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(57) **Abrégé/Abstract:**

Proteins that bind to IGF-II and/or IGF-IIIE and methods of using such proteins are described. Also disclosed are new methods for treatment of cancer. Cancer is treated by administration of an IGF-II/IGF-IIIE binding protein and an additional therapy such as a growth hormone/growth hormone releasing hormone pathway modulator or epidermal growth factor receptor inhibitor.



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(54) Title: IGF-II/GF-III BINDING PROTEINS

(57) Abstract: Proteins that bind to IGF-II and/or IGF-III and methods of using such proteins are described. Also disclosed are new methods for treatment of cancer. Cancer is treated by administration of an IGF-II/IGF-III binding protein and an additional therapy such as a growth hormone/growth hormone releasing hormone pathway modulator or epidermal growth factor receptor inhibitor.



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IGF-II/IGF-IIe BINDING PROTEINS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 61/051,956, filed on May 9, 2008, U.S. Application Serial No. 61/053,427, filed on May 15, 2008, and
5 U.S. Application Serial No. 61/163,180, filed on March 25, 2009. The disclosures of the prior applications are considered part of (and are incorporated by reference in) the disclosure of this application.

BACKGROUND

The insulin-like growth factor family of polypeptides plays a key role in normal
10 growth and development. Altered expression of components such as IGF II, of the IGF system are implicated in the development and maintenance of the malignant phenotype in many tumor types, suggesting that agents targeting this system may have potential as anti-cancer therapeutics. A number of pathways have been identified that can contribute to increased IGF-II secretion by tumors; these include a loss of genomic imprinting of the
15 maternal IGF-II allele, loss of heterozygosity with paternal allelic duplication, and/or loss of transcriptional control. Such increased secretion allows for greater proliferation, protection from apoptosis and metastatic potential of a cancer, especially as the receptors that specifically bind the IGF-II, the IGF-I receptor (IGF-1R) and an isoform of the insulin receptor (IR-A), are typically up-regulated in tumor cells. The increased
20 production of IGF-II is further exacerbated by the down-regulation of the mannose-6-phosphate receptor, a third type of IGF-II receptor that appears to be central for the clearance of IGF-II from the circulation. Local levels of IGF-II may also be elevated by changes in the expression of specific IGF-II binding proteins secreted by the tumor, or as a result of increased protease activity produced by the tumors.

25 Even though it is implicated in the pathogenesis of 50% of cancers, there are limited therapeutic agents available that specifically target the IGF signaling axis although there are many research efforts underway to remedy this. It has recently been demonstrated that the efficacy of EGF receptor antagonists in a model of breast cancer treatment is limited by the rapid development of resistance via the IGF system.

Discovery or development of therapeutics that interfere with the IGF system is complicated by the finding that most IGF-I receptors appear to form hybrid receptors with the IR-A, the isoform of the insulin receptor that binds both IGF-II and insulin with high affinity. Therefore, therapeutic targeting of the IGF-IR with tyrosine kinase inhibitors or antibodies may also block insulin signaling and cause diabetes, and recent reports have indicated this to be the case. Toxicity problems with the IGF-IR small molecule kinase antagonists in primate studies have also been reported.

In normal circulation, 95% of IGF-I and II are bound to six high-affinity IGF binding proteins (IGFBPs). The major serum-binding protein is IGFBP-3, which forms a trimeric complex with acid labile protein (ALS). Normally IGF-II is synthesized as a 156 amino acid (aa) precursor protein, known as pro-IGF-II. This protein includes an 87 aa C-terminal region known as the E-domain, and is thus referred to as "IGF-II-E." Herein, the construct comprising amino acids 1-104, which encompass the E domain, is referred to as "IGF-II-E". Proteolytic steps release the mature 67 aa IGF-II polypeptide. In the literature "long" or "big" forms of IGF-II are also sometimes referred to forms in which only portions of the E domain are cleaved. Sometimes the long or big forms are also referred to as IGF-II-E even though they may contain only parts of the E domain as opposed to the complete E domain. In many tumors there is increased production of IGF-II, due mainly to loss of imprinting at a genomic level, or decreased levels of binding proteins due to increased protease activity produced by the tumors that allow for increased bioavailability of free IGF-II. In a recent IGF II mouse model, offspring mice with loss of imprinting characteristics, mated with Apc⁺/Min mice have shown greatly enhance tumorigenesis. Many tumors lack the enzymatic machinery for processing IGF-II-E to the mature 7.5kDa protein and thus predominately secrete IGF-II-E. This long IGF-II ligand has a 21 amino acid extension and defective glycosylation at threonine 75 and therefore cannot bind ALS, allowing greater "free IGF-II" to activate the IR or IGF-I R, potentiating neoplastic growth and, in some cancers, causing hypoglycemia.

Therefore, there exists a need to design antibodies which bind to IGF-II and IGF-II-E as a therapeutic for IGF dependent cancers. Furthermore, combinations of therapies often provide enhanced, or even synergistic, therapeutic results.

SUMMARY

Immunotherapeutic treatment of cancer holds many advantages over traditional therapies such as surgery, radiotherapy and chemotherapy, as the therapeutic agent, such as the cytokine, antibodies or antibody-like moieties can be highly specific for the tumorous cell. Antibodies that block or interfere with naturally occurring events such as signal events associated with the EGFR pathway have been shown to be effective in reducing the growth of several tumor types. We have evaluated a number of colorectal cancer cell lines and all express not only the IGF-I receptor but have upregulated expression of IR-A. The action of binding of IGF-II to the IR-A predominately results in mitogenic signaling and metastasis, exacerbating the malignant phenotype. A therapeutic targeting IGF-II would block the action of this ligand by inhibiting binding to both receptors, without causing down regulation of the IR and the possible complications of hypoglycemia. Further, a therapeutic targeting IGF-IIIE in addition to IGF-II would target tumors that do not process IGF-IIIE further. Development of an antibody that specifically binds IGF-II and IGF-IIIE would also alleviate the issue of toxicity demonstrated with kinase antagonists. Further, a therapeutic targeting only IGF-IIIE and not IGF-II would also be of value.

Accordingly, this disclosure relates, *inter alia*, to proteins that bind to both or either, IGF-II and IGF-IIIE, herein referred to as “IGF-II/IGF-IIIE binding proteins” and methods of identifying and using such proteins. These proteins include antibodies and antibody fragments (e.g., primate antibodies and Fabs, especially human antibodies and Fabs) that bind to and/or inhibit both IGF-II and IGF-IIIE consequential binding. The IGF-II/IGF-IIIE binding proteins can be used in the treatment of diseases, particularly human disease, such as cancer, in which excess or inappropriate activity of IGF-II and/or IGF-IIIE features. In many cases, the proteins have tolerable low or no toxicity.

In one aspect, the disclosure features a protein (e.g., an isolated protein) that binds to IGF-II and IGF-IIIE (e.g., human IGF-II and IGF-IIIE) and includes at least one immunoglobulin variable region. For example, the protein includes a heavy chain (HC) immunoglobulin variable domain sequence and a light chain (LC) immunoglobulin variable domain sequence. In one embodiment, the protein binds to and inhibits IGF-II and IGF-IIIE consequential binding, e.g., human IGF-II and/or IGF-IIIE. In another

embodiment, the protein binds to and/or inhibits only IGF IIE but not IGF-II consequential binding.

The protein can include one or more of the following characteristics: (a) a human CDR or human framework region; (b) the HC immunoglobulin variable domain sequence comprises one or more (e.g., one, two, or three) CDRs that are at least 85, 88, 90, 92, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a LC variable domain described herein; (c) the LC immunoglobulin variable domain sequence comprises one or more (e.g., one, two, or three) CDRs that are at least 85, 88, 90, 92, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a HC variable domain described herein; (d) the LC immunoglobulin variable domain sequence is at least 85, 88, 90, 92, 94, 95, 96, 97, 98, 99, or 100% identical to a LC variable domain described herein; (e) the HC immunoglobulin variable domain sequence is at least 85, 88, 90, 92, 94, 95, 96, 97, 98, 99, or 100% identical to a HC variable domain described herein; (f) the protein binds an epitope bound by a protein described herein, or an epitope that overlaps with such epitope; and (g) a primate CDR or primate framework region.

The protein can bind to human IGF-II and IGF-IIE, e.g., human IGF-II and/or IGF-IIE, with a binding affinity of at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} and 10^{11} M⁻¹. In one embodiment, the protein binds to human IGF-II and/or IGF-IIE with a K_{off} slower than 1×10^{-3} , 5×10^{-4} s⁻¹, or 1×10^{-4} s⁻¹. In one embodiment, the protein binds to human IGF-II and/or IGF-IIE with a K_{on} faster than 1×10^2 , 1×10^3 , or 5×10^3 M⁻¹s⁻¹. In one embodiment, the protein inhibits both human IGF-II and human IGF-IIE activity, e.g., with a K_i of less than 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , and 10^{-10} M. In one embodiment, the protein inhibits either human IGF-II or human IGF-IIE activity, e.g., with a K_i of less than 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , and 10^{-10} M. The protein can have, for example, an IC₅₀ of less than 100 nM, 10 nM or 1 nM. For example, the protein may modulate IGF-I receptor (IGF-1R) and an isoform of the insulin receptor (IR-A) activity, as well as IGF-II and/or IGF-IIE. The protein may inhibit IGF-1R, IR-A, and IGF-II and IGF-IIE activity. The affinity of the protein for human IGF-II and/or IGF-IIE can be characterized by a K_D of less than 100 nM, less than 10 nM, or less than 1 nM.

In a preferred embodiment, the protein is a human antibody having the light and heavy chains of antibodies picked from the list comprising M0033-E05, M0063-F02,

M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and M0072-G06. In a preferred embodiment, the protein is a human antibody having its heavy chain picked from the list comprising: M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and M0072-G06. In a preferred embodiment, the protein is a human antibody having its light chain picked from the list comprising: M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and M0072-G06. In a preferred embodiment, the protein is a human antibody having one or more (e.g., one, two, or three) heavy chain CDRs picked from the corresponding CDRs of the list of heavy chains comprising M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and M0072-G06. In a preferred embodiment, the protein is a human antibody having one or more (e.g., one, two, or three) light chain CDRs picked from the corresponding CDRs of the list of heavy chains comprising M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and M0072-G06.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain. In another, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the protein is an IgG., e.g., IgG1, IgG2, IgG3, or IgG4. The protein can be a soluble Fab (sFab). In other implementations the protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA + VH::CH1, HSA::LC + VH::CH1, or other appropriate construction.

In one embodiment, the protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions. In one embodiment, the protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions. In one embodiment, the protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. “Primate” includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibbons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In some embodiments, the affinity of the primate antibody for human IGF-II and/or IGF-IIIE is characterized by a K_D of less than 1 nM.

In certain embodiments, the protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In certain embodiments, the protein may be capable of binding to tumor cells expressing IGF-II and/or IGF-IIIE, e.g., to colorectal cell lines SW1116 (Grade A), SW480 (Grade B), HT29, SW480, Caco2, HCT116, SW620 (all Grade C), and COLO 205 (Grade D); breast cancer cell lines MCF-7 and 4T1; uterine cancer cell line SKUT-1 (mesodermal tumor), rhabdomyosarcoma cell lines, and hepatocellular carcinoma cell lines HepG2, HuH7 and Hep3B.

In one embodiment, protein is physically associated with a nanoparticle, and can be used to guide a nanoparticle to a cell expressing IGF-II and/or IGF-IIIE on the cell surface.

A binding protein described herein can be provided as a pharmaceutical composition, e.g., including a pharmaceutically acceptable carrier. The composition can be at least 10, 20, 30, 50, 75, 85, 90, 95, 98, 99, or 99.9% free of other protein species.

In another aspect, the disclosure features a method of detecting IGF-II and/or IGF-IIIE in a sample. The method includes: contacting the sample with an IGF-II/IGF-IIIE binding protein; and detecting an interaction between the protein and the IGF-II and/or IGF-IIIE, if present. In some embodiments, the protein includes a detectable label. An IGF-II/IGF-IIIE binding protein can be used to detect IGF-II and/or IGF-IIIE in a subject. The method includes: administering an IGF-II/IGF-IIIE binding protein to a subject; and detecting the protein in the subject. In some embodiments, the protein further includes a detectable label. For example, the detecting comprises imaging the subject.

In another aspect, the disclosure features a method of modulating IGF-II and/or IGF-IIIE activity. The method includes: contacting IGF-II and/or IGF-IIIE with an IGF-II/IGF-IIIE binding protein (e.g., in a human subject), thereby modulating IGF-II and/or IGF-IIIE activity.

5 In another aspect, the disclosure features a method of treating cancer (e.g., metastatic cancer). The method includes: administering, to a subject, an IGF-II/IGF-IIIE binding protein in an amount sufficient to treat a cancer in the subject. For example, the cancer is head and neck cancer, oral cavity cancer, laryngeal cancer, chondrosarcoma, breast cancer, laryngeal cancer, colorectal cancer, liver cancer, ovarian cancer, testicular
10 carcinoma, melanoma, or brain tumors (e.g., astrocytomas, glioblastomas, gliomas).

IGF-II/IGF-IIIE binding proteins are useful to modulate metastatic activity in a subject. The protein can be administered, to the subject, an IGF-II/IGF-IIIE binding protein in an amount effective to modulate metastatic activity. For example, the protein inhibits one or more of: tumor growth, tumor embolism, tumor mobility, tumor
15 invasiveness, and cancer cell proliferation. The method can further include providing to the subject a second therapy that is an anti-cancer therapy, e.g., administration of a chemotherapeutic, e.g., an agent that antagonizes signaling through a VEGF pathway, e.g., bevacizumab. In one embodiment, the second therapy includes administering 5-FU, leucovorin, and/or irinotecan. In one embodiment, the second therapy includes
20 administering a Tie1 inhibitor (e.g., an anti-Tie1 antibody). Other exemplary therapeutic methods that include administering an IGF-II/IGF-IIIE binding protein are described below. An IGF-II/IGF-IIIE binding protein described herein can be administered in combination with one or more other IGF-II or IGF-IIIE inhibitors, e.g., small molecule inhibitors, e.g., broad specificity inhibitors. In another embodiment, the one or more
25 IGF-II or IGF-IIIE inhibitors include another IGF-II/IGF-IIIE binding protein. The IGF-II/IGF-IIIE binding protein can be administered in combination with an additional anti-cancer therapy, e.g., that is an epidermal growth factor (EGF) pathway inhibitor. The EGF pathway inhibitor may be an EGF receptor (EGFR) inhibitor, such as a small molecule inhibitor (such as erlotinib (e.g., TARCEVA®) or gefitinib (e.g., IRESSA®)),
30 or an antibody that binds to EGFR, such as cetuximab (e.g., ERBITUX®). Additional inhibitors of the pathway include PD1 53035, SU5271, and ZD1 839.

IGF-II/IGF-IIIE binding proteins are useful for targeted delivery of an agent to a subject (e.g., a subject who has or is suspected of having a tumor), e.g., to direct the agent to a tumor in the subject. For example, an IGF-II/IGF-IIIE binding protein that is coupled to an anti-tumor agent (such as a chemotherapeutic, toxin, drug, or a radionuclide (e.g.,
5 ¹³¹I, ⁹⁰Y, ¹⁷⁷Lu)) can be administered to a subject who has or is suspected of having a tumor.

In another aspect, the disclosure features a method of imaging a subject. The method includes administering an IGF-II/IGF-IIIE binding protein to the subject. In some embodiments, the protein is one that does not substantially inhibit IGF-II or IGF-IIIE
10 activity. The IGF-II/IGF-IIIE binding protein may include a detectable label (e.g., a radionuclide or an MRI-detectable label). In one embodiment, the subject has or is suspected of having a tumor. The method is useful for cancer diagnosis, intraoperative tumor detection, post-operative tumor detection, or monitoring tumor invasive activity.

In one aspect, the disclosure features the use of an IGF-II/IGF-IIIE binding protein
15 described herein for the manufacture of a medicament for the treatment of a disorder described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer).

One pathway that is a good candidate for anticancer drug development is the growth hormone/insulin-like growth factor (GH/IGF) axis. In vitro and in vivo studies of
20 rodent and primate model systems illustrate that GH and IGF-I can induce mammary epithelial cell proliferation and differentiation while blocking apoptosis. Receptors for GH have been identified in mouse, rat, monkey, and human mammary stroma. While the majority of receptors are found on stromal cells, mammary epithelial cells also express GH receptor (GHR). The IGFs, their receptors and binding proteins are found during all
25 stages of normal mammary gland development in all mammalian species examined to date. Kleinberg et al. have shown that GH, acting through its receptors on mammary stromal cells, induces IGF-I that can act in paracrine fashion to stimulate parenchymal proliferation and differentiation.

Further, the GH/IGF axis is important for the growth of advanced human breast
30 cancers. Several independent laboratories have observed GHRs in human breast cancer cells. Pollak et al. have reported that breast cancers derived from MCF-7 cells grow

more slowly in mice homozygous for lit relative to control mice. Lit mice harbor a missense mutation resulting in loss of function of the pituitary GH-releasing hormone receptor and secondary suppression of GH and IGF-I. Schally et al. have published studies recently that demonstrate an inhibitory effect of GH-releasing hormone (GHRH) antagonists on the growth of human mammary xenografts in nude mice. GHRH antagonists decreased IGF-I levels both in serum and tumors of treated animals. Pollak et al. have shown that mice expressing a bovine GH antagonist develop fewer mammary tumors when exposed to dimethylbenzanthracene compared with control mice. Friend et al. have shown that the GH antagonist pegvisomant (SOMAVERT®) inhibits the growth of human breast cancer cells grown as xenografts in immunodeficient mice.

Accordingly, in one aspect, the invention provides methods for treating (*e.g.*, ameliorating at least one symptom of cancer by administering an IGF-II/IGF-IIE binding protein and a growth hormone/growth hormone releasing hormone pathway modulator.

In one embodiment, the IGF-II/IGF-IIE binding protein is administered for a period prior to the administration of the growth hormone/growth hormone releasing hormone pathway modulator. The period of IGF-II/IGF-IIE binding protein administration may be less than one day (*e.g.*, less than one hour, or about 1, 2, 3, 4, 6, 8, 12, 18, or 24 hours) or range from 1 day up to 35 days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 21, 28, 30, or 35 days, or any day or range in between) prior to the first administration of the growth hormone/growth hormone releasing hormone pathway modulator. The period of IGF-II/IGF-IIE binding protein administration may be followed by a hiatus period during which neither the IGF-II/IGF-IIE binding protein nor the growth hormone/growth hormone releasing hormone inhibitor are administered. The hiatus period may be less than one day (*e.g.*, less than one hour, or about 1, 2, 3, 4, 6, 8, 12, 18, or 24 hours) or range from 1 day up to 35 days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 21, 28, 30, or 35 days, or any day or range in between) prior to the first administration of the growth hormone/growth hormone releasing hormone pathway modulator.

In another embodiment, the IGF-II/IGF-IIE binding protein is administered following first administration of the growth hormone/growth hormone releasing hormone pathway modulator. The period between first administration of the growth

hormone/growth hormone releasing hormone pathway modulator and administration of the IGF-II/IGF-IIE binding protein may be less than one day (e.g., less than one hour, or about 1, 2, 3, 4, 6, 8, 12, 18, or 24 hours) or range from 1 day up to 35 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 21, 28, 30, or 35 days, or any day or range in between).

5 In some embodiments, administration of the IGF-II/IGF-IIE binding protein is continued following the first administration of the growth hormone/growth hormone releasing hormone pathway modulator, while in other embodiments, the administration of the IGF-II/IGF-IIE binding protein is discontinued upon initiation of growth
10 hormone/growth hormone releasing hormone pathway modulator administration. In some embodiments in which administration of the IGF-II/IGF-IIE binding protein is discontinued following initiation of administration of the growth hormone/growth hormone releasing hormone pathway modulator, administration of the IGF-II/IGF-IIE binding protein is not re-initiated.

In some embodiments, the combination therapy disclosed herein may be
15 administered in a series (two or more) of cycles. For example, in configurations in which the IGF-II/IGF-IIE binding protein is administered for a period prior to the initiation of administration of the growth hormone/growth hormone releasing hormone pathway modulator and the IGF-II/IGF-IIE binding protein is discontinued upon, or following, the initiation of growth hormone/growth hormone releasing hormone pathway modulator
20 administration, the IGF-II/IGF-IIE binding protein may be reinitiated following administration or completion of administration of the growth hormone/growth hormone releasing hormone pathway modulator.

In some embodiments, the combination therapy disclosed herein utilizes a schedule of alternating agents. For example, the IGF-II/IGF-IIE binding protein is
25 administered for a period, followed by administration of a growth hormone/growth hormone releasing hormone pathway modulator, followed by further administration of the IGF-II/IGF-IIE binding protein, followed by another administration of the growth hormone/growth hormone releasing hormone pathway modulator, etc. The converse schedule may also be used (growth hormone/growth hormone releasing hormone
30 pathway modulator, then IGF-II/IGF-IIE binding protein, then growth hormone/growth hormone releasing hormone pathway modulator, then IGF-II/IGF-IIE binding protein,

etc.). In some embodiments, the administration of one agent is discontinued prior to the administration of the other agent.

In some embodiments, the IGF-II/IGF-IIIE binding protein and the growth hormone/growth hormone releasing hormone pathway modulator are each administered in an amount effective to individually ameliorate at least one symptom of cancer in the subject or otherwise treat or prevent the disorder in a subject. In other embodiments, the IGF-II/IGF-IIIE binding protein and the growth hormone/growth hormone releasing hormone pathway modulator are each administered in an amount that is less than an amount that is individually effective to ameliorate at least one symptom of cancer in the subject or otherwise treat or prevent the disorder in a subject. In some embodiments, the growth hormone/growth hormone releasing hormone pathway modulator is administered in an amount that is less than an amount that is individually effective to ameliorate at least one symptom of cancer in the subject or otherwise treat or prevent the disorder in a subject. In some embodiments, the IGF-II/IGF-IIIE binding protein is administered in an amount that is less than an amount that is individually effective to ameliorate at least one symptom of cancer in the subject or otherwise treat or prevent the disorder in a subject. In some embodiments, the IGF-II/IGF-IIIE binding protein and the growth hormone/growth hormone releasing hormone pathway modulator are administered in synergistically effective amounts (*e.g.*, amounts which, when compared to either compound administered alone, result in a synergistic effect).

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURES 1A and 1B show polypeptide folds as determined by the crystallographic analysis of a complex of IGF-II with M0064-F02 Fab (as described in Example 8 below). Helices are indicated by curled ribbons and beta sheets by broad arrows.

FIGURES 2A and 2B give typical profiles obtained from SPR affinity measurements of one of the antibodies interacting with the Binding Proteins BP2 and BP4. (A) data for M0063-F02, (B) data for M0064-E04 candidate antibody.

FIGURE 3 is a graph illustrating the effects of DX-2647 on Hep3B colony formation.

FIGURE 4 is a schematic depicting the pro-IGF II precursor.

FIGURES 5A and 5B: FIGURE 5A demonstrates that DX-2647 competes with receptors/IGFBPs for binding to IGF-II and IGF-IIE. FIGURE 5B summarizes the results.

FIGURES 6A and 6B: FIGURE 6A is a graph showing the effects of DX-2647 on HepG2 colony formation. FIGURE 6B represents tabular data for three independent colony formation experiments.

FIGURE 7 is a bar graph showing the effects of DX-2647 on cell viability of SKUT-1 cells.

FIGURE 8 is a bar graph showing the effects of DX-2647 on Colo205 cell proliferation in complete medium containing 10% FBS.

FIGURE 9 is a bar graph showing the effects of DX-2647 on IGF-II induced Colo205 cell proliferation.

FIGURE 10 is a bar graph showing the effects of DX-2647 on HepG2 anchorage dependent colony formation.

FIGURE 11 is a line graph depicting the effects of DX-2647 on Hep3B tumor growth in a xenograft model.

DETAILED DESCRIPTION

Definitions

For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are defined here. Other terms are defined as they appear in the specification.

The singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise.

The term “agonist”, as used herein, is meant to refer to an agent that mimics or up-regulates (e.g., potentiates or supplements) the bioactivity of a protein. An agonist can be a wild-type protein or derivative thereof having at least one bioactivity of the wild-type protein. An agonist can also be a compound that upregulates expression of a gene or which increases at least one bioactivity of a protein. An agonist can also be a compound which increases the interaction of a polypeptide with another molecule, e.g., a target peptide or nucleic acid.

“Antagonist” as used herein is meant to refer to an agent that downregulates (e.g., suppresses or inhibits) at least one bioactivity of a protein. An antagonist can be a compound which inhibits or decreases the interaction between a protein and another molecule, e.g., a target peptide or enzyme substrate. An antagonist can also be a compound that downregulates expression of a gene or which reduces the amount of expressed protein present.

The term “antibody” refers to a protein that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term “antibody” encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, F(ab')₂, Fd fragments, Fv fragments, scFv, and domain antibodies (dAb) fragments (de Wildt et al., Eur J Immunol. 1996; **26**(3):629-39.)) as well as complete antibodies. An antibody can have the structural features of IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). Antibodies may be from any source, but primate (human and non-human primate) and primatized are preferred.

The VH and VL regions can be further subdivided into regions of hypervariability, termed “complementarity determining regions” (“CDRs”), interspersed with regions that are more conserved, termed “framework regions” (“FRs”). The extent of the framework region and CDRs have been defined (see, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) *J. Mol. Biol.*

196:901-917). Kabat definitions are used herein. Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

As used herein, an “immunoglobulin variable domain sequence” refers to an amino acid sequence which can form the structure of an immunoglobulin variable domain such that one or more CDR regions are positioned in a conformation suitable for an antigen binding site. For example, the sequence may include all or part of the amino acid sequence of a naturally-occurring variable domain. For example, the sequence may omit one, two or more N- or C-terminal amino acids, internal amino acids, may include one or more insertions or additional terminal amino acids, or may include other alterations. In one embodiment, a polypeptide that includes immunoglobulin variable domain sequence can associate with another immunoglobulin variable domain sequence to form an antigen binding site, e.g., a structure that preferentially interacts with IGF-II and/or IGF-IIIE.

The VH or VL chain of the antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. In IgGs, the heavy chain constant region includes three immunoglobulin domains, CH1, CH2 and CH3. The light chain constant region includes a CL domain. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The light chains of the immunoglobulin may be of types kappa or lambda. In one embodiment, the antibody is glycosylated. An antibody can be functional for antibody-dependent cytotoxicity and/or complement-mediated cytotoxicity.

One or more regions of an antibody can be human or effectively human. For example, one or more of the variable regions can be human or effectively human. For example, one or more of the CDRs can be human, e.g., HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3. Each of the light chain CDRs can be

human. HC CDR3 can be human. One or more of the framework regions can be human, e.g., FR1, FR2, FR3, and FR4 of the HC or LC. For example, the Fc region can be human. In one embodiment, all the framework regions are human, e.g., derived from a human somatic cell, e.g., a hematopoietic cell that produces immunoglobulins or a non-hematopoietic cell. In one embodiment, the human sequences are germline sequences, e.g., encoded by a germline nucleic acid. In one embodiment, the framework (FR) residues of a selected Fab can be converted to the amino-acid type of the corresponding residue in the most similar primate germline gene, especially the human germline gene. One or more of the constant regions can be human or effectively human. For example, at least 70, 75, 80, 85, 90, 92, 95, 98, or 100% of an immunoglobulin variable domain, the constant region, the constant domains (CH1, CH2, CH3, CL1), or the entire antibody can be human or effectively human.

All or part of an antibody can be encoded by an immunoglobulin gene or a segment thereof. Exemplary human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the many immunoglobulin variable region genes. Full-length immunoglobulin “light chains” (about 25 kDa or about 214 amino acids) are encoded by a variable region gene at the NH₂-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH-terminus. Full-length immunoglobulin “heavy chains” (about 50 kDa or about 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids). The length of human HC varies considerably because HC CDR3 varies from about 3 amino-acid residues to over 35 amino-acid residues.

The term “antigen-binding fragment” of a full length antibody refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to a target of interest. Examples of binding fragments encompassed within the term “antigen-binding fragment” of a full length antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment

consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR) that retains functionality.

Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules known as single chain Fv (scFv). See e.g., US patents 5,260,203, 4,946,778, and 4,881,175; Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883.

Antibody fragments can be obtained using any appropriate technique including conventional techniques known to those with skill in the art. The term “monospecific antibody” refers to an antibody that displays a single binding specificity and affinity for a particular target, e.g., epitope. This term includes a “monoclonal antibody” or “monoclonal antibody composition,” which as used herein refers to a preparation of antibodies or fragments thereof of single molecular composition, irrespective of how the antibody was generated.

As used herein, “binding affinity” refers to the apparent association constant or K_A . The K_A is the reciprocal of the dissociation constant (K_D). A binding protein may, for example, have a binding affinity of at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} and 10^{11} M^{-1} for a particular target molecule, e.g., IGF-II and/or IGF-IIE. Higher affinity binding of a binding protein to a first target relative to a second target can be indicated by a higher K_A (or a smaller numerical value K_D) for binding the first target than the K_A (or numerical value K_D) for binding the second target. In such cases, the binding protein has specificity for the first target (e.g., a protein in a first conformation or mimic thereof) relative to the second target (e.g., the same protein in a second conformation or mimic thereof; or a second protein). Differences in binding affinity (e.g., for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 91, 100, 500, 1000, 10,000 or 10^5 fold.

Binding affinity can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (e.g., using a fluorescence assay). Exemplary conditions for evaluating

binding affinity are in HBS-P buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 0.005% (v/v) Surfactant P20). These techniques can be used to measure the concentration of bound and free binding protein as a function of binding protein (or target) concentration. The concentration of bound binding protein ([Bound]) is related to the concentration of free binding protein ([Free]) and the concentration of binding sites for the binding protein on the target where (N) is the number of binding sites per target molecule by the following equation:

$$[\text{Bound}] = N \cdot [\text{Free}] / ((1/K_A) + [\text{Free}]).$$

It is not always necessary to make an exact determination of K_A , though, since sometimes it is sufficient to obtain a quantitative measurement of affinity, e.g., determined using a method such as ELISA or FACS analysis, is proportional to K_A , and thus can be used for comparisons, such as determining whether a higher affinity is, e.g., 2-fold higher, to obtain a qualitative measurement of affinity, or to obtain an inference of affinity, e.g., by activity in a functional assay, e.g., an *in vitro* or *in vivo* assay.

The term “binding protein” refers to a protein that can interact with a target molecule. This term is used interchangeably with “ligand.” An “IGF-II/IGF-IIE binding protein” refers to a protein that can interact with both IGF-II and IGF-IIE, and includes, in particular, proteins that preferentially interact with and/or inhibit both IGF-II and IGF-IIE. For example, the IGF-II/IGF-IIE binding protein is an antibody. Likewise, an “IGF-IIE binding protein” refers to a protein that can interact with only IGF-IIE, and includes, in particular, proteins that preferentially interact with and/or inhibit only IGF-IIE.

The term “cancer” is meant to refer to an abnormal cell or cells, or a mass of tissue. The growth of these cells or tissues exceeds and is uncoordinated with that of the normal tissues or cells, and persists in the same excessive manner after cessation of the stimuli which evoked the change. These neoplastic tissues or cells show a lack of structural organization and coordination relative to normal tissues or cells which may result in a mass of tissues or cells which can be either benign or malignant. As used herein, cancer includes any neoplasm. This includes, but is not limited to, melanoma, adenocarcinoma, malignant glioma, prostatic carcinoma, kidney carcinoma, bladder carcinoma, pancreatic carcinoma, thyroid carcinoma, lung carcinoma, colon carcinoma,

rectal carcinoma, brain carcinoma, liver carcinoma, breast carcinoma, ovary carcinoma, bone cancer, and the like.

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). It is possible for many framework and CDR amino acid residues to include one or more conservative substitutions. An IGF-II/IGF-IIIE binding protein may have mutations (e.g., at least one, two, or four, and/or less than 15, 10, 5, or 3) relative to a binding protein described herein (e.g., a conservative or non-essential amino acid substitutions), which do not have a substantial effect on protein function. Whether or not a particular substitution will be tolerated, i.e., will not adversely affect biological properties, such as binding activity can be predicted, e.g., by evaluating whether the mutation is conservative or by the method of Bowie, et al. (1990) *Science* 247:1306-1310.

Motif sequences for biopolymers can include positions which can be varied amino acids. For example, the symbol “X” in such a context generally refers to any amino acid (e.g., any of the twenty natural amino acids or any of the nineteen non-cysteine amino acids). Other allowed amino acids can also be indicated for example, using parentheses and slashes. For example, “(A/W/F/N/Q)” means that alanine, tryptophan, phenylalanine, asparagine, and glutamine are allowed at that particular position.

An “effectively human” immunoglobulin variable region is an immunoglobulin variable region that includes a sufficient number of human framework amino acid positions such that the immunoglobulin variable region does not elicit an immunogenic response in a normal human. An “effectively human” antibody is an antibody that includes a sufficient number of human amino acid positions such that the antibody does not elicit an immunogenic response in a normal human.

An “epitope” refers to the site on a target compound that is bound by a binding protein (e.g., an antibody such as a Fab or full length antibody). In the case where the target compound is a protein, the site can be entirely composed of amino acid components, entirely composed of chemical modifications of amino acids of the protein (e.g., glycosyl moieties), or composed of combinations thereof. Overlapping epitopes include at least one common amino acid residue, glycosyl group, phosphate group, sulfate group, or other molecular feature.

The term “growth hormone/growth hormone releasing hormone pathway modulator” refers to any compound or molecule that can modulate the activity of a component of the growth hormone/growth hormone releasing hormone pathway, e.g., an agonist or antagonist.

Calculations of “homology” or “sequence identity” between two sequences (the terms are used interchangeably herein) are performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The optimal alignment is determined as the best score using the GAP program in the GCG software package with a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid “identity” is equivalent to amino acid or nucleic acid “homology”). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences.

In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 92%, 95%, 97%, 98%, or 100% of the length of the reference sequence. For example, the reference sequence may be the length of the immunoglobulin variable domain sequence.

A “humanized” immunoglobulin variable region is an immunoglobulin variable region that is modified to include a sufficient number of human framework amino acid positions such that the immunoglobulin variable region does not elicit an immunogenic response in a normal human. Descriptions of “humanized” immunoglobulins include, for
5 example, US 6,407,213 and US 5,693,762.

As used herein, the term “hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions” describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-
10 6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: (1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); (2) medium
15 stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; (3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; and (4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very
20 high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified. The disclosure includes nucleic acids that hybridize with low, medium, high, or very high stringency to a nucleic acid described herein or to a complement thereof, e.g., nucleic acids encoding a binding protein described herein. The nucleic acids can be the same length or within 30, 20, or 10% of the length of the
25 reference nucleic acid. The nucleic acid can correspond to a region encoding an immunoglobulin variable domain sequence described herein.

An “isolated composition” refers to a composition that is removed from at least 90% of at least one component of a natural sample from which the isolated composition can be obtained. Compositions produced artificially or naturally can be “compositions of
30 at least” a certain degree of purity if the species or population of species of interests is at least 5, 10, 25, 50, 75, 80, 90, 92, 95, 98, or 99% pure on a weight-weight basis.

The term “modulator” refers to a polypeptide, nucleic acid, macromolecule, complex, molecule, small molecule, compound, species or the like (naturally-occurring or non-naturally-occurring), or an extract made from biological materials such as bacteria, plants, fungi, or animal cells or tissues, that may be capable of causing modulation.

5 Modulators may be evaluated for potential activity as inhibitors or activators (directly or indirectly) of a functional property, biological activity or process, or combination of them, (e.g., agonist, partial antagonist, partial agonist, inverse agonist, antagonist, anti-microbial agents, inhibitors of microbial infection or proliferation, and the like) by inclusion in assays. In such assays, many modulators may be screened at one time. The
10 activity of a modulator may be known, unknown or partially known.

A “non-essential” amino acid residue is a residue that can be altered from the wild-type sequence of the binding agent, e.g., the antibody, without abolishing or more preferably, without substantially altering a biological activity, whereas changing an “essential” amino acid residue results in a substantial loss of activity.

15 A “patient”, “subject” or “host” to be treated by the subject method may mean either a human or non-human animal.

As used herein, the term “substantially identical” (or “substantially homologous”) is used herein to refer to a first amino acid or nucleic acid sequence that contains a sufficient number of identical or equivalent (e.g., with a similar side chain, e.g.,
20 conserved amino acid substitutions) amino acid residues or nucleotides to a second amino acid or nucleic acid sequence such that the first and second amino acid or nucleic acid sequences have (or encode proteins having) similar activities, e.g., a binding activity, a binding preference, or a biological activity. In the case of antibodies, the second antibody has the same specificity and has at least 50%, at least 25%, or at least 10% of the affinity
25 relative to the same antigen.

