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Endometrial Cancers Harboring Mutated Fibroblast Growth Factor Receptor 2 Protein Are Successfully Treated With a New Small Tyrosine Kinase Inhibitor in an Orthotopic Mouse Model

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Objectives: AL3818 (anlotinib) is a receptor tyrosine kinase inhibitor targeting vascular endothelial growth factor receptors (VEGFR1, VEGFR2/KDR, and VEGFR3), stem cell factor receptor (C-kit), platelet-derived growth factor (PDGFR β), and fibroblast growth factor receptors (FGFR1, FGFR2, and FGFR3). This study evaluates the efficacy of AL3818 studying tumor regression in an orthotopic murine endometrial cancer model.

Methods: We tested the cytotoxicity of AL3818 on a panel of 7 human endometrial cancer cell lines expressing either wild-type or mutant FGFR2 and also assessed the in vivo antitumor efficacy in a murine, orthotopic AN3CA endometrial cancer model. AL3818 was administered daily per os either alone or in combination with carboplatin and paclitaxel, which represent the current standard of adjuvant care for endometrial cancer.

Results: AL3818 significantly reduces AN3CA cell number in vitro, characterized by high expression of a mutated FGFR2 protein. Daily oral administration of AL3818 (5 mg/kg) resulted in a complete response in 55% of animals treated and in a reduced tumor volume, as well as decreased tumor weights of AN3CA tumors by 94% and 96%, respectively, following a 29-day treatment cycle. Whereas carboplatin and paclitaxel failed to alter tumor growth, the combination with AL3818 did not seem to exhibit a superior effect when compared with AL3818 treatment alone.

Conclusions: AL3818 shows superior efficacy for the treatment of endometrial cancer irrespective to conventional carboplatin and paclitaxel combination and warrants further investigation.

Key Words: Carboplatin and paclitaxel, Endometrial cancer, Mutant FGFR2, Orthotopic murine model, Tyrosine kinase inhibitor

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Uterine epithelial adenocarcinoma is the most common of all uterine malignancies and is the most common gynecologic cancer in the United States diagnosed, with an estimated 60,130 new cases and 10,920 deaths in 2017.¹ Endometrial cancer (EC) occurs mainly in postmenopausal women. The prognosis remains favorable for patients diagnosed at an early stage (International Federation of Gynecology and Obstetrics stage I–II), with an overall 5-year relative survival of 81.7% but unfortunately has steadily decreased since 1975 (88%).²

The most common surgical treatment for all stages of EC is total hysterectomy, which also may include bilateral salpingo-oophorectomy. Women diagnosed as having a low-grade endometrioid tumor usually do not require any additional treatment following surgery.³ However, 10% to 15% of women with a presurgical diagnosis of a grade I EC will harbor higher-grade lesions within their tumors and require additional therapies to help prevent a rapid recurrence.⁴ Treatments usually include platinum-based chemotherapy in combination with paclitaxel and/or doxorubicin,⁵ radiotherapy, and hormonal therapy either alone or in combination with the above.⁶ The optimum therapeutic regimen is yet to be established as the overall 5-year survival rate of patients following their first recurrence is only 19.2%.⁴

In recent years, the molecular characterization of EC has identified potential therapeutic targets including vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR). Both signaling pathways have been reported to be deregulated in EC.^{7,8} Activating somatic mutations of FGFR2 were identified in 30% of EC cell lines and in 10% of primary ECs.⁹ Mutations were essentially associated with a type I endometrioid histologic subtype (48/466 cases).¹⁰ The inhibition of the mutated FGFR2 decreases EC cell survival.⁷ Previous studies using either a multikinase inhibitor (ie, dicitinib) against VEGFR, PDGFR, FGFR, c-Kit, and FLT3 or a more selective pan-FGFR inhibitor (ie, NVP-BGJ398) promoted the reduction of the tumor size in vivo in a subcutaneous xenograft mouse model of EC harboring either wild-type or mutated FGFR2.⁸

In this study, we propose to evaluate the efficacy of AL3818 (anlotinib), a newly developed small-molecule receptor tyrosine kinase inhibitor, as a potent growth inhibitor of human EC cell lines in accordance with their expression of either wild-type or mutant FGFR2. AL3818 was found to be a potent inhibitor of VEGFR1 to VEGFR3, FGFR1 to FGFR3, c-Kit, and PDGFR β .¹¹ Also, we demonstrate the safety and anticancer efficacy of AL3818 in an orthotopic mouse model of human EC harboring mutant FGFR2 expression.

MATERIALS AND METHODS

Materials

Carboplatin (CARBOplatin Injection; Hospira, Lake Forest, IL) and paclitaxel (Paclitaxel Injection, USP; Hospira)

were obtained from the University of Utah hospital pharmacy. AL3818 was provided by Advanchem Laboratories LLC (Moorpark, CA). Dulbecco modified Eagle medium: nutrient mixture F-12 (DMEM/F12), Eagle minimum essential medium, minimum essential medium, RPMI-1640, and McCoy 5a were obtained from American Type Culture Collection (Manassas, VA). Fetal bovine serum (FBS) (Hyclone) was purchased from (GE Healthcare Lifesciences, Marlborough, MA).

Cell Lines

AN3CA, HEC1B, HEC1A, and KLE cells were purchased from American Type Culture Collection. MFE280 and MFE296 cells were obtained from Dr Johnathan M. Lancaster (Moffitt Cancer Center, Tampa, FL). Ishikawa cell line was kindly donated by Dr Victoria Bae-Jump (University of North Carolina, Chapel Hill, NC).

