

GanetespiB, A Unique Resorcinolic Hsp90 Inhibitor, Exhibits Potent Antitumor Activity and A Superior Safety Profile in Preclinical Models

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Abstract (#B105)

Targeted inhibition of the molecular chaperone heat shock protein 90 (Hsp90) results in the simultaneous blockade of multiple oncogenic signaling pathways and has thus emerged as an attractive strategy for the development of novel cancer therapeutics. GanetespiB (STA-9090) is a unique resorcinolic inhibitor of Hsp90 currently in clinical trials for a number of human cancers. Here we describe the key interaction of ganetespiB with a number of amino acid residues in the ATP binding pocket of Hsp90 which results in high affinity binding. GanetespiB exhibits potent *in vitro* cytotoxicity in a range of solid and hematological tumor cell lines. By using a novel isotope-labeling scheme and LC-MS/MS detection technique, we have determined that Hsp90 occupancy by ganetespiB in cancer cells is relatively fast under saturating conditions, reaching equilibrium within 5 minutes of ganetespiB exposure. *In vivo*, ganetespiB demonstrated strong single-agent activity in solid and hematological xenograft models, as evidenced by significant tumor growth inhibition and/or regression. Of note, evaluation of the microregional activity of ganetespiB in tumor xenografts showed that ganetespiB efficiently distributed throughout tumor tissue, including hypoxic regions >150 μm from the microvasculature, to inhibit proliferation and induce apoptosis. Most importantly, ganetespiB showed no evidence of cardiac or liver toxicity and exhibited minimal potential risk for CNS or ocular toxicities. Taken together, this preclinical activity profile suggests that ganetespiB may have broad application for a variety of human malignancies and mechanistic and safety advantages over other Hsp90 inhibitors.

Introduction

About GanetespiB
GanetespiB is a potent, next-generation, small-molecule heat shock protein 90 (Hsp90) inhibitor, being developed for treating multiple solid tumor and hematologic cancers. GanetespiB was discovered and developed internally at Synta and has a chemical structure unrelated to the first-generation, ansamycin family of Hsp90 inhibitors such as 17-AAG or 17-DMAG. In preclinical studies, ganetespiB has shown potency up to 100 times greater than the first-generation Hsp90 inhibitors as well as activity against a wider range of kinases. In *in vitro* and *in vivo* models, ganetespiB has shown potent activity against a wide range of cancer types, including lung, prostate, colon, breast, gastric, pancreatic, gastrointestinal stromal tumors, melanoma, acute myeloid leukemia, chronic myeloid leukemia, Burkitt's lymphoma, diffuse large B-cell lymphoma, and multiple myeloma - as well as potent activity against cancers resistant to imatinib (Gleevec®), sunitinib (Sutent®), erlotinib (Tarceva®), and dasatinib (Sprycel®).

Mechanism of Action
GanetespiB potently inhibits Hsp90, a chaperone protein required for the proper folding and activation of other cellular proteins, particularly kinases. Many of these "client proteins", such as AKT, BCR-ABL, BRAF, KIT, MET, EGFR, FLT3, HER2, PDGFR, VEGFR, have been shown to be critical to cancer cell growth, proliferation, and survival. In preclinical studies, inhibiting Hsp90 causes the degradation of multiple client proteins and leads to cancer cell death. Because mutated kinases which no longer respond to treatment with kinase inhibitors remain dependent on Hsp90 for their activity, inhibiting Hsp90 offers the potential for treating cancers that have become resistant to targeted therapies such as kinase inhibitors. We believe that inhibiting kinases indirectly, by disrupting the chaperone activities of Hsp90, provides two advantages: first, a means to simultaneously attack multiple cancer-promoting kinases; and second, an ability to kill tumor cells with mutated kinases that have lost responsiveness to a direct kinase inhibitor.

Chemical Structure of GanetespiB and Co-Crystal Structure of GanetespiB and Hsp90 N-Terminus

GanetespiB is a novel resorcinolic triazolone compound that is structurally distinct to the first-generation ansamycin Hsp90 inhibitors. With a molecular weight of 364.4, ganetespiB is considerably smaller than the ansamycin class, and most of the newer, second generation Hsp90 inhibitors. GanetespiB is relatively hydrophobic, with a cLogP value of 3.3. GanetespiB exhibits competitive binding to the ATP pocket at the N-terminus of Hsp90.

