

Spotlight on Clinical Response

***BRAF* and *NRAS* mutations in melanoma: potential relationships to clinical response to HSP90 inhibitors**

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Abstract

Oncogenic *BRAF* and *NRAS* mutations are frequent in malignant melanoma. *BRAF* that is activated by the common V600E and other mutations, as well as by upstream *NRAS* mutations, has been shown to require the molecular chaperone heat shock protein 90 (HSP90) for stabilization and is depleted by the HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG). Here, we explore the possible relationship between tumor *BRAF* and *NRAS* mutations and clinical response to 17-AAG in six patients with metastatic malignant melanoma who received pharmacologically active doses of 17-AAG as part of a phase I clinical trial. One patient with disease stabilization for 49 months had a ^{G13D}*NRAS* mutation and ^{WT}*BRAF*. A second patient who had stable disease for 15 months had a ^{V600E}*BRAF* mutation and ^{WT}*NRAS*. These preliminary results suggest that *BRAF* and *NRAS* mutation status should be determined in prospective phase II studies of HSP90 inhibitors in melanoma. [Mol Cancer Ther 2008;7(4):737–9]

Introduction

Heat shock protein 90 (HSP90) is a highly conserved molecular chaperone regulating stabilization, activation, and degradation of client proteins. The list of HSP90 client proteins includes CRAF, ERB-B2, BCR-ABL, CDK4, AKT, mutant p53, MET, h-TERT, and HIF1- α , which are known to contribute to the multiple hallmark traits of malignancy (1, 2).

Several HSP90 inhibitors are now in clinical development. 17-Allylamino-17-demethoxygeldanamycin (17-AAG) is a benzoquinone ansamycin that inhibits HSP90 by competing with ATP at the nucleotide binding site in the amino-terminal domain of the chaperone. Inhibition of HSP90 by 17-AAG leads to the degradation of multiple client proteins by the ubiquitin-proteasome pathway (1–3). Multiple phase I trials of 17-AAG have been carried out and it is possible to reach pharmacologically relevant drug concentrations and show client protein depletion with a variety of dosing schedules (4–8).

Malignant melanoma represents an increasing health problem. The global incidence of melanoma ranges between 2.3/100,000 and 2.6/100,000 (9). Treatment options for metastatic disease include biochemotherapy (10), with modest results. Hence, there is an urgent need to explore new molecularly targeted agents. Mutations of *BRAF* and *NRAS* genes have been shown in ~53% to 66% and 9% to 29% of all melanomas, respectively (11–14). Mutant *BRAF* and *NRAS* can activate the downstream MEK1/2-ERK1/2 oncogenic signal transduction pathway independently (11, 15). Interestingly, most melanomas have either *BRAF* or *NRAS* mutations, but not both (11–14). The most common ^{V600E}*BRAF* mutant has been shown to be a client protein of HSP90 (16, 17). Cell lines exposed to HSP90 inhibitors show a depletion of ^{V600E}*BRAF* but not ^{WT}*BRAF* (16, 17). In fact, most but not all *BRAF* mutant forms were hypersensitive to 17-AAG depletion, as was wild-type *BRAF* activated by mutant *NRAS* (16, 17). In addition, wild-type CRAF is an HSP90 client protein that is depleted as effectively as mutant/activated *BRAF* (16, 17). Taken together, the results explain why no obvious relationship was observed between *NRAS* and *BRAF* mutation status and sensitivity to 17-AAG in melanoma cell lines (16, 17). Nevertheless, it is important to extend the preclinical studies to explore any possible relationship between *BRAF* and *NRAS* mutation status and response to 17-AAG in patients with melanoma.

Materials and Methods

A phase I trial in which 17-AAG was administered weekly was conducted under the auspices of Cancer Research UK at the Royal Marsden Hospital in a collaborative project between the Cancer Research UK and the Cancer Therapy Evaluation Program at the National Cancer Institute. The protocol was reviewed by the Cancer Research UK protocol review committee and the clinical research and ethics

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committees of the Royal Marsden Hospital. All patients signed informed consent prior to entry into the study.

