Published in final edited form as:
Clin Cancer Res. 2010 May 1; 16(9): 2562–2570. doi:10.1158/1078-0432.CCR-10-0017.

EphA2 Targeted Chemotherapy Using an Antibody Drug Conjugate in Endometrial Carcinoma

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Abstract

Purpose—EphA2 overexpression is frequently observed in endometrial cancers, and is predictive of poor clinical outcome. Here, we utilize an antibody drug conjugate (MEDI-547) composed of a fully human monoclonal antibody against both human and murine EphA2 (1C1) and the tubulin polymerization inhibitor, monomethylauristatin F (MMAF).

Experimental design—EphA2 expression was examined in endometrial cancer cell lines by Western Blot. Specificity of MEDI-547 was examined by antibody degradation and internalization assays. Viability and apoptosis were investigated in endometrial cancer cell lines and orthotopic tumor models.

Results—EphA2 was expressed in the Hec-1A and Ishikawa cells, but was absent in the SPEC-2 cells. Antibody degradation and internalization assays showed that the antibody drug conjugate

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decreased EphA2 protein levels and was internalized in EphA2 positive cells (Hec-1A and Ishikawa). Moreover, *in vitro* cytotoxicity and apoptosis assays demonstrated that the antibody drug conjugate decreased viability and increased apoptosis of Hec-1A and Ishikawa cells. *In vivo* therapy experiments in mouse orthotopic models with this antibody drug conjugate resulted in 86 to 88% growth inhibition ($P < 0.001$) in the orthotopic Hec-1A and Ishikawa models compared to controls. Moreover, the mice treated with this antibody drug conjugate had a lower incidence of distant metastasis compared with controls. The anti-tumor effects of the therapy were related to decreased proliferation and increased apoptosis of tumor and associated endothelial cells.

Conclusions—The preclinical data for endometrial cancer treatment using MEDI-547 demonstrate substantial anti-tumor activity.

Keywords

EphA2 Receptor; Antibody Drug Conjugates; Targeted Therapy; Endometrial Cancer

Introduction

Endometrial cancer is the most common gynecological malignancy and the fourth most common cancer in North American and European women (1). Overall prognosis for these women is excellent as the majority of patients present with early-stage disease that is confined to the uterus at the time of hysterectomy, leading to 5-year survival rates of greater than 70% (2). Unfortunately, women with recurrent or advanced-stage disease have a much poorer prognosis, with a median survival of approximately 12 months (3). The mainstay of treatment for these women remains systemic therapy in the form of hormonal agents or cytotoxic chemotherapy, and while some responses have been documented, the treatment programs are associated with intolerable side-effects and infrequent durable remission (4). Therefore, newer and more effective targeted therapies are urgently needed.

Efforts to improve the cytotoxic action of monoclonal antibodies (mAbs) and consequently their therapeutic effectiveness have focused on conjugates with highly toxic substances, including radio-isotopes and cytotoxic agents (5). These conjugates can deliver a toxic load selectively to the tumor site while generally sparing normal tissues. To date, just two radioimmunoconjugates (ibritumomab tiuxetan and tositumomab) and one chemo-immunoconjugate (gemtuzumab ozogamicin) have been approved in the U.S. for limited use in refractory hematologic cancers (6-8). Thus, mAb conjugates can be a viable approach to killing tumor cells in hematological malignancies.

The Eph receptors are the largest family of tyrosine kinases and are divided into two subclasses based on interaction with their ligands, ephrin-A and ephrin-B (9). There is growing evidence that several Eph receptors play critical roles in cancer development and progression (10). Moreover, high expression of EphA2 was detected in human endometrial carcinoma, which was significantly associated with poor clinical outcome (11). In normal adult tissues, EphA2 expression is either absent or present at low levels in only a few epithelial tissues (9); therefore, EphA2 could be an ideal therapeutic target in patients with advanced or recurrent endometrial cancer.

Recently, an EphA2 targeted antibody drug conjugate with a fully human mAb (1C1) has been developed that selectively binds both the human and rodent EphA2 receptor. This antibody is conjugated to the microtubule inhibitor monomethylauristatin F (MMAF) using a stable maleimidocaproyl (mc) linker (MEDI-547) (12-14). The purpose of our study was to determine the biological activity of this antibody drug conjugate in endometrial cancer using orthotopic endometrial cancer models.

Materials and Methods

Cell lines and cultures

Endometrial cell lines Ishikawa, Hec-1A and KLE were maintained and propagated in MEM (Ishikawa), McCoy's 5A (Hec-1A), and 1:1 DMEM:F12 (KLE) media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin (Life Technologies) at 37°C (15). The human papillary serous endometrial carcinoma cell line SPEC-2 was maintained in a 1:1 mixture of Dulbecco's modified Eagle's minimum essential medium (DMEM) and Ham's F-12 medium supplemented with 10% FBS, sodium pyruvate, non-essential amino acids, L-glutamine, and a two-fold vitamin solution (Gibco Laboratories, Gaithersburg, MD) (16). The Ishikawa and Hec-1A cell lines were obtained from Dr. Russell Broaddus (M. D. Anderson Cancer Center, Houston, TX) and SPEC-2 from Dr. Isaiah J. Fidler (M. D. Anderson Cancer Center, Houston, TX). The KLE cell line was obtained from the American Type Culture Collection (Rockville, MD). All experiments were conducted at 70 to 80% confluent cultures. Before *in vivo* injection, cells were trypsinized, centrifuged at 1,000 rpm for 7 min at 4°C, washed twice with HBSS, and resuspended in HBSS for intrauterine injections. Both cell lines were tested and found to be negative for murine antigen reactivity and Mycoplasma species before injection into mice.

Antibodies and antibody drug conjugates

1C1 (fully human monoclonal antibody recognizing both human and murine EphA2), control IgG-mcMMAF (non-binding specific IgG monoclonal antibody conjugated to MMAF via the mc linker), and MEDI-547 (1C1 conjugated to MMAF via the mc linker) were provided by MedImmune, LLC (Gaithersburg, MD). The antibody description and the details of the conjugation reaction have been described previously (12).

