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# Phase 0 and window of opportunity clinical trial design in neuro-oncology: a RANO review

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**Abstract**

Glioblastoma is a devastating disease with poor prognosis. Few effective chemotherapeutics are currently available, and much effort has been expended to identify new drugs capable of slowing tumor progression. The phase 0 trial design was developed to facilitate early identification of promising agents for cancer that should undergo accelerated approval. This design features an early in-human study that enrolls a small number of patients who receive subtherapeutic doses of medication with the goals of describing pharmacokinetics through drug blood level measurements and determining intratumoral concentrations of the investigational compound as well as pharmacodynamics by studying the biochemical and physiological effects of drugs. In neuro-oncology, however, the presence of the blood–brain barrier and difficulty in obtaining brain tumor tissue warrant a separate set of considerations. In this paper, we critically reviewed the protocols used in all brain tumor related in-human phase 0 and phase 0–like ("window of opportunity") studies between 1993 and 2018, as well as ongoing clinical trials, and identified major challenges in trial design as applied to central nervous system tumors that include surgical specimen collection and storage, brain tumor drug level analysis, and confirmation of drug action. We therefore propose that phase 0 trials in neuro-oncology should include (i) only patients in whom a resection of the tumor is planned, (ii) use of clinical doses of an investigational agent, (iii) tissue sampling from enhancing and non-enhancing portions of the tumor, and (iv) assessment of drug-specific target effects. Standardization of clinical protocols for phase 0/window of opportunity studies can help accelerate the development of effective treatments for glioblastoma.

## Key Points

1. Most clinical investigations of novel drugs for gliomas do not account for the ability of these drugs to access their targets in the CNS.
2. Incorporation of elements of phase 0/window of opportunity clinical trial designs into neuro-oncology trials will permit a greater understanding of the potential for a novel agent to generate meaningful clinical responses.

## Importance of the Study

Few effective chemotherapeutics are currently available for treating glioblastoma despite extensive clinical investigation of a multitude of compounds. The presence of a blood–brain barrier makes it challenging to rely on the pharmacokinetics and pharmacodynamics that are generated from investigations in systemic cancers. Phase 0/window of opportunity clinical trial designs can be used to determine the intratumoral concentrations of the investigational compound as well as pharmacodynamics by studying the biochemical and physiological effects of drugs in tumor tissue. This report provides specific consensus guidance regarding the use of Phase 0-like/window of opportunity clinical trial designs in NeuroOncology with the goal of evaluating drug-treated brain tumor tissue to help guide therapeutic development and minimize the risk of undertaking futile efficacy-oriented clinical trials.

Glioblastoma is the most common malignant primary brain neoplasm.[1] The median survival after initial diagnosis is less than one year without treatment.[2] Surgical resection alone is insufficient to control tumor progression given that glioblastoma is an infiltrative disease. The addition of radiation and chemotherapy significantly improves median patient survival to 14–16 months in clinical trial populations,[3] and this may be further extended by use of tumor-treating fields.[4] Despite intensive research and numerous clinical trials, no chemotherapeutic drug except temozolomide has been proven effective at unequivocally slowing the relentless growth of this devastating neoplasm in a randomized clinical trial. Facilitating early clinical testing of promising targets can have a meaningful impact on improving our ability to conduct trials on agents that hold real promise for survival benefit.

There have been significant advances made in oncologic drug discovery in the last several decades. The path that a drug has to take from the laboratory through to FDA approval has remained unchanged, however.[5] With only 5–10% of new molecules advancing past initial stages of development, there is a great need to develop protocols that would allow early efficient testing of adequate drug penetration and sufficient biological efficacy of novel targeted agents.[6] An important goal of these studies is to obtain signals that suggest promise for further studies or that indicate futility for compounds that are unlikely to be effective.

New scientific approaches and regulatory guidelines have been proposed to shorten the drug development timeline by streamlining clinical models that test drug distribution and biological effects. One new approach, which has been called a "phase 0 trial," is driven by incorporation of systemic and ideally intratumoral pharmacokinetic and pharmacodynamic parameters into an early-phase study design.[7] Phase 0 studies can take various forms but typically refer to non-therapeutic, first-in-human studies enrolling a small number of patients (typically 10–12), involving limited drug exposures (often as a microdose) and incorporating pre- and post-drug tissue biopsies (Table 1).[8,9] A significant step in the direction of enabling phase 0 trial designs was the FDA's announcement of the exploratory IND (investigational new drug) mechanism in 2006.

