Inhibition of type 1 insulin-like growth factor receptor (IGF-IR) signaling has been proposed as a potential means of optimizing anticancer therapy in a number of tumor systems (1–3). We have previously shown that interrupting the function of IGF-IR using the human IGF-IR monoclonal antibody A12 markedly decreases tumor size in human xenograft models of androgen-dependent and androgen-independent prostate cancer (4). In addition, blocking IGF-IR activation with A12 has been shown to induce cell cycle G1 arrest in androgen-dependent prostate tumors and G2-M arrest in androgen-independent prostate tumors (4). Therefore, inhibition of IGF-IR signaling with A12 might enhance or suppress the activity of cell cycle–specific chemotherapy if transition to cell cycle checkpoints is blocked. Because tumor recovery from radiation and chemotherapy involves activation of the IGF-IR, A12 may also be an attractive adjunct to these modalities (5).

Docetaxel, a member of the taxane family, is one of the newer potent anti–solid tumor agents currently undergoing extensive laboratory and clinical investigations (6). Docetaxel is a microtubulin active drug that causes cancer cells to arrest at the G2-M cell cycle transition and ultimately to undergo apoptosis (7, 8). The antimitotic mechanisms of docetaxel are not fully understood; yet, studies have shown that docetaxel induces expression of tumor suppressor gene p53 and cyclin-dependent kinase inhibitor p27/Kip-1 and phosphorylation of the antiapoptotic protein Bcl-2 (9, 10). Clinical trials have shown that docetaxel has significant activity against androgen-independent prostate cancers (11, 12). It is the only drug to date shown to improve survival in patients with androgen-independent prostate cancer (13, 14). Although significant, the median gain in life expectancy in patients with androgen-independent prostate cancer treated with docetaxel is only 2 to 3 months (15). Some phase I studies have suggested that docetaxel in combination with other tumor-targeting cytotoxic
agents, such as calcitriol, may extend life expectancy much further (12).

Because the IGF-IR signaling pathways are important in tumor growth and also are suggested as one of the possible mechanisms by which tumor cells can survive taxane treatment (5), we postulated that the IGF-IR would be a potential therapeutic target as combined therapy with docetaxel for advanced prostate cancer. In the current study, we tested whether the addition of the IGF-IR antibody A12 would augment or inhibit the activity of docetaxel using two established human prostate tumor xenograft models: the androgen-independent model LuCaP 35V and the osseous model LuCaP 23.1 (16).

Materials and Methods

Anti-IGF-IR antibody and docetaxel. A12 is a fully human antibody antagonist to the human IGF-IR. The generation and characterization of A12 has been previously described (3). Docetaxel was purchased from Aventis Pharmaceuticals (Bridgewater, NJ).

In vivo study of AI LuCaP 35V tumors. Tumor bits (20-30 mm³) of human androgen-independent prostate tumor xenograft LuCaP 35V were implanted s.c. into 6-week-old castrated severe combined immunodeficient mice as previously described (4). Fifty castrated mice were used in this study. When the implanted tumor was observed to reach a volume of ~100 mm³, animals were randomized into five groups (10 mice per group). Group 1 animals received human IgG treatment and were designated as controls. Group 2 animals received docetaxel treatment at a dose of 10 mg/kg. Group 3 animals received docetaxel treatment at a dose of 20 mg/kg. Group 4 animals received treatment of 10 mg/kg docetaxel and 40 mg/kg A12. Group 5 animals received treatment of 20 mg/kg docetaxel and 40 mg/kg A12. All treatments were given i.p. Docetaxel was given once a week. A12 was given thrice a week. All animals were treated for 4 weeks and monitored for additional 4 weeks before euthanization. Tumors were measured twice weekly. Tumor volume was estimated by the formula: volume = \( L \times W^2 / 2 \). Animals were weighed twice a week. Blood samples were collected from orbital sinus weekly. Serum was separated, and prostate-specific antigen (PSA) level was determined using the IMx Total PSA Assay (Abbott Laboratories, Abbott Park, IL). Bromodeoxyuridine (BrdUrd) was injected into the tumors 1 hour before the animals were euthanized for evaluation of in vivo tumor cell proliferation rate.

