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REVIEW

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Glioma drug development benefits from emerging phase 0 and window-of-opportunity trial paradigm

Shenzhong Jiang[1*] and Chunlong Zhong[1*]

Abstract

Clinical drug development is fundamentally difficult for rare and difficult-to-treat solid tumors, for example, glioma. Glioblastoma (GBM), an invariably fatal primary brain tumor, poses a significant challenge in the realm of effective treatments, necessitating an accelerated approach to innovative drug discovery. Investigators keep requiring a process toward obtaining more reliable early-stage signals related to drug activity and a process toward translating those signals into clinical benefits efficiently in late-stage drug development. Besides, these processes could increase the likelihood of benefit in late-stage settings at a lower cost and encourage more opportunities for drug development against other rare and difficult-to-treat cancers. Phase 0 and window-of-opportunity design has been advocated for glioma, aiming to identify and eliminate ineffective therapies early in the specific drug development process, thereby enhancing overall trial quality. However, challenges persist in implementing this trial design including obtaining pre-treatment samples, establishing accurate methodological platforms and biostatistical pipelines, and identifying novel biomarkers based on both clinical and multi-omics information to predict long-term drug responses. In this review, we encapsulate current evidence regarding the window-of-opportunity design in glioma, advocating for its recognition as a standard paradigm in new drug development.

Highlights

Duration between phase 2 to 3 trial on glioma is long and most phase 3 trials fail.
Phase 0 and window-of-opportunity studies should both fulfill PK and PD analysis.
Phase 0 and window-of-opportunity trial helps to avoid ineffective therapies and promote new drug discovery.
Conduction of phase 0 and window-of-opportunity trials needs a comprehensive coordination from a study team.
Obtaining pretreatment samples and identify surrogate biomarkers are still challenging.

Keywords Phase 0, Window-of-opportunity trial, Glioma, Early-stage trial, Drug development

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Gliomas are the most common primary central nervous system (CNS) tumors and Glioblastoma (GBM) stands as the most lethal among them. Despite the application of standard and aggressive therapies, the overall survival (OS) for patients remains disheartening [1, 2]. Over the past two decades, the translation of findings from basic and preclinical research into promising treatment methods approved by the United States Food and Drug Administration (FDA) has been limited and challenging. While radiotherapy (RT), chemotherapy (temozolomide (TMZ)), and anti-angiogenesis therapy (bevacizumab) are conventionally utilized in glioma treatment, tumor-treating fields (TTFs) have emerged in clinical trials, showcasing extended OS. However, real-world cost-effectiveness concerns and cautious determination of specific populations are essential considerations. Although conventional immunotherapy, exemplified by immune checkpoint inhibitors (ICIs), has demonstrated remarkable efficacy in various solid tumors such as melanoma, non-small cell lung cancer (NSCLC), and urothelial carcinoma, its clinical benefits are less pronounced in glioma [3, 4]. The key reason is the immunosuppressive tumor microenvironment (TME) of glioma and intratumoral heterogeneity [5]. Addressing this heterogeneity and developing novel GBM-specific yet accurate models for personalized treatments are imperative for both preclinical and clinical studies [6, 7].

Given the deficiency in new drug discovery for GBM, there is ongoing debate regarding the need for improvement in the clinical trial landscape. Notably, the average duration from the initiation of phase II trials to the conclusion of phase III trials stands at 7.2 years, with over 91% of phase III trials for GBM proving unsuccessful [8–10]. In response, clinicians have instituted the early stages of phase 0 or window-of-opportunity trials to expeditiously identify potentially efficacious drugs for advancement to later-stage trials and elucidate their pharmacokinetic (PK) characteristics [8]. In this case, the timely exclusion of ineffective drugs ensures optimal cost-effectiveness. The judicious and ethical design of the study contributes to maintaining the quality of the trial. While phase 0 and window-of-opportunity trials exhibit similarities and disparities. Both can transpire before, during, or even subsequent to early-phase studies. However, a window-of-opportunity trial harbors therapeutic intent that aligns before, alongside, or immediately after phase 1 studies (Fig. 1). Its objective is to ascertain the maximum tolerated dose (MTD), ensure a comprehensive understanding of PK before formally establishing a treatment regimen, and ultimately decide whether to progress to further phase 1 or phase 2 trials.

Pharmacokinetics and pharmacodynamics pave the way for these early clinical trials

GBM evolves and propagates within an organ, impeding accessibility of tumor cells to most systematically administered agents due to the presence of the blood-brain barrier (BBB), which is constituted by an intricate network of endothelial and glial cells that obstruct the majority of systematically administered agents from reaching the CNS compartment [11, 12]. Insufficient penetration into the central nervous system is frequently cited as the primary cause of trial failures in neuro-oncology [13].

The assessment of PK and pharmacodynamics (PD) associated with a novel therapy, be it molecularly targeted or otherwise, constitutes the cornerstone of innovative therapy development. In the context of non-CNS cancers, the response of blood PK often serves as a robust indicator of drug exposure at the targeted site of disease, while PD markers in blood and/or tumor tissue offer confirmation of the anticipated metabolic or biological effects. In CNS cancers, paradoxically, PK values lack substantial significance as indicators for brain exposure since the majority of systematically administered therapeutics exhibit limited penetration into the brain [14]. Even purportedly "brain-penetrant" therapeutics have not consistently undergone evaluation for their efficacy in entering intact brain tissue, largely due to restricted assessments conducted within preclinical models involving the implantation of a tumor in the brain of a rodent [15].

