

## Abstract

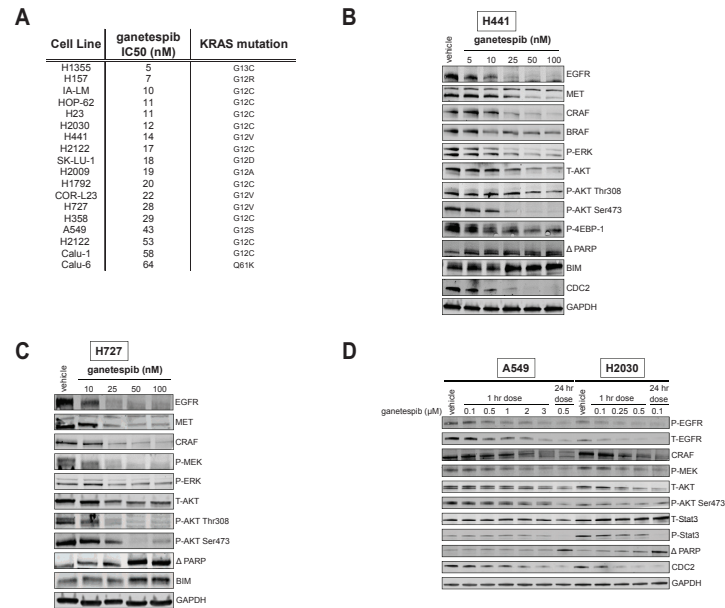
**Background:** Mutant KRAS is detected in 20-25% of non-small cell lung carcinomas (NSCLC) and represents one of the most common oncogenic drivers of this disease. NSCLC tumors with oncogenic KRAS respond poorly to currently available therapies necessitating the pursuit of new treatment strategies. Heat shock protein 90 (Hsp90) is a molecular chaperone required for the maturation and stability of hundreds of client proteins, many of which are known oncogenic drivers or effectors of such proteins. Inactivation of Hsp90 results in the simultaneous inhibition of multiple oncogenic signaling pathways, making Hsp90 a highly attractive therapeutic target. Ganetespib is a second generation, small molecule Hsp90 inhibitor currently being evaluated in multiple clinical trials. Recent results from a Phase 2 trial with ganetespib revealed that >60% of patients with KRAS mutant NSCLC exhibited tumor shrinkage at 8 weeks, indicating that ganetespib has promising potential to benefit this disease.

**Aims:** To further understand the actions of ganetespib in mutant KRAS NSCLC tumors, preclinical studies were executed in a diverse panel of KRAS mutant NSCLC cell lines to: (1) Investigate whether ganetespib is effective in suppressing critical cell signaling nodes responsible for KRAS-driven NSCLC cell survival and (2) Assess whether ganetespib can synergize with both clinical agents targeted against these signaling nodes and standard of care chemotherapies.

**Results:** Ganetespib displayed potent anticancer activity across 15 KRAS mutant NSCLC cell lines assayed *in vitro*, with an average IC50 of 24 nM. Combining ganetespib with anti-mitotics, alkylating agents or topoisomerase inhibitors resulted in an increase in cell death of up to 1.5, 1.4 and 2.6 fold, respectively, versus monotherapy. At the molecular level, ganetespib induced the destabilization of several KRAS substrates, including BRAF and CRAF, leading to inactivation of their downstream effectors followed by programmed cell death. Ganetespib effectively suppressed the growth of human KRAS mutant NSCLC tumor xenografts *in vivo*; however, ganetespib did not induce tumor regression. In light of this, we sought to investigate whether inhibitors targeting KRAS driven signaling nodes would confer greater sensitivity to ganetespib. *In vitro*, combinations of low dose ganetespib with either MEK or PI3K/mTOR inhibitors consistently resulted in greater activity than monotherapy, up to 2.3 and 1.7 fold, respectively. Furthermore, ganetespib suppressed activating feedback loops that occur in response to MEK and PI3K/mTOR inhibition, providing a rationale for the enhanced combinatorial activity. To validate these results, *in vivo* combinations were performed with ganetespib and a PI3K/mTOR inhibitor in KRAS mutant NSCLC xenografts. While both agents promoted tumor shrinkage on their own, considerable improvement in tumor growth inhibition was observed in the combination arm.

