Claims

1. An isolated bispecific FN3 domain containing molecule comprising a first fibronectin type III (FN3) domain and a second FN3 domain, wherein the first FN3 domain specifically binds epidermal growth factor receptor (EGFR) and blocks binding of epidermal growth factor (EGF) to EGFR, and the second FN3 domain specifically binds hepatocyte growth factor receptor (c-Met), and blocks binding of hepatocyte growth factor (HGF) to c-Met, and wherein the first FN3 domain comprises an amino acid sequence at least 87% identical to the amino acid sequence of SEQ ID NO: 27, and the second FN3 domain comprises an amino acid sequence at least 83% identical to the amino acid sequence of SEQ ID NO: 41.

2. The bispecific molecule of claim 1, wherein:

a) the first FN3 domain inhibits EGF-induced EGFR phosphorylation at EGFR residue Tyrosine 1173 with an IC50 value of less than about 2.5 x 10-6 M when measured in A431 cells using 50 ng/mL human EGF, and the second FN3 domain inhibits HGF- induced c-Met phosphorylation at c-Met residue Tyrosine 1349 with an IC50 value of less than about 1.5 x 10-6 M when measured in NCI-H441 cells using 100 ng/mL human HGF;

b) the first FN3 domain inhibits EGF-induced EGFR phosphorylation at EGFR residue Tyrosine 1173 with an IC50 value between about 1.8 x 10-8 M and about 2.5 x 10-6 M when measured in A431 cells using 50 ng/mL human EGF, and the second FN3 domain inhibits HGF- induced c-Met phosphorylation at c-Met residue Tyrosine 1349 with an IC50 value between about 4 x 10-9 M and about 1.5 x 10-6 M when measured in NCI-H441 cells using 100 ng/mL human HGF;

c) the first FN3 domain binds human EGFR with a dissociation constant (KD ) of less than about 1x10-8 M, and the second FN3 domain binds human c-Met with a KD of less than about 5 x10-8 M, wherein the KD is measured using surface plasmon resonance; or

d) the first FN3 domain binds human EGFR with a KD of between about 2x10-10 to about 1x10-8 M, and the second FN3 domain binds human c-Met with a KD of between about 3x10-10 to about 5 x10-8 M, wherein the KD is measured using surface plasmon resonance; and/or

e) the bispecific molecule inhibits NCI-H292 cell proliferation with an IC50 value that is at least 30-fold less when compared to the IC50 value of inhibition of NCI-H292 cell growth with a mixture of the first FN3 domain and the second FN3 domain, wherein the cell proliferation is induced with 10% FBS containing 7.5 ng/mL HGF; and/or

f) the bispecific molecule:

i) inhibits EGF-induced EGFR phosphorylation at EGFR residue Tyrosine 1173 with an IC50 value of less than about 8x10-7 M when measured in NCI-H292 cells using 50 ng/mL human EGF;

ii) inhibits HGF-induced c-Met phosphorylation at c-Met residue Tyrosine 1349 with an IC50 value of less than about 8.4x10-7 M when measured in NCI-H441 cells using 100 ng/mL human HGF;

iii) inhibits HGF-induced NCI-H292 cell proliferation with an IC50 value of less than about 9.5x10-6 M, wherein the cell proliferation is induced with 10% FBS containing 7.5 ng HGF;

iv) binds EGFR with a KD of less than about 2.0x10-8 M; or

v) binds c-Met with a KD of less than about 2.0x10-8 M, wherein the KD is measured using surface plasmon resonance as described in Example 3 and Example 5.

3. The bispecific molecule of claim 1 or 2, wherein:

a) the first FN3 domain comprises the amino acid sequence of SEQ ID NO: 107 or 108, and the second FN3 domain comprises the amino acid sequence of SEQ ID NO: 114;

b) the first FN3 domain binds EGFR with one or more amino acid residues corresponding to residues D23, F27, Y28, V77 and G85 of P54AR4-83v2 (SEQ ID NO: 27), optionally wherein the second FN3 domain binds c-Met with one or more amino acid residues corresponding to residues R34, F38, M72 and 179 in P114AR7P95-A3 (SEQ ID NO: 41);

