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Pharmacodynamic Endpoints as Clinical Trial Objectives to Answer Important Questions in Oncology Drug Development

Ralph E. Parchment¹ and James H. Doroshow²

¹Clinical Pharmacodynamics Program, Applied/Developmental Research Directorate, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702

²Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Maryland 20892

Drug Mechanism of Action: Fundamental Knowledge for Developing Therapeutics

Since the earliest days of oncology drug development, mechanism of action (MOA) has served as a guiding principle for: (i) selecting experimental agents with novel MOAs to advance into clinical trials, (ii) setting dose schedules of investigational agents, (iii) selecting patients who enrich early clinical trial populations with potential responders, and (iv) combining drugs with non-cross-resistant MOAs to generate new regimens. The use of pharmacodynamics (PD) during clinical drug development primarily involves the strategy and timing of using MOA knowledge to complement, but not supplant, clinical information in decision making—a form of “rational drug development” that measures the actual molecular target response instead of predicting it from estimates of drug concentration in the tissue [1-3]. Drug development is generally more likely to succeed if the preclinical MOA has been confirmed in patient tumors, so it seems illogical to accept the preclinical MOA *carte blanche* when clinical confirmation in different genomic contexts is possible. Conversely, development is more likely to stall if the MOA of the investigational agent in human patients and preclinical models is different yet this difference never recognized. Thus, in a wide variety of scenarios, the use of PD tools to confirm the preclinical MOA in patients enhances the drug development process.

Because performing invasive biopsies for MOA and other clinical PD studies is a research activity but not a diagnostic one, this process cannot inform the medical treatment of an

Corresponding Author: James H. Doroshow, Division of Cancer Treatment and Diagnosis, National Cancer Institute, NIH, Bldg. 31, Room 3A-44, 31 Center Drive, Bethesda, Maryland 20892. Phone: (301) 496-42919, doroshoj@mail.nih.gov.

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individual patient. Thus, it is ethically imperative that the value and utility of the acquired MOA knowledge justify the increased risk to the patient from the research biopsies. The crux of the matter is that this knowledge is valuable and useful only when it answers key questions and correctly informs the development of an investigational agent [4], so the only ethical PD studies are those conducted with methodology of sufficient quality to provide reliable and relevant measurements. The primary ethical responsibility for the clinical PD study lies with the team that will collect and analyze the research biopsy samples to obtain this knowledge. As described in detail in the following sections, well-designed PD studies that use robust, fit-for-purpose measurement tools to confirm drug MOA in human tumors create an ever-expanding platform of translational and clinical knowledge that is sufficiently reliable for conceiving, building, and prioritizing new clinical trials and drug regimens.

High-Value Applications of Clinical PD in Oncology Drug Development

Clinical PD assessment usually begins by seeking proof-of-mechanism (POM) evidence in tumor biopsy samples. This fundamental knowledge of the full PD response can be applied in many ways to advance and enhance oncology drug development, including: (i) optimal scheduling of targeted agents based on molecular response rather than plasma pharmacokinetics or toxicity; (ii) using the biologically effective dose (BED) rather than maximum tolerated dose (MTD) to achieve a safe yet maximum effect on the molecular target in tumor; (iii) creating new, mechanistically based drug combinations capable of directly and safely treating the multiple signaling defects that drive many malignancies; and (iv) developing protein biomarkers as improved diagnostics for patient selection. Building on the preceding articles in this issue of *Seminars*, the following sections describe several of the potentially high-impact applications of clinical PD.

Proof of Clinical Mechanism of Action

Clinical POM is usually the first application of PD measurements in oncology developmental therapeutics because verifying the MOA of a new compound as it emerges from preclinical development is a foundational step toward its full pharmacological characterization. The inability to confirm the MOA of an investigational agent—or actually disproving the MOA by observing efficacy in the absence of molecular target modulation—means that further development of the agent must be entirely empirical. Additionally, neither the optimization of dosing schedule nor the discovery and evaluation of candidate drug combinations using PD biomarkers is possible without establishing this fundamental property.

This *Seminars* edition is focused on molecular PD, so POM herein refers to confirming that an investigational agent engages its intended molecular target, resulting in the desired alteration of target function. For first-in-human studies, this will most likely be the mechanism of action assigned to the investigational agent during preclinical development. If efficacious, a drug's action on its molecular target should trigger sequential biochemical, cellular, and, ultimately, multi-cellular physiological responses, with the latter manifesting as a clinical response. The sequential steps of an unfolding PD response can be divided into

primary (1°), secondary (2°), and tertiary (3°) PD effects, and each of these effect levels requires different PD biomarkers and likely different time points for study.

The 1° PD effect is the first action of a drug on the biological system; for example, the clinical POM for imatinib was assay-based evidence of reversible inhibition of Bcr-Abl kinase activity [5]. A 1° PD effect is considered evidence of target engagement, i.e., proof that the drug is interacting with a target and affecting its function. Without this step, no other molecular, biochemical, or physiological changes should occur; if they do, then one must conclude that there are off-target drug effects—i.e., engagement of unintended target(s). The 1° PD effect can be any change in the reaction product of any targeted enzyme: for example, an autophosphorylation site of a receptor tyrosine kinase or a phosphoprotein product of a tyrosine kinase. The NCI conducted the first Phase 0 clinical trial in oncology, under the exploratory investigational new drug (xIND) mechanism, using a measured decrease in the reaction product of poly(ADP-ribose) polymerase (PARP) 1/2 catalytic activity to demonstrate the MOA of veliparib [6].

In addition to examining 1° effects by measuring changes in activity of the drug target, POM studies often include evaluation of predicted drug-induced 2° effects: i.e., the biochemical changes occurring immediately downstream of the intended molecular target, such as a reduction in phospho-ERK levels after drug inhibition of Raf kinase activity. Subsequent cell biological or physiological responses to these biochemical consequences of target engagement are termed 3° PD effects and include, for example, drug effects on cell cycle progression, apoptosis, effector T cell-mediated tumor cell cytolysis, and tumor cell migration/invasiveness.

A foundational principle of dose-response is the reversible binding of an agent to its molecular target, the extent of which is determined in real time by measuring the unbound (“free”) drug concentration in the microenvironment surrounding the molecular target. The subject of pharmacokinetics (PK) involves the understanding of this unbound drug concentration at the target as a function of elapsed time since dose administration, and this concentration is determined by the combined effects of absorption, distribution, metabolism, and elimination (ADME). The unbound drug concentration at the molecular target is the variable that connects pharmacodynamics to pharmacokinetics, and because of this connection, establishing a PK/PD relationship is an important breakthrough in the understanding of dose-response. Unfortunately, it is highly impractical to measure unbound drug concentrations in the microenvironment or even at a macroscopic level in tumor biopsies, and it is clinically impossible to apply the dense sampling strategy of systemic PK studies to repeated measurements of drug level in tumor for deriving concentration × time profiles. Thus, PK studies often settle for the much more practical measurement of drug levels in plasma, and then assume that tissue and plasma drug concentrations equilibrate rapidly, without actually measuring drug levels in tumor. There is, however, an alternative approach that balances feasibility with scientific rigor; establishing a relationship between plasma drug concentration and 1° PD biomarker response provides some confidence that plasma concentrations of drug can be used as surrogate measurements of drug concentration in the tumor tissue.

