The Biology Behind

Combining Cytotoxics and 17-Allylamino, 17-Demethoxygeldanamycin: Sequence and Tumor Biology Matters

Commentary re: P. Münster *et al.*, Modulation of Hsp90 Function by Ansamycins Sensitizes Breast Cancer Cells to Chemotherapy-induced Apoptosis in an RB- and Schedule-dependent Manner. Clin. Cancer Res., *7:* 2228–2236, 2001.

Edward A. Sausville[1]

Developmental Therapeutics Program, National Cancer Institute, Rockville, Maryland 20852

A surprising outcome of the search for agents that might target signaling systems that drive cancer cell growth has been the empirical observation that many of these drugs appear to potentiate the effect of conventional therapeutic agents. For example, herceptin, in both preclinical (1) and clinical (2) circumstances, potentiates DNA-directed and microtubule-directed drugs. Certain anti-epidermal growth factor-receptor-directed monoclonal antibodies behave similarly (3). This phenomenon is observed with a variety of other "small molecule" protein kinase antagonists, including flavopiridol (4), UCN-01 (5), and Iressa (6), as well as other classes of agents targeting cell signaling functions, including proteosome inhibitors (7). The implications of these findings are that, in addition to being assessed for activity in their own right, strategies for development of these agents might reasonably include efforts to detect useful augmentation of chemotherapy. Despite this favorably expanded menu of development possibilities, this outcome actually creates a number of complications.

First, except for a few noteworthy and admittedly incompletely understood examples (*e.g.,* the capacity of UCN-01 to inhibit *chk1*, one regulator of the DNA damage checkpoint; Refs. 8 and 9), we do not understand the basis underlying the signaling molecules' chemopotentiating effect. Although it is true in general terms that the pathways affected by the inhibitors feed into the regulation of cell survival pathways, *e.g.,* through the activation of phosphatidylinositol 3′-kinase and *akt*-mediated phosphorylation of *bad* (10) or the down-regulation of cell survival gene expression (11), these general modifiers of the cell-death response lead to no clear roadmap for exploiting these development possibilities. The "subtargets" relevant to the signaling agents acting as potentiators of the chemotherapy effect may be inconsistently related to the activities of the agent on its "primary" target. The economics of "filling in the boxes" by testing all signaling agents *versus* all drugs in all tumors is overtly prohibitive. Second, certain aspects of the signaling

agent action may lead to cell-cycle arrest, and, thus, theoretically antagonize the action of agents that may require some level of proliferative activity to maximally elicit cytotoxicity.

In this issue of *Clinical Cancer Research*, Münster *et al.* (12) provide several important experiments that begin to clarify these issues with respect to 17-allyl-amino 17-demethoxygeldanamycin (17AAG; NSC330507). This agent entered into clinical trials sponsored by the National Cancer Institute, Rockville, MD, with an intent to clarify the potential of benzoquinoid ansamycins to act as useful antitumor agents. The prototypic compounds in the series, herbimycin and geldanamycin, were found in the 1980s to be agents that reversed the transformed phenotype of cells driven by v-*src* family members (13), and for a time herbimycin was considered a tyrosine kinase inhibitor, until several laboratories clarified that herbimycin and geldanamycin did not directly inhibit *src* family kinases but, apparently, caused their accelerated turnover in drug-treated cells, with actual decreased mass of a variety of tyrosine kinases including *src, lck, erbB*1*,* and *erbB*2*,* among others (14–17). A unifying mechanism that explained these results was provided by Whitesell *et al.* (18), who identified that derivatized geldanamycin analogues bound to the ubiquitously expressed the cellular chaperone molecule *hsp*90.[2]

The heat shock proteins include a variety of members that, as the name implies, are modulated in their expression not only by heat but by nutrient deprivation, oxidative, and other stressful circumstances where protein denaturation might occur (19). A unifying biochemical activity on the part of *hsp*90 is catalyzing the proper folding and maturation of a number of protein substrates, including many members of the tyrosine kinase family of cell signaling molecules (Fig. 1*A*). Without proper *hsp*90 function, the abnormal conformations of these *hsp*90 partners are ubiquitinated and targeted for proteosomal degradation. The benzoquinoid ansamycins, including herbimycin, geldanamycin, and 17AAG, bind to *hsp*90 and cause the displacement and degradation of the client proteins (20, 21). Indeed, elegant structural studies (22) have confirmed that the benzoquinoid ansamycins bind to the NH_2-terminal domain of *hsp*90, and these studies provided an additional basis for constructing derivatives at the 17 position. An additional set of functions for *hsp*90 is illustrated in Fig. 1*B*, and emphasizes a distinct role as a "docking station" in the cytoplasm for a variety of important