Following our University of Washington Institutional Animal Care and Use Committee–approved protocol, animals in the control group were euthanized at an earlier time point when tumors reached a volume of 1,000 mm³. After euthanization, tumors were collected and halved. A portion of the tumors were fixed in 10% neutral buffer formalin and embedded in paraffin. The remaining portion of the tumors was separated into single cells mechanically by mincing and filtering through 70-μm nylon sieves.

Apoptosis and cell cycle assay. Apoptosis and cell cycle were measured by terminal deoxynucleotidyl transferase–mediated nick end labeling assay and propidium iodide staining using the ApoDirect kit (BD Biosciences, San Jose, CA) as previously described (4). Cells (1 × 10⁶) from the single-cell suspension of tumors were fixed with 10% neutral buffer formalin followed by 70% ethanol at −20°C for 30 minutes. After several washes, cells were permeabilized with 0.1% Triton X-100 and incubated with FITC-conjugated dUTP and terminal deoxynucleotidyl transferase enzyme at 37°C for 1 hour, followed by an incubation with propidium iodide/RNase buffer (100 μg/mL of propidium iodide, 50 μg/mL RNase) at room temperature for 60 minutes. Samples were analyzed by flow cytometry using a BD FACScan. Data were analyzed with CellQuest software.

Evaluation of BrdUrd incorporation. Five-micrometer tumor sections were deparaffinized and rehydrated. Antigens were retrieved with 0.01 mol/L citric acid (pH 6) at 95°C for 10 minutes. After quenched endogenous peroxidase activity and blocked with 1.5% goat serum, sections were incubated with mouse anti-BrdUrd antibody (1 μg/mL) or control mouse IgG for 1 hour followed by sequential incubation at room temperature with biotinylated goat anti-mouse IgG for 30 minutes, peroxidase-labeled avidin for 15 minutes (Santa Cruz Biotechnology, Santa Cruz, CA), and diaminobenzidine/hydrogen peroxide chromogen substrate (Vector Laboratories, Burlingame, CA). Sections were counterstained with hematoxylin (Sigma, St. Louis, MO) and mounted with permount (Fisher Scientific, Fair Lawn, NJ). Slides were examined under a Zeiss Microscope, and digital images were obtained. Ten random views were evaluated from each section. Rate of BrdUrd uptake was calculated by the number of BrdUrd-positive nuclei divided by the total number of nuclei.

Androgen-dependent intrasosseous study. Osseous LuCaP 23.1 human prostate tumor bits (20-30 mm³) were mechanically digested as previously described (17). Viable LuCaP 23.1 cells (2.5 × 10⁵) were injected into the tibiae of 6- to 8-week-old severe combined immunodeficient mice. Twenty-one mice randomized into three groups were used for this study. After tumor injection, serum PSA was monitored weekly. Treatment started when serum PSA level reached 5 to 10 ng/mL, an indication of tumor growth. Group 1 received control vehicle saline buffer. Group 2 received 20 mg/kg of docetaxel i.p. once a week for 4 weeks. Group 3 received 20 mg/kg of docetaxel once a week and 40 μg/kg of A12 i.p. thrice a week for 4 weeks. To determine whether the response to treatment was osteoblastic or osteolytic, bone density was obtained by Deka-scan and X-rays of the animals at the end point of all treatments.

Measurement of serum A12. Serum A12 was quantitated using a human IgG binding ELISA. Goat anti-human IgG (Sigma) was immobilized in 96-well plates and blocked with 1% skim milk/PBS. Mouse serum samples were tittered onto the plates, and bound human IgG was detected with horseradish peroxidase–conjugated goat anti-human IgG secondary antibody (Jackson ImmunoResearch, Bar Harbor, MN). Signal was visualized using TMB detection reagents (KPL, Guithersburg, MD), and absorbance was measured at 405 nm. A12 were quantitated using a standard curve.