Sequences similar or homologous (e.g., at least about 85% sequence identity) to the sequences disclosed herein are also part of this application. In some embodiments, the sequence identity can be about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher. In addition, substantial identity exists when the nucleic acid
30 segments hybridize under selective hybridization conditions (e.g., highly stringent

hybridization conditions), to the complement of the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

Statistical significance can be determined by any art known method. Exemplary statistical tests include: the Students T-test, Mann Whitney U non-parametric test, and Wilcoxon non-parametric statistical test. Some statistically significant relationships have a P value of less than 0.05 or 0.02. Particular binding proteins may show a difference, e.g., in specificity or binding, which are statistically significant (e.g., P value < 0.05 or 0.02). The terms “induce”, “inhibit”, “potentiate”, “elevate”, “increase”, “decrease” or the like, e.g., which denote distinguishable qualitative or quantitative differences between two states, and may refer to a difference, e.g., a statistically significant difference, between the two states.

The term “treat” or “treatment” refers to the application or administration of an agent, alone or in combination with one or more other agents (e.g., a second agent) to a subject, e.g., a patient, e.g., a patient who has a disorder (e.g., a disorder as described herein), a symptom of a disorder or a predisposition for a disorder, e.g., to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. Treating a cell refers to a reduction in an activity of a cell, e.g., ability of an endothelial cell to form tubes or vessels. A reduction does not necessarily require a total elimination of activity, but a reduction, e.g., a statistically significant reduction, in the activity or the number of cells.

IGF-II/IGF-IIE Binding Proteins

The disclosure provides proteins that bind to both or either IGF-II and/or IGF-IIE (e.g., human IGF-II and/or IGF-IIE) and include at least one immunoglobulin variable region. For example, the IGF-II/IGF-IIE binding protein includes a heavy chain (HC) immunoglobulin variable domain sequence and a light chain (LC) immunoglobulin variable domain sequence. A number of exemplary IGF-II/IGF-IIE and IGF-IIE binding proteins are described herein.

The IGF-II/IGF-IIE binding protein may be an isolated protein (e.g., at least 70, 80, 90, 95, or 99% free of other proteins).

The IGF-II/IGF-IIE binding protein may additionally inhibit both IGF-II and IGF-IIE, e.g., human IGF-II and IGF-IIE.

In one aspect, the disclosure features a protein (e.g., an isolated protein) that binds to IGF-II and IGF-IIE (e.g., human IGF-II and IGF-IIE) and includes at least one immunoglobulin variable region. For example, the protein includes a heavy chain (HC) immunoglobulin variable domain sequence and a light chain (LC) immunoglobulin variable domain sequence. In one embodiment, the protein binds to and inhibits IGF-II and IGF-IIE, e.g., human IGF-II and/or IGF-IIE.

The protein can include one or more of the following characteristics: (a) a human CDR or human framework region; (b) the HC immunoglobulin variable domain sequence comprises one or more CDRs that are at least 85, 88, 90, 92, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a LC variable domain described herein; (c) the LC immunoglobulin variable domain sequence comprises one or more CDRs that are at least 85, 88, 90, 92, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a HC variable domain described herein; (d) the LC immunoglobulin variable domain sequence is at least 85, 88, 90, 92, 94, 95, 96, 97, 98, 99, or 100% identical to a LC variable domain described herein; (e) the HC immunoglobulin variable domain sequence is at least 85, 88, 90, 92, 94, 95, 96, 97, 98, 99, or 100% identical to a HC variable domain described herein; (f) the protein binds an epitope bound by a protein described herein, or an epitope that overlaps with such epitope; and (g) a primate CDR or primate framework region.

In certain embodiments, the protein binds the following epitope of IGF-II, or a fragment thereof:

TXCGGXLVXXLXXXXXXXXXXFXXXXPXXRVXXRSGXVEEXCFRXXXXXXXXXXY

wherein X is any amino acid.

More particularly, the protein may bind the following sequence of IGF-II, or a fragment thereof:

SETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPA

wherein the non-bolded residues may be substituted with conservative mutations.

The protein can bind to human IGF-II and IGF-IIE, e.g., human IGF-II and/or IGF-IIE, with a binding affinity of at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} and 10^{11} M⁻¹. In one embodiment, the protein binds to human IGF-II and/or IGF-IIE with a K_{off} slower than 1×10^{-3} , 5×10^{-4} s⁻¹, or 1×10^{-4} s⁻¹. In one embodiment, the protein binds to human

IGF-II and/or IGF-IIIE with a K_{on} faster than 1×10^2 , 1×10^3 , or $5 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$. In one embodiment, the protein inhibits human human IGF-II and IGF-IIIE activity, e.g., with a K_i of less than 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , and 10^{-10} M . The protein can have, for example, an IC_{50} of less than 100 nM, 10 nM or 1 nM. For example, the protein may modulate

5 IGF-I receptor (IGF-1R) and an isoform of the insulin receptor (IR-A) activity, as well as IGF-II and IGF-IIIE. The protein may inhibit IGF-1R, IR-A, and IGF-II and IGF-IIIE activity. The affinity of the protein for human IGF-II and/or IGF-IIIE can be characterized by a K_D of less than 100 nM, less than 10 nM, or less than 1 nM.

IGF-II/IGF-IIIE binding proteins may be antibodies. IGF-II/IGF-IIIE binding

10 antibodies may have their HC and LC variable domain sequences included in a single polypeptide (e.g., scFv), or on different polypeptides (e.g., IgG or Fab).

In a preferred embodiment, the protein is a human antibody having the light and heavy chains of antibodies picked from the list comprising M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and

15 M0072-G06. In a preferred embodiment, the protein is a human antibody having its heavy chain picked from the list comprising: M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and M0072-G06. In a preferred embodiment, the protein is a human antibody having its light chain picked from the list comprising: M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03,

20 M0070-H08, M0072-C06, M0072-E03, and M0072-G06. In a preferred embodiment, the protein is a human antibody having one or more heavy chain CDRs picked from the corresponding CDRs of the list of heavy chains comprising M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and M0072-G06. In a preferred embodiment, the protein is a human antibody having one or

25 more light chain CDRs picked from the corresponding CDRs of the list of heavy chains comprising M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and M0072-G06.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain. In another, the HC and LC variable domain sequences are

30 components of different polypeptide chains. For example, the protein is an IgG., e.g., IgG1, IgG2, IgG3, or IgG4. The protein can be a soluble Fab. In other implementations

the protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA + VH::CH1, HSA::LC + VH::CH1, or other appropriate construction.

In one embodiment, the protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions. In one embodiment, the protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions. In one embodiment, the protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibbons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In some embodiments, the affinity of the primate antibody for human IGF-II and IGF-IIIE is characterized by a K_D of less than 1 nM.

In certain embodiments, the protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In certain embodiments, the protein may be capable of binding to tumor cells expressing IGF-II and/or IGF-IIIE, e.g., to colorectal cell lines SW1116 (Grade A), SW480 (Grade B), HT29*, HT29, SW480, CaCO2, HCT116, SW620 (all Grade C), and COLO 205 (Grade D); breast cancer cell lines MCF-7* and 4T1; uterine cancer cell line SKUT-1 (mesodermal tumor), rhabdomyosarcoma cell lines, and hepatocellular carcinoma cell lines HepG2, HuH7 and Hep3B.

IGF-II and IGF-IIE

Exemplary IGF-II and IGF-IIE sequences against which IGF-II/IGF-IIE binding proteins may be developed can include the human or mouse IGF-II and IGF-IIE amino acid sequence, a sequence that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to one of these sequences, or a fragment thereof, e.g., a fragment without the signal sequence or prodomain. The human and mouse IGF-II and IGF-IIE amino acid sequences, and the mRNA sequences encoding them, are illustrated below.

IGF-II

10 >insulin-like growth factor II [human, small cell lung cancer cell line T3M-11, mRNA, 1322 nt] (truncated from ACCESSION S77035)

gctta ccgccccagt gagaccctgt gcggcgggga gctggtggac accctccagt
tcgtctgtgg ggaccgcggcttctacttca gcaggccgc aagccgtgtg agccgtcgca
gccgtggcat cgttgaggagtgtgtttcc gcagctgtga cctggccctc ctggagacgt
15 actgtgctac ccccgccaagtccgag

>insulin-like growth factor II; IGF-II [Homo sapiens]. (truncated from ACCESSION AAB34155)

ayrpsetlccgelvdtlqfvcgdrfyfsrpasrvsrrsrgiveeccfrscdlalletycatpakse

20

>Mus musculus insulin-like growth factor 2, mRNA (cDNA clone MGC:60598 IMAGE:30013295), complete cds. (truncated from ACCESSION BC053489)

gc ttacggcccc ggagagactctgtgcggagg ggagcttggt gacacgcttc
agtttgtctg ttccgaccgc ggcttctacttcagcaggcc ttcaagccgt gccaacgctc
25 gcagccgtgg catcgtggaa gagtgtgtgttccgcagctg cgacctggcc ctccctggaga
catactgtgc cccccccgcc aagtccgag

>Igf2 protein [Mus musculus]. (truncated from ACCESSION AAH53489)

aygpgetlccgelvdtlqfvcsdrgfyfsrpssranrrsrgiveeccfrscdlalletycatpakse

30

IGF-IIIE

>insulin-like growth factor II [human, small cell lung cancer cell line T3M-11, mRNA,
1322 nt] (truncated from ACCESSION S77035)

```

atgggaa tcccaatggg gaagtcgatgctggtgcttc tcaccttctt ggccttcgcc
5  tcgtgctgca ttgctgctta ccgccccagtgagaccctgt gcggcgggga gctggtggac
accctccagt tcgtctgtgg ggaccgcggcttctacttca gcaggcccg c aagccgtgtg
agccgtcgca gccgtggcat cgttgaggagtgtgtttcc gcagctgtga cctggccctc
ctggagacgt actgtgctac ccccgccaagtccgagaggg acgtgtcgac ccctccgacc
gtgcttccgg acaacttccc cagataccccgtgggcaagt tcttccaata tgacacctgg
10 aagcagtcca ccagcgcct gcgcaggggacctgcctgccc tcctgcgtgc ccgccggggt
cacgtgctcg ccaaggagct cgaggcgttcaggagaggcca aacgtcaccg tcccctgatt
gctctacca cccaagaccc cgcccacggggggcgcccccc cagagatggc cagcaatcgg
aag

```

15 >insulin-like growth factor II; IGF-II [Homo sapiens]. (ACCESSION AAB34155)

```

mgipmgksml vlltflafas cciaayrpse tlcggelvd t lqfvcgdr gf yfsrparvsrrsrgiveec cfrscdlall
etycatpaks erdvstpptv lpdnfpryp v gkffqydtwkqstqrlrrgl pallrarrgh vlakeleafr eakrhrplia
lptqdpahgg appemasnrk

```

>Mus musculus insulin-like growth factor 2, mRNA (cDNA clone MGC:60598 IMAGE:30013295), complete cds. (truncated from ACCESSION BC053489)

```

atgg ggatcccagt ggggaagtcg atgttggtgcttctcatctc tttggccttc
gccttgtgct gcatcgctgc ttacggcccc ggagagactctgtgcggagg ggagcttggt
5  gacacgcttc agtttgtctg ttcggaccgc ggcttctacttcagcaggcc ttcaagccgt
gccaaccgtc gcagccgtgg catcgtggaa gagtgctgcttccgcagctg cgacctggcc
ctcctggaga catactgtgc ccccccgcc aagtccgagagggacgtgtc tacctctcag
gccgtacttc cggacgactt cccagatac cccgtgggcaagttcttcca atatgacacc
tgagagacagt ccgcgggacg cctgcgcaga ggctgcctgccctcctgcg tgcccgccgg
10 ggtcgcatgc ttgccaaaga gctcaaagag ttcagagaggccaaacgtca tcgtcccttg
atcgtgttac cacccaaaga ccccgccac gggggagcctcttcggagat gtccagcaac
catcag

```

>IgF2 protein [Mus musculus]. (ACCESSION AAH53489)

```

15  mgipvgksml vllislafal cciaaygpge tleggclvdt lqfvcdrgrf yfsrpssranrrsrgiveec cfrscdlall
etycatpaks erdvstsqav lpddfprypv gkffqydtwrqsagrlrrgl pallrarrgr mlakelkefr eakrhrpliv
lppkdpahgg assemssnhq

```

Display Libraries

A display library is a collection of entities; each entity includes an accessible

20 polypeptide component and a recoverable component that encodes or identifies the polypeptide component. The polypeptide component is varied so that different amino acid sequences are represented. The polypeptide component can be of any length, e.g., from three amino acids to over 300 amino acids. A display library entity can include more than one polypeptide component, for example, the two polypeptide chains of an

25 sFab. In one exemplary implementation, a display library can be used to identify proteins that bind to both IGF-II and IGF-IIE. In a selection, the polypeptide component of each member of the library is probed with IGF-II and/or IGF-IIE or fragment thereof) and if the polypeptide component binds to the IGF-II and/or IGF-IIE, the display library member is identified, typically by retention on a support.

Retained display library members are recovered from the support and analyzed. The analysis can include amplification and a subsequent selection under similar or dissimilar conditions. For example, positive and negative selections can be alternated. The analysis can also include determining the amino acid sequence of the polypeptide component and purification of the polypeptide component for detailed characterization.

A variety of formats can be used for display libraries. Examples include the following.

Phage Display: The protein component is typically covalently linked to a bacteriophage coat protein. The linkage results from translation of a nucleic acid encoding the protein component fused to the coat protein. The linkage can include a flexible peptide linker, a protease site, or an amino acid incorporated as a result of suppression of a stop codon. Phage display is described, for example, in U.S. 5,223,409; Smith (1985) *Science* 228:1315-1317; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; WO 90/02809; de Haard *et al.* (1999) *J. Biol. Chem* 274:18218-30; Hoogenboom *et al.* (1998) *Immunotechnology* 4:1-20; Hoogenboom *et al.* (2000) *Immunol Today* 2:371-8 and Hoet *et al.* (2005) *Nat Biotechnol.* **23**(3)344-8. Bacteriophage displaying the protein component can be grown and harvested using standard phage preparatory methods, e.g., PEG precipitation from growth media. After selection of individual display phages, the nucleic acid encoding the selected protein components can be isolated from cells infected with the selected phages or from the phage themselves, after amplification. Individual colonies or plaques can be picked, the nucleic acid isolated and sequenced.

Other Display Formats. Other display formats include cell based display (see, e.g., WO 03/029456), protein-nucleic acid fusions (see, e.g., US 6,207,446), ribosome display (See, e.g., Mattheakis *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:9022 and Hanes *et al.* (2000) *Nat Biotechnol.* 18:1287-92; Hanes *et al.* (2000) *Methods Enzymol.* 328:404-30; and Schaffitzel *et al.* (1999) *J Immunol Methods.* 231(1-2):119-35), and *E. coli* periplasmic display (*J Immunol Methods.* 2005 Nov 22;PMID: 16337958).

Scaffolds. Scaffolds useful for display include: antibodies (e.g., Fab fragments, single chain Fv molecules (scFV), single domain antibodies, camelid antibodies, and camelized antibodies); T-cell receptors; MHC proteins; extracellular domains (e.g.,

fibronectin Type III repeats, EGF repeats); protease inhibitors (e.g., Kunitz domains, ecotin, BPTI, and so forth); TPR repeats; trifoil structures; zinc finger domains; DNA-binding proteins; particularly monomeric DNA binding proteins; RNA binding proteins; enzymes, e.g., proteases (particularly inactivated proteases), RNase; chaperones, e.g.,
5 thiorodoxin and heat shock proteins; intracellular signaling domains (such as SH2 and SH3 domains); linear and constrained peptides; and linear peptide substrates. Display libraries can include synthetic and/or natural diversity. See, e.g., US 2004-0005709.

Display technology can also be used to obtain binding proteins (e.g., antibodies) that bind particular epitopes of a target. This can be done, for example, by using
10 competing non-target molecules that lack the particular epitope or are mutated within the epitope, e.g., with alanine. Such non-target molecules can be used in a negative selection procedure as described below, as competing molecules when binding a display library to the target, or as a pre-elution agent, e.g., to capture in a wash solution dissociating display library members that are not specific to the target.

15 **Iterative Selection.** In one preferred embodiment, display library technology is used in an iterative mode. A first display library is used to identify one or more binding proteins for a target. These identified binding proteins are then varied using a mutagenesis method to form a second display library. Higher affinity binding proteins are then selected from the second library, e.g., by using higher stringency or more
20 competitive binding and washing conditions.

In some implementations, the mutagenesis is targeted to regions at the binding interface. If, for example, the identified binding proteins are antibodies, then mutagenesis can be directed to the CDR regions of the heavy or light chains as described herein. Further, mutagenesis can be directed to framework regions near or adjacent to the
25 CDRs. In the case of antibodies, mutagenesis can also be limited to one or a few of the CDRs, e.g., to make precise step-wise improvements. Exemplary mutagenesis techniques include: error-prone PCR, recombination, DNA shuffling, site-directed mutagenesis and cassette mutagenesis.

In one example of iterative selection, the methods described herein are used to
30 first identify a protein from a display library that binds both IGF-II and IGF-IIIE, with at least a minimal binding specificity for a target or a minimal activity, e.g., an equilibrium

dissociation constant for binding of less than 1 nM, 10 nM, or 100 nM. The nucleic acid sequence encoding the initial identified proteins are used as a template nucleic acid for the introduction of variations, e.g., to identify a second protein that has enhanced properties (e.g., binding affinity, kinetics, or stability) relative to the initial protein.

5 **Off-Rate Selection.** Since a slow dissociation rate can be predictive of high affinity, particularly with respect to interactions between polypeptides and their targets, the methods described herein can be used to isolate binding proteins with a desired (e.g., reduced) kinetic dissociation rate for a binding interaction to a target.

10 To select for slow dissociating binding proteins from a display library, the library is contacted to an immobilized target. The immobilized target is then washed with a first solution that removes non-specifically or weakly bound biomolecules. Then the bound binding proteins are eluted with a second solution that includes a saturating amount of free target or a target specific high-affinity competing monoclonal antibody, i.e., replicates of the target that are not attached to the particle. The free target binds to
15 biomolecules that dissociate from the target. Rebinding is effectively prevented by the saturating amount of free target relative to the much lower concentration of immobilized target.

20 The second solution can have solution conditions that are substantially physiological or that are stringent. Typically, the solution conditions of the second solution are identical to the solution conditions of the first solution. Fractions of the second solution are collected in temporal order to distinguish early from late fractions. Later fractions include biomolecules that dissociate at a slower rate from the target than biomolecules in the early fractions.

25 Further, it is also possible to recover display library members that remain bound to the target even after extended incubation. These can either be dissociated using chaotropic conditions or can be amplified while attached to the target. For example, phage bound to the target can be contacted to bacterial cells.

30 **Selecting or Screening for Specificity.** The display library screening methods described herein can include a selection or screening process that discards display library members that bind to a non-target molecule. Examples of non-target molecules include streptavidin on magnetic beads, blocking agents such as bovine serum albumin, non-fat

bovine milk, soy protein, any capturing or target immobilizing monoclonal antibody, or non-transfected cells which do not express the target.

In one implementation, a so-called “negative selection” step is used to discriminate between the target and related non-target molecule and a related, but distinct non-target molecules. The display library or a pool thereof is contacted to the non-target molecule. Members of the sample that do not bind the non-target are collected and used in subsequent selections for binding to the target molecule or even for subsequent negative selections. The negative selection step can be prior to or after selecting library members that bind to the target molecule.

In another implementation, a screening step is used. After display library members are isolated for binding to the target molecule, each isolated library member is tested for its ability to bind to a non-target molecule (e.g., a non-target listed above). For example, a high-throughput ELISA screen can be used to obtain this data. The ELISA screen can also be used to obtain quantitative data for binding of each library member to the target as well as for cross species reactivity to related targets or subunits of the target (e.g., IGF-II and/or IGF-IIIE) and also under different condition such as pH6 or pH 7.5. The non-target and target binding data are compared (e.g., using a computer and software) to identify library members that specifically bind to the target.

Other Exemplary Expression Libraries

Other types of collections of proteins (e.g., expression libraries) can be used to identify proteins with a particular property (e.g., ability to bind IGF-II and IGF-IIIE), including, e.g., protein arrays of antibodies (see, e.g., De Wildt et al. (2000) Nat. Biotechnol. 18:989-994), lambda gt11 libraries, two-hybrid libraries and so forth.

Exemplary Libraries

It is possible to immunize a non-human primate and recover primate antibody genes that can be displayed on phage (see below). From such a library, one can select antibodies that bind the antigen used in immunization. See, for example, Vaccine. (2003) 22(2):257-67 or Immunogenetics. (2005) 57(10):730-8. Thus one could obtain primate antibodies that bind and inhibit IGF-II and IGF-IIIE by immunizing a chimpanzee or macaque and using a variety of means to select or screen for primate antibodies that bind

and inhibit IGF-II and IGF-IIIE. One can also make chimeras of primatized Fabs with human constant regions, see Curr Opin Mol Ther. (2004) 6(6):675-83. "PRIMATIZED antibodies, genetically engineered from cynomolgus macaque monkey and human components, are structurally indistinguishable from human antibodies. They may, therefore, be less likely to cause adverse reactions in humans, making them potentially suited for long-term, chronic treatment" Curr Opin Investig Drugs. (2001) 2(5):635-8.

One exemplary type of library presents a diverse pool of polypeptides, each of which includes an immunoglobulin domain, e.g., an immunoglobulin variable domain. Of interest are display libraries where the members of the library include primate or "primatized" (e.g., such as human, non-human primate or "humanized") immunoglobulin domains (e.g., immunoglobulin variable domains) or chimeric primatized Fabs with human constant regions. Human or humanized immunoglobulin domain libraries may be used to identify human or "humanized" antibodies that, for example, recognize human antigens. Because the constant and framework regions of the antibody are human, these antibodies may avoid themselves being recognized and targeted as antigens when administered to humans. The constant regions may also be optimized to recruit effector functions of the human immune system. The in vitro display selection process surmounts the inability of a normal human immune system to generate antibodies against self-antigens.

A typical antibody display library displays a polypeptide that includes a VH domain and a VL domain. An "immunoglobulin domain" refers to a domain from the variable or constant domain of immunoglobulin molecules. Immunoglobulin domains typically contain two β -sheets formed of about seven β -strands, and a conserved disulphide bond (see, e.g., A. F. Williams and A. N. Barclay, 1988, *Ann. Rev. Immunol.* 6:381-405). The display library can display the antibody as a Fab fragment (e.g., using two polypeptide chains) or a single chain Fv (e.g., using a single polypeptide chain). Other formats can also be used.

As in the case of the Fab and other formats, the displayed antibody can include one or more constant regions as part of a light and/or heavy chain. In one embodiment, each chain includes one constant region, e.g., as in the case of a Fab. In other embodiments, additional constant regions are displayed.

Antibody libraries can be constructed by a number of processes (see, e.g., de Haard et al., 1999, *J. Biol. Chem.* 274:18218-30; Hoogenboom et al., 1998, *Immunotechnology* 4:1-20; Hoogenboom et al., 2000, *Immunol. Today* 21:371-378, and Hoet et al. (2005) *Nat Biotechnol.* **23**(3)344-8. Further, elements of each process can be combined with those of other processes. The processes can be used such that variation is introduced into a single immunoglobulin domain (e.g., VH or VL) or into multiple immunoglobulin domains (e.g., VH and VL). The variation can be introduced into an immunoglobulin variable domain, e.g., in the region of one or more of CDR1, CDR2, CDR3, FR1, FR2, FR3, and FR4, referring to such regions of either and both of heavy and light chain variable domains. The variation(s) may be introduced into all three CDRs of a given variable domain, or into CDR1 and CDR2, e.g., of a heavy chain variable domain. Any combination is feasible. In one process, antibody libraries are constructed by inserting diverse oligonucleotides that encode CDRs into the corresponding regions of the nucleic acid. The oligonucleotides can be synthesized using monomeric nucleotides or trinucleotides. For example, Knappik et al., 2000, *J. Mol. Biol.* 296:57-86 describe a method for constructing CDR encoding oligonucleotides using trinucleotide synthesis and a template with engineered restriction sites for accepting the oligonucleotides.

In another process, an animal, e.g., a rodent, is immunized with IGF-II and IGF-III. The animal is optionally boosted with the antigen to further stimulate the response. Then spleen cells are isolated from the animal, and nucleic acid encoding VH and/or VL domains is amplified and cloned for expression in the display library.

In yet another process, antibody libraries are constructed from nucleic acid amplified from naïve germline immunoglobulin genes. The amplified nucleic acid includes nucleic acid encoding the VH and/or VL domain. Sources of immunoglobulin-encoding nucleic acids are described below. Amplification can include PCR, e.g., with primers that anneal to the conserved constant region, or another amplification method.

Nucleic acid encoding immunoglobulin domains can be obtained from the immune cells of, e.g., a primate (e.g., a human), mouse, rabbit, camel, or rodent. In one example, the cells are selected for a particular property. B cells at various stages of maturity can be selected. In another example, the B cells are naïve.

In one embodiment, fluorescent-activated cell sorting (FACS) is used to sort B cells that express surface-bound IgM, IgD, or IgG molecules. Further, B cells expressing different isotypes of IgG can be isolated. In another preferred embodiment, the B or T cells are cultured in vitro. The cells can be stimulated in vitro, e.g., by culturing with
5 feeder cells or by adding mitogens or other modulatory reagents, such as antibodies to CD40, CD40 ligand or CD20, phorbol myristate acetate, bacterial lipopolysaccharide, concanavalin A, phytohemagglutinin, or pokeweed mitogen.

In another embodiment, the cells are isolated from a subject that has a disease of condition described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast
10 cancer), an inflammatory disease (e.g., synovitis, atherosclerosis), rheumatoid arthritis, osteoarthritis, an ocular condition (e.g., macular degeneration), diabetes, Alzheimer's Disease, cerebral ischemia, endometriosis, fibrin-invasive activity, angiogenesis, or capillary tube formation. In another embodiment, the cells are isolated from a transgenic non-human animal that includes a human immunoglobulin locus.

15 In one preferred embodiment, the cells have activated a program of somatic hypermutation. Cells can be stimulated to undergo somatic mutagenesis of immunoglobulin genes, for example, by treatment with anti-immunoglobulin, anti-CD40, and anti-CD38 antibodies (see, e.g., Bergthorsdottir et al., 2001, *J. Immunol.* 166:2228). In another embodiment, the cells are naïve.

20 The nucleic acid encoding an immunoglobulin variable domain can be isolated from a natural repertoire by the following exemplary method. First, RNA is isolated from the immune cell. Full length (i.e., capped) mRNAs are separated (e.g., by degrading uncapped RNAs with calf intestinal phosphatase). The cap is then removed with tobacco acid pyrophosphatase and reverse transcription is used to produce the
25 cDNAs.

The reverse transcription of the first (antisense) strand can be done in any manner with any suitable primer. See, e.g., de Haard et al., 1999, *J. Biol. Chem.* 274:18218-30. The primer binding region can be constant among different immunoglobulins, e.g., in order to reverse transcribe different isotypes of immunoglobulin. The primer binding
30 region can also be specific to a particular isotype of immunoglobulin. Typically, the primer is specific for a region that is 3' to a sequence encoding at least one CDR. In

another embodiment, poly-dT primers may be used (and may be preferred for the heavy-chain genes).

A synthetic sequence can be ligated to the 3' end of the reverse transcribed strand. The synthetic sequence can be used as a primer binding site for binding of the forward primer during PCR amplification after reverse transcription. The use of the synthetic sequence can obviate the need to use a pool of different forward primers to fully capture the available diversity.

The variable domain-encoding gene is then amplified, e.g., using one or more rounds. If multiple rounds are used, nested primers can be used for increased fidelity. The amplified nucleic acid is then cloned into a display library vector.

Secondary Screening Methods

After selecting candidate library members that bind to a target, each candidate library member can be further analyzed, e.g., to further characterize its binding properties for the target, e.g., IGF-II and/or IGF-IIIE, or for binding to other protein, e.g., another metalloproteinase. Each candidate library member can be subjected to one or more secondary screening assays. The assay can be for a binding property, a catalytic property, an inhibitory property, a physiological property (e.g., cytotoxicity, renal clearance, immunogenicity), a structural property (e.g., stability, conformation, oligomerization state) or another functional property. The same assay can be used repeatedly, but with varying conditions, e.g., to determine pH, ionic, or thermal sensitivities.

As appropriate, the assays can use a display library member directly, a recombinant polypeptide produced from the nucleic acid encoding the selected polypeptide, or a synthetic peptide synthesized based on the sequence of the selected polypeptide. In the case of selected Fabs, the Fabs can be evaluated or can be modified and produced as intact IgG proteins. Exemplary assays for binding properties include the following.

ELISA. Binding proteins can be evaluated using an ELISA assay. For example, each protein is contacted to a microtitre plate whose bottom surface has been coated with the target, e.g., a limiting amount of the target. The plate is washed with buffer to

remove non-specifically bound polypeptides. Then the amount of the binding protein bound to the target on the plate is determined by probing the plate with an antibody that can recognize the binding protein, e.g., a tag or constant portion of the binding protein. The antibody is linked to a detection system (e.g., an enzyme such as alkaline phosphatase or horse radish peroxidase (HRP) which produces a colorimetric product when appropriate substrates are provided).

Homogeneous Binding Assays. The ability of a binding protein described herein to bind a target can be analyzed using a homogenous assay, i.e., after all components of the assay are added, additional fluid manipulations are not required. For example, fluorescence resonance energy transfer (FRET) can be used as a homogenous assay (see, for example, Lakowicz et al., U.S. Patent No. 5,631,169; Stavrianopoulos, et al., U.S. Patent No. 4,868,103). A fluorophore label on the first molecule (e.g., the molecule identified in the fraction) is selected such that its emitted fluorescent energy can be absorbed by a fluorescent label on a second molecule (e.g., the target) if the second molecule is in proximity to the first molecule. The fluorescent label on the second molecule fluoresces when it absorbs to the transferred energy. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. A binding event that is configured for monitoring by FRET can be conveniently measured through standard fluorometric detection means, e.g., using a fluorimeter. By titrating the amount of the first or second binding molecule, a binding curve can be generated to estimate the equilibrium binding constant.

Another example of a homogenous assay is ALPHASCREEN™ (Packard Bioscience, Meriden CT). ALPHASCREEN™ uses two labeled beads. One bead generates singlet oxygen when excited by a laser. The other bead generates a light signal when singlet oxygen diffuses from the first bead and collides with it. The signal is only generated when the two beads are in proximity. One bead can be attached to the display library member, the other to the target. Signals are measured to determine the extent of binding.

Surface Plasmon Resonance (SPR). The interaction of binding protein and a target can be analyzed using SPR. SPR or Biomolecular Interaction Analysis (BIA) detects biospecific interactions in real time, without labeling any of the interactants. Changes in the mass at the binding surface (indicative of a binding event) of the BIA chip result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)). The changes in the refractivity generate a detectable signal, which are measured as an indication of real-time reactions between biological molecules. Methods for using SPR are described, for example, in U.S. Patent No. 5,641,640; Raether, 1988, *Surface Plasmons* Springer Verlag; Sjolander and Urbaniczky, 1991, *Anal. Chem.* 63:2338-2345; Szabo et al., 1995, *Curr. Opin. Struct. Biol.* 5:699-705 and on-line resources provide by BIAcore International AB (Uppsala, Sweden).

Information from SPR can be used to provide an accurate and quantitative measure of the equilibrium dissociation constant (K_D), and kinetic parameters, including K_{on} and K_{off} , for the binding of a binding protein to a target. Such data can be used to compare different biomolecules. For example, selected proteins from an expression library can be compared to identify proteins that have high affinity for the target or that have a slow K_{off} . This information can also be used to develop structure-activity relationships (SAR). For example, the kinetic and equilibrium binding parameters of matured versions of a parent protein can be compared to the parameters of the parent protein. Variant amino acids at given positions can be identified that correlate with particular binding parameters, e.g., high affinity and slow K_{off} . This information can be combined with structural modeling (e.g., using homology modeling, energy minimization, or structure determination by x-ray crystallography or NMR). As a result, an understanding of the physical interaction between the protein and its target can be formulated and used to guide other design processes.

Cellular Assays. Binding proteins can be screened for ability to bind to cells which transiently or stably express and display the target of interest on the cell surface. For example, IGF-II/IGF-IIIE binding proteins can be fluorescently labeled and binding to IGF-II and/or IGF-IIIE in the presence or absence of antagonistic antibody can be detected by a change in fluorescence intensity using flow cytometry e.g., a FACS machine.

Other Exemplary Methods for Obtaining IGF-II/IGF-IIE Binding Antibodies

In addition to the use of display libraries, other methods can be used to obtain an IGF-II/IGF-IIE binding antibody. For example, IGF-II and/or IGF-IIE protein or a region thereof can be used as an antigen in a non-human animal, e.g., a rodent.

5 In one embodiment, the non-human animal includes at least a part of a human immunoglobulin gene. For example, it is possible to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci. Using the hybridoma technology, antigen-specific monoclonal antibodies (Mabs) derived from the genes with the desired specificity may be produced and selected. See, e.g.,
10 XENOMOUSE™, Green et al., 1994, *Nat. Gen.* 7:13-21; U.S. 2003-0070185, WO 96/34096, published Oct. 31, 1996, and PCT Application No. PCT/US96/05928, filed Apr. 29, 1996.

In another embodiment, a monoclonal antibody is obtained from the non-human animal, and then modified, e.g., humanized or deimmunized. Winter describes a CDR-grafting method that may be used to prepare the humanized antibodies (UK Patent
15 Application GB 2188638A, filed on March 26, 1987; US Patent No. 5,225,539. All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized
20 antibody to a predetermined antigen.

Humanized antibodies can be generated by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986,
25 *BioTechniques* 4:214, and by Queen et al. US Patent Nos. 5,585,089, US 5,693,761 and US 5,693,762. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Numerous sources of such nucleic acid are available. For example, nucleic acids may be obtained from a hybridoma producing an
30 antibody against a predetermined target, as described above. The recombinant DNA

encoding the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

Reducing Immunogenicity of IGF-II/IGF-IIIE Binding Proteins

Immunoglobulin IGF-II/IGF-IIIE binding proteins (e.g., IgG or Fab IGF-II/IGF-IIIE binding proteins) may be modified to reduce immunogenicity. Reduced immunogenicity is desirable in IGF-II/IGF-IIIE binding proteins intended for use as therapeutics, as it reduces the chance that the subject will develop an immune response against the therapeutic molecule. Techniques useful for reducing immunogenicity of IGF-II/IGF-IIIE binding proteins include deletion/modification of potential human T cell epitopes and ‘germlining’ of sequences outside of the CDRs (e.g., framework and Fc).

An IGF-II/IGF-IIIE- binding antibody may be modified by specific deletion of human T cell epitopes or "deimmunization" by the methods disclosed in WO 98/52976 and WO 00/34317. Briefly, the heavy and light chain variable regions of an antibody are analyzed for peptides that bind to MHC Class II; these peptides represent potential T-cell epitopes (as defined in WO 98/52976 and WO 00/34317). For detection of potential T-cell epitopes, a computer modeling approach termed “peptide threading” can be applied, and in addition a database of human MHC class II binding peptides can be searched for motifs present in the VH and VL sequences, as described in WO 98/52976 and WO 00/34317. These motifs bind to any of the 18 major MHC class II DR allotypes, and thus constitute potential T cell epitopes. Potential T-cell epitopes detected can be eliminated by substituting small numbers of amino acid residues in the variable regions, or preferably, by single amino acid substitutions. As far as possible conservative substitutions are made, often but not exclusively, an amino acid common at this position in human germline antibody sequences may be used. Human germline sequences are disclosed in Tomlinson, I.A. et al., 1992, *J. Mol. Biol.* 227:776-798; Cook, G. P. et al., 1995, *Immunol. Today* Vol. 16 (5): 237-242; Chothia, D. et al., 1992, *J. Mol. Bio.* 227:799-817. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, I.A. et al. MRC Centre for Protein Engineering, Cambridge, UK). After the deimmunizing changes are identified, nucleic acids encoding V_H and V_L can be constructed by mutagenesis or other synthetic methods (e.g., de novo synthesis, cassette replacement, and so forth).

Mutagenized variable sequence can, optionally, be fused to a human constant region, e.g., human IgG1 or κ constant regions.

In some cases a potential T cell epitope will include residues which are known or predicted to be important for antibody function. For example, potential T cell epitopes are usually biased towards the CDRs. In addition, potential T cell epitopes can occur in framework residues important for antibody structure and binding. Changes to eliminate these potential epitopes will in some cases require more scrutiny, e.g., by making and testing chains with and without the change. Where possible, potential T cell epitopes that overlap the CDRs were eliminated by substitutions outside the CDRs. In some cases, an alteration within a CDR is the only option, and thus variants with and without this substitution should be tested. In other cases, the substitution required to remove a potential T cell epitope is at a residue position within the framework that might be critical for antibody binding. In these cases, variants with and without this substitution should be tested. Thus, in some cases several variant deimmunized heavy and light chain variable regions were designed and various heavy/light chain combinations tested in order to identify the optimal deimmunized antibody. The choice of the final deimmunized antibody can then be made by considering the binding affinity of the different variants in conjunction with the extent of deimmunization, i.e., the number of potential T cell epitopes remaining in the variable region. Deimmunization can be used to modify any antibody, e.g., an antibody that includes a non-human sequence, e.g., a synthetic antibody, a murine antibody other non-human monoclonal antibody, or an antibody isolated from a display library.

