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PRECLINICAL STUDIES

# Alofanib, an allosteric FGFR2 inhibitor, has potent effects on ovarian cancer growth in preclinical studies

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**Summay** *Purpose* Early data suggest that combining FGFR2 inhibitors with platinum-containing cytotoxic agents for the treatment of epithelial ovarian cancer may yield increased antitumor activity. We investigated antitumor activity of alofanib (RPT835), a novel allosteric FGFR2 inhibitor, in ovarian cancer in vitro and in vivo. *Methods* Equal amounts of ovarian cancer cell (SKOV3) lysates were analyzed for FGFR1–3 protein expression using Wes. To assess the efficacy of alofanib on FGF-mediated cell proliferation, SKOV3 cells were incubated and were treated with serially diluted alofanib. Basic FGF was added at a concentration of 25 ng/ml. Control wells were left untreated. Cell growth inhibition was determined using Promega's Cell Titer-Glo® assay. Immunocompromised mice were used for xenotransplantation of SKOV3 cancer cells. Seventy animals with measurable tumors were selected on day 10 and randomized into control groups (no treatment or chemotherapy alone (paclitaxel + carboplatin) and treatment groups (alofanib orally or intravenously (different dose levels) in combination with chemotherapy). Measurements of tumor volume (mm3) were performed by digital calipers every 3 days during 31 days after tumor inoculation. Number of tumor vessels and Ki-67 index were calculated. *Results* SKOV3 cells express FGFR1 and FGFR2 but not FGFR3. Basic FGF increased proliferation of the ovarian cancer cells in untreated control group ($P = 0.001$). Alofanib inhibited growth of FGFR2-expressing SKOV3 cells with GI50 value of 0.37 μmol/L. Treatment with alofanib in combination with paclitaxel/carboplatin resulted in tumor growth delay phenotype in all treatment groups compared to control non-treatment groups. Compound exhibited a dose-dependent effect on tumor growth. Daily intravenous regimen of alofanib (total maximum dose per week was 350 mg/kg) demonstrated significant effect (inhibiting growth by 80 % and by 53 % in comparison with vehicle and chemotherapy group alone, respectively ($P < 0.001$). Alofanib decreased number of vessels in tumor (−49 %; $P < 0.0001$) and number of Ki-67-positive SKOV3 cells (−42 %, $P < 0.05$). There were tumor necrosis and cell degeneration in alofanib group. *Conclusions* We suggest that FGFR2 inhibition has potent effects on ovarian cancer growth in preclinical studies.

**Keywords** Alofanib · RPT835 · FGFR2 · Allosteric inhibitor · Ovarian cancer · Preclinical studies

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## Background

Ovarian cancer is the leading cause of death from gynecologic tumors and the fifth most lethal malignancy in women in the world [1]. The treatment approaches have not been changed during the last decades and the prognosis remains poor with 5-year survival rates in the range of 40 % for stage III and 18 %

for stage IV disease [2]. Papillary serous histology accounts for approximately 75 % of ovarian cancers, mucinous and endometrioid for 10 % each, followed by clear cell tumors, Brenner tumors (transitional cell), and undifferentiated carcinomas [3].

The standard treatment of ovarian cancer includes surgery and chemotherapy approaches. The first-line chemotherapy has not been changed for more than 15 years. Standard chemotherapy consists of a combination of paclitaxel and carboplatin [4]. Despite the activity of the first-line therapy, which gives response rates up to 80 %, the majority of patients die of their recurrent disease [5].

Fibroblast growth factor receptor 2 (FGFR2) is receptor with tyrosine-kinase activity. Phosphorylation of FGFR2 and its substrate, FRS2, triggers recruitment of GRB2, GAB1, PIK3R1 and SOS1, and mediates activation of RAS, MAPK1/ERK2, MAPK3/ERK1 and the MAP kinase signaling pathway, as well as of the AKT1 signaling pathway [6, 7]. FGFR2 signaling is down-regulated by ubiquitination, internalization and degradation. Mutations that lead to constitutive kinase activation or impair normal FGFR2 maturation, internalization and degradation lead to aberrant signaling. Overexpressed FGFR2 promotes activation of STAT1.

