

## Phase 1 Trials Today

p0010 All commercially available anticancer agents must have undergone phase 1 investigation as part of their clinical development. As the evolution of novel anticancer drugs evolved from primarily cytotoxic agents to targeted therapies, clinical investigators have developed novel Phase 1 trial designs and endpoints. It is estimated that approximately 500 anticancer agents will present to the clinical arena within the next decade. It is a well-known fact that one of the most important components of conducting phase 1 trials is eligible patient availability. As the number of commercially available agents for several tumor types has increased, as well as the number of patients treated off-protocol in community settings, the availability of patient resources has become a challenge.

p0020 As a result, it is important to conduct efficient and effective trials by maximizing data acquisition while minimizing patient numbers. Previously, standard phase 1 trials used large patient numbers and cohorts. Now, it is the norm to use well-thought-out trials minimizing patient numbers and cohorts by using alternative designs and carefully selecting the starting dose. Once thought of only as an alternative to hospice with no significant benefit, treatment on a phase 1 trial is now viewed as an additional therapeutic option. The overall clinical benefit of phase 1 trials is approximately 45%, with highly variable response rates, depending on the type of agent and the Phase I trial under investigation (1). Ethically, the intent of all clinical studies, for both the patient and physician alike, is therapeutic (2–4). A better understanding of the compound(s) under investigation and the various types of phase 1 clinical trials available will assist the investigator in determining at what point and for which patient-specific phase 1–clinical trials should be considered.

### s0010 **Types of Phase I Clinical Trials**

p0030 Phase I clinical trials are the first stage of drug testing in human subjects. These studies play a vital role in the development of novel therapeutics. Phase I studies are typically designed to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of a novel agent. Novel cancer therapeutics are usually offered to patients with advanced cancer who have had other types of therapy and who have few, if any, remaining treatment options. In addition,

due to several tumor types with limited current treatment options which could impact favorably on patient survival, it is considered ethical to treat patients with metastatic disease in phase 1 trials by using novel agents in combination with standard therapies; this is especially true if the standard therapies demonstrated therapeutic success in previous clinical investigations.

While the primary stated objective of a typical phase 1 study is to determine the optimal dose of a novel therapeutic for use in subsequent studies, several different types of phase 1 clinical trials exist. A brief overview is given here of several phase 1 trials that meet specific needs for clinical early drug development.

### Single Ascending Dose

Single ascending dose (SAD) studies are those in which groups, or cohorts, of up to six patients are given a small dose of the drug and observed for a specific period of time. If the cohort does not exhibit any adverse side effects, a new group of patients is then given a higher dose. This continues until intolerable side effects are observed. Once such side effects occur, the cohort may be expanded to determine if more side effects are observed. If an unacceptable number of intolerable side effects occur, the dose will be lowered and tested with more patients. The highest dose administered to a patient on a phase 1 trial is referred to as the maximum administered dose. The dose that is as high as possible but still tolerable for patients is said to be the maximum tolerated dose (MTD).

Often, SAD clinical trials can be categorized as being first-in-human, first-in-class, or a combination of the two. As the name implies, first-in-human clinical trials are those that are conducted for the first time in a human patient. In order to be tested in humans, a drug typically has to first show promise of activity in the laboratory and in animals. Normally, a small (approximately 20) group of patients will be selected for inclusion into a first-in-human phase 1 study. First-in-human studies are almost always done in a single ascending dose manner. The objective of the first-in-human phase 1 trial is to find a suitable safe dose, (the MTD) for use in later studies that will more thoroughly examine efficacy. Once the MTD has been determined in a phase 1 SAD study, later-phase studies can be designed and multiple ascending dose studies can be performed.

p0070 First-in-class studies examine novel drugs that are uniquely manufactured or based on a new target or indication. Such therapeutics are typically innovative, novel, and no other pharmaceutical products are currently approved for the same therapeutic indication; hence, they have no pharmaceutical substitute.

### s0030 **Multiple Ascending Dose**

p0080 Multiple ascending dose (MAD) studies are conducted to better understand the pharmacokinetics/pharmacodynamics of a drug. The primary purpose of a MAD study is not to determine the MTD, but rather to examine the biologic effects of the drug. In these studies, a group of patients receives a low dose of the drug and the dose is subsequently escalated up to a predetermined level. Specimens (of blood, and/or other fluids) are collected at various time points and analyzed to understand how the drug is processed within the body.

### s0040 **Method and Model**

p0090 MeMo trials are studies that are done in anticipation of a phase 1 clinical trial. Typically done for “targeted” agents, these trials help in the development of a pharmacodynamic endpoint. It may help identify either a direct tissue or a surrogate tissue marker. This assists in determining if the marker can be measured within the tissue and also helps to refine the assay needed for pharmacodynamic measurement.

### s0050 **Phase 1 Trials Using Radiolabeled Tracer Doses**

p0100 The use of radiolabeled experimental agents has become an increasingly important factor in drug development. In preclinical studies, radiolabeled compounds are frequently used in the laboratory to understand the distribution, metabolic fate, and localization of experimental drugs both *in vitro* and *in vivo*. Clinical studies performed as part of phase 1 trials, or in support of them, may also involve the administration of small doses of radiolabeled compounds, called tracers, to healthy human volunteers or to patients to better understand the mechanisms of drug action.

p0110 Radiolabeled tracers are synthesized by replacing one or more atoms of an experimental drug agent with a radioisotope. Radioisotopes must have a suitably long half-life in order to allow for imaging or detection in biologic samples. Examples of commonly-used isotopes for detection in tissue or blood samples include carbon ( $^{14}\text{C}$ ), hydrogen ( $^3\text{H}$ ), sulfur ( $^{35}\text{S}$ ), and iodine ( $^{125}\text{I}$ ). Isotopes which are commonly used in imaging, specifically in positron emission tomography (PET) scanning, include fluorine ( $^{18}\text{F}$ ), carbon ( $^{11}\text{C}$ ), and oxygen ( $^{15}\text{O}$ ).

p0120 Radiolabeled compounds have allowed researchers to study many aspects of a drug's behavior *in vivo* (5). Evaluation of the mass balance of a drug can be performed to better understand how much of an applied dose is recovered with respect to time. The metabolism of the drug can be extensively studied to determine if any metabolites might represent a potential toxicologic hazard to the patient. Advances in clinical imaging have greatly impacted drug discovery and development in recent years (6). Clinical imaging studies

using labeled drug have the potential to facilitate early clinical pharmacokinetic/pharmacodynamic assessments, including target interaction and modulation (7). This is particularly useful in patients where there are no direct measures of pharmacokinetics/pharmacodynamics throughout the tissues of the body and at the target.

Studies using a method called “microdosing” offer the prospect of taking a drug directly into human studies by administering extremely low doses of radiolabeled agent. Microdosing studies may also be referred to as phase 0 studies. By using only very tiny amounts of radiolabeled drug, researchers use microdosing to establish the likely pharmacologic dose and thereby determine the first dose for a subsequent phase 1 study. However, microdosing is not without controversy among researchers in drug development (5). Concern has been raised that microdosing may not accurately predict the behavior of clinical doses. It has also been suggested that nonlinearities may be induced when binding, metabolizing, or eliminating systems become saturated, thus resulting in differences between low and high doses.

### **Drug/Food Metabolic Interaction Studies**

The U.S. Food and Drug Administration (FDA) has recommended the metabolism of an investigational new drug be defined during drug development and that interactions with other drugs be explored as part of an adequate assessment of its safety and effectiveness (8). Medicines are often used concomitantly with other drugs, and some degree of drug–drug interaction often occurs with concomitant use. Although only a small proportion of this interaction is clinically significant, it sometimes causes serious adverse reactions.

Concomitant medications can abruptly alter metabolic routes of absorption and elimination. The risk of a drug–drug interaction depends on the number of drugs used, the tendency of particular drugs to interact, and the amount of drug taken. Types of drug–drug interactions include duplication, opposition (antagonism), and alteration of what the body does to one or both drugs. Observed changes arising from metabolic drug–drug interactions can be substantial—an order of magnitude or more decrease or increase in the blood and tissue concentrations of a drug or metabolite—and can include formation of toxic metabolites or increased exposure to a toxic parent compound. In addition to potential interactions of an experimental agent with other drugs, it has long been recognized that some foods and drugs, when taken during the same period of time, can alter metabolism or cause serious adverse events.

Therefore, early on in the drug development process, appropriate efforts should be made to predict the nature and degree of potential interactions so that patients will not be adversely affected. The cytochrome P450 (CYP450) family of enzymes is an important group of enzymes found in the liver that plays a large role in metabolizing drugs. Many metabolic routes of elimination, including most of those occurring via the CYP450 family of enzymes, can be inhibited, activated, or induced by concomitant drug treatment. The FDA has recommended detailed studies be performed with the major CYP450 enzymes (CYP1A2, 2C9, 2C19, 2D6,

2E1, and 3A4). Typically, preclinical testing is performed to investigate the effects of an agent on metabolic factors, such as CYP450, and of inhibition or induction potential. If in vitro experiments reveal the potential for drug–drug interaction, in vivo experiments usually will follow. Therefore, phase 1 clinical trials often include testing for the ability of an experimental agent to affect CYP450 and a determination of whether the agent causes a change in concentration of other drugs as a result. With the combination of in vitro studies and in vivo studies in support of phase 1 clinical trials, the potential for drug–drug interactions can be studied early in the development process, with further study of observed interactions assessed later in the process, if necessary.