IGF-II/IGF-IIIE binding antibodies are “germlined” by reverting one or more non-germline amino acids in framework regions to corresponding germline amino acids of the antibody, so long as binding properties are substantially retained. Similar methods can also be used in the constant region, e.g., in constant immunoglobulin domains.

Antibodies that bind to both IGF-II and IGF-IIIE e.g., an antibody described herein, may be modified in order to make the variable regions of the antibody more similar to one or more germline sequences. For example, an antibody can include one, two, three, or more amino acid substitutions, e.g., in a framework, CDR, or constant region, to make it more similar to a reference germline sequence. One exemplary

germlining method can include identifying one or more germline sequences that are similar (e.g., most similar in a particular database) to the sequence of the isolated antibody. Mutations (at the amino acid level) are then made in the isolated antibody, either incrementally or in combination with other mutations. For example, a nucleic acid library that includes sequences encoding some or all possible germline mutations is made. The mutated antibodies are then evaluated, e.g., to identify an antibody that has one or more additional germline residues relative to the isolated antibody and that is still useful (e.g., has a functional activity). In one embodiment, as many germline residues are introduced into an isolated antibody as possible.

In one embodiment, mutagenesis is used to substitute or insert one or more germline residues into a framework and/or constant region. For example, a germline framework and/or constant region residue can be from a germline sequence that is similar (e.g., most similar) to the non-variable region being modified. After mutagenesis, activity (e.g., binding or other functional activity) of the antibody can be evaluated to determine if the germline residue or residues are tolerated (i.e., do not abrogate activity). Similar mutagenesis can be performed in the framework regions.

Selecting a germline sequence can be performed in different ways. For example, a germline sequence can be selected if it meets a predetermined criteria for selectivity or similarity, e.g., at least a certain percentage identity, e.g., at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 99.5% identity. The selection can be performed using at least 2, 3, 5, or 10 germline sequences. In the case of CDR1 and CDR2, identifying a similar germline sequence can include selecting one such sequence. In the case of CDR3, identifying a similar germline sequence can include selecting one such sequence, but may including using two germline sequences that separately contribute to the amino-terminal portion and the carboxy-terminal portion. In other implementations more than one or two germline sequences are used, e.g., to form a consensus sequence.

In one embodiment, with respect to a particular reference variable domain sequence, e.g., a sequence described herein, a related variable domain sequence has at least 30, 40, 50, 60, 70, 80, 90, 95 or 100% of the CDR amino acid positions that are not identical to residues in the reference CDR sequences, residues that are identical to

residues at corresponding positions in a human germline sequence (i.e., an amino acid sequence encoded by a human germline nucleic acid).

In one embodiment, with respect to a particular reference variable domain sequence, e.g., a sequence described herein, a related variable domain sequence has at least 30, 50, 60, 70, 80, 90 or 100% of the FR regions identical to FR sequence from a human germline sequence, e.g., a germline sequence related to the reference variable domain sequence.

Accordingly, it is possible to isolate an antibody which has similar activity to a given antibody of interest, but is more similar to one or more germline sequences, particularly one or more human germline sequences. For example, an antibody can be at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 99.5% identical to a germline sequence in a region outside the CDRs (e.g., framework regions). Further, an antibody can include at least 1, 2, 3, 4, or 5 germline residues in a CDR region, the germline residue being from a germline sequence of similar (e.g., most similar) to the variable region being modified. Germline sequences of primary interest are human germline sequences. The activity of the antibody (e.g., the binding activity as measured by K_A) can be within a factor of 100, 10, 5, 2, 0.5, 0.1, and 0.001 of the original antibody.

Germline sequences of human immunoglobulin genes have been determined and are available from a number of sources, including the INTERNATIONAL IMMUNOGENETICS INFORMATION SYSTEM® (IMGT), available via the world wide web at imgt.cines.fr, and the V BASE directory (compiled by Tomlinson, I.A. et al. MRC Centre for Protein Engineering, Cambridge, UK, available via the world wide web at vbase.mrc-cpe.cam.ac.uk).

Exemplary germline reference sequences for V_{kappa} include: O12/O2, O18/O8, A20, A30, L14, L1, L15, L4/18a, L5/L19, L8, L23, L9, L24, L11, L12, O11/O1, A17, A1, A18, A2, A19/A3, A23, A27, A11, L2/L16, L6, L20, L25, B3, B2, A26/A10, and A14. See, e.g., Tomlinson et al., 1995, *EMBO J.* 14(18):4628-3.

A germline reference sequence for the HC variable domain can be based on a sequence that has particular canonical structures, e.g., 1-3 structures in the H1 and H2 hypervariable loops. The canonical structures of hypervariable loops of an immunoglobulin variable domain can be inferred from its sequence, as described in

Chothia et al., 1992, *J. Mol. Biol.* 227:799-817; Tomlinson et al., 1992, *J. Mol. Biol.* 227:776-798); and Tomlinson et al., 1995, *EMBO J.* 14(18):4628-38. Exemplary sequences with a 1-3 structure include: DP-1, DP-8, DP-12, DP-2, DP-25, DP-15, DP-7, DP-4, DP-31, DP-32, DP-33, DP-35, DP-40, 7-2, hv3005, hv3005f3, DP-46, DP-47, DP-58, DP-49, DP-50, DP-51, DP-53, and DP-54.

Protein Production

Standard recombinant nucleic acid methods can be used to express a protein that binds to both IGF-II and IGF-IIIE (or growth hormone/growth hormone releasing hormone pathway modulators that are in protein form). Generally, a nucleic acid sequence encoding the protein is cloned into a nucleic acid expression vector. Of course, if the protein includes multiple polypeptide chains, each chain can be cloned into an expression vector, e.g., the same or different vectors, that are expressed in the same or different cells.

Antibody Production. Some antibodies, e.g., Fabs, can be produced in bacterial cells, e.g., *E. coli* cells. For example, if the Fab is encoded by sequences in a phage display vector that includes a suppressible stop codon between the display entity and a bacteriophage protein (or fragment thereof), the vector nucleic acid can be transferred into a bacterial cell that cannot suppress a stop codon. In this case, the Fab is not fused to the gene III protein and is secreted into the periplasm and/or media.

Antibodies can also be produced in eukaryotic cells. In one embodiment, the antibodies (e.g., scFv's) are expressed in a yeast cell such as *Pichia* (see, e.g., Powers et al., 2001, *J. Immunol. Methods.* 251:123-35), *Hansenula*, or *Saccharomyces*.

In one preferred embodiment, antibodies are produced in mammalian cells. Preferred mammalian host cells for expressing the clone antibodies or antigen-binding fragments thereof include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, 1982, *Mol. Biol.* 159:601-621), lymphocytic cell lines, e.g., NS0 myeloma cells and SP2 cells, COS cells, HEK293T cells (*J. Immunol. Methods* (2004) 289(1-2):65-80.), and a cell

from a transgenic animal, e.g., a transgenic mammal. For example, the cell is a mammary epithelial cell.

In addition to the nucleic acid sequence encoding the diversified immunoglobulin domain, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Patent Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in *dhfr*⁻ host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

In an exemplary system for recombinant expression of an antibody, or antigen-binding portion thereof, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into *dhfr*⁻ CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G coupled matrix.

For antibodies that include an Fc domain, the antibody production system may produce antibodies in which the Fc region is glycosylated. For example, the Fc domain

of IgG molecules is glycosylated at asparagine 297 in the CH2 domain. This asparagine is the site for modification with biantennary-type oligosaccharides. It has been demonstrated that this glycosylation is required for effector functions mediated by Fcγ receptors and complement C1q (Burton and Woof, 1992, *Adv. Immunol.* 51:1-84; Jefferis et al., 1998, *Immunol. Rev.* 163:59-76). In one embodiment, the Fc domain is produced in a mammalian expression system that appropriately glycosylates the residue corresponding to asparagine 297. The Fc domain can also include other eukaryotic post-translational modifications.

Antibodies can also be produced by a transgenic animal. For example, U.S. Patent No. 5,849,992 describes a method of expressing an antibody in the mammary gland of a transgenic mammal. A transgene is constructed that includes a milk-specific promoter and nucleic acids encoding the antibody of interest and a signal sequence for secretion. The milk produced by females of such transgenic mammals includes, secreted therein, the antibody of interest. The antibody can be purified from the milk, or for some applications, used directly.

Characterization of IGF-II/IGF-IIE Binding Proteins

EC₅₀ (Effective Concentration 50%) value for that binding protein. Within a series or group of binding proteins, those having lower IC₅₀ or EC₅₀ values are considered more potent inhibitors of IGF-II or IGF-IIE than those binding proteins having higher IC₅₀ or EC₅₀ values. Exemplary binding proteins have an IC₅₀ value of less than 800 nM, 400 nM, 100 nM, 25 nM, 5 nM, or 1 nM, e.g., as measured in an in vitro assay for inhibition of IGF-II or IGF-IIE activity when the IGF-II or IGF-IIE is at 2 pM.

IGF-II/IGF-IIE binding proteins may also be characterized with reference to the activity of IGF-II/IGF-IIE on its substrates.

The binding proteins can also be evaluated for selectivity toward IGF-II and/or IGF-IIE. For example, an IGF-II/IGF-IIE binding protein can be assayed for its potency toward IGF-II and/or IGF-IIE and a panel of IGF-II's and an IC₅₀ value or EC₅₀ value can be determined for each IGF. In one embodiment, a compound that demonstrates a low IC₅₀ value or EC₅₀ value for the IGF-II or IGF-IIE, and a higher IC₅₀ value or EC₅₀ value,

e.g., at least 2-, 5-, or 10- fold higher, for another IGF within the test panel is considered to be selective toward IGF-II and/or IGF-IIE.

IGF-II/IGF-IIE binding proteins can be evaluated for their ability to inhibit IGF-II and/or IGF-IIE in a cell based assay. The expansion of tumor cells inside a three-
5 dimensional collagen-matrix can be significantly enhanced in response to IGF-II and/or IGF-IIE overexpression (Hotary et al., 2003 Cell 114:33-45). Addition of an IGF-II/IGF-IIE binding protein to this assay can be used to determine the inhibitory properties and other characteristics of the protein.

A pharmacokinetics study in rat, mice, or monkey can be performed with IGF-II/IGF-IIE binding proteins for determining IGF-II and/or IGF-IIE half-life in the serum.
10 Likewise, the effect of the binding protein can be assessed *in vivo*, e.g., in an animal model for a disease, for use as a therapeutic, for example, to treat a disease or condition described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), an inflammatory disease (e.g., synovitis, atherosclerosis), rheumatoid arthritis, osteoarthritis,
15 an ocular condition (e.g., macular degeneration), diabetes, Alzheimer's Disease, cerebral ischemia, endometriosis, fibrin-invasive activity, angiogenesis, or capillary tube formation.

Pharmaceutical Compositions

In another aspect, the disclosure provides compositions, e.g., pharmaceutically
20 acceptable compositions or pharmaceutical compositions, which include an IGF-II/IGF-IIE -binding protein, e.g., an antibody molecule, other polypeptide or peptide identified as binding to IGF-II and IGF-IIE, or growth hormone/growth hormone releasing hormone pathway modulators, as described herein. The IGF-II/IGF-IIE binding protein or growth hormone/growth hormone releasing hormone pathway modulator can be formulated
25 together with a pharmaceutically acceptable carrier. Pharmaceutical compositions include therapeutic compositions and diagnostic compositions, e.g., compositions that include labeled IGF-II/IGF-IIE binding proteins for *in vivo* imaging.

A pharmaceutically acceptable carrier includes any and all solvents, dispersion
media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying
30 agents, and the like that are physiologically compatible. Preferably, the carrier is suitable

for intravenous, intramuscular, subcutaneous, parenteral, spinal, or epidermal administration (e.g., by injection or infusion), although carriers suitable for inhalation and intranasal administration are also contemplated. Depending on the route of administration, the IGF-II/IGF-IIIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

A pharmaceutically acceptable salt is a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see e.g., Berge, S.M., et al., 1977, *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous, and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids, and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium, and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine, and the like.

The compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The form can depend on the intended mode of administration and therapeutic application. Many compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for administration of humans with antibodies. An exemplary mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In one embodiment, the IGF-II/IGF-IIIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator is administered by intravenous infusion or injection. In another preferred embodiment, the IGF-II/IGF-IIIE binding protein and/or growth hormone/growth

hormone releasing hormone pathway modulator is administered by intramuscular or subcutaneous injection.

The phrases “parenteral administration” and “administered parenterally” as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the binding protein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

An IGF-II/IGF-IIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator can be administered by a variety of methods, although for many applications, the preferred route/mode of administration is intravenous injection or infusion. For example, for therapeutic applications, the IGF-II/IGF-IIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m² or 7 to 25 mg/m². The route and/or mode of administration will vary depending upon the desired results. In certain

embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are available. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., 1978, Marcel Dekker, Inc., New York.

Pharmaceutical compositions can be administered with medical devices. For example, in one embodiment, a pharmaceutical composition disclosed herein can be administered with a device, e.g., a needleless hypodermic injection device, a pump, or implant.

In certain embodiments, an IGF-II/IGF-IIe binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds disclosed herein cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Patent Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties that are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., V.V. Ranade, 1989, *J. Clin. Pharmacol.* 29:685).

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms can be dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to

be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody disclosed herein is 0.1-20 mg/kg, more preferably 1-10 mg/kg. An anti-IGF-II/IGF-IIIE antibody and/or growth hormone/growth hormone releasing hormone pathway modulator can be administered, e.g., by intravenous infusion, e.g., at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m² or about 5 to 30 mg/m². For binding proteins smaller in molecular weight than an antibody, appropriate amounts can be proportionally less. Dosage values may vary with the type and severity of the condition to be alleviated. For a particular subject, specific dosage regimens can be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

The pharmaceutical compositions disclosed herein may include a “therapeutically effective amount” or a “prophylactically effective amount” of an IGF-II/IGF-IIIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator disclosed herein. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects.

A “therapeutically effective dosage” preferably modulates a measurable parameter, e.g., levels of circulating IgG antibodies by a statistically significant degree or at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to modulate a measurable parameter, e.g., a disease-associated parameter, can be evaluated in an animal model system predictive of efficacy in human disorders and conditions, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), an inflammatory disease (e.g., synovitis, atherosclerosis), rheumatoid

arthritis, osteoarthritis, an ocular condition (e.g., macular degeneration), diabetes, Alzheimer's Disease, cerebral ischemia, endometriosis, fibrin-invasive activity, angiogenesis, or capillary tube formation. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to modulate a parameter in vitro.

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, because a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Stabilization and Retention

In one embodiment, an IGF-II/IGF-IIIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator is physically associated with a moiety that improves its stabilization and/or retention in circulation, e.g., in blood, serum, lymph, or other tissues, e.g., by at least 1.5, 2, 5, 10, or 50 fold. For example, an IGF-II/IGF-IIIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator can be associated with a polymer, e.g., a substantially non-antigenic polymers, such as polyalkylene oxides or polyethylene oxides. Suitable polymers will vary substantially by weight. Polymers having molecular number average weights ranging from about 200 to about 35,000 (or about 1,000 to about 15,000, and 2,000 to about 12,500) can be used. For example, an IGF-II/IGF-IIIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator can be conjugated to a water soluble polymer, e.g., hydrophilic polyvinyl polymers, e.g. polyvinylalcohol and polyvinylpyrrolidone. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

An IGF-II/IGF-IIIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator can also be associated with a carrier protein, e.g., a serum albumin, such as a human serum albumin. For example, a translational fusion

can be used to associate the carrier protein with the IGF-II/IGF-IIIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator.

Growth Hormone/Growth Hormone Releasing Hormone Pathway Modulator

A growth hormone/growth hormone releasing hormone pathway modulator can act on (e.g., indirectly (e.g., by steric effects) or directly (e.g., by interacting with, e.g., binding to)) a growth hormone/growth hormone releasing hormone pathway component. Components of the growth hormone/growth hormone releasing hormone pathway and their activities are described in Lin-Su, K. and Wajnrajch, M.P., 2002, Rev. Endocr. Metab. Disord. 3:313-323; Salvatori, R. 2004, Rev Endocr. Metab. Disord. .5:15-23 and Schally, A.V., 2008, Nat. Clin. Pract. Endocrinol. Metab. 4:33-43. Examples of growth hormone/growth hormone releasing hormone pathway component include, but are not limited to, growth hormone and its receptor, growth hormone releasing hormone and its receptor, somatostatin (growth hormone inhibiting hormone (GHIH) or somatotropin) and IGF-1.

Growth hormone/growth hormone releasing hormone pathway modulators that can be used include, but are not limited to, an antibody (which includes antibody fragments, as described herein), a polypeptide, a small molecule and an aptamer. Examples of polypeptides include a receptor fragment (e.g., soluble receptor, e.g., an Fc-fusion of a soluble receptor fragment) and a ligand fragment (e.g., an Fc-fusion of a soluble receptor fragment).

Examples of growth hormone/growth hormone releasing hormone pathway modulators include, but are not limited to, growth-hormone-releasing hormone antagonists such as those described in Schally, A.V., 2008, Nat. Clin. Pract. Endocrinol. Metab. 4:33-43 and Schulz, et al. 2006, Eur. J. Cancer 42:2390-2396 and growth hormone receptor antagonists such as SOMAVERT® (pegvisomant).

Anti-growth hormone/growth hormone releasing hormone pathway components can be one or more of the following: human, humanized, non-immunogenic, isolated, monoclonal, and recombinant.

Growth hormone/growth hormone releasing hormone pathway components include monoclonal antibodies that can be used to develop bifunctional antibodies where

there are two independent antigenic binding sites on each immunoglobulin molecule. This technology is known in the art and has been disclosed in the literature (*Thromb. Res. Suppl. X*: 83, 1990). Additionally, bispecific antibodies can also be constructed from single chain antibodies. This technology is known in the art and has been disclosed, for example, by A. George (The Second Annual IBC International Conference on Antibody Engineering, Dec. 16-18, 1991, San Diego Calif.).

The growth hormone/growth hormone releasing hormone pathway component modulators can be a panel of antibodies capable of inhibiting two or more growth hormone/growth hormone releasing hormone pathway components. As used herein, the term "panel" denotes a combination of two or more antibodies having different specificities. The antibodies may be specific for different antigens or for different epitopes on a single antigen. In some embodiments, monoclonal antibodies (MAbs) are preferred.

The growth hormone/growth hormone releasing hormone pathway modulators (e.g., anti-receptor monoclonal antibodies) may also be used as targeting agents for the delivery of compounds of therapeutic interest. Such compounds include, but are not limited to, toxins, cytostatic compounds, or proenzymes whose potential function can be to activate endogenous proenzymes, to activate proenzymes added from exogenous sources, or to activate enzyme cleavage sites on prodrugs.

In some embodiments, the growth hormone/growth hormone releasing hormone pathway modulator can be a growth hormone receptor analog or fragment, e.g., a soluble form of a growth hormone receptor, e.g., the soluble form of the receptor binds growth hormone receptor and antagonizes growth hormone binding to a growth hormone receptor. For example, the receptor fragment can be an Fc fusion protein.

For example, the growth hormone/growth hormone releasing hormone pathway modulator can be SOMAVERT® (pegvisomant), an analog of human growth hormone (GH) that has been structurally altered to act as a GH receptor antagonist.

Pegvisomant is a protein of recombinant DNA origin containing 191 amino acid residues to which several polyethylene glycol (PEG) polymers are covalently bound (predominantly 4 to 6 PEG/protein molecule). The molecular weight of the protein of pegvisomant is 21,998 Daltons. The molecular weight of the PEG portion of pegvisomant

is approximately 5000 Daltons. The predominant molecular weights of pegvisomant are thus approximately 42,000, 47,000, and 52,000 Daltons. The schematic shows the amino acid sequence of the pegvisomant protein (PEG polymers are shown attached to the 5 most probable attachment sites). Pegvisomant is synthesized by a specific strain of Escherichia coli bacteria that has been genetically modified by the addition of a plasmid that carries a gene for GH receptor antagonist. Biological potency is determined using a cell proliferation bioassay

Additional growth hormone/growth hormone releasing hormone pathway modulator compounds include SANDOSTATIN® (octreotide acetate, Novartis), which mimics somatostatin pharmacologically.

Kits

An IGF-II/IGF-IIIE binding protein described herein can be provided in a kit, e.g., as a component of a kit. For example, the kit includes (a) an IGF-II/IGF-IIIE binding protein, e.g., a composition that includes an IGF-II/IGF-IIIE binding protein, and, optionally (b) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of an IGF-II/IGF-IIIE binding protein for the methods described herein.

The informational material of the kits is not limited in its form. In one embodiment, the informational material can include information about production of the compound, molecular weight of the compound, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to using the binding protein to treat, prevent, or diagnosis of disorders and conditions, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer).

In one embodiment, the informational material can include instructions to administer an IGF-II/IGF-IIIE binding protein in a suitable manner to perform the methods described herein, e.g., in a suitable dose, dosage form, or mode of administration (e.g., a dose, dosage form, or mode of administration described herein). In another embodiment, the informational material can include instructions to administer an IGF-II/IGF-IIIE binding protein to a suitable subject, e.g., a human, e.g., a human having, or at risk for, a disorder or condition described herein, e.g., a cancer (e.g., metastatic cancer,

e.g., metastatic breast cancer). For example, the material can include instructions to administer an IGF-II/IGF-IIIE binding protein to a patient with a disorder or condition described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer). The informational material of the kits is not limited in its form. In many cases, the informational material, e.g., instructions, is provided in print but may also be in other formats, such as computer readable material.

An IGF-II/IGF-IIIE binding protein can be provided in any form, e.g., liquid, dried or lyophilized form. It is preferred that an IGF-II/IGF-IIIE binding protein be substantially pure and/or sterile. When an IGF-II/IGF-IIIE binding protein is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When an IGF-II/IGF-IIIE binding protein is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, e.g., sterile water or buffer, can optionally be provided in the kit.

The kit can include one or more containers for the composition containing an IGF-II/IGF-IIIE binding protein. In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example, the composition can be contained in a bottle, vial, or syringe, and the informational material can be contained association with the container. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more unit dosage forms (e.g., a dosage form described herein) of an IGF-II/IGF-IIIE binding protein. For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a single unit dose of an IGF-II/IGF-IIIE binding protein. The containers of the kits can be air tight, waterproof (e.g., impermeable to changes in moisture or evaporation), and/or light-tight.

Combinations of IGF-II/IGF-IIIE binding protein and growth hormone/growth hormone releasing hormone pathway modulators described herein can be provided in a kit, e.g., as a component of a kit. For example, the kit includes (a) an IGF-II/IGF-IIIE binding protein, e.g., a composition that includes an IGF-II/IGF-IIIE binding protein, (b) a

growth hormone/growth hormone releasing hormone pathway modulator, e.g. a composition that includes a growth hormone/growth hormone releasing hormone pathway modulator; and, optionally (c) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of the combination for the methods described herein.

The informational material of the kits is not limited in its form. In one embodiment, the informational material can include information about production of the compound, molecular weight of the compound, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to using the combination to treat, prevent, or diagnosis of disorders and conditions, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer).

In one embodiment, the informational material can include instructions to administer an IGF-II/IGF-IIE binding protein and growth hormone/growth hormone releasing hormone pathway modulator in a suitable manner to perform the methods described herein, e.g., in a suitable dose, dosage form, or mode of administration (e.g., a dose, dosage form, or mode of administration described herein). In another embodiment, the informational material can include instructions to administer an IGF-II/IGF-IIE binding protein and growth hormone/growth hormone releasing hormone pathway modulator to a suitable subject, e.g., a human, e.g., a human having, or at risk for, a disorder or condition described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer). For example, the material can include instructions to administer an IGF-II/IGF-IIE binding protein and growth hormone/growth hormone releasing hormone pathway modulator to a patient with a disorder or condition described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer). The informational material of the kits is not limited in its form. In many cases, the informational material, e.g., instructions, is provided in print but may also be in other formats, such as computer readable material.

An IGF-II/IGF-IIE binding protein or growth hormone/growth hormone releasing hormone pathway modulator can be provided in any form, e.g., liquid, dried or lyophilized form. It is preferred that an IGF-II/IGF-IIE binding protein or growth

hormone/growth hormone releasing hormone pathway modulator be substantially pure and/or sterile. When an IGF-II/IGF-IIE binding protein or growth hormone/growth hormone releasing hormone pathway modulator is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When an IGF-II/IGF-IIE binding protein or growth hormone/growth hormone releasing hormone pathway modulator is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, e.g., sterile water or buffer, can optionally be provided in the kit.

The kit can include one or more containers for the composition containing an IGF-II/IGF-IIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator. In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example, the composition can be contained in a bottle, vial, or syringe, and the informational material can be contained association with the container. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more unit dosage forms (e.g., a dosage form described herein) of an IGF-II/IGF-IIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator. For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a single unit dose of an IGF-II/IGF-IIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator. The containers of the kits can be air tight, waterproof (e.g., impermeable to changes in moisture or evaporation), and/or light-tight.

The kit optionally includes a device suitable for administration of the composition, e.g., a syringe, inhalant, dropper (e.g., eye dropper), swab (e.g., a cotton swab or wooden swab), or any such delivery device. In one embodiment, the device is an implantable device that dispenses metered doses of the binding protein. The disclosure also features a method of providing a kit, e.g., by combining components described herein.

Treatments

Proteins that bind to both IGF-II/IGF-IIE, and identified by the method described herein and/or detailed herein have therapeutic and prophylactic utilities, particularly in human subjects. These binding proteins are administered to a subject to treat, prevent, and/or diagnose a variety of disorders, including e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), or even to cells in culture, e.g., *in vitro* or *ex vivo*. Treating includes administering an amount effective to alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. The treatment may also delay onset, e.g., prevent onset, or prevent deterioration of a disease or condition.

Exemplary disorders include a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer).

As used herein, an amount of a target-binding agent effective to prevent a disorder, or a prophylactically effective amount of the binding agent refers to an amount of a target binding agent, e.g., an IGF-II/IGF-IIE binding protein, e.g., an anti-IGF-II/IGF-IIE antibody described herein, which is effective, upon single- or multiple-dose administration to the subject, for preventing or delaying the occurrence of the onset or recurrence of a disorder, e.g., a disorder described herein.

Methods of administering IGF-II/IGF-IIE binding proteins and other agents are also described in "Pharmaceutical Compositions." Suitable dosages of the molecules used can depend on the age and weight of the subject and the particular drug used. The binding proteins can be used as competitive agents to inhibit, reduce an undesirable interaction, e.g., between a natural or pathological agent and the IGF-II/IGF-IIE. The dose of the IGF-II/IGF-IIE binding protein can be the amount sufficient to block 90%, 95%, 99%, or 99.9% of the activity of IGF-II/IGF-IIE in the patient, especially at the site of disease. Depending on the disease, this may require 0.1, 1.0, 3.0, 6.0, or 10.0 mg/Kg. For an IgG having a molecular mass of 150,000 g/mole (two binding sites), these doses correspond to approximately 18 nM, 180 nM, 540 nM, 1.08 μ M, and 1.8 μ M of binding sites for a 5 L blood volume.

In one embodiment, the IGF-II/IGF-IIE binding proteins are used to inhibit an activity (e.g., inhibit at least one activity of, reduce proliferation, migration, growth or

viability) of a cell, e.g., a cancer cell *in vivo*. The binding proteins can be used by themselves or conjugated to an agent, e.g., a cytotoxic drug, cytotoxin enzyme, or radioisotope. This method includes: administering the binding protein alone or attached to an agent (e.g., a cytotoxic drug), to a subject requiring such treatment. For example,
5 IGF-II/IGF-IIE binding proteins that do not substantially inhibit IGF-II/IGF-IIE may be used to deliver nanoparticles containing agents, such as toxins, to IGF-II/IGF-IIE associated cells or tissues, e.g., tumors.

Proteins that bind to both IGF-II/IGF-IIE, and identified by the method described herein and/or detailed herein, in combination with growth hormone/growth hormone
10 releasing hormone pathway modulators, have therapeutic and prophylactic utilities, particularly in human subjects. These binding proteins and modulators are administered to a subject to treat, prevent, and/or diagnose a variety of disorders, including e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), or even to cells in culture, e.g. *in vitro* or *ex vivo*. Treating includes administering an amount effective to alleviate,
15 relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. The treatment may also delay onset, e.g., prevent onset, or prevent deterioration of a disease or condition.

Exemplary disorders include a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer).

20 As used herein, an amount of an target-binding agent effective to prevent a disorder, or a prophylactically effective amount of the binding agent refers to an amount of a target binding agent, e.g., an IGF-II/IGF-IIE binding protein, e.g., an anti- IGF-II/IGF-IIE antibody described herein, and/or growth hormone/growth hormone releasing hormone pathway modulator which is effective, upon single- or multiple-dose
25 administration to the subject, for preventing or delaying the occurrence of the onset or recurrence of a disorder, e.g., a disorder described herein.

Methods of administering IGF-II/IGF-IIE binding proteins, growth hormone/growth hormone releasing hormone pathway modulators, and other agents are also described in "Pharmaceutical Compositions". Suitable dosages of the molecules
30 used can depend on the age and weight of the subject and the particular drug used. The binding proteins can be used as competitive agents to inhibit, reduce an undesirable

interaction, e.g., between a natural or pathological agent and the IGF-II/IGF-IIIE and/or growth hormone/growth hormone releasing hormone pathway modulator. The dose of the IGF-II/IGF-IIIE binding protein can be the amount sufficient to block 90%, 95%, 99%, or 99.9% of the activity of IGF-II/IGF-IIIE in the patient, especially at the site of disease. Depending on the disease, this may require 0.1, 1.0, 3.0, 6.0, or 10.0 mg/Kg. For an IgG having a molecular mass of 150,000 g/mole (two binding sites), these doses correspond to approximately 18 nM, 180 nM, 540 nM, 1.08 μ M, and 1.8 μ M of binding sites for a 5 L blood volume.

Exemplary therapeutically effective dosages of SOMAVERT® range from about 5 to about 50 U of activity, given in daily doses to a subject.

Exemplary therapeutically effective dosages of SANDOSTATIN® include a depot formulation available in 10, 20 and 30 mg doses, generally administered monthly, and injections of 50, 100 or 500 mcg doses, generally administered two to three times per day.

Dosages considerations for both SOMAVERT® and SANDOSTATIN® are described in the Physician's Desk Reference, 62nd Edition (2008).

In one embodiment, the IGF-II/IGF-IIIE binding proteins and growth hormone/growth hormone releasing hormone pathway modulators are used to inhibit an activity (e.g., inhibit at least one activity of, reduce proliferation, migration, growth or viability) of a cell, e.g., a cancer cell *in vivo*. The binding proteins can be used by themselves or conjugated to an agent, e.g., a cytotoxic drug, cytotoxin enzyme, or radioisotope. This method includes: administering the binding protein alone or attached to an agent (e.g., a cytotoxic drug), to a subject requiring such treatment. For example, IGF-II/IGF-IIIE binding proteins that do not substantially inhibit IGF-II/IGF-IIIE may be used to deliver nanoparticles containing agents, such as toxins, to IGF-II/IGF-IIIE associated cells or tissues, e.g., tumors.

Because the IGF-II/IGF-IIIE binding proteins recognize IGF-II/IGF-IIIE - expressing cells and can bind to cells that are associated with (e.g., in proximity of or intermingled with) cancer cells, e.g., cancerous lung, liver, colon, breast, ovarian, epidermal, laryngeal, and cartilage cells, and particularly metastatic cells thereof, IGF-II/IGF-IIIE binding proteins can be used to inhibit (e.g., inhibit at least one activity,

reduce growth and proliferation, or kill) any such cells and inhibit carcinogenesis. Reducing IGF-II/IGF-IIE activity near a cancer can indirectly inhibit (e.g., inhibit at least one activity, reduce growth and proliferation, or kill) the cancer cells which may be dependent on the IGF-II/IGF-IIE activity for metastasis, activation of growth factors, and so forth.

Likewise, because the growth hormone/growth hormone releasing hormone pathway modulators recognize growth hormone/growth hormone releasing hormone pathway component -expressing cells and can bind to cells that are associated with (e.g., in proximity of or intermingled with) cancer cells, e.g., cancerous lung, liver, colon, breast, ovarian, epidermal, laryngeal, and cartilage cells, and particularly metastatic cells thereof, growth hormone/growth hormone releasing hormone pathway modulators can be used to inhibit (e.g., inhibit at least one activity, reduce growth and proliferation, or kill) any such cells and inhibit carcinogenesis. Reducing growth hormone/growth hormone releasing hormone pathway component activity near a cancer can indirectly inhibit (e.g., inhibit at least one activity, reduce growth and proliferation, or kill) the cancer cells which may be dependent on the activity for metastasis, activation of growth factors, and so forth. Alternatively, the binding proteins bind to cells in the vicinity of the cancerous cells, but are sufficiently close to the cancerous cells to directly or indirectly inhibit (e.g., inhibit at least one activity, reduce growth and proliferation, or kill) the cancers cells. Thus, the IGF-II/IGF-IIE binding proteins (e.g., modified with a toxin, e.g., a cytotoxin) can be used to selectively inhibit cells in cancerous tissue (including the cancerous cells themselves and cells associated with or invading the cancer).

The binding proteins may be used to deliver an agent (e.g., any of a variety of cytotoxic and therapeutic drugs) to cells and tissues where IGF-II/IGF-IIE is present. Exemplary agents include a compound emitting radiation, molecules of plants, fungal, or bacterial origin, biological proteins, and mixtures thereof. The cytotoxic drugs can be intracellularly acting cytotoxic drugs, such as toxins short range radiation emitters, e.g., short range, high energy α -emitters.

To target IGF-II/IGF-IIE expressing cells, particularly cancerous cells, a prodrug system can be used. For example, a first binding protein is conjugated with a prodrug which is activated only when in close proximity with a prodrug activator. The prodrug

activator is conjugated with a second binding protein, preferably one which binds to a non competing site on the target molecule. Whether two binding proteins bind to competing or non competing binding sites can be determined by conventional competitive binding assays. Exemplary drug prodrug pairs are described in Blakely et al., (1996) Cancer Research, 56:3287 3292.

The IGF-II/IGF-IIIE binding proteins can be used directly *in vivo* to eliminate antigen-expressing cells via natural complement-dependent cytotoxicity (CDC) or antibody dependent cellular cytotoxicity (ADCC). The binding proteins described herein can include complement binding effector domain, such as the Fc portions from IgG1, -2, or -3 or corresponding portions of IgM which bind complement. In one embodiment, a population of target cells is ex vivo treated with a binding agent described herein and appropriate effector cells. The treatment can be supplemented by the addition of complement or serum containing complement. Further, phagocytosis of target cells coated with a binding protein described herein can be improved by binding of complement proteins. In another embodiment target, cells coated with the binding protein which includes a complement binding effector domain are lysed by complement.

Methods of administering IGF-II/IGF-IIIE binding proteins and growth hormone/growth hormone releasing hormone pathway modulators are described in "Pharmaceutical Compositions." Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used. The binding proteins can be used as competitive agents to inhibit or reduce an undesirable interaction, e.g., between a natural or pathological agent and the IGF-II/IGF-IIIE and/or growth hormone/growth hormone releasing hormone pathway modulators.

The IGF-II/IGF-IIIE binding protein can be used to deliver macro and micromolecules, e.g., a gene into the cell for gene therapy purposes into the endothelium or epithelium and target only those tissues expressing the IGF-II/IGF-IIIE. The binding proteins may be used to deliver a variety of cytotoxic drugs including therapeutic drugs, a compound emitting radiation, molecules of plants, fungal, or bacterial origin, biological proteins, and mixtures thereof. The cytotoxic drugs can be intracellularly acting cytotoxic drugs, such as short range radiation emitters, including, for example, short range, high energy α emitters, as described herein.

In the case of polypeptide toxins, recombinant nucleic acid techniques can be used to construct a nucleic acid that encodes the binding protein (e.g., antibody or antigen-binding fragment thereof) and the cytotoxin (or a polypeptide component thereof) as translational fusions. The recombinant nucleic acid is then expressed, e.g., in cells and the encoded fusion polypeptide isolated.

Alternatively, the IGF-II/IGF-IIIE binding protein can be coupled to high energy radiation emitters, for example, a radioisotope, such as ^{131}I , a γ -emitter, which, when localized at a site, results in a killing of several cell diameters. See, e.g., S.E. Order, "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy", *Monoclonal Antibodies for Cancer Detection and Therapy*, R.W. Baldwin et al. (eds.), pp 303 316 (Academic Press 1985). Other suitable radioisotopes include α emitters, such as ^{212}Bi , ^{213}Bi , and ^{211}At , and β emitters, such as ^{186}Re and ^{90}Y . Moreover, ^{177}Lu may also be used as both an imaging and cytotoxic agent.