FGFR2 amplification and high-level polysomy may be a promising molecular target for serous ovarian cancer. FGFR2 amplification was found in 17.1 % of patients with advanced serous ovarian cancer [8]. High-level polysomy was observed in 57.1 % of patients. Mostly, FGFR2 abnormalities were detected in metastases compared with primary tumor or relapse. Early data suggest that combining FGFR2 inhibitors with platinum-containing cytotoxic agents for the treatment of epithelial ovarian cancer may yield increased antitumor activity [9].

Alofanib (RPT835) is a predominantly FGFR2-selective allosteric small-molecular inhibitor that shows promising antiangiogenic and antitumor activities in preclinical studies [10, 11]. We investigated antitumor activity of alofanib in ovarian cancer in vitro and in vivo.

## Materials and methods

### Cells and culture conditions

Ovarian carcinoma SKOV3 cell line was obtained from the American Type Culture Collection (ATCC, HTB-77) and cultured in McCoy's 5a medium (ATCC), supplemented with fetal bovine serum to a final concentration of 10 % (ATCC). Subculturing was performed by cells trypsinization with 0.25 % Trypsin-EDTA and 1: 6 split for every subsequent passage. Cell line was cultured at 37 °C / 5 % CO2 in humidified incubator.

To assess the efficacy of alofanib on FGF-mediated cell proliferation, cells were treated with serially diluted compound. Six hours after dosing, FGF2 (BD Bioscience) was added at a concentration of 25 ng/ml. Negative control cells were left untreated. Cell growth inhibition was determined using Cell Titer-Glo® (Promega) assay. To differentiate between a cytostatic and cytotoxic drug effect, the concentration that causes 50 % growth inhibition (GI50) was determined by correcting for the cell count at time zero (time of treatment) and plotting data as percentage of growth relative to vehicle-treated cells. The MTT assay was used to assess the cell sensitivity to chemotherapeutic agents as described previously [12].

### In vitro study testing FGFR2 expression in SKOV3 cell line

SKOV3 cells were lysed in NP-40 lysis buffer (10 mM Tris-Cl, pH 7.5, 140 mM NaCl, 1 % NP-40) containing protease inhibitors (Pierce # 78,430). Adherent cells were lysed in a T-75 flask using 1.5mLs of lysis buffer per flask. The cells were covered with lysis buffer and frozen at −20 °C for approx. 20 min. The flasks were thawed and the lysate was removed and aliquoted in 200 uL aliquots per 1.5 mL tube. Sonication was performed at an amplitude of 40 followed by pelleting cell debris by centrifugation at 10,000 rpm at 4 °C for 10 min. The cleared lysates were removed and used for analysis.

**Table 1** Design of xenograft study 1 (alofanib, oral route)

| Group | N | Tested compounds | | | |
|---|---|---|---|---|---|
| | | Agent | Dose, mg/kg | Route | Schedule |
| 1 | 10 | vehicle | - | - | - |
| 2 | 10 | paclitaxel | 10 | intravenously | first day of each week, 1–3 weeks |
| | | carboplatin | 50 | intravenously | first day, once |
| 3 | 10 | alofanib | 50 | orally | daily, 1–3 weeks |
| | | paclitaxel | 10 | intravenously | first day of each week, 1–3 weeks |
| | | carboplatin | 50 | intravenously | first day, once |

All samples were prepared for loading on the Wes by mixing four volumes of lysate with one volume of 5× fluorescent master mix. Diluted samples were heated to 95 °C for 5 min., cooled and centrifuged briefly then loaded on the Wes plate.

Primary antibodies were purchased (anti-FGFR1 (rabbit monoclonal, Cell Signaling Technology #9740), anti-FGFR2 (rabbit polyclonal, Santa Cruz sc-122), anti-FGFR3 (mouse monoclonal, Santa Cruz sc-13,121), anti-Rabbit-HRP (ProteinSimple, #042–206) and anti-Mouse-HRP (ProteinSimple, #042–205) antibodies were used as secondary antibodies.