Western blot

The preparation of cultured cell lysates have been described previously (17-18). Briefly, protein concentrations were determined using a BCA Protein Assay Reagent kit (Pierce Biotechnology, Rockford, IL) and aliquots of 20 μg protein were subjected to gel electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gels. The proteins were then transferred to a nitrocellulose membrane (Millipore, Bedford, MA), and then incubated overnight at 4°C with primary antibody (mouse anti-human/mouse EphA2 monoclonal antibody [clone D7, Upstate, Lake Placid, NY]), after washing with TBST. The membranes were incubated with 1 μg/mL horseradish peroxidase (HRP)-conjugated horse anti-mouse IgG (Amersham, Piscataway, NJ). HRP was visualized by use of an enhanced chemiluminescence detection kit (Pierce) (18-19).

Antibody internalization

Procedures for antibody internalization after treatment with MEDI-547 in Hec-1A and Ishikawa cells were performed as described previously (12). Briefly, viable cells (0.5×10^6) were aliquoted into wells of a 96-well plate in 100 μl of growth media. The cells were centrifuged at 1500 RPM for 5 minutes and labeled with primary antibody drug conjugates by resuspension in 100 μl PBS containing 5 μg of MEDI-547 or control IgG-mcMMAF and incubated for 30 minutes at 4°C. Cells were then washed twice with PBS and cell-surface-bound primary antibody drug conjugates were allowed to internalize by resuspending the cells in 100 μl of growth media and incubation at 37°C / 5% CO_2 for 30 minutes or on ice as negative control. Subsequent to internalization, cells were fixed (4% paraformaldehyde, 20 minutes at room temperature), permeabilized (0.5% Triton X-100, 5 minutes at room temperature). Cells were then labeled with secondary AlexaFluor 488 goat anti-human IgG

Ab (Biosource) by resuspension in 100 μl PBS + 2% FBS containing 1 ug of secondary antibody and incubated for 30 minutes at 4°C (12).

Cytotoxicity assay

The cytotoxic effects of 1C1, control IgG-mcMMAF and MEDI-547 were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide uptake (MTT) assay as described previously (20).

Analysis of cell apoptosis

The relative percentage of apoptotic cells was assessed at three time points (24hr, 48hr, and 72hr) using the Annexin V-FITC apoptosis Detection Kit-1 (BD Pharmingen, San Diego, CA) according to the manufacturer's protocol. Briefly, Hec-1A and Ishikawa cells were washed twice in PBS, and the pellet was resuspended in annexin V binding buffer at a concentration of 10^6 cells/ml. Annexin V FITC and propidium iodide (PI) were added (5 μl to each per 10^5 cells). Samples were mixed gently and incubated for 15 min at room temperature in the dark before fluorescence activated cell sorter (FACS) analysis.

Animal care and orthotopic implantation of tumor cells

Female athymic mice (NCr-*nu*) were purchased from the National Cancer Institute-Frederick Cancer Research and Development Center. The mice were housed and maintained under specific pathogen-free conditions in facilities approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with the current regulations and standards of the U.S. Department of Agriculture, the U.S. Department of Health and Human Services, and the NIH. All studies were approved and supervised by the University of Texas M.D. Anderson Cancer Center Institutional Animal Care and Use Committee. The mice used in these experiments were 8 to 12 weeks old. To produce tumors, Hec-1A and Ishikawa cells (both 4.0×10^6 cells per 50 μL HBSS; Life Technologies Invitrogen) (15) or SPEC-2 cells (2.0×10^6 cells per 50 μL HBSS) (16) were injected into the mice. Before injection, mice were anesthetized with isoflurane inhalation (Baxter, Deerfield, IL, USA) 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 (15). The incision was then closed with staples. Mice (n = 10 per group) were monitored daily for adverse effects of therapy and were sacrificed when any of the mice seemed moribund. Total body weight, tumor incidence and mass, and the number and location of tumor nodules were recorded. Tumors were fixed in formalin and embedded in paraffin or snap frozen in optimal cutting temperature (OCT) media in liquid nitrogen.

Therapy for established endometrial tumors in nude mice

The antibody drug conjugate was dosed at weekly 3 mg/kg i.p. injections (14). Therapy experiments were designed using human endometrial cancer cell lines, Hec-1A, Ishikawa, and SPEC-2. Following cell line injection, mice were randomized into four treatment groups: (a) control, 200 μL PBS (i.p., once a week); (b) 1C1, 3 mg/kg in 200 μL PBS (i.p., once a week); (c) control IgG-mcMMAF, 3 mg/kg in 200 μL PBS (i.p., once a week); and (d) MEDI-547, 3 mg/kg in 200 μL PBS (i.p., once a week). Therapy was initiated 2 weeks following cell line injection (15). Mice were monitored for adverse effects and sacrificed by cervical dislocation 6 to 7 weeks following initiation of treatment.

Immunohistochemistry

Procedures for immunohistochemical analysis of EphA2 and PCNA were performed as described previously (18, 21).

Immunofluorescence double staining for CD31 and terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL)

Frozen tissues were used for CD31/TUNEL immunofluorescence double staining as described previously (22). The quantification of apoptotic endothelial cells was calculated by the number of apoptotic endothelial cells in 10 random fields at ×200 magnification.

Statistical methods

For animal experiments, 10 mice were assigned per treatment group. This sample size gave 80% power to detect a 50% reduction in tumor weight at a 5% level of statistical significance. Mouse and tumor weights and the number of tumor nodules for each group were compared using analysis of variance (ANOVA) and post-hoc analysis (Dunnett's method). Normality was tested by Kolmogrov-Smirnov test. Variables not meeting the criteria for normality were interrogated using the Mann-Whitney rank sum test using the Statistical Package for the Social Sciences (SPSS, Inc.). A P value of < 0.05 was considered statistically significant.

Results

EphA2 degradation and internalization of MEDI-547

Among the endometrial cancer cell lines tested, EphA2 protein expression was detected in the KLE, Hec-1A, and Ishikawa cells, but was absent in the SPEC-2 cells (Fig. 1*A*). Since agonist antibodies can cause internalization and degradation of EphA2, we first asked whether MEDI-547 would affect EphA2 levels. EphA2 expression showed the greatest decrease following treatment with 1C1 or MEDI-547 compared with control (PBS) or control IgG -mcMMAF in Hec-1A and Ishikawa cells at 48h after treatment, and gradual return of expression by 96 h (Fig. 1*B*). After stimulation of the EphA2 receptor on Hec-1A and Ishikawa cells with MEDI-547 for 20 minutes, antibody internalization was detected by immunofluorescence (Fig. 1*C*). These results were not observed in the EphA2 negative SKMel 28 cells (data not shown).

