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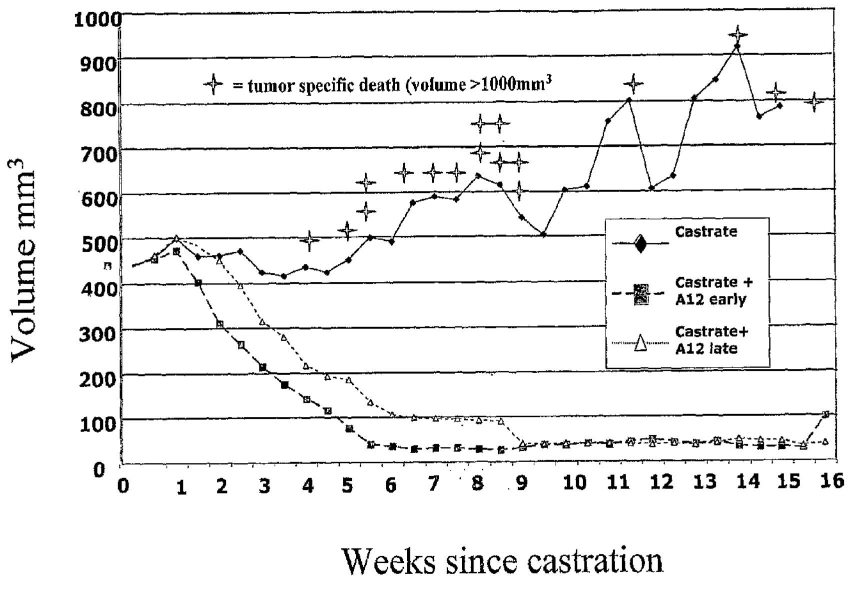
(54) Title: IGF-IR ANTAGONISTS AS ADJUVANTS FOR TREATMENT OF PROSTATE CANCER



(57) Abstract: The present invention relates to a method of treating prostate cancer with androgen deprivation therapy and an insulin-like growth factor receptor (IGF-IR) antagonist. Although the response rate of prostate cancer to androgen deprivation therapy (ADT) is high, surviving cancer cells invariably become androgen independent (AI) and tumor growth follows. The invention inhibits or delays transition of androgen dependent cancer to androgen independent cancer, significantly decreases risk of recurrence, and improves treatment outcome.

WO 2007/092453 A2

IGF-IR ANTAGONISTS AS ADJUVANTS FOR TREATMENT OF PROSTATE CANCER

FEDERAL FUNDING

[0001] The present invention was made in part with United States Government support under Grant No. CA85859 from the National Institutes of Health. and Grant No. W81XWH-04-1-0912 from the Department of Defense. Accordingly, the United States Government has certain rights in this invention.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority to U.S. Application No.60/765,072, filed February 3, 2006, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention relates to a method of treating prostate cancer with androgen deprivation therapy and an insulin-like growth factor receptor (IGF-IR) antagonist. The method inhibits or delays transition of androgen dependent cancer to androgen independent cancer and significantly decreases risk of recurrence and improves treatment outcome.

BACKGROUND OF THE INVENTION

[0004] Prostate cancer is the most common nonskin cancer and second most common cause of cancer mortality in US men. Most prostate cancer is initially androgen dependent (AD). Prostate cancer cells initially require androgen for continued proliferation. Response to ablation of testosterone through androgen deprivation therapy (ADT), either surgically (orchiectomy) or medically (GnRH agonists or estrogens), leads to rapid induction of apoptosis of sensitive prostate cancer cells. The positive response rate is about 86% based on decrease in prostate specific antigen (PSA) and stabilization or decrease in tumor volume. The cell death that occurs generally takes place within the first few days to a week. However, the positive response is followed by a period of growth arrest in which remaining cells tend not to die. After 18-36 months following hormone ablation, growth recurs in 90% of cases. Invariably, surviving cancer cells become androgen independent or unresponsive, and androgen-independent (AI) tumor growth follows. Since ADT is initially very effective, a therapy that could take advantage of the benefits of ADT and extend or enhance its effects would be of great benefit.

[0005] Androgen independence appears to arise by a variety of mechanisms. Mutations in the androgen receptor gene are rare at diagnosis, but increase after exposure to the anti-androgen flutamide. However, these mutations do not occur in the majority of patients and do not explain most cases of hormone-refractory disease. High levels of bcl-2 are seen with greater frequency in advanced disease as compared to localized disease. Thus, the ability to induce apoptosis diminishes as the disease progresses. The proliferation of cells harboring mutations of the tumor suppressor gene p53, the loss of TGF-β receptors, and the expression of peptide growth factors likely play a role in the development of a hormone-refractory state. However, these processes do not explain the rapidity and frequency of development.

[0006] The insulin-like growth factor receptor (IGF-IR) is a ubiquitous transmembrane tyrosine kinase receptor that is essential for normal fetal and post-natal growth and development. IGF-IR can stimulate cell proliferation, cell differentiation, changes in cell size, and protect cells from apoptosis. It has also been considered to be quasi-obligatory for cell transformation (reviewed in Adams et al., *Cell. Mol. Life Sci.* 57:1050-93 (2000); Baserga, *Oncogene* 19:5574-81 (2000)). IGF-IR is located on the cell surface of most cell types and serves as the signaling molecule for growth factors IGF-I and IGF-II (collectively termed henceforth IGFs). IGF-IR also binds insulin, albeit at three orders of magnitude lower affinity than it binds to IGFs. IGF-IR is a pre-formed hetero-tetramer containing two alpha and two beta chains covalently linked by disulfide bonds. The receptor subunits are synthesized as part of a single polypeptide chain of 180kd, which is then proteolytically processed into alpha (130kd) and beta (95kd) subunits. The entire alpha chain is extracellular and contains the site for ligand binding. The beta chain possesses the transmembrane domain, the tyrosine kinase domain, and a C-terminal extension that is necessary for cell differentiation and transformation, but is dispensable for mitogen signaling and protection from apoptosis.

[0007] IGF-IR is highly similar to the insulin receptor (IR), particularly within the beta chain sequence (70% homology). Because of this homology, recent studies have demonstrated that these receptors can form hybrids containing one IR dimer and one IGF-IR dimer (Pandini et al., *Clin. Canc. Res.* 5:1935-19 (1999)). The formation of hybrids occurs in both normal and transformed cells and the hybrid content is dependent upon the concentration of the two homodimer receptors (IR and IGF-IR) within the cell. In one study of 39 breast cancer specimens, although both IR and IGF-IR were over-expressed in all tumor samples, hybrid receptor content consistently exceeded the levels of both homo-receptors by approximately 3-fold (Pandini et al., *Clin. Canc. Res.* 5:1935-44 (1999)). Although hybrid receptors are composed of IR and IGF-IR pairs, the hybrids bind selectively to IGFs, with affinity similar to that of IGF-IR, and only weakly bind insulin (Siddle and Soos, The IGF System. Humana Press. pp. 199-225. 1999). These hybrids therefore can bind IGFs and transduce signals in both normal and transformed cells.

[0008] Endocrine expression of IGF-I is regulated primarily by growth hormone and produced in the liver, but recent evidence suggests that many other tissue types are also capable of expressing IGF-I. This ligand is therefore subjected to endocrine and paracrine regulation, as well as autocrine in the case of many types of tumor cells (Yu, H. and Rohan, J., *J. Natl. Cancer Inst.* 92:1472-89 (2000)).

[0009] The androgen receptor (AR) consists of 3 functional and structural domains: an N-terminal (modulatory) domain; a DNA binding domain (Interpro Accession No. IPR001628) that mediates specific binding to target DNA sequences (ligand-responsive elements); and a hormone binding domain. The N-terminal domain (NTD) is unique to the androgen receptors and spans approximately the first 530 residues; the highly-conserved DNA-binding domain is smaller (around 65 residues) and occupies the central portion of the protein; and the hormone ligand binding domain (LBD) lies at the receptor C-terminus. In the absence of ligand, steroid hormone receptors are thought to be weakly associated with nuclear components; hormone binding greatly increases receptor affinity. The interaction among androgen receptor (AR), androgen, and prostate cancer is complex. Distribution of AR between the nucleus and cytoplasm is affected by androgen and androgen withdrawal. For example, AR immunoreactivity is observed only in the nuclei of LuCaP 35 cells grown in intact male mice, but strong immunoreactivity is observed in the cytoplasm and nuclei of LuCaP 35 grown in intact male mice and subsequently castrated.

SUMMARY OF THE INVENTION

[0010] This invention relates to treatment of androgen dependent tumors such as prostate cancer. Prostate tumors are typically stimulated by androgens such as testosterone, and exhibit androgen dependent (AD) growth. Therefore, treatment of prostate cancer typically involves therapy that deprives prostate cancer cells of androgen. However, a large proportion of prostate cancers eventually transition to androgen independence (AI). It has been discovered that administration of an IGF-IR antagonist in combination with androgen deprivation therapy (ADT) inhibits or prevents transition of AD tumors to AI tumors.

[0011] Accordingly, the invention provides a method of treatment of an androgen dependent cancer by administering androgen deprivation therapy and an IGF-IR antagonist. In an embodiment of the invention, the androgen dependent cancer is prostate cancer.

[0012] According to the invention, the IGF-IR antagonist can be an extracellular antagonist or an intracellular antagonist and more than one antagonist may be employed. More generally, the invention relates to inhibition of the IFG-IR signal transduction and to modulation of component of the pathway so as to inhibit transition of tumor cells from AD to AI. Extracellular antagonists include, but are not limited to proteins or other biological molecules that bind to IGF-IR or its ligand (IGF). In certain embodiments of the invention, the extracellular antagonist inhibits binding of IGF-IR to IGF. In one embodiment, the binding protein is an antibody, such as, for example, IMC-A12. In another embodiment, the binding protein is a soluble ligand binding fragment of IGF-IR. Intracellular IGF-IR antagonists can be biological molecules, but are usually small molecules. In an embodiment of the invention, the IGF-IR antagonist is a small molecule selected from AG1024, NVP-AEW541, and BMS-554417.

[0013] The effectiveness of various antagonists to inhibit IGF-IR signal transduction can be observed, for example, by assaying the state of IGF-IR signal transduction pathway components. In one embodiment, inhibition of IGF-IR is observed in the reduced phosphorylation of Akt. In another embodiment, inhibition of IGF-IR signaling is observed in the reduced expression of survivin or tubulin β-peptide (TUBB).

[0014] An IGF-IR antagonist of the invention is used with any form of ADT. In an embodiment of the invention, ADT comprises orchiectomy. In another embodiment of the invention, ADT comprises administration of a luteinizing hormone-releasing hormone analog. In another embodiment, ADT comprises administration of an antiandrogen. In yet another embodiment, an adrenal androgen inhibitor is administered. According to the invention, two or more methods of ADT can be combined.

[0015] The invention further provides for inhibition of signaling through Akt. Accordingly, the invention includes administration of modulators of signal transduction proteins that activate Akt. In one embodiment, such a modulator is an antagonist of EGFR.

[0016] According to the invention, an IGF-IR antagonist is administered as an adjuvant for ADT. In one embodiment, ADT and administration of an IGF-IR antagonist are initiated at about the same time. In another embodiment, ADT is initiated first, and an IGF-IR antagonist is administered before the androgen-independent cancer becomes androgen-independent. The invention further provides for use of anti-neoplastic agents with ADT and IGF-IR antagonist administration. In an embodiment of the invention, an IGF-IR antagonist and an ADT agent are used together as a neoadjuvant for surgical or radiation treatment of prostate cancer.

[0017] The invention also provides compositions comprising an IGF-IR antagonist and an ADT agent in a dosage form.

BRIEF DESCRIPTION OF THE FIGURES

[0018] Figure 1 depicts a study in which LuCap35 subcutaneous xenografts in SCID mice were observed. All mice were castrated when the average tumor size reached 400 mm^3. The control group of mice received castration alone. In two other groups, IMC-A12 was administered three times per week starting one or two weeks after castration.

[0019] Figure 2 depicts levels of PSA in the castrated control mice and in castrated mice treated with IMC-A12 starting one (early) or two (late) weeks after castration.

[0020] Figure 3 depicts the distribution of androgen receptor (AR) in response to stimulation of IGF-IR with IGF and/or antagonism of IGF-IR with IMC-A12. Levels of cytoplasm and nuclear AR were assessed by Western Blots.

[0021] Figure 4 depicts the effect of an IGF-IR antagonist (IMC-A12) on the distribution of androgen receptor (AR) in androgen dependent xenograft tumors of LuCaP 35 cells in intact mice (left column) and androgen independent xenograft tumors of LuCaP 35V cells in castrated mice (right column).

[0022] Figure 5 depicts the correlation between AR score and tumor volume. $R = 0.66$, $p < 0.01$. Castrate only values are in the open circles and Castrate + A12 early and late values are in the closed circles. Values are the mean value for 100 nuclei graded per tumor.

