*Minireview*

# Sequential Tumor Biopsies in Early Phase Clinical Trials of Anticancer Agents for Pharmacodynamic Evaluation[1]

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## Abstract

***Purpose*: In the setting of target-based anticancer drug development, it is critical to establish that the observed preclinical activity can be attributed to modulation of the intended target in early phase trials in human subjects. This paradigm of target modulation allows us to determine a Phase II or III dose (optimal biochemical/biological modulatory dose) that may not necessarily be the maximum tolerated dose. A major obstacle to target-based (often cytostatic) drug development has been obtaining relevant tumor tissue during clinical trials of these novel agents for laboratory analysis of the putative marker of drug effect.**

***Experimental Design:* From 1989 to present, we have completed seven clinical trials in which the end point was a biochemical or biological modulatory dose in human tumor tissues (not surrogate tissue). Eligibility enrollment required that patients have a biopsiable lesion either with computerized tomography (CT) guidance or direct visualization and consent to sequential (pre and posttreatment) biopsies.**

***Results:* A total of 192 biopsies were performed in 107 patients. All but 8 patients had sequential pre and posttreatment biopsies. Seventy-eight (73%) of the 107 patients had liver lesion biopsies. In eight patients, either one or both biopsies contained insufficient viable tumor tissue or no tumor tissue at all for analysis. Of a total of 99 patients in whom we attempted to obtain paired biopsies, a total of 87 (88%) were successful. Reasons for failure included patient refusal for a second biopsy ($n = 2$), vasovagal reaction with first biopsy precluding a second biopsy ($n = 1$), subcapsular hepatic bleeding ($n = 1$), and most commonly obtaining necrotic tumor, fibrous, or normal tissue in one of the two sequential biopsies ($n = 8$).**

***Conclusions*: This is the first and largest reported series demonstrating that with adequate precautions and experience, sequential tumor biopsies are feasible and safe during early phase clinical trials.**

## Introduction

The discovery of a plethora of target-based antineoplastic compounds has opened up an era of new opportunities and extraordinary challenges in drug development. Although the molecular or cellular target for many of these new agents has been defined *in vitro*, little data exists to demonstrate their biological relevance in patients. In this era of rational drug design of target-based anticancer drugs, it is critical to establish that the observed preclinical activity can be attributed to modulation of the target. In particular, it is desirable to demonstrate effect in humans during early drug development. This paradigm of target modulation allows us to determine a Phase II or III dose that may not necessarily be the MTD[3] (1, 2). The optimal biochemical/biological modulatory dose would thus be determined based on the relevant target inhibition (3). The main obstacle to target-based (often cytostatic) drug development has been 2-fold: (*a*) obtaining relevant tumor tissue during clinical trials of these novel agents for laboratory analysis of the putative marker of drug effect; and (*b*) validating the laboratory assay and demonstrating that it is feasible, reproducible, and correlates with the intended drug effect. This report outlines our experience with sequential tumor biopsies, at CWRU, in target-based drug development trials.

## Patients and Methods

From 1989 to present, we have completed seven clinical trials in which the end point was a biochemical or biological modulatory dose in human tumor tissues (Refs. 3–9; Table 1). Eligibility enrollment required that patients have a biopsiable lesion either with CT guidance or direct visualization of skin lesions and consent to sequential biopsies. Biopsiable lesions that were considered included hepatic metastases and pelvic tumors, all of which were accessible by CT guidance; CT or directly accessible lymph nodes; and superficial involvement of the head and neck, chest wall, or skin, which were accessible percutaneously or by CT/magnetic resonance guidance. Before accrual, all imaging studies were reviewed by our interventional radiologist to determine the safety and accessibility of the lesion. Hypervascular lesions were excluded by performing a bolus injection of contrast material with repet-

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[3] The abbreviations used are: MTD, maximum tolerated dose; CWRU, Case Western Reserve University; CT, computerized tomography; AGT, alkylguanine DNA alkyltransferase; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; BG, Benzylguanine.