In vitro sensitivity of endometrial cancer cells to MEDI-547

We next tested the effect of MEDI-547 on *in vitro* viability of the EphA2 positive Hec-1A and Ishikawa cells. The effect of MEDI-547 was tested at doses ranging from 10 to 50,000 ng/mL. In the Hec-1A cells, compared to controls (either 1C1 or control IgG-mcMMAF), growth was significantly inhibited by MEDI-547 in a dose-dependent manner (Fig. 2*A*). Similar results were noted with the Ishikawa cells (Fig. S1*A*). But there was no effect in EPhA2-negative SPEC-2 cells (Fig. S2). Given the decrease in cell viability following treatment, we next asked whether the effects were apoptotic in nature. Treatment with MEDI-547 demonstrated a significant increase in apoptosis compared to treatment with the controls in the Hec-1A ($P < 0.05$, Fig. 2*B*), and Ishikawa ($P < 0.05$, Fig. S1*B*) cells.

In vivo tumor growth inhibition of MEDI-547 treated endometrial carcinoma

On the basis of the observed effects for cytotoxicity and apoptosis on endometrial cancer cells, we next performed several *in vivo* experiments using an orthotopic mouse model of uterine cancer to examine the potential therapeutic efficacy of MEDI-547. Mice injected with either Hec-1A or Ishikawa were assigned to one of four groups (n = 10 mice per group): 1) PBS; 2) 1C1, 3 mg/kg once a week; 3) control IgG-mcMMAF, 3 mg/kg once a

week; or 4) MEDI-547, 3 mg/kg once a week. Following 5-6 weeks of therapy, the mice were sacrificed and necropsies were performed. Fig. 4*B* showed that the primary tumor of endometrial carcinoma indeed developed in the uterine cavity and had metastases to ovary. Prolonged MEDI-547 therapy led to a significant reduction in tumor growth in the Hec-1A and Ishikawa models compared with control (PBS), 1C1, or control IgG-mcMMAF (each $P < 0.01$, Fig. 3*A* and *B*, respectively). We next asked whether the MEDI-547 has anti-angiogenic effects *in vivo* since it also recognizes murine EphA2 (18). To address this question, we used the EphA2 negative SPEC-2 cells (Fig. 1*A*). The tumor vasculature is known to express higher EphA2 levels compared to normal endothelial cells (18). Although the mean tumor weight of MEDI-547 treated group was lower than the other groups, the difference did not reach statistical significance (Fig. 3*C*). No obvious signs of toxicity were observed in the treatment groups (i.e. body weight loss).

In the Hec-1A model, metastases developed to the pelvis, omentum, mesentery, porta hepatis, perisplenic area, liver, para-aortic lymph nodes, and diaphragm. We also examined whether MEDI-547 therapy could reduce metastatic spread. When we assessed the number of metastases by site (n = 10 per group), treatment with MEDI-547 resulted in substantially lower frequency of metastases compared with the 1C1 or control IgG-mcMMAF controls, especially in mesentery, liver, and para-aortic nodes (Fig. 4*A*). And in a representative sample, histologic slides showed that the endometrial cancer had developed from the uterine cavity (Fig. 4*B*) and showed ovarian metastasis (Fig. 4*C*).

Effect of MEDI-547 on cell proliferation, apoptosis, and anti-angiogenesis

To determine potential mechanisms underlying the anti-tumor activity of MEDI-547 treatment, we examined the effect of MEDI-547 therapy on tumor cell proliferation by using PCNA staining. In the Hec-1A model, tumor cell proliferation was significantly reduced following MEDI-547 treatment compared with controls ($P < 0.05$, Fig. 5). Similar results were noted in the Ishikawa model ($P < 0.05$, Fig. S3). TUNEL staining revealed that MEDI-547 treatment also resulted in significantly increased apoptosis in the Hec-1A ($P < 0.05$, Fig. 5) and Ishikawa ($P < 0.05$, Fig. S3) models. To test for potential direct effects on the tumor vasculature, we performed dual immunofluorescence (CD31/TUNEL) staining following short-term treatment. There was a significant increase in endothelial cell apoptosis in the MEDI-547 group in both Hec-1A ($P < 0.05$, Fig. 5) and Ishikawa ($P < 0.05$, Fig. S3) models. These data suggest that the MEDI-547 therapy exerts anti-tumor effects by both direct and indirect effects on tumor growth. Moreover, in the EphA2-negative SPEC-2 model, while tumor cell PCNA and TUNEL expression was not significantly lower, there was significantly greater endothelial cell apoptosis with MEDI-547 treatment ($P < 0.05$, data not shown).

Discussion

The identification of effective therapies for the treatment of endometrial cancer is critical. Although current therapies have included various chemotherapeutics, radiation and hormonal therapy recent reports suggest that biologics such as bevacizumab or cetuximab may be efficacious in endometrial cancer (23) (24). The EphA2 antibody drug conjugate (MEDI-547) selectively delivers a tubulin polymerization inhibitor to EphA2 expressing tumors resulting in cell death and the inhibition of tumor growth.

The key findings from this study are that MEDI-547, effectively inhibits uterine cancer growth in orthotopic xenograft tumor models without overt signs of toxicity (i.e. weight loss). Our data indicate that MEDI-547 is internalized by the EphA2-positive cells, resulting in cytotoxicity. Moreover, MEDI-547 demonstrates therapeutic activity in orthotopic animal models by both direct and possibly indirect effects on tumor cells.

The microtubule inhibitors, paclitaxel and docetaxel, in combination therapy, are used to treat endometrial cancer patients and have shown some promise. We would anticipate that MEDI-547 treatment may be useful for endometrial cancer patients by delivery of the microtubule inhibitor (MMAF) to the tumor. Derivatives of MMAF have been successfully used as potent cytotoxic agents that are delivered by conjugated antibodies (25). Various linker and auristatin combinations can specifically kill tumor cells after internalization through cell surface antigens, such as CD30 (26), CD70 (27), and CD79 (28). The covalent linkage is highly stable until the antibody-drug conjugate enters lysosomal vesicles, which allows for drug release in the cytoplasm and the disruption of microtubule polymerization (29-31). In addition to endometrial cancer, MEDI-547 was previously reported to effectively inhibit the growth of human prostate cancer xenografts (12).

We have recently reported the use of a novel orthotopic model for uterine cancer that recapitulates the pattern of spread seen in endometrial cancer patients (15). A major drawback of using subcutaneous xenograft models is that the microenvironment is quite different compare to tumors grown in the native organ (32-33). In the current study, we used the orthotopic uterine cancer model using Hec-1A or Ishikawa cells. Following tumor growth within the uterus, there was spread to multiple locations in the abdominal cavity. MEDI-547 treatment was effective in reducing metastatic spread as well as primary tumor and total tumor weight (Fig. 4).