Overview features of window-of-opportunity trials

The window-of-opportunity trials have several principles. The first is short treatment duration. Their treatment duration should be short and limited to a few days and weeks to avoid delaying curative treatment. Additionally, investigations would consider the PK and mechanisms of action of the compound to determine the final duration. In PK stage, the investigated compound should be administered long enough to reach a steady state to generate meaningful results. Modulation of phosphoproteins is generally achieved shortly after drug administration if the drug's blood levels are appropriate. However, for better evaluation of molecular profiles like gene/protein expression or individual immune response, a longer period maybe required. The ideal time to implement a window-of-opportunity design is during the preparatory phase between diagnosis and standard treatment. Therefore, the interval could be allocated for staging procedures, essential physical examinations and the planning of chemotherapy, radiotherapy or immunotherapy [16]. Although the interval between clinical diagnosis and standard treatment is not yet precisely defined, a timeframe of within four weeks is preferable [17, 18] (Fig. 1).

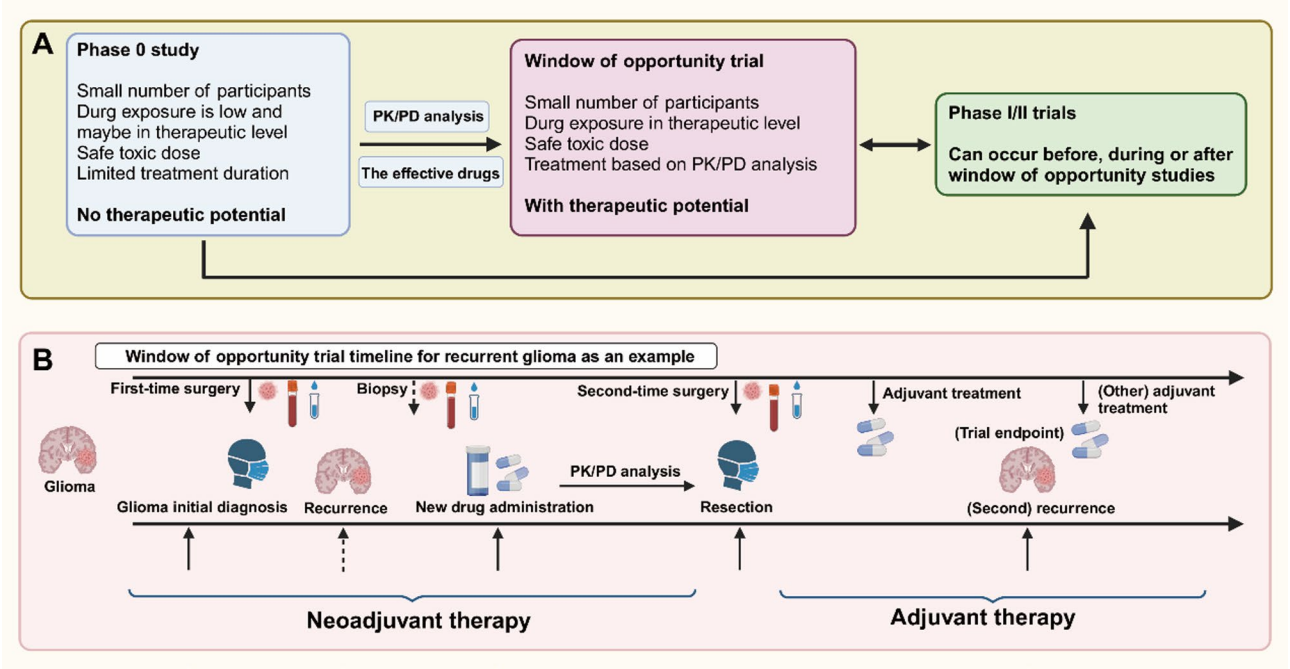

Fig. 1 The overview of phase 0 and window-of-opportunity study for glioma. (A) Phase 0 and window-of-opportunity studies exhibit both differences and similarities. Phase 0 studies involve minimal drug exposure, ensuring a safe toxic dose, while exposure in the window-of-opportunity trial may reach therapeutic levels, with limited treatment duration. The primary goal of a phase 0 study is not therapeutic. Effective drugs identified through PD/PD analysis may progress to a window-of-opportunity trial. In the latter, drug exposure is assessed at therapeutic levels, with careful consideration of safety margins. Treatment duration is determined based on prior PK/PD analyses. Both trials involve a small number of participants, given their early-stage nature. Typically, phase I/II studies follow phase 0, but they can occur before, during, or after window-of-opportunity studies. (**B**) We present the key procedures and sample collection in the development of a window-of-opportunity trial for recurrent glioma. Before the first recurrence, surgeons can obtain tumor and other samples during the initial surgery after diagnosis. Following the first recurrence, before the second resection, new drugs are administered based on PK/PD analysis, with the treatment duration determined. Biopsy samples may be collected during this phase. Adjuvant treatment follows, employing the same or different drugs until the second recurrence. This entire process highlights that the window-of-opportunity trial provides opportunities to obtain pre-new-drug treatment samples for further analysis or comparison. It heavily relies on PK/PD results and selectively advances drugs with therapeutic potential to subsequent stages or higher-level clinical trials, optimizing efficiency in terms of energy, time, and cost. Abbreviations: PK, pharmacokinetic; PD, pharmacodynamics