Statistical analysis. Differences among treatment groups were assessed by a one-way ANOVA. Statistical significance between means of two paired groups was assayed using Student’s t-test with Bonferroni correction; 95% confidence interval (P < 0.05) was considered significant. Stepwise regression analysis for calculating tumor growth rate was done using Statview 5.0 (CalaBasas, CA).

cDNA microarray analysis. Custom Prostate Expression Data Base cDNA microarrays were constructed as previously described using clones derived from the Prostate Expression Data Base, a sequence repository of human prostate expressed sequence tag data available to the public (http://www.pedb.org; refs. 4, 18, 19). The inserts of individual cDNA clones were amplified by PCR, purified, and spotted in duplicates onto glass microscope slides (Gold Seal, BD Biosciences) with GeneMachine OmniGrid 100. Methods of labeling with Cy3 and Cy5 fluorescent dyes, hybridization to the microarray slides, and array processing were as described (20).

Five tumors were pooled in each experimental group. RNA was prepared from the pooled tumors using Trizol (Invitrogen, San Diego, CA). cDNA was synthesized using the Ambion MessageAmp II Amplification kit. Hybridization probes were labeled, and quality control of the array experiments was done as described previously (20). Differences in gene expression associated with treatment groups were determined using the SAM procedure (http://www-stat.stanford.edu/~tibs/SAM/) with a false discovery rate of <0.05% considered significant (21).

Real-time reverse transcription-PCR. A standard PCR fragment of the target cDNA was purified. A series of dilutions of the standards from 10 ng/μL to 10⁻³ pg/μL were used for real-time reverse transcription-PCR to generate the standard curves. One microgram 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 reverse transcription-PCR was done 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). Reverse transcription-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.

**Results**

**IGF-IR antibody A12 augments the inhibitory effect of docetaxel on tumor growth.** Two doses of docetaxel were chosen for this study. The dose of 20 mg/kg had been shown in our preliminary studies to be a dose that had maximum suppression on tumor growth without significant toxicity to mice. A higher dose (30 mg/kg) did not show a greater rate of tumor suppression but did result in significant toxicities to animals, including weight loss and mortality (data not shown). We specifically chose 10 mg/kg as a suboptimal dose to show synergy with A12 in tumor suppression. The optimal dose of 40 μg/kg of A12 has been previously determined (3).

The LuCaP 35V xenograft tumors grew aggressively with an average increase in volume of 362.0 ± 72.0 mm³/wk in animals that received control human IgG (Fig. 1A); all animals had to be sacrificed within 3 weeks after treatment initiation, due to tumor volumes exceeding 1,000 mm³. When animals were treated with 40 μg/kg A12 alone, tumor growth rate was reduced to 192.7 ± 35.6 mm³/wk during treatment. However, after stopping treatment, 50% of the animals have to be sacrificed at follow-up week 2, and all animals have to be sacrificed at follow-up week 4, due to recovery of tumor growth. When animals were treated with a suboptimal dose of docetaxel (10 mg/kg), tumor growth rate was reduced to an average of 29.6 ± 6.1 mm³/wk. When treatment included 10 mg/kg of docetaxel in combination with A12, tumor growth rate was further reduced significantly to an average of 7.9 ± 1.0 mm³/wk (Fig. 1B; P < 0.01). In addition, after termination of all therapy, the inhibitory effect of docetaxel combined with A12 persisted, whereas tumor growth recurred significantly in animals that had received docetaxel alone (Fig. 1B; P < 0.01).

When animals were treated with a high dose of docetaxel (20 mg/kg), tumor growth was significantly inhibited during the 4-week treatment period compared with 10 mg/kg of docetaxel (Fig. 1B; P < 0.001). The combination of 20 mg/kg of docetaxel with A12 did not significantly reduce tumor volume compared with 20 mg/kg docetaxel alone during the 4-week treatment period. However, following treatment cessation, tumor growth significantly recurred at an average rate of 32.0 ± 16.1 mm³/wk in animals that had received 20 mg/kg docetaxel alone (P < 0.01), whereas there was no tumor growth in animals that had received 20 mg/kg of docetaxel combined with A12. The posttreatment suppression of tumor growth persisted for at least 4 weeks to the point when the study was terminated. Together, these results suggest that, for a given dose of docetaxel, combined treatment with A12 can enhance the inhibitory effect of docetaxel on tumor growth during treatment and after treatment follow-ups.

