQTKEIK.

[0236] 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.

Compositions and Methods of the Invention

[0237] The present disclosure includes a crystalline form and a crystal structure of FGFR3 complexed with an anti-FGFR3 antibody, and methods of using the FGFR3:anti-FGFR3 antibody crystal structure and structural coordinates to identify homologous proteins and to design or identify agents that can modulate the function of FGFR3 or the FGFR3:anti-FGFR3 antibody complex. The present disclosure also includes the three-dimensional configuration of points derived from the structure coordinates of at least a portion of an FGFR3 molecule or molecular complex, as well as structurally equivalent configurations, as described below. The three-dimensional configuration includes points derived
from structure coordinates of, e.g., the FGFR3-anti-FGFR3 antibody complex, representing the locations of a plurality of the amino acids defining the FGFR3-anti-FGFR3 antibody complex binding site.

[0238] In some embodiments, the three-dimensional configuration includes points derived from structure coordinates representing the locations of the backbone atoms of a plurality of amino acids defining the FGFR3-anti-FGFR3 antibody complex or the FGFR3 binding site of, e.g., the FGFR3-anti-FGFR3 antibody complex. Alternatively, the three-dimensional configuration includes points derived from structure coordinates representing the locations of the side chain and the backbone atoms (other than hydrogens) of a plurality of the amino acids defining the FGFR3-anti-FGFR3 antibody complex.

[0239] The disclosure also includes the scalable three-dimensional configuration of points derived from structure coordinates of molecules or molecular complexes that are structurally homologous to FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex, as well as structurally equivalent configurations. Structurally homologous molecules or molecular complexes are defined below. Advantageously, structurally homologous molecules can be identified using the structure coordinates of the FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex or extracellular fragment(s) of FGFR3 according to a method of the disclosure.

[0240] The configurations of points in space derived from structure coordinates according to the disclosure can be visualized as, for example, a holographic image, a stereogram, a model, or a computer-displayed image, and the disclosure thus includes such images, diagrams or models.

[0241] The crystal structure and structural coordinates can be used in methods, for example, for obtaining structural information of a related molecule, and for identifying and designing agents that modulate FGFR3, or the FGFR3-antifGFR3 antibody complex.

[0242] In some embodiments, the FGFR3 binding site may comprise, consist essentially of, or consist of one or more of the amino acid residues 154, 155, 158, 159, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 202, 205, 207, 210, 212, 214, 216, 217, 241, 246, 247, 248, 278, 279, 280, 281, 282, 283, 314, 315, 316, 317 and/or 318, or mixtures thereof, of FGFR3-IIib polypeptide (e.g., human FGFR3-IIib disclosed in (UniProKB/Swiss-Prot accession number P22607_2), or equivalent residues of FGFR3-IIic polypeptide (e.g., human FGFR3-IIic disclosed in UniProKB/Swiss-Prot accession number P22607). In some embodiments, the FGFR3 binding site may comprise, consist essentially of, or consist of one or more of the amino acid residues 158, 159, 160, 161, 170, 171, 172, 173, 175, 205, 207, and/or 315, or mixtures thereof, of FGFR3-IIib polypeptide, or equivalent residues of FGFR3-IIic polypeptide. In some embodiments, the FGFR3 binding site may comprise, consist essentially of, or consist of one or more of the amino acid residues 158, 170, 171, 172, 173, 175, and/or 315, or mixtures thereof, of FGFR3-IIic polypeptide, or equivalent residues of FGFR3-IIlc polypeptide.

FGFR3 Polypeptides, Polynucleotides and Variants Thereof

[0243] FGFR3 nucleic acid and amino acid sequences are known in the art. Nucleic acid sequence encoding the FGFR3 can be designed using the amino acid sequence of the desired region of FGFR3. 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). 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)):

<table>
<thead>
<tr>
<th>FGFR3-IIib</th>
<th>FGFR3-IIic</th>
</tr>
</thead>
<tbody>
<tr>
<td>R248C</td>
<td>R248C</td>
</tr>
<tr>
<td>S249C</td>
<td>S249C</td>
</tr>
<tr>
<td>G372C</td>
<td>G372C</td>
</tr>
<tr>
<td>Y373C</td>
<td>Y373C</td>
</tr>
<tr>
<td>G380R</td>
<td>G380R</td>
</tr>
<tr>
<td>K650E</td>
<td>K650E</td>
</tr>
</tbody>
</table>

[0244] The present disclosure also includes a polypeptides comprising, consisting essentially of, or consisting of a portion or fragment of the FGFR3, and polynucleotides encoding the polypeptides.

[0245] An embodiment of a polypeptide fragment comprises, consists essentially of, or consists of any of amino acid residue starting from amino acid residue 154 to amino acid residue 164 and ending at amino acid residue 178 to amino acid 283 of human FGFR3 (e.g., human UniProKB/Swiss-Prot accession number P22607_2 (human FGFR3-IIib)). An embodiment of a polypeptide fragment comprises, consists essentially of, or consists of any of amino acid residue 154 to amino acid residue 177, amino acid residue 202 to amino acid residue 217, amino acid residue 241 to amino acid residue 248, amino acid residue 278 to amino acid residue 283 and/or amino acid residue 314 to amino acid residue 318. An embodiment of a polypeptide fragment comprises, consists essentially of, or consists of any of amino acid residue 164 to amino acid residue 164 to residue 178, residue 269 to residue 283 and/or residue 154 to residue 318. In some embodiments, the polypeptide portion has the ability to bind to FGFR3 ligand.

[0246] The present disclosure also includes variants of FGFR3. Variants include those polypeptides that have amino acid substitutions, deletions, and additions (such as at least 1, 2, 3, 4, 5, 6, 7, 8, 9 10, or more amino acid substitutions, deletions and additions). In some embodiments, the variant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 10 or more conservative substitutions (relative to a reference sequence, such as a human FGFR3 reference sequence). Amino acid substitutions can be made for example to replace cysteines and eliminate formation of disulfide bonds. Amino acid substitutions can also be made to change proteolytic cleavage sites. Other variants can be made at the FGFR3 inhibitor binding site. In other embodiments, the variants of the FGFR3 bind FGFR3 ligand with the same or higher affinity than the wild type FGFR3. In some embodiments, the variants of the FGFR3 bind an FGFR3 inhibitor (e.g. anti-FGFR3 antibody) with the same or higher affinity that the wild type FGFR3.

Fusion Proteins

[0247] FGFR3 polypeptides, variants, or structural homologs or portions thereof, may be fused to a heterologous polypeptide or compound. The heterologous polypeptide is a polypeptide that has a different function than that of the
FGFR3. Examples of heterologous polypeptide include polypeptides that may act as carriers, may extend half life, may act as epitope tags, may provide ways to detect or purify the fusion protein. Heterologous polypeptide include K.H, albumin, salvage receptor binding epitopes, immunoglobulin constant regions, and peptide tags. Peptide tags useful for detection or purification include FLAG, g3 protein, polyhistidine tags, hemagglutinin from influenza virus, T7 tag, S tag, Strep tag, chloramphenicol acetyl transferase, biotin, glutathione-S transferase, green fluorescent protein and maltose binding protein. Compounds that can be combined with the FGFR3, variants or structural homolog or portions thereof, include radioactive labels, protecting groups, and carbohydrate or lipid moieties.

Polynucleotides, Vectors and Host Cells

FGFR3, variants or fragments thereof can be prepared by introducing appropriate nucleotide changes into DNA encoding FGFR3, or by synthesis of the desired polypeptide variants.

Polynucleotide sequences encoding the polypeptides described herein can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from appropriate source cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides or variant polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in a host cell. 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 (in particular when the vector is inserted into a prokaryotic cell), 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 a 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 pheno- typic 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.

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.

Either constitutive or inducible promoters can be used in the present invention, in accordance with the needs of a particular situation, which can be ascertained by one skilled in the art. 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 a polypeptide described herein by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of choice. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. However, heterologous promoters are preferred, 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 polypeptides or variant polypeptides (Siebenlist et al. (1980) Cell 20: 269) using linkers or adaptors to supply any required restriction sites.

In embodiments, each cistron within a recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptide across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the polypeptide encoding 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, lpp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP.

Prokaryotic host cells suitable for expressing polypeptides include *Archea* 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* marcescens, *Klebsiella*, *Proteus*, *Shigella*, *Rhizobia*, *Vitreoscilla*, or *Paracoccus*. Preferably, gram-negative cells are used. Preferably the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

Besides prokaryotic host cells, eukaryotic host cell systems are also well established in the art. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* S9, as well as plants and plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); Chinese hamster ovary cells-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 (1980)); mouse sertoli cells (TM4, Matther, Biol. Reprod., 23:243-251 (1980)); and mouse mammary tumor (M60562, ATCC CCL51).
Polypeptide Production

Host cells are transformed or transfected 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.

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, CaPO4 precipitation and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

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 lauria broth (LB) plus necessary nutrient supplements. In preferred 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, diithoerythritol 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, protein expression is induced under conditions suitable for the activation of the promoter. For example, if a PhoA promoter is used for controlling transcription, the transformed host cells may be cultured in a phosphate-limiting medium for induction. A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

Eukaryotic host cells are cultured under conditions suitable for expression of the FGFR3 and/or KD polypeptides. The host cells used to produce the polypeptides 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 one or more of Ham et al., 1979, Meth. Enz. 58:44, Barnes et al., 1980, Anal. Biochem. 102: 255, U.S. Pat. No. 4,767,704, U.S. Pat. No. 4,657,866, U.S. Pat. No. 4,927,762, U.S. Pat. No. 4,560,655, or U.S. Pat. No. 5,122,469, WO 90/103430, WO 87/00195, and U.S. Pat. No. 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 GENTAMYCINTM), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Other 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.

Polypeptides described herein are expressed in a host cell may be secreted into and/or recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such methods 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 therefrom. 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 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; gel filtration using, for example, Sephadex G-75; hydrophobic affinity resins, ligand affinity using a suitable antigen immobilized on a matrix and Western blot assay.

Polypeptides that are produced may be purified to obtain preparations that are substantially homogeneous for further assays and uses. 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.

Antibody Production

For recombinant production of an antibody, 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.
Generating Antibodies Using Prokaryotic Host Cells:

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 Curter et al., U.S. Pat. No. 5,648,237.

In addition, plasmid 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-galactosidase and lactose promoter systems, a tryptophan (trp) promoter system and hybrid promoters such as the tac or the trp 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 openly 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, PecB, OmpA and MTP. 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 cytoplasmic conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits. Probas and Pluckthun Gene, 159:203 (1995).

The present invention provides an expression system in which the quantitative ratio of expressed polypeptide components can be modulated in order to maximize the yield of secreted and properly assembled antibodies of the invention. Such modulation is accomplished at least in part by simultaneously modulating translational strengths for the polypeptide components.

One technique for modulating translational strength is disclosed in Simmons et al., U.S. Pat. No. 5,840,523. It utilizes variants of the translational initiation region (TIR) within a cistron. For a given TIR, a series of amino acid or nucleic acid sequence variants can be created with a range of translational strengths, thereby providing a convenient means by which to adjust this factor for the desired expression level of the specific chain. TIR variants can be generated by conventional mutagenesis techniques that result in codon changes which can alter the amino acid sequence, although
silent changes in the nucleotide sequence are preferred. Alterations in the TIR can include, for example, alterations in the number or spacing of Shine-Dalgarno sequences, along with alterations in the signal sequence. One method for generating mutant signal sequences is the generation of a “codon bank” at the beginning of a coding sequence that does not change the amino acid sequence of the signal sequence (i.e., the changes are silent). This can be accomplished by changing the third nucleotide position of each codon; additionally, some amino acids, such as leucine, serine, and arginine, have multiple first and second positions that can add complexity in making the bank. This method of mutagenesis is described in detail in Yansura et al. (1992) METHODS: A Companion to Methods in Enzymol. 4:151-158.

[0281] Preferably, a set of vectors is generated with a range of TIR strengths for each cistron therein. This limited set provides a comparison of expression levels of each chain as well as the yield of the desired antibody products under various TIR strength combinations. TIR strengths can be determined by quantifying the expression level of a reporter gene as described in detail in Simmons et al. U.S. Pat. No. 5,840,523. Based on the translational strength comparison, the desired individual TIRs are selected to be combined in the expression vector constructs of the invention.

[0282] Prokaryotic host cells suitable for expressing antibodies of the invention include Archaeabacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include Escherichia (e.g., E. coli), Bacillli (e.g., B. subtilis), Enterobacteria, Pseudomonas species (e.g., P. aeruginosa), Salmonella typhimurium, Serratia marcescens, Klebsiella, Proteus, Shigella, Rhizoba, 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 ΔlhuA (ΔttnA) purR lac Iq lacLΔ AmpTΔ(mpPCP-FeP) degPM1 kanR (U.S. Pat. No. 5,693,659). Other strains and derivatives thereof, such as E. coli 294 (ATCC 31,446), E. coli 3, E. coli 1, 1776 (ATCC 31,537) and E. coli RY208 (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, Boss 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 pKVN410 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.

[0283] Antibody Production

[0284] 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.

[0285] 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.

[0286] 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 includeuria 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.

[0287] 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.

[0288] 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.

[0289] 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 polypeptide. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. Preferably, the phosphate-limiting medium is the C.M.R.A.P medium (see, for 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.

[0289] 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.

[0291] 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 has no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

[0292] 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 OD50 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.

[0293] 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 peptidylprolil 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. Bio Chem. 274:19601-19605; Georgiou et al., U.S. Pat. No. 6,083,715; Georgiou et al., U.S. Pat. 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.

[0294] 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 Ml, 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. Pat. No. 5,264,365; Georgiou et al., U.S. Pat. No. 5,508,192; Hara et al., Microbial Drug Resistance, 2:63-72 (1996).

[0295] 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.

[0296] Antibody Purification

[0297] In one embodiment, the antibody protein produced herein is further purified to obtain preparations that are substantially homogeneous for further assays and uses. 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, Sephade G-75.

[0298] 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 aureus which binds with a high affinity to the Fe 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 silica 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.

[0299] 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.

[0300] Generating antibodies using eukaryotic host cells:

[0301] The vector components generally include, but are not limited to, one or more of the following:

[0302] (i) Signal Sequence Component

[0303] (ii) Origin of Replication

[0304] (iii) Selection Gene Component

[0305] The DNA for such precursor region is ligated in reading frame to DNA encoding the antibody.

[0306] (ii) Origin of Replication

[0307] 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.

[0308] (iii) Selection Gene Component

[0309] 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.

[0310] 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.

[0311] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to trap the antibody nucleic acid, such as DHFR, thymidine kinase, methotrexine-I and -II, preferably primate methotrexine genes, adenosine deaminase, ornithine decarboxylase, etc.

[0312] 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 (Mex), 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 aminoglycoside antibiotic, e.g., kanamycin, neomycin, or G418, see U.S. Pat. No. 4,965,199.

(iv) Promoter Component

Expression 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 all eukaryotic 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 the CAAT 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 poloma 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 described in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes et al., Nature 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. 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 polynuclea 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 untranscribed 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 eukaryotic 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)); baboon 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, Mathe, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (COS, ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CCL 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 (M1T06562, ATCC CCL 51); TRl 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) Cultivating the Host Cells

The host cells used to produce an antibody of this invention may be cultivated 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 cultivating 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. Pat. No. 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 adenine 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.

**[0327]** (ix) Purification of Antibody

**[0328]** 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.

**[0329]** 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 isoform of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human y1, y2, or y4 heavy chains (Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)). Protein G is recommended for all mouse isotopes and for human y3 (Guss et al., EMBOJ. 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechnically stable matrices such as controlled pore glass or poly(styrene-dvinyl)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 5B™ resin (J.T. Baker, Phillipsburg, N.J.) 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.

**[0330]** 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 0.02-0.25M salt).

**[0331]** Activity Assays

**[0332]** The antibodies can be characterized for their physical/chemical properties and biological functions by various assays known in the art.

**[0333]** The purified immunoglobulins 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.

**[0334]** In certain embodiments of the invention, the immunoglobulins produced herein are analyzed for their biological activity. In some embodiments, the immunoglobulins 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.

**[0335]** 2. Crystals and Crystal Structures

**[0336]** The present disclosure provides crystals of an FGFR3 anti-FGFR3 antibody complex as well as the crystal structure of FGFR3 anti-FGFR3 antibody as determined therefrom. In some embodiments, the crystals are formed from an FGFR3 sequence comprising sequence

**[0337]** ADPDGVDMTPGATPRMPDIKLLAV-PAANTVRFRCPAAGNPTISWKLKNG REFGERHRIGG-LIKHLIQWQLSVMESVPSDRCNYG-TCVVENKFGISRQTYTLIDLERSPH RPIQLAGPLLQANQTVLGSVDVEFICHVKYS-DAQPHIQWKLHVNGSKVGDGTPYTVTL KSWISESVERAVRNLAVSNERDGGEYLCRATN-FIGVAEKFWLVSHPRAEEELVEA DEAGSV (SEQ ID NO:272) and an anti-FGFR3 antibody.

**[0338]** In some embodiments, the crystals are formed from an FGFR3 sequence comprising sequence

**[0339]** ADPDGVDMTPGATPRMPDIKLLAV-PAANTVRFRCPAAGNPTISWKLKNG REFGERHRIGG-LIKHLIQWQLSVMESVPSDRCNYG-TCVVENKFGISRQTYTLIDLERSPH RPIQLAGPLLQANQTVLGSVDVEFICHVKYS-DAQPHIQWKLHVNGSKVGDGTPYTVTL KSWISESVERAVRNLAVSNERDGGEYLCRATN-FIGVAEKFWLVSHPRAEEELVEA DEAGSV (SEQ ID NO:273) and an anti-FGFR3 antibody.

**[0340]** In some embodiments, the anti-FGFR3 antibody comprises a light chain variable region comprising HVR-L-1, HVR-L-2, HVR-L-3, wherein each, in order, comprises SEQ ID NO:4, 5, 6, and/or a heavy chain variable region comprising HVR-H1, HVR-H2, and HVR-H3, wherein each, in order, contains SEQ ID NO:1, 2, 3. In some embodiments, the anti-FGFR3 antibody comprises a light chain variable region comprising sequence

**[0341]** In some embodiments, the anti-FGFR3 antibody comprises any antibody disclosed herein or disclosed in co-pending co-owned U.S. Ser. No.Filed Mar. 24, 2010 (attorney docket P4294R1).

**[0342]** In a specific embodiment, the structure of FGFR3 complexed with an anti-FGFR3 antibody was solved by...
molecular replacement with the program PHASER. The crystals belonged to space group P212121 with cell parameters of 

\[a = 58.5 \text{ Å}, \quad b = 99.3 \text{ Å}, \quad c = 143.7 \text{ Å}\].

[0343] The crystals are useful to provide the crystal structure and to provide a stable form of the molecule for storage.

[0344] Each of the constituent amino acids of FGFR3:anti-FGFR3 antibody is defined by a set of structure coordinates as set forth in Table 6. The term “structure coordinates” refers to Cartesian coordinates derived from mathematical equations related to the patterns obtained on diffraction of a monochromatic beam of X-rays by the atoms (scattering centers) of a FGFR3 and FGFR3:anti-FGFR3 antibody, in crystal form. The diffraction data are used to calculate an electron density map of the repeating unit of the crystal. The electron density maps are then used to establish the positions of the individual atoms of the FGFR3, anti-FGFR3 antibody and FGFR3:anti-FGFR3 antibody complex.

[0345] Slight variations in structure coordinates can be generated by mathematically manipulating the FGFR3 and FGFR3:anti-FGFR3 antibody complex structure coordinates. For example, the structure coordinates as set forth in Table 6 could be manipulated by crystallographic permutations of the structure coordinates, fractionalization of the structure coordinates, integer additions or subtractions to sets of the structure coordinates, inversion of the structure coordinates, or any combination of the above. Alternatively, modifications in the crystal structure due to mutations, additions, substitutions, deletions, and combinations thereof, of amino acids, or other changes in any of the components that make up the crystal, could also yield variations in structure coordinates. Such slight variations in the individual coordinates will have little effect on overall shape. If such variations are within an acceptable standard error as compared to the original coordinates, the resulting three-dimensional shape is considered to be structurally equivalent. Structural equivalence is described in more detail below.