The goal of a phase 0 study is to examine the pharmacological effects of the compound on patient tumors at an early stage of drug development. In assessing the drug's penetration into tumor tissue and modulation of its target(s) in an early stage of its development, the results can identify whether a candidate agent's trajectory is suitable for acceleration or that agent's clinical study should be held pending further preclinical optimization.[10] Since subtherapeutic exposure of drugs or therapeutic exposure for a limited number of doses is typically employed, the risk to the patient from the study agent is extraordinarily low,[11] as is the likelihood of benefit, however (see next paragraph). Still, this trial design shortens the preclinical stage of drug development by providing in vivo information from patients and their tumors, which is critical for drug development and could not be obtained via any other mechanism.[8]

Execution of a phase 0 clinical trial requires many considerations. Target selection must be optimized with appropriate preclinical biochemical and animal modeling. Pharmacokinetic assays to determine drug concentrations must be validated to provide a consistent assessment of drug content in tissues. The risks to the patient are less than conventional early-phase investigation, owing to the non-therapeutic nature of the regimen (when microdosing is used), but include risks associated with tumor or tissue sampling and the potential delay of participation in therapeutic clinical trials unless patients are allowed to stay on the experimental agent in seamless phase 0 to 1/2 transitions. From an ethical standpoint, their enrollment in a non-therapeutic drug study is justified by collective benefit of early human data on a prospective agent and its utility in accelerating subsequent phase 1, 2, or 3 studies.[11]

One of the major objectives of a phase 0 study is to demonstrate the biochemical effect of drug exposure, ie,

**Table 1** Characteristics of a classically defined phase 0 clinical trial

| |
|---|
| First-in-human |
| Small number of patients (<15) |
| Limited time of drug exposure (<7 days) |
| Microdosing |
| No therapeutic intent |
| Sampling of target tissue before and after drug exposure |

alteration in pathway activity as a result of drug action. This evaluation is optimally coupled to measurement of drug levels within the tumor to distinguish circumstances where a drug fails to exert its biological effect due to low tumor concentrations versus instances where high tumor levels are achieved but the drug does not successfully interact with its intended target in vivo. Such determination requires drug administration at a dose level that is expected to be effective.

In neuro-oncology, there are special considerations with respect to implementation of the phase 0 study design. The presence of the blood–brain barrier creates a separate physiological compartment that many molecules cannot cross.[12] Therefore, serum drug levels are unlikely to reflect drug exposure of the tumor.[13] Consequently, microdosing is also not a practical approach in neuro-oncology, as such low doses are likely to confound efforts to measure intratumoral concentrations. Furthermore, frequently only a limited number of tissue samples can be obtained safely, and potential complications such as hemorrhage can have a devastating outcome, more so than in non-CNS tumors. Therefore, each sample that is obtained for the study needs to be strategically planned.

The goal of this report is to extensively review previously published phase 0 or phase 0–like ("window of opportunity") clinical studies performed in the setting of glioblastoma that included evaluation of tumor pharmacodynamics of a therapeutic drug. Our goals were to critically analyze the protocols used in each study and to derive guidelines for future phase 0 studies applicable specifically to the development of therapeutics for CNS disease.

## Methods

This project was developed within the scope of the Response Assessment in Neuro-Oncology (RANO) working group and endorsed by its steering committee (including the following authors: M.A.V., M.v.d.B., S.C., P.W.).

### Search Methodology

A literature search of PubMed and EMBASE was conducted to include all studies up to December 2019. The specific search terms included in various combinations "phase 0," "phase 1," "phase 2," "glioblastoma," "glioma," "malignant brain tumors," "human brain tumor tissue," "pharmacokinetics," and "pharmacodynamics." Studies were limited to those involving drugs, and not biologics (eg, antibodies, engineered proteins, viral vectors, oncolytic viruses). The search results were filtered and restricted to studies or clinical trials in humans with abstracts and full texts, excluding reports that were limited to conference or congress abstracts. After the search was completed, the abstract of each identified publication was reviewed to determine relevance. From these studies, we selected literature that included analysis of drug levels or drug effect in patient tumor tissue. All of these studies were obtained and their reference lists were reviewed. Excluded from analysis were review articles, editorials, and individual case reports and animal studies. We eliminated any duplicate subject cohorts reported in more than one publication. Additionally, a search in the ClinicalTrials.gov registry returned a list of studies that met our above-mentioned search criteria, and the available ongoing trial information. Additional trials were identified by the personal knowledge of each of the authors.

### Data Extraction

Using a predesigned data extraction sheet, 2 reviewers (D.K. and H.B-R.) extracted the data from included studies. A third reviewer (M.A.V.) reviewed the search results and extracted data. Summary data that were extracted from the selected studies included the following: the journal name, the first author's name, country, searching database, search terms, language limitation, additional retrieval, study sample and design, patient numbers, drug that was used in the study, dose of the drug, systemic dose of drug used in other studies, the schedule of the drug administration prior to the surgery, drug blood level, the level of the drug in tumor tissue, and physiologic effect of the drug in the tumor tissue.