---

[2] The abbreviations used are: *hsp*90, heat shock protein 90; 17AAG, 17-allylamino-17-demethoxygeldanamycin; Rb, retinoblastoma.
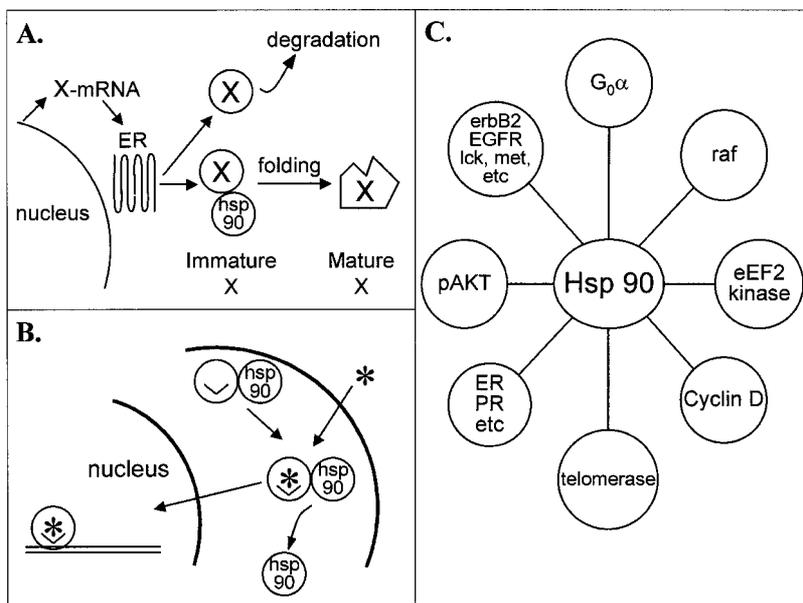
Fig. 1 Schematic of *hsp*90 functions. *A,* one important role is the action of *hsp*90 to catalyze the proper folding of newly synthesized client proteins into a stable conformation. In the absence of *hsp*90 action, the protein may be subject to rapid degradation. *B,* an additional role is to stabilize certain receptor proteins, *e.g.,* estrogen, progesterone, in the cytoplasm. Binding of their respective ligands causes dissociation from *hsp*90, with migration of the ligand-bound receptor to the nucleus. *C,* a partial and incomplete list of *hsp90* client proteins.

regulators of gene expression, including steroid hormone-family binding proteins including the estrogen, progesterone, and androgen receptors and the aryl hydrocarbon receptor. In these cases, binding of the receptors' cognate ligands causes dissociation from *hsp*90, with migration to the nucleus to allow nuclear activities. Fig. 1*C* indicates the range of potential client proteins for *hsp*90 modulation as discussed in the voluminous recent literature that is beyond the scope of this commentary. A legitimate concern is that, because of the multiplicity of functions influenced by *hsp*90, a basis for selectivity may not exist in relation to the effects in normal tissues. However, it is possible that tumors uniquely driven by some *hsp*90 client proteins, including many tyrosine kinase-driven cell types, might actually be selectively sensitive to the agent.

Geldanamycin had modest evidence of an antitumor effect in conventional xenograft models (23) but prohibitive liver toxicity in preclinical toxicology models. 17-AAG emerged from a conscious effort to define geldanamycin analogues with continued evidence of antiproliferative potential, retention of the ability to modulate *hsp*90 client, and an acceptable toxicology profile. Initial Phase I trials of a variety of schedules are ongoing or have been completed (24–26).

Münster *et al.* (27) had contributed previously to our understanding of geldanamycin and 17-AAG action by emphasizing that, in a series of breast cancer cell lines, the presence of a functional *Rb* tumor suppressor gene was associated with the arrest of ansamycin-treated cells in G_1, with some evidence of mammary epithelial cell differentiation. The mechanism for this effect remains to be understood in detail, but initial studies are concordant with an effect of ansamycins to decrease cyclin D elaboration by down-regulating growth factor-derived signals proceeding through the *akt* pathway (28). The resultant loss of CDK4 activity would naturally lead to z block in G_1. In contrast, cells with defective *Rb* exposed to ansamycins seem to arrest in G_2 with an innate propensity to undergo apoptosis (27). The

gratifying implication of the present studies of Münster *et al.* (12) is that this may be exploitable with the clinically useful drug paclitaxel, because cells exposed to taxane and then exposed to 17AAG show enhanced apoptosis. An important finding for capitalizing on this result is that, in *Rb*-competent cells, this schedule of administration was important, and that the reverse sequence of drug exposure actually seemed to protect from taxane-induced cell death by blocking cells in G_1. Cells with mutated *Rb* did not show schedule dependence. In contrast to paclitaxel, 17AAG enhanced the cytotoxicity of doxorubicin without reference to sequence of administration or *Rb* status.