EphA2 protein has been reported to be overexpressed in approximately 50% of ovarian, prostate, colon, and brain cancers (34). Whereas the expression of EphA2 receptor in human cancer has been well established, little is known about the normal adult tissue expression and function (12). Imaging studies using a radiolabeled version of the highly selective anti-EphA2 antibody 1C1, revealed preferential distribution to tumors with no accumulation in any normal mouse or rat normal tissues (35), suggesting that targeting of the MEDI-547 to normal mouse tissues is minimal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Dr. Robert Langley for helpful discussions. We also thank Donna Reynolds for assistance with immunohistochemistry. AMN and RLS are supported by the US National Cancer Institute (NCI) Department of Health and Human Services (DHHS) National Institute of Health (NIH) T32 Training Grant (T32 CA 101642). Part of this work was supported by the University of Texas M. D. Anderson Cancer Center Specialized Programs of Research Excellence (SPORE) (P50CA083639 and P50CA098258), the Marcus Foundation, and the Betty Anne Asche Murray Distinguished Professorship.

References

1. Jemal A, Murray T, Ward E, et al. Cancer statistics. CA Cancer J Clin. 2005; 2005; 55:10–30. [PubMed: 15661684]
2. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet. 2005; 366:491–505. [PubMed: 16084259]
3. Obel JC, Friberg G, Fleming GF. Chemotherapy in endometrial cancer. Clin Adv Hematol Oncol. 2006; 4:459–68. [PubMed: 16981669]
4. Humber CE, Tierney JF, Symonds RP, et al. Chemotherapy for advanced, recurrent or metastatic endometrial cancer: a systematic review of Cochrane collaboration. Ann Oncol. 2007; 18:409–20. [PubMed: 17150999]
5. Strome SE, Sausville EA, Mann D. A mechanistic perspective of monoclonal antibodies in cancer therapy beyond target-related effects. Oncologist. 2007; 12:1084–95. [PubMed: 17914078]

6. Nowakowski GS, Witzig TE. Radioimmunotherapy for B-cell non-Hodgkin lymphoma. Clin Adv Hematol Oncol. 2006; 4:225–31. [PubMed: 16728934]
7. Cheson BD. Radioimmunotherapy of non-Hodgkin lymphomas. Blood. 2003; 101:391–8. [PubMed: 12393555]
8. van Der Velden VH, te Marvelde JG, Hoogeveen PG, et al. Targeting of the CD33-calicheamicin immunoconjugate Mylotarg (CMA-676) in acute myeloid leukemia: in vivo and in vitro saturation and internalization by leukemic and normal myeloid cells. Blood. 2001; 97:3197–204. [PubMed: 11342449]
9. Walker-Daniels J, Hess AR, Hendrix MJ, Kinch MS. Differential regulation of EphA2 in normal and malignant cells. Am J Pathol. 2003; 162:1037–42. [PubMed: 12651595]
10. Pasquale EB. Eph receptor signalling casts a wide net on cell behaviour. Nat Rev Mol Cell Biol. 2005; 6:462–75. [PubMed: 15928710]
11. Kamat AA, Coffey D, Merritt WM, et al. EphA2 overexpression is associated with lack of hormone receptor expression and poor outcome in endometrial cancer. Cancer. 2009; 115:2684–92. [PubMed: 19396818]
12. Jackson D, Gooya J, Mao S, et al. A human antibody-drug conjugate targeting EphA2 inhibits tumor growth in vivo. Cancer Res. 2008; 68:9367–74. [PubMed: 19010911]
13. Doronina SO, Mendelsohn BA, Bovee TD, et al. Enhanced activity of monomethylauristatin F through monoclonal antibody delivery: effects of linker technology on efficacy and toxicity. Bioconjug Chem. 2006; 17:114–24. [PubMed: 16417259]
14. Lee JW, Han HD, Shahzad MM, et al. EphA2 immunoconjugate as molecularly targeted chemotherapy for ovarian carcinoma. J Natl Cancer Inst. 2009; 101:1193–205. [PubMed: 19641174]
15. Kamat AA, Merritt WM, Coffey D, et al. Clinical and biological significance of vascular endothelial growth factor in endometrial cancer. Clin Cancer Res. 2007; 13:7487–95. [PubMed: 18094433]
16. Berry KK, Siegal GP, Boyd JA, Singh RK, Fidler IJ. Development of a metastatic model for human endometrial carcinoma using orthotopic implantation in nude mice. Int J Oncol. 1994; 4:1163–71. [PubMed: 21567033]
17. Halder J, Kamat AA, Landen CN Jr. et al. Focal adhesion kinase targeting using in vivo short interfering RNA delivery in neutral liposomes for ovarian carcinoma therapy. Clin Cancer Res. 2006; 12:4916–24. [PubMed: 16914580]
18. Landen CN Jr. Lu C, Han LY, et al. Efficacy and antivascular effects of EphA2 reduction with an agonistic antibody in ovarian cancer. J Natl Cancer Inst. 2006; 98:1558–70. [PubMed: 17077358]
19. Landen CN, Kinch MS, Sood AK. EphA2 as a target for ovarian cancer therapy. Expert Opin Ther Targets. 2005; 9:1179–87. [PubMed: 16300469]
20. Shishodia S, Majumdar S, Banerjee S, Aggarwal BB. Ursolic acid inhibits nuclear factor-kappaB activation induced by carcinogenic agents through suppression of IkappaBalpha kinase and p65 phosphorylation: correlation with down-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1. Cancer Res. 2003; 63:4375–83. [PubMed: 12907607]
21. Apte SM, Fan D, Killion JJ, Fidler IJ. Targeting the platelet-derived growth factor receptor in antivascular therapy for human ovarian carcinoma. Clin Cancer Res. 2004; 10:897–908. [PubMed: 14871965]
22. Baker CH, Kedar D, McCarty MF, et al. Blockade of epidermal growth factor receptor signaling on tumor cells and tumor-associated endothelial cells for therapy of human carcinomas. Am J Pathol. 2002; 161:929–38. [PubMed: 12213721]
23. Wright JD, Powell MA, Rader JS, Mutch DG, Gibb RK. Bevacizumab therapy in patients with recurrent uterine neoplasms. Anticancer Res. 2007; 27:3525–8. [PubMed: 17972512]
24. Takahashi K, Saga Y, Mizukami H, et al. Cetuximab inhibits growth, peritoneal dissemination, and lymph node and lung metastasis of endometrial cancer, and prolongs host survival. Int J Oncol. 2009; 35:725–9. [PubMed: 19724908]
25. Doronina SO, Toki BE, Torgov MY, et al. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat Biotechnol. 2003; 21:778–84. [PubMed: 12778055]