[0023] Figure 6 depicts gene expression changes between two time periods for subcutaneous A12-treated tumors. Out of 3170 unique genes on the array with sufficient data to test, there were 21 up-regulated (including many androgen-regulated, denoted by "*") and 41 down-regulated with ≤10% q-value in the late time period when tumors began to recur compared to the early time period.

[0024] Figure 7A depicts the correlation between survivin copy number score and tumor volume ($r= 0.66$, $p \leq 0.01$). Figure 7B depicts the correlation between tubulin beta peptide 3 copy number score and tumor volume ($r = 0.59$, $p \leq 0.01$). Castrate only values are in the open circles and Castrate + A12 early and late values are in the closed circles, Each value is the mean of three PCR runs.

DETAILED DESCRIPTION OF THE INVENTION

[0025] It has been discovered that inhibitors of IGF-IR are useful in therapies for treatment of prostate cancer. In particular, administration of an IGF-IR antagonist in combination with androgen deprivation therapy (ADT) results in improved treatment outcome relative to ADT alone.

[0026] It has been observed that androgens up-regulate insulin-like growth factor-I receptor expression and may sensitize prostate cancer to the effects of IGF-I. Similarly, the transition to androgen independence that is observed in prostate cancer cells can result from adaptations of the cell that increase androgen receptor signaling such as increased levels of AR that make the cell sensitive to low levels of circulating androgen or AR mutations allowing activation by nonandrogen steroids. Indeed, evidence demonstrates that IGF-I signaling can actually mediate AR translocation to the nucleus of tumor cells and lead to up-regulation of AR-dependent genes. In this fashion, it is proposed that IGF-I can promote the conversion of androgen-dependent prostate cancer to androgen-independent, following hormone ablation therapy, by promoting AR signaling in the absence of circulating levels of androgen. Recent data from men and from human prostate xenografts has also shown that current methods of androgen ablation fail to decrease prostatic androgens to levels that no longer result in activation of the androgen receptor. The prostate may actually be able to synthesize DHT from several precursor steroids and possibly acetate.

[0027] It therefore follows that inhibition of IGF-I signaling concomitant with hormone ablation therapy may prevent or prolong the time until conversion of prostate cancer to androgen-independent disease, significantly delaying the onset of recurrence. Antagonists of IGF-IR may therefore be an effective adjuvant therapy to androgen deprivation strategies to treat newly diagnosed and locally advanced or metastatic hormone-dependent prostate cancer.

[0028] The use of IGF-IR antagonists with androgen withdrawal also has the potential to block IGF mediated recovery from apoptosis. Mechanisms by which IGF-IR can abrogate apoptosis include inhibition of ras-raf-map kinase, PI3 kinase including mTOR and forkhead signaling, and 14-3-3. Another mechanism by which IGF-IR inhibition can prolong the effects of androgen withdrawal is by maintaining the tumor in cell cycle arrest following initial apoptosis.

[0029] Previous studies have demonstrated that IGF-IR antagonists can have a positive effect when used to treat xenografts of both androgen dependent and androgen independent prostate cancers. Growth of the xenografts, while slowed, was not arrested or reversed. It has now been discovered that antagonists of IGF-IR are particularly useful for treatment of prostate cancer when administered with androgen deprivation therapy (ADT). Typically, prostate tumors transition to androgen independence, and become insensitive to ADT. As has been previously observed, such androgen insensitive tumors tend not to show strong responses to IGF-IR antagonists. However, as demonstrated herein, the time for progression of prostate tumors from AD to AI is significantly prolonged by a therapy that combines ADT with administration of an IGF-IR antagonist. During that extended period, the tumors diminish in size, and PSA levels are reduced. The combined therapy reduces the high risk of recurrence that is seen with ADT alone, and reduces the risk that metastatic cancer will develop. Treatment with an IGF-IR antagonists is also advantageous for treatment of advanced prostate cancer in which metastases potentially are present or have been diagnosed.

[0030] In models incorporating prostate cancer cells, AR translocation from cytoplasm to nucleus is observed to be induced not only by androgen stimulation, but also, though to a lesser extent, by IGF-IR stimulation. Even in the presence of androgen, AR translocation in the presence of androgen and IGF is reduced by an IGF-IR antagonist.

[0031] In the prostate, following castration, low levels of androgens are still detectable. It is also reported that expression of IGF-IR, which signals through Akt, first decreases in response to castration, but then increases, and further that growth factor stimulation of Akt enhances AR signaling to low levels of androgen.

[0032] As demonstrated herein, treatment with an IGF-IR antagonist significantly delays regrowth of tumors in castrated mice. Further, there is a good correlation between decreased nuclear AR and decreased tumor volume. This suggests that inhibition of IGF-IR signaling plays a considerable role in inhibiting AR driven tumor progression. In the experiments described herein, IGF-IR signaling is inhibited using an antibody designated A12, that binds to IGF-IR. Previous experiments with A12 and similar antibodies show that there is decreased phosphorylation (*i.e.*, activation) of a various signal transduction molecules, including ERK and MAPK, and particularly Akt. The effect of inhibition of IGF-IR has been observed in a variety of tumor cell types, including the M12 prostate tumor line (Wu, J.D. et al., 2005, *Clin. Cancer Res.* 11:3065-74) and MCF7 breast cancer cells (Burtrum, D. et al., 2003, *Cancer Res.* 63:8912-21). Thus, it should be appreciated that the same or similar adjuvant activity observed herein for an IGF-IR antagonist would be observed for agents that exert the same or similar effect on Akt activation.

[0033] Treatment with an IGF-IR antagonist is observed to result in inhibition of AR translocation to the nucleus. The inhibition can be observed histochemically or by fluorescence microscopy, as well as in reduced expression levels of AR induced genes. Two genes associated with resistance to castration, survivin and tubulin β-peptide are regulated by IGF-IR through Akt activation. Expression of the genes is suppressed in castrated mice treated with an IGF-IR antagonist as compared to castration alone. Similar inhibitory effects on AR translocation and Akt activated gene expression would be observed in response to an Akt specific inhibitor or an antagonist of another signal transduction pathway involving Akt to a significant degree.

[0034] A variety of IGF-IR antagonists can be used according to the invention. The IGF-IR antagonists can be extracellular antagonists or intracellular antagonists. The extracellular and intracellular IGF-IR antagonists can be biological molecules, small molecules, or any other substance that inhibits activation of IGF-IR, for example by interaction with the extracellular binding region of the receptor (*i.e.*, extracellular antagonist), by inhibiting phosphorylation of the intracellular tyrosine kinase domain of IGF-IR, or by inhibiting interaction with of activation of any other cellular component involved in the IGF-IR signaling pathway, thereby ultimately inhibiting gene activation or cellular proliferation.

[0035] In an embodiment of the present invention, an extracellular IGF-IR antagonist interacts with the extracellular ligand binding region of the receptor through sufficient physical or chemical interaction between the antagonist and the extracellular binding region of the receptor, such that binding of IGF-IR and its ligand (IGF) is blocked and tyrosine kinase activity of the receptor is inhibited. One of skill in the art would appreciate that examples of such chemical interactions, which include association or bonding, are known in the art and include covalent bonding, ionic bonding, hydrogen bonding, and the like between the antagonist and the extracellular binding region. In an embodiment of the invention, the extracellular IGF-IR antagonist is a biological molecule. Biological molecules include, but are not limited to, antibodies or antibody fragments that bind to IGF-IR. In another embodiment, the IGF-IR antagonist can be a small molecule that blocks ligand binding to IGF-IR. In another embodiment, the extracellular antagonist is a substance that sequesters or degrades IGF-IR ligands. One example is a soluble extracellular fragment of IGF-IR that binds to IGF. Another example of such a substance is an IGF binding protein (IGFBP) that can bind to IGF such as to limit IGF receptor activation, such as, for example, IGFBP-1, IGFBP-2, and IGFBP-3. In another embodiment of the invention, a small molecule inhibitor binds to the ligand binding domain of IGF-IR and blocks binding and receptor activation by an IGF-IR ligand.

[0036] Although not wishing to be bound by theory, it is thought that the extracellular IGF-IR antagonist inhibits all signal transduction cascades initiated by the conformation changes in the extracellular region of the IGF-IR following IGF-IR activation. This inhibition includes surface IGF-IR as well as those IGF-IR that have been internalized within a cell. For example, it is thought that activated receptor tyrosine kinases (RTKs) can be internalized via a clathrin-coated pit into an endosome, while still maintaining their signaling activity. Following internalization, such receptors are either recycled back to the cell surface or degraded in the endosome or lysosome.

[0037] Another way to inhibit IGF-IR mediated signal transduction is by down-regulation IGF-IR expression. In an embodiment of the invention, an IGF-IR antagonist binds to the receptor and promotes receptor internalization and degradation. In another embodiment, an IGF-IR antagonist reduces expression of the receptor.

[0038] Biological molecules, in the context of the present invention, include all amino acids, nucleotides, lipids and polymers of monosaccharides that generally have a molecular weight greater than 650 D. Thus, biological molecules include, for example, oligopeptides, polypeptides, peptides, and proteins, oligonucleotides and polynucleotides such as, for example, DNA and RNA, and oligosaccharides and polysaccharides. Biological molecules further include derivatives of any of the molecules described above. For example, derivatives of biological molecules include lipids and glycosylation derivatives or oligopeptides, polypeptides, peptides, and proteins. Derivatives of biological molecules further include lipid derivatives of oligosaccharides and polysaccharides, *e.g.* lipopolysaccharides. Most typically, biological molecules are antibodies or functional derivatives thereof.

[0039] Small molecules include organic compounds, such as heterocycles, peptides, saccharides, steroids, and the like, organometallic compounds, salts of organic compounds and organometallic compounds, and inorganic compounds. Atoms in a small molecule are linked together via covalent and ionic bonds; the former is typical for small organic compounds such as small molecule tyrosine kinase inhibitors and the latter is typical of small inorganic compounds. The arrangement of atoms in a small organic molecule may represent a chain, *e.g.* a carbon-carbon chain or carbon-heteroatom chain or may represent a ring containing carbon atoms, *e.g.* benzene or a polycyclic system, or a combination of carbon and heteroatoms, *i.e.*, heterocycles such as a pyrimidine or quinazoline. Although small molecules can have any molecular weight they generally include molecules that would otherwise be considered biological molecules, except their molecular weight is not greater than 650 D. Small molecules include both compounds found in nature, such as hormones, neurotransmitters, nucleotides, amino acids, sugars, lipids, and their derivatives as well as compounds made synthetically, either by traditional organic synthesis, bio-mediated synthesis, or a combination thereof. *See e.g.* Ganesan, *Drug Discov. Today* 7(1): 47-55 (Jan. 2002); Lou, *Drug Discov. Today*, 6(24): 1288-1294 (Dec. 2001). The compounds may be modified to enhance efficacy, stability, pharmaceutical compatibility, and the like.

[0040] The intracellular IGF-IR antagonists can be biological molecules, such as mutant receptor subunits, intracellular binding proteins (*e.g.*, intracellularly expressed fragments of antibodies) and the like. In a preferred embodiment, the intracellular antagonists are small molecules. The small molecule inhibitors include but are not limited to small molecules that modify or block the ATP binding domain, substrate binding regions, or kinase domain of IGF-IR. The small molecule inhibitors also include substances that are inhibitors of other components of the IGF-IR signal transduction pathway, including, but not limited to, ras-mitogen activated protein kinase (MAPK) pathway, and the phospatidylinositol-3 kinase (PI3K)-Akt pathway.

[0041] To identify antagonists, small molecule libraries can be screened for inhibitory activity using high-throughput biochemical, enzymatic, or cell based assays. The assays can be formulated to detect the ability of a test compound to inhibit binding of IGF-IR to IGF-IR ligands or substrate IRS-1 or to inhibit the formation of functional receptors from IGF-IR dimers. The intracellular IGF-IR antagonist may inhibit the tyrosine kinase activity of IGF-IR by binding to or inhibiting activation of the intracellular region bearing a kinase domain or by binding to or inhibiting activation of any intracellular protein involved in the signaling pathway of IGF-IR. Small molecule antagonists of IGF-IR include, for example, the insulin-like growth factor-I receptor selective kinase inhibitors NVP-AEW541 (García-Echeverría,

C. et al., 2004, *Cancer Cell* 5:231-9) and NVP-ADW742 (Mitsiades, C. et al., 2004, *Cancer Cell* 5:221-30), INSM-18 (Insmed Incorporated), which selectively inhibits IGF-IR and HER2, and the tyrosine kinase inhibitor tryphostins AG1024 and AG1034 (Párrizas, M. et al., 1997, *Endocrinology* 138:1427-33) which inhibit phosphorylation by blocking substrate binding and have a significantly lower IC_{50} for inhibition of IFG-IR phosphorylation than for IR phosphorylation. The cyclolignan derivative picropodophyllin (PPP) is another IGF-IR antagonist that inhibits IGF-IR phosphorylation without interfering with IR activity (Girnita, A. et al., 2004, *Cancer Res.* 64:236-42). Other small molecule IGF-IR antagonists include the benzimidazol derivatives BMS-536924 (Wittman, M. et al., 2005, *J. Med. Chem.* 48:5639-43) and BMS-554417 (Haluska P. et al., 2006, *CancerRes.* 66:362-71), which inhibit IGF-IR and IR almost equipotently. For compounds that inhibit receptors in addition to IGF-IR, it should be noted that IC_{50} values measured *in vitro* in direct binding assays may not reflect IC_{50} values measured *ex vivo* or *in vivo* (*i.e.*, in intact cells or organisms). For example, where it is desired to avoid inhibition of IR, a compound that inhibits IR *in vitro* may not significantly affect the activity of the receptor when used *in vivo* at a concentration that effectively inhibits IGF-IR.