These studies have a number of ramifications for clinical studies. First, although we do not consider *Rb* status as a basis for stratifying patient entry into early phase trials, this might be a case where such information, at the very least, would be usefully correlated with the outcome if not actually be an entry criterion. A corollary concern to this however, is that *Rb* status may actually incompletely define cells with enhanced apoptotic susceptibility after exposure to ansamycins. One might imagine that, in addition to *Rb* status, defining the "context" of tumor dependence on the actions of a critical *hsp*90 client protein would also need to occur before optimal use of the compound. Second, although Münster and colleagues clearly indicate the importance of sequence for *Rb*-competent tumors, how sequence duration and timing will be approached in patients will be an important variable to explore—one might imagine different cohorts of patient treated at different intervals after exposure to taxanes, and imagine further that one interval may not be suitable for all patients. Preclinical studies may be useful to clarify this, but still the relevance of these primarily mouse models (which, in general, cycle rapidly) to real clinical cancer could be questioned. Third, assays have been developed to assess the occurrence of taxane-induced effect including apoptosis in biopsies from treated patients (29); perhaps an additional end point of paclitaxel-plus-17AAG trials should be the

scoring of apoptosis indicators to contribute to evidence that a particular schedule is achieving the hoped-for end point. This would have to be balanced against the practicality of obtaining multiple biopsy specimens. Last, 17AAG is a quinone, as are certain chemotherapeutic agents, notably doxorubicin. Augmentation of end-organ toxicities not related to cell cycling must be carefully and prospectively evaluated as to whether quinone-related metabolizing systems are relevant to the action of 17AAG is unclear at this point (30, 31).

This last issue emphasizes that, although the current results are of great empirical interest and importance in defining a potential therapeutic opportunity, we still do not know the mechanism by which 17AAG augments the cytotoxic action of either paclitaxel or doxorubicin. Recent studies have clarified that paclitaxel kills cells by at least two different mechanisms: at very low concentrations, apoptosis is induced with little evidence of M-phase block; whereas, at higher concentrations, manifest M-phase block occurs with evidence of activation of *raf* kinase (32). As *raf* is a prime example of an *hsp*90 client protein, perhaps 17AAG-induced deregulation of this activity might be a proapoptotic stimulus. Alternately, perhaps another, yet to be defined *hsp*90 client molecule participates in regulating the assembly of the mitotic apparatus or the function of spindle checkpoints. The importance of this mechanistic information will be in offering yet another avenue for selecting patients who might best benefit from this combination.

New therapeutic opportunities will continue to emerge as we define agents with selective effects on pathways of importance to the economy of tumors. Unless we have the luxury of an absolutely specific, tumor-related target, *e.g.,* a p210[bcr-abl], these therapies invariably will be directed against targets which also have varying degrees of function in normal cells. Developing strategies that emphasize those aspects of tumor biology that maximize the possibility of achieving a therapeutic "window" will be a key aspect of combining these drugs of the future with both conventional and investigational agents. This will be a challenge in diagnosis and patient selection, as well as in clinical treatment. The results of Münster *et al.* (12) highlighted in this issue are an important step in approaching this issue rationally in the case of the ansamycins.

References

1. Baselga, J., Norton, L., Albanell, J., Kim, Y. M., and Mendelsohn, J. Recombinant humanized anti-HER2antibody (herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing breast cancer xenografts. Cancer Res., *58:* 2825–2831, 1998.

2. Slamon, D. J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., Baselga, J., and Norton, L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast carrcinoma that overexpresses HER2. N. Engl. J. Med., *344:* 783–792, 2001.

3. Ciardiello, F., Bianco, R., Damiano, V., De Lorenzo, S., Pepe, S., De Placido, S., Fan, Z., Mendelsohn, J., Bianco, A. R., and Tortora, G. Antitumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225. Clin. Cancer Res., *5:* 909–916, 1999.

4. Bible, K. C., and Kaufmann, S. H., Cytotoxic synergy between flavopiridol (NSC 649890, L86–8275) and various antineoplastic agents: the importance of sequence of administration. Cancer Res., *57:* 3375–3380, 1997.

