Toward better early-phase brain tumor clinical trials: A reappraisal of current methods and proposals for future strategies[1]

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Although no optimal treatment is currently available for malignant brain tumors, as the molecular mechanisms underlying brain tumor development have been delineated, new chemotherapeutic agents that act directly on specific molecular targets have become available. Defining a specific molecular target raises the possibility that the molecular effects of a given agent can be analyzed in patients in a clinical trial. Specifically, whereas standard phase I and II clinical trials classically determine the safety and efficacy of agents by using indirect global end points, these new biological agents afford the opportunity to incorporate molecular end points into phase I and II clinical trials to determine whether the agent under investigation is actually doing what it was intended to do. This work presents avenues for improving current brain tumor clinical trial designs based on the molecular specificity of new agents and the unique features of brain tumors. Specifically, the authors recommend brain-applicable phase I and II clinical trial strategies that take advantage of the targeted nature of new agents to maximize information about their efficacy, toxicity, and molecular effects.
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[3]Abbreviations used are as follows: ABD, appropriate biological dose; MTD, maximum tolerated dose; PDGFR, platelet-derived growth factor receptor.

The outcomes of cases of malignant brain tumor have changed little during the past 30 years. For glioblastoma multiforme, the most common primary brain tumor, the median survival with maximal therapy is 1 year, and only 15% of patients are alive 2 years after diagnosis (Walker et al., 1985). However, in the past decade, major advances have been made in elucidating the molecular mechanisms underlying brain tumor formation and progression (Hanahan and Weinberg, 2000; Kleihues and Cavenee, 2000). As a consequence of this new information, a variety of molecular pathways have been identified as potential targets for therapy, and new anticancer agents that act directly on these specific tumor-related molecular pathways are becoming increasingly available (Table 1). Compared with the "classic" cytotoxic chemotherapeutic drugs (e.g., carmustine and cisplatin), the modes of action of which are largely nonspecific, these newer "molecular" or "biological" agents specifically activate or, more commonly, inhibit proteins that contribute to the malignant phenotype of brain tumors. Because they attack the underlying cause of the disease, there is great optimism in the field of neuro-oncology that these molecularly targeted approaches will have a significant impact on the survival of patients with gliomas.

For this potential to be realized, it is incumbent upon clinical investigators to carefully consider the design of early-phase clinical trials. Specifically, whereas standard phase I and II clinical trials classically determine the safety and efficacy of agents by using indirect global end points, these new biological agents provide the opportunity to incorporate molecular end points into phase I and II clinical trials to determine whether the agent under investigation is actually doing what it was intended to do (Dowlati et al., 2001; Eisenhauer, 1998; Gelmon et al., 1999).

Although several authors have outlined the problems inherent in incorporating molecular analyses into the evaluation of the toxicity and efficacy of these new targeted agents in the clinical setting, the complexity of

Table 1. Molecular targets in glioma

	Molecular alteration	Literature	Compound
Growth factors	↑ PDGF/PDGFR	Guha et al., 1995	STI 571 (Novartis)
			PTK 787 (Novartis)
	↑ EGF/EGFR	Feldkamp et al., 1999; Bigner et al., 1990;	PK1-166 (Novartis)
		Libermann et al., 1985; Nagane et al., 1996;	ZD1839 (AstraZeneca)
		Mishima et al., 2001	C225Ab (Imclone)
Intracellular circuits	↑ Ras	Holland et al., 2000; Guha et al., 1997;	SCH 66336 (Schering Plough)
		Ding et al., 2001; Sonoda et al., 2001a	R115777 (Jansen)
			Ad-Y28 (Introgen)
Cell-cycle control	↓ P16/P15/CDK/RB	Henson et al., 1994; James et al., 1988;	Delta 24 (UT MDACC)
	pathway	Ichimura et al., 1996; Ueki et al., 1996	
Apoptosis	↓ P53	Frankel et al., 1992; Lang et al., 1994;	Ad-p53 (Introgen)
		Nigro et al., 1989; Bogler et al., 1999;	ONYX-015 (ONYX)
		Merritt et al., 2001	CP31398 (Pfizer)
	↑ Mdm2	Reifenberger et al., 1993	—
Survival factors	↑ PI3 kinase/AKT/PTEN	Holland et al., 2000; Sano et al., 1999;	Ad-PTEN (Canji)
		Davies et al., 1998; Steck et al., 1997;	RAD001 (Novartis)
		Smith et al., 2001; Sonoda et al., 2001a, 2001b	
Angiogenesis	↑ VEGF/VEGFR	Ke et al., 2000; Mendel et al., 2000	SU6668 (Sugen)
Invasion	↑ Matrix metalloproteases		Marimastat (Schering Plough)

brain tumors warrants particular attention (Eisenhauer, 1998; Gelmon et al., 1999; Mauro et al., 2002). The purpose of this article is to provide perspective on this challenge and to suggest changes in current early-phase brain tumor clinical trial designs. Specifically, we propose several brain-applicable phase I and phase II trial designs that incorporate molecular end points while also permitting more standard assessments of toxicity and efficacy.

