Preclinical Activity Profile and Therapeutic Efficacy of the HSP90 Inhibitor Ganetespib in Triple-Negative Breast Cancer

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Abstract

Purpose: Treatment options for patients with triple-negative breast cancer (TNBC) are largely limited to systemic chemotherapies, which have shown disappointing efficacy in the metastatic setting. Here, we undertook a comprehensive evaluation of the activity of ganetespib, a potent inhibitor of HSP90, in this malignancy.

Experimental Design: The antitumor and antimetastatic activity of ganetespib was investigated using TNBC cell lines and xenograft models. Combinatorial drug analyses were performed with chemotherapeutic agents and concomitant effects on DNA damage and cell-cycle disruption were assessed in vitro; antitumor efficacy was assessed in vivo. Metabolic and objective tumor responses were evaluated in patients with metastatic TNBC undergoing ganetespib treatment.

Results: Ganetespib simultaneously deactivated multiple oncogenic pathways to potently reduce cell viability in TNBC cell lines, and suppressed lung metastases in experimental models. Ganetespib potentiated the cytotoxic activity of doxorubicin via enhanced DNA damage and mitotic arrest, conferring superior efficacy to the doxorubicin–cyclophosphamide regimen in TNBC xenografts. Ganetespib also promoted mitotic catastrophe and apoptosis in combination with taxanes in vitro, and these effects translated to significantly improved combinatorial activity in vivo. Marked tumor shrinkage of metastatic lung and lymphatic lesions were seen in patients on ganetespib monotherapy.

Conclusion: The preclinical activity profile and clinical evidence of tumor regression suggest that ganetespib offers considerable promise as a new therapeutic candidate to target TNBC.

Introduction

Breast cancer remains the leading cause of cancer-related mortality in women worldwide (1). Despite a remarkable degree of heterogeneity, human breast tumors have been classified into at least five “intrinsic” subtypes on the basis of discrete molecular signatures identified through gene expression profiling (2–5). These include normal breast-like, the hormone receptor-positive (estrogen and progesterone receptors; ER and PR) luminal A and luminal B subtypes, human EGF receptor 2 (HER2)-positive, and basal-like. Within this stratification, triple-negative breast cancers (TNBC) represent an orphan grouping of tumors characterized by an absence of ER, PR, and HER2 expression that primarily fall within the basal-like subtype; however, the two definitions are not strictly synonymous (6, 7).

Although the incidence of TNBC is 10% to 20%, these cancers show a disproportionate mortality for patients with breast cancer (6). TNBCs more frequently affect younger women, are generally of higher grade and poorly differentiated at presentation, and are biologically aggressive (6–8). Despite being relatively chemo-sensitive to neoadjuvant treatment, patients with TNBC have a higher likelihood of visceral metastatic disease and rapid relapse (9–11). In contrast with effective tailored treatments for luminal or HER2-amplified tumors, the absence of defined molecular targets in TNBC has restricted therapeutic options to systemic chemotherapies in the adjuvant or metastatic setting (6). Consequently, these cancers remain high risk with particularly unfavorable prognoses (7, 12).

The activity of HSP90, a ubiquitously expressed molecular chaperone required for the structural maturation and function of numerous cellular client proteins (13), is often exploited by cancer cells during tumorigenesis to promote aberrant proliferative, survival, angiogenic, and/or metastatic...
potential (14, 15). Pharmacologic blockade of HSP90 has emerged as a promising treatment strategy for breast cancer, in which a number of client proteins have been implicated in the pathogenesis of the disease, including ER, PR, and HER2 (16). Moreover, clinical benefit has been observed following the addition of the first-generation HSP90 inhibitor tanezumycin to trastuzumab in HER2-positive metastatic breast cancer (17), providing important proof-of-concept support for improved patient outcomes following the inclusion of an HSP90 inhibitor to established treatment regimens.

Ganetespib is a potent next-generation inhibitor of HSP90 with favorable pharmacologic and safety characteristics (18). Ganetespib is structurally distinct, relatively hydrophobic, and considerably smaller in size compared with the prototypical ansamycin class of HSP90 inhibitors (including tanezumycin). These features promote extensive penetration, distribution, and retention of the compound throughout solid tumors in in vivo models (18). Because of its chemical design, ganetespib also lacks the characteristic hepatotoxicity that hampered the clinical application of the ansamycins. As predicted by its robust preclinical activity against a range of cancer models including lung, prostate, and leukemia (19–23), a maturing clinical profile has revealed clear evidence of therapeutic efficacy in human xenograft tumor models (18). Moreover, we present clinical evidence in preclinical models of TNBC, and can potentiate the activity of standard-of-care chemotherapeutics both in vitro and in vivo. Furthermore, we present clinical evidence of tumor responses in patients with metastatic disease undergoing ganetespib monotherapy. Taken together, these findings establish ganetespib as a valid point for therapeutic intervention in TNBC and provide a rationale for the design of ganetespib-based treatments for individuals with this disease.