5. Monks, A., Harris, E. D., Vaigro-Wolff, A., Hose, C. D., Connelly, J. W., and Sausville, E. A. UCN-01 enhances the *in vitro* toxicity of clinical agents in human tumor cell lines. Investig. New Drugs, *18:* 95–107, 2000.

6. Sirotnak, F. M., Zakowski, M. F., Miller, V. A., Scher, H. I., and Kris, M. G. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhjanced by coadministration of ZD1839 (Iressa), and inhibitor of EGFR tyrosine kinase. Clin. Cancer Res., *6:* 4885–4892, 2000.

7. Cusack, J. C., Jr., Liu, R., Houston, M., Abendroth, K., Elliott, P. J., Adams, J., and Baldwin, A. S., Jr. Enhanced chemosensitivity to CPT-11 with proteosome inhibitor PS-341: implications for systemic nuclear factor-κB inhibition. Cancer Res., *61:* 3535–3540, 2001.

8. Graves, P. R., Yu, L., Schwarz, J. K., Gales, J., Sausville, E. A., O'Connor, P. M., and Piwnica-Worms, H. The chk1 protein kinase and the Cdc25C regulatory pathways are targets of the anticancer agent UCN-01. J. Biol. Chem., *275:* 5600–5605, 2000.

9. Busby, E. C., Leistritz, D. F., Abraham, R. T., Karnitz, L. M., and Sarkaria, J. N. The radiosensitizing agent 7-hydroxystaurosporine(UCN-01) inhibits the DNA damage checkpoint kinase hChk1. Cancer Res., *60:* 2108–2112, 2000.

10. Datta, S. R., Dudek, T. X., Masters, S., Fu, H., Gotoh, Y., and Greenburg, M. E. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell, *91:* 231–241, 1997.

11. Kitada, S., Zapata, J. M., Andreeff, M., and Reed, J. C., Protein kinase inhibitors flavopiridol and 7-hydroxy-staurosporine down-regulate antiapoptosis proteins in B-cell chronic lymphocytic leukemia. Blood, *96:* 393–397, 2000.

12. Münster, P. N., Basso, A., Solit, D., Norton, L., and Rosen, N. Modulation of Hsp 90 function by ansamycins sensitizes breast cancer cells to chemotherapy-induced apoptosis in an RB and schedule dependent manner. Clin. Cancer Res., *7:* 2228–2236, 2001.

13. Uehara, Y., Hori, M., Takeuchi, T., and Umezawa, H. Phenotypic change from transformed to normal induced by benzoquinoid ansamycins accompanies inactivation of p60src in rat kidney cells infected with Rous sarcoma virus. Mol. Cell Biol., *6:* 2198–2206, 1986.

14. Miller, P., Schnur, R. C., Barbacci, E., Moyer, M. P., and Moyer, J. D. Binding of benzoquinoid ansamycins to p100 correlates with their ability to deplete the *erb*B2 gene product p185. Biochem. Biophys. Res. Commun., *201:* 1313–1319, 1994.

15. Miller, P., DiOrio, C., Moyer, M., Schnur, R. C., Bruskin, A., Cullen, W., and Moyer, J. D. Depletion of the *erb*B-2 gene product p185 by benzoquinoid ansamycins. Cancer Res., *54:* 2724–2730, 1994.

16. Sepp-Lorenzino, L., Ma, Z., Lebwohl, D. E., Vinitsky, A., and Rosen, N. Herbimycin A induces the 20S proteosome and ubiquitin-dependnet degradation of receptor tyrsosine kinases. J. Biol. Chem., *270:* 16580–16587, 1995.

17. Mimnaugh, E. G., Chavany, C., and Neckers, L., Polyubiquitination and proteosomal degradation of the p185c-erbB-2 receptor protein-tyrosine kinase induced by geldanamycin. J. Biol. Chem., *271:* 22796–22801, 1996.

18. Whitesell, L., Mimnaugh, E. G., De Costa, B., Myers, C. E., and Neckers, L. M. Inhibiton of heat shock protein HSP90-pp60v-src heteroprotein complex formation by bexzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc. Natl. Acad. Sci. USA, *91:* 8324–8328, 1994.

19. Pearl, L. H., and Prodromou, C. Structure and *in vivo* function of Hsp 90. Curr. Opin. Struct. Biol., *10:* 46–51, 2000.

20. Schulte, T. W., Blagosklonny, M. V., Romanova, L., Mushinski, J. F., Monia, B. P., Johnston, J. F., Nguyen, P., Trepel, J., and Neckers, L. M. Destabilization of raf-1 by geldanamycin leads to disruption of the Raf-1-MEK-mitogen activated protein kinase signalling pathway. Mol. Cell. Biol., *16:* 5839–5845, 1996.

