

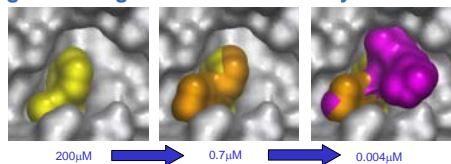
Hsp90 inhibition by AT13387 modulates growth factor and cytokine stimulated cell signalling in multiple cell lines.

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Abstract

The utility of Hsp90 inhibitors currently under clinical investigation is limited by their poor pharmaceutical properties and dose limiting toxicities which may be secondary to off-target effects. The identification of a small molecule inhibitor with improved drug-like properties would be of significant value in clinical development. AT13387 a recently identified clinical candidate is a potent (800pM), novel, small molecule inhibitor of Hsp90 discovered by high-throughput X-ray crystallography and fragment-derived chemistry. The effects of Hsp90 inhibition on tumour related signal transduction pathways in multiple growth factor and cytokine stimulated cell lines have been analyzed. AT13387 downregulated multiple Hsp90 client proteins involved in growth and survival of myeloma and breast cancer cell lines. In order to test if Hsp90 clients were down-regulated sufficiently to affect key cell signalling pathways, cells were stimulated with epidermal growth factor, phorbol ester and cytokines such as interleukin 6 in the presence or absence of AT13387, and the phosphorylation status of specific signalling pathway molecules was assessed. Constitutive, as well as growth factor and cytokine-induced phosphorylation of AKT, ERK and Stat3 were all inhibited by AT13387 in multiple tumour cell lines. There were cell line dependent differences in pathway activation by the various stimuli tested. Cell type-dependent differences in Hsp90 client modulation were also observed. AT13387 also caused a decrease in multiple myeloma cell adhesion to cultured bone marrow stromal cells and resulted in induction of apoptosis. AT13387 treatment also induced a cell cycle arrest in multiple tumour cell lines and gave good in vivo efficacy in mouse xenograft models. In conclusion the Hsp90 inhibitor AT13387 negatively regulated multiple signal transduction pathways in growth factor and cytokine stimulated cell lines, an effect which was correlated with inhibition of survival and or growth of these tumor lines.

Figure 1. Fragment based discovery of AT13387



A screen of drug-like fragments identified >10 chemotypes which bound in the active site