### Assessment of tumor angiogenesis and ovarian cancer cell activity in vivo

Tumor specimens were fixed in neutral formalin and embedded in paraffin for H&E staining, histological and immunohistochemical analysis. Immunohistochemical study was performed using immunoperoxidase assay with rabbit antibodies to CD31 (Abcam, ab28364) and Ki-67 (Dako, M7240). *EnVision* + and *DAB*+ kits (Dako) were used for identification. The results of staining were analyzed by the number of microvessels per field of view (Nikon 80i microscope, ×200).

### Xenograft studies

Immunocompromised nude mice (8- to 12-week old females) were purchased from the Harlan laboratories. All animal procedures and maintenance were conducted in accordance with the institutional guidelines.

SKOV3 cells were mixed (1:1 volume) with Matrigel (BD Biosciences) and cell line suspension (50 % Matrigel) was subcutaneously injected ($1.5 \times 10^6$ cells per injection) in animal flank area to ensure successful tumor initiation and tumor growth measurements. Injection performed at respective day 0.

Seventy immunocompromised animals were used for SKOV3 xenotransplantation and sixty animals with measurable tumors were selected on day 5 and used for subsequent experiments. Administration of compound (or control) started on day 5 after inoculation when measurable tumor growth was detected in all animals.

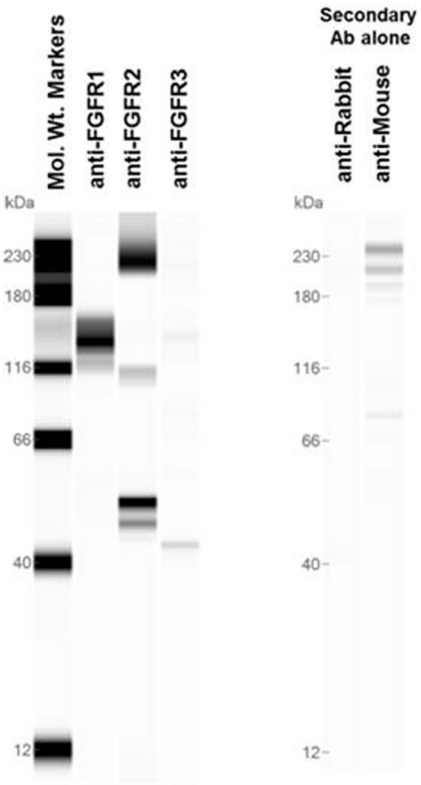


**Fig. 1** Analysis of expression of different FGF receptors in SKOV3 cell line. Whole cell lysates were analyzed by Western blot analysis by probing for FGFR1, FGFR2 and FGFR3 protein expression. FGFR1 and FGFR2 expression was found. The right panel shows the result of probing extracts with the HRP-conjugated secondary antibody alone. The exposure shown is a much higher exposure than used for the primary antibodies

In first study mice were randomized to receive alofanib orally in combination with chemotherapy (paclitaxel + carboplatin) or chemotherapy alone (Table 1).

In second study mice were randomized to receive alofanib intravenously in combination with chemotherapy (paclitaxel + carboplatin) in different settings (Table 2).

**Table 2** Design of xenograft study 2 (alofanib intravenously)

| Group | N | Tested Compounds | | | |
|---|---|---|---|---|---|
| | | Agent | Dose, mg/kg | Route | Schedule |
| 1 | 10 | vehicle | - | - | - |
| 2 | 10 | paclitaxel | 10 | intravenously | first day of each week, 1–3 weeks |
| | | carboplatin | 50 | intravenously | first day, once |
| 3 | 10 | alofanib | 50 | orally | daily, 1–3 weeks |
| | | paclitaxel | 10 | intravenously | first day of each week, 1–3 weeks |
| | | carboplatin | 50 | intravenously | first day, once |

Measurements of tumor volume ($mm^3$) were performed by digital calipers every 3 days during 31 days after tumor inoculation. Animals were weighed daily for the first five days of the study and twice weekly thereafter. Acceptable toxicity was defined as group mean body weight loss of less than 20 % during the study and not more than one treatment related death among ten treated animals in each study.

