Biologic Effects of Platelet-Derived Growth Factor Receptor α Blockade in Uterine Cancer

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Introduction

Uterine cancer is the most common gynecologic malignancy and the fourth most common cancer in North American women (1). Although overall outcome for these women is quite good, patients with recurrent or advanced-stage disease have a much poorer prognosis, with a median survival of 12 months (2). The mainstay of treatment for women with advanced disease remains systemic therapy in the form of hormonal agents or cytotoxic chemotherapy. Although tumor response to therapy has been documented, the treatments are associated with intolerable side effects and infrequent durable remission (3). Therefore, new and more effective targeted therapies are needed.

Platelet-derived growth factor receptor α (PDGFRα) is a type III receptor tyrosine kinase that can be activated by PDGF-AA, PDGF-AB, PDGF-BB, and PDGF-CC (4, 5). These growth factors are dimeric molecules composed of disulfide-linked polypeptide chains that bind to 2 receptors simultaneously and induce receptor dimerization, autophosphorylation, and intracellular signaling. Activated receptors induce a variety of cellular signals that act to prevent apoptosis, stimulate mitogenesis, and promote cellular chemotaxis (6, 7). These effects are mediated through several well-characterized downstream signaling cascades, including the Ras/mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase (PI3-K)/AKT pathway (8, 9). PDGFRα is critical to early development as mice homozygous for a null mutation die during embryogenesis (10). In the adult, PDGFs function in wound healing by activating mitogenesis, chemotaxis, and protein synthesis of PDGFR-positive fibroblast and smooth muscle cells (11). Although these mesenchymal cells are considered to be

Abstract

**Purpose:** Platelet-derived growth factor receptor α (PDGFRα) expression is frequently observed in many kinds of cancer and is a candidate for therapeutic targeting. This preclinical study evaluated the biologic significance of PDGFRα and PDGFRα blockade (using a fully humanized monoclonal antibody, 3G3) in uterine cancer.

**Experimental Design:** Expression of PDGFRα was examined in uterine cancer clinical samples and cell lines, and biologic effects of PDGFRα inhibition were evaluated using in vitro (cell viability, apoptosis, and invasion) and in vivo (orthotopic) models of uterine cancer.

**Results:** PDGFRα was highly expressed and activated in uterine cancer samples and cell lines. Treatment with 3G3 resulted in substantial inhibition of PDGFRα phosphorylation and of downstream signaling molecules AKT and mitogen-activated protein kinase (MAPK). Cell viability and invasive potential of uterine cancer cells were also inhibited by 3G3 treatment. In orthotopic mouse models of uterine cancer, 3G3 monotherapy had significant antitumor effects in the PDGFRα-positive models (Hec-1A, Ishikawa, Spec-2) but not in the PDGFRα-negative model (OVCA432). Greater therapeutic effects were observed for 3G3 in combination with chemotherapy than for either drug alone in the PDGFRα-positive models. The antitumor effects of therapy were related to increased apoptosis and decreased proliferation and angiogenesis.

**Conclusions:** These findings identify PDGFRα as an attractive target for therapeutic development in uterine cancer. *Clin Cancer Res;* 20(10); 1–11. ©2014 AACR.

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-13-2507
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Platelet-derived growth factor receptor \( \alpha \) (PDGFR\( \alpha \)) expression is frequently observed in many kinds of cancer and is a candidate for therapeutic targeting. Here, we evaluated the biologic significance of PDGFR\( \alpha \) and PDGFR\( \alpha \) blockade using a fully humanized monoclonal antibody, IMC-3G3 (3G3) in uterine cancer. PDGFR\( \alpha \) was expressed in uterine cancer cells, and its blockade with 3G3 resulted in inhibition of PDGFR\( \alpha \) phosphorylation and of downstream signaling molecules AKT and mitogen-activated protein kinase (MAPK). Cell viability and invasive potential of uterine cancer cells were also inhibited by treatment of 3G3. Moreover, greater therapeutic effects were observed for 3G3 in combination with chemotherapy than for either drug alone in orthotopic mouse models of uterine cancer. These findings identify PDGFR\( \alpha \) as an attractive therapeutic target for uterine cancer.