Full characterization of a molecular drug response includes the evaluation of PD biomarkers for each of the 1°, 2°, and 3° effect levels that compose the complete drug response. The PD study of a single dose of an investigational agent will usually reveal a change in molecular target status within minutes to hours of dose administration, with biochemical modulation occurring throughout the window of time that molecular target function is sufficiently inhibited. Cellular consequences may be measurable if the single dose is expected to be potentially therapeutic, but the lower doses used for pharmacology studies might not modulate cellular biochemistry for a long enough duration to affect cell biology or physiology. Multiple doses of a reversible inhibitor may be required to detect changes in 3° PD biomarkers because prolonged, perhaps even continually sustained modulation of molecular target function may be required to alter cellular behavior. There is thus the conundrum that single-dose studies provide a purer profile of PD response over time than multiple-dose studies that superimpose 1°, 2°, and 3° drug effects, but unless the single dose shows clinical efficacy, it should not be expected to elicit a robust 3° biomarker response. This is the most likely explanation for the paucity of successful proof of concept (POC) studies associating molecular target modulation with efficacy. With targeted agents, a successful POC study will need to be built upon multiple milestones: POM from a 1° PD biomarker response shortly after administration of the first dose; reproducing the 1° PD biomarker response after multiple doses; a sustainable and perhaps increasing 2° PD biomarker response throughout dosing; and, finally, the appearance of 3° PD biomarkers prior to clinically detectable responses. Because it is neither ethical nor practical to make all of these biomarker measurements in the same patients, the PD biomarker analysis in responding patients should be used for the POC analysis, while the PD biomarker response in non-responding patients should be useful in identifying where the uncoupling of 1°, 2°, and 3° drug effects occurs.

Of unique importance in oncology, it should be noted that clinical PD concepts are much more difficult to apply to two classes of oncology drugs: i) chemotherapeutic drugs that cause cytotoxic damage persisting after the drug is cleared from the tissue and ii) drugs that bind irreversibly to molecular targets, exhibiting slow turnover rates. With these mechanisms, each dose may add to the accumulating effects of previous doses. Although PD biomarkers may reveal the actual duration of drug effect and its reversal so that chemotherapy dose scheduling can be optimized, it is unclear if this laboratory-based approach would offer any advantages over optimized scheduling of chemotherapeutics based on end-organ tolerance and organ recovery from toxicity. However, this issue of *Seminars* included examples of using clinical PD not only to study targeted agents with reversible binding to their intended molecular targets but also to guide the development of chemotherapeutic regimens, especially when combined with intended modulators of drug effect.

The pharmacology of some oncology drugs is not contained entirely within a single target cell. For example, by disrupting binding between a receptor on a cytotoxic T-lymphocyte (CTL) and its ligand on a tumor cell (e.g., PD1:PD-L1), immune checkpoint inhibitors reverse the suppression of the CTL response to tumor neoantigens. Drug action then causes activation of perforin-mediated killing of the adjacent tumor cell. The trans-cellular pharmacology of the drug begins on the tumor cell, with the targeting of PD-L1, but then

moves into the adjacent CTL, and ultimately returns to the tumor cell, where a cell death program is activated. Measuring the 1°, 2°, and 3° PD effects of this class of agents requires multiplexed microscopy to precisely locate the wave of PD biomarkers moving across adjacent cells. The new field of immunopharmacodynamics is important for building mechanism-based drug combinations on top of immune oncology therapeutic backbones (described in “Immunopharmacodynamics for evaluating mechanism of action and developing immunotherapy combinations” within this issue). There will most likely be development of additional therapeutic drug classes exhibiting transcellular pharmacology, where the intended molecular drug target and the cellular consequences of altering the function of that target occur in different but neighboring cells.

Not every agent has a single molecular target, as some are “multi-target drugs” that affect several molecular targets with approximately equal potency. Notable examples of multi-target drugs in current clinical use include sunitinib, crizotinib, and axitinib, and multi-target drug discovery is an emerging field focused on the intentional, rather than accidental, discovery of new agents that modulate several targets. PD evaluation of these multi-target drugs is quite complex because some of the 2° and 3° PD effects occur in signaling pathways that converge, interact, or feedback upon the others. PD biomarker assays provide a tool to determine which targets are actually affected by these drugs in patients, which may vary by tumor genotype and histology. A related area arises when a new targeted agent causes unexpected biochemical changes; such off-target effects on unintended molecular targets are important, but unexpected drug MOA is beyond the scope of this issue of *Seminars*.

PD-Guided Optimization of Biologically Effective Dose Scheduling (Dosage Regimens)

After results from clinical POM studies have confirmed the expected MOA of an investigational drug, the PD biomarkers of this MOA can then be used as tools for establishing optimized dosage regimens (dose and schedule). Historically, dosage regimens have been optimized in one of two ways. The first approach has been to compare the tolerability of different schedules of drug administration and to select as optimal the one that administers the largest total dose per cycle with acceptable organ toxicity. This method essentially applies the approach for developing cytotoxic chemotherapeutics to new classes of targeted agents and assumes that the response of normal organ function is related mechanistically to the intended drug action in tumor. This approach also appears to abandon the idea that somatic mutations should result in greater drug potency in malignant tissue as compared to healthy tissue containing the normal genome. The second approach has been to administer a drug as frequently as is needed (reasonably *bid*, but no more than *qid*) to maintain blood or plasma drug concentrations above a preset threshold value. This approach uses drug concentration in venous blood as a surrogate measurement of tumor drug concentration and therefore assumes that drug levels in plasma and tumor tissue rapidly equilibrate. While this is a reasonable assumption for healthy tissue with fully functional microvasculature, the known defects in tumor vasculature and endothelial function that result in low and irregular tumor perfusion call into question whether plasma levels can be used as surrogates of tumor drug levels, especially as a rapidly equilibrating surrogate of the drug concentration $\times$ time profile in the microenvironment of the molecular target.