Clinical Trial End Points: Systemic Versus Molecular

When considered from the perspective of tumor biology, the end points of classic phase I and phase II clinical trials are indirect. These indirect measures traditionally provide a valid assessment of cytotoxic chemotherapeutic agents because the therapeutic targets of these agents are ill-defined. Thus, classic phase I trials determine the optimal therapeutic dose of an anticancer agent by identifying the MTD[3] using an acceptable level of systemic or neurologic toxicity as the end point. This trial design is based on the premise that there usually is a positive correlation between higher doses of cytotoxic agents and tumor kill. Although chronic dosing with lower drug doses may be beneficial in tumors with low replication rates, these types of drug schedules are typically discovered well after initial high-dose toxicity monitoring. Traditional phase I trials generally do not include determinations of whether the "optimal" dose actually alters the biology of the tumor or whether this dose results in adequate drug levels within the tumor. Indeed, for gliomas where the blood-brain barrier might be a significant obstacle to drug delivery, these types of tumor measurements may be useful.

Similarly, classic phase II trials generally determine drug efficacy by assessing tumor response based on measures of tumor regression—evaluating decreases in the size of the mass (or sometimes stabilization of a progressing mass) as determined by CT or MRI radiographic studies. Agents that produce a radiographic response are considered effective, whereas those that do not are abandoned. For cytotoxic drugs, such an assessment is often adequate, as tumor regression has unsurprisingly been shown to correlate with prolonged survival (Eisenhauer, 1998). However, these indirect radiographic end points provide no information regarding specific cellular effects of the agent on the tumor or the extent of the changes that occur within the tumor. Moreover, for tumors that do not demonstrate a radiographic response, these indirect end points provide no insight into the cause of failure: Was the problem with drug delivery? Did the drug not exert its desired molecular effect? Or, did the molecular effect not influence tumor growth as intended?

Table 1 outlines some of the newer biological agents that are currently available for clinical testing. In contrast to cytotoxic agents, each of these agents typically attacks a specific molecular target and the effects of the agent can be determined at the molecular level. This suggests that the end points of clinical trials evaluating the new targeted agents can be direct: the specific molecular effect of the agent on the tumor can be measured (Dowlati et al., 2001; Eisenhauer, 1998; Gelmon et al., 1999; Mauro et al., 2002; Spiro et al., 1999; Szende et al., 2001; Plecha et al., 1997). For example, in the evaluation of receptor tyrosine kinase inhibitors (such as STI571), direct measurements of receptor activity (such as PDGFR phosphorylation) within tumor specimens can be made. These measurements of the biological effect of an agent can be

used to augment determinations of appropriate doses and assessments of efficacy. Thus, for phase I trials, measuring drug-induced alterations in the molecular target may be used to determine the most efficacious dose and schedule for a drug directly (Eisenhauer, 1998; Gelmon et al., 1999). Rather than determine appropriate doses based on indirect measures of systemic toxicity, direct measures of the dose that alter the molecular target can be made. Similarly, for phase II trials, efficacy can be determined by directly measuring alterations in molecular end points in conjunction with indirectly assessing radiographic response.

Value of Molecular End Points

Direct measurements of molecular end points provide several advantages over the more indirect assessments. First, the goal of classic phase I trials is to identify the MTD, the dose that produces clinically acceptable toxicity given the incurable nature of the disease. In contrast, incorporating molecular end points would allow for assessments of whether a particular dose of a drug actually alters the tumor biology—the end point that is most critical to therapy. Drug doses can be increased until the desired molecular effect is achieved. Thus, phase I trials can determine what has been referred to as the *appropriate biological dose* (ABD), the *optimal biological dose* (OBD), or the *maximal target inhibitory dose* (MTID) (Eisenhauer et al., 1998). As pointed out by several investigators (Dowlati et al., 2001; Eisenhauer et al., 1998; Gelmon et al., 1999; Spiro et al., 1999), the ABD may be most suitable for targeted molecular anticancer agents because molecular alterations may occur well below the MTD. This is important because it may not be necessary to push a drug to toxicity so long as the molecular target is affected. Of course, defining the ABD raises its own concerns. Indeed, determining the amount of inhibition (or activation) of a molecular target that is required for a biologically relevant response may not be a trivial undertaking. Moreover, the level or activity of a particular target may vary from tumor to tumor, and the drug dose that achieves 100% inhibition (or some other suitable percentage of inhibition) may also vary. Thus, the ABD may best be viewed as the highest dose among those that cause the desired degree of target inhibition.

Second, because the molecular pathways subserving gliomagenesis are capable of cross talk and/or overlap with each other, it is unlikely that an agent that interferes with only one pathway will produce a measurable decrease in tumor cell proliferation (Hanahan and Weinberg, 2000). Consequently, in phase II trials, many of these highly specific biological agents will likely produce little effect on tumor growth when given alone (Eisenhauer, 1998; Gelmon et al., 1999; Hanahan and Weinberg, 2000). Consequently, many of these agents will be judged ineffective based on standard radiographic response criteria. In other words, for many of these targeted agents, the response of a tumor to the agent may be evident only as a molecular change. Therefore, the identification of effective anticancer agents may rest strictly on determining whether the drug hits its molecular target. Agents that produce molecular responses become good candidates for further investigation, whereas agents that do not are excluded. Taking a molecular approach may avoid prematurely eliminating agents that do not meet traditional response criteria but that effectively alter an important molecular pathway underlying tumor growth.

