Send Orders for Reprints to reprints@benthamscience.ae

Current Cancer Drug Targets, 2018, *18*, 1-9

RESEARCH ARTICLE

Targeting FGFR with BGJ398 in Breast Cancer: Effect on Tumor Growth and Metastasis

Ana Sahores[a], María May[a], Gonzalo R. Sequeira[a], Cynthia Fuentes[a], Britta Jacobsen[b], Claudia Lanari[a], Caroline A. Lamb[a*]

[a]*Instituto de Biología y Medicina Experimental (IBYME), CONICET, Buenos Aires, Argentina;* [b]*Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

ARTICLE HISTORY

Received: July 13, 2017
Revised: September 29, 2017
Accepted: November 14, 2017

DOI:
10.2174/1568009618666171214114706

Abstract: ***Background*:** Endocrine resistance and metastatic dissemination comprise major clinical challenges for breast cancer treatment. The fibroblast growth factor receptor family (FGFR) consists of four tyrosine kinase transmembrane receptors, involved in key biological processes. Genomic alterations in FGFR have been identified in advanced breast cancer and thus, FGFR are an attractive therapeutic target. However, the efficacy of FGFR inhibitors on *in vivo* tumor growth is still controversial.

***Objective*:** The purpose of this study was to evaluate the role of FGFR in tumor growth and breast cancer progression.

***Method*:** Cell proliferation was assessed by ^{3}H-thymidine uptake and cell counting in primary cultures of endocrine resistant mammary carcinomas and a human cell line, respectively. Tumor transplants and cell injections were used to determine *in vivo* growth and spontaneous metastasis. FGFR1-3 and αSMA expression were evaluated on primary tumors by immunohistochemistry.

***Results*:** Antiprogestin resistant murine transplants and a human xenograft express high levels of total FGFR1-3. *In vitro* treatment with the FGFR inhibitor, BGJ398, impaired cell proliferation of resistant variants versus vehicle. *In vivo*, versus control, BGJ398 treatment decreased one out of four resistant tumors, however all tumors showed a decreased epithelial/stromal ratio. Finally, in a model of hormone resistant mammary cancer that spontaneously metastasizes to the lung, BGJ398 decreased the number of mice with lung metastasis.

***Conclusion*:** FGFR inhibitors are promising tools that require further investigation to identify sensitive tumors. These studies suggest that targeting FGFR combined with other targeted therapies will be useful to impair breast cancer progression.

Keywords: Breast cancer, FGFR, FGFR inhibitors, BGJ398, endocrine resistance, tumor progression.

1. INTRODUCTION

Breast cancer is the second leading cause of cancer death among women in developed countries [1]. Approximately 70% of human breast cancers express estrogen receptor α (ERα) and progesterone receptor (PR) at the time of diagnosis [2] and endocrine therapies are usually the standard treatment. Despite expressing hormone receptors, most patients develop resistance with time [3], and if the disease progresses, cancer cells may disseminate to distant sites.

The fibroblast growth factor family (FGFR) comprises four transmembrane receptors with cytoplasmic tyrosine kinase domains [4] which, upon FGF binding, transduce extracellular signals to a variety of intracellular signaling cascades, such as RAS-ERK, PI3K-AKT, IP3-Ca^{2+} and DAG-PKC [5]. These pathways are involved in the regulation of cell survival, proliferation, differentiation and motility during embryogenesis and carcinogenesis [6-8]. Multiple genomic alterations in FGFR leading to increased pathway activation have been identified in breast cancer [9-14] and FGFR appear as an attractive therapeutic target for endocrine resistant breast cancers.

The evidence supporting a role of the FGFR pathway in breast cancer led to the development of FGFR-blocking agents. BGJ398 (infigratinib) is a selective small molecule compound that occupies the ATP-binding pocket in the kinase domain of FGFR1 to 3, and inhibits the receptors at low nM levels (IC50 value ≤20 nM), but is less effective on FGFR4 [15]. The antiproliferative effect of this drug has proven to be specific for cell lines with an activated FGFR pathway [15, 16]. To date, despite promising tumor growth inhibitory effects in preclinical studies and in patients carrying FGFR amplifications in non-small cell lung cancer or bladder/urothelial cancers treated with FGFR inhibitors [16, 17], limited therapeutic effects were observed in breast cancer patients treated with BGJ398 [18].

*Address correspondence to this author at the IBYME-CONICET, Vuelta de Obligado 2490, C1428ADN Buenos Aires, Argentina; Tel: +54-11-4783-2869, Fax: +54-11-4786-2564; E-mail: carolinealamb@gmail.com

1568-0096/18 $58.00+.00

In this study, to gain further insight on the role of FGFR in tumor growth and breast cancer progression, we inhibited FGFR activity in endocrine resistant mammary carcinomas and evaluated tumor growth and metastatic spread.

2. MATERIALS AND METHODS

2.1. Animals

Two-month-old virgin female BALB/c mice (IBYME Animal Facility) and NOD/LtSz-scid/IL-2Rgamma null mice (NSG; Jackson Labs, Bar Harbor, Maine) were used. Animal care and manipulation were in agreement with Institutional guidelines and the 8th Edition of *Guide for the Care and Use of Laboratory Animals* [19].

2.2. Murine Tumors

In previous studies, medroxyprogesterone acetate (MPA) induced mainly ductal mammary carcinomas that express ERα and PR [20] isoforms A (PRA) and B (PRB). Tumors with a high PRA/PRB ratio respond to different antiprogestins such as: mifepristone (MFP), onapristone and lonaprisan, while tumors with the opposite ratio are unresponsive and thus, considered endocrine resistant [21]. In this study, three independent endocrine resistant tumors that arose in different animals were used: C4-2-HI, C7-HI and, 59-HI [22, 23]. Tumors were dissected into small pieces and transplanted into the inguinal flank of two-month-old BALB/c mice.

2.3. Human Cell Line

T47D-YB cells, engineered to express only PRB, were a kind gift of *Dr. K. Horwitz* (University of Colorado, [24, 25]). This cell line is unresponsive to antiprogestin and antiestrogen therapy [21, 25]. These cells are maintained in 10% fetal calf serum (FCS) DMEM/F12 medium, supplemented with 1 nM insulin (Denver, Buenos Aires) and G418 200 µg/ml (InvivoGen, San Diego, CA). This cell line was validated in 2017 by Genetica DNA Laboratories, Inc. (Cincinnati, OH) by short tandem repeat profiling.

2.4. Reagents and Antibodies

MFP was purchased from Sigma Aldrich (St Louis, MI). BGJ398 was purchased from Selleckchem and was prepared as 10 µM DMSO stocks. For *in vivo* experiments, BGJ398 was diluted in saline solution.

Antibodies against FGFR1 (sc-121), FGFR2 (sc-122) and FGFR3 (sc-123) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody specificity has been validated in previous work from our lab [26, 27]. Smooth muscle actin alpha (αSMA; ab-5694) was obtained from Abcam (Cambridge, MA). Secondary antibodies were from Vector Laboratories (Burlingame, CA).

2.5. Primary Cultures

Primary cultures of epithelial cells from mammary carcinomas were performed as previously described [28]. Epithelial cells were isolated by differential sedimentation and plated with 10% FCS.