## Results

### FGFR2 expression in SKOV3 cell line

Equal amounts of cell lysates were analyzed for FGFR1, FGFR2 and FGFR3 protein expression. FGFR1 antibody detected a single 140 kDa protein consist with the known molecular weight of FGFR1 (Fig. 1). The FGFR2 antibody detected several proteins of ~50 kDa, 110 kDa and 240 kDa. The 110 kDa and 50 kDa species consist with FGFR2 proteins. FGFR3 antibody did not detect any proteins. Thus, we concluded that SKOV3 cells express FGFR1 and FGFR2.

### Alofanib reduced proliferation of ovarian cancer cells

Alofanib was evaluated for antiproliferative activity against the human ovarian cancer FGFR-expressing cell line in FGF-mediated signaling model. FGF2 significantly increased proliferation of the ovarian cancer cells in untreated control group ($P = 0.001$). Cells were treated with increasing concentrations of alofanib ranging from 0 to 1 μmol/L. Compound significantly inhibited FGF-triggered cell proliferation in dose-dependent manner ($P < 0.001$). To find out whether alofanib could demonstrate cytotoxic activity in vitro, we examined the effect of compound on SKOV3 cell line by MTT assay. Alofanib displayed low cytotoxic effect on ovarian cancer cells. Compound inhibited growth of SKOV3 cells with GI50 value of 0.37 μmol/L (Fig. 2a).

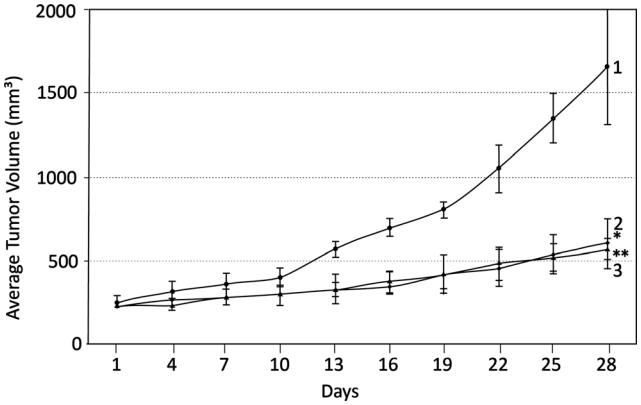


**Fig. 3** The combination of oral alofanib and paclitaxel/carboplatin produced a greater inhibitory effect on xenograft growth compared with vehicle group (1). There was no significant difference in tumor size between animals treated with combination of oral alofanib and chemotherapy (3) or chemotherapy alone (2). The tumor growth curve was drawn according to the tumor volume and time after implantation. Data shown are mean ± SD of tumor volume for each group ($n = 30$)

We assessed the status of apoptotic markers and found marked mitochondrial-mediated apoptosis with cleavage of caspase 3 and PARP in cells (Fig. 2b). The expression of Bcl-2 was analyzed and major changes in expression of this protein were observed following treatment in SKOV3 cell line.

### Alofanib enhanced the efficacy of chemotherapeutic agents and suppressed angiogenesis in ovarian cancer mouse model

To investigate whether alofanib could enhance the efficacy of chemotherapeutic agents in vivo, we established the FGFR2-

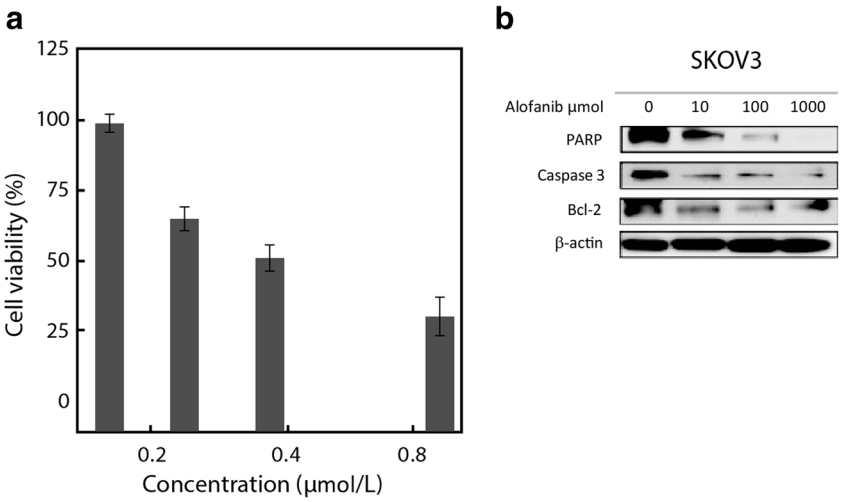


**Fig. 2** Dose response effect of alofanib was evaluated in ovarian cancer cells. A, SKOV3 ovarian cancer cells were exposed to alofanib and then calculated. Data are presented as means (± SE). B, Cells were treated with alofanib (10, 100, and 1000 μM) for 72 h and whole-cell lysates were immunoblotted with cleaved caspase 3, cleaved PARP, and Bcl-2 antibodies