### s0070 **Organ Dysfunction Studies**

p0170 The desirable and undesirable effects of a drug arising from its concentrations at the sites of action are usually related either to the amount administered (dose) or to the resulting blood concentrations (accumulation), which are affected by its absorption, distribution, metabolism and/or excretion. Elimination of a drug or its metabolites occurs either by metabolism, usually by the liver, or by excretion, usually by the kidneys and liver.

p0180 Although clinical trials for drug approval are often conducted in patients with normal hepatic and renal function, patients in clinical practice, especially those with cancer, may have compromised organ function because of underlying disease or from other causes such as aging, diabetes, infectious and autoimmune diseases, or drug-related toxicities (9). In general, drugs are approved and marketed with limited or no information on the pharmacokinetics and/or pharmacodynamics of the drugs in patients with organ dysfunction. Inadequate information in the drug label or in the scientific literature about the starting dose for organ dysfunction patients is of great concern to the treating physician who must manage the risk-benefit of these agents in patients with serious co-morbidities.

p0190 Phase 1 hepatic and renal dysfunction studies have been defined as clinical pharmacokinetic and pharmacodynamic experiments that represent prospective attempts to collect clinically useful dosing information from a difficult to study patient population and to formulate dosing recommendations based on these data (10). Phase 1 studies in organ dysfunction patients are typically carried out after a recommended dose has been established in an unimpaired patient population, and efficacy and safety have been established in Phase 2 studies. It has been recommended that organ dysfunction studies be designed in the form of a formal dose-escalation phase 1 study, with a complete pharmacokinetic and toxicity profile as end points (9). The primary goal of the phase 1 study in an organ-impaired population should be to determine if the pharmacokinetics are altered to such an extent that the dosage requires adjustment, based on degree of organ dysfunction, from the dose established in the unimpaired population.

p0200 Due to the uniqueness of eligible patients, these studies are typically conducted as multisite studies to complete them in a timely and efficient fashion. In 1999, the National Cancer Institute (NCI) developed an Organ Dysfunction Working Group (ODWG), composed of approximately 12 to 15 phase 1 sites. The

ODWG has successfully completed evaluation of oxaliplatin and imatinib (STI-571) in the renal and hepatic impaired populations (11–15). In addition, several additional agents are currently undergoing evaluation.

### **Thorough QT Phase 1 studies**

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p0210 Adverse effects on cardiac health have become one of the most common causes of product withdrawal from the market. As a result, regulatory authorities around the world have recently placed greater emphasis on cardiac safety. In May of 2005, The FDA endorsed the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) E14 document entitled: *The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs* (16). The FDA has indicated that new agents are expected to receive a clinical electrocardiographic evaluation, beginning early in clinical development, typically including a single, phase 1 trial designed to evaluate their effect on cardiac repolarization. This type of study has been referred to as a “thorough QT” study. A thorough QT study is described as a single trial dedicated to evaluating the effect a drug has on cardiac repolarization as a way to predict the risk of sudden death. According to the ICH E14 guideline, thorough QT phase 1 studies are to be performed to show that new investigational drugs do not change cardiac repolarization. The FDA’s regulatory guidance recommends a thorough QT phase 1 study to be conducted regardless of pre-clinical cardiac findings. When a thorough QT study is not feasible for other reasons, which may be the case in certain therapeutic areas such as oncology, alternative approaches are recommended, such as expanding the number and timings of electrocardiogram (ECG) recordings in other clinical studies in patients.

### **Dose-Scheduling Studies**

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p0220 Inefficient dose-scheduling can lead to treatment failure and the inadequate development of a potentially promising therapeutic. Unlike the typical phase 1 clinical trial designed to determine the MTD, an investigator may be interested in determining how often (i.e., how many administrations of a schedule) an agent could be safely administered to determine the long-term toxicity due to cumulative effects. Dose-scheduling studies are designed to determine the optimal administration schedule for an investigational agent.

p0230 Dose-scheduling studies can be combined with dose-finding studies or be completely separate studies. Pharmacokinetic and safety data obtained during a phase 1 dose-finding study may suggest it is feasible to increase the dose and/or reduce the frequency of administration of an agent, therefore indicating a dose-scheduling study is warranted.

### **Combination Studies**

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p0240 The typical phase 1 dose-finding study is designed to determine the MTD of a single, novel agent. However, an increasing number of patients, particularly in oncology, are being treated with drug combinations. The goal of a two-agent dose-finding trial is to find

the maximally tolerated dose of a dose combination (or combinations). Combination studies can be performed to determine the optimal dose and schedule of experimental drugs combined with standard chemotherapies and also of novel drugs combined together.

p0250 At the time that a combination study is designed, the monotherapy MTD doses of the individual agents under investigation are usually known. As a result, a minimal number of dose levels are typically needed to achieve the recommended dose of both drugs in combination. In the past, combination studies were routinely performed as single arm trials. However, recent novel designs, which include targeted and standard therapies, have been designed with several arms with differing standard therapies in combination with the novel agent under investigation (17). Such a design has been shown to expedite the identification of the combination MTD.

p0260 A common design for dose-finding studies with multiple agents is to investigate a single dose, or a small number of doses, of one agent and multiple doses of the second agent. If the study is combining a novel agent with a standard chemotherapy, the dose of the novel agent is usually varied while the standard chemotherapy is held to a single or a few doses.

## Phase 1 Cancer Clinical Trial Designs

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p0270 Phase 1 cancer clinical trials offer great excitement and hope for clinician and patients alike as they often represent the end result of many years of preclinical work and new target identification. These trials are typically small, usually enrolling about 20 patients. However, this number can be quite variable depending on the trial design and the number of dose escalations, or cohorts, needed to determine the MTD. Many phase 1 clinical trial design methods have been proposed, and there is currently no consensus among the scientific, medical, and statistical communities on how best to perform these studies in humans.

## Traditional Design

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p0280 The most-commonly used design, often referred to as the traditional or "3 + 3" design, begins by assigning three patients in a cohort at a designated dose level, often one tenth the lethal dose ( $LD_{10}$ ) in mice, scaled up to humans (18). Doses to be assigned are predefined by the investigators, based on preclinical data and clinical experience with similar agents, if it exists. One method of assigning successive dose levels uses a set of "increasing decreasing" Fibonacci dose level increments, usually 100%, 67%, 50%, 40%, and 33% for each dose level thereafter (19–21). These increments are added to each dose to get the next dose level. For example, the second dose level is 100% more than the first, the third dose level is 67% more than the second, the fourth dose level is 50% more than the third, and so on.

p0290 The decision whether to escalate to the next higher dose, expand a cohort, or de-escalate to a lower dose is made based on the toxicity information received from each three-patient cohort (Figure 47-1). If none of the three patients experience a

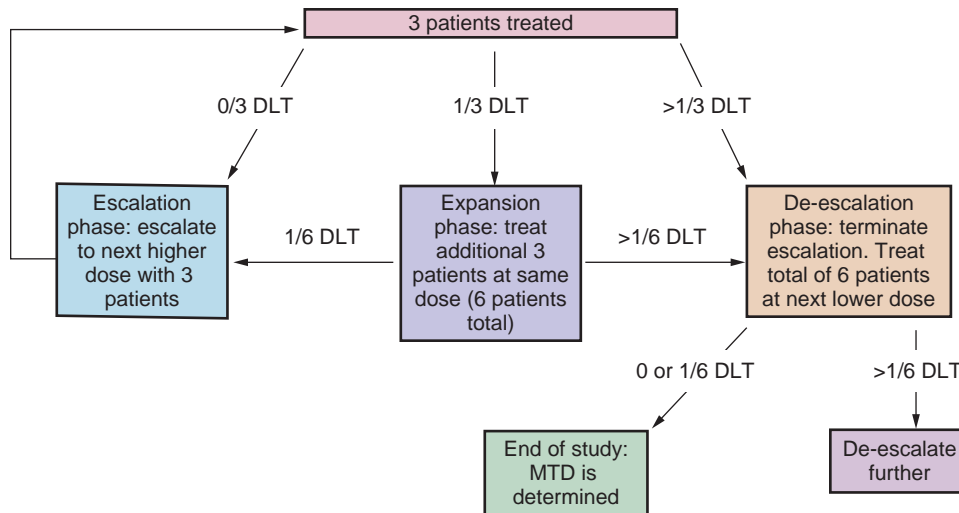
dose-limiting toxicity (DLT), the study proceeds to another cohort of three patients at a higher dose level (escalation phase). DLTs are predefined, and often include unacceptable drug-related toxicities as defined in grading scales commonly used in oncology, such as the NCI's Common Terminology Criteria for Adverse Events (CTCAE). If one out of the three patients treated on a dose level cohort experience a DLT, up to an additional three patients (for a total of six) are treated at that dose level (expansion phase). If none of the additional patients experience a DLT, the dose will escalate. If at least two patients experience a DLT, the MTD is said to have been exceeded and the maximum administered dose (MAD) has been defined. An additional three patients will be tested at the next lowest dose level if there were only three patients previously treated at that level (de-escalation phase). In this particular case, the MTD is therefore defined as the highest dose level for which no more than one patient out of six experiences a DLT. Table 47-1 shows the different dose escalation/de-escalation decisions associated with toxicity outcomes at a given dose for an example of the 3+3 design.

## Modifications to Traditional Design

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p0300 A fundamental conflict in phase 1 trial design exists between escalating too quickly, resulting in the potential exposure of patients to excessive toxicity, and escalating too slowly, resulting in the treatment of patients at doses too low to have to be efficacious (19). A major criticism of the traditional phase 1 design is that the potential exists for many patients to be treated at subtherapeutic dose levels. In addition, the length of time these studies often take can inhibit the ability to rapidly bring new agents to subsequent phase 2 and phase 3 studies. Several variations of the traditional design have been developed to reduce the number of patients treated at doses below the biologically active level and to improve upon the precision of the MTD definition. Some of the most commonly used types of modified traditional designs include those proposed by Storer (22) and by Simon (18).

