An overview of currently available anti-insulin-like growth factor I receptor antibodies

Yu Kusada[1, 2], Yoko Fujita-Yamaguchi[1, 2,*]

[1] *Department of Applied Biochemistry, Tokai University School of Engineering, Hiratsuka, Kanagawa, Japan;*

[2] *Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Japan.*

SUMMARY **A number of studies during the last two decades revealed that the insulin-like growth factor I receptor (IGFIR) is an attractive target for cancer molecular therapy. Different molecular strategies have been developed and evaluated in experimental systems, and one such strategy involves anti-IGFIR antibodies, which have been rigorously tested for their therapeutic potential over the last 5-6 years. This mini-review thus introduces currently available IGFIR antibodies with a particular emphasis on epitope mapping and anti-IGFIR antibody-induced cancer growth inhibition.**

Key Words: Antibody, cancer therapy, epitope specificity, hormone, insulin-like growth factor I receptor

Antibody engineering for use in cancer therapy

The ground-breaking establishment of monoclonal antibody (mAb)-production technology (*1*) was followed by the use of recombinant DNA technology in antibody engineering (*2*), which laid the groundwork for major advances in producing a variety of antibodies as therapeutics to treat patients with various diseases including cancer. Production of therapeutic antibodies, however, requires humanization of murine antibodies in order to reduce their immunogenicity in humans. Chimeric antibodies with mouse variable regions and human constant regions were constructed (*3*,*4*), but were found to still be immunogenic. Further improvements in producing therapeutic antibodies include complementarity-determining region (CDR) grafting of a murine antibody onto a human variable-domain framework (*5*), screening of recombinant antibody libraries (*6*), and human antibody production from transgenic animals having human immunoglobulin gene loci (*7*).

Antibody-based therapeutics has emerged as an important component of therapies for an increasing number of human malignancies. Rituximab (anti-CD20) was the first FDA-approved agent for treatment of cancer, specifically non-Hodgkins lymphoma, in 1997. Herceptin (Trastuzumab; anti-HER2/neu), which was approved for clinical use in 1998, has successfully been used to treat metastatic breast cancer. These earlier studies encouraged screening of new and more effective target molecules expressed on various malignant cells by a number of laboratories and companies (*8*,*9*). Since accumulating evidence suggests that IGFIR is involved in mitogenic and anti-apoptotic effects of a variety of cancer cell lines, IGFIR is a potentially worthwhile molecular target (*10*-*13*).

IGFIR axis

The ligands for IGFIR are IGF- I and II, which consist of 70 and 67 amino acids, respectively. They share 62% identity and also show structural homology to proinsulin (*14*). IGF-I is synthesized in the liver under the regulation of growth hormone and secreted into the bloodstream (endocrine action). IGFs also act in an autocrine/paracrine manner in peripheral tissues (*15*). Both ligands bind to IGFIR with equally high affinity, which leads to growth promotion and inhibition of apoptosis. IGFIR is a transmembrane glycoprotein consisting of two α subunits and two β subunits that are linked by disulfide bonds. The α subunit is completely extracellular and responsible for ligand-binding while the β subunit is a transmembrane protein whose cytoplasmic domain carries tyrosine protein kinase activity (*16*). The cytoplasmic domain

**Correspondence to:* Department of Applied Biochemistry, Tokai University School of Engineering, 1117 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan;
e-mail: yamaguch@keyaki.cc.u-tokai.ac.jp

Received December 16, 2007
Accepted December 23, 2007

of the β subunit contains tyrosine residues, which are auto-phosphorylated after ligand stimulation, that act as docking sites for several substrates including insulin receptor substrates (IRSs) and Shc (*17*). Following these events, down-stream signal molecules including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt (*18*) are activated, leading to cell proliferation and attenuation of apoptosis. Furthermore, the actions of IGFs are regulated by the presence of IGF-binding proteins (IGF-BPs) 1-6 that are found in the circulation and extracellular fluids (*17*).