PSA is a commonly used clinical variable to assess prostate tumor growth (21, 22). We, thus, measured serum levels of PSA in animals during and after the treatments. As shown in Fig. 1C, except in animals treated with 10 mg/kg of docetaxel alone, no significant change in serum levels of PSA was seen during the 4-week treatment in the other groups of animals, reflecting the suppressed tumor growth. After treatment termination, serum PSA levels significantly increased in animals treated with docetaxel alone (P < 0.05) and remained unchanged or even decreased in animals treated with docetaxel in combination

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**Fig. 1.** Effects of A12, docetaxel, and docetaxel combined with A12 (40 mg/kg) on LuCaP 35V xenograft tumor growth during and posttreatment. Tumor bits of LuCaP 35V were implanted s.c. into castrated severe combined immunodeficient mice and allowed to grow to ~100 mm³. Groups of animals were treated with control vehicle saline buffer, docetaxel (10 or 20 mg/kg body weight), or docetaxel and A12 (40 μg/kg) thrice a week. All treatment stopped at week 4, and animals were monitored for posttreatment response for four more weeks. Tumor size was measured twice a week, and tumor volume was estimated by the formula: volume = L × W²/2. A, tumor growth in animals treated with control human IgG or A12. B, tumor growth in animals during and after treatment (Rx) of 10 mg/kg of docetaxel (Doc 10), 20 mg/kg of docetaxel (Doc 20), Doc 10 + A12, and Doc 20 + A12. C, serum PSA levels reflect the differences in tumor growth. Serum was separated from blood by centrifugation, and levels of PSA were determined using the IMx Total PSA assay. Points, mean; bars, SE.
with A12 at experiment end points. These data are in agreement with the continued posttreatment inhibition of tumor growth in animals that had received docetaxel combined with A12.

**Induction of apoptosis by docetaxel in combination with A12.** We investigated the effect of docetaxel combined with A12 on cell cycle and cell survival after treatment cessation using terminal deoxynucleotidyl transferase–mediated nick end labeling assay and propidium iodide staining. In animals that had received A12 or docetaxel alone, no apoptosis was found in tumors at 4 weeks posttreatment (Fig. 2A). Instead, following treatment cessation, tumor growth recurred, and tumor cells proceeded to a normal control cell cycle index of G1, S, and G2-M phases, respectively, at 71.0 ± 1.4%, 6.2 ± 2.5%, and 21.0 ± 3.7% in the majority of animals (88-100%). On the contrary, in animals that had received docetaxel in combination with A12, tumor cells failed to proceed to normal cell cycle progression after therapy stopped; apoptosis or preapoptotic G0-G1 cell cycle arrest was found in tumors in a significant percentage of animals (77.8-100%). The average apoptotic events in these apoptosis-positive tumors occurred at an index of 15.0 ± 4.3% (data not shown).

**Enhanced inhibition of cell proliferation by docetaxel in combination with A12.** To further evaluate posttreatment tumor cell proliferation, paraffin sections of tumors were stained with anti-BrdUrd antibody. No significant difference in posttreatment BrdUrd uptake in tumors was found between docetaxel-treated animals and animals in the control group (Fig. 2B). A significant suppression in posttreatment BrdUrd uptake was shown in animals that had received combined treatment of docetaxel and A12 (Fig. 2B; P < 0.01). These data are consistent with the above observations of cell cycle and apoptosis, suggesting that A12 significantly enhanced the cytotoxic effects of docetaxel and, in turn, reduced tumor cell survival and proliferation.

**Differential regulation of gene expression in tumors treated with docetaxel combined with A12 versus docetaxel alone.** To determine potential mechanisms for the markedly enhanced effect of docetaxel by A12, we first examined IGF-IR expression in all harvested tumors by immunohistochemistry and flow cytometry analysis. There was no difference in surface IGF-IR expression among all the treatment groups or compared with the control group (data not shown). This suggests that the enhanced effect of docetaxel by A12 is unlikely, or in part, due to A12 induced down-regulation of IGF-IR expression, which is consistent with our previous observation in the LuCaP 35V xenografts (4). We next compared differences in posttreatment gene expression in tumors from animals that had received 20 mg/kg of docetaxel and 20 mg/kg of docetaxel combined with A12, using cDNA microarray analyses. Based on SAM analyses, 49 genes were identified as differentially expressed in tumors that received combined treatment of docetaxel and A12 compared with those received docetaxel alone, with >2-fold change and <10% false discovery rate (data not shown). Because the effects of docetaxel and docetaxel combined with A12 on tumors showed differences in apoptosis and cell proliferation, we have since identified 13 of the 49 genes that are potentially involved in regulation of apoptosis or cell cycle (Table 1). All 13 genes were at least 2-fold different between the two treatments and had a false discovery rate of <0.02%. Nine genes were down-regulated, and four genes were up-regulated in the docetaxel combined with A12-treated tumors compared with docetaxel alone–treated tumors. We validated these expression differences in selected genes by real-time reverse transcription-PCR and compared their expression to which in tumors with A12 treatment alone (Fig. 3).