In addition, proving that an agent can alter its targeted molecular pathway is the cornerstone of molecular-based therapy. Specifically, it is becoming increasingly possible to characterize the molecular changes that occur within individual brain tumors, and indeed, it is likely that tumors will be classified by their molecular profile in addition to descriptive histopathology (Louis et al., 2001). In this context, the development of targeted agents affords the opportunity to customize or tailor treatments for individual patients based on the molecular profile of their specific brain tumors. The common practice of using anticancer drugs for all tumors within a histologic type or across types will become increasingly less appealing as the ability to define particular molecular changes increases and the armamentarium of specific agents is augmented. In this context, the success of a molecular-based therapeutic approach rests on proving that an anticancer agent is capable of altering the molecular target against which it was designed. Obtaining such a proof will require that clinical trials of brain tumor therapies incorporate measurements of the molecular effects of the agent on the tumor (Dowlati et al., 2001; Eisenhauer, 1998; Gelmon et al., 1999; Spiro et al., 1999; Szende et al., 2001; Plecha et al., 1997).

Finally, incorporating molecular targets may also improve clinical evaluations of cytotoxic agents. Critical to this approach is the concept that many cytotoxic drugs are most effective when they induce apoptosis (rather than other less sensitive forms of cell death, such as necrosis) within tumor cells. Measuring and quantifying a molecular change such as apoptosis during clinical trials of cytotoxic agents may help to eliminate trial design biases that plague early-phase trials (Gilbert, 2000).

The Challenge

The challenge of phase I brain tumor clinical trials of these new targeted agents is to determine how best to incorporate assessments of molecular end points to evaluate drug dosing, while also determining the safety profile of the compound. For phase II trials, the challenge is to determine the efficacy of the agent when given at the optimal biological dose by defining the relationship between tumor response and changes in its molecular targets. This correlation will be required to fully determine the activity of a new agent and its potential use in combination with other agents. Although awareness has increased for the need to integrate molecular analyses of tumor specimens into classic phase I and phase II trials, the incorporation of these end points into brain tumor trials has lagged behind other tumor types (Dowlati et al., 2001; Eisenhauer, 1998; Gelmon et al., 1999; Mendel et al., 2000; Plecha et al., 1997; Sabiers et al., 1993; Spiro et al., 1999; Szende et al., 2001). Understanding the requirements of these types of studies and the impact on trial design may

help to speed the development of molecular-based clinical trials for brain tumors.

Requirements for Molecular Clinical Trial and Questions of Design

Tissue Acquisition

Clinical trials with molecular end points require tumor tissue for analysis. Although noninvasive methods for assessing biological effects, such as PET or MRI of molecular probes (Gelmon et al., 1999), are being developed, noninvasive methods of assessment at the molecular level remain elusive. Once developed, these techniques will require validation through tissue analysis. Use of surrogate tissues, such as peripheral leukocytes or skin biopsies (Gelmon et al., 1999), may not reflect the true effect of the agent on the target tissue. This consideration is particularly important for assessing brain tumors in which the blood-brain barrier or blood-tumor barrier, as well as other issues of drug delivery, may significantly alter the pharmacology of tumor tissue. Tissue sampling after drug treatment should be incorporated into clinical protocols if molecular end points are to be assessed optimally . The most direct approach incorporates preoperative administration of drug followed by determinations of drug levels and target effects in the tumor (Friedman et al., 1998; Gelmon et al., 1999). Ultimately, optimization of dosing and tissue acquisition will need to be determined for each agent used.

Brain tumor tissue acquisition is a significant hurdle given the potential complications associated with invasive brain procedures. Tissue can be acquired either by stereotactic biopsy or open craniotomy. Taking serial biopsies before and after treatment is most desirable (Dowlati et al., 2001; Plecha et al., 1997; Szende et al., 2001), but may not be feasible for many brain tumor trials. Another potential limitation is the typically small amount of tissue obtained by needle biopsies, which may not provide an adequate sample for analysis given the heterogeneity of gliomas. For example, the nonenhancing invasive portion of a glioblastoma multiforme may have a different molecular response to therapy than does the enhancing solid tumor portion (Plecha et al., 1997). Although schemes can be devised to sample the various parts of a tumor, another solution for obtaining brain tumor tissue is to perform resections that maintain the histologic integrity of the specimen. In our experience, this is best achieved with en bloc resection techniques in which tumors are removed by circumferential dissection around the enhancing mass rather than by "inside-out" techniques. The en bloc approach provides a large amount of tissue for analysis and preserves the tumor architecture such that histologic variations within a tumor can be visualized and specific areas of tumor can be selected for analyzing drug effects.