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*Table 1* Completed trials at CWRU using sequential CT-guided biopsies of solid tumors for Phase I/II drug development

| Study | No. pts[a] on trial (requiring biopsy) | No. pts having biopsies | No. of biopsies | Location of biopsies | Primary tumor | Pharmacodynamic end point | Success rate for obtaining paired biopsies containing tumor |
|---|---|---|---|---|---|---|---|
| Phase I trial of tiazofurin and BCNU (Ref. 6) | 21 (ns) | 10 | 18 | Liver 4<br>LN 4<br>Abdo wall 1<br>Subcut 1 | Colorectal 8<br>Melanoma 1<br>SCLC 1 | Depletion of nicotinamide and ATP with tiazofurin. No correlation was seen. | 7 of 10<br>3 pts with either normal or necrotic tissue on one of the two biopsies |
| Phase I trial of topotecan as 72-h weekly infusion (Ref. 8) | 22 (ns) | 8 | 8 (pretreatment only) | Liver 4<br>LN 3<br>Pelvic mass 1 | Colorectal 8 | No correlation between pretreatment topoisomerase levels and response was seen. | 6 of 8<br>2 fibrotic specimens |
| Phase I trial of streptozotocin and BCNU (Ref. 7) | 15 (10 requiring biopsy) | 10 | 19 | Liver 9<br>LN 1 | Colon 10 | Streptozotocin decreased alkyltransferase levels but incompletely. | 9 of 10<br>1 vasovagal reaction |
| Phase I trial of $O^6$BG and BCNU (Ref. 3) | 53 (38 requiring biopsy) | 38 | 62 | Liver 28<br>Breast 3<br>LN 2<br>Lung 1<br>Abd. mass 2<br>Cheat wall 1<br>Spleen 1 | Breast 5<br>Colorectal 22<br>Lungs 2<br>Renal 1<br>Gastric 3<br>Sinus 1<br>UNK pr. 2<br>Pancreas 1<br>Soft tissue sarcoma 1 | Increasing doses of $O^6$-benzylguanine resulted in total depletion of alkyltransferase. | 31 of 38<br>5 necrotic tissue<br>1 caps hemorrhage<br>1 pt refused second biopsy |
| Phase I trial of temozolomide (Ref. 4) | 22 (16 requiring biopsy) | 16 | 31 | Liver 15<br>LN 1 | Colorectal 8<br>Sinus 1<br>Pancreas 2<br>Melanoma 1<br>Small int 1<br>Breast 2<br>Unk pr 1 | Temozolamide depletion of alkyltransferase was demonstrated but with residual alkyltransferase activity. | 15 of 16<br>1 pt refused second biopsy |
| Phase I trial of paclitaxel and concurrent radiation in head and neck cancer (Ref. 5) | 24 (ns) | 4 | 12 (3 each) | H + N Cancer 4 | H + N Cancer 4 | Correlation of paclitaxel dose and mitotic arrest was seen. | 4 of 4 |
| Phase II trial of irinotecan in colon cancer (Ref. 9) | 21 (all require biopsy) | 21 | 42 | Liver 18<br>Pelvic mass 2<br>LN 1 | Colon 21 | Determination of topoisomerase levels as predictors of response to irinotecan. Results pending. | 21 of 21 |
| Total | — | 107 | 192 | Liver 78<br>LN 12<br>Pelvic/abdo mass 5<br>H + N 4<br>Breast 3<br>Chest/abdo wall 2<br>s.c. 1<br>Splenic 1<br>Lung 1 | Colorectal 77<br>Breast 7<br>H + N 4<br>Pancreas 3<br>Unknown primary 3<br>Gastric 3<br>Melanoma 2<br>Lung 2<br>Sinus 2<br>Renal 1<br>SCLC 1<br>Small intestinal 1<br>Soft tissue sarcoma 1 | | 87 of 99 (88%)<br>(excluding the 8 pts who were planned to receive pretreatment biopsy only) |

[a] Pt, patient; ns, not stated in protocol; SCLC, small cell lung cancer.

itive scans over the site of tumor target. Those lesions that demonstrated hypervascularity relative to adjacent tissue were excluded. A total of 107 patients were biopsied in these seven trials. One trial required biopsies of all patients. In another trial, biopsies were required of the first 38 enrolled patients to determine the biochemical modulatory dose of the investigational agent; additional enrollment then proceeded without biopsies. In the other five trials, sequential tumor biopsy was performed in a limited number of patients at or around the MTD.