Using tumor material available from the phase I study, we looked for any relationship between *BRAF* and *NRAS* mutation status and disease stabilization in patients with melanoma. Pretreatment paraffin-embedded biopsies were used for analysis. Eight-micrometer sections of paraffin-embedded melanoma samples were stained with Fast Red after deparaffinizing. Regions of tumor tissue were obtained by laser capture microdissection using the Pixel II LM system (Arcturus Engineering, Inc.) and the CapSure HS LCM (Arcturus Engineering). Captured tumor regions were digested in 25 μ L of proteinase K buffer following a standard protocol at 55°C overnight. After overnight incubation, the samples were incubated at 95°C for 10 min to inactivate the proteinase K. Ten microliters of the DNA solution was added as a template and sequential PCR was done to amplify exon 15 of *BRAF* and exon 2 of *NRAS* by previously validated and published methods (17). As this was a retrospective analysis, it was unfortunately not possible to analyze the expression of mutant/wild-type *BRAF* or *NRAS* protein by Western blots due to the lack of appropriate biopsy material.

Results

At doses of 320 to 450 mg/m²/wk, 12 patients were treated. Six of these patients had metastatic malignant melanoma and had plasma concentrations in excess of the levels needed to inhibit human tumor xenografts in animal models (18). Pretreatment and posttreatment tumor biopsies showed target inhibition. Radiologic response was evaluated by computerized tomography scans done every 8 weeks or earlier if clinically indicated. No patient had a complete or partial response. However, patients progressed over variable (1–49 months) periods of time while receiving 17-AAG (4). This is consistent with results from human melanoma xenograft models which show that 17-AAG used as a single agent causes tumor growth delay rather than regression (19).

Table 1 summarizes the *NRAS* and *BRAF* mutation status and time to progression in the six patients with malignant melanoma who received therapeutic doses of 17-AAG. Figure 1 shows representative traces of the patients' wild-type or mutant *BRAF* and *NRAS* status. Three patients had a ^{V600E}*BRAF* mutation whereas three patients had wild-type ^{WT}*BRAF*. One patient out of the six had a ^{G13D}*NRAS* mutation, whereas five patients had ^{WT}*NRAS*. The *NRAS* and *BRAF* mutations were mutually exclusive. Two patients were wild-type for both genes. It was interesting to note that the only patient who had an *NRAS* mutation (^{G13D}*NRAS*) together with ^{WT}*BRAF* had a prolonged time to progression of 49 months, which is in excess of what is clinically predicted (20). A second patient who had a time to progression of 15 months had ^{V600E}*BRAF* and ^{WT}*NRAS*. Patients who had melanomas with ^{WT}*BRAF*/^{WT}*NRAS* mutational status all progressed within 1 to 1.5 months while receiving 17-AAG.

Table 1. BRAF and NRAS mutations in melanoma specimens: relationship with time to progression

Patient no.	BRAF	NRAS	Time to progression while on 17-AAG (mo)
20	WT	G13D	49
24	V600E	WT	2
25	V600E	WT	1.5
26	WT	WT	1.5
27	V600E	WT	15
28	WT	WT	1

NOTE: Six patients with metastatic malignant melanoma were treated with 17-AAG at doses of 320 to 450 mg/m²/wk. The time to progression on treatment along with *BRAF* and *NRAS* mutation status are summarized.