Translational Relevance

Triple-negative breast cancer (TNBC) represents a molecularly heterogeneous subgrouping of high-risk breast tumors characterized by aggressive behavior, poor prognosis, and a lack of targeted therapeutic options. This study shows that ganetespib, a selective HSP90 inhibitor, has potent cytotoxic and antimitastatic activity in preclinical models of TNBC, and can potentiate the efficacy of standard of care chemotherapeutics both in vitro and in vivo. Moreover, we present clinical evidence of tumor responses in patients with metastatic disease undergoing ganetespib monotherapy. Taken together, these findings establish ganetespib as a valid point for therapeutic intervention in TNBC and provide a rationale for the design of ganetespib-based treatments for individuals with this disease.

Materials and Methods

Cell lines, antibodies, and reagents

The MDA-MB-231, MDA-MB-468, and BT-20 cell lines were obtained from the American Type Culture Collection, were authenticated by DNA typing (26), and used within 6 months of receipt. Primary antibodies were purchased from Cell Signaling Technology with the exception of CRAF, cyclin E, and glycerinaldehyde-3-phosphate dehydrogenase (GAPDH) antibodies (Santa Cruz Biotechnology). Ganetespib (Fig. 1A) was synthetized by Synta Pharmaceuticals Corp. Paclitaxel and docetaxel were purchased from LC Laboratories, doxorubicin and cyclophosphamide from Sigma-Aldrich.

Western blotting

Following in vitro assays, cells were disrupted in lysis buffer on ice for 10 minutes. Lysates were clarified by centrifugation, resolved by SDS-PAGE, transferred to nitrocellulose (Bio-Rad), blocked with StartingBlock T20 (Thermo Scientific) and immunoblotted with the indicated antibodies. Antibody–antigen complexes were visualized using an Odyssey system (LI-COR Biosciences).

Reverse phase protein array

Cell lines were treated with dimethyl sulfoxide (DMSO; control) or ganetespib (250 nmol/L) for 24 hours. Lysates were prepared as recommended by MD Anderson Cancer Center (Houston, TX), arrayed on nitrocellulose-coated FAST slides (Whatman), and probed for a standard list of antibodies (27).

Invasion assay

MDA-MB-231 cells (4 x 10⁴) were seeded into the upper chamber of a Transwell system (Millipore) coated with type I collagen in serum-free medium. Medium containing 10% FBS was added to the lower chambers. Cells were treated with vehicle or 100 nmol/L ganetespib for 24 hours in the presence of mitomycin C (10 µg/mL) to control for proliferative effects. Experiments were performed in quadruplicate. Migrated cells were stained with CyQUANT (Invitrogen) and read on a fluorescence plate reader.

In vivo xenograft tumor models

Female CB.17/SCID mice (Charles River Laboratories) 7 to 12 weeks of age were maintained in a pathogen-free environment and all procedures were approved by the Synta Pharmaceuticals Corp. Institutional Animal Care and Use Committee. MDA-MB-231 (5 x 10⁴) cells were subcutaneously implanted into severe combined immunodeficient (SCID) mice. Mice bearing tumors (~200 mm³) were randomized into groups of eight. Animals were treated with...
ganetespib (100 mg/kg), or doxorubicin (2 mg/kg) plus cyclophosphamide (100 mg/kg), alone or in combination, on a weekly schedule. Tumor-bearing animals were also dosed with single agent or combination treatment using ganetespib and docetaxel (4 mg/kg) or paclitaxel (10 mg/kg). Tumor growth inhibition was determined as described previously (23).

**Metastasis assays**

For the experimental metastasis model, luciferase-labeled 4T1 cells (4T1-LUC; $5 \times 10^5$ cells per mouse) were injected into 8-week-old female BALB/c mice through the tail vein. Animals were randomized into two groups 24 hours after inoculation and treated with vehicle or 100 nmol/L ganetespib. Viable cells that migrated through the collagen after 24 hours were stained with CyQUANT DNA dye and quantification (relative fluorescence units; RFU) is presented as averages ± SD.