The Storer BD design uses a two-stage approach (22). In the first stage, only a single patient is entered at each dose level. Dose escalation continues with one-patient cohorts until a DLT is observed. Accrual to the second stage then begins at one lower dose level and follows the traditional (three-patient cohort) design. Such a scheme allows fewer patients to be treated at dose levels less likely to be efficacious. Storer also proposed defining the MTD by fitting the first-course toxicity data to a logistic dose-toxicity curve and letting the MTD be defined as the dose level associated with a target DLT rate (e.g., 20%–30%; 22). This allows for a more precise MTD definition.

Simon described three types of accelerated titration designs that were modifications of the traditional design (referred to by Simon as Design 1; 18). The Simon Design 2 is similar to the Storer design in that it uses single-patient cohorts during the initial stage, but the switch to the second stage (the traditional design) occurs when either the first instance of first-course DLT is observed or if two patients exhibit grade 2 toxicity, as defined by the CTCAE, during their first course of treatment (18). The Simon Design 3 mimics Design 2, except for the incorporation of



**FIGURE 47-1** The traditional “3+3” phase 1 clinical trial design. The initial three-patient cohort begins at a predefined dose. If no dose-limiting toxicities (DLTs) are observed, escalation to the next higher dose will occur. If a single DLT is observed, expansion of the cohort to a total of six patients occurs. If more than one DLT is observed, de-escalation to the next lowest dose will occur for a total of six patients treated at that dose. Termination of the study will occur if more than one DLT is observed at the starting dose. The maximum tolerated dose (MTD) is defined as the highest dose level for which no more than one patient out of six experiences a DLT.

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more rapid dose-escalation by using double-dose steps during the single patient cohort stage. Finally, the Simon Design 4 is similar to the Design 3, except switching to the second (three-patient cohort) stage may occur when either the first instance of a DLT occurs or the second instance of grade 2 toxicity is observed in *any* course of treatment. The three Simon accelerated titration designs also allow for inpatient dose escalation, permitting escalation for an individual patient if toxicity during their previous course was less than grade 2 as defined by the CTCAE and did not result in a DLT. Accelerated titration designs have become very popular, as they can dramatically reduce the number of patients required, shorten the duration of the trial, and provide a great deal of information about cumulative toxicity, interpatient variability, and steepness of the dose-toxicity curve (23). Most important, they provide all patients a maximum opportunity to be treated at a therapeutic dose. In reviewing several accelerated titration design phase 1 trials, it was identified that the advantages of its use from a prospective perspective were a minimal amount of patients needed to reach the MTD, a lower percentage of patients treated at potentially sub-therapeutic doses or with an ineffective agent, and cost containment (24–28). However, accelerated titration designs did not expedite

the completion of studies overall, relative to traditional designs when compared with matching studies done in high-throughput phase 1 centers.

### Cytotoxic Versus Targeted Design

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One of the assumptions inherent in the traditional phase 1 design is that both toxicity and clinical benefit will increase as the dose of an agent increases. For cytotoxic therapeutic agents, this assumption usually holds true. Recently, however, several agents have been developed that target specific tumor characteristics, such as receptors, and these agents may not follow the standard efficacy/toxicity model. Specifically, targeted agents may demonstrate a plateau on the dose-efficacy curve, meaning higher doses will not improve clinical benefit. In addition, toxicity occurring with the use of these agents, if it occurs at all, may not necessarily increase as the dose increases. For drugs of this type, determining the MTD may not be feasible or useful. For targeted agents that do not produce immediate or consistent drug-related toxicity, three categories of alternative endpoints have been considered: (1) measuring inhibition of a target, (2) plasma drug levels that are biologically relevant, and (3) surrogate markers of biologic activity in nontumoral tissues (29).

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Several phase 1 trial designs have been developed for studies examining targeted, noncytotoxic agents (30–32). Hunsberger et al. (30) proposed several designs that are based on the assumption that there is a binary (positive or negative) response that is measured in each patient after treatment with an agent; this response indicates whether or not the desired effect has been achieved. The simplest of these designs mimics the traditional 3+3 design, but adapts it to examine response rather than toxicity. The goal of this design is to recommend the lowest dose meeting a predefined level of activity (response) for further testing. Dose escalation occurs when a predefined number of responses are not observed. Dose de-escalation will occur if the predefined level of responses has been exceeded.

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### Pharmacokinetically Guided Dose-Escalation Method

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The pharmacokinetically guided dose-escalation (PGDE) method of clinical trial design was proposed by Collins et al. as a more

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**10010 Table 47-1** Dose Escalation/De-escalation Decisions Associated with Toxicity Outcomes at Given Dose For Popular Version of 3+3 Design

No. Patients with Dose-Limiting Toxicity	Decision
0/3	Escalate one level
1/3	Treat 3 more at same level
1/3+0/3*	Escalate one level
1/3+1/3*	Stop and chose previous dose as the MTD
1/3 + (2/3 or 3/3)*	Stop and chose previous dose as the MTD
2/3 or 3/3	Stop and chose previous dose as the MTD

\* Note that those rows with number of toxicities equal to 1/3+ t/3 (for t=0,...,3) corresponds to situations in which one toxicity is observed in the first cohort of three patients enrolled at the current dose and t toxicities are observed in the second cohort of patients enrolled at that dose.

informative and efficient alternative to the traditional design (19,33). The authors retrospectively analyzed the results of several phase 1 studies of chemotherapeutic agents, and demonstrated that observed toxicity was not a function of the dose administered to the patient, but rather was a function of the area under the curve (AUC) of plasma drug concentration measured over time of exposure. The PGDE phase 1 clinical trial design targets the AUC associated with the mouse  $LD_{10}$ . Patients are treated at one tenth of the mouse  $LD_{10}$ , as in the traditional method, but escalation to the next dose and subsequent doses is based on the distance of the observed AUC in humans to the target mouse  $LD_{10}$  AUC. The retrospective analysis performed by Collins et al. indicated that the sample size of phase 1 clinical trials could be reduced by up to as much as 50% by using the PGDE over the traditional design (19). Although several studies have reported success using the PGDE design, it is still not widely used in the drug development community (34). One reason for the lack of use is the presence of large interpatient variability in AUC for the same administered dose (23). For some drugs (e.g., antimetabolites and vinca alkaloids), toxicity is a function of exposure time rather than AUC, and the use of a PGDE design is not justified (35). Finally, the requirement of real-time pharmacokinetic monitoring inherent in the PGDE design has been considered a limitation to its use (34,36). Pharmacokinetic correlative studies, however, have become standard measurements in almost all oncology phase 1 trials as they help to better understand phase 1 trial outcomes.

### Continual Reassessment Method

O'Quigley et al. (37) proposed the continual reassessment method (CRM) as an alternative phase 1 study design. This phase 1 design utilizes formal statistical methods of dose-toxicity modeling to guide dose escalation. The CRM is considered superior by many because it allows the use of toxicity information gained at earlier time points of the study to assign subsequent doses. The CRM design is considered less likely to treat patients at toxic doses, and more likely to treat patients at doses considered efficacious (38). The CRM, as originally designed, works by fitting a dose-toxicity curve to the available toxicity data and assigns subsequent patients to the dose most likely to be associated with a predefined target toxicity level. Therefore, the MTD is defined as the dose estimated to produce a desired predefined toxicity rate. The estimated dose-toxicity curve is refit after the outcome of each individual patient is determined, and the next patient is assigned the dose estimated to be nearest the MTD based on the new data (38). Due to its complexity, involvement of a capable statistician is necessary in the design and execution of a CRM-designed clinical trial.

### Statistical Considerations of Phase 1 Studies

There are many designs available to estimate the MTD, as discussed previously. Two of the main design types used in practice are either algorithmic in nature (e.g., the previously described 3+3 design) or model-based designs (i.e., designs based on a statistical model). The purpose of the 3+3 design is not to produce accurate estimates of the probability of toxicity at a given dose but to quickly

identify a dose level that does not exhibit too much toxicity. An alternative to algorithmic approaches such as the 3+3 design and one more amenable to the goal of precisely estimating (i.e., estimating with more certainty) the MTD are model-based methods. The conceptual frameworks for most model-based phase 1 designs are Bayesian in nature. Bayesian designs treat the probability that a patient will experience toxicity at a given dose as a quantity about which the investigator has some degree of uncertainty. Moreover, this uncertainty is quantified via probability. The Bayesian framework provides a means by which one can learn about the toxicity rates at the different doses, and naturally make decisions based on the data observed in a sequential manner.

Using these model-based designs requires that the investigator *explicitly* specify a target probability of toxicity. The target probability of toxicity represents the rate of toxicity acceptable to the investigator. (The 3+3 design has an *implicit* target rate of toxicity of approximately 17%.) For compounds associated with very severe life-threatening toxicities, the target probability may be set by the investigator at 0.10 (i.e., 10%), whereas for other compounds with more mild toxicities it may be acceptable to set the target probability of toxicity at 0.35. As with algorithmic designs, patients are sequentially enrolled into the trial in cohorts of patients. After each cohort of patients has been evaluated for toxicity, the decision to escalate, stay or de-escalate from the current dose is based on the dose that has the expected probability of toxicity closest to the target toxicity.

An important advantage of model-based phase 1 designs is that they allow one to combine information from patients treated at different dose levels, that is, to "borrow strength," to more reliably predict what may occur at a particular dose given to a future patient. A second advantage is the ability to adjust the target probability of toxicity to match the characteristics of the compound under investigation. A third advantage of model-based methods is, unlike the 3+3 design, that the cohort size is not limited to three patients, and more important, a variable cohort size may be used. Although one could argue that algorithmic designs can also use alternative cohort sizes, the complication associated with changing the cohort size when using "X+X" algorithmic approaches (i.e., 2+2, 4+4, 5+5, etc.) is that the implicit targeted rate toxicity changes with the size of the cohort. We should note that there are other algorithmic designs which do not tie the implicit target toxicity rate to the cohort size but these methods are very rarely used and tend to place too many patients on doses which are too toxic (reviewed [39]).