26. Francisco JA, Cerveny CG, Meyer DL, et al. cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. Blood. 2003; 102:1458–65. [PubMed: 12714494]
27. Law CL, Gordon KA, Toki BE, et al. Lymphocyte activation antigen CD70 expressed by renal cell carcinoma is a potential therapeutic target for anti-CD70 antibody-drug conjugates. Cancer Res. 2006; 66:2328–37. [PubMed: 16489038]
28. Ricart AD, Tolcher AW. Technology insight: cytotoxic drug immunoconjugates for cancer therapy. Nat Clin Pract Oncol. 2007; 4:245–55. [PubMed: 17392715]
29. Sutherland MS, Sanderson RJ, Gordon KA, et al. Lysosomal trafficking and cysteine protease metabolism confer target-specific cytotoxicity by peptide-linked anti-CD30-auristatin conjugates. J Biol Chem. 2006; 281:10540–7. [PubMed: 16484228]
30. Jeffrey SC, Torgov MY, Andreyka JB, et al. Design, synthesis, and in vitro evaluation of dipeptide-based antibody minor groove binder conjugates. J Med Chem. 2005; 48:1344–58. [PubMed: 15743178]
31. Jeffrey SC, Andreyka JB, Bernhardt SX, et al. Development and properties of beta-glucuronide linkers for monoclonal antibody-drug conjugates. Bioconjug Chem. 2006; 17:831–40. [PubMed: 16704224]
32. Sausville EA, Burger AM. Contributions of human tumor xenografts to anticancer drug development. Cancer Res. 2006; 66:3351–4. discussion 4. [PubMed: 16585151]
33. Dai D, Holmes AM, Nguyen T, et al. A potential synergistic anticancer effect of paclitaxel and amifostine on endometrial cancer. Cancer Res. 2005; 65:9517–24. [PubMed: 16230417]
34. Surawska H, Ma PC, Salgia R. The role of ephrins and Eph receptors in cancer. Cytokine Growth Factor Rev. 2004; 15:419–33. [PubMed: 15561600]
35. Cai W, Ebrahimnejad A, Chen K, et al. Quantitative radioimmunoPET imaging of EphA2 in tumor-bearing mice. Eur J Nucl Med Mol Imaging. 2007; 34:2024–36. [PubMed: 17673999]

Statement of Translational Relevance

EphA2 overexpression is frequently observed in endometrial cancers, and is predictive of poor clinical outcome. Due to the relatively low expression in normal adult tissues, EphA2 may represent a novel target for molecularly targeted delivery of cytotoxic agents. Here, we utilize an antibody drug conjugate (MEDI-547) composed of a fully human monoclonal antibody against both human and murine EphA2 (1C1) and the tubulin polymerization inhibitor, monomethylauristatin F (MMAF). MEDI-547 specifically bound to and was internalized by EphA2-positive cells (Hec-1A and Ishikawa). Moreover, *in vitro* cytotoxicity and apoptosis assays demonstrated that MEDI-547 decreased viability and increased apoptosis of Hec-1A and Ishikawa cells. *In vivo* therapy experiments in mouse orthotopic models with MEDI-547 resulted in significant growth inhibition in the orthotopic Hec-1A and Ishikawa models.