**Comet assay**

MDA-MB-231 cells were seeded in six-well plates and incubated at 37°C, 5% CO₂ for 24 hours before the addition of vehicle or ganetespib to the culture. The assay was performed according to the manufacturer’s instructions (Trevigen, Inc.). Microscopic images were obtained using a Nikon Eclipse TE-2000 microscope 48 hours after drug addition.

**Immunofluorescent staining**

BT-20 cells were plated overnight onto chamber slides before 1-hour pulse treatment with 1 µmol/L ganetespib, 600 nmol/L doxorubicin, 50 nmol/L docetaxel, or combinations thereof, before washout and reculturing in regular growth medium. At the 24- and 48-hour time point, slides were fixed and permeabilized.
Patient A had failed six lines of chemotherapy regimens before enrollment into an ongoing phase I dose-escalation trial of twice weekly single-agent ganetespib in solid tumors (NCT00688116), and received ganetespib at a dose of 114 mg/m². Patient B had previously received six cycles of adjuvant chemotherapy before progression 2 years later and is currently enrolled in an open-label phase II trial evaluating ganetespib monotherapy in metastatic breast cancer (NCT01677455). Both patients were required to provide written informed consent before enrollment. The trials are being conducted in accordance with the Declaration of Helsinki and were approved by the Ethics Committee at each participating institution. Both studies are sponsored by Synta Pharmaceuticals Corp.

Results

Ganetespib displays potent antitumor activity in TNBC models

Ganetespib sensitivity was evaluated in a panel of 13 TNBC lines, in which it reduced cell viability in all cases with low nanomolar potency (Supplementary Table S1). Expression changes in HSP90 client proteins and signaling pathways associated with breast cancer progression were then examined. In BT-20 cells, ganetespib treatment resulted in a dose-dependent destabilization of EGFR, IGF-1R, MET, and CRAF (Fig. 1B) accompanied by inactivation of downstream effectors (phosphorylated STAT3) and mammalian target of rapamycin (mTOR) signaling (4E-BP1, ribosomal protein S6). Interestingly, although levels of total AKT protein were only minimally affected by ganetespib exposure, more robust destabilization of phosphorylated AKT (p-AKT) was observed, suggesting that AKT activity was effectively downregulated by drug treatment. These findings were supported by an extensive protein array analysis using BT-20, MDA-MB-231, and MDA-MB-468 cells, showing congruent alterations in mitogen-activated protein kinase (MAPK), AKT, and mTOR signaling and cell-cycle regulation, along with apoptotic induction (Table 1). Such coordinate impacts on multiple signaling cascades conferred by HSP90 inhibition accounts for the potent cytotoxic activity of ganetespib in TNBC cancer lines.

Ganetespib inhibits the migratory and invasive capacity of MDA-MB-231 cells in vitro

Tumor cell migration and invasion are essential for metastatic potential; therefore the ability of ganetespib to modulate these processes was investigated. In a standard wound healing assay, MDA-MB-231 cells exhibited a high migratory capacity that was blocked with low-dose ganetespib treatment (Supplementary Fig. S1). When seeded onto type 1 collagen-coated plates, MDA-MB-231 showed reduced E-cadherin expression, increased FAK expression and activity, and secretion of matrix metalloproteinase 9 (MMP9; Fig. 1C), consistent with this substrate promoting a more motile phenotype. Ganetespib exposure suppressed this FAK signaling and MMP9 production and inhibited the chemotactic invasion of MDA-MB-231 cells through collagen-coated Transwells (Fig. 1D)

