

Cancer Chemotherapy Targeted at Reactivating the Expression of Epigenetically Inactivated Genes

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As we enter the era of molecularly targeted therapy, drug trials will take on a new face. Specifically, if a drug is designed to perform a specific biochemical action and that action should result in other specific effects, these effects can be objectively monitored in tumor tissue before and after such therapy as tumor response criteria. In this way, we will immediately know if the desired effects were achieved. If these biochemical effects are achieved and the tumor does not respond by standard criteria, then the trial is essentially over. If the biochemical effects are not achieved, then pharmacologic measures or other agents can be altered to achieve the desired effects. In the current issue of the *Journal of Clinical Oncology*, Chan et al¹ have reported an example of such an approach. This study is the first to demonstrate that treatment with azacitidine can produce demethylation of specific DNA sites in tumors. The investigators examined the methylation status of Epstein-Barr viral (EBV) promoters pre- and post-treatment with azacitidine in five patients with EBV-associated tumors (nasopharyngeal carcinoma and lymphoma), and demonstrated substantial demethylation in the target tissue of all examined viral promoters.

These viral promoters regulate the expression of viral proteins which engender strong cytotoxic T cell (CD8+) responses in seropositive individuals. The viral promoter regions are known to be methylated in tumors, resulting in a lack of viral gene expression and in turn allowing the tumor to escape from immune recognition. However, despite the changes in tumor cell viral promoter DNA methylation, Chan et al¹ found no significant changes in tumor expression of viral antigens, and no clinical responses were seen. In an analogous situation, tumor suppressor genes have undergone tumor-acquired promoter methylation in a variety of solid tumors leading to their loss of expression and function in tumors, resulting in the malignant pheno-

type. In fact, a large variety of tumors have multiple genes methylated, associated with a loss of gene expression.² Thus, the Chan study is a model for future trials in solid tumors in which the methylation status and re-expression of tumor suppressor genes are monitored. In preparation for this, many preclinical studies in the past 3 years have shown that in vitro tissue culture, human tumor cells treated with azacitidine or decitabine will show demethylation and subsequent re-expression of a variety of key tumor suppressor genes and other genes whose loss of expression is involved in cancer pathogenesis.^{2,3}

Azacitidine (5-azacitidine) and decitabine (5-aza-2'-deoxycytidine) were originally synthesized in 1964 by Piskala et al⁴ and Pliml et al,⁵ and are the two most extensively studied agents that prevent DNA methylation. Despite this long history, the optimal role for these agents in the treatment of malignancy remains to be defined. Numerous clinical trials have evaluated the efficacy of azacitidine in hematologic malignancies. As a single agent, it has demonstrated activity in both pediatric and adult relapsed or refractory acute myelogenous leukemia (AML).⁶⁻¹⁰ In combination chemotherapy, azacytidine has been effectively used as first-line therapy in AML.⁶ However, no adequately powered phase III studies have defined the utility of azacytidine in the treatment of AML.

Several phase II trials have also demonstrated that single-agent azacitidine has activity in myelodysplastic syndrome (MDS).^{11,12} Recently, the Cancer and Leukemia Group B reported the results of a phase III comparison of azacytidine and best supportive care in MDS. Azacitidine resulted in significantly superior response rates (60% v 5%), quality of life, and overall median survival (18 months v 11 months).¹³

A number of small phase II trials of single-agent azacitidine utilizing a variety of doses and schedules have also been performed in solid tumors, including breast, gastroin-

testinal, lung, kidney, testicular, melanoma, sarcoma, and non-Hodgkin's and Hodgkin's lymphomas. Although occasional responses have been observed, results are uniformly disappointing, and no further large-scale studies in solid tumors have been pursued.¹⁴⁻¹⁹

Similar results have been seen with decitabine. As a single agent, it has activity in refractory or relapsed adult AML and pediatric leukemias, as well as MDS.^{20,21} Unlike azacitidine, decitabine has also shown efficacy in chronic myelogenous leukemia in either the accelerated phase or blast crisis.²² In addition, decitabine has been studied in a wide variety of solid tumors, but unfortunately has been inactive.²³⁻²⁷

Despite years of clinical study with azacitidine and decitabine, the precise mechanism of action responsible for their antitumor effects remains unclear. While both agents prevent DNA methylation, they also have direct cytotoxic effects as a result of incorporation into DNA and RNA.²⁸ Few clinical studies have utilized molecular end points to evaluate the effectiveness of these drugs as inhibitors of methylation. In 1982, Ley et al²⁹ were able to show that treatment with azacitidine caused hypomethylation of the γ -globin gene and induction of fetal hemoglobin production in a patient with β -thalassemia. More recently, it has been shown that decitabine treatment produces a decrease in p15 methylation in the bone marrow of patients with MDS, which was associated with clinical response.²¹ However, a similar phase I study of a low-dose prolonged exposure to decitabine in patients with hematologic malignancies found no correlation between methylation of p15, before or after treatment, and clinical response.³⁰

In the Chan study,¹ post-treatment assessments of promoter methylation and tumor antigen expression were made within 72 hours of the last infusion of the first cycle of therapy. Given the limited understanding of the pharmacokinetics of this schedule of azacitidine, this may not represent the optimal time point and may not have allowed for evaluation of the effects of more protracted treatment with azacitidine. By design, determination of the methylation status of only a small number of EBV promoters was attempted. Were there methylation changes in other genes including tumor suppressor genes inactivated in the tumors as well as changes in genes inactivated by methylation as part of normal differentiation? Likewise, were such genes re-expressed? Future studies may utilize cDNA microarray to globally assess re-expression of previously silenced genes following treatment with DNA methylation inhibitors, and may provide a more comprehensive assessment of the effects of these agents. Lastly, while substantial promoter demethylation was induced by azacitidine in this study, expression of viral antigens and tumor response were not achieved. Given the long history of unimpressive clinical activity against solid tumors with these agents, it is likely that combination therapy will be required to achieve meaningful clinical response. There is substantial information

indicating that the continued role of DNA methylation coupled with histone deacetylation leads to a loss of gene expression in tumors.³ In this regard, acetylated histones provide for "opening" of DNA to facilitate transcription. Thus, a logical next step is to combine demethylation agents with histone deacetylase inhibitors to achieve gene re-expression. Preclinical data suggest that the combination of an inhibitor of DNA methylation and an inhibitor of histone deacetylase have synergistic effects on gene expression.^{2,3} The logical extension of this work are clinical trials of such combinations, and currently at least two phase I studies of combinations of these inhibitors are underway, both of which monitor gene re-expression in the target tumor tissues after treatment. In future trials, the approach used in the Chan study of assessing the effect in the target tissue—loss of methylation coupled with demonstration of protein re-expression—can likewise be used to monitor the response to treatment. As more targeted therapies for cancer are developed, molecular assessment of the desired effects represents new, highly-specific, important objective response criteria to add to classic measurements of tumor response.

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