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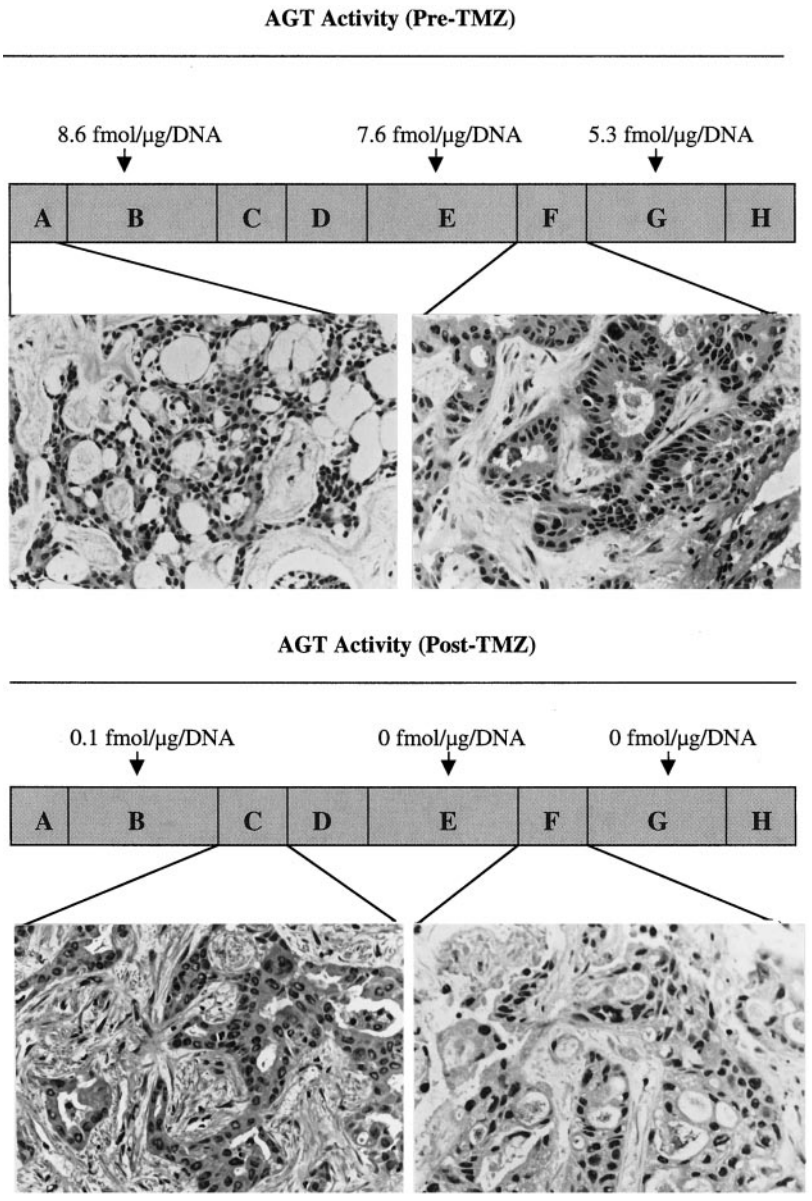


*Fig. 1* AGT activity in a biopsy from a liver metastasis in a colon cancer patient. *A*, pre-temozolomide (*TMZ*) administration; *B*, post-TMZ treatment. Temozolomide causes total depletion of AGT activity.