Although model-based designs have been available since the early 1990s, these methods have not gained as wide an audience as biostatisticians would like. This is because it can be difficult to explain these methods to nonstatisticians and difficult to implement (40). These difficulties are being addressed by making computer code available to investigators and by providing innovative designs which target endpoints other than the typical endpoint in a classical implementation of a phase 1 oncology design. Some of these innovative designs are discussed in the following sections.

One interesting innovation is modeling time to toxicity as opposed to toxicity as a binary outcome. Using this strategy, each cohort of patients does not have to be completely followed before

the next patient or group is assigned a dose (unlike traditional designs for phase 1 clinical trials). This design has most appeal in cases where one wishes to evaluate the safety of a new compound over a long period of time (i.e., 3 months or longer). Using a traditional approach would result in trials that are excessively long. A new method, called the time-to-event continual reassessment method (41), allows patients to be entered into a trial before all patients currently enrolled have been completely observed. Using this method, a trial enrolling 24 patients in cohorts of size 3 and utilizing a toxicity assessment window of 90 days, which would take 3 years to complete using a traditional method, could be reduced to 15 months using the time-to-event method.

p0420 Another innovation includes phase 1 trials focused on schedule finding rather than dose finding. A new method based on determining the maximum-tolerated schedule (MTS) rather than a conventional MTD addresses this type of trial design (42). The method accounts for a patient's entire sequence of administrations, with the overall hazard of toxicity modeled as the sum of a sequence of hazards, each associated with one administration.

p0430 New developments that have received much research interest are adaptive Bayesian methods for dose-finding in phase 1/2 clinical trials based on tradeoffs between the probabilities of treatment efficacy and toxicity. Studies include O'Quigley, Hughes, and Fenton (2001); Ivanova (2003); Braun (2002); and Thall and Cook (2005; 43–46). These methods are most effective when toxicity and efficacy are not correlated through dose or only correlated through dose for a subset of doses. These trials are fundamentally different from the typical phase 1 oncology trial where toxicity is assumed to be correlated with efficacy through dose.

p0440 An interesting advance in phase 1/2 dose finding includes the work by Bekele and Shen (2005) in which they propose a new Bayesian approach to dose-finding in oncology trials by jointly modeling a binary toxicity outcome and a continuous biomarker expression outcome (47). They applied their method to a clinical trial of a new gene therapy for bladder cancer patients. In this trial, the biomarker expression indicates biologic activity of the new therapy. For ethical reasons, the trial is conducted sequentially, with the dose for each successive patient chosen using both toxicity and activity data from patients previously treated in the trial. The modeling framework they use naturally incorporates correlation between the binary toxicity and continuous activity outcome via a latent Gaussian variable. They show that the design reliably chooses the preferred dose using both toxicity and expression outcomes under various clinical scenarios.

p0450 The work by Bekele and Thall, in which toxicity is modeled as a set of ordinal toxicity outcomes, was successfully used to model the relationship between toxicity and dose in a phase 1 trial of gemcitabine for soft-tissue sarcoma (48). This approach was taken because in phase 1 oncology trials of new cytotoxic agents, patients typically are at risk of several qualitatively different toxicities, each occurring at several possible severity levels. The oncologists planning the trial desired to account for differences in importance among several toxicities, and they wanted the dose-finding method to use the information that a low-grade toxicity observed at a given dose is a warning that a higher grade of that toxicity is likely to occur at a higher dose. Because conventional

methods do not address these issues, they developed a Bayesian method for dose-finding based on a set of correlated, ordinal-valued toxicities with severity levels that vary with dose. They also developed a method for eliciting the set of weights quantifying the clinical importance of each level of each type of toxicity, and the physicians' target total toxicity burden.

## Pharmacodynamic Markers in Phase I Studies: Tissue Analysis

s0180

### Overview of Pharmacodynamic Markers in Tissues

s0190

In recent years, there has been significant progress in the development of drug-targeted therapies, particularly those that target receptor tyrosine kinases (RTKs; 49,50). The rapid emergence of hundreds of molecular agents against numerous targets offer greater anticancer efficacy with fewer side effects. Despite these recent advances, assessing the effects of these agents individually or in combination, or combined with conventional therapies, has created significant challenges for basic scientists and clinical investigators to effectively integrate molecular targeted therapies into clinical practice (51). Because the number of possible drug–target combinations is limitless, better strategies are needed to understand the pharmacodynamic effects of investigational agents in tumors (52). One of the most informative approaches is to implement correlative tissue-based analyses in clinical studies (53). Although tumor biopsies are not accessible in every cancer type, data obtained from correlative studies may help determine (1) whether an agent hit its intended target, (2) whether the target inhibition is transient or stable to induce apoptosis in specific cell types (e.g., in endothelial or tumor cells), (3) optimization of dosing or scheduling, (4) biomarkers that indicate which patients are most likely or least likely to respond, and (5) mechanisms of actions and resistance of single agents and their combinations. This section discusses the development of reliable assays for quantifying pharmacodynamic effects in tissues, the effects of different agents on various markers and their correlation with clinical outcome, and issues that pose challenges for incorporating tumor tissue analysis into clinical trials.

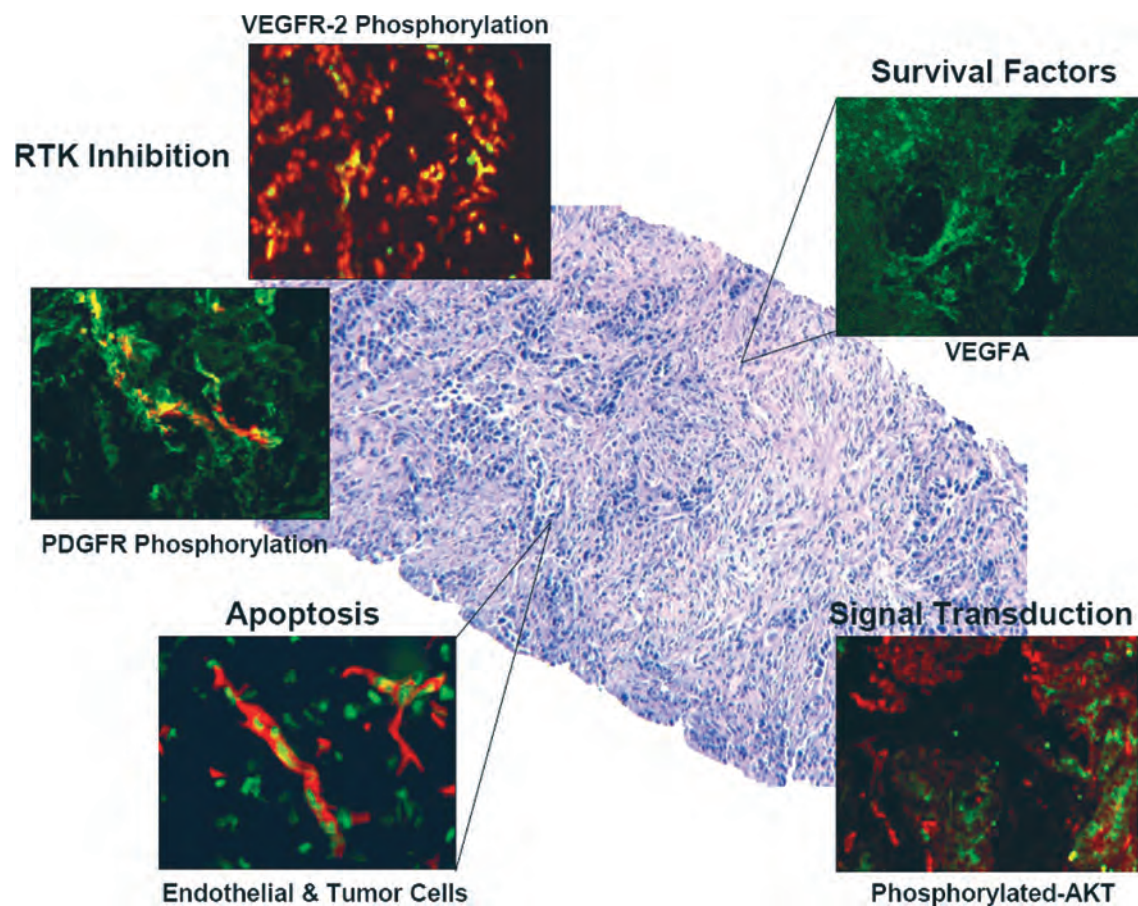
p0460

### Quantitative Analysis of Pharmacodynamic Markers in Tissues

s0200

Investigators typically rely on immunohistochemistry assays to measure the pharmacodynamic effects of molecular targeted therapies in tissues. Most studies use chromogenic or immunoperoxidase staining, which are semi-quantitative in nature and have other limitations (54). In contrast, immunofluorescence detection methods can provide simultaneous labeling of multiple proteins in one sample and a quantitative assessment using a continuous scale (55). Recent research efforts have focused on the development of immunofluorescence-based assays to quantify protein expression patterns and apoptosis in tissues for phase 1 studies (Figure 47-2; 56,57). Initially, this work focused on developing a method to detect apoptosis in endothelial cells, which requires