[0042] Antisense oligodeoxynucleotides, antisense RNAs and small inhibitory RNAs (siRNA) provide for targeted degradation of mRNA, thus preventing the translation of proteins. Accordingly, expression of receptor tyrosine kinases and other proteins critical for IGF signaling can be inhibited. The ability of antisense oligonucleotides to suppress gene expression was discovered more than 25 yr ago (Zamecnik and Stephenson, 1978, *Proc. Natl. Acad. Sci. USA.* 75:280–84). Antisense oligonucleotides base pair with mRNA and pre-mRNAs and can potentially interfere with several steps of RNA processing and message translation, including splicing, polyadenylation, export, stability, and protein translation (Sazani and Kole, 2003, *J. Clin. Invest.* 112:481–86). However, the two most powerful and widely used antisense strategies are the degradation of mRNA or pre-mRNA via RNaseH and the alteration of splicing via targeting aberrant splice junctions. RNaseH recognizes DNA/RNA heteroduplexes and cleaves the RNA approximately midway between the 5' and 3' ends of the DNA oligonucleotide. Inhibition of IGF-IR by antisense oligonucleotides is exemplified in Wraight, *Nat. Biotechnol.* 18:521-6.

[0043] Innate RNA-mediated mechanisms can regulate mRNA stability, message translation, and chromatin organization (Mello and Conte, 2004, *Nature.* 431:338–42).

Furthermore, exogenously introduced long double-stranded RNA (dsRNA) is an effective tool for gene silencing in a variety of lower organisms. However, in mammals, long dsRNAs elicit highly toxic responses that are related to the effects of viral infection and interferon production (Williams, 1997, *Biochem. Soc. Trans.* 25:509–13). To avoid this, Elbashir and colleagues (Elbashir, et al., 2001, *Nature.* 411:494–98) initiated the use of siRNAs composed of 19-mer duplexes with 5' phosphates and 2 base 3' overhangs on each strand, which selectively degrade targeted mRNAs upon introduction into cells.

[0044] The action of interfering dsRNA in mammals usually involves two enzymatic steps. First, Dicer, an RNase III–type enzyme, cleaves dsRNA to 21–23-mer siRNA segments. Then, RNA-induced silencing complex (RISC) unwinds the RNA duplex, pairs one strand with a complementary region in a cognate mRNA, and initiates cleavage at a site 10 nucleotides upstream of the 5' end of the siRNA strand (Hannon, 2002, *Nature.* 418:244–51). Short, chemically synthesized siRNAs in the 19–22 mer range do not require the Dicer step and can enter the RISC machinery directly. It should be noted that either strand of an RNA duplex can potentially be loaded onto the RISC complex, but the composition of the oligonucleotide can affect the choice of strands. Thus, to attain selective degradation of a particular mRNA target, the duplex should favor loading of the antisense strand component by having relatively weak base pairing at its 5' end (Khvorova, 2003, *Cell* 115:209–16). Exogenous siRNAs can be provided as synthesized oligonucleotides or expressed from plasmid or viral vectors (Paddison and Hannon, 2003, *Curr. Opin. Mol. Ther.* 5:217–24). In the latter case, precursor molecules are usually expressed as short hairpin RNAs (shRNAs) containing loops of 4–8 nucleotides and stems of 19–30 nucleotides; these are then cleaved by Dicer to form functional siRNAs.

[0045] Anti-IGF-IR antibodies to be used according to the present invention exhibit one or more of following properties:

[0046] 1) The antibodies bind to the external domain of IGF-IR and inhibit binding of IGF-I or IGF-II to IGF-IR. Inhibition can be determined, for example, by a direct binding assay using purified or membrane bound receptor. In this embodiment, the antibodies of the present invention, or fragments thereof, preferably bind IGF-IR at least as strongly as the natural ligands of IGF-IR (IGF-I and IGF-II).

[0047] 2) The antibodies neutralize IGF-IR. Binding of a ligand, *e.g.*, IGF-I or IGF-II, to an external, extracellular domain of IGF-IR stimulates autophosphorylation of the beta subunit and phosphorylation of IFG-IR substrates, including MAPK, Akt, and IRS-1.

[0048] Neutralization of IGF-IR includes inhibition, diminution, inactivation and/or disruption of one or more of these activities normally associated with signal transduction. Neutralization can be determined *in vivo*, *ex vivo*, or *in vitro* using, for example, tissues, cultured cell, or purified cellular components. Neutralization includes inhibition of IGF-IR / IR heterodimers as well as IGF-IR homodimers. Thus, neutralizing IGF-IR has various effects, including inhibition, diminution, inactivation and/or disruption of growth (proliferation and differentiation), angiogenesis (blood vessel recruitment, invasion, and metastasis), and cell motility and metastasis (cell adhesion and invasiveness).

[0049] One measure of IGF-IR neutralization is inhibition of the tyrosine kinase activity of the receptor. Tyrosine kinase inhibition can be determined using well-known methods; for example, by measuring the autophosphorylation level of recombinant kinase receptor, and/or phosphorylation of natural or synthetic substrates. Thus, phosphorylation assays are useful in determining neutralizing antibodies in the context of the present invention. Phosphorylation can be detected, for example, using an antibody specific for phosphotyrosine in an ELISA assay or on a western blot. Some assays for tyrosine kinase activity are described in Panek et al., 1997, *J. Pharmacol. Exp. Thera.* 283: 1433-44 and Batley et al., 1998, *Life Sci.* 62:143-50. Antibodies of the invention cause a decrease in tyrosine phosphorylation of IGF-IR of at least about 75%, preferably at least about 85%, and more preferably at least about 90% in cells that respond to ligand.

[0050] Another measure of IGF-IR neutralization is inhibition of phosphorylation of downstream substrates of IGF-IR. Accordingly, the level of phosphorylation of MAPK, Akt, or IRS-1 can be measured. The decrease in substrate phosphorylation is at least about 50%, preferably at least about 65%, more preferably at least about 80%.

[0051] In addition, methods for detection of protein expression can be utilized to determine IGF-IR neutralization, wherein the proteins being measured are regulated by IGF-IR tyrosine kinase activity. An example of such a protein that is associated with cancer progression and drug resistance is survivin, which is a member of the inhibitor of apoptosis (IAP) family. While survivin regulation is complex and mediated by more than one pathway, regulation mediated by Akt and increased by IGF-1 has been demonstrated. See, e.g., Zhang et al., 2005, *Oncogene*, 24:2474-82. Methods for analyzing gene expression include immunohistochemistry (IHC) for detection of protein expression, fluorescence in situ hybridization (FISH) for detection of gene amplification, competitive radioligand binding assays, solid matrix blotting techniques, such as Northern and Southern blots, reverse transcriptase polymerase chain reaction (RT-PCR) and ELISA. See, e.g., Grandis et al., 1996, *Cancer*, 78:1284-92; Shimizu et al., 1994, *Japan J. Cancer Res.*, 85:567-71; Sauter et al., 1996, *Am. J. Path.*, 148:1047-53; Collins, 1995, *Glia* 15:289-96; Radinsky et al., 1995, *Clin. Cancer Res.* 1:19-31; Petrides et al., 1990, *Cancer Res.* 50:3934-39; Hoffmann et al., 1997, *Anticancer Res.* 17:4419-26; Wikstrand et al., 1995, *Cancer Res.* 55:3140-48.

[0052] *Ex vivo* assays can also be utilized to determine IGF-IR neutralization. For example, receptor tyrosine kinase inhibition can be observed by mitogenic assays using cell lines stimulated with receptor ligand in the presence and absence of inhibitor. The MCF7 breast cancer line (American Type Culture Collection (ATCC), Rockville, MD) is such a cell line that expresses IGF-IR and is stimulated by IGF-I or IGF-II. Another method involves testing for inhibition of growth of IGF-IR -expressing tumor cells or cells transfected to express IGF-IR. Inhibition can also be observed using tumor models, for example, human tumor cells injected into a mouse.

[0053] The antibodies of the present invention are not limited by any particular mechanism of IGF-IR neutralization. The anti-IGF-IR antibodies of the present invention can bind externally to the IGF-IR cell surface receptor, block binding of ligand (e.g., IGF-I or IGF-II) and subsequent signal transduction mediated via the receptor-associated tyrosine kinase, and prevent phosphorylation of the IGF-IR and other downstream proteins in the signal transduction cascade.

[0054] 3) The antibodies down modulate IGF-IR. The amount of IGF-IR present on the surface of a cell depends on receptor protein production, internalization, and degradation. The amount of IGF-IR present on the surface of a cell can be measured indirectly, by detecting internalization of the receptor or a molecule bound to the receptor. For example, receptor internalization can be measured by contacting cells that express IGF-IR with a labeled antibody. Membrane-bound antibody is then stripped, collected and counted. Internalized antibody is determined by lysing the cells and detecting label in the lysates.

[0055] Another way is to directly measure the amount of the receptor present on the cell following treatment with an anti-IGF-IR antibody or other substance, for example, by fluorescence-activated cell-sorting analysis of cells stained for surface expression of IGF-IR. Stained cells are incubated at 37°C and fluorescence intensity measured over time. As a control, part of the stained population can be incubated at 4°C (conditions under which receptor internalization is halted).

[0056] Cell surface IGF-IR can be detected and measured using a different antibody that is specific for IGF-IR and that does not block or compete with binding of the antibody being tested. (Burtrum, et al., 2003, *Cancer Res.* 63:8912-21) Treatment of an IGF-IR expressing cell with an antibody of the invention results in reduction of cell surface IGF-IR. In a preferred embodiment, the reduction is at least about 70%, more preferably at least about 80%, and even more preferably at least about 90% in response to treatment with an antibody of the invention. A significant decrease can be observed in as little as four hours.

[0057] Another measure of down-modulation is reduction of the total receptor protein present in a cell, and reflects degradation of internal receptors. Accordingly, treatment of cells (particularly cancer cells) with antibodies of the invention results in a reduction in total cellular IGF-IR. In a preferred embodiment, the reduction is at least about 70%, more preferably at least about 80%, and even more preferably at least about 90%.

[0058] For treatment of human subjects, the antibodies are preferably human antibodies, but can also be humanized or chimeric antibodies. One preferred human antibody that binds to IGF-IR is A12 (See, WO2005016970). Another preferred human antibody is 2F8 (See, WO2005016970). Useful antibodies further include anti-IGF-IR antibodies that compete with IMC-A12 or IMC-2F8 for binding to IGF-IR, as well as antibodies that bind to other epitopes (*i.e.*, antibodies that bind to other epitopes and exhibit properties as previously described such as ligand blocking, receptor internalization, etc., but do not compete with IMC-A12 or IMC-2F8). Other nonlimiting examples of neutralizing anti-IGF-IR antibodies useful according to the invention are described by Wang et al. (WO 2003/1000008; US 2004/0018191) and Singh et al. (WO 2003/106621; US 2003/0235582). The nucleotide and amino acid sequences of several antibodies mentioned herein are indexed in Table 1.

Table 1. SEQ ID NOS for Antibody Variable Domains and CDRs (nucleotide / amino acid)

Antibody Name	VH	CDRH1	CDRH2	CDRH3	VL	CDRL1	CDRL2	CDRL3
A12	1/2	13/14	15/16	17/18	9/10	25/26	27/28	29/30
2F8	1/2	13/14	15/16	17/18	5/6	19/20	21/22	23/24
11F8	37/38	31/32	33/34	35/36	45/46	39/40	41/42	43/44
C225	47/48				49/50			

[0059] Antibodies that can be used according to the invention include complete immunoglobulins, antigen binding fragments of immunoglobulins, as well as antigen binding proteins that comprise antigen binding domains of immunoglobulins. Antigen binding fragments of immunoglobulins include, for example, Fab, Fab', and F(ab')$_2$. Other antibody formats have been developed which retain binding specificity, but have other characteristics that may be desirable, including for example, bispecificity, multivalence (more than two binding sites), compact size (e.g., binding domains alone).