The second is primary endpoint should be a molecular or functional imaging parameter as a surrogate marker of treatment efficacy impacting OS and progression-free survival (PFS). The cut-off value for binary endpoints should be sufficiently precise to distinguish responders from non-responders, closely linked to clinical outcomes. While continuous endpoint like Ki-67 was also suitable for specific exploratory analyses, of note, Ki-67 cannot universally serve as a surrogate marker for all compounds and cancers [19, 20]. Other molecular endpoints, such as decreased phosphorylation of the targeted kinase receptor or modulation of specific cell cycle regulators, may also be promising candidates for surrogate markers when investigating compounds, though they still require standardization, validation, and central analysis [21, 22]. When the primary endpoint is based on comparing pre- and post-treatment biopsies, paired biopsies should be conducted under identical conditions and procedures to mitigate the effects of tumor heterogeneity and procedure-induced modifications. Pathological response, combined with the quantification of viable residual tumor cells in the surgical specimen, could correlate with long-term outcomes and might serve as a valid endpoint [23]. The Response Evaluation Criteria in Solid Tumors (RECIST) criteria have been extensively utilized in clinical trials involving solid tumors; however, anatomic imaging endpoints are impractical due to the abbreviated treatment duration [16, 24–26]. Functional imaging modalities, such as 2-[fluorine-18]-fluoro-2-deoxy-D-glucose positron emission tomography (18 F-FDG-PET), also serve as primary endpoints to identify early metabolic alterations [16, 21]. Similarly, it is advisable to predefine safety endpoints according to early stopping rules, with a particular emphasis on surgical safety assessed by an independent committee [16, 21]. Additionally, the toxicity profile, MTD, and recommended dose of the investigational compound should be thoroughly characterized before commencing the window study.

Moreover, they should incorporate endpoints encompassing molecular analyses such as

immunohistochemistry (IHC), staining, DNA/RNA investigations, gene expression profiling, or functional imaging techniques. Furthermore, certification of molecular analyses and quality control, along with a centralized review of imaging and standard treatment procedures, is indispensable. Functional imaging measurements heavily rely on imaging standardization levels. Therefore, imaging guidelines should be established beforehand to ensure standardized imaging acquisition [27]. Additionally, the proposed standard treatment must be clearly defined and subjected to rigorous quality control measures.

Overview features of phase 0 clinical trials

Phase 0 studies are acknowledged as pivotal in the development of targeted therapies, particularly where the therapeutic effect hinges on the successful delivery of the drug to the tissue target and instigating a PD response. Consequently, therapies that prove ineffective in modulating the tissue target and eliciting a PD response are promptly eliminated through phase 0 studies [28, 29]. In the realm of neuro-oncology, both "phase 0" and "window-of-opportunity" trials involve administering a potential drug for a brief duration between the "window" of treatment and surgery, followed by the collection of samples to evaluate PD effects. Despite some commonalities, there exist distinctions. Traditional phase 0 trials encompass non-therapeutic microdoses, whereas window-of-opportunity trials involve therapeutic doses albeit for a brief duration [30]. The use of therapeutic doses in window-of-opportunity trials is aimed at ensuring adequate drug penetrance, facilitating a successful assessment of the PD effects of the drug. In the context of neuro-oncology, a phase 0 trial can adopt a window-of-opportunity trial approach by prescribing a higher dose level to ensure BBB penetrance. Typically, the MTD is employed for a brief period to mitigate potential toxicities [31]. Due to the considerable similarities in design, phase 0 and window-of-opportunity trials are occasionally used interchangeably.

Overall, phase 0 trials entail the inclusion of a limited cohort exposed to a singular regimen of a low, non-toxic dosage of a pharmaceutical agent for a constrained duration (typically less than 7 days). The primary objective of phase 0 trials is to scrutinize the PK and PD characteristics of a pharmaceutical agent of considerable interest; typically, these trials do not harbor therapeutic intent. Similarly, but different, a window-of-opportunity trial assesses both PK and PD features while investigating doses and schedules postulated to contribute to a therapeutic effect. The transition from phase 0 to window-of-opportunity studies is plausible; when a drug in the phase 0 stage, or micro-dosing study, exhibits promise, the treatment can progress to the subsequent stage involving higher therapeutic doses.

Sample acquisition in phase 0/window-of-opportunity trials

A crucial procedural stage involves the acquisition of tumor samples prior to initiating treatment, serving as a biological control mechanism to comprehensively grasp the intricacies of the tumor landscape (Fig. 2). While challenges consist in these tissue-based studies, on one hand, samples are not uniformly obtained in accordance with contemporary neurosurgical practices preceding therapeutic interventions. On the other hand, the nomenclature employed to delineate the window-of-opportunity trials lacks uniformity, thereby contributing to technical disparities. Moreover, obtaining tumor tissues from recurrent diseases may appear ostensibly uncomplicated, the duration between sample acquisition and subsequent genotoxic therapies spans several weeks, if not months [32–34]. Evidently, the tumor immune profile, TME, cell state, and response sensitivity undergo substantial alterations during this interim [35]. In light of this, it is advisable to advocate and expand biopsies before the intervention. Stereotactic needle biopsies (SNBs) constitute a routine clinical practice, and although there exists some risk of intracranial hemorrhage, judicious patient stratification enables its performance as an outpatient procedure [36, 37].

Biopsies and surrogate tissues are consistently procured as a baseline before the administration of the investigational drug at a non-therapeutic dose. Subsequently, post-treatment blood samples can also be obtained for PK analyses. Prior to formal resection and post-treatment, there remain viable opportunities to acquire samples through SNB for CNS tumors, thereby facilitating the assessment of PK. For GBM patients, the sampled tissues exhibit notable diversities. For instance, cerebrospinal fluid (CSF) samples can be obtained as surrogate tissue to assess BBB penetrance [38]. During craniotomy, it is advisable to procure tumor samples from the BBB-permeable contrast-enhancing tumor, representing the most active tumor region in gliomas [39–41]as well as from the more diffuse and BBB-intact non-enhancing tumor to capture heterogeneity in the intratumoral TME and BBB penetrance [42].