[0346] It should be noted that slight variations in individual structure coordinates of the FGFR3, anti-FGFR3 antibody and FGFR3:anti-FGFR3 antibody complex would not be expected to significantly alter the nature of chemical entities such as ligands that could associate with a binding site or other structural features of FGFR3. In this context, the phrase “associating with” refers to a condition of proximity between a ligand and a FGFR3 molecule or portions thereof. The association may be non-covalent, wherein the juxtaposition is energetically favored by hydrogen bonding, van der Waals forces, and/or electrostatic interactions, or it may be covalent.

[0347] FGFR3 residues that form a binding site for a modulator (e.g., an antagonist or agonist) of FGFR3 are described in the present application. The identification of a binding site for a modulator on FGFR3 can be used to design new classes of FGFR3 modulators, such as antagonists, agonists, and like agents, having therapeutic applications, such as, for treating cancer.

[0348] 3. Structurally Equivalent Crystal Structures

[0349] Various computational analyses can be used to determine whether a molecule or portions of the molecule defining structure features are “structurally equivalent,” defined in terms of its three-dimensional structure, to all or part of an activated unbound FGFR3 or bound to an inhibitor, such as an anti-FGFR3 antibody, or FGFR3 present in FGFR3:anti-FGFR3 antibody complex. Such analyses may be carried out in current software applications, such as the Molecular Similarity application of QUANTA (Molecular Simulations IIc., San Diego, Calif.), Version 4.1, and as described in the accompanying User’s Guide.

[0350] The Molecular Similarity application permits comparisons between different structures, different conformations of the same structure, and different parts of the same structure. A procedure used in Molecular Similarity to compare structures comprises: 1) loading the structures to be compared; 2) defining the atom equivalences in these structures; 3) performing a fitting operation; and 4) analyzing the results.

[0351] One structure is identified as the target (i.e., the fixed structure); all remaining structures are working structures (i.e., moving structures). Since atom equivalency within QUANTA is defined by user input, for the purpose of this disclosure equivalent atoms are defined as protein backbone atoms (N, Calpha, C, and O) for all conserved residues between the two structures being compared. A conserved residue is defined as a residue that is structurally or functionally equivalent. Only rigid fitting operations are considered.

[0352] When a rigid fitting method is used, the working structure is translated and rotated to obtain an optimum fit with the target structure. The fitting operation uses an algorithm that computes the optimum translation and rotation to be applied to the moving structure, such that the root mean square difference of the fit over the specified pairs of equivalent atoms is an absolute minimum. This number, given in Angstroms, is reported by QUANTA.

[0353] Structurally equivalent crystal structures have portions of the two molecules that are substantially identical, within an acceptable margin of error. The margin of error can be calculated by methods known to those of skill in the art. In some embodiments, any molecule or molecular complex or any portion thereof, that has a root mean square deviation of conserved backbone atoms (N, Calpha, C, O) of less than about 0.70 Å, preferably 0.5 Å. For example, structurally equivalent molecules or molecular complexes are those that are defined by the entire set of structure coordinates listed in Table 7 or as a root mean square deviation from the conserved backbone atoms of those amino acids of not more than 0.70 Å, preferably 0.5 Å. The term “root mean square deviation” means the square root of the arithmetic mean of the squares of the deviations. It is a way to express the deviation or variation from a trend or object. For purposes of this disclosure, the “root mean square deviation” defines the variation in the backbone of a protein from the backbone of FGFR3 complex (as defined by the structure coordinates of the complex as described herein) or a defining structural feature thereof.

[0354] 4. Structurally Homologous Molecules, Molecular Complexes, and Crystal Structures

[0355] Structure coordinates can be used to aid in obtaining structural information about another crystalized molecule or molecular complex. The method of the disclosure allows determination of at least a portion of the three-dimensional structure of molecules or molecular complexes that contain one or more structural features that are similar to structural features of at least a portion of the FGFR3, anti-FGFR3 antibody, or FGFR3:anti-FGFR3 antibody complex. These molecules are referred to herein as “structurally homologous” to FGFR3, anti-FGFR3 antibody, or FGFR3:anti-FGFR3 antibody complex. Similar structural features can include, for example, regions of amino acid identity, conserved active site
or binding site motifs, and similarly arranged secondary structural elements (for example, binding sites for FGFR3 ligand on FGFR3).

Optionally, structural homology is determined by aligning the residues of the two amino acid sequences to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. Two amino acid sequences are compared using the BLAST program, version 2.0.9, of the BLAST 2 search algorithm, as described by Tatusova et al., and available at http://www.ncbi.nlm.nih.gov/BLAST/. Preferably, the default values for all BLAST 2 search parameters are used, including matrix=BLOSUM62; open gap penalty=-11, extension gap penalty=-1, gap x dropoff=-50, expect=10, wordsize=3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, structural similarity is referred to as “identity.” In some embodiments, a structurally homologous molecule is a protein that has an amino acid sequence having at least 80% identity with a wild type or recombinant amino acid sequence of FGFR3, in some embodiments human FGFR3-IIIb or human FGFR3-IIIc. More preferably, a protein that is structurally homologous to FGFR3 includes at least one contiguous stretch of at least 50 amino acids that has at least 80% amino acid sequence identity with the analogous portion of the wild type or recombinant FGFR3. Methods for generating structural information about the structurally homologous molecule or molecular complex are well known and include, for example, molecular replacement techniques.

Therefore, in another embodiment this disclosure provides a method of utilizing molecular replacement to obtain structural information about a molecule or molecular complex whose structure is unknown comprising:

(a) generating an X-ray diffraction pattern from a crystallized molecule or molecular complex of unknown or incompletely known structure; and

(b) applying at least a portion of the structural coordinates of FGFR3 complex to the X-ray diffraction pattern to generate a three-dimensional electron density map of the molecule or molecular complex whose structure is unknown or incompletely known.

By using molecular replacement, all or part of the structure coordinates of FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex as provided by this disclosure can be used to determine the unsolved structure of a crystallized molecule or molecular complex more quickly and efficiently than attempting to determine such information ab initio. Coordinates of structural features of FGFR3 can be utilized including that of trypsin-like serine protease domain.

Molecular replacement can provide an accurate estimation of the phases for an unknown or incompletely known structure. Phases are one factor in equations that are used to solve crystal structures, and this factor cannot be determined directly. Obtaining accurate values for the phases, by methods other than molecular replacement, can be a time-consuming process that involves iterative cycles of approximations and refinements and greatly hinders the solution of crystal structures. However, when the crystal structure of a protein containing at least a structurally homologous portion has been solved, molecular replacement using the known structure provide a useful estimate of the phases for the unknown or incompletely known structure.

Thus, this method involves generating a preliminary model of a molecule or molecular complex whose structure coordinates are unknown, by orienting and positioning the relevant portion of the FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex within the unit cell of the crystal of the unknown molecule or molecular complex. This orientation or positioning is conducted so as best to account for the observed X-ray diffraction pattern of the crystal of the molecule or molecular complex whose structure is unknown. Phases can then be calculated from this model and combined with the observed X-ray diffraction pattern amplitudes to generate an electron density map of the structure. This map, in turn, can be subjected to established and well-known model building and structure refinement techniques to provide a final, accurate structure of the unknown crystallized molecule or molecular complex (see for example, Lattman, 1985. Methods in Enzymology 115:55-77).

Structural information about a portion of any crystallized molecule or molecular complex that is sufficiently structurally homologous to a portion of FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex can be solved by this method. In addition to a molecule that shares one or more structural features with the FGFR3, such as the extracellular domain and binding site, two or three immunoglobulin-like domains (IgD1-3) and an acid box, and/or FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex as described above, a molecule that has similar bioactivity, such as the same ligand binding activity as FGFR3 and/or anti-FGFR3 antibody, may also be sufficiently structurally homologous to a portion of the FGFR3 and/or anti-FGFR3 antibody to permit use of the structure coordinates of FGFR3:anti-FGFR3 antibody to solve its crystal structure or identify structural features that are similar to those identified in the FGFR3 described herein. It will be appreciated that amino acid residues in the structurally homologous molecule identified as corresponding to the FGFR3 structural feature may have different amino acid numbering.

In one embodiment of the disclosure, the method of molecular replacement is utilized to obtain structural information about a molecule or molecular complex, wherein the molecule or molecular complex includes at least one FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex subunit or homolog. In the context of the present disclosure, a “structural homolog” of the FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex is a protein that contains one or more amino acid substitutions, deletions, additions, or rearrangements with respect to the amino acid sequence of FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex but that, when folded into its native conformation, exhibits or is reasonably expected to exhibit at least a portion of the tertiary (three-dimensional) structure of at least a portion of FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex. A portion of the FGFR3 includes the binding site for an FGFR3 inhibitor.

A heavy atom derivative of FGFR3 is also included as a FGFR3 homolog. The term “heavy atom derivative” refers to derivatives of the FGFR3, anti-FGFR3 antibody, or FGFR3-anti-FGFR3 antibody complex produced by chemically modifying a crystal of FGFR3 or both. In practice, a crystal is soaked in a solution containing heavy metal atom salts, or organometallic compounds, e.g., lead chloride, gold
thiomalate, thiomersal or uranyl acetate, which can diffuse through the crystal and bind to the surface of the protein. The location(s) of the bound heavy metal atom(s) can be determined by X-ray diffraction analysis of the soaked crystal. This information, in turn, is used to generate the phase information used to construct three-dimensional structure of the protein (Blundell, et al., 1976, Protein Crystallography, Academic Press, San Diego, Calif.).

Variants may be prepared, for example, by expression of FGFR3 cDNA previously altered in its coding sequence by oligonucleotide-directed mutagenesis as described herein. Variants may also be generated by site-specific incorporation of unnatural amino acids into FGFR3 proteins using known biosynthetic methods (Noren, et al., 1989, Science 244:182-88). In this method, the codon encoding the amino acid of interest in wild-type FGFR3 is replaced by a "blank" nonsense codon, TAG, using oligonucleotide-directed mutagenesis. A suppressor tRNA directed against this codon is then chemically aminocylated in vitro with the desired unnatural amino acid. The aminocylated tRNA is then added to an in vitro translation system to yield a mutant FGFR3 with the site-specific incorporated unnatural amino acid.

For example, structurally homologous molecules can contain deletions or additions of one or more contiguous or noncontiguous amino acids, such as a loop or a domain. Structurally homologous molecules also include "modified" FGFR3, anti-FGFR3 antibody, or FGFR3 anti-FGFR3 antibody complex that have been chemically or enzymatically derivatized at one or more constituent amino acid, including side chain modifications, backbone modifications, and N- and C-terminal modifications including acetylation, hydroxylation, methylation, amidation, and the attachment of carbohydrate or lipid moieties, cofactors, and like modifications. It will be appreciated that amino acid residues in the structurally homologous molecule identified as corresponding to activated FGFR3 or other structural feature of the FGFR3 may have different amino acid numbering.

The structure coordinates of FGFR3 are also particularly useful to solve or model the structure of crystals of FGFR3, anti-FGFR3 antibody, or FGFR3 anti-FGFR3 antibody complex homologs, which are co-complexed with a variety of ligands (e.g., a ligand binding the antagonist binding site). This approach enables the determination of the optimal sites for interaction between ligand and candidate FGFR3 ligands. Potential sites for modification within the various binding sites (such as an FGFR3 binding site) of the molecule can also be identified. This information provides an additional tool for determining more efficient binding interactions, for example, increased hydrophobic or polar interactions, between FGFR3 and a ligand. For example, high-resolution X-ray diffraction data collected from crystals exposed to different types of solvent allows the determination of where each type of solvent molecule resides. Small molecules that bind tightly to those sites can then be designed and synthesized and tested for their FGFR3 affinity, and/or inhibition activity.

All of the complexes referred to above may be studied using well-known X-ray diffraction techniques and may be refined versus 1.5-3.5 Å resolution X-ray data to an R-factor of about 0.30 or less using computer software, such as X-PLOR (Yale University, distributed by Molecular Simulations, Inc.) (see for example, Blundell, et al. 1976. Protein Crystallography, Academic Press, San Diego, Calif., and Methods in Enzymology, Vol. 114 & 115, H. W. Wyckoff et al., eds., Academic Press (1985)). This information may thus be used to optimize known FGFR3 modulators, and more importantly, to design new FGFR3 modulators.

The disclosure also includes the unique three-dimensional configuration defined by a set of points defined by the structure coordinates for a molecule or molecular complex structurally homologous to FGFR3, anti-FGFR3 antibody, or FGFR3 anti-FGFR3 antibody complex as determined using the method of the present disclosure, structurally equivalent configurations, and magnetic storage media including such set of structure coordinates.

Homology Modeling

Using homology modeling, a computer model of a homolog, e.g., an FGFR3 homolog, can be built or refined without crystallizing the homolog. First, a preliminary model of the homolog is created by sequence alignment with FGFR3, anti-FGFR3 antibody, or FGFR3 anti-FGFR3 antibody complex secondary structure prediction, the screening of structural libraries, or any combination of those techniques. Computational software may be used to carry out the sequence alignments and the secondary structure predictions. Structural incoherences, e.g., structural fragments around insertions and deletions, can be modeled by screening a structural library for peptides of the desired length and with a suitable conformation. For prediction of the side chain conformation, a side chain rotamer library may be employed. If the homolog has been crystallized, the final homology model can be used to solve the crystal structure of the homolog by molecular replacement, as described above. Next, the preliminary model is subjected to energy minimization to yield an energy-minimized model. The energy-minimized model may contain regions where stereochemistry restraints are violated, in which case such regions are remodeled to obtain a final homology model. The homology model is positioned according to the results of molecular replacement, and subjected to further refinement including molecular dynamics calculations.

Methods for Identification of Modulators of FGFR3

Potent and selective ligands that modulate activity (agonists and antagonists) can be identified using the threedimensional model of the FGFR3 using the coordinates of Table 6. In some embodiments, the three-dimensional model of the binding site on FGFR3 and/or other structural features are produced using the coordinates of Table 6. Using this model, candidate ligands that interact with the FGFR3, e.g., the FGFR3 binding site, are assessed for the desired characteristics (e.g., interaction with FGFR3) by fitting against the model, and the result of the interactions is predicted. Agents predicted to be molecules capable of modulating the activity of FGFR3 can then be further screened or confirmed against known bioassays. For example, the ability of an agent to inhibit the effects of FGFR3 can be measured using assays known in the art. Using the modeling information and the assays described, one can identify agents that possess FGFR3-modulating properties. Modulators of FGFR3 of the present disclosure can include compounds or agents having, for example, inhibitory activity.

Ligands which can interact with FGFR3 can also be identified using commercially available modeling software, such as docking programs, in which the solved crystal structure coordinates of Table 6 can be computationally represented and screened against a large virtual library of small molecules.
molecules or virtual fragment molecules for interaction with a site of interest, such as the FGFR3 binding site. Preferred small molecules or fragments identified in this way can be synthesized and contacted with the FGFR3. The resulting molecular complex or association can be further analyzed by, for example, NMR or X-ray co-crystallography, to optimize the FGFR3 ligand interaction and/or desired biological activity. Fragment-based drug discovery methods are known and computational tools for their use are commercially available, for example “SAR by NMR” (Shakers, S. B., et al., Science, 1996, 274, 1531-1534). “Segments of Active Structures” (www.stronmix.com; Niemaber, V. L., et al., Nat. Biotechnol., 2000, 18, 1105-1108), and “Dynamic Combinatorial X-ray Crystallography” (e.g., permitting self-selection by the protein molecule of self-assembling fragments; Congreve, M. S., et al., Angew. Chem., Int. Ed., 2003, 42, 4479-4482). Still other molecular modeling, and like methods are discussed below and in the Examples.

[0376] In another embodiment, a candidate modulator can be identified using a biological assay such as binding to FGFR3, modulation (e.g., inhibition) of FGFR3 ligand activation of FGFR3, modulation (e.g., inhibition) of FGFR3 biological activity. The candidate modulator can then serve as a model to design similar agents and/or to modify the candidate modulator for example, to improve characteristics such as binding to FGFR3. Design or modification of candidate modulators can be accomplished using the crystal structure coordinates and available software.

[0377] Binding Site and Other Structural Features

[0378] The present disclosure provides information inter alia about the shape and structure of a binding site of FGFR3 in the presence of an inhibitor (anti-FGFR3 antibody). Binding sites are of significant utility in fields such as drug discovery. The association of natural ligands or substrates with the binding sites of their corresponding receptors or enzymes is the basis of many biological mechanisms of action. Similarly, many drugs exert their biological effects through association with the binding sites of receptors and enzymes. Such associations may occur with all or any part of the binding site. An understanding of such associations helps lead to the design of drugs having more favorable associations with their target, and thus improved biological effects. Therefore, this information is valuable in designing potential modulators of FGFR3 binding sites, as discussed in more detail below.

[0379] The amino acid constituents of a FGFR3 binding site as defined herein are positioned in three dimensions. The structural coordinates of FGFR3 with a bound inhibitor are in Table 6. In one aspect, the structure coordinates defining a binding site of FGFR3 include structure coordinates of all atoms in the constituent amino acids; in another aspect, the structure coordinates of a binding site include structure coordinates of just the backbone atoms of the constituent atoms. FGFR3 that is bound to an inhibitor has a different conformation than when inhibitor is not bound. In the bound state, a number of amino acid residues form a pocket.

[0380] The FGFR3 binding site may be defined by those amino acids whose backbone atoms are situated within about 5 Å of one or more constituent atoms of a bound ligand.

[0381] Rational Drug Design

[0382] Computational techniques can be used to screen, identify, select, design ligands, and combinations thereof, capable of associating with FGFR3 or structurally homologous molecules. Candidate modulators of FGFR3 may be identified using functional assays, such as binding to FGFR3, and novel modulators designed based on the structure of the candidate molecules so identified. Knowledge of the structure coordinates for FGFR3 permits, for example, the design, the identification of synthetic compounds, and like processes, and the design, the identification of other molecules and like processes, that have a shape complementary to the conformation of the FGFR3 binding sites. In particular, computational techniques can be used to identify or design ligands, such as agonists and/or antagonists, that associate with a FGFR3 binding site. Antagonists may bind to or interfere with all or a portion of an active site of FGFR3, and can be competitive, non-competitive, or uncompetitive inhibitors. Once identified and screened for biological activity, these agonists, antagonists, and combinations thereof, may be used therapeutically and/or prophylactically, for example, to block FGFR3 activity and thus prevent the onset and/or further progression of diseases associated with FGFR3 activity. Structure-activity data for analogues of ligands that bind to or interfere with FGFR3 binding sites can also be obtained computationally.

[0383] In some embodiments, agonists or antagonists can be designed to include components that preserve and/or strengthen the interactions. Such antagonists or agonists would include components that are able to interact, for example, hydrogen bond with the charged amino acids found in, e.g., either an antagonist binding site of FGFR3 (activated or unactivated, bound to substrate or unbound to substrate) or FGFR3 bound to an inhibitor or both.

[0384] In some embodiments, for FGFR3, antagonist or agonist molecules are designed or selected that can interact with at least one or all amino acid residues that comprise, consist essentially of, or consist of at least one amino acid residue corresponding to an amino acid residue in one or more of the binding site, or mixtures thereof.

[0385] Comparison of the binding site on FGFR3 to analogous sites of related receptors will direct design of inhibitors that favor FGFR3 over the related receptors. The crystal structures of other related receptors, if they are available can be utilized to maximize fit and/or interaction with FGFR3 binding site and minimize the fit and/or interactions with amino acids in the corresponding positions in other receptors.

[0386] Data stored in a machine-readable storage medium that is capable of displaying a graphical three-dimensional representation of the structure of FGFR3 or a structurally homologous molecule or molecular complex, as identified herein, or portions thereof may thus be advantageously used for drug discovery. The structure coordinates of the ligand are used to generate a three-dimensional image that can be computationally fit to the three-dimensional image of FGFR3 and anti-FGFR3 antibody, or a structurally homologous molecule. The three-dimensional molecular structure encoded by the data in the data storage medium can then be computationally evaluated for its ability to associate with ligands. When the molecular structures encoded by the data is displayed in a graphical three-dimensional representation on a computer screen, the protein structure can also be visually inspected for potential association with ligands.