Figure 2. Inhibition of tumour cell proliferation

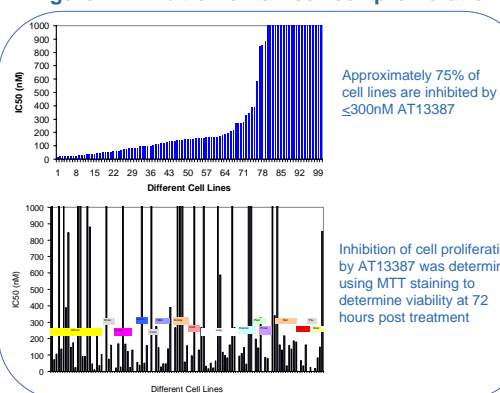


Figure 3. AT13387 modulation of Hsp90 clients

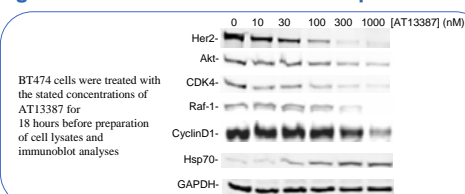


Figure 4. Duration of AT13387 Action

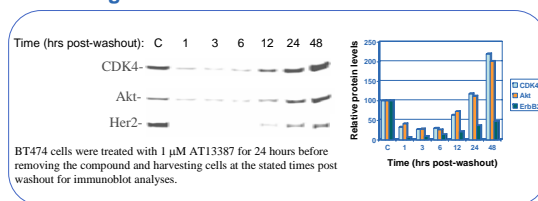


Figure 5. Effects of AT13387 on Signal Transduction

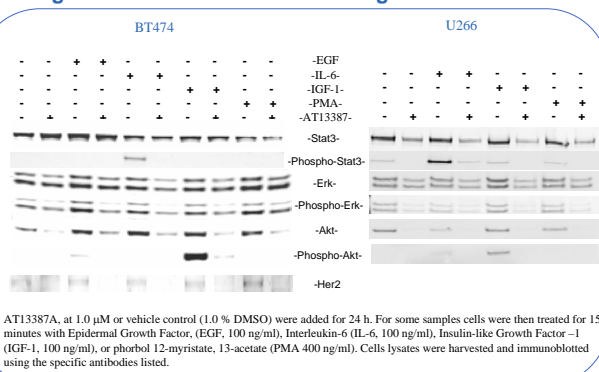


Figure 6. Effects of AT13387 on Cell Cycle Progression

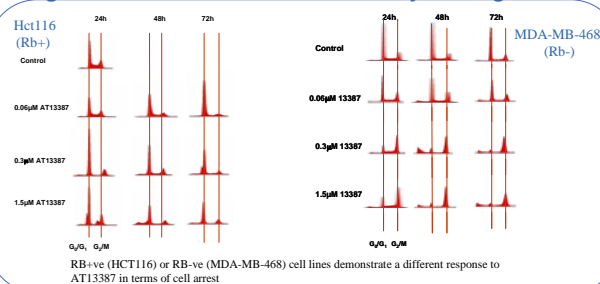


Figure 7. AT13387 modulates Akt pathway regulators and induces apoptotic markers

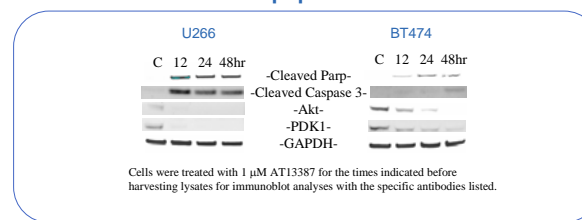


Figure 8. AT13387 Inhibits Multiple Myeloma Cell Adhesion to Stromal Cells

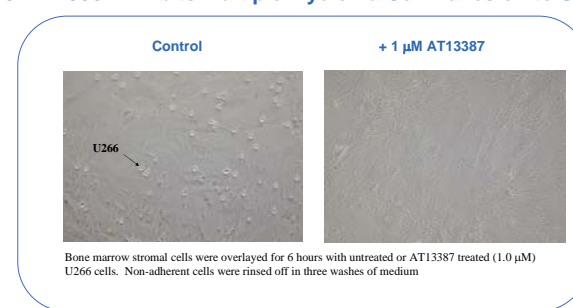
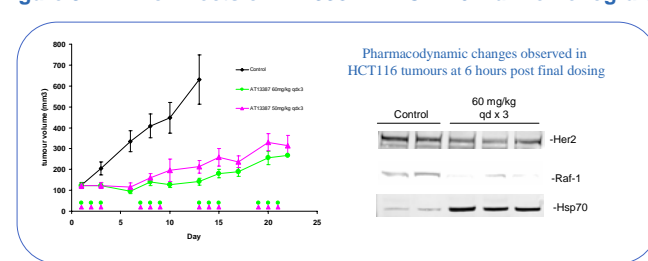


Figure 9. In Vivo Effects of AT13387 in HCT116 Murine Xenografts



Conclusion

- AT13387 is a potent small molecule inhibitor of HSP90 generated using the Astex fragment-based discovery platform
- AT13387 affects multiple signalling pathways important for a wide variety of cellular processes including:
 - cellular proliferation
 - cell survival
 - cell-cell interactions important for supporting survival of tumour cells in their microenvironment
- AT13387 inhibits the proliferation of a wide spectrum of cancer cell lines with IC50s ≤ 300 nM and has potent anti-tumour activity in mouse xenograft models
- AT13387 is in preclinical development with IND filing scheduled for late 2007