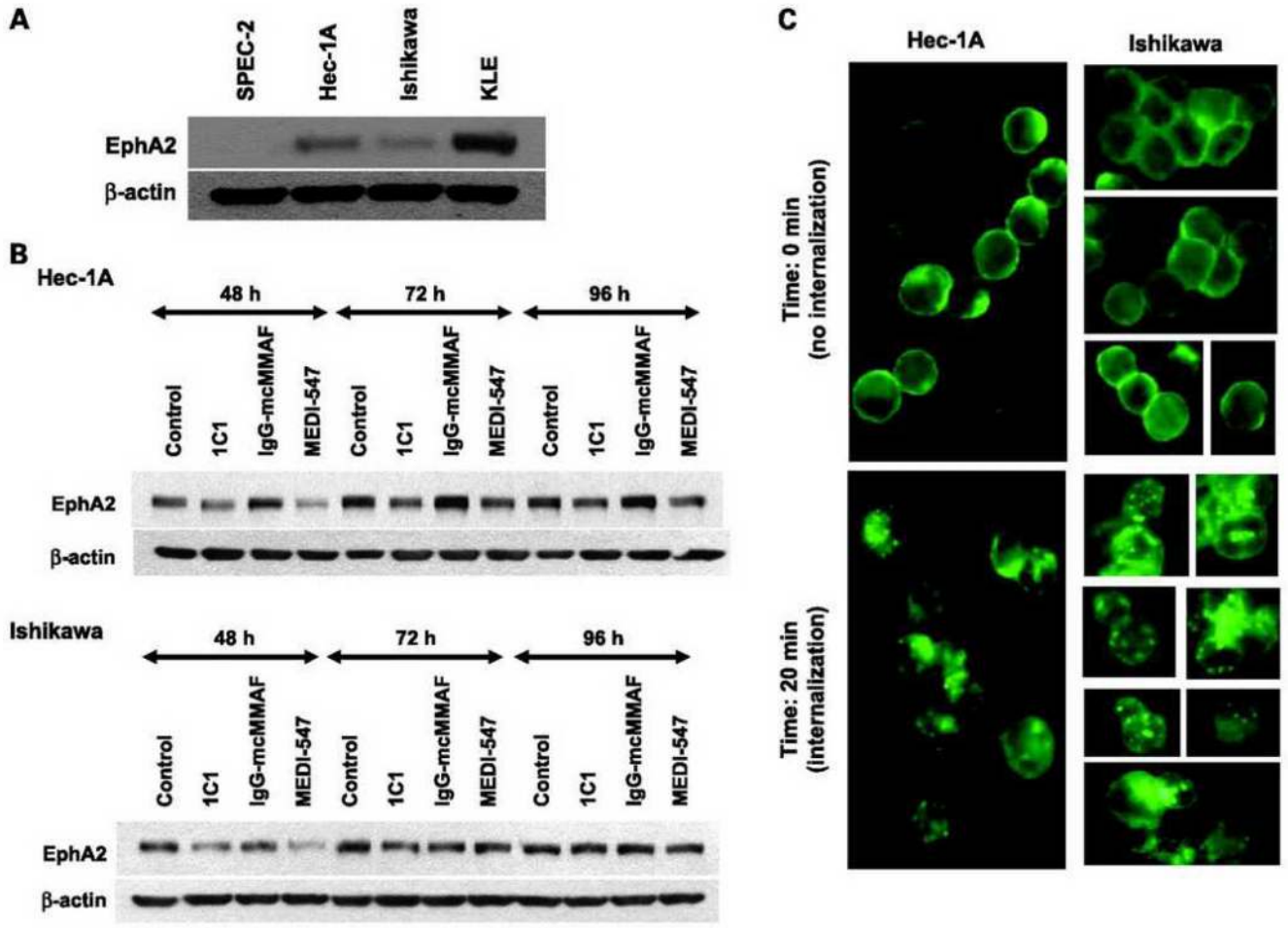

Figure 1.

EphA2 expression in endometrial cancer cell lines and the assessment of antibody degradation and internalization of EphA2 antibody drug conjugates. *A*, EphA2 expression in several endometrial cancer cells was assessed by Western blot. *B*, Effect of 1C1 or MEDI-547 on EphA2 levels in Hec-1A and Ishikawa cells was assessed by Western blot. Cells were treated with PBS (control), 1C1, control IgG-mcMMAF, or MEDI-547 (each at 100 ng/mL) for 48, 72, or 96hrs. *C*, Internalization of EphA2 on Hec-1A and Ishikawa cells treated with MEDI-547. Cells were immunostained for EphA2 with phosphate-buffered saline (PBS) containing MEDI-547. (5 μg) and then incubated for 20 minutes at 4°C. The experiment was performed three times.

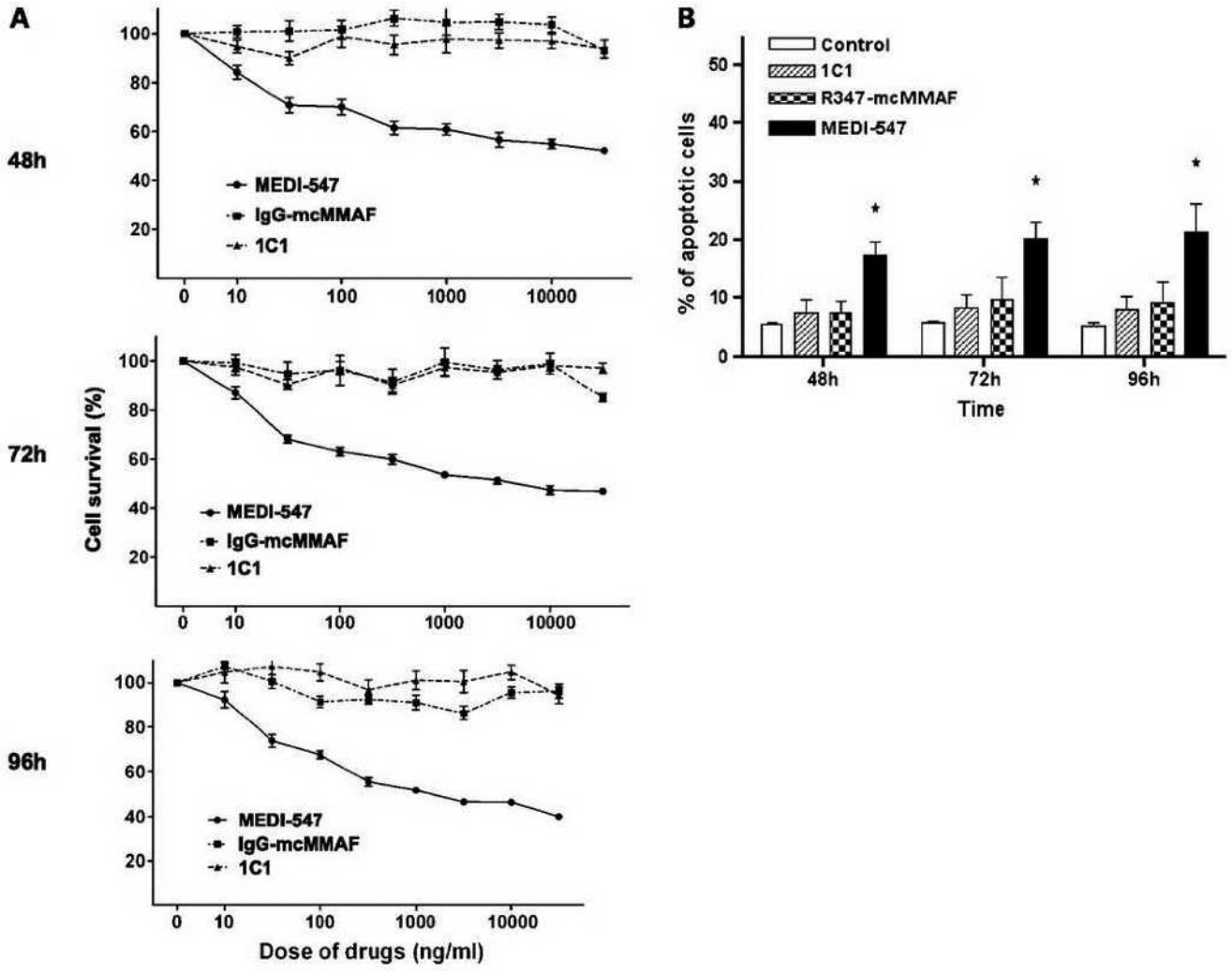

Figure 2.

Effect of MEDI-547 on *in vitro* tumor cell viability. *A*, Viability of cultured Hec-1A cells after treatment with 1C1 or antibody drug conjugates (IgG-mcMMAF or MEDI-547). Viability was assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at 48, 72, or 96 hours after treatment with 1C1, control IgG-mcMMAF, or MEDI-547 at 10 - 50,000 ng/mL. Results were confirmed with duplicate experiments. Bars represent S.E. *B*, Apoptotic cells as detected by flow cytometry. Induction of apoptosis in Hec-1A cells after treatment with 500ng/ml of PBS (control), 1C1, IgG-mcMMAF, or MEDI-547 for 24 h to 72h. Apoptosis was examined by the Annexin V and propidium iodide dual staining assay, as detected by flow cytometry. The ratio of positive-staining cells was calculated and presented as mean ± SD. **P* < 0.01. Each experiment was preformed two times, with similar results in both experiments.