[0387] One embodiment of the method of drug design involves evaluating the potential association of a candidate ligand with FGFR3 or a structurally homologous molecule or homologous complex, particularly with at least one amino acid residue in a binding site (e.g., a binding site) of the FGFR3 or a portion of the binding site. The method of drug design thus includes computationally evaluating the potential of a selected ligand to associate with any of the molecules or
molecular complexes set forth above. This method includes the steps of: (a) employing computational means, for example, such as a programable computer including the appropriate software known in the art or as disclosed herein, to perform a fitting operation between the selected ligand and a ligand binding site or a subsite of the ligand binding site of the molecule or molecular complex; and (b) analyzing the results of the fitting operation to quantify the association between the ligand and the ligand binding site. Optionally, the method further comprises analyzing the ability of the selected ligand to interact with amino acids in the FGFR3 binding site and/or subsite. The method may also further comprise optimizing the fit of the ligand for the binding site of FGFR3 as compared to other receptors. Optionally, the selected ligand can be synthesized, co-crystallized with FGFR3, and further modifications to selected ligand can be made to enhance inhibitory activity or fit in the binding pocket. In addition as described previously, portions of anti-FGFR3 antibody that bind to FGFR3 can be modified and utilized in the method described herein. Other structural features of the FGFR3 and/or FGFR3-anti-FGFR3 antibody complex can also be analyzed in the same manner.

[0388] In another embodiment, the method of drug design involves computer-assisted design of ligand that associates with FGFR3, its homologs, or portions thereof. Ligands can be designed in a step-wise fashion, one fragment at a time, or may be designed as a whole de novo. Ligands can be designed based on the structure of molecules that can modulate at least one biological function of FGFR3, such as anti-FGFR3 antibody and other naturally occurring inhibitors of FGFR3. In addition, the inhibitors can be modeled on other known inhibitors of receptors, such as FGFRs.

[0389] In some embodiments, to be a viable drug candidate, the ligand identified or designed according to the method must be capable of structurally associating with at least part of a FGFR3 binding site (e.g., a FGFR3 binding site), and must be able, sterically and energetically, to assume a conformation that allows it to associate with the FGFR3 binding site. Non-covalent molecular interactions important in this association include hydrogen bonding, van der Waals interactions, hydrophobic interactions, and/or electrostatic interactions. In some embodiments, an agent may contact at least one amino acid position in the FGFR3 binding site (e.g., a binding site for an inhibitor, such as anti-FGFR3 antibody. Conformational considerations include the overall three-dimensional structure and orientation of the ligand in relation to the ligand binding site, and the spacing between various functional groups of a ligand that directly interact with the FGFR3 binding site or homologs thereof.

[0390] Optionally, the potential binding of a ligand to a FGFR3 binding site is analyzed using computer modeling techniques prior to the actual synthesis and testing of the ligand. If these computational experiments suggest insufficient interaction and association between it and the FGFR3 binding site, testing of the ligand is obviated. However, if computer modeling indicates a strong interaction, the molecule may then be synthesized and tested for its ability to bind to or interfere with a FGFR3 binding site. Binding assays to determine if a compound actually modulates FGFR3 activity can also be performed and are well known in the art.

[0391] Several methods can be used to screen ligands or fragments for the ability to associate with a FGFR3 binding site (e.g., an antagonist binding site). This process may begin by visual inspection of, for example, a FGFR3 binding site on the computer screen based on the FGFR3 structure coordinates or other coordinates which define a similar shape generated from the machine-readable storage medium. Selected ligands may then be positioned in a variety of orientations, or docked, within the binding site. Docking may be accomplished using software such as QUANTA and SYBYL, followed by energy minimization and molecular dynamics with standard molecular mechanics force fields, such as CHARMM and AMBER.


[0394] Once a compound has been designed or selected by the above methods, the efficiency with which that ligand may bind to or interfere with a FGFR3 binding site may be tested and optimized by computational evaluation. FGFR3 binding site ligands may interact with the binding site in more than one conformation that is similar in overall binding energy. In those cases, the deformation energy of binding is taken to be the difference between the free energy of the ligand and the average energy of the conformations observed when the ligand binds to the protein.

[0395] A ligand designed or selected as binding to or interfering with a FGFR3 binding site may be further computationally optimized so that in its bound state it would preferably lack repulsive electrostatic interaction with the target enzyme and with the surrounding water molecules. Such non-complementary electrostatic interactions include repulsive charge-charge, dipole-dipole, and charge-dipole interactions.

[0396] Specific computer software is available to evaluate compound deformation energy and electrostatic interactions. Examples of programs designed for such uses include: Gaussian 94, revision C (M. J. Frisch, Gaussian, Inc., Pittsburgh, Pa.); AMBER, version 4.1 (P. A. Kollman, University of California at San Francisco); QUANTA/CHARMM (Molecular Simulations, Inc., San Diego, Calif.); Insight II/Discover (Molecular Simulations, Inc., San Diego, Calif.); DelPhi (Molecular Simulations, Inc., San Diego, Calif.); and AMSON (Quantum Chemistry Program Exchange, Indiana University). These programs can be implemented, for instance, using a Silicon Graphics workstation, such as an Indigo2 with IMPACT graphics. Other hardware systems and software packages will be known to those skilled in the art.

[0397] Another approach encompassed by this disclosure is the computational screening of small molecule databases
for ligands or compounds that can bind in whole, or in part, to a FGFR3 binding site whether in bound or unbound conformation. In this screening, the quality of fit of such ligands to the binding site may be judged either by shape complementarity or by estimated interaction energy (Meng et al., 1992. J. Comp. Chem., 13:505-24). In addition, these small molecule databases can be screened for the ability to interact with the amino acids in the FGFR3 binding site as identified herein.

[0398] A compound that is identified or designed as a result of any of these methods can be obtained (or synthesized) and tested for its biological activity, for example, binding and/or inhibition of FGFR3 activity.

[0399] Another method involves assessing agents that are antagonists or agonists of the FGFR3 receptor. A method comprises applying at least a portion of the crystallography coordinates of Table 6 to a computer algorithm that generates a three-dimensional model of a FGFR3:anti-FGFR3 antibody complex or the FGFR3 suitable for designing molecules that are antagonists or agonists and searching a molecular structure database to identify potential antagonists or agonists. In some embodiments, a portion of the structural coordinates of Table 6 that define a structural feature, for example, all or a portion of a binding site (e.g., an antagonist binding site) for an inhibitor on FGFR3. The method may further comprise synthesizing or obtaining the agonist or antagonist and contacting the agonist or antagonist with the FGFR3 and selecting the antagonist or agonist that modulates the FGFR3 activity compared to a control without the agonist or antagonists and/or selecting the agonist or antagonist that binds to the FGFR3.

[0400] A compound that is identified or designed as a result of any of these methods can be obtained (or synthesized) and tested for its biological activity, for example, binding to FGFR3 and/or modulation of FGFR3 activity.


[0402] Transformation of the structure coordinates for all or a portion of FGFR3, anti-FGFR3 antibody or the FGFR3:anti-FGFR3 antibody complex, or one of its ligand binding sites, or structurally homologous molecules as defined below, or for the structural equivalents of any of these molecules or molecular complexes as defined above, into three-dimensional graphical representations of the molecule or complex can be conveniently achieved through the use of commercially-available software.

[0403] The disclosure thus further provides a machine-readable storage medium including a data storage material encoded with machine-readable data wherein a machine programmed with instructions for using said data displays a graphical three-dimensional representation of any of the molecule or molecular complexes of this disclosure that have been described above. In a preferred embodiment, the machine-readable storage medium includes a data storage material encoded with machine-readable data wherein a machine programmed with instructions for using the aforementioned data displays a graphical three-dimensional representation of a molecule or molecular complex including all or any parts of an unbound FGFR3, a FGFR3 ligand binding site for an inhibitor or pseudo substrate, or FGFR3-like ligand binding site, anti-FGFR3 antibody, FGFR3:anti-FGFR3 antibody complex as defined above. In an alternative embodiment, the machine-readable storage medium includes a data storage material encoded with machine-readable data wherein a machine programmed with instructions for using the data displays a graphical three-dimensional representation of a molecule or molecular complex as root mean square deviation from the atoms of the amino acids of not more than 0.05 Å.

[0404] In an alternative embodiment, the machine-readable data storage medium includes a data storage material encoded with a first set of machine readable data which includes the Fourier transform of structure coordinates, and wherein a machine programmed with instructions for using the data is combined with a second set of machine readable data including the X-ray diffraction pattern of a molecule or molecular complex to determine at least a portion of the structure coordinates corresponding to the second set of machine readable data.

[0405] For example, a system for reading a data storage medium may include a computer including a central processing unit ("CPU"), a working memory which may be, for example, RAM (random access memory) or "core" memory, mass storage memory (such as one or more disk drives or CD-ROM drives), one or more display devices (e.g., cathode ray tube ("CRT") displays, light emitting diode ("LED") displays, liquid crystal displays ("LCDs"), electroluminescent displays, vacuum fluorescent displays, field emission displays ("FEDs"), plasma displays, projection panels, etc.), one or more input devices (e.g., keyboards, microphones, mice, track balls, touch pads, etc.), one or more input lines, and one or more output lines, all of which are interconnected by a conventional bidirectional system bus. The system may be a stand-alone computer, or may be networked (e.g., through local area networks, wide area networks, intranets, extranets, or the internet) to other systems (e.g., computers, hosts, servers, etc.). The system may also include additional computer controlled devices such as consumer electronics and appliances.

[0406] Input hardware may be coupled to the computer by input lines and may be implemented in a variety of ways. Machine-readable data of this disclosure may be inputted via the use of a modem or modems connected by a telephone line or dedicated data line. Alternatively or additionally, the input hardware may include CD-ROM drives or disk drives. In conjunction with a display terminal, a keyboard may also be used as an input device.

[0407] Output hardware may be coupled to the computer by output lines and may similarly be implemented by conventional devices. By way of example, the output hardware may include a display device for displaying a graphical representation of a binding site of this disclosure using a program such as QUANTA as described herein. Output hardware might also include a printer, so that hard copy output may be produced, or a disk drive, to store system output for later use.

[0408] In operation, a CPU coordinates the use of the various input and output devices, coordinates data accesses from mass storage devices, accesses to and from working memory, and determines the sequence of data processing steps. A number of programs may be used to process the machine-readable data of this disclosure. Such programs are discussed in reference to the computational methods of drug discovery as described herein. References to components of the hardware system are included as appropriate throughout the following description of the data storage medium.

[0409] Machine-readable storage devices useful in the present disclosure include, but are not limited to, magnetic devices, electrical devices, optical devices, and combinations thereof. Examples of such data storage devices include, but
are not limited to, hard disk devices, CD devices, digital video disk devices, floppy disk devices, removable hard disk devices, magneto-optic disk devices, magnetic tape devices, flash memory devices, bubble memory devices, holographic storage devices, and any other mass storage peripheral device. It should be understood that these storage devices include necessary hardware (e.g., drives, controllers, power supplies, etc.) as well as any necessary media (e.g., disks, flash cards, etc.) to enable the storage of data.

[0410] Therapeutic Use

[0411] FGFR3 modulator compounds obtained by methods of the invention are useful in a variety of therapeutic settings. For example, FGFR3 antagonists designed or identified using the crystal structure of FGFR3 complex can be used to treat disorders or conditions where inhibition or prevention of FGFR3 binding or activity is indicated.

[0412] Likewise, FGFR3 agonists designed or identified using the binding site and/or crystal structures provided herein can be used to treat disorders or conditions where induction or stimulation of FGFR3 is indicated.

[0413] An indication can be, for example, inhibition or stimulation of FGFR3 activation and the concomitant activation of a complex set of intracellular pathways that lead to cell growth in a variety of cell types. Yet another indication can be, for example, inhibition or stimulation of the FGFR3 signaling pathway. Still yet another indication can be, for example, inhibition or stimulation of invasive tumor growth and metastasis.

[0414] 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

Materials and Methods

Cell Lines and Cell Culture

[0415] The cell line RT4 was obtained from American Type Cell Culture Collection. Cell lines RT112, OPM2 and Ba/F3 were purchased from German Collection of Microorganisms and Cell Cultures (DSMZ, Germany). Multiple myeloma cell line KMS11 was kindly provided by Dr. Takemi Otsuki at Kawasaki Medical School (Japan). Bladder cancer cell line TCC-97-7 was a generous gift from Dr. Margaret Knowles at St. James’s University Hospital (Leeds, UK). UMUC-14 cell line was obtained from Dr. H. B. Grossman (currently at University of Texas M.D. Anderson Cancer Center, TX). The cells were maintained with RPMI medium supplemented with 10% fetal bovine serum (FBS) (Sigma), 100 U/ml penicillin, 0.1 mg/ml streptomycin and 1-glutamine under conditions of 5% CO2 at 37° C. FGFR35249C Dimerization Studies

[0416] UMUC-14 cells were grown in cysteine-free medium, treated with R3Mab or DTNB for 3 hr, and cell lysates were subject to immunoblot analysis under reducing or non-reducing conditions. For in vitro dimerization studies, FGFR3-L1B5249C (residues 143-374) was cloned into pAE067A vector and expressed in Tn7i Pro cells. The recombinant protein was purified through Ni-NTA column followed by Superdex 5200 column. Dimeric FGFR35249C was eluted in 25 mM Tris (pH 7.5) and 300 mM NaCl. R3Mab (1 μM) was incubated with FGFR35249C dimer (0.1 μM) at 37°C. under the following conditions: 100 mM KH2PO4 (pH 7.5), 25 μM DTT, 1 mM EDTA and 0.75 mg/ml BSA. Aliquots of the reaction were taken at indicated time points and the reaction was stopped by adding sample buffer without β-mercaptoethanol. Dimer-monomer was analyzed by immunoblot.

Xenograft Studies

[0417] All studies were approved by Genentech’s Institutional Animal Care and Use Committee. Female nu/nu mice or CB17 severe combined immunodeficiency (SCID) mice, 6-8 weeks of age, were purchased from Charles River Laboratory (Hollister, Calif.). Female athymic nude mice were obtained from the National Cancer Institute-Frederick Cancer Center. Mice were maintained under specific pathogen-free conditions. RT112 shRNA stable cells (7×10⁴), RT112 (7×10⁴), Ba/F3-FGFR35249C (5×10⁴), OPM2 (15×10⁴), or KMS11 cells (20×10⁴) were implanted subcutaneously into the flank of mice in a volume of 0.2 ml in HBSS/matrigel (1:1 v/v, BD Biosciences). UMUC-14 cells (5×10⁴) were implanted without matrigel. Tumors were measured twice weekly using a caliper, and tumor volume was calculated using the formula: V=0.5 a×b², where a and b are the length and width of the tumor, respectively. When the mean tumor volume reached 150-200 mm³, mice were randomized into groups of 10 and were treated twice weekly with intraperitoneal (i.p) injection of R3Mab (0.3-50 mg/kg), or a control human IgG1 diluted in HBSS. Control animals were given vehicle (HBSS) alone.

Statistics

[0418] Pooled data are expressed as means±SEM. Unpaired Student’s t tests (2-tailed) were used for comparison between two groups. A value of P≤0.05 was considered statistically significant in all experiments.

Generation of FGFR3 shRNA Stable Cells

[0419] Three independent FGFR3 shRNA were cloned into pHUSH vector as described (50). The sequence for FGFR3 shRNAs used in the studies is as follows:

shRNA2: (SEQ ID NO: 192)
5'-GATCCGGAGATCAGTGCTGGATTCGATCATTCAAGAGATGATGCAGCTTGATGCTTTTTGGAAA; shRNA4: (SEQ ID NO: 193)
5'-GATCCGGAGATCAGTGCTGGATTCGATCATTCAAGAGATGATGCAGCTTGATGCTTTTTGGAAA; shRNA6: (SEQ ID NO: 194)
5'-GATCCGGAGATCAGTGCTGGATTCGATCATTCAAGAGATGATGCAGCTTGATGCTTTTTGGAAA.

All constructs were confirmed by sequencing. EGFP control shRNA was described in our previous study (50). The shRNA containing retrovirus was produced by co-transfecting GP2-293 packaging cells (Clontech Laboratories, Mountain View, Calif.) with VSV-G (Clontech Laboratories) and pHUSH-FGFR3 shRNA constructs, and viral supernatants were harvested 72 hr after transfection, and cleared of cell debris by centrifugation for transduction experiment.
[0420] RT112 cells were maintained in RPMI 1640 medium containing tetracycline-free FBS (Clontech Laboratories), and transduced with retroviral supernatant in the presence of 4 µg/ml polybrene. 72 hours after infection, 2 µg/ml puromycin (Clontech Laboratories) was added to the medium to select stable clones expressing shRNA. Stable cells were isolated, treated with 0.1 or 1 µg/ml doxycycline (Clontech Laboratories) for 4 days, and inducible knockdown of FGFR3 protein expression was assessed by Western blotting analysis. Cell cycle analyses were performed as described (51).

Selecting Phage Antibodies Specific for FGFR3

[0421] Human phage antibody libraries with synthetic diversities in the selected complementary determining regions (H1, H2, H3, L3), mimicking the natural diversity of human IgG repertoire were used for panning. The Fab fragments were displayed bivalently on the surface of M13 bacteriophage particles (52). His-tagged IgD2-D3 of human FGFR3-IIIB and Mc were used as antigens. 96-well Maxisorp immunoplates (Nunc) were coated overnight at 4°C with FGFR3-IIIB-His protein or FGFR3-IIIC-His protein (10 µg/ml) and blocked for 1 hour with PBST buffer (PBS with 0.05% Tween 20) supplemented with 1% BSA. The antibody phage libraries were added and incubated overnight at room temperature (RT). The plates were washed with PBST buffer and bound phage were eluted with 50 mM HCl and 500 mM NaCl for 30 minutes and neutralized with equal volume of 1M Tris base. Recovered phages were amplified in E. coli XL-1 blue cells. During subsequent selection rounds, the incubation time of the phage antibodies was decreased to 2 hours and the stringency of plate washing was gradually increased (53). Unique and specific phage antibodies that bind to both Mb and IIc isoforms of FGFR3 were identified by phage ELISA and DNA sequencing. Out of 400 clones screened, four were selected to reformat to full length IgGs by cloning VL and VH regions of individual clones into LPG3 and LPG4 vectors, respectively, transiently expressed in mammalian cells, and purified with protein A columns (54). Clone 184.6 was selected for affinity maturation.

[0422] For affinity maturation, phagemid displaying monovalent Fab on the surface of M13 bacteriophage (52) served as the library template for grafting light chain (VL) and heavy chain (VH) variable domains of the phage Ab. Stop codons was incorporated in CDR-L3. A soft randomization strategy was adopted for affinity maturation as described (53). Two different combinations of CDR loops, H1/H2/L3, H3/L3, or L1/L2/L3 were selected for randomization. For selecting affinity-matured clones, phage libraries were sorted against FGFR3-IIIB or IIIC-His protein, subjected to plate sorting for the first round and followed by four rounds of solution phase sorting as described (52). After five rounds of panning, a high-throughput single-point competitive phage ELISA was used to rapidly screen for high-affinity clones as described (55). Clones with low ratio of the absorbance at 450 nm in the presence of 10 nM FGFR3-His to that in the absence of FGFR3-His were chosen for further characterization.

