

#P2-84 Multi-Targeted Activity of the Hsp90 Inhibitor Ganetespib (STA-9090) in Prostate Cancer Cells

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Abstract

Background: Heat Shock Protein 90 (Hsp90) is emerging as an important target in cancer therapy because its inactivation results in the simultaneous blockade of multiple oncogenic signaling pathways and sensitizes cancer cells to other chemotherapeutic agents. Ganetespib (formerly STA-9090) is a potent inhibitor of heat shock protein 90 (Hsp90) that is structurally unrelated to earlier Hsp90 inhibitors such as 17-AAG, and has shown superior activity to these agents in preclinical studies. Ganetespib is currently being studied in 11 Phase 2 trials, where it has demonstrated encouraging clinical activity and is well tolerated. In light of this, we sought to determine the potency of ganetespib in prostate cancer (PCa) cells given the importance of several Hsp90 client proteins in mediating PCa progression.

Results: We examined the effectiveness of ganetespib or the first generation Hsp90 inhibitor 17-AAG in both hormone-dependent (LNCaP, 22Rv1) and hormone-independent (PC-3, DU-145) PCa cell lines. Ganetespib displayed low nanomolar activity regardless of the cell's AR status, with IC50's 3-7 fold less than 17-AAG. In the treated cultures, ganetespib increased the population of apoptotic (Annexin V positive) cells, whose appearance paralleled the dose-dependent degradation of the anti-apoptotic protein Mcl-1. In all of the cell lines, the master cell cycle regulator Cdk1 and the DNA damage checkpoint protein Chk1 were completely destabilized by ganetespib exposure. This led to the cells arresting in G2/M. Interestingly, expression of a distinct isoform of Chk2 was enhanced in response to the down-regulation of Chk1, suggesting a potential feedback loop. We also evaluated the stability of several client proteins (AR, IGF-1R, EGFR, RAF1 and JAK2) and their effectors responsible for PCa progression in response to ganetespib and observed significant degradation/inactivation, albeit with variable kinetics.

Conclusions: Ganetespib is a highly potent Hsp90 inhibitor that displays preclinical activity in a panel of prostate cancer cell lines due to its ability to target the key signaling components required for PCa cell growth, survival and cell division. Thus, further investigation of ganetespib as a new treatment for patients with prostate cancer is warranted.

Ganetespib Displays Potent Anticancer Activity in Prostate Cancer Cells Independent of AR Status

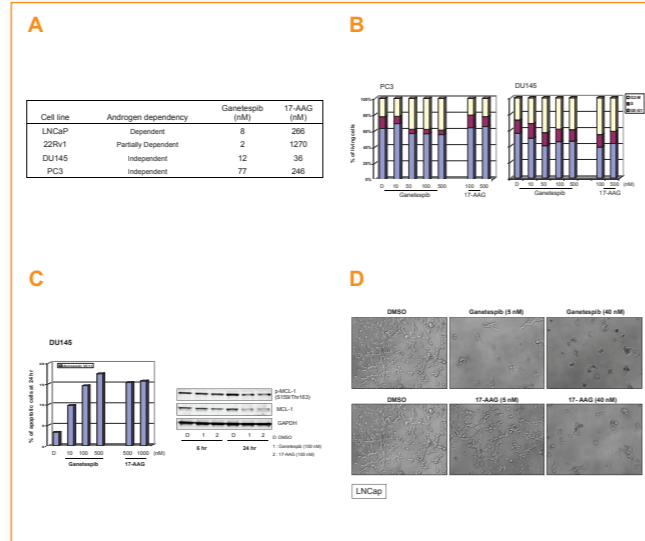


Figure 1. (A) Ganetespib has activity against both AR-dependent and AR-independent prostate cancer cell lines. Indicated cell lines were treated with ganetespib for 72 hr and cytotoxicity determined by CellTiter-Glo. (B) Cell cycle analysis after treatment with ganetespib. (C-D) Ganetespib induces apoptosis in PCa cells. (C) Prostate cancer cells were treated with ganetespib or 17-AAG and analyzed by FACS for Annexin V staining or Western blot for expression of total/phospho MCL-1, an antiapoptotic Bcl-2 family protein. (D) Viability observed by microscopy 72 hr after drug exposure.