**Method of Biopsy and Determination of Tumor Presence.** All patients were monitored for 24 h after biopsy in the General Clinical Research Center. The 14-gauge cutting needle that we use produces a 5- to 10-mm long 1.5-mm cylindrical core that yields an average of 20–30 mg of core tissue (Fig. 1). We have demonstrated previously that whereas most institutions use 18-guage needles for routine biopsies, 14-guage needles result in significantly higher yield of tumor tissue without a significant increase in complications. By targeting the outer rim of tumor deposits, rather than the central portion of the tumor, necrotic areas of tumor are mostly avoided. The tissue core is immediately frozen in liquid nitrogen and later carefully labeled and divided into multiple sections for alternate determination of the relevant biological parameter and tissue histology. We have developed a method by which the core biopsy sample is divided into 4–11 sections. The tumor samples obtained by CT-guided or percutaneous cutting needle biopsies are frozen immediately in liquid nitrogen. They are then directly transferred to the laboratory where the core biopsy sample is divided on a metal plate kept cold with dry ice. The tissue section immediately adjacent to a biopsy portion used for biochemical analysis is examined by light microscopy. As shown in Fig. 1, histological sections are required to select the tumor-containing portion of the biopsy for determination of the measured biological end point. In this sample, sections A and F were analyzed histologically to show tumor presence. All molecular and biochemical assays were performed on the piece of tissue inward and immediately adjacent to sections A and F, meaning sections B and E. This guaranteed that the assays were done on nonnecrotic tumor tissue. If tumor was not documented on sections A and F, the

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analyses were not performed on sections B and E. In cases where immunohistochemistry was also being performed, section D was used for that purpose. Section C was analyzed histologically, additionally confirming that sections B, D, and E contained viable tumor tissue.

**Validation of Assays from *in Vitro* Models to Xenografts to the Clinic.** Our experience with the clinical development of *O*-6-benzylguanine and the biochemical modulation of AGT is illustrative of vigorous target assay validation in conjunction with a Phase I trial (3). We have also developed a similar strategy in the clinical development of topoisomerase I inhibitors in early phase trials of these agents (9, 10). Part of the validation process for the assays being used requires demonstration of reproducibility in cell lines and human tumor xenograft models, as well as observation of the changes in the assays with the experimental agent. In addition, for our ongoing studies at CWRU, we attempt to perform the assays on human tumor tissue obtained from the tissue procurement center to demonstrate feasibility and reproducibility before initiating trials in our patients.

AGT is a repair enzyme that repairs alkyl adducts at the O6 position of guanine. Each AGT molecule removes one adduct through covalent binding of the alkyl group to the cysteine residue at the amino acid number 145 (11, 12). During this process, irreversible inactivation of the protein occurs, and synthesis of new molecules is required to regenerate AGT activity. The "suicide protein" properties of AGT make it a unique target for biochemical modulation. *In vitro* and human xenograft studies have shown a close correlation between AGT depletion and enhancement of alkylating agent activity (13). AGT depletion for 12–18 h is needed for the enhancement of response (14, 15). We and others have demonstrated increased AGT activity in many solid tumors (16–19). Human tumor xenografts with high AGT activity are similarly resistant to BCNU (20, 21). BG is a potent AGT-inactivating agent (22). In cell lines, as well as human tumor xenografts models, sequential BG and BCNU administration caused significant tumor inhibition (23, 24). Methodologies used for AGT assessment have included an enzyme assay requiring 50–250 μg of protein of tumor tissue. This technique has been tested in *in vitro* tumor assays as well as xenograft models. Modulation of AGT by BG has been shown using this assay in both cell lines and xenografts, and AGT depletion correlates with BCNU activity. We thus demonstrated in a Phase I dose-escalation trial, with a biochemical end point, that BG at a dose of 120 mg/m$^2$ completely depletes AGT in tumor tissue with biochemical assay performed (3). Patients had pre- and post-BG tumor measurements in this study. Interestingly, the use of peripheral blood mononuclear cells to measure AGT modulation by BG fails to predict its depletion in tumors (3). We have similar experience with the development of tumor measurements of topoisomerase I and II (9, 10). We have demonstrated correlation between topoisomerase I levels and cytotoxicity to irinotecan and topotecan in both *in vitro* and xenograft models. The assay was extensively tested and validated and later taken to our early phase trials of these agents with sequential tumor biopsies.