[0423] Clones 184.6.1, 184.6.21, 184.6.49, 184.6.51, 184.6.58, 184.6.62 and 184.6.92 significantly reduced viability of Ba/F3-FGFR3-IIIB, Ba/F3-FGFR3-IIIC and Ba/F3-FGFR3-S249C cell lines, and clone 184.6.52 significantly reduced the viability of the Ba/F3-FGFR3-S249C cell line. The increased inhibitory activity ranged from about 50-fold (clone 184.6.52) to about 100-fold (clones 184.6.1, 184.6.21, 184.6.49, 184.6.51, 184.6.58, 184.6.62 and 184.6.92) greater than parent clone 184.6, depending on the cell line assayed. Binding kinetics of clones 184.6.1, 184.6.58, and 184.6.62 to FGFR3-IIIB and FGFR3-IIIC were determined using BIAcore as follows:

<table>
<thead>
<tr>
<th>FGFR3-IIIB KD (M)</th>
<th>FGFR3-IIIC KD (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>184.6</td>
<td>3.80E-08</td>
</tr>
<tr>
<td>184.6.1</td>
<td>1.20E-10</td>
</tr>
<tr>
<td>184.6.49</td>
<td>1.90E-10</td>
</tr>
<tr>
<td>184.6.51</td>
<td>1.10E-07</td>
</tr>
<tr>
<td>184.6.58</td>
<td>8.80E-10</td>
</tr>
<tr>
<td>184.6.62</td>
<td>2.24E-09</td>
</tr>
</tbody>
</table>

[0424] Clone 184.6.1 was selected. A sequence modification, N54S, was introduced into HVR 12 at residue 54, to improve manufacturability, creating clone 184.6.1N54S. Clones 184.6.1 and 184.6.1N54S displayed comparable binding kinetics (measured in Biacore assays) and comparable activity in the Ba/F3 cell viability assay. Additional HVR 12 variants were generated: N54S was introduced in clone 184.6.58 and N54G, N54A, or N54Q were introduced in clone 184.6.1 and 184.6.58. These clones showed comparable activity in the Ba/F3 cell viability assay to parent clones 184.6.1 or 184.6.58.

[0425] Another sequence modification, D30E, was introduced into HVR 11 of clone 184.6.1N54S, creating clone 184.6.1N54SDE. Clone 184.6.1N54SDE and clone 184.6.1N54S showed comparable binding kinetics and comparable activity in the Ba/F3 cell viability assay to parent clones 184.6.1 or 184.6.58.

[0426] As used herein, “R3Mab” refers to anti-FGFR3 antibody clones 184.6.1N54S, 184.6.1, or 184.6. Clone 184.6.1N54S was used in figures and experiments referencing “R3Mab”, except in the experiments leading to the results shown in the following figures (for which the antibody used is shown in parentheses): FGFR, 9B (clone 184.6.1), 10 (clone 184.6), 11A and B (clone 184.6), 13 (clone 184.6.1), 14A (clone 184.6.1), 14B, G, and H (clone 184.6), 19 (clone 184.6.1), and 22B and C (clone 184.6.1).

BIAcore/Plate Surface Plasmon Resonance (SRP) Analysis to Determine Antibody Binding Affinities

[0427] Binding affinities of R3Mab to FGFR3 were measured by Biacore/SPR using a BIAcore™-3000 instrument as described (52) with the following modifications. R3Mab was directly coated on CM5 biosensor chips to achieve approximately 400 response units (RU). For kinetic measurement, two-fold serial dilutions of FGFR3-IIIB or IIIC-His protein (starting from 0.67 nM) were injected in PBST buffer at 25°C with a flow rate of 30 µl/minute. Association rates (Kon, per mol/s) and dissociation rates (Koff, per s) were calculated using a simple one-one Langmuir binding model (BIAcore Evaluation Software version 3.2). The equilibrium dissociation constant (Kd, per mol) was calculated as the ratio of Koff/Kon.

[0428] Binding affinities of mouse hybridoma antibodies to FGFR3 were measured by Biacore/SPR as follows. Human FGFR3-IIIB or Mc were coupled onto three different flow cells (FC), FC2, FC3 and FC4, of a BIAcore™ CM5 sensor chip to achieve the response unit (RU) about 50 RU. Immobilization was achieved by random coupling through amino groups
using a protocol provided by the manufacturer. Sensorgrams were recorded for binding of hybridoma-derived anti-FGFR3 murine IgG or the Fab fragment to these surfaces at 25°C. By injection of a series of solutions ranging from 250 nM to 0.48 nM in 2-fold increments at a flow rate of 300 min. Between each injection, 10 mM Glycine-HCl pH 1.7 served as the buffer to regenerate the sensor chip. The signal from the reference cell (FC1) was subtracted from the observed sensorgram at FC2, FC3 and FC4. Kinetic constants were calculated by nonlinear regression fitting of the data according to a 1:1 Langmuir binding model using BLAcore evaluation software (version 3.2) supplied by the manufacturer.

ELISA Binding Studies

[0429] cDNAs encoding the extracellular domains (ECD) of human FGFR1-IIIb, Iib, Fgfr2-IIIb and Me, and FGFR4 were cloned into pRK-based vector to generate human FGFR-human Fe chimeric proteins. The recombinant proteins were produced by transiently transfected Chinese hamster ovary (CHO) cells and purified via protein A affinity chromatography. To test binding of antibodies to human FGFRs, Maxisorp 96-well plates (Nunc) were coated overnight at 4°C with 50 μl of 2 μg/ml of FGFR ECD-human Fe chimeric proteins. After blocking with phosphate-buffered saline (PBS)/3% BSA, FGFR3 antibody was added and incubated at RT for 2 hours. Specifically bound FGFR3 antibody was detected using an HRP-conjugated anti-human Fab and the TMB peroxidase colorigenic substrate (KPL, Gaithersburg, Md.).

[0430] To test the effect of antibodies to FGFR3 on FGF/FGFR3 interaction, FGFR3-Fe chimeric proteins were captured on Maxisorp plates coated with anti-human immunoglobulin Fc fragment-specific antibody (Jackson Immunoresearch, West Grove, Pa.). After wash, increasing amount of FGFR3 antibody was added to the plate and incubated for 30 minutes. Then, FGFI or FGF9 and heparin were added for incubation at RT for 2 hours. The plates were washed and incubated for 1 hour with biotinylated FGFI-specific polyclonal antibody (BAF232) or biotinylated FGFI antibody (BAF273, R&D Systems), followed by detection with streptavidin-HRP and TMB.

Generation of Ba/F3-FGFR3 Stable Cells cDNA encoding full-length human FGFR3-IIIb or IIIc was cloned into pQ CXIP vector (Clontech Laboratories, Mountain View, Calif.) to generate pQ CXIP-FGFR3-IIIb or Mc. Specific mutations, i.e., R248C, S249C, G372C, Y375C and K652E, were introduced into the cDNA via QuikChange (Stratagene, La Jolla, Calif.). To generate Ba/F3 stable cells expressing wild type or mutant FGFR3, various pQ CXIP-FGFR3 constructs were co-transfected into packaging cells GP2-293 with VSV-G plasmid (Clontech Laboratories). After selection with 2 μg/ml puromycin for two weeks, cells expressing wild type or mutant FGFR3 were stained with Phycoerythrin-conjugated anti-human FGFR3 mAb (FAB766P, R&D Systems), and selected through fluorescence-activated cell sorting (FACS) for functional assays. For cell proliferation assay in 96-well micro-titer plate, the following cell density was used: For cells expressing wild type FGFR3-IIIb and FGFR3-K652E: 5000 cells/well; for the rest: 10000 cells/well. Cells were seeded in RPMI 1640 medium supplemented with 10% fetal bovine serum, 10 ng/ml FGF1 plus 10 μg/ml heparin (Sigma-Aldrich, St. Louis, Mo.). R3Mab was added at indicated concentration and mouse hybridoma FGFR3 antibodies were added at 2000 to 0.49 ng/ml (in four-fold serial dilutions) in the FGFR3-IIIb experiment and 5000 to 1.2 ng/ml (in four-fold serial dilutions) in the FGFR3-IIIc experiment. After incubation for 72 hours, cell viability was assessed with CellTiter-Glo (Promega, Madison, Wis.).

Cell Proliferation Assay

[0431] For proliferation assays for RT112, RT4 and TCC-97-7 cells, 3000 cells/well were seeded into 96-well micro-titer plate and were allowed to adhere overnight. The medium was then replaced with low serum medium (0.5% FBS) with control or R3Mab at concentrations indicated. Following 4 days incubation, 1 μCi of [Methyl-3H] thymidine (PerkinElmer, Waltham, Mass.) was added to each well, and incubated for additional 16 hours. Cells were transferred to Uni-Filters using Packard Filtermate Harvester, and [3H] thymidine incorporated into the genomic DNA of growing cells was measured using TopCount (PerkinElmer). In some cases, cell viability was assessed with CellTiter-Glo (Promega) following incubation with antibodies for 4 days. Values are presented as means+/SE of quadruplets.

Clonal Growth Assay

[0432] The effect of R3Mab on cell clonogenicity was assessed following a previously described protocol (50). In brief, 400 UMUC-14 cells were seeded into 6-well plate in DMEM medium supplemented with 10% fetal bovine serum to allow adhesion overnight. Then R3Mab or control antibody diluted in 0.1% BSA medium was added to a final concentration of 10 μg/ml. Equal volume of 0.1% BSA medium alone (Mock) was used as another control. The cells were incubated for about 12 days until cells in control groups formed sufficiently large colonies. Colonies were stained with 0.5% crystal violet, and the number and size of colonies were quantitated using GelCount (Oxford, UK). The number of colonies larger than 120 μm in diameter was presented as means+/SEM (n=12).

Immunoprecipitation and Immunoblotting Analyses

[0433] To study the effect of antibodies on FGF signaling, cells were starved in serum-free medium overnight prior to the beginning of treatment. Cells were incubated with either antibodies diluted in 0.1% BSA (w/v), RPMI 1640 medium, or with 0.1% BSA medium alone (Mock). After 3 hours at 37°C, FGF1 (final concentration of 15 ng/ml) and heparin (final concentration of 5-10 μg/ml) were added to half of the samples. As controls, a similar volume of heparin alone was added to the other half of samples. The incubation was continued for 10 min. Supernatants were removed by aspiration, and cells were washed with ice-cold PBS, then lysed in RIPA buffer (Upstate, Charlottesville, Va.) supplemented with 1 mM sodium orthovanadate and Complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, Ind.). The lysates were cleared of insoluble materials by centrifugation.

[0434] FGFR3 was immunoprecipitated using a rabbit polyclonal antibody (sc-123, Santa Cruz Biotechnology, Santa Cruz, Calif.) and analyzed by sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. Phosphorylated FGFR3 was assessed with a monoclonal antibody against phospho-tyrosine (4G10, Upstate). Total FGFR3 was probed with a monoclonal antibody against FGFR3 (sc-13121, Santa Cruz Biotechnology). Phosphory-
lation and activation of FGFR3 signaling pathway were probed using the following antibodies: anti-FGFR
\( ^{37} \) (Amersham Pharmacia Biotech, Piscataway, N.J.).

Antibody Epitope Mapping

[0435] To determine the epitope of R3Mab, 13 overlapping peptides, each of 15 amino acids in length, were synthesized to cover the extracellular domain of human FGFR3 from residues 138 to 310. The peptides were biotinylated at the C-terminus, and captured on streptavidin plates (Pierce, Rockford, Ill.) overnight. After blocking with PBS/3% BSA, the plates were incubated with R3Mab and detected using an HRP-conjugated anti-human IgG (Jackson Immunoresearch) and the TMB peroxidase colorogenic substrate (KPL, Gaithersburg, Md.).

[0436] Mouse anti-human FGFR3 hybridoma antibodies 1G6, 6G1, and 15B2 were tested in ELISA assay to identify their binding epitopes. 1G6, 6G1 and 15B2 bind to human FGFR IgD2-IgD3 (both IIIb and IIIc isoforms), whereas 5B8 only binds IgD2-IgD3 of human FGFR3-IIB. In a competition assay, 1G6, 6G1 and 15B2 competed with each other to bind human FGFR3, suggesting that 1G6, 6G1 and 15B2 have overlapping epitopes. None of the hybridoma antibodies competed with phage antibody 184.6, suggesting that the hybridoma antibodies have distinct epitope(s) from 184.6.

Preparation and Molecular Cloning of Mouse Anti-FGFR3 Antibodies 1G6, 6G1, and 15B2

[0437] BALB/c mice were immunized 12 times with 2.0 µg of FGFR3-IIBb (hFGFR3-IIBb/Fc Chimera), from R&D Systems, catalog #1264-FL, or CYHO26511, or with 2.0 µg of FGFR3-IIBC (hFGFR3-IIBC/Fc Chimera), from R&D Systems, catalog #766-FL, resuspended in monophosphoryl lipid A (trehalose dicaryonymycolate adjuvant) (Corixa, Hamilton, Mont.) in hind footpad twice a week. Three days after final boost, popliteal lymph nodes were fused with mouse myeloma cell line P3X63Ag.1.1, via electroporation (Hybrimate, Cyto Pulse Sciences, Glen Burnie, Md.). Fused hybridoma cells were selected from unfused popliteal node or myeloma cells using hypoxanthin

aminopterin-thymidine (HAT) selection in Medium D from the ClonCell® hybridoma selection kit (StemCell Technologies, Inc., Vancouver, BC, Canada). Culture supernatants were initially screened for its ability to bind to FGFR3-IIBb and FGFR3-IIBC by ELISA, and hybridomas of interest were subsequently screened for its ability to stain by FACS on transfected FGFR3-IIBb Ba/F cells and control Ba/F, as well as antibody blocking activity. Selected hybridomas were then cloned by limiting dilution.

[0438] Total RNA was extracted from hybridoma cells producing the mouse anti human FGFR3 monoclonal antibody 1G6 and 15B2, using RNeasy Mini Kit (Qiagen, Germany). The variable light (VL) and variable heavy (VH) domains were amplified using RT-PCR with the following degenerate primers:

<table>
<thead>
<tr>
<th>Light chain (LC) forward:</th>
<th>(SEQ ID NO: 195)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-GCCAGATACATCTGCTCTACGTC-3'</td>
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<table>
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<tr>
<th>Heavy chain (HC) forward:</th>
<th>(SEQ ID NO: 196)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-GATCGACCATATCGTGACGTCCTCC-3'</td>
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<tr>
<th>Light chain (LC) forward:</th>
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<tr>
<th>Heavy chain (HC) forward:</th>
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<tr>
<th>Light chain (LC) forward:</th>
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<td>5'-GATCGACCATATCGTGACGTCCTCC-3'</td>
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[0439] Light chain and Heavy chain reverse primer for all three clones are as follows:

<table>
<thead>
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<th>(SEQ ID NO: 201)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-TTTDDAKYCTGGCTGTACC-3'</td>
<td></td>
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<table>
<thead>
<tr>
<th>Heavy chain reverse:</th>
<th>(SEQ ID NO: 202)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-ACGOTGGGCCCTCTGTGACGTCCTGCC-3'</td>
<td></td>
</tr>
</tbody>
</table>

[0440] The forward primers were specific for the N-terminal amino acid sequence of the VL and VH region. The I.C and I.H reverse primers were designed to anneal to a region in the constant light (CL) and constant heavy domain I (CHI), respectively, which are highly conserved across species.

[0441] Amplified VL was cloned into a pRK mammalian cell expression vector (Shields et al., 2000 J. Biol. Chem. 276:659) containing the human kappa constant domain. Amplified VH was inserted to a pRK mammalian cell expression vector encoding the full-length human IgG1 constant domain. The sequence of the heavy and light chains was determined using conventional methods.

Crystallization, Structure Determination and Refinement

[0442] The human FGFR3-IIBb ECD (residues 143-374) was cloned into pAcGP67A vector (BD Bioscience, San Jose, Calif.), produced in Tni Pro cells and purified using Ni-NTA column followed by size exclusion chromatography. The R3Mab Fab was expressed in E. coli and purified sequentially over a protein G affinity column, an SP sepharose column and a Superdex 75 column. The R3Mab Fab had the following sequence:

<table>
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<td>D1GMTGSSSSDEASVGGTIVTTKCASQDQSTAVANYQKQPKPSLNLSS</td>
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</tbody>
</table>
[0443] Fab-FGFR3 complex was generated by incubating the Fab with an excess of FGFR3 ECD, and the complex was then deglycosylated and purified over a Superdex-200 sizing column in 20 mM TrisCl pH 7.5 and 200 mM NaCl buffer. The complex-containing fractions were pooled and concentrated to 20 mg/ml and used in crystallization trials. Crystals used in the structure determination were grown at 4°C from the following condition: 0.1 M sodium cacodylate pH 6.5, 40% PEG and 5% PEG8000 using vapor diffusion method. Data was processed using HKL2000 and Scalepack (56). The structure was solved with molecular replacement using program Phaser (57) and the coordinates of 1R3Y (FGFR3) and 1NZ8 (Fab-fragment). The model was completed using program Coot (58) and the structure refined to R/Rfree of 20.4% 24.3% with program Refmac (59). Coordinates and structure factors were deposited in the Protein Data Bank with accession code 5GRW and are also disclosed in U.S. Ser. No. 61/163,222, filed on Mar. 25, 2009, the contents of which is hereby incorporated by reference.

ADCC Assay

[0444] Human PBMCs were isolated by Ficoll gradient centrifugation of heparinized blood, and ADCC was measured using the multiple myeloma cell lines OPM2 or KMS11 or bladder cancer cell lines RT112 or UMUC-14 as target and PBMCs as effector cells at a 1:100 target:effector ratio. The target cells (10,000 cells/well) were treated with R3Mab or with control human IgG1 for 4 hours at 37°C. Cytotoxicity was determined by measuring LDH release using the CytoTox-ONE Homogeneous Membrane Integrity Assay following manufacturer's instructions (Promega, Madison, Wis.). The results are expressed as percentage of specific cytolyis using the formula: Cytotoxicity (%) = [Experimental lysis - Experimental spontaneous lysis]/(Target maximum lysis - Target spontaneous lysis)] x 100, where spontaneous lysis is the nonspecific cytolyis in the absence of antibody, and target maximum lysis is induced by 1% Triton X-100.

Results

[0445] Inducible shRNA Knockdown of FGFR3 Attenuates Bladder Cancer Growth In Vivo

[0446] As a prelude to assessing the importance of FGFR3 for bladder tumor growth in vivo, we examined the effect of FGFR3 knockdown in vitro. Several FGFR3 small interfering (si) RNAs effectively downregulated FGFR3 in bladder cancer cell lines expressing either WT (RT112, RT4, SW780) or mutant (UMUC-14, S240C mutation) FGFR3. FGFR3 knockdown in all four cell lines markedly suppressed proliferation in culture (FIG. 15). Next, we generated stable RT112 cell lines expressing doxycycline-inducible FGFR3 shRNA. Induction of three independent FGFR3 shRNAs by doxycycline diminished FGFR3 expression, whereas induction of a control shRNA targeting EGFP had no effect (FIG. 7A). In the absence of exogenous GGF, doxycycline treatment reduced [3H]-thymidine incorporation by cells expressing different FGFR3 shRNAs, but not control shRNA (FIG. 7B), confirming that FGFR3 knockdown inhibits proliferation. Further analysis of exponentially growing RT112 cells revealed that FGFR3 knockdown by doxycycline markedly and specifically reduced the percentage of cells in the S and G2 phases of the cell cycle, with a concomitant increase of cells in G1 phase (FIG. 7C). Similar effect was observed with two other FGFR3 shRNAs (FIG. 16A). No significant numbers of cells with a sub-diploid DNA content were detected, suggesting no change in apoptosis levels. Hence, the inhibitory effect of FGFR3 knockdown on the proliferation of RT112 cells is mainly due to attenuation of cell cycle progression.

[0447] We next evaluated the effect of FGFR3 knockdown on the growth of pre-established RT112 tumor xenografts in mice. FGFR3 knockdown substantially and specifically suppressed tumor growth (FIG. 7D, top panels and FIG. 16B). Analysis of day 45 tumor samples confirmed effective FGFR3 knockdown upon doxycycline induction of FGFR3 shRNA as compared to control shRNA (FIG. 7D, bottom panels). These results demonstrate that FGFR3 is critically important both in vitro and in vivo for the growth of RT112 bladder cancer cells.