c) the first FN3 domain comprises

i) an FG loop comprising the amino acid sequence of SEQ ID NO: 179 or the amino acid sequence of SEQ ID NO: 180; and

ii) a BC loop comprising the amino acid sequence of SEQ ID NO: 181; andthe second FN3 domain comprises

i) a C strand and a CD loop comprising the amino acid sequence of SEQ ID NO: 184; and

ii) an F strand and an FG loop comprising the amino acid sequence of SEQ ID NO: 185;

d) the first FN3 domain comprises the amino acid sequence of SEQ ID NO: 182 or the amino acid sequence of SEQ ID NO: 183; and the second FN3 domain comprises the amino acid sequence of SEQ ID NO: 186;

e) the first FN3 domain and/or the second FN3 domain comprises substitutions at one or more residue positions corresponding to positions 11, 14, 17, 37, 46, 73 and 86 in Tencon (SEQ ID NO: 1), optionally wherein the first FN3 domain comprises the amino acid sequence of SEQ ID NO: 187; and the second FN3 domain comprises the amino acid sequence of SEQ ID NO: 188;

f) the first FN3 domain comprises the sequence shown in one of SEQ ID NOs: 18-29, 107-110, 122-137 or 194-211, and the second FN3 domain comprises the sequence shown in one of SEQ ID NOs: 32-49, 111-114 or 212-223, optionally wherein the first FN3 domain and the second FN3 domain comprises the amino acid sequence of SEQ ID NOs: 27 and 32, respectively; 27 and 41, respectively; 27 and 40, respectively; 27 and 33, respectively; 107 and 41, respectively; 107 and 40, respectively; 107 and 33, respectively; 107 and 32, respectively; 107 and 114, respectively; 108 and 111, respectively; 108 and 112, respectively; 108 and 113, respectively; 108 and 114, respectively; 109 and 111, respectively; 109 and 112, respectively; 109 and 113, respectively; 109 and 114, respectively; 110 and 111, respectively; 110 and 112, respectively; 110 and 113, respectively; or 110 and 114, respectively,for example wherein the first FN3 domain and/or the second FN3 domain comprises one or more substitutions corresponding to substitutions L17A, N46V and E86I in Tencon (SEQ ID NO: 1); and/or

g) the first FN3 domain and/or the second FN3 domain is isolated from a library designed based on Tencon sequence of SEQ ID NO: 1.

4. The bispecific molecule of any of the claims 1-3:

a) wherein the first FN3 domain and the second FN3 domain are coupled by a linker, optionally wherein the linker comprises the amino acid sequence shown in one of SEQ ID NOs: 78-84 or 224;

b) comprising the amino acid sequence shown in SEQ ID NOs: 50-72, 106, 118-121, 138-165 or 190-193, and optionally comprising a methionine (Met) linked to the N-terminus of the molecule, optionally further comprising a cysteine linked to the C-terminus of the molecule; and/or

c) coupled to a half-life extending moiety, optionally wherein the half-life extending moiety is an albumin binding molecule, a polyethylene glygol (PEG), an albumin, an albumin variant, or at least a portion of an Fc region of an immunoglobulin and optionally wherein the half-life extending moiety is the albumin variant of SEQ ID NO: 189.

5. An isolated fibronectin type III (FN3) domain that specifically binds epidermal growth factor receptor (EGFR) and blocks binding of epidermal growth factor (EGF) to EGFR, wherein the FN3 domain is isolated from a library designed based on the Tencon amino acid sequence of SEQ ID NO: 1, wherein:

a) the FN3 domain comprises an amino acid sequence at least 87% identical to the amino acid sequence of SEQ ID NO: 27; and optionally

b) the FN3 domain binds EGFR with one or more amino acid residues corresponding to residues D23, F27, Y28, V77 and G85 of P54AR4-83v2 (SEQ ID NO: 27);

c) the FN3 domain comprises

i) an FG loop comprising the amino acid sequence of SEQ ID NO: 179 or the amino acid sequence of SEQ ID NO: 180; and

ii) a BC loop comprising the amino acid sequence of SEQ ID NO: 181, and optionally comprises the amino acid sequence of SEQ ID NO: 182 or the amino acid sequence of SEQ ID NO: 183;

d) the FN3 domain comprises the sequence shown in one of SEQ ID NOs: 18-29, 107-110, 122-137 or 194-211; and/or

e) the FN3 domain comprises substitutions at one or more residue positions corresponding to positions 11, 14, 17, 37, 46, 73, and 86 in Tencon (SEQ ID NO: 1), optionally wherein the FN3 domain comprises one or more substitutions corresponding to substitutions L17A, N46V, and E86I in the Tencon amino acid sequence of SEQ ID NO: 1.