2.6. *In vitro* Studies

^{3}H-thymidine uptake [28] and cell counting assays [26] were performed as previously described. In ^{3}H-thymidine uptake assays, primary cell cultures were starved in 1% charcoal stripped FCS (chFCS) for 24 hrs and treated for 48 hrs with experimental solutions (BGJ398: 1-10 nM). In cell counting assays, cells were starved in 1% chFCS for 24 hrs and then treated for 5 days with BGJ398: 1-10 nM.

2.7. *In vivo* Assays

Murine tumors were transplanted by subcutaneous (*sc*) injection into the inguinal flank of BALB/c mice. In the xenograft studies, T47D-YB ($6x10^6$) cells were inoculated orthotopically into mammary gland (#4) of NSG mice pretreated with 17-β-estradiol (0.25 mg pellets; [21, 25, 26, 29]). Tumor growth was measured twice a week using a *Vernier* caliper and tumor areas were calculated as length x width. MFP pellets (6 mg) were implanted *sc* [29] eight days after tumor transplants. Intravenous (iv) BGJ398 treatment consisted of retro-orbital injections (30 mg/kg/every two days). At the end of the experiments, mice were euthanized, lungs and tumors excised, weighed and fixed in 10% buffered formalin, and embedded in paraffin for histological evaluation. Tumor histology and incidence of lung metastasis was evaluated in H&E stained slides by a pathologist (MM).

2.8. Immunohistochemistry (IHC)

IHC of murine and human xenografts were carried out as described previously [27]. Briefly, formalin-fixed paraffin-embedded tissue sections were dewaxed and hydrated. Antigen retrieval was achieved by boiling tissue sections for 50 min in 10 mM Sodium Citrate buffer (pH6), and slides were then reacted with the primary antibodies ON at 4°C followed by 1 hr at room temperature of the biotynilated secondary antibodies. The avidin/biotin peroxidase complex technique was used (Vectastain Elite ABC kit; Vector), and the slides were developed under microscopic control with liquid 3-3'diaminobenzidine and substrate chromogen system (Dako, Agilent Technologies). Primary and secondary antibodies were used at 1/100 and 1/400 dilutions, respectively. αSMA quantification was obtained by counting at least five random sections of each tumor sample. Areas with positive staining were measured using the ImageJ software and expressed as a percent of the total area for each image. The mitotic and apoptotic indexes were determined by counting the number of cells undergoing mitosis/apoptosis in a 100X high power field (5 fields were quantified for each tumor, N=6). For FGFR1-3 quantification, cytoplasmic, and nuclear staining were differentially evaluated and expressed as percentage of stained cells (0-100%) times the intensity (0-3), in a scale ranging from 0 to 300 by a pathologist (MM). The score was graded for positive samples in a low/high scoring system, considering <100 or >100, respectively.

2.9. Statistical Analysis

One-way ANOVA followed by *Tukey t*-test was used to compare means of multiple samples and the *Student's t*-test was used to compare the means of two different experimental

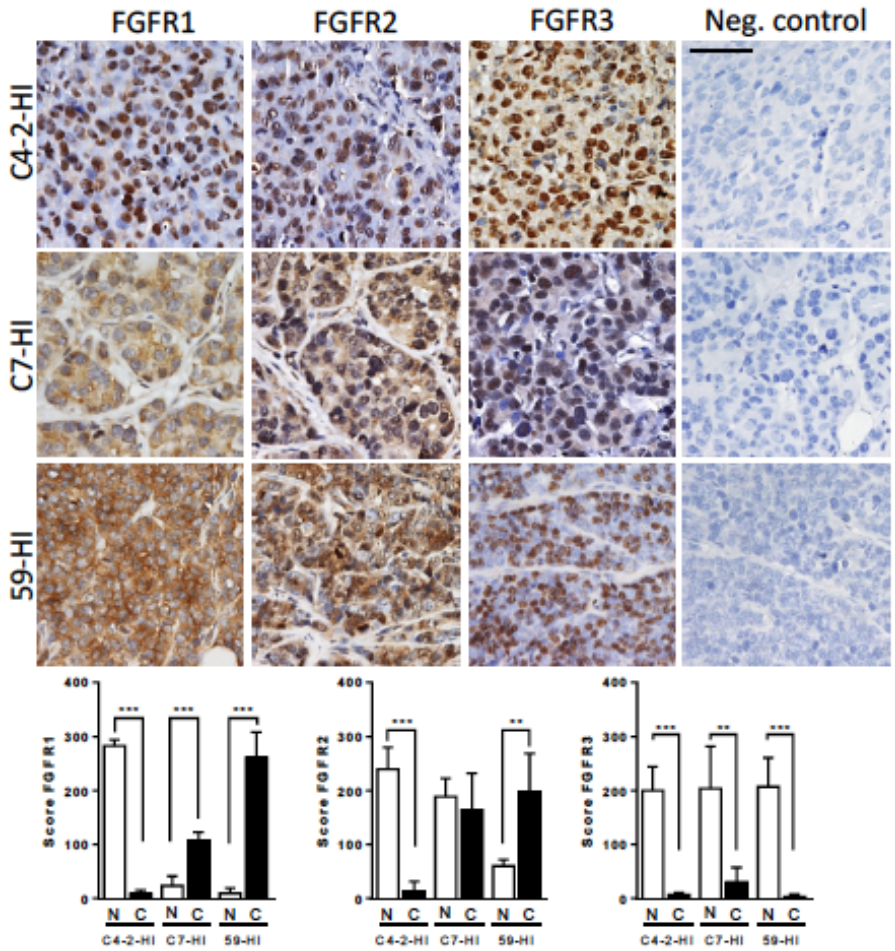


Fig. (1). Immunohistochemical staining and score for FGFR1-3 in endocrine resistant murine mammary tumors C4-2-HI, C7-HI and 59-HI. Nuclei were counterstained with hematoxylin. FGFR1-3 were quantified and nuclear (N) and cytosolic (C) staining was expressed as a score as detailed in *Materials and Methods*. A negative control (omitting the primary antibody) is shown in the column on the right. Scale bar: 50 µm. Statistics: *t Test*: (***): p<0.001; (**): p<0.01.

groups. The slopes of the tumor growth curves were compared using a linear regression analysis followed by slope comparison. *In vivo* experiments were performed at least twice and IHC assays three times. *Chi* squared was used to compare number of animals with metastasis. All values are expressed as mean ± SD and significant differences are indicated with asterisks: (*) p<0.05; (**) p<0.01 and (***) p<0.001.

3. RESULTS

3.1. FGFR1-3 Expression in Endocrine Resistant Experimental Mammary Carcinomas

We first evaluated FGFR1-3 expression in three endocrine resistant murine mammary carcinomas (C4-2-HI, C7-HI and 59-HI) derived from tumors that arose in different animals, to determine if an anti-FGFR therapy is a plausible treatment for these tumors. We found positive staining with a high total score (>100) for FGFR 1-3 with differences in receptor localization among tumor variants. Despite differences in nuclear or cytosolic localization of FGFR1 and 2, C4-2-HI, C7-HI and, 59-HI tumors shared a strong nuclear staining for FGFR3 and, similar high total FGFR levels (Fig. **1**).