Ganetespib suppresses experimental and spontaneous 4T1 lung metastasis

Two well-defined syngeneic metastasis models were used to validate the in vitro migration results (29, 30). In the first, mice were inoculated with 4T1-LUC via the tail vein and treated for 3 weeks with vehicle or ganetespib. Upon necropsy, lungs were removed and stained with India ink to confirm the presence of macroscopic metastatic lesions (Supplementary Fig. S2A). Representative images of metastatic burden are presented in Figure 2A, showing a clear reduction in the number and size of pulmonary lesions following ganetespib treatment. Indeed, quantification revealed that the numbers of metastatic lung nodules were significantly reduced (P = 0.015) in drug-treated animals (Fig. 2A, right). Furthermore, the degree of tumor formation in the lungs after 3 weeks in vehicle-treated mice resulted in a doubling of lung weight. Although this measure is not a strict correlate of metastatic burden, ganetespib treatment significantly reduced weights to levels comparable with normal lungs (Fig. 2B). Because ganetespib had no effect on normal lung weight, these data suggest that the compound was directly suppressing metastatic tumor growth and this was confirmed by measuring lung tissue reporter activity, which showed that ganetespib significantly reduced luciferase levels compared with control animals (Fig. 2C). In the second model, spontaneous metastasis of 4T1 cells to the lung was measured following orthotopic implantation into the mammary fat pads of animals. Although only modest reductions in primary tumor growth were seen after four weekly doses of ganetespib (Supplementary Fig. S2B), ganetespib treatment resulted in a highly significant decrease in metastatic lung colonization as determined by luciferase reporter activity (Fig. 2D). It is important to note that, although commonly used as a quantitative and reliable measure of total metastatic burden, luciferase activity more accurately represents an indirect measurement of the viable metastasized tumor cell population. Consistent with these data, histologic examination of lung tissue showed prominent foci of neoplastic cells in 3 of 3 vehicle-treated mice (representative image shown in Fig. 2D). In contrast, small subpleural foci of tumor cells were only observed in 1 of 3 ganetespib-treated animals examined.

Clinical activity of ganetespib in TNBC

Ganetespib is undergoing clinical evaluation in multiple human trials, including patients with advanced and metastatic breast cancer. In this regard, we have observed...
Table 1. Fold-changes in protein expression following ganetespib treatment in TNBC lines using reverse phase protein array analysis

<table>
<thead>
<tr>
<th>Cellular target</th>
<th>Protein</th>
<th>BT-20</th>
<th>MDA-MB-231</th>
<th>MDA-MB-468</th>
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<td>Receptor tyrosine kinases</td>
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<td>AKT signaling</td>
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<td></td>
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<tr>
<td></td>
<td>Cyclin B1</td>
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<td>+1.4</td>
<td>+1.4</td>
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<tr>
<td>Apoptosis</td>
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<td>+1.6</td>
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</tr>
<tr>
<td></td>
<td>BIM</td>
<td>+1.3</td>
<td>+1.6</td>
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<td>ACC (pS79)</td>
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<td>Claudin-7</td>
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<td>−1.4</td>
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cmpelling anecdotal evidence of single-agent clinical activity in TNBC as shown in the computed tomography (CT) and positron emission tomography (PET) scans presented in Fig. 3. Patient A, 39 years old, initially presented with stage III invasive ductal carcinoma. At the time of enrollment in an ongoing phase I study (NCT00688116), her disease had progressed with multiple liver, bilateral pulmonary, and bone metastases following six prior chemotherapy regimens. Ganetespib was administered as monotherapy at a dose of 114 mg/m² twice weekly. The patient achieved an unconfirmed response after four cycles (16 weeks) of treatment including the marked shrinkage of metastatic lung lesions (Fig. 3A). An overall objective response was not confirmed as the patient was taken off-study due to brain metastases at the end of cycle 5.

Patient B (Fig. 3B) is a 68-year-old female with metastatic TNBC, originally diagnosed with poorly differentiated ductal carcinoma, currently enrolled in a phase II ganetespib monotherapy trial (ENCHANT; NCT01677455). In 2011, the patient received six cycles of adjuvant FEC therapy (5-fluorouracil, epirubicin, and cyclophosphamide) but progressed in 2013 with multiple axillary and supraclavicular lymph node deposits and pulmonary metastases. Within 3 weeks of monotherapy ganetespib treatment, tumor shrinkage was observed in all target lesions, as evidenced by the striking reductions in standard uptake values in PET imaging (Fig. 3B, top). Moreover, after 6 weeks on therapy, the patient achieved a confirmed partial response accompanied by marked axillary lymph node tumor shrinkage (Fig. 3B; CT scans, bottom). The patient remains on study, with treatment duration of 5 months to date.