Structural and functional relationships with respect to the insulin receptor

Molecular cloning of human IGFIR cDNA (*19*) revealed sequence homology with the insulin receptor (IR). Although both IR and IGFIR signaling pathways overlap, IR and IGFIR mainly play distinct roles in metabolic and mitogenic pathways, respectively. IGFIR is overexpressed in a variety of cancers in which IGFIR signaling plays an important role in proliferation, anti-apoptosis, and tumorigenesis (*20*). IGFIR is also a key mediator of hormone-independent progression in prostate cancer cell lines (*21*). In addition, IGFIR can dimerize with IR, resulting in an IR/IGFIR hybrid receptor. This IR/IGFIR hybrid receptor also acts as a growth receptor through stimulation by IGF-I or IGF-II (*22,23*).

Production of monoclonal antibodies against human IGFIR

The first anti-IGFIR mAb, αIR-3, was obtained from mice immunized by IR purified from human placentae that had to have contained IGFIR as a contaminant (*24*). αIR-3 was thus a kind of by-product that has since proven extremely useful. Later, more anti-IGFIR mAbs were produced using a variety of antigens including purified human placental IGFIR (*25,26*), purified ecto-IGFIR (*27*), and IGF-IR-overexpressing cells (*28*).

IGF-I binding domains and epitope mapping for anti-IGFIR mAbs

Over the last two decades, studies on binding sites for ligands and mAbs took advantage of two structurally-related receptors, in that receptor chimeras in which IGFIR and IR domains were shuffled within the framework of IR or IGFIR were recombinantly expressed in order to test the reactivity of the grafted domain. A good example for this is the study by Gustafson and Rutter (*29*), which identified the cysteine-rich domains of IR (230-285) and IGFIR (223-274) as primary determinants of hormone binding specificity. Consequent works by Mynarcik *et al.* (*30*), Whittaker *et al*. (*31*), and Keyhanfar *et al*. (*32*) more precisely mapped the IGF-I binding site to the cysteine-rich domain on IGFIR using IR/IGFIR chimeras and point mutational analysis, but suggested other residues, especially Phe^{701}, also play critical roles in ligand binding. Interestingly, the IGFIR ectodomain (L1-cysteine rich-L2 domain), produced and structurally determined by X-ray crystallography, was unable to bind to the ligand (*33*). This may indicate that although the cysteine-rich domain contains the IGF-I binding site, the entire α subunit connected to the extracellular domain of the β subunit may be necessary to exhibit ligand-binding activity.

In addition to the aforementioned approach, strategies commonly used to categorize various mAbs obtained include screening the effects of mAbs on IGF-I or -II binding to IGFIR and on cell growth as usually determined by DNA synthesis. Table 1 summarizes various anti-IGFIR mAbs whose epitopes and ligand binding effects have been reported. Siddle and his colleagues developed several mAbs and characterized their epitopes using domain-shuffled chimera receptors (*28*) and reported further analysis of epitope mapping as well as their effects on ligand binding (*34*). For example, 16-13 and 26-3, which bind to respective

Table 1. Summary of each anti-IGFIR mAb-epitope on the α subunit of IGFIR and effect on ligand binding

Number	Name	Epitope	Effect on ligand (IGF-I)-binding	References
1	1H7	440-514	inhibition	Li *et al*. (*25*) Kusada *et al*. (*35*)
2	3B7	62-184	stimulation	Xiong *et al*. (*26*) Kusada *et al*. (*35*)
3	αIR-3	223-274	inhibition	Kull *et al*. (*24*) Gustafson *et al*. (*29*)
4	24-31	283-440	no effect	Schumacher *et al*. (*34*)
5	17-69	514-586	inhibition	Schumacher *et al*. (*34*)
6	24-55	440-514	inhibition	Schumacher *et al*. (*34*)
7	24-60	184-283	inhibition	Schumacher *et al*. (*34*)
8	24-57	440-514	inhibition	Schumacher *et al*. (*34*)
9	16-13	62-184	stimulation	Soos *et al*. (*28*)
10	26-3	283-440	stimulation	Schumacher *et al*. (*34*)
11	7C2	131-315	inhibition	Keyhanfar *et al*. (*32*)
12	9E11	131-315	inhibition	Keyhanfar *et al*. (*32*)