Generation of a Blocking Anti-FGFR3 Monoclonal Antibody

[0448] To examine further the importance of FGFR3 in tumor growth and to explore the potential of this receptor as a therapeutic target, we developed an antagonistic anti-FGFR3 monoclonal antibody (dubbed R3Mab) using a phage display approach. We selected this particular antibody based on its ability to block both ligand binding and dimerization by FGFR3, and its unique capacity to inhibit not only WT FGFR3 but also the most prevalent cancer-associated mutants of this receptor (see below). R3Mab targets the extracellular IgD2 and IgD3 domains of FGFR3, which are necessary and sufficient for FGF binding (4). R3Mab bound both the IIIb and Mc isofoms of human FGFR3, but showed no detectable binding to FGFR1, FGFR2 or FGFR4 (FIG. 8A). Biacore analysis indicated that R3Mab had similar apparent affinity to murine, cynomolgus monkey and human FGFR3-IIIe (data not shown). The affinity of R3Mab to human FGFR3 is shown in Table 2.

<p>| TABLE 2 |
| Affinity of R3Mab to human FGFR3 determined by Biacore analysis. |</p>
<table>
<thead>
<tr>
<th>R3 Ab captured on chip</th>
<th>Human FGFR3 ECD</th>
<th>kD(1/M)</th>
<th>kD(1/s)</th>
<th>kD(M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIIb</td>
<td>1.8E+06</td>
<td>2.0E+04</td>
<td>1.1E+10</td>
<td></td>
</tr>
<tr>
<td>IIIc</td>
<td>1.9E+04</td>
<td>3.2E+04</td>
<td>3.52E-09</td>
<td></td>
</tr>
</tbody>
</table>

[0449] We next tested the ability of R3Mab to block FGFR3 binding to FGFI and FGF9. R3Mab strongly inhibited binding of FGFI to FGFR3-IIIb and -IIIc, with half-maximal inhibitory concentrations (IC50) of 0.7 nM and 1.7 nM, respectively (FIG. 8B,C). Similarly, R3Mab efficiently
blocked FGF9 binding to FGFRIIIb and -IIIc, with an IC_{50} of 1.1 nM and 1.3 nM, respectively (FIG. 8D,E).

R3Mab Inhibits WT FGFRIII and its Most Prevalent Cancer-Associated Mutant Variants

To examine whether R3Mab inhibits cell proliferation driven by WT or mutant FGFRIII, we took advantage of the observation that ectopic FGFRIII expression in murine pro-B cell Ba/F3 confers interleukin (IL)-3-independent, FGF1-dependent proliferation and survival (29). In the absence of FGF1 and IL-3, Ba/F3 cells stably expressing WT FGFRIII were not viable, while FGF1 greatly enhanced their proliferation (FIG. 9A). R3Mab specifically blocked FGF1-stimulated Ba/F3-FGFR2 cell proliferation in a dose-dependent manner (FIG. 9A). We next evaluated the impact of R3Mab on FGFRIII signaling in these cells. FGF1 induced phosphorylation and activation of FGFRIII and concomitant activation of p44/42 MAPK, while R3Mab effectively suppressed the activation of both molecules (FIG. 9B).

In bladder cancer, somatic activating mutations in FGFRIII cluster within the linker region between IgD2 and IgD3, the extracellular juxtamembrane domain, or the kinase domain (FIG. 9C). The extracellular missense substitutions most often give rise to an unpaired cysteine, leading to ligand-independent dimerization of FGFRIII. These mutations cause markedly different levels of constitutive FGFRIII activation, possibly owing to a differential impact on the orientation of the cytoplasmic kinase domain (30, 31). The most frequent mutations are S249C, Y375C, R248C, G372C, and K652E, which together account for 98% of all FGFRIII mutations in bladder cancer (32). We reasoned that an optimal therapeutic agent should block not only the WT FGFRIII protein, which is overexpressed in certain cancers, but also the most prevalent tumor-associated FGFRIII mutants. To assess R3Mab further, we generated Ba/F3 cells stably expressing each of the five most common FGFRIII mutant variants. All mutants were expressed at similar levels at the cell surface, and the cysteine mutants dimerized spontaneously without ligand (data not shown). Cell lines expressing different cysteine mutants exhibited a variable growth response to FGF1, consistent with earlier findings (30, 31). As previously reported (33), cells expressing FGFRIII^{S249C} displayed constitutive, ligand-independent proliferation, and were not responsive to FGF1 (FIG. 9D). Similarly, the most frequent mutation, FGFRIII^{S249C}, conferred ligand-independent proliferation (FIG. 9E). Remarkably, R3Mab suppressed constitutive proliferation driven by either mutant (FIG. 9D,E). Cells expressing the juxtamembrane domain mutations FGFRIII^{G237C} (FIG. 9F) or FGFRIII^{S377C} (FIG. 9G) required FGF1 for proliferation, and their growth was completely blocked by R3Mab. Cells expressing FGFRIII^{R205S2} showed weak ligand-independent proliferation and significant growth response to FGF1 (33). R3Mab did not affect the weak basal activity of FGFRIII^{R205S2} (data not shown), but nearly abolished ligand-induced proliferation mediated by this mutant (FIG. 9F). Hence, R3Mab has a unique capacity to inhibit both WT and prevalent cancer-associated mutants of FGFRIII. Moreover, R3Mab did not display detectable agonist activity.

As a separate effort, we generated and characterized multiple mouse-anti-human FGFRIII hybridoma antibodies. None of the hybridoma antibodies could inhibit all the cancer-linked FGFRIII mutants we tested (FIG. 17), nor did they share overlapping epitopes with R3Mab.

Moreover, all of the hybridoma antibodies showed agonist activity, strongly stimulating proliferation of cancer-linked FGFRIII mutants R248C and S249C, and showing some stimulation of proliferation of mutants Y375C and G370C. The hybridoma antibodies showed differential levels of antagonist and agonism, depending on the FGFRIII mutant tested, as follows:

<table>
<thead>
<tr>
<th>FGFR3-IIb</th>
<th>1G6</th>
<th>6G1</th>
<th>15B2</th>
</tr>
</thead>
<tbody>
<tr>
<td>wildtype</td>
<td>inhibition</td>
<td>inhibition</td>
<td>inhibition</td>
</tr>
<tr>
<td>R248C</td>
<td>2X stimulation</td>
<td>4-5X stimulation</td>
<td>3-4X stimulation</td>
</tr>
<tr>
<td>R248C</td>
<td>2X stimulation</td>
<td>4-5X stimulation</td>
<td>4-5X stimulation</td>
</tr>
<tr>
<td>Y375C</td>
<td>1.2-1.5X</td>
<td>1.2-1.5X</td>
<td>1.2-1.5X</td>
</tr>
<tr>
<td>K652E</td>
<td>50% inhibition</td>
<td>60-70% inhibition</td>
<td>inhibition</td>
</tr>
<tr>
<td>inhibition</td>
<td>inhibition</td>
<td>inhibition</td>
<td></td>
</tr>
<tr>
<td>no effect</td>
<td>20-30% inhibition</td>
<td>10-20% inhibition</td>
<td></td>
</tr>
</tbody>
</table>

Thus, the hybridoma antibodies showed unpredictable differential effect on Ba/F3 cells cell proliferation driven by various FGFRIII mutants.

Characterization of Mouse-Anti-Human FGFRIII Hybridoma Antibodies

(1) In an assay to test ability of anti-FGFRIII murine hybridoma antibodies to inhibit FGF1 binding to human FGFRIII-IIb and IIIc isoforms, antibodies 1G6, 6G1 and 15B2 were able to block binding of FGF1 to human FGFRIII-IIb and IIIc isoforms in a dose-dependent manner. When tested across an antibody concentration range of about 2000 to 0.49 ng/ml, antibodies 1G6, 6G1 and 15B2 blocked FGF1 binding to FGFRIII-IIb with IC50 values of 0.69, 0.87 and 0.72 nM. When tested across an antibody concentration range of about 5000 to 1.2 ng/ml, antibodies 1G6, 6G1 and 15B2 blocked FGF1 binding to FGFRIII-IIc with IC50 values of 0.57, 3.4 and 0.7 nM, respectively.

(2) In an assay to test ability of anti-FGF1 murine hybridoma antibodies to inhibit FGF1 binding to human FGFRIII-IIb and IIIc isoforms, antibodies 1G6, 6G1 and 15B2 efficiently blocked binding of FGF1 to human FGFRIII-IIb and IIIc isoforms in a dose-dependent manner. When tested across an antibody concentration range of about 2000 to 0.49 ng/ml, antibodies 1G6, 6G1 and 15B2 blocked FGF1 binding to FGFRIII-IIb with IC50 values of 0.13, 0.16, and 0.07 nM, respectively. When tested across an antibody concentration range of about 5000 to 1.2 ng/ml, antibodies 1G6, 6G1 and 15B2 blocked FGF1 binding to FGFRIII-IIc with IC50 values of 0.13, 0.11, and 0.07 nM, respectively.

(3) The binding affinity of full-length anti-FGF1 murine hybridoma antibodies 1G6, 6G1 and 15B2 was determined using Biacore analysis. The results of this analysis are shown in Table 3.
TABLE 3

<table>
<thead>
<tr>
<th>Antibody</th>
<th>kon (10⁻⁴ M⁻¹ s⁻¹)</th>
<th>koff (10⁻⁵ M⁻¹ s⁻¹)</th>
<th>Kd (nM)</th>
<th>kon (10⁻⁴ M⁻¹ s⁻¹)</th>
<th>koff (10⁻⁵ M⁻¹ s⁻¹)</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1G6 mgG</td>
<td>2.2</td>
<td>3.1</td>
<td>1.4</td>
<td>2.2</td>
<td>2.8</td>
<td>1.3</td>
</tr>
<tr>
<td>6G1 mgG</td>
<td>2.7</td>
<td>3.8</td>
<td>1.4</td>
<td>2.6</td>
<td>3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>15B2 mgG</td>
<td>4.1</td>
<td>29</td>
<td>7.1</td>
<td>3.5</td>
<td>39</td>
<td>11.1</td>
</tr>
</tbody>
</table>

[0458] In an assay to test ability of anti-FGFR3 murine hybridoma antibodies to inhibit Ba/F3 cell proliferation driven by human FGFR3-IIIb or Mc, antibodies 1G6, 6G1 and 15B2 were able to block Ba/F3 cell proliferation driven by human FGFR3-IIIb or IIIc in a dose-dependent manner. When tested across an antibody concentration range of about 0.01 to 100 μg/ml, antibodies 1G6, 6G1 and 15B2 blocked Ba/F3 cell proliferation driven by FGFR3-IIIb with IC50 values of 3-5 nM, 3 nM, and 6-8 nM, respectively, and blocked Ba/F3 cell proliferation driven by FGFR3-IIIc with IC50 values of 10-35 nM, 24 nM, and 60 nM, respectively.

[0459] In an assay to test ability of anti-FGFR3 murine hybridoma antibodies to inhibit FGFR1-induced signaling in Ba/F3 cells expressing human FGFR-IIIb, antibodies 1G6, 6G1 and 15B2 were able to block FGFR1-induced signaling in Ba/F3 cells expressing human FGFR3-IIIb in a dose-dependent manner when tested across an antibody concentration range of about 0.25 to 6.75 μg/ml. 25 ng/ml of FGFR1 was used in this experiment. In the absence of FGFR1, antibody treatment had no effect on FGFR3 activation.

[0460] In an assay to test ability of anti-FGFR3 murine hybridoma antibodies to inhibit FGFR1-induced signaling in Ba/F3 cells expressing human FGFR-IIIc, antibodies 1G6, 6G1 and 15B2 were able to block FGFR1-induced signaling in Ba/F3 cells expressing human FGFR3-IIIc in a dose-dependent manner when tested across an antibody concentration range of about 0.25 to 6.75 μg/ml. 25 ng/ml of FGFR1 was used in this experiment. In the absence of FGFR1, antibody treatment had no effect on FGFR3 activation.

Structured Basis for the Interaction of R3Mab with FGFR3

[0461] To gain insight into R3Mab’s mode of interaction with FGFR3, we synthesized a panel of 13 overlapping peptides spanning the FGFR3-IIIb IgD2 and D3 regions and tested their binding to R3Mab. Peptides 3 (residues 164-178) and 11 (residues 269-283) showed specific binding to R3Mab, with peptide 3 having a stronger interaction (FIG. 10A), indicating that the corresponding regions on FGFR3 are critical for recognition by R3Mab. Previous crystallographic studies of FGFR1 in complex with FGFR2 identified critical receptor residues engaged in direct binding to FGF and heparin as well as in receptor dimerization (34). Alignment of FGFR3 peptides 3 and 11 with the functionally important sites in FGFR1 revealed that these peptides encompass corresponding FGFR1 residues essential for direct FGFR2 binding, receptor dimerization, as well as interaction with heparin (FIG. 10B). These data indicate that the epitope of R3Mab on FGFR3 overlaps with receptor residues engaged in ligand association and receptor-receptor interaction.

[0462] We next crystallized the complex between the Fab fragment of R3Mab and the extracellular IgD2-D3 region of human FGFR3-IIIb, and determined the X-ray structure at 2.1 Å resolution (FIG. 10C, D; Table 4). In this complex, approximately 1400 Å² and 1500 Å² of solvent-accessible surface areas are buried on FGFR3 and the Fab, respectively. About 80% of the buried interface involves IgD2, while the remainder entails the linker and IgD3 regions. On the Fab side of the complex, about 40% of the buried interface involve complementarity-determining region (CDR)-H3, 20% CDR-H2, 20% CDR-L2, and minor contributions are from other CDRs and framework residues. Notably, amino acids (AAs) from CDR-H3 form two β-strands, which extend the β-sheet of IgD2 (FIG. 10D). The Fab interacts with AAs that constitute the FGF binding site of FGFR3 as well as residues that form the receptor dimerization interface, as previously identified in various dimeric FGF:FGFR complexes (e.g., PDB code 1CVS, 34; and FIG. 10C, areas in grey/crosshatched and dark grey). The interaction interfaces identified by crystallography were fully consistent with the peptide-based data (FIG. 18A, B). Together, these results reveal how R3Mab inhibits ligand binding, and further suggest that binding of R3Mab to FGFR3 may prevent receptor dimerization. FGFR3 amino acids that contact R3Mab are shown in Table 5. Crystallographic coordinates for this structure are deposited in the Protein Data Bank with accession code 3GWW and shown in Table 6.

TABLE 5

<table>
<thead>
<tr>
<th>Residues in FGFR3 that are in contact with R3Mab</th>
<th>Residue</th>
<th>Buried surface of residue in the interface</th>
</tr>
</thead>
<tbody>
<tr>
<td>THR 154</td>
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<td></td>
</tr>
<tr>
<td>ARG 155</td>
<td>16.50</td>
<td></td>
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<tr>
<td>ARG 158</td>
<td>105.40</td>
<td></td>
</tr>
<tr>
<td>MET 159</td>
<td>6.00</td>
<td></td>
</tr>
<tr>
<td>LYS 161</td>
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<td></td>
</tr>
<tr>
<td>LYS 162</td>
<td>1.70</td>
<td></td>
</tr>
<tr>
<td>LEU 163</td>
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<td></td>
</tr>
<tr>
<td>LEU 164</td>
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<tr>
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</tr>
<tr>
<td>VAL 166</td>
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<tr>
<td>PRO 167</td>
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TABLE 4  Summary of crystallographic analysis

<table>
<thead>
<tr>
<th>Data collection</th>
<th>FGFR3-IIIc: R3Mab Fab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>P2₁2₁2₁</td>
</tr>
<tr>
<td>Cell parameters</td>
<td>a = 58.5 Å, b = 99.3 Å, c = 143.7 Å</td>
</tr>
<tr>
<td>Resolution (Å)</td>
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</tr>
<tr>
<td>Rmerge (%)</td>
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</tr>
<tr>
<td>Number of reflections</td>
<td>284508</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>99.99 (100.0)</td>
</tr>
<tr>
<td>Refinement</td>
<td></td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>20.21</td>
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<tr>
<td>Number of reflections</td>
<td>46714</td>
</tr>
<tr>
<td>Final R, Rmerge (F &gt; 0)</td>
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</tr>
<tr>
<td>Number of non-H atoms</td>
<td>5270</td>
</tr>
<tr>
<td>Number of Amino Acids</td>
<td>650</td>
</tr>
<tr>
<td>Sulfate</td>
<td>1</td>
</tr>
<tr>
<td>Sugar</td>
<td>1</td>
</tr>
<tr>
<td>Solvent atoms</td>
<td>274</td>
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<tr>
<td>R.m.s. bond (Å)</td>
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<tr>
<td>R.m.s. angle (°)</td>
<td>1.4</td>
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</table>

R<sub>merge</sub> = Σ||I<sub>o</sub> - I<sub>c</sub>|| / ΣI<sub>c</sub> is the average intensity of symmetry related observations of a unique reflection.

Numbers in parentheses refer to the highest resolution shell.

R<sub>merge</sub> = Σ||F<sub>o</sub> - F<sub>c</sub>|| / ΣF<sub>c</sub> is calculated as R<sub>c</sub> but for 5% of the reflections excluded from all refinement.

[0463] We compared the R3Mab-FGFR3 structure with a previously published structure of FGFR3-IIIc in complex with FGF1 (4, 35) (FIG. 10E, 10F). Superposition of the antibody-receptor and ligand-receptor complexes revealed that there are no major conformational differences within the individual receptor domains, except in the region that distinguishes FGFR3-IIIc from FGFR3-IIIb; however, the orientation of IgD3 relative to IgD2 was drastically different (FIG. 10E, white and grey; FIG. 10F, white and white-grey). Since the relative positions of IgD2 and IgD3 are critical for ligand binding, the alternate conformation adopted by IgD3 upon R3Mab binding may provide an additional mechanism to prevent ligand interaction with FGFR3.

R3Mab Inhibits Endogenous WT and Mutant FGFR3 in Bladder Cancer Cells

[0464] To assess whether R3Mab could suppress FGFR3 function in bladder cancer cells, we first examined RT112 and RT4 cell lines, which express WT FGFR3. R3Mab strongly inhibited [3H]-thymidine incorporation by RT112 cells (FIG. 11A) and exerted a significant, though more moderate suppression of RT4 cell proliferation (FIG. 19A). To investigate R3Mab’s effect on FGFR3 activation, we examined the phosphorylation of FGFR3 in RT112 cells. Consistent with the results in Ba/F3-FGFR3 cells (FIG. 9B), R3Mab markedly attenuated FGF1-induced FGFR3 phosphorylation (FIG. 11B). We next examined phosphorylation of FRS2α, AKT, and p44/42 MAPK, three downstream mediators of FGFR3 signaling. FGF1 strongly activated these molecules in RT112 cells, while R3Mab significantly diminished this activation (FIG. 11B). Similarly, R3Mab suppressed FGF1-induced phosphorylation of FGFR3 and MAPK in RT4 cells (FIG. 19B).

[0465] We next investigated whether R3Mab could inhibit activation of endogenous mutant FGFR3 in human bladder cancer cells. S249C is the most frequent FGFR3 mutation in bladder cancer (FIG. 3C). Two available cell lines, UMUC-14 and TCC-97-7, carry a mutated FGFR3<sup>S249C</sup> allele (Ref. 36 and data not shown). Although R3Mab did not affect the exponential growth of UMUC-14 cells in culture (data not shown), it significantly reduced the clonal growth of these cells (FIG. 11C). Specifically, R3Mab decreased the number of colonies larger than 120 μm in diameter approximately by 77% as compared with control antibody (FIG. 11D). Furthermore, R3Mab inhibited [3H]-thymidine incorporation by TCC-97-7 cells in culture (FIG. 19C).

[0466] The S249C mutation is reported to result in ligand-independent activation of FGFR3 (26, 30). Indeed, FGFR3<sup>S249C</sup> was constitutively phosphorylated irrespective of FGF1 treatment in UMUC-14 cells and TCC-97-7 cells, while R3Mab reduced constitutive phosphorylation of FGFR3<sup>S249C</sup> as compared with control antibody in both cell lines (FIGS. 11E, 19D).

