Combining the HSP90 inhibitor, AT13387, with vemurafenib delays the emergence of resistance in a preclinical model of BRAF^{V600E} mutant melanoma.

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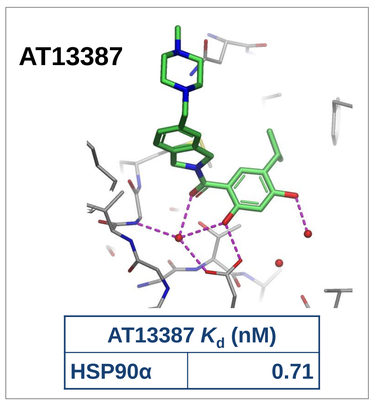
INTRODUCTION

Patients with BRAF^{V600E}-driven melanoma can be treated with BRAF inhibitors such as vemurafenib. Despite the rapid initial response, the median duration of response to vemurafenib is only 5-6 months. Resistance develops via multiple mechanisms including reactivation of the ERK and AKT pathways. Signalling proteins in these pathways are HSP90 clients and we are investigating HSP90 inhibition as a potential mechanism for overcoming BRAF inhibitor resistance.

AT13387 is a fragment-derived, potent HSP90 inhibitor being tested in clinical trials. We showed previously that AT13387 was active in both vemurafenib-sensitive and -resistant preclinical melanoma models. Co-treatment of A375 cells (BRAF^{V600E}) with AT13387 and vemurafenib *in vitro* also prevented the emergence of resistant clones.

Here, we demonstrate that addition of AT13387 to vemurafenib treatment can delay the emergence of resistance *in vivo* using a SK-MEL-28 (BRAF^{V600E}) xenograft model.



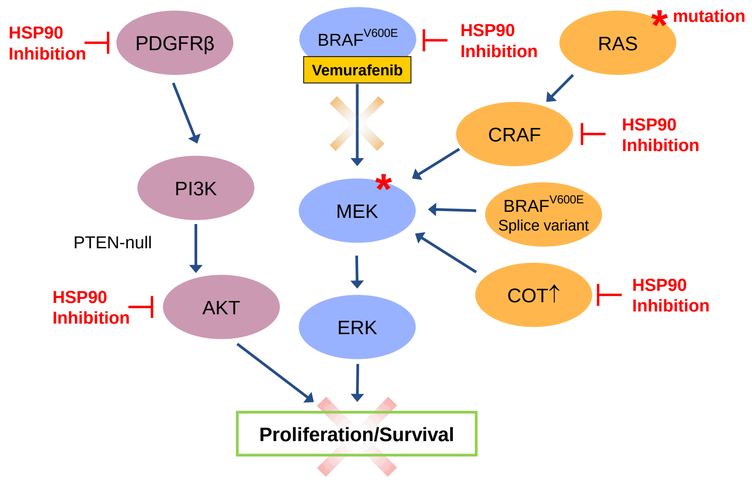
AT13387 K_d (nM)	
HSP90α	0.71

Tumour-bearing mice were treated with AT13387, vemurafenib, or the combination of both. While treatment with vemurafenib alone led to emergence of resistant tumours, no such regrowth was observed when the treatment with vemurafenib was combined with AT13387.

In addition, we show that the tumour cells with acquired vemurafenib-resistance remained sensitive to AT13387.

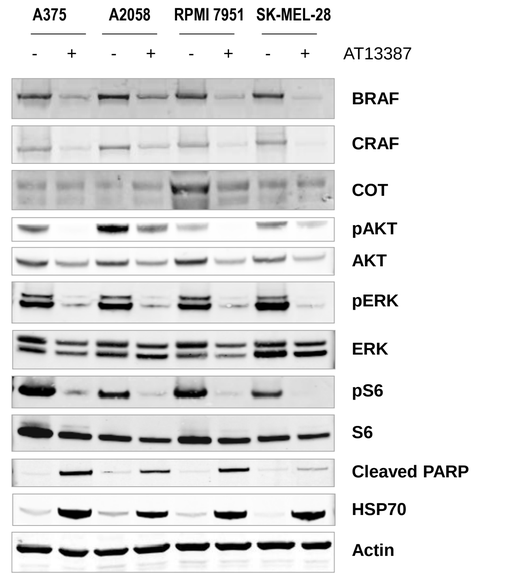
Vemurafenib resistance mechanisms and effects of AT13387 *in vitro*

AT13387 inhibits the proliferation of vemurafenib-sensitive and -resistant melanoma cell lines



Cell line	BRAF	Vemurafenib Sensitivity	Inhibition of Proliferation IC_{50} (nM)	
			Vemurafenib	AT13387
SK-MEL-28	V600E	S	340	73
A375	V600E	S	87	22
A2058	V600E	R (PTEN null)	1700	34
RPMI-7951	V600E	R (COT ↑)	>10000	30
SK-MEL-2	WT	R	>10000	45

Cell viability determined using Alamar Blue following 3-day incubation with AT13387 or vemurafenib.