3.2. BGJ398 Treatment Inhibits Cell Proliferation of Primary Cultures from Resistant Murine Mammary Tumors

Given that all our tested endocrine resistant tumors express high levels of FGFR1-3 and to explore the hypothesis that these tumors may be sensitive to an anti-FGFR therapy, we investigated the effect of blocking the FGFR pathway on cell proliferation. In primary cultures, the selective FGFR inhibitor BGJ398 reduced baseline cell proliferation in epithelial cells from all three endocrine resistant variants (Fig. **2**). Collectively, these results suggest that the FGFR pathway has a proliferative role in endocrine resistant tumors.

3.3. Mild Effect of BGJ398 Treatment on Endocrine Resistant Tumor Growth

We next evaluated if BGJ398 treatment is a viable therapeutic approach for endocrine resistant tumors. We analyzed tumor growth upon BGJ398 treatment in the three endocrine resistant tumors and found that tumor growth was unaffected in two of these variants while a reduction in tumor growth rate and final tumor burden was determined in the C7-HI variant (Fig. **3A**). Growth inhibition was accompanied by a

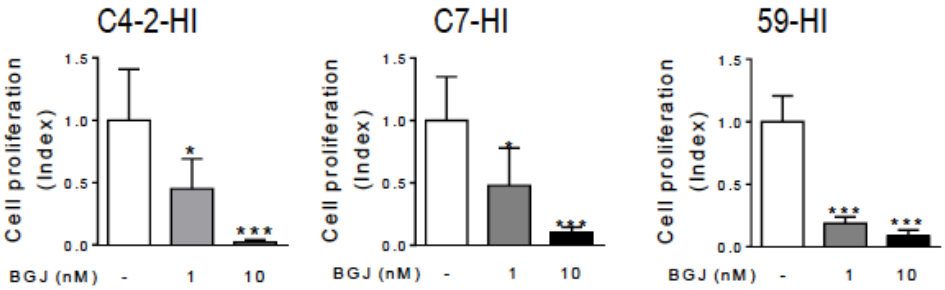


Fig. (2). Effect of BGJ398 treatment on epithelial cell proliferation in primary cell cultures of C4-2-HI, C7-HI and 59-HI murine tumors. Cell proliferation was measured by [H]-thymidine uptake. Primary cell cultures were starved in 1% chFCS for 24 hrs and treated for 48 hrs with experimental solutions (BGJ398: 1-10 nM). Treatments were performed in octuplicates. A representative experiment of three is shown. Statistics: ANOVA followed by *Tukey's* multiple comparisons test: (***): p<0.001; (*): p<0.05.

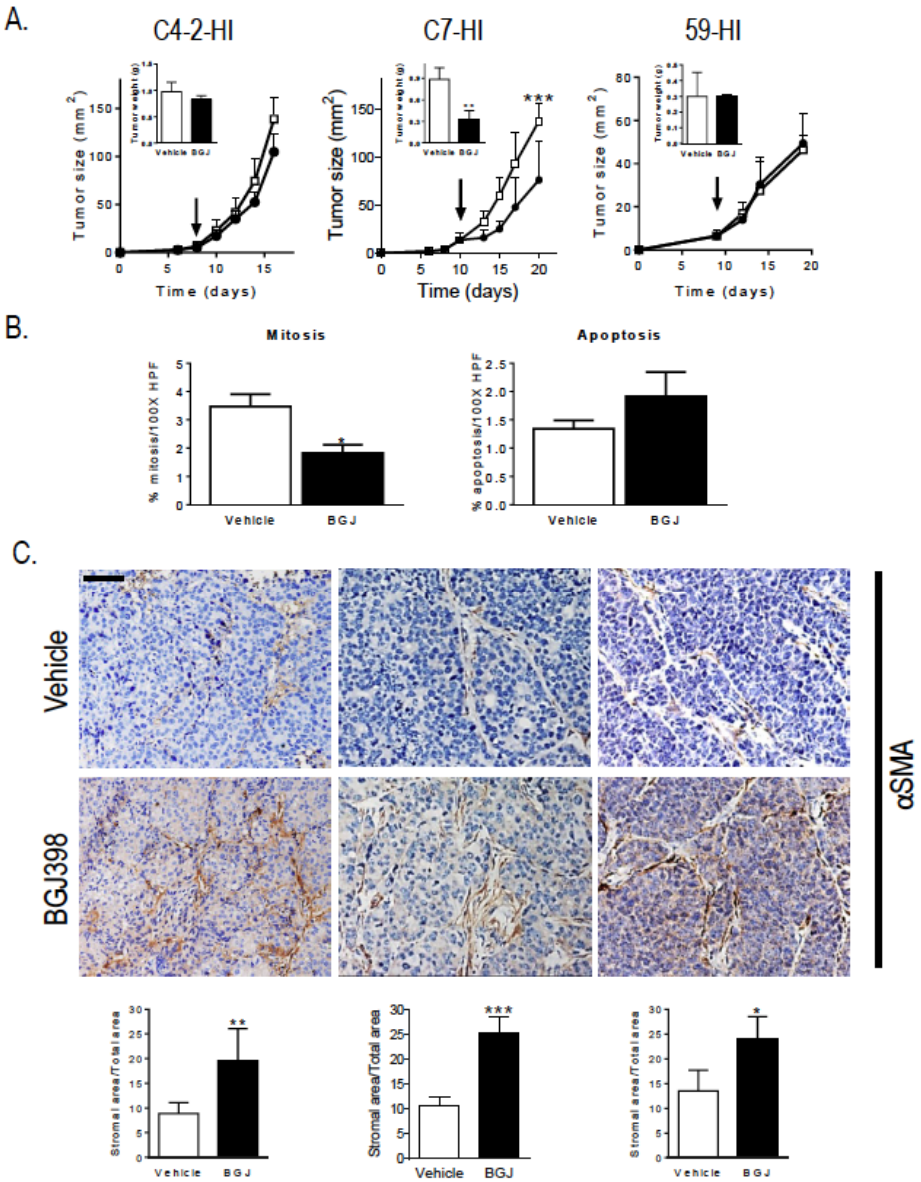


Fig. (3). Effect of BGJ398 treatment on tumor growth and αSMA expression of endocrine resistant murine mammary carcinomas C4-2-HI, C7-HI and 59-HI. A: Tumor growth curves and tumor weight at the end of the experiment in vehicle (open square) or BGJ398-treated (black circle; 30 mg/kg/every two days, iv) mice. The arrow shows when treatment started. B: Quantification of mitosis and apoptosis in untreated or BGJ-treated C7-HI tumors as described in *Materials and Methods*. HPF: high power field. C: Immunohistochemical staining and quantification for αSMA expression, in vehicle or BGJ398-treated tumors at the end of the experiment. Scale bar: 100 μm. Statistics: Lineal regression and slope comparison. *t Test* when comparing groups: (***): p<0.001; (**): p<0.01; (*): p<0.05.

significant reduction in the mitotic index (Fig. **3B**). Interestingly, BGJ398 treatment induced changes in tumor composition; the stromal area increased with a concomitant decrease in the tumor cells. These rearrangements were confirmed by an increase in αSMA staining of stromal cells in the three resistant variants (Fig. **3C**).

Altogether, our findings indicate that, the efficacy of BGJ398 on tumor size was less than what was expected according to the *in vitro* data, although it did reduce the epithelial/stromal ratio, thus diminishing the total number of tumor cells.