If the time window between biopsy and resection is notably narrow, there may be insufficient time for an exhaustive investigation of the agent's bioactivity [43]. In window-of-opportunity trials, a pre-treatment biopsy can be reserved for patients exhibiting good physical function and possessing tumors smaller than 50 ml. The median doubling time for tumors typically spans 21–22 days [43]. Consequently, planned resection typically occurs 3–4 weeks subsequent to biopsy and drug administration within the designated time window. The median assessment of tumor growth may not adequately reflect the characteristics of all patients, particularly

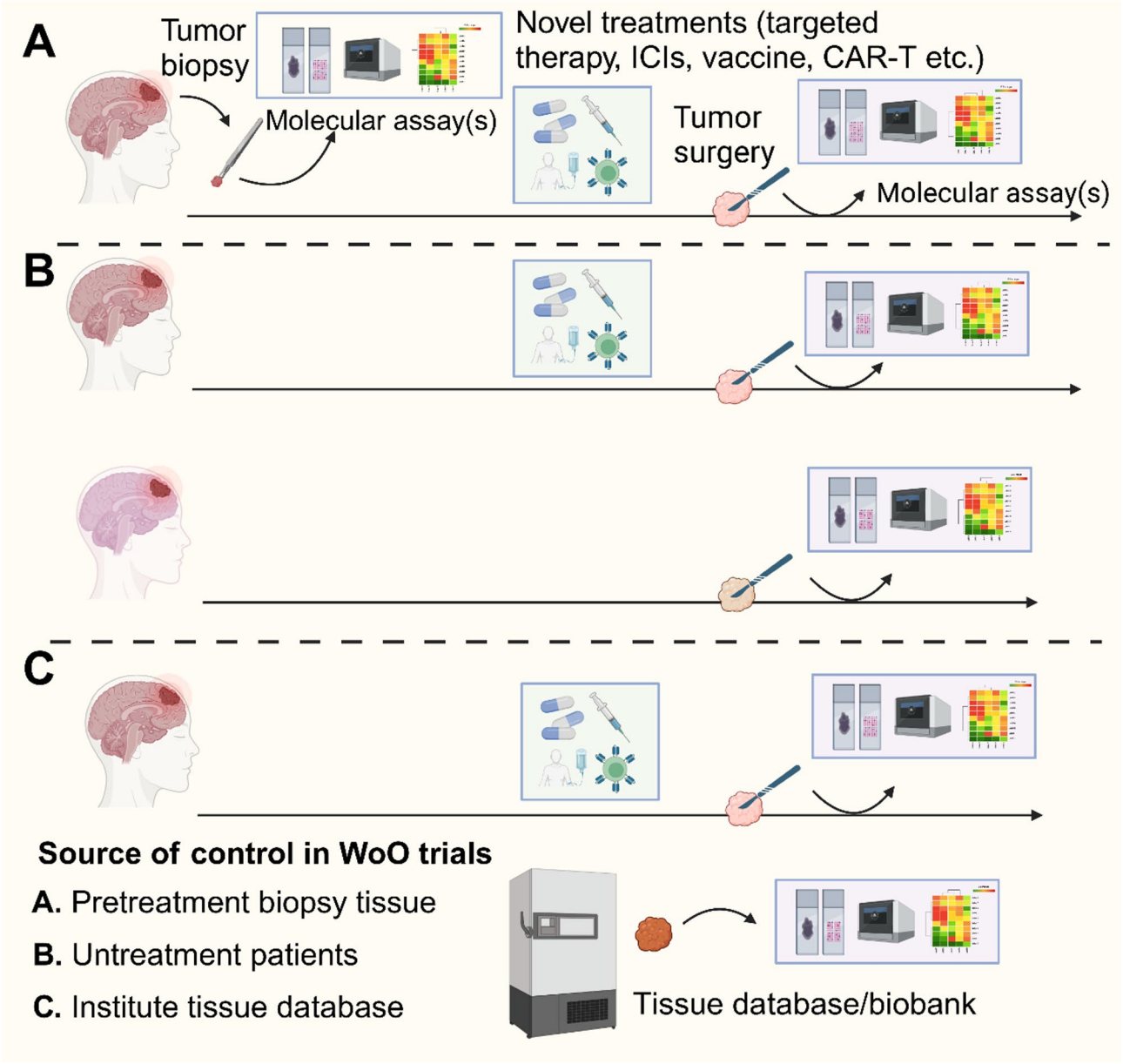

Fig. 2 The source of control for window-of-opportunity trial. (A) The control group is derived from pretreatment biopsy specimens. (B) The control group is from untreated patients. (C) The control group is from cohort/institute tissue biobank. Abbreviations: ICIs, immune checkpoint inhibitors; CAR-T, chimeric antigen receptor T cell; WoO, window-of-opportunity

those susceptible to rapid tumor growth. In such cases, prompt surgery accompanied by diagnostic imaging and cellular proliferation markers is imperative. Although biopsy facilitates sample acquisition before treatment and resection, given the evident intratumoral heterogeneity of gliomas, the biopsy tissues should be of sufficient size to enable accurate detection of histological features and to be representative. Additionally, comparing similar regions of the samples from biopsy and resection is advisable to mitigate the possibility that histological changes resulting from treatment are derived from intratumoral heterogeneity [44].

Molecular assay in phase 0/window-of-opportunity trials

In above part, it was stated that predefined molecular assay should be considered as a crucial component for the desired molecular outcome of window-of-opportunity studies, here we will give more details. When selecting the optimal assay, one fundamental assay that should be incorporated in all studies is a simple assessment of drug levels in the tissue. Indeed, a primary objective of window-of-opportunity trials is to evaluate the therapeutic concentration of the drug in the tumor following its administration at the dosage and schedule intended

for later-stage trials [45]. Moreover, molecular assays can determine whether the drug is interacting with its target(s), particularly when the target is known and an assay for assessing target engagement is available [46, 47]. It is noteworthy that many molecular assays utilized in preclinical laboratory testing can be applied in window-of-opportunity trials. These include IHC [48, 49] western blot (WB) [47]in-suit hybridization [50]cytometry by time-of-flight (CyTOF) [51] and single-cell RNA sequencing (scRNA-seq) [51, 52] (Fig. 3). Preclinical animal studies of the novel agent can establish assays for target engagement and potentially translate these findings into clinical specimens. Beyond verifying efficacy through enhancement in animal survival, another crucial application of preclinical studies is broadening the assay spectrum to clinical specimens [53].