“classic” targets for PDGFs, tumor cells have also been shown to express PDGFRs, providing evidence for the involvement of this receptor in tumorigenesis (12).

Tumors reported to express PDGFR\( \alpha \) include ovarian (13), prostate (14), breast (15), lung (16), and melanoma (17). Also, tumor PDGFR\( \alpha \) expression has been associated with disease progression in renal cell cancer (18), diminished survival in ovarian cancer (13), lymph node metastasis in breast cancer (19), and bone metastases in prostate cancer (20). The extracellular region of the receptor consists of 5 immunoglobulin-like domains whereas the intracellular part is a tyrosine kinase domain. The ligand-binding sites of the receptors are located in the first 3 immunoglobulin-like extracellular domains (6, 7, 21, 22). Recently, a fully humanized monoclonal antibody to PDGFR\( \alpha \), called IMC-3G3 (ImClone Systems), was developed. The epitopes for 3G3 spatially overlap the PDGF-AA and PDGF-BB binding sites, and strong affinity for PDGFR\( \alpha \) was confirmed. The 3G3 antibody has been shown to inhibit ligand-induced dimerization and autophosphorylation and promote PDGFR internalization after prolonged treatment (21). Moreover, therapeutic efficacy with 3G3 has been shown in several malignancies, including preclinical models of leiomyosarcoma and glioblastoma (22). However, in uterine cancer, the role of PDGFR\( \alpha \) and the effect of its blockade have not yet been determined. The purpose of our study was to determine the biologic and therapeutic effects of PDGFR\( \alpha \) blockade in uterine cancer models.

Materials and Methods

Cell lines and cultures

Uterine cancer cell lines (HEC-265, SPEC-2, Hec-1A, Ishikawa, RL95-2, AN3CA, SK-LIT-2, and KLE) and ovarian cancer cell lines (SKOV3, OVCA432, OVCAR5, A2780, and HeyA8) were obtained from the MD Anderson Characterized Cell Line Core Facility (Houston, TX), which supplies authenticated cell lines. Cells were maintained in specific culture medium. The cell lines were routinely tested to confirm the absence of mycoplasma, and all experiments were performed with cell lines at 60% to 80% confluence. Before in vivo injection, cells were trypsinized, centrifuged at 1,100 rpm for 5 minutes at 4°C, washed twice with DPBS, and resuspended in HBSS for intrauterine injections. The cell lines were routinely tested to confirm the absence of mycoplasma, and all experiments were performed with cell lines at 60% to 80% confluence.

Reagents

IMC-3G3 (3G3; neutralizing fully human immunoglobulin G monoclonal antibody to PDGFR\( \alpha \)) was provided by ImClone Systems. Additional details about the development of this antibody have been described previously (21). Paclitaxel, docetaxel, and cisplatin (cis-diaminedichloroplatinum, CDDP) were purchased from the MD Anderson Cancer Center pharmacy.

Western blot

Preparation of cell and tumor tissue lysates has been described previously (23). Protein concentrations were determined using a BCA Protein Assay Reagent Kit (Pierce Biotech), and aliquots of 30 \( \mu \)g protein were subjected to gel electrophoresis on 8% or 10% SDS-PAGE gels. Transfer to membranes and immunoblotting were performed as described previously (23). The following antibodies were used for Western blot analyses: PDGFR\( \alpha \) (Cell Signaling; #3174), phospho-PDGFR\( \alpha \left( Y^{762} \right) \); Invitrogen; #44-1010), P44/42 MAPK and phospho-P44/42 MAPK (T202/Y204; Cell Signaling, #9102, #9101), and AKT and p-AKT (pS473; Cell Signaling, #9272, #9746, #9271). Receptor and downstream signaling molecule phosphorylation assays were done as described previously (21).

Briefly, cells were seeded in 6-well tissue culture plates (1 \( \times \) 10\(^5\) cells per well) and allowed to grow overnight. The cells were then rendered quiescent by serum deprivation, treated with monoclonal antibodies for 2 hours at 37°C, and then stimulated with human PDGF-AA (R&D Systems) for 10 minutes at 37°C. Afterward, cell lysates were analyzed by SDS-PAGE and Western blotting with the antibodies mentioned above.