[0060] Single chain antibodies comprise two variable domains lack some or all of the constant domains of the whole antibodies from which they are derived. Therefore, they can overcome some of the problems associated with the use of whole antibodies. For example, single-chain antibodies tend to be free of certain undesired interactions between heavy-chain constant regions and other biological molecules. Additionally, single-chain antibodies are considerably smaller than whole antibodies and can have greater permeability than whole antibodies, allowing single-chain antibodies to localize and bind to target antigen-binding sites more efficiently. Furthermore, the relatively small size of single-chain antibodies makes them less likely to provoke an unwanted immune response in a recipient than whole antibodies.

[0061] Multiple single chain antibodies, each single chain having one V_H and one V_L domain covalently linked by a first peptide linker, can be covalently linked by at least one or more peptide linker to form a multivalent single chain antibodies, which can be monospecific or multispecific. Each chain of a multivalent single chain antibody includes a variable light chain fragment and a variable heavy chain fragment, and is linked by a peptide linker to at least one other chain. The peptide linker is composed of at least fifteen amino acid residues. The maximum number of amino acid residues is about one hundred.

[0062] Two single chain antibodies can be combined to form a diabody, also known as a bivalent dimer. Diabodies have two chains and two binding sites, and can be monospecific or bispecific. Each chain of the diabody includes a V_H domain connected to a V_L domain. The domains are connected with linkers that are short enough to prevent pairing between domains on the same chain, thus driving the pairing between complementary domains on different chains to recreate the two antigen-binding sites. Similarly, three single chain antibodies can be combined to form a triabody, also known as a trivalent trimer. Triabodies are constructed with the amino acid terminus of a V_L or V_H domain directly fused to the carboxyl terminus of a V_L or V_H domain (*i.e.*, without any linker sequence). Triabodies can be monospecific, bispecific or trispecific. Bispecific antibodies that are bivalent for each antigen binding site have also been developed. For example, Zhu (WO 01/90192) describes an antibody with four binding sites that otherwise has the structure of, and retains the effector functions of, a naturally occurring antibody. Zhu (WO 2006/020258) discloses a bispecific antibody that incorporates two diabodies and Ig constant regions.

[0063] Thus, antibodies of the invention and fragments thereof include, but are not limited to, naturally occurring antibodies, bivalent fragments such as $(Fab')_2$, monovalent fragments such as Fab, single chain antibodies, single chain Fv (scFv), single domain antibodies, multivalent single chain antibodies, diabodies, triabodies, and the like that bind specifically with antigens.

[0064] IGF-IR antogonists are exemplified herein by IMC-A12, a human monoclonal antibody that binds to the extracellular domain of IGF and blocks binding of IGF. Properties of IMC-A12 and a similar human antibody are provided in International Publication WO 2005/016970.

[0065] Effects of IGF-IR antagonists of the invention on androgen dependent prostate cancer cells include one or more of the following. 1) IGF can mediate AR activation or translocation in the absence of androgen. IGF-IR antagonists of the invention block IGF mediated translocation. 2) IGF-IR antagonists mediate enhance cell killing or inhibition of tumor cell proliferation. 3) AR mediated androgen receptor activated gene expression is reduced. Genes demonstrating AR mediated expression include, for example, PSA and TMPRSS2 (a transmembrane serine protease).

[0066] According to the invention, an IGF-IR antagonist is administered to a subject having prostate cancer in coincidence with androgen deprivation therapy (ADT; also call hormonal therapy). The goal of ADT is to lower levels of the male hormones (androgens, such as testosterone) in the body. Androgens, produced mainly in the testicles, can actually stimulate prostate cancer cells to grow. Lowering androgen levels can usually make prostate cancers shrink or grow more slowly.

[0067] ADT is used in several situations: as first-line (initial) therapy for patients unable to have surgery or radiation or that can't be cured by these treatments because the cancer has already spread beyond the prostate gland; after initial treatment, such as surgery or radiation therapy, if the cancer remains or comes back; as an addition (adjuvant) to radiation therapy as initial treatment in certain groups of men at high risk for cancer recurrence; and before surgery or radiation (neoadjuvant therapy), in an attempt to shrink the cancer and make the other treatment more effective. According to the invention, an IGF-IR antagonist is administered in conjunction with ADT in any situation where ADT would otherwise be employed. The IGF-IR antagonist is an adjuvant that enhances and/or prolongs the effect of ADT.

[0068] There are several methods used for ADT. Orchiectomy involves removal of the testicles, where more than 90% of the androgens, mostly testosterone, are produced. With this source removed, most prostate cancers shrink. Although permanent and resulting in a variety of undesirable side effects generally related to changing levels of hormones in the body, orchiectomy is probably the least expensive and simplest way to reduce androgen production and can be done as a simple outpatient procedure.

[0069] Luteinizing hormone-releasing hormone (LHRH) analogs (also called LHRH agonists) lower testosterone levels as effectively as orchiectomy by decreasing the androgens, mainly testosterone, produced by the testicles. LHRH analogs are injected or placed as small implants under the skin and are given either monthly or every 3, 4, 6, or 12 months. Examples of LHRH analogs include leuprolide, goserelin, and triptorelin. Possible side effects of LHRH analogs are similar to those of orchiectomy, and are largely due to changes in hormone levels.

[0070] Antiandrogens block the body's ability to use any androgens. Even after orchiectomy or during treatment with LHRH analogs, a small amount of androgens is still produced by the adrenal glands. Drugs of this type include flutamide, bicalutamide, and nilutamide. These drugs are usually taken daily as pills.

[0071] Antiandrogen treatment is often combined with orchiectomy or LHRH analogs. This combination is called combined androgen blockade (CAB). Further, an antiandrogen may be added if treatment with orchiectomy or an LHRH analog is no longer working by itself. Several recent studies have compared the effectiveness of antiandrogens alone with that of LHRH agonists. Most found no difference in survival rates, but a few found antiandrogens to be slightly less effective.

[0072] Side effects of antiandrogens in patients already treated by orchiectomy or with LHRH agonists are usually not serious. Diarrhea is the major side effect, although nausea, liver problems, and tiredness can also occur. The major difference from LHRH agonists is that antiandrogens have fewer sexual side effects and allow maintenance of libido and potency if used alone.

[0073] Adrenal androgen inhibitors can be administered because the low level of androgens produced by the adrenal glands may be sufficient to provide continued stimulation. Following androgen ablation, a subset of prostate cancer cells can become hypersensitive to androgens and the adrenal gland is the source of 5 to 10% of peripheral testosterone. The two most commonly used agents to inhibit adrenal androgen production are aminoglutethimide and ketoconazole.

[0074] Other examples of androgen-suppressing drugs include diethylstilbestrol (DES), megesterol acetate, cyproterone acetate, and prednisone Estrogens were once the main alternative to orchiectomy for men with advanced prostate cancer, but because of their possible side effects, which include blood clots and breast enlargement, estrogens have been largely replaced by LHRH analogs and antiandrogens.

[0075] According to the invention, a course of treatment with an IGF-IR antagonist is administered starting before, at the time of, or after initiation of ADT. The course of administration of an IGF-IR antagonist should coincide with ADT, but the coincidence need not be complete. For example, the IGF-IR antagonist can be administered any time during remission resulting from androgen withdrawal. In an embodiment of the invention, the IGF-IR antagonist is administered within 24 months of androgen withdrawal for treatment of a primary or metastatic tumors. In another embodiment, the IGF-IR antagonist is administered within 18 months of androgen withdrawal. In an embodiment of the invention, the IGF-IR antagonist is administered during or near the end of the cell death period observed upon ADT treatment, and will still prevent or delay the subsequent outgrowth of AI cells. In an embodiment of the invention, administration of the IGF-IR antagonist is initiated within two weeks of androgen withdrawal. In another embodiment, administration is begun within one week of androgen withdrawal.

[0076] IGF-IR antagonists of the invention can be administered with antagonists that neutralize other receptors involved in tumor growth. Of particular interest are receptors involved in a signal transduction pathway includes Akt. For example, signal transduction through EGFR or HER2 (erbB2) is thought to involve Akt activation. Accordingly, IGF-IR antagonists of the invention may be combined with intracellular or extracellular antagonists of EGFR or HER2.

[0077] Antagonists of EGFR or HER2 include antigen-binding proteins that bind to the extracellular domain of EGFR or HER2 and block binding of one or more ligands and/or neutralize ligand-induced activation. The antagonists also include antibodies or other binding proteins that bind to a ligand of EGFR and inhibits binding of EGFR to the ligand. Ligands for EGFR include, for example, EGF, TGF-α, amphiregulin, heparin-binding EGF (HB-EGF) and betacellulin. EGF and TGF-α are thought to be the main endogenous ligands that result in EGFR-mediated stimulation, although TGF-α has been shown to be more potent in promoting angiogenesis. EGFR antagonists also include substances that inhibit EGFR dimerization with other EGFR receptor subunits (*i.e.*, EGFR homodimers) or heterodimerization with other growth factor receptors (*e.g.*, HER2). EGFR antagonists further include biological molecules and small molecules, such as synthetic kinase inhibitors that act directly on the cytoplasmic domain of EGFR to inhibit EGFR-mediated signal transduction. Erbitux® (cetuximab; C225) is an example of an EGFR antagonist antibody that binds to EGFR and blocks ligand binding. Erbitux® is a chimeric IgG1 antibody having murine variable domains of M225 (See, e.g., WO 96/40210) and human constant domains. A human anti-EGFR antibody designated 11F8 is disclosed by Zhu (WO 2005/090407). Other anti-EGFR antibodies include EMD 72000 (matuzumab), Vectibix™ (panitumumab; ABX-EGF), TheraCIM (nimotuzumab), and Hu-Max-EGFR (zalutumumab). An example of a small molecule EGFR antagonist is IRESSA™ (ZD1939), which is a quinozaline derivative that functions as an ATP-mimetic to inhibit EGFR. *See* U.S. Patent No. 5,616,582 (Zeneca Limited). Another example of a small molecule EGFR antagonist is TARCEVA™ (OSI-774), which is a 4-(substitutedphenylamino)quinozaline derivative [6,7-Bis(2-methoxy-ethoxy)-quinazolin-4-yl]- (3-ethynyl-phenyl)amine hydrochloride] EGFR inhibitor. *See* WO 96/30347 (Pfizer Inc.); Moyer *et al.*, *Cancer Res.*, 57: 4838-48 (1997); Pollack *et al.*, *J. Pharmacol.*, 291: 739-48 (1999). TARCEVA™ may function by inhibiting phosphorylation of EGFR and its downstream PI3/Akt and MAP (mitogen activated protein) kinase signal transduction pathways resulting in p27-mediated cell-cycle arrest. *See* Hidalgo *et al.*, Abstract 281 presented at the 37th Annual Meeting of ASCO, San Francisco, CA, 12-15 May 2001.

[0078] While the antagonists can be administered separately, in certain instances, it can be desirable to combine the functions of two antagonists into a single molecule, such as a bispecific antibody or a dual inhibitor. Bispecific antibodies can be engineered to combine IGF-IR specificity with specificity for a different RTK or other cell surface molecule. Combinations of IGF-IR specificity with EGFR specificity of HER2 specificity are of particular interest. An example of a bispecific antibody that binds to IGF-IR and EGFR is provided by Zhu (WO 2006/020258). Similarly, small molecules that inhibit IGF-IR and a second cellular component are available, or can be screened for. For example as mentioned above, INSM-18 (Insmed/University of California San Franscisco) inhibits IGF-IR and HER2/neu.

[0079] Another aspect of the present invention relates to pharmaceutical compositions containing the antagonists of the present invention or a pharmaceutically acceptable salt, hydrate or pro-drug thereof, in combination with a pharmaceutically acceptable carrier. Such compositions may be separate compositions of the IGF-IR antagonist and the ADT agent or a single composition containing both.

[0080] The compositions of the present invention may be in solid or liquid form, in solution or in suspension. Routes of administration include, for example, oral, parenteral (intravenous, intraperitoneal, subcutaneous, or intramuscular), topical, transdermal and by inhalation.

[0081] For oral administration, the IGF-IR antagonist may be administered, for example, in liquid form with an inert diluent or assimilable carrier, or incorporated into a solid dosage form. Examples of oral liquid and solid dosage forms include, for example, solutions, suspensions, syrups, emulsions, tablets, lozenges, capsules (including soft gelatin capsules), and the like. Oral dosage forms may be formulated as sustained release products using, for example, a coating to delay disintegration or to control diffusion of the active compound. Where necessary, the compositions may also include a solubilizing agent.

[0082] Examples of injectable dosage forms include sterile injectable liquids, including, for example, solutions, emulsions and suspensions. Injectable dosage forms further include solids such as sterile powders that are reconstituted, dissolved or suspended in a liquid prior to injection. Sterile injectable solutions are prepared by incorporating the EGF-IR antagonist and/or the ADT agent in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Carriers typically include, for example, sterile water, saline, injectable organic esters, peanut oil, vegetable oil, and the like. Buffering agents, preservatives, and the like can be included in the administerable forms. Sterile formulations can be prepared by heating, irradiation, microfiltration, and/or by addition of various antibacterial and antifungal agents, such as, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like.