Furthermore, the heterogeneity of these molecular events necessitates careful consideration of control tissue for comparing molecular outcomes. While some molecular outcomes may not demand control tissue, the baseline levels of most molecular targets exhibit significant variability across tumors. Ideally, comparing untreated to treated tissue is desirable. However, as mentioned above, obtaining untreated tissue is challenging in patients with brain tumors, as it necessitates a biopsy before and after treatment. Many innovative approaches have been developed for acquiring tissues for molecular assays, and several of them have been explored in window studies.

Perioperative trials on brain tumors

For most patients with brain tumors, diagnostic tumor biopsies and safe maximal tumor resections typically occur during the same neurosurgical procedure, providing a limited window-of-opportunity for PD evaluation. However, many patients experience tumor recurrence and undergo a second surgery, as recurrent brain tumors often lack effective second-line treatments.

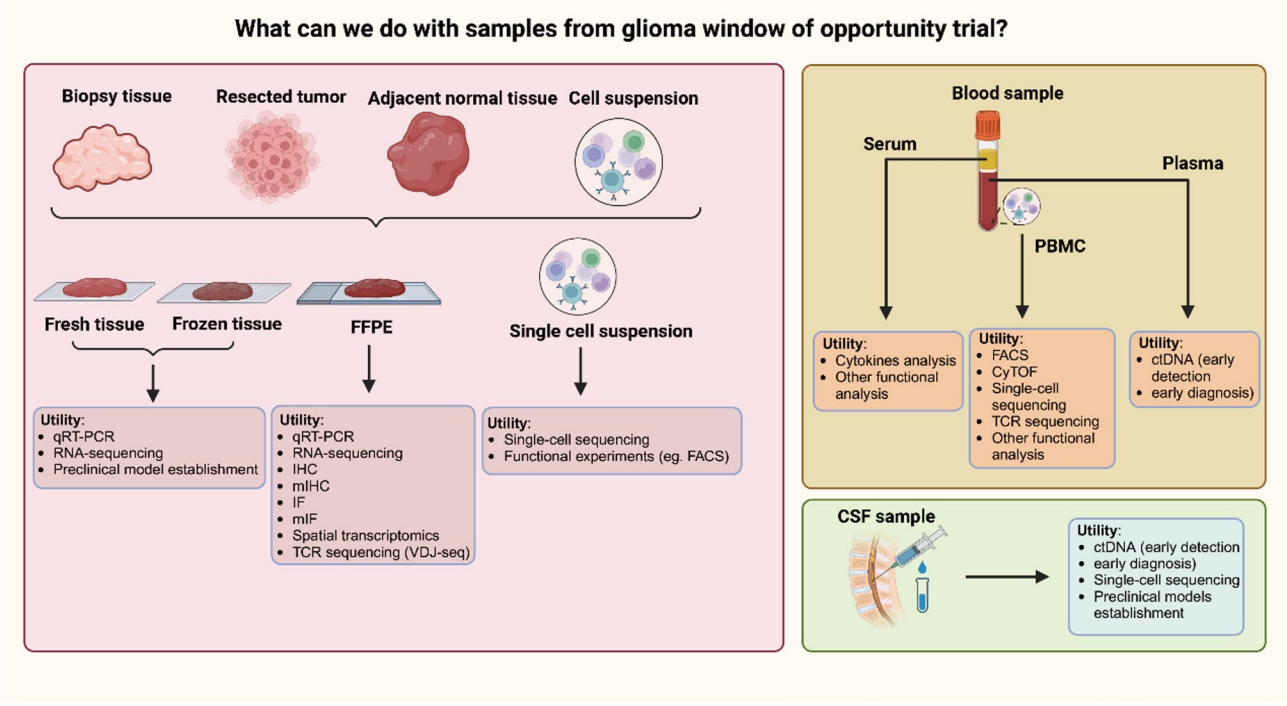

Fig. 3 Samples obtained from window-of-opportunity trial for sequencing and functional analysis. Window-of-opportunity studies provide unique opportunities to obtain multiple samples and tissues before, during, and after the administration of a target drug—an undertaking often challenging in most unresectable cancers. Tumor samples, biopsy tissues, adjacent normal tissue, and single-cell suspensions, both pre- and post-drug treatment, can be preserved in various manners tailored to desired downstream analyses. Specifically, resected tissues can be categorized as fresh tissues, frozen tissues, FFPE, and single-cell suspensions. Fresh, frozen, and FFPE tissues can facilitate sequencing techniques like RNA-sequencing, spatial transcriptomics, and TCR sequencing, as well as functional experiments such as RT-qPCR, IHC, IHC, IF, mIF, and preclinical model establishment. Viably cryopreserved enzymatically digested single-cell suspensions enable single-cell sequencing and partial functional experiments like FACS. Peripheral blood samples, collected at sequential timepoints, can be fractionated into plasma, serum, and PBMCs. These samples are valuable downstream for ctDNA analysis, isolated cytokines, functional experiments including FACS and CyTOF, and single-cell sequencing, along with TCR sequencing. Notably, CSF, specific and accessible for brain tumor tissues, offers the identification of ctDNA for early detection and diagnosis. CSF samples also facilitate single-cell analysis and provide the potential for generating preclinical models using cells captured from CSF. Abbreviations: FFPE, formalin-fixed and paraffin-embedded; qRT-PCR, quantitative real-time PCR; IHC, immunohistochemistry; mIHC, multiplex immunohistochemistry; IF, immunofluorescence; mIF, multiplex immunofluorescence; TCR, T cell receptor; FACS, fluorescence activating cell sorter; CyTOF, cytometry by time of flight; ctDNA, circulating tumor DNA; PBMC, peripheral blood mononuclear cells; CSF, cerebrospinal fluid