p0470



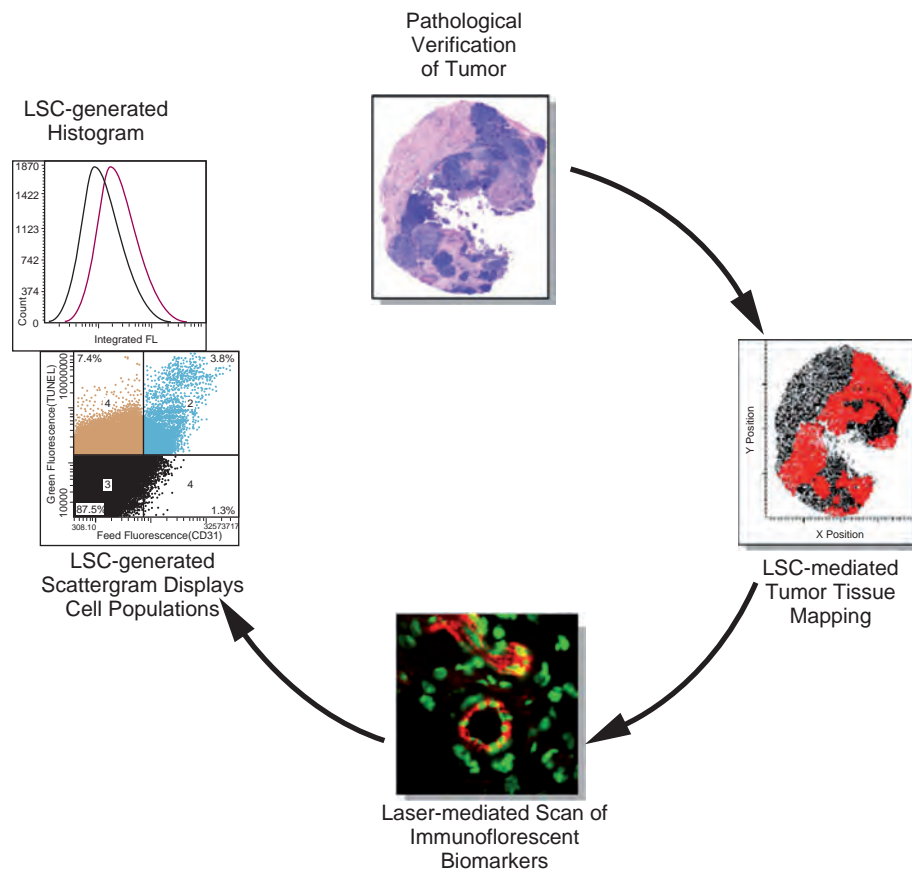
**FIGURE 47-2 PHARMACODYNAMIC ANALYSIS OF MOLECULAR TARGETED THERAPIES IN TUMOR TISSUES.** Correlative tissue studies may help determine the pharmacodynamic effects of targeted therapies on receptor tyrosine kinase phosphorylation, growth factors, signal transduction, and apoptosis in phase 1 studies. Immunofluorescence detection permits the analysis of biomarkers in specific cell types (e.g., phosphorylation of platelet-derived growth factor receptor- $\beta$  [PDGFR- $\beta$ ]) in endothelial cells. Measuring endpoints that include target or pathway inhibition linked to apoptosis may provide better evidence of the biologic effects of the drug in the tumor and correlation with clinical outcome. *Red*, endothelium; *green*, protein expression or terminal deoxynucleotidyl transferase-dUTP nick end labeling (TUNEL); *yellow*, colocalization of endothelium and protein or TUNEL.

three fluorochromes to visualize the total cell nuclei, endothelial cells, and terminal deoxynucleotidyl transferase-dUTP nick end labeling (TUNEL)-positive cells (58). Hence, multiple labeling techniques can facilitate visualization of specific cell types by eye as a result of colocalization of different fluorochromes (Figure 47-2). However, manual quantification is limited to enumerating “positive” and “negative” cells in random microscopic fields using a categorical score and may not be able to detect subtle, but significant changes (59).

Various platform technologies have been developed to facilitate quantitative in situ assessment of protein expression (60). Most of these systems are designed for standard immunohistochemistry assays using chromogenic substrates. Measuring the pharmacodynamic effects of molecular targeted therapies requires the ability to detect specific cell types (e.g., endothelial cells) and quantify their protein expression patterns. One platform technology capable of quantifying multiple fluorochromes in fixed tissue specimens is the laser scanning cytometer (LSC). The LSC platform is an automated analysis system described as a cross between a flow and a static image cytometer. Lasers are used to simultaneously excite different fluorochromes in cellular specimens that emit discrete wavelengths detected by a set of photomultiplier tubes.

Together these features permit the ability to generate high-content stoichiometric data on heterogeneous populations of large numbers of cells. Thus, the LSC is used much like a flow cytometer to obtain multicolor immunofluorescence intensity information on fixed specimens.

Several phase 1 studies have incorporated LSC-mediated analysis to determine drug-target interactions, effects on downstream signaling pathways, and rates of apoptosis in skin and tumor tissues (55–57,61). Because the LSC is a platform technology, many different applications can be developed to exploit its inherent capabilities. Research efforts have been focused on developing specific tissue-based applications using LSC technology in an attempt to standardize the methodology for consistent data generation that can be compared between different tissue specimens and molecular targeted therapies. Although LSC-mediated data acquisition is automated, the process requires a systematic interactive approach to maintain high quality control standards and ensure consistent data generation (Figure 47-3). Pharmacodynamic data generated using a process to analyze markers in entire tumor tissue cross sections has consistently provided biologic evidence of the effects of targeted therapies and correlation with clinical outcome (55,62,63). A summary of phase 1 studies of targeted therapies



**FIGURE 47-3** Quantitative analysis of pharmacodynamic effects in tissues using laser scanning cytometer (LSC) technology. Pathologic verification of biopsy samples is essential for mapping tumor regions and excluding normal and necrotic regions from the analysis. Lasers detect individual cells within the mapped region of interest based on immunofluorescence staining. LSC-generated scattergrams display the percentage of cell populations based on user defined gating using controls (e.g., apoptotic endothelial cells). Alternatively, protein expression levels (e.g., phosphorylated VEGF receptor-2) measured by mean fluorescent intensity may be determined as shown in the histogram. (Immunofluorescent image courtesy of Eaton Publishing, Westborough, MA.)

f0030

that have incorporated pharmacodynamic analysis of tissues, their effects on various pathways and correlation with clinical outcome are shown in Table 47-2.

### s0210 **Pharmacodynamic Analysis of Receptor Tyrosine Kinase Targeted Therapies**

p0500 Aberrant expression of cell-surface RTKs (e.g., epidermal growth factor receptor [EGFR]) plays a pivotal role in the progression of cancer (64). Drugs that target RTKs are designed to block the intrinsic enzymatic activity that catalyzes the transfer of the gamma-phosphate of ATP to tyrosine residues in protein substrates (65). Inhibiting phosphorylation of these tyrosine residues prevents downstream signaling events, which affect cellular function (e.g., proliferation, differentiation, migration or apoptosis; 66). Thus, the ability to measure phosphorylation status and signal transduction pathways has become an important pharmacodynamic endpoint in clinical studies.

### s0220 **Zd1839**

p0510 ZD1839 (Iressa, Gefitinib) was the first in a new class of small, molecular-targeted therapies against EGFR to gain market approval (based on two phase 2 studies) for non-small cell lung cancer (NSCLC; 67,68). Although the phase 2 studies did not incorporate correlative tissue studies, two different phase 1 studies of ZD1839 demonstrated that pharmacodynamic endpoints

can be measured in both tumor and skin tissues. In a metastatic colorectal cancer trial, total EGFR was detected by immunohistochemistry in all 16 pretreatment tumor biopsies (69). Total EGFR levels remained unchanged in seven of ten patients after treatment with ZD1839 whereas the other three demonstrated a decrease. Interestingly, two of the patients whose EGFR levels decreased displayed a large increase in apoptosis. Phosphorylation of EGFR was also measured, however, it was reported that detection was reproducible in only one patient and that this patient displayed complete inhibition after treatment with ZD1839. Other downstream markers of the EGFR signaling pathway were measured including phosphorylation of AKT and extracellular receptor kinase (ERK), p27<sup>Kip1</sup>, and  $\beta$ -catenin expression. Phosphorylated-AKT and ERK (in tumor cells) were decreased in two and one patients, respectively. However, phosphorylated-ERK in tumor stromal fibroblasts was lower in five of nine patients after ZD1839 therapy. Daneshmand and coworkers suggested that it is likely other patients had activated EGFR and AKT but at levels that were below the detection limit of standard immunohistochemistry. The cyclin-dependent protein kinase p27<sup>Kip1</sup> was not detected in seven of nine patients, in part because EGFR activation promotes degradation of p27<sup>Kip1</sup> (70). After ZD1839 therapy, levels of p27<sup>Kip1</sup> increased in two patients, whose tumors also displayed an increase in apoptosis. ZD1839 treatment did not affect  $\beta$ -catenin expression in three of the paired samples that were evaluated. The proliferation index, measured by Ki67, was the only marker in this study that significantly correlated with change in tumor burden. Interestingly, the two patients who

10020 **Table 47-2** Analysis of Pharmacodynamic Markers in Tissues from Phase 1 Clinical Studies