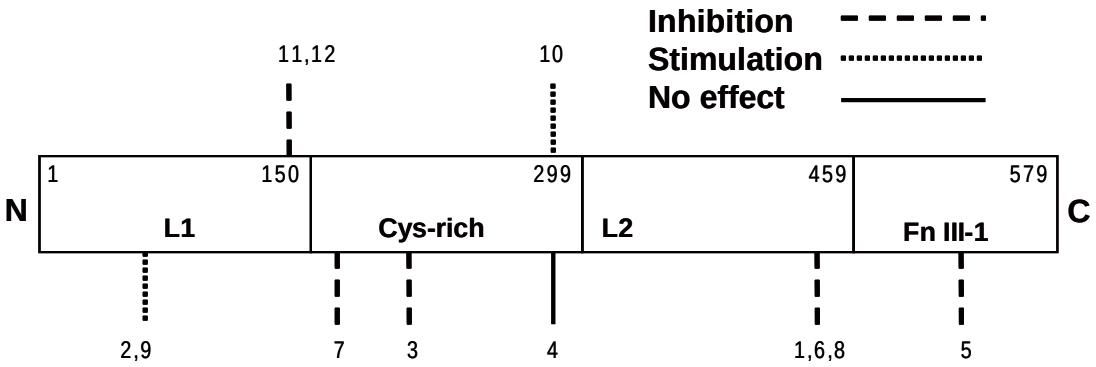

Figure 1. Schematic representation of the IGFIR α subunit (1-579) in relation to epitopes of anti-IGFIR mAbs. "N" and "C" indicate the N terminus and C terminus of the IGFIR α subunit (1-579), respectively. "L", "Cys-rich", and "Fn III" are respectively the leucine-rich repeat, cysteine-rich domain and fibronectin III repeat domain. Anti-IGFIR mAbs listed in Table 1 are marked with their corresponding numbers at their epitope sites (amino acids of N-termini in the regions recognized by each mAb). The effects of anti-IGFIR mAbs on ligand binding are shown by bars; the dashed line indicates inhibition, the dotted line indicates stimulation, and the solid line indicates no effect.

Table 2. Summary of therapeutic antibodies targeting IGFIR

Clone name	Generation technology	References
scFv-Fc	Recombinant chimeric antibody derived from mAb	Li *et al*. (*44*)
CP-751,871	Transgenic mouse producing human antibodies	Cohen *et al*. (*47*)
A12	Phage display screening	Burtrum *et al*. (*48*)
19D12	Transgenic mouse producing human antibodies	Wang *et al*. (*52*)
h7C10	Recombinant humanized antibody derived from mAb	Goetsch *et al*. (*53*)

62-184 and 283-440 residues on the IGFIR α subunit, are able to stimulate IGF-I binding whereas 24-60 and 24-57, which recognize respective 184-283 and 440-514 residues of the α subunit, almost completely inhibit IGF-I binding. The ligand-binding inhibition by 24-60 is consistent with the notion that it competitively binds to the IGF-I binding domain. Ligand-binding inhibitory mAbs, whose epitopes are identified to be regions other than the IGF-I binding domain (cysteine-rich domain), most likely either induce conformational changes in IGFIR upon binding or induce steric hindrance, resulting in low ligand-binding ability. In the case of ligand-binding stimulatory mAbs, however, conformational changes in the receptor caused by mAbs must be responsible for the observed higher binding ability of IGFIR. Our laboratory recently determined the epitopes of anti-human placental IGFIR mAbs, 1H7 and 3B7 (*35*), by competitive inhibition assays using mAbs (24-57 and 16-13) produced by Soos *et al*. (*28,34*). 1H7 and 3B7 exhibited opposite effects on ligand binding; that is, 1H7 inhibits ligand binding whereas 3B7 stimulates it (*25,26*). The competitive inhibition study demonstrated that 1H7 recognizes 440-514 regions (since it competes with 24-57) whereas 3B7 binds to 62-184 regions (since it competes with 16-13) on the α subunit (*35*).

