Inhibiting Aurora Kinases Reduces Tumor Growth and Suppresses Tumor Recurrence after Chemotherapy in Patient-Derived Triple-Negative Breast Cancer Xenografts

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Abstract

Triple-negative breast cancers (TNBC) have an aggressive phenotype with a relatively high rate of recurrence and poor overall survival. To date, there is no approved targeted therapy for TNBCs. Aurora kinases act as regulators of mammalian cell division. They are important for cell-cycle progression and are frequently overexpressed or mutated in human tumors, including breast cancer. In this study, we investigated the therapeutic potential of targeting Aurora kinases in preclinical models of human breast cancers using a pan-inhibitor of Aurora kinases, AS703569. In vitro, AS703569 was tested in 15 human breast cancer cell lines. TNBC cell lines were more sensitive to AS703569 than were other types of breast cancer cells. Inhibition of proliferation was associated with cell-cycle arrest, aneuploidy, and apoptosis. In vivo, AS703569 administered alone significantly inhibited tumor growth in seven of 11 patient-derived breast cancer xenografts. Treatment with AS703569 was associated with a decrease of phospho-histone H3 expression. Finally, AS703569 combined to doxorubicin–cyclophosphamide significantly inhibited in vivo tumor recurrence, suggesting that Aurora kinase inhibitors could be used both in monotherapy and in combination settings. In conclusion, these data indicate that targeting Aurora kinases could represent a new effective approach for TNBC treatment. Mol Cancer Ther; 11(12): 2693–703. ©2012 AACR.

Introduction

Triple-negative breast cancers (TNBC), which lack expression of estrogen receptors (ER), progesterone receptors (PR), and EGF receptor 2 (HER2), account for 15% of breast tumors in Europe and an even higher percentage of breast cancer in women of African descent. TNBC tumors have a relatively high rate of recurrence, distant metastases, and poor overall survival (1). TNBC is responsible for a large proportion of breast cancer deaths, despite its relatively small proportion among all breast cancers, due to its generally aggressive clinical course. The standard-of-care is chemotherapy, although recent research suggests a sound rationale for the use of targeted agents with antitumor and/or antiangiogenic activity such as receptor tyrosine kinase inhibitors. The absence of tumor-specific treatment options in this cancer subset underscores the critical need to develop a better understanding of the biology of this disease, as well as to advance treatment strategies for these patients (1).

Signaling pathway abnormalities commonly reported in TNBCs involve the regulatory mechanisms of cellular proliferation, differentiation, p21-mediated cell signaling, and G1–S checkpoint controls (2, 3).

The Aurora kinase proteins are serine/threonine kinases that act as regulators of mammalian cell division. Aurora A localizes to centrosomes/spindle poles and is required for spindle assembly, whereas Aurora B is a chromosome passenger protein required for phosphorylation of histone H3, chromosome segregation, and cytokinesis (4–7).

Aurora A and B have been implicated in tumor formation and progression (6, 8) and are overexpressed in a variety of cell lines (4, 9). Relatively high expression of Aurora A and B has been shown in small patient cohorts in several tumor types, including breast, lung, colon,
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Mol Cancer Ther; 11(12) December 2012

prostate, pancreas, liver, skin, stomach, rectum, esophagus, endometrium, cervix, bladder, ovary, and thyroid cancers (4–6, 10). In breast cancer, the expression of Aurora kinase A has been found to be predictive of survival in a cohort of more than 600 primary tumors (7).

Given their pivotal role in mitosis and overexpression in cancers, Aurora kinases have become attractive targets. A number of inhibitors displaying differential inhibitory activities toward the 3 family members have since then been developed and used to understand the functional role of Aurora kinases in mitotic progression. Aurora kinase inhibitors induce tetraploidy and polyploidy as a result of aberrant mitosis. The effect of the Aurora kinase inhibitors is therefore unique in that tumor cells do not undergo cell-cycle arrest after drug treatment. Rather, they are catastrophically driven forward and from aberrant mitosis, which leads to cell death.

In vivo studies with several agents targeting Aurora kinases have shown promising results, with tumor growth being inhibited in a number of models (8, 11, 12). The therapeutic potential of Aurora-based targeted therapy is also being assessed in clinical trials (13), although none of them have specifically addressed TNBCs.