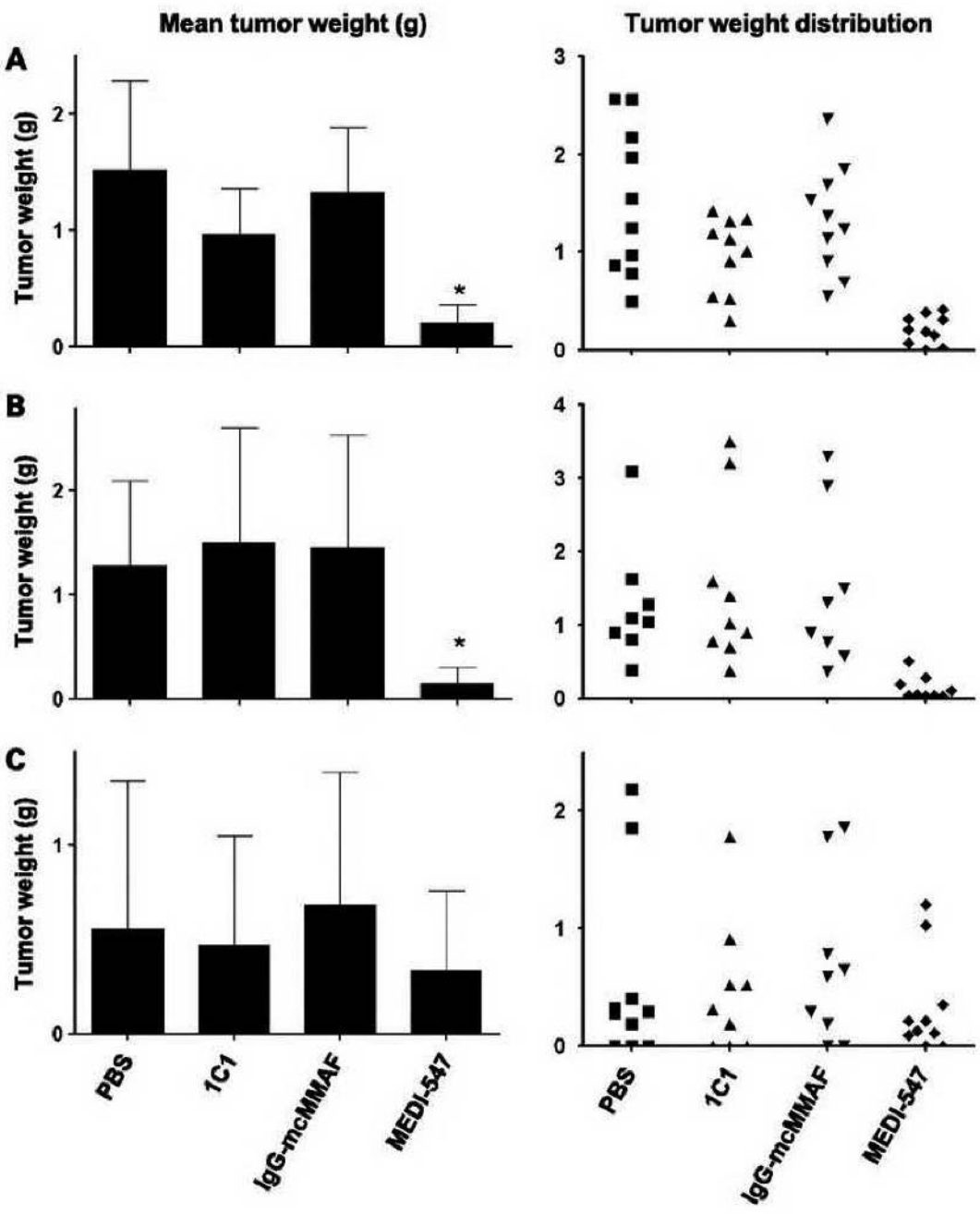

Figure 3.

Effect of MEDI-547 therapy on endometrial cancer growth. Mice inoculated with Hec-1A *A*, Ishikawa *B*, or SPEC-2 *C*, received PBS (control), 1C1, control IgG-mcMMAF, or MEDI-547 (each at 3 mg/kg) after 2 weeks following cell line injection. Animals from all groups were sacrificed when control animals became moribund (5-6 weeks after initiating therapy depending on the cell line used). All tumors were harvested; mean tumor weight and its distribution were recorded. Columns, mean tumor weights for each samples group; bars, SD. *$P < 0.01$