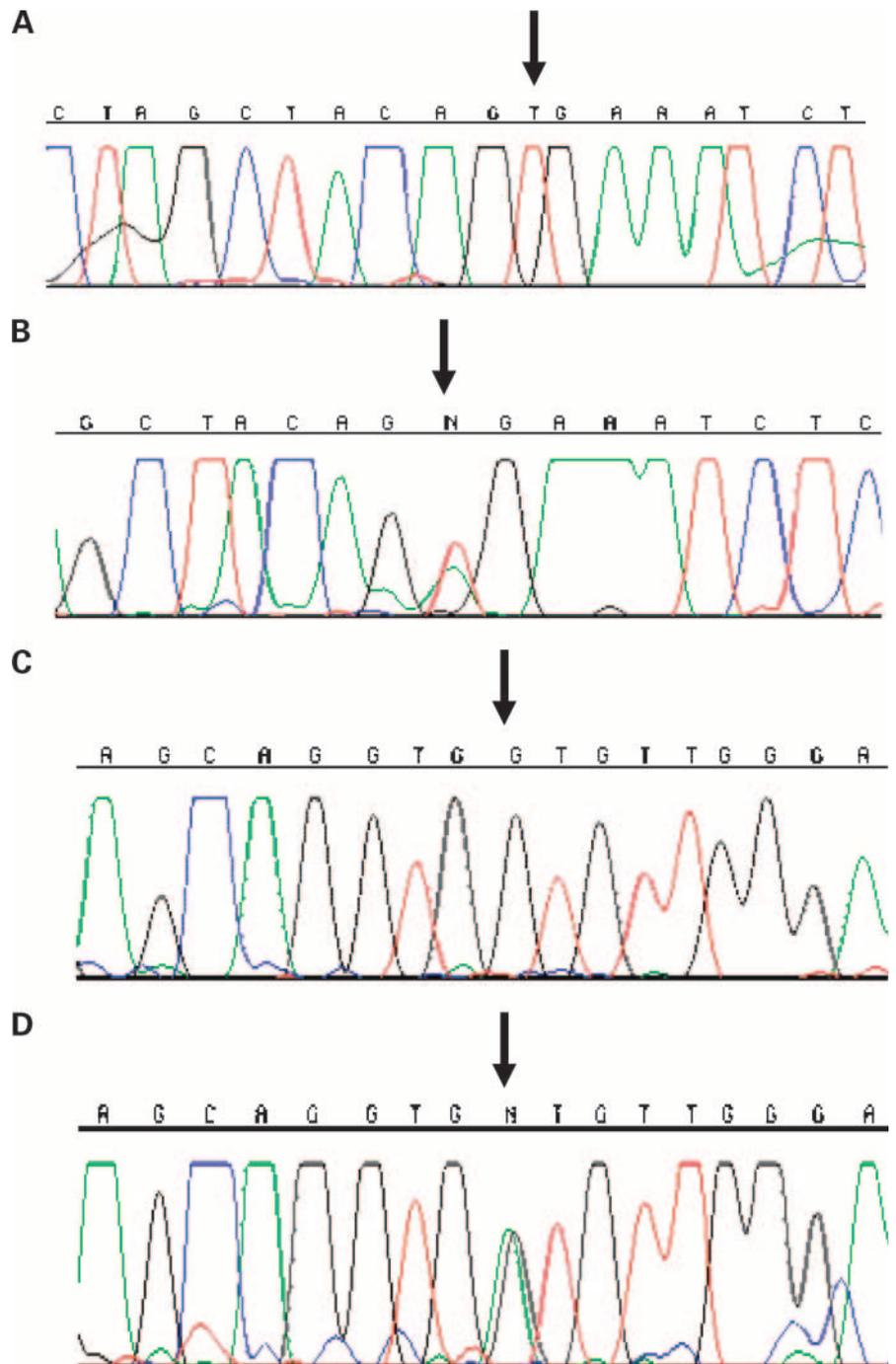
Conclusion

To conclude, in this small study of six patients with malignant melanoma treated with pharmacologically active doses of 17-AAG, two patients with prolonged disease stabilization had either ^{V600E}*BRAF* or ^{G13D}*NRAS* mutations. Although the numbers are small, we suggest further prospective analysis of *BRAF* and *NRAS* mutations in all phase II trials of HSP90 inhibitors in malignant melanoma.

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Figure 1. Representative traces of mutation status. **A**, patient 28 ^{WT}*BRAF* exon 15, codon 600: valine (GTG, arrow). **B**, patient 27 ^{V600E}*BRAF* exon 15, codon 600, showing a valine → glutamic acid substitution (GTG to GAG, arrow). **C**, patient 25 ^{WT}*NRAS* exon 2, codon 13 (GGT, arrow). **D**, patient 20 ^{G13D}*NRAS* exon 2, codon 13 showing a glycine → aspartic acid substitution (GGT to GAT, arrow).



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