[0083] For topical administration, IGF-IR antagonists and the ADT agents of the present invention can be administered separately or together, for example, in the form of gels, creams, or ointments, or paints. Typical carriers for such application include hydrophobic or hydrophilic bases, oleaginous or alcoholic liquids, and dry powders. IGF-IR antagonists and ADT agents may also be incorporated in a gel or matrix base for application in a patch, optionally providing for controlled release of compound through a transdermal barrier. IGF-IR antagonists and ADT agents can also be formulated by known methods for rectal administration.

[0084] For administration by inhalation, IGF-IR antagonists and ADT agents of the present invention may be dissolved or suspended in, or adsorbed onto, a suitable carrier for use in a nebulizer, aerosol, or dry powder inhaler.

[0085] Suitable dosages can be determined by a physician or qualified medical professional, and depend on factors such as the nature of the illness being treated, the route of administration, the duration of treatment, and the condition of the patient. The IGF-IR antagonists and ADT agents may be administered as frequently as necessary in order to obtain the desired therapeutic effect. Frequency of administration will depend, for example, on the nature of the dosage form used. One of skill in the art would understand that dosages and frequency of treatment depend on the tolerance of the individual patient and on the pharmacological and pharmacokinetic properties of blocking or inhibitory agent used. Ideally, one wishes to achieve saturable pharmacokinetics for the agent used. A loading dose for an anti-IGF-IR antibody can range, for example, from about 10 to about 1000 mg/m^2, preferably from about 200 to about 400 mg/m^2. This can be followed by several additional daily or weekly dosages ranging, for example, from about 200 to about 400 mg/m^2. An exemplary dosage of an IGF-IR antibody is 400 mg/m^2 loading and 250 mg/m^2 weekly infusion. (For conversions between mg/kg and mg/m^2 for humans and other mammals, see Freireich, E.J. et al., 1966, Cancer Chemother. Rep. 50:219-44.) The patient is monitored for side effects and the treatment is stopped when such side effects are severe. Effective dosages of the ADT agents are well known in the art.

[0086] One of skill in the art would also know how to monitor the progress of the treatment in order to determine an effective dose. For prostate cancer, one such way is to monitor PSA levels. Another is to monitor prostatic acid phosphatase (PAP). Other ways to monitor prostate cancers include ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and the like. Tissue samples can also be examined for expression and cellular distribution of AR, as well as expression of survivin and/or TUBB.

[0087] In certain embodiments of the invention, treatments combining administration of IGF-IR antagonists with ADT can employ with one or more anti-neoplastic agents. For example, as noted above, ADT is often employed as a neoadjuvant for radiation treatment of prostate tumors. When the anti-neoplastic agent is radiation, the source of the radiation can be either external (external beam radiation therapy – EBRT) or internal (brachytherapy – BT) to the patient being treated.

[0088] The anti-neoplastic agent can be an alkylating agent or an anti-metabolite. Examples of alkylating agents include, but are not limited to, cisplatin, cyclophosphamide, melphalan, and dacarbazine. Examples of anti-metabolites include, but not limited to, doxorubicin, daunorubicin, and paclitaxel, gemcitabine.

[0089] Useful anti-neoplastic agents also include mitotic inibitors, such as taxanes docetaxel and paclitaxil. Topoisomerase inhibitors are another class of anti-neoplastic agents that can be used in combination with antibodies of the invention. These include inhibitors of topoisomerase I or topoisomerase II. Topoisomerase I inhibitors include irinotecan (CPT-11), aminocamptothecin, camptothecin, DX-8951f, topotecan. Topoisomerase II inhibitors include etoposide (VP-16), and teniposide (VM-26). Other substances are currently being evaluated with respect to topoisomerase inhibitory activity and effectiveness as anti-neoplastic agents. In a preferred embodiment, the topoisomerase inhibitor is irinotecan (CPT-11).

[0090] Throughout this application, various publications, reference texts, textbooks, technical manuals, patents, and patent applications are referred to. The teachings and disclosures of these publications, patents, patent applications and other documents in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which the present invention pertains.

[0091] It is to be understood and expected that variations in the principles of invention herein disclosed may be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

[0092] The following examples further illustrate the invention, but should not be construed to limit the scope of the invention in any way. Detailed descriptions of conventional methods, such as those employed in the construction of vectors and plasmids, and expression of antibodies and antibody fragments can be obtained from numerous publications, including Sambrook, J et al., (1989) Molecular Cloning: A Laboratory Manual, 2^{nd} ed., Cold Spring Harbor Laboratory Press; Coligan, J. et al. (1994) Current Protocols in Immunology, Wiley & Sons, Incorporated; Enna, S.J. et al. (1991) Current Protocols in

Pharmacology, Wiley & Sons, Bonifacino, J.S. et al. (1999) Current Protocols in Cell Biology, Wiley & Sons. All references mentioned herein are incorporated in their entirety.

EXAMPLES

[0093] **Antagonism of IGF-IR inhibits tumor regrowth following ADT.**

[0094] A preclinical model was developed to test the efficacy of inhibition of IGF-IR signaling using a human monoclonal IGF-IR antibody (IMC-A12) with castration on recurrence of prostate cancer following castration. For the study, a xenograft of LuCaP 35, an androgen responsive human prostate cancer cell line, was implanted subcutaneously into the flank of male SCID mice. LuCaP 35 can transition to an androgen-independent state and can be used to evaluate molecular changes associated with this process. At first, PSA levels drop and tumor volume decreases, but after a period of 60-120 days, regrowth of tumors is observed. LuCaP 35 has metastatic potential and results in mixed bone lesions. LuCaP 35 grown in intact male mice is androgen sensitive and responds to androgen withdrawal in the manner that is usually seen in patients.

[0095] LuCaP 35 cells were implanted subcutaneously into the flank of male SCID mice. When the tumors reached a volume of ca. 400mm^3, the mice were castrated and divided into three groups of 20 animals each. Group 1 controls received castration alone, Group 2 received castration and IMC-A12 intraperitoneally three times a week for 14 days starting 7 days after castration and Group 3 received IMC-A12 for14 days beginning 14 days after castration. After 14 days of IMC-A12 no further therapy was administered. The timing of A12 administration for 2 weeks beginning either 1 or 2 weeks after castration was based on published data with the LuCaP 35 cell line indicating that maximum castration-induced apoptosis occurs within four days of castration (Corey, E. et al., 2003, *Prostate* 99:392-401). Since inhibition of IGF-IR signaling can cause cell cycle arrest and prevent cells from undergoing apoptosis, it was decided to start administration of A12 when apoptosis was "complete" following castration (Corey et al., 2003; Tennant, M. et al., 2003, *Prostate*, 56:115-22).

[0096] Blood samples were collected from orbital sinus weekly. The serum was separated and PSA levels were determined using the IMx Total PSA Assay (Abbott Laboratories, Abbott Park, IL). Tumors were measured twice weekly and tumor volume was estimated by the formula: volume = L X W2/2. Mice were sacrificed if tumors reached 1000mm^3 or when animal weight loss exceeded 20% of initial body weight. BrdU was injected i.p. into the mice 1 h before animals were sacrificed in order to determine in vivo tumor cell proliferation rate.

[0097] Upon castration, tumor growth was initially halted in all mice. (Fig. 1) In mice treated with IMC-A12, tumor volume decreased over the course of the study and there were no tumor specific deaths. In the untreated cohort, an increase in average tumor volume was evident by week 5, with tumor specific deaths (sacrificed) beginning in the fourth week and continuing through the study. Note that the plot of average tumor volume is artificially depressed for mice that did not receive IMC-A12 as each death removed a large tumor from the averaged tumor set.

[0098] PSA levels were monitored in the LuCaP 35 xenograft mice. All mice responded initially to hormone ablation and a similar drop in PSA levels was observed in the first week following castration (Fig. 2). In mice treated by castration alone, after the initial drop, PSA levels then increased over the course of the study starting at about the second week. In contrast, PSA levels in castrated mice that were treated with IMC-A12 did not rise, but remained near baseline.

[0099] This study demonstrates that blocking IGF-IR signaling and expression after castration with IGF-IR antibody, IMC-A12, results in a significantly greater decrease in tumor volume than castration alone, $p < 0.001$, and significantly prolongs the time to AI tumor regrowth as determined by tumor volume and an increase in PSA, $p < 0.001$.

[0100] In control animals treated by castration alone, tumor growth stopped for about four weeks, but increased thereafter. Among animals treated by castration alone more than half were sacrificed due to tumor growth by 9 weeks following castration and most animals had been sacrificed by the end of 16 weeks. In contrast, all animals which received IMC-A12 were alive after 16 weeks.

[0101] The *in vivo* results presented demonstrate the effectiveness of inhibition of IGF-IR signal transduction. Notably, the IGF-IR antagonist was administered over the course of 14 days, and then halted. In a separate study in which A12 was administered in a similar manner, some tumor regrowth was observed late in the study following administration of A12. Two of 40 Group 2 and 3 animals had to be sacrificed because of tumor volume by the end of the study. Maintenance doses of an IGF-IR antagonist would prolong the time to tumor regrowth indefinitely.

[0102] To investigate whether there was a relationship between reduction in tumor volume in A12 treated tumors and AR translocation, AR immunohistochemistry was performed on tumors from each of the three groups, as shown in Fig. 5. A nuclear AR staining score was assigned to 100 nuclei from each tumor. Nuclei were scored blindly by two individuals and the mean of the two scores was counted as the score for that tissue. There is a significant positive correlation between tumor volume and nuclear AR intensity, $r = 0.66$, $p \leq 0.01$.

[0103] **Antagonism of IGF-IR inhibits AR translocation.**

[0104] The effect of an stimulation and antagonism of IGF-IR on androgen receptor localization was assessed. LuCaP 35 cells were cultured with or without IGF-1 stimulation, in the presence of absence of IMC-A12. (Fig. 3) Cytoplasmic and nuclear extracts were prepared from treated cells and assessed by PAGE. The level of ERK was used to equalize loading of lanes. In cells stimulated with IGF-1, IMC-A12 caused a reduction in the proportion of androgen receptor observed in the nucleus.

[0105] Androgen receptor translocation was also assessed by immunohistochemistry. (Fig. 4). LuCaP 35 (AD) xenograft tumors were grown in intact male and LuCaP 35V (AI) xenograft tumors were grown in castrated mice. Test mice were treated with IMC-A12. Serial sections of the tumors were prepared and stained with an AR specific antibody. In intact control mice, AR in androgen dependent LuCaP 35 tissue was localized predominantly in the nucleus. In tissue from test animals treated with IMC-A12, AR staining was observed in the cytoplasm. In castrated control mice, AR in androgen independent LuCaP 35v cells was distributed between nucleus and cytoplasm. In tissue from test animals treated with IMC-A12, AR staining was predominantly in the cytoplasm.

[0106] In a similar experiment, the localization of AR was studied by fluorescence microscopy in tissue culture. Treatment with 10^{-8}M DHT resulted in a significant redistribution of AR from cytoplasm to nucleus. Treatment with IGF-1 alone resulted in a partial redistribution of AR to the nucleus, and IMC-A12 completely reversed that effect.

[0107] **Antagonism of IGF-IR inhibits AR dependent gene expression.**

[0108] Survivin, which is an inhibitor of apoptosis, is strongly expressed in several human prostate cancer cell lines. In cell lines with intact androgen receptors, androgen stimulation with DHT increases survivin expression. Survivin expression is also observed to be mediated by AKT as IGF induced AKT signaling increases survivin expression even in AR-negative cell lines. A gene chip experiment to detect differential expression of survivin indicates that survivin expression is reduced upon treatment with IMC-A12.

[0109] Custom cDNA microarrays were constructed as previously described [ref] using clones derived from the Prostate Expression Data Base (PEDB), a sequence repository of human prostate expressed sequence tag (EST) data available to the public. (Nelson, P.S. et al., 2002, *Nucl. Acids Res.* 30:218-20). Methods of labeling with Cy3 and Cy5 fluorescent dyes, hybridization to the microarray slides, and array processing were as described (Tusher, V. et al., 2001, *Proc. Natl. Acad. Sci. U.S.A.* 98:5116-21).