In the current studies, we investigated the efficacy of a pan-Aurora kinase inhibitor, AS703569 (14), in preclinical models of human breast cancers. We report that AS703569 has potent antitumor activity in TNBC cell lines, associated with endoreduplication and apoptosis. This antitumor activity was confirmed in vivo using patient-derived breast cancer xenografts.

Materials and Methods

Compound

Inhibition of Aurora kinases was achieved using the small-molecule pan-Aurora kinase inhibitor, AS703569 (previously named R763), an orally potent ATP competitive inhibitor. In vitro biochemical assays showed that AS703569 inhibited Aurora kinases A, B, and C with IC50 values of 4.0, 4.8, and 6.8 nmol/L, respectively (Fig. 1A; ref. 14). The selectivity of AS703569 assessed in a panel of cell-based kinase assays, was previously published (14).

In vitro experiments were carried out using dilutions from a 2 nmol/L stock of compound dissolved in dimethyl sulfoxide (DMSO). For in vivo studies, AS703569 was suspended in sterile 0.9% NaCl solution and administered by oral gavage.

Human breast cancer cell lines

The following cell lines were obtained from American Type Culture Collection (ATCC) and suspended in sterile 0.9% NaCl solution and administered by oral gavage:

- Prostate (DU145, 22Rv1, PC3, PC3M, LnCap)
- Pancreatic (AsPC-1, MiaPaCa-2)
- Liver (HepG2, Hep3B)
- Skin (A431, A431V, Hs578T)
- Stomach (MKN45, MKN7, MKN28, MKN45)
- Rectum (HCT116, HT29, SW480, SW620, Caco-2)
- Esophagus (KATO-3, OE33, OE33A)
- Endometrium (IEX1, IEX2, IEX3, IEX4, IEX5)
- Cervix (HeLa, SiHa, HLRCC, CaSki, HPV18, HPV16)
- Bladder (RT4, T24, T24B)
- Ovary (CaOv, SKOV3, DOV and OVCAR-3, OVCAR-5, OVCAR-8)

These cell lines were grown in Dulbecco’s Modified Eagle’s Medium F12 with 12 mg/mL sodium bicarbonate, 10% FBS, 10 mmol/L HEPES, 10 μg/mL insulin (all from Life Technologies), and 10–12 mol/L estradiol (Sigma-Aldrich). The HBCx-17, HBCx-15, and HBCx-8 TNBC cell lines were obtained from 3 patient-derived breast cancer xenografts (15, 16).

Cell proliferation assay

The ATPlite assay was used as a surrogate for detection of cell count/proliferation. Intracellular ATP concentrations were determined using the ATPlite Luminescence ATP Detection Assay System (Perkin Elmer), following manufacturer’s instructions. Briefly, cells were incubated in the presence of serial dilutions of AS703569 (range from 0.001 to 100 μmol/L). After 96 hours in culture, the growth medium was replaced with 100 μL PBS. Cell lysis buffer (50 μL) was added and mixed for 5 minutes, 50 μL of ATPLite substrate reagent was then added and mixed for 5 minutes. After incubating for 10 minutes in the dark, plates were measured for luminescence on a Victor-5 1428 Multilabel HTS counter (Perkin Elmer). Results were displayed as relative light units.

Cell-cycle and apoptosis analysis

Cells were plated on 6-well tissue culture dishes and allowed to grow overnight to a density of 40% to 50% confluence. They were then treated with various concentrations of inhibitor and harvested after 48, 72, and 96 hours. At the time of harvest, cells were washed once with PBS and trypsinized. Cells were harvested in 1 mL of complete medium and pelleted for 5 minutes at 600 × g. Cells were fixed and permeabilized with the addition of 100% ice-cold methanol and incubated on ice for 30 minutes. Cells were pelleted and washed twice in 1% bovine serum albumin (BSA; Fisher Scientific)/PBS and split into 2 tubes, one to measure cell cycle by propidium iodide (PI) staining and the other to measure apoptosis by staining of cleaved caspase-3. Cells for cell-cycle analyses were resuspended in PI/RNase (Becton Dickenson) and incubated for 15 minutes at room temperature and then analyzed by flow cytometry using a Guava EasyCyte instrument (Guava). Cells to be analyzed for the presence of cleaved caspase-3 were stained with Alexa-488 conjugated cleaved caspase-3 (Asp175) antibody (Cell Signaling Technologies) followed by flow cytometric analysis (Guava EasyCyte).