Cell Cultures

AN3CA, HEC1B, and MFE280 cells were maintained in Eagle minimum essential medium supplemented with 10% FBS. HEC1A, KLE, and MFE296 cells were cultured in McCoy 5a, DMEM/F12, or RPMI-1640, respectively, supplemented with 10% FBS. Ishikawa cells were maintained in minimum essential medium supplemented with 5% FBS, 1% nonessential amino acids. In all media, 100 U/mL penicillin and 100 μ g/mL of streptomycin were added.

Cell Number Determination Assay

AN3CA (1.2×10^4 cells/well), HEC1B (10^4 cells/well), MFE280 (10^4 cells/well), HEC1A (10^4 cells/well), KLE (1.2×10^4 cells/well), MFE296 (8×10^3 cells/well), and Ishikawa cells (6×10^3 cells/well) were seeded in 96-well plates and incubated for 24 hours at 37 °C in 5% CO₂. Cells were treated with AL3818 (range of concentrations from 0 to 100 μ M) for 72 hours. Following drug treatments, cell numbers were determined by sulforhodamine B (SRB) assay.¹² The cells were fixed using trichloroacetic acid (10%) and 96-well plates were processed as described by Vichai and Kirtikara.¹² Measurements were taken from 3 independent experiments conducted in triplicate.

Western Blotting

Cells were lysed in RIPA buffer. The lysates were cleared by centrifugation at 20,000g for 10 minutes, boiled in Laemmli buffer, subjected to polyacrylamide gel electrophoresis 4% to 20% gradient sodium dodecyl sulfate–polyacrylamide gel (Bio-Rad Laboratories, Inc, Hercules, CA), and analyzed by Western blotting using polyvinylidene difluoride membrane (Bio-Rad Laboratories, Inc). Expression of FGFR2 and β -actin was assessed with anti-FGFR2 (Cell Signaling Technology, Beverly, MA) and anti- β -actin (Sigma-Aldrich Ltd).

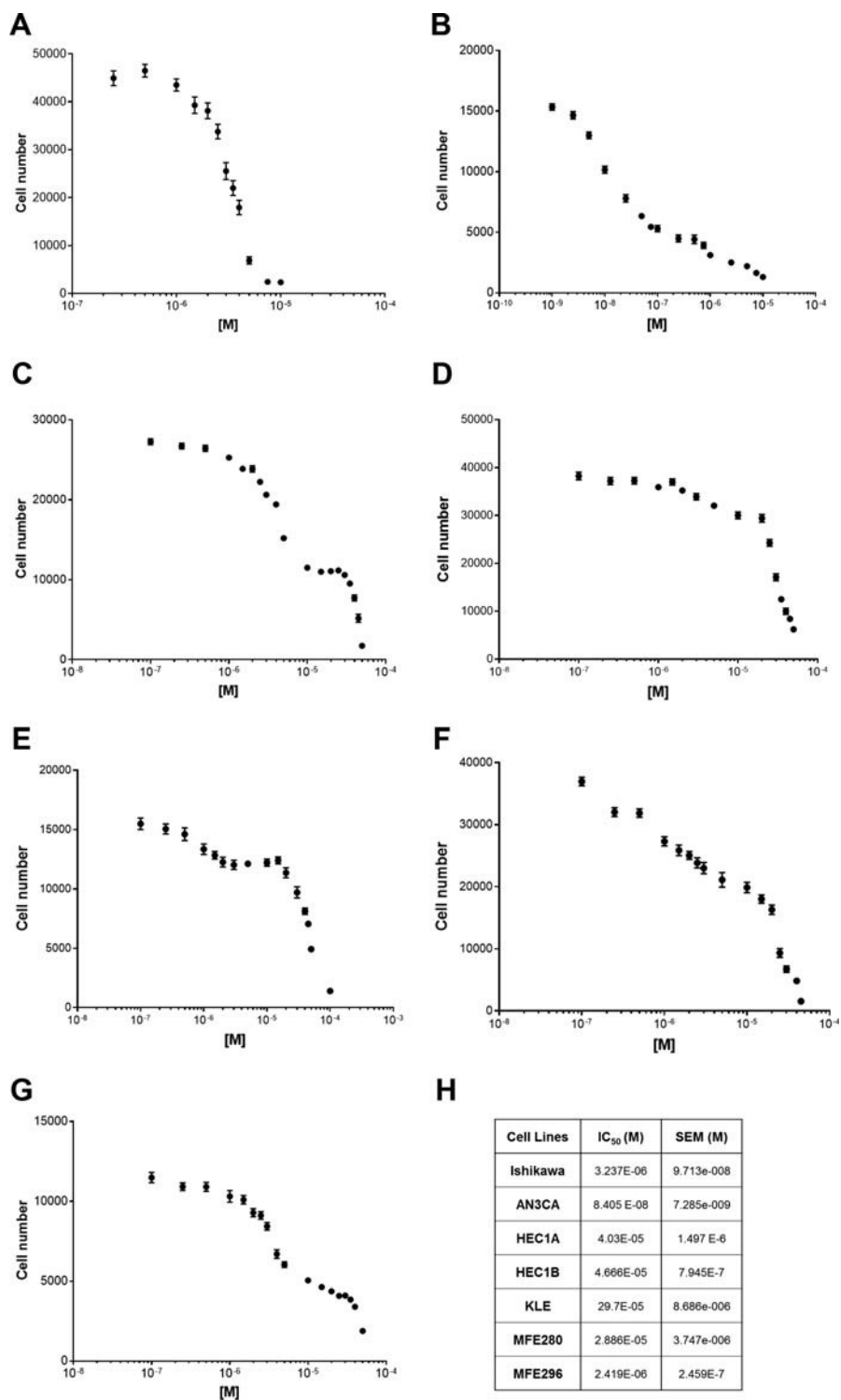


FIGURE 1. Effect of AL3818 on cell viability of a panel of 7 EC cells. Cells were treated with a range of concentrations of AL3818 and after 72-hour incubation; cell number was determined by SRB assay. Response to AL3818 treatment of EC cells as shown in (A) Ishikawa cells, (B) AN3CA cells, (C). HEC1A cells, (D) HEC1B cells, (E) KLE cells, (F) MFE280 cells, and (G) MFE296 cells. H, Table showing half maximal inhibitory concentration (IC₅₀) value of AL3818 for each cell line. Data are expressed as mean ± SEM (n = 3).