## Results

### Clinical Studies

Twenty-two publications[14–35] were identified that examined drug pharmacodynamics and/or pharmacokinetics in patients with glioblastoma. They are presented in chronological order, according to date of publication, and summarized in Table 2.

Eleven (50%) studies included patients with glioblastoma only, 9 (41%) with any World Health Organization (WHO) grade III or IV glioma, and 2 (9%) with any brain malignancy (primary or metastatic). Seventeen (77%) studies included patients with recurrent tumors only, 3 (14%) with newly diagnosed only, 1 (4%) with either; and 1 (4%) study did not specify the timing of disease. The studies were relatively small, with most occurring in the phase I setting. The maximum patient number was 30 and the smallest study included 3 patients. The average sample size was 12.

Therapeutics that have been subjected to tissue-based pharmacokinetic or pharmacodynamic evaluations had a variety of mechanisms of action, ranging from conventional cytotoxic agents to more recently developed targeted agents. Five (23%) studies investigated conventional cytotoxic chemotherapy agents, while 15 (68%) investigated agents that were targeted against specific cell surface receptors or signaling cascades. Two (9%) studies investigated an agent that reduces $O^6$-methylguanine-DNA methyltransferase (MGMT) activity ($O^6$-benzylguanine [O6-BG]). Twelve (54%) studies included multiple doses of the study agent prior to surgical sampling, whereas 8 (36%) studies provided drug in a single dose only prior to surgery, and 1 (4%) study with 2 drugs involved multiple doses of one drug and a single dose of the second drug prior to surgery. One (4%) study involved the use of

**Table 2** Summary of studies (1993–2019) that examined tumor tissue post administration of the study drug

| Study | # of Patients | Pathology | Drug | Dose | Relationship to Clinical Dose | Schedule Prior to Surgery | Tissue Samples | Drug Level Assessment | Biological Assessment/ Effect | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| Bergenheim 1993[14] | 16 | Astrocytoma, glioblastoma, ependymoma, metastases | Estramustine phosphate | 280 mg | Subclinical by 50% | One dose orally 14 h before surgery | Enhancing tumor and cystic fluid | Retained in tumor | N/A | Inhomogeneous pathology; differential oral absorption |
| Friedman 1998[15] | 30 | Newly diagnosed or recurrent anaplastic astrocytoma, glioblastoma | $O^6$-benzyl guanine | Dose escalation100 mg/ $m^2$ | Clinical | One dose 18 h before surgery | Enhancing tumor | Variable | MGMT concentration | |
| Zucchetti 1999[16] | 8 | Recurrent glioblastoma | Daunorubicin, liposomal | 50 mg, 1 h infusion | Subclinical | 24 h prior to surgery, 48 h in 1 patient | Central (enhancing), peripheral, and adjacent brain tissue | Variable similar concentration in tumor periphery and surrounding brain | N/A | |
| Albrecht 2001[17] | 8 | Newly diagnosed glioblastoma, gliosarcoma, anaplastic astrocytoma, adenocarcinoma | Daunorubicin, liposomal | 2 mg/kg i.v. over 30 min | Supraclinical | Surgery 12–50 h after the dose | Central (enhancing), peripheral and adjacent brain tissue | Variable tumor levels | N/A | |
| Lassman 2005[18] | 12 | Recurrent anaplastic astrocytoma, glioblastoma | Erlotinib or Gefitinib | 150 mg PO daily or 500 mg PO daily (respectively) | Clinical for erlotinib; supraclinical for gefitinib | Daily for 8 days prior to surgery | Enhancing tumor | ~10% for erlotinib ~220–370% for gefitinib | Phospho-EGFR (western blot) | Poor penetration of drug; increased levels of pEGFR |
| Weingart 2007[19] | 14 | Recurrent glioblastoma or anaplastic astrocytoma | $O^6$-benzyl guanine | 120 mg/ $m^2$ bolus, followed by an infusion of 30 mg/ $m^2$ | Clinical | Infusion for 48 h prior to surgery | Enhancing tumor | N/A | MGMT activity | MGMT inhibition study with carmustine wafers |
| Kuhn 2007[20] | 6 | Recurrent malignant glioma | Temsirolimus | 170–250 mg i.v. | Clinical | One dose 2 hours prior to surgery | Enhancing tumor | Comparable or higher than blood levels | N/A | |
| Blakeley 2009[21] | 4 | Recurrent malignant gliomas | Methotrexate | 12 g/ $m^2$, infused over 4 h | Clinical | N/A; Sampling during drug infusion | N/A | Continuous sampling Variable levels—higher in enhancing tumor | N/A | Microdialysis study |