Radioimmunotherapy (RIT) using antibodies labeled with ^{131}I , ^{90}Y , and ^{177}Lu is under intense clinical investigation. There are significant differences in the physical characteristics of these three nuclides and as a result, the choice of radionuclide is very critical in order to deliver maximum radiation dose to a tissue of interest. The higher beta energy particles of ^{90}Y may be good for bulky tumors. The relatively low energy beta particles of ^{131}I are ideal, but in vivo dehalogenation of radioiodinated molecules is a major disadvantage for internalizing antibody. In contrast, ^{177}Lu has low energy beta particle with only 0.2-0.3 mm range and delivers much lower radiation dose to bone marrow compared to ^{90}Y . In addition, due to longer physical half-life (compared to ^{90}Y), the residence times are higher. As a result, higher activities (more mCi amounts) of ^{177}Lu labeled agents can be administered with comparatively less radiation dose to marrow. There have been several clinical studies investigating the use of ^{177}Lu labeled antibodies in the treatment of various cancers. (Mulligan T et al., 1995, *Clin. Canc. Res.* 1: 1447-1454; Meredith RF, et al., 1996, *J. Nucl. Med.* 37:1491-1496; Alvarez RD, et al., 1997, *Gynecol. Oncol.* 65: 94-101).

Exemplary Diseases and Conditions

The IGF-II/IGF-IIIE binding proteins described herein are useful to treat diseases or conditions in which IGF-II and/or IGF-IIIE activity implicated, e.g., a disease or condition described herein, or to treat one or more symptoms associated therewith. In
5 some embodiments, the IGF-II/IGF-IIIE binding protein (e.g., IGF-II/IGF-IIIE binding IgG or Fab) inhibits IGF-II and/or IGF-IIIE activity.

Examples of such diseases and conditions include a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer). A therapeutically effective amount of a IGF-II/IGF-IIIE binding protein is administered to a subject having or suspected of having a
10 disorder in which IGF-II/IGF-IIIE activity is implicated, thereby treating (e.g., ameliorating or improving a symptom or feature of a disorder, slowing, stabilizing or halting disease progression) the disorder.

The IGF-II/IGF-IIIE binding protein is administered in a therapeutically effective amount. A therapeutically effective amount of an IGF-II/IGF-IIIE binding protein is the
15 amount which is effective, upon single or multiple dose administration to a subject, in treating a subject, e.g., curing, alleviating, relieving or improving at least one symptom of a disorder in a subject to a degree beyond that expected in the absence of such treatment. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the
20 compound to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects.

The IGF-II/IGF-IIIE binding proteins and growth hormone/growth hormone releasing hormone pathway modulators described herein are useful to treat diseases or
25 conditions in which IGF-II and/or IGF-IIIE activity and growth hormone/growth hormone releasing hormone pathway activity are implicated, e.g., a disease or condition described herein, or to treat one or more symptoms associated therewith.

Examples of such diseases and conditions include a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer). A therapeutically effective amount of a IGF-II/IGF-IIIE binding protein and growth hormone/growth hormone releasing hormone
30 pathway modulators is administered in combination to a subject having or suspected of

having a disorder in which IGF-II/IGF-IIIE activity is implicated, thereby treating (*e.g.*, ameliorating or improving a symptom or feature of a disorder, slowing, stabilizing or halting disease progression) the disorder.

The IGF-II/IGF-IIIE binding protein and growth hormone/growth hormone releasing hormone pathway modulators are administered in a therapeutically effective amount. A therapeutically effective amount of an IGF-II/IGF-IIIE binding protein or growth hormone/growth hormone releasing hormone pathway modulator is the amount which is effective, upon single or multiple dose administration to a subject, in treating a subject, *e.g.*, curing, alleviating, relieving or improving at least one symptom of a disorder in a subject to a degree beyond that expected in the absence of such treatment. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects.

A therapeutically effective amount can be administered, typically an amount of the compound which is effective, upon single or multiple dose administration to a subject, in treating a subject, *e.g.*, curing, alleviating, relieving or improving at least one symptom of a disorder in a subject to a degree beyond that expected in the absence of such treatment. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects. A therapeutically effective dosage preferably modulates a measurable parameter, favorably, relative to untreated subjects. The ability of a compound to inhibit a measurable parameter can be evaluated in an animal model system predictive of efficacy in a human disorder.

Dosage regimens can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or

increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Cancer

The disclosure provides methods of treating (e.g., slowing, eliminating, or reversing tumor growth, preventing or reducing, either in number or size, metastases, reducing or eliminating tumor cell invasiveness, providing an increased interval to tumor progression, or increasing disease-free survival time) cancer (e.g., breast cancer, head and neck cancer, oral cavity cancer, laryngeal cancer, colorectal cancer, liver cancer, chondrosarcoma, ovarian cancer, testicular carcinoma, melanoma, brain tumors (e.g., astrocytomas, glioblastomas, gliomas)) by administering an effective amount of an IGF-II/IGF-IIIE binding protein (e.g., an anti-IGF-II/IGF-IIIE IgG or Fab). In some embodiments, the IGF-II/IGF-IIIE binding protein inhibits IGF-II/IGF-IIIE activity.

In certain embodiments, the IGF-II/IGF-IIIE binding protein is administered as a single agent treatment. In other embodiments, the IGF-II/IGF-IIIE binding protein is administered in combination with an additional anti-cancer agent.

Also provided are methods of preventing or reducing risk of developing cancer, by administering an effective amount of an IGF-II/IGF-IIIE binding protein to a subject at risk of developing cancer, thereby reducing the subject's risk of developing a cancer.

The disclosure further provides methods of modulating (e.g., reducing or preventing) angiogenesis at a tumor site by administering an effective amount of an IGF-II/IGF-IIIE binding protein, thereby reducing or preventing angiogenesis at the tumor site. The IGF-II/IGF-IIIE binding protein may be administered as a single agent therapy or in combination with additional agents.

The disclosed methods are useful in the prevention and treatment of solid tumors, soft tissue tumors, and metastases thereof. Solid tumors include malignancies (e.g., sarcomas, adenocarcinomas, and carcinomas) of the various organ systems, such as those

of lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary (e.g., renal, urothelial, or testicular tumors) tracts, pharynx, prostate, and ovary. Exemplary adenocarcinomas include colorectal cancers, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, and cancer of the small intestine. Additional exemplary solid tumors include: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, gastrointestinal system carcinomas, colon carcinoma, pancreatic cancer, breast cancer, genitourinary system carcinomas, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, endocrine system carcinomas, testicular tumor, lung carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma. Metastases of the aforementioned cancers can also be treated or prevented in accordance with the methods described herein.

Guidance for determination of a therapeutically effective amount for treatment of cancer may be obtained by reference to *in vivo* models of the cancer to be treated. For example, the amount of an IGF-II/IGF-IIe binding protein that is a therapeutically effective amount in a rodent or Libechov minipig model of cancer may be used to guide the selection of a dose that is a therapeutically effective amount. A number of rodent models of human cancers are available, including nude mouse/tumor xenograft systems (e.g., melanoma xenografts; see, e.g., Trikha et al. Cancer Research 62:2824-2833 (2002)) and murine models of breast cancer or glioma (e.g., Kuperwasser et al., Cancer Research 65, 6130-6138, (2005); Bradford et al., Br J Neurosurg. 3(2):197-210 (1989)). A melanoblastoma-bearing Libechov minipig (MeLiM) is available as an animal model of melanoma (e.g., Boisgard et al., Eur J Nucl Med Mol Imaging 30(6):826-34 (2003)).

The disclosure provides methods of treating (e.g., slowing, eliminating, or reversing tumor growth, preventing or reducing, either in number or size, metastases, reducing or eliminating tumor cell invasiveness, providing an increased interval to tumor progression, or increasing disease-free survival time) cancer (e.g., breast cancer, head and neck cancer, oral cavity cancer, laryngeal cancer, chondrosarcoma, ovarian cancer, testicular carcinoma, melanoma, brain tumors (e.g., astrocytomas, glioblastomas, gliomas)) by administering an effective amount of an IGF-II/IGF-IIE binding protein (e.g., an anti-IGF-II/IGF-IIE IgG or Fab) and growth hormone/growth hormone releasing hormone pathway modulators, such as SOMAVERT®.

Also provided are methods of preventing or reducing risk of developing cancer, by administering effective amounts of an IGF-II/IGF-IIE binding protein and a growth hormone/growth hormone releasing hormone pathway modulator to a subject at risk of developing cancer, thereby reducing the subject's risk of developing a cancer.

The disclosed methods are useful in the prevention and treatment of solid tumors, soft tissue tumors, and metastases thereof. Solid tumors include malignancies (e.g., sarcomas, adenocarcinomas, and carcinomas) of the various organ systems, such as those of lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary (e.g., renal, urothelial, or testicular tumors) tracts, pharynx, prostate, and ovary. Exemplary adenocarcinomas include colorectal cancers, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, and cancer of the small intestine. Additional exemplary solid tumors include: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, gastrointestinal system carcinomas, colon carcinoma, pancreatic cancer, breast cancer, genitourinary system carcinomas, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, endocrine system carcinomas, testicular tumor, lung carcinoma, small cell lung carcinoma, non-small cell lung

carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma. Metastases of the aforementioned cancers can also be treated or prevented in accordance with the methods described herein.

Guidance for determination of a therapeutically effective amount for treatment of cancer may be obtained by reference to *in vivo* models of the cancer to be treated. For example, the amount of an IGF-II/IGF-IIIE binding protein or growth hormone/growth hormone releasing hormone pathway modulator that is a therapeutically effective amount in a rodent or Libechov minipig model of cancer may be used to guide the selection of a dose that is a therapeutically effective amount. A number of rodent models of human cancers are available, including nude mouse/tumor xenograft systems (e.g., melanoma xenografts; see, e.g., Trikha et al. Cancer Research 62:2824-2833 (2002)) and murine models of breast cancer or glioma (e.g., Kuperwasser et al., Cancer Research 65, 6130-6138, (2005); Bradford et al., Br J Neurosurg. 3(2):197-210 (1989)). A melanoblastoma-bearing Libechov minipig (MeLiM) is available as an animal model of melanoma (e.g., Boisgard et al., Eur J Nucl Med Mol Imaging 30(6):826-34 (2003)).

Combination Therapies

The IGF-II/IGF-IIIE binding proteins described herein, e.g., anti- IGF-II/IGF-IIIE Fabs or IgGs, can be administered in combination with one or more of the other therapies for treating a disease or condition associated with IGF-II/IGF-IIIE activity, e.g., a disease or condition described herein. For example, an IGF-II/IGF-IIIE binding protein can be used therapeutically or prophylactically with surgery, an IGF-II inhibitor, e.g., a small molecule inhibitor, another anti- IGF-II/IGF-IIIE Fab or IgG (e.g., another Fab or IgG described herein), another IGF-II inhibitor, a peptide inhibitor, or small molecule inhibitor. Examples of IGF-II inhibitors that can be used in combination therapy with an IGF-II/IGF-IIIE binding protein described herein include anti-IGF-II antibodies that cross react with the IGF-I and IGF-II (see, e.g., WO2007118214, WO2007070432, EP1505075, US20060165695, WO2005028515 , WO2005027970 , WO2005018671) as well as anti-IGF-II antibodies that react only with IGF-II (see, e.g., WO2007118214).

One or more small-molecule IGF-II/IGF-IIE inhibitors can be used in combination with one or more IGF-II/IGF-IIE binding proteins described herein. For example, the combination can result in a lower dose of the small-molecule inhibitor being needed, such that side effects are reduced.

5 The IGF-II/IGF-IIE binding proteins described herein can be administered in combination with one or more of the other therapies for treating cancers, including, but not limited to: surgery; radiation therapy, and chemotherapy. For example, proteins that inhibit IGF-II or that inhibit a downstream event of IGF-II/IGF-IIE activity can also be used in combination with other anti-cancer therapies, such as radiation therapy,
10 chemotherapy, surgery, or administration of a second agent. For example, the second agent can be a Tie-1 inhibitor (e.g., Tie-1 binding proteins; see e.g., U.S. Ser. No. 11/199,739 and PCT/US2005/0284, both filed August 9, 2005). As another example, the second agent can be one that targets or negatively regulates the VEGF signaling pathway. Examples of this latter class include VEGF antagonists (e.g., anti-VEGF antibodies such
15 as bevacizumab) and VEGF receptor antagonists (e.g., anti-VEGF receptor antibodies). One particularly preferred combination includes bevacizumab. The combination can further include 5-FU and leucovorin, and/or irinotecan.

 Further, the IGF-II/IGF-IIE binding proteins described herein can be administered in combination with one or more epidermal growth factor (EGF) pathway inhibitors, e.g.,
20 for the treatment of cancer. The EGF pathway inhibitor may be an EGF receptor (EGFR) inhibitor, such as a small molecule inhibitor (such as erlotinib (e.g., TARCEVA®) or gefitinib (e.g., IRESSA®)), or an antibody that binds to EGFR, such as cetuximab (e.g., ERBITUX®). Additional inhibitors of the pathway include PD1 53035, SU5271, and ZD1 839. In some embodiments, the EGF pathway inhibitor is erlotinib.

25 The term “combination” refers to the use of the two or more agents or therapies to treat the same patient, wherein the use or action of the agents or therapies overlap in time. The agents or therapies can be administered at the same time (e.g., as a single formulation that is administered to a patient or as two separate formulations administered
 concurrently) or sequentially in any order. Sequential administrations are administrations
30 that are given at different times. The time between administration of the one agent and another agent can be minutes, hours, days, or weeks. The use of an IGF-II/IGF-IIE

binding protein described herein can also be used to reduce the dosage of another therapy, e.g., to reduce the side-effects associated with another agent that is being administered, e.g., to reduce the side-effects of an anti-VEGF antibody such as bevacizumab. Accordingly, a combination can include administering a second agent at a dosage at least 10, 20, 30, or 50% lower than would be used in the absence of the IGF-II/IGF-IIIE binding protein.

The second agent or therapy can also be another anti-cancer agent or therapy. Nonlimiting examples of anti-cancer agents include, e.g., anti-angiogenic agents, anti-microtubule agents, topoisomerase inhibitors, antimetabolites, mitotic inhibitors, alkylating agents, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis, radiation, and antibodies against other tumor-associated antigens (including naked antibodies, immunotoxins and radioconjugates). Examples of the particular classes of anti-cancer agents are provided in detail as follows: anti-angiogenic agents, e.g., VEGF pathway antagonists (agents that targets or negatively regulate the VEGF signaling pathway) including VEGF inhibitors (e.g., agents that directly inhibit VEGF (e.g., VEGF-A, -B, or -C), such as by binding VEGF (e.g., anti-VEGF antibodies such as bevacizumab (AVASTIN®) or ranibizumab, or other inhibitors such as pegaptanib, ranibizumab, NEOVASTAT®, AE-941, VEGF Trap, and PI-88)), modulators of VEGF expression (e.g., INGN-241, oral tetrathiomolybdate, 2-methoxyestradiol, 2-methoxyestradiol nanocrystal dispersion, bevasiranib sodium, PTC-299, Veglin), inhibitors of a VEGF receptor (e.g., KDR or VEGF receptor III (Flt4), for example anti-KDR antibodies, VEGFR2 antibodies such as CDP-791, IMC-1121B, VEGFR2 blockers such as CT-322), modulators of VEGFR expression (e.g., VEGFR1 expression modulator Sirna-027) or inhibitors of VEGF receptor downstream signaling. In some embodiments, the VEGF antagonist agent is bevacizumab, pegaptanib, ranibizumab, sorafenib, sunitinib, NEOVASTAT®, AE-941, VEGF Trap, pazopanib, vandetanib, vatalanib, cediranib, fenretinide, squalamine, INGN-241, oral tetrathiomolybdate, tetrathiomolybdate, Panzem NCD, 2-methoxyestradiol, AEE-788, AG-013958, bevasiranib sodium, AMG-706, axitinib, BIBF-1120, CDP-791, CP-547632, PI-88, SU-14813, SU-6668, XL-647, XL-999, IMC-1121B, ABT-869, BAY-57-9352, BAY-73-4506, BMS-582664, CEP-7055, CHIR-265, CT-322, CX-3542, E-

7080, ENMD-1198, OSI-930, PTC-299, Sirna-027, TKI-258, Veglin, XL-184, or ZK-304709; antitubulin/antimicrotubule agent, e.g., paclitaxel, vincristine, vinblastine, vindesine, vinorelbin, taxotere; topoisomerase I inhibitors, e.g., irinotecan, topotecan, camptothecin, doxorubicin, etoposide, mitoxantrone, daunorubicin, idarubicin, teniposide, amsacrine, epirubicin, merbarone, piroxantrone hydrochloride; antimetabolites, e.g., 5-fluorouracil (5-FU), methotrexate, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, cytarabine/Ara-C, trimetrexate, gemcitabine, acivicin, alanosine, pyrazofurin, N-Phosphoracetyl-L-Aspartate=PALA, pentostatin, 5-azacitidine, 5-Aza 2'-deoxycytidine, ara-A, cladribine, 5 - fluorouridine, FUDR, tiazofurin, N-[5-[N-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-N-methylamino]-2-thenoyl]-L-glutamic acid; alkylating agents, e.g., cisplatin, carboplatin, mitomycin C, BCNU=Carmustine, melphalan, thiotepa, busulfan, chlorambucil, plicamycin, dacarbazine, ifosfamide phosphate, cyclophosphamide, nitrogen mustard, uracil mustard, pipobroman, 4-ipomeanol; agents acting via other mechanisms of action, e.g., dihydrolenperone, spiromustine, and desipeptide; biological response modifiers, e.g., to enhance anti-tumor responses, such as interferon; apoptotic agents, such as actinomycin D; and anti-hormones, for example anti-estrogens such as tamoxifen or, for example antiandrogens such as 4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-(trifluoromethyl) propionanilide.

The additional agent or therapy can also be a vascular disrupting agent (VDA). VDAs useful in the instant invention disrupt existing vasculature, particularly the abnormal vasculature associated with angiogenesis-related disorders (e.g., neoplastic disorders). Exemplary VDAs include combretastatins and combretastatin-related compounds, colchicine and colchicine-related compounds, flavone-related compounds, dolastatin 10 derivatives, other microtubule disrupting agents and agents which target abnormal vasculature.

Combretastatin-related compounds include combretastatin A-4 disodium phosphate (CA4P), combretastatin analogs such as (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenyl]-L-serinamide hydrochloride (AVE8062, Sanofi-Aventis), and combretastatin A-1 prodrugs such as CA-1-P (also known as OXI4503, Oxigene).

Colchicine-related compounds include N-acetylcolchinol (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl dihydrogenphosphate (ZD6126, AstraZeneca).

Flavone-related compounds include flavone acetic acid (FAA) and tricyclic analogues of FAA, such as 5,6-dimethylxanthenone-4-acetic acid (DMXAA, also known as AS1404, Antisoma).

Dolastatin 10-related compounds include soblidotin (*N*²-(*N,N*-dimethyl-L-valyl)-*N*-[(1*S*,2*R*)-2-methoxy-4-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(2-phenylethyl)]amino]propyl]-1-pyrrolidinyl]-1-[(*S*)-1-methylpropyl]-4-oxobutyl]-*N*-methyl-L-valinamide, also known as TZT1027).

Additional VDAs include BNC105 (Bionomics Ltd.) and microtubule disrupting agents such as MPC-6827 (Myriad Genetics), CYT997 (Cytopia Ltd.).

A combination therapy can include administering an agent that reduces the side effects of other therapies. The agent can be an agent that reduces the side effects of anti-cancer treatments. For example, the agent can be leucovorin.

The IGF-II/IGF-IIIE binding proteins described herein, e.g., anti-IGF-II/IGF-IIIE Fabs or IgGs, are administered in combination with a growth hormone/growth hormone releasing hormone pathway modulator, and optionally one or more of the other therapies for treating a disease or condition associated with IGF-II/IGF-IIIE activity and growth hormone/growth hormone releasing hormone pathway activity, e.g., a disease or condition described herein. For example, an IGF-II/IGF-IIIE binding protein and growth hormone/growth hormone releasing hormone pathway modulator can be used therapeutically or prophylactically with surgery, an IGF-II inhibitor, e.g., a small molecule inhibitor, another anti-IGF-II/IGF-IIIE Fab or IgG (e.g., another Fab or IgG described herein), another IGF-II inhibitor, a peptide inhibitor, or small molecule inhibitor.

The IGF-II/IGF-IIIE binding proteins and growth hormone/growth hormone releasing hormone pathway modulators described herein can be administered in combination with one or more of the other therapies for treating cancers, including, but not limited to: surgery; radiation therapy, and chemotherapy. For example, proteins that inhibit IGF-II or that inhibit a downstream event of IGF-II/IGF-IIIE activity can also be

used in combination with other anti-cancer therapies, such as radiation therapy, chemotherapy, surgery, or administration of a second agent. For example, the second agent can be a Tie-1 inhibitor (e.g., Tie-1 binding proteins; see e.g., U.S. Ser. No. 11/199,739 and PCT/US2005/0284, both filed August 9, 2005). As another example, the second agent can be one that targets or negatively regulates the VEGF signaling pathway. Examples of this latter class include VEGF antagonists (e.g., anti-VEGF antibodies such as bevacizumab) and VEGF receptor antagonists (e.g., anti-VEGF receptor antibodies). One particularly preferred combination includes bevacizumab. The combination can further include 5-FU and leucovorin, and/or irinotecan.

Further, the IGF-II/IGF-IIIE binding proteins and growth hormone/growth hormone releasing hormone pathway modulators described herein can be administered in combination with one or more epidermal growth factor (EGF) pathway inhibitors, e.g., for the treatment of cancer. The EGF pathway inhibitor may be an EGF receptor (EGFR) inhibitor, such as a small molecule inhibitor (such as erlotinib (e.g., TARCEVA®) or gefitinib (e.g., IRESSA®)), or an antibody that binds to EGFR, such as cetuximab (e.g., ERBITUX®). Additional inhibitors of the pathway include PDI 53035, SUS271, and ZDI 839. In some embodiments, the EGF pathway inhibitor is erlotinib.

The term “combination” refers to the use of the two or more agents or therapies to treat the same patient, wherein the use or action of the agents or therapies overlap in time. The agents or therapies can be administered at the same time (e.g., as a single formulation that is administered to a patient or as two separate formulations administered concurrently) or sequentially in any order. Sequential administrations are administrations that are given at different times. The time between administration of the one agent and another agent can be minutes, hours, days, or weeks. The use of an IGF-II/IGF-IIIE binding protein in combination with a growth hormone/growth hormone releasing hormone pathway modulator described herein can also be used to reduce the dosage of the modulator (and vice versa), or, to reduce the side-effects associated with yet another agent that is being administered, e.g., to reduce the side-effects of an anti-VEGF antibody such as bevacizumab. Accordingly, a combination can include administering a second agent at a dosage at least 10, 20, 30, or 50% lower than would be used in the absence of

the IGF-II/IGF-IIIE binding protein and/or growth hormone/growth hormone releasing hormone pathway modulator.

The third agent or therapy, in addition to the IGF-II/IGF-IIIE and growth hormone/growth hormone releasing hormone pathway modulator combination can also be another anti-cancer agent or therapy. Nonlimiting examples of anti-cancer agents include, e.g., anti-angiogenic agents, anti-microtubule agents, topoisomerase inhibitors, antimetabolites, mitotic inhibitors, alkylating agents, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis, radiation, and antibodies against other tumor-associated antigens (including naked antibodies, immunotoxins and radioconjugates). Examples of the particular classes of anti-cancer agents are provided in detail as follows: anti-angiogenic agents, e.g., VEGF pathway antagonists (agents that targets or negatively regulate the VEGF signaling pathway) including VEGF inhibitors (e.g., agents that directly inhibit VEGF (e.g., VEGF-A, -B, or -C), such as by binding VEGF (*e.g.*, anti-VEGF antibodies such as bevacizumab (AVASTIN®) or ranibizumab, or other inhibitors such as pegaptanib, ranibizumab, NEOVASTAT®, AE-941, VEGF Trap, and PI-88)), modulators of VEGF expression (*e.g.*, INGN-241, oral tetrathiomolybdate, 2-methoxyestradiol, 2-methoxyestradiol nanocrystal dispersion, bevasiranib sodium, PTC-299, Veglin), inhibitors of a VEGF receptor (e.g., KDR or VEGF receptor III (Flt4), for example anti-KDR antibodies, VEGFR2 antibodies such as CDP-791, IMC-1121B, VEGFR2 blockers such as CT-322), modulators of VEGFR expression (*e.g.*, VEGFR1 expression modulator Sirna-027) or inhibitors of VEGF receptor downstream signaling. In some embodiments, the VEGF antagonist agent is bevacizumab, pegaptanib, ranibizumab, sorafenib, sunitinib, NEOVASTAT®, AE-941, VEGF Trap, pazopanib, vandetanib, vatalanib, cediranib, fenretinide, squalamine, INGN-241, oral tetrathiomolybdate, tetrathiomolybdate, Panzem NCD, 2-methoxyestradiol, AEE-788, AG-013958, bevasiranib sodium, AMG-706, axitinib, BIBF-1120, CDP-791, CP-547632, PI-88, SU-14813, SU-6668, XL-647, XL-999, IMC-1121B, ABT-869, BAY-57-9352, BAY-73-4506, BMS-582664, CEP-7055, CHIR-265, CT-322, CX-3542, E-7080, ENMD-1198, OSI-930, PTC-299, Sirna-027, TKI-258, Veglin, XL-184, or ZK-304709; antitubulin/antimicrotubule agent, e.g., paclitaxel, vincristine, vinblastine, vindesine,

vinorelbin, taxotere; topoisomerase I inhibitors, e.g., irinotecan, topotecan, camptothecin, doxorubicin, etoposide, mitoxantrone, daunorubicin, idarubicin, teniposide, amsacrine, epirubicin, merbarone, piroxantrone hydrochloride; antimetabolites, e.g., 5-fluorouracil (5-FU), methotrexate, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, cytarabine/Ara-C, trimetrexate, gemcitabine, acivicin, alanosine, pyrazofurin, N-Phosphoracetyl-L-Asparate=PALA, pentostatin, 5-azacitidine, 5-Aza 2'-deoxycytidine, ara-A, cladribine, 5 - fluorouridine, FUDR, tiazofurin, N-[5-[N-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-N-methylamino]-2-thenoyl]-L-glutamic acid; alkylating agents, e.g., cisplatin, carboplatin, mitomycin C, BCNU=Carmustine, melphalan, thiotepa, busulfan, chlorambucil, plicamycin, dacarbazine, ifosfamide phosphate, cyclophosphamide, nitrogen mustard, uracil mustard, pipobroman, 4-ipomeanol; agents acting via other mechanisms of action, e.g., dihydrolenperone, spiromustine, and desipeptide; biological response modifiers, e.g., to enhance anti-tumor responses, such as interferon; apoptotic agents, such as actinomycin D; and anti-hormones, for example anti-estrogens such as tamoxifen or, for example antiandrogens such as 4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-(trifluoromethyl) propionanilide.

The additional agent or therapy can also be a vascular disrupting agent (VDA). VDAs useful in the instant invention disrupt existing vasculature, particularly the abnormal vasculature associated with angiogenesis-related disorders (e.g., neoplastic disorders). Exemplary VDAs include combretastatins and combretastatin-related compounds, colchicine and colchicine-related compounds, flavone-related compounds, dolastatin 10 derivatives, other microtubule disrupting agents and agents which target abnormal vasculature.

Combretastatin-related compounds include combretastatin A-4 disodium phosphate (CA4P), combretastatin analogs such as (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenyl]-L-serinamide hydrochloride (AVE8062, Sanofi-Aventis), and combretastatin A-1 prodrugs such as CA-1-P (also known as OXI4503, Oxigene).

Colchicine-related compounds include N-acetylcolchinol (5*S*)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*]cyclohepten-3-yl dihydrogenphosphate (ZD6126, AstraZeneca).

Flavone-related compounds include flavone acetic acid (FAA) and tricyclic analogues of FAA, such as 5,6-dimethylxanthenone-4-acetic acid (DMXAA, also known as AS1404, Antisoma).

Dolastatin 10-related compounds include soblidotin (N^2 -(*N,N*-dimethyl-L-valyl)-*N*-[(1*S*,2*R*)-2-methoxy-4-[(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(2-phenylethyl)]amino]propyl]-1-pyrrolidinyl]-1-[(*S*)-1-methylpropyl]-4-oxobutyl]-*N*-methyl-L-valinamide, also known as TZT1027).

Additional VDAs include BNC105 (Bionomics Ltd.) and microtubule disrupting agents such as MPC-6827 (Myriad Genetics), CYT997 (Cytopia Ltd.).

A combination therapy can include administering an agent that reduces the side effects of other therapies. The agent can be an agent that reduces the side effects of anti-cancer treatments. For example, the agent can be leucovorin.

Combination therapies that include administering an IGF-II/IGF-IIE binding protein or other binding protein described herein and a growth hormone/growth hormone releasing hormone pathway modulator can also be used to treat a subject having or at risk for another angiogenesis related disorder (e.g., a disorder other than cancer, e.g., disorders that include undesired endothelial cell proliferation or undesirable inflammation, e.g., rheumatoid arthritis).

Diagnostic Uses

Proteins that bind to IGF-II/IGF-IIE and identified by the method described herein and/or detailed herein have *in vitro* and *in vivo* diagnostic utilities. The IGF-II/IGF-IIE binding proteins described herein (e.g., the proteins that bind and inhibit, or the proteins that bind but do not inhibit IGF-II/IGF-IIE) can be used, e.g., for *in vivo* imaging, e.g., during a course of treatment for a disease or condition in which IGF-II and/or IGF-IIE is active, e.g., a disease or condition described herein, or in diagnosing a disease or condition described herein.

In one aspect, the disclosure provides a diagnostic method for detecting the presence of IGF-II and/or IGF-IIE, *in vitro* or *in vivo* (e.g., *in vivo* imaging in a subject). The method can include localizing IGF-II and/or IGF-IIE within a subject or within a sample from a subject. With respect to sample evaluation, the method can include, for

example: (i) contacting a sample with IGF-II/IGF-IIE binding protein; and (ii) detecting location of the IGF-II/IGF-IIE binding protein in the sample.

An IGF-II/IGF-IIE binding protein can also be used to determine the qualitative or quantitative level of expression of IGF-II and/or IGF-IIE in a sample. The method can also include contacting a reference sample (e.g., a control sample) with the binding protein, and determining a corresponding assessment of the reference sample. A change, e.g., a statistically significant change, in the formation of the complex in the sample or subject relative to the control sample or subject can be indicative of the presence of IGF-II and/or IGF-IIE in the sample. In one embodiment, the IGF-II/IGF-IIE binding protein does not cross react with another metalloproteinase.

The IGF-II/IGF-IIE binding proteins are also useful for *in vivo* tumor imaging. Better clinical endpoints are needed to monitor the efficacy of drugs that are designed to block enzymatic function (Zucker et al, 2001, Nature Medicine 7:655- 656). Imaging of tumors *in vivo* by using labeled IGF-II/IGF-IIE binding proteins could be of help to target the delivery of the binding protein to tumors for cancer diagnosis, intraoperative tumor detection, and for investigations of drug delivery and tumor physiology. IGF-II/IGF-IIE binding proteins can be used to monitor native enzymatic activity *in vivo* at invasive sites. Another exemplary method includes: (i) administering the IGF-II/IGF-IIE binding protein to a subject; and (iii) detecting location of the IGF-II/IGF-IIE binding protein in the subject. The detecting can include determining location or time of formation of the complex.

The IGF-II/IGF-IIE binding protein can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials.

Complex formation between the IGF-II/IGF-IIE binding protein and IGF-II and/or IGF-IIE can be detected by evaluating the binding protein bound to the IGF-II/IGF-IIE or unbound binding protein. Conventional detection assays can be used, e.g., an enzyme-linked immunosorbent assays (ELISA), a radioimmunoassay (RIA) or tissue immunohistochemistry. Further to labeling the IGF-II/IGF-IIE binding protein, the presence of IGF-II and/or IGF-IIE can be assayed in a sample by a competition

immunoassay utilizing standards labeled with a detectable substance and an unlabeled IGF-II/IGF-IIE binding protein. In one example of this assay, the biological sample, the labeled standards, and the IGF-II/IGF-IIE binding protein are combined and the amount of labeled standard bound to the unlabeled binding protein is determined. The amount of IGF-II and/or IGF-IIE in the sample is inversely proportional to the amount of labeled standard bound to the IGF-II/IGF-IIE binding protein.

Fluorophore and chromophore labeled proteins can be prepared. Because antibodies and other proteins absorb light having wavelengths up to about 310 nm, the fluorescent moieties should be selected to have substantial absorption at wavelengths above 310 nm and preferably above 400 nm. A variety of suitable fluoreseers and chromophores are described by Stryer, 1968, *Science* 162:526 and Brand, L. et al., 1972, *Annu. Rev. Biochem.* 41:843 868. The proteins can be labeled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Patent Nos. 3,940,475, 4,289,747, and 4,376,110. One group of fluoreseers having a number of the desirable properties described above is the xanthene dyes, which include the fluoresceins and rhodamines. Another group of fluorescent compounds are the naphthylamines. Once labeled with a fluorophore or chromophore, the protein can be used to detect the presence or localization of the IGF-II and/or IGF-IIE in a sample, e.g., using fluorescent microscopy (such as confocal or deconvolution microscopy).

Histological Analysis. Immunohistochemistry can be performed using the proteins described herein. For example, in the case of an antibody, the antibody can be synthesized with a label (such as a purification or epitope tag), or can be detectably labeled, e.g., by conjugating a label or label-binding group. For example, a chelator can be attached to the antibody. The antibody is then contacted to a histological preparation, e.g., a fixed section of tissue that is on a microscope slide. After an incubation for binding, the preparation is washed to remove unbound antibody. The preparation is then analyzed, e.g., using microscopy, to identify if the antibody bound to the preparation.

Of course, the antibody (or other polypeptide or peptide) can be unlabeled at the time of binding. After binding and washing, the antibody is labeled in order to render it detectable.

Protein Arrays. The IGF-II/IGF-IIIE binding protein can also be immobilized on a protein array. The protein array can be used as a diagnostic tool, e.g., to screen medical samples (such as isolated cells, blood, sera, biopsies, and the like). Of course, the protein array can also include other binding proteins, e.g., that bind to IGF-II and/or IGF-IIIE or to other target molecules.

Methods of producing polypeptide arrays are described, e.g., in De Wildt et al., 2000, *Nat. Biotechnol.* 18:989-994; Lueking et al., 1999, *Anal. Biochem.* 270:103-111; Ge, 2000, *Nucleic Acids Res.* 28, e3, I-VII; MacBeath and Schreiber, 2000, *Science* 289:1760-1763; WO 01/40803 and WO 99/51773A1. Polypeptides for the array can be spotted at high speed, e.g., using commercially available robotic apparatus, e.g., from Genetic Microsystems or BioRobotics. The array substrate can be, for example, nitrocellulose, plastic, glass, e.g., surface-modified glass. The array can also include a porous matrix, e.g., acrylamide, agarose, or another polymer.

For example, the array can be an array of antibodies, e.g., as described in De Wildt, *supra*. Cells that produce the proteins can be grown on a filter in an arrayed format. Polypeptide production is induced, and the expressed polypeptides are immobilized to the filter at the location of the cell. A protein array can be contacted with a labeled target to determine the extent of binding of the target to each immobilized polypeptide. Information about the extent of binding at each address of the array can be stored as a profile, e.g., in a computer database. The protein array can be produced in replicates and used to compare binding profiles, e.g., of a target and a non-target.

FACS (Fluorescence Activated Cell Sorting). The IGF-II/IGF-IIIE binding protein can be used to label cells, e.g., cells in a sample (e.g., a patient sample). The binding protein is also attached (or attachable) to a fluorescent compound. The cells can then be sorted using fluorescence activated cell sorter (e.g., using a sorter available from Becton Dickinson Immunocytometry Systems, San Jose CA; see also U.S. Patent Nos. 5,627,037; 5,030,002; and 5,137,809). As cells pass through the sorter, a laser beam excites the fluorescent compound while a detector counts cells that pass through and determines whether a fluorescent compound is attached to the cell by detecting fluorescence. The amount of label bound to each cell can be quantified and analyzed to characterize the sample.

The sorter can also deflect the cell and separate cells bound by the binding protein from those cells not bound by the binding protein. The separated cells can be cultured and/or characterized.

In Vivo Imaging. Also featured is a method for detecting the presence of an IGF-II and/or IGF-IIIE expressing tissues *in vivo*. The method includes (i) administering to a subject (e.g., a patient having, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer) an anti- IGF-II/IGF-IIIE antibody, conjugated to a detectable marker; (ii) exposing the subject to a means for detecting said detectable marker to the IGF-II and/or IGF-IIIE expressing tissues or cells. For example, the subject is imaged, e.g., by NMR or other tomographic means.

Examples of labels useful for diagnostic imaging include radiolabels such as ^{131}I , ^{111}In , ^{123}I , $^{99\text{m}}\text{Tc}$, ^{32}P , ^{125}I , ^3H , ^{14}C , and ^{188}Rh , fluorescent labels such as fluorescein and rhodamine, nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography (“PET”) scanner, chemiluminescers such as luciferin, and enzymatic markers such as peroxidase or phosphatase. Short range radiation emitters, such as isotopes detectable by short range detector probes can also be employed. The protein can be labeled with such reagents; for example, see Wensel and Meares, 1983, *Radioimmunoimaging and Radioimmunotherapy*, Elsevier, New York for techniques relating to the radiolabeling of antibodies and D. Colcher et al., 1986, *Meth. Enzymol.* 121: 802 816.