TABLE 1. Endometrial cancer cell line mutations and origin

Cell Line	Mutations		Origin	References
HEC1A	KRAS	G12D	Moderately differentiated papillary adenocarcinoma	Korch et al ¹³
	PIK3CA	G1049R		
	FGFR2	WT		
HEC1B	KRAS	G12D	Adenocarcinoma group of cells that sustained a stationary period of 135–190 culture days	Korch et al ¹³
	PIK3CA	G1049R		
	FGFR2	WT		
KLE	FGFR2	WT	Peritoneal metastases	Korch et al ¹³ and Weigelt et al ¹⁴
AN3CA	FGFR2	K310R-N550K	Lymph node metastases	Korch et al ¹³ and Weigelt et al ¹⁴
	PI3KR1	R557_K561 > Q		
	PTEN	R130fs		
Ishikawa	PI3KR1	L570P	Moderately differentiated, stage 2, endometrial adenocarcinoma	Weigelt et al ¹⁴
	FGFR2	WT		
	PTEN	V317fs; V290fs		
MFE280	FGFR2	S252W	Recurrent poorly differentiated EC	Krakstad et al ¹⁵
	PIK3CA	H1047Y; I391M		
MFE296	PIK3CA	I20M; P539R	Moderately differentiated EC	Weigelt et al ¹⁴ and Krakstad et al ¹⁵
	FGFR2	N550K		
	TEN	R130Q; N323fs		

Orthotopic Murine Endometrial Cancer Xenograft Model and Treatment

Animals were housed under standard conditions following guidelines of approved protocols by the Institutional Animal Care and Use Committee at the University of Utah. AN3CA tumor chunk generation is described in Supplementary Table 1, <http://links.lww.com/IGC/A557>. Tumor development was tracked in 3 additional mice, and AN3CA tumors were measured 2 weeks following implantation after necropsy using digital calipers. AN3CA tumor chunks were implanted orthotopically into the upper left uterine horn of 8-week-old female nude (nu/nu) mice (n = 37). Size of tumor chunks was large enough to infill the uterine horn preventing migration within the uterus. Mice were monitored daily for 15 days before drug treatments were initiated. Mice were randomly assigned into 4 experimental groups as follows: control (dimethyl sulfoxide 1%) (n = 4), AL3818 (5 mg/kg per day) (n = 11), carboplatin and paclitaxel (33 and 20 mg/kg per week, respectively) (n = 9), and combination of AL3818, carboplatin, and paclitaxel at a dosage similar to the single treatments (n = 13). AL3818 was solubilized in dimethyl sulfoxide 1% and administered daily through oral gavage. Carboplatin and paclitaxel were diluted to their respective dosage with saline (NaCl 0.9%) and administered weekly through intraperitoneal injection. Mice were monitored and weighted daily over the course of 29 days. Animals were killed on day 29, and tumors were collected and weighted. Tumors were measured

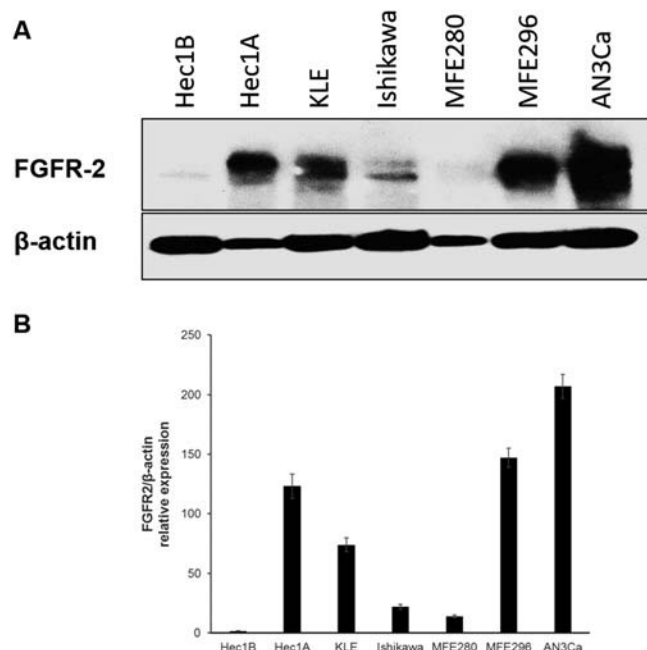


FIGURE 2. Expression of FGFR2 across a panel of EC cell lines. A, Western blot of FGFR2 and β-actin. B, Normalized expression of FGFR2 over β-actin.

using electronic calipers, and tumor volume was calculated according to the following formula: length \times width \times height) / 2. Tumor tissues were immunohistochemically stained with hematoxylin-eosin, Ki67, and CD31 (ARUP, Salt Lake City, UT).

Statistical Analysis

Data were analyzed using a 1-way analysis of variance, where $P < 0.05$ is required for statistical significance.