21. Nimmanapalli, R., O'Bryan, E., and Bhalla, K. Geldanamycin and its analog 17-allylamino-17-demethoxygeldanamycin lowers Bcr-Abl levels and induces apoptosis and differentiation of Bcr-Abl-positive human leukemic blasts. Cancer Res., *61:* 1799–1804, 2001.

22. Stebbins, C. E., Russo, A. A., Schneider, C., Rosen, N., Hartl, F. U., and Pavletich, N. P. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell, *89:* 239–250, 1997.

23. Whitesell, L., Shifrin, D., Schwab, G., and Neckers, L. M. Benzoquinoid ansamycins possess selective tumoricidal activity unrelated to src kinase inhibition. Cancer Res., *52:* 1721–1728, 1992.

24. Wilson, R. H., Takimoto, C. H. Agnew, E. B., Morrison, G., Grollman, F., Thomas, R. R., Saif, M. W., Hopkins, J., Allegra, C., Grochow, L., Szabo, E., Hamilton, J. M., Monhan, B. P., Neckers, L., and Grem, J. L. Phase I pharmacological study of 17-(allylamino)-17-demethoxygeldanamycin (AAG) in adult patients with advanced solid tumors. Proc. Am. Soc. Clin. Oncol., *20:* 82a, 2001.

25. Banerji, U., O'Donnell, A., Scurr, M., Benson, C., Hanwell, J., Clark, S., Raynaud, F., Turner, A., Walton, M., Workman, P., and Judson, I. Phase I trial of the heat shock protein 90(HSP90) inhibitor 17-allylamino-17-demethoxygeldanamycin (17aag). Pharmacoklinetic (PK) profile and pharmacodynamic endpoints. Proc. Am. Soc. Clin. Oncol., *20:* 82a, 2001.

26. Münster, P. N., Tong, W., Schwartz, L., Larson, S., Keneson, K., De La Cruz, A., Rosen, N., and Scher, H. Phase I trial of 17-(allylamino)-17-Demethoxygeldanamycin (17AAG) in patients (pts) with advanced solid malignancies. Proc. Am. Soc. Clin. Oncol., *20:* 83a, 2001.

27. Münster, P. N., Srethapakdi, M., Moasser, M. M., and Rosen, N. Inhibition of heat shock protein 90 function by ansamycins causes the morphological and functional differentiation of breast cancer cells. Cancer Res., *61:* 2945–2952, 2001.

28. Muise-Helmericks, R. C., Grimes, H. L., Bellacosa, A., Malstrom, S. E., Tsichlis, P. N., and Rosen, N. Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/Akt dependent pathway. J. Biol. Chem., *273:* 29864–29872, 1998.

29. Symmans, W. F., Volm, M. D., Shapiro, R. L., Perkins, A. B., Kim, A. Y., Demaria, S., Yee, H. T., McMullen, H., Oratz, R., Klein, P., Formenti, S. C., and Muggia, F. Paclitaxel-induced apoptosis and mitotic arrest assessed by serial fine needle aspiration: implications for early prediction of breast cancer response to neoadjuvant treatment. Clin. Cancer Res., *6:* 4610–4617, 2000.

30. Brunton, V. G., Steele, G., Lewis, A. D., and Workman, P. Geldanamycin-induced cytotoxicity in human colon-cancer cell lines: evidence against the involvement of c-src or DT-diaphorase. Cancer Chemother. Pharmacol., *41:* 417–422, 1998.

31. Kelland, L. R., Sharp, S. Y., Rogers, P. M., Myers, T. G., and Workman, P. DT-diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. J. Natl. Cancer Inst., *91:* 1940–1949, 1999.

32. Torres, K., and Horwitz, S. B. Mechanisms of Taxol-induced cell death are concentration dependent. Cancer Res., *58:* 3620–3626, 1998.

Clinical Cancer Research

Combining Cytotoxics and 17-Allylamino, 17-Demethoxygeldanamycin: Sequence and Tumor Biology Matters: Commentary re: P. Münster et al., Modulation of Hsp90 Function by Ansamycins Sensitizes Breast Cancer Cells to C hemotherapy-induced Apoptosis in an RB- and Schedule-dependent Manner. Clin. Cancer Res., 7: 2228−2236, 2001.

Edward A. Sausville

Updated version	Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/7/8/2155

Cited articles	This article cites 31 articles, 21 of which you can access for free at: http://clincancerres.aacrjournals.org/content/7/8/2155.full#ref-list-1
Citing articles	This article has been cited by 11 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/7/8/2155.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/7/8/2155. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.