Of the down-regulated genes, TUBB and BIRC5 are of particular interest. Overexpression of TUBB has been shown to result in resistance to docetaxel (23); increased expression of BIRC5 (survivin) has been shown to be associated with aggressive prostate cancer and resistance to antiandrogen therapy (24, 25). Here, we show that A12 treatment alone down-regulated TUBB and survivin expression, which may account for possible mechanisms of A12 augmenting the effect of docetaxel. Furthermore, TUBB is an IGF-IR–regulated gene that is involved with IGF-IR–mediated transformation (26). Of the four up-regulated genes, IGFBP3 has been shown to inhibit IGF ligand signaling as well as to induce apoptosis in prostate tumor cells in a ligand-dependent manner (27–30).

**Posttreatment serum levels of A12.** We measured posttreatment serum levels of A12 in animals that had received
docetaxel combined with A12. Serum A12 levels declined 100-fold 2 weeks after treatment cessation (Fig. 4A). Serum A12 was detected only at a very low level in animals at posttreatment week 4 (Fig. 4A). These data suggest that posttreatment serum residual A12 may in part contribute to the prolonged inhibition of tumor growth.

**Pharmacotoxicity evaluation.** Although A12 has >95% cross-reactivity with murine IGF-IR,6 no abnormal daily activity or behavior changes were apparent in animals treated with combined reagents or docetaxel alone compared with control tumor bearing. No significant effect on kidney cells was observed in any treatment group by both cell cycle and apoptosis assays (data not shown). No significant change in body weight was observed among treatment groups (Fig. 4B). These observations suggest that combined treatment of docetaxel and A12 did not display significant toxicity in animals.

**A12 enhances the inhibitory effects of docetaxel on osseous human prostate cancer xenografts.** We further investigated how the combined treatment of docetaxel and A12 would affect prostate tumor growth in a bone environment, using the established osseous prostate cancer xenograft model LuCaP23.1 (17). During treatment, docetaxel alone or docetaxel combined with A12 significantly inhibited LuCaP 23.1 tumor growth as reflected by suppression of serum PSA levels (Fig. 5A), with no significant difference between the two treatments. However, after treatment cessation, serum PSA began to increase significantly in animals that had been treated with docetaxel alone, indicating a regrowth of the tumor, whereas a continued suppression of serum PSA level was shown in animals that received combined treatment, indicating a prolonged period of posttreatment tumor quiescence. Serum PSA levels were shown to correlate with bone density and radiographed tumor bone sizes (Fig. 5B). As measured at week 5, the average bone density in the control, docetaxel 20, and docetaxel 20 combined with A12 treated animals was 0.112 ± 0.01, 0.09 ± 0.02, and 0.05 ± 0.009 (mean ± SE), respectively. There was an apparent trend towards a decrease in bone density with treatment.

**Discussion**

Recent studies have shown that treatment of androgen-independent prostate cancer with docetaxel results in a significant prolongation of life (11, 12, 18). Improving the efficacy of docetaxel by inhibiting prosurvival pathways or enhancing docetaxel effect on apoptosis has implications for treatment of prostate cancer and many other malignancies (13, 14). In this preclinical study, we showed that blocking signaling through

### Table 1. Posttreatment differential gene expression in docetaxel + A12–treated tumors compared with docetaxel alone–treated tumors