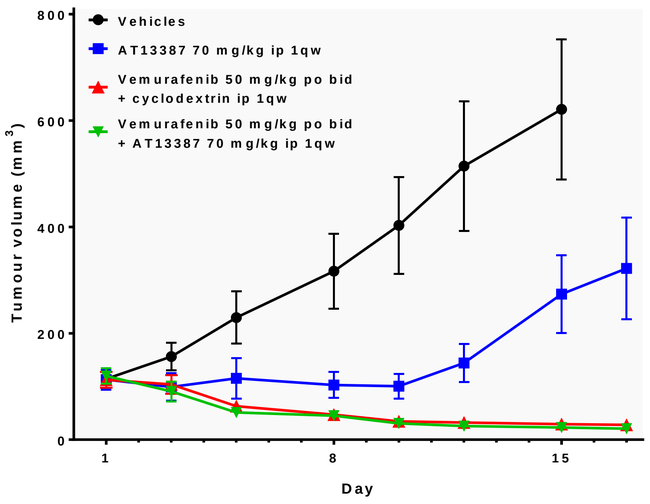
Cells were treated with 1 μM AT13387 for 24 hours then lysed for protein analysis by Western blot.

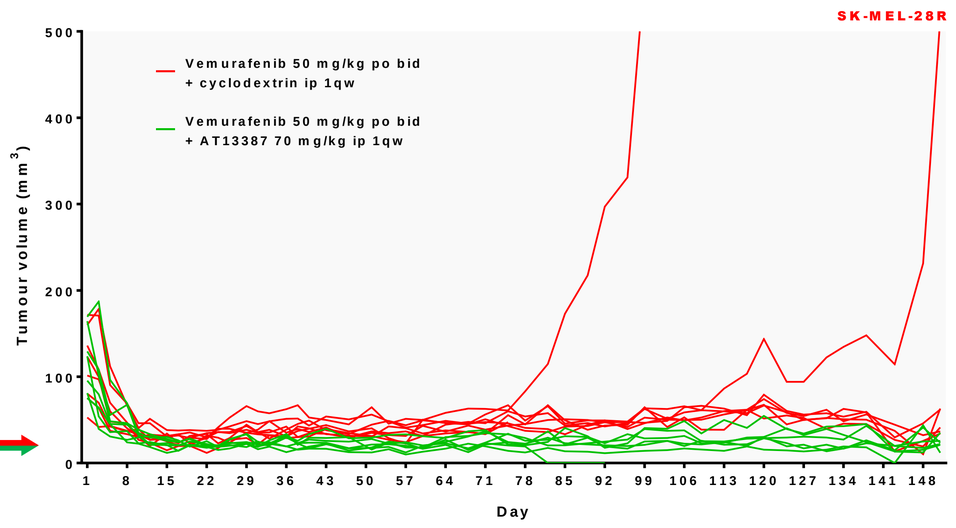
AT13387 inhibited the proliferation of a number of melanoma cell lines, including those resistant to vemurafenib through COT upregulation (RPMI-7951) or loss of PTEN (A2058). AT13387 treatment depleted BRAF, CRAF, COT and AKT, resulting in inhibition of the ERK and AKT signaling pathways.