Ganetespib Targets Multiple Oncogenic Hsp90 Client Proteins in Prostate Cancer Cells

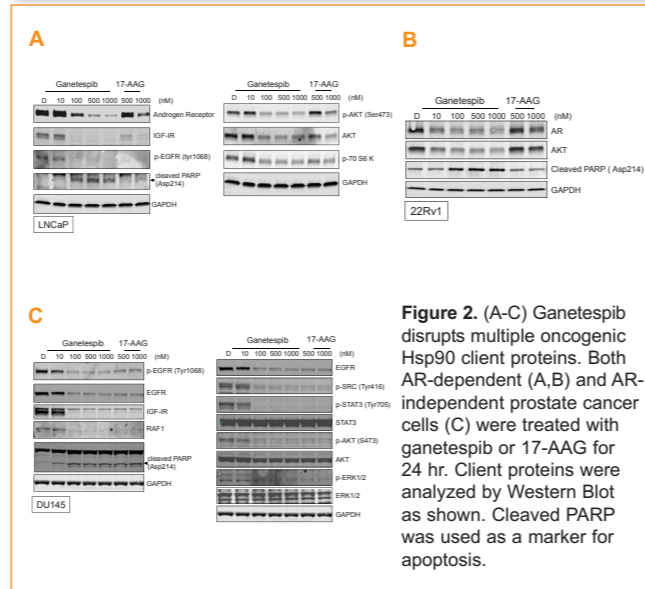


Figure 2. (A-C) Ganetespib disrupts multiple oncogenic Hsp90 client proteins. Both AR-dependent (A,B) and AR-independent prostate cancer cells (C) were treated with ganetespib or 17-AAG for 24 hr. Client proteins were analyzed by Western Blot as shown. Cleaved PARP was used as a marker for apoptosis.

Kinetics of Hsp90 Client Protein Degradation by Ganetespib

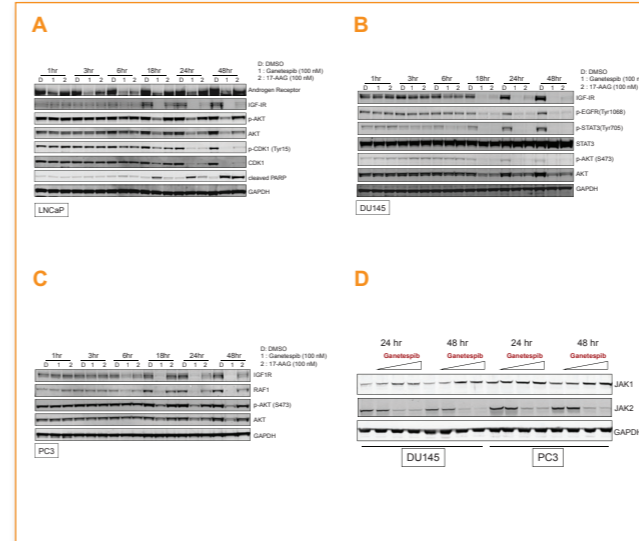


Figure 3. (A-D) Kinetic responses of Hsp90 client proteins after treatment with ganetespib in prostate cancer cells. Prostate cancer cells were exposed to ganetespib or 17-AAG for indicated amount of drug and time. Client protein expression was analyzed by Western Blot. Ganetespib destabilizes client proteins for at least 48 hours in all cell lines, and shows greater potency than 17-AAG in LNCaP and PC3 cells.