In vivo efficacy studies

Female Swiss nude mice, 10-week-old, were purchased from Charles River and maintained under specific pathogen-free conditions. Their care and housing were in accordance with Institutional Guidelines as put forth by the French Ethical Committee. Human breast cancer xenografts were established from primary patient’s surgical specimens by grafting tumor fragments into the interscapular fat pad of nude mice and maintained through in vivo passages as previously described (16). Adriamycin (doxorubicin) and cyclophosphamide (Endoxan) were
purchased from Teva Pharmaceuticals and Baxter, respectively. Docetaxel was purchased from Sanofi. Adriamycin (2 mg/kg), cyclophosphamide (100 mg/kg), and docetaxel (20 mg/kg) were administered by the intraperitoneal route at 3-week intervals.

AS703569 was administered once per week by oral gavage at its maximum tolerated dose (MTD) of 50 mg/kg, which was determined by a tolerance study (Supplementary Materials and Methods). Tumor growth inhibition (TGI) and tumor growth delay (GD) were calculated as detailed in Supplementary Methods and Materials.

Real-time PCR amplification

Extraction of total RNA and synthesis of cDNA were conducted as previously described from 1 μg total RNA.
Ki67 and MDR1 transcripts were quantified using real-time quantitative reverse transcription PCR. The nucleotide and probe sequences and the conditions for the PCR were previously described for Ki67 and MDR1 (16, 18). Normalization, reference genes, and quantification are described elsewhere (19) and in Supplementary Methods and Materials.

Pharmacodynamic study and immunohistochemistry of xenograft tumor tissues

Xenoengrafted tumors HBCx-5, HBCx-10, and HBCx-15 were evaluated for expression of phospho-histone H3 Ser10 (pHistH3) as a marker of Aurora kinase B activity. Tumor-bearing mice were treated with a single administration of AS703569 (50 mg/kg) or buffer vehicle when tumors had reached a volume of approximately 150 to 200 mm³. Mice were euthanized at 6, 24, 48, 72 hours, and 21 days posttreatment. Two control groups are included in the kinetics study (named control 6 h and control 72 h). Four animals were included in each time point. Tumor tissue samples were fixed in 10% neutral-buffered formalin and paraffin-embedded using routine protocols with a tissue microarray (780–2845) was denatured for 8 minutes at 85 °C, followed by aBRCA2-mutated.

Histological analysis of the interscapular fat pad and immunostaining (immunohistochemistry) of residual tumor cells

To analyze residual tumor cells after tumor remission, mice were euthanized when tumors had completely regressed (~60 days after the chemotherapy treatment). The interscapular fat pad was excised, fixed, and paraffin-embedded. Semiserial 4-μm sections were cut every 250 μm until exhaustion of the tissue. Ziehl–Nielsen staining was used to characterize intracellular brown pigments. Ki67 immunohistochemistry (IHC) was conducted using a rabbit monoclonal anti-human Ki67 antibody (clone SP6) and paraffin-embedded sections using routine protocols with a Sakura Tissue Tek VIP Vacuum Infiltration Processor (Sakura) and a Leica EG 1150 Embedding Center (Leica Microsystèmes SAS). Further details on tissue microarray techniques, antibodies, and morphometry are included in Supplementary Methods and Materials.

Results

AS703569 potently inhibited cell growth of TNBCs in vitro

To determine the effects of AS703569 on tumor cell viability, a panel of 15 cell lines, representing the various subtypes of human breast cancers, was treated with increasing concentrations of AS703569 for 96 hours. Eight cell lines were triple-negative, 3 cell lines were positive for HER2, and 4 were triple-positive (ER⁺, PR⁺, and HER2⁺). p53 was mutated in 10 cell lines (7 TNBCs) and wild-type (wt) in 5 cell lines (1 TNBC). The 50% inhibitory concentrations of cell viability (IC₅₀) at 96 hours, determined by ATPliite assay, are shown in Table 1. The triple-negative cell lines were relatively more sensitive to AS703569, with IC₅₀ values between 0.004 and 0.694 μmol/L, whereas the ER⁺/PR⁺ and HER2⁺ cell lines were more resistant to AS703569, with IC₅₀ between 2.52 and 7.37 μmol/L. The difference between these 2 groups was statistically significant (Fig. 1B; unpaired Student t test, P < 0.0001).