Although dose-response is a fundamental principle in pharmacology, it is an incomplete concept unless it includes the duration of drug response over time, which is important to determine when seeking maximum effect. When drug response is clinically evident and measureable, the time of onset of maximum drug response is easy to establish, and obtaining dose-response data is straightforward. But when the response is molecular (sub-clinical), as occurs in many clinical trials of single-agent investigational oncology therapies in advanced disease, dose-response will require the measurement of PD biomarkers of MOA. However, a large but short-lived drug effect on the molecular target could be insufficient for achieving clinical efficacy, and thus, determining the *duration of molecular effect* is critical (Figure 1). The key measurement is the period of time that a single dose of drug suppresses molecular target function at or below a certain level.

But how should that minimum target response level be established? Using tumor models that are addicted to a drug's molecular target for survival, the minimum target response level can be defined as the level of target suppression that results from administering the first dose of an efficacious dosage regimen in that model. In other words, what is the extent and duration of the 1° PD biomarker change resulting from a single dose of the minimal dosage regimen that is efficacious? This is complicated, however, by the fact that the entire dosage regimen must be administered to achieve efficacy; repeated dosing is required for continuous suppression of molecular target function at or below the set point required for efficacy (POC), including during dosing intervals. The hypothesis is that the level of target modulation resulting from a single dose of that dosage regimen will achieve efficacy if it is maintained by sufficiently frequent repeated dosing. For example, a single dose of a tyrosine kinase inhibitor (TKI) may provide 4-6 hours of suppression of the tyrosine kinase (TK) activity of a particular receptor to < 50% of normal activity (POM). However, if continuous suppression of enzyme TK activity by at least 50% is required for efficacy, then the TKI must be administered several times per day to prevent target recovery between doses. Thus, in this PD-guided method of dosage regimen optimization, molecular target control dictates the dose and schedule—a stark contrast to administering the maximum dose tolerated by dose-limiting healthy tissues. With targeted therapy, the desired physiological effect of drug action is not end-organ toxicity but, rather, a certain combination of magnitude and duration of the molecular drug effect that yields continuous control of target function.

Dose scheduling is important because continuous molecular target control requires dosing intervals spaced closely enough together to prevent the recovery of target activity and thereby achieve optimal efficacy (Figure 1). The concept of molecular targeted therapy incorporates the principle of biologically effective dose, meaning the lowest dose associated with efficacy. By analogy, a “molecular BED” can be defined as the lowest dose associated with the required level of molecular target control. This concept provides a basis for distinguishing targeted agents that are effective in controlling their molecular targets from those that are not. Following that logic further, the concept also provides a way to use Phase I and II trials to identify molecularly active drugs despite the overwhelming number of advanced cases often harboring multiple signaling defects. In this situation, single-agent molecularly targeted therapy should not be expected to control enough of those abnormalities to result in tumor regression, even though the drugs capable of controlling their respective targets might cause tumor regression when combined to treat cancers with

those precise abnormalities. Thus, the complete concept of a PD-guided, efficacy-optimized dosing regimen combines dose scheduling with BED to avoid intervals of molecular target recovery. Unfortunately, this understanding of the extent and duration of PD biomarker change sufficient for efficacy is rarely established during preclinical development, so clinical dose scheduling resorts to one of the two conventional strategies described above. Instead, PD biomarker changes provide a useful measurement for optimizing dose schedule and demonstrating continuous molecular target control—a developmental therapeutics concept that could be called Pharmacodynamics-Guided Biologically Effective Dose Scheduling (PGDS).

Focusing developmental therapeutics of targeted agents on the concept of optimized BED instead of MTD will require measurements of drug target engagement, and the downstream biochemical and cellular responses, that are just as reliable and quantifiable as current clinical and laboratory procedures used for assessing dose-limiting toxicities. The following case studies illustrate the use of such robust PD biomarker assays.

Case Study 1: Inhibitors of PARP1/2 Catalytic Activity

PARP1/2 inhibitors comprise a chemically diverse family of compounds that possess two independent MOAs: inhibiting the PARP enzymatic activity that produces PARylated proteins (inhibition of “catalytic activity”) and stabilizing PARP1/2 binding to sites of DNA damage (“PARP trapping”). The individual PARP inhibitors (PARPi) differ in their relative propensities to act primarily via catalytic inhibition or trapping, with both MOAs exhibited by olaparib, the only FDA-approved PARPi to date [7-10]. One investigational PARPi, veliparib, potentially inhibits PARP catalytic activity but shows very weak potency in trapping PARP [7], so this drug represents nearly a pure inhibitor of PARP enzymatic activity except at very high dose levels where trapping may also occur. It entered clinical development accompanied by a validated PD biomarker assay for the product of PARP enzymatic activity, PARylated macromolecules (referred to here as “PAR”), and by fit-for-purpose evidence from xenograft studies that replicated as many aspects of a clinical trial as possible in the preclinical models, including core needle biopsy sampling of xenografted tumors, standard operating procedure (SOP)–governed specimen handling and assay execution, dose levels and a time frame for tumor biopsy relative to dosing that could be replicated clinically, and demonstration of a significant change in PAR biomarker levels following administration of a single oral dose of veliparib [11]. The PAR biomarker and validated assay were used to uncover the extent and duration of PARP1/2 inhibition in advanced solid tumor patients following a single oral dose of veliparib [6]. Significant decreases in tumor PAR content compared to baseline levels occurred within 4-7 hours of oral dosing, as predicted by the preclinical modeling of the clinical trial, yet PAR levels completely recovered in some, but not all, patients 24 hours after the single dose. Based on these results, *bid* dose scheduling of veliparib was recommended for maintaining continuous control of PARP1/2 catalytic activity throughout the treatment cycle, i.e., maintaining tumor PAR levels at 50% of baseline levels in > 90% of the treated patients. The PD biomarker-guided *bid* dosing schedule from the Phase 0 trial has been used in more than fifty Phase I and II clinical trials combining veliparib with chemotherapeutic agents (Table 1). Assay performance has been robust, dose-dependent drug effects on PAR levels reproducible, and assay-derived

conclusions consistent across trials (for further details, see the article by Ferry-Galow et al. in this issue). To date, the dose-response of PAR levels to veliparib appears to be similar in the presence or absence of a chemotherapeutic agent, despite the potentially confounding effects of cytotoxic agent-induced PARP1/2 activity. When combined with chemotherapy, this schedule achieves sustained inhibition of PARP catalytic activity and has shown promising clinical results in triple negative breast cancer (combined with irinotecan [12]), metastatic breast cancer (combined with carboplatin [13]), and AML (combined with temozolomide or with carboplatin/topotecan [14, 15]).