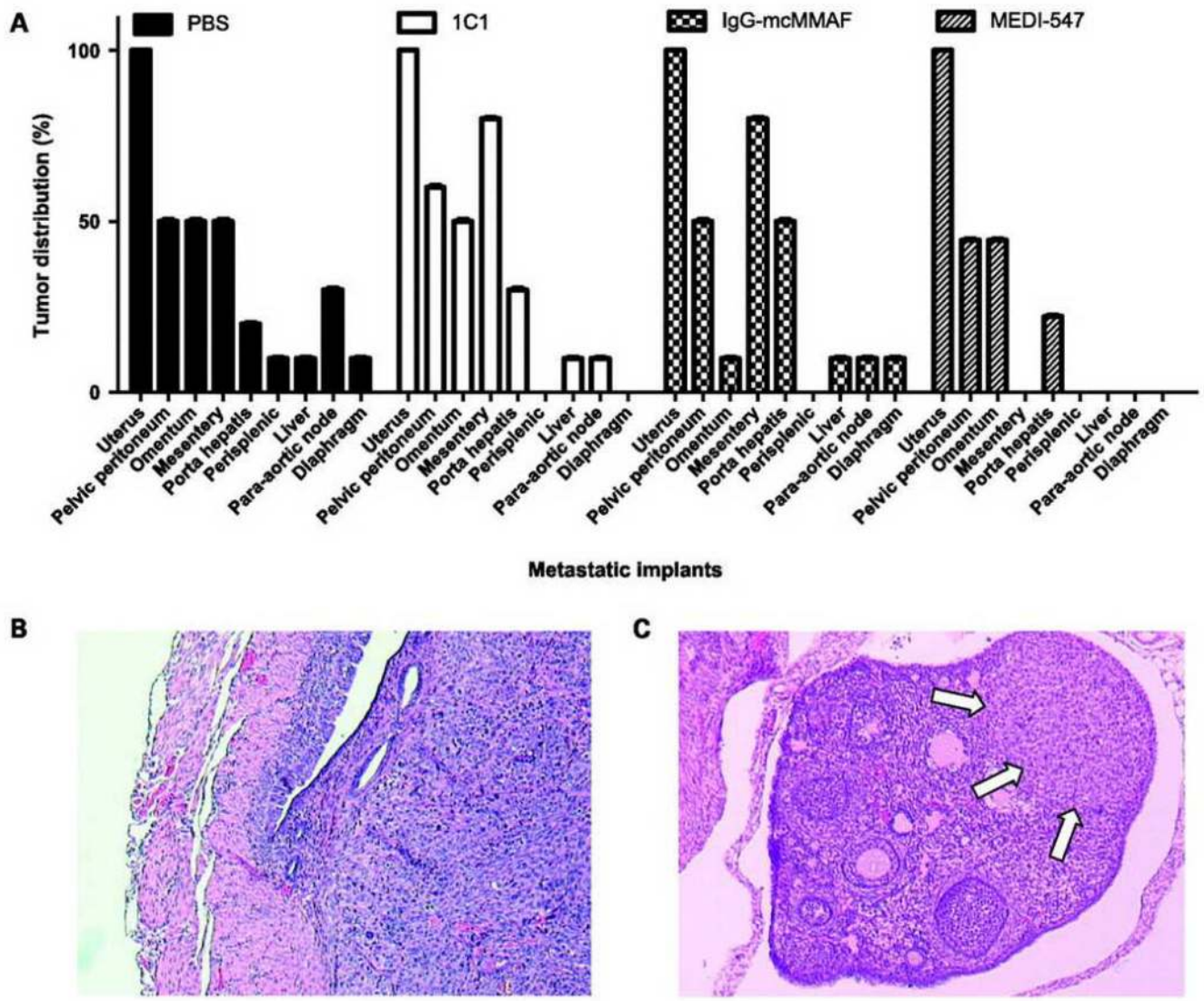

Figure 4.

Tumor distribution of Hec1A mouse model. *A*, MEDI-547 treated group had relatively less loci of distant metastasis, especially mesentery, liver, and para-aortic nodes. Histologic slides showed that the endometrial cancer had developed from the uterine cavity *B*, and showed ovarian metastasis (arrowheads) *C*.

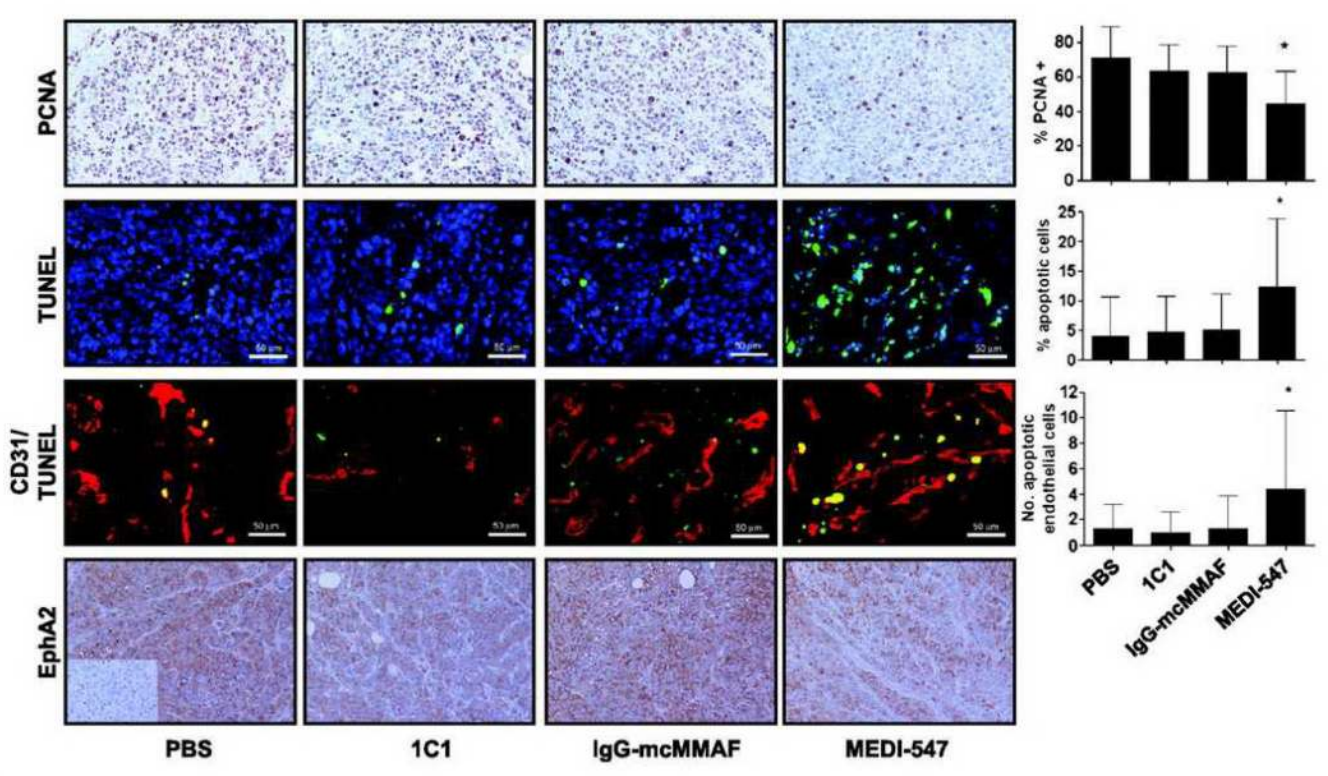

Figure 5.

In vivo effects of MEDI-547 on proliferation and apoptosis of tumor or endothelial cells in the Hec-1A model. *A*, Tumor sections were stained with PCNA to reveal tumor cell proliferation. PCNA-positive cells were counted and graphed. **P* < 0.05. *B*, Tumor sections were stained with Hoechst (blue) and TUNEL (green) using double immunofluorescence staining. The tumor cells undergoing apoptosis show green fluorescence. *C*, Tumor sections were stained with CD31 (red) and TUNEL (green) using double immunofluorescence staining. Colocalization of endothelial cells undergoing apoptosis show yellow fluorescence. *D*, EphA2 expression in the tumor tissues taken at the time of sacrifice (three days after injection of last dose) was assessed by immunohistochemistry. **Inset** in PBS panel represents a negative control. Error bars represent SD. **P* < 0.05.