AS703569 induces endoduplication and apoptosis

Inhibition of Aurora kinase B, or both A and B, results in early exit from mitosis without cell division, leading to endoreduplication and polyploidy. The latter is defined as an accumulation in DNA content (>4N). To evaluate the association between polyploidy and the antitumor

<table>
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<th>ER</th>
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Abbreviations: mut, mutated; neg, negative; pos, positive. *BRCA2-mutated.
activity of AS703569, compound-induced changes in cell-cycle progression were investigated in 7 breast cancer cell lines representing different intrinsic subtypes of breast cancer.

Figure 1C depicts representative data in the TNBC cell line MDA-MB-231 and ER⁺ cell line MCF-7. Consistent with the anti-proliferative data described in Table 1 and Fig. 1B, AS703569 caused G2–M arrest and accumulation of DNA content, accompanied by apoptosis, at relatively low concentrations (10 and 100 nmol/L) in the MDA-MB-231 cell line. In the MCF-7 cell line, G2–M arrest and polyploidy were observed at higher concentrations but led to lower levels of apoptosis than those seen in the MDA-MB-231 cells. In both cell lines, polyploidy was observed starting from 48 hours, which increased over time to 96 hours, similar to the apoptotic effect. To confirm this phenotypic effect of AS703569 in patient-derived models with limited passages, AS703569-induced changes in cell-cycle progression were evaluated in 2 cell lines derived from the TNBC xenografts used in this study, HBCx-8 and HBCx-17 (Fig. 1C). In both of these cell lines, polyploidy (>4N content) was observed starting at 72 hours with 100 nmol/L AS703569. This concentration is known to be achievable in the blood of mice at the dose administered in this study based on the pharmacokinetic properties of the compound (data not shown). Endoreduplication was also observed for the AS703569-resistant MDA-MB-361, SKBR-3, and ZR75-1 cell lines (data not shown).

In addition to cell-cycle effects, we investigated whether treatment with AS703569 resulted in apoptosis. MDA-MB-231 and MCF-7 cell lines were treated with different concentrations of the compound for up to 96 hours, and cleaved caspase-3 was measured by flow cytometric analysis (Fig. 1D). The percentages of apoptotic cells in both cell lines increased with increasing duration of exposure to AS703569, with a relatively greater percentage of apoptotic cells seen in the TNBC cell line. Similarly, higher percentage of apoptosis was observed in TNBC MDA-MB-468 cells at lower concentrations of AS703569, compared with non-TNBC MDA-MB-361, BT474, SKBR-3, and ZR75-1 cells (data not shown).

AS703569 significantly inhibited growth of patient-derived breast cancer xenografts

To test the effects of Aurora kinase inhibition in vivo, the efficacy of AS703569 as a single agent at its MTD (50 mg/kg once a week) was evaluated in 11 human primary breast cancer xenografts (HBCx, 10 of them triple-negative and 1 HER2⁺), previously characterized for their IHC profile and their response to standard chemotherapies, (15, 16, 20). AS703569 had significant antitumor activity in 7 xenograft models (40%–80% TGI compared with the vehicle-treated control group, \( P < 0.005; \) responders, Table 2). Five breast cancer xenograft models were resistant or low responder (TGI not significantly different or lower than 40%). No correlation was found with the expression of proteins involved in the cell-cycle control such as PTEN, Rb, or p53. One human breast
cancer xenograft carried both p53 and BRCA2 mutations (15) and showed a moderate response to AS703569 with a TGI of 40% and a target/control (T/C) growth delay of 2-fold. Importantly, AS703569 was also active in the xenograft models that were resistant to docetaxel (Table 2). An example is illustrated in Fig. 2A and B: treatment with AS703569 during 6 weeks resulted in TGI of 80% in the HBCx-10 xenograft (Fig. 2A), whereas treatment with docetaxel (20 mg/kg given every 3 weeks) did not change the HBCx-10 tumor growth curve (Fig. 2B). To determine whether the effect of AS703569 in vivo was correlated to the intrinsic expression of Aurora kinase genes and other mitotic spindle checkpoint genes, RT-PCR analyses were conducted to quantify the expression of AURKA, AURKB, AURKC, PLK1 (polo family protein kinase), TPX2 (microtubule-associated protein homolog), NEK2 (NIMA-related kinase 2), and NDC80 (kinetochore complex component; Fig. 2C). The expression of the Myc oncogene was also determined as possible factor related to the resistance to Aurora kinase inhibitors (21). Results showed that AURKA and AURKB genes were highly expressed (Ct ~ 26) in all xenograft models, showing increased expression of AURKB in low-responder/resistant models compared with responders (P = 0.0385, unpaired t test; Fig. 2C). AURKC was weakly expressed or not expressed (Ct > 32). The expression of PLK1, TPX2, NEK, NDC80, and MYC genes was relatively high in all models, with no significant differences between responder and resistant xenografts (unpaired t test).