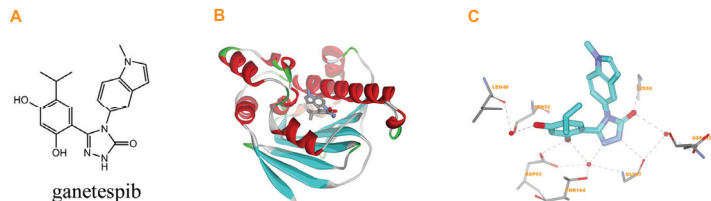


Figure 1. Chemical structure of ganetespiB and its co-crystal structure with Hsp90 N-terminal. A, Chemical structure of ganetespiB. B, Crystallographic complex of ganetespiB in the Hsp90 N-terminal. C, Hydrogen bond interactions between ganetespiB with amino acid residues in the Hsp90 N-terminal ATP binding pocket.

- The co-crystal structure of ganetespiB bound to Hsp90 confirmed important hydrogen bonding interactions involving the resorcinolic hydroxyl group with Asp⁹⁰ and the carbonyl group of triazolone with Lys⁹¹.
- In ganetespiB, the 2-hydroxyl of resorcinol is within hydrogen bond distance to both oxygen atoms of the carboxylic group in Asp⁹⁰, resulting in a substantially stronger interaction.
- The N¹ of triazolone forms a water-bridged hydrogen bond with Asp⁹⁰ to provide additional hydrogen bonding. Water bridge hydrogen bonds between 4-hydroxyl of resorcinol and Leu⁹⁴ and Ser⁹⁵ were found to be critical for binding efficiency.
- The hydrazinocarboxamide group of triazolone in ganetespiB is of particular structural importance. In addition to the direct hydrogen bond with Lys⁹¹, it forms a unique hydrogen bond with Gly⁹², a distinguishing feature from the ansamycin analogs.

Determining Hsp90 Occupancy in Cells Treated with GanetespiB

We have determined Hsp90 occupancy by ganetespiB in the NSCLC cell line NCI-H1975 using a quantitative occupancy assay. This method involved titration of increasing concentrations of ganetespiB to cells in culture, prior to lysis and incubation with saturating concentrations of ganetespiB-D3 to unoccupied binding sites. The ratio of Hsp90-bound ganetespiB/ganetespiB-D3 was measured by LC-MS/MS. Occupancy values showed that ganetespiB binding to Hsp90 saturated between 64 and 128 nM. Kinetic analysis showed that the association of ganetespiB with Hsp90 was relatively fast under saturating conditions, reaching equilibrium within 5 min of ganetespiB exposure.

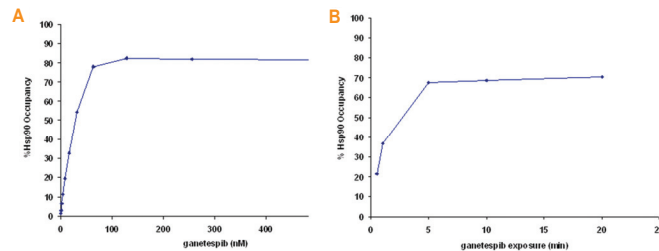


Figure 2. Hsp90 occupies Hsp90 ATP-binding pockets and induces apoptosis in human cancer cells *in vitro*. A, Hsp90 occupancy determined by the ratio of ganetespiB and ganetespiB-D3 in NCI-H1975 cells. B, Kinetics of ganetespiB dissociation as measured by Hsp90 occupancy in NCI-H1975 cells.

GanetespiB Displays Potent Activity Against Drug-Resistant Tumor Phenotypes *In Vitro*

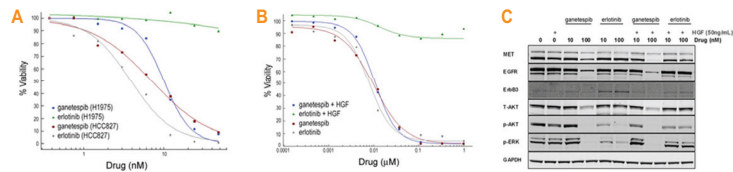


Figure 3. GanetespiB exhibits potency against erlotinib-resistant NSCLC tumor phenotypes *in vitro*. A, NCI-H1975 and HCC827 cells were treated with increasing concentrations of ganetespiB or erlotinib and cell viability was assessed after 72 h. B, HCC827 cells were seeded in the presence or absence of HGF (50ng/mL) for 24 h, then exposed to graded concentrations of ganetespiB or erlotinib for 72 h. Cell viability was measured by AlamarBlue. C, HCC827 cells seeded with or without 50 ng/mL HGF for 24 h were treated with 0, 10 or 100 nM ganetespiB or erlotinib for an additional 24 h. The levels of MET, EGFR, ErbB3, total (T-) AKT, p-AKT, p-ERK1/2, and GAPDH analyzed by western blot.