Agent <sup>Reference</sup>	Target	PD Markers	Change in Expression on Therapy	Correlation	Response
ZD1839 <sup>69</sup> (Iressa, Gefitinib) Metastatic Colorectal Cancer	EGFR TKI	EGFR pEGFR pAKT pERK p27 Beta-Catenin TUNEL Ki67	7 of 10 No Change, 3 Decrease 1 of 16 Decrease 2 of 10 Decrease 5 of 9 Decrease in Tumor Fibroblasts 2 of 9 increase in Nucleus 2 of 9 No Change 6 of 10 Increase, 2 No Change, 2 Decrease 8 of 10 Decrease*	None None None None None None None None With tumor burden	None
ZD1839 <sup>71</sup> (Iressa, Gefitinib) Metastatic Breast Cancer	EGFR TKI	<i>Tumor</i> ERBB2 EGFR	No Change No Change	None None	2 PR, 7 SD
ZD1839 <sup>72</sup> (Iressa, Gefitinib) Advanced Solid Tumors	EGFR TKI	<i>Skin</i> EGFR pEGFR pMAPK pSTAT3 p27 TUNEL Ki67	No change Decrease* Decrease* Increase* Increase* Increase* Increase* Decrease*	None Compared to Pretreatment Compared to Pretreatment Compared to Pretreatment Compared to Pretreatment Compared to Pretreatment Compared to Pretreatment	7 SD
EMD 72000 <sup>132</sup> Advanced Solid Tumors	EGFR mAB	<i>Skin</i> EGFR p-EGFR pMAPK pSTAT3 TGF-alpha p27 Ki67	No change Decrease* Decrease* Increase* No change Increase* Decrease*	None Compared to Pretreatment Compared to Pretreatment Compared to Pretreatment None Compared to Pretreatment Compared to Pretreatment	5 PR, 6 SD, 1 MR
SU5416 <sup>133</sup> Advanced Solid Tumors	VEGFR-2 TKI	<i>Tumor</i> MVD	5 or 19 Increase	None	4 SD
SU6668 <sup>55, 61</sup> Advanced Solid Tumors	VEGFR-2 and PDGFR TKI	<i>Tumor</i> pVEGFR-2 EC & TC pPDGFR EC TUNEL TC TUNEL MVD	1 of 6 Decrease 1 of 6 TC Decrease No Change No Change No Change	Transient target inhibition Transient target inhibition Transient target inhibition Transient target inhibition Transient Target Inhibition	None
Endostatin <sup>134</sup> advanced solid tumors	Endogenous angiogenesis inhibitor	<i>Tumor</i> MVD CD31 + Ki67 vWF + TUNEL <i>Skin</i> MVD CD31 + Ki67 vWF + TUNEL	No Change No Change Rare Event No Change Slight Decrease Rare Event	None None None None None None	SD
Endostatin <sup>56, 75</sup> advanced solid tumors	Endogenous angiogenesis inhibitor	<i>Tumor</i> MVD CD31 + TUNEL BCL-2 HIF-1 TC + TUNEL	Decrease* Increase* No Change No Change No Change	With optimal dose & response With optimal dose & response None None None	1 PR, 1 SD
CI-1040 <sup>78</sup> Advanced Solid Tumors	MAPK 1 & 2	<i>Tumor</i> pERK	Decrease	Target Inhibition, Median 73%	1 PR, 19 SD

**Table 47-2** Analysis of Pharmacodynamic Markers in Tissues from Phase 1 Clinical Studies—Continued

Agent <sup>Reference</sup>	Target	PD Markers	Change in Expression on Therapy	Correlation	Response
PS-341 <sup>82</sup> (Bortezomib) Advanced Solid Tumors	PS-341 Reversible Proteasome Inhibitor	<i>Tumor</i> p27	1.27-fold Increase	Confirmed Therapeutic Response	1 PR, 1 SD
BMS-214662 <sup>86</sup> Advanced Solid Tumors	Farnesyltransferase Inhibitor of H-ras K-ras	<i>Tumor</i> Total & p-MAPK  Total & p-AKT p27 Ki67 TUNEL Caspase 3 & 9	None  None None None Increase* Increase*	None  None None None With Dose and Schedule With Dose and Schedule	5 SD
17-AAG <sup>135</sup> Advanced solid tumors	HSP90 - ATP Inhibitor	<i>Tumor</i> HSP70  CDK4 RAF-1	Increase  Decrease Decrease	Identified optimal schedule	2SD
Ad5CMV-p53 <sup>136</sup> Advanced solid tumors	Adenoviral Vector Containing Wild-Type p53 Gene	<i>Tumor</i> p53 <i>Skin</i> p53	6 of 7 Increase  1 of 4 Increase	Confirmed delivery  Confirmed delivery	1SD

MR, major response; PR, partial response; SD, stable disease; TKI, tyrosine kinase inhibitor.  
\* Significant change.

had the largest decrease in Ki67 also showed the largest increase in apoptosis. In another phase 1 study of ZD1839 in metastatic breast cancer, comparison of pre- and post-treatment ERBB2 and EGFR values was not statistically significant between the subgroups of patients regarding responsiveness to treatment (71).

Serial skin biopsies have been analyzed as potential surrogate tissues for monitoring the biologic effects of molecular targeted therapies. A phase 1 study of ZD1839 in advanced solid malignancies incorporated skin, but not tumor, biopsies to determine effects on EGFR signaling (72). Levels of phosphorylated-EGFR expression were completely inhibited, however, no changes in total EGFR expression were observed after treatment. Other downstream markers in the EGFR network were affected by ZD1839 including phosphorylated-Ras-mitogen-activated protein kinase (MAPK) and STAT3, Ki67, p27<sup>kip1</sup>, and apoptosis (Table 47-2). Although significant changes were observed in almost all of the markers when comparing pre- and post-treatment skin biopsies, none of the changes correlated with dose or clinical response.

## Pharmacodynamic Analysis of Signal Transduction Inhibitors and Other Targets

### Endostatin

Endostatin is a COOH-terminal cleavage fragment of collagen XVIII that can function as an endogenous inhibitor of tumor angiogenesis (73,74). Endostatin's receptor(s) has not been isolated, and its mechanism of action remains obscure. Low levels of apoptosis were observed in a phase 1 dose-finding study (15–600 mg/m<sup>2</sup>) of endostatin, and it was apparent that more sensitive quantitative methods were required (57,75). Using the LSC-mediated quantitative analysis technique, it was demonstrated that the average level of apoptosis significantly

increased from 0.2% to 1.1% after endostatin therapy (56). Although the levels of apoptosis were low, a fivefold increase is comparable to tumor cell lines exposed to cytotoxic agents in vitro. In addition, significant decreases in microvessel density were observed following endostatin treatment. Effects on the vasculature were quantified using an innovative technique to enumerate microvessels in an entire tissue cross-section. Other parameters quantified by LSC that did not show significant changes with dose were BCL-2 and hypoxia-inducible factor-1 $\alpha$ . Intriguingly, the changes in endothelial cell apoptosis and microvessel density were consistent with significant decreases in blood flow measured by positron emission tomography. Together, these data strongly suggested that endostatin had optimal biologic activity at doses of 250 mg/m<sup>2</sup> in this cohort of patients (56).

### Ci-1040

MAPK plays a key role in mediating growth-promoting signals from multiple growth factor receptors. Specifically, MAPK catalyzes the phosphorylation of its substrates, ERK-1 and ERK-2 (76). CI-1040 is a highly potent and selective inhibitor of both MAPK isoforms as evidenced by an IC<sub>50</sub> (concentration associated with 50% inhibition of MEK) of 17 nmol/L against purified MEK1 (77). In a phase 1 study of CI-1040 (78), reduced levels of phosphorylated-ERK in tumor tissue of 50% were used as part of the decision to continue clinical development of the compound. This development objective was achieved based on the 73% median decrease in phosphorylated-ERK observed in ten patients.

### Ps-341

The 26S proteasome is a multicatalytic protease that selectively degrades polyubiquitinated proteins, primarily short-lived intracellular

p0580 proteins that regulate cell cycle, tumor growth, and survival (79,80). Inhibition of the 26S proteasome results in the disruption of cell cycle checkpoints and apoptosis pathways. PS-341 is a potent and specific (Ki 0.6 nmol/L) reversible proteasome inhibitor that was rapidly approved (4.5 years) for the treatment of multiple myeloma (81) and is under clinical development for solid tumors. A phase 1 study was conducted to evaluate pharmacodynamic endpoints comparing two different schedules (82). Unfortunately, tumor tissue was limited and only one evaluable patient demonstrated a 27-fold increase in p27<sup>Kip1</sup> expression after PS-341 treatment. These data combined with other pharmacodynamic data obtained from peripheral blood mononuclear cells correlated with 70% inhibition in proteasome activity and provided evidence of PS-341-induced biologic effects in the tumor.

#### s0270 **BMS-214662**

p0560 Ras proteins play a key role in a large number of cellular processes including growth, differentiation, apoptosis, membrane trafficking, and cytoskeletal organization (83,84). After synthesis, these proteins are post-transcriptionally modified by the farnesyltransferase enzyme to a more hydrophobic state that permits its localization to the cytoplasmic membrane to mediate RTK signaling. BMS-214662 is a small-molecule inhibitor of human farnesyltransferase of both H-ras and K-ras, with an IC<sub>50</sub> of 1.3 and 8.4 nM and an IC<sub>90</sub> of 18 and 108 nM, respectively (85). Taberero and coworkers assessed the effects of BMS-214662 on signal transduction proteins hypothesized to play a key role in signaling in tumor tissues (86). Surprisingly, BMS-214662 did not induce changes in total MAPK or AKT, phosphorylation of MAPK and AKT, p27<sup>Kip1</sup>, or Ki67. In contrast, a strong increase in tumor cell apoptosis was observed as evident from an increased expression of cleaved caspase 3 and 9, and TUNEL. Together, these data suggest that BMS-214662 acts through a Ras-independent pathway similar to other farnesyltransferase enzymes (87,88). Although the levels of apoptosis correlated with schedule and dose, there was no direct correlation with clinical benefit. These data are consistent with the fact that no objective tumor responses were observed.

#### s0280 **Challenges and Perspectives**

p0570 There are many challenges to successfully incorporating tissue analysis in the design of a clinical study. Acquiring the tissue alone requires the commitment of the sponsor, scientists, oncologists, interventional radiologists, committees, and patients. Standardization of tumor sampling and tissue procurement is critical to ensure that quality tumor tissue is being evaluated. A lack of quantitative standardization among different assays may lead to unintentional interpretation and variability between laboratories. Other issues that may impact interpretation of pharmacodynamic data are intra- and intertumor heterogeneity, tissue microenvironment (skin vs. tumor), compensatory mechanisms, and timing of biopsies after initiation of therapy and after the last dose. It is worth emphasizing that few studies have attempted to link target or pathway inhibition with tumor cell apoptosis. It is possible that some agents may demonstrate transient target inhibition, but fail to induce apoptosis (55). Thus, measuring pharmacodynamic endpoints that include target or pathway inhibition linked to cellular fate (e.g., apoptosis) may provide better evidence of the biologic effects of the drug in the tumor.