Consequently, perioperative trials with investigational agents are commonly conducted during recurrence procedures. This presents a crucial opportunity for study participation and enables the assessment of efficacy surrogates such as OS and PFS. As previously mentioned, perioperative trials in neuro-oncology have progressed to randomized settings before surgical treatment [54–56]. The primary advantage of randomization is that patients in both arms have identical inclusion and exclusion criteria, ensuring comparable baseline characteristics before surgery. When analyzing tissue-based comparisons between different treatment arms, randomization instills a higher level of confidence in the results, irrespective of the sample size. Indeed, many investigational therapies necessitate combinations to target the immune suppressive TME or to infiltrate activated T cells into the tumor. Utilizing a neoadjuvant clinical trial platform approach could expedite evaluations, share controls, and harmonize inclusion and exclusion criteria, as well as tissue-based evaluations [57–60]. Beyond considerations for developing and validating PD assays, proper perioperative trials can effectively eliminate ineffective drugs that fail to penetrate into the tumor or engage their targets. Consequently, perioperative trials enhance enthusiasm for developing new agents by meticulously documenting target engagement and additional molecular and cellular effects.

Drugs selection and pharmacodynamics

Not all drugs under investigation are suitable for phase 0 trials, and not all phase 0 trials represent the first in human studies. Candidate drugs selected for phase 0 trials should adhere to the following criteria: (1) there exists an aspect of the drug's mechanism of action that remains unknown; (2) PD considerations are pivotal for the successful development of the drug; (3) preclinical investigations indicate an association between the drug target and an anti-tumor effect; (4) the investigated drug possesses a broad therapeutic window; (5) modulation of the drug target is expected at nontoxic doses and over a short duration of exposure (typically no longer than 7 days); (6) target modulation is likely to be discerned with a small sample size of patients (usually no more than 15 patients). The selection of "promising" agents for phase 0 studies should be evaluated in the specific context of the proposed agents. A phase 0 trial can be conducted for a single agent when it meets the following criteria: (1) evaluating drug efficacy through biopsies obtained pre- and post-exposure; (2) obtaining biomarkers associated with drug effects in tumors, blood, or other surrogate tissues; (3) determining a very safe but potentially effective starting dose based on the number of patients. In the case of combination treatment strategies, investigators often integrate two targeted agents, a targeted agent with a conventional cytotoxic agent, or a targeted agent with novel immunotherapy. Additionally, phase 0 studies can assist in determining the relative schedule and sequence for monotherapy or combined therapy. Interestingly, "promising" agents can be defined in various ways, but they typically share common characteristics: (1) being first-in-class molecules; (2) having previously unexplored mechanisms in brain tumors; (3) demonstrating validated effects in diseases other than brain tumors; (4) theoretically, possessing mechanistic or toxicity characteristics well-suited for combined drug therapy. The process of drug selection is accompanied by the identification of a suitable biomarker-based PD assay to evaluate drug effects.

Obtaining tumor samples before drug exposure is particularly challenging for CNS tumors due to the heightened risks, costs, time consumption, and the absence of surrogate tissues corresponding to brain tumors. In such phase 0 trials, baseline comparator samples are typically set as prior drug exposure samples. Consequently, phase 0 studies for brain tumor patients to obtain planned biopsies before drug exposure are limited. While these limitations are likely unavoidable in phase 0 trials for brain tumor patients, it is advisable to optimize the study design by initially assessing PD endpoints in a reference population of matched samples from the initial diagnosis and recurrence. Only biomarkers demonstrating stable expressional and functional profiles should be selected as PD endpoints.

Phase 0/window-of-opportunity trials require more biostatistical considerations

Phase 0 trials typically involve limited sample sizes, posing challenges in estimating criteria that incorporate a PD response. In the initial introduction of phase 0 trials, Kummar et al. presented preliminary statistical guidelines and example designs for dichotomized PD, and subsequently [28]Murgo et al. refined the statistical designs by providing calculations to ensure the criteria of PD response along with analysis methods [29]. Rubinstein et al. later contributed to the field by generating new study design estimates for different PD response rates, introducing Simon Optimal and Minimax designs [61]. However, phase 0 trials for gliomas can be viewed as exploratory analyses, and as such, a prior power analysis to determine sample sizes is lacking [31, 42]62– [64]. The justification for the low sample size lies in its feasibility [64]. Response criteria may be established based on prior preclinical or clinical data, or by simply assessing if there is a relative change compared to archived tissue without a predefined threshold (e.g., one-fold difference) [62, 63]. Nevertheless, the relatively recent development of phase 0 studies in GBM may be conducive to the exploration and implementation of novel statistical innovations.