In vivo investigation of the effect of AT13387, alone or in combination with vemurafenib, on SK-MEL-28 BRAF^{V600E} melanoma model

AT13387 delays the emergence of resistant tumours when combined with vemurafenib

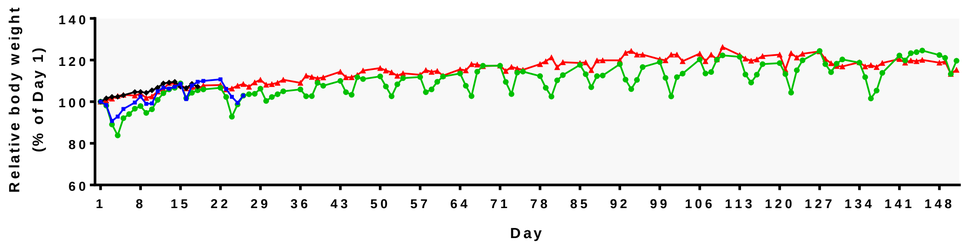
Comparison of vemurafenib monotherapy and vemurafenib + AT13387 combination treatment.





Male SCID mice bearing subcutaneous SK-MEL-28 xenograft tumours were treated with AT13387 (dissolved in 17.5% 2HP-β-cyclodextrin solution, 70 mg/kg ip once weekly), vemurafenib (suspended in 5% DMSO, 95% methylcellulose, 50 mg/kg po twice a day) or combination. Above left: Tumour graphs showing mean and SEM of at least 7 animals per group. Above right: Lines represent individual animals. Right: median relative body weight of the animals during this study.

Median relative body weight

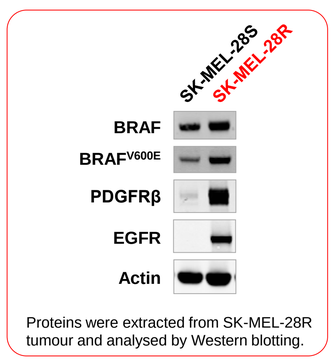


SK-MEL-28 xenograft tumours were initially sensitive to vemurafenib but 2 tumours relapsed within 5 months.

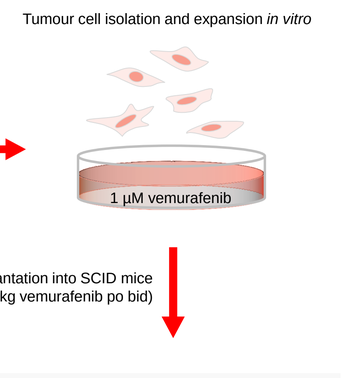
Continuous treatment combining vemurafenib with AT13387 prevented such re-growth during this study period. This combination treatment was well-tolerated.

Cells with acquired vemurafenib resistance remain sensitive to AT13387 *in vivo*

SK-MEL-28R tumour

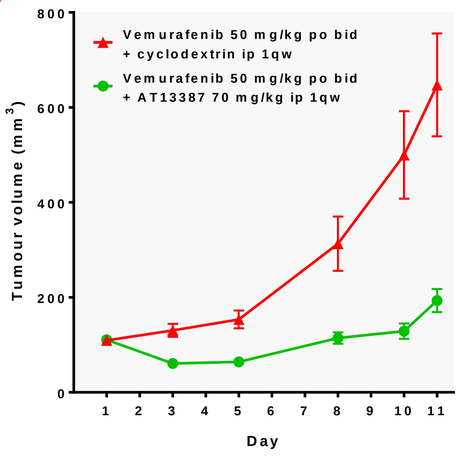


Proteins were extracted from SK-MEL-28R tumour and analysed by Western blotting.



Cells were isolated from a section of fresh SK-MEL-28R tumour by collegenase IV digestion and cultured *in vitro*. The cells were maintained in the presence of 1 μM vemurafenib.

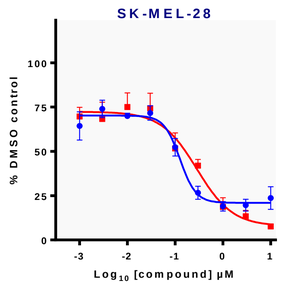
Xenograft was established in SCID mice which were treated with vemurafenib (50 mg/kg po bid) throughout the study. AT13387 treatment (70 mg/kg ip once weekly) was started when the mean tumour volume was approximately 100 mm^3.

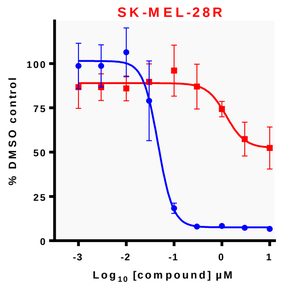


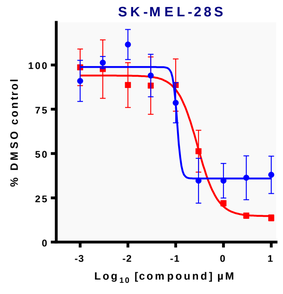
SK-MEL-28R tumours retained BRAF^{V600E}, expressed PDGFRβ and EGFR and were resistant to vemurafenib while sensitive to AT13387 *in vivo*.