**Table 2** Continued

| Study | # of Patients | Pathology | Drug | Dose | Relationship to Clinical Dose | Schedule Prior to Surgery | Tissue Samples | Drug Level Assessment | Biological Assessment/ Effect | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| Razis 2009[22] | 20 | Newly diagnosed glioblastoma | Imatinib | 400 mg orally b.i.d. for 7 days | Clinical | b.i.d. for 7 days prior to surgery | Enhancing tumor | N/A | Downstream kinase activation | Also looked at MRI at day 7 of therapy |
| Galanis 2009[23] | 66 (5 surgical) | Recurrent glioblastoma | Vorinostat | 200 mg b.i.d. | Clinical | 6 doses prior to surgery (3 days) | Enhancing tumor | N/A | Post treatment increase in acetylation of histones H2B and H3 and H4, upregulation of E-cadherin | Modest single agent clinical activity |
| Hegi 2011[24] | 22 | Recurrent glioblastoma | Gefitinib | 500 mg daily | Clinical | Daily for 5 days prior to surgery | Enhancing tumor | 4.1 μg/g | Phosphorylation-specific assays of EGFR pathway | EGFR dephosphorylated; no downstream effects observed |
| Gilbert 2012[25] | 12 | Recurrent glioblastoma or gliosarcoma | Cilengitide | 500 mg and 2000 mg | Clinical and subclinical | Three doses on 8, 4, and 1 day prior to surgery | Enhancing tumor | Higher than blood levels | N/A | Showed that the drug penetrates into enhancing tumor |
| Reardon 2012[26] | 10 | Recurrent glioblastoma | Ridaforlimus | Phase I, 12.5 mg and 15 mg | Subclinical | Surgery after 4 doses | Enhancing tumor | N/A | pS6 kinase, downstream of mTOR | Reduced activation of pS6 kinase |
| Drappatz 2013[27] | 9 | Recurrent glioblastoma, anaplastic astrocytoma, anaplastic oligodendroglioma | GRN1005 (peptide-drug conjugate with paclitaxel) | Phase I, 30 mg/m$^2$ | Subclinical (order of magnitude below MTD) | Surgery 6 h after infusion | Enhancing tumor | Intratumoral concentration, paclitaxel–cytotoxic concentrations | N/A | |
| Wen 2014[28] | 3 | Recurrent glioblastoma, anaplastic glioma | Temsirolimus + erlotinib | 15 mg weekly + 150 mg daily | Near clinical for temsirolimus, clinical for erlotinib | Erlotinib: daily for 5–7 days prior to surgery; temsirolimus: 1 dose 3–24 hours prior to surgery | Enhancing tumor | Variable levels | pS6 levels—similar pre and post treatment | Small samples limited the ability to perform complete analysis |
| Raizer 2016[29] | 9 | Recurrent high-grade gliomas | Bortezomib | Phase II 1.7 mg/m$^2$ i.v. on days 1, 4, and 8 | Clinical | Daily for 8 or 9 days prior to surgery | Enhancing tumor before and after treatment, normal brain controls | Higher levels in tumor vs plasma | NFkB Ia concentration the same pre and post treatment, by IF | Drug accumulates but has no physiologic effect |

**Table 2** Continued

| Study | # of Patients | Pathology | Drug | Dose | Relationship to Clinical Dose | Schedule Prior to Surgery | Tissue Samples | Drug Level Assessment | Biological Assessment/ Effect | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| Butowski 2016[30] | 13 | Recurrent glioblastoma | PLX3397 | 1000 mg PO daily | Clinical | Daily for 8 days prior to surgery | Enhancing tumor | Greater than 0.1 μM | Circulating biomarkers, and IHC | Accumulates in the tumor but demonstrates no efficacy |
| Xu 2016[31] | 21 (11 surgical) | Newly diagnosed glioblastoma or anaplastic astrocytoma | RO4929097 | 10–20 mg PO daily | Clinical and subclinical (dose escalation) | Daily for 7 days prior to surgery | Enhancing and non-enhancing tumor | Variable, 0.33 to 0.73 micromol/L; higher in contrast enhancing tumor | Downregulation of multiple aspects of Notch signaling in enhancing tissue | Evidence of biological activity in enhancing tumor tissue; evidence of tumor escape mechanism |
| Batchelor 2017[32] | 4 | Recurrent glioblastoma | Tandutinib | 500 mg PO b.i.d. | Near clinical, MTD was 600 mg b.i.d. | Surgery on day 8, 6 h after last dose | Enhancing tumor | 6.5–26.2 times the plasma concentration | Circulating biomarkers | Accumulates in tumor, but no clinical benefit (markers and survival) |
| Sanai 2018[33] | 20 | Recurrent glioblastoma | AZD1775 | 100, 200, and 400 mg | Clinical and subclinical | 4, 8, or 24 h prior to surgery | Enhancing tumor | 3.8–40.4 times the plasma concentration | IHC, markers of checkpoint disruption | Accumulates in tumor and exerts expected physiological effect |
| Wen 2019[34] | 65 (15 surgical) | Recurrent glioblastoma | Buparlisib | 100 mg | Clinical | Daily for 7 to 13 days prior to surgery | Enhancing tumor and non-enhancing tumor infiltrated brain | Tumor to plasma geometric mean ratio of 1.0 (0.18–8.44) | Decrease in $pAKT^{S473}$ in 6/15 patients | Did not meet its efficacy endpoint |
| Tien 2019[35] | 12 | Recurrent glioblastoma | Ribociclib | 900 mg | MTD | Daily for 5 days prior to surgery | Enhancing tumor and non-enhancing tumor infiltrated brain | Median unbound tumor to plasma ratio of 3.77 | Decline in phospho-RB in 6/12 patients | Accumulates in tumor and exerts expected physiological effect |