### The AS703569 inhibitor induces a strong and reversible reduction of phosphorylated histone H3 levels in vivo

To evaluate the pharmacodynamics of AS703569 in vivo, we measured the levels of phosphorylated histone H3 (pHisH3) in 3 breast cancer xenografts as a measure of Aurora kinase B inhibition. The 3 breast cancer xenografts were the responders HBCx-5 and HBCx-10 (HER2+ and TN, respectively) and the nonresponder HBCx-15 (TN). Four animals of each breast cancer xenograft were treated with a single administration of AS703569 (50 mg/kg), and tumors were collected from mice at various times after treatment: 6, 2, 4, 72 hours, and 21 days and analyzed by IHC.

The kinetics of pHisH3 expression is shown in Fig. 3A. In untreated tumors, 8% to 9% of tumor cells were stained. Six hours after treatment, pHisH3 immunostaining was decreased dramatically in the 3 xenograft tumors. Twenty-four hours after treatment, pHisH3 expression returned to control levels. No significant treatment effects were observed at time points beyond 24 hours. An example of the decrease in pHisH3 IHC is shown in Fig. 3B: a field from 2 each independent tumors of the HBCx-10 xenograft, untreated, or at 6

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![Graph A](image1.png)  ![Graph B](image2.png)  ![Graph C](image3.png)

**Figure 2.** A, antitumor activity of AS703569 (50 mg/kg once/wk for 6 weeks) in the HBCx-10 xenograft. B, HBCx-10 tumor growth after docetaxel treatment (20 mg/kg given at days 1 and 22). C, RT-PCR expression analysis of AURKA, AURKB, AURKC, MYC, PLK1, TPX2, NEK2, and NDC80 genes. Expression values are represented as a ratio to the TBP (TATA-binding protein) gene expression.
and 24 hours after treatment. Six hours after the AS703569 inhibitor treatment, no positive cells were detected in treated tumors, whereas at the 24-hour time point, pHisH3 expression was similar to that of untreated tumors.

Inhibition of tumor recurrence in a basal-like breast cancer xenograft

We next addressed the question whether Aurora kinase inhibition could prevent tumor recurrence after conventional therapy, using the HBCx-10 xenograft that is sensitive to Adriamycin/cyclophosphamide (AC) and has a high frequency of tumor relapse (22). In the control group, mice were treated with one administration of AC (Adriamycin, 2 mg/kg; cyclophosphamide, 100 mg/kg), and by day 60, 7 of 10 animals showed complete tumor regression as observed by local palpation (Fig. 4A). In the control group, one mouse was euthanized during tumor remission for histologic analysis and 9 animals were followed and tumor regrowth was monitored until ethical euthanasia. In the second group, mice were treated with one administration of AC followed by 8 weeks of treatment with AS703569. Tumor recurrence was seen in 2 mice, whereas no tumor regrowth was observed in 5 mice after 305 days. Kaplan–Meier survival analysis (Fig. 4B) shows that mice treated with AC alone (n = 10, black line) have decreased survival compared with mice treated with AC and AS703569 (n = 7, red line). Histologic analysis of the mouse interscapular fat pad (group AC alone) euthanized during tumor remission revealed the presence of numerous small islands of tumor cells (Fig. 4C), as confirmed by in situ hybridization with a specific human Alu probe (Fig. 4D and E). To determine whether these residual tumor cells were resting or proliferating cells, we conducted Ki67 staining, which showed a relatively high proliferation status of the cancer cells (Fig. 4F and G). Immunostaining for pHisH3 (Fig. 4H and I) revealed that expression in these residual cells was similar to that seen in the HBCx-10 xenograft before chemotherapy (Fig. 3D), indicating Aurora kinase activity in residual cancer cells. One mouse in the AC + AS703569 group was submitted for histologic examination of the interscapular fat pad at day 305 (Fig. 4J). The analysis revealed the presence of a small fibrous nodule containing several necrotic and mineralized foci within the fat pad with no residual tumor cells as confirmed by a negative Alu