R3Mab Inhibits Dimer Formation by FGFR3<sup>S249C</sup>

[0467] The ability of R3Mab to inhibit constitutive FGFR3<sup>S249C</sup> signaling and proliferation in bladder cancer cells was surprising, considering that this mutant can undergo disulfide-linked, ligand-independent dimerization (26, 30). To explore how R3Mab inhibits FGFR3<sup>S249C</sup>, we examined the effect of R3Mab on the oligomeric state of this mutant in UMUC-14 cells. Under reducing conditions, FGFR3<sup>S249C</sup> migrated as a single band of ~97 kDa, consistent with monomeric size (FIG. 12A). Under non-reducing conditions, in cells treated with control antibody a large fraction of FGFR3<sup>S249C</sup> appeared as a band of ~200 kDa, regardless of FGF1 addition, indicating a constitutive dimeric state (FIG. 12A). R3Mab treatment substantially decreased the amount of dimers, with a concomitant increase in monomers (FIG. 12A). Consistently, R3Mab decreased the level of FGFR3<sup>S249C</sup> dimers in TCC-97-7 cells irrespective of FGF1 treatment (FIG. 19E).

[0468] How does R3Mab decrease the FGFR3<sup>S249C</sup> dimer levels in bladder cancer cells? One potential explanation is that it may disrupt the FGFR3<sup>S249C</sup> dimer through antibody-induced FGFR3 internalization and trafficking through endosomes or lysosomes. We tested this possibility by pharmacologically intervening with endocytosis. R3Mab nonetheless decreased the amount of dimer in UMUC-14 cells pre-treated with various endocytosis inhibitors, despite substantial blockade of FGFR3<sup>S249C</sup> internalization (FIG. 20A, B). Thus, dimer disruption by R3Mab is independent of endocytosis. Another possible explanation is that cellular FGFR3<sup>S249C</sup> may exist in a dynamic monomer-dimer equilibrium; accordingly, binding of R3Mab to monomeric FGFR3<sup>S249C</sup> could prevent dimer formation and thereby shift the equilibrium toward the monomeric state. To examine this possibility, we used the non-cell-permeating agent 5,5’-Dithiobis-2-nitrobenzoic acid (DTNB), which selectively reacts with and blocks free sulfhydryl groups of unpaired cysteines (37). Treatment of UMUC-14 cells with DTNB led to the accumulation of FGFR3<sup>S249C</sup> monomers at the expense of dimers (FIG. 12B), indicating that FGFR3<sup>S249C</sup> exists in a dynamic equilibrium between monomers and dimers.

[0469] To test whether R3Mab affects this equilibrium, we generated a soluble recombinant protein comprising the IgD2-D3 domains of FGFR3<sup>S249C</sup> and isolated the dimers by size exclusion chromatography. We incubated the dimers with buffer or antibodies in the presence of a very low concentration of reducing agent (25 μM of DTDT), and analyzed the oligomeric state of the receptor by SDS-PAGE under non-reducing conditions. R3Mab significantly accelerated the appearance of a ~25 kDa band representing monomeric FGFR3<sup>S249C</sup> at the expense of the ~50 kDa dimer, as com-
pared with mock or antibody controls (FIG. 12C); indeed, by 2 hr the decrease in dimers was substantially more complete in the presence of R3Mab. These results indicate that R3Mab shifts the equilibrium between the monomeric and dimeric states of FGFR3<sup>524G6C</sup> in favor of the monomer.

R3Mab does not Promote FGFR3 Down-Regulation

**[0470]** We examined the effect of R3Mab (clone 184.6.1) and anti-FGFR3 hybridoma antibodies on FGFR3 down-regulation by analyzing FGFR3 internalization and degradation in FGFR3 antibody-treated cells. Bladder cancer cell lines expressing wild type FGFR3 (RT112) or mutated FGFR3 (S249C in TCC97-7) were treated with R3Mab or hybridoma antibodies IgG6 or 6G1 for 4 to 24 hours, then cell lysates were harvested for western blot analysis of total FGFR3 levels. Treatment with R3Mab did not reduce FGFR3 levels, while treatment with hybridoma mabs 1G6 and 6G1 significantly reduced FGFR3 levels. These results suggested that R3Mab did not promote FGFR3 down-regulation while mabs 1G6 and 6G1 did promote FGFR3 receptor internalization and down regulation. In a separate experiment, surface FGFR3 levels were examined using FACS analysis. After 24 hours of R3Mab (clone 184.6.1) treatment of UMUC-14 cells (containing FGFR3 S249C mutation), cell surface FGFR3 levels slightly increased. These results demonstrate that R3Mab treatment did not promote FGFR3 down-regulation.

R3Mab Inhibits Growth and FGFR3 Signaling in Multiple Tumor Models

**[0471]** Next, we examined the effect of R3Mab on the growth of bladder cancer cells in vivo. We injected nu/nu mice with RT112 cells (which express WT FGFR3), allowed tumors to grow to a mean volume of ~150 mm<sup>3</sup>, and dosed the animals twice weekly with vehicle or R3Mab. Compared with vehicle control at day 27, R3Mab treatment at 5 or 50 mg/kg suppressed tumor growth by about 41% or 73% respectively (FIG. 13A). Analysis of tumor lysates collected 48 hr or 72 hr after treatment showed that R3Mab markedly decreased the level of phosphorylated FRS2α (FIG. 13B). Intriguingly, total FRS2α protein levels were also lower in R3Mab-treated tumors, suggesting that FGFR3 inhibition may further lead to down-regulation of FRS2α. R3Mab also lowered the amount of phosphorylated MAPK in tumors, without affecting total MAPK levels (FIG. 13B). Thus, R3Mab inhibits growth of RT112 tumor xenografts in conjunction with blocking signaling by WT FGFR3.

**[0472]** We next investigated the effect of R3Mab on growth of xenografts expressing mutant FGFR3. R3Mab treatment profoundly attenuated the progression of Ba/F3-FGFR3<sup>524G6C</sup> tumors (FIG. 13C). Moreover, R3Mab significantly inhibited growth of UMUC-14 bladder carcinoma xenografts (FIG. 13D). To evaluate whether R3Mab impacts FGFR3<sup>524G6C</sup> activation in vivo, we assessed the level of FGFR3<sup>524G6C</sup> dimer in tumor lysates collected 24 hr or 72 hr after treatment. Under non-reducing conditions, the amount of FGFR3<sup>524G6C</sup> dimer was substantially lower in R3Mab treated tumors as compared with control group, whereas total FGFR3<sup>524G6C</sup> levels, as judged by the amount detected under reducing conditions, showed little change (FIG. 13E). No apparent accumulation of FGFR3<sup>524G6C</sup> monomer was observed in tumor lysates, in contrast to the results in cell culture (FIG. 13E vs. 12A). This could be due to the weak detection sensitivity for monomeric FGFR3 under non-reducing conditions by the rabbit polyclonal anti-FGFR3 antibody used in this study (FIG. 21). Importantly, R3Mab also significantly inhibited the phospho-rylation and activation of MAPK in UMUC-14 tumors (FIG. 13E), suggesting that R3Mab inhibits the activity of FGFR3<sup>524G6C</sup> in vivo. We did not observe any significant weight loss or other gross abnormalities in any of the in vivo studies. Furthermore, in a safety study conducted in mice, R3Mab, which binds with similar affinity to both human and murine FGFR3, did not exert any discernable toxicity in any organs, including bladder (data not shown). Together, these data indicate that multiple exposures to R3Mab are well tolerated in mouse.

Anti-Tumor Activity of R3Mab in Multiple Myeloma Xenograft Models Involves ADCC

**[0473]** To assess whether R3Mab might harbor therapeutic potential for multiple myeloma, we first tested the effect of R3Mab on the proliferation and survival of three (4, 14)+ cell lines in culture. UTMC-2 cells carry WT FGFR3, while OPM2 and KMS11 harbor a K650E and Y373C substitution, respectively (7). In culture, R3Mab abrogated FGFR- and GAV-induced proliferation of UTMC-2 cells completely (FIG. 22A). R3Mab modestly inhibited the growth of OPM2 cells, but had no apparent effect on the proliferation of KMS11 cells (FIG. 22B, C). Since UTMC-2 cells do not form tumors in mice, we evaluated the efficacy of R3Mab against OPM2 and KMS11 tumors. R3Mab almost completely abolished xenograft tumor growth of both cell lines (FIG. 14A, B).

**[0474]** The marked difference in activity of R3Mab against OPM2 and KMS11 tumor cells in vitro and in vivo suggested the possibility that R3Mab may be capable of supporting Fe-mediated immune effector functions against these FGFR3-overexpressing tumors. Both cell lines express high levels of CD55 and CD59 (data not shown), two inhibitors of the complement pathway; accordingly, no complement-dependent cytotoxicity was observed (data not shown). We then focused on ADCC. ADCC occurs when an antibody binds to its antigen on a target cell, and via its Fc region, engages Fc receptors (FcγRs) expressed on immune effector cells (38). To test ADCC in vivo, we incubated KMS11 or OPM2 cells with freshly isolated human peripheral blood mononuclear cells (PBMC) in the presence of R3Mab or control antibody. R3Mab mediated significant PBMC cytolysis activity against both myeloma cell lines (FIG. 14C, D). By contrast, R3Mab did not support cytolysis of bladder cancer RT112 or UMUC-14 cells (FIG. 14E, F). As measured by Scatchard analysis, the multiple myeloma cells express substantially more cell-surface FGFR3 than the bladder carcinoma cell lines (~5-6 fold more receptors per cell; FIG. 23A, B).

**[0475]** To address the contribution of ADCC to the activity of R3Mab in vivo, we introduced the previously characterized D265A/N297A (DANA) mutation into the antibody’s Fc domain. This dual substitution in the Fc domain of an antibody abolishes its binding to FcγRs (39), preventing recruitment of immune effector cells. The DANA mutation did not alter R3Mab binding to FGFR3 or inhibition of FGFR3 activity in vitro, nor did it change the pharmacokinetics of R3Mab in mice (data not shown); however, it substantially abolished in vivo activity against OPM2 or KMS11 xenografts (FIG. 14G, H). By contrast, the DANA mutation did not alter the anti-tumor activity of R3Mab towards RT112 and UMUC-14 bladder cancer xenografts (FIG. 24A, B). Together, these results suggest that Fc-dependent ADCC plays an important role in the efficacy of R3Mab against OPM2 and KMS11 multiple myeloma xenografts.
Additional Xenograft Studies

0476 R3Mab (clone 184.6.1N54S) was further characterized as follows:

0477 (a) R3Mab was tested for in vivo efficacy using a tumor xenograft model based on a liver cancer cell line (11th?). When tested at an antibody concentration of 5 mg/kg and 30 mg/kg, R3Mab significantly inhibited tumor growth in vivo. Tumor growth was inhibited about 50% compared to tumor growth in control animals.

0478 (b) R3Mab was tested for in vivo efficacy using a tumor xenograft model based on a breast cancer cell line (CaI-51) which expressed FGFR3. Results from this efficacy study showed that the R3Mab antibody was capable of inhibiting tumors in vivo when tested at antibody concentration range of about 1 mg/kg to 100 mg/kg. Tumor growth was inhibited about 30% compared to tumor growth in control animals.

Discussion

0479 The association of FGFR3 overexpression with poor prognosis in (4, 14)+ multiple myeloma patients and the transforming activity of activated FGFR3 in several experimental models have established FGFR3 as an important oncogenic driver and hence a potential therapeutic target in this hematologic malignancy. By contrast, despite reports of a high frequency of mutation and/or overexpression of FGFR3 in bladder carcinoma (24, 25, 40), a critical role for FGFR3 signaling in this epithelial malignancy has not been established in vivo. Moreover, the therapeutic potential of FGFR3 inhibition in bladder cancer has yet to be defined. Here we show that genetic or pharmacological intervention with FGFR3 inhibits growth of several human bladder cancer xenografts in mice. These results demonstrate that FGFR3 function is critical for tumor growth in this setting, underscoring the potential importance of this receptor as an oncogenic driver and therapeutic target in bladder cancer. Blockade of FGFR3 function inhibited growth of xenografts expressing either WT or mutant FGFR3 alike, suggesting that both forms of the receptor may contribute significantly to bladder tumor progression. Although much less frequently than in bladder cancer, FGFR3 mutations or overexpression have been identified in other solid tumor malignancies, including cervical carcinoma (40), hepatocellular carcinoma (41) and nonsmall cell lung cancer (42, 43), suggesting a potential contribution of FGFR3 to additional types of epithelial cancer.

0480 The apparent involvement of FGFR3 in diverse malignancies identifies this receptor as an intriguing candidate for targeted therapy. While small molecule compounds that can inhibit FGFR3 kinase activity have been described (18-22, 44), the close homology of the kinase domains within the FGFR family has hampered the development of FGFR3-selective inhibitors. The lack of selectivity of the reported inhibitors makes it difficult to discern the relative contribution of FGFR3 to the biology of specific cancer types; furthermore, it may carry safety liabilities, including maximal dose levels and thus limiting optimal inhibition of FGFR3. Therefore, to achieve selective and specific targeting of FGFR3, we turned to an antibody-based strategy. We reasoned that an optimal therapeutic antibody should be capable of blocking not only the WT but also the prevailing cancer-linked mutants of FGFR3. Furthermore, given that dimerization of FGFR3 is critical for its activation, an antibody that not only blocks ligand binding but also interferes with receptor dimerization could be superior. Additional desirable properties would include the ability to support Fc-mediated effector function and the long serum half-life conferred by the natural framework of a full-length antibody. We focused our screening and engineering efforts to identify an antibody molecule that combines all of these features, leading to the generation of R3Mab. Binding studies demonstrated the ability of R3Mab to compete with FGFR ligands for interaction with both the IIb and IIC isoforms of FGFR3. Further experiments with transfected BaF3 cell lines confirmed the remarkable ability of R3Mab to block both WT and prevalent cancer-associated FGFR3 mutants. In addition, R3Mab exerted significant anti-tumor activity in several xenograft models of bladder cancer expressing either WT FGFR3 or FGFR3*624C*, which is the most common mutant of the receptor in this disease. Pharmacodynamic studies suggested that the anti-tumor activity R3Mab in these models is based on inhibition of FGFR3 signaling, evident by diminished phosphorylation of its downstream mediators FRS2α and MAPK. These data further reinforce the conclusion that FGFR3 is required for bladder tumor progression, as demonstrated by our FGFR3 shRNA studies.

0481 FGFR3 mutations in bladder cancer represent one of the most frequent oncogenic alterations of a protein kinase in solid tumor malignancies, reminiscent of the common mutation of B-Raf in melanoma (45). Most of the activating mutations in FGFR3 give rise to an unpaired cysteine, leading to ligand-independent receptor dimerization and to various degrees of constitutive activation. A previous study using a monoclonal anti-FGFR3 Fab fragment indicated differential inhibitory activity against specific FGFR3 mutants (46); however, the molecular basis for this variable effect was not investigated. Compared with monovalent antibody fragments, bivalent antibodies have the capacity to induce the clustering of antigens, and in the case of receptor tyrosine kinases, may cause receptor oligomerization and activation. Despite its full-length, bivalent configuration, R3Mab displayed universal inhibition of WT FGFR3 and of a wide spectrum of FGFR3 mutants, including variants that are ligand-dependent (FGFR3*624C*, FGFR3*575C), constitutively active (FGFR3*284Y, FGFR3*284C), or both (FGFR3*284Y*575C). These results raise the question: How does R3Mab antagonize both WT and various FGFR3 mutants, including disulfide-linked variants?

0482 Based on sequence alignment with FGFR1, the peptide epitope recognized by R3Mab overlaps with FGFR3 residues involved in binding to ligand and heparin, as well as receptor dimerization. This conclusion was confirmed by crystallographic studies of the complex between R3Mab and the extracellular regions of FGFR3. The X-ray structure revealed that the antibody binds to regions of IgD2 and IgD3 that are critical for ligand-receptor interaction as well as receptor-receptor contact. Thus, R3Mab may block WT FGFR3 both by competing for ligand binding and by preventing receptor dimerization. R3Mab may employ a similar mechanism to inhibit FGFR3*575C, which has low constitutive activity, but requires ligand for full activation. Furthermore, R3Mab binding changes the relative orientation of FGFR IgD3 with respect to IgD2. This finding raises the formal possibility that the antibody might also inhibit receptor activation by forcing a conformation that is not conducive to signal transduction—a notion that requires further study.

0483 To gain better insight into how R3Mab blocks FGFR3 variants possessing an unpaired cysteine, we ana-
alyzed the most common mutant, FGFR3\textsuperscript{524-526C}, in greater detail. Experiments with the free-sulfhydryl blocker DTNB indicated a dynamic equilibrium between the monomeric and dimeric state of FGFR3\textsuperscript{524-526C}. Similar equilibrium between oxidized and reduced states modulated by endogenous redox regulators has been reported for NMDA receptors (46). Inhibition of bladder cancer cells expressing FGFR3\textsuperscript{524-526C} with R3Mab led to a decline in the amount of receptor dimers and a concomitant increase in the level of monomers. Moreover, the purified IgD2-D3 fragment of FGFR3\textsuperscript{524-526C} formed dimers in solution; when incubated with R3Mab, the dimers steadily disappeared while monomeric FGFR3\textsuperscript{524-526C} accumulated. Taken together with the structural analysis, these results suggest that R3Mab captures monomeric FGFR3\textsuperscript{524-526C} and hinders its dimerization. Over time, R3Mab shifts the equilibrium towards the monomeric state, blocking constitutive receptor activity. This mechanism might also explain how R3Mab inhibits other cysteine mutants of FGFR3.

[0484] Another important finding of this study was the potent anti-tumor activity of R3Mab against the t(4;14)+ multiple myeloma cell lines OPM2 and KMS11 in vivo. By contrast, R3Mab had modest to minimal impact on proliferation or survival of these cells in culture. OPM2 and KMS11 cells express relatively high cell surface levels of FGFR3 (5-6 fold higher than RT112 and UMUC-14 bladder carcinoma cells). These higher antigen densities may permit R3Mab to support efficient recruitment of FcγRI-bearing immune effector cells and activation of ADCC. Indeed, in the presence of human PBMC, R3Mab mediated cytolyis of OPM2 and KMS11 cells, but not RT112 or UMUC-14 bladder cancer cells. Moreover, the DANA mutant version of R3Mab, which is incapable of FcγR binding, had no effect on KMS11 or OPM2 growth in vivo, but still suppressed growth of RT112 and UMUC-14 tumors similarly to R3Mab. Together, these data indicate that R3Mab has a dual mechanism of anti-tumor activity: (a) In cells expressing lower surface levels of WT or mutant FGFR3, it blocks ligand-dependent or constitutive signaling; (b) In cells expressing relatively high surface FGFR3 levels, it induces ADCC.

[0485] Our results also raise some new questions. First, it is unknown why the bladder cancer cell lines tested in this study display variable sensitivity to R3Mab. Such differential response, which is common for targeted therapy, may be a reflection of the distinct genetic make-up of individual tumors. Indeed, Her2-positive breast cancer cells show variable sensitivity to anti-Her2 antibody (48), as do various cancer cells in response to anti-EGFR antibody (49). In this context, development of additional in vivo models for bladder cancer with WT and mutant FGFR3 is urgently needed to assess sensitivity to FGFR3 molecules in animals. Moreover, elucidation of predictive biomarkers may help identify patients who can optimally benefit from FGFR3-targeted therapy. Secondly, because R3Mab did not induce tumor regression in the models we examined, future studies should explore whether R3Mab can cooperate with established therapeutic agents.

[0486] In conclusion, our findings implicate both WT and mutant FGFR3 as important for bladder cancer growth, thus expanding the in vivo oncogenic involvement of this receptor from hematologic to epithelial malignancy. Furthermore, our results demonstrate that both WT and mutant FGFR3 can be effectively targeted in tumors with a full-length antibody that combines the ability to block ligand binding, receptor dimerization and signaling, as well as to promote tumor cell lysis by ADCC. These results provide a strong rationale for investigating antibody-based, FGFR3-targeted therapies in diverse malignancies associated with this receptor.

PARTIAL REFERENCE LIST


[0546] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention.