RESULTS

Effect of AL3818 on Cell Number

The effect of AL3818 on cell numbers of 7 EC cell lines, namely, HEC1A, HEC1B, KLE, Ishikawa, MFE280, MFE296, and AN3CA cells, was evaluated in vitro using SRB assays over a period of 72 hours. The EC cell lines showed differential sensitivity to AL3818 (Figs. 1A–G); AN3CA cells appeared the most sensitive (Fig. 1F) with an IC_{50} value of 84 nM (Fig. 1H). The other cell lines were approximately 28- to 550-fold less sensitive to AL3818. HEC1B had an IC_{50} value

of 46 μ M, and MFE296 cells were sensitive to AL3818, with an IC_{50} value of 2.9 μ M compared with 3.2, 28.9, 29, and 40 μ M for Ishikawa, MFE280, KLE, and HEC1A, respectively (Fig. 1H).

Overexpression of FGFR2 in AN3CA Cells

As shown in Table 1, the EC cell lines considered in this study harbor coexistent mutations in genes encoding proteins that are essential for the control of cell proliferation, inhibition of apoptosis, and metastasis such as activating mutations in KRAS, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PI3KCA), and FGFR2, as well as inactivating mutations in phosphatidylinositol 3-kinase regulatory subunit α gene and phosphatase and tensin homolog (PTEN). Western blot analysis of FGFR2 expression across all EC cell lines assessed in this study showed that AN3CA cells had the highest expression (Fig. 2A). When compared with other cell lines, the expression of FGFR2 protein in AN3CA cells was 1.4-, 1.7-, 2.8-, 9.5-, 14.8-, and 162.1-fold higher than in MFE296, HEC1A, KLE, Ishikawa, MFE280, and HEC1B cells, respectively (Fig. 2B). Endometrial cancer cell lines such as MFE296 and AN3CA cells with an activating N549K

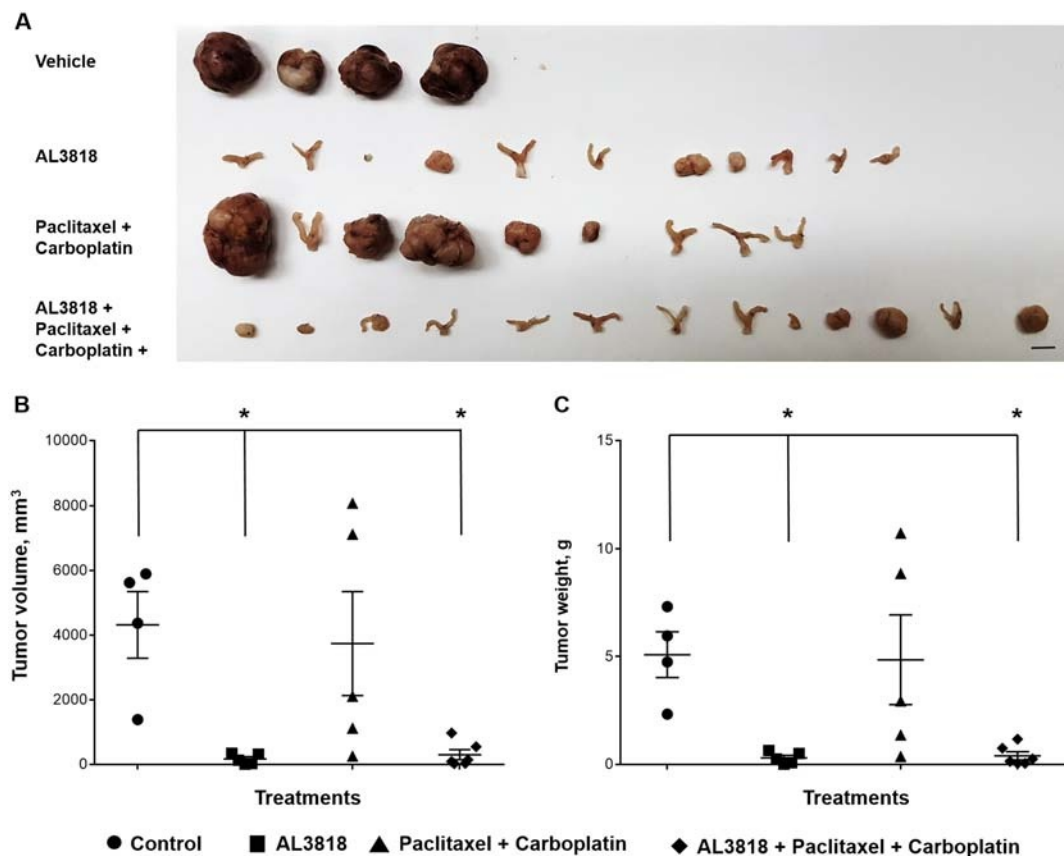


FIGURE 3. Effect of AL3818 on orthotopic AN3CA xenograft tumor growth. A, Representative images of mouse uteri and EC tumors (scale bar, 1 cm). B, Tumor volume. C, Tumor weight. Mice with orthotopic AN3CA xenografts were treated for 29 days with either AL3818 alone (5 mg/kg, orally, daily), carboplatin (33 mg/kg, intraperitoneal, weekly), and paclitaxel (20 mg/kg, intraperitoneal, weekly) or a combination of AL3818, carboplatin, and paclitaxel. Data were analyzed by a 2-way analysis of variance coupled with a Bonferroni post hoc test. *Significantly decreased when compared with vehicle control ($P < 0.05$).

mutation seemed to be the most sensitive to AL3818 treatment (Table 1, Fig. 1). AN3CA's increased sensitivity may be explained by the high level of FGFR2 expression and 2 FGFR2-activating mutations (K310R/N549K). The efficacy of AL3818 was further assessed in vivo in an orthotopic murine EC model.