Case Study 2: Inhibitors of MET Tyrosine Kinase Activity

Clinical development of investigational small-molecule inhibitors of MET began in 2005-2006, and new MET inhibitors continue to enter clinical development, with two new entries in 2016 ([16], search term “cMET”). Many of these agents are small molecules intended to inhibit the tyrosine kinase activity of MET, which results from binding of the HGF ligand or from constitutive activity due to abnormal activation or levels of expression. MET TK enzymatic activity is initiated by auto- or transphosphorylation of tyrosine residues 1234 and 1235 [17-23], so the tumor ratio of pY¹²³⁵-MET:total MET can serve as a PD biomarker of the MET TK response. We have found that daily dosing of PF-02341066, the active ingredient in crizotinib, achieves growth stasis in a MET-addicted human tumor xenograft model, which was associated with a transient suppression of molecular target function by 65-85% [24]. This reduction in activated MET provided evidence for a molecular BED; however, MET TK activity fully recovered during the 24-hour period prior to subsequent dosing, explaining why this dosage regimen achieved tumor stasis but not regression. Daily treatment with another MET TKI (PHA-665752) confirmed the association between tumor stasis and a transient, ~80% inhibition of MET signaling. These data illustrate the value of PGDS, as conceptualized by Figure 1, and the study exemplified an experimental strategy for identifying optimized dosage regimens of molecularly targeted therapies.

Obviously, increased and more sustained inhibition of MET kinase activity will be required to achieve tumor regression, which could be accomplished with more frequent dosing and/or higher doses that increase the residence time of inhibitory concentrations of drug in the tissue. Preclinical studies are underway to develop an optimized dose schedule that will maintain continuous, ~80% suppression of MET TK activity throughout the treatment period. These studies directly test the POC hypothesis that optimized MET TKI dosage regimens that achieve equal PD effects will also achieve equal efficacies (i.e., levels of tumor regression). These data support use of PD biomarker assays to guide optimization of dose and schedule to achieve sustained inhibition of MET based on biologically effective dose rather than the traditional maximum tolerated dose. This pharmacodynamic approach could be useful for identifying “best-in-class” targeted agents by directly comparing the *in vivo* efficacy of their molecular BED regimens. The clinical development of MET TKIs would benefit from a similar PD assessment of cancers harboring active MET signaling, the data from which would provide a basis for optimized dose scheduling of MET TKIs in patients.

Developing Targeted Drug Combinations to Treat Molecularly Complex Diseases such as Pancreatic Cancer

In contrast to cancers such as Ph+ CML [25] and retinoblastoma [26] that are caused by one or two genetic events, many commonly occurring advanced cancers contain genomes harboring multiple abnormalities; some of these are driver mutations, while others are passenger mutations. The important questions for cancer therapeutics include: (a) how many driver abnormalities are present? (b) must all of these abnormalities be treated to achieve tumor control? and (c) how many drugs will need to be combined to cover all of the relevant abnormalities? In an elegant analysis of the genomic profiles of twenty-four patient tumors, Jones et al. reported that advanced pancreatic adenocarcinomas contain an average of 63 driver mutations and other abnormalities [27]. All of the drivers mapped to twelve signaling pathways and processes, and six processes/pathways were altered in 100% of the cases: the G1/S phase transition in the cell cycle, apoptosis, and biochemical signaling of the SHH, KRAS, TGF- β , and Wnt pathways [27]. These are fundamental biological processes and signaling pathways that are utilized by normal tissues as well, so targeting these six pathways would likely cause multiple toxicities. However, the genetic aberrations in these pathways were discovered as somatic changes, and because they are not found in normal tissues, each abnormality represents a potential drug target with a wide safety margin and good therapeutic index.

This disease presents a challenge to current developmental therapeutics concepts and strategies: how does one develop a combination regimen from individual targeted agents that treat the minimum number of critical pathways? Because tumor response seems unlikely to be achieved by controlling just one of the six pathways, one would predict that empirical clinical trials of single agents would fail to discover active agents based on tumor response. However, clinical pharmacology trials assessing PD biomarker responses to single doses of candidate targeted agents could identify those that control one or more of these pathways at the molecular level, and a combination regimen to explore tumor response could then be formed based on the PGDS of the molecularly active single agents. Given enough drug selectivity for the somatic mutations in these tumors, such a rationally constructed combination regimen might be safe enough to administer. Regardless, PD biomarkers can assist in distinguishing drugs that are truly inactive, because they do not inhibit the intended molecular target, from those that affect the intended target but would be considered “inactive” by standard clinical criteria because they cannot treat enough driver mutations to produce a tumor response. Only clinical PD results could lead to a drug combination capable both of targeting the complex molecular landscape of an advanced solid tumor harboring multiple defects and of controlling enough abnormal signaling to achieve efficacy, even if this combination were composed of drugs that had failed to show efficacy as single agents precisely because of these myriad complex genomic abnormalities. The traditional, empirical approach only forms new combination regimens from drugs that show single-agent activity, and this approach would therefore fail to identify these aforementioned opportunities for combination therapies.

PD Guidance for Integrating Targeted Therapy and Immune Oncology into New Combinations

Combining drugs that act upon different cell types of a tumor, so called “multi-cellular pharmacology,” represents another setting in which clinical PD studies may be more efficient than empirical clinical trials for understanding the pharmacodynamics of drug-drug interactions, developing the most effective combination regimens, and avoiding antagonism. When developing combinations of kinase inhibitors and immune oncology agents, there may be desirable drug effects of the small molecule in one cell type but undesirable effects in another. The traditional approach to drug combination regimens is combining two to three drugs that are each active individually, with the hypothesis that these agents will act in concert on the malignant cell. But, what if those drugs act in multiple cell types and possess MOA that exert opposing therapeutic effects in the different cell types? For example, the success of trametinib and dabrafenib in melanoma established RAF/MEK/ERK signaling as an important target for small molecule therapy in this disease [28, 29]. However, MEK/ERK signaling also plays a role in T cell proliferation during antigen response [30-33], so the important developmental therapeutics question is: will the addition of MEK inhibitors to immune checkpoint inhibitors tip the balance toward better response rates due to further slowing of melanoma growth, or toward worse response rates due to inhibited T cell responses after release from immune checkpoints? Preclinical studies contribute further complexity to the question; MEK inhibition upregulates PD-L1 expression in triple negative breast cancer models but suppresses it in melanoma models [34, 35]. Through the use of informative PD biomarkers, it should be possible to answer this MOA question with small clinical pharmacology studies that not only provide confirmation of the preclinical MOA but also exclude the possibility of antagonism due to adverse PD interactions between drugs. Favorable PD findings would provide a rationale for POC clinical trials to confirm that the desired molecular mechanism translates into improved clinical efficacy and effectiveness.