IF, immunofluorescence; IHC, immunohistochemistry; MTD, maximum tolerated dose; mTOR, mammalian target of rapamycin; NFkB = nuclear factor kappa B; RB, retinoblastoma.

microdialysis, and the study drug was given continuously during this form of monitoring.

Eleven (50%) studies were performed using a dose of drug that was found to be the maximum tolerated dose or the usual clinical dose in prior studies. Three (14%) studies involved dose escalation and hence provided either subclinical or clinical doses prior to surgery. Six (27%) studies provided subclinical doses, and 2 (9%) studies provided doses higher than the conventional doses used for other indications.

Tissue samples were obtained from enhancing tumor in 21 (95%) studies, non-enhancing tumor in 6 (27%) studies, and from cyst fluid in 1 (4%) study. One (4%) study involved microdialysis, and no tissue samples were obtained. Drug levels were assessed in tumor and/or tumor-infiltrated brain in 17 (77%) studies. Biological assessments of drug activity in tumor tissue were performed in 15 (68%) studies.

### Ongoing Clinical Trials

A list of 14 clinical trials that include the collection of tumor specimens after a short course of preoperative treatment and that were open at the time of manuscript writing (February 2020) is presented in Table 3.

Nine (64%) trials enroll patients with glioblastoma only, and 4 (29%) permit any high-grade glioma; 1 (7%) trial is open for meningiomas. Nearly all include patients in the recurrent setting only. One (7%) trial includes patients with brain metastases from solid tumors, in addition to gliomas. There is a large variability in presurgical regimens of experimental drug administration, and 9 of the studies do not specify the exact dose of the drug that they intend to use, although for most of those it is because the tissue-based study is within the context of a phase I dose escalation design.

All clinical trials are collecting blood samples to characterize the pharmacokinetics of the study drug and 4 (29%) explicitly mention that tumor samples will be obtained and analyzed for tumor drug levels. Out of these, only one study will sample different tumor components, such as enhancing and non-enhancing tumor compartments. The majority of these trials are designed to assess biological impact of the drug on the tumor. Seven (50%) studies specify that pharmacodynamic evidence of drug action will be evaluated in the study. Methods vary among different studies and are tailored to the mechanism of the drug action. Some of the employed techniques include immunohistochemistry assessment of phosphorylation levels of key proteins, activation of apoptosis pathways, Ki67 staining to assess tumor proliferative activity, and assessment of lymphocyte infiltration in studies assessing immunotherapy drugs.

## Discussion

In this review, we identified 21 published studies that assessed tissue-based pharmacokinetic and pharmacodynamic parameters of experimental chemotherapeutics used to treat patients with brain tumors. While these studies were not identified as "phase 0" at the time of publication, they meet many, but not all, of the classical criteria for this designation. Notably, these studies were published over a 25-year time period; hence, there has been fewer than one published "phase 0" study per year in neuro-oncology despite previous calls for more of these types of trials.[36,37] Given the known challenges associated with systemic delivery of therapeutics to the brain, it should not come as a surprise that the paucity of investigations into the pharmacodynamics of brain tumor–targeted experimental therapeutics is associated with the overall lack of success in therapeutic development in this field. Indeed, the lack of phase 0 investigations may even be predictive of the general failure to make substantial progress in finding effective treatments for gliomas.

An important point to consider with respect to brain tumor tissue collection is the amount and quality of the tissue allocated for the study and the mode of tissue preservation. Stereotactic tumor biopsies typically provide limited amounts of tissue that may not be sufficient for accurate drug level analysis, tissue preservation for immunohistochemistry, and biochemical studies. One example of a study that was limited in its ability to provide meaningful information on tissue distribution was published by Wen et al.[28] Their disappointing experience with this trial led them to adjust future protocols to ensure that sufficient tissue is obtained in a higher percentage of patients. Indeed, we believe that this experience supports a requirement for neuro-oncology phase 0 studies to be conducted in the setting of a planned tumor resection only.