Because acquiring a large intact specimen requires a craniotomy, research designs that incorporate craniotomy into the drug evaluation are needed (Friedman et al., 1998). Eligibility criteria for many phase I and phase II trials must include patients who have surgically accessible lesions. Incorporating surgical resection into the enrollment criteria of clinical trials is a new paradigm. Indeed, most trials do not mandate, or even consider, surgical resection in their design; this new approach must become commonplace in the future. A potential problem relates to drug administration before surgery, possibly increasing drug-related intra- or postoperative complications. Effects on coagulation and immune responses are some of the critical concerns. Likewise, adverse systemic effects of the drug (e.g., cardiac and respiratory effects) must be considered prior to anesthesia administration. For scientific validity, the timing of drug administration relative to tumor acquisition must also be carefully appraised in light of drug pharmacokinetics.

Molecular Assays

The paradigm of serially sampling tissue during clinical trials mandates knowing what molecular end points are to be assessed. Obviously, but most importantly, a validated assay must exist to assess the targeted molecular function. Determining the best assay(s) to delineate molecular function may be the most critical aspect of new trial designs. Even when the molecular action of a drug is known, it can be challenging to develop a clinically applicable assay of drug function. The molecular effects of treatments are often enhanced with in vitro systems and may not translate into assays that are applicable to tissue obtained in clinical trials. For example, assessments of receptor tyrosine kinase inhibitors (such as STI 571) may require determining the amount of phosphorylation of the targeted receptor (such as PDGFR). These assays are often more complex than those typically performed in most clinical laboratories. Thus, coordinating clinical trials with specialized research laboratories is required to complete quality tissue assays.

The definition of a *significant molecular effect* must be determined before embarking on the clinical trial. In other words, if an agent is to inhibit a molecular target, what level of inhibition defines success? Obviously complete (100%) inhibition would be definitive and desirable, but for particular agents, lower levels of inhibition may be adequate to produce a biological impact. Preclinical evaluations, especially in animal models (see below), will be helpful to determine the success criteria to be used in a study.

Tissue handling, processing, and quality control are also critical to reliable molecular assessments. It will be important, for example, to carefully control or at least monitor the time between specimen removal and processing. For assays of genetic alterations requiring analyses of DNA, stringent tissue processing may not be necessary. However, for evaluations that assess changes in mRNA profiles (such as cDNA microarray studies), and especially assays of protein modifications (such as phosphorylation), stringent quality control will be needed. Standardization of tissue processing to prevent degradation of the targeted moiety must be assured. These issues may have an impact on the choice of control tissue (see below).

These issues may also mandate the implementation of new procedures to monitor and provide quality assurance. In particular, "intraoperative research nurses" may be needed to track, record, and verify that tissue is handled properly.

Just as clinical research nurses are invaluable for assessing, recording, and verifying common toxicities and protocol adherence, positions for professional personnel who monitor operative specimens may need to be established.

Assays that measure the direct effect of a drug are preferable to those that measure downstream effects (Eisenhauer, 1998). For example, for drugs that block farnesylation of the Ras protein, an assay that directly measures this function may be more appropriate than one that measures levels of mitogen-activated protein kinase, a downstream effector of Ras. When assays of direct function are not available, other end points can be used with the recognition that indirect measurements may complicate interpretation of results. For example, interpretation of apoptosis induction, as measured by staining with TdT-mediated dUTP nick-end labeling, is complicated by the baseline variability of apoptosis within each specimen when comparing different tumor specimens. Nevertheless, when taken in conjunction with upstream end points, a downstream effect, such as apoptosis, may be a highly valuable end point.

Finally, molecular assays that determine changes within a tissue specimen on a cell-by-cell basis will likely prove to be more useful than assays that determine global changes within the tissue. Assays of global changes, such as Western blotting, in which tissue architecture is lost and individual cells are not assessed, provide insight into molecular events within a population of cells, but do not determine the percentage of cells within a specimen that exhibit the specific change being assessed. They also cannot identify variations in response based on differences in tumor architecture or tumor heterogeneity. Histologic assays, such as immunohistochemistry or in situ hybridization in which tissue architecture is maintained, can provide a cell-by-cell analysis. Global assays, however, are more versatile and more readily available than histologically based assays.

Requirements for Control Tissue

The molecular inter- and intratumoral variability of gliomas mandates careful consideration of controls in the design of clinical trials that assess molecular end points. Although standard phase I and phase II clinical trials do not incorporate controls, to accurately determine drug-induced changes in human tumors, developing controls that define the baseline function or level of the targeted molecule is an important aspect of evaluating new agents. Although comparing an untreated specimen to a treated specimen from the same patient is most desirable, limitations of serial tissue acquisition may preclude such an approach. However, the value of serial biopsy may outweigh such risk so that it may be argued that serial biopsies should be incorporated into all clinical trial designs. However, when this approach cannot be used, an alternative is to compare results from cohorts of treated and untreated patients.

These complex clinical trials will undoubtedly require more sophisticated statistical methods than are currently used in order to account for baseline variability and to determine the significance of molecular changes. Indeed, it is likely that depending on the variability of the target and the magnitude of the desired change, the number of patients required for early-phase clinical trials may increase. However, the value of the information should outweigh the effort, time, and cost of enrolling more patients.