Pharmacodynamic analysis of tumor tissues can provide direct proof of whether an investigational agent affected its intended target and downstream consequences on signal transduction and apoptosis, however, they are also limited. Recent studies have demonstrated that skin may serve as a surrogate tissue to confirm drug-target inhibition, signal transduction, and kinetics in clinical studies. However, analysis of biomarkers in tumor tissues may better represent the biologic effects of a targeted therapy as tumor cells often respond differently compared to normal cells. More quantitative studies are needed to identify reliable biomarkers and their correlation between the effects in skin, tumor, and clinical outcome. Another promising surrogate source that could potentially be used to assess the effects of targeted agents is the circulating tumor cell or endothelial cell. These cells may better represent the tumor microenvironment and are now being routinely isolated for a variety of applications (88). Our ongoing research efforts are aimed at developing assays to analyze the pharmacodynamic effects of circulating tumor and endothelial cells. Furthermore, pharmacodynamic studies in tumor tissue may also identify the genomic and proteomic profile of the population with the greatest chance to benefit from treatment. For example, the therapeutic activity of Herceptin would likely have been missed if patients had not been pre-selected based on their HER2 status.

Clearly, there is a need for better strategies to assess the effects of molecular targeted therapies early in clinical development. For example, in a phase 1 trial of bevacizumab, no objective responses were observed out of 25 patients (89). It was not until a series of randomized phase 2 and 3 trials over a period of more than 5 years that the clinical activity of bevacizumab was established. However, it is generally not practical to perform large randomized trials for drugs without evidence of biologic activity early in their development, and therefore, many promising drugs may not be developed. Given the large number of targeted therapies entering clinical testing, it is crucial that phase 1 studies incorporate correlative endpoints to determine optimal dosing and scheduling for phase 2 and 3 trials. Ultimately, clinical development of targeted therapies would benefit if the recommended dose was identified early and actually known to inhibit the target for which it was designed.

#### **Imaging Techniques in Phase 1 Studies**

A variety of imaging techniques can play an important role in phase 1 studies of anticancer drugs when used as an objectively measured indicator of a biologic/pathobiologic process or pharmacologic response to treatment (i.e., as a biomarker [90]). Imaging biomarkers can be used to determine if the drug is hitting the target, if it has the anticipated biologic activity, and can also provide an early indication of whether or not the new agent has clinical activity (91). The information provided by imaging biomarkers, taken together with information from molecular biomarkers and clinical pharmacology, provide the input required to determine how aggressively to pursue development of a particular drug or a backup drug for a given target. For example, if there is no evidence for the anticipated biologic activity of a drug candidate, evidence that the drug did not hit the target (or only at an insufficient level) would support

development of a back-up drug. However, if the candidate drug does hit the target, it would not make sense to pursue development of a backup drug. In addition, imaging biomarkers can assist in the selection of the dose and/or schedule for phase 2 studies (92).

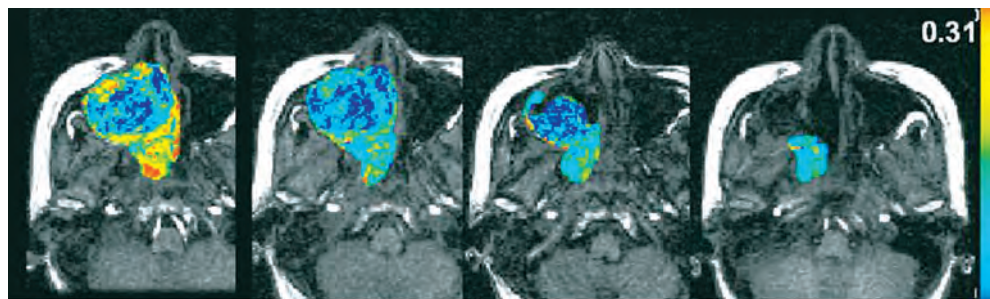
p0610 The ability to detect labeled drug at thousand-fold lower concentrations than needed to produce pharmacodynamic effects makes nuclear medicine the best suited modality for determining if the drug is hitting the target (93). Small molecules can be labeled with the positron emitting nuclides  $^{11}\text{C}$  or  $^{18}\text{F}$  and larger molecules (e.g., proteins or antibodies) can be labeled with the positron emitting nuclides  $^{124}\text{I}$  or  $^{64}\text{Cu}$  (94–97). Alternately, other specific receptor ligands can be labeled with radionuclides and used to measure receptor occupancy (98). Although none of these methods have been used to a great extent in phase 1 oncology studies to date, they could provide a powerful approach for obtaining information about how much drug reaches the target. It should be noted that the Pharmacodynamic/Pharmacokinetic Technologies Advisory Committee of Cancer Research United Kingdom recently argued that measuring downstream biologic effects are likely to be more cost-effective than those that measure specific molecular targets (99). This concern may be most relevant for small therapeutic molecules, since each molecule may require unique labeling methods and some may not be amenable to radiolabeling. However, the methods used for labeling biomolecules are more general, so may be more readily applied to a variety of drugs. This is of particular importance given the greater concern regarding delivery of macromolecules to solid tumors (100).

p0620 A number of imaging modalities can be used to determine if the drug has the anticipated biologic activity. The more commonly used methods are dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and [ $^{18}\text{F}$ ] fluoro-2-deoxyglucose (FDG) positron emission tomography (PET). Other promising imaging biomarkers under development for oncology include  $^{11}\text{C}$ -thymidine, FLT or FMAU for proliferation (101), Annexin-V or specific caspase-3 activity tracers for apoptosis (102,103), diffusion MRI for cellularity (104,105), and labeled RGD (106),  $\alpha_v\beta_3$  (107,108), or tracers for other specific vascular targets (109). These pharmacodynamic biomarkers promise to provide a valuable set of measures of downstream biologic effects for nearly any molecular target.

p0630 DCE-MRI, which uses a commonly used contrast agent (gadopentetate dimeglumine), has been implemented in several phase 1 studies to quantify the effects of antivascular agents on the tumor blood supply within hours to days after the start of treatment (110). Various analytic approaches have been used to quantify variables reflecting blood flow (F), vessel permeability surface area (PS), and the contrast distribution volume (generally assumed to reflect extravascular-extracellular space) from DCE-MRI data (111). Generally, treatment-induced changes in these variables reflect changes in F and/or PS (112). Substantial decreases in F and/or PS are induced both by vascular targeting agents (113–115) and by tyrosine kinase inhibitors (116,117). For VEGF-targeted agents, it appears that a substantial decrease in F and/or PS is necessary, but not sufficient for a significant reduction in tumor size (116,117). Interestingly, for vascular targeting agents, a similar reduction in F and/or PS is not associated with a reduction in tumor size. It is also worth noting that initial use of DCE-MRI in phase 1 studies were single-center studies, at sites

with considerable DCE-MRI expertise, raising concern about the ability to use this approach more generally (92). However, one recent study included three centers, without specific DCE-MRI expertise at all sites, demonstrating that the methodology can be standardized to yield consistent results in an early clinical trial at multiple institutions (116,118,119). In the study, DCE-MRI was used to help measure the pharmacodynamic response to acute dosing of AG-013736, a novel angiogenesis inhibitor, to identify suitable markers of biologic activity to assist in optimizing the dose and schedule of therapy. Thirty-six patients with advanced solid tumors were treated with various doses of AG-013736. In addition to standard measures of objective disease response and pharmacokinetic analysis, DCE-MRI scans were acquired at baseline and repeated cycle 1, day 2 after the scheduled morning dose of the AG-013736 in 26 patients. Indicators of a vascular response, such as the volume transfer constant ( $K^{\text{trans}}$ ) and initial area under the curve (IAUC), were calculated to assess the effect of treatment on tumor vascular function. In this study evaluable vascular response data was obtained in 17 (65%) of 26 patients. An example of a scan in a responding patient with adenocarcinoma is shown in Figure 47-4. A linear correlation was found in which the percentage change from baseline to day 2 in  $K^{\text{trans}}$  and IAUC was inversely proportional to AG-013736 exposure (Figure 47-5). Using a conservative a priori assumption that a more than 50% decrease in  $K^{\text{trans}}$  was indicative of an objective vascular response, a 50% decrease in  $K^{\text{trans}}$  was achieved and corresponded to a plasma  $\text{AUC}_{0-24}$  of more than 200 (ng.hour/mL). Hence, although a sufficient decrease in tumor vascular parameters was observed at a dose chosen for further phase 2 testing by conventional toxicity criteria, the day 2 vascular response measured using DCE-MRI appears to be a useful indicator of drug pharmacology. Further research will be needed to determine if it is a suitable marker for predicting clinical activity.