Current available phase 0/window-of-opportunity trials on glioma

Drawing from a comprehensive review conducted by the Response Assessment in Neuro-Oncology (RANO) working group, the authors initially identified 22 publications pertinent to PD/PK analyses among GBM patients [42]. It is worth noting that not all of these publications align with the criteria of phase 0 or early phase 1 window-of-opportunity trials. A good example is a randomized perioperative phase 1, window-of-opportunity trial (NCT03343197) of 49 patients with isocitrate dehydrogenase-mutant (IDH-mt) gliomas, 2 types of IDH-inhibitors vorasidenib and ivosidenib were administrated as trial agents to study dose-specific PD and IDH-related metabolic pathways [65]. There were 28 (+7) treatment window for the patients to be administrated with the intervention prior surgery and postoperative treatment was also allowed until disease progression or unacceptable toxicity. The concentration of 2-hydroxyglutarate (2HG) in resected tumors was the primary endpoint for the trial. The investigators found 2HG was significantly reduced in IDH inhibitors group over the control group, 92.6% in vorasidenib 50 mg q.d level and 91.1% in ivosidenib 500 mg q.d level respectively. Tumor/blood ratio was higher for vorasidenib vs. ivosidenib. Then the objective response rate (ORR) for vorasidenib 50 mg q.d was 42.9% vs. 10% for ivosidenib 10 mg q.d, 12.5% for ivosidenib 250 mg bid, 35.7% for ivosidenib 500 mg q.d. Both the IDH inhibitors were well-tolerated. The promising results were also instrumental in accelerating the success of final phase 3 clinical trial on vorasidenib [66]. While there are also some other examples of phase 0/window-of-opportunity trials on glioma [31, 63, 64]67– [79] (Table 1). The PerSurge (NOA-30) trial is a multicenter, phase 2, randomized, double-blind, placebo-controlled superiority window-of-opportunity study evaluating perampanel, an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor inhibitor, in 66 progressive and recurrent GBM patients scheduled for surgical resection [74, 80, 81]. Patients are randomized 1:1 to receive perampanel (escalated to 10 mg/day) or placebo for 60 days (30 days pre- and post-surgery). Co-primary endpoints were to assess tumor-neuron connectivity via single nucleus RNA sequencing (snRNA-seq) and AI-based T2/ fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) tumor growth rate changes pre-surgery. Secondary endpoints included contrast-enhanced MRI volumes, health-related quality of life (EORTC QLQ-C30/BN20), cognitive function (MMSE), seizure frequency, PFS, and OS. Besides, translational analyses would explore biomarkers, perampanel tissue concentration levels, and network connectivity via proteomics and sequencing. This trial is still ongoing and results would be expected in the future. Conclusively, PerSurge tests perampanel's potential to disrupt neuron-glioma synapses hence to reduce tumor proliferation and invasion supported by preclinical evidence [80, 81]. Positive results could inform cancer neuroscience principles and support larger confirmatory trials for GBM network-targeted therapies. Balinda et al. in a phase 0 window-of-opportunity trial (NCT03995706) evaluated sacituzumab govitecan (SG) that selectively delivers SN-38 to tumors to inhibit tumor growth in 13 breast cancer with brain metastasis and 12 recurrent GBM patients undergoing craniotomy [75]. Patients received 10 mg/kg SG intravenously preoperatively and resumed post-recovery on days 1 and 8 of 21-day cycles. The first aim was to assess intratumoral SN-38 concentration, the secondary aim was to investigate PFS, OS, and safety profile. Intracranial activity was confirmed in xenograft models. SG inhibited tumor growth and prolonged OS in xenografts. Median tissue SN-38 concentration was 104.5 ng/g for recurrent GBM, the PFS, OS and ORR was 2/9.5 months and 29% respectively. Grade ≥ 3 AEs included neutropenia (28%). SG exhibits robust tumor penetration and promising intracranial activity with acceptable toxicity, paving the way for phase II trial (NCT04559230) [75]. Another phase 1 trial (NCT01849146) evaluated Wee1 oral small-molecule inhibitor adavosertib combined with RT and TMZ in newly diagnosed GBM using a 3 + 3 dose-escalation design across concurrent, adjuvant, and combination cohorts ($n = 57$) [76]. Intratumoral drug distribution (IDD) was assessed in recurrent GBM ($n = 14$) via tissue homogenates and microdialysis. MTD were 200 mg/day (concurrent RT/TMZ) and 425 mg/day (adjuvant TMZ), but combination yielded unacceptable dose-limiting toxicities (50% rate). IDD showed superior penetration in contrast-enhancing (median 644–3576 ng/g) versus non-enhancing tissue. Median PFS was 8.5 months. Adavosertib exhibited excessive toxicity in concurrent settings; recommended phase II dose is 425 mg/day with adjuvant TMZ. Complementary PK informs future window-of-opportunity designs [76]. A recent phase 0 surgical window-of-opportunity trial (NCT03107780) evaluated the murine double minute homolog 2 (MDM2) inhibitor avtemadlin in 21 patients with recurrent TP53 wild-type GBM. Eligible patients received navtemadlin at 120 mg ($n = 10$) or 240 mg ($n = 11$) daily for 2 days before surgery, followed by 240 mg post-surgery until tumor progression or unaccepted toxicity [77]. The primary endpoint was set as assessing navtemadlin intratumoral concentration via liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), secondary endpoints included treatment safety, PD via IHC, RNA-seq, issue-based cyclic immunofluorescence (t-CyCIF) and survival data. Patient-derived GBM neurospheres were used for in vitro functional