Role of Animal Models

The importance of clinically applicable molecular assays suggests that a new paradigm for preclinical animal studies of brain tumors is needed. Much effort has gone into developing animal models that mimic human brain tumors (for review, see Van Dyke and Jacks, 2002, and Begemann et al., 2002). Most preclinical studies use animal models to assess the efficacy of new drugs by determining whether the agent improves animal survival or slows tumor growth (Fig. 1) (Houghton et al., 1998). However, animal models are not commonly used to assess the molecular effects of an agent on a tumor or to develop specifically applicable clinical assays to measure the molecular effects of a particular agent in situ (Feldkamp et al., 2001; Kilic et al., 2000). In this context, we believe that the optimal use of animal models may be to study the molecular effects of agents on in situ brain tumor systems and, most importantly, to use these experimental systems to develop assays that can directly translate results from animal brain tumor tissue to clinically acquired surgical brain tumor specimens (Fig. 1). Kilic et al. (2000) demonstrated the feasibility of such an approach in their evaluation of the effects of STI571 on PDGFR phosphorylation in glioblastoma xenografts grown subcutaneously in nude mice. Similarly, Feldkamp et al. (2001) evaluated the downstream effects of blocking Ras using the farnesyl transferase inhibitor SCH66336 in xenografts grown subcutaneously in nude mice. Rather than simply evaluating animal survival, direct effects of the drugs on tumor specimens were demonstrated in these studies. Similar approaches using intracranial tumor models are needed to better mimic the biology of human brain tumors.

Phase I and Phase II Trial Designs

Given the complexities of incorporating molecular end points into early-phase clinical trials, what are the starting models that are used for phase I or II clinical trials of brain tumors and that seek to include biological end points? One approach was used in a trial of O^6-benzylguanine on O^6-alkylguanine alkyltransferase (Friedman et al., 1998). Enrolled patients were treated 18 h before surgery with i.v. O^6-benzylguanine. Resected tumors were frozen and evaluated for alkylguanine alkyltransferase activity. With this approach, the direct effects of the drug on the target were measured in brain tumor specimens from patients.

A more complex approach, but one that determines both molecular and long-term toxicity, was exemplified by a recent phase I trial of adenoviral-mediated *P53* gene therapy (Lang et al., 2000). The goal in this trial was to determine the biological effect of intratumoral administration of a replication-deficient type 5 adenovirus vector