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<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 28

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
20 25

<210> SEQ ID NO 29
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 29

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
20 25

<210> SEQ ID NO 30
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 30

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
20 25

<210> SEQ ID NO 31
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide
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<400> SEQUENCE: 31

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1     5      10     15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20    25     30

<210> SEQ ID NO 32
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 32

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1     5      10     15

Ser Leu Arg Leu Ser Cys Ala Ala Ser
20    25

<210> SEQ ID NO 33
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 33

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1     5      10     15

Ser Leu Arg Leu Ser Cys Ala Ala Ser
20    25

<210> SEQ ID NO 34
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 34

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1     5      10     15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20    25     30

<210> SEQ ID NO 35
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 35

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1     5      10     15

Ser Leu Arg Leu Ser Cys Ala Ala Ser
20    25
<210> SEQ ID NO 36
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 36

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

20 25

<210> SEQ ID NO 37
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 37

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

20 25

<210> SEQ ID NO 38
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 38

Asp Ile Gln Met Thr Gln Ser Pro Ser Leu Ser Ala Ser Val Gly

1  5  10  15

Asp Arg Val Thr Ile Thr Cys

20

<210> SEQ ID NO 39
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 39

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly

1  5  10  15

Glu Pro Ala Ser Ile Ser Cys

20

<210> SEQ ID NO 40
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 40
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Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys
20

<210> SEQ ID NO 41
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 41
Asp Ile Val Met Thr Gln Ser Pro Ser Leu Ala Val Ser Leu Gly
1 5 10 15 Glu Arg Ala Thr Ile Asn Cys
20

<210> SEQ ID NO 42
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15 Asp Arg Val Thr Ile Thr Cys
20

<210> SEQ ID NO 43
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 43
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> SEQ ID NO 44
<211> LENGTH: 32
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide
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Gly Val Pro Ser Arg Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr
1 5 10 15 Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
20 25 30

<210> SEQ ID NO 45
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 45

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1  5  10

<210> SEQ ID NO 46
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 46

Glu Val Gln Leu Val Glu Ser Gly Gln Leu Val Gln Pro Gly Gly
1  5  10  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser
20  25

<210> SEQ ID NO 47
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 47

Trp Val Arg Gln Ala Pro Gly Lys Leu Glu Trp Val
1  5  10

<210> SEQ ID NO 48
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 48

Gly Phe Thr Phe Ser Thr Gly Ile Ser
1  5  10

<210> SEQ ID NO 49
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 49

Gly Arg Ile Tyr Pro Leu Tyr Gly Ser Thr His Tyr Ala Asp Ser Val
1  5  10  15

Lys Gly
peptide

Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1 5 10 15
Val Met Asp Tyr
20

Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1 5 10

Ser Ala Ser Phe Leu Tyr Ser
1 5

Gln Gln Thr Tyr Thr Thr Ser Leu Thr
1 5

Gly Phe Thr Phe Thr Ser Thr Gly Ile Ser
1 5 10
Gly Arg Ile Tyr Pro Tyr Asp Asp Ser Phe Tyr Tyr Ala Asp Ser Val
1    5    10    35

Lys Gly

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
  <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 56

Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1    5    10    15

Val Met Asp Tyr
20

<210> SEQ ID NO 57
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
  <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 57

Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1    5    10

<210> SEQ ID NO 58
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
  <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 58

Ser Ala Ser Phe Leu Tyr Ser
1    5

<210> SEQ ID NO 59
<211> LENGTH: 9
<212> TYPE: PRT
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<220> FEATURE:
  <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 59

Gln Gln Ser Tyr Thr Thr Pro Leu Thr
1    5

<210> SEQ ID NO 60
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
  <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 60

Gly Phe Thr Phe Thr Ser Thr Gly Ile Ser
1    5    10
<210> SEQ ID NO 61
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURES:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 61
Gly Arg Ile Tyr Pro Thr Asn Gly Ser Thr Asn Tyr Ala Asp Ser Val
 1  5 10 15
Lys Gly

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: PRT
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<220> FEATURES:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 62
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
 1  5 10 15
Val Met Asp Tyr
 20

<210> SEQ ID NO 63
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURES:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 63
Arg Ala Ser Gln Val Ile Asp Ile Ser Leu Ala
 1  5 10

<210> SEQ ID NO 64
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<400> SEQUENCE: 64
Gly Ala Ser Thr Leu Ala Ser
 1  5

<210> SEQ ID NO 65
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
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Gln Gln Ser Ala Ala Asp Pro Tyr Thr
 1  5
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 66

Gly Phe Ser Phe Thr Gly Thr Gly Ile Ser
1   5       10

<210> SEQ ID NO 67
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 67

Gly Ser Ile Tyr Pro Tyr Phe Ala Thr Lys Asn Tyr Ala Asp Ser Val
1   5       10      15

Lys Gly

<210> SEQ ID NO 68
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 68

Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1   5       10      15

Val Met Asp Tyr
20

<210> SEQ ID NO 69
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 69

Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1   5       10

<210> SEQ ID NO 70
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 70

Ser Ala Ser Phe Leu Tyr Ser
1   5

<210> SEQ ID NO 71
<211> LENGTH: 9
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<210> SEQ ID NO 71
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 71

Gln Gln Ser Tyr Thr Thr Pro Pro Thr 15

<210> SEQ ID NO 72
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 72

Gly Phe Thr Phe Tyr Thr Thr Gly Ile Ser 10

<210> SEQ ID NO 73
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 73

Gly Arg Ile Tyr Pro Ala Phe Gly Ser Ser Ile Tyr Ala Asp Ser Val 15

Lys Gly

<210> SEQ ID NO 74
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 74

Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr 15

Val Met Asp Tyr

<210> SEQ ID NO 75
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 75

Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala

<210> SEQ ID NO 76
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
FEATURE: OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 76
Ser Ala Ser Phe Leu Tyr Ser
1 5

<210> SEQ ID NO 77
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 77
Gln Glu Thr Tyr Ser Ala Glu Pro Thr
1 5

<210> SEQ ID NO 78
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 78
Gly Phe Ser Phe Trp Ser Thr Gly Ile Ser
1 5 10

<210> SEQ ID NO 79
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 79
Gly Arg Ile Tyr Pro Ser Ser Ala Thr Thr Asn Tyr Ala Asp Ser Val
1 5 10 15
Lys Gly

<210> SEQ ID NO 80
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 80
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1 5 10 15
Val Met Asp Tyr
20
peptide

<400> SEQUENCE: 81
Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1  5 10

<210> SEQ ID NO 82
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 82
Ser Ala Ser Phe Leu Tyr Ser
1  5

<210> SEQ ID NO 83
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 83
Gln Gln Ser Tyr Ser His Gln Ser Thr
1  5

<210> SEQ ID NO 84
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 84
Gly Phe Thr Phe Thr Ser Thr Ile Ser
1  5 10

<210> SEQ ID NO 85
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 85
Gly Arg Ile Tyr Pro Thr Ser Gly Ser Thr Asn Tyr Ala Asp Ser Val
1  5 10 15
Lys Gly

<210> SEQ ID NO 86
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 86
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
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 Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr 
1  5  10  15
 Val Met Asp Tyr
 20

 Arg Ala Ser Gln Asp Val Asp Thr Ser Leu Ala
1  5  10

 Ser Ala Ser Phe Leu Tyr Ser
1  5

 Gln Gln Ser Thr Gly His Pro Gln Thr
1  5

 Gly Phe Thr Phe Thr Ser Thr Gly Ile Ser
1  5  10
<ORGANISM: Artificial Sequence>

<SEQUENCE: 97>
Gly Arg Ile Tyr Pro Thr Ala Gly Ser Thr Asn Tyr Ala Asp Ser Val
1   5   10  15
Lys Gly

<SEQUENCE: 98>
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1   5   10  15
Val Met Asp Tyr
20

<SEQUENCE: 99>
Arg Ala Ser Gln Asp Val Asp Thr Ser Leu Ala
1   5   10

<SEQUENCE: 100>
Ser Ala Ser Phe Leu Tyr Ser
1   5

<SEQUENCE: 101>
Gln Gln Ser Thr Gly His Pro Gln Thr
1   5

<SEQUENCE: 102>
Gly Arg Ile Tyr Pro Thr Ala Gly Ser Thr Asn Tyr Ala Asp Ser Val
1   5   10  15
Lys Gly

<SEQUENCE: 98>
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1   5   10  15
Val Met Asp Tyr
20

<SEQUENCE: 99>
Arg Ala Ser Gln Asp Val Asp Thr Ser Leu Ala
1   5   10

<SEQUENCE: 100>
Ser Ala Ser Phe Leu Tyr Ser
1   5

<SEQUENCE: 101>
Gln Gln Ser Thr Gly His Pro Gln Thr
1   5

<SEQUENCE: 102>
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 102

Gly Phe Thr Phe Thr Ser Thr Gly Ile Ser
1     5     10

<210> SEQ ID NO 103
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 103

Gly Arg Ile Tyr Pro Thr Gln Gly Ser Thr Aen Tyr Ala Asp Ser Val
1     5     10     15

Lys Gly

<210> SEQ ID NO 104
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 104

Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1     5     10     15

Val Met Asp Tyr
20

<210> SEQ ID NO 105
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 105

Arg Ala Ser Gln Asp Val Asp Thr Ser Leu Ala
1     5

<210> SEQ ID NO 106
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 106

Ser Ala Ser Phe Leu Tyr Ser
1     5

<210> SEQ ID NO 107
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 107
Gln Gln Ser Thr Gly His Pro Gln Thr
 1  5

<210> SEQ ID NO 108
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 108
Gly Phe Thr Phe Thr Ser Thr Gly Ile Ser
 1  5 10

<210> SEQ ID NO 109
<211> LENGTH: 18
<212> TYPE: PRT
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 109
Gly Arg Ile Tyr Pro Thr Ser Gly Ser Thr Asn Tyr Ala Asp Ser Val
 1  5 10 15

Lys Gly

<210> SEQ ID NO 110
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 110
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
 1  5 10 15

Val Met Asp Tyr
 20

<210> SEQ ID NO 111
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 111
Arg Ala Ser Gln Val Val Asp Thr Ser Leu Ala
 1  5 10

<210> SEQ ID NO 112
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 112
Ser Ala Ser Ser Leu Ala Ser
1 5

<210> SEQ ID NO 113
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 113
Gln Gln Gly Ala Gly Asn Pro Tyr Thr
1 5

<210> SEQ ID NO 114
<211> LENGTH: 10
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 114
Gly Phe Thr Phe Thr Ser Thr Gly Ile Ser
1 5 10

<210> SEQ ID NO 115
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 115
Gly Arg Ile Tyr Pro Thr Gly Gly Ser Thr Asn Tyr Ala Asp Ser Val
1 5 10 15
Lys Gly

<210> SEQ ID NO 116
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 116
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1 5 10 15
Val Met Asp Tyr
20

<210> SEQ ID NO 117
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 117
Arg Ala Ser Gln Val Val Asp Thr Ser Leu Ala
<210> SEQ ID NO 118
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 118
Ser Ala Ser Ser Leu Ala Ser
1  5

<210> SEQ ID NO 119
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 119
Gln Glu Gly Ala Gly Asn Pro Tyr Thr
1  5

<210> SEQ ID NO 120
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 120
Gly Phe Thr Phe Thr Ser Thr Gly Ile Ser
1  5

<210> SEQ ID NO 121
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 121
Gly Arg Ile Tyr Pro Thr Ala Gly Ser Thr Asn Tyr Ala Asp Ser Val
1  5  10

Lys Gly

<210> SEQ ID NO 122
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 122
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1  5  10

Val Met Asp Tyr
20
Arg Ala Ser Gln Val Val Asp Thr Ser Leu Ala
1   5   10

Ser Ala Ser Ser Leu Ala Ser
1   5

Gln Gln Gly Ala Gly Asn Pro Tyr Thr
1   5

Gly Phe Thr Phe Thr Ser Thr Gly Ile Ser
1   5   10

Gly Arg Ile Tyr Pro Thr Gln Gly Ser Thr Asn Tyr Ala Asp Ser Val
1   5   10   15

Lys Gly
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1  5  10  15
Val Met Asp Tyr
20

Arg Ala Ser Gln Val Val Asp Thr Ser Leu Ala
1  5  10

Ser Ala Ser Ser Leu Ala Ser
1  5

Gln Gln Gly Ala Gly Asn Pro Tyr Thr
1  5

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1  5  10  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Ser Thr
20  25  30
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Gln Trp Val
35  40  45
Gly Arg Ile Tyr Pro Thr Ser Gly Ser Thr Asn Tyr Ala Asp Ser Val
50  55  60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65  70  75  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85  90  95
 Ala Arg Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr
100 105 110
Glu Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
115 120 125

<210> SEQ ID NO 133
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 133
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asp Thr Ser
20  25  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35  40  45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50  55  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65  70  75  80
Glu Asp Phe Ala Thr Tyr Tyr Tyr Cys Gln Gln Ser Thr Gly His Pro Gln
85  90  95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> SEQ ID NO 134
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 134
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Ser Thr
20  25  30
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35  40  45
 Ala Arg Ile Tyr Pro Thr Asn Gly Ser Thr Asn Tyr Ala Asp Ser Val
50  55  60
 Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65  70  75  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85  90  95
 Ala Arg Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr
100 105 110
Glu Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
<210> SEQ ID NO 135
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 135

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
Aasp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asp Ile Ser
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Gly Leu Leu Ile
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Ser Arg Phe Ser Gly
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Ala Gly Asn Pro Tyr
Thr Phe Gly Gln Gly Thr Val Val Glu Ile Lys Arg

<210> SEQ ID NO 136
<211> LENGTH: 126
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 136

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
Ser Leu Arg Leu Val Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Thr
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
Ala Arg Ile Tyr Pro Leu Tyr Gly Ser Thr His Tyr Ala Asp Ser Val
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
Ala Arg Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr
Glu Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Leu Val

<210> SEQ ID NO 137
<211> LENGTH: 129
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide
<400> SEQUENCE: 137
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1    5    10    15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
 20   25    30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35   40    45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
 50   55    60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65   70    75    80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Thr Tyr Thr Ser Leu
 85   90    95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100  105

<210> SEQ ID NO 138
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 138
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1    5    10    15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Ser Thr
 20   25    30
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35   40    45
Gly Arg Ile Tyr Pro Thr Ser Gly Ser Thr Asn Tyr Ala Asp Ser Val
 50   55    60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
 65   70    75    80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85   90    95
Ala Arg Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr
100  105    110
Glu Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
115  120  125

<210> SEQ ID NO 139
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 139
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1    5    10    15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Glu Thr Ser
 20   25    30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
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<212> TYPE: PRT
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 140
Arg Ala Ser Gln Asp Val Glu Thr Ser Leu Ala
1   5   10

<210> SEQ ID NO 141
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 141
Ser Ala Ser Phe Leu Tyr Ser
1   5

<210> SEQ ID NO 142
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 142
Gln Gln Ser Thr Gly His Pro Gln Thr
1   5

<210> SEQ ID NO 143
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 143
Gly Phe Thr Phe Thr Ser Thr Gly Ile Ser
1   5   10

<210> SEQ ID NO 144
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
peptide

<400>  SEQUENCE: 144

Gly  Arg  Ile  Tyr  Pro  Thr  Ser  Gly  Ser  Thr  Aen  Tyr  Ala  Asp  Ser  Val
1    5    10   15

Lys  Gly

<410>  SEQ ID NO: 145
<411>  LENGTH: 20
<412>  TYPE: PRT
<413>  ORGANISM: Artificial Sequence
<420>  FEATURE:
<421>  OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400>  SEQUENCE: 145

Ala  Arg  Thr  Tyr  Gly  Ile  Tyr  Asp  Leu  Tyr  Val  Asp  Tyr  Thr  Glu  Tyr
1    5    10   15

Val  Met  Asp  Tyr
20

<410>  SEQ ID NO: 146
<411>  LENGTH: 11
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<422>  LOCATION: (5) (5)
<423>  OTHER INFORMATION: Val or Asp
<420>  FEATURE:
<421>  NAME/KEY: MOD_RES
<422>  LOCATION: (6) (6)
<423>  OTHER INFORMATION: Val or Ile
<420>  FEATURE:
<421>  NAME/KEY: MOD_RES
<422>  LOCATION: (7) (7)
<423>  OTHER INFORMATION: Asp, Glu or Ser
<420>  FEATURE:
<421>  NAME/KEY: MOD_RES
<422>  LOCATION: (8) (8)
<423>  OTHER INFORMATION: Thr or Ile
<420>  FEATURE:
<421>  NAME/KEY: MOD_RES
<422>  LOCATION: (9) (9)
<423>  OTHER INFORMATION: Ala or Ser
<420>  FEATURE:
<421>  NAME/KEY: MOD_RES
<422>  LOCATION: (10) (10)
<423>  OTHER INFORMATION: Val or Leu

<400>  SEQUENCE: 146

Arg  Ala  Ser  Glu  Xaa  Xaa  Xaa  Xaa  Xaa  Xaa  Xaa  Ala
1    5    10

<410>  SEQ ID NO: 147
<411>  LENGTH: 7
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<421>  OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<420>  FEATURE:
<421>  NAME/KEY: MOD_RES
<422>  LOCATION: (1) (1)
<423>  OTHER INFORMATION: Ser or Gly
<420>  FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6) (6)
<223> OTHER INFORMATION: Ala or Tyr

<400> SEQUENCE: 147
Xaa Ala Ser Phe Leu Xaa Ser
1 5

<210> SEQ ID NO 148
<211> LENGTH: 9
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<223> OTHER INFORMATION: Gly, Ser or Thr
<220> FEATURE:
<221> NAME/KEY: MOD_RES
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<223> OTHER INFORMATION: Thr, Tyr or Ala
<220> FEATURE:
<221> NAME/KEY: MOD_RES
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<223> OTHER INFORMATION: Gly, Ser, Thr or Ala
<220> FEATURE:
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<222> LOCATION: (6) (6)
<223> OTHER INFORMATION: Ala, His, Asp, Thr or Asn
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7) (7)
<223> OTHER INFORMATION: Gln, Pro or Ser
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8) (8)
<223> OTHER INFORMATION: Ser, Tyr, Leu, Pro or Gln

<400> SEQUENCE: 148
Gln Gln Xaa Xaa Xaa Xaa Xaa Xaa Thr
1 5

<210> SEQ ID NO 149
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<223> OTHER INFORMATION: Ser, Gly or Thr

<400> SEQUENCE: 149
Gly Phe Xaa Phe Xaa Xaa Thr Gly Ile Ser
1 5 10

<210> SEQ ID NO 150
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Tyr, His, Asn or Ile
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Gly Arg Ile Tyr Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr Ala Asp Ser Val
1   5   10   15
Lys Gly

<410> SEQ ID NO 151
<411> LENGTH: 20
<412> TYPE: PRT
<413> ORGANISM: Artificial Sequence
<420> FEATURE:
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<400> SEQUENCE: 151
Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr Glu Tyr
1   5   10   15
Val Met Asp Tyr
20

<410> SEQ ID NO 152
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Arg Ala Ser Gln Xaa Val Xaa Xaa Xaa Val Ala
1 5 10

<210> SEQ ID NO 153
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<400> SEQUENCE: 153
Gln Gln Xaa Xaa Xaa Xaa Xaa Xaa Thr
1 5

<210> SEQ ID NO 154
<211> LENGTH: 16
<212> TYPE: PRT
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<400> SEQUENCE: 154
Gly Arg Ile Tyr Pro Xaa Xaa Gly Ser Thr Xaa Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> SEQ ID NO 155
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
Ser Ala Ser Ser Ser Val Ser Tyr Met His
1  5  10

Leu Ala Ser Gln Thr Ile Gly Thr Trp Leu Ala
1  5  10

Thr Trp Ile Tyr Asp Thr Ser Ile Leu Ala Ser
1  5  10

Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser
1  5  10

Leu Leu Ile Tyr Ala Ala Thr Ser Leu Ala Asp
1  5  10

Gln Gln Trp Thr Ser Asn Pro Leu Thr
1  5
<400> SEQUENCE: 162
Gln Gln Trp Ser Ser Tyr Pro Pro Thr
1   5

<210> SEQ ID NO 163
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 163
Gln Gln Leu Tyr Ser Pro Pro Trp Thr
1   5

<210> SEQ ID NO 164
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 164
Gly Tyr Ser Phe Thr Asp Tyr Asn Met Tyr
1   5   10

<210> SEQ ID NO 165
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 165
Gly Tyr Val Phe Thr His Tyr Asn Met Tyr
1   5   10