Pharmacokinetic and pharmacodynamic approaches have been used to assess drug delivery, including assessment of tumor and CSF drug levels and assessment of drug effects. Tumor tissue drug levels were measured in 16 studies. Three studies obtained samples from different areas of the brain tumor, including from solid tumor and tumor-adjacent brain tissue,[16,17] or enhancing and non-enhancing tumor components,[21] whereas others relied on samples from solid tumor tissue only. Given the unique therapeutic challenge in neuro-oncology posed by the presence of the blood–brain barrier, it is important to obtain samples from both enhancing and non-enhancing tumor components, as the concentration of the drug, and hence effectiveness of therapy, may be substantially different in those two areas. These two areas are illustrated with use of relevant MR images in Figure 1. For example, a phase 0/1 study of a Notch inhibitor in newly diagnosed WHO grade III or IV glioma showed that the levels in non-enhancing and enhancing tumor differed substantially.[31] In two other studies that evaluated adjacent brain tissue, similar drug levels were observed within tumor and in normal brain.[16,17] One study used microdialysis to evaluate drug distribution in enhancing and non-enhancing portions of the tumor, and noted very different pharmacokinetics with slower drug distribution and lower peaked levels in non-enhancing areas of the tumor.[21] These disparate results may reflect differences in the chemistry of the drugs evaluated in the various studies but support the need to evaluate both tumor core and tumor-infiltrated brain to paint a complete picture of drug distribution. Most notably, these studies do not account for the challenge of differentiating intravascular drug from that which is truly within the tumor interstitial

**Table 3** Summary of ongoing clinical trials that obtain tumor tissue for analysis as part of their protocols

| Study | Phase Number | Number of Patients | Recruitment Status | Pathology | Drug | Dose | Schedule Prior to Surgery | Specimens | Physiologic Effect |
|---|---|---|---|---|---|---|---|---|---|
| NCT01849146 | Phase I | 36 | Recruiting | Recurrent glioblastoma | Adavosertib | Not specified | Not specified | Tumor, blood | pRb expression, Ki67, pCDC2, cleaved caspase 3 |
| NCT01986348 | Phase II | 110 | Active, not recruiting | Recurrent gliomas | Selinexor | 60 mg twice per week | 3 doses prior to surgery | Tumor, blood | |
| NCT02101905 | Pilot | 33 | Active, not recruiting | Recurrent high-grade glioma, EGFR amplified | Lapatinib | MTD | Days −2, and 0, last dose 3 h prior to surgery | Tumor | Total and phospho-EGFR, Ki67 |
| NCT02133183 | Phase I | 40 | Active, not recruiting | Recurrent glioblastoma | Sapanisertib | MTD | Not specified | Enhancing and non-enhancing tumor | IHC for pS6, p4EBP, pmTOR, and AKTpSer473; pharmacokinetics in enhancing tumor |
| NCT02337686 | Phase II | 18 | Active, not recruiting | Recurrent glioblastoma | Pembrolizumab | 200 mg i.v. | 2 doses q3weeks | Tumor, blood | Polyfunctional effector T cells:Treg ratio |
| NCT02525692 | Phase II | 76 | Recruiting | Recurrent glioblastoma ± H3 K27M mutation | ONC201 | Not specified | Not specified | Tumor | Not specified |
| NCT02630030 | Phase 0 | 3 | Completed | Recurrent or progressive glioblastoma | Ixazomib | Not specified | Oral dose 3 h prior to surgery | Tumor | Pharmacokinetics in tumor tissue |
| NCT02850744 | Phase II | 10 | Terminated | Recurrent glioblastoma | PQR309 | 80 mg | For 3 consecutive days prior to surgery | Tumor, CSF, skin | Pharmacokinetics in cerebrospinal fluid |
| NCT02852655 | Pilot | 35 | Active, not recruiting | Glioblastoma | Pembrolizumab | | | Tumor, blood | Tumor infiltrating T-lymphocyte density |
| NCT02933736 | Early phase I | 48 | Unknown | Meningioma | Ribociclib | 900 mg/d | 5 doses then 3 cohorts: 2–4, 4–8, 22–26 h after admin | Enhancing and non-enhancing tumor, CSF | Glioblastoma cohort published[35] |
| NCT02981940 | Phase II | 36 | Active, not recruiting | Recurrent glioblastoma | Abemaciclib | b.i.d. | Short preoperative course | Enhancing tumor | pRB expression level |
| NCT03122197 | Phase 0/I | 42 | Recruiting | Recurrent gliomas | Letrozole | 2–12 mg/d | 1–5 days preop and continuing postop | Enhancing tumor | Area under the curve in tumor tissue |
| NCT03027388 | Phase II | 20 | Recruiting | Recurrent gliomas | LBIOO | 2.33 mg/m$^2$ | 1–12 h prior to surgery | Tumor tissues | Pharmacokinetics parameters in tumor tissues |
| NCT03107780 | Phase 0/I | 86 | Recruiting | Recurrent glioblastoma | AMG-232 | Dose escalation | 2 doses prior to surgery (q.d. dosing) | Enhancing and nonenhancing tumor | Pharmacokinetics parameters in tumor tissue |