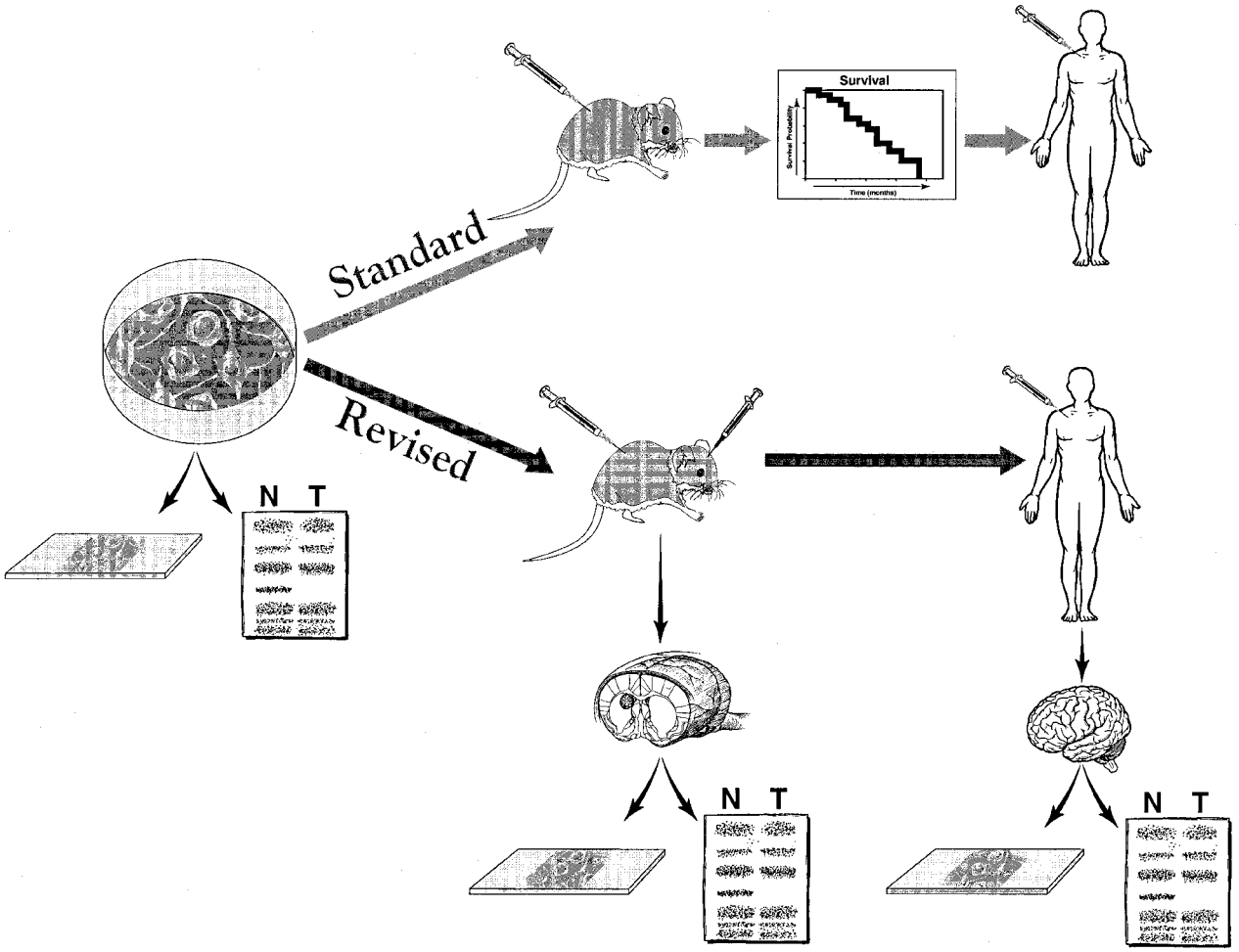

Fig. 1. Use of animal models in preclinical testing. Preclinical analyses typically use animals to determine efficacy in survival studies. Animal models may also be useful for defining assays that demonstrate molecular effects of the agent. These assays can be translated to clinical studies.

containing cDNA from the *P53* gene (Ad-p53), while also determining the clinical toxicity customarily assessed in standard phase I trials. A 2-stage surgical approach was undertaken in which patients underwent a stereotactic biopsy and injection of an Ad-p53 vector via a catheter implanted in the center of the tumor. Several days after injection, the tumor was resected en bloc in an open craniotomy approach without disturbing the catheter. Tissue from a pretreatment biopsy allowed *P53* mutational status to be assessed in the tumor before treatment. The posttreatment surgical specimen was immunohistochemically analyzed for *P53* gene expression with the catheter serving as a reference for the site of Ad-p53 delivery. After tumor resection, the walls of the postresection tumor cavity (which contained infiltrating tumor cells) were injected with Ad-p53 so that patients could be followed up to determine clinical toxicity related to Ad-p53 and treatment efficacy. The dose of Ad-p53 was escalated among 4 cohorts, permitting determination of an ABD (based on a gene expression end point) and an MTD (based on a clinical toxicity end point).

For phase I clinical trials, we believe this paradigm can be used as a working model for any trial that seeks to incorporate molecular end points, including those that do not require intratumoral administration (Fig. 2). In the ideal design (Fig. 2A), patients with surgically accessible tumors undergo stereotactic biopsy to confirm diagnosis and, most importantly, to obtain tissue to establish a baseline evaluation of the molecular target to be assessed after treatment. After this baseline is established, the experimental drug is given before tumor resection. At the completion of the drug treatment, open craniotomy and en bloc resection of the drug-treated tumor is performed to provide architecturally intact tissue for analysis of pharmacokinetic and drug-related molecular changes. After recovery from surgery, the experimental drug is given to determine clinical toxicity according to standard phase I criteria. This 2-stage model represents the ideal approach because it allows comparisons to be made between treated and untreated specimens from the same patient and also allows for conventional toxicity assessments. However, issues of enrollment and cost exist because of the need for 2 procedures.

Other more simplified and less invasive approaches can also be considered (Fig. 2B and 2C). Baseline biopsies may be avoided using an alternative approach in which patients