Figure 3. A, kinetic histogram showing pHisH3 expression (IHC) in tumor sections at different time points after a single administration of AS703569 (n = 4 mice for each time point). B, pHisH3 immunostaining in the HBCx-10 xenograft in 2 untreated tumors and 6 and 24 hours after AS703569 administration.
staining (Fig. 4K). Necrotic areas were surrounded by a rim of lipofuscin-containing mouse macrophages (Fig. 4L).

Discussion

As TNBCs have high proliferation levels, antimitotic agents such as Aurora kinase inhibitors could represent a new class of targeted therapy. We report here the antitumor activity of the pan-Aurora kinase inhibitor (AS703569) in the triple-negative subtype of breast cancer. In vitro data collected from human breast cancer cell lines showed that TNBC cell lines were more sensitive to the AS703569 inhibitor than the ER/PR or HER2⁺ cell lines. This sensitivity was not dependent on p53 status, in line with a previous report of in vitro sensitivity of lung, colon, and prostate cancer cell lines to AS703569 (14). No correlation with Aurora kinases and Ki67 expression was observed (data not shown). In a previous study, the activity of an Aurora kinase B inhibitor (AZD1152) in human breast cancer cell lines was reported, with no selective antitumor activity for triple-negative cells (23). Our data indicate that both triple-negative and ER⁺ cell lines exposed to AS703569 showed endoreduplication, whereas induction of apoptosis seemed to be greater in the triple-negative cells (from 50% to 80%). The mechanisms of the AS703569 poor activity in HER2 or ER⁺ cells in vitro are unknown and could be related to genes differentially expressed within the panel of breast cancer cell lines. Another possibility regarding the sensitivity of TNBC cell lines to AS703569 may, in part, be due to an off-target effect of the compound.

As for the cell lines, the sensitivity to Aurora kinase inhibition in vivo did not appear to be dependent on p53 mutational status. This relationship is not completely discerned in the literature, with different results being
reported depending on tumor cell type and the Aurora kinase inhibitor used. Cells compromised for p53 checkpoint function were more likely to induce endoreduplication and apoptosis in response to the inhibitor VX-680 (24). Conversely, leukemia cell lines with wild-type p53 were more sensitive to the Aurora kinase inhibitor ZM447439 than cell lines with mutant p53, suggesting that a p53-dependent post-mitotic checkpoint may be important in determining cell fate after exposure to Aurora kinases inhibition (25). This question has not been specifically addressed in the literature for breast cancers. Interestingly, the HBCx-17 xenograft that carries mutations in both p53 and BRCA2 genes (15) was moderately sensitive to AS703569, suggesting that a combination of these factors might not be sufficient to increase sensitivity to Aurora kinase inhibitors, as it was shown in vitro (26).

General mechanisms of tumor cell resistance to Aurora kinase inhibitors have not been identified yet, although in vitro studies suggest that mutations of the targeted Aurora kinases and overexpression of drug resistance genes may be involved (27, 28).

The absence or low response observed in 4 xenograft models cannot be related to the PgR expression, as none of the models tested in this study express the MDR1 gene. As Aurora kinases are known to be dramatically upregulated in highly proliferating cells, the proliferation rate of the tumor (i.e., the number of mitotic cells exposed to Aurora kinase inhibition) could potentially determine sensitivity. Our data indicate that a high proliferation index alone is not sufficient to determine sensitivity to AS703569 and that other genes/pathway may be more important in regulating the response to Aurora kinase inhibition.