AL3818 Decreases Tumor Growth in Orthotopic Murine EC Model

Before initiation of treatment, the average tumor volume was calculated as $8.4 \pm 1.5 \text{ mm}^3$ based on tumor sizes in control mice. After 29 days of drug treatment, the average tumor volume of the vehicle-only (saline)-treated group ($n = 4$, control) was $4315 \pm 894 \text{ mm}^3$, whereas the average weight was $5.01 \pm 1 \text{ g}$. Within the AL3818-treated (5 mg/kg) group ($n = 11$), tumor size was found to be reduced 26-fold, with an average tumor volume of $164.8 \pm 70 \text{ mm}^3$, when compared with the control group (Figs. 3A, B). Tumor weights were consistent with tumor sizes with average tumor weights of $0.3 \pm 0.12 \text{ g}$ for this group (Fig. 3C). Following treatment with AL3818, only 5 of the 11 mice were found to have residual tumor burden at the end of the treatment period. The histopathologic analysis of uterine tissues from these mice showed no residual disease, suggesting a complete clinical and pathological response to AL3818 treatment in these animals. Treatment response to conventional carboplatin and paclitaxel chemotherapy was found to be more heterogeneous. Nine mice received a cumulative dose of carboplatin and paclitaxel of 99 and 60 mg/kg, respectively, administered intraperitoneal weekly over 3 weeks (Fig. 3A). Four mice had a complete response without any residual tumor mass detectable on gross examination, as well as upon histopathologic analyses, whereas 5 mice exhibited tumor growth progression similar in size to the tumors in the control group. The average tumor volume in this treatment group was $3734 \pm 1611 \text{ mm}^3$, whereas the average tumor weight was $4.85 \pm 2.1 \text{ g}$ (Figs. 3B, C). The combination of AL3818 with carboplatin and paclitaxel does not seem to potentiate the effect of AL3818-only treatment. Approximately half (53%) of the mice ($n = 7/13$) in the combination group showed a complete response compared with most animals (63%) observed in the AL3818-only treatment group ($n = 7/11$) (Fig. 3A). The tumor volume in the combination treatment

group was reduced 14.5-fold when compared with the control group. The average tumor volume was $297 \pm 155 \text{ mm}^3$ (Fig. 3B), and the average tumor weight $0.4 \pm 0.19 \text{ g}$ (Fig. 3C). Also, carboplatin and paclitaxel and AL3818 either alone or in combination with carboplatin and paclitaxel did not have a significant impact on total animal body weights, suggesting that treatments were tolerable and safe as administered (Fig. 4).

AL3818 Treatment Reduces Tumor Cell Proliferation and Inhibits Neoangiogenesis

To assess vascular density within tumor tissues, immunohistochemical CD31 staining was performed. Furthermore, Ki67 stains were used as a marker of cell proliferation (Fig. 5A). Treatment with AL3818 decreased neoangiogenesis by 48.5% and cell proliferation by 27% when compared with the control group (Figs. 5B, C). Furthermore, carboplatin and paclitaxel treatment decreased cell proliferation by 18%, but did not alter the formation of blood vessels (Figs. 5B, C). Finally, the combination of AL3818, carboplatin, and paclitaxel treatment exhibited a similar decrease in blood vessel formation and cell proliferation by 20% (Figs. 5B, C). The combination of AL3818 treatment with carboplatin and paclitaxel failed to show any additive or synergistic effect, suggesting that tyrosine kinase inhibitor targeting mutant FGFR2-bearing tumors may be more efficient and could be considered as a first-line therapy rather than traditional cytostatic treatment consisting of a platinum/taxane combination.

DISCUSSION

We have assessed a multitargeted tyrosine kinase inhibitor, AL3818, targeting FGFR1 to FGFR4, c-Kit, PDGFR β , and VEGFR1 to VEGFR3 in ECs. Based on cell number determination assays, we showed that AL3818 exhibited cell number reduction in cell lines expressing a mutated FGFR2 protein. In AN3CA cells carrying a high expression of FGFR2-K310R/N550K-activating mutation, AL3818 treatment led to a more significant reduction in cell numbers when compared with other EC cell lines harboring FGFR2 wild-type or single mutations. Furthermore, we showed that a daily oral dose of AL3818 was sufficient to significantly ($P < 0.05$) reduce tumor growth in an orthotopic murine model of EC and achieved

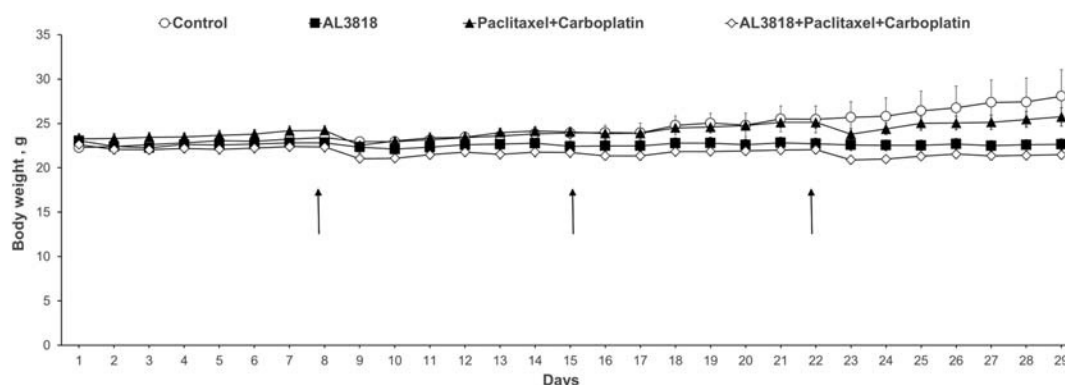


FIGURE 4. Animal body weights of AN3CA tumor-bearing mice tracked over a 29-day treatment period. Arrows indicate times of treatment with paclitaxel and carboplatin.

a complete response with no detectable tumor for 55% of animals dosed daily.