<210> SEQ ID NO 166
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 166
Gly Tyr Ala Phe Thr Ser Tyr Asn Met Tyr
1   5   10

<210> SEQ ID NO 167
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 167
Trp Ile Gly Tyr Ile Glu Pro Tyr Asn Gly Gly Thr Ser Tyr Asn Gln
1   5   10   15
Lys Phe Lys Gly
20

<210> SEQ ID NO 168
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 168
Trp Ile Gly Tyr Ile Glu Pro Tyr Asn Gly Gly Thr Ser Tyr Asn Gln
1   5   10   15
Lys Phe Lys Gly
20
<210> SEQ ID NO 169
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 169

Trp Ile Gly Tyr Ile Asp Pro Tyr Ile Gly Gly Thr Ser Tyr Asn Gln
1   5   10  15
Lys Phe Lys Gly
20

<210> SEQ ID NO 170
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 170

Ala Ser Pro Asn Tyr Tyr Asp Ser Ser Pro Phe Ala Tyr
1   5  10

<210> SEQ ID NO 171
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 171

Ala Arg Gly Gln Gly Pro Asp Phe Asp Val
1   5  10

<210> SEQ ID NO 172
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 172

Ala Arg Trp Gly Asp Tyr Asp Val Gly Ala Met Asp Tyr
1   5  10

<210> SEQ ID NO 173
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 173

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20  25  30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35  40  45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50  55  60
Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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Glu Asp Phe Ala Thr Tyr Tyr Cys Gin Gin His Tyr Thr Thr Pro Pro
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<400> SEQUENCE: 174

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly 1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Val Ser Thr Ala 20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile 35 40 45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro 65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro 85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys 100 105

<210> SEQ ID NO 175
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 175

Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln 1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 20 25 30

<210> SEQ ID NO 176
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 176

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 1 5 10

<210> SEQ ID NO 177
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 177
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1     5     10    15
Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Ala Thr Tyr Tyr Cys
20    25    30

<210> SEQ ID NO 178
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 178

Arg Phe Thr Ile Ser Arg Asp Ser Lys Asn Thr Leu Tyr Leu Gln
1     5     10    15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20    25    30

<210> SEQ ID NO 179
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 179

Leu Ala Val Pro Ala Ala Asn Thr Val Arg Phe Arg Cys Pro Ala
1     5     10    15

<210> SEQ ID NO 180
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 180

Ser Asp Val Glu Phe His Cys Lys Val Tyr Ser Asp Ala Gln Pro
1     5     10    15

<210> SEQ ID NO 181
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 181

His Ala Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser
1     5     10    15

<210> SEQ ID NO 182
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 182

Ser Asn Glu Phe Met Cys Lys Val Tyr Ser Asp Pro Gln Pro
1     5     10    15

<210> SEQ ID NO 183
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 183
Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp
1 5 10

<210> SEQ ID NO 184
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 184

Glu Val Gln Leu Val Glu Ser Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser
20 25

<210> SEQ ID NO 185
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 185

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
1 5 10

<210> SEQ ID NO 186
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 186

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

<210> SEQ ID NO 187
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 187

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 188
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 188
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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1  5  10  15
Asp Arg Val Thr Ile Thr Cys
20

<210> SEQ ID NO 189
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 189
Trp Tyr Gln Gln Gly Gly Lys Leu Pro Lys Leu Leu Ile Tyr
1  5  10  15

<210> SEQ ID NO 190
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 190
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1  5  10  15
Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
20  25  30

<210> SEQ ID NO 191
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 191
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1  5  10

<210> SEQ ID NO 192
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 192
gatccoccga tcaagctgcg gcatcattca agagatgatg cgcagctg atgctttttt 60
                      ggaaa  65

<210> SEQ ID NO 193
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 193
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gatcctgc acaacctcga ctactatca agagatagta gtgagcttg tgatattaatt 60
ggaa 65

<210> SEQ ID NO 194
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide

<400> SEQUENCE: 194

gatcctgcaac ctgagacta acaagatcttg tagtagtcga ggtttttttt 60
ggaa 65

<210> SEQ ID NO 195
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 195

gtcagatagc gtcccacmc artctccwgc 30

<210> SEQ ID NO 196
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 196

gatcagccta cgctgagatc carygcarc artctgg 37

<210> SEQ ID NO 197
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 197

gtcagatagc gtgctgacmc artctcc 27

<210> SEQ ID NO 199
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 199

gatcagccta cgctgagatc carygcarc artctgg 37

<210> SEQ ID NO 199
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer
<400> SEQUENCE: 199

gtacgatatc cagatgcacmc artctcc

27

<210> SEQ ID NO 200
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer
<400> SEQUENCE: 200

gatcgaagta cgctgagac cgtycgarc artctgg

37

<210> SEQ ID NO 201
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer
<400> SEQUENCE: 201

tttdakytcc agcttgctac c

21

<210> SEQ ID NO 202
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer
<400> SEQUENCE: 202

acagttgggcc cttgtggag gctgmrgaga cdgtgashrd rgt

43

<210> SEQ ID NO 203
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide
<400> SEQUENCE: 203

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
1      5      10

<210> SEQ ID NO 204
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide
<400> SEQUENCE: 204

Arg Val Thr Ile Thr Ala Aep Thr Ser Thr Ser Thr Ala Tyr Met Glu
1      5      10      15

Leu Ser Ser Leu Arg Ser Glu Thr Ala Val Tyr Tyr Cys Ala Arg
20      25      30
Trp Gly Gln Gly Thr Val Thr Val Ser Ser
1 5 10

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
1 5 10

Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu
1 5 10 15
Leu Ser Ser Leu Arg Ser Glu Asp Ala Val Tyr Tyr Cys Ala Arg
20 25 30

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
1 5 10

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
1 5 10

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
1 5 10
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu
1 5 10 15
Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
20 25 30

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
1 5 10

Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu
1 5 10 15
Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 215

Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Gly
1      5      10

<210> SEQ ID NO 216
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 216

Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1      5      10      15
Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20     25     30

<210> SEQ ID NO 217
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 217

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1      5      10

<210> SEQ ID NO 218
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 218

Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
1      5      10

<210> SEQ ID NO 219
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 219

Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1      5      10      15
Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20     25     30

<210> SEQ ID NO 220
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 220

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
  1   5   10

<210> SEQ ID NO 221
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 221

Trp Ile Arg Gln Pro Gly Lys Gly Leu Glu Trp Ile
  1   5   10

<210> SEQ ID NO 222
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 222

Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
  1   5   10   15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
  20  25  30

<210> SEQ ID NO 223
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 223

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
  1   5   10

<210> SEQ ID NO 224
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 224

Trp Ile Arg Gln Pro Gly Lys Gly Leu Glu Trp Ile
  1   5   10

<210> SEQ ID NO 225
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 225
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Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1 5 10 15
Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
20 25 30

<210> SEQ ID NO 226
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 226

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 227
<211> LENGTH: 14
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 227

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> SEQ ID NO 228
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 228

Arg Phe Thr Ile Ser Arg Asp Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> SEQ ID NO 229
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 229

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 230
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 230
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
1 5 10

<210> SEQ ID NO 231
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 231
Arg Phe Thr Ile Ser Arg Asp Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> SEQ ID NO 232
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 232
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 233
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 233
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
1 5 10

<210> SEQ ID NO 234
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 234
Arg Phe Thr Ile Ser Arg Asp Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> SEQ ID NO 235
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 235
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
<210> SEQ ID NO 236
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 236

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

1  5  10

<210> SEQ ID NO 237
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 237

Arg Phe Thr Ile Ser Arg Asp Aen Ser Lys Aen Thr Leu Tyr Leu Gln

1  5  10  15

Met Aen Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

20  25  30

<210> SEQ ID NO 238
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 238

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

1  5  10

<210> SEQ ID NO 239
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 239

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser

1  5  10

<210> SEQ ID NO 240
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 240

Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Aen Thr Ala Tyr Leu Gln

1  5  10  15

Met Aen Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg

20  25  30
<210> SEQ ID NO 241
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 241

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1   5   10

<210> SEQ ID NO 242
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 242

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
1   5   10

<210> SEQ ID NO 243
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 243

Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1   5   10   15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg
20  25  30

<210> SEQ ID NO 244
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 244

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1   5   10

<210> SEQ ID NO 245
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 245

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
1   5   10

<210> SEQ ID NO 246
<211> LENGTH: 31

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<210> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 251

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
  1   5

<210> SEQ ID NO 252
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 252

Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
  1   5  10

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 20  25  30

<210> SEQ ID NO 253
<211> LENGTH: 11
<212> TYPE: PRT
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<400> SEQUENCE: 253

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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<210> SEQ ID NO 254
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 254

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
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<210> SEQ ID NO 255
<211> LENGTH: 31
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<400> SEQUENCE: 255

Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
  1   5  10

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 20  25  30

<210> SEQ ID NO 256
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<220> FEATURES:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 256

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 257
<211> LENGTH: 13
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<400> SEQUENCE: 257

Trp Val Arg Gln Ala Pro Gly Lys Leu Glu Trp Val
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<210> SEQ ID NO 258
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<400> SEQUENCE: 258

Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

<210> SEQ ID NO 259
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<212> TYPE: PRT
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<400> SEQUENCE: 259

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 260
<211> LENGTH: 15
<212> TYPE: PRT
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<400> SEQUENCE: 260

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
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<400> SEQUENCE: 261
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
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| 20 | 25 | 30 |

<210> SEQ ID NO 262
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 262
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
| 1 | 5 | 10 |

<210> SEQ ID NO 263
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 263
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Ile Tyr
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<210> SEQ ID NO 264
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Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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<210> SEQ ID NO 266
<211> LENGTH: 15
<212> TYPE: PRT
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**SEQ ID NO**: 271  
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<210> SEQ ID NO 272
<211> LENGTH: 235
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 272

Ala Asp Pro Asp Thr Gly Val Asp Thr Gly Ala Pro Tyr Trp Thr Arg
1   5   10  15

Pro Glu Arg Met Asp Lys Leu Leu Ala Val Pro Ala Ala Asn Thr
20  25  30  35

Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro Thr Pro Ser Ile Ser
35  40  45  50

Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu His Arg Ile Gly Gly
55  60  65  70

Ile Lys Leu Arg His Gln Gln Trp Ser Leu Val Met Glu Ser Val Val
85  90  95 100

Pro Ser Asp Arg Gly Asn Tyr Thr Cys Val Val Glu Asn Lys Phe Gly
110 115 120

Ser Ile Arg Gln Thr Tyr Thr Leu Asp Val Leu Glu Arg Ser Pro His
125 130 135 140

Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Gln Thr Ala Val Leu
145 150 155 160

Gly Ser Asp Val Glu Phe His Cys Lys Val Tyr Ser Asp Ala Gln Pro
165 170 175 180

His Ile Gln Trp Leu Lys His Val Glu Val Asn Gly Ser Lys Val Gly
185 190 195 200

Pro Asp Glu Gly Thr Pro Tyr Val Thr Val Leu Lys Ser Trp Ile Ser Glu
205 210 215 220

Ser Val Glu Ala Asp Val Arg Leu Arg Leu Ala Asn Val Ser Glu Arg
225 230 235

Asp Gly Gly Glu Tyr Leu Cys Arg Ala Thr Asn Phe Ile Gly Val Ala

Glu Lys Ala Phe Trp Leu Ser Val His Gly Pro Arg Ala Ala Glu

Glu Leu Val Glu Ala Asp Glu Ala Gly Ser Val

<210> SEQ ID NO 273
<211> LENGTH: 241
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 273

Ala Asp Pro Asp Thr Gly Val Asp Thr Gly Ala Pro Tyr Trp Thr Arg
1   5   10  15

Pro Glu Arg Met Asp Lys Leu Leu Ala Val Pro Ala Ala Asn Thr
20  25  30  35

Val Arg Phe Arg Cys Pro Ala Ala Gly Asn Pro Thr Pro Ser Ile Ser
40  45

Trp Leu Lys Asn Gly Arg Glu Phe Arg Gly Glu His Arg Ile Gly Gly
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**<210> SEQ ID NO 274**

**<211> LENGTH: 108**

**<212> TYPE: PRT**

**<213> ORGANISM: Artificial Sequence**

**<220> FEATURE: **

**<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide**

**<400> SEQUENCE: 274**

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala 20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile 35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg 100 105

**<210> SEQ ID NO 275**

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**<212> TYPE: PRT**

**<213> ORGANISM: Artificial Sequence**

**<220> FEATURE: **

**<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide**
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Glu Val Glu Leu Val Val Ser Gly Gly Gly Gly Leu Val Glu Val Pro Gly Gly
1     5      10     15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Ser Thr
20    25     30
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35    40     45
Gly Arg Ile Tyr Pro Thr Asn Gly Ser Thr Asn Tyr Ala Asp Ser Val
50    55     60
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65    70     75     80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Cys
85     90    95
Ala Arg Ala Arg Thr Tyr Gly Ile Tyr Asp Leu Tyr Val Asp Tyr Thr
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Glu Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
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<210> SEQ ID NO: 276
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE: OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 276
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1     5      10     15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20    25     30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35    40     45
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50    55     60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65    70     75     80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Thr Pro Pro
85     90    95
Thr Phe Gly Gln Gly Thr Tyr Val Glu Ile Lys Arg Thr Val Ala Ala
100  105    110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115  120    125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130  135    140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Glu Ser Gly Asn Ser Gln
145  150    155     160
Glu Ser Val Thr Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165    170    175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180  185    190
Ala Cys Glu Val Thr His Glu Gly Leu Ser Ser Pro Val Thr Lys Ser
195  200    205
What is claimed is:

1. A crystal of FGFR3 complexed with an anti-FGFR3 antibody comprising a human FGFR3 comprising sequence of SEQ ID NO:272 or conservative substitutions thereof, and complexed with an anti-FGFR3 antibody comprising (a) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:274 or conservative substitutions thereof, and (b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:275 or conservative substitutions thereof.

2. Crystalline form of a complex of FGFR3 and an anti-FGFR3 antibody, space group P2_1_2_1, with cell parameters of a=58.5 Å, b=99.3 Å and c=143.7 Å.

3. A crystal of a 1:1 complex of FGFR3 and an anti-FGFR3 antibody having a space group having a space group symmetry of P2_1_2_1, and comprising a unit cell having the dimensions of a, b, and c, wherein a=58.5 Å, b=99.3 Å and c=143.7 Å.

4. A co-crystal of FGFR3 with an anti-FGFR3 antibody having the three-dimensional coordinates of Table 6.

5. A crystal of claim 4, wherein the crystal diffractions X-rays for the determination of atomic coordinates to a resolution of 5 Å or better.

6. A composition comprising a crystal of any of claims 1-5, and a carrier.

7. A molecule or molecular complex comprising at least a portion of the binding site of FGFR3 or conservative substi-
tution thereof, wherein the binding site comprises at least one amino acid residue corresponding to residues 158, 170, 171, 173, 175, and/or 315 or mixtures thereof, the binding site defined by a set of points having a root mean square deviation of less than about 0.70 Å from points representing the backbone atoms of the amino acids as represented by the structure coordinates listed in Table 6.

8. The molecule or molecular complex of claim 7, wherein the binding site comprises at least one amino acid residue corresponding to residues 158, 159, 169, 170, 171, 173, 175, 205, 207, and/or 315 or mixtures thereof.

9. The molecule or molecular complex of claim 7, wherein the binding site comprises at least one amino acid residue corresponding to residues 154, 155, 158, 159, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 202, 205, 207, 210, 212, 214, 216, 217, 241, 246, 247, 248, 278, 279, 280, 281, 282, 283, 314, 315, 316, 317 and/or 318 or mixtures thereof.

10. A computer-implemented method for causing a display of a graphical three-dimensional representation of the structure of a portion of a crystal of FGFR3 complexed with an anti-FGFR3 antibody, or structural homologs thereof, wherein the method comprises:

causing said display of said graphical three-dimensional representation by a computer system programmed with instructions for transforming structure coordinates into said graphical three-dimensional representation of said structure and for displaying said graphical three-dimensional representation,

wherein said graphical three-dimensional representation is generated by transforming said structure coordinates into said graphical three-dimensional representation of said structure,

wherein said structure coordinates comprise structure coordinates of the backbone atoms of the portion of the crystal,

wherein the portion of the crystal comprises an FGFR3 binding site, and

wherein the crystal has the space group symmetry P2₁2₁2₁.

11. The computer-implemented method of claim 10, wherein the FGFR3-anti-FGFR3 antibody crystal comprises a polypeptide comprising an amino acid sequence shown in Table 6 or conservative substitution thereof, and further comprises an antibody comprising (a) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:274 or conservative substitutions thereof, and (b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:275 or conservative substitutions thereof.

12. The computer-implemented method of claim 10, wherein the structure coordinates are defined in Table 6.

13. The computer-implemented method of claim 10, wherein the structure coordinates comprise the structure coordinates of the backbone atoms of the amino acid residues corresponding to residues 158, 170, 171, 173, 175, and/or 315 of FGFR3.


15. The computer-implemented method of claim 10, wherein the structure coordinates are determined by homology modeling.

16. A machine-readable data storage medium comprising a data storage material encoded with machine-readable instructions for:

(a) transforming data into a graphical three-dimensional representation for the structure of a portion of a crystal of FGFR3 complexed with an anti-FGFR3 antibody, or structural homologs thereof, and

(b) causing the display of said graphical three-dimensional representation,

wherein said data comprise structure coordinates of the backbone atoms of the amino acids defining an FGFR3 binding site, and wherein the crystal or structural homolog has the space group symmetry P2₁2₁2₁.

17. A computer system for displaying a three-dimensional graphical representation for the structure of a portion of a crystal of FGFR3 complexed with an anti-FGFR3 antibody, or structural homologs thereof, comprising:

(a) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said data comprise structure coordinates of the backbone atoms of the amino acids defining an FGFR3 binding site, wherein the crystal or structural homolog has the space group symmetry P2₁2₁2₁;

(b) a working memory;

(c) a central processing unit coupled to said working memory and to said machine-readable data storage medium for processing said machine-readable data into said three-dimensional graphical representation; and

(d) a display coupled to said central processing unit for displaying said three-dimensional graphical representation.

18. A method for obtaining structural information about a molecule or molecular complex comprising applying at least a portion of the structure coordinates of a FGFR3 complexed with an anti-FGFR3 antibody to an X-ray diffraction pattern of the molecule or molecular complex’s crystal structure to cause the generation of a three-dimensional electron density map of at least a portion of the molecule or molecular complex,

wherein the FGFR3-anti-FGFR3 antibody crystal comprises a polypeptide comprising an amino acid sequence of SEQ ID NO:272 or conservative substitution thereof, and further comprises an antibody comprising (a) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:274 or conservative substitutions thereof, and (b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:275 or conservative substitutions thereof;

wherein the FGFR3-anti-FGFR3 antibody crystal comprises a polypeptide comprising an amino acid sequence of SEQ ID NO:272 or conservative substitution thereof, and further comprises an antibody comprising (a) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:274 or conservative substitutions thereof, and (b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:275 or conservative substitutions thereof;

wherein the binding site comprises at least one amino acid residue corresponding to residues 158, 170, 171, 173, 175, and/or 315 or mixtures thereof.

19. A method of screening for molecules that may be antagonists or agonists of FGFR3 comprising:

(a) computationally screening agents against a three-dimensional model to identify potential antagonists or agonists of FGFR3;

(b) the three-dimensional model comprises a three-dimensional model of at least a portion of a crystal of a FGFR3 complexed with an anti-FGFR3 antibody;

wherein the three-dimensional model is generated from at least a portion of the structure coordinates of the crystal.
by a computer algorithm for generating a three-dimensional model of the crystal useful for identifying agents that are potential antagonists or agonists of FGFR3; wherein the FGFR3: anti-FGFR3 antibody crystal comprises a polypeptide comprising an amino acid sequence SEQ ID NO:272 or conservative substitution thereof, and further comprises an antibody comprising (a) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:274 or conservative substitutions thereof, and (b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:275 or conservative substitutions thereof; and wherein the FGFR3:anti-FGFR3 antibody crystal diffractions x-rams for the determination of atomic coordinates to a resolution of 5 Å or better.