The binding protein can be labeled with a radioactive isotope (such as ^{14}C , ^3H , ^{35}S , ^{125}I , ^{32}P , ^{131}I). A radiolabeled binding protein can be used for diagnostic tests, e.g., an *in vitro* assay. The specific activity of a isotopically-labeled binding protein depends upon the half life, the isotopic purity of the radioactive label, and how the label is incorporated into the antibody.

In the case of a radiolabeled binding protein, the binding protein is administered to the patient, is localized to cells bearing the antigen with which the binding protein reacts, and is detected or “imaged” *in vivo* using known techniques such as radionuclear scanning using e.g., a gamma camera or emission tomography. See e.g., A.R. Bradwell et al., “Developments in Antibody Imaging”, *Monoclonal Antibodies for Cancer Detection and Therapy*, R.W. Baldwin et al., (eds.), pp 65 85 (Academic Press 1985).

Alternatively, a positron emission transaxial tomography scanner, such as designated Pet VI located at Brookhaven National Laboratory, can be used where the radiolabel emits positrons (e.g., ^{11}C , ^{18}F , ^{15}O , and ^{13}N).

MRI Contrast Agents. Magnetic Resonance Imaging (MRI) uses NMR to visualize internal features of living subject, and is useful for prognosis, diagnosis, treatment, and surgery. MRI can be used without radioactive tracer compounds for obvious benefit. Some MRI techniques are summarized in EP-A-0 502 814. Generally, the differences related to relaxation time constants T1 and T2 of water protons in different environments is used to generate an image. However, these differences can be insufficient to provide sharp high resolution images.

The differences in these relaxation time constants can be enhanced by contrast agents. Examples of such contrast agents include a number of magnetic agents paramagnetic agents (which primarily alter T1) and ferromagnetic or superparamagnetic (which primarily alter T2 response). Chelates (e.g., EDTA, DTPA and NTA chelates) can be used to attach (and reduce toxicity) of some paramagnetic substances (e.g., Fe^{+3} , Mn^{+2} , Gd^{+3}). Other agents can be in the form of particles, e.g., less than 10 μm to about 10 nm in diameter). Particles can have ferromagnetic, antiferromagnetic, or superparamagnetic properties. Particles can include, e.g., magnetite (Fe_3O_4), $\gamma\text{-Fe}_2\text{O}_3$, ferrites, and other magnetic mineral compounds of transition elements. Magnetic particles may include: one or more magnetic crystals with and without nonmagnetic material. The nonmagnetic material can include synthetic or natural polymers (such as sepharose, dextran, dextrin, starch and the like).

The IGF-II/IGF-IIe binding protein can also be labeled with an indicating group containing of the NMR active ^{19}F atom, or a plurality of such atoms inasmuch as (i) substantially all of naturally abundant fluorine atoms are the ^{19}F isotope and, thus, substantially all fluorine containing compounds are NMR active; (ii) many chemically active polyfluorinated compounds such as trifluoroacetic anhydride are commercially available at relatively low cost; and (iii) many fluorinated compounds have been found medically acceptable for use in humans such as the perfluorinated polyethers utilized to carry oxygen as hemoglobin replacements. After permitting such time for incubation, a whole body MRI is carried out using an apparatus such as one of those described by

Pykett, 1982, *Sci. Am.* 246:78 88 to locate and image tissues expressing IGF-II and/or IGF-IIIE.

EXEMPLIFICATIONS

The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

EXAMPLE 1: Selection and Screening of Anti-IGF-II/IGF-IIIE Antibodies from Libraries

Phage displaying Fabs were first placed in contact with magnetic streptavidin beads to deplete those which might bind to streptavidin beads. Non-binding phage were then placed in contact with biotinylated IGF-IIIE (amino acids 1-104) immobilized on streptavidin magnetic beads. Unbound phage were washed away and beads with bound phage placed with *E. coli* cells for propagation of phage. Propagated phage were placed in contact with magnetic streptavidin beads and with biotinylated IGF-I immobilized on streptavidin beads for depletion purposes. Unbound phage were placed in contact with biotinylated IGF-IIIE (amino acids 1-104) immobilized on streptavidin magnetic beads as before. Unbound phage were washed away and the whole process repeated for one more cycle. Gene III removal was then performed on propagated output phage. sFab ELISAs were then performed using IGF-IIIE (amino acids 1-104), IGF-II (amino acids 1-67), IGF-I and streptavidin as targets. Those sFab binding IGF-IIIE and IGF-II, but not IGF-I or streptavidin were then pursued. sFabs were screened for inhibition on IGF-II or IGF-IIIE stimulated Ba/F3 cell proliferation using the following materials and methods:

Cell Culture and Materials:

Ba/F3 cells were cultured in complete medium (90% RPMI 1640 + 10% FBS + 10 ng/ml IL-3 + 2 mM L-Alanyl-Glutamine + 1X Pen/Strep). Cells at passage 6 to 15 were used for the high throughput cell proliferation assay. IGF-II (67aa), IGF-IIIE (104aa) were at 10 µg/ml in PBS, kept at -70°C. IL-4 was purchased from R&D system, Cat#: 404-ML, and anti-IGF-II antibody was purchased from R&D system, Cat#:

MAB292. 34 sFabs from batch 1 and batch 2 in a median scale purification, all in PBS, were screened.

Screening Procedure:

IGF-II was prepared at concentration of 400ng/ml; IGF-IIIE at 800ng/ml; sFab at 200 µg/ml in PBS. 25 µl of IGF-II or IGF-IIIE was preincubated with 25 µl of sFab in 96-well plate, triplicate for each sFab, 30 min at room temperature in a total volume of 50 µl/well. The cells were prepared as follows:

- Harvest Ba/F3 cells by centrifuging at 1100 rpm for 5 min.
- Remove supernatant, and resuspend cells in 20 ml of PBS.
- Count cell density by mixing 10 µl of cell suspension with 10 µl of trypan blue solution and loading 10 µl to a hemocytometer to count cell density and viability.
- Spin down cells from PBS solution at 1100 rpm for 5 min.
- Remove supernatant, and resuspend cells in culture medium without IL-3 at 2×10^6 cells/ml.
- Add 25 µl of cell suspension to each well of the prepared 96-well plate to make the final cell density at 5×10^4 cells/well.
- Add 25 µl of IL-4 at 200ng/ml in IL-3 free medium to each well to make the final con. 50 ng/ml.
- The total volume is 100 µl per well. Neutralizing antibody from R&D at 50 µg/ml as positive control; no treatment as negative control. The final concentration of sFab: 50 µg/ml (1 µM)
- Incubate the plate at 37°C, 5% CO₂ for 72 h.

The MTS assay was performed as follows:

- Add 20 µl of CellTiter 96 Aqueous One Solution Reagent to each well
- Incubate at 37°C, 5% CO₂ for additional 4 h.
- Read plate for absorbance at a microplate spectrophotometer at wavelength 490 nm.

There were 2 Fabs from the first batch and 6 Fabs from the second batch that showed significant inhibitory effect on both IGF-II and IGF-IIIE stimulated Ba/F3 cell proliferation. These 8 Fabs, M0068-E03, M0072-C06, M0064-F02, M0072-G06, M0072-E03, M0070 H08, M0064-E04 and M0063-F02 were further evaluated for IC₅₀ determination.

EXAMPLE 2: IC₅₀ Determination of Anti-IGF-II/IGF-IIIE Fabs

The IC₅₀ values of the 8 sFabs for inhibition on IGF-II or IGF-IIIE stimulated Ba/F3 cell proliferation were determined as follows:

Cell Culture and Materials:

Ba/F3 cells were cultured in complete medium (90% RPMI 1640 + 10% FBS + 10 ng/ml IL-3 + 2 mM L-Alanyl-Glutamine + 1X Pen/Strep). Cells at passage 24 were used for cell proliferation assay. IGF-II (67aa), IGF-IIIE (104aa) were at 10 µg/ml in PBS and kept at -70°C. IL-4 was purchased from R&D system, Cat#: 404-ML, and anti-IGF-II antibody was purchased from R&D system, Cat#: MAB292. The 8 sFabs from Example 1 were subjected to medium scale purification, in PBS.

Procedure:

- Prepare IGF-II at concentration of 400ng/ml; IGF-IIIE at 800ng/ml; sFab at 200 µg/ml in PBS.
- Pre-incubate 25 µl of IGF-II or IGF-IIIE with 25 µl of 1:2 serially diluted sFab in a 96-well plate (50µg/ml down to 0), triplicate for each dose of each sFab, incubate for 30 min at room temperature in a total volume of 50 µl/well.
- Prepare cells:
 - Harvest Ba/F3 cells by centrifuging at 1100 rpm for 5 min.
 - Remove supernatant, and resuspend cells in 20 ml of PBS.
 - Count cell density by mixing 10 µl of cell suspension with 10 µl of trypan blue solution and loading 10 µl to a hemocytometer to count cell density and viability.
 - Spin down cells from PBS solution at 1100 rpm for 5 min.
 - Remove supernatant, and resuspend cells in culture medium without IL-3 at 2 X 10⁶ cells/ml.
 - Add 25 µl of cell suspension to each well of the prepared 96-well plate to make the final cell density at 5 X 10⁴ cells/well.
 - Add 25 µl of IL-4 at 200ng/ml in IL-3 free medium to each well to make the final con. 50 ng/ml.
 - The total volume is 100 µl per well.
 - Incubate the plate at 37°C, 5% CO₂ for 72 h.

MTS Assay:

- Add 20 µl of CellTiter 96 Aqueous One Solution Reagent to each well
- Incubate at 37°C, 5% CO₂ for additional 4 h.
- Read plate for absorbance at a microplate spectrophotometer at wavelength 490 nm.

An inhibitory effect of anti-IGF-II/IIIE sFabs was observed for 6 out of 8 Fabs for cell proliferation in a dose dependent manner with IC₅₀ values shown in Table 2. Fab 72E03 showed some inhibition, but not as significant as others in IGF-IIIE stimulated cell proliferation. The IC₅₀ value of 72E03 was not calculated due to low potency. Fab 70H08 did not show significant inhibition.

Table 2: IC₅₀ Values of the 6 Fabs which showed inhibitory effect on both IGF-II and IGF-IIIE stimulated cell proliferation.

IC ₅₀ μg/ml	M0072-G06	M0063-F02	M0064-F02	M0064-E04	M0068-E03	M0072-C06
IGF-II	8.7 ± 7.17	5.56 ± 2.61	0.68 ± 0.31	3.04 ± 1.89	2.27 ± 0.71	5.12 ± 3.25
IGF-IIIE	2.5 ± 0.87	2.04 ± 0.5	3.12 ± 1.44	5.46 ± 2.9	5.74 ± 3.43	2.3 ± 0.59

An inhibitory effect of most sFabs at 50 μg/ml on IGF-I stimulated cell proliferation was observed. Neu-IGF-I antibody showed potent inhibition; Neu-IGF-II antibody and all other sFabs except for 70H08 also showed about 30% inhibition.

In conclusion, 6 sFabs (M0072-G06, M0063-F02, M0064-F02, M0064-E0, M0068-E03 and M0072-C06) demonstrated significant inhibitory effect on both IGF-II and IGF-IIIE stimulated Ba/F3 cell proliferation. Further, all soluble Fabs except M0070-H08 at 50 μg/ml showed more or less inhibition (~30%) on IGF-I stimulated cell proliferation.

EXAMPLE 3: DNA and Amino Acid Sequences of Anti-IGF-II/IGF-IIIE Fabs

Exemplary Fabs that bind to both human IGF-II/IGF-IIIE were identified as described above and designated as: M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, and M0072-G06.

The DNA sequences of these Fab light chain variable regions (LV), light chain constant regions (LC), heavy chain variable regions (HV) and heavy chain constant regions (HC) are shown in Table 3:

Table 3: DNA sequences of anti-IGF-II/IGF-IIIE Fabs

>M0063-F02 (R0032-A01) LV

CAAGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
CCGGGCAAGTCAGCCCATTAACACATATTTAAATTGGTATCAGCAGAGACCAGGGAAAGCCCCTAGGATCC
TGATCTATACTTTCATCCACTTTGCAAAGTGGGGTCCCATCAAGGTTTCAAGTGGCAGTGGATCTGGGACAGAT
TTCATCTCACCATCAGCAGTGTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTTT
5 CCCTCTCACTTTTCGGCGGAGGGACCAAGGTGGAGATCAAA

>M0063-F02 (R0032-A01) LC

CGAACTGTGGCTGCACCATCTGTCTTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTC
TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCTCC
10 AATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACC
CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAG
CTCGCCCGTACAAAGAGCTTCAACAGGGGAGAGTGTTAATAA

>M0063-F02 (R0032-A01) HV

GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTACAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTAAGTACGAGATGGATTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTGTTATCTCTTCTTCTGGTGGCGGTACTATTTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCT
AGAGACAACCTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACCGCCATGTATTA
CTGTGCGAGAGGCCGGACCCTATACGGAGGTGCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCT
20 CAAGC

>M0063-F02 (R0032-A01) HC

GCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAGTCAAGCGCCCTGACCA
25 GCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCTAGCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTG
CCCTCTTCTAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
CAAGAAAGTTGAGCCCAAATCTTGTGCGGCCGCACATCATCACCATCACGGGGCCGCAGAACAAAAAC
TCATCTCAGAAGAGGATCTGAATGGGGCCGCAGAGGCTAGTTCTGCTAGTAACGCG

>M0064-E04 (R0032-A03) LV

CAAGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
CCAGGCGAGTCACGACATTAGCAACTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCC
TGATTTATGCTGCATCCCGTTTGCAAAGTGGGGTCCCATCAAGGTTTCAAGTGGCGGTGGATCTGGGACAGAT
35 TTCAGTCTCACCATCAGCAGTCTGCAAGCTGAAGATTTTGCAACTTATTACTGTCAACAGAGTTACAGTTT
CCCTCGAACTTTTGGCCAGGGGACCAACCTGGAGATCAAA

>M0064-E04 (R0032-A03) LC

CGAACTGTGGCTGCACCATCTGTCTTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTC
TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCTCC
40 AATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGTAAGGACAGCACCTACAGCCTCAGCAGCACC
CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAG
CTCGCCCGTACAAAGAGCTTCAACAGGGGAGAGTGTTAATAA

>M0064-E04 (R0032-A03) HV

GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTACAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTGTTTACGATATGAATTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTTCTATCTCTTCTTCTGGTGGCGGTACTCTTTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCT
AGAGACAACCTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCTGTGTATTA
50 CTGTGCGAGAGACTCTGATACCAGTTCTTATTACTGGTACTACGATCTCTGGGGCCGCGGCACCCCTGGTCA
CCGTCTCAAGC

>M0064-E04 (R0032-A03) HC

GCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAGTCAAGCGCCCTGACCA
55 GCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCTAGCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTG
CCCTCTTCTAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA

CAAGAAAGTTGAGCCCAAATCTTGTGCGGCCGCACATCATCATCACCATCACGGGGCCGCAGAACAAAAAC
TCATCTCAGAAGAGGATCTGAATGGGGCCGCAGAGGCTAGTTCTGCTAGTAACGCGTGATGA

5 >M0033-E05 (R0032-A05) LV
CAAGACATCCAGATGACCCAGTCTCCATCCTCACTGTCTGCATCTGTTGGAGACAGAGTCACCATCAGTTG
TCGGGCAAGTCAGGGCATTACCAATTATTTAGTCTGGTTTCAGCAGAAACCAGGGAAAGCCCCTAGGCTCC
TGATCTATGATGCCTCCACTTTGGAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAT
TTCACCTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTGTCAACAGGCTGACGGTTT
10 CCCTCTCACTTTTCGGCGAGGGGACCAAGGTGGAGATGAAA

>M0033-E05 (R0032-A05) LC
CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTC
TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCC
15 AATCGGGTAACTCCCAGGAGAGTGTACACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACC
CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAG
CTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAATAA

>M0033-E05 (R0032-A05) HV
20 GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTCATTACTCTATGTGGTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTTATATCGGTCCTTCTGGTGGCCATACTCGTTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCT
AGAGACAACCTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTA
CTGTGCTAGAGGGCTATATTATTATGATAGTAGCGTGACTCATGCCTTTGATCTCTGGGGCCAAGGGA
25 CAATGGTCACCGTCTCAAGC

>M0033-E05 (R0032-A05) HC
GCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCA
30 GCGGCGTCCACACCTTCCCGGCTGTCTTACAGTCTAGCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTG
CCCTCTTCTAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
CAAGAAAGTTGAGCCCAAATCTTGTGCGGCCGCACATCATCATCACCATCACGGGGCCGCAGAACAAAAAC
TCATCTCAGAAGAGGATCTGAATGGGGCCGCAGAGGCTAGTTCTGCTAGTAACGCGTGATGA

35 >M0070-H08 (R0032-A07) LV
CAAGACATCCAGATGACCCAGTCTCCGTCCCTGTCTGCATCTGCAGGAGACAGAGTCACCATCACTTG
CCGGGCAAGTCAGAGCATTAGCAGTTATTTAAATTGGTATCAGCAGAAACCAGGAAAAGCCCCTAACCTCC
TGATCTATACTACATCCAATTTACAAGGTGGGGTCCCATCAAGGTTTCAGTGGCAGTGGATCTGGGACAGAT
40 TTCACCTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGGGTTACAGTTT
CCCTCTCACTTTTCGGCGGAGGGACCAAGGTAGAGATCAAA

>M0070-H08 (R0032-A07) LC
CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTC
45 TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCC
AATCGGGTAACTCCCAGGAGAGTGTACACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACC
CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAACTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAG
CTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAATAA

50 >M0070-H08 (R0032-A07) HV
GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTCGTTACTGGATGATTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTTCTATCCGTTCTTCTGGCGAGACTAAGTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCTAGA
GACAACTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTACTG
55 TGCGAGAGGCCCTTTAAGCGATTACTATGATAGTAGTGGTTATTACTTTGATGCTTTTGATATCTGGGGCC
AAGGGACAATGGTCACCGTCTCAAGC

>M0070-H08 (R0032-A07) HC
 GCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC
 CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCA
 GCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCTAGCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTG
 5 CCCTCTTCTAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
 CAAGAAAGTTGAGCCCAAATCTTGTGCGGCCGCACATCATCATCACCATCACGGGGCCGCAGAACAAAAAC
 TCATCTCAGAAGAGGATCTGAATGGGGCCGCAGAGGCTAGTTCTGCTAGTAACGCG

10 >M0072-C06 (R0032-A09) LV
 CAAGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
 CCGGGCAAGTCAGAGTATTCGCAACTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAACTCC
 TGATCTATGCTGCATCCAAGTTGGAAGACGGGGTCCCATCAAGATTCAGTGGCAGTGGAACCTGGGACAGAT
 TTTACTCTCACCATCAGAAGTCTGCAACCTGAAGATTTTGCAAGTTATTTCTGTCAACAGAGCTACTCTAG
 15 TCCAGGGATCACTTTTCGGCCCTGGGACCAAGGTGGAGATCAAA
 >M0072-C06 (R0032-A09) LC
 CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTC
 TGTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCC
 AATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACC
 20 CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAG
 CTCGCCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAATAA

>M0072-C06 (R0032-A09) HV
 GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTACAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
 25 TTCCGGATTCACTTTCTCTGCTTACATTATGACTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
 TTTCTTCTATCTCTCCTTCTGGTGGCTATACTGTTTATGCTGACTCCGTAAAGGTCGCTTCACTATCTCT
 AGAGACAACCTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTATATTA
 CTGTGCGAGAGACTCGGGGTTTCGGGGACCCCTTTGACTACTGGGGCCAAGGGACAATGGTCACCGTCTCAA
 30 GC
 >M0072-C06 (R0032-A09) HC
 GCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC
 CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCA
 GCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCTAGCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTG
 35 CCCTCTTCTAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
 CAAGAAAGTTGAGCCCAAATCTTGTGCGGCCGCACATCATCATCACCATCACGGGGCCGCAGAACAAAAAC
 TCATCTCAGAAGAGGATCTGAATGGGGCCGCAGAGGCTAGTTCTGCTAGTAACGCGTGATGA

40 >M0064-F02 (R0032-A11) LV
 CAAGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
 CCGGGCAAGTCAGAGCATTAGCAATTACTTGAATTGGTATCAACAGAAACCAGGTAAAGCCCCTAAGCTCC
 TGATCTATACTGCATCCACTTTGCAGAGTGGGGTCCCATCAAGGTTTCAAGTGGCAGTGCATCTGGGACAGAC
 TTTACTCTCACCATCAACAGTCTGCAACCTGAAGATTTTGCTACTTACTCCTGTCAACAGAGTTACAATTC
 45 CCCCTGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAA
 >M0064-F02 (R0032-A11) LC
 CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTC
 TGTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCC
 50 AATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACC
 CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAG
 CTCGCCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAATAA

>M0064-F02 (R0032-A11) HV
 55 GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTACAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
 TTCCGGATTCACTTTCTCTAATTACATTATGTGGTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
 TTTCTGTTATCTCTTCTTCTGGTGGCATGACTCGTTATGCTGACTCCGTAAAGGTCGCTTCACTATCTCT

AGAGACAACCTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTA
CTGTGCGAGAGATAACGGTGACTACGTAGGCGAAAAAGGTTTTGATATCTGGGGCCAAGGGACAATGGTCA
CCGTCTCAAGC

5 >M0064-F02 (R0032-A11) HC
GCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCA
GCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCTAGCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTG
CCCTCTTCTAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
10 CAAGAAAGTTGAGCCCAAATCTTGTGCGGCCGCACATCATCATCACCATCACGGGGCCGCAGAACAAAAAC
TCATCTCAGAAGAGGATCTGAATGGGGCCGCAGAGGCTAGTTCTGCTAGTAACGCGTGATGA

>M0068-E03 (R0032-C01) LV
15 CAAGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
CCGGGCAAGTCAGAGCATTAACACTTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGGTCC
TGATCCATGCTGCATCCACTTTGGAAAGTGGGGTGCCATCAAGGTTTCAAGTGGCAGTGGATCTGCGACAGAA
TTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTTCTACTGTCAACAGAGTTACAGTGT
GCCCTTCACTTTTCGGCCCTGGGACCAGACTGTCTAGCAAA

20 >M0068-E03 (R0032-C01) LC
CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTC
TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCC
AATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACC
25 CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAG
CTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAATAA

>M0068-E03 (R0032-C01) HV
GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTTACGCTCTTTTACGTCTTTCTTGCGCTGC
30 TTCCGGATTCACTTTTCTCTGAGTACGTTATGGCTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTTCTATCGTTTCTTCTGGTGGCTATACTAAGTATGCTGACTCCGTTAAAGGTCGCTTCACTATCTCT
AGAGACAACCTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTA
CTGTGCAAAAGATATGACTTACAGTGGGGATGCTTTTGTATGTCTGGGGCCAAGGGACAATGGTCACCGTCT
CAAGC

35 >M0068-E03 (R0032-C01) HC
GCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCA
GCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCTAGCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTG
40 CCCTCTTCTAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
CAAGAAAGTTGAGCCCAAATCTTGTGCGGCCGCACATCATCATCACCATCACGGGGCCGCAGAACAAAAAC
TCATCTCAGAAGAGGATCTGAATGGGGCCGCAGAGGCTAGTTCTGCTAGTAACGCGTGATGA

45 >M0072-E03 (R0032-C03) LV
CAAGACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
CCGGGCCAGTCAGAGTATTAGTAGCTGGTTGGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCC
TGATCTATAAGGCGTCTAGTTTAGAAAGTGGGGTCCCATCAAGGTTTCAAGCGCAGTGGATCTGGGACAGAA
50 TTCACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTATTACTGCCAACAGTATAATAGTTA
TCCGTGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAA

>M0072-E03 (R0032-C03) LC
CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTC
55 TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCC
AATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACC

CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAG
CTCGCCCGTCCACAAAGAGCTTCAACAGGGGAGAGTGTTAATAA

>M0072-E03 (R0032-C03) HV

5 GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTGAGTACGAGATGTCTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTTCTATCTATTCTTCTGGTGGCTGGACTAAGTATACTGACTCCGTAAAGGTCGCTTCACTATCTCT
AGAGACAACCTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTA
10 CTGTGCGAAAGGTGTACACTATGATAGTAGTGGCCTTCCTATTGACTGGTACTTCGATCTCTGGGGCCGTG
GCACCCTGGTCACCGTCTCAAGC

>M0072-E03 (R0032-C03) HC

GCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCA
15 GCGGCGTCCACACCTTCCCGGTGTCTACAGTCTAGCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTG
CCCTCTTCTAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
CAAGAAAGTTGAGCCCAAATCTTGTGCGGCCGCACATCATCACCATCACGGGGCCGCAGAACAAAAAC
TCATCTCAGAAGAGGATCTGAATGGGGCCGCAGAGGCTAGTTCTGCTAGTAACGCG

20

>M0072-G06 (R0032-C05) LV

CAAGACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
CCGGGCCAGTCAGACAATTAGTAGCTGGCTGGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGTTGA
TGATCTATAAGGCGGCTAGTTTAGGAAGTGAGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAG
25 TTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTTCAACTTACTACTGCCAACAGTACAAAACCTTA
TCCCGTCACTTTTGGCCAGGGGACCAGGCTGGAGATCAAA

>M0072-G06 (R0032-C05) LC

CGAACTGTGGCTGCACCATCTGTCTTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTC
30 TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCC
AATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACC
CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAG
CTCGCCCGTCCACAAAGAGCTTCAACAGGGGAGAGTGTTAATAA

35

>M0072-G06 (R0032-C05) HV

GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGC
TTCCGGATTCACTTTCTCTGAGTACGTTATGTGGTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGG
TTTCTGTTATCTCTCCTTCTGGTGGCTATACTGTTTATGCTGACTCCGTAAAGGTCGCTTCACTATCTCT
AGAGACAACCTCTAAGAATACTCTCTACTTGCAGATGAACAGTTTAAGGGCTGAGGACACGGCCGTGTATTA
40 CTGTGCGAGAGATCGGGGGGAGCTACTACCCTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCAA
GC

>M0072-G06 (R0032-C05) HC

GCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC
45 CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCA
GCGGCGTCCACACCTTCCCGGTGTCTACAGTCTAGCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTG
CCCTCTTCTAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGA
CAAGAAAGTTGAGCCCAAATCTTGTGCGGCCGCACATCATCACCATCACGGGGCCGCAGAACAAAAAC
TCATCTCAGAAGAGGATCTGAATGGGGCCGCAGAGGCTAGTTCTGCTAGTAACGCGTGATGA

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The amino acid sequences of exemplary Fab LV, LC, HV and HC regions that bind to and inhibit human IGF-II and IGF-IIIE, the DNA sequence of which are provided in Table 3, are shown in Table 4:.

Table 4: Amino Acid sequences of anti-IGF-II/IGF-III Fabs

>M0063-F02-R0032-A01-LV
 QDIQMTQSPSSLSASVGDRVITITCRASQPINTYLNWYQQRPGKAPRILIIYTSSTLQSGVPSRFSGSGSGTD
 FTLTISSVQPEDFATYYCQQSYSFPLTFGGGTKVEIK
 5 >M0063-F02-R0032-A01-LC
 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST
 LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC..
 >M0063-F02-R0032-A01-HV
 EVQLLES GGGLVQPGGSLRLS CAASGFTFSKYEMDWVRQAPGKGLEWVSVISSSGGGTIYADSVKGRFTIS
 10 RDNSKNTLYLQMNSLRAEDTAMYYCARGRTLYGGAFDIWGQGTMTVTVSS
 >M0063-F02-R0032-A01-HC
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
 PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNAAEASSASNA

 15 >M0064-E04-R0032-A03-LV
 QDIQMTQSPSSLSASVGDRVITITQASHDISNYLNWYQQKPGKAPKLLIYAASRLQSGVPSRFSGGGSGTD
 FSLTISSLQAEDFATYYCQQSYSPRTFGQGTNLEIK
 >M0064-E04-R0032-A03-LC
 20 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST
 LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC..
 >M0064-E04-R0032-A03-HV
 EVQLLES GGGLVQPGGSLRLS CAASGFTFSVYDMNWVRQAPGKGLEWVSSISSSGGGTLYADSVKGRFTIS
 RDNSKNTLYLQMNSLRAEDTAVYYCARDSDTSSYYWYYDLWGRGTLVTVSS
 25 >M0064-E04-R0032-A03-HC
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
 PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNAAEASSASNA

 30 >M0033-E05-R0032-A05-LV
 QDIQMTQSPSSLSASVGDRVITISCRASQGITNYLVWFQQKPGKAPRLLIYDASTLESVPSRFSGSGSGTD
 FTLTISSLQPEDFATYYCQQADGFPLTFGEGTKVEMK
 >M0033-E05-R0032-A05-LC
 35 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST
 LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC..
 >M0033-E05-R0032-A05-HV
 EVQLLES GGGLVQPGGSLRLS CAASGFTFSHYSMWWVRQAPGKGLEWVSYIGPSGGHTRYADSVKGRFTIS
 RDNSKNTLYLQMNSLRAEDTAVYYCARGLYYYDSSSVTHAFDLWGQGTMTVTVSS
 40 >M0033-E05-R0032-A05-HC
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
 PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNAAEASSASNA

 45 >M0070-H08-R0032-A07-LV
 QDIQMTQSPSSLSASAGDRVITITCRASQSISSYLNWYQQKPGKAPNLLIYTTSNLQGGVPSRFSGSGSGTD
 FTLTISSLQPEDFATYYCQQGYSFPLTFGGGTKVEIK
 >M0070-H08-R0032-A07-LC
 50 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST
 LTLKADYEKHKLYACEVTHQGLSSPVTKSFNRGEC..
 >M0070-H08-R0032-A07-HV
 EVQLLES GGGLVQPGGSLRLS CAASGFTFSRYWMIWVRQAPGKGLEWVSSIRSSGETKYADSVKGRFTISR
 DNSKNTLYLQMNSLRAEDTAVYYCARGPLSDYYDSSGYFDADFIDWGQGTMTVTVSS
 55 >M0070-H08-R0032-A07-HC

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNGAAEASSASNA

5

>M0072-C06-R0032-A09-LV
QDIQMTQSPSSLSASVGDRVTITCRASQSIRNYLNWYQQKPGKAPKLLIYAASKLEDGVPSRFSGSGTGT
FTLTIRSLQPEDFASYFCQQSYSSPGITFGPGTKVEIK

10

>M0072-C06-R0032-A09-LC
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST
LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC..

>M0072-C06-R0032-A09-HV

EVQLLESQGGGLVQPGGSLRLSCAASGFTFSAYIMTWVRQAPGKGLEWVSSISPSGGYTVYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARDSGFGDPFDYWGQGTMTVTVSS

15

>M0072-C06-R0032-A09-HC
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNGAAEASSASNA

20

>M0064-F02-R0032-A11-LV
QDIQMTQSPSSLSASVGDRVTITCRASQSIISNYLNWYQQKPGKAPKLLIYTASTLQSGVPSRFSGSASGTD
FTLTINSLQPEDFATYSCQQSYNSPWTFGQGTKEIK

>M0064-F02-R0032-A11-LC

25

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST
LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC..

>M0064-F02-R0032-A11-HV

EVQLLESQGGGLVQPGGSLRLSCAASGFTFSNYIMWWVRQAPGKGLEWVSVISSSGMTRYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARDNGDYVGEKGFDIWGQGTMTVTVSS

30

>M0064-F02-R0032-A11-HC
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNGAAEASSASNA

35

>M0068-E03-R0032-C01-LV
QDIQMTQSPSSLSASVGDRVTITCRASQSINTYLNWYQQKPGKAPKVLIIHAASTLESGVPSRFSGSGSATE
FTLTISSLQPEDFATFYCQQSYSVPFTEFGPGTRLSSK

>M0068-E03-R0032-C01-LC

40

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST
LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC..

>M0068-E03-R0032-C01-HV

EVQLLESQGGGLVQPGGSLRLSCAASGFTFSEYVMAWVRQAPGKGLEWVSSIVSSGGYTKYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAKDMTYSGDAFDVWGQGTMTVTVSS

45

>M0068-E03-R0032-C01-HC
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNGAAEASSASNA

50

>M0072-E03-R0032-C03-LV
QDIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASSLESGVPSRFSGSGSGTE
FTLTISSLQPDDEFATYYCQQYNSYPWTFGQGTKEIK

>M0072-E03-R0032-C03-LC

55

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST
LTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC..

>M0072-E03-R0032-C03-HV

EVQLLES GGG LVQPGGSLRLSCAASGFTFSEYEMSWVRQAPGKGLEWVSSIYSSGGWTKYTDSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCAKGVHYDSSGLPIDWYFDLWGRGTLVTVSS

>M0072-E03-R0032-C03-HC

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNGAAEASSASNA

>M0072-G06-R0032-C05-LV

QDIQMTQSPSTLSASVGDRVTITCRASQTISWLAHYQQKPGKAPKLMYKAASLGSEVPSRFSGSGSGTE
FTLTISLQPEDFSTYYCQQYKTYPTVTFGQGRLEIK

>M0072-G06-R0032-C05-LC

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC..

>M0072-G06-R0032-C05-HV

EVQLLES GGG LVQPGGSLRLSCAASGFTFSEYVMWWVRQAPGKGLEWVSVISPSGGYTIVYADSVKGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARDRGGATTLDYWGQGTLLVTVSS

>M0072-G06-R0032-C05-HC

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNGAAEASSASNA

EXAMPLE 4: Germlining and Production of Anti-IGF-II/IGF-IIe IgGs

2 IgG's were germlined. Both were derived from VK1_02 DPK9/02. M0064-E04 required 4 changes overall 3 in the light chain proper and 1 in JK5 and M0064-F02 required 3 changes in the light chain proper and none in JK1.

The amino acid changes made in the germlining are illustrated below:

M0064-E04:

>VK1_02 DPK9/02

Length = 95; Score = 174 bits (442), Expect = 7e-48
Identities = 86/95 (90%), Positives = 89/95 (93%)

Query: 2 DIQMTQSPSSLSASVGDRVTITC QASHDISNYLN WYQQKPGKAPKLLIY AASRLQS
GVPS 61

DIQMTQSPSSLSASVGDRVTITC +AS IS+YLN WYQQKPGKAPKLLIY AAS LQS
GVPS

Sbjct: 1 DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS
GVPS 60

Query: 62 RFSGGGSGTDFSLTISSLQAEDFATYYC QQSYSEFP 96
RFSG GSGTDF+LTISSLQ EDFATYYC QQSYS P

Sbjct: 61 RFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTP 95

>JK5

Length = 12; Score = 24.3 bits (51), Expect = 4e-05
Identities = 10/11 (90%), Positives = 10/11 (90%)

Query: 98 T FGQGTNLEIK 108

T FGQGT LEIK
 Sbjct: 2 T FGQGTRLEIK 12

5 **M0064-F02-LV**

>VK1_O2 DPK9/O2

10 Length = 95; Score = 178 bits (451), Expect = 6e-49
 Identities = 87/95 (91%), Positives = 92/95 (96%)

Query: 2 DIQMTQSPSSLSASVGDRVITC RASQSIISNYLN WYQQKPGKAPKLLIY TASTLQS
 GVPS 61
 DIQMTQSPSSLSASVGDRVITC RASQSIIS+YLN WYQQKPGKAPKLLIY AS+LQS
 15 GVPS
 Sbjct: 1 DIQMTQSPSSLSASVGDRVITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS
 GVPS 60

20 Query: 62 RFSGSASGTDFTLTINSLQPEDFATYSC QQSYNSP 96
 RFSGS SGTDFTLTI+SLQPEDFATY C QQSY++P
 Sbjct: 61 RFSGSGSGTDFTLTISLQPEDFATYYC QQSYSTP 95

>JK1

25 Length = 12; Score = 30.4 bits (67), Expect = 6e-07
 Identities = 12/12 (100%), Positives = 12/12 (100%)

Query: 97 WT FGQGTKVEIK 108
 WT FGQGTKVEIK
 30 Sbjct: 1 WT FGQGTKVEIK 12

EXAMPLE 5: Affinity Measurement of Selected Anti-IGF-II/IGF-IIE Fabs and IgGs

Table 5: Affinity measurements of Fabs by SPR (Flexchip) (in duplicate)

Original isolate name	IGF-II	IGF-IIE		IGF-II	IGF-IIE
M0033-E05	4.3 E-09	8.7E-10		5.5 E-09	4.4 E-09
M0063-F02	7.7 E-10	1.2 E-10		1.6 E-09	2.0 E-10
M0064-E04	1.2 E-10	9.3 E-11		2.5 E-10	1.2 E-10
M0064-F02	4.5 E-10	3.2 E-10		1.2 E-09	2.9 E-10
M0068-E03	9.3 E-10	6.6 E-10		----	----
M0070-H08	3.3 E-09	3.6 E-10		1.1 E-09	3.9 E-10
M0072-C06	5.2 E-09	5.2 E-10		7.0 E-10	---
M0072-E03	7.5 E-10	9.6 E-11		3.0 E-10	2.2 E-10
M0072-G06	5.8 E-10	5.6 E-10		1.4 E-09	8.9 E-10

--- = No second affinity measurement obtained

5

Table 6: Affinity measurements of IgGs as measured by SPR (BIAcore®)

Non-germlined	IGF-II	IGF-IIE	Germlined	IGF-II	IGF-IIE
M0064-F02	0.14 nM	32 pM	X0008-A01	23 pM	< 23 pM
M0064-E04	0.52 nM	0.24 nM	X0005-H01	0.52 nM	0.26 nM

nM = E-09 pM = E-12

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EXAMPLE 6: IC₅₀ Determination of Selected Anti-IGF-II/IGF-IIE IgGs

M0063-F02

The purpose of this study was to test the large production of M0063-F02 IgG (parental) for its inhibition on IGF-II or IGF-IIE stimulated Ba/F3 cell proliferation and IC₅₀ determination.