As described above, αIR-3, which inhibits IGF-I binding, recognizes the cysteine-rich domain that was determined to be the IGF-I binding site (*29*). 1H7 mAb binds to an epitope other than the IGF-I binding site, indicating that 1H7 induces conformational changes in the receptor or causes steric hindrance. There are conflicting reports regarding the ligand-binding domain (cysteine-rich domain). Delafontaine *et al*. prepared anti-IGFIR polyclonal Abs by immunizing rabbit with peptide fragments of the IGFIR α subunit (*36*). They reported that any Abs recognizing the cysteine rich domain did not interfere with IGF-I binding, but one group of Ab, RAB6, that recognizes the 38-44 residues near the N-terminus of the α subunit, inhibited IGF-I binding. The question of whether or not the cysteine-rich domain is the major binding site for IGF-I is still unresolved. However the antibodies described by Delafontaine *et al*. are polyclonal and showed weak affinity for native receptor. Therefore it is inappropriate to compare these antibodies with other mAbs. The mAbs described above are summarized in Table 1 and in Figure 1, where each epitope of mAbs and its effect on ligand binding are shown with respect to the structure of the IGFIR α subunit.

Recombinant IGFIR antibodies for cancer therapy

Since the well-studied anti-IGFIR mAb, αIR-3, was shown to inhibit the growth of human cancer cells *in vitro* and *in vivo* (*37-39*), several other groups have reported on the potential for using anti-IGFIR mAbs to develop cancer therapeutics (*40-43*). With the advancement of recombinant antibody technologies, more therapeutic anti-IGFIR mAbs have been developed (Table 2). Li *et al*. first produced a chimeric IGFIR antibody consisting of a single chain variable

fragment (scFv) derived from mAb 1H7 and the human IgG_1 Fc region (*44*). This recombinant antibody, named IGFIR scFv-Fc, was shown to inhibit growth of the human breast cancer cell line MCF7 *in vitro* and *in vivo*. Sachdev *et al*. revealed that scFv-Fc has an agonistic effect on MCF-7 cells but that the long-time treatment of MCF-7 cells with scFv-Fc down-regulated IGFIR, resulting in the cancer cells becoming refractory to ligand stimulation (*45*). Breast cancer tumor growth *in vivo* was inhibited by scFv-Fc in two different systems, MCF-7 (*45*) and T61 (*46*). In combination with Tamoxifen, αIGFIR scFv-Fc treatment suppressed the growth of T61 tumors *in vivo* more significantly than scFv-Fc treatment alone (*46*). Cohen *et al*. produced an anti-IGFIR mAb called CP-751,871 from transgenic mice and demonstrated that this mAb inhibits tumor growth alone or in combination with chemotherapy *in vivo* (*47*). A fully human anti-IGFIR mAb, A12, that was prepared by screening of a phage displayed human Fab library exhibited tumor growth inhibition on breast, colon, pancreatic, and prostate cancer cell lines *in vivo* (*48,49*). A12 was also tested for its efficacy when used in combination with chemotherapy or radiotherapy (*50,51*). Another fully human antibody 19D12, which was produced from transgenic mice by Wang *et al*. (*52*), was found to significantly inhibit tumor growth *in vivo* as a single agent. Goetsch *et al*. produced a recombinant humanized anti-IGFIR antibody, h7C10 (*53*). This antibody showed *in vivo* antitumor efficacy as a single agent against established breast (MCF-7) and non-small cell lung cancer (A549) xenografts when administered intraperitoneally (*53*). Ligand-independent down-regulation of both IGFIR and hybrid receptors (IR-A or IR-B/IGFIR) was demonstrated upon long-term incubation of cells expressing IR-A/IGFIR or IR-B/IGFIR with h7C10 (*54*), indicating that this mAb is a potent inhibitor of both IGFIR and hybrid receptors.

A major mechanism for anti-cancer growth by IGFIR antibodies

Although several therapeutic strategies for targeting IGFIR, including antisense RNA and tyrosine kinase inhibitors, have been developed (*55-57*), monoclonal antibody therapy has emerged as the most promising approach for anti-cancer applications. What follows is a brief summary of how therapeutic anti-IGFIR antibodies work, as is illustrated in Figure 2. Most anti-IGFIR antibodies developed for cancer therapy thus far seem to down-regulate (internalize and degrade) IGFIR, thereby making cancer cells insensitive to ligand stimulation (*45-49,52,53*). Anti-IGFIR antibodies induce receptor degradation mainly *via* endosomal- and lysosomal-pathways (*45*). The receptor degradation not only causes the loss of cell-sensitivity to IGFs resulting in growth inhibition but also induces apoptosis resulting in cell death. This receptor degradation effect is attributable

