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The HSP90 inhibitor, AT13387, demonstrates potent anti-tumor activity in both imatinib-sensitive and -resistant gastrointestinal stromal tumor models

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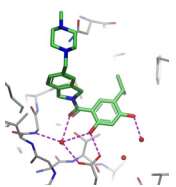
INTRODUCTION

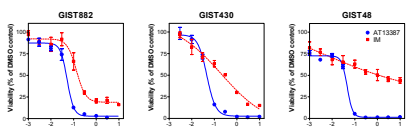
Activating mutations in the receptor tyrosine kinase, KIT, are found in the majority of gastrointestinal stromal tumors (GIST) and further secondary resistance mutations in KIT frequently arise upon treatment with tyrosine kinase inhibitors such as imatinib. KIT and its mutant forms are clients of HSP90 and it has been suggested that HSP90 inhibition might be a valuable treatment option for GIST, which would be equally effective on imatinib-sensitive and -resistant clones that may coexist within a patient.

AT13387 is a fragment-derived, potent HSP90 inhibitor, which is currently being tested in clinical trials. To evaluate its anti-tumor activity against GIST, AT13387 was tested in both imatinib-sensitive (GIST882, GIST-PSW) and -resistant (GIST430, GIST48) *in vitro* and *in vivo* GIST models.



AT13387 inhibits proliferation of GIST cell lines

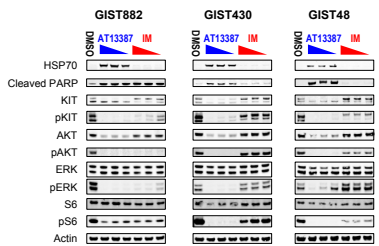
Cell Line	KIT Mutation		IC_{50} (nM)			
	Primary	Secondary	AT13387	17-AAG	Imatinib	Sunitinib
GIST882	Exon 13 K642E	none	82	140	111	26
GIST430	Exon 11 Δ560 – 576	Exon 13 V654A	34	110	46%I at 300 nM	37
GIST48	Exon 11 V560D	Exon 17 D820A	55	97	>1000	>10,000



Proliferation data.

- AT13387 inhibited the proliferation of all three cell lines at sub-100 nM, regardless of secondary KIT mutations leading to TKI resistance.
- Cells were incubated with AT13387 or imatinib (IM) for 7 days and viability assessed by Alamar Blue.
- GIST cell lines were from J Fletcher, The Brigham and Women's Hospital.

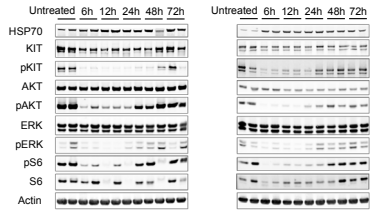
AT13387 inhibits KIT signaling in GIST cell lines



Effects of AT13387 on KIT signaling in imatinib-sensitive and -resistant GIST cell lines.

- AT13387 depleted KIT and inhibited KIT signaling in all three GIST cell lines. It also caused cleavage of PARP.
- Imatinib caused levels of KIT signaling inhibition and PARP cleavage comparable to AT13387 in the GIST882 cell line only.
- GIST882, GIST430 and GIST48 cells were incubated with 1, 0.5 or 0.25 μM of AT13387 or imatinib for 24 hours. Samples were analyzed by Western immunoblotting.

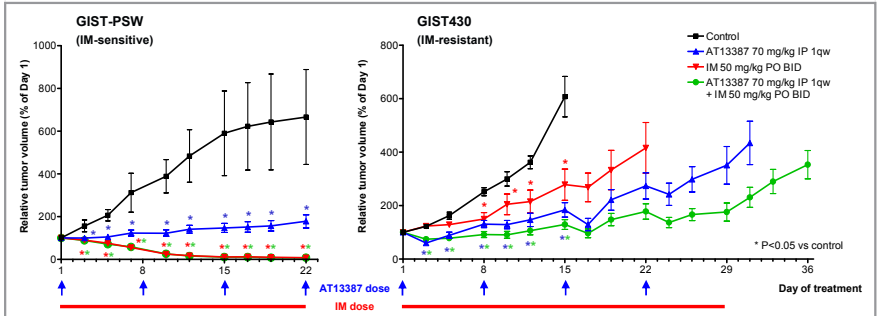
Inhibition of KIT signaling in GIST xenografts



Duration of effect of AT13387 treatment on KIT signaling in imatinib-sensitive and -resistant GIST xenografts.