6. An isolated fibronectin type III (FN3) domain that specifically binds hepatocyte growth factor receptor (c-Met) and blocks binding of hepatocyte growth factor (HGF) to c-Met, wherein:

a) the FN3 domain comprises an amino acid sequence at least 83% identical to the amino acid sequence of SEQ ID NO: 41; and optionally

b) the FN3 domain binds c-Met with one or more amino acid residues corresponding to residues R34, F38, M72 and 179 in P114AR7P95-A3 (SEQ ID NO: 41);

c) the FN3 domain comprises:

i) a C strand and a CD loop comprising the amino acid sequence of SEQ ID NO: 184; and

ii) an F strand and an FG loop comprising the amino acid sequence of SEQ ID NO: 185, and optionally comprises the amino acid sequence of SEQ ID NO: 186;

d) the FN3 domain comprises the sequence shown in one of SEQ ID NOs: 32-49, 111-114 or 212-223; optionally wherein the FN3 domain comprises substitutions at one or more residue positions corresponding to positions 11, 14, 17, 37, 46, 73, and 86 of the Tencon amino acid sequence of SEQ ID NO: 1, optionally wherein the FN3 domain comprises one or more substitutions corresponding to substitutions L17A, N46V, and E86I of the Tencon amino acid sequence of SEQ ID NO: 1; and/or

e) the FN3 domain is isolated from a library designed based on the Tencon amino acid sequence of SEQ ID NO: 1.

7. An isolated polynucleotide encoding:

a) the bispecific molecule of claim 1;

b) the FN3 domain of claim 5; or

c) FN3 domain of claim 6.

8. A vector comprising the polynucleotide of claim 7.

9. An isolated host cell comprising the vector of claim 9.

10. A method of producing:

a) a bispecific molecule, comprising culturing the isolated host cell of claim 9, as dependent on claim 7a), under conditions such that the bispecific molecule is expressed, and purifying the bispecific molecule;

b) an FN3 domain that specifically binds EGFR, comprising culturing the isolated host cell of claim 9, as dependent on claim 7b), under conditions such that the FN3 domain that specifically binds EGFR is expressed, and purifying the FN3 domain; or

c) an FN3 domain specifically binding c-Met, comprising culturing the isolated host cell of claim 9, as dependent on claim 7c), under conditions such that the FN3 domain specifically binding c-Met is expressed, and purifying the FN3 domain specifically binding c-Met.

11. A pharmaceutical composition comprising:

a)

i) the bispecific molecule of any of the claims of 1-4; or

ii) the FN3 domain of claim 5 or 6; and

c) a pharmaceutically acceptable carrier.

12. A bispecific FN3 domain containing molecule of any of the claims 1-4, an isolated fibronectin type III (FN3) domain that specifically binds epidermal growth factor receptor (EGFR) of claim 5, an isolated fibronectin type III (FN3) domain that specifically binds hepatocyte growth factor receptor (c-Met) of claim 6, or a composition of claim 11 for use in therapy.

13. A bispecific FN3 domain containing molecule according to any of the claims 1-4, an isolated fibronectin type III (FN3) domain that specifically binds epidermal growth factor receptor (EGFR) according to claim 5, an isolated fibronectin type III (FN3) domain that specifically binds hepatocyte growth factor receptor (c-Met) according to claim 6, or a composition of claim 11 for use in treating cancer, optionally wherein:

a) the cancer is an epithelial cell cancer, breast cancer, ovarian cancer, lung cancer, non-small cell lung cancer (NSCLC), lung adenocarcinoma, small cell lung cancer, colorectal cancer, anal cancer, prostate cancer, kidney cancer, bladder cancer, head and neck cancer, pharynx cancer, cancer of the nose, pancreatic cancer, skin cancer, oral cancer, cancer of the tongue, esophageal cancer, vaginal cancer, cervical cancer, cancer of the spleen, testicular cancer, gastric cancer, cancer of the thymus, colon cancer, thyroid cancer, liver cancer, hepatocellular carcinoma (HCC) or sporadic or hereditary papillary renal cell carcinoma (PRCC); and/or

b) the subject is resistant or has acquired resistance to treatment with erlotinib, gefitinib, afatinib, CO-1686, AZD9192, or cetuximab.