Successful PD Biomarkers as Leads for Improved Molecular Diagnostics

Clinical PD biomarkers are measured using assay formats and instrumentation similar to those used for diagnostic biomarkers, including immunoassays, histochemistry/immunohistochemistry, and circulating tumor cell analysis. In fact, prioritizing the development of clinical PD biomarker assays for these clinical instrument platforms was a key strategic decision when the NCI PD program was launched in 2005. An important goal of the NCI PD program is enabling the research community to access validated, clinically suitable, fit-for-purpose biomarker assays, and utilizing existing clinical instrumentation and common clinical methodology was expected to facilitate more rapid and widespread adoption of the assays than requiring major capital expense for niche instrumentation.

It is often argued that the investment required to develop, validate, prove suitability of, and launch a new PD biomarker assay into the developmental therapeutics community is large compared to its low potential revenue, which stems from the short, non-commercial life span of a PD assay (use of which is usually limited to Phase I and II of development). However, there is considerable cost from negative Phase II and III trials caused by poor understanding of drug MOA and poorly performing laboratory tests that result in the failure to accurately select appropriate patients. An unexplored application of robust clinical PD biomarker

assays is their use as diagnostic tests for selecting patients for targeted therapies. The baseline measurement of a PD biomarker in an untreated patient essentially provides evidence for the presence of the molecular drug target prior to treatment. When the baseline measurement is a biomarker of molecular target function (for example, pY¹²³⁵-MET indicating active signal transduction), then this positive assay is also identifying patients with a chance of responding to a particular targeted therapy. Although this approach may be unable to distinguish tumors addicted to the signaling axis from those that express the biomarker as a meaningless passenger alteration, it will nonetheless enrich a trial population of patients with individuals who have the greatest possibility of responding. For example, it would be unproductive to use MET TKIs to treat patients with tumors harboring high levels of unphosphorylated MET (i.e., tumors positive for MET but negative for pY¹²³⁵-MET) because the assay results indicate that MET kinase signaling is inactive. Converting PD biomarkers of molecular target activity into companion diagnostic tests for classes of targeted agents would generate a revenue stream lasting years after drug approval—in contrast to the historically short life span of a PD assay. This could be another use of recently generated monoclonal antibodies for quantifying the PD response of ATR and MET [36, 37].

However, fundamental differences between PD assays and diagnostic assays should be noted, including those involving the purpose of the test, the timing of tumor sampling relative to drug dosing, and the regulations governing the use of the assay results. PD is the study of drug response, which means that nothing can be studied until after drug administration, and assays of PD biomarkers can only be conducted on tumor specimens obtained from a patient already treated with a drug. Importantly, the physician has already decided what drug to use in treating that patient before obtaining the PD results, so medical treatment is not influenced by the PD measurement. Instead, the patient's treatment is governed by an IRB-approved clinical protocol, and the PD assay is conducted under the rules of that protocol. In contrast, the intended purpose of a diagnostic laboratory assay is to influence medical treatment of the patient, and therefore, it must be performed by a certified laboratory (e.g., CLIA-certified) according to regulations regarding diagnostic laboratory testing and medical devices (e.g., Laboratory Developed Test guidance documents).

The important distinction between a PD assay and a diagnostic assay is not in assay methodology itself but, rather, in the use of the assay results. For example, the same pY¹²³⁵-MET assay could be used either to quantify drug suppression of MET signaling after drug administration (PD) or to select for MET TKI therapy only patients with tumors that harbor constitutive MET signaling before drug administration (diagnostic). Note that TKI therapy for adaptive or acquired resistance to current therapy may require diagnostic assays of tumor biopsies obtained after starting treatment with the first drug in order to decide whether to add a second drug (e.g., in cases where induction of MET TK signaling occurs in response to EGFR TKI therapy [38, 39]). The assay of pMET in this situation is still diagnostic, because the results of the assay will be used for the medical decision of whether to treat the patient with an added MET TKI.

Incorporating Pharmacodynamic Studies into Clinical Trial Design

The required specificity of a PD biomarker measurement must be balanced with existing knowledge about the expression and response of the molecular target in clinical disease. First-in-human evaluation of a new PD biomarker may start with a broader, less specific measurement that then evolves into a much more specific measurement of MOA as follow-on studies build on the emerging knowledge base. For example, a Phase I trial might use extracts of optional biopsies from the dose escalation stage (Phase Ia) for sandwich immunoassays of the PD biomarker, with the objective of identifying and establishing a basic PD biomarker response, followed by use of formalin-fixed, paraffin-embedded sections of mandatory biopsies from the dose expansion stage (Phase Ib) for a microscopy assay to verify that the PD biomarker change is occurring within the malignant cell population.

Note that neither assay is ideal. The sandwich immunoassay provides a very specific biomarker measurement because it utilizes two antibody epitopes (one to capture the biomarker and a second to probe its function), but it cannot distinguish the biomarker changes occurring in malignant cells from those occurring in the normal cells that are also found in the tumor biopsy and thus extracted together with the tumor cells. While data from extraction assays can demonstrate a PD response, one cannot conclude from these data that the response is occurring in the tumor cell population. In contrast, the microscopy assay allows for identification of the cellular origin(s) of the biomarker signal but uses a single antibody (or other probe) to a single epitope (or binding site). Thus, this approach cannot distinguish the intact protein from its cross-reactive degradation fragments or differentiate between conserved sequences that are shared with other isoforms or highly related proteins. For example, some commonly used PD biomarkers of signal transduction are actually measurements of phosphorylation in multiple proteins: the pSer⁴⁷³ epitope found in activated Akt1, Akt2, and Akt3, each of which mediates very different biological functions; the Thr-Glu-Tyr sequence in ERK1 (pT²⁰²EpY²⁰⁴) and ERK2 (pT¹⁸⁵EpY¹⁸⁷), which is bis-phosphorylated by the dual kinase activity of MEK1/2; and the pY¹⁵ epitope shared by CDK1, CDK2, and CDK3. Because the amino acid sequence of a signal transduction site is often highly conserved, or even identical, among isozymes, distinguishing the isozyme(s) affected by drug treatment requires the specificity of a sandwich immunoassay of tissue extracts. On the other hand, microscopy can demonstrate that the PD response is occurring in the malignant cells, but it cannot identify which isozymes have been phosphorylated.

