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New Agents—Noncytotoxins, Antiangiogenesis

By Edward A. Sausville, MD, PhD, Session Chair

Abstract: **Traditional cytotoxic agents, which cause death of tumor cells in tissue culture or in vivo animal models, have been the mainstay of agents available for clinical trials and have led to useful and relatively safe agents for clinical use. However, the continuing improvement of our understanding of the biochemical pathways that give rise to the malignant phenotype have defined new targets for antineoplastic agents that may have a cytostatic, rather than cytotoxic, end point. The hope is that such agents may have a greatly improved therapeutic index, allowing their long-term and perhaps indefinite use. These agents would be of particular value in the treatment of disease diagnosed by molecular markers in a tissue at risk or as an adjuvant treatment after primary ablative therapy. Inhibitors of signal transduction pathways and antiangiogenic agents are two such categories of agent that do not have primary cytotoxicity for the tumor cell. 17-Allylamino 17-demethoxygeldanamycin (17AAG; NSC 330507) and rapamycin analog WAY-130779 (NSC 683864) represent novel signal transduction inhibitors that will enter or have just entered clinical trial, which illustrate the thinking that encourages clinical trials with this type of agent.**

THIS SESSION WILL EXPLORE initial efforts to define the clinical value of noncytotoxic approaches to cancer therapeutics. Current cancer drugs that have proven safe and effective in a way that leads to regulatory approval are, in large measure, cytotoxic compounds, the activity of which was first detected in tumor screening systems in vivo, such as the murine leukemias, L1210 and P388, or murine solid tumors. Yet, these drugs use a relatively limited repertoire of mechanisms, for the most part targeting either some aspect of DNA (eg, structural integrity or synthesis) or tubulin. These agents have a set of stereotypical side effects, including leukopenia and mucositis. Moreover, it is increasingly recognized that the success of these agents in causing cell death does not directly relate to their affecting an immediate target, eg, topoisomerases or nucleotide-synthesizing systems, but rather in inducing the activity of cell death pathways, the action of which leads to apoptosis or programmed cell death. In that regard, interaction with traditional cancer drug targets is necessary, but not sufficient for a useful effect of these agents.

There is no question that clinically used, traditional cytotoxic agents have saved lives either through their direct use in advanced disease or through adjuvant treatment programs after primary therapy. However, increasing definition of the regulatory pathways perturbed in tumor cells has raised the question of whether specific targeting of these deregulated pathways would define agents that do not necessarily result in tumor-cell death, but decrease the rate of tumor-cell proliferation in a way that will be clinically useful. An additional hope is that agents directed at these deregulated pathways will have an altered spectrum of toxicities, with ideally little toxicity for nonproliferating normal cells. Tolerable or essentially nonexistent toxicity when administered for protracted periods, perhaps chronically, will be a necessary feature if these molecules are to achieve their broadest utility. A differing school of thought would hold that cytostatic agents of the various types to be described later may actually magnify or augment the action of cytotoxic drugs by diminishing the capacity of the cell to repair damage evoked by cytotoxic agents. This point of view would propose that cytostatic agents might be best intermittently used as modulators to enhance the action of cytotoxic agents.

Noncytotoxic agents under active development have the common property of generally not causing overt tumor-cell kill after brief exposure of cells in conventional screening assays for growth inhibition in vitro. This is in contrast to classical cytotoxic agents, for which a relatively brief exposure is frequently sufficient to evoke significant cell kill manifest, for example, in 18 to 24 hours after drug exposure. In many cases, brief exposure to noncytotoxic agents may lead to protracted cytostasis, which can in some cases be reversed by drug removal even after several hours or days in cell culture. Noncytotoxic agents include those

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that interrupt the propagation of growth factor–related signals, which result in a block to cell-cycle progression or entry into the cell cycle; differentiation agents, which induce gene activity that causes cells to exit from the cell cycle, with or without apoptosis; antimetastatic agents, intended to target tumor-cell motility, invasion, and the appropriate microenvironment for growth; and antiangiogenic agents, which attack not the tumor cells directly, but the supporting network of blood vessels necessary for progression of a tumor from a microscopic to macroscopic growth.

The presentations to be focused on in this session will emphasize signal transduction blockers and antiangiogenic agents. Examples of agents early in clinical development will be presented in the remainder of this section to illustrate features common to thinking about signal transduction blockers. A subsequent module by Dr Michael Cooper will contain an update of different types of signal transduction blockers that have proceeded to more advanced clinical trials. The final module by Dr James Pluda will address recent approaches to inhibition of tumor endothelial cell proliferation. Both modules will touch on the difficulty in designing clinical trials that efficiently capture valuable clinical evidence of cytostatic activity.

Figure 1 presents a greatly simplified view of the controls operating to regulate cell proliferation. Growth factor receptors respond to external stimuli to activate intermediary signal transduction cascades. Among the best characterized systems of growth factor receptors are the tyrosine kinase–activating receptors, which include epidermal growth factor receptor (EGF-R; c-*erb*B1) and c-*erb*B2. Transduction of signals results in expression of genes critical to entry into the cell cycle, such as the *myc* family genes and cyclin D gene products. The latter activate the cyclin-dependent kinase (CDK) family of cell-cycle regulatory enzymes CDKs 4 or 6 and CDK2 to promote progression through G1 and entry into S phase. The action of endogenous inhibitors of CDK action (p16 and p21 families) diminishes. Phosphorylation of the tumor-suppressor gene pRB, first detected as the basis for hereditary retinoblastoma, occurs. Following pRB phosphorylation, the E2F family of transcription factors is activated, which leads to elaboration of enzymes necessary for completion of DNA synthesis, ultimately followed by mitosis after the action of CDK1, which triggers entry into mitosis.