Cell viability assay

Cytotoxic effects of 3G3 compared with human immunoglobulin G (HmgG) and with no treatment, with or without chemotherapy, were determined by the MTT uptake assay as described previously (24). Cells were plated on 96-well plates (7,000 per well for Ishikawa, Hec-1A, and KLE; 10,000 per well for Spec-2) in triplicate and incubated overnight at 37°C and 5% CO\(_2\). After incubation, cells were washed, serum-free medium was added, and cells were treated with PBS (control), HmgG, and 3G3. After 6 hours, regular media or media-containing chemotherapeutic agents (paclitaxel, docetaxel, and cisplatin) were added.
After 72 hours (Ishikawa, Hec-1A, or Spec-2) or 96 hours (KLE), cell viability was determined.

### Apoptosis assay

The relative percentage of apoptotic cells was assessed by phycoerythrin (PE) Annexin V and 7-amino-actinomycin (7-AAD) staining (BD Biosciences), as previously described (24). Briefly, uterine cancer cells (1 × 10^5 cells/mL) were pelleted and washed twice in PBS and resuspended in a binding buffer containing PE Annexin V and 7-AAD (5 µL per 10^5 cells). Samples were incubated in the dark for 15 minutes at room temperature before being analyzed by flow cytometry.

### Cell invasion assay

Cell invasion assays have been previously described (15). Briefly, cells were treated with control, HmIgG, or 3G3 for 6 hours. Cells were then reconstituted in serum-free medium (1 × 10^6 cells/mL) and 100 µL added to inserts coated with a defined matrix consisting of human laminin, type IV collagen, and gelatin. Inserts were then transferred to wells filled with serum-containing media. Cells were then

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**Figure 1.** Expression of PDGFR<sub>α</sub> and anti-phosphorylation effect of its blockade in cancer cells. **A,** Western blot analysis of PDGFR<sub>α</sub> expression in uterine and ovarian cancer cell lines. **B,** in vitro effect of 3G3 on PDGFR<sub>α</sub>, pPDGFR<sub>α</sub>, and its downstream targets. Cells (Hec-1A) were treated with 3G3 or nonspecific human immunoglobulin G (HmIgG) for 24 hours and then stimulated with 1 nmol/L PDGF-AA for 10 minutes. Cell lysates were analyzed by Western blot analysis with antibodies against PDGFR<sub>α</sub>, pPDGFR<sub>α</sub>, P44/42 MAPK, p-P44/42 MAPK, AKT, and p-AKT. **C,** Western blot analysis of expression of PDGFR<sub>α</sub> downstream targets in RL95-2 cells. RL95-2 cells were treated with 3G3 or PDGF-AA as mentioned above. Loading of an equal amount of sample per gel lane was confirmed by β-actin expression.
Figure 2. In vitro effects of 3G3 or the combination of 3G3 and chemotherapy on cell viability. A, MTT cell viability assay of Hec-1A and RL95-2 cells. Viability was assessed with the MTT assay at 72 hours after PDGF-AA stimulation or 3G3 + PDGF-AA stimulation. "3G3 + PDGF-AA" means pretreatment with 3G3 before PDGF-AA stimulation (red line), and "PDGF-AA + 3G3" means cotreatment with PDGF-AA and 3G3 at the same time. B, MTT cell viability assay of Ishikawa, Hec-1A, Spec-2, and KLE cells after 3G3 treatment combined with chemotherapy. C, IC50 reduction ratio of chemotherapy combined with 3G3 treatment. All experiments were statistically analyzed on the basis of 3 repeated experiments. Error bars, SEM; *, *P < 0.05.
allowed to invade for 24 hours at 37°C. Cells that had migrated into the bottom wells were collected, fixed, stained, and counted by light microscopy. Cells were counted in 10 random fields (×200 final magnification) and the average number of cells determined.

**Animal care and orthotopic implantation of tumor cells**

Female athymic nude mice (NCr-nu) were purchased from the National Cancer Institute–Frederick Cancer Research and Development Center and housed in specific pathogen-free conditions. They were cared for in accordance with guidelines set forth by the Association for Assessment and Accreditation of Laboratory Animal Care International and the U.S. PHS Policy on Humane Care and Use of Laboratory Animals, and all studies were approved and supervised by the MD Anderson Cancer Center Institutional Animal Care and Use Committee.