Phase 0 Clinical Trials: Bridging the PD Data Gap Between Preclinical and Clinical Studies

Phase I clinical trials are not the only venue for conducting POM studies using PD biomarkers. The FDA promulgated a guidance on novel clinical trial designs for answering pharmacological questions about investigational agents in order to accelerate development of these agents [40]. The Exploratory IND Studies guidance allows the human testing of a non-therapeutic, non-toxic “microdose” in order to gain key pharmacology knowledge—pharmacodynamic and pharmacokinetic—that will inform early clinical development. Because xIND trials are envisioned to precede Phase I trials, they have been called “Phase 0 clinical trials,” and they are intended to bridge the knowledge gap between preclinical models and human cancer [41-43]. Patient participation in Phase 0 trials lasts 2-4 weeks, so

participation is possible while waiting to enroll in Phase I/II treatment trials. Importantly, by definition, Phase 0 trials are trials without therapeutic intent, so Phase 0 clinical pharmacology studies of dose and dosage regimens must seek to avoid drug-induced physiological changes, whether related to efficacy or toxicity. The purpose of such studies is only to obtain critical pharmacology data that will inform clinical development of an investigational agent, so there are important and unique ethical considerations and informed consent processes that accompany these types of trials [44]. Clearly, investigational drugs with either cytotoxic MOAs or undefined MOAs are not candidates for Phase 0 trials, so their initial clinical PD evaluation must wait until first-in-human Phase I trials. However, as described above in the case study of PARP inhibitors, Phase 0 trials have been useful in optimizing dose schedules for drugs that modulate chemotherapeutic effectiveness.

Having a pharmacological endpoint should not be misunderstood to mean that IND standards are lower for Phase 0 trials than for therapeutic trials. The abbreviated toxicology studies required for supporting an xIND submission must provide evidence that the planned dosages are reasonably expected not to cause any toxicities. Because PD characterization is the primary objective of a Phase 0 trial, the PD biomarker assays are included in the IND application, and they must be analytically valid, suitable for use with the proposed clinical specimen type (core needle biopsy, excisional biopsy, etc.), and proven to be fit-for-purpose. Fitness-for-purpose can be demonstrated by successfully replicating the Phase 0 trial protocol—both design and procedures—in a preclinical model system and using the validated PD assays to accomplish the proposed objective of the Phase 0 trial: demonstrating a molecular target response to a single dose of drug. The fit-for-purpose study is critical for identifying the window of time for tumor sampling in the clinical protocol, which is the time frame in the fit-for-purpose study that yields the greatest probability of measuring the largest, statistically significant change in the PD biomarker(s).

Phase I and II Trials: Correlating Pharmacodynamic and Clinical Responses

The Phase 0 clinical trial setting is well-suited for POM studies evaluating biomarkers of 1° and 2° PD effects of non-toxic microdoses of single agents and drug combinations, as well as for optimizing the molecular BED schedule to set a dosing interval that will achieve the desired extent and duration of molecular target control. In contrast, POC studies seek to establish a relationship between the PD response of the molecular target(s) and drug efficacy. Because such studies seek to link molecular effect with tumor response, they are clinical trials with therapeutic intent that evaluate full-strength doses and carry the risk of toxic side effects; thus, POC analysis would not make sense in a Phase 0 trial setting, and instead, Phase I and II clinical trials provide suitable venues for conducting POC studies.

There is a trade-off between Phase I and II trials as venues for PD studies. On the one hand, Phase II trials offer a higher probability of clinical response to correlate with PD response, yet these studies offer a much lower probability of obtaining tumor biopsy samples for PD studies because of low participation in the optional biopsy procedure. On the other hand, tumor biopsy samples are much more likely to be obtained from Phase I trials, yet the accrual of patients with advanced stage disease of multiple histologies and genomes results in very low clinical response rates, especially without patient selection criteria, and therefore

no opportunity to establish POC. All things considered, one good strategy for reliably establishing POM and POC within the time frame of an early clinical trial program begins with a Phase 0 trial to demonstrate clinical suitability of the PD biomarker measurement, molecular target engagement/MOA, and the time course of biomarker changes, as well as to inform dose scheduling during Phase I. PD testing during Phase Ia will then confirm that the dosing schedule adequately controls molecular target function, and such analysis can also detect a possible dose–PD response relationship. Once the PD response has been detected using the optimal sampling time determined from the preclinical fitness-for-purpose studies, Phase Ia is also a good setting for establishing the time course of PD biomarker responses in tumors sampled at different time points after dosing. These data can be obtained either by randomizing the individual patients in a dose level cohort to different times of tumor sampling or by assigning each dose level cohort to a particular sampling time. Finally, a Phase Ib expansion cohort for patients with molecularly or histologically defined disease that likely harbors functioning drug target offers the best chance for establishing POC via quantified PD biomarker responses. Some Phase I designs wait to conduct clinical PD studies until MTD establishment or Phase Ib expansion, but this approach carries two significant risks: (i) it misses the chance to understand dose-response and to determine the molecular BED—as dose escalation has been guided by dose-limiting toxicity—and (ii) the first clinical experience with the PD biomarker and its assay is occurring too late in clinical development for adjustments to be made if the initial experience proves it unsuitable for clinical use.

To be related as cause and effect, the PD biomarkers of 1°, 2°, and 3° drug effects must occur sequentially in time, so longitudinal clinical sampling is required to detect these changes and confirm the expected sequence of events connecting drug exposure, MOA, and efficacy. Most clinical trials allow a maximum of 2–3 biopsy procedures per patient. Thus, the usual design of a clinical PD study begins by establishing that a drug modulates its intended target (i.e., the 1° PD effect), and once demonstrated, this is followed by analyses of changes in either pathway signaling or cellular behavior. However, if the investigational agent is already known to be clinically active, the PD study could begin by establishing a 3° biomarker and its association with clinical response before working backward to identify the biochemical link between the cellular response and drug target modulation.

Into Uncharted Waters with Robust Tools: What the Future Needs from Clinical PD

This issue of *Seminars* contains a collection of clinical PD studies exemplifying the highest standards in study design, methodology, and application, but the careful reader will notice opportunities for improved clinical PD evaluations in several areas:

1. New measurement technologies that combine the best aspects of current methods. Different methods offer trade-offs because of their advantages and disadvantages. Currently, there is a need for new measurement technologies capable of combining high-specificity measurements (e.g., probing more than one region of an individual, full-length protein molecule) with geographic precision in the tumor sample (i.e., identifying

the cellular sources of the PD biomarker signal and the relative geographic position of those cellular sources). This might be a form of “sandwich microscopy” or perhaps an extension of imaging mass spectrometry that includes analysis of full length proteins for isoforms and post-translational modifications. Fluorescence microscopy is currently limited by dye chemistry; thus, new fluorescent tags with improved quantum yield (brightness) and tunable, very narrow spectra would dramatically increase the plex size of multiplex microscopy and, therefore, the breadth of the molecular signaling analysis.