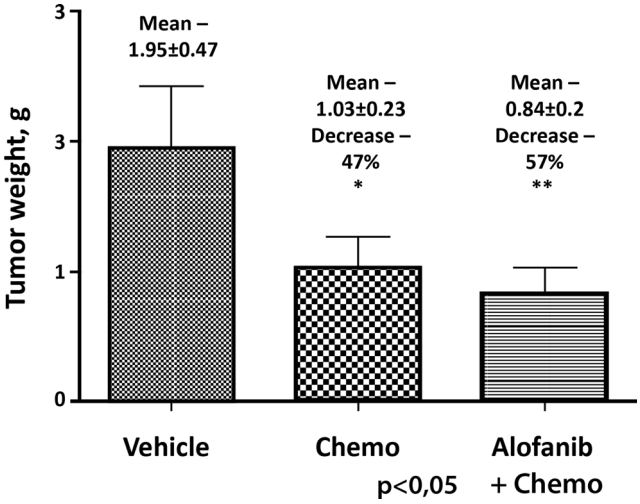


**Fig. 4** Effect of alofanib on the weight of tumors. Tumor growth inhibition as measured by the tumor weight at time of harvest

overexpressing SKOV3 cell xenograft model in nude mice. The combination of oral alofanib and paclitaxel/carboplatin produced a greater inhibitory effect on xenograft growth compared with vehicle group ($P < 0.05$; Fig. 3), and the inhibition rate was more than 63 %. Unfortunately, there was no significant difference in tumor size between animals treated with oral alofanib in combination with chemotherapy or chemotherapy alone ($P > 0.05$). Means of tumor volumes were 1655.4 ± 376.0, 565.0 ± 63.1 and 598.3 ± 149.4 mm3 in vehicle, chemotherapy and combination groups, respectively. Nevertheless, mean weight of tumors in combination group was lower by 10 % compared with chemotherapy group and was less than 2-fold compared with vehicle (all $P < 0.05$, Fig. 4). Studying the structure of the tumor, we found that there were tumor necrosis and cell degeneration in alofanib group in comparison with chemotherapy alone (Fig. 5).

Moreover, alofanib significantly decreased number of vessels in tumor (−49 %; $P < 0.0001$) and number of Ki-67-positive SKOV3 cells (−42 %, $P < 0.05$, Fig. 6). Vascular changes were presented as significant engorgement of vessels and vascular congestion not stained with antibodies to CD31 ("dead" microvessels). In some tumors we observed endothelial cell death with a yield of red blood cells in the tumor tissue (Fig. 7). Extensive hemorrhages were also found.

In the group treated with carboplatin and paclitaxel vascular changes were found in 19.1 % of tumors only.

Treatment with intravenous alofanib in combination with paclitaxel/carboplatin demonstrated significant tumor growth delay phenotype in all treatment groups compared to control non-treatment groups in Study 2 (Fig. 8). Alofanib exhibited a dose-dependent effect on tumor growth. Daily intravenous regimen of alofanib (total maximum dose per week was 350 mg/kg) demonstrated dramatic effect (inhibiting growth by 80 % ($P < 0.001$). Means of tumor volumes were 1425 ± 93.2, 671 ± 52.2, 586 ± 65.1, and 315 ± 36.6 in vehicle, alofanib once a week, alofanib biweekly and daily alofanib groups, respectively. The curve reached a plateau and there was no tumor growth from Day 19 in daily alofanib group. These indicated that intravenous alofanib enhances the efficacy of substrate conventional chemotherapeutic agents in FGFR2-overexpressing cell xenografts. Importantly, we did not find any death or decrease of body weight in the four groups, suggesting that the combination regimen did not result in increased toxicity.

