

Beyond HER2 and Hormonal Agents: The Heat Shock Protein 90 Inhibitor Ganetespib as a Potential New Breast Cancer Therapy

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Abstract

Background: Ganetespib is a fully synthetic and selective inhibitor of heat shock protein 90 (HSP90), a molecular chaperone recognized as a key facilitator of breast cancer initiation, progression and metastasis.

Methods: Preclinical activity of ganetespib across the four major breast cancer subtypes and inflammatory breast cancer was assessed *in vitro* and *in vivo*. Modulation of cell proliferation and viability was determined both in monolayer and three-dimensional cultures. HSP90 client protein expression and activity was monitored by Western blot and protein array. To recapitulate clinical dosing, kinetics of client protein destabilization were measured following short exposures to drug *in vitro*. Anticancer activity of ganetespib was further investigated *in vivo* using breast cancer xenografts.

Results: Ganetespib displayed potent, low nanomolar activity in luminal (A and B), basal (A and B) and inflammatory breast cancer cell lines grown as monolayers *in vitro*. BT-474 (HER2 amplified) luminal cells grown as mammospheres in 3D were equally as sensitive to ganetespib as those grown in monolayer. In luminal cells, ganetespib simultaneously disrupted multiple signaling components including the estrogen and progesterone receptor, several receptor and non-receptor tyrosine kinases, as well as the MAPK pathway. Further, ganetespib effectively inhibited AKT, PDK1 and SGK3 activity in PIK3CA mutant cells suggesting that HSP90 is essential for both AKT-dependent and AKT-independent signaling. Clinically relevant exposure times to ganetespib *in vitro* resulted in potent, long term destabilization of HER2. In the basal-like breast cancer cell line MDA-MB-231, enriched in CD44+CD24- stem like cells that commonly display chemotherapeutic resistance and activated JAK2/STAT3 signaling, ganetespib (50 nM) induced significant degradation of JAK2 concordant with loss of both tyrosine and serine phosphorylation of STAT3, followed by cell death. The potent anticancer activity *in vitro* translated *in vivo*, where ganetespib was effective in modulating breast cancer xenograft growth as a single agent in both luminal and basal-like breast cancer models. Finally, ganetespib has demonstrated encouraging signs of clinical activity in breast cancer patients, including confirmed partial responses in both a triple negative breast cancer patient and a HER2 positive breast cancer patient.

Conclusions: Ganetespib is a highly potent HSP90 inhibitor that displays preclinical activity in breast cancer due to its ability to simultaneously perturb multiple oncogenic signaling pathways.

Ganetespib displays potent anticancer activity in breast cancer cells

Cell Line	Subtype	ER	PR	HER2	Ganetespib (IC50, nM)
SUM149	IBC	--	--	--	13
OCUB-M	Basal	--	--	--	39
MDA-MB-468	Basal A	--	--	--	27
HCC70	Basal A	--	--	--	114
BT20	Basal A	--	--	--	28
MDA-MB-231	Basal B	--	--	--	24
SK-BR-3	Luminal	--	--	+	25
BT-474	Luminal	+	+	+	13
MCF7	Luminal	+	+	--	25
T47D	Luminal	+	+	--	15

Figure 1. Ganetespib induces cell death across all breast cancer subtypes *in vitro* regardless of HER2, ER and PR status.

Ganetespib is effective in HER2+ luminal breast cancer cells

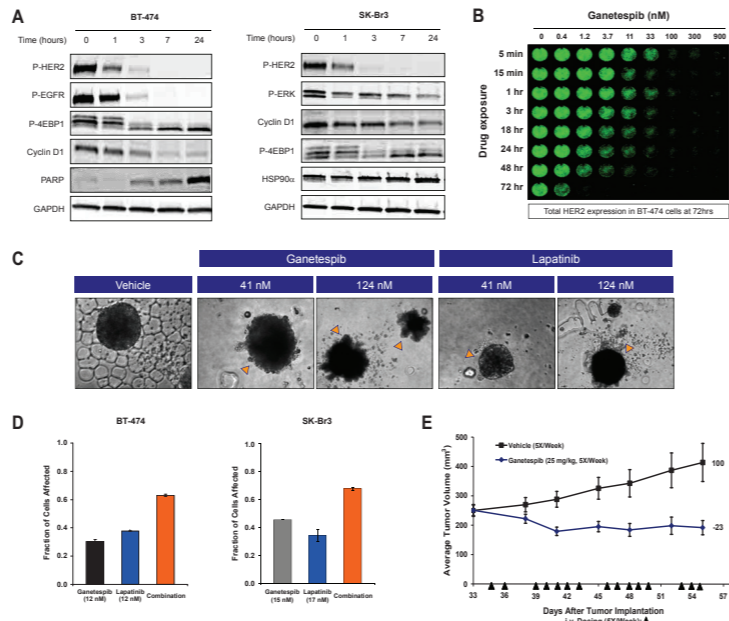


Figure 2. (A-B) Ganetespib induces the rapid and persistent destabilization of HER2 in BT-474 and SK-BR3 breast cancer cells. (C) Ganetespib kills BT-474 cells grown as mammospheres in 3D culture. (D) Synergy between ganetespib and lapatinib in BT-474 and SK-BR3 cells. (E) Ganetespib treatment induced tumor regression in a BT-474 breast cancer xenograft model.