Table 1 Current phase 0/early I, window-of-opportunity trials on glioma

Study	Status	Patients	Target drugs	Dose	Schedule and resection	Sample types	Drug level assessment	Biological relevance	Clinical finding and relevance	Registration number	References
Weingart et al., 2007	Finished	rGBM or anaplastic astrocytoma ($n=14$)	Carmustine	120 mg/m2, followed by an infusion of 30 mg/m2	48 h before final resection	Blood, tumor	Blood and tumor drug concentration, AGT activity	To evaluate MGMT activity	Role of carmustine to inhibit MGMT	NA	[67]
Kuhn et al., 2007	finished	recurrent glioma ($n=6$)	Temsirolimus	170–250 mg	1 dose 2 h before final resection	Blood, tumor	LC-MS/MS, blood and tumor drug concentration	Temsirolimus is an ester of sirolimus (rapamycin) that shows anti-glioma efficacy in cell line and orthotopic models	PK and PD analysis	NA	[68]
Reardon et al., 2012	Finished	rGBM ($n=10$)	Ridaforlimus	12.5 mg and 15 mg	4 doses before final resection	Blood, tumor	LC-MS/MS, blood and tumor drug concentration, mTOR activity assessment with p4E-BP1 in blood	pS6 kinase, downstream of mTOR	Efficacy assessed by imaging, safety profile	NA	[69]
Drappatz et al., 2013	Finished	rGBM, anaplastic astrocytoma and oligodendroglioma ($n=9$)	GRN1005	30 mg/m2	6 h before final resection	Blood, tumor	LC-MS/MS, blood and tumor drug concentration, WB to determine LRP-1 expression	GRN1005 binds to LRP-1 and enters brain effectively	Determine MTD, PK and PD analysis	NA	[70]
Xu et al., 2016	Finished	nGBM and anaplastic astrocytoma ($n=21$)	RO4929097	10–20 mg daily	7 days before final resection	Both enhancing and non-enhancing tumor, blood	LC-MS/MS, qRT-PCR, IHC, morphometry, RNA sequencing, and FACS, blood and tumor drug concentration	Down-regulation of Notch signaling in enhancing tumor	PK and PD analysis in enhancing tumor and the tumor escape mechanism	NCT01119599	[71]
Sanai et al., 2018	Finished	rGBM ($n=20$)	AZD1775	100, 200, 400 mg	4 h, 8 h, 24 h respectively before final resection	Blood, tumor	LC-MS/MS, IHC, DNA sequencing, blood and tumor drug concentration	markers of checkpoint disruption	determine MTD, PK and PD analysis	NCT02207010	[63]

Table 1 (continued)

Study	Status	Patients	Target drugs	Dose	Schedule and resection	Sample types	Drug level assessment	Biological relevance	Clinical finding and relevance	Registration number	References
Tien et al., 2019	Finished	rGBM ($n=12$)	Ribociclib	900 mg	5 days daily before final resection	Both enhancing and non-enhancing tumor, blood	AGT activity, blood and tumor drug concentration	Decline in phospho-RB in half patients	Determine MTD, PK and PD analysis	NCT02933736	[31]
Quillin et al., 2020	finished	rGBM ($n=3$)	ixazomib	4.0 mg	3 h before final resection	Blood and enhancing tumor	LC-MS/MS, blood and tumor drug concentration	Ixazomib is the second-generation proteasome inhibitor which showed efficacy against multiple myeloma	Safety profile	NCT02630030	[64]
Arrillaga-Romany et al., 2020	Finished	rGBM ($n=6$)	ONC201	625 mg once a week	8 days before final resection	Blood and enhancing tumor	LC-MS/MS, tumor drug concentration, rat model validation in blood, tumor and other organs drug concentration	ONC201 as DRD2 antagonist penetrates BBB	PFS, OS, AEs, imaging data	NCT02525692 (part)	[72]
Moreno et al., 2023	Finished	high-grade glioma ($n=20$)	Trotabresib	30 mg daily	on day 1–4 before surgery	Blood, CSF, both enhancing and non-enhancing tumor	Blood, tumor and CSF drug concentration, RNA-seq for markers investigation	The bromodomain and extraterminal protein inhibitor trotabresib shows antitumor activity to solid tumors	PK/PD analysis, safety profile, anti-tumor immunity, PFS	NCT04047303	[73]

Table 1 (continued)

Study	Status	Patients	Target drugs	Dose	Schedule and resection	Sample types	Drug level assessment	Biological relevance	Clinical finding and relevance	Registration number	References
Heuer et al., 2024	Unfinished	rGBM ($n=66$)	Perampanel	2 mg and dose expansion till 10 mg without severe adverse events	30 days before surgery and 30 days after surgery	Blood, CSF, tumor	Imaging-based screen and molecular analysis	Perampanel is FDA approved AMPA inhibitor that reduce the interaction between glioma and neuron	tumor cell network and imaging-based tumor growth evaluation, adverse events, PFS and OS	2023-503938-52-00 30.11.2023	[74]
Fresnedo et al., 2023	Unfinished	rGBM ($n=18$)	Adipose-derived mesenchymal stem cells	2 ml fibrinogen, 1 ml thrombin, 1 ml cell mixture	Intra-cavitary treatment after surgery	Blood, CSF, tumor	Blood biochemistry, molecular analysis, ctDNA sequencing, sample IHC	Mesenchymal stem cells migrate and colocaliaze with tumor cells that remain in the tumor border; they are hypoimmunogenic and possess immunomodulatory properties	toxicity profile, OS and PFS	NCT05789394	[78]
Balinda et al., 2024	Finished	rGBM ($n=12$)	Sacituzumab govitecan	10 mg sacituzumab govitecan	Given one day before surgery and continued on day 1, 8 and 21 after surgery	blood, CSF, tumor	PK/PD analysis with xenograft model data, sample immunohistochemistry, molecular analysis, high resolution mass spectrometry for blood, tumor, CSF samples	sacituzumab govitecan is an antibody-drug conjugate targets Trop-2 for selectively delivering SN-38	OS, PFS and AEs	NCT03995706	[75]
Cain et al., 2024	Unfinished	IDH1-mt glioma ($n=15$)	safusidenib	250 mg safusidenib	250 mg safusidenib twice daily up to 28 days before surgery, and adjuvant safusidenib after surgery	blood, CSF, tumor	PK/PD analysis like PK concentrations, 2-HG changes and ctDNA analysis in blood and CSF, multi-omics sequencing, and other laboratory tests	Safusidenib is an oral IDH1 small-molecule inhibitor	AEs, imaging response, PFS, time to treatment failure, duration of response	NCT05577416	[79]

Table 1 (continued)

Study	