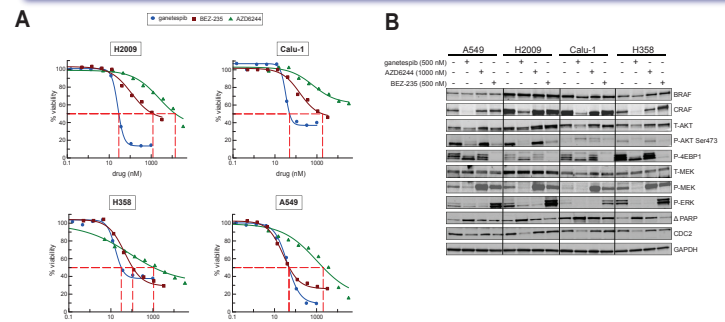
**Conclusions:** Ganetespib, a potent inhibitor of Hsp90, has shown encouraging evidence of clinical activity, including tumor shrinkage in patients with KRAS mutant NSCLC. *In vitro*, ganetespib exhibited potent anticancer activity in NSCLC cells harboring a diverse spectrum of KRAS mutations due in part to degradation and inactivation of critical KRAS signaling effectors. Combination with targeted therapies that overlap with these signaling nodes led to enhanced anticancer activity *in vitro* and in mouse models of KRAS mutant NSCLC. Taken together, these results could have interesting clinical utility in patients with KRAS mutant NSCLC.

## Loss of client protein expression and viability by ganetespib in KRAS mutant NSCLC cells



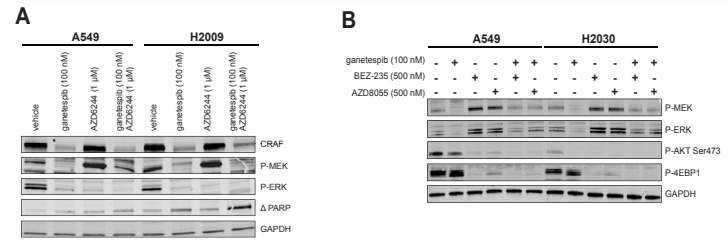
**Figure 1.** (A) Cells were treated with ganetespib for 72 hr and IC50 values were determined by quantification of ATP. (B-C) Hsp90 inhibition by ganetespib leads to simultaneous destabilization of multiple oncogenic signaling nodes and activation of apoptotic cell death. Cells were treated with the indicated concentrations of ganetespib for 24 hours followed by Western blot analysis. (D) A clinically relevant exposure of ganetespib is sufficient to suppress oncogenic signaling.

## Ganetespib simultaneously abrogates MAPK and PI3K/mTOR signaling



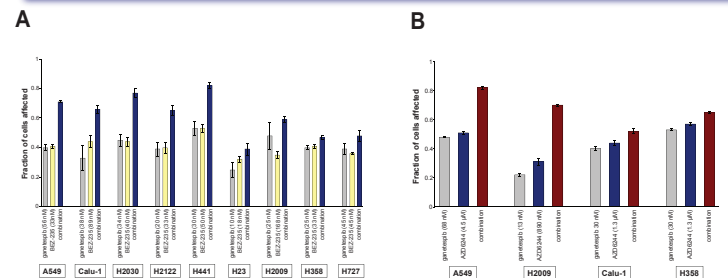
**Figure 2.** (A) Cells were treated with ganetespib, AZD6244, or BEZ-235 for 72 hours and cell viability was measured by quantification of ATP. (B) In contrast to MEK and PI3K/mTOR inhibitors, ganetespib does not induce accumulation of activated MEK or ERK. Cells were treated with the indicated concentrations of ganetespib, AZD6244, or BEZ-235 for 24 hours followed by Western blot analysis.