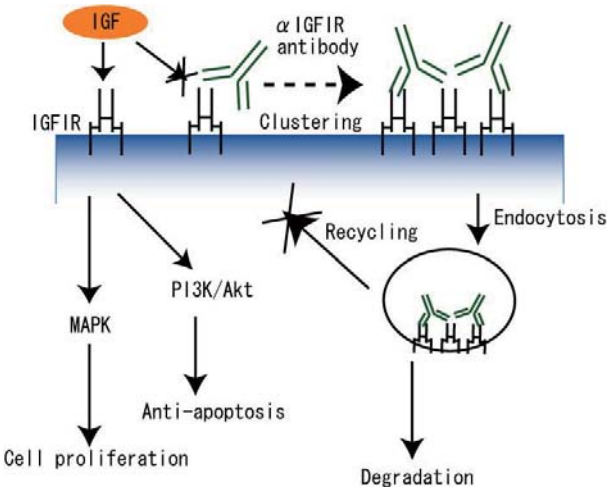

Figure 2. Schematic illustration for IGF signaling and IGFI receptor degradation in IGFIR-expressing cells. When ligands, IGF-I or IGF-II, bind to the receptor, down-stream signaling molecules are activated, leading to cell proliferation and counteracting apoptosis through MAPK and PI3K/Akt pathways. Anti-IGFIR antibody treatment not only prevents ligand-binding but also causes receptor-clustering followed by degradation through endosomal/lysosomal pathways.

to the multivalency of antibodies such as IgG or scFv-Fc, since monomeric anti-IGFIR Fab fragments were not able to trigger receptor degradation (*52*). Receptor down-regulation is very effective in cancer cells overexpressing IGFIRs. Anti-IGFIR antibodies are not believed to down-regulate IGFIR in normal cells since anti-IGFIR antibodies that cross-react to mouse IGFIR did not cause any significant side effects in mice. This observation is consistent with the notion that anti-IGFIR antibody-induced down-regulation occurs only in cancer cells overexpressing IGFIRs but not in normal cells that express lower levels of IGFIRs (*49*).

Anti-IGFIR antibodies also down-regulated IR (*58*). IR can form a heterodimer with IGFIR, resulting in IR/IGFIR hybrid receptors. IR exists in two isoforms of IR-A and IR-B (*17*). Since IR-A is expressed predominantly in cancer cell lines and cancerous tissues, IR-A/IGFIR hybrid receptor may exist as a major type in cancer cells. Both IR-A holo-receptor and IR-A/IGFIR hybrid receptor have high affinity for IGF-II, thus having more of a growth effect than a metabolic effect (*23,59*). Zhang *et al.* recently reported that down-regulation of IGFIR by small interfering RNA increases sensitivity of breast cancer cells to insulin (*60*). Because IR also activates signaling pathways similar to IGFIR in cancer cells, agents targeting both receptors may be necessary to disrupt the malignant phenotype regulated by this growth factor system. Thus, IR-A targeted antibodies will be the next generation of antibodies to be developed.

Concluding remarks

Over the last decade, significant progress has been made in the development of anti-IGFIR antibodies for

therapeutic use. Several are now undergoing clinical trials. As these trials move forward, they should elucidate whether disruption of IGFIR signaling results in relevant clinical outcomes.