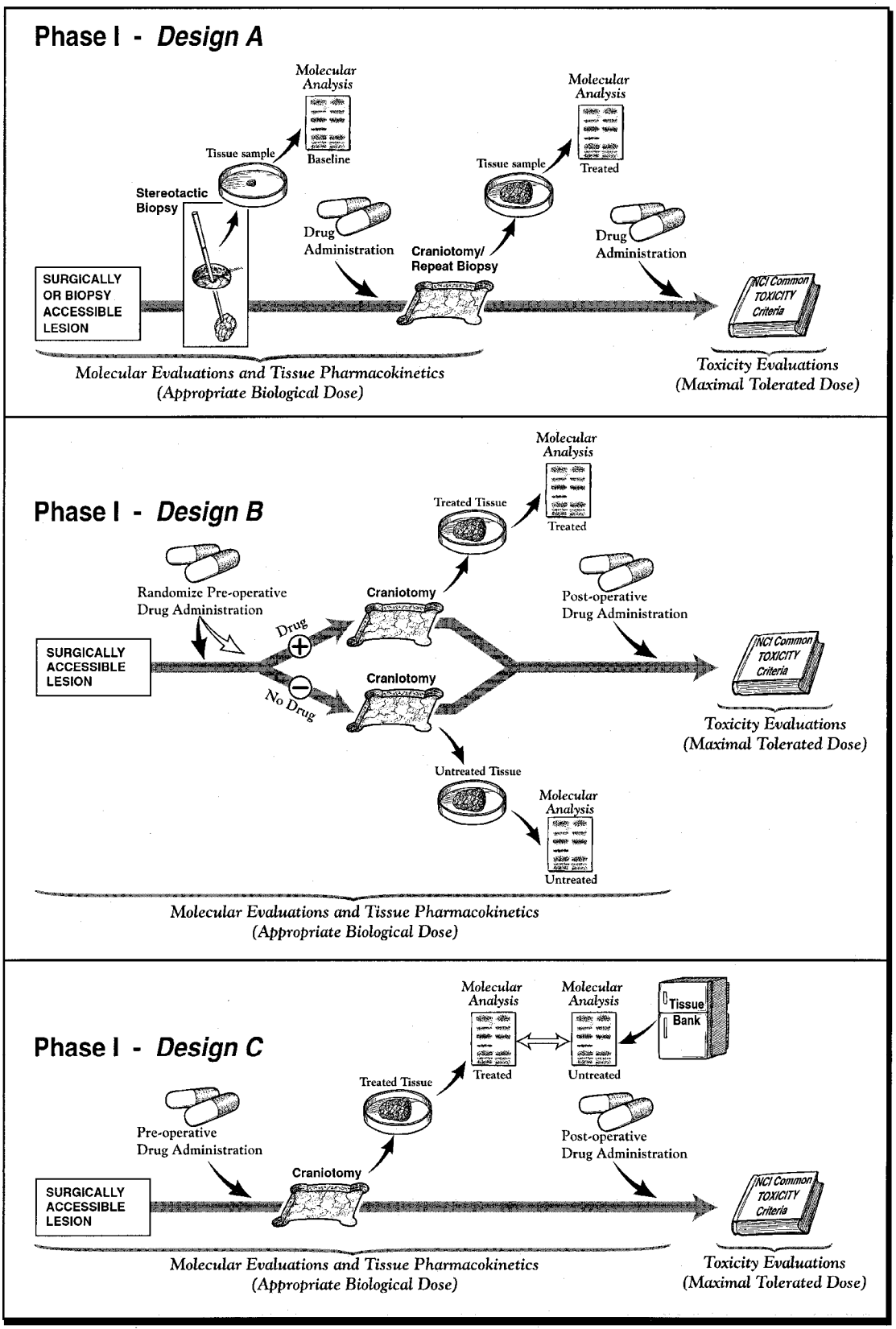

Fig. 2. Proposed designs for phase I clinical trials of agents for which molecular targets can be tested. A. Optimal design. A biopsy can be used to determine the baseline value of the target prior to treatment. Patients are then treated with drug, and the effects of the agent on the tumor are determined in a posttreatment surgical specimen. B. Avoidance of pretreatment biopsy design. Patients are randomized to receive drug or not. Posttreatment tumor reaction allows comparisons to be made between untreated specimen (controls) and treated specimens. C. Alternative design. Control specimens may be obtained from specimens in a tumor bank.

are randomized to a preoperative drug administration group or to a placebo/no-drug group (Fig. 2B). Patients in both groups undergo surgical resection, and the tumor specimens from the untreated cohort serve as controls for the specimens from the drug-treated group (Fig. 2B). (Alternatively, untreated specimens may be obtained from patients who meet eligibility criteria but decline the treatment regimen.) The number of patients randomized to the untreated group depends on the anticipated variability of the particular molecular change being studied. After surgery, all patients are given the agent and are followed for clinical toxicity using standard criteria. This design has the advantage of requiring only one surgical procedure and may be more acceptable to patients and institutional review boards. It suffers from its dependence on population analyses to determine the baseline variability of the targeted molecule and will require enrolling more patients per cohort. As an alternative, baseline values for a molecular effect could be determined from tissue obtained from banked specimens (Fig. 2C). This design would eliminate the need for randomizing the administration of the drug but suffers from relying on historic controls, the handling, processing, and quality of which may not be the same as prospectively obtained tissue.

When drugs with no established toxicity profiles are evaluated in phase I trials, open craniotomy after drug administration may not be advisable due to the potential for adverse events, particularly impaired hemostasis, wound healing, and immune responses. These problems can be avoided by enrolling patients in a staggered design such that enrollment in a nonsurgical cohort preceded enrollment in the surgical cohort. Once a particular dose level is shown to be nontoxic, the patient would be entered into the surgical cohort. Patients with resectable and unresectable tumors could all be entered into the trial, with unresectable patients preceding resectable patients at each dose level.

For phase II trials, similar paradigms can be used. Just as phase I trials with molecular end points must retain the goal of assessing clinical toxicity, phase II trials must retain the goal of determining clinical efficacy (radiographic response or progression-free survival) of the phase I–defined dose while incorporating measurement of biological efficacy (molecular end points). This goal can also be achieved through a 2-stage approach. In the first stage, patients who are eligible for resection are randomized (similar to the method described for phase I trials) to receive either the experimental drug or the placebo/no drug prior to resection. After drug treatment, both groups undergo open craniotomy and en bloc tumor removal to provide a tissue specimen for molecular analyses. Thus, the tissue acquired from the patients who receive no preoperative drug treatment serves as a control for the specimens acquired from the patients who receive the drug (Fig. 3). Biological efficacy is determined by accruing a statistically significant number of patients who demonstrate a desired molecular response. From a statistical perspective, this molecular response is viewed as