- HSP70 was induced for at least 72 hours in both models. KIT protein levels and KIT signaling were reduced for 24 hours.
- Animals bearing subcutaneous GIST-PSW (KIT exon 11 del) or GIST-430 tumors were intraperitoneally treated with 70 mg/kg of AT13387 once and sacrificed at indicated time points. Tumors were analyzed by Western immunoblotting. Two tumors out of four are shown in the immunoblots.

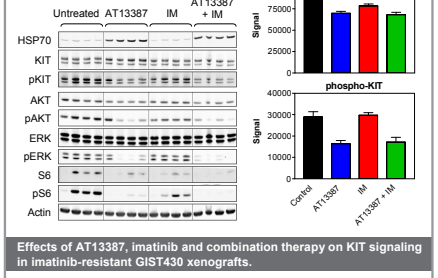
AT13387 inhibits growth of imatinib-sensitive and -resistant GIST xenografts *in vivo*



Anti-tumor activity of AT13387 and imatinib as single-agents and in combination against imatinib-sensitive and -resistant GIST xenografts.

- AT13387 (70 mg/kg IP once a week) significantly reduced the growth of both GIST-PSW and GIST430 xenografts.
- As expected, imatinib (50 mg/kg PO twice a day) caused regression of GIST-PSW but not GIST430 xenografts.
- Combining AT13387 and imatinib enhanced tumor growth inhibition over either of the monotherapies in the GIST430 model.

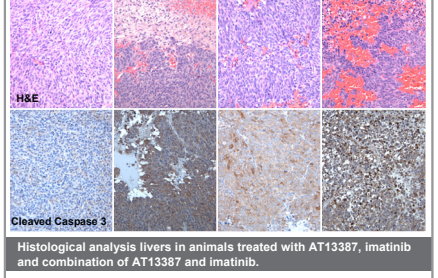
Inhibition of KIT signaling in GIST xenografts



Effects of AT13387, imatinib and combination therapy on KIT signaling in imatinib-resistant GIST430 xenografts.

- AT13387 as monotherapy or combination with imatinib inhibited KIT signaling in GIST430 tumor xenografts. Imatinib alone had little effect on KIT signaling.
- Pharmacodynamics were investigated on Day 2 of the tumor growth study by Western immunobloting (left) and MSD quantification (right).

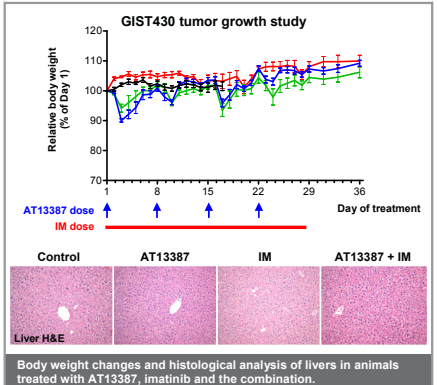
Histology of GIST430 xenograft



Histological analysis livers in animals treated with AT13387, imatinib and combination of AT13387 and imatinib.

- GIST430 tumors were analyzed by H&E staining (top) and IHC for cleaved caspase 3 (bottom).
- Extensive necrotic areas were observed with AT13387 treatments.
- Staining for cleaved caspase 3 was increased when AT13387 was combined with imatinib.

Tolerability of AT13387 and imatinib combination



Body weight changes and histological analysis of livers in animals treated with AT13387, imatinib and the combination.

- Animals in the tumor growth study tolerated AT13387 and imatinib combination therapy (top).
- No signs of necrosis were observed in H&E-stained livers of the animals at the end of tumor growth study (bottom).

SUMMARY AND CONCLUSIONS

- AT13387 has significant anti-tumor activity against imatinib-sensitive and –resistant GIST *in vitro* and *in vivo*.
- A combination of AT13387 and imatinib significantly enhanced tumor growth inhibition over either of the monotherapies in the GIST430 xenograft.
- The combination of imatinib and AT13387 was well tolerated and no histological signs of hepatotoxicity were observed in these mouse preclinical models.
- These data strongly support the clinical testing of AT13387 as monotherapy and in combination with tyrosine kinase inhibitors in GIST.
- AT13387 is currently being evaluated in combination with imatinib in a Phase II trial in GIST (NCT01294202).

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