We established that the effect of AL3818 on cell number was reduced in EC cell lines expressing FGFR2 wild-type such as HEC1A, HEC1B, and KLE, except Ishikawa cells harboring sensitivity to AL3818 in the lower μM range. However, when considering mutation status of FGFR2 protein, approximately 10% to 12% of ECs are associated with mutations that confer a constitutive activation of FGFR2.^{7,10} The mutation S252W identified in MFE280 is located in the extracellular domain and increases the affinity for fibroblast growth factor, whereas N550K mutation observed in MFE296 and AN3CA cells is located in the kinase domain preventing the autoinhibitory conformation of FGFR2.¹⁶ AN3CA also carries an FGFR2 K310R mutation whose function remains uncharacterized.⁹ We found that AL3818 was most effective against AN3CA cells harboring double FGFR2 N550K/

K310R mutations. However, in EC cell lines such as MFE280 (S252W) and MFE296 (N550K) harboring only a single FGFR2 mutation, AL3818 effect on cell number was lower when compared with the response in AN3CA cells. This observation was previously described with other FGFR2 inhibitors.^{7,8} Also, the unique sensitivity of AN3CA to FGFR2 inhibitors has been observed with several other compounds such as PD173074, dovitinib, AZD4547, and NVP-BGJ398.^{7,8,17,18} However, the mechanisms of action for these inhibitors remain elusive. Moreover, the level of expression of FGFR2 protein differs largely across EC cell lines. The highest FGFR2 expression was observed in AN3CA cells, higher than in other FGFR2 mutated cell lines, MFE280 and MFE296 cells, and wild-type FGFR2 in HEC1A, KLE, Ishikawa, and HEC1B cells. It is likely that the high level of expression of mutated FGFR2 in AN3CA cells renders these cells dependent on the FGFR2 pathway for proliferation.

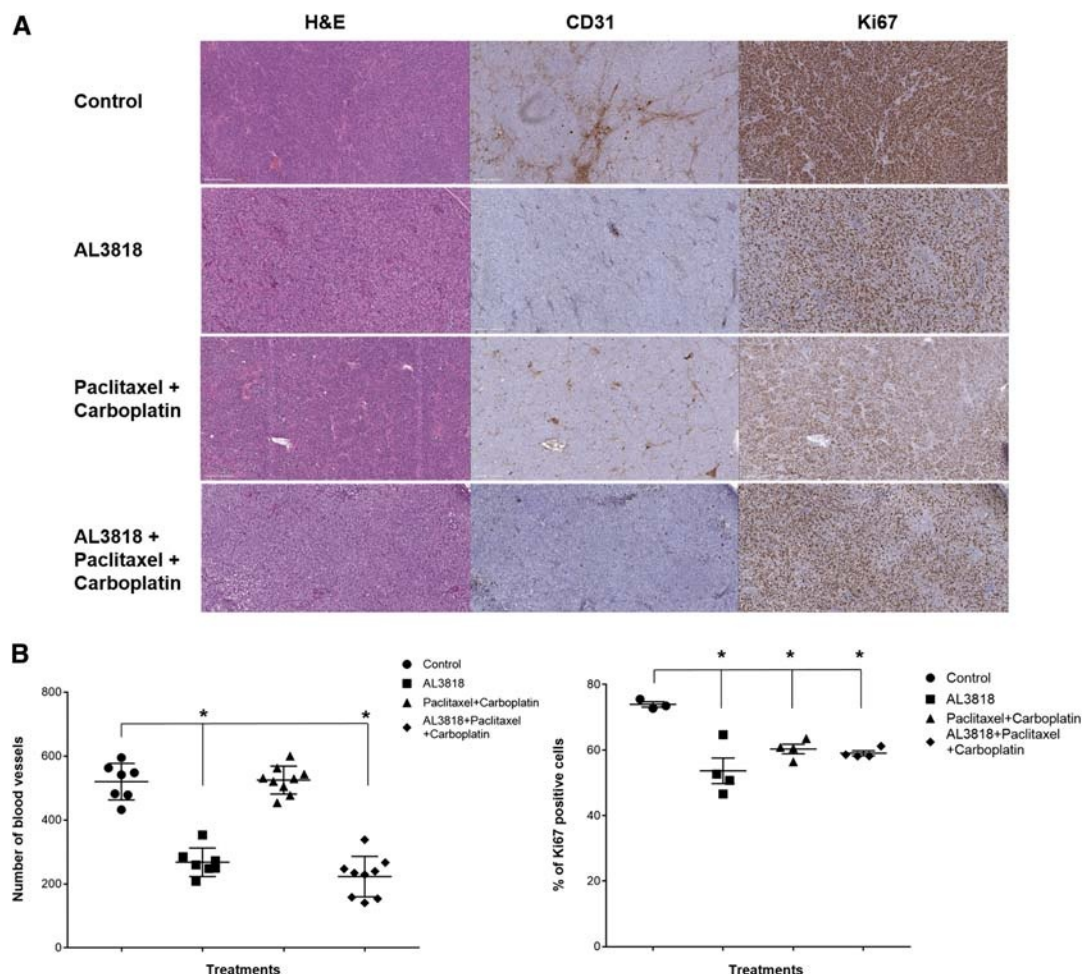


FIGURE 5. AL3818 treatment decreases neoangiogenesis and tumor cell proliferation. A, Hematoxylin-eosin, CD31, and Ki67 staining of representative tumor sections from each treatment group. B, Quantification of the number of blood vessel in each treatment group. Tissue sections stained with CD31 antibody were scanned, and number of blood vessels estimated from 5 randomly selected areas. C, Quantification of the number of Ki67-positive cells in each treatment group. Tissue sections stained with Ki67 antibody were scanned using Aperio Scancope (Leica Biosystems), and the number of Ki67-positive cells was calculated as an average from 5 randomly selected areas across each slide. *A statistically significant decrease was noted when compared with tissue from untreated vehicle control groups (* $P < 0.05$).