## Discussion

FGF2, or basic FGF, and its transmembrane tyrosine kinase receptors make up a large, complex family of signaling molecules involved in several physiologic processes, and the dysregulation of these molecules has been associated with cancer

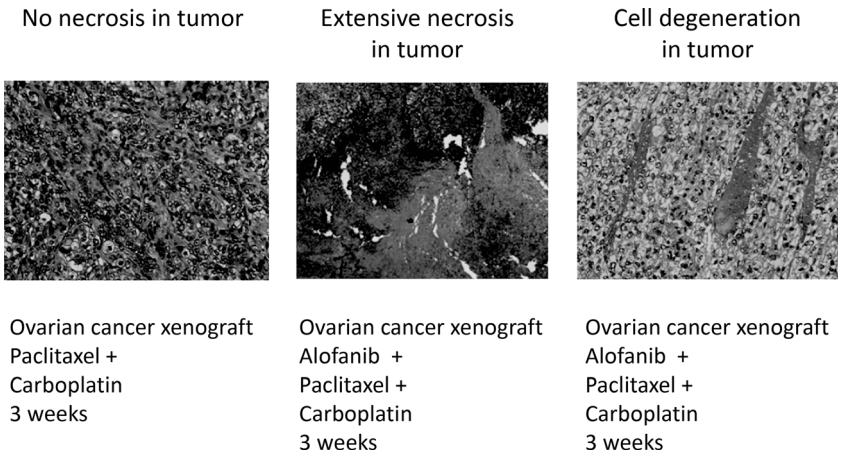


**Fig. 5** Alofanib induced necrosis and cell degeneration in tumor. All pictures were taken at original magnification (200×)

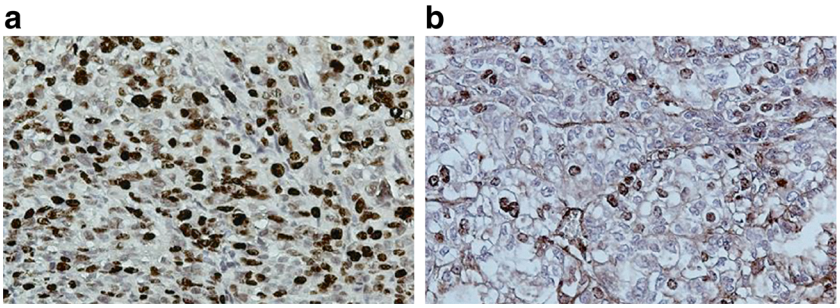


**Fig. 6** Ki-67 index was significantly reduced in mice treated with alofanib (**b**) in comparision with chemotherapy alone (**a**). Immunohistochemical study was performed using immunoperoxidase assay with rabbit antibodies to Ki-67 (Dako). The results of staining were analyzed (Nikon 80i microscope, ×200)

development [13]. Basic FGF belongs to a family of ubiquitously expressed ligands that bind to the extracellular domain of FGFRs, initiating a signal transduction cascade that promotes cell proliferation, motility, and angiogenesis.

In this study, we have shown that alofanib, an allosteric inhibitor of extracellular domain of FGFR2, demonstrated its efficacy against SKOV3 ovarian cancer cell line with expression of FGFR2. Of note, the function of the FGFR2 in ovarian cancer was previously unclear. In our study we found that SKOV3 cells highly express FGFR1 and FGFR2. Basic FGF significantly impacts on proliferation of SKOV3, and FGF/FGFR could be important pathway in development of ovarian cancer.

The results from in vitro study revealed that alofanib induced mainly apoptosis with cleavage of caspase 3, PARP and Bcl-2 in SKOV3 cell line. Cytotoxic effect of compound on ovarian cancer cells was low.