15

Cell Culture and Materials:

Ba/F3 cells were cultured in complete medium (90% RPMI 1640 + 10% FBS + 10 ng/ml IL-3 + 2 mM L-Alanyl-Glutamine + 1X Pen/Strep). Cells at passage 41 were used for proliferation assay. IGF-II (67aa), IGF-IIE (104aa) were at 10 µg/ml in PBS and kept at -70°C. IL-4 was purchased from R&D system, Cat#: 404-ML, and anti-IGF-II

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antibody was purchased from R&D system, Cat#: MAB292. The IgF M0063-F02 was at 6.1mg/ml. Guava ViaCount Reagent, Cat# 4000-0041, was purchased.

Procedure:

- Prepare IGF-II at concentration of 400 ng/ml; IGF-IIIE at 800 ng/ml; IgG at 120 μ g/ml in PBS.
- Pre-incubate 25 μ l of IGF-II or IGF-IIIE with 25 μ l of serially diluted IgGs (final concentration from 30 μ g/ml down to 0) in a 96-well plate, triplicate for each dose, for 30 min at room temperature in a total volume of 50 μ l/well.
- Prepare cells:
 - Harvest Ba/F3 cells by centrifuging at 1100 rpm for 5 min.
 - Remove supernatant, and resuspend cells in 20 ml of PBS.
 - Count cell density by mixing 10 μ l of cell suspension with 10 μ l of trypan blue solution and loading 10 μ l to a hemocytometer to count cell density and viability.
 - Spin down cells from PBS solution at 1100 rpm for 5 min.
 - Remove supernatant, and resuspend cells in culture medium without IL3 at 4×10^5 cells/ml.
 - Add 25 μ l of cell suspension to each well of the prepared 96-well plate to make the final cell density at 1×10^4 cells/well.
 - Add 25 μ l of IL-4 at 200ng/ml in IL-3 free medium to each well to make the final con. 50 ng/ml.
 - The total volume is 100 μ l per well.
 - Incubate the plate at 37°C, 5% CO₂ for 48 h.

Guava ViaCount Assay:

- Centrifuge the 96-well plate, resuspend cells into 200 μ l of Guava ViaCount reagent, mix and incubate for 5 minutes. Transfer into a round-bottom 96-well plate.
- Guava ViaCount Analysis.
- Calculate the IC₅₀ by using the following equation in Sigmaplot:

$$f=y0-((a*x)/(IC_{50}+x)).$$

M0063-F02 IgG demonstrated inhibition on IGF-II and IGF-IIIE stimulated Ba/F3 proliferation in a dose dependent manner. The M0063-F02 IgG inhibited both IGF-II and IGF-IIIE stimulated cell proliferation with IC₅₀ values at 2 and 0.85 nM respectively.

M0064-E04 IgG and M0063-F02 IgG (Parental):

The purpose of this study was to test the 2 IgG candidates for inhibition on IGF-II or IGF-IIIE stimulated Ba/F3 cell proliferation and IC₅₀ value comparison.

Cell Culture and Materials:

Ba/F3 cells were cultured in complete medium (90% RPMI 1640 + 10% FBS + 10 ng/ml IL-3 + 2 mM L-Alanyl-Glutamine + 1X Pen/Strep). Cells at passage 22 were used for proliferation assay.

IGF-II (67aa), IGF-IIE (104aa) were at 10 µg/ml in PBS and kept at -70°C. IL-4 was purchased from R&D system, Cat#: 404-ML, and anti-IGF-II antibody was purchased from R&D system, Cat#: MAB292. Anti-IGF-II antibody: R&D system, Cat#: MAB292.

Procedure:

- Prepare IGF-II at concentration of 400ng/ml; IGF-IIE at 800ng/ml; IgG at 200 µg/ml in PBS.
- Pre-incubate of 25 µl of IGF-II or IGF-IIE with 25 µl of 1:2 serial diluted IgGs (from 50µg/ml down to 0) in a 96-well plate, triplicate for each dose, incubate for 30 min at room temperature in a total volume of 50 µl/well.
- Prepare cells:
 - Harvest Ba/F3 cells by centrifuging at 1100 rpm for 5 min.
 - Remove supernatant, and resuspend cells in 20 ml of PBS.
 - Count cell density by mixing 10 µl of cell suspension with 10 µl of trypan blue solution and loading 10 µl to a hemocytometer to count cell density and viability.
 - Spin down cells from PBS solution at 1100 rpm for 5 min.
 - Remove supernatant, and resuspend cells in culture medium without IL-3 at 2×10^6 cells/ml.
 - Add 25 µl of cell suspension to each well of the prepared 96-well plate to make the final cell density at 5×10^4 cells/well.
 - Add 25 µl of IL-4 at 200 ng/ml in IL-3 free medium to each well to make the final con. 50 ng/ml.
 - The total volume is 100 µl per well.
 - Incubate the plate at 37°C, 5% CO₂ for 48 h.

Guava ViaCount Assay:

- Mix each well of the 96well plate, take 20 µl of cell sample from each well to a new 96well plate, add 180 µl of Guava ViaCount reagent to each well, mix and incubate for 5 minutes.
- Guava ViaCount Analysis.

Both IgGs demonstrated inhibition on IGF-II; IGF-IIE stimulated Ba/F3 proliferation in a dose dependent manner. Neither IgG showed a significant effect on IGF-I stimulated cell proliferation. The M0063-F02 IgG inhibited both IGF-II and IGF-

IIE stimulated cell proliferation with IC_{50} values at ~7 and 10 nM respectively, and the M0064-E04 IgG inhibited both IGF-II and IGF-IIE stimulated cell proliferation with IC_{50} values at ~19 and 130 nM respectively.

5 **EXAMPLE 7: In Vitro Studies Using Anti-IGF-II/IGF-IIE Binding Proteins**

IGF-1R Phospho-assay in MCF-7 Cells

We tested the inhibitory effect of M0063-F02 IgG (parental) on IGF-II and/ or IGF-IIE induced IGF-1R phosphorylation in MCF-7 cell lines.

MCF-7: breast cancer cell line cells were cultured in MEM media with 10% FBS,
10 0.1mM NEAA, 1mM Na Pyruvate, 0.01mg/ml bovine Insulin and 1X Pen/Strep. Anti-IGF-1R antibody was purchased from Upstate, Cat# 05-656 and anti-Phospho-IGF-IR antibody was purchased from Cell Signaling. Cat# 3024. MCF-7 (P15) were cultured in complete medium in 6-well plate at 1×10^6 cells/well at 37°C, 5%CO₂ incubator O/N. The cells were then starved with basal-MEM media for 6 hrs and were treated in batches
15 as follows:

1. No treatment
2. Cells were treated with 10 nM IGF-II for 20 min
3. Cells were treated with 10 nM IGF-IIE for 20 min
4. Cells were pre-treated with 40 nM M0063-F02 IgG for 30 min then IGF-II
20 10 nM was added for 20min
5. Cells were pre-treated with 40 nM M0063-F02 IgG for 30 min then IGF-IIE 10 nM was added for 20 min
6. Pre-mixed M0063-F02 40 nM with IGF-II 10nM for 30 min, then added to cells
- 25 7. Pre-mixed M0063-F02 40 nM with IGF-IIE 10nM for 30 min, then added to cells
8. F02 40nM, IGF-II 10 nM were added to cells simultaneously, treated for 20 min
- 30 9. M0063-F02 40nM, IGF-IIE 10 nM were added to cells simultaneously, treated for 20 min

10. Cells were pre-treated with 40 nM A02 IgG for 30 min then IGF-II 10 nM was added for 20min

11. Cells were pre-treated with 40 nM A02 IgG for 30 min then IGF-IIE 10 nM was added for 20min

5 The cells were washed once with ice-cold PBS containing 1 mM sodium orthovanadate. Cells were lysed with 1 ml of RIPA buffer with protease inhibitor cocktail, and incubated for 10 min on ice. The lysates were spun at 14,000 rpm for 10 min to get rid of the cell debris. Cell lysates were immunoprecipitated with anti-IGF-1R antibody at 2 ug/ml, 20 μ l agarose beads at 4°C, O/N, immunoprecipitates were collected and washed 3 times
10 with 1ml RIPA buffer. 12 μ l of 2X electrophoresis sample buffer was added.

Western blotting: Samples were heated at 70°C, water bath for 10 min, then loaded samples into 15-well 4-12% Bis-Tris gel. Transferred the resolved proteins to a 0.45 μ m PVDF membrane. The membrane was blocked with 5% BSA-PBST (0.05% Tween 20) at room temperature for 1 hr and probed with anti-phospho-IGF-1R Ab at
15 1:1000 dilution in 3% BSA-PBS-T O/N 40°C. The membrane was washed 3 times with PBS. Subsequently, the blot was probed with anti-Rabbit-IgG-HRP at 1:5000 dilution in 3% BSA-PBST for 1 hr at room temperature and washed 3 times with PBS. The blot was developed with Supersignal west Femto Maximum Sensitivity Substrate (Pierce 1859022&23) The membrane was stripped and blocked with 5% BSA-PBST (0.05%
20 Tween 20) at room temperature for 1 hr. It was probed with anti-IGF-1R Ab at 1:3000 dilution in 3% BSA-PBS-T O/N at 40°C. The membrane was washed 3 times with PBS. Subsequently, the blot was probed with anti-mouse IgG-HRP at 1:5000 dilution in 3% BSA-PBST for 1 hr at room temperature and washed 3 times with PBS. The blot was developed with Supersignal west Femto Maximum Sensitivity Substrate (Pierce
25 1859022&23)

Preliminary results demonstrated that stimulation of MCF-7 cells with IGF-II or IGF-IIE induced IGF-1R phosphorylation under serum free condition. M0063-F02 IgG at 40 nM showed an inhibitory effect on both IGF-II or IGF-IIE induced IGF-1R phosphorylation in MCF-7 cells. The similar inhibitory activity was observed in 3
30 different protocols: preincubation of the cells with antibody; premixing of the antibody with IGF-II or IGF-IIE and simultaneous addition of IGF-II/IIE and the antibody to cells.

A02 as an IgG control showed no effect on IGF-II or IGF-IIIE induced IGF-1R phosphorylation.

M0064-E04 and M0064-F02 IgG Comparison in IGF-1R phosphorylation assay

We tested the comparative inhibitory effect of M0064-F02 and M0064-E04 IgG on IGF-II and/ or IGF-IIIE induced IGF-1R phosphorylation in MCF-7 cell lines.

MCF-7 breast cancer cell line cells were cultured in MEM media with 10% FBS, 0.1mM NEAA, 1mM Na Pyruvate, 0.01mg/ml bovine Insulin and 1X Pen/Strep. Anti-IGF-1R antibody was purchased from Upstate, Cat# 05-656 and anti-Phospho-IGF-IR antibody was purchased from Cell Signaling. Cat# 3024. MCF-7 (P20) cells were harvested and seeded in complete medium in 6-well plate at 1×10^6 cells/well at 37°C, 5% CO₂ incubator O/N. The cells were then starved with basal-MEM media for 6 hrs. The cells were treated in batches as follows:

- No treatment
- Cells were treated for 20 min with 10 nM IGF-II
- Cells were treated for 20min with IGF-II 10 nM plus different dose of M0064-E04, from 40 nM down to 0.16 nM
- Cells were treated for 20min with IGF-II 10 nM plus different dose of M0064-F02, from 40 nM down to 0.16 nM
- Cells were treated for 20min with IGF-II 10 nM plus IgG A2 40 nM as negative control

The cells were washed once with ice-cold PBS containing 1 mM sodium orthovanadate. Cells were lysed with 1 ml of RIPA buffer with protease inhibitor cocktail, and incubated for 10 min on ice. The lysates were spun at 14,000 rpm for 10 min to get rid of the cell debris. Cell lysates were immunoprecipitated with anti-IGF-1R antibody at 2ug/ml, 20ul agarose beads at 4°C, O/N, immunoprecipitates were collected and washed 3 times with 1ml RIPA buffer. Twelve ul of 2X electrophoresis sample buffer was added.

Western blotting: heated samples at 70°C, water bath for 10 min, then loaded samples into 15-well 4-12% Bis-Tris gel. The resolved proteins were transferred to a 0.45 um PVDF membrane. The membrane was blocked with 5% BSA-PBST (0.05% Tween 20) at room temperature for 1 hr, and probed with anti-phospho-IGF-1R Ab at

1:1000 dilution in 3% BSA-PBS-T O/N 40°C. The membrane was washed 3 times with PBS. Subsequently, the blot was probed with anti-Rabbit-IgG-HRP at 1:5000 dilution in 3% BSA-PBST for 1 hr at room temperature, and washed 3 times with PBS. The blot was developed with Supersignal west Femto Maximum Sensitivity Substrate (Pierce 1859022&23). The membrane was stripped and blocked with 5% BSA-PBST (0.05% Tween 20) at room temperature for 1 hr, and probed with anti-IGF-1R Ab at 1:3000 dilution in 3% BSA-PBS-T o/N 40°C. The membrane was washed 3 times with PBS. Subsequently, the blot was probed with anti-mouse IgG-HRP at 1:5000 dilution in 3% BSA-PBST for 1 hr at room temperature, and washed 3 times with PBS. The blot was developed with Supersignal west Femto Maximum Sensitivity Substrate (Pierce 1859022&23)

Preliminary results demonstrated that stimulation of MCF-7 cells with IGF-II induced IGF-1R phosphorylation under serum free condition. M0064-E04 and M0064-F02 IgGs showed inhibitory effects on IGF-II induced IGF-1R phosphorylation in MCF-7 cells in a dose dependent manner. The similar inhibitory potency was observed between those two antibodies. A02 as an IgG negative control showed no effect on IGF-II induced IGF-1R phosphorylation.

EXAMPLE 8: Crystallography and epitopic mapping

The crystallographic structure of IGF-II with M0064-F02 was determined in order to characterize the epitopic region of the IGF2 to which the antibody binds. Crystals were obtained using 1 – 10 mg/mL IGF-II with the M0064-F02 Fab in a molar ratio of 2:1 in mid-weight PEG conditions, pH ~5 with either Ca⁺⁺ or Li⁺⁺ as additives.

Crystallization statistics were as follows:

Cell: 50.22 106.67 110.89 90.00 90.00 90.00

Space Group: P2₁2₁2₁

Number of Atoms: 4050 (233 water molecules)

% Solvent: 52.67

 for atomic model: 33.85

Sigma(B): 9.21

Resolution: 49.21-2.40

Reported R factor: 0.185

Rfree: 0.261

Number max possible refls: 24010 Actual: 22775

Completeness: 94.9%

5 Correlation factor: 0.9254

The Fab structure was solved using molecular replacement with pdb #1igf and the IGF-II structure solved using pdb 2v5p.

Views of the structure are depicted in FIGURES 1A and 1B. One valley appears to be important in the Fab surface for binding to IGF-II. One encloses the residues Cys9 through Gly11 and buries the Cys9-Cys47 disulfide bridge and with a bump also residue Phe48. The second valley is on the other side of the Tyr103H bulge and the residues here line the top of the valley but are not found deep within. This valley has a negative electrostatic potential, but there are no positively charged residues that delve into this area to offset this charge. There are two Arg residues (37 and 38) with their side chains pointing into space that do not make H-bonds nor ionic interactions with Asp102H and the further buried Glu106H and Asp99H residues. The closest contacts seem to be the N epsilon of Arg34 to the Val35 backbone nitrogen at 4.4A and the carboxylic acid group of Asp102 at 4.7A.

The Met57H residue seems to be covered on three sides by mostly charged residues (Glu44, Glu45, Arg49), but the charges are all pointing away from the Met and only the aliphatic carbons seem to contribute to the binding surface, which also includes the uncharged Phe48.

The most prominent feature on the Fab is the finger bulge made by Tyr103H. Although this sticks a bit like a finger into IGF-II, there is still a gap or hole left between the two molecular surfaces that might be filled to a certain extent by a larger residue such as Trp. It is a hydrophobic pocket on the IGF-II surface made up of Tyr59, Phe26, Leu17, Leu13, Val43, Val14.

Table 7 below shows the partial sequence of IGF-II, the bolded amino acids being those that have been shown through crystallographic studies to be involved with the binding of the Fab:

EXAMPLE 9: Affinity in Solution measurements for Fabs binding to IGF-II and IGF-IIE

Competition SPR (BIAcore®) analysis was utilised

- 1) to demonstrate that Fab fragments isolated from phage display library specifically inhibited binding of IGF-II and IGF-IIE ligands to immobilized full-length human insulin receptor-A ectodomain (huIR-A ECD) and
- 2) to estimate in solution affinities of these Fabs.

Method

Human IR-A ECD comprising residues 1-914 of the mature protein together with a C-terminal myc epitope tag, was isolated and purified from bioreactor cultures of stably transfected Lec 8 cells (a glycosylation mutant derived from CHO-K1 cells) as described methodology (J Struct Biol. 1999 Mar;125(1):11-8.)

Competition SPR (BIAcore®) was performed under conditions of partially mass-transport limited conditions according to previously described methodology (Nieba et al., 1996). Approximately 16,700 relative response units (RU) of huIR-A (-exon11) ectodomain was coupled by standard amine chemistry to a BIAcore® CM5 chip sensor. Uncoated flow-cell surface was used as a reference. Each binding/regeneration cycle between IGF-II ligands and immobilized huIR-A ECD was performed at 25°C with a constant flow rate of 30 ul/min in HBS-EP+ running buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.05% Tween-20). Regeneration of the surface was achieved by injection of 30 ul NaCitrate/NaCl pH 4.5. Initially, 60 ul samples containing increasing amounts of IGF-II (or IGF-IIE) ligand (typically increasing two-fold from 0.5 to 8 nM) in HBS-EP+ buffer were injected over the immobilized huIR-A ECD and their overall binding responses (5 sec after injection stopped) used to establish standard binding curve. To demonstrate inhibition of IGF-II binding by Fab fragments and to derive affinity in solution values (K_D), the IGF-II (or IGF-IIE) ligand was pre-incubated with Fab fragments at different concentrations in a constant final volume of 120 ul for at least 1 h at 25°C before injection. 60 ul samples of these equilibrated mixtures were injected over immobilized huIR-A ECD and the overall binding response generated by binding of free IGF-II ligands to huIR-A ECD recorded 5 seconds after injection stopped.

Binding data were evaluated using the BIACORE® T100 Evaluation software (GE Healthcare) whereby these overall binding responses together with standard curve described above were used to derive concentrations of free IGF-II ligand in solution at equilibrium (Req). These Req estimates were subsequently plotted against total concentrations of Fab used and the resulting inhibition curve was utilised to calculate dissociation constant (K_D).

Results:

Table 8: Affinity in solution (KD's) estimates of Fabs (BIACORE® T100)

Original Isolate name	Measurement#1 (KD nM)		Measurement#2 (KD nM)	
	IGF-II	IGF-IIE	IGF-II	IGF-IIE
M0033-E05	4.32E-09	8.21E-09	-----	-----
M0063-F02	1.17E-09	2.78E-09	8.58E-10	1.47E-09
M0064-E04	6.08E-10	4.29E-10	1.11E-09	4.24E-10
M0064-F02	1.03E-09	1.91E-09	5.03E-10	3.52E-10
M0068-E03	5.76E-09	5.55E-09	-----	-----
M0070-H08	3.24E-09	4.83E-09	-----	-----
M0072-C06	1.64E-09	3.95E-09	2.52E-09	1.05E-09
M0072-E03	2.62E-09	5.32E-09	-----	-----
M0072-G06	3.99E-09	6.36E-09	-----	-----
M0080-G03	No significant binding	2.93E-08	No significant binding	2.12E-08
M0073-C11	No significant binding	2.02E-08	No significant binding	1.91E-08

Results demonstrated that most of these Fabs inhibited binding of IGF-II and IGF-IIE to the huIR-A ECD receptor. Two Fabs shown here (M0080-G03 and M0073-C11) inhibited binding of IGF-IIE only whilst no inhibition of IGF-II binding was observed, thus demonstrating that binding of these two Fabs was specific to IGF-IIE.

EXAMPLE 10. Anti-IGFII (and IGF-IIIE) antibody competition assay against Binding proteins (BP2 and BP4)

IGF binding proteins (BPs) play a major role in modulating the actions of IGF-I and IGF-II (and IGF-IIIE) on cells. They can either enhance or inhibit the action of IGF on cells. IGF BP2 preferentially binds IGF-II over IGF-I and is secreted by a variety of cells. Similarly, IGF BP4 acts as a scavenger of IGFs and is an inhibitor of IGF action.

In this example, the candidate IgG's interaction with BP2 and BP4 and their competitive binding to IGF-II and IGF-IIIE was investigated.

Method:

BIACORE® T100 and a CM5 chip coated with Protein G were utilised to set up following assay. Approximately 1000 RU of Protein G' was immobilized on a CM5 chip using standard amine chemistry immobilization method.

Entire assay consisted of three sequential injection of the following four reagents:

1) Candidate IgG (parental) was injected onto Protein G' at 10 ug/ml at 5 ul/min for 180 sec. This typically resulted in a capture of ~3000 RU of IgG.

2) IGF-II ligand (at 50 nM) was then injected at 5 ul/min for 90 sec. This allowed for binding of IGF-II to the captured antibody.

3) BP2 or BP4 (also at 50 nM) were then injected at 30 ul/min for 90 sec. Significant binding response upon injection of BP was indicative of candidate antibody and BP binding to IGF-II in non-competitive manner.

4) Protein G' surface was finally regenerated by injection of 10 mM Glycine pH1.5 - injected at 30ul/min for 60 sec.

FIGURE 2A gives a typical binding profile obtained for one of the candidate antibodies. Under these experimental conditions, M0063-F02 appeared to bind to IGFII in a non-competitive manner with respect to BP4.

FIGURE 2B shows competition binding data for M0064-E04 candidate antibody under these conditions. Following controls were included to ascertain these binding results (refer to legend – inset bottom left)

1) no IGF-II injected (instead inject Biacore running buffer only)

Confirmed that BP2/BP4 do not bind captured antibody

2) no BP2 or BP4 was injected after capturing IGF-II

Established the baseline for IGF-II dissociation from antibody

5 3) control antibody that does not bind IGF-II (w02 murine antibody)

to confirm IGF-II and/or BP2/BP4 do not significantly interact with either Protein G' or chip surface.

The following table shows the summary for these competition binding results
for candidate antibodies against IGF-II ligand with respect to BP2, BP4 under these
experimental conditions.

Table 9. Scoring system scales:

5 = candidate IgG competes strongly with BP for binding to IGF-II ligand

0 = candidate IgG does not competes significantly with BP for binding to IGF-II ligand

	IGF-II		IGF-IIIE	
Candidate Antibody	BP2	BP4	BP2	BP4
M0063-F02	1	0	1	1
M0064-E04	5	5	4	4
M0064-F02	4	4	4	4
M0070-H08	3	2	2	1
M0072-C06	3	2	2	1
M0072-E03	4	4	3	3
M0072-G06	2	1	2	1
M0080-G03	<i>does not bind to IGF-II</i>		5	5
566A-M0073-C11	<i>does not bind to IGF-II</i>		4	4

In summary:

IGFII ligand competition:

Under the experimental conditions tested, M0064-E04 IgG appears to be best competing antibody with BP2 and BP4 for binding to IGFII ligand, while M0064-F02 and M0072-E03 are the next two best antibodies.

EXAMPLE 11: Anti-IGF-II/IGF-IIE IgG and GH Receptor Antagonist Combination Study in Xenograft Models

Immunocompromised mice were implanted with either an isolated cell suspension of Colo205 cancer cells or a Colo205 derived tumor fragment. Tumors were allowed to grow to a size of approximately 100mm³. Mice bearing tumors were separated into 4 groups (8-12 mice per group), and treatments and treatment schedules as listed in Table 10 were tested:

Table 10

Group #	Treatment	Schedule
1	PBS Control	
2	Anti-IGF-II/IGF-IIE Antibody (DX-2647)	10mg/kg IP q2d
3	Growth Hormone Antagonist (e.g. SOMAVERT®)	Dose administered is dependent upon drug used; for SOMAVERT® recommended dose is 60mg/kg IP q2d
4	Anti-IGF-II/IGF-IIE Antibody (DX-2647) + Growth Hormone Antagonist (e.g. SOMAVERT®)	10mg/kg IP q2d Dose administered is dependent upon drug used; for SOMAVERT® recommended dose is 60mg/kg IP q2d

Tumor volumes were determined by caliper measurements twice weekly. Animals were weighed to monitor any drug-related toxicities. No significant tumor growth inhibition was observed under the treatment conditions and schedules tested using this cancer model.

Similar experiments were performed using HT-29 cells. Treatments and treatment schedules as listed in Table 11 were tested:

Table 11

Group #	Treatment	Schedule
1	PBS Control	10 ml/kg q2d

2	Anti-IGF-II/IGF-IIE Antibody (DX-2647)	10mg/kg IP q2d
3	Growth Hormone Antagonist (e.g. pegvisomant)	Dose administered is dependent upon drug used; for pegvisomant recommended dose is 60mg/kg IP q2d
4	Anti-IGF-II/IGF-IIE Antibody (DX-2647) + Growth Hormone Antagonist (e.g. pegvisomant)	10mg/kg IP q2d Dose administered is dependent upon drug used; for pegvisomant recommended dose is 60mg/kg IP q2d
5	Paclitaxel – Positive Control	25 mg/kg Q2Dx5

No significant tumor growth inhibition was observed under the treatment conditions and schedules tested using this cancer model.

5 In addition, we will run a mouse xenograft study using implanted SKUT-1 cells (leiomyosarcoma cell line). Mice bearing these tumors will be treated with the Anti-IGF-II Antibody at 10mg/kg IP q2d used as a monotherapy.

EXAMPLE 12: Exemplary IGF II / IGF IIE Inhibitory Binding Proteins

10 **DX-2647**

DX-2647 is an exemplary IGF II/ IGF IIE inhibitory antibody. DX-2647 is germlined from 566A-M0064-F02 parental clone. The DNA and amino acid sequences of DX-2647 are as follows.

>DX-2647 LV+LC

15 DIQMTQSPSSLSASVGDRVITITCRASQSI SNYLNWYQQKPGKAPKLLIYTASTLQSGVPSRFSGSGSG
 TDFTLTISSLQPEDFATYYCQQSYNSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
 NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP
 VTKSFNRGEC

20 **>DX-2647 HV+HC**

 EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYIMWWVRQAPGKGLEWVSVISSSGGGTLYADSVKGRF
 TISRDN SKNTLYLQMNSLRAEDTAVYYCARDNGDYVGEKGFDIWGQGTMTVTVSSASTKGPSVFPLAPS
 SKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYI
 CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS

HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGS
FFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

5 **>DX-2647 LV+LC**

GACATCCAGATGACCCAGTCCCCCAGCTCCCTGTCTGCTTCCGTGGGCGACCGGGTGACCATCACCTG
CCGGGCCTCCCAGTCCATCTCCAACCTACCTGAACTGGTATCAGCAGAAGCCTGGCAAGGCCCTAAGC
TGCTGATCTACACCGCCTCTACACTGCAGTCTGGAGTCCCTTCCAGGTTCTCCGGCTCCGGCAGCGGC
ACCGACTTCACCCTGACCATCTCCTCCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCAGCAGTC
10 CTACAACTCCCCTTGGACCTTCGGCCAGGGCACCAAGGTGGAGATCAAGCGGACCGTGGCCGCTCCTT
CCGTGTTTATCTTCCCTCCCTCCGACGAGCAGCTGAAATCCGGCACTGCCAGCGTGGTCTGCCTGCTG
AACAACTTCTACCCTCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACTC
CCAGGAATCCGTCACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCCAGCACCTTGACCCTGT
CCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCTCCCCC
15 GTGACCAAGTCCTTCAACCGGGGCGAGTGC

>DX-2647 HV+HC

GAGGTGCAATTGCTGGAGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCTCCCTGCGGCTGTCTTGCGC
CGCCTCCGGCTTCACCTTCTCCAACCTACATCATGTGGTGGGTGCGGCAGGCTCCTGGAAAGGGCCTCG
20 AGTGGGTGTCCGTGATCTCCAGCTCCGGGGGAGGAACACTGTACGCCGACTCCGTGAAGGGCCGGTTC
ACCATCTCCAGAGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACAC
CGCCGTGTACTACTGCGCCAGGGACAACGGCGACTACGTGGGCGAGAAGGGCTTCGACATCTGGGGCC
AGGGCACAATGGTGACCGTGTCTTCCGCCTCCACCAAGGGCCCTTCCGTGTTCCCGCTAGCACCTTCC
TCCAAGTCCACCTCTGGCGGCACCGCCGCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGT
25 GACCGTGAGCTGGAACCTCTGGCGCCCTGACCTCCGGCGTGCATACCTTCCCTGCCGTGCTGCAGTCCT
CCGGCCTGTACTCCCTGTCTTCCGTGGTGACAGTGCCTTCTCCTCCCTGGGCACCCAGACCTACATC
TGCAACGTGAACCACAAGCCTTCCAACACCAAGGTGGACAAGCGGGTGGAGCCTAAGTCCTGCGACAA
GACCCACACCTGCCCTCCCTGCCCTGCCCCCTGAGCTGCTGGGCGGACCCTCCGTGTTCTGTCTCCCTC
CTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAGGTGACCTGCGTGGTGGTGGACGTGTCC
30 CACGAGGACCCAGAGGTGAAGTTTAATTGGTATGTGGACGGCGTGGAGGTCCACAACGCCAAGACCAA
GCCTCGGGAGGAACAGTACAACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACT
GGCTGAACGGCAAGGAATACAAGTGCAAAGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACC
ATCTCCAAGGCCAAGGGCCAGCCTCGCGAGCCTCAGGTGTACACCCTGCCTCCTAGCCGGGAGGAAAT
GACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGT
35 GGGAGTCCAACGGCCAGCCTGAGAACAACCTACAAGACCACCCCTCCTGTGCTGGACTCCGACGGCTCC
TTCTTCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCTGTCTC
CGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGAGCCCTGGCAAG

The light and heavy chain framework, CDR and constant region sequences are as follows. A protein containing the 6 CDRs from DX-2647 can be used in the compositions and methods described herein.

LV-FR1	LV-CDR1	LV-FR2	LV-CDR2	LV-FR3
DIQMTQSPSSLSASVGDRVTITC	RASQSI SNYLN	WYQQKPGKAPKLLIY	TASTLQS	GVPSRFSGSGSGTDFTLTIS SLQPEDFATYYC

5

LV-CDR3	LV-FR4	L-Constant
QQSYNSPWT	FGQGTKVEIK	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC

HV-FR1	HV-CDR1	HV-FR2	HV-CDR2	HV-FR3
EVQLLES GGG LVQPGGSLRLSCAASGFTFS	NYIMW	WVRQAPGKGLEWVS	VISSSGGGTLYADSVKG	RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR

HV-CDR3	HV-FR4	H-Constant
DNGDYVGEKGF DI	WGQGTMTVSS	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTV PSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCVMHEALHNHYTQKSLSLSPGK

LV-AA

10 DIQMTQSPSSLSASVGDRVTITCRASQSI SNYLNWYQQKPGKAPKLLIYTASTLQSGVPSRFSGSGSG
TDFTLTIS SLQPEDFATYYCQQSYNSPWTFGQGTKVEIK

HV-AA

15 EVQLLES GGG LVQPGGSLRLSCAASGFTFSNYIMWWVRQAPGKGLEWVSVISSSGGGTLYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCARDNGDYVGEKGF DIWGQGTMTVSS

Differences between the germlined and non-germlined LV-FR3 region are shown below.

	LV-FR3
germlined	GVPSRFSGSGSGTDFTLTIS SLQPEDFATYYC
Non-germined	GVPSRFSGS A SGTDFTLT I NSLQPEDFATY S C
	Bold are non-germlined amino acids
	found in parental 566A-M0064-F02

	Fab.
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DX-2655

DX-2655 is an exemplary IGF II/ IGF IIE inhibitory antibody. DX-2655 is
germlined from 566A-M0064-E04 parental clone. The DNA and amino acid sequences
of DX-2655 are as follows.

>566A-X0009-D01 (DX-2655) LV+LC

DIQMTQSPSSLSASVGDRVTITCQASHDISNYLNWYQQKPGKAPKLLIYAASRLQSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQSYSPRTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEEKHKVYACEVTHQGLSSP
VTKSFNRGEC

>566A-X0009-D01 (DX-2655) HV+HC

EVQLLESGGGLVQPGGSLRLSCAASGFTFSVYDMNWVRQAPGKGLEWVSSISSSGGGTLYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCARDSDTSSYYWYYDLWGRGTLTVSSASTKGPSVFPLAPS
SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYI
CNVNHKPSNTKVDKRVKPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

>566A-X0009-D01 (DX-2655) LV+LC

GATATCCAGATGACCCAGTCCCCAGCTCCCTGTCCGCTAGCGTGGGCGACCGGGTGACCATCACCTG
CCAGGCCTCCCACGACATCTCCAACCTGAACTGGTATCAGCAGAAGCCTGGCAAGGCCCTAAGC
TGCTGATCTACGCCGCCAGCAGACTGCAGTCCGGCGTCCCTAGCCGGTTCTCCGGCTCCGGCAGCGGC
ACCGACTTCACCCTGACCATCTCCTCCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCAGCAGTC
CTACTCCTTCCCTCGGACCTTCGGCCAGGGCACCCGGCTGGAGATCAAGCGGACCGTGGCCGCTCCTT
CCGTGTTTATCTTCCCTCCCTCCGACGAGCAGCTGAAGAGCGGCACAGCCAGCGTCGTGTGCCTGCTG
AACAACTTCTACCCTCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACTC
CCAGGAATCCGTCACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCTCCACCCTGACCCTGT
CCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCT
GTGACCAAGTCCTTCAACCGGGGCGAGTGC

>566A-X0009-D01 (DX-2655) HV+HC

GAGGTGCAATTGCTGGAGTCTGGCGGCGGACTGGTGCAGCCTGGCGGGCTCCCTGCGGGCTGTCCTGCGC
CGCTCCGGCTTCACCTTCTCCGTGTACGACATGAACTGGGTGCGGCAGGCTCCTGGAAAGGGCCTCG
AGTGGGTGTCCTCCATCTCCAGCTCCGGGGGAGGAACACTGTACGCCGACTCCGTGAAGGGCCGGTTC
5 ACCATCTCCAGAGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACAC
CGCCGTGTACTACTGCGCCAGGGACTCCGACACCTCCTCCTACTACTGGTACTACGACCTGTGGGGCA
GGGGCACCCCTGGTGACCGTGTCTTCCGCCTCCACCAAGGGCCCTTCCGTGTTCCCGCTAGCACCTTCC
TCCAAGTCCACCTCTGGCGGCACCGCCGCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGT
GACCGTGAGCTGGAACCTCTGGCGCCCTGACCTCCGGCGTGCATACCTTCCCTGCCGTGCTGCAGTCCT
10 CCGGCCTGTACTCCCTGTCTTCCGTGGTGACAGTGCCTTCTCCTCCCTGGGCACCCAGACCTACATC
TGCAACGTGAACCACAAGCCTTCCAACACCAAGGTGGACAAGCGGGTGGAGCCTAAGTCCTGCGACAA
GACCCACACCTGCCCTCCCTGCCCTGCCCCTGAGCTGCTGGGCGGACCCTCCGTGTTCTGTTCCTC
CTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAGGTGACCTGCGTGGTGGTGGACGTGTCC
CACGAGGACCCAGAGGTGAAGTTTAATTGGTATGTGGACGGCGTGGAGGTCCACAACGCCAAGACCAA
15 GCCTCGGGAGGAACAGTACAACCTCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACT
GGCTGAACGGCAAGGAATACAAGTGCAAAGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACC
ATCTCCAAGGCCAAGGGCCAGCCTCGCGAGCCTCAGGTGTACACCCTGCCTCCTAGCCGGGAGGAAAT
GACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGT
GGGAGTCCAACGGCCAGCCTGAGAACAAC TACAAGACCACCCCTCCTGTGCTGGACTCCGACGGCTCC
20 TTCTTCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTC
CGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGAGCCCTGGCAAG

LV-CDR1	LV-FR2	LV-CDR2	LV-FR3
QASHDISNYLN	WYQQKPGKAPKLLIY	AASRLQS	GVPSRFSGSGSGTDFTLTITSSLPEDFATYYC

LV-CDR3	LV-FR4	L-Constant
QQSYSPRT	FGQGTRLEIK	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

HV-FR1	HV-CDR1	HV-FR2	HV-CDR2	HV-FR3
EVQLLESGGGLVQPGGSLRLSCAASGFTFS	VYDMN	WVRQAPGKGLEWVS	SISSSGGGTLYADSVKG	RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR

25

HV-CDR3	HV-FR4	H-Constant
DSDTSSYYWYYDL	WGRGTLVTVSS	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVE PKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

Differences between the germlined and non-germlined LV-FR3 region are shown below.