<table>
<thead>
<tr>
<th>HUGO Name</th>
<th>GO function</th>
<th>Fold change</th>
<th>False discovery rate (%)</th>
</tr>
</thead>
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<tr>
<td><strong>Down-regulated genes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC2</td>
<td>Cell division cycle 2</td>
<td>Cytokinesis; mitosis</td>
<td>3.0</td>
</tr>
<tr>
<td>CDC6</td>
<td>CDC6 cell division cycle 6 homologue</td>
<td>Negative regulation of cell proliferation</td>
<td>2.2</td>
</tr>
<tr>
<td>CCNA2</td>
<td>Cyclin A2</td>
<td>Regulation of CDK activity</td>
<td>2.1</td>
</tr>
<tr>
<td>MYBL2</td>
<td>V-myb myeloblastosis viral oncogene homologue (avian)-like 2</td>
<td>Antiapoptosis; development; regulation of cell cycle</td>
<td>3.2</td>
</tr>
<tr>
<td>TUBB</td>
<td>Tubulin β polypeptide</td>
<td>Microtubule-based movement; taxane resistance</td>
<td>2.3</td>
</tr>
<tr>
<td>K-Alpha-1</td>
<td>Tubulin α ubiquitous</td>
<td>Microtubule-based movement; taxane resistance</td>
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<tr>
<td><strong>Up-regulated genes</strong></td>
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<td>TOB1</td>
<td>Transducer of ERBB21</td>
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<td>Cyclin G2</td>
<td>Cell cycle checkpoint</td>
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<td>BIRC3</td>
<td>Baculoviral IAP repeat-containing 3</td>
<td>Antiapoptosis; cell surface receptor–linked signal transduction</td>
<td>2.2</td>
</tr>
</tbody>
</table>

6 Ludwig, unpublished data.

![Fig. 3. Real-time reverse transcription-PCR showing the relative expression levels of TUBB, survivin, and MyBL2 in tumors received combined treatment of docetaxel and A12 compared with those that received docetaxel or A12 alone. Columns, mean; bars, SE.](image-url)
the IGF-IR could enhance the therapeutic efficacy of docetaxel. The addition of A12 improved androgen-independent prostate tumor responses at two doses of docetaxel, the lower of which has shown less effective in inhibiting tumor growth in the current and other in vivo studies (31). In animals treated with docetaxel alone, androgen-independent tumor growth resumed almost immediately upon discontinuing therapy, and by 4 weeks, cell cycle kinetics and BrdUrd uptake were equivalent to the controls. In contrast, when A12 was given in conjunction with docetaxel, posttreatment suppression of androgen-independent tumor growth was significantly prolonged with ongoing apoptosis even up to 4 weeks after treatment was stopped. In the osseous human prostate xenograft model LuCaP 23.1, the combination of A12 and docetaxel also produced a greater and more prolonged decrease in tumor growth than docetaxel alone.

We have previously reported that A12 given as a single agent inhibits tumor growth in the LuCaP35V by inducing cell cycle G2–M arrest, rather than by induction of cell cycle G1 arrest or apoptosis (4). In the present report, A12 enhanced the apoptotic effect induced by docetaxel. Multiple studies have shown that signaling through the IGF-IR with subsequent activation of antiapoptotic pathways is a mechanism for recovery of tumor cells from chemotherapeutic and radiation therapies, including taxanes (5). Although multiple antiapoptotic pathways are stimulated by activation of the IGF-IR, the phosphatidylinositol 3-kinase (PI3K)/Akt pathway is most prominent (32). We have previously shown that A12 inhibits the PI3K/Akt pathway in the LuCaP 35V xenograft (4). Therefore, the mechanism by which A12 prolongs or accentuates the effect of docetaxel seems to be, at least in part, by inhibition of antiapoptotic pathways that permit recovery from docetaxel treatment. It should also be noted that persistence of circulating A12 in serum due to the long half-life of antibody therapies may in part contribute to the prolonged inhibition of tumor growth. We measured posttreatment serum levels of A12 in animals that had received docetaxel combined with A12. Serum A12 was detectable in animals through posttreatment week 4 (Fig. 4A).