To produce tumors, Hec-1A and Ishikawa cells (both 4.0 × 10⁶ cells per 50 μL HBSS) or Spec-2 cells (2.0 × 10⁶ cells per 50 μL HBSS; ref. 24) were injected into the mice. Before injection, mice were anesthetized with isoflurane inhalation (Baxter), and a 0.5-cm incision was made in the right lower flank to optimize exposure to the right uterine horn. The distal portion of the horn was then identified and pulled to the incision for exposure. A single-cell suspension of 50 μL was then injected into the lumen of the uterine horn. The injection site was closely monitored during and following injection to ensure that no spillage occurred into the peritoneal cavity (25). The incision was then closed with staples. Mice were monitored daily for adverse effects of therapy, and all were euthanized when any of the mice seemed moribund.

**Therapy for established uterine tumors in nude mice**

To assess tumor growth, treatment began 2 weeks after injection of tumor cells. Mice were randomly divided into 4 groups (n = 10 mice per group): (i) control PBS, (ii) 3G3, (iii) paclitaxel (Ishikawa, Hec-1A, and OVCA432) or docetaxel (Spec-2), and (iv) 3G3 combined with chemotherapy (paclitaxel or docetaxel).

Antibody 3G3 was dosed using 60 mg/kg intraperitoneal injection twice weekly with an initial loading dose of 214 mg/kg (21). Chemotherapy was injected into the peritoneal cavity once a week at a dose of 100 μg/mouse (paclitaxel) or 30 μg/mouse (docetaxel). Mice were euthanized after they became moribund (typically 6 to 7 weeks after injection).
depending on tumor cell type). Tumor weight, number of tumor nodules, and distribution of tumors were recorded. Tumor tissue used in this study was obtained at the time of necropsy and immersed in optimum cutting temperature medium for frozen slide preparations. Tumor specimens were also fixed in formalin for paraffin slide preparation.

**Immunohistochemical staining**

Expression of PDGFRα (Santa Cruz Biotechnology; #sc-338) and pPDGFRα (pY720; #sc-12910) was examined in clinical samples (10 normal endometrium, 10 endometrial polyp, and 20 endometrial cancers). For immunohistochemistry, staining was graded from 0 (no staining) to 3 (strong staining), based on the intensity and number of positive cells, by a single pathologist. Immunohistochemical analysis for CD31 and Ki67 was performed as previously described (26). To quantify microvessel density (MVD), the number of blood vessels staining positive for CD31 was recorded in 5 random fields at ×200 magnification for each sample. For Ki67, the number of positive cells and the total number of cells were counted in 5 random fields at ×200 magnification for each sample. Terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) staining was performed on frozen tissue using a Promega Kit (Promega), and cells were counted in 5 random fields at ×200 magnification (26).

**Microarray analysis of PDGFR downstream genes**

We analyzed the effect of 3G3 on differentially down-regulated genes in the Hec-1A and RL95-2 cells using the Illumina cDNA microarray platform. Briefly, Hec-1A and RL95-2 cells were treated with 3G3 or PDGF-AA or the combination for 24 hours. For microarray hybridization, total RNA was extracted from treated and untreated cells using a mirVana RNA isolation labeling kit (Ambion). We used 750 ng of total RNA to label and hybridize the cells according to Illumina’s protocols. After we scanned bead chips (Sentrix HumanHT-12 v3, Illumina) using an Illumina BeadArray reader, we normalized the microarray data using the quantile normalization method with the Linear Models for Microarray Data software program in the R language. The level of expression of each gene was transformed into a log₂ base before additional analysis was performed. Genes that were differentially expressed in control and treated cells were identified using a random variance t test; gene expression differences were considered statistically significant if \( P < 0.001 \). A stringent significance threshold was used to limit the number of significant changes.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Effect of PDGFRα blockade on cell invasion in Ishikawa (A), Hec-1A (B), and Spec-2 cells (C). PDGF-AA was used to activate PDGFRα expression. Statistical analysis was based on 3 repeated experiments. Error bars, SEM. *, \( P < 0.05 \) compared with control. ***, \( P < 0.05 \) compared with serum deprivation (SD) condition.
Inhibition of PDGFRα in Uterine Cancer

**A**

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<th>PBS</th>
<th>3G3</th>
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**B**

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<th>Paclitaxel</th>
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**Graphs**

- Tumor weight (g) comparisons across different treatments.
- Immunohistochemical staining for PDGFRα, pPDGFRα, pAKT, pMAPK, K67, CD31, TUNEL. 