**Ganetespib potentiates the cytotoxic activity of doxorubicin via enhanced DNA damage and mitotic arrest**

Systemic chemotherapy, typically involving anthracycline/taxane–based regimens, represents the current standard of care approach for patients with TNBC (7). Accordingly, we examined the potential for improved...
therapeutic benefit by combining ganetespib with such agents. Concurrent administration of approximately IC_{50} doses of ganetespib (20 nmol/L) and doxorubicin (133 nmol/L) resulted in complete killing of MDA-MB-231 cells and similar combinatorial benefit was seen in the BT-20 line (data not shown). Doxorubicin induces DNA damage, and many DNA-repair components are HSP90 client proteins (31). To examine the hypothesis that ganetespib cotreatment augmented drug-induced effects on DNA, we measured the extent of DNA damage in MDA-MB-231 cells following ganetespib, doxorubicin, or combination treatment using the comet assay (Supplementary Fig. S3A). A low dose of doxorubicin (50 nmol/L) induced minor DNA damage with comet "tail" formation occurring in around 20% of the cells, whereas single-agent ganetespib at the same concentration had a minimal effect on DNA integrity (Fig. 4A). In contrast, the combination of ganetespib with doxorubicin resulted in a notable increase in DNA damage as evidenced by tail formation in 70% of cells (Fig. 4A).

We examined the stability of DNA repair and checkpoint proteins following single agent or combinatory treatment (Fig. 4B). Doxorubicin induced expression of the DNA damage-repair proteins p-BRCA1 and RAD51 coordinate with an increase in CDK1 and p21, two proteins involved in G_{1}→S checkpoint control. Ganetespib suppressed the DNA
damage and checkpoint response prompted by doxorubicin while simultaneously abrogating expression of the G2-M checkpoint regulators CHK1 and p-WEE1, both of which are important for preventing entry into mitosis (Fig. 4B). The loss of checkpoint control suggested that combination treatment may enhance the G2-M phase accumulation induced by doxorubicin treatment alone, and this was confirmed by cell-cycle analysis in the BT-20 line (Supplementary Fig. S3B). This finding was further validated by microscopic examination of BT-20 cells, which showed that the ganetespib plus doxorubicin combination substantially increased the proportion of mitotic cells 24 hours posttreatment (Fig. 4C). Thus, deregulated entry into mitosis with unrepaired DNA damage provides a mechanistic rationale for the increased apoptotic response seen with combination treatment.

Ganetespib sensitizes TNBC xenograft tumors to chemotherapy

In clinical practice, doxorubicin is commonly administered in the adjuvant setting. We examined whether the addition of ganetespib to the clinically relevant doxorubicin–cyclophosphamide regimen would translate to improved efficacy in vivo using mice bearing established MDA-MB-231 xenografts. We have previously determined that the highest nonseverely toxic dose of ganetespib on a weekly dosing regimen is 150 mg/kg (18). As shown in Figure 4D, weekly administration of a suboptimal dose of ganetespib (100 mg/kg) inhibited tumor growth by 26% (T/C value, 74%), whereas treatment with the doxorubicin (2 mg/kg) + cyclophosphamide (100 mg/kg) doublet resulted in 70% tumor growth inhibition. Even at this efficacious dose, cotreatment with ganetespib improved the antitumor response to 89% growth inhibition.

Combined ganetespib–docetaxel exposure triggers mitotic catastrophe and apoptosis

The therapeutic benefit of taxanes for TNBC has become realized in recent years (32) and we have previously reported that ganetespib displays strong synergistic activity with these types of agents in models of NSCLC (19). Here, we found that combining ganetespib with docetaxel produced enhanced cytotoxic effects in TNBC cells that were associated with potentiated mitotic catastrophe. Docetaxel alone had minimal effects on the expression levels of CDK1 (phosphorylated or total), CDK4, CHK1, or p-WEE1 in BT-20 cells; however, cotreatment with ganetespib effectively targeted these checkpoint proteins for degradation (Fig. 5A). Apoptotic induction was confirmed by dose-dependent increases in cleaved caspase-7 and PARP expression. Interestingly, this was accompanied by elevations in the phosphorylated form of histone H2AX, a sensitive indicator of DNA double-strand break formation. Of relevance,
H2AX phosphorylation may serve as a marker of chromosomal damage arising as a consequence of mitotic catastrophe (33). Immunofluorescent staining revealed that the combination of ganetespib and docetaxel indeed induced a catastrophic mitotic phenotype, as evidenced by enlarged micro- and multinucleated cells and multipolar mitosis, in BT-20 cells 48 hours posttreatment (Fig. 5B).