3.4. *In vitro* and *in vivo* Differential Effect of BGJ398 in the Human Endocrine Resistant T47D-YB Cell Line

Next, we tested the effects of FGFR inhibition in human luminal endocrine resistant T47D-YB breast cancer cells that specifically express PRB [21, 30]. In tumor xenograft sections, we found high total FGFR1-3 expression levels with a mainly nuclear localization for FGFR2 and cytosolic localization of FGFR1 and 3 (Fig. **4A**). As determined for the murine resistant variants, BGJ398 inhibited cell proliferation in culture (Fig. **4B**). *In vivo*, BGJ398 treatment induced no significant effects on tumor size (Fig. **4C**). However, there was a strong increase in the stromal area of treated tumors in detriment of the total number of tumor cells versus control tumors (Fig. **4D**).

3.5. BGJ398 Inhibits MFP-induced Lung Metastasis of Hormone Resistant C7-HI Tumors

The FGFR pathway is not only involved in cell proliferation, survival, and differentiation but also in migration and invasion (reviewed in [31]). Taking into account FGFR blockage induced tumor remodeling and that it partially reduced C7-HI primary tumor growth, we examined whether BGJ398 treatment had an impact on distant metastasis on this same model. C7-HI tumors give rise to spontaneous lung metastasis within 6-8 weeks of tumor transplantation. It is an antiprogestin resistant variant and treatment with MFP augments metastatic incidence even more [32]. Given the tumor's high growth rate, we surgically removed primary tumors on day 20. This allowed us to extend the experimental scheme and distinguish consolidated lung metastasis without exceeding the ethical limit of tumor burden (Fig. **5A**). Five doses of BGJ398 were administered before and after surgery. The blockage of FGFR signaling reduced C7-HI tumor growth as expected, while MFP, alone or combined with BGJ398, did not affect the final primary tumor burden (Fig. **5B**). Regarding dissemination, the % of mice with lung metastasis were higher in the MFP group compared to the control group (Fig. **5C**). Also, BGJ398-treated mice displayed a positive trend toward a lower number of lung foci compared to the control group (p=0.1541). Interestingly, the inhibitor could reduce the pro-metastatic effect of MFP, given that the combined therapy (MFP+BGJ398) significantly reduced the metastatic dissemination compared to the MFP group.

4. DISCUSSION

In this study, using different endocrine resistant luminal murine and human breast cancer models, we established that hormone resistant mammary carcinomas express high levels of FGFR1-3. All tumors proved to be responsive to FGFR inhibitors *in vitro*. *In vivo*, a delay in tumor growth was observed only in C7-HI BGJ398-treated tumors. However, an increase in stromal tissue in relation to the epithelial tumor areas was observed even in tumors that did not show a significant change in tumor burden. Moreover, a therapeutic effect was observed when impaired tumor dissemination occurred in BGJ398-treated tumors. Altogether, our findings indicate that blocking the FGFR pathway is a promising therapeutic alternative to delay breast cancer progression, although new highly-potent drugs should be developed to overcome *in vivo* low drug efficacy on primary tumor growth.

Various studies support the role of the FGFR pathway in endocrine resistant breast cancer [33-35]. FGFR1 gene amplification has been reported in approximately 10-14% of breast cancers [33, 36] and overexpression has been associated with poor survival and early relapse [33, 37]. We found that hormone resistant tumor variants expressed high total FGFR1 levels. Our results are in accordance with Turner *et al.* that hypothesize that high levels of FGFR1 lead to a constitutive activated pathway which could explain endocrine resistance even in the absence of the ligand [33]. Moreover, taking into consideration recent results from our laboratory, in which hormone resistant tumors express high levels of FGF2 (unpublished results), and that this ligand has a higher affinity for FGFR1 than to any of the other receptors [38], we propose that an elevated FGFR1 expression may be the result of a positive regulation in response to high FGF2 levels. Further work needs to be done to determine if the high FGFR1 expression in hormone resistant tumors is due to genetic amplification.

FGFR2 gene aberrations have been reported in approximately 11% of breast cancers [39], and single nucleotide polymorphisms in *FGFR2* have been associated to a higher risk of sporadic postmenopausal breast cancer [12]. We have previously reported that ligand-activated nuclear FGFR2 together with PR, bind to progesterone responsive elements in the *MYC* promoter, which in turn induce cell proliferation in hormone responsive murine tumors and in the T47D cell line [26]. In this study, we found that most of the endocrine resistant tumor variants still expressing high levels of PR, display high nuclear FGFR2, suggesting the involvement of constitutive activating mechanisms promoting tumor growth and endocrine resistance.

High expression levels of FGFR have been detected in breast cancer samples and, herein we found that different endocrine resistant tumors express high FGFR1-3 levels, potentially rendering them susceptible to anti-FGFR therapy. Data from our laboratory and others demonstrate that FGFR can localize to the nucleus where it can influence gene expression which may, in turn alter breast cancer progression [27, 40-43]. Based on our results, high total nuclear scores seem to be essential to achieve at least a small effect and we cannot associate nuclear or cytosolic FGFR localization to BGJ398 responsiveness.

Besides high FGFR expression in hormone resistant variants, we recently demonstrated that these variants display a basal activation of FGFR downstream proteins, such as FRS2, AKT, and PKCα (unpublished results). This suggests

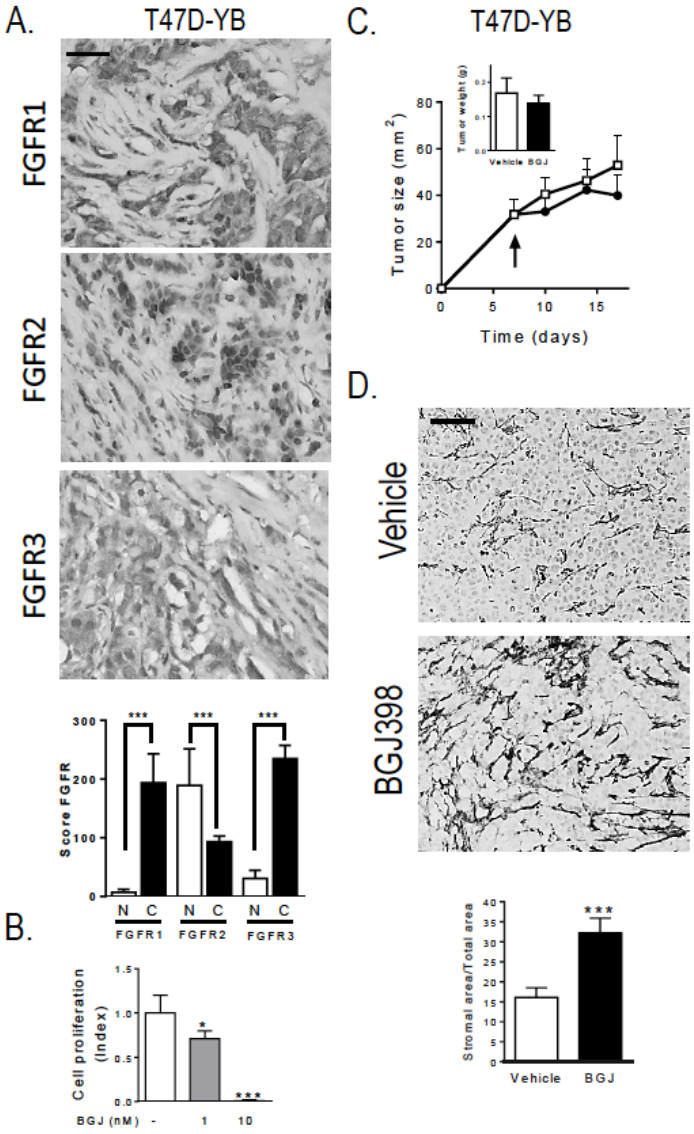
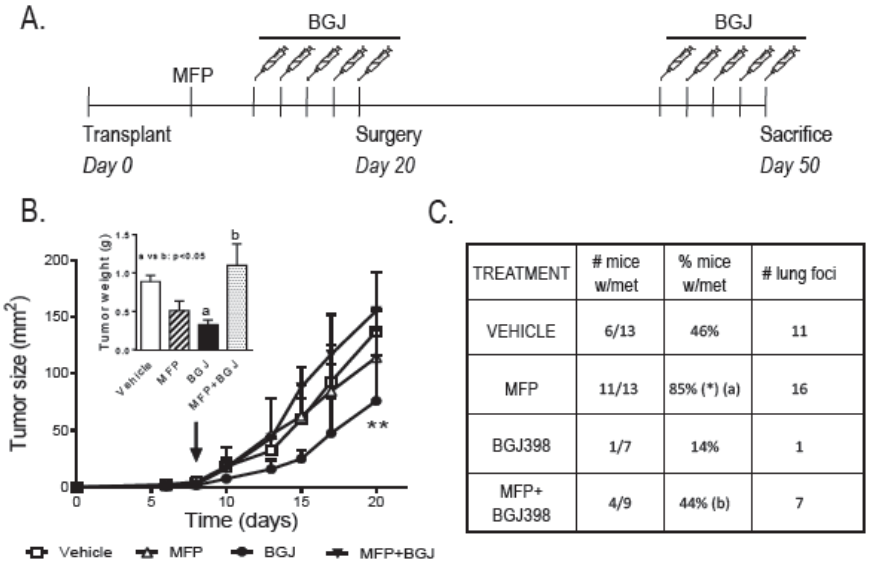