## Results

A total of 192 biopsies were performed in 107 patients (Table 1). All but 8 patients had sequential pre and posttreatment biopsies. Seventy-eight (73%) of the 107 patients had liver lesion biopsies. Twelve patients had lymph-node biopsies of which 8 were under CT guidance, and the remaining 4 were under direct surgical visualization. Pelvic and intra-abdominal lesions were biopsied in 5 patients. In one trial, patients with head and neck cancer were biopsied. Breast, chest/abdominal wall, s.c. tumor, spleen, and lung comprised the remaining sites of biopsy.

**Complications, Tolerability, and Success Rate for Obtaining Paired Tumor Biopsies.** With careful patient selection, including exclusion of patients with any abnormality on coagulation profile, the stopping of any medication that may alter platelet function, baseline CT dynamic scan to exclude highly vascularized lesions, access to the General Clinical Research Center for close postbiopsy patient monitoring, and coordination by our research nurses, we have not encountered any significant complications from these procedures. One patient had minor vaso-vagal reactions during the CT-guided liver biopsies. Twelve patients experienced local pain at the site of biopsy for several h, relieved by simple analgesics. One episode of subcapsular hemorrhage occurred on a CT-guided liver biopsy, which did not require additional intervention but which precluded a second biopsy. In several patients, either one or both biopsies contained insufficient viable tumor tissue or no tumor tissue at all for analysis. Of a total of 99 patients in whom we attempted to obtain paired biopsies (before and after treatment), a total of 87 (88%) were successful. Reasons for failure included patient refusal for a second biopsy ($n = 2$), vasovagal reaction with first biopsy precluding a second biopsy ($n = 1$), subcapsular hepatic bleeding ($n = 1$), and most commonly obtaining necrotic tumor, fibrous, or normal tissue in one of the two sequential biopsies ($n = 8$).

## Discussion

The key end point in Phase I trials for targeted therapies should evolve from the current idea of a MTD in normal tissue to a more suitable end point of the dose required to maximally inhibit the relevant target in tumor tissue. Measures of target inhibition (preferably in tumor tissue) may be a more relevant end point for Phase I trials where optimal dosing is the goal.

Peripheral blood mononuclear cells, which are the most accessible tissue and possess many receptors and signaling pathways, have been used frequently over the past decade. Demonstrating a "biologically effective dose" in peripheral blood mononuclear cells has been suggested as a method of guiding dose escalation in Phase I trials. However, the use of blood cells may not be relevant to what is occurring in the tumor, as we have demonstrated in our Phase I trial of *O*-6-benzylguanine. At the very least, if blood cells are to be used to evaluate the magnitude of target inhibition, there ought to be evidence from preclinical models that target inhibition in blood cells is a valid marker for efficacy. This is often not the case; furthermore, species specificity of molecular inhibitors may make this type of assessment difficult. No data to date has shown that modulation of a target in peripheral blood cells

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*Table 2* Ongoing Phase I/II trials of solid tumors at CWRU requiring sequential tumor biopsies for pharmacodynamic end points

| Trial | Tumor type | Pharmacodynamic end points |
|---|---|---|
| Phase I pharmacodynamic trial of SU5416 | Advanced solid tumors | Microvessel density, apoptotic rate, and proliferative rate |
| Phase Ib/II trial of SU5416 and doxorubicin in inflammatory breast cancer | Inflammatory breast cancer | Microvessel density, apoptotic rate, and proliferative rate |
| Phase Ib/II trial of SU5416 and paclitaxel in head and neck cancer | Head and neck cancer | Microvessel density, apoptotic rate, and proliferative rate |
| Phase I trial of fenretinide, cisplatin, and paclitaxel | Advanced solid tumors | Apoptosis, retinoid receptor function, and TIG3 expression |
| Phase II trial of $O^6$BG and BCNU in malignant melanoma | Malignant melanoma | Depletion of alkylguanine DNA alkyltransferase |
| Phase I trial of CI-1033 | Colon cancer | Expression and activation of the epidermal growth factor receptor, ERK1/ERK2,[a] and AKT pathways |

[a] ERK, extracellular signal-regulated kinase.

predicts the same modulation in tumors. Therefore, obtaining tumor would be the optimal tissue to demonstrate a biological effect. Indeed, many current Phase I trials of anticancer agents with variable mechanisms of action are using tumor biopsies for pharmacodynamic studies. Table 2 illustrates the ongoing trials at our institution requiring sequential tumor biopsies.