**Statistical Significance**

- * Indicates p < 0.05
- ** Indicates p < 0.01
- *** Indicates p < 0.001
false-positive findings. In addition, the NetWalker software program was used for gene network analysis (27). In accordance with Minimum Information About a Microarray Experiment (MIAME) guidelines, we deposited the microarray data into the GEO repository with accession number GSE55483.

Statistical analyses
Continuous variables were assessed for normal distribution (Kolmogorov–Smirnov test) and expressed as appropriate (mean with SEM or median with range). A one-way ANOVA test with post hoc (Bonferroni adjustment) comparison or Kruskal–Wallis test with multiple comparisons (Wilcoxon rank-sum test with Bonferroni correction) was performed to determine the statistical significance as appropriate. Categorical variables were evaluated with use of the Fisher exact test. Three replicates were taken to monitor the performance of each experiment. We repeated experiments independently at least 3 times for statistical analysis. P < 0.05 was considered statistically significant. IBM SPSS Statistics 21.0 (IBM SPSS, Inc.) was used for all statistical analyses.

Results
Basal expression of PDGFRα and anti-phosphorylation effect of its blockade
We first examined PDGFRα expression in uterine and ovarian cancer cell lines. All cells tested showed PDGFRα expression, except RL95-2 and OVCA432 cells (Fig. 1A). The effects on PDGFR-induced intracellular signaling by anti-PDGFRα antibody 3G3 were determined using the PDGFRα-positive cells (Hec-1A) and PDGFRα-negative cells (RL95-2 and OVCA432). An early event in the signaling process is receptor autophosphorylation; therefore, 3G3 was tested for its ability to inhibit ligand-induced receptor tyrosine phosphorylation (Fig. 1B). PDGF-AA at 1 nmol/L concentration increased PDGFRα tyrosine phosphorylation. A higher concentration of ligand (PDGF-AA; 10 nmol/L) resulted in less receptor phosphorylation (data not shown), possibly because of ligand-induced degradation. The 3G3 antibody, at a concentration of 2 μg/mL or higher, inhibited PDGF-AA-induced receptor phosphorylation. As expected, PDGFRα blockade resulted in decreased phosphorylation of downstream proteins MAPK and AKT (Fig. 1B) in Hec-1A cells, but no effects of 3G3 on AKT, p-AKT, MAPK, and p-MAPK were noted in PDGFRα-null cells (Fig. 1C). To determine PDGFRα expression in cancer and stromal populations, immunohistochemical staining of normal endometrium, endometrial polyps, and endometrial cancer samples was performed. There was higher expression of total and phospho-PDGFRα in cancer tissues than in the benign controls (Supplementary Fig. S1). Moreover, while there was some staining in the stroma, the predominant staining was present in the cancer cells (Supplementary Fig. S1).

In vitro effect of PDGFRα blockade
Before testing the in vivo biologic effect of PDGFRα blockade, we tested the in vitro effects on uterine cancer cell lines. To confirm the effect of 3G3, cell lines were tested at doses ranging from 0.1 to 200 μg/mL. Cell viability was inhibited by 3G3 alone in Hec-1A but not in RL95-2 cells (Fig. 2A). We also examined the effects of PDGFRα blockade using the combination of 3G3 and chemotherapy (paclitaxel, docetaxel, and cisplatin; Fig. 2B). Treatment with 3G3 enhanced the cytotoxicity of chemotherapy in Ishikawa, Hec-1A, and Spec-2 cells but not in KLE cells. In the Hec-1A and Spec-2 cell lines, combining all types of chemotherapy with 3G3 resulted in lower cell viabilities than were seen when the chemotherapy agents were combined with HmIgG or control. Statistical analysis showed that 3G3 enhanced the sensitivity to all chemotherapy drugs tested in the Ishikawa, Hec-1A, and Spec-2 cells, but still not in KLE cells (Fig. 2C).