	LV-FR3
germlined	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
Non-germlined	GVPSRFSG GG SGTDF S LTISSLQ A EDFATYYC
	Bold are non-germlined amino acids
	found in parental 566A-M0064-E04
	Fab.

5 Differences between the germlined and non-germlined LV-FR4 region are shown below.

	LV-FR4
germlined	FGQGTRLEIK
Non-germlined	FGQGT N LEIK
	Bold = non
	germlined
	aa found
	in M064-E04

EXAMPLE 13: Effects of IGF II / IGF IIE Inhibitory Binding Protein on Hep3B Cells in Colony Formation Assay

The ability of DX-2647 to inhibit colony formation of Hep3B cells was tested. DX-2647 was tested at two concentrations. The results are shown in FIGURE 3. DX-2647 inhibited colony formation in a dose-dependent manner.

Anchorage Dependent Colony Formation Assay: Cells were seeded into a six-well plate at a cell density of 500 – 1000 cells per well in culture medium containing 10% FBS and DX-2647 at various concentrations, and placed in a 37°C incubator with 5% CO₂ for 1 week to form colonies. Colonies >50 cells were scored in three independent experiments, each with triplicate dishes.

EXAMPLE 14: Effects of IGF II / IGF IIE Inhibitory Binding Protein in Rhabdomyosarcoma Xenograft Models

DX-2647 will be evaluated against three rhabdomyosarcomas (Rh10, Rh28 and Rh41). These three rhabdomyosarcomas have previously been tested with an antibody that is currently being used in clinical trials and which is directed against the Insulin-like Growth Factor-I receptor (IGF-IR). The IGF-IR binds not only IGF-I but also IGF-II. Rh28 has shown the best response to this anti-IGF-IR antibody while Rh10 and Rh41 have shown intermediate responses. As a 'negative' control, EW-8 xenografts that demonstrate no response to the anti-IGF-IR antibody will be used.

In vivo tumor growth inhibition studies: CB17SC-M *scid*^{-/-} female mice (Taconic Farms, Germantown NY), are used to propagate subcutaneously implanted sarcomas (Ewing, rhabdomyosarcoma). All mice are maintained under barrier conditions and experiments are conducted using protocols and conditions approved by the institutional animal care and use committee. Ten (10) mice per treatment group will be used. Tumor volumes (cm³) and responses are determined using activity measures as follows.

Response and Event Definitions for RMS Xenograft Models. For individual tumors, progressive disease (PD) is defined as < 50% regression from initial volume during the study period and > 25% increase in initial volume at the end of study period. Stable disease (SD) is defined as < 50% regression from initial volume during the study period and ≤ 25% increase in initial volume at the end of the study. Partial response (PR) is defined as a tumor volume regression ≥ 50% for at least one time point but with measurable tumor (≥ 0.10 cm³). Complete response (CR) is defined as a disappearance of measurable tumor mass (< 0.10 cm³) for at least one time point. A complete response is considered maintained (MCR) if the tumor volume was < 0.10 cm³ at the end of the study period. For treatment groups only, if the tumor response is PD, then PD is further classified into PD1 or PD2 based on the tumor growth delay (TGD) value (described below).

Event-free survival: An event in the xenograft models is defined as a quadrupling of tumor volume from the initial tumor volume. Event-free survival is defined as the time interval from initiation of study to the first event or to the end of the study period for

tumors that did not quadruple in volume. The time to event is determined using interpolation (described below).

Summary statistics and analysis methods. For treatment groups only, a tumor growth delay (TGD) value is used to further classify mice with PD as PD1 or PD2. TGD values are calculated based on the number of days to event. For each individual mouse that has PD and has an event in the treatment group, a TGD value is calculated by dividing the time to event for that mouse by the median time to event in the respective control group. Median times to event are estimated based on the Kaplan-Meier event-free survival distribution. If a mouse has a TGD value ≤ 1.5 , that mouse is considered PD1. If the TGD value is > 1.5 , the mouse is considered PD2. Mice that have PD but do not have an event at the end of the study are coded as PD2.

Tumor Volume T/C value: Relative tumor volumes (RTV) for control (C) and treatment (T) mice are calculated at day 21 or when all mice in the control and treated groups still have measurable tumor volumes (if less than 21 days). The mean relative tumor volumes for control and treatment mice for each study are then calculated and the T/C value is the mean RTV for the treatment group divided by the mean RTV for the control group. For the tumor volume T/C response measure, agents producing a T/C of $\leq 15\%$ are considered highly active, those with a mean tumor volume T/C of $\leq 45\%$ but $> 15\%$ are considered to have intermediate activity, and those with mean T/C values $> 45\%$ are considered to have low levels of activity.

EFS T/C value: An EFS T/C value is defined by the ratio of the median time to event of the treatment group and the median time to event of the respective control group. All of the control RMS xenograft lines events within 6 weeks. For the EFS T/C measure, agents or combinations are considered highly active if they meet three criteria: a) an EFS T/C > 2 ; b) a significant difference in EFS distributions ($p \leq 0.050$), and c) a net reduction in median tumor volume for animals in the treated group at the end of treatment as compared to at treatment initiation.

Interpolation method: The time to event interpolation formula for solid tumor xenografts is shown in equation (1)

$$t_x = t_1 + (t_2 - t_1) \ln(V_e/V_1) / \ln(V_2/V_1) \quad (1)$$

where t_x is the interpolated day to event, t_1 is the lower observation day bracketing the event, t_2 is the upper observation day bracketing the event,

V_1 is the tumor volume on day t_1 ,

V_2 is the tumor volume on day t_2

and V_e is the event threshold (4 times initial tumor volume for solid tumor xenografts).

Statistical Methods: The exact log-rank test, as implemented using PROC STATXACT® for SAS, is used to compare event-free survival distributions between treatment and control groups. P-values are two-sided and are not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation: DX-2647 is to be given at 20 mg/kg IP every other day (Q2DxN) and as a control an irrelevant IgG1 antibody (DX-2647 is an IgG1 antibody) will also be administered at 20 mg/kg IP every other day (Q2DxN).

EXAMPLE 15: Isolation and characterization of a phage-derived human monoclonal antibody against IGF-II

Introduction

Insulin-like growth factor-II (IGF-II) is a maternally imprinted embryonic growth factor that can deliver a mitogenic signal through both the IGF-I receptor (IGF-IR) and an alternatively spliced form of the insulin receptor (IR-A). The mature form of IGF-II arises following post-translational processing, including O-glycosylation and endoproteolysis, of the pro-IGF-II precursor (FIGURE 4). Elevated expression of IGF-II, in part the result of loss of imprinting, is observed in a variety of human malignancies including cancer of the breast, colon and liver. This may be accompanied by the secretion of aberrantly processed pro-IGF-II isoforms with novel properties by some tumour types.

As IGF-II exerts its biological effects by binding to and activating both the IGF-IR and IR-A, neutralising the biological activity of this growth factor directly is an attractive therapeutic option. Here we describe the isolation and characterisation of a phage-derived human monoclonal antibody against IGF-II.

Results

A human Fab-on-phage display library was screened with biotinylated IGF-IIIE. Over 200 individual clones were isolated and subsequently characterised for specificity (IGF-II vs. IGF-IIIE), affinity and ability to block ligand binding to receptor (IGF-IR and IR-A). Lead Fabs were re-formatted, transiently expressed as full-length IgG1 proteins and subject to further characterisation. One IgG was codon-optimised and germlined, with the H and L chain cDNAs subsequently cloned into the mammalian expression vector, pEE12.4 (Lonza Biologics). The construct encoding this protein designated DX-2647 was stably expressed following transfection of CHOK1SV cells (Lonza Biologics) and selection in the presence of methionine sulfoximine.

DX-2647 binds IGF-II and IGF-IIIE with high affinity

The affinity of DX-2647 for recombinant isoforms of IGF-II and IGF-IIIE was established using surface plasmon resonance (SPR) with the IgG “captured” onto an anti-human Fc Ig-coupled chip, followed by injection of ligand analyte. Affinity measurements revealed that DX-2647 bound both IGF-II and IGF-IIIE with very high affinity (KD values of 23 pM and <23 pM, respectively, data not shown).

The binding data for IGF-II were:

1:1 binding model

$k_a = 1.06 \text{ E6 M}^{-1}\text{s}^{-1}$

$k_d = 2.38 \text{ E-5 s}^{-1}$

Kd = 22.6 Pm

Chi2 = 1.34

The binding data for IGF-IIIE were:

1:1 binding model

$k_a = 2.06 \text{ E6 M}^{-1}\text{s}^{-1}$

$k_d \text{ less than } 2 \text{ E-5 s}^{-1}$

Kd less than 23 pM

Chi2 = 1.03

The binding data for IGF-I were:

1:1 binding model

$k_a = 5.8E6 \text{ M}^{-1}\text{s}^{-1}$

$k_d = 0.1 \text{ s}^{-1}$

5 $K_D = 17 \text{ nM}$

IGF-II and the related growth factor, IGF-I, share 70% homology at the amino acid level. SPR was used to demonstrate that, while DX-2647 bound IGF-I, it did so with a K_D value that was three orders of magnitude weaker than for IGF-II (23 pM vs. 28 nM, data not shown).

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DX-2647 does not bind IGF-II when complexed with other proteins

While the IGF-IR and IR-A are the principal mediators of the biological activity of IGF-II, the protein can bind with high affinity to a number of other proteins that include a family of six serum IGF-binding proteins (IGFBP 1-6), and the cell-surface mannose-6-phosphate receptor (IGF-IIR). A number of different assay formats employing SPR were used to assess whether DX-2647 could still bind IGF-II or IGF-IIIE when complexed with other proteins, or vice versa. In the example shown (FIGURE 5A), the Fc-captured human antibody binds free IGF-II, but does not bind a pre-formed complex of IGF-II and IGFBP-1, or IGFBP-1 alone. FIGURE 5B summarizes the results.

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From the summary provided in FIGURES 5A and 5B, it can be seen that, under the experimental conditions used, while DX-2647 blocks the binding of both IGF-II and IGF-IIIE to all three cell-surface receptors for IGF-II, its ability to bind both ligands when they are complexed with the IGFBPs assayed is inhibited. This latter observation may be beneficial with respect to the pharmacokinetic profile of DX-2647 *in vivo*.

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DX-2647 inhibits colony formation and augments induction of cell death

In preliminary studies we found that DX-2647 blocked ligand-induced receptor activation and cellular proliferation in a number of cell types. We then asked if DX-2647

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was inhibitory in a benchmark assay for tumour cell growth *in vitro*, the ability to grow under anchorage-independent conditions.

As depicted in FIGURE 6A, DX-2647 strongly inhibited the ability of HepG2 human hepatoma cells to form colonies when grown in soft agar. Cells were seeded in growth medium plus 10% FCS in soft agar in the presence of increasing concentrations of antibody. Colonies > 50 cells were counted after two weeks. Cells were seeded in growth medium plus 10% FCS in soft agar in the presence of increasing concentrations of antibody. Colonies > 50 cells were counted after two weeks. DX-2647 inhibited anchorage-independent growth of HepG2 cells. The mean IC₅₀ value for the experiment shown in FIGURE 6B was 1.5 nM $\pm$ 0.74 standard error.

Furthermore, DX-2647 potently enhanced cisplatin (CDDP)-induced cellular cytotoxicity in human SKUT-1 myosarcoma cell grown in 20% fetal calf serum (FCS) (FIGURE 7). SKUT-1 cells were seeded in growth medium plus 20% FCS across 96-well plates in the presence of increasing concentration of CDDP and the indicated concentration of DX-2647 or control antibody. Cell viability was measured 48 hours later using CellTitreBlue (Promega). % values indicate the decrease in cell death over CDDP alone.

Resolution of the crystal complex of the parental Fab of DX-2647 and IGF-II

During the course of this work we crystallised IGF-II in complex with M0064-F02, the parental, non-germlined Fab from which DX-2647 was derived. This Fab binds IGF-II with seven-fold less affinity (0.14 nM) than DX-2647. The co-crystal complex was solved to 2.4°A resolution. See FIGURES 1A and 1B.

EXAMPLE 16: Studies of DX-2647 in Hepatocellular Carcinoma Models

The effects of DX-2647 alone or in combination with SOMAVERT® and/or erlotinib are evaluated. Studies are performed in HepG2 or Hep3B cell xenografts per one of the following protocols. For combination treatments, the same dose and timing for each agent are used as for the monotherapy with the given agent.

1. Cell line: **HepG2** HCC cells implanted as 2×10^6 cells in 0.1 ml PBS

Mice: Female Balb/C nude

Groups:	1. vehicle		IP	Q2DxN
	2. SOMAVERT®	60 mg/kg	SC	Q2DxN
5	3. DX-2647	20 mg/kg	IP	Q2DxN
	4. doxorubicin	3 mg/kg	IP	Q2DxN
	5. SOMAVERT® + DX-2647			
	7. erlotinib + DX-2647			
	8. erlotinib + DX-2647 + SOMAVERT®			

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2. Cell line: **Hep3B** HCC cells implanted as 5×10^6 cells in 0.1 ml PBS

Mice: Female Balb/C nude

Groups:	1. vehicle		IP	Q2DxN
	2. SOMAVERT®	60 mg/kg	SC	Q2DxN
15	3. DX-2647	20 mg/kg	IP	Q2DxN
	4. doxorubicin	3 mg/kg	IP	Q2DxN
	5. Somavert + DX-2647			
	6. erlotinib	50 mg/kg	PO	QDx14
	7. erlotinib + DX-2647			
20	8. erlotinib + DX-2647 + SOMAVERT®			

3. Cell line: **Hep3B** HCC cells implanted as 1 mm³ tumor fragments

Mice: Female athymic nude (nu/nu, Harlan)

Groups:	1. vehicle		IP	Q2DxN
25	2. DX-2647	20 mg/kg	IP	Q2DxN
	3. A2 Ab	20 mg/kg	IP	Q2DxN
	4. doxorubicin	3 mg/kg	IP	Q4Dx3

A2 Ab is an isotype (IgG1) negative control antibody.

EXAMPLE 17: DX-2647 Inhibits Proliferation of Colo205 Cells

To further demonstrate the effect of DX-2647 on tumor cell proliferation, Colo205 cells were grown in complete growth medium containing 10% FBS, and treated with or without DX-2647 or isotype control antibody. Colo205 cells were seeded into a 96-well plate at 8×10^3 per well in complete medium with or without 2 μ M DX-2647 or isotype control antibody (Ctrl. IgG). The plate was placed in a 37°C incubator with 5% CO₂ for 4 days. Proliferation was measured by CellTiter 96 _{Aqueous} One Solution Reagent (Promega). DX-2647 at 2 μ M significantly inhibited Colo205 proliferation in complete growth medium (FIGURE 8). Colo205 cells have two cell populations in culture vessels. We demonstrated here that DX-2647 inhibited proliferation of both adherent and suspension cell populations.

We also demonstrated that DX-2647 inhibited IGF-II induced Colo205 cell proliferation (FIGURE 9). In this experiment, cells were cultured in serum free medium containing 2 nM IGF-II. Cells were treated with or without DX-2647 for 96 h. Neutralizing IGF-II antibody (R&D Systems) was used at 333 nM as a positive control. DX-2647 showed maximum potency in inhibiting IGF-II stimulated Colo205 proliferation at concentrations as low as 10 nM. In this experiment, Colo205 cells were seeded into a 96-well plate at 8×10^3 per well in serum free medium in the presence or absence of 2 nM IGF-II, and treated with or without DX-2647 at a concentration ranging from 1 nM to 100 nM. IGF-II neutralizing antibody from R&D at 333 nM was used as a positive control. The plate was placed in a 37°C incubator with 5% CO₂ for 4 days. Proliferation was measured by CellTiter 96 _{Aqueous} One Solution Reagent (Promega).

EXAMPLE 18: DX-2647 Inhibits Exogenous IGF-II Induced IGF-1R**Phosphorylation**

Upon ligand binding, IGF-1R undergoes autophosphorylation on tyrosine residues of the two β - subunits. To determine if DX-2647 binds to IGF-II and thus blocks IGF-II-induced IGF-1R phosphorylation, we tested several cell lines including two hepatocellular carcinoma cell lines: HepG2 and Hep3B, and one colorectal cancer cell line Colo205. HT-29 and MCF-7 were also tested with the non-germlined counterpart of DX-2647 (Example 7 and data not shown).

DX-2647 inhibited IGF-II (2.6 nM)-induced IGF-1R phosphorylation in a dose-dependent manner. Partial inhibition was observed at 0.3 nM of DX-2647 treatment, and complete inhibition was observed at 3 nM in HepG2 cells (data not shown). To perform the experiments, HepG2 cells were seeded and cultured for 24 h, and then serum starved overnight followed by treatment with DX-2647 plus IGF-II for 10 min. After being washed twice with ice-cold PBS containing 1 mM sodium orthovanadate, cells were lysed and immunoprecipitated with anti-IGF-IR antibody. Immunoprecipitated proteins were resolved in a 4-12% Bis-tris gel, and transferred to a PVDF membrane. The membrane was probed with anti-phospho-IGF-IR (p-IGF-1R) antibody first, and then stripped and re-probed with anti-IGF-IR antibody for detection of total IGF-1R proteins.

Similar results were observed in Hep3B cells, with partial inhibition at 0.1 nM and complete inhibition at 1 nM (data not shown). Inhibitory effect of DX-2647 on IGF-IR phosphorylation was also observed in Colo205 cells with a higher effective concentration of 10 nM (data not shown). DX-2647 binds to IGF-I at much lower affinity compared with binding to IGF-II based on BIAcore analysis. As a result, DX-2647 at 10 nM failed to show significant inhibition on IGF-I induced IGF-IR phosphorylation. Higher concentrations of DX-2647 may be required to obtain an inhibitory effect against IGF-I. To perform the experiments,

Hep3B cells were seeded and cultured for 24 h, and then serum starved overnight followed by treatment with DX-2647 plus IGF-I or IGF-II for 10 min. After being washed twice with ice-cold PBS containing 1 mM sodium orthovanadate, cells were lysed and immunoprecipitated with anti-IGF-IR antibody. Immunoprecipitated proteins were resolved in a 4-12% Bis-tris gel, and transferred to a PVDF membrane. The membrane was probed with anti-phospho-IGF-IR (p-IGF-1R) antibody first, and then stripped and re-probed with anti-IGF-IR antibody for detection of total IGF-1R proteins.

EXAMPLE 19: DX-2647 Inhibits Endogenous IGF-II Induced IGF-1R Phosphorylation

Hep3B cells synthesize and secrete IGF-II which in turn binds IGF-1R and induces IGF-1R phosphorylation in an autocrine fashion. The autocrine IGF-II induced IGF-1R phosphorylation can be detected by IP/WB analysis after 48 h serum starvation

of cells. DX-2647 at 10 nM showed significant inhibitory effect on autocrine IGF-II induced IGF-1R phosphorylation (data not shown). Similar result was observed with 24 h treatment of DX-2647 (data not shown). To perform the experiments, Hep3B cells were seeded and cultured for 24 h. After cells were washed twice with PBS, fresh medium
 5 containing no FBS was added with or without DX-2647 at 10 nM, and cells were then incubated for additional 48 h. After being washed twice with ice-cold PBS containing 1 mM sodium orthovanadate, cells were lysed and immunoprecipitated with anti-IGF-IR antibody. Immunoprecipitated proteins were resolved in a 4-12% Bis-tris gel, and transferred to a PVDF membrane. The membrane was probed with anti-phospho-IGF-IR
 10 (p-IGF-1R) antibody first, and then stripped and re-probed with anti-IGF-IR antibody for detection of total IGF-1R proteins.

EXAMPLE 20: DX-2647 Inhibits Anchorage Dependent Colony Formation of HepG2 Cells

15 DX-2647 at 1 nM significantly inhibited the colony-forming ability of HepG2 cells. HepG2 cells were seeded into a 6-well plate at 1000 cells/well in 2 ml of culture medium containing 10% FBS. Cells were treated with or without DX-2647 for 7 days. Isotype control IgG (Ctrl. IgG) was used at 100 nM. After incubation cells were fixed and stained with crystal violet blue. Five pictures were taken randomly from each well and
 20 data were analyzed using the MetaMorph software. The results are summarized graphically in FIGURE 10.

EXAMPLE 21: Studies of DX-2647 in Hep3B Hepatocellular Carcinoma Model

The effects of DX-2647 in a tumor model were evaluated. Studies were
 25 performed in Hep3B cell xenografts per the following protocol. For combination treatments, the same dose and timing for each agent are used as for the monotherapy with the given agent.

Cell line: **Hep3B** HCC cells implanted as 5×10^6 cells in 0.1 ml PBS

Mice: Female Balb/C nude

Groups: 1. PBS vehicle

IP Q2DxN

	2. Pegvisomant	60 mg/kg	SC	Q2DxN
	3. DX-2647	20 mg/kg	IP	Q2DxN
	4. doxorubicin	3 mg/kg	IV	Q4Dx3
	5. Pegvisomant + DX-2647			
5	6. erlotinib	50 mg/kg	PO	QDx14
	7. erlotinib + DX-2647			
	8. erlotinib + DX-2647 + Pegvisomant			

FIGURE 11 shows results for the treatments listed above. The results shown in
 10 FIGURE 11 are based on adjusted data from which data outliers (more than 2 standard
 deviations from the means) were eliminated.

REFERENCES

The contents of all cited references including literature references, issued patents,
 published or non-published patent applications cited throughout this application as well
 15 as those listed below are hereby expressly incorporated by reference in their entireties. In
 case of conflict, the present application, including any definitions herein, will control.

EQUIVALENTS

A number of embodiments of the invention have been described. Nevertheless, it
 will be understood that various modifications may be made without departing from the
 20 spirit and scope of the invention. Accordingly, other embodiments are within the scope
 of the following claims.

WHAT IS CLAIMED IS:

1. An isolated protein that binds IGF II and /or IGF IIE wherein the binding is characterized by an affinity of at least 10^9 M^{-1} .

5 2. An isolated protein comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence, wherein the heavy chain immunoglobulin variable domain sequence comprises the amino acid sequence of the heavy chain variable domain of M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03 or M0072-G06, the
10 light chain immunoglobulin variable domain sequence comprises the amino acid sequence of the light chain variable domain of M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, or M0072-G06, and the protein binds to and inhibits both IGF-II and IGF-IIE.

15 3. An isolated protein comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence, wherein the heavy chain immunoglobulin variable domain sequence comprises the amino acid sequence of the heavy chain variable domain of M0080-G03 and M0073-C11, the light chain immunoglobulin variable domain sequence comprises the amino acid sequence of
20 the light chain variable domain of M0080-G03 and M0073-C11 and the protein binds to and inhibits IGF-IIE but not IGF-II.

4. An isolated protein capable of specifically binding to the following consensus sequence or a functional fragment thereof:

25

TXCGGXLVXXLXXXXXXXXXXFXXXXPXXRVXXSRGXVEEXCFRXXXXXXXXXXY

wherein X is any amino acid.

30

5. The isolated protein of claim 4, wherein the protein is capable of specifically binding to the following consensus sequence or a functional fragment thereof:

SETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPA.

6. A method for treating cancer in a subject, the method comprising administering an IGF-II/IGF-IIE binding protein to the subject.

7. The method of claim 6 further comprising administering a growth hormone/growth hormone releasing hormone pathway modulator to the subject.

8. The method of claim 7, wherein the IGF-II/IGF-IIE binding protein is administered for a period prior to the administration of the growth hormone/growth hormone releasing hormone pathway modulator.

9. The method of claim 7, wherein the IGF-II/IGF-IIE binding protein is administered following first administration of the growth hormone/growth hormone releasing hormone pathway modulator.

10. The method of claim 6, wherein the IGF-II/IGF-IIE binding protein is an antibody.

11. The method of claim 10, wherein the IGF-II/IGF-IIE binding protein comprises a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence, wherein the heavy chain immunoglobulin variable domain sequence comprises the amino acid sequence of the heavy chain variable domain of M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03 or M0072-G06, the light chain immunoglobulin variable domain sequence comprises the amino acid sequence of the light chain variable domain of M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03, or M0072-G06, and the protein binds to and inhibits both IGF-II and IGF-IIE.

12. The method of claim 6, wherein the method further comprises monitoring the subject.

13. The method of claim 12, wherein the monitoring is for one or more of:
5 reduction in tumor size; reduction in a cancer marker; reduction in the appearance of a new lesion; reduction in the appearance of a new disease-related symptom; decrease of size of soft tissue mass; stabilization of size of soft tissue mass; or any parameter related to improvement in clinical outcome.

10 14. The method of claim 6, wherein the subject is a mammal.

15. The method of claim 14, wherein the mammal is a human.

16. A kit comprising
15 (a) an IGF-II/IGF-IIIE binding protein;
(b) a growth hormone/growth hormone releasing hormone pathway modulator;
and
(c) instructions for use in accordance with a method for treating cancer in a subject.

20 17. A method for treating cancer in a subject, the method comprising:
administering an effective amount of an IGF-II/IGF-IIIE binding protein to a subject having cancer, wherein the IGF-II/IGF-IIIE binding protein comprises a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin
25 variable domain sequence, wherein the heavy chain immunoglobulin variable domain sequence comprises the amino acid sequence of the heavy chain variable domain of M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-C06, M0072-E03 or M0072-G06, the light chain immunoglobulin variable domain sequence comprises the amino acid sequence of the light chain variable domain of
30 M0033-E05, M0063-F02, M0064-E04, M0064-F02, M0068-E03, M0070-H08, M0072-

C06, M0072-E03, or M0072-G06, and the protein binds to and inhibits both IGF-II and IGF-III.

18. The method of claim 17 further comprising administering an effective amount
5 of a growth hormone/growth hormone releasing hormone pathway modulator to the
subject.

19. The method of claim 18, wherein said growth hormone/growth hormone
releasing hormone pathway modulator is a growth hormone receptor antagonist.

10

20. The method of claim 19, wherein the growth hormone receptor antagonist is
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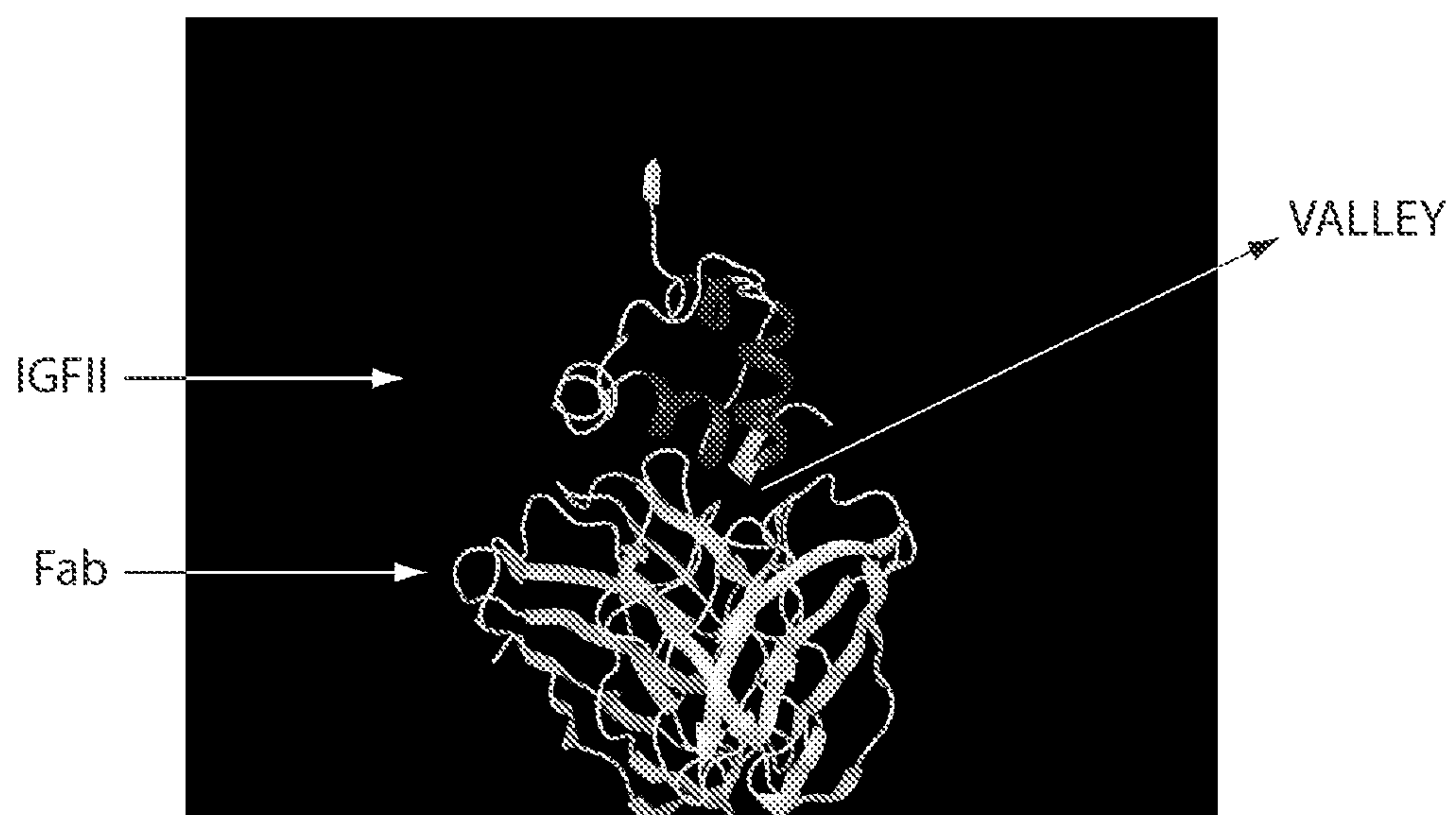


Figure 1A

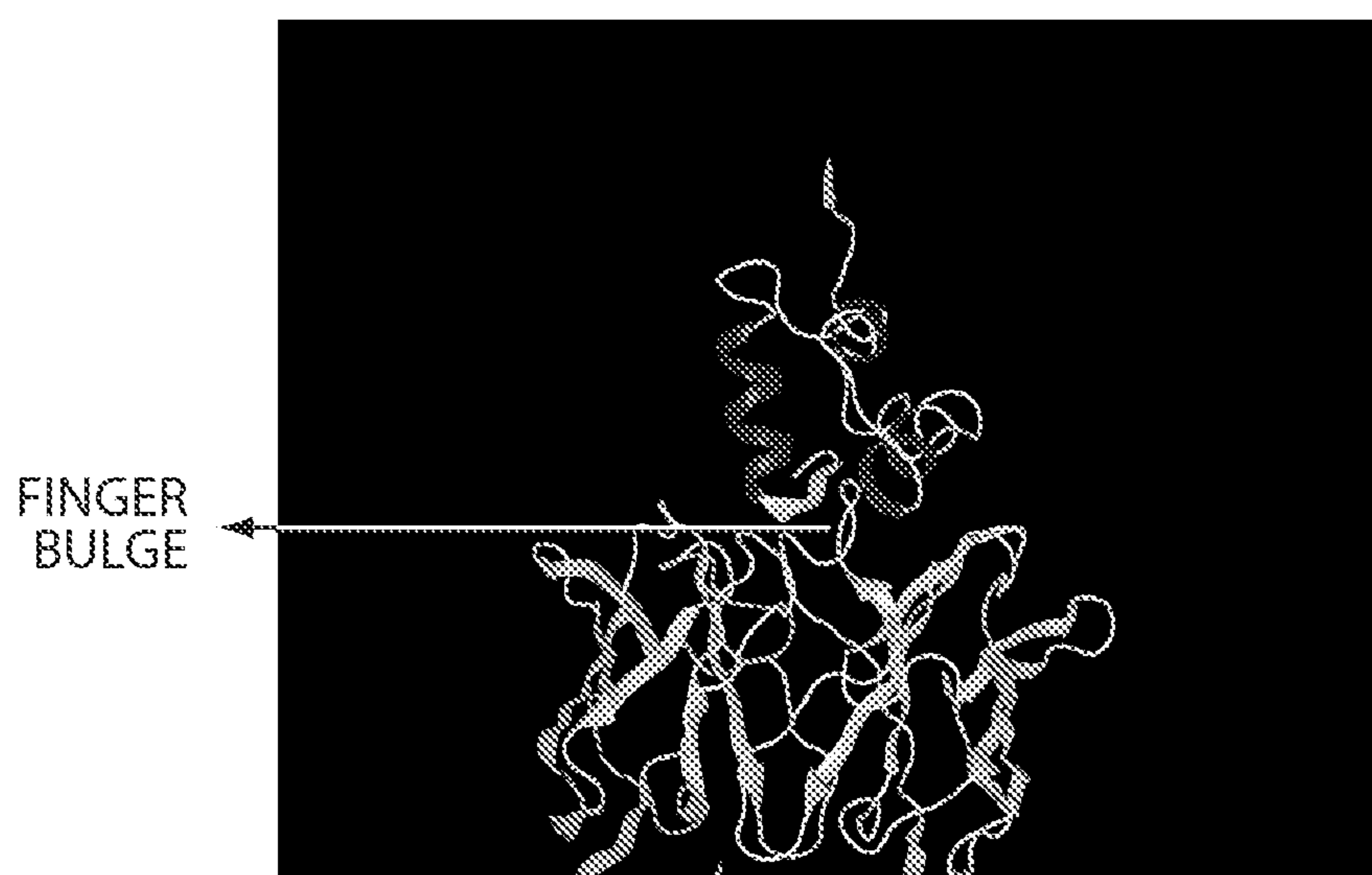


Figure 1B

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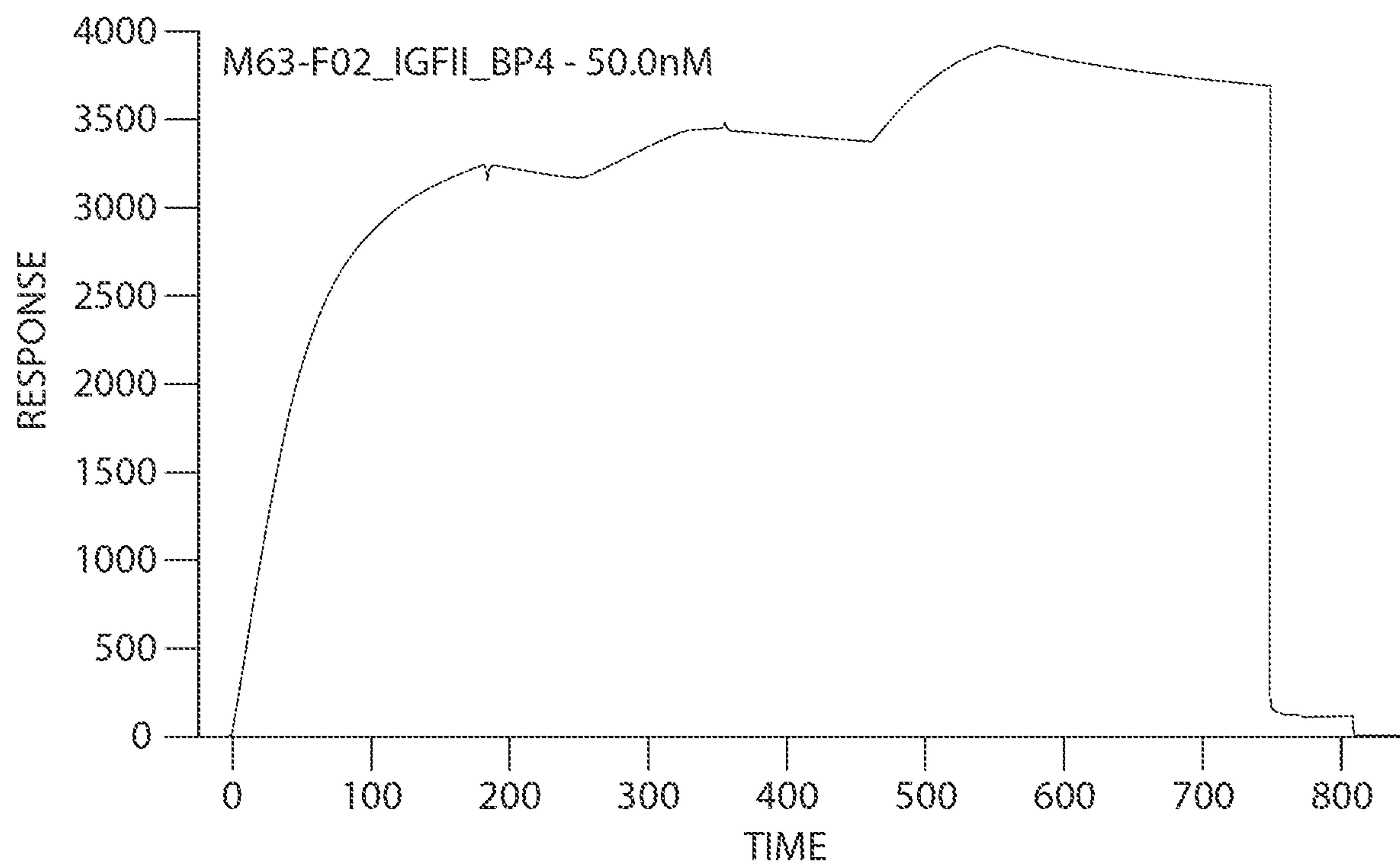