## Ganetespib suppresses treatment-induced activation of feedback pathways by MEK and PI3K/mTOR inhibitors



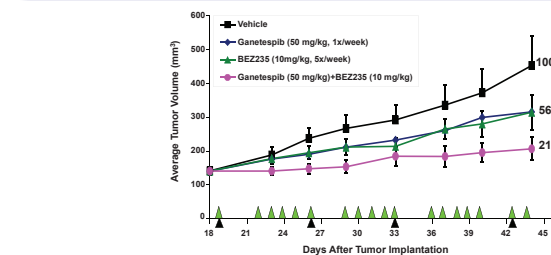
**Figure 3.** Cells were treated with the indicated concentrations of ganetespib either alone or in combination with (A) MEK inhibitor AZD6244 or (B) mTOR inhibitor AZD8055, or PI3K/mTOR inhibitor BEZ-235 for 24 hours. The effects on oncogenic signaling were assessed by Western blot analysis.

## MEK and PI3K/mTOR inhibitors enhance the activity of ganetespib in KRAS mutant NSCLC cells



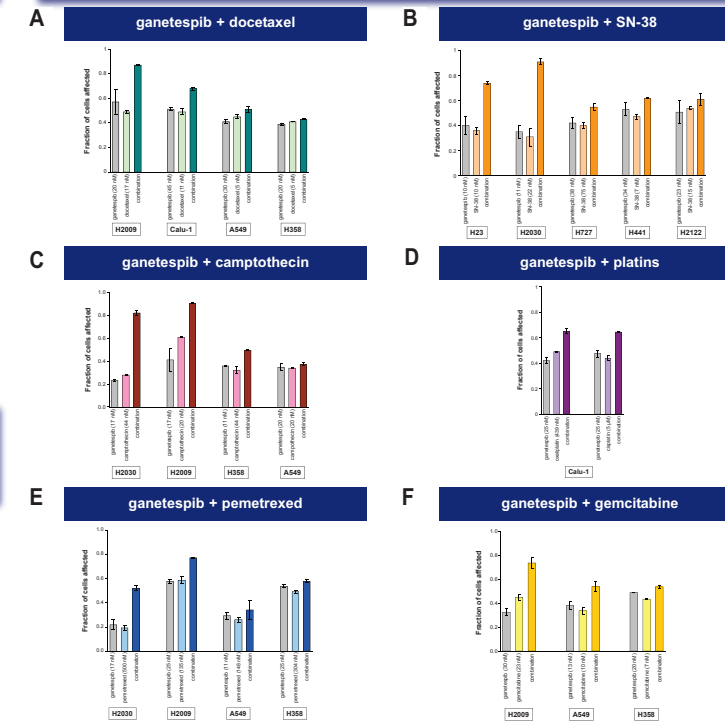
**Figure 4.** Ganetespib was combined with the PI3K/mTOR inhibitor, BEZ-235, (A) or the MEK inhibitor AZD6244 (B) for 72 hours. The effect on cell viability was determined by quantification of ATP.

## PI3K/mTOR inhibitors enhance the antitumor activity of ganetespib *in vivo*



**Figure 5.** Combination of ganetespib with BEZ-235 in A549 NSCLC xenografts.

## Standard of care chemotherapeutics sensitize KRAS mutant NSCLC cells to ganetespib



**Figure 6.** Ganetespib was combined with docetaxel (A), SN-38 (B), camptothecin (C), platin (D), pemetrexed (E), or gemcitabine (F). The level of viability was determined by quantification of ATP after 72 hours for docetaxel, camptothecin, and platin and after 96 hours for pemetrexed and gemcitabine.

## Conclusions

- Ganetespib promotes destabilization of multiple oncogenic signaling proteins and is potently cytotoxic in KRAS mutant NSCLC cells.
- Ganetespib simultaneously disrupts multiple nodes of KRAS driven signaling resulting in enhanced apoptosis compared to MEK or PI3K/mTOR inhibitors.
- Combining ganetespib with MEK or mTOR inhibitors blocks feedback induced accumulation of activated MEK and ERK contributing to enhanced cytotoxicity *in vitro* and *in vivo*.
- Traditional chemotherapies enhance the anticancer activity of ganetespib, likely due to ganetespib-mediated inhibition of DNA repair and/or chemoresistance pathways.