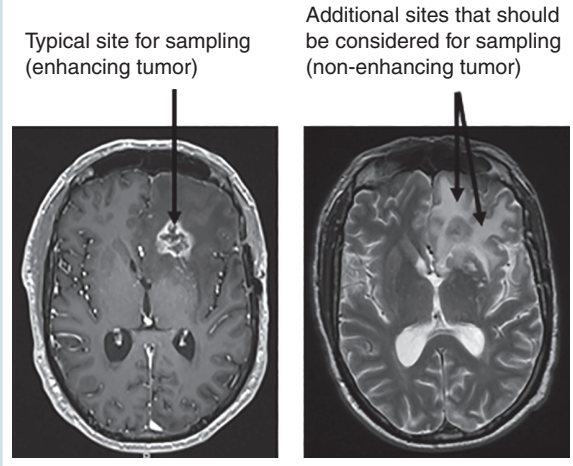


**Fig. 1** MR images that demonstrate the enhancing (left, T1 weighted MRI with contrast) and non-enhancing (right, T2 weighted MRI) regions of a recurrent glioblastoma.

space.[13,38–40] Nonetheless, in the context of gliomas, which have both solid and brain-infiltrating components, complete assessment of drug penetration/effects must include sampling of both enhancing and non-enhancing tumor tissue. Simultaneously, for early stage trials of systemically delivered therapeutics in neuro-oncology patients, it is always necessary to also evaluate peripheral pharmacokinetics at the same time as CNS pharmacokinetic measurements are obtained, even when the systemic pharmacokinetics for the same dose have been well established in other cancers. It has been well documented that the peripheral pharmacokinetics of some therapeutics can be impacted by certain classes of drugs used extensively in the neuro-oncology patient population (eg, liver enzyme-inducing anti-epileptic drugs).[41]

Because of the challenges associated with interpretation of drug level measurements alone in clinical tissues, a more compelling argument for proof of delivery comes in the form of pharmacodynamic assays. Few studies included an evaluation of drug effect on tumor tissue, and many of those that did presented a more complete, yet complex, picture. For example, while gefinitib appeared to be capable of inhibiting phosphorylation of epidermal growth factor receptor (EGFR) in enhancing glioma tissue, the critical parts of the downstream signal transduction pathway were unaffected.[24] This result is instructive in that it suggests that the clinical failure of this signal transduction approach may have been due to a complex biology more so than drug delivery. Similar observations were reported in other studies where analysis of activation of downstream pathway revealed levels of phosphorylated signal transduction proteins that were similar to those observed in tumors that were naïve to the drug.[26,28,34] Yet studies that included the use of tissue-based assays of drug effect were in the minority—most studies were not designed to provide, or were not capable of providing,[28,31] treated tissues for mechanistic analyses.

Another limitation to some of the studies performed to date is lack of relevant baseline (control) data that would be essential for interpreting the experimental result. For example, the studies that evaluated the utility of $O^6$-BG were not designed to provide a baseline assessment of MGMT activity (either directly or indirectly via assessment of MGMT promoter methylation assay).[15,19] Similarly, studies of signal transduction inhibitors ideally should include an assessment of target activity prior to treatment. Reardon et al used archival specimens from tumors that were treatment naïve, whereas subsequent medical treatments may have changed tumor phenotype at recurrence.[26] This reliance on what may be an outdated specimen for baseline assessments is one of the challenges inherent to the field of neuro-oncology. Another way to approach this issue is to randomize patients with respect to presurgical treatment followed by surgery with tissue harvesting for assessment of relevant treatment targets. In this manner, tumor not exposed to drug can be compared with tumor treated with the drug, with the caveat that these tumors are not derived from the same patient.