Fig. (4). FGFR1-3 expression, effect of BGJ398 treatment and αSMA expression in T47D-YB human cell line. A: Immunohistochemical staining and score for FGFR1-3 in endocrine resistant T47D-YB human xenograft. Nuclei were counterstained with hematoxylin. N: nuclear, C: cytosolic. Scale bar: 50 µm. Statistics:*t Test*: (***): p<0.001. B: Effect of BGJ398 treatment on T47D-YB cell proliferation. Cell proliferation was measured by total cell counting. T47D-YB cells were starved in 1% chFCS for 24 hrs and treated for 5 days with the experimental solutions (BGJ398: 1-10 nM). Treatments were performed in quadruplicates. A representative experiment of three is shown. Statistics: ANOVA followed by *Tukey's* multiple comparisons test: (***): p<0.001; (*): p<0.05. C: Effect of BGJ398 treatment on tumor growth expression in endocrine resistant T47D-YB human xenografts. Tumor growth curves and tumor weight at the end of the experiment in vehicle (open square) or BGJ398-treated (black circle; 30 mg/kg/every two days, iv) mice. The arrow shows when treatment started. D: Immunohistochemical staining and quantification for αSMA expression, in vehicle or BGJ398-treated tumors. Scale bar: 100µm. Statistics: *t Test*: (***): p<0.001.

that high FGF2 expression in endocrine resistant variants induce constitutive activation of the FGFR pathway, promoting tumor growth and endocrine resistance.

Considering that nM concentrations of BGJ398 induced a significant inhibition in cell proliferation in experimental conditions and that recent results from our laboratory demonstrate an inhibition in downstream effectors of the FGFR pathway, pERK and pAKT (Giulianelli *et al*, submitted), we expected to find strong *in vivo* responses using BGJ398. Despite using similar time schedules as those reported by others [16], mild effects on tumor growth were detected and were not improved when we tested different doses (18 mg/kg/day and 30 mg/kg/every two days) and administration schedules (oral gavage *vs* iv; data not shown). Our results are in line with the controversial data available regarding the effectiveness of FGFR inhibitors for breast cancer treatment [18, 44] where, despite the promising results that emerge mainly from preclinical *in vitro* studies, these do not seem to be sufficient to accurately predict the benefits of using FGFR inhibitors in clinical practice.

BGJ398 inhibited tumor growth in preclinical models of bladder, gastric, endometrial, and colon cancer bearing FGFR alterations [15, 16, 45, 46]. In breast cancer, *in vitro* studies with the *FGFR1*-amplified MDA-MB-134 cell line,



TREATMENT	# mice w/met	% mice w/met	# lung foci
VEHICLE	6/13	46%	11
MFP	11/13	85% (*) (a)	16
BGJ398	1/7	14%	1
MFP+ BGJ398	4/9	44% (b)	7

Fig. (5). Effect of BGJ398 on tumor metastasis in the endocrine resistant, metastatic C7-HI mammary carcinoma. A: Experimental scheme. B: Tumor growth curves of C7-HI tumors treated with vehicle, Mifepristone (MFP, 6 mg sc pellet), BGJ398 (30 mg/kg/every two days, iv) and MFP+BGJ398 until surgery (day 20). The arrow shows when treatment started. One representative experiment of two is shown. C: Quantification of lung metastatic foci (met). Statistics: Lineal regression and slope comparison: (**): $p<0.01$. To compare the % of mice with met: *Chi* Squared; a vs b and (*): $p<0.05$.

displayed an activated FGFR pathway that was inhibited by BGJ398 treatment [15, 16]. *In vivo*, BGJ398 induced complete tumor regression using an inducible Wnt1/ iFGFR1-driven breast cancer model [17] and impaired pulmonary tumor outgrowth in mice injected with the D2.A1 metastatic murine mammary tumor cell line [47]. However, recent data from the first clinical trial of the BGJ398 inhibitor in patients with advanced solid tumors bearing FGFR genetic alterations (ClinicalTrials.gov identifier NCT01004224), show that patients with squamous cell lung and bladder/urothelial cancer have clinical benefits after treatment, while no objective responses were observed in breast cancer patients [18]. In this study, we show that overall, BGJ398 induces mild effects on tumor growth and suggest that *in vivo:* either the drug is not fully accessible to the tumor cells or that it is not able to counteract the promoting role of the stroma which is not present in the *in vitro* settings. Taking into account that we found an increase in the amount of stromal tissue, indirectly diminishing the number of tumor cells, it seems that these agents may exert a beneficial effect that might be even improved in combined treatments. In line with our results, Holdman *et al.* described that FGFR inhibition with BGJ398 induced changes in the stromal microenvironment of regressing breast cancer tumors, causing an increase in the percentage of αSMA+ cells [17].