Hence, targeting this pathway addiction with AL3818 promotes higher cytotoxicity when compared with other EC cell lines.

We compared the treatment efficacy of AL3818 to a conventional, platinum-containing chemotherapeutic regimen for treatment of EC, and we further assessed the combination of AL3818 with carboplatin and paclitaxel in this orthotopic AN3CA EC model. Mice were treated daily with AL3818 (5 mg/kg), whereas carboplatin (33 mg/kg) and paclitaxel (20 mg/kg) were administered by intraperitoneal injection once a week for 3 weeks. The cumulative dose of carboplatin and paclitaxel was chosen as equivalent to a human dose of 300 and 180 mg/m², respectively.¹⁹ We observed that treatments were well tolerated as indicated by only a slight decrease in total animal body weights (<7%) 24 hours following injection of carboplatin and paclitaxel either alone or in combination with AL3818. Animals recovered to their preinjection weights within 3 days. Also, no weight loss was observed in animals receiving daily AL3818 doses. Treatment was initiated in 10- to 12-week-old animals after strain-specific development and physiological weight gain stabilized. Patients with advanced and recurrent EC have a rather poor prognosis, and chemotherapy often remains as the main treatment option.²⁰ The Gynecologic Oncology Group Protocol 209 clinical trial assessed the efficacy of a combination of carboplatin and paclitaxel for advanced EC^{5,21} and demonstrated a similar efficacy and a lower toxicity.²¹ In our study, all the tumors responded to AL3818 treatment as 6 animals had a complete response with no detectable tumor and 5 mice with an average tumor size 26-fold smaller than in the ones observed in the control group. The treatment was further associated with a decrease in neoangiogenesis and cancer cell proliferation. AL3818 is a multityrosine kinase inhibitor and was demonstrated to inhibit VEGFR2 and VEGFR3 in vitro,¹¹ suggesting that it may inhibit angiogenesis in vivo.

The effects of a combination of carboplatin and paclitaxel were more contrasted with complete responses observed in 4 of 9 animals, partial responses in 3 of 9 animals, and 2 of 9 animals with tumor volumes similar to the control group. Our findings seem to mimic an observation that has been made in patients with advanced EC where the response to chemotherapy was modest with rapidly developing resistance to treatment.²² It has been shown previously that the activation of FGFR signaling pathways does promote chemotherapy resistance.²³ However, the combination of AL3818 with carboplatin and paclitaxel showed similar efficacy to AL3818-only treatment. In this group, 7 animals had no detectable tumor, and 6 animals did bear tumors with an average size 17-fold smaller than the tumors observed in the control group. Based on this initial animal study, we demonstrated that AL3818 is a safe and potent therapeutic agent for the personalized treatment of EC when considering each patient's FGFR2 mutation and expression status. Additional in vivo studies are still necessary to assess dose response and a combination with additional chemotherapeutic or immunotherapeutic agents, as well as determining the underlying mechanisms of action for AL3818 in these various settings. Several clinical trials have used specific or multityrosine kinase inhibitors to target the FGFR signaling pathway in various cancer types.²⁴ Dovitinib, a

multityrosine kinase inhibitor, showed clinical benefits, but independent of FGFR2 mutation status.²⁵ Brivanib, a VEGFR tyrosine kinase inhibitor and weak FGFR inhibitor, also demonstrated clinical benefits.²⁶ However, in both cases, effects may have been mediated through antiangiogenic mechanisms.^{25,26} Specific FGFR inhibitors including BGJ398,²⁷ AZD4547,²⁸ and JNJ-42756493²⁹ are currently being assessed and seem to show efficacy for the treatment of various cancers. The safety and pharmacokinetics of AL3818 are currently also being evaluated in a phase 1B/2A clinical study for patients with gynecological malignancies including ECs, and initial results reported a dose of 12 mg to be well tolerated.³⁰ The potential relationship between drug response and the FGFR2 mutational status in EC gives hope that these types of agents will become a viable alternative to current treatment approaches of EC supporting the goal of improved patient survival with decreased or more easily managed toxicities.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin*. 2017;67:7–30.
2. Howlander N, Noone A, Krapcho M, et al. SEER Cancer Statistics Review, 1975–2013. Bethesda, MD: National Cancer Institute. Based on November 2015 SEER data submission, posted to the SEER Web site, Available at: https://seer.cancer.gov/csr/1975_2013/. Accessed April 2016.
3. DeSantis CE, Lin CC, Mariotto AB, et al. Cancer treatment and survivorship statistics, 2014. *CA Cancer J Clin*. 2014;64:252–271.
4. Sorbe B, Juresta C, Ahlin C. Natural history of recurrences in endometrial carcinoma. *Oncol Lett*. 2014;8:1800–1806.
5. Miller D, Filiaci V, Fleming G, et al. Late-breaking abstract 1: randomized phase III noninferiority trial of first line chemotherapy for metastatic or recurrent endometrial carcinoma: a Gynecologic Oncology Group study. *Gynecol Oncol*. 2012;125:771.
6. Rauh-Hain JA, Del Carmen MG. Treatment for advanced and recurrent endometrial carcinoma: combined modalities. *Oncologist*. 2010;15:852–861.
7. Dutt A, Salvesen HB, Chen TH, et al. Drug-sensitive FGFR2 mutations in endometrial carcinoma. *Proc Natl Acad Sci U S A*. 2008;105:8713–8717.
8. Konecny GE, Kolarova T, O'Brien NA, et al. Activity of the fibroblast growth factor receptor inhibitors dovitinib (TKI258) and NVP-BGJ398 in human endometrial cancer cells. *Mol Cancer Ther*. 2013;12:632–642.
9. Pollock PM, Gartside MG, Dejeza LC, et al. Frequent activating FGFR2 mutations in endometrial carcinomas parallel germline mutations associated with craniosynostosis and skeletal dysplasia syndromes. *Oncogene*. 2007;26:7158–7162.
10. Byron SA, Gartside M, Powell MA, et al. FGFR2 point mutations in 466 endometrioid endometrial tumors: relationship