Ganetespib in combination with taxanesconfers superior efficacy in TNBC xenografts

Finally, we evaluated ganetespib efficacy in combination with docetaxel and paclitaxel in the same TNBC xenograft model shown in Fig. 4. As single agents, docetaxel (4 mg/kg) and paclitaxel (10 mg/kg) each induced a similar degree of tumor growth inhibition (T/C values, 57%; Fig. 5C). Concurrent treatment of either drug with ganetespib (100 mg/kg) resulted in a significant enhancement in tumor suppression (T/C values, 12%). All regimens were well tolerated, with no significant loss of body weight or toxicity observed (data not shown). Of note, ganetespib also markedly enhanced the antitumor activity of eribulin, a nontaxane microtubule disruptor, in MDA-MB-231 TNBC tumors (Supplementary Fig. S4). Taken together, these data show that the combination of ganetespib with a
Discussion

In recent years, the development of highly selective, molecularly targeted agents has transformed breast cancer treatment, particularly for individuals with endocrine or HER2-driven disease. Unfortunately, such advancements have proven elusive for TNBC, a heterogeneous collection of orphan status tumors that lack a unifying or defining vulnerability to serve as druggable molecular target. In addition, the absence of reliable predictive biomarkers, combined with the disappointing efficacy of conventional chemotherapy, highlights an urgent need for alternate, multimodal treatment options for patients with TNBC. To date, a variety of potential oncogenic drivers and molecular processes have been incompletely validated in this disease (6). Interestingly, many of these are established client proteins of HSP90, including the cell surface receptors EGFR, KIT, and IGF-1R as well as critical mediators of the RAS/RAF/ERK, PI3K/AKT, and mTOR tumorigenic signaling pathways (7). Exposure of TNBC cell lines to ganetespib resulted in the potent and simultaneous destabilization of the EGFR, AKT, and mTOR signaling axes, culminating in low nanomolar cytotoxicity values \( \text{in vitro} \) and robust tumor growth suppression in xenograft models. These observations are in concordance with two earlier reports that have provided similar preclinical evidence for the sensitivity of TNBC cells to HSP90 inhibition (34, 35). In both of those studies, novel HSP90 inhibitory agents were assessed; however, neither has reached a comparable stage of clinical development as ganetespib. In addition, this report is the first to describe efficacy in rationally designed combination studies and provides anecdotal evidence of tumor responses in patients with TNBC who are currently on therapy.
A distinct clinicopathologic feature of TNBC is the hematogenous spread of metastases, showing preferential dissemination to the lungs and brain rather than to bone or soft tissues (7). Our in vivo results indicated that ganetespib exhibited robust ant metastatic activity and, in particular, the strong impact on spontaneous lung metastasis in the orthotopic model seemed not to be related to significant suppression of primary tumor growth. In addition, in the lung colonization assay, lung weights were significantly increased in tumor-bearing animals—likely due not only to tumor cell burden but also fluid retention, inflammation, and other side effects of embolization. Ganetespib treatment attenuated this increase in lung volume yet had no effect on lung weights in non–tumor bearing animals. This is consistent with previously reported results in NSCLC xenograft models in which ganetespib displayed high penetrance and retention in tumors but rapid clearance and a short half-life in normal organs (21). Taken together, these data underscore the high degree of selectivity of ganetespib for tumor over normal tissue, a characteristic of targeted HSP90 inhibitors that contributes to improved therapeutic windows for cancer treatment (36). Of particular note, however, the first-generation HSP90 inhibitor tanespimycin has been shown to potentiate MDA-MB-231 bone metastasis in preclinical xenograft models, indirectly mediated via aberrant activation of osteoclastogenesis (37). Although this effect has not manifested in clinical trials of tanespimycin, and bone metastases are atypical in TNBC, this potential counterindication for HSP90 inhibitor therapy remains an important consideration for the clinical application of this group of investigational agents.

Significantly, we have presented compelling evidence of visceral metastatic tumor responses to ganetespib therapy in the clinical setting. Patient A was heavily pretreated and had progressed on six prior chemotherapeutic regimens. Despite this, four cycles of ganetespib monotherapy resulted in a marked tumor response and discernible shrinkage of large metastatic lung lesions. Similarly, patient B had recurrent disease 2 years after initial adjuvant chemotherapy and yet achieved a partial response within 6 weeks of starting ganetespib treatment. Such dramatic responses suggest that these tumors were acutely reliant on the chaperoning function of HSP90, with one or more HSP90-dependent signaling pathways driving the growth and survival of the malignancies. Given the remarkable molecular heterogeneity of TNBC, and the pleiotropic effects of HSP90 inhibition, the challenge remains to identify which specific client proteins may ultimately serve as predictive biomarkers for those cancers likely to respond to HSP90 inhibitor treatment. Overall, however, these observations highlight the therapeutic potential of ganetespib for treatment of metastatic disease in patients with TNBC.