It is well known that endocrine resistance and metastatic dissemination are key players in tumor progression. Aberrant FGFR signaling may promote metastatic spread (reviewed in [48]) and *FGFR1* amplification, which frequently leads to FGFR1 overexpression, has been reported in metastatic breast cancer [33]. C7-HI is a PR+, endocrine resistant and metastatic tumor variant. In this study, we found that it also expresses high levels of FGFR1-3, and that blocking FGFR activity inhibited cell proliferation, tumor growth, and MFP-induced lung metastasis. Little published data are available regarding the effects of BGJ398 on tumor spread. In bladder cancer, Cheng *et al.* have reported that impairing FGFR signaling with BGJ398 strongly suppressed invasion and metastasis without altering primary tumor growth. Their results indicate that FGFR1 plays a crucial role in invasion and metastasis while FGFR3 drives cell proliferation. In line with our results in metastatic mammary cancer cells, FGFR blockage inhibited pulmonary tumor outgrowths [47]. BGJ398 has mainly cytostatic effects [49], and this may explain its limited impact on tumor regression, although stromal remodeling and cell differentiation may account for a reduction in the invasive capacity.

In conclusion, our results reveal that in breast cancer, FGFR inhibitors are promising tools that need further improvement and substantiate the idea of targeting FGFR combined with other targeted therapies to impair breast cancer progression.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

This work was supported by Instituto Nacional del Cáncer (2016-2017), Agencia Nacional de Promoción de Ciencia y Tecnología (ANAPCYT; PICT 2015/1022, PICT 2012/

1244, PID 2012/84), CONICET (PIP 603, 2013-15), Fundación Roemmers, Fundación René Barón, Fundación Williams and Fundación Fiorini. Fundación Sales supported the study until March 2014. AS, MM, and GRS are CONICET fellows, and CL and CAL are members of the Research Career.

ACKNOWLEDGEMENTS

CL and CAL conceived and designed the experiments. AS, CF, GS, and CAL performed the experiments. AS, MM, CL, and CAL analyzed the data. BJ contributed with intellectual content and was involved in revising the manuscript.

The authors are very grateful to *Dr. K. Horwitz*, University of Colorado, for kindly sharing the T47D-YB cells. Technical assistance with animal manipulation was provided by technician *B. Luna*. We thank Annabelle Nelson for reading and correcting the manuscript.

REFERENCES

[1] Torre, L. A.; Bray, F.; Siegel, R. L.; Ferlay, J.; Lortet-Tieulent, J.; Jemal, A., Global cancer statistics, 2012. CA Cancer J Clin 2015, 65, 87-108.

[2] Santen, R. J.; Manni, A.; Harvey, H.; Redmond, C., Endocrine treatment of breast cancer in women. Endocr Rev 1990, 11, 221-265.

[3] Normanno, N.; Di Maio, M.; De Maio, E.; De Luca, A.; de Matteis, A.; Giordano, A.; Perrone, F., Mechanisms of endocrine resistance and novel therapeutic strategies in breast cancer. Endocr Relat Cancer 2005, 12, 721-747.

[4] Eswarakumar, V. P.; Lax, I.; Schlessinger, J., Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 2005, 16, 139-149.

[5] Brooks, A. N.; Kilgour, E.; Smith, P. D., Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. Clin Cancer Res 2012, 18, 1855-1862.

[6] Turner, N.; Grose, R., Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 2010, 10, 116-129.

[7] Katoh, Y.; Katoh, M., FGFR2-related pathogenesis and FGFR2-targeted therapeutics. Int J Mol Med 2009, 23, 307-311.

[8] Kelleher, F. C.; O'Sullivan, H.; Smyth, E.; McDermott, R.; Viterbo, A., Fibroblast growth factor receptors, developmental corruption and malignant disease. Carcinogenesis 2013, 34, 2198-2205.

[9] Turner, N.; Lambros, M. B.; Horlings, H. M.; Pearson, A.; Sharpe, R.; Natrajan, R.; Geyer, F. C.; van Kouwenhove, M.; Kreike, B.; Mackay, A.; Ashworth, A.; van de Vijver, M. J.; Reis-Filho, J. S., Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. Oncogene 2010, 29, 2013-2023.

[10] Jaakkola, S.; Salmikangas, P.; Nylund, S.; Partanen, J.; Armstrong, E.; Pyrhonen, S.; Lehtovirta, P.; and Nevanlinna, H., Amplification of fgfr4 gene in human breast and gynecological cancers. Int J Cancer 1993, 54, 378-382.

[11] Easton, D.F.; Pooley, K. A.; Dunning, A. M.; Pharoah, P. D.; Thompson, D.; Ballinger, D. G.; Struewing, J. P.; Morrison, J.; Field, H.; Luben, R.; Wareham, N.; Ahmed, S.; Healey, C. S.; Bowman, R.; Meyer, K. B.; Haiman, C. A.; Kolonel, L. K.; Henderson, B. E.; Le Marchand, L.; Brennan, P.; Sangrajrang, S.; Gaborieau, V.; Odefrey, F.; Shen, C. Y.; Wu, P. E.; Wang, H. C.; Eccles, D.; Evans, D. G.; Peto, J.; Fletcher, O.; Johnson, N.; Seal, S.; Stratton, M. R.; Rahman, N.; Chenevix-Trench, G.; Bojesen, S. E.; Nordestgaard, B. G; Axelsson, C. K.; Garcia-Closas, M.; Brinton, L.; Chanock, S.; Lissowska, J.; Peplonska, B.; Nevanlinna, H.; Fagerholm, R.; Eerola, H.; Kang, D.; Yoo, K. Y.; Noh, D. Y.; Ahn, S. H.; Hunter, D. J.; Hankinson, S. E.; Cox, D. G.; Hall, P.; Wedren, S.; Liu, J.; Low, Y. L.; Bogdanova, N.; Schurmann, P.; Dork, T.; Tollenaar, R. A.; Jacobi, C. E.; Devilee, P.; Klijn, J. G.; Sigurdson, A. J.; Doody, M. M.; Alexander, B. H.; Zhang, J.; Cox, A.; Brock, I. W.; MacPherson, G.; Reed, M. W.; Couch, F. J.; Goode, E. L.; Olson, J. E.; Meijers-Heijboer, H.; van den, O. A.; Uitterlinden, A.; Rivadeneira, F.; Milne, R. L.; Ribas, G.; Gonzalez-Neira, A.; Benitez, J.; Hopper, J. L.; McCredie, M.; Southey, M.; Giles, G. G.; Schroen, C.; Justenhoven, C.; Brauch, H.; Hamann, U.; Ko, Y. D.; Spurdle, A. B.; Beesley, J.; Chen, X.; Mannermaa, A.; Kosma, V. M.; Kataja, V.; Hartikainen, J.; Day, N. E.; Cox, D. R.; Ponder, B. A., Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007, 447, 1087-1093.

[12] Hunter, D. J.; Kraft, P.; Jacobs, K. B.; Cox, D. G.; Yeager, M.; Hankinson, S. E.; Wacholder, S.; Wang, Z.; Welch, R.; Hutchinson, A.; Wang, J.; Yu, K.; Chatterjee, N.; Orr, N.; Willett, W. C.; Colditz, G. A.; Ziegler, R. G.; Berg, C. D.; Buys, S. S.; McCarty, C. A.; Feigelson, H. S.; Calle, E. E.; Thun, M. J.; Hayes, R. B.; Tucker, M.; Gerhard, D. S.; Fraumeni, J. F. Jr.; Hoover, R. N.; Thomas, G.; Chanock, S. J., A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 2007, 39, 870-874.