- We compared the activity of ganetespiB and erlotinib using the NCI-H1975 cell line, which expresses a mutationally activated and erlotinib-resistant EGFR^{L858R/T790M} mutation, and erlotinib-sensitive HCC827 cells, which express EGFR^{WT}.
- Resistance to EGFR inhibitors may also emerge through alternative oncogenic mechanisms. Hepatocyte growth factor (HGF) can induce TKI resistance in lung tumors with EGFR-activating mutations by independently activating and restoring PI3K/AKT signaling via phosphorylation of c-MET. Both ganetespiB and erlotinib were highly potent in non-stimulated HCC827 cells, with IC₅₀ values of approximately 10 nM. Importantly, while HGF-treated cells did not respond to erlotinib, ganetespiB retained its potency in the presence of the growth factor.
- GanetespiB treatment promoted the down-regulation of client protein levels in both the absence and presence of HGF, resulting in the complete loss of AKT and ERK activity. Erlotinib exposure was capable of inactivating AKT and ERK in the absence of HGF, but was ineffective in the presence of the growth factor.

GanetespiB Exhibits Potent *In Vivo* Activity in Both Solid and Hematological Xenograft Models

To determine whether the effects of ganetespiB *in vitro* translated to antitumor efficacy *in vivo*, the activity of ganetespiB was evaluated using a variety of doses and schedules in a series of xenograft models.

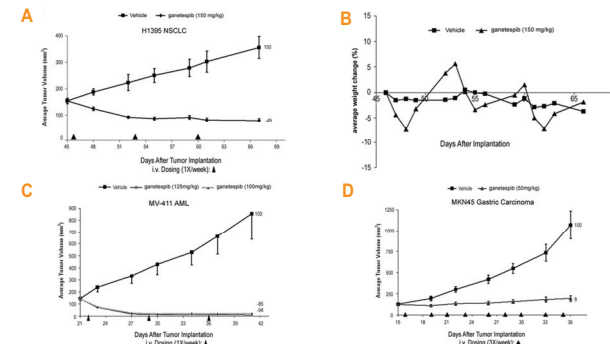


Figure 4. GanetespiB exhibits potent antitumor efficacy in oncogene-driven xenograft models of solid and hematological malignancies. A, SCID mice bearing established NCI-H1395 NSCLC xenografts were i.v. dosed with ganetespiB at 150 mg/kg once weekly. B, GanetespiB was administered i.v. weekly at 150 mg/kg. Body weights were measured 5 times per week. C, SCID mice bearing established MV-411 AML xenografts were i.v. dosed with ganetespiB at 100 and 125mg weekly. D, Mice bearing established MKN45 gastric carcinoma xenografts were i.v. dosed with ganetespiB at 50 mg/kg three times per week.

GanetespiB Penetrates Hypoxic Regions of Tumors *In Vivo*

Tumor penetration and the microregional activity of ganetespiB were assessed in NCI-H1975 tumor xenografts. A single 125 mg/kg dose of ganetespiB was administered i.v. to established NCI-H1975 xenografts and tumors (n = 8/group) were removed 24 h after treatment.

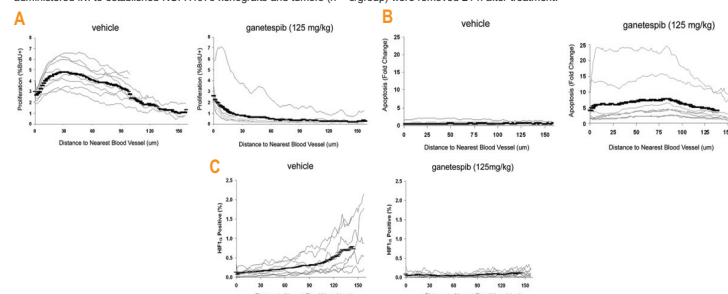


Figure 5. Tumor penetration and microregional activity of ganetespiB. Immunohistochemistry was performed on cryosections for markers of proliferation (bromodeoxyuridine) and apoptosis (TUNEL). A, Proliferation was mapped in relation to distance from the nearest CD31⁺ endothelial cells. 100% of tumor cells were located <170 μm from the nearest blood vessels. B, Apoptosis was mapped in relation to distance from the nearest CD31⁺ cells. C, HIF-1α expression was mapped in relation to distance from the nearest CD31⁺ cells.