Inhibition of PI3K/mTOR Pathway Improves Action of Ganetespib

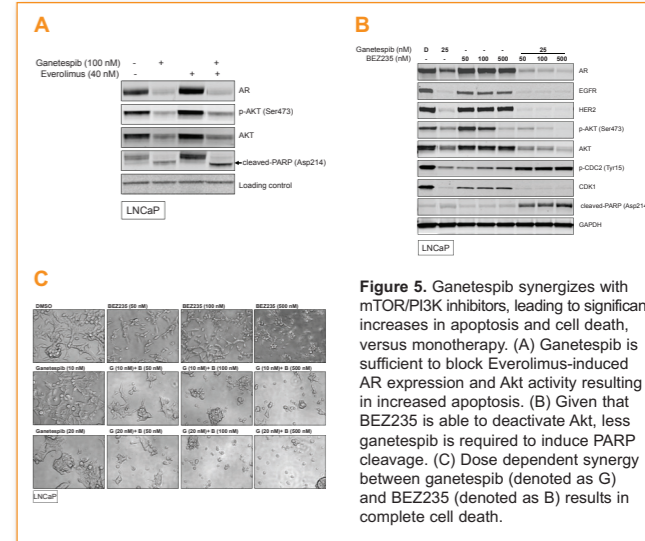


Figure 5. Ganetespib synergizes with mTOR/PI3K inhibitors, leading to significant increases in apoptosis and cell death, versus monotherapy. (A) Ganetespib is sufficient to block Everolimus-induced AR expression and Akt activity resulting in increased apoptosis. (B) Given that BEZ235 is able to deactivate Akt, less ganetespib is required to induce PARP cleavage. (C) Dose dependent synergy between ganetespib (denoted as G) and BEZ235 (denoted as B) results in complete cell death.

Ganetespib Suppresses Tumor Growth in a Prostate Cancer Xenograft Model

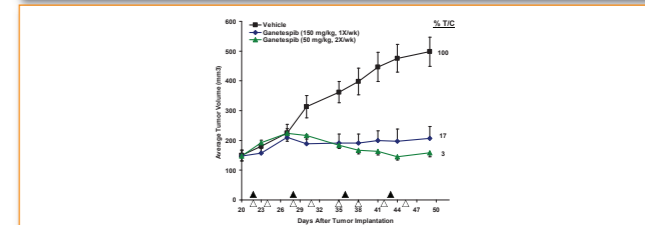


Figure 6. PC3 xenografts were implanted in Nude mice, followed by treatment with ganetespib once a week (150 mg/kg) or twice a week (50 mg/kg) for 4 weeks. Ganetespib displays potent single agent activity versus vehicle, with %T/C values of 17, 3 respectively.

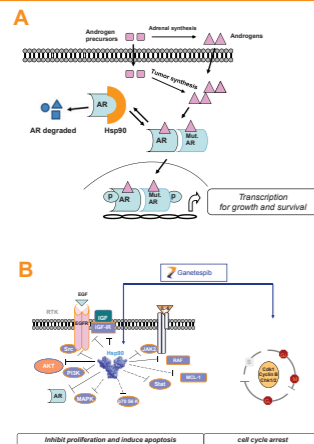
Conclusions

- Ganetespib displays potent nonclinical anticancer activity in prostate cancer cells, *in vitro* and *in vivo*, regardless of androgen receptor status.
- Multiple oncogenic Hsp90 client proteins, including androgen receptor, IGF-1R, EGFR, RAF1, CHK1, JAK2 and their downstream signaling pathways are destabilized by ganetespib.
- Ganetespib disrupts cell cycle and DNA repair checkpoints. Interestingly, downregulation of CHK1 by ganetespib results in upregulation of CHK2.
- Ganetespib synergizes with CHK, mTOR and dual mTOR/PI3K inhibitors resulting in enhanced apoptosis and cell death, compared to monotherapy.
- An investigator-sponsored Phase 2 clinical study for ganetespib in patients with metastatic hormone-resistant prostate cancer is ongoing.



For further information on Ganetespib: www.syntapharma.com

Introduction



Disruption of androgen receptor and mitogenic signaling by ganetespib leads to growth arrest and apoptosis.

(A) AR signaling is crucial for prostate tumor initiation and progression. The AR-Hsp90 interaction maintains AR in a high-affinity ligand-binding conformation, which is necessary for efficient response to hormone.

(B) Inhibition of Hsp90 by ganetespib results in the degradation of several key oncoproteins required for cell survival, proliferation and cell cycle progression.