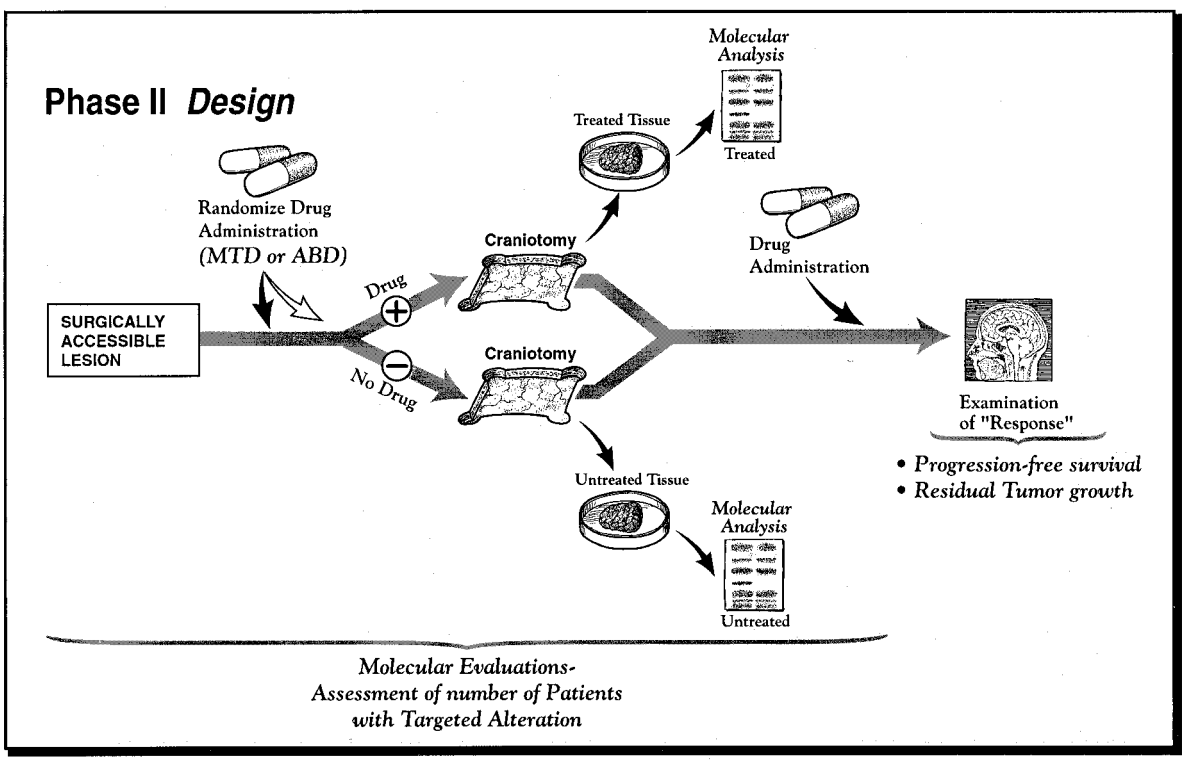

Fig. 3. Proposed design for phase II clinical trials of agents for which molecular targets can be tested. Similar to phase I trials, phase II trials can include a preoperative treatment followed by surgical acquisition of a posttreatment specimen for molecular and pharmacokinetic analyses. Randomization can be used to provide baseline comparisons. After surgery, patients are again given the drug, and standard clinical assessments of efficacy, such as progression-free survival, can be measured.

being similar to a radiographic response. The *desired molecular response* is defined in phase I studies, with a goal of objectivity.

After recovery from craniotomy, all patients would then receive the study drug and would be evaluated to determine clinical efficacy as part of the more standard phase II trial approach. Therefore, biological efficacy could be correlated with clinical efficacy. Obviously, incorporating surgical removal has the advantage that all patients would have a verified tumor mass. However, because different degrees of resection would be performed, criteria for assessing clinical efficacy would need to be established. In our opinion, because most tumors will undergo gross total or near-total resection of enhancing tumor, response measurements (radiographic assessments of tumor reduction) might not be possible. Consequently, clinical efficacy with this approach might best be assessed based on determination of time to recurrence and progression-free survival. Precedent for the use of progression-free survival in brain tumor studies has already been established (Hess et al., 1999). Traditional measures of response, if deemed necessary, could be determined in a cohort of patients who did not undergo resection or who had measurable disease after resection.

Conclusion

The development of targeted brain tumor therapeutic agents provides a unique opportunity for rational clinical trial designs that assess the direct effects of the agent of interest on the tumor. Clinical trial design deserves significant effort as it is the cornerstone for the rational application of these new agents. The complexity of the proposed methods suggests that clinical trials of new targeted agents will require collaboration between neuro-oncologists, neurosurgeons, neuropathologists, and basic-science investigators. Neuro-oncologists will continue in their primary role of giving drugs and monitoring patients enrolled in clinical trials. In contrast, neurosurgeons will plan appropriate methods of safe tissue acquisition and determine parameters for drug administration relative to tissue acquisition. Neuropathologists will provide histologic diagnoses and verification that tested samples contain specific tumor qualities. Laboratory-based scientists will be critical for developing appropriate assays of drug activity that can be used for surgical specimens. It is anticipated that, in the future, the agents given to patients will be based on the molecular signature of their individual tumors. Therefore, through rational and innovative clinical trials that incorporate molecular end points, agents with the greatest biological activity against specific molecular targets will be identified and efficacious therapies can be designed.

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