Figure 2A

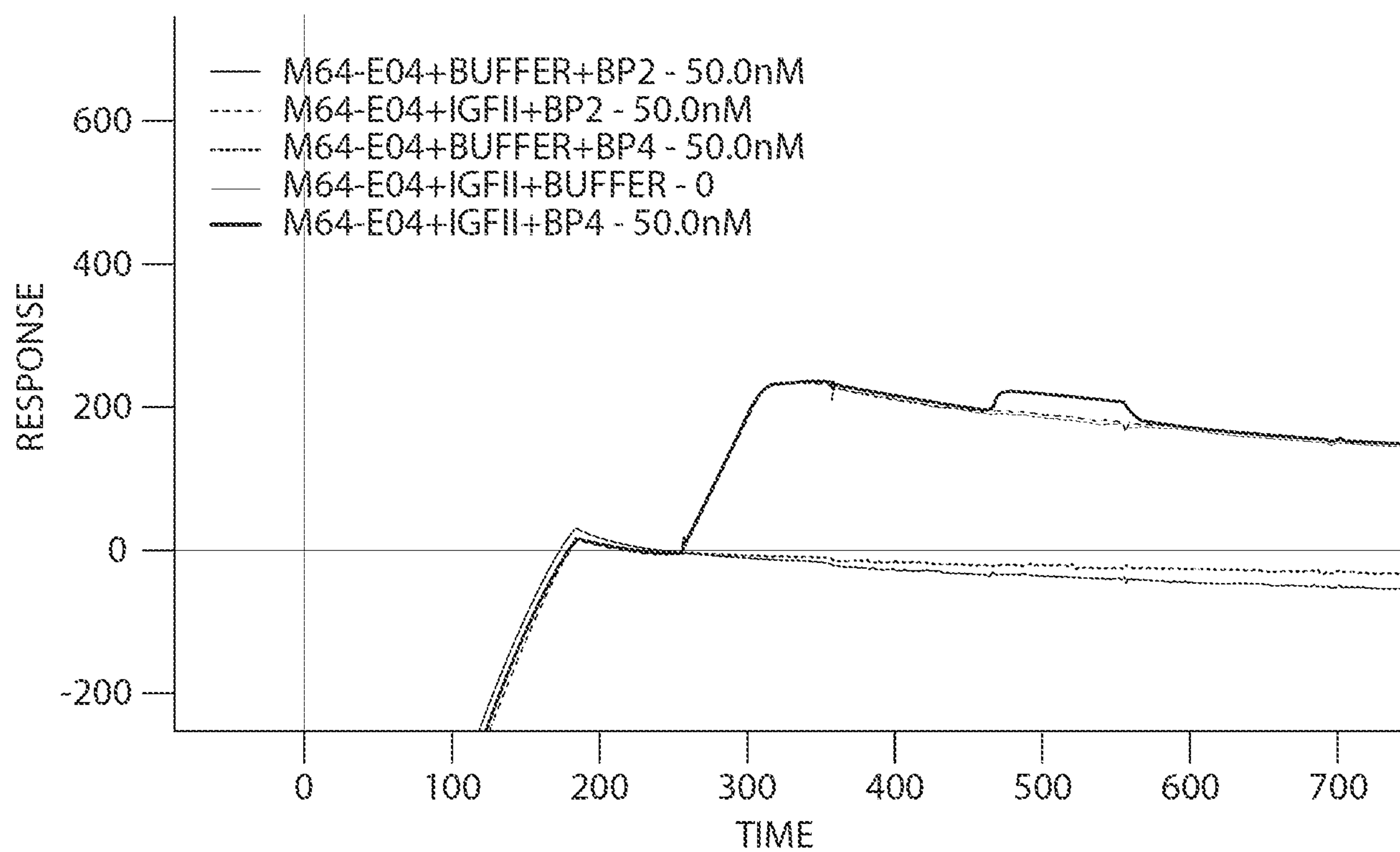


Figure 2B

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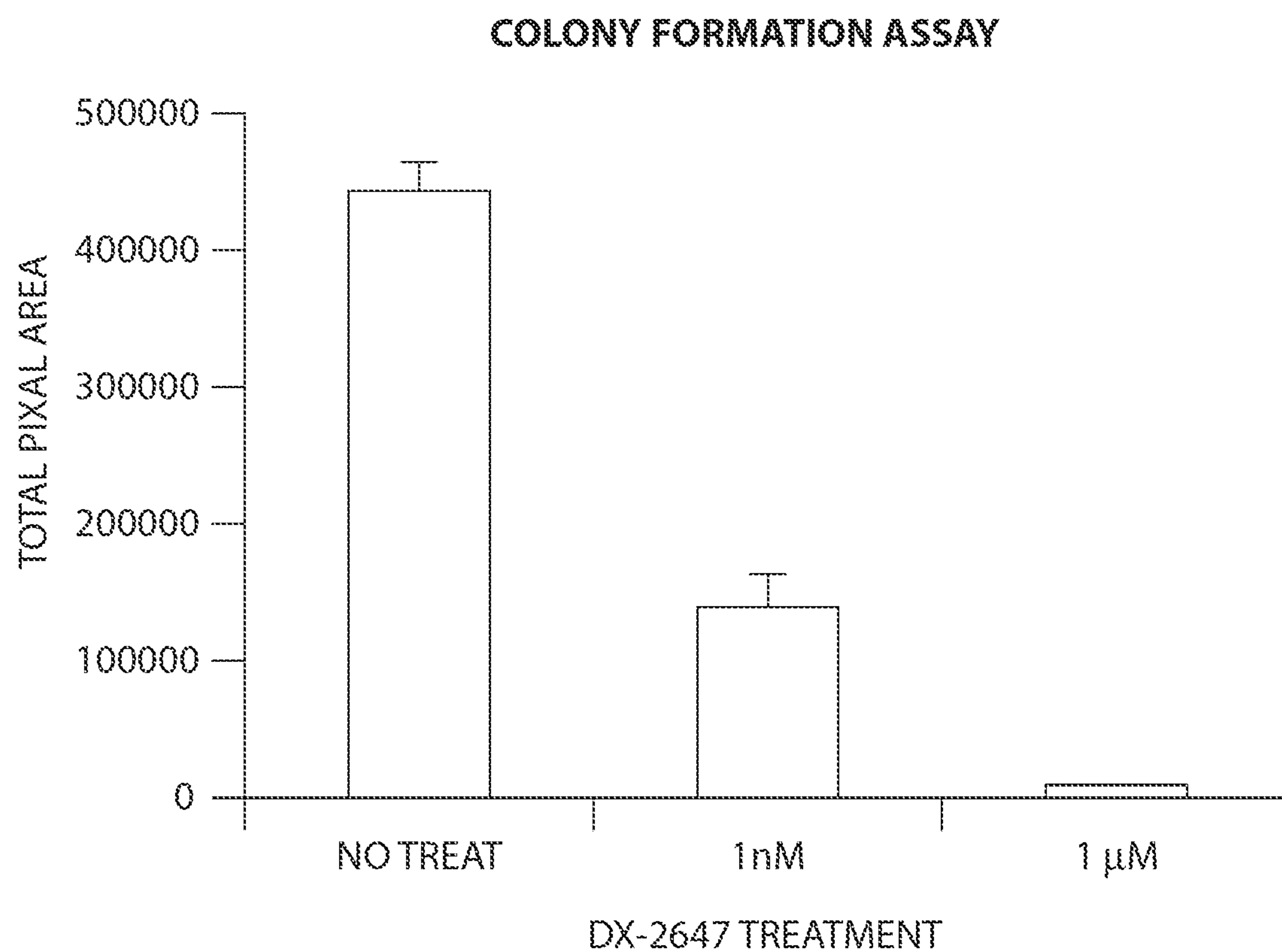


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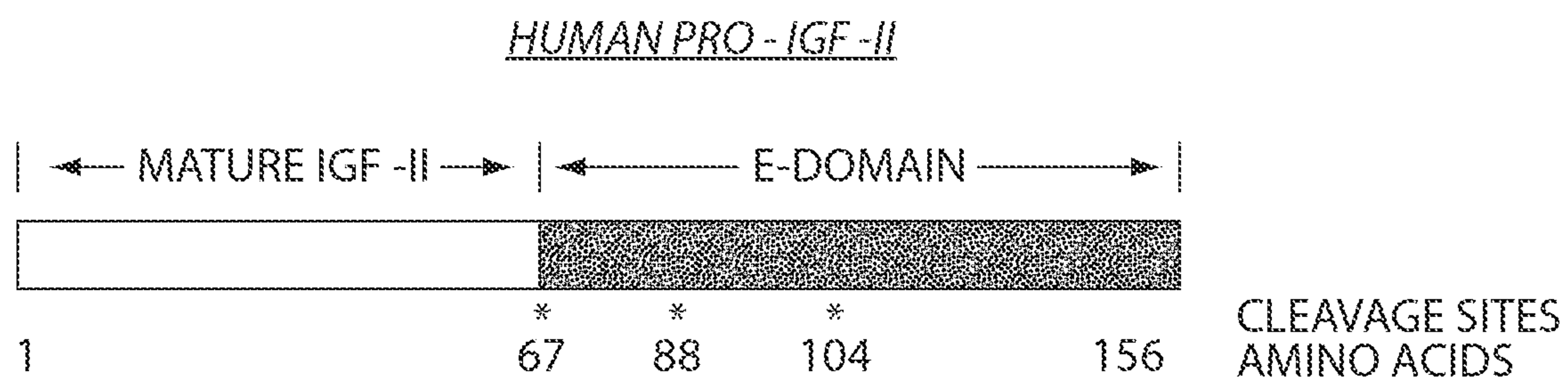


Figure 4

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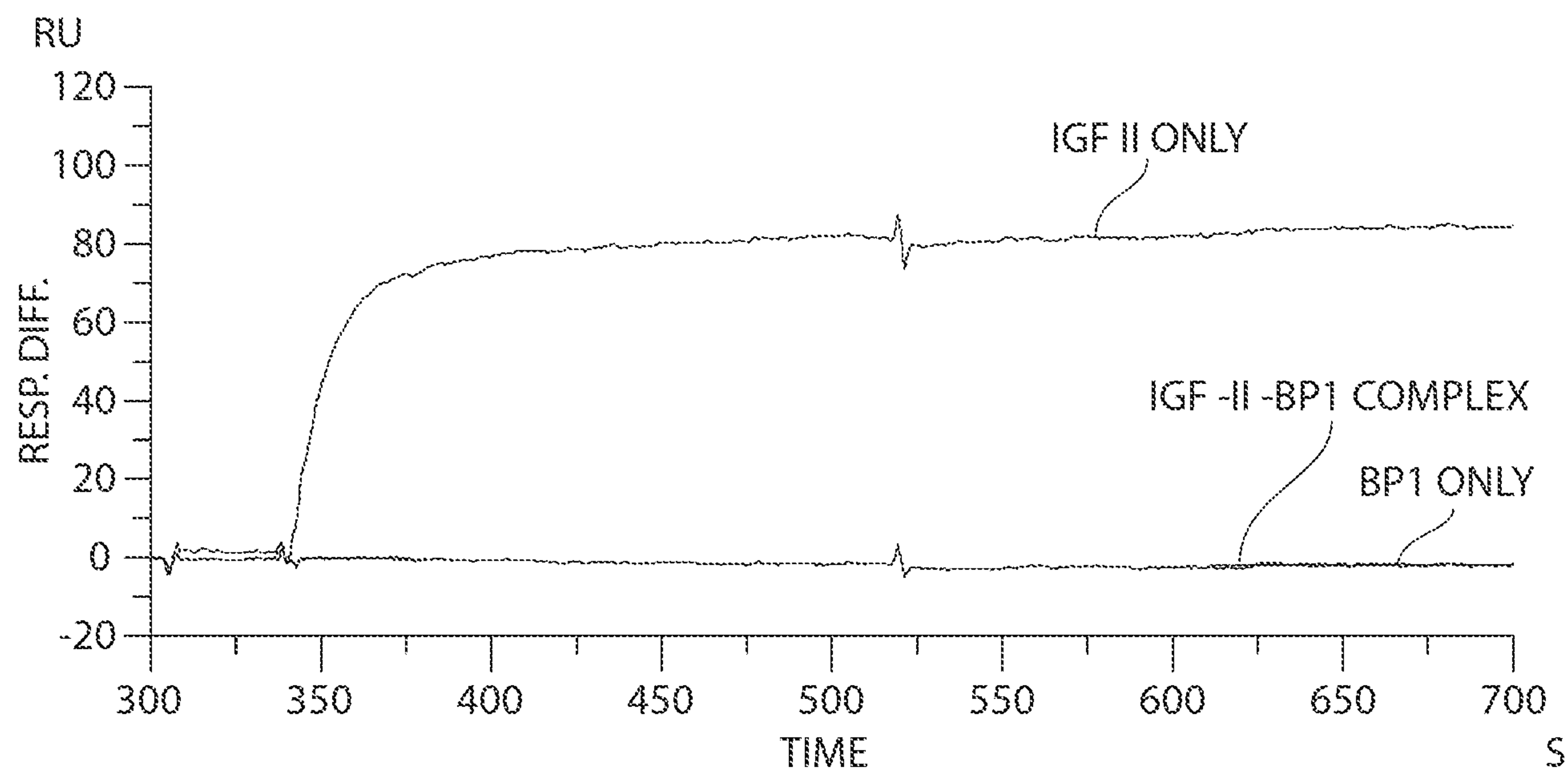


Figure 5A

RECEPTOR/BP	DX-2647
IGF-IR	YES
IGF-IIR	YES
IR-A	YES
IGFBP-1	YES
IGFBP-2	YES
IGFBP-3	ND
IGFBP-4	YES
IGFBP-5	ND
IGFBP-6	YES

ND: NOT DETERMINED

Figure 5B

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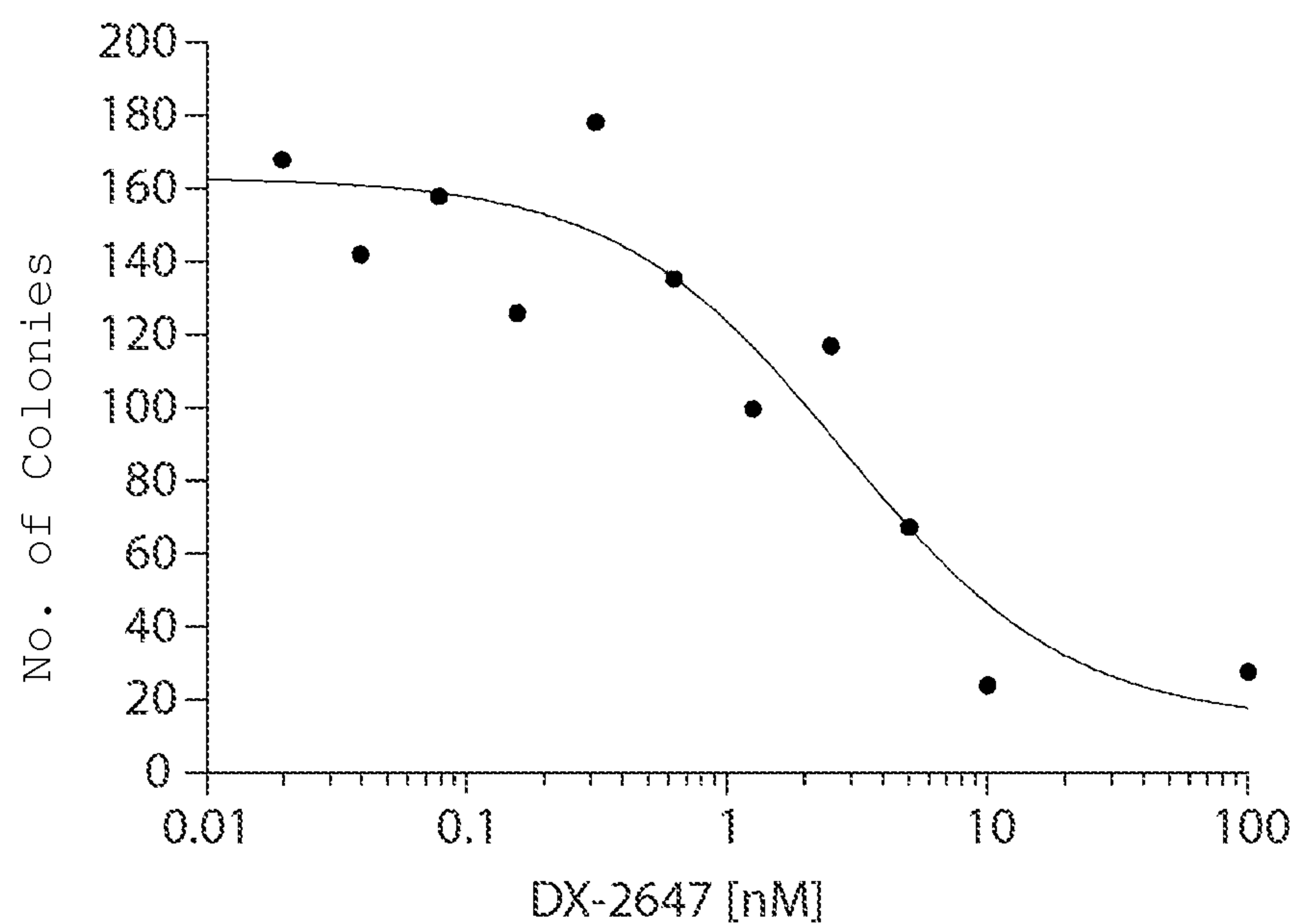


Figure 6A

IgG	Mean IC ₅₀ (nM)	S.E. ^a	n ^b
DX-2647	1.5	0.74	3

S.E.^a : STANDARD ERRORn^b: NUMBER OF EXPERIMENTS

Figure 6B

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SKUT-1 CELL VIABILITY FOLLOWING 48 HOUR TREATMENT

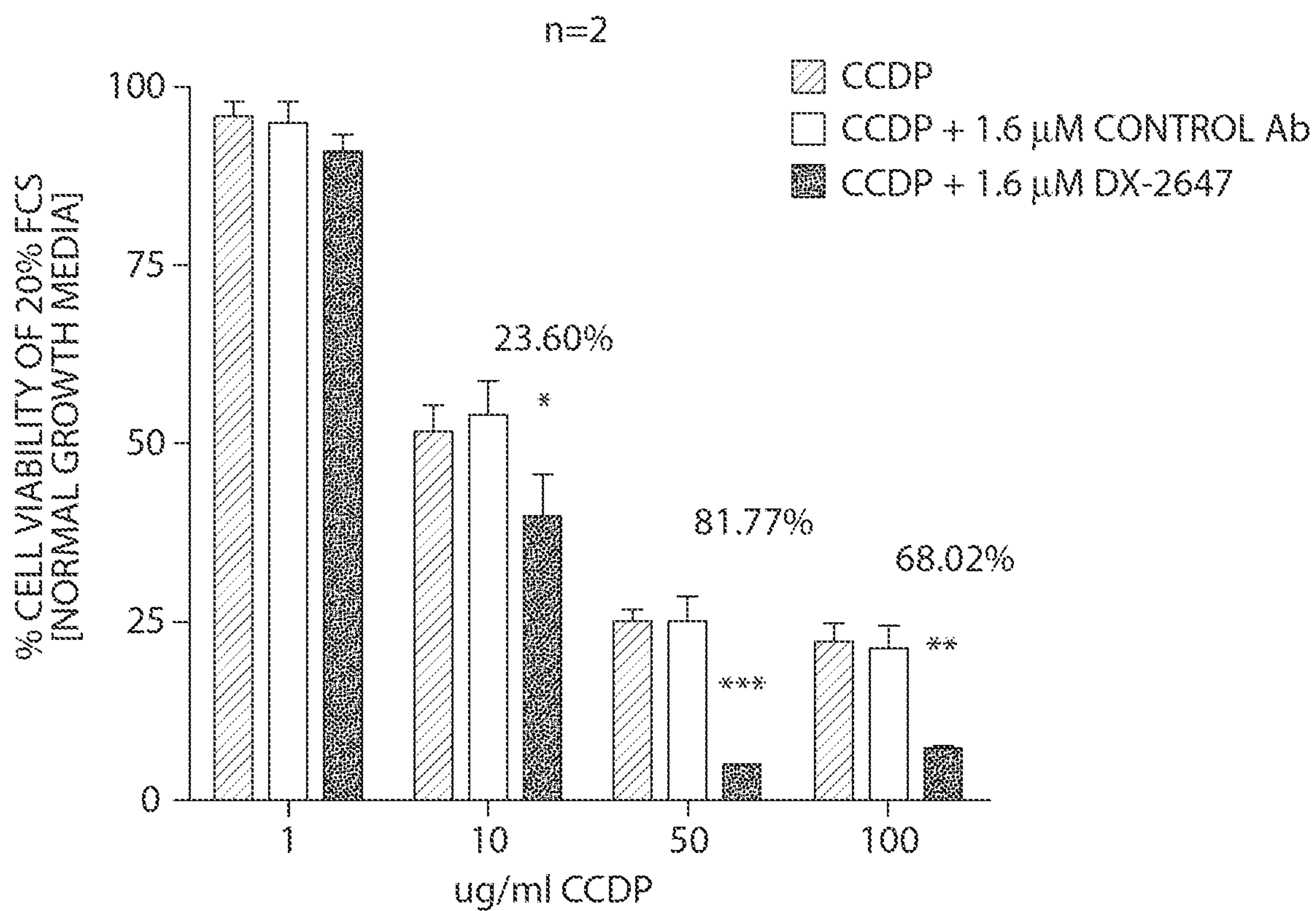


Figure 7

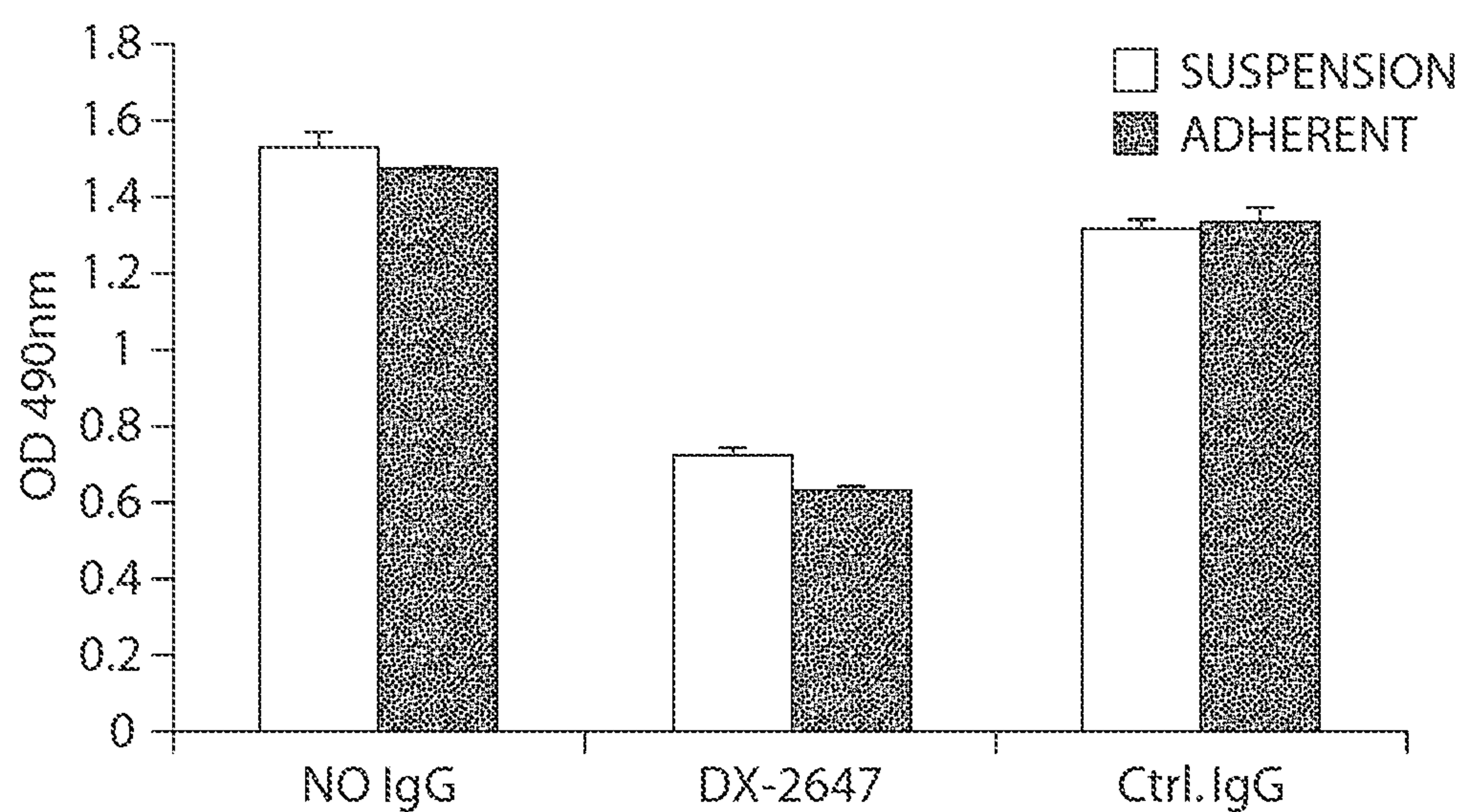


Figure 8

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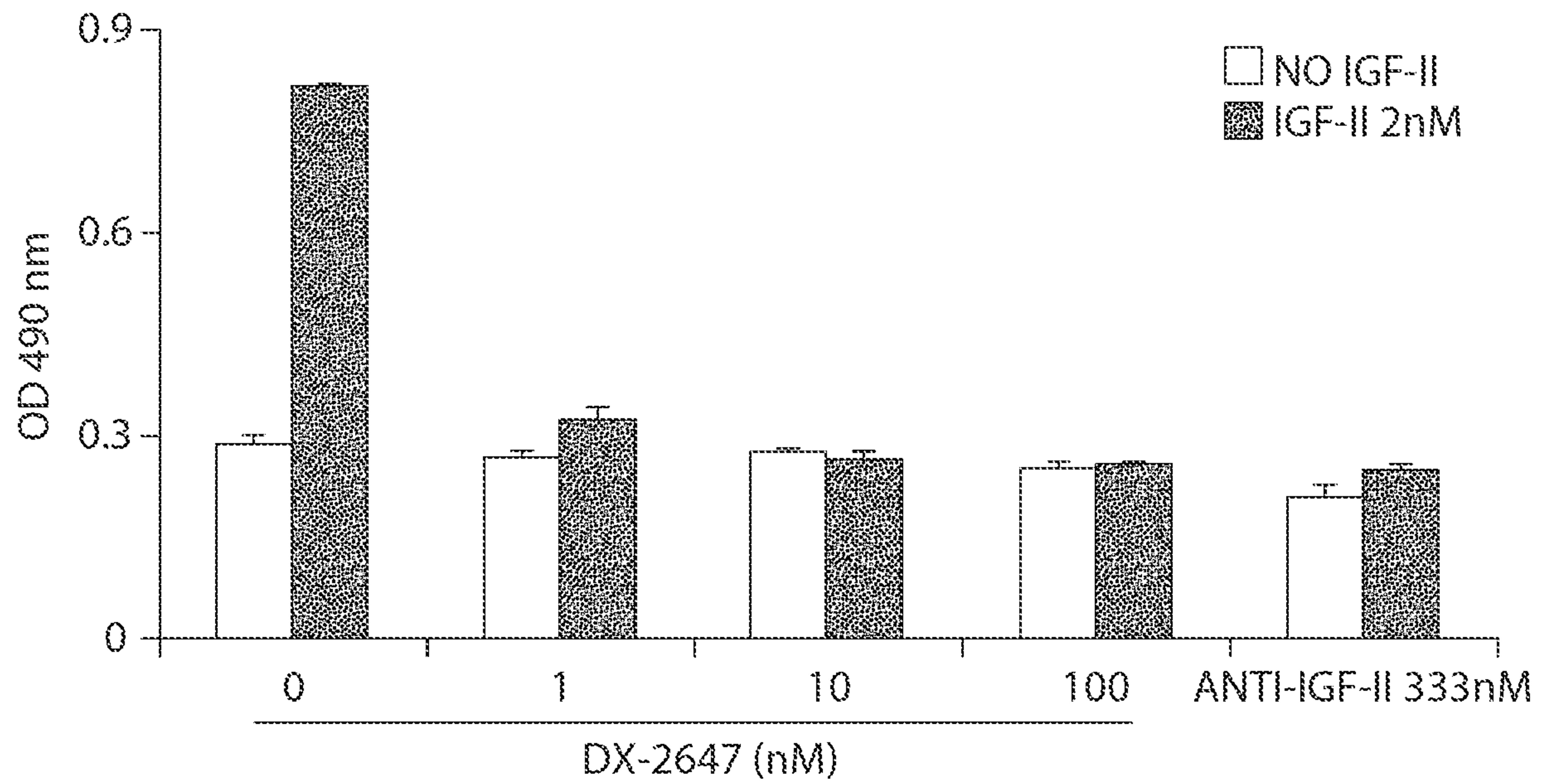


Figure 9

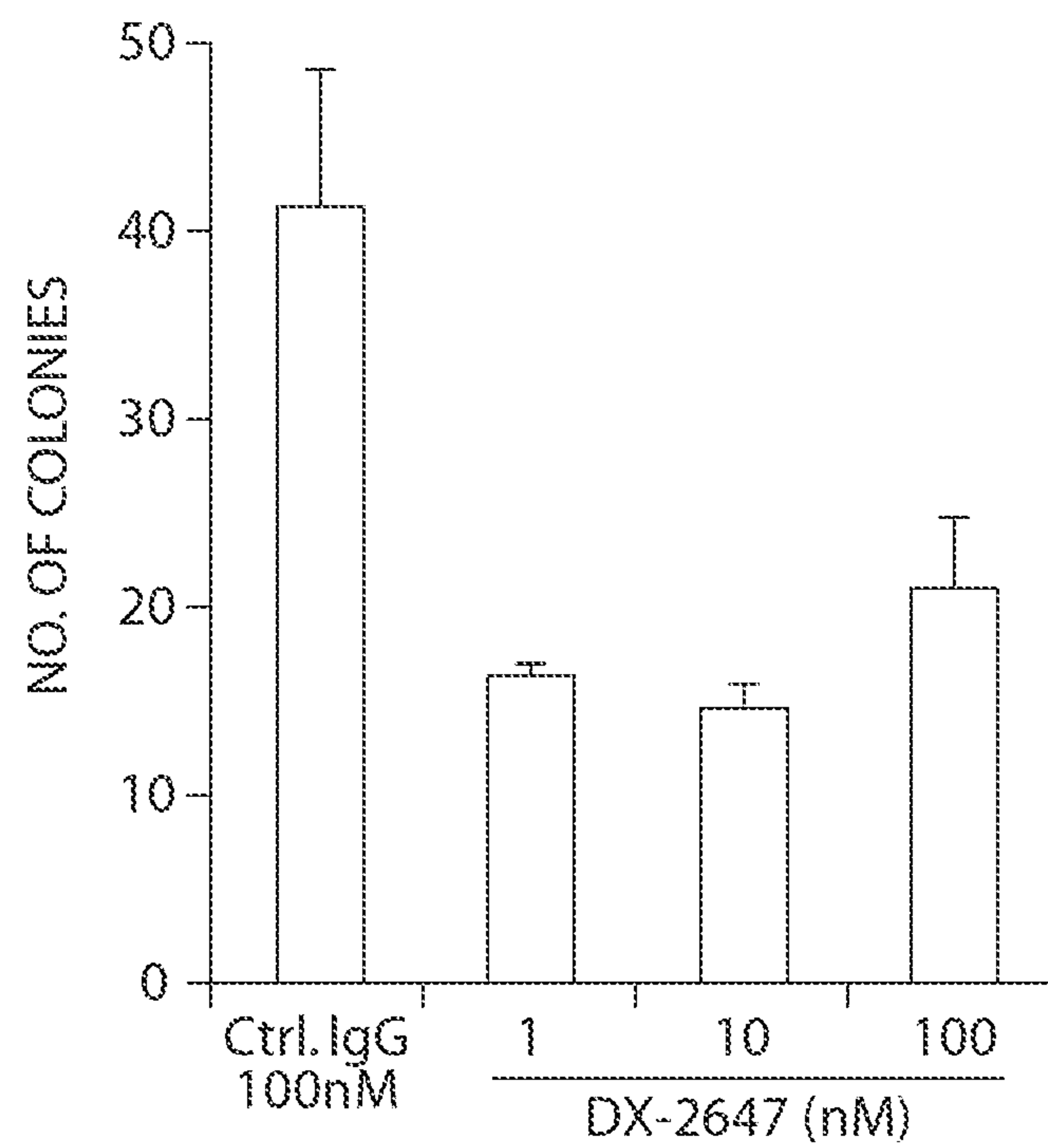


Figure 10

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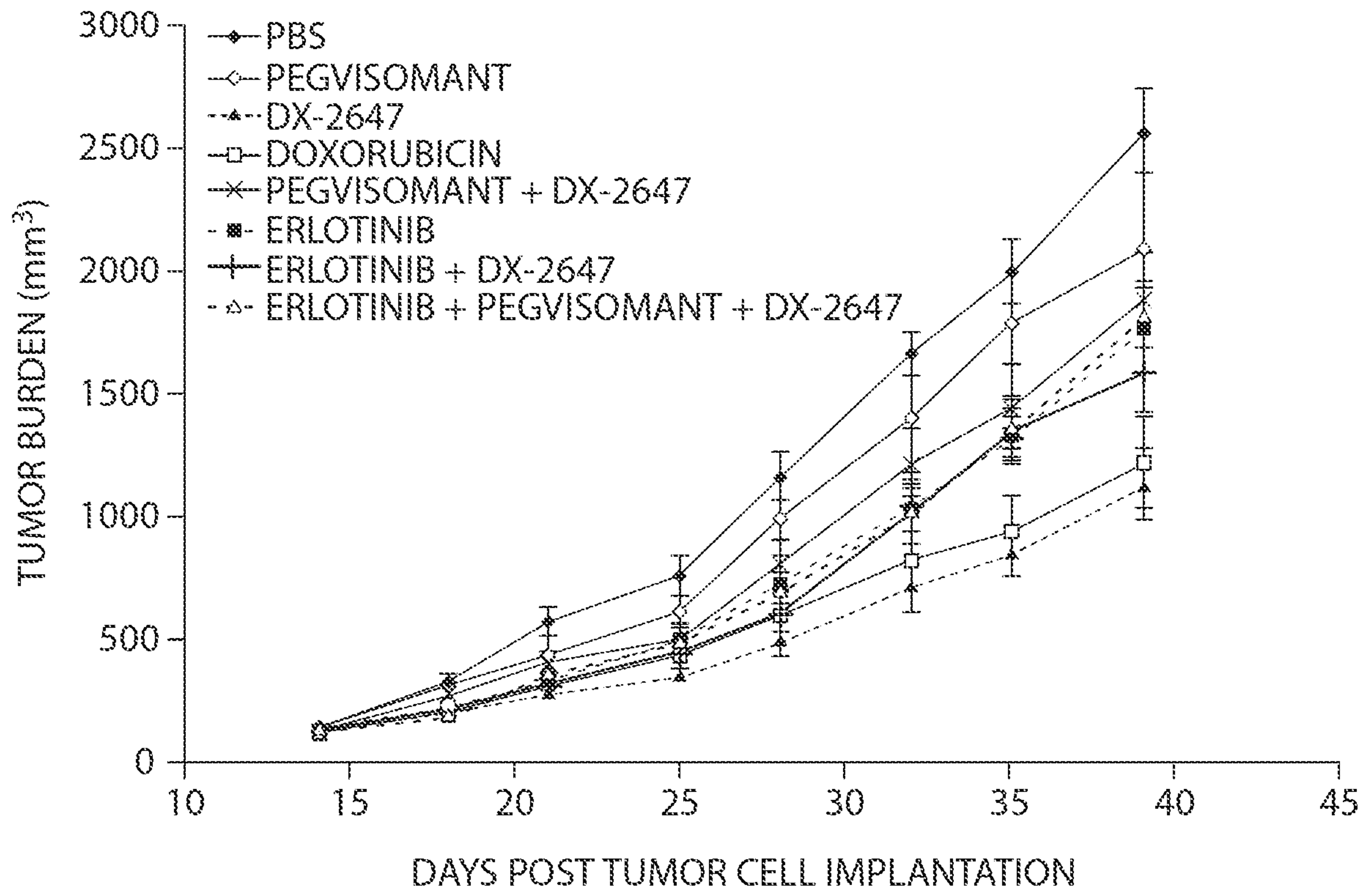


Figure 11