Aim of these xenograft studies was to increase efficacy of paclitaxel and carboplatin adding FGFR2 inhibitor. It has been known that FGFR2 inhibition may enhance antitumor activity of platinum-containing cytotoxic regimens [9]. As expected, intravenous alofanib significantly in dose-dependent manner potentiated the efficiency of the combination of paclitaxel and carboplatin. Daily intravenous regimen resulted in a 3-fold tumor growth delay in comparison with vehicle, and the inhibition rate of 80 % had been achieved. We examined treatment effects on tumor cell proliferation by calculating the proliferative index after immunohistochemical staining for Ki-67 in tumors collected at necropsy at the end of the therapy experiments. The proliferative index has been significantly reduced in mice treated with alofanib.

Angiogenic pathways and the tumor microvasculature are attractive targets for cancer treatment, as supported by the numerous ongoing trials in this arena in a variety of solid malignancies, including ovarian cancer [14–16]. In our study, we demonstrated marked antiangiogenic activity in ovarian carcinoma model using combination of alofanib and chemotherapy. Alofanib decreased number of vessels on 49 % and initiated vascular changes in tumors. There was no animal death caused by alofanib.

Taken together, these data support the concept of testing FGFR2 inhibition in patients with ovarian cancer. The question also arises as to whether adding a alofanib to standard chemotherapy could improve outcome in patients

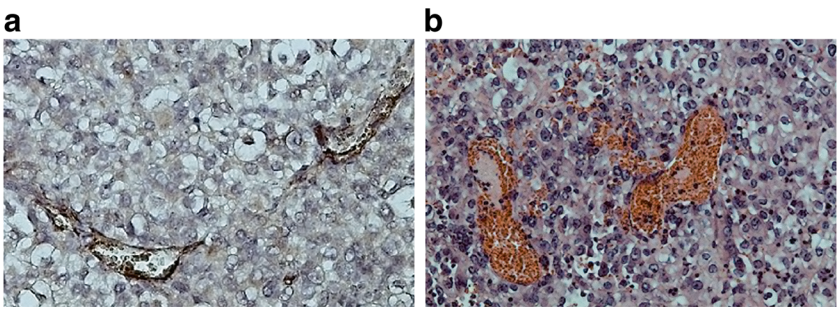


**Fig. 7** Alofanib inhibits tumor angiogenesis. Microvessel density (CD31) were detected by IHC staining in SKOV3-inoculated mice treated with chemotherapy alone (**a**) or in combination with alofanib (**b**). Vascular changes were presented as significant engorgement of vessels and vascular congestion not stained with antibodies to CD31. In some tumors we observed endothelial cell death with a yield of red blood cells in the tumor tissue All pictures were taken at original magnification (200×)

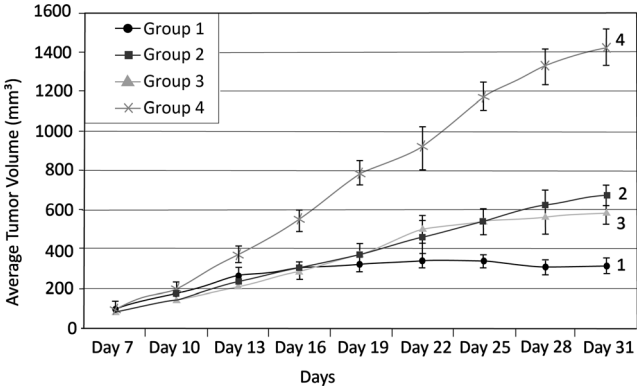


**Fig. 8** Co-treatment of paclitaxel/carboplatin regimen in combination with alofanib demonstrated significant tumor grow delay phenotype in all treatment groups compared to control non-treatment group (Group 4). Daily regimen of alofanib (Group 1) demonstrated maximum effect (−80 %; no tumor growth). Means of tumor volumes were 1425 ± 93.2, 671 ± 52.2, 586 ± 65.1, and 315 ± 36.6 in vehicle (4), alofanib once a week (2), alofanib twice a week (3) and daily alofanib (4) groups, respectively

who have already progressed on prior platinum-based therapy. Protocol of Phase IB/first in human study of alofanib in patients with platinum resistant ovarian cancer was developed at the ECCO-AACR-EORTC-ESMO Workshop on Methods in Clinical Cancer Research ("Flims17"). This clinical trial will be initiated in the near future.

**Compliance with ethical standards**

**Conflict of interest** I. Tsimafeyeu, M. Byakhov and S. Tjulandin have ownership interest in Ruspharmtech LLC. Evgenia Gavrilova is an employee of Ruspharmtech LLC.