After treatment termination, apoptosis and cell cycle arrest continued in tumors from the combined treatment of A12 and docetaxel. Gene profiling analyses indicated that the posttreatment prolonged effects of combined reagents may largely be due to the continuation of A12 enhanced down-regulation of.
cell cycle progression – or/and cell survival –associated genes and up-regulation of cell cycle –negative regulators or/and proapoptotic genes. Several gene products that positively regulate cell cycle progression (CDC2, CDC6, CCNA2, and CDC25B; refs. 33 – 35) or cell survival (BIRC5 and MYBL2; refs. 24, 36) were significantly down-regulated in tumors pretreated with A12 and docetaxel compared with docetaxel alone, whereas other genes that negatively regulate cell cycle progression or survival were significantly up-regulated (e.g., TOB1, CCNG2, and IGFBP3; refs. 28, 30, 37, 38). These changes in posttreatment gene expression after the combined therapy are consistent with the failure of these tumor cells to reenter the cell cycle and eventually to undergo apoptosis. Interestingly, some of these down-regulated genes, such as CDC6, CCNA2, BIRC5, and CDC25B, were also found down-regulated in tumors treated with A12 alone (39), further suggesting that the posttreatment prolonged inhibition of tumor growth is due to the augmented effect of A12 in suppressing or mitigating the activity of cell cycle/survival–related genes during treatment. Two genes, TUBB and K-Alpha-1 (23, 40), that are specifically related to docetaxel resistance are down-regulated following cessation of combined treatment, suggesting that blocking IGFR with A12 may potentially abrogate resistance mechanisms and allow a prolonged chemotherapeutic effect. Whether the down-regulation of these genes is simply an indication of the failure of tumors to reenter the cell cycle or represents specific therapy-related effects is the focus of ongoing studies.

Docetaxel induces cell death by multiple mechanisms, many of which might be augmented by suppression of IGFR signaling. Prerequisite for taxane cytotoxicity is initial perturbation of microtubule dynamics followed by induction of mitotic arrest and ultimately activation of the mitochondrial pathway of apoptosis. Resistance to taxanes may result from interference with each of the steps to induction of apoptosis. These include modulation of the isoforms of the taxane target β-tubulin (41), up-regulation of cell cycle regulatory protein p21Cip1 (42), inhibition of proapoptotic BAD, and up-regulation of prosurvival pathways involving Bcl-2 and PI3K. IGFR signaling rescues cells from apoptotic stress by maintaining Bcl-2 levels, suppressing BAD and up-regulating PI3K activation (32, 43). Induction of the IGFR-interacting protein RACK induces p21, p27, and G0-G1 arrest, all of which would be predicted to mediate resistance to taxanes (44). Previous work from our group shows that A12 treatment alone induces G0-M arrest; the combination of A12 and docetaxel may induce mitotic catastrophe in tumor cells and fail to reenter cell cycle even after treatment cessation, which ultimately resulted in cell death. We have also shown previously that A12 treatment suppresses PI3K activity, suggesting that the cell cycle regulatory pathways and blockade of PI3K are among the most relevant to A12 enhancement of docetaxel cytotoxicity. The ability of A12 to both enhance initial chemotherapy responses and induce a persistent reduction in tumor growth with ongoing spontaneous apoptosis is unique among agents used to sensitize cells to taxanes. How this effect is mediated, either through prolonged down-regulation of IGFR expression, inhibition of drug efflux, or inhibition of tumor angiogenesis (45), will have important implications for its use in patients with advanced prostate cancer.

In summary, in this study, we have shown that addition of an IGFR antibody A12 to docetaxel results in a significant increase in antitumor activity in two human prostate cancer xenograft models. The effect seems to be due to A12-enhanced down-regulation of cell cycle progression/cell survival–associated genes and/or up-regulation of proapoptotic genes that results in an inhibition of recovery from apoptosis induced by docetaxel. No obvious toxicity was seen from the combination therapy that was not observed in our previous studies with A12 as a single agent (4). Furthermore, this study supports the potential for future clinical trials in androgen-independent and bone-metastasized prostate cancer with combinations of an approved chemotherapeutic agent and an inhibitor of IGFR signaling, such as the human monoclonal A12 antibody.

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Cancer Therapy: Preclinical


Clinical Cancer Research

Combined In vivo Effect of A12, a Type 1 Insulin-Like Growth Factor Receptor Antibody, and Docetaxel against Prostate Cancer Tumors

Jennifer D. Wu, Kathy Haugk, Ilsa Coleman, et al.


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