We next assessed whether PDGFRα blockade could promote tumor cell apoptosis. Although we did not find significant changes in apoptosis after co-treatment with 3G3 and PDGFAA in Hec-1A and RL95-2 cells (Fig. 3A), pretreatment with 3G3 resulted in significantly increased tumor apoptosis in Hec-1A cells but not in RL95-2 cells (Fig. 3A). Furthermore, combination of 3G3 with chemotherapy resulted in significantly increased tumor cell apoptosis in Ishikawa, Hec-1A, and Spec-2 cells but not in KLE cells (Fig. 3B). These results paralleled the results of the cell viability assay. We also examined the effect of PDGFRα blockade on cancer cell invasion. Serum deprivation decreased invasive ability, and PDGF-AA restored invasion in Ishikawa and Hec-1A cells. PDGFRα blockade with 3G3 resulted in significantly decreased cell invasion compared with controls in Ishikawa, Hec-1A, and Spec-2 cells (Fig. 4).

Therapeutic effect of PDGFRα blockade
On the basis of the in vitro data, we next assessed in vivo effects of PDGFRα blockade. Two weeks after orthotopic injection of tumor cells into the uterine horn, mice were randomly allocated to 1 of 4 treatment groups. Mice were euthanized when animals in any group became moribund (4–5 weeks after initiating therapy, depending on the cell line used). All tumors were harvested; mean tumor weight, number of nodules, and their distribution were recorded. Columns mean tumor weights for each group; Error bars, SEM. *P < 0.05; **P < 0.01 compared with control group; ***P < 0.05 compared with chemotherapy-alone group. B, in vivo biologic effects of PDGFRα blockade on expression of PDGFRα, pPDGFRα, p-AKT, and p-MAPK; tumor cell proliferation (Ki67); angiogenesis (CD31); and apoptosis (TUNEL). Immunohistochemical stains were performed on Hec-1A cell-bearing mice. Error bars, SEM. *P < 0.05 compared with control; **P < 0.05 compared with chemotherapy-alone group.
moribund. Both 3G3 alone and chemotherapy alone resulted in significant growth inhibition compared with PBS, as expected (89.8% and 86.6%, respectively, in Ishikawa cells; 50.9% and 71.7% in Hec-1A; 71.4% and 65.9% in Spec-2; Fig. 5A). The combination of 3G3 and chemotherapy resulted in the greatest tumor reduction compared with PBS (98.8% in Ishikawa, *P* = 0.0004; 84.5% in Hec-1A, *P* < 0.0001; 93.2% in Spec-2, *P* = 0.011). To identify the biologic effects of PDGFRα blockade, we examined PDGFRα, phospho-PDGFRα, p-AKT, and p-MAPK expression, tumor cell proliferation (Ki67), tumor-associated MVD (CD31 staining), and tumor cell apoptosis (TUNEL; Fig. 5B and Supplementary Fig. S2). PDGFRα expression, especially the phosphorylated form, was significantly reduced in the 3G3 treatment groups. Moreover, there was reduced expression of p-AKT and p-MAPK in the 3G3 treatment groups. Tumor cell proliferation was significantly reduced in the 3G3 group, chemotherapy group, and the combination group compared with the PBS group (20.3%, 21.2%, and 39.3% reduction, respectively, in the Hec-1A model, *P* < 0.0001; Fig. 5B). The decrease in the combination group was greatest and statistically significant in comparison to the 3G3- and paclitaxel-only groups (*P* = 0.001 and 0.0004, respectively; Fig. 5B). MVD was also significantly reduced in the 3G3, paclitaxel, and combination groups (53.1%, 63.65%, and 77.9% reduction, respectively, *P* < 0.01; Fig. 5B), and the combination group showed significantly lower MVD compared with the PBS group in Hec-1A cells (P < 0.0001; Fig. 5B). Combination 3G3 and paclitaxel treatment resulted in the greatest increase in tumor cell apoptosis compared with the 3G3-only, paclitaxel-only, and PBS groups (*P* < 0.0001; Fig. 5B). Similar observations were found in the Ishikawa and Spec-2 models (Supplementary Fig. S2).

To determine whether PDGFRα expression was necessary for the 3G3 therapeutic response, we carried out an experiment with the OVCA432 model (this model was used as the RL95-2 cell line is not tumorigenic in mouse models). In the OVCA432 model, no significant antitumor effect of 3G3 was observed (Fig. 5A).