14. A bispecific FN3 domain containing molecule according to any of the claims 1-4 or a composition of claim 11 for use in treating cancer, optionally wherein:

a) the cancer is associated with an EGFR activating mutation, an EGFR gene amplification, a c-Met activating mutation or a c-Met gene amplification, optionally wherein the EGFR activating mutation is G719A, G719X (X being any amino acid), L861X (X being any amino acid), L858R, E746K, L747S, E749Q, A750P, A755V, V765M, L858P or T790M substitution, deletion of E746-A750, deletion of R748-P753, insertion of Ala (A) between M766 and A767, insertion of Ser, Val and Ala (SVA) between S768 and V769, and insertion of Asn and Ser (NS) between P772 and H773; and/or

b) the treating comprises administering a second therapeutic agent, optionally wherein the second therapeutic agent is:

i) a chemotherapeutic agent or a targeted anti-cancer therapy, optionally cisplatin, vinblastine, a tyrosine kinase inhibitor of EGFR, c-Met, HER2, HER3, HER4 or VEGFR, erlotinib,gefitinib or afatinib; and/or

ii) administered simultaneously, sequentially or separately from the bispecific molecule.

15. Use of a bispecific FN3 domain containing molecule according to any of the claims 1-4, an isolated fibronectin type III (FN3) domain that specifically binds epidermal growth factor receptor (EGFR) according to claim 5, an isolated fibronectin type III (FN3) domain that specifically binds hepatocyte growth factor receptor (c-Met) according to claim 6, or a composition of claim 11 in the manufacture of a medicament for the treatment of cancer, optionally wherein:

a) the cancer is an epithelial cell cancer, breast cancer, ovarian cancer, lung cancer, non-small cell lung cancer (NSCLC), lung adenocarcinoma, small cell lung cancer, colorectal cancer, anal cancer, prostate cancer, kidney cancer, bladder cancer, head and neck cancer, pharynx cancer, cancer of the nose, pancreatic cancer, skin cancer, oral cancer, cancer of the tongue, esophageal cancer, vaginal cancer, cervical cancer, cancer of the spleen, testicular cancer,

gastric cancer, cancer of thymus, colon cancer, thyroid cancer, liver cancer, hepatocellular carcinoma (HCC) or sporadic or hereditary papillary renal cell carcinoma (PRCC); and/or

b) the subject is resistant or has acquired resistance to treatment with erlotinib, gefitinib, afatinib, CO-1686, AZD9192, or cetuximab.

16. Use of a bispecific FN3 domain containing molecule according to any of the claims 1-4 or a composition of claim 11 in the manufacture of a medicament for the treatment of cancer, optionally wherein:

a) the cancer is associated with an EGFR activating mutation, an EGFR gene amplification, a c-Met activating mutation or a c-Met gene amplification, optionally wherein the EGFR activating mutation is G719A, G719X (X being any amino acid), L861X (X being any amino acid), L858R, E746K, L747S, E749Q, A750P, A755V, V765M, L858P or T790M substitution, deletion of E746-A750, deletion of R748-P753, insertion of Ala (A) between M766 and A767, insertion of Ser, Val and Ala (SVA) between S768 and V769, and insertion of Asn and Ser (NS) between P772 and H773; and/or

b) the treatment involves a second therapeutic agent; or

c) the treatment involves a second therapeutic agent which is to be administered simultaneously, sequentially or separately from the medicament; or

d) the treatment involves a second therapeutic agent which is to be administered simultaneously, sequentially or separately from the medicament and wherein the second therapeutic agent is a chemotherapeutic agent or a targeted anti-cancer therapy, optionally cisplatin, vinblastine, a tyrosine kinase inhibitor of EGFR, c-Met, HER2, HER3, HER4 or VEGFR, erlotinib, gefitinib or afatinib.