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**Ethical approval** All applicable international and institutional guidelines for the care and use of animals were followed.

## References

1. Siegel R, Kimberly DM, Jemal A (2016) Cancer statistics, 2016. CA Cancer J Clin 66:7–30
2. Heintz AP, Odicino F, Maisonneuve P et al (2006) Carcinoma of the ovary. FIGO 26th annual report on the results of treatment in gynecological cancer. Int J Gynaecol Obstet 95(Supervision 1): S161–S192
3. Scully R, Young RH, Clement PB (1998) Surface epithelial-stromal tumors and serous tumors. In: Tumors of the ovary, maldeveloped gonads, fallopian tube, and broad ligament. District of Columbia: Armed Forces Institute of Pathology, Washington, p. 51
4. NCCN Clinical Practice Guidelines. Ovarian cancer ver.1 2016 https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf
5. Klar M, Hasenburg A, Hasanov M, Hilpert F, Meier W, Pfisterer J et al (2016) Prognostic factors in young ovarian cancer patients: an analysis of four prospective phase III intergroup trials of the AGO study group. GINECO and NSGO Eur J Cancer 66:114–124
6. Tsimafeyeu I, Khasanova A, Stepanova E, Gordiev M, Khochenkov D, Naumova A et al (2016) FGFR2 overexpression predicts survival outcome in patients with metastatic papillary renal cell carcinoma. Clin Transl Oncol 5 [Epub ahead of print]. doi:10.1007/s12094-016-1524-y
7. Luo Y, Yang C, Jin C, Xie R, Wang F, McKeehan WL (2009 Sep) Novel phosphotyrosine targets of FGFR2IIIb signaling. Cell Signal 21(9):1370–1378
8. Tyulyandina A, Tsimafeyeu I, Demidova I, Gikalo M, Tjulandin S FGFR2 amplification in serous ovarian cancer. Cancer Res 76(14 Supplement):4586–4586
9. Cole C, Lau S, Backen A, Clamp A, Rushton G, Dive C et al (2010 Sep 1) Inhibition of FGFR2 and FGFR1 increases cisplatin sensitivity in ovarian cancer. Cancer Biol Ther 10(5):495–504
10. Tsimafeyeu I, Ludes-Meyers J, Stepanova E, Daeyaert F, Kochenkov D, Joose JB et al (2016) Targeting FGFR2 with alofanib (RPT835) shows potent activity in tumour models. Eur J Cancer 61:20–28
11. Tsimafeyeu I, Daeyaert F, Joos JB, Aken KV, Ludes-Meyers J, Byakhov M et al (2016) Molecular modeling, de novo design and synthesis of a novel, extracellular binding fibroblast growth factor receptor 2 inhibitor alofanib (RPT835). Med Chem 12(4):303–317
12. Chen L, Wu X, Ruan J, Liang Y, Ding Y, Shi Z et al (2004) Screening novel, potent multidrug-resistant modulators from imidazole derivatives. Oncol Res 14:355–362
13. Tsimafeyeu I, Zaveleva E, Stepanova E, Low W (2013) OM-RCA-01, a novel humanized monoclonal antibody targeting fibroblast growth factor receptor 1, in renal cell carcinoma model. Investig New Drugs 31(6):1436–1443
14. Eichbaum M, Mayer C, Eickhoff R, Bischofs E, Gebauer G, Fehm T et al (2011) The PACOVAR-trial: a phase I/II study of pazopanib (GW786034) and cyclophosphamide in patients with platinum-resistant recurrent, pre-treated ovarian cancer. BMC Cancer 11:453
15. Garcia AA, Hirte H, Fleming G, Yang D, Tsao-Wei DD, Roman L et al (2008) Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret hospital phase II consortia. J Clin Oncol 26:76–82
16. Previs RA, Armaiz-Pena GN, Lin YG, Davis AN, Pradeep S, Dalton HJ et al (2015) Dual metronomic chemotherapy with Nab-paclitaxel and Topotecan has potent antiangiogenic activity in ovarian cancer. Mol Cancer Ther 14(12):2677–2686