References

1. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975; 256:495-497.
2. Cabilly S, Riggs AD, Pande H, Shively JE, Holmes WE, Rey M, Perry LJ, Wetzel R, Heyneker HL. Generation of antibody activity from immunoglobulin polypeptide chains produced in Escherichia coli. Proc Natl Acad Sci U S A 1984; 81:3273-3277.
3. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. Proc Natl Acad Sci U S A 1984; 81:6851-6855.
4. Boulianne GL, Hozumi N, Shulman MJ. Production of functional chimaeric mouse/human antibody. Nature 1984; 312:643-646.
5. Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 1986; 321:522-525.
6. Hoogenboom HR. Selecting and screening recombinant antibody libraries. Nat Biotechnol 2005; 23:1105-1116.
7. Lonberg N. Human antibodies from transgenic animals. Nat Biotechnol 2005; 23:1117-1125.
8. Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. Nat Biotechnol 2005; 23:1147-1157.
9. Reichert JM, Rosensweig CJ, Faden LB, Dewitz MC. Monoclonal antibody successes in the clinic. Nat Biotechnol 2005; 23:1073-1078.
10. Haddad T, Yee D. Targeting the insulin-like growth factor axis as a cancer therapy. Future Oncol 2006; 2:101-110.
11. Ibrahim YH, Yee D. Insulin-like growth factor-I and breast cancer therapy. Clin Cancer Res 2005; 11:944-950.
12. Yee D. Targeting insulin-like growth factor pathways. Br J Cancer 2006; 94:465-468.
13. Miller BS, Yee D. Type I insulin-like growth factor receptor as a therapeutic target in cancer. Cancer Res 2005; 65:10123-10127.
14. Daughaday WH, Rotwein P. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. Endocr Rev 1989; 10:68-91.
15. Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B, LeRoith D. Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc Natl Acad Sci U S A 1999; 96:7324-7329.
16. LeRoith D, Werner H, Beitner-Johnson D, Roberts CT Jr. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev 1995; 16:143-163.
17. LeRoith D, Roberts CT Jr. The insulin-like growth factor system and cancer. Cancer Lett 2003; 195:127-137.
18. Samani AA, Yakar S, LeRoith D, Brodt P. The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocr Rev. 2007; 28:20-47.
19. Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramachandran J, Fujita-Yamaguchi Y. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J 1986; 5:2503-2512.
20. Larsson O, Girnita A, Girnita L. Role of insulin-like growth factor 1 receptor signaling in cancer. Br J Cancer 2005; 92:2097-2101.
21. Pandini G, Mineo R, Frasca F, Roberts CT Jr, Marcelli M, Vigneri R, Belfiore A. Androgens up-regulate the insulin-like growth factor-I receptor in prostate cancer cells. Cancer Res 2005; 65:1849-1857.
22. Pandini G, Vigneri R, Costantino A, Frasca F, Ippolito A, Fujita-Yamaguchi Y, Siddle K, Goldfine ID, Belfiore A. Insulin and insulin-like growth factor-I (IGF-I) receptor overexpression in breast cancers leads to insulin/IGF-I hybrid receptor overexpression: evidence for a second mechanism of IGF-I signaling. Clin Cancer Res 1999; 5:1935-1944.
23. Pandini G, Frasca, F, Mineo R, Sciacca L, Vigneri R, Belfiore A. Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. J Biol Chem 2002; 277:39684-39695.
24. Kull FC Jr, Jacobs S, Su YF, Svoboda ME, Van Wyk JJ, Cuatrecasas P. Monoclonal antibodies to receptors for insulin and somatomedin-C. J Biol Chem 1983; 258:6561-6566.
25. Li SL, Kato J, Paz IB, Kasuya J, Fujita-Yamaguchi Y. Two new monoclonal antibodies against the alpha subunit of the human insulin-like growth factor-I receptor. Biochem Biophys Res Commun 1993; 196:92-98.
26. Xiong L, Kasuya J, Li SL, Kato J, Fujita-Yamaguchi Y. Growth-stimulatory monoclonal antibodies against human insulin-like growth factor I receptor. Proc Natl Acad Sci U S A 1992; 89:5356-5360.
27. Keyhanfar M, Forbes, BE, Cosgrove, LJ, Wallace, JC, Booker GW. Production and characterization of monoclonal antibodies against insulin-like growth factor type 1 receptor (IGF-1R). Hybridoma 2006; 25:230-237.
28. Soos MA, Field CE, Lammers R, Ullrich A, Zhang B, Roth RA, Andersen AS, Kjeldsen T, Siddle K. A panel of monoclonal antibodies for the type I insulin-like growth factor receptor. Epitope mapping, effects on ligand binding, and biological activity. J Biol Chem 1992; 267:12955-12963.
29. Gustafson TA, Rutter WJ. The cysteine-rich domains of the insulin and insulin-like growth factor I receptors are primary determinants of hormone binding specificity. Evidence from receptor chimeras. J Biol Chem 1990; 265:18663-18667.
30. Mynarcik DC, Williams PF, Schaffer L, Yu GQ, Whittaker J. Identification of common ligand binding determinants of the insulin and insulin-like growth factor 1 receptors. J Biol Chem 1997; 272:18650-18655.
31. Whittaker J, Groth AV, Mynarcik DC, Pluzek L, Gadsbøll VL, Whittaker LJ. Alanine scanning mutagenesis of a type 1 insulin-like growth factor receptor ligand binding site. J Biol Chem 2001; 276:43980-433986.
32. Keyhanfar M, Booker GW, Whittaker J, Wallace JC, Forbes BE. Precise mapping of an IGF-I-binding site on the IGF-1R. Biochem J 2007; 401:269-277.
33. Garrett TP, McKern NM, Lou M, Frenkel MJ, Bentley JD, Lovrecz GO, Elleman TC, Cosgrove LJ, Ward CW. Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor. Nature 1998; 394:395-399.
34. Schumacher R, Soos MA, Schlessinger J, Brandenburg D, Siddle K, Ullrich A. Signaling-competent receptor chimeras allow mapping of major insulin receptor binding domain determinants. J Biol Chem 1993; 268:1087-1094.
35. Kusada Y, Morizono T, Matsumoto-Takasaki A, Sakai K, Sato S, Asanuma H, Takayanagi A, Fujita-Yamaguchi