[0110] Three tumors were pooled in each experimental group. To provide a reference standard RNA for use on cDNA microarrays, equal amounts of total RNA were isolated and pooled from LNCaP, DU145, PC3, and CWR22rV1 cell lines (American Type Culture Collection, Manassas, VA) growing at log phase in dye-free RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS; Life Technologies, Rockville, MD). Total RNA was isolated from the pooled tumors and cell lines using Trizol (Invitrogen, SanDiego, CA). mRNA was amplified one round using the Ambion MessageAmp™ II Amplification Kit (Ambion Inc, Austin, TX), and sample quality and quantity were assessed by agarose gel electrophoresis and absorbance at A260. Hybridization probes were labeled and quality control of the array experiments was performed as described previously (Tusher, V. et al., 2001). Differences in gene expression associated with treatment groups were determined using the SAM procedure (Chu, G., Narasimhan, B., Tibshirani, R. & Tusher, V., 2002, Significance analysis of microarrays (sam) software, Stanford University) with a false discovery rate (FDR) of $\leq$10% considered significant(37). Similarities between samples were assessed by unsupervised, hierarchical clustering of genes and samples using Cluster 3.0 software (de Hoon et al., 2004, Bioinformatics 20:1453-4) and viewed by TreeView (Page, R.D., 1996, Comput. Appl. Biosci. 12:357-8).

[0111] Survivin and TUBB were also assayed by PCR using primers and methods previously described (Wu, J. et al., 2006, *Clin. Cancer Res.* 12:6153-60). A standard PCR fragment of the target cDNA was purified. A series of dilutions of the standards from 10ng/μl to 10^{-3} pg/μl were used for real-time RT-PCR to generate the standard curves. One μg of total RNA from each group of pooled tumor was used for first-strand cDNA synthesis using Superscript First Strand Synthesis System (Invitrogen). Real-time RT-PCR was performed in 20 μl of reaction mixture consisted of 1 μl of first strand of cDNA, specific primers sets, and Lightcycler FastStart DNA Master Plus SYBR Green using a Roche Lightcycler following the manufacturer's protocol (Roche, Nutley, NJ). RT-PCR products were subjected to melting curve analysis on Lightcycler software v3.5. The amplicon sizes were confirmed by agarose gel electrophoresis. Each sample was assayed in duplicate.

[0112] Castration combined with an IGF-IR antagonist is associated with a decrease in AR gene expression until recurrence of tumor. RNA samples from tumors harvested in each group at the time frames noted in Table 2 were analyzed on cDNA microarrays. No genes were found to be significantly altered between the time periods for group 1 (castration alone) when tested by two sample t-test in SAM (q-value ≥100 %) In addition, unsupervised, hierarchical clustering of known androgen-regulated genes did not segregate the two time periods. This may not be surprising since many of the animals in this group had PSA recurrence and increased nuclear AR scores compared to Groups 2 and 3 by day 40. In contrast, there were significant gene expression changes between the two time periods of A12-treated tumors. Out of 3170 unique genes on the array with sufficient data to test, there were 21 up-regulated (including many androgen-regulated) and 41 down-regulated with ≤10% q-value in the late time period when tumors began to recur compared to the early time period (Fig. 6) Furthermore, unsupervised, hierarchical clustering of known androgen-regulated genes clearly differentiated the A12-treated, two time periods into two separate clusters. These data indicate that nuclear AR expression is associated with AR transcriptional activity and prostate cancer progression through AR activation.

Table 2. cDNA Arrays at Each Time Point		
	Days Post Castration	
	20-60	70-150
Group 1 (castration)	3	3
Group 2 (castration + A12 early)	2	2
Group 3 (castration + A12 late)	1	1

[0113] Expression of survivin and β Tubulin is significantly decreased by an IGF-IR antagonist. The microarray studies determined that survivin expression was decreased in the tumors treated with A12 antibody. As depicted in Fig. 7A, Qt-RT PCR on RNA extracted from tumors demonstrates a significant positive correlation between survivin copy number and tumor volume, $r = 0.66$, $p \leq 0.01$. A second gene recently implicated in IGF-IR induced tumor formation is ß-tubulin, TUBB (O'Connor, R., 2003, *Horm. Metab. Res.* 35:771-7; Geller, J. et al., 1984, *J. Urol.* 132:693-700). TUBB was shown to be decreased in the microarrays and as shown in Fig. 7B, was shown in tumor specimens to correlate positively with tumor volume, r = 0.59, p ≤0.01, and to be significantly decreased in groups 2 and 3 compared to group 1. A third gene that was not different over time on the microarrays in group 1 but was decreased in the two early time periods in the group 2 and 3 animals was PSA. The change in PSA expression was confirmed by a similar pattern in the serum PSA levels.

[0114] **Proliferation and Apoptosis**

[0115] Apoptosis was determined by terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay and propidium (PI) staining using the Apop-Direct kit (BD BioScience) as previously described (Wu, J.D. et al., 2005, *Clin. Cancer Res.*, 11:3065-74). Briefly, 1×10^6 cells from the single-cell suspension were fixed with 10% neutral buffer formalin (NBF) followed by 70% ethanol alcohol at –20°C for 30 min. After several washes, cells were permeablized with 0.1% Triton X-100 and incubated with FITC-conjugated dUTP and terminal deoxynucleotidyl transferase enzyme -(TdT) at 37°C for 1 h, followed by an incubation with PI/RNase buffer (100 μg/ml of PI, 50 μg/ml RNase) at room temperature for 60 min. Samples were analyzed by flow cytometry using a BD FACscan. Data were analyzed with CellQuestPRO software. Apoptosis was also determined using by TUNEL assay on formalin fixed tissue using the Apop-Tag kit (Millipore Co, MA) following manufacturer's recommendations. Apoptotic cells were determined per 300 cells per tissue slide.

[0116] As shown in Table 3, proliferation was significantly greater in the Group 1 tumors compared to Group 2 and 3, p ≤0.01. In contrast, apoptosis as determined by TUNEL staining was higher in the Group 1 compared to Groups 2 and 3, Table 3.

Table 3. Apoptosis and BRDU Uptake
** p < 0.001 compared to castrate group.

Treatment Group	Apoptosis (TUNEL) +/- SEM	BRDU +/- SEM
Castrate	6.58 +/- 1.41	27.74 +/- 1.93
Castrate + A12 early	1.29 +/- 0.49 **	17.78 +/- 2.74 **
Castrate + A12 late	1.16 +/- 0.37 **	12.36 +/- 1.75 **

1. A method of inhibiting growth of an androgen dependent cancer comprising administering androgen deprivation therapy and an IGF-IR antagonist.

2. The method of Claim 1, wherein the androgen dependent cancer is prostate cancer.

3. The method of Claim 1, wherein androgen deprivation therapy and the IGF-IR antagonist are initiated at about the same time.

4. The method of Claim 1, wherein the IGF-IR antagonist is initiated after androgen deprivation therapy and before the androgen dependent cancer becomes androgen independent.

5. The method of Claim 1, wherein the IGF-IR antagonist is an extracellular antagonist.

6. The method of Claim 5, wherein the extracellular antagonist is an antibody that binds to IGF-IR.

7. The method of Claim 5, wherein the extracellular antagonist binds to a ligand of IGF-IR.

8. The method of Claim 1, wherein the IGF-IR antagonist down-regulates IGF-IR.

9. The method of Claim 8, wherein the IGF-IR antagonist is a siRNA or an antisense RNA.

10. The method of Claim 5, wherein the extracellular antagonist is IMC-A12.

11. The method of Claim 1, wherein the IGF-IR antagonist is an intracellular antagonist.

12. The method of Claim 11, wherein the intracellular antagonist is a small molecule.

13. The method of Claim 11, wherein the intracellular antagonist is selected from the group consisting of AG1024, NVP-AEW541, and BMS-554417.

14. The method of Claim 1, wherein the androgen deprivation therapy comprises administering a luteinizing hormone-releasing hormone (LHRH) analog.

15. The method of Claim 1, wherein the androgen deprivation therapy comprises administering anti-androgen treatment.

16. The method of Claim 1, wherein the androgen deprivation therapy comprises administering an adrenal androgen inhibitor.

17. The method of Claim 1, wherein the androgen deprivation therapy is orchiectomy.

18. The method of Claim 1, which further comprises administering an Akt antagonist.

19. The method of Claim 18, wherein the Akt antagonist is an EGFR antagonist.

20. The method of Claim 1, wherein the androgen deprivation therapy and the IGF-IR antagonist are administered with an anti-neoplastic agent.

21. The method of Claim 20, wherein the anti-neoplastic agent is radiation.

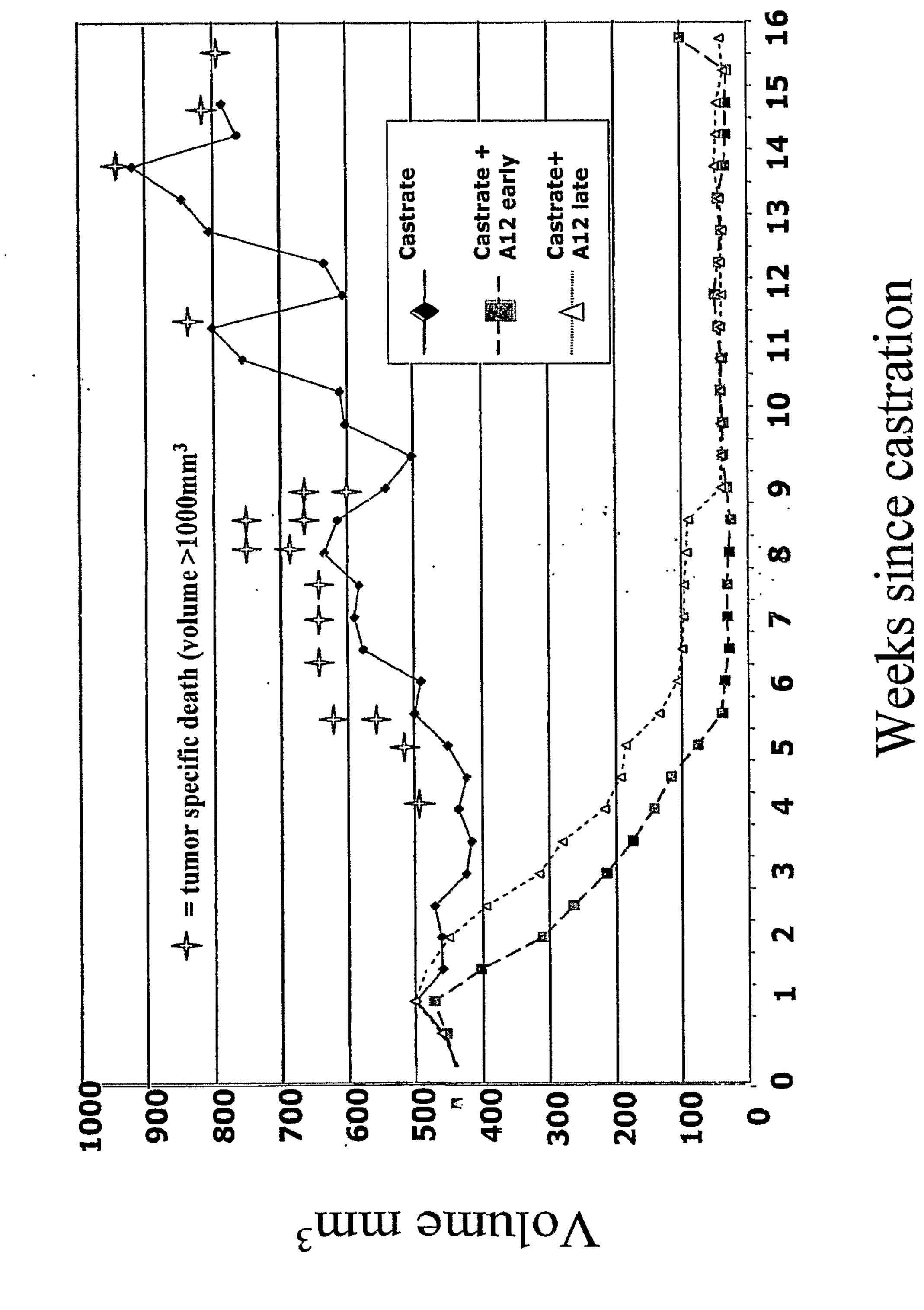

= tumor specific death (volume >1000mm3
Castrate
Castrate + A12 early
Castrate+ A12 late
Volume mm3
1000
900
800
700
600
500
400
300
200
100
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks since castration