Degradation of ER and PR by ganetespib in luminal breast cancer cells

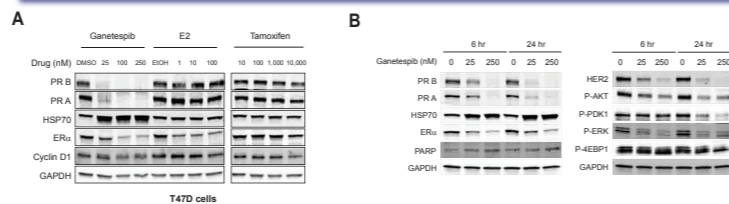


Figure 3. (A-B) Ganetespib rapidly abrogates ER, PR and HER2 expression in a dose-dependent manner in T47D cells.

Ganetespib synergizes with doxorubicin and lapatinib in inflammatory breast cancer cells

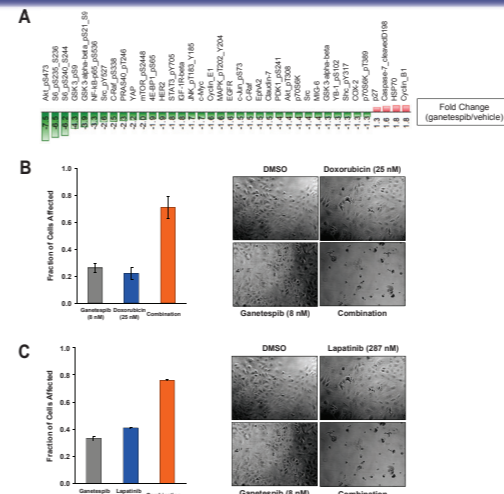


Figure 4. (A) Ganetespib destabilizes and deactivates multiple oncogenic signaling pathways simultaneously to induce apoptosis in the inflammatory breast cancer cell line SUM149, as determined by reverse phase protein array. (B-C) Ganetespib synergizes with front line therapeutic agents, doxorubicin and lapatinib, in SUM149 cells.

Ganetespib inhibits AKT dependent and independent signaling

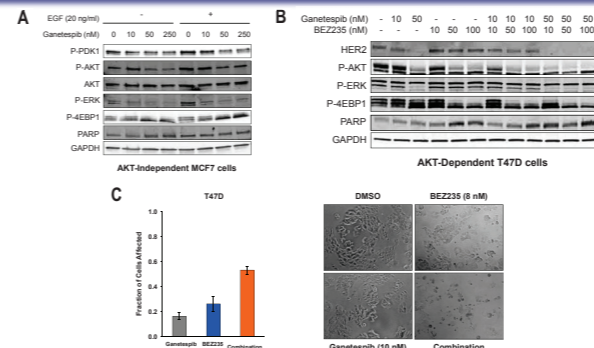


Figure 5. (A) Inhibition of AKT independent, PDK1 dependent signaling in MCF7 cells by ganetespib. (B-C) Ganetespib inhibits AKT activity and downstream signaling in AKT-dependent T47D cells and synergizes with BEZ235.

Inhibition of Hsp90, but not JAK2, kills pSTAT3+ TNBC cells

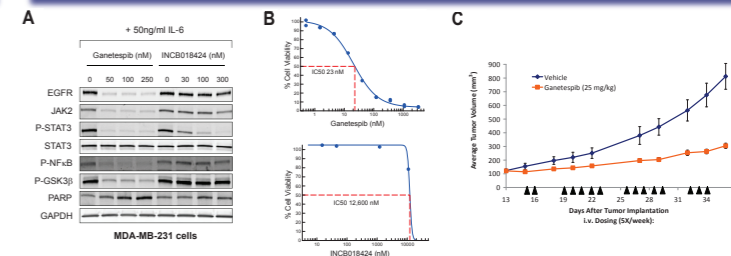
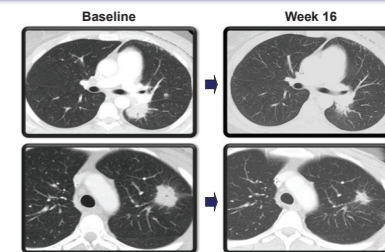


Figure 6. (A) Destabilization of JAK2 by ganetespib (50-250 nM) or inhibition of JAK2 activity by INCB018424 (30-300 nM) results in the loss of IL-6 mediated STAT3 activation in MDA-MB-231 cells. (B) Inhibition of JAK2 by INCB018424 is not sufficient to induce cell death in the CD44+ MDA-MB-231 cell line. In contrast, the multitargeted actions of ganetespib induce the loss of cell viability. (C) Ganetespib slows MDA-MB-231 xenograft tumor growth.

Ganetespib displays clinical signs of activity in TNBC

Ganetespib Case Study

- A 39-year-old white female with triple negative breast cancer
- Initial diagnosis: March 2007, Stage III invasive ductal carcinoma
- Progressed on 7 prior chemotherapeutic regimens
- Enrolled in November 2010, ganetespib dosing at 144 mg/m² twice weekly
- Patient achieved partial response following 4 cycles of ganetespib treatment
- Treatment tolerated well with mild/moderate toxicities



Summary

- Ganetespib is a potent small molecule Hsp90 inhibitor with *in vitro* and *in vivo* activity in multiple subtypes of breast cancer
- Ganetespib induces the degradation of multiple, clinically validated breast cancer oncoproteins including ER, PR and HER2
- Multitargeted actions of ganetespib are essential to elicit cell death in pSTAT3 positive TNBC cells as targeted inhibition of JAK2 is not sufficient
- Ganetespib sensitizes breast cancer cells to both standard of care therapy as well as HER2 and mTOR/PI3K inhibitors
- Ganetespib displays signs of activity in TNBC patients