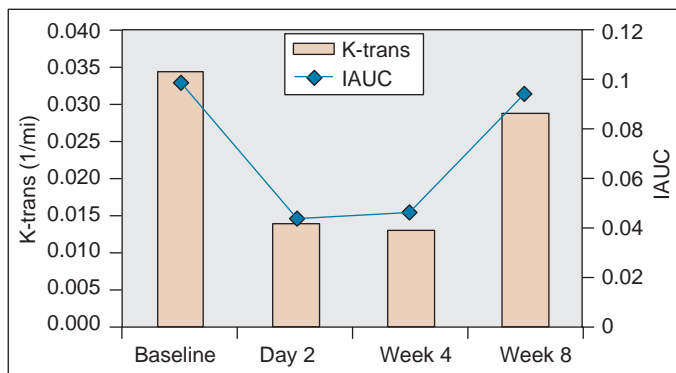
p0640 FDG-PET uses an  $^{18}\text{F}$ -labeled glucose analog (FDG) that is transported into cells by GLUT-1 and GLUT-3, phosphorylated by hexokinase and, since FDG-6-P is a poor substrate for glucose-6-phosphatase, there is little dephosphorylation and the radioactivity is trapped in the cell (120–122). Glucose metabolism is quantified as the activity in the tumor, normally restricted to the region of highest activity, relative to the amount of activity injected and patient's body weight, the so-called standardized uptake value (SUV). The potential for FDG-PET to assess drug-induced biologic effects prior to a change in tumor size was illustrated clearly in patients with advanced gastrointestinal stromal tumors treated with imatinib mesylate (123). Tumor FDG activity decreased markedly from baseline as early as 24 hours after a single dose of imatinib in all patients demonstrating a response by CT or MRI weeks later. Conversely, increased tumor FDG activity, activity at new sites, or both were seen in all patients with disease progression evident at a later date by conventional means. It should be noted that it has not been established whether these dramatic early decreases in FDG activity are due to decreased glucose metabolism (generally associated with viable tumor cells) or decreased glucose transport due to translocation of the glucose transporters from the cell membrane to the cytosol (124). Moreover, although a variety of treatment regimens result in reduced FDG activity following the



**FIGURE 47-4** Representative dynamic contrast-enhanced magnetic resonance images from a patient with adenoid cystic carcinoma showing a decline in tumor perfusion after exposure to AG-013736. The tumor initial area under the curve (IAUC) values are mapped over the tumor region (shown quantitatively in the graphs below).

f0040

Baseline Day 2 Week 4 Week 8

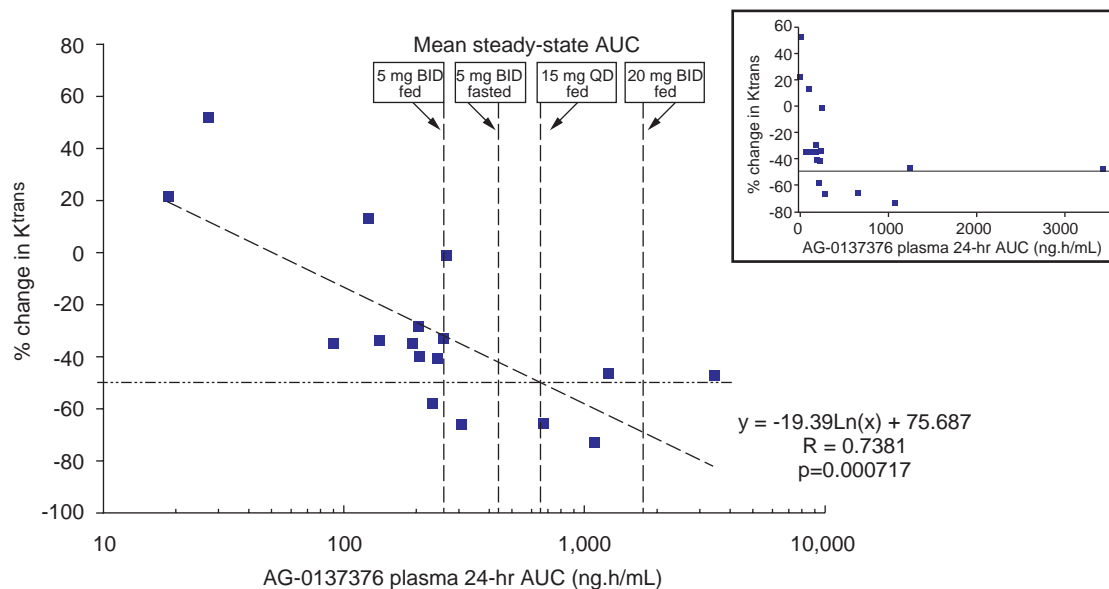


first cycle of therapy, after macrophage activity (which can result in increased FDG uptake) has subsided, yet before response is evaluable by standard methods (120), such dramatic effects are not generally observed so early after treatment. Nonetheless, FDG-PET shows considerable promise to provide an indication of decreased tumor viability prior to conventional methods and may provide a valuable downstream biomarker for biologic activity in phase 1 trials.

Although it is not reasonable to expect clinical efficacy in the advanced-stage patients entered into phase 1 trials and assessment of clinical response is not a primary focus of phase 1 trials, any indication that the drug/target impacts tumor growth is beneficial. Typically, tumor burden is assessed using computed tomography (CT) or MRI data. The method most commonly used to assess clinical effect is based on response evaluation criteria in solid tumors (RECIST), which was put forth in

p0650

Change in dce-MRI  $K^{trans}$  vs AG-013736 plasma exposure



**FIGURE 47-5** Correlation between plasma exposure of AG-013736 and change in  $K^{trans}$  in patients undergoing serial dynamic contrast-enhanced magnetic resonance imaging. The mean plasma concentrations of AG-013736 obtained in each dosing cohort are shown over the plot as a point of reference.

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2000 as a simpler way to measure the response of tumors to experimental treatments (125). It should be noted that, in practice, RECIST are generally modified to address some of the concerns raised by the International Cancer Imaging Society (ICIS) regarding the strengths and weaknesses of using the RECIST criteria and what other issues should potentially be added to a response criterion (126). Nonetheless, even with these changes, concerns remain regarding RECIST especially in the context of early-phase trials (127). One point of particular concern is whether the single longest tumor dimension, determined in an axial plane, accurately represents changes in tumor burden since most tumors grow and regress irregularly (128). Another concern is how relevant the categoric response assessments (complete response, partial response, stable disease, and progressive disease), which were originally based on the error in oncologists' physical measurements of solid spheres arranged in random size order on a soft mattress and covered with a layer of foam rubber, are in the context of early-phase trials (129,130). It seems an alternative model, where response is considered a continuous variable, the change in tumor size (estimated as the single longest dimension, the cross-product of the longest dimension, and the perpendicular longest dimension or volume) after treatment (131), would be much more useful for evaluating clinical effect in phase 1 trials.

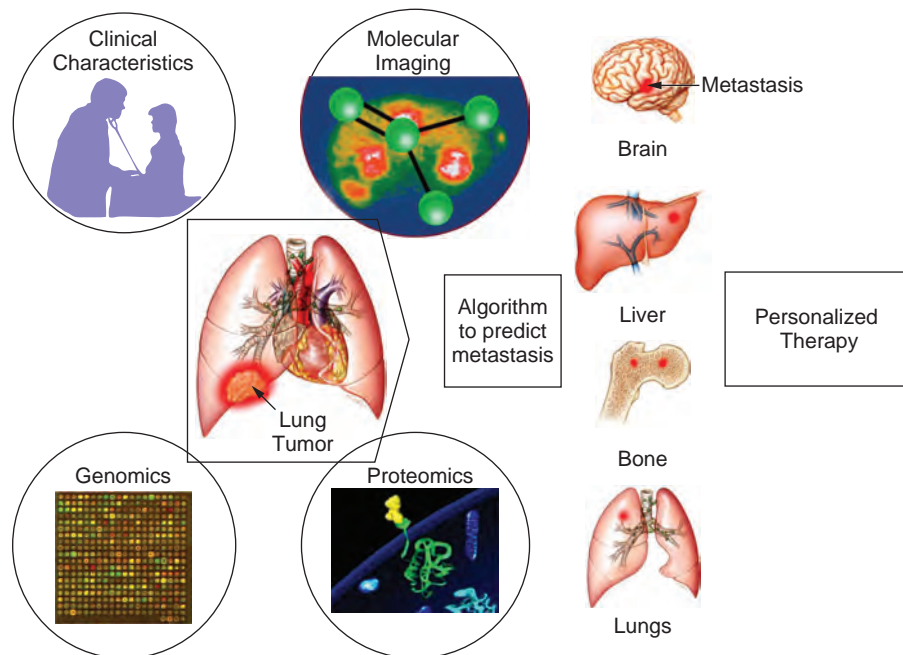
patient care, and local and systemic therapies, most deaths from cancer are still due to metastasis that are resistant to conventional treatment. Novel therapeutic approaches are critically needed if we are to impact positively on patient outcome. Phase 1 studies are the critical link in targeting cancer, since they represent the first translation of years of laboratory/preclinical studies to the patient.

As drug development has evolved to a more tumor-targeted, or tumor-specific focus, so has the evolution of phase 1 trials moved from the more generic, mathematical modeling to the more rational design. In addition, it is increasingly being recognized that incorporation of select endpoints relative to patient eligibility in phase 1 trials are needed to more effectively and efficiently develop drugs clinically. Our current classification of most cancers is still based in large part on tissue type, tumor size, nodal status, and metastatic sites.

The future of tumor classifications is evolving as measurements such as molecular classifiers, genomics and proteomics become increasingly utilized not only preclinically, but also in the clinical arena to help better understand the molecular pathways that are aberrant in any given cancer cell (Figure 47-6). Several phase 1 designs are incorporating these tools, not so much as response predictors, but to help determine feasibility and to develop diagnostic/predictive tools for future clinical use. The hope is that a more personalized approach to clinical care will increase the efficacy of treatment, while decreasing its toxicity and cost. The result is the development of phase 1 trials aimed not only at defining dose and safety, but also at assisting in target validation. The latter will become increasingly important as we develop newer methods for targeting cancer.

**Conclusion**

Cancer remains a major public health problem. Despite significant improvements in diagnosis, surgical techniques, general



**FIGURE 47-6** An algorithm of relevant proteomic, clinical, and imaging factors plus genomic factors all indicating a poor prognosis (metastasis) and best choice of molecular-targeted chemotherapy.

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