Fig. 1

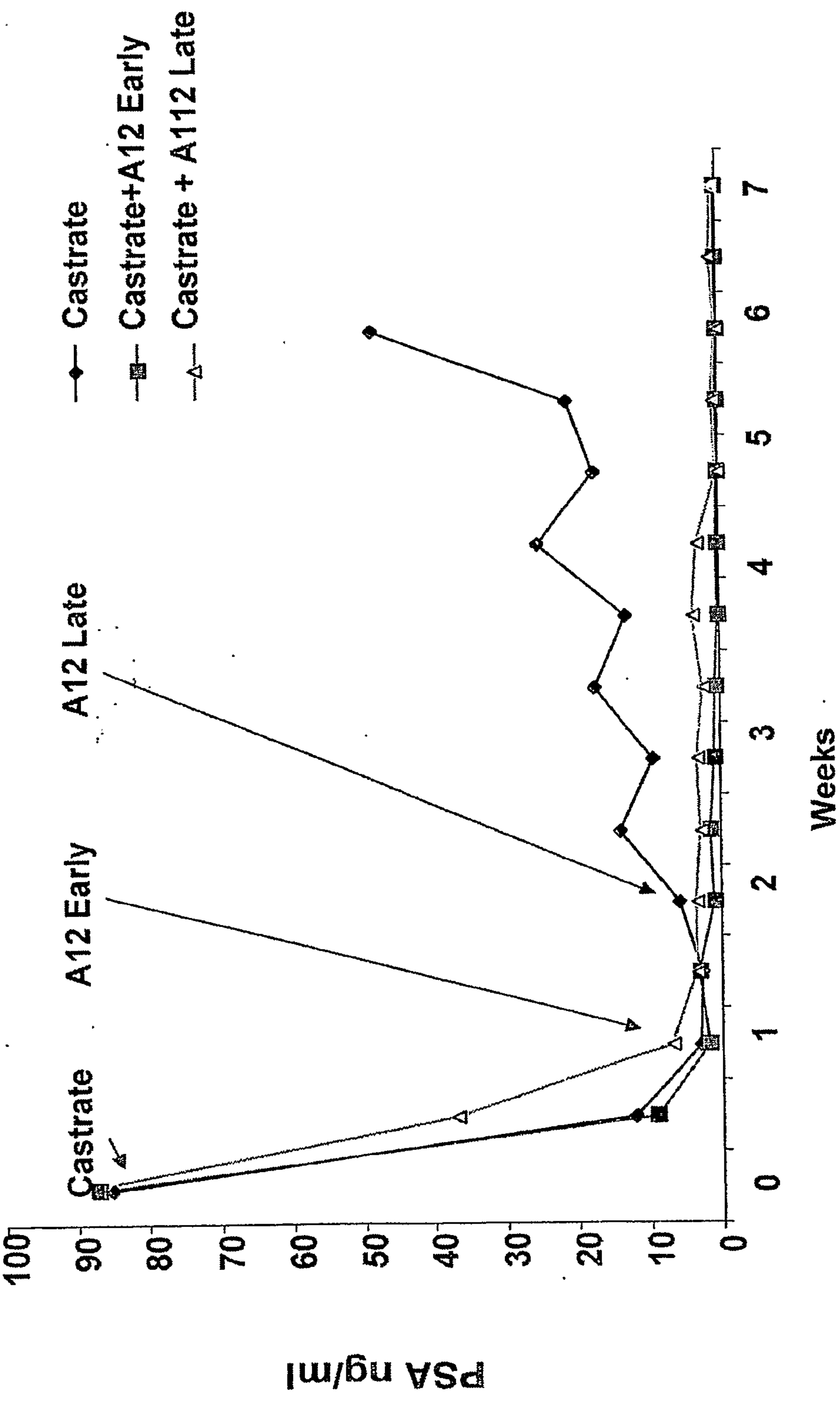

Castrate
Castrate+A12 Early
Castrate + A112 Late
Castrate
A12 Early
A12 Late
PSA ng/ml
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Weeks

Fig. 2

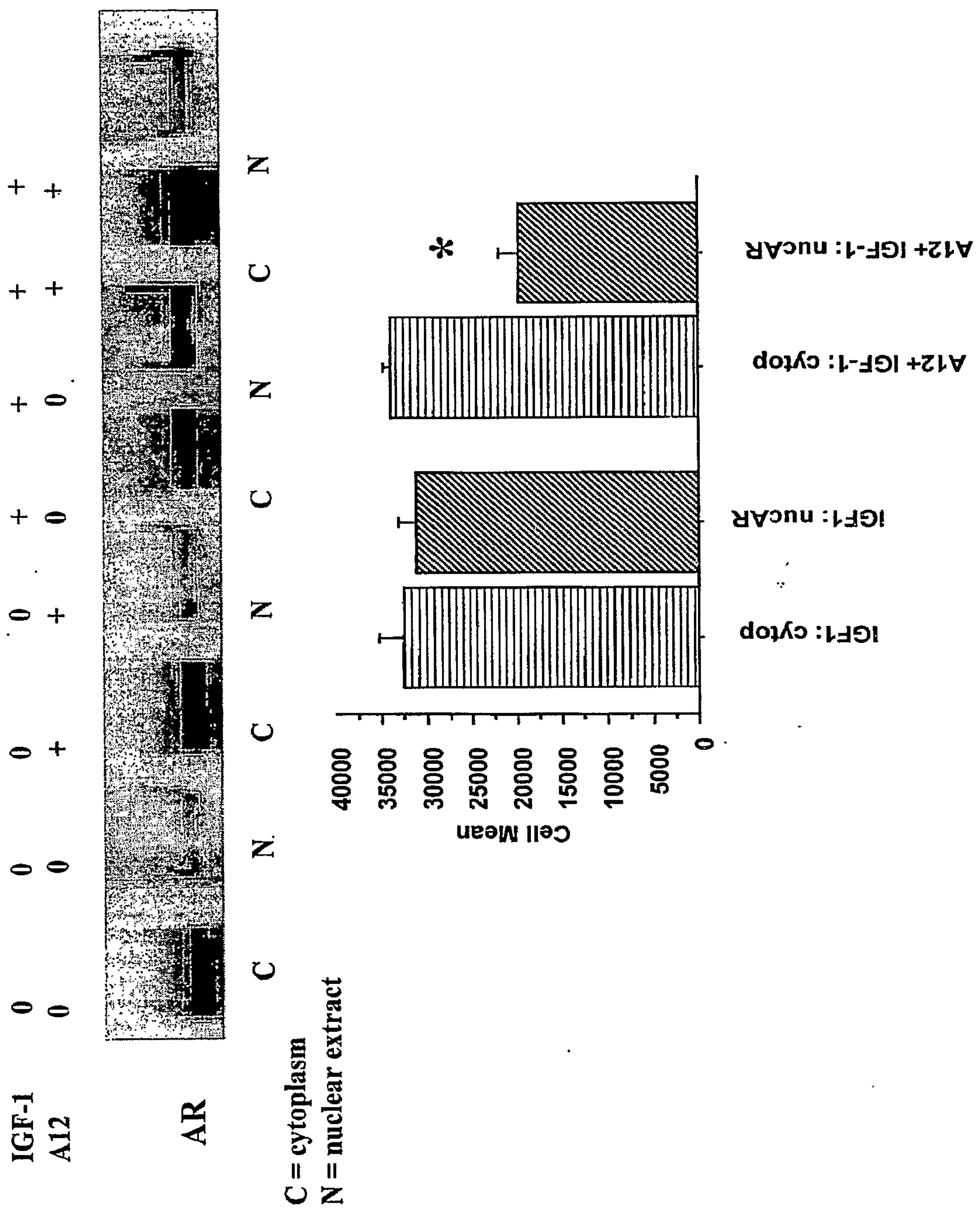

IGF-1 0 0 0 0 + + + +
A12 0 0 + + 0 0 + +
AR
C N C N C N C N
C = cytoplasm
N = nuclear extract
Cell Mean
40000
35000
30000
25000
20000
15000
10000
5000
0
IGF1: cytop
IGF1: nucAR
A12+ IGF-1: cytop
A12+ IGF-1: nucAR
*