At CWRU, we have centered our drug development program around obtaining tumor tissue during Phase I drug development. We have demonstrated in a significant number of patients that with suitable precautions and experience, tumor tissue can safely be obtained under CT guidance. Careful patient selection in terms of biopsy-accessible tumors and close collaboration with our interventional radiologist allows a high success rate of obtaining paired tumor tissue samples, pre and posttreatment. Although many different tumor types can and should be evaluated to determine the heterogeneity of the end point in different histologies, colon cancer with liver metastases, in our hands, is the most accessible for this approach. One other approach to obtaining tumor tissue, currently being investigated by us and others, has been the preoperative administration of drug and subsequent analysis of the drug concentration and target activity in the tumor at timed, planned surgical intervention (*e.g.*, palliative nephrectomy, craniotomy, etc.). Many tumor types are managed with preoperative biopsies (control sample); for these cases, a comparison with nondrug-treated controls is possible.

The use of a 14-guage needle is of importance to obtain a sufficient amount of tissue and to maintain histological architecture. Most institutions use an 18-guage needle for routine CT-guided biopsies. Our group had performed a study previously of 190 sequential liver biopsies in Yorkshire pigs using 14-, 18-, and 20-guage needles (25). This study showed that 14-guage needles recovers an average DNA content per sample of 40.38 micrograms *versus* only 12.18 micrograms for 18-guage needles. The ratio of blood loss to amount of DNA recovered did not differ among the different caliber needles. Furthermore, we had also demonstrated previously that 14-guage Tru-Cut needles result in preservation of nodal tissue architecture in cases of lymphoma (26). The maintenance of tissue architecture may be important when the pharmacodynamic end point uses an immunohistochemical technique.

The validation of the biological end point and assay is critical before commencing this approach with human subjects. Too often, laboratory tests are being done blindly during these trials. We have demonstrated that the paradigm of incorporating preclinical data from i*n vitro* studies to xenografts and then to human subjects is feasible. Careful review and optimal use of preclinical studies are mandatory to the development of early clinical trials with a biological/biochemical end point. The target measured, the type of assay, and the timing of the assay to be done (time to obtain tumor tissue) should all be based on relevant preclinical data (*e.g.*, depletion of AGT occurs within the first few h, and therefore, the second biopsy is performed within the first 20 h of drug delivery). In contrast, the effects of SU5416 have been demonstrated to occur within weeks (27), and therefore, the timing of the second biopsy is being performed at 2 months in our ongoing study of this agent. Validation of assays to be performed on the core biopsy sample remains a challenge. Indeed, many assays fail to demonstrate acceptable coefficients of variability. In addition, for many assays, no "range" has been determined. Specifically, it is unclear how these values may vary among different tumor types, as well as different histologies. Variations may also exist between metastatic and primary tumor sites.

In our studies, there was clear consent presentation of the biopsies. Physicians, nurses, and radiologists were all intimately involved with safety issues. Furthermore, before opening each study, our Phase I group evaluated the indication for the biopsies and made sure there was scientific rationale for the procedure. Patients generally accepted the procedure knowing that it would benefit the scientific community.

The are many challenges to successfully incorporating tumor tissue analysis into the design of a clinical trial. Overcoming these challenges requires the commitment of oncologists, interventional radiologists, pathologists, laboratory scientists, human subject protection committees, and patients; substantial additional funding; and standardization of tissue sampling to ensure the tumor is being measured. Nevertheless, once done, all subsequent trials and drug development would benefit from the knowledge that the recommended dose was correct and inhibited the target for which it was designed.

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