[13] Stacey, S. N.; Manolescu, A.; Sulem, P.; Thorlacius, S.; Gudjonsson, S. A.; Jonsson, G. F.; Jakobsdottir, M.; Bergthorsson, J. T.; Gudmundsson, J.; Aben, K. K.; Strobbe, L. J.; Swinkels, D. W.; van Engelenburg, K. C.; Henderson, B. E.; Kolonel, L. N.; Le Marchand, L.; Millastre, E.; Andres, R.; Saez, B.; Lambea, J.; Godino, J.; Polo, E.; Tres, A.; Picelli, S.; Rantala, J.; Margolin, S.; Jonsson, T.; Sigurdsson, H.; Jonsdottir, T.; Hrafnkelsson, J.; Johannsson, J.; Sveinsson, T.; Myrdal, G.; Grimsson, H. N.; Sveinsdottir, S. G.; Alexiusdottir, K.; Saemundsdottir, J.; Sigurdsson, A.; Kostic, J.; Gudmundsson, L.; Kristjansson, K.; Masson, G.; Fackenthal, J. D.; Adebamowo, C.; Ogundiran, T.; Olopade, O. I.; Haiman, C. A.; Lindblom, A.; Mayordomo, J. I.; Kiemeney, L. A.; Gulcher, J. R.; Rafnar, T.; Thorsteinsdottir, U.; Johannsson, O. T.; Kong, A.; Stefansson, K., Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet 2008, 40, 703-706.

[14] Bange, J.; Prechtl, D.; Cheburkin, Y.; Specht, K.; Harbeck, N.; Schmitt, M.; Knyazeva, T.; Muller, S.; Gartner, S.; Sures, I.; Wang, H.; Imyanitov, E.; Haring, H. U.; Knayzev, P.; Iacobelli, S.; Hofler, H.; Ullrich, A. Cancer progression and tumor cell motility are associated with the FGFR4 Arg(388) allele. Cancer Res 2002, 62, 840-847.

[15] Guagnano, V.; Furet, P.; Spanka, C.; Bordas, V.; Le Douget, M.; Stamm, C.; Brueggen, J.; Jensen, M. R.; Schnell, C.; Schmid, H.; Wartmann, M.; Berghausen, J.; Drueckes, P.; Zimmerlin, A.; Bussiere, D.; Murray, J.; Graus, P. D., Discovery of 3-(2,6-dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamin o]-pyrimidin-4-yl}-1-methyl-urea (NVP-BGJ398), a potent and selective inhibitor of the fibroblast growth factor receptor family of receptor tyrosine kinase. J Med Chem 2011, 54, 7066-7083.

[16] Guagnano,V., Kauffmann,A., Wohrle,S., Stamm,C., Ito,M., Barys,L., Pornon,A., Yao,Y., Li,F., Zhang,Y., Chen,Z., Wilson,C.J., Bordas,V., Le Douget,M., Gaither,L.A., Borawski,J., Monahan,J.E., Venkatesan,K., Brummendorf,T., Thomas,D.M., Garcia-Echeverria,C., Hofmann,F., Sellers,W.R., Graus-Porta,D. FGFR genetic alterations predict for sensitivity to NVP-BGJ398, a selective pan-FGFR inhibitor. Cancer Discov 2012, 2, 1118-1133.

[17] Holdman, X. B.; Welte, T.; Rajapakshe, K.; Pond, A.; Coarfa, C.; Mo, Q.; Huang, S.; Hilsenbeck, S. G.; Edwards, D. P.; Zhang, X.; Rosen, J. M., Upregulation of EGFR signaling is correlated with tumor stroma remodeling and tumor recurrence in FGFR1-driven breast cancer. Breast Cancer Res 2015, 17, 141.

[18] Nogova, L.; Sequist, L. V.; Perez Garcia, J. M.; Andre, F.; Delord, J. P.; Hidalgo, M.; Schellens, J. H.; Cassier, P. A.; Camidge, D. R.; Schuler, M.; Vaishampayan, U.; Burris, H.; Tian, G. G.; Campone, M.; Wainberg, Z. A.; Lim, W. T.; LoRusso, P.; Shapiro, G. I.; Parker, K.; Chen, X.; Choudhury, S.; Ringeisen, F.; Graus-Porta, D.; Porter, D.; Isaacs, R.; Buettner, R.; Wolf, J., Evaluation of BGJ398, a Fibroblast Growth Factor Receptor 1-3 Kinase Inhibitor, in Patients With Advanced Solid Tumors Harboring Genetic Alterations in Fibroblast Growth Factor Receptors: Results of a Global Phase I, Dose-Escalation and Dose-Expansion Study. J Clin Oncol 2017, 35, 157-165.

[19] National Research Council. Guide for the Care and Use of Laboratory Animals. 8th Edition. Washington, DC: National Academies Press 2011.

[20] Lanari, C.; Lamb, C. A.; Fabris, V. T.; Helguero, L. A.; Soldati, R.; Bottino, M. C.; Giulianelli, S.; Cerliani, J. P.; Wargon, V.; Molinolo, A., The MPA mouse breast cancer model: evidence for a role

of progesterone receptors in breast cancer. Endocr Relat Cancer 2009, 16, 333-350.

[21] Wargon, V.; Riggio, M.; Giulianelli, S.; Sequeira, G. R.; Rojas, P.; May, M.; Polo, M. L.; Gorostiaga, M. A.; Jacobsen, B.; Molinolo, A.; Novaro, V.; Lanari, C., Progestin and antiprogestin responsiveness in breast cancer is driven by the PRA/PRB ratio via AIB1 or SMRT recruitment to the CCND1 and MYC promoters. Int J Cancer 2015, 136, 2680-2692.

[22] Wargon, V.; Helguero, L. A.; Bolado, J.; Rojas, P.; Novaro, V.; Molinolo, A.; Lanari, C., Reversal of antiprogestin resistance and progesterone receptor isoform ratio in acquired resistant mammary carcinomas. Breast Cancer Res Treat 2009, 116, 449-460.

[23] Wargon, V.; Fernandez, S. V.; Goin, M.; Giulianelli, S.; Russo, J.; Lanari, C., Hypermethylation of the progesterone receptor A in constitutive antiprogestin-resistant mouse mammary carcinomas. Breast Cancer Res Treat 2011, 126, 319-332.

[24] Jacobsen, B. M.; Richer, J. K.; Schittone, S. A.; Horwitz, K. B., New Human Breast Cancer Cells to Study Progesterone Receptor Isoform Ratio Effects and Ligand-independent Gene Regulation. J Biol Chem 2002, 277, 27793-27800.

[25] Sartorius, C. A.; Shen, T.; Horwitz, K. B., Progesterone receptors A and B differentially affect the growth of estrogen-dependent human breast tumor xenografts. Breast Cancer Res Treat 2003, 79, 287-299.

[26] Cerliani, J. P.; Guillardoy, T.; Giulianelli, S.; Vaque, J. P.; Gutkind, J. S.; Vanzulli, S. I.; Martins, R.; Zeitlin, E.; Lamb, C. A.; Lanari, C, Interaction between FGFR-2, STAT5, and Progesterone Receptors in Breast. Cancer Cancer Res 2011, 71, 3720-3731.

[27] Cerliani, J. P.; Vanzulli, S. I.; Pinero, C. P.; Bottino, M. C.; Sahores, A.; Nuñez, M.; Varchetta, R.; Martins, R.; Zeitlin, E.; Hewitt, S. M.; Molinolo, A. A.; Lanari, C.; Lamb, C. A., Associated expressions of FGFR-2 and FGFR-3: from mouse mammary gland physiology to human breast cancer. Breast Cancer Res Treat 2012, 133, 997-1008.