2. Improved tumor sampling methods Because targeted therapies are usually effective only when administered as multi-dose courses of treatment, drug PD studies need to measure not only the magnitude but also the time course of response to fully understand the MOA and optimize dose scheduling. The ultimate PD study would generate an “effect $\times$ time” profile analogous to the “concentration $\times$ time” profile in pharmacokinetics. Dense, longitudinal sampling of tumor—akin to venous blood sampling for PK studies—would be needed to generate this time course of PD response, but this is impossible with current tumor biopsy methods and procedures. Precision oncology may turn out to be a driving force behind the development of new approaches for tumor sampling. One might envision individual biopsy procedures being replaced with central access points or in-place guide needles for repeated tumor sampling. There is already considerable R&D effort in the design and development of smart biopsy needles containing sensors that will provide physicians with real-time feedback about the characteristics of the tissue through which the biopsy needle is passing [45-48].
3. Biomarkers of tissue quality for measuring the impact of pre-analytical variables Given the inherent instability of many important biomarkers during specimen collection, handling, and storage (especially, for example, phosphorylation sites in proteins), sample preservation methods are critical for controlling pre-analytical variables and obtaining valid assay results [24, 49-53]. However, there are currently few measures that indicate tissue quality and, therefore, a sample's suitability for biomarker assessment and clinical reporting. The Tissue Quality Index (TQI) is a metric that has been proposed for judging the quality of a fixed tissue specimen in paraffin block, and early attempts to develop a TQI for microscopy-based evaluations have been promising [50, 54]. It is likely that TQI measurements will become more sophisticated as they begin to incorporate emerging results from proteomic studies identifying proteins with the largest and fastest changes in response to cold ischemia [55, 56]. Post-extraction markers of tissue quality and viable tumor content are also needed for assays that use tissue extracts and thereby destroy morphology, but there is little R&D activity in this area. Particular analytes with levels that always correlate to viable tumor cell content are needed to replace

non-specific measures of total protein or ubiquitous proteins that are currently used to normalize biomarker measurements across biopsy specimens and their extracts.

In closing, an important point made by all of the studies in this issue is that valuable PD results come from thorough, well-conducted clinical studies using measurements designed to assess changes in PD biomarkers that point conclusively to drug action on the intended molecular target. Although it is tempting to use whatever R&D reagents are already available to measure some signal from a tumor biopsy sample, the need for specific MOA information to accelerate development of new drugs and drug combinations should motivate us to design rigorous assays of the most important drug effects and to interpret the resulting data within the limits of the assay measurement. As described recently [4], the study of clinical PD in oncology must be distinct from academic exercises aiming to generate data about “laboratory correlates” that one's colleagues just happen to be able to measure. The latter is exemplified by a physician PI combing the laboratories of the local institution to locate a scientist colleague who can measure something (*anything*) relevant to their clinical protocol. No doubt, there have been poorly conducted PD studies using mechanistically uninformative and sometimes even unrelated endpoints, and when coupled with unreliable assays, such studies rightfully create skepticism about the value of clinical PD in the developmental therapeutics community. Improperly conducted PD studies generate misleading information about drug MOA that can compromise thinking about trial design, prioritization of studies, and patient selection for many years to come. However, when practiced appropriately, clinical PD at its best is the elucidation of the actual MOA of an oncology drug in patients; the use of biochemical and cellular changes to connect drug action at its intended molecular target to clinical efficacy carries high value for pharmacology, developmental therapeutics, and drug evaluation. We hope that this issue of *Seminars* will prompt not only high-quality clinical uses of PD biomarkers of drug action and the acceleration of oncology drug development but also improved understanding of the role of each clinical and laboratory specialty in achieving successful clinical PD studies.

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Figure 1.

Pharmacodynamics-guided biologically effective dose scheduling maximizes the clinical efficacy of targeted therapies through continual suppression of PD biomarker function. **A)** While treatment at dose levels below the biologically effective dose does not sufficiently control tumor growth (green), even treatment with the BED may result in mere growth stasis (blue) if the dosing schedule is not optimized through the use of a PD biomarker. In contrast, PGDS produces a strong clinical response, resulting in tumor regression (orange). **B)** PGDS (orange) yields a dosage regimen capable of sufficiently suppressing the biological function of a biomarker at a specified level, even during dosing intervals. In contrast, even frequent administration of dose levels < BED does not adequately suppress biomarker function (green), and non-PGDS-optimized administration of the BED (blue) allows for unacceptably high levels of biomarker function recovery during dosing intervals.

Table 1

Multi-agent clinical trials utilizing the PD-guided dosing regimen established for the PARP inhibitor veliparib. A *bid* dosage regimen of veliparib was recommended for clinical development based on Phase 0 clinical trial results showing recovery of tumor PARP1/2 activity in some patients within 24 hours of dosing. The *bid* regimen maintains suppression of PARP1/2 catalytic activity in most patients and has been adopted in combination regimens currently in clinical development.

NCTC Protocol ID	Sponsor	Phase	Oncology Drugs Combined with Veliparib	Disease	Status
NCT00535119	NCI	I	Carboplatin; Paclitaxel	Advanced Solid Cancer	Completed
NCT00553189	NCI	I	Topotecan Hydrochloride	Solid Tumors and Lymphomas	Completed
NCT00576654	NCI	I	Irinotecan Hydrochloride	Metastatic, Unresectable Cancer	Recruiting
NCT00588991	NCI	I	Topotecan; Carboplatin	Relapsed or Refractory Acute Leukemia, High-Risk Myelodysplasia, or Aggressive Myeloproliferative Disorders	Active, not recruiting
NCT00740805	NCI	I	Cyclophosphamide; Doxorubicin Hydrochloride	Metastatic or Unresectable Solid Tumors or Non-Hodgkin Lymphoma	Active, not recruiting
NCT00770471	Sidney Kimmel Comprehensive Cancer Center	I & II	Temozolomide; Radiation	Newly Diagnosed Glioblastoma Multiforme	Completed
NCT00804908	AbbVie (prior sponsor, Abbott)	II	Temozolomide	Metastatic Melanoma	Completed
NCT00892736	NCI	I	none	Refractory Malignant Solid Tumors	Active, not recruiting
NCT00946335	NCI	I	Temozolomide	Recurrent or Refractory CNS Tumors	Completed
NCT00989651	NCI	I	Carboplatin; Paclitaxel; Bevacizumab	Newly Diagnosed Stage II-IV Ovarian Epithelial, Fallopian Tube, or Primary Peritoneal Cancer	Recruiting
NCT00994071	NCI	I	Temozolomide	Recurrent/Refractory CNS Tumors	Completed
NCT01009788	Steven Isakoff, MD, PhD	II	Temozolomide	Metastatic Breast Cancer and BRCA1/2 Breast Cancer	Active, not recruiting
NCT01017640	NCI	I	Mitomycin C	Metastatic, Unresectable, or Recurrent Solid Tumors	Active, not recruiting
NCT01026493	Radiation Therapy Oncology Group	I & II	Temozolomide	Recurrent Glioblastoma	Active, not recruiting
NCT01051596	Georgetown University	II	Temozolomide	Colorectal Cancer	Completed
NCT01063816	AbbVie (prior sponsor, Abbott)	I	Carboplatin; Gemcitabine	Advanced Solid Tumors	Completed
NCT01085422	AbbVie (prior sponsor, Abbott)	I	Temozolomide	Metastatic Prostate Cancer	Completed
NCT01104259	University of Washington	I	Cisplatin; Vinorelbine Ditartrate	Recurrent and/or Metastatic Breast Cancer	Active, not recruiting
NCT01123876	AbbVie (prior sponsor, Abbott)	I	FOLFIRI	Solid Tumors	Completed