In vitro characterisation of the SK-MEL-28R cells

AT13387 inhibits melanoma cells with acquired resistance to vemurafenib during *in vivo* treatment



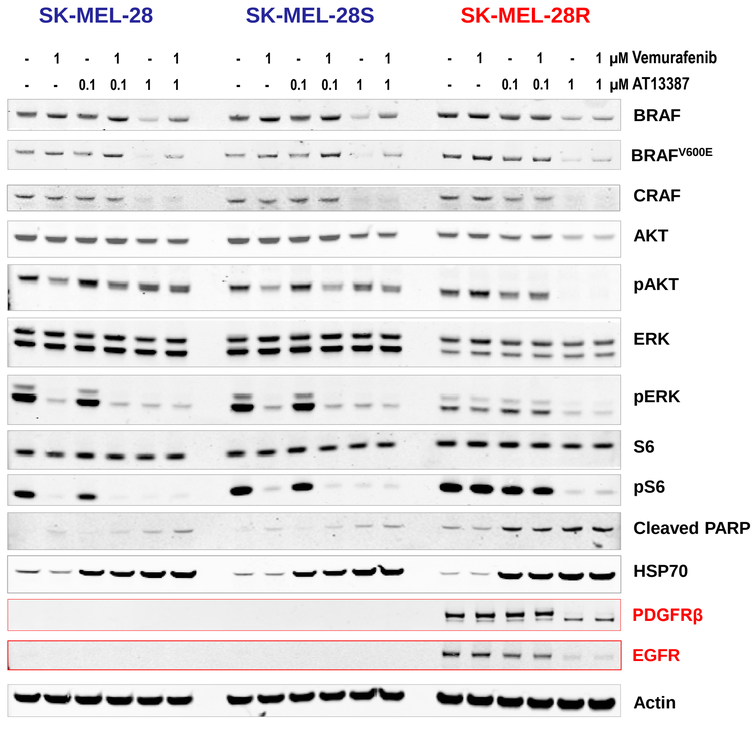




SK-MEL-28 cell line, cell populations isolated from vemurafenib-naïve xenograft (SK-MEL-28S) and from xenograft that acquired resistance to vemurafenib (SK-MEL-28R) were analysed in parallel.

Viability assay was performed using Alamar Blue following 3-day incubation with AT13387 or vemurafenib.

Signaling was investigated by incubating cells with the indicated concentration of the drugs for 24 hours, and analysing the protein levels in the cell lysates.



SK-MEL-28R cells showed decreased sensitivity to vemurafenib *in vitro* while remaining sensitive to AT13387. Signalling in these cells was unaffected by 1 μM vemurafenib (no reduction in pAKT, pERK and pS6).

These cells expressed PDGFRβ and EGFR, which were depleted, along with BRAF and CRAF, upon treatment with 1 μM AT13387. Overall response to AT13387 appeared increased (complete inhibition of pAKT and increased PARP cleavage) in SK-MEL-28R cells compared to the vemurafenib-naïve cell populations.

SUMMARY AND CONCLUSIONS

- Continuous dual treatment with AT13387 and vemurafenib delayed the emergence of resistant tumours in the SK-MEL-28 (BRAF^{V600E}) xenograft model.
- Vemurafenib monotherapy resulted in relapsed tumours. One which was characterised further retained BRAF^{V600E} and upregulated PDGFRβ and EGFR. The cell population isolated from the vemurafenib-resistant tumour was sensitive to AT13387. PDGFRβ, EGFR and AKT in these cells were depleted by AT13387 treatment *in vitro*.
- AT13387 is currently being evaluated in a number of Phase II clinical trials in combination with targeted therapy.
- These data, together with our previously reported findings, strongly support the clinical testing of AT13387, particularly in combination with BRAF inhibitors in melanoma. Such combination used as first-line therapy has the potential to prolong the initial duration of response and delay disease progression. In addition, AT13387 monotherapy can be effective on tumours that have relapsed or progressed during treatment with BRAF inhibitors.

Poster presented at World Melanoma 2013, Hamburg, 17th-20th July 2013.