Recently, an extensive integrated analysis of primary breast cancers across multiple platforms identified the high expression of DNA repair proteins as characteristic of basal-like (predominantly TNBC) tumors (5). In this context, the ability of ganetespib to sensitize TNBC cells to DNA-damaging chemotherapeutics provides a rationale for inclusion of this agent in novel combinatorial strategies for this phenotypic background. Anthracycline-based therapies constitute the standard treatment approach for most patients with TNBC, but these protocols are not curative for advanced disease (7). As shown here, ganetespib potentiated the cytotoxic activity of doxorubicin, in part through modulation of the DNA damage response machinery and elevated DNA damage. In this regard, HSP90 is known to play a critical role in DNA repair and genome stability (31) and a number of repair components and essential checkpoint regulators are themselves client proteins (38, 39). Indeed, the concomitant impairment of G1–S checkpoint activation with abrogation of G2–M control conferred by ganetespib cotreatment promoted an accumulation of tumor cells into M phase arrest. It is reasonable to suggest that the genotoxic stress induced by aberrant entry into mitosis with unrepaird DNA damage was sufficient to account for the increased cell death observed following combination ganetespib–doxorubicin exposure. Of interest, doxorubicin also functions as a topoisomerase II inhibitor and HSP90 blockade can enhance the activity of such poisons through disruption of a protective HSP90-topoisomerase II complex (40, 41). Furthermore, the doxorubicin–cyclophosphamide doublet forms the basis of most systemic TNBC therapies and the addition of ganetespib to this regimen conferred superior efficacy in MDA-MB-231 xenografts. This result is consistent with the premise that HSP90 inhibitors may best be exploited in the clinical setting as chemotherapeutic sensitizers to provide effective antitumor activity, block repair/resistance mechanisms, and reduce treatment-related toxicities (14).

A finding with further clinical relevance was the capacity of ganetespib to significantly improve the therapeutic indices of taxanes and other antimitotic agents in TNBC tumor models. Synergistic combinatorial benefit between HSP90 inhibitors and taxanes has been described in a number of cancer models (42–46), suggesting that the nonoverlapping yet complementary mechanisms of action of these agents are conserved across tumor types. We have previously found that ganetespib displays strong synergistic activity with both paclitaxel and docetaxel in NSCLC (19) based on loss of prosurvival signaling and impacts on the cell-cycle machinery that enhance the microtubule disruption activity of the taxanes. Here, we extended these mechanistic observations to include a role for ganetespib in exacerbating mitotic catastrophe, as has been reported for breast cancer cells following docetaxel exposure (47). Moreover, recent analysis of our global, randomized phase IIb/III study in NSCLC (GALAXY-1; NCT01348126) showed that the combination of ganetespib plus docetaxel improved both progression-free and overall survival in patients with adenocarcinoma compared with docetaxel alone (48). For TNBC specifically, the addition of a taxane to anthracycline-based regimens has been shown to confer therapeutic benefit for patients (6, 49), and meta-analysis of 12 randomized clinical trials revealed that adjuvant docetaxel-based chemotherapy was associated with improvements in disease-free and overall survival.
survival compared with regimens lacking taxanes (50). In light of all these considerations, evaluation of the ganetespib plus docetaxel combination in patients with TNBC is currently being planned.

In summary, the preclinical and clinical activity profiles presented here validate targeted HSP90 inhibition as a promising strategy for therapeutic intervention in TNBC and establish a framework for the design of future ganetespib-based therapies to address an urgent unmet medical need in this malignancy. Furthermore, the findings provide strong support for the potential use of ganetespib, both as monotherapy and as part of novel combinatorial strategies, for patients with breast cancer with triple-negative disease.

Disclosure of Potential Conflicts of Interest
D.A. Proia is Director, N. Spector has honoraria, M. Nagai is principal scientist, J. Acquaviva is scientist II, R.C. Bates is senior scientific writer, and I. El-Hariry is Vice President Clinical Research at Synta Pharmaceuticals Corp. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

References
utility for analysis of primary leukemia specimens and hematopoietic stem cells. Mol Cancer Ther 2006;5:2512–21.


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