- with MSI, KRAS, PIK3CA, CTNNB1 mutations and clinicopathological features. *PLoS One*. 2012;7:e30801.
11. Sun Y, Niu W, Du F, et al. Safety, pharmacokinetics, and antitumor properties of anlotinib, an oral multi-target tyrosine kinase inhibitor, in patients with advanced refractory solid tumors. *J Hematol Oncol*. 2016;9:105.
 12. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc*. 2006;1:1112–1116.
 13. Korch C, Spillman MA, Jackson TA, et al. DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination. *Gynecol Oncol*. 2012;127:241–248.
 14. Weigelt B, Warne PH, Lambros MB, et al. PI3K pathway dependencies in endometrioid endometrial cancer cell lines. *Clin Cancer Res*. 2013;19:3533–3544.
 15. Krakstad C, Birkeland E, Seidel D, et al. High-throughput mutation profiling of primary and metastatic endometrial cancers identifies KRAS, FGFR2 and PIK3CA to be frequently mutated. *PLoS One*. 2012;7:e52795.
 16. Chen H, Ma J, Li W, et al. A molecular brake in the kinase hinge region regulates the activity of receptor tyrosine kinases. *Mol Cell*. 2007;27:717–730.
 17. Kwak Y, Cho H, Hur W, et al. Antitumor effects and mechanisms of AZD4547 on FGFR2-deregulated endometrial cancer cells. *Mol Cancer Ther*. 2015;14:2292–2302.
 18. Guagnano V, Kauffmann A, Wöhrle S, et al. FGFR genetic alterations predict for sensitivity to NVP-BGJ398, a selective pan-FGFR inhibitor. *Cancer Discov*. 2012;2:1118–1133.
 19. Pectasides D, Xiros N, Papaxoinis G, et al. Carboplatin and paclitaxel in advanced or metastatic endometrial cancer. *Gynecol Oncol*. 2008;109:250–254.
 20. Bestvina CM, Fleming GF. Chemotherapy for endometrial cancer in adjuvant and advanced disease settings. *Oncologist*. 2016;21:1250–1259.
 21. Simpkins F, Drake R, Escobar PF, et al. A phase II trial of paclitaxel, carboplatin, and bevacizumab in advanced and recurrent endometrial carcinoma (EMCA). *Gynecol Oncol*. 2015;136:240–245.
 22. Moxley KM, McMeekin DS. Endometrial carcinoma: a review of chemotherapy, drug resistance, and the search for new agents. *Oncologist*. 2010;15:1026–1033.
 23. Sugimoto K, Miyata Y, Nakayama T, et al. Fibroblast growth factor-2 facilitates the growth and chemo-resistance of leukemia cells in the bone marrow by modulating osteoblast functions. *Sci Rep*. 2016;6:30779.
 24. Shaw AT, Hsu PP, Awad MM, et al. Tyrosine kinase gene rearrangements in epithelial malignancies. *Nat Rev Cancer*. 2013;13:772–787.
 25. Konecny GE, Finkler N, Garcia AA, et al. Second-line dovitinib (TKI258) in patients with FGFR2-mutated or FGFR2-non-mutated advanced or metastatic endometrial cancer: a non-randomised, open-label, two-group, two-stage, phase 2 study. *Lancet Oncol*. 2015;16:686–694.
 26. Powell MA, Sill MW, Goodfellow PJ, et al. A phase II trial of brivanib in recurrent or persistent endometrial cancer: an NRG Oncology/Gynecologic Oncology Group Study. *Gynecol Oncol*. 2014;135:38–43.
 27. Sequist LV, Cassier P, Varga A, et al. Phase I study of BGJ398, a selective pan-FGFR inhibitor in genetically preselected advanced solid tumors. Presented at the American Association for Cancer Research Annual Meeting 2014; April 5–9, 2014; San Diego, CA. Abstract CT326.
 28. Saka H, Kitagawa C, Kogure Y, et al. Safety, tolerability and pharmacokinetics of the fibroblast growth factor receptor inhibitor AZD4547 in Japanese patients with advanced solid tumours: a phase I study. *Invest New Drugs*. 2017;35:451–462.
 29. Tabernero J, Bahleda R, Dienstmann R, et al. Phase I dose-escalation study of JNJ-42756493, an oral pan-fibroblast growth factor receptor inhibitor, in patients with advanced solid tumors. *J Clin Oncol*. 2015;33:3401–3408.
 30. Werner TL, Kannapel E, Chen J, et al. Safety and PK results from a phase IB study of AL3818 (anlotinib) hydrochloride in subjects with ovarian, cervical, and endometrial cancers. *J Clin Oncol*. 2017;35:e17071.