**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 G13DNRAS mutation and WT BRAF. A second patient who had stable disease for 15 months had a V600EBRAF mutation and WTNRAS. 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-a, 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 V600EBRAF mutant has been shown to be a client protein of HSP90 (16, 17). Cell lines exposed to HSP90 inhibitors show a depletion of V600EBRAF but not V600ENRAS. 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
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 μL 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 V600EBRAF mutation whereas three patients had wild-type WT BRAF. One patient out of the six had a G13DNRAS 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 (G13DNRAS) 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 V600EBRAF 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.

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 V600EBRAF or G13DNRAS 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.

References

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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