[28] Lamb, C.; Simian, M.; Molinolo, A.; Pazos, P.; Lanari, C., Regulation of cell growth of a progestin-dependent murine mammary carcinoma in vitro: progesterone receptor involvement in serum or growth factor-induced cell proliferation. J Steroid Biochem Mol Biol 1999, 70, 133-142.

[29] Sahores, A.; Luque, G. M.; Wargon, V.; May, M.; Molinolo, A.; Becu-Villalobos, D.; Lanari,C., Lamb,C.A. Novel, low cost, highly effective, handmade steroid pellets for experimental studies. PLoS ONE 2013, 8, e64049.

[30] Sartorius, C. A.; Groshong, S. D.; Miller, L. A.; Powell, R. L.; Tung, L.; Takimoto, G. S.; Horwitz, K. B., New T47D breast cancer cell lines for the independent study of progesterone B- and A-receptors: only antiprogestin-occupied B- receptors are switched to transcriptional agonists by cAMP. Cancer Res 1994, 54, 3868-3877.

[31] Andre, F.; Cortes, J., Rationale for targeting fibroblast growth factor receptor signaling in breast cancer. Breast Cancer Res Treat 2015, 150, 1-8.

[32] Álvarez, M.; Giulianelli, S.; Riggio, M.; Sequeira, G.; May, M.; Novaro, V.; Sahores, A.; Lamb, C.; Lanari, C., The ratio of progesterone receptor isoform A to B determines the effect of antiprogestins on preclinical models of breast cancer metastasis. San Antonio Breast Cancer Symposium 2015, P2-05-27.

[33] Turner, N.; Pearson, A.; Sharpe, R.; Lambros, M.; Geyer, F.; Lopez-Garcia, M. A.; Natrajan, R.; Marchio, C.; Iorns, E.; Mackay, A.; Gillett, C.; Grigoriadis, A.; Tutt, A.; Reis-Filho, J. S.; Ashworth, A., FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer Res 2010, 70, 2085-2094.

[34] Meijer, D.; Sieuwerts, A. M.; Look, M. P.; van Agthoven, T.; Foekens, J. A.; Dorssers, L. C., Fibroblast growth factor receptor 4 predicts failure on tamoxifen therapy in patients with recurrent breast cancer. Endocr Relat Cancer 2008, 15, 101-111.

[35] Azuma, K.; Tsurutani, J.; Sakai, K.; Kaneda, H.; Fujisaka, Y.; Takeda, M.; Watatani, M.; Arao, T.; Satoh, T.; Okamoto, I.; Kurata, T.; Nishio, K.; Nakagawa, K., Switching addictions between HER2 and FGFR2 in HER2-positive breast tumor cells: FGFR2 as a potential target for salvage after lapatinib failure. Biochem Biophys Res Commun 2011, 407, 219-224.

[36] Shi, Y. J.; Tsang, J. Y.; Ni, Y. B.; Chan, S. K.; Chan, K. F.; Tse, G. M., FGFR1 is an adverse outcome indicator for luminal A breast cancers. Oncotarget 2015, FALTAN LAS HOJAS

[37] Elbauomy, E. S.; Green, A. R.; Lambros, M. B.; Turner, N. C.; Grainge, M. J.; Powe, D.; Ellis, I. O.; Reis-Filho, J. S., FGFR1 amplification in breast carcinomas: a chromogenic in situ hybridisation analysis. Breast Cancer Res 2007, 9, R23.

[38] Ibrahimi, O. A.; Zhang, F.; Hrstka, S. C.; Mohammadi, M.; Linhardt, R. J., Kinetic model for FGF, FGFR, and proteoglycan signal transduction complex assembly. Biochemistry 2004, 43, 4724-4730.

[39] Adnane, J.; Gaudray, P.; Dionne, C. A.; Crumley, G.; Jaye, M.; Schlessinger, J.; Jeanteur, P.; Birnbaum, D.; Theillet, C., BEK and FLG, two receptors to members of the FGF family, are amplified in subsets of human breast cancers. Oncogene 1991, 6, 659-663.

[40] Zammit, C.; Barnard, R.; Gomm, J.; Coope, R.; Shousha, S.; Coombes, C.; Johnston, C., Altered intracellular localization of fibroblast growth factor receptor 3 in human breast cancer. J Pathol 2001, 194, 27-34.

[41] Sun, S.; Jiang, Y.; Zhang, G.; Song, H.; Zhang, X.; Zhang, Y.; Liang, X.; Sun, Q.; Pang, D., Increased expression of fibroblastic growth factor receptor 2 is correlated with poor prognosis in patients with breast cancer. J Surg Oncol 2012, 105, 773-779.

[42] Brown, W. S.; Tan, L.; Smith, A.; Gray, N. S.; Wendt, M. K., Covalent Targeting of Fibroblast Growth Factor Receptor Inhibits Metastatic Breast Cancer. Mol Cancer Ther 2016, 15, 2096-2106.

[43] Stachowiak, M. K.; Stachowiak, E. K., Evidence-Based Theory for Integrated Genome Regulation of Ontogeny-An Unprecedented Role of Nuclear FGFR1 Signaling. J Cell Physiol 2016, 231, 1199-1218.

[44] Schram, A. M.; Voss, M. H.; Hyman, D. M., Genome-Driven Paradigm for the Development of Selective Fibroblast Growth Factor Receptor Inhibitors. J Clin Oncol 2017, 35, 131-134.

[45] Konecny, G. E.; Kolarova, T.; O' Brien, N. A.; Winterhoff, B.; Yang, G.; Qi, J.; Qi, Z.; Venkatesan, N.; Ayala, R.; Luo, T.; Finn, R. S.; Kristof, J.; Galderisi, C.; Porta, D. G.; Anderson, L.; Shi, M. M.; Yovine, A.; Slamon, D. J., 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.

[46] Acquaviva, J.; He, S.; Zhang, C.; Jimenez, J. P.; Nagai, M.; Sang, J.; Sequeira, M.; Smith, D. L.; Ogawa, L. S.; Inoue, T.; Tatsuta, N.; Knowles, M. A.; Bates, R. C.; Proia, D. A., FGFR3 translocations in bladder cancer: differential sensitivity to HSP90 inhibition based on drug metabolism. Mol Cancer Res 2014, 12, 1042-1054.

[47] Wendt, M. K.; Taylor, M. A.; Schiemann, B. J.; Sossey-Alaoui, K.; Schiemann, W. P., Fibroblast growth factor receptor splice variants are stable markers of oncogenic transforming growth factor beta1 signaling in metastatic breast cancers. Breast Cancer Res 2014, 16, R24.

[48] Katoh, M., FGFR inhibitors: Effects on cancer cells, tumor microenvironment and whole-body homeostasis. Int J Mol Med 2016, 38, 3-15.

[49] Cheng, T.; Roth, B.; Choi, W.; Black, P. C.; Dinney, C.; McConkey, D .J., Fibroblast growth factor receptors-1 and -3 play distinct roles in the regulation of bladder cancer growth and metastasis: implications for therapeutic targeting. PLoS ONE 2013, 8, e57284.