Y. Construction and characterization of single-chain antibodies against human insulin-like growth factor-I receptor from hybridomas producing 1H7 or 3B7 monoclonal antibody. J Biochem (Tokyo) in press.
36. Delafontaine P, Ku L, Ververis JJ, Cohen C, Runge MS, Alexander RW. Epitope mapping of the alpha-chain of the insulin-like growth factor I receptor using antipeptide antibodies. J Mol Cell Cardiol 1994; 26:1659-1673.
37. Arteaga CL, Kitten LJ, Coronado EB, Jacobs S, Kull FC Jr, Allred DC, Osborne CK. Blockade of the type I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. J Clin Invest 1989; 84:1418-1423.
38. Gansler T, Furlanetto R, Gramling TS, Robinson KA, Blocker N, Buse MG, Sens DA, Garvin AJ. Antibody to type I insulinlike growth factor receptor inhibits growth of Wilms' tumor in culture and in athymic mice. Am J Pathol 1989; 135:961-966.
39. Arteaga CL, Osborne CK. Growth inhibition of human breast cancer cells *in vitro* with an antibody against the type I somatomedin receptor. Cancer Res 1989; 49:6237-6241.
40. Cara JF, Stuart CA, Furlanetto RW. A monoclonal antibody to the type 1 insulin-Like growth factor and insulin receptors stimulates deoxyribonucleic acid synthesis in human and murine fibroblasts. Endocrinology 1988; 123:1341-1347.
41. Morgan DO, Roth RA. Identification of a monoclonal antibody which can distinguish between two distinct species of the type I receptor for insulin-like growth factor. Biochem Biophys Res Commun 1986; 138:1341-1347.
42. Hailey J, Maxwell E, Koukouras K, Bishop WR, Pachter JA, Wand Y. Neutralizing anti-insulin-like growth factor receptor 1 antibodies inhibit receptor function and induce receptor degradation in tumor cells. Mol Cancer Ther 2002; 1:1349-1353.
43. Maloney EK, McLaughlin JL, Dagdigian NE, Garrett LM, Connors KM, Zhou XM, Blättler WA, Chittenden T, Singh R. An anti-insulin-like growth factor I receptor antibody that is a potent inhibitor of cancer cell proliferation. Cancer Res 2003; 63:5073-5083.
44. Li SL, Liang SJ, Guo N, Wu AM, Fujita-Yamaguchi Y. Single-chain antibodies against human insulin-like growth factor I receptor: expression, purification, and effect on tumor growth. Cancer Immunol Immunother 2000; 49:243-252.
45. Sachdev D, Li SL, Hartell JS, Fujita-Yamaguchi Y, Miller JS, Yee D. A chimeric humanized single-chain antibody against the type I insulin-like growth factor (IGF) receptor renders breast cancer cells refractory to the mitogenic effects of IGF-I. Cancer Res 2003; 63:627-635.
46. Ye JJ, Liang SJ, Guo N, Li SL, Wu AM, Giannini S, Sachdev D, Yee D, Brunner N, Ikle D, Fujita-Yamaguchi Y. Combined effects of tamoxifen and a chimeric humanized single chain antibody against the type I IGF receptor on breast tumor growth *in vivo*. Horm Metab Res 2003; 35:836-842.
47. Cohen BD, Baker DA, Soderstrom C, Tkalcevic G, Rossi AM, Miller PE, Tengowski MW, Wang F, Gualberto A, Beebe JS, Moyer JD. Combination therapy enhances the inhibition of tumor growth with the fully human anti-type 1 insulin-like growth factor receptor monoclonal antibody CP-751,871. Clin Cancer Res 2005; 11:2063-2073.