Importantly, AS703569 was effective in models resistant to docetaxel, indicating the therapeutic potential of targeting the Aurora kinase family in this subtype of tumors, especially recurrent or advanced breast cancers.

One of the key effector proteins regulated by Aurora B kinase is the RBL protein (29). However, the RBL protein expression was not related to the in vivo response of the AS703569 inhibitor in this study, which is in line with previous reports showing that RBL status alone does not correlate with sensitivity to Aurora kinase inhibitors in vivo (21, 30). The finding that Aurora kinase genes A and B were highly expressed in breast cancer xenografts is consistent with previous works that showed high level of these genes in aggressive breast cancers than in benign tumors (19). A high AURKB expression was correlated to in vivo response to AS703569. A recent study showed that expression of Aurora A and B in lung cancer cell lines were weak predictors of response to Aurora kinase inhibitor PF-03814735 (21). Interestingly, the same authors reported a higher response to PF-03814735 in lung tumors with amplified or overexpressed MYC. That was not the case in our study, suggesting that the relationship between MYC and the in vivo response to Aurora kinase inhibitors could be specific to lung cancers.

Consistent with other studies, pHisH3 reduction was rapid and reversible (31, 32). Interestingly, pHisH3 reduction was also observed in a xenograft model that did not respond to AS703569, suggesting that inhibition of Aurora kinases activity alone may not be sufficient to arrest cell proliferation in resistant tumors and these might escape treatment through alternative pathways. In addition, this result suggests that a decreased expression of pHisH3, the phenotype of Aurora B inhibition, is not predictive of tumor response in vivo.

In the responding tumor xenograft models, tumor growth was not completely abolished, despite the strong inhibition of pHisH3 induced by AS703569. This is consistent with other preclinical studies showing that Aurora kinases inhibitors alone do not induce tumor regression in solid cancers (10, 21, 31–33). Treatment with the Aurora kinase inhibitors PF-03814735 and MK-0457 delayed the growth of colon and ovarian xenografts (31, 32). Similarly, treatment with MK-5108 had antitumor activity in the HCT116 and SW48 xenografts, without tumor remissions (33). As Aurora kinase inhibitors have had relatively excellent activity against cancers with high mitotic index such as acute myeloid leukemia and certain lymphomas (34), highly proliferating solid tumors such as TNBCs could potentially be sensitive to Aurora kinase inhibition. Our results indicate that targeting Aurora kinases is not sufficient to abolish tumor growth in highly proliferating breast cancers, presumably because tumor cells in these tumors may not be addicted to Aurora kinases for their proliferation and survival.

To address the question whether targeting Aurora kinases could inhibit tumor recurrence in patient-derived xenograft models, we used a TNBC cancer xenograft characterized by an initial response to AC followed by high frequency of local tumor relapse (22). The positive staining for Ki67 and pHisH3 markers in residual cancer cells that have survived chemotherapy indicates that these cells were still proliferating and had Aurora kinase activity. AS703569 in combination with an anthracycline-based treatment reduced tumor recurrence. Other studies have reported combination therapies of Aurora kinases inhibitors with cytotoxic anticancer agents, such as docetaxel and cisplatin (33, 35). Although obtained in only one breast tumor model, this preliminary result suggests that targeting Aurora kinases in the adjuvant setting may improve response to anthracyclines in advanced TNBCs.

Over a dozen small-molecule Aurora kinase inhibitors have entered clinical development (36, 37). As single agents, the Aurora kinase inhibitors seem only to have a future in patients with leukemias and lymphomas, whereas the responses observed in patients with solid tumors have been disappointing, with disease stabilization as the best response. Future studies with Aurora kinase inhibitors should focus on the possibility of combining these agents with chemotherapy or other targeted anticancer agents.
In summary, we have presented a preclinical study with cancer cell lines and patient-derived tumor xenografts indicating for the first time that targeting Aurora kinases has an antitumor effect in human TNBCs.

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

Authors’ Contributions
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc): A. Clark, F. Assayag, S. Chateau-Joubert, J.-L. Servely, J.-J. Fontaine, X. Liu, P. de Cremoux, I. Bieche

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