Another challenge associated with phase 0 trials, in general, which is likely to be even more challenging in neuro-oncology trials, is that of appropriate statistical powering of pharmacodynamic analyses. For conventional phase 0 studies, there is a well-recognized problem that the small patient sample size can risk underpowering of the analysis of any study endpoints.[42] For neuro-oncology trials, this risk is even higher due to the limited sampling of target tissues that can be performed, usually at only one time point and without same-patient, pretreatment control tissue samples. In addition, the challenges of tissue heterogeneity of response are likely to be larger in brain tumors than in their systemic counterparts due to the presence of a blood–tumor barrier that can provide variable permeability to most agents. Finally, it can be challenging to determine what magnitude of pharmacodynamic response needs to be observed in order to properly power the analysis. As shown in several trials that evaluated pharmacodynamic responses, the correlation between pharmacodynamic and clinical responses in neuro-oncology trials has been poor. Perhaps a better strategy is to dichotomize results for go/no-go decision making—that is, lack of evidence of any target-specific biological effect should eliminate the agent from further evaluation (at least via the systemic route of administration). While the presence of an effect, even if substantial, is not a guarantee of clinical activity, at least it is an indicator of the ability of the agent to impact on tumor tissue.

Some phase 0/window of opportunity trials involve use of presurgical treatment only with the explicitly stated intent to determine the biological, but not clinical, impact of a novel therapy. The use of any therapeutic in a cancer patient is often defined as a "regimen," and so a pharmacodynamic-only study design may result in the patient being excluded from subsequent trials due to the number of prior regimens. In neuro-oncology, the use of a pharmacodynamic-only trial design is rare; but in line with these types of trials that are used in systemic cancer, a trial that intends to collect pharmacodynamic data only and that is unlikely to produce a drug-induced physiological

impact on efficacy or toxicity should not be considered a "regimen."[43]

Overall, the experience to date suggests that several key components must be present in a phase 0 clinical trial in neuro-oncology in order to identify systemically administered therapeutics that are capable of crossing the blood–brain or blood–tumor barrier, accumulate in tumor tissue, and exert pharmacodynamic effects on tumor biology. Table 4 summarizes the major components of phase 0 clinical trial design specifically pertaining to phase 0/window of opportunity clinical trial design in neuro-oncology. Specifically, when protocols include tumor tissue analysis of drug levels, these assessments should be performed in a variety of tumor subenvironments (enhancing and non-enhancing, central and peripheral, and tumor-adjacent areas). Sampling from these separate areas is not expected to add time to the tumor resection procedure as they are already regions that are either removed or visually assessed by the neurosurgeon in the course of the operation. The assessment of tumor drug levels alone without a parallel effort to assess the activity of the drug on tumor tissue, however, should be discouraged as drug levels are only one important variable potentially impacting on the overall efficacy of a study drug for CNS tumor patients. Other factors must be taken into account, including drug kinetics, binding to serum or tissue proteins, timing of sample collection with respect to last dose, and tissue sample contamination with intravascular drug, to name a few. Ideally, all phase 0 trials in neuro-oncology should include measurements of the biological effects of the drug, including demonstration of the effect of the drug on cell viability and proliferation potential, but mostly focused on validation of drug-specific target effects. These assays should be supported by robust preclinical studies that confirm their validity and reliability in the in vivo setting, and they may be supplemented by techniques to perform noninvasive, imaging-based evaluations of drug effect on tumor tissue.[44,45] The study protocol should also include a discussion of what constitutes a positive or negative result with the use of each assay, and these thresholds should be discussed in the study report. Finally, the tissue requirements (volume, timing between collection and assay) for successful implementation of the assay in the clinical setting need to be specified. Ultimately, challenges associated with systemic therapeutic delivery to brain tumors, particularly their infiltrative components that are protected by the blood–brain barrier, rise to the level of making treated tissue-based assessments essential for successful therapeutic development, unless such assessments are contraindicated by patient safety concerns.

**Table 4** Key components proposed for phase 0/window of opportunity trials in neuro-oncology

| | |
|---|---|
| Patients undergoing a planned tumor resection | |
| Use clinical dose of drug | |
| Perform comprehensive tumor drug level measurement | |
| | Enhancing tumor component |
| | Non-enhancing tumor component |
| | Tumor-adjacent areas |
| | Consider microdialysis for compounds suitable for this method |
| Always evaluate the biological effect of the drug | |
| | Cell viability |
| | Cell proliferation |
| | Drug-specific target(s) |

## Conclusions

The phase 0 clinical trial approach is an underutilized strategy for the development of systemically administered therapeutics in neuro-oncology. Few trials incorporate tissue-based assessment of drug penetration and pharmacodynamics in a field where there are unique and substantial biological barriers that prevent drug access to tumor and tumor-infiltrated brain. In addition, there has been substantial heterogeneity in pharmacodynamic approaches, and some of the strategies available for the development of therapeutics for systemic cancers are not appropriate for gliomas. Tissue-based assessments of biological effects of treatment should be strongly supported early in the course of the clinical development of novel therapeutics.

## Keywords

clinical trial | glioblastoma | pharmacodynamics | pharmacokinetics | phase 0

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