48. Burtrum D, Zhu Z, Lu D, *et al*. A fully human monoclonal antibody to the insulin-like growth factor I receptor blocks ligand-dependent signaling and inhibits human tumor growth *in vivo*. Cancer Res 2003; 63:8912-8921.
49. Wu JD, Odman A, Higgins LM, Haugk K, Vessella R, Ludwig DL, Plymate, SR. *In vivo* effects of the human type I insulin-like growth factor receptor antibody A12 on androgen-dependent and androgen independent xenograft human prostate tumors. Clin Cancer Res 2005; 11:3065-3074.
50. Wu JD, Haugk K, Coleman I, Woodke L, Vessella R, Nelson P, Montgomery RB, Ludwig DL, Plymate SR. Combined *in vivo* effect of A12, a type 1 insulin-like growth factor receptor antibody, and docetaxel against prostate cancer tumors. Clin Cancer Res 2006; 12:6153-6160.
51. Allen GW, Saba C, Armstrong EA, Huang SM, Benavente S, Ludwig DL, Hicklin DJ, Harari PM. Insulin-like growth factor-I receptor signaling blockade combined with radiation. Cancer Res 2007; 67:1155-1162.
52. Wang Y, Hailey J, Williams D, *et al*. Inhibition of insulin-like growth factor-I receptor (IGF-IR) signaling and tumor cell growth by a fully human neutralizing anti-IGF-IR antibody. Mol Cancer Ther 2005; 4:1214-1221.
53. Goetsch L, Gonzalez A, Leger O, Beck A, Pauwels PJ, Haeuw JF, Corvaia N. A recombinant humanized anti-insulin-like growth factor receptor type I antibody (h7C10) enhances the antitumor activity of vinorelbine and anti-epidermal growth factor receptor therapy against human cancer xenografts. Int J Cancer 2005; 113:316-328.
54. Pandini G, Wurch T, Akla B, Corvaia N, Belfiore A, Goetsch L. Functional responses and *in vivo* anti-tumour activity of h7C10: a humanised monoclonal antibody with neutralising activity against the insulin-like growth factor-1 (IGF-1) receptor and insulin/IGF-1 hybrid receptors. Eur J Cancer 2007; 43:1318-1327.
55. Neuenschwander S, Roberts CT Jr, LeRoith D. Growth inhibition of MCF7 breast cancer cells by stable expression of an insulin-like growth factor I receptor antisense ribonucleic acid. Endocrinology 1995; 136:4298-4303.
56. Hopfner M, Sutter AP, Huether A, Baradari V, Scherubl H. Tyrosine kinase of insulin-like growth factor receptor as target for novel treatment and prevention strategies of colorectal cancer. World J Gastroenterol 2006; 12:5635-5643.
57. Warshamana-Greene GS, Litz J, Buchdunger E, Hofmann F, Garcia-Echeverría C, Krystal GW. The insulin-like growth factor-I (IGF-I) receptor kinase inhibitor NVP-ADW742, in combination with STI571, delineates a spectrum of dependence of small cell lung cancer on IGF-I and stem cell factor signaling. Mol Cancer Ther 2004; 3:527-535.
58. Sachdev D, Singh R, Fujita-Yamaguchi Y, Yee D. Down-regulation of insulin receptor by antibodies against the type I insulin-like growth factor receptor: implications for anti-insulin-like growth factor therapy in breast cancer. Cancer Res 2006; 66:2391-2402.
59. Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, Goldfine ID, Belfiore A, Vigneri R. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol 1999; 19:3278-3288.
60. Zhang H, Pelzer AM, Kiang DT, Yee D. Down-regulation of type I insulin-like growth factor receptor increases sensitivity of breast cancer cells to insulin. Cancer Res 2007; 67:391-397.