- A single dose of ganetespiB at 125mg/kg dramatically reduced cellular proliferation throughout the tumors, with the maximal effect occurring 24 h following treatment.
- A concomitant induction of tumor cell apoptosis occurred within 24 h.
- Increased HIF-1α staining as a function of distance confirmed the hypoxic gradient that existed within the tumors and, importantly, this Hsp90 client protein was potently suppressed *in vivo* following treatment with ganetespiB.
- These results provide strong evidence that ganetespiB efficiently distributed within the extravascular compartment, including the hypoxic regions >150 μm from the microvasculature, resulting in sustained inhibition of proliferation and induction of apoptosis throughout the tumors

GanetespiB Exhibits A Favorable Safety Profile

The hepatotoxicity profile of ganetespiB was evaluated in male SD rats based on changes in the liver enzymes AST and ALT and immunohistochemical staining. Cardiovascular effects of escalating doses ganetespiB on electrophysiological (PQ, QRS, RR and QTc) and mechanical (left ventricular developed pressure) properties were evaluated in isolated New Zealand white rabbit hearts.

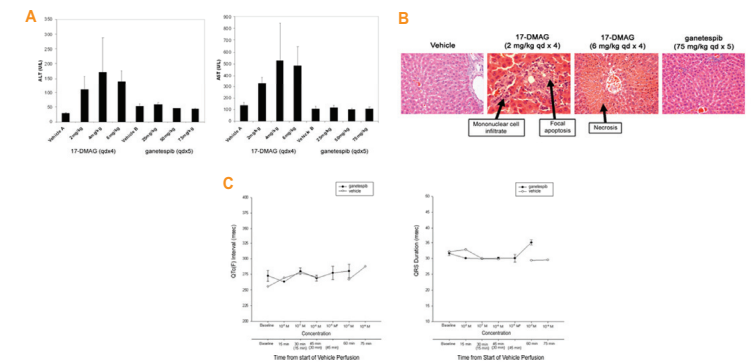


Figure 6. Hepatotoxicity induced by 17-DMAG, but not ganetespiB, in a repeated dose toxicity study. A, Male SD rats (n = 3-5 rats/group) were treated with repeated administration of 17-DMAG at 2, 4 and 6 mg/kg for 4 days (qd x 4) or ganetespiB at 25, 50 and 75 mg/kg for 5 days (qd x 5). Plasma levels of the liver enzymes AST and ALT were measured. B, Hematoxylin and eosin staining (H&E) of liver cross sections from vehicle control, 17-DMAG (2 and 6 mg/kg), or ganetespiB (75 mg/kg) treated animals. C, Effects of escalating doses of ganetespiB and vehicle (0.1% DMSO in Krebs) on the QTc(F) interval (left panel) and QRS interval (right panel) in male rabbit hearts.

- No changes in the levels of either enzyme were observed in the ganetespiB-treated animals, even at the highest dose of 75 mg/kg, which is higher than the efficacious dose range for this compound. In stark contrast, a dose-dependent and marked elevation of ALT (293% - 510%) and AST (149% - 296%) was seen with 17-DMAG treated rats, at doses 12.5 times lower than ganetespiB.
- Histologic analysis revealed that livers of animals treated with 17-DMAG at the lowest 2 mg/kg dose showed patchy and focal hepatocytic apoptosis with mild mononuclear cell infiltration. At the 6 mg/kg dose, the lesions were diffuse and severe, including larger areas of coagulative hepatocytic necrosis. In accordance with the lack of enzymatic induction, there were no discernable morphologic changes in the hepatocytes of animals treated with ganetespiB.
- There was no change in the QTc(F) intervals at concentrations of ganetespiB between 10⁻⁶ to 10⁻¹⁰ M when compared to baseline or vehicle. Similarly, there was no change in the QRS duration after exposure to concentrations of ganetespiB ranging from 10⁻⁶ to 10⁻¹⁰ M, when compared to baseline or vehicle.
- An increase in the duration of the QRS was noted after exposure to the 10⁻⁶ M concentration. At 10⁻⁶ M, the highest concentration tested, ganetespiB dose in the NCI-H1975 tumor penetration studies.
- Other cardiac electrophysiological parameters and mechanical properties, including left ventricular developed pressure, were not significantly altered following exposure to ganetespiB, while expected physiological changes with the positive control quinidine were observed.

Conclusions

In summary, we have developed and characterized ganetespiB, a unique small-molecule inhibitor of Hsp90, that

- exhibits potent and sustained antitumor effects in a broad range of malignancies both *in vitro* and *in vivo*.
- retains its potency against tumor phenotypes that confer drug resistance to agents currently in use in the clinic.
- displays optimal pharmacological properties including high tumor penetration and retention and a favorable safety profile that may predict for a wide therapeutic index.

Accordingly, ganetespiB represents an exciting new targeted agent for the treatment of human cancer and is currently being evaluated in multiple Phase 2 clinical trials and a Phase 2/3 randomized trial in combination with docetaxel in NSCLC. For ganetespiB clinical trial information, please visit: <http://www.syntapharma.com/ClinicalTrialHome.aspx>



For further information on ganetespiB: www.syntapharma.com