NCTC Protocol ID	Sponsor	Phase	Oncology Drugs Combined with Veliparib	Disease	Status
NCT01139970	NCI	I	Temozolomide	Acute Leukemia	Active, not recruiting
NCT01145430	NCI	I	Pegylated Liposomal Doxorubicin Hydrochloride	Recurrent Ovarian Cancer, Fallopian Tube Cancer, or Primary Peritoneal Cancer or Metastatic Breast Cancer	Recruiting
NCT01149083	NCI	II	Carboplatin	Stage III or Stage IV Breast Cancer	Active, not recruiting
NCT01154426	NCI	I	Gemcitabine Hydrochloride	Advanced Solid Tumors	Completed
NCT01193140	Abbott	II	Temozolomide	Solid Tumors	Completed
NCT01205828	Georgetown University	II	Temozolomide	Liver Cancer	Terminated
NCT01233505	NCI	I	Oxaliplatin; Capecitabine	Advanced Solid Tumors	Terminated
NCT01251874	NCI	I	Carboplatin	HER2-Negative Metastatic Breast Cancer	Active, not recruiting
NCT01264432	NCI	I	Radiation	Advanced Solid Malignancies With Peritoneal Carcinomatosis, Epithelial Ovarian, Fallopian, or Primary Peritoneal Cancer	Active, not recruiting
NCT01266447	NCI	II	Topotecan Hydrochloride; Filgrastim; Pegfilgrastim	Persistent or Recurrent Cervical Cancer	Completed
NCT01281150	NCI	I	Paclitaxel; Carboplatin	Locally Advanced or Metastatic Solid Tumors	Completed
NCT01282333	NCI	I	Cisplatin; Gemcitabine Hydrochloride	Advanced Biliary, Pancreatic, Urothelial, or NSCLC	Terminated
NCT01326702	NCI	I & II	Bendamustine Hydrochloride; Rituximab	Relapsed or Refractory Lymphoma, Multiple Myeloma, or Solid Tumors	Completed
NCT01366144	NCI	I	Paclitaxel; Carboplatin	Solid Tumors That Are Metastatic or Cannot Be Removed by Surgery and Liver or Kidney Dysfunction	Recruiting
NCT01386385	NCI	I & II	Radiation; Carboplatin; Paclitaxel	Unresectable Stage III NSCLC	Suspended
NCT01434316	NCI	I	Dinaciclib	Advanced Solid Tumors	Recruiting
NCT01472783	Vejle Hospital	I & II	none	Relapsed Ovarian Cancer with BRCA Mutation	Active, not recruiting
NCT01477489	University of Michigan Cancer Center	I	Radiation	Inflammatory or Loco-regionally Recurrent Breast Cancer	Active, not recruiting
NCT01489865	Georgetown University	I & II	FOLFOX6	Metastatic Pancreatic Cancer	Recruiting
NCT01495351	Alberta Health Services	I	Bortezomib; Dexamehasone	Relapsed Refractory Myeloma	Unknown
NCT01514201	NCI	I & II	Temozolomide	Diffuse Pontine Glioma	Active, not recruiting
NCT01540565	NCI	II	none	Persistent or Recurrent Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer	Active, not recruiting
NCT01576172	NCI	II	Abiraterone Acetate; Prednisone	Metastatic Hormone-Resistant Prostate Cancer	Active, not recruiting

NCTC Protocol ID	Sponsor	Phase	Oncology Drugs Combined with Veliparib	Disease	Status
NCT01585805	NCI	II	Gemcitabine Hydrochloride; Cisplatin	Locally Advanced or Metastatic Pancreatic Cancer	Recruiting
NCT01617928	AbbVie (prior sponsor, Abbott)	I	Carboplatin; Paclitaxel	Solid Tumors	Completed
NCT01618357	Richard Zellars	I	Radiation	Pre-Operative Breast Cancer	Suspended
NCT01638546	NCI	II	Temozolomide	Relapsed or Refractory SCLC	Active, not recruiting
NCT01642251	NCI	I & II	Cisplatin; Etoposide	Extensive Stage SCLC or Metastatic Large Cell Neuroendocrine NSCLC	Active, not recruiting
NCT01690598	Vejle Hospital	I & II	Topotecan	Relapsed Ovarian Cancer with Negative or Unknown BRCA Status	Completed
NCT01711541	NCI	I & II	Carboplatin; Cisplatin; Fluorouracil; Hydroxyurea; Paclitaxel; Radiation	Stage IV Head and Neck Cancer	Recruiting
NCT01749397	NCI	I	Floxuridine	Metastatic Epithelial Ovarian, Primary Peritoneal Cavity, or Fallopian Tube Cancer	Recruiting
NCT01818063	Thomas Jefferson University	II	Carboplatin; Paclitaxel; Doxorubicin; Cyclophosphamide	Stage IIB-IIIc Breast Cancer	Suspended
NCT01908478	Cedars-Sinai Medical Center	I	Gemcitabine; Radiation	Locally Advanced, Unresectable Pancreatic Cancer	Recruiting
NCT02152982	NCI	II & III	Temozolomide	Newly Diagnosed Glioblastoma Multiforme	Recruiting
NCT02158507	University of Alabama at Birmingham	N/A	Lapatinib	Metastatic TNBC	Recruiting
NCT02210663	AbbVie	I	none	Advanced Solid Tumors	Active, not recruiting
NCT02289690	AbbVie	I	Carboplatin; Etoposide	Treatment-naïve ED SCLC (Extensive stage Disease Small Cell Lung Cancer)	Recruiting
NCT02483104	AbbVie	I	Carboplatin; Paclitaxel	Ovarian Cancer	Active, not recruiting
NCT02595905	NCI	II	Cisplatin	Stage IV Triple-Negative and/or BRCA Mutation-Associated Breast Cancer	Recruiting
NCT02723864	NCI	I	VX-970; Cisplatin	Refractory Solid Tumors	Recruiting