Editorial

Can O^6-Alkylguanine-DNA Alkyltransferase Depletion Enhance Alkylator Activity in the Clinic?

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Resistance to chemotherapy remains an essential reason for the failure to cure patients with a diverse spectrum of malignancies. Malignant tumors, particularly those arising in adults, demonstrate a broad range of *de novo* or acquired mechanisms of resistance to virtually all chemotherapeutic agents in clinical use. Accordingly, efforts are under way in numerous laboratory and clinical programs to define these mechanisms of resistance and devise strategies to either reverse or bypass this resistance.

An extensive series of preclinical and clinical studies have demonstrated previously that the DNA repair protein AGT[2] is responsible for resistance to alkylnitrosoureas and methylating agents (1, 2). AGT removes chloroethylation or methylation damage from the O^6 position of DNA guanines before cell injury and death. The high incidence of AGT activity in virtually all human tumors, as well as recent clinical trials showing an inverse relationship between AGT levels and survival in patients with malignant glioma receiving BCNU therapy, provides the rationale for strategies designed to deplete tumor AGT levels before therapy with BCNU. O^6-BG is an AGT substrate that inactivates AGT and enhances alkylnitrosourea activity both *in vitro* and *in vivo*. Accordingly, clinical trials have now emerged that are designed to evaluate the maximum tolerated dose of alkylnitrosoureas or methylating agents given in combination with O^6-BG (3).

In this issue of *Clinical Cancer Research*, Schilsky *et al.* (4) report a Phase I trial for patients with histologically confirmed advanced solid tumors or lymphoma who have previously failed standard therapy and for whom no standard therapy was available. The patients were treated with O^6-BG as a 1-h i.v. infusion, and blood was collected for pharmacokinetic and pharmacodynamic studies. Patients were given a 2-week period for the drug effects to dissipate. They were then treated again with O^6-BG, followed 1 h later by BCNU. This trial, which was based on an extremely sound and rigorous foundation of detailed *in vitro* and *in vivo* studies as described above, is an important step in determining the toxicity of this combination. AGT is present in virtually all normal human tissues, and there has been concern in the scientific community that the toxicity of BCNU (and indeed any other nitrosourea or methylating agent) would be greatly enhanced when administered after O^6-BG-mediated depletion of AGT. This has proven to be the case because the BCNU dose that can safely be administered is 40 mg/m^2, approximately one-fifth of the dose given in the absence of AGT depletion. Dose-limiting toxicity was bone marrow suppression, which is not surprising in light of previous work demonstrating the role of AGT in protecting hematopoietic cells from nitrosoureas. Other toxicities seen were of less consequence.

An important lesson from this trial (4), aside from the actual determination of the maximum tolerated dose of BCNU in combination with O^6-BG, is the folly of seeking surrogate markers for demonstration of AGT suppression. This study (4) initially used quantitation of AGT in peripheral blood mononuclear cells as a marker for AGT depletion. Unfortunately, the study clearly showed that peripheral blood mononuclear cells cannot serve as an adequate surrogate for the assessment of biochemical events occurring in the tumors. These investigators (4) initially concluded that an O^6-BG dose was sufficient to completely suppress AGT activity; however, other investigators using extraneural or central nervous system tumors precisely demonstrate that higher doses of O^6-BG were necessary. In fact, those data, which were made available to Schilsky *et al.* (4), led to the use of higher doses of O^6-BG so that the study could define a more accurate dose of BCNU to use. It is clear that future studies using biochemical modulators such as O^6-BG or others will have to rely on actual tumor measurements in the absence of convincing data that surrogate tissues can be used (5).

Finally, the most critical question remains regarding the therapeutic gain that will be seen on administering O^6-BG plus BCNU in combination. The current Phase I trial (4) failed to demonstrate any complete or partial response, which is disappointing. Nevertheless, only classical Phase II trials of O^6-BG plus BCNU in patients who are clearly refractory to nitrosoureas and treated at the appropriate doses of BCNU and O^6-BG will provide the data necessary to determine whether BCNU resistance can be reversed. It is possible that we have merely lowered the dose of BCNU when given with O^6-BG due to toxicity and that the combination will not result in an increase in the therapeutic index. Studies in murine models using human tumors have been extremely positive, but there are differences in both the metabolism and kinetics of AGT depletion in mice *versus* humans. However, even if O^6-BG plus BCNU fails to reverse resistance in nitrosourea-resistant tumors because of either alternative mechanisms of resistance or the use of homeopathic doses of BCNU, other strategies may prove more successful, such as the use of regionally administered O^6-BG to minimize systemic AGT depletion. Trials are now in progress at the University of Chicago, Case Western University, Duke University, and Johns Hopkins University that are rigorously designed to demonstrate the benefits of O^6-BG plus BCNU in alkylnitrosourea-resistant tumors and may prove to be the first

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[2] The abbreviations used are: AGT, O^6-alkylguanine-DNA alkyltransferase; O^6-BG, O^6-benzylguanine; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.

demonstration of an effective strategy for chemotherapy-resistant neoplasms.

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