Fig. 3

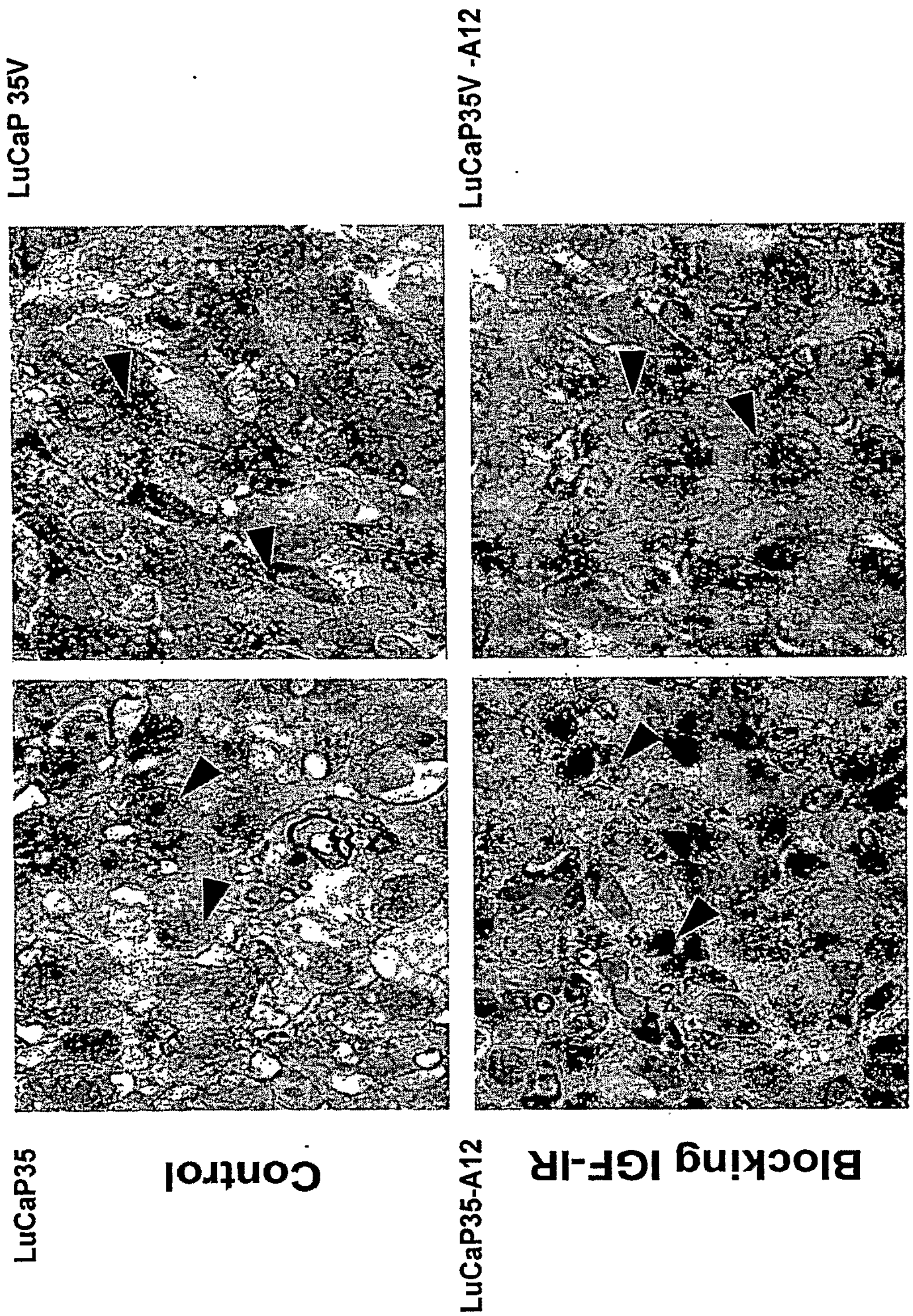

LuCaP 35V
LuCaP35V -A12
LuCaP35
Control
LuCaP35-A12
Blocking IGF-IR

Fig. 4

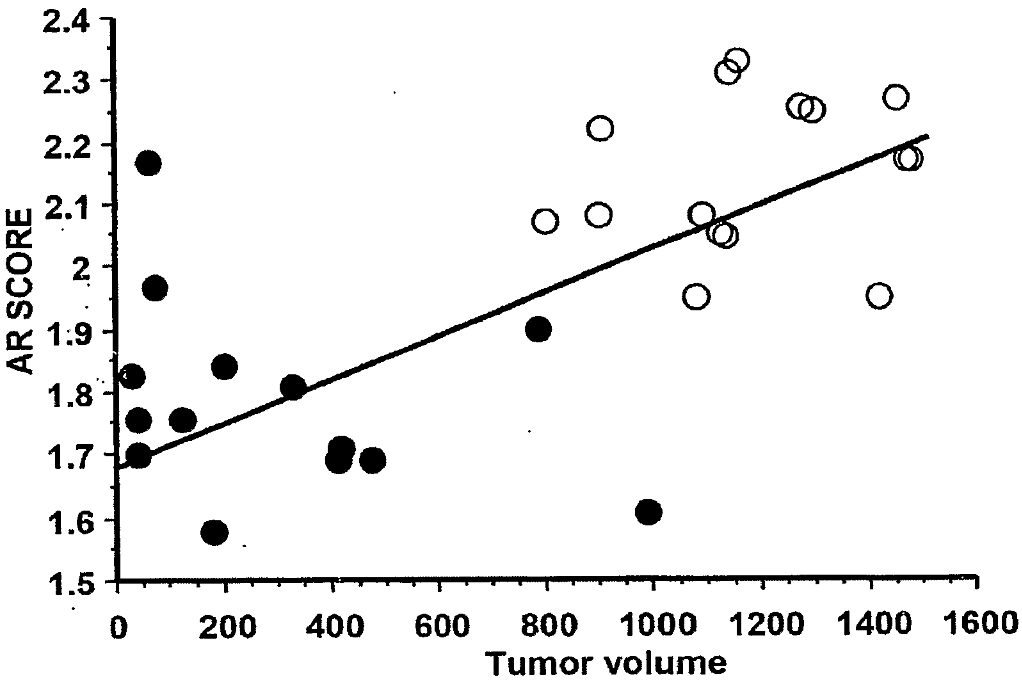
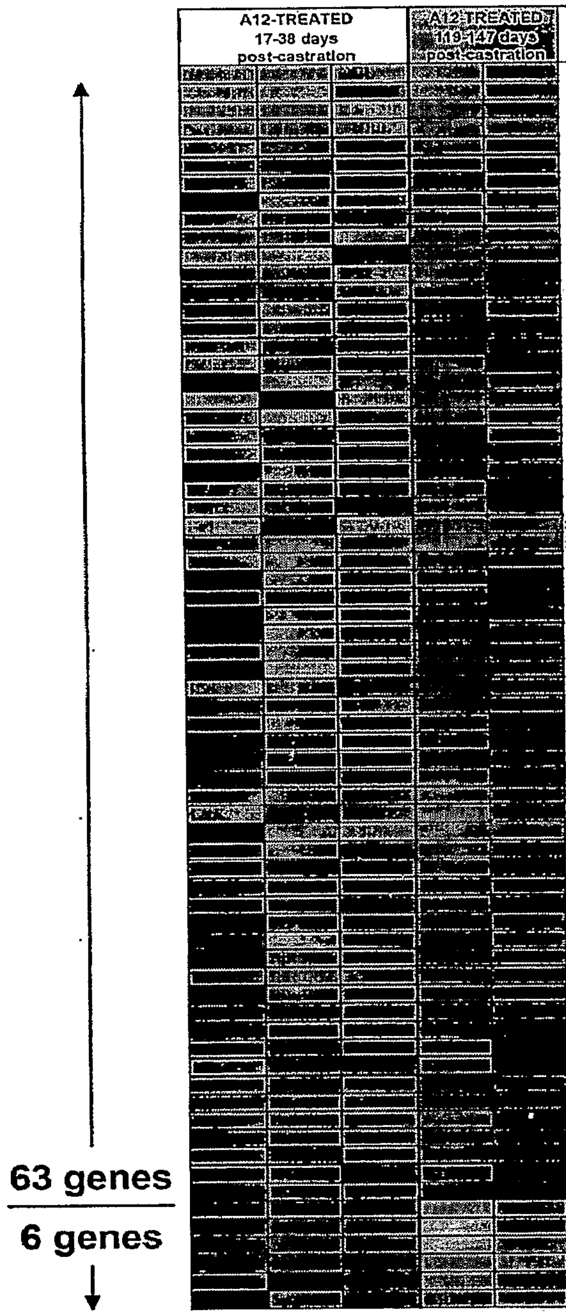

2.4
2.3
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
AR SCORE
0
200
400
600
800
1000
1200
1400
1600
Tumor volume

Fig. 5



HUGO	NAME	AVERAGE FOLD RATIO	
CYP1B1	Cytochrome P450 family 1 subfamily B polypeptide 1	15.4	
KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	7.9	
LDHA	Lactate dehydrogenase A	7.0	
KLK3	Kallikrein 3 (prostate specific antigen)	6.3	*
MGC15937	Glycine-N-acyltransferase-like 1	5.8	
KLK2	Kallikrein 2 prostatic	5.3	
OACT2	O-acyltransferase (membrane bound) domain containing 2	5.3	*
DKFZP566M114	Chromosome 4 open reading frame 13	5.2	
ACSL3	Acyl-CoA synthetase long-chain family member 3	5.1	
ALDOC	Aldolase C fructose-bisphosphate	5.0	*
CGI-121	CGI-121 protein	4.9	
KIAA0830	KIAA0830 protein	4.8	
GDF15	Growth differentiation factor 15	4.7	
MAOA	Monoamine oxidase A	4.5	
SMURF1	SMAD specific E3 ubiquitin protein ligase 1	4.4	
FKBP5	FK506 binding protein 5	4.4	*
ODC1	Ornithine decarboxylase 1	4.3	*
ITM2B	Integral membrane protein 2B	4.2	
PTN	Pleiotrophin	4.2	
CDC2	Cell division cycle 2 G1 to S and G2 to M	4.2	
GPR34	G protein-coupled receptor 34	4.1	
DNAJB1	DnaJ (Hsp40) homolog subfamily B member 1	4.0	*
PART1	Prostate androgen-regulated transcript 1	4.0	
DKFZP564B167	Brain protein 44	3.9	
KIAA0460	KIAA0460	3.8	
AMACR	Alpha-methylacyl-CoA racemase	3.8	
P4HA1	Procollagen-proline 2-oxoglutarate 4-dioxygenase alpha polypeptide I	3.8	
HSPA8	Heat shock 70kDa protein 8	3.8	
RODH	Hydroxysteroid (17-beta) dehydrogenase 6	3.7	
UBE2C	Ubiquitin-conjugating enzyme E2C	3.7	*
ADH1B	Alcohol dehydrogenase IB (class I) beta polypeptide	3.6	
GALNT7	polypeptide N-acetylgalactosaminyltransferase 7	3.6	
CKB	Creatine kinase brain	3.6	
ARID4B	AT rich interactive domain 4B (RBP1- like)	3.5	
DBI	Diazepam binding inhibitor	3.4	
EEF1A2	Eukaryotic translation elongation factor 1 alpha 2	3.4	*
STK39	Serine threonine kinase 39 (STE20/SPS1 homolog yeast)	3.4	
MAPRE1	Microtubule-associated protein RP/EB family member 1	3.4	*
RAB3B	RAB3B member RAS oncogene family	3.4	
ACLY	ATP citrate lyase	3.3	
FDFT1	Farnesyl-diphosphate farnesyltransferase 1	3.3	*
RDH11	Retinol dehydrogenase 11 (all-trans and 9-cis)	3.3	
GABRB3	Gamma-aminobutyric acid (GABA) A receptor beta 3	3.2	*
CXCR4	Chemokine (C-X-C motif) receptor 4	3.2	
PGK1	Phosphoglycerate kinase 1	3.2	
IL1R1	Interleukin 1 receptor type I	3.1	
HRMT1L2	HMT1 hnRNP methyltransferase-like 2 (S. cerevisiae)	3.1	
CTDSP1	CTD small phosphatase 1	3.1	
IGHG1	Immunoglobulin heavy constant gamma 1 (G1m marker)	3.0	
DDIT4	DNA-damage-inducible transcript 4	3.0	
ALDH1A3	Aldehyde dehydrogenase 1 family member A3	3.0	*
MRPL37	Mitochondrial ribosomal protein L37	3.0	
Sprn	Shadow of prion protein homolog (zebrafish)	2.9	
WRN	Werner syndrome	2.9	
MYBL2	V-myb myeloblastosis viral oncogene homolog (avian)-like 2	2.9	
NDRG1	N-myc downstream regulated gene 1	2.9	
PYGL	Phosphorylase glycogen; liver	2.8	*
MAP2K1	Mitogen-activated protein kinase kinase 1	2.8	
ATP5L	ATP synthase H+ transporting mitochondrial F0 complex subunit g	2.8	
CHPT1	Choline phosphotransferase 1	2.8	
MAGEA1	Melanoma antigen family A 1 (directs expression of antigen MZ2-E)	2.7	
RNP24	Transmembrane emp24 domain trafficking protein 2	2.7	
MEP50	WD repeat domain 77	2.7	
MALAT1	Metastasis associated lung adenocarcinoma transcript 1	-16.9	
QSCN6	Quiescin Q6	-5.5	
SPP1	Secreted phosphoprotein 1	-5.5	
LOC200916	Ribosomal protein L22-like 1	-4.9	
CCL1	Chemokine (C-C motif) ligand 1	-4.2	
ZAK	Hypothetical protein LOC339751	-4.1	

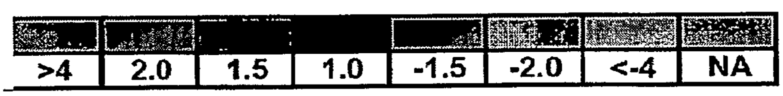
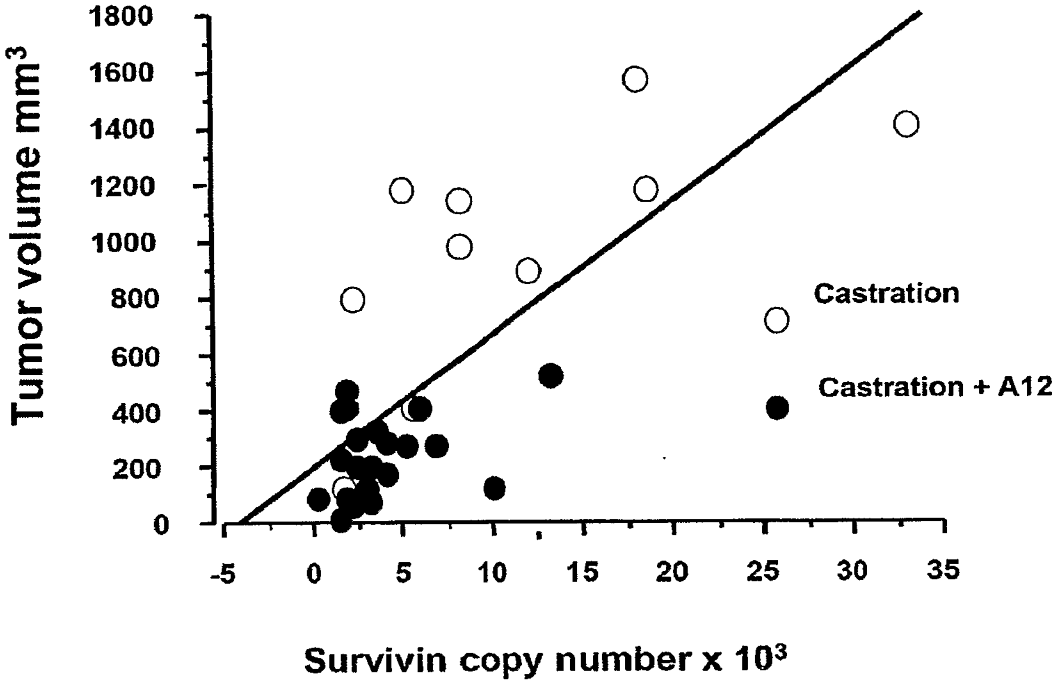


Fig. 6

A.


Tumor volume mm3
1800
1600
1400
1200
1000
800
600
400
200
0
-5
0
5
10
15
20
25
30
35
Survivin copy number x 10³
Castration
Castration + A12

B.

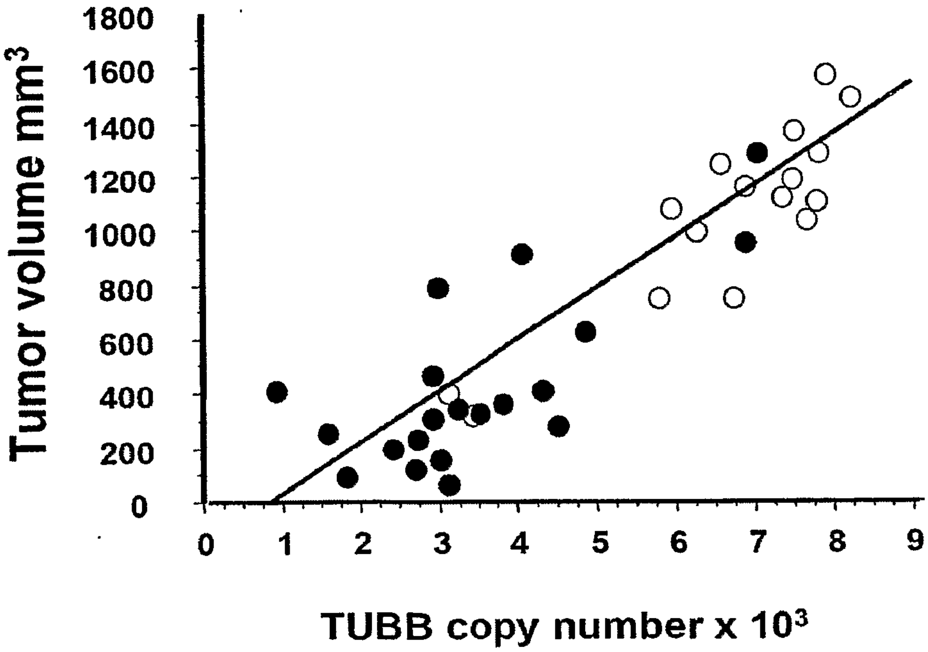

Tumor volume mm3
1800
1600
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
6
7
8
9
TUBB copy number x 10³

Fig. 7