Because the *in vivo* effects of 3G3 therapy were greater than the observed *in vitro* effects, we considered the possibility of indirect effects on the tumor microenvironment via reduced production of angiogenesis factors by cancer cells. To address this possibility, we performed microarray analysis of PDGFRα-positive and -negative cells following treatment with 3G3. Integrated pathway analysis showed that several proangiogenic genes (e.g., VEGFA, HDAC7, ITG, and VCAN) were downregulated in PDGFRα-positive cells but not in negative cells (Fig. 6).

**Discussion**

The key findings from this study are that PDGFRα blockade effectively inhibits growth in PDGFRα-positive uterine cancer models. PDGFRα has been considered an important target in other cancers, including ovarian (13), prostate (14), breast (28), and lung (16). PDGFR/PDGFRβ signaling plays a relevant role in cancer biology because of not only direct effects on tumor cells but also paracrine effects mediated by PDGFRα expression on stromal cells (29–31). Here,
while there were modest effects with 3G3 in vitro, the effects were more substantial in vivo. The differences in the in vitro versus in vivo effects are likely explained by modulation of proangiogenic genes in PDGFRα-positive cells following 3G3 treatment. Collectively, these data support both direct and indirect effects of the 3G3 antibody in uterine cancer models.

Antibody-based approaches may have some advantages over small molecules, including greater target specificity, less off-target toxicity, and an additional capacity for immune-mediated cytotoxicity (32). The 3G3 antibody is a neutralizing monoclonal antibody to PDGFRα and does not cross-react with the β-form of the receptor. 3G3 blocks both PDGF-AA and PDGF-BB ligands from binding to PDGFRα and receptor autophosphorylation. In addition to these functions, 3G3 has been shown to inhibit phosphorylation of downstream signaling molecules AKT and MAPK (21). AKT and MAPK have also been shown to play a role in response to chemotherapy in uterine cancer (33). The enhanced efficacy of 3G3 in combination with chemotherapy is likely due to blockade of these pathways (34). 3G3 treatment effectively inhibited autophosphorylation of receptor and phosphorylation of downstream molecules MAPK and AKT in PDGFRα-positive cells, but not in PDGFRα-null cells, suggesting that PDGFRα expression on cancer cells was an important determinant of response to 3G3 (35).

To date, clinical results with 3G3 reveal a favorable safety and therapeutic profile in patients with advanced solid tumors and lymphomas (31). On the basis of these preclinical and early clinical findings, several phase II clinical trials of 3G3 in advanced solid malignancies are either underway or in development. In the future, combination studies of PDGFR-targeting therapeutics with anti-angiogenic agents may take further advantage of the potential interplay among tumor, stromal, and angiogenic factors that appear to be a hallmark of PDGFR signaling.

In summary, we demonstrated that inhibition of PDGFRα with 3G3 is an effective approach for treatment of uterine cancer in orthotopic animal models. PDGFRα blockade decreased tumor angiogenesis, cell survival, and tumor growth, and these effects might be related to modulated AKT and MAPK activity. These data suggest that this monoclonal antibody to PDGFRα may be useful for the treatment of uterine tumors that express PDGFRα.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
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Acknowledgments
The authors thank Sunita Patterson of the MD Anderson Department of Scientific Publications for the helpful editing.

Grant Support
This study was supported by NIH (CA109298, P50 CA098258, P50 CA083639, CA128797, U54 CA151668, Institutional Core Grant CA016672), Ovarian Cancer Research Fund, Inc. (Program Project Development Grant), U.S. Department of Defense (OC073399, OC093146), Betty Anne Arche Murray Distinguished Professorship, the Gilder Foundation, and the RCK Foundation. J. Botsford-Miller, B. Zand, and H.J. Dalton are supported by an NCI-DHHS-NIH T32 Training Grant (T32 CA101642). J.-W. Roh is supported by Basic Science Research Program of Korea funded by the Ministry of Education (NRF-2012R1A1A0301474).

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Received September 17, 2013; revised March 3, 2014; accepted March 5, 2014; published OnlineFirst March 14, 2014.
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*Clin Cancer Res*  Published OnlineFirst March 14, 2014.

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