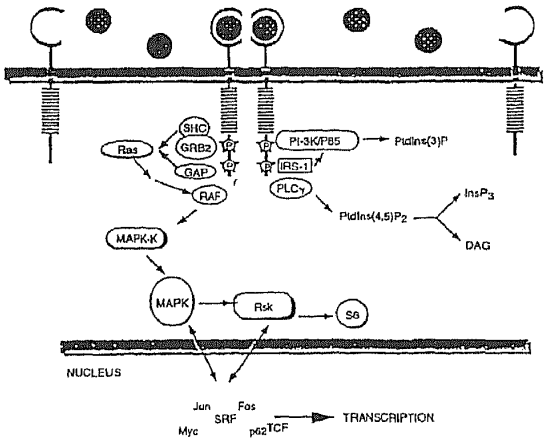


Fig 1. Growth factor–mediated signals targeted by cytostatic agents. In this simplified depiction, growth factors combine with their cognate receptors and cause dimerization, with activation of protein tyrosine kinase activity. The phosphorylated tyrosine kinase receptor recruits adaptor molecules, including shc, and grb2, to activate *ras* through influence on the GTPase activator proteins (GAPs). *Ras* in turn directly interacts with *raf*, to augment gene activity through the MAP kinase pathway (MAPK-K, MAPK). Phosphorylation on tyrosine of activated receptors activates phospholipase C, resulting in calcium mobilization by inositol tris phosphate (InsP3) and the potential for protein kinase C activation by diacylglycerol (DAG). Note that additional types of receptors not depicted here, eg, 7-transmembrane receptor-G-protein–coupled receptors can activate calcium mobilization by a distinct phospholipase.

The importance of this pathway to tumorigenesis is illustrated by the fact that substantial numbers of patients with common adult malignancies have lesions in this pathway. For example, substantial fractions of breast and ovarian tumors overexpress c-*erb*B2; 80% of small-cell lung tumors have inactivation of pRB, and of those that do not, a high frequency of deletions of the endogenous inhibitors to cell-cycle progression can be demonstrated. Thus, agents that would return these altered pathophysiologic features of tumor cells toward normal are attractive in that they are addressing the pathophysiologic basis for tumor-cell growth.

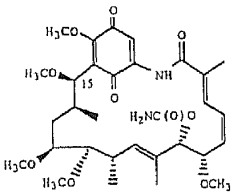
17-ALLYLAMINO 17-DEMETHOXYGELDANAMYCIN

This agent is a derivative of the benzoquinoid ansamycin, geldanamycin. The parent compound, geldanamycin, is similar to the related ansamy-

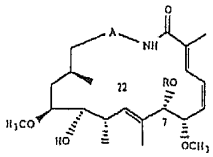
cin, herbimycin (Fig 2), in its ability to cause reversion of fibroblasts transformed by the viral oncogene v-*src* to a more normal phenotype under noncytotoxic conditions.[1] This feature was extended to include cells transformed by a number of tyrosine kinase oncogenes, including c-*erb*B2. Since the content of tyrosine phosphates was decreased in drug-treated cells, both herbimycin and geldanamycin were used for many years as nonspecific inhibitors of tyrosine kinases in laboratory experiments. However, efforts to define direct inhibition of tyrosine kinases in biochemical reactions outside of cells failed to demonstrate potent tyrosine kinase inhibitory capacity. Instead, closer scrutiny of the effects of geldanamycin in living cells showed that the reason for apparent diminution of tyrosine kinase activity was that the actual mass of several tyrosine kinases was decreased in drug-treated cells, and subsequent experiments demonstrated accelerated turnover with degradation of newly synthesized tyrosine kinases.[2-4]

The molecular basis for this behavior became apparent when Whitesell et al[5] demonstrated that geldanamycin actually formed a high-affinity complex with the heat-shock protein (hsp) 90, and thus, inhibited the formation of complexes between hsp and the oncoproteins, including the *src* family oncoproteins. Hsps of various molecular weights had long been defined as cellular proteins whose level increased in response to a variety of nonspecific stresses, including heat, osmotic, toxic, and other stresses. The hsp90 family act physiologically as "chaperones" to effect proper folding and cellular localization of tyrosine kinases. Thus, binding of geldanamycin to hsp90 interferes with the proper localization and folding of the oncoprotein. The improperly chaperoned tyrosine kinase is rapidly degraded, with loss of its signaling function. Interestingly, hsp90 forms complexes not only with tyrosine kinases, but a variety of other molecules important to tumorigenesis, including c-*raf* (an intermediary kinase in the MAP kinase signaling pathway), several steroid hormone receptors, and the nuclear oncoprotein, p53. Thus, geldanamycin can potentially interfere with several signaling systems at once by altering the normal association of these molecules with their chaperones.

Initial efforts to define antitumor activity on the part of geldanamycin met with, at best, limited success, as the drug was toxic at doses that afforded little or no efficacy. Efforts to develop approaches to treat large animals with geldanamycin failed due to hepatic toxicity. Consideration of several analogs led to 17-allylamino 17-demthoxygeldanamycin (17AAG; NSC 330507). This drug has shown consistent evidence of activity, eg, when administered to the MEXF 276 melanoma model, T/C (ratio of tumor weight in treated as compared with control animals) of 6% to 12% were

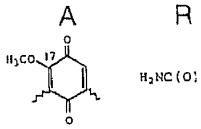


Herbimycin

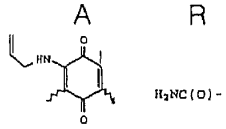


Geldanamycin Ring System

Fig 2. Geldanamycin, 17AAG, and herbimycin.



Geldanamycin
NSC 122750



17-allylamino, 17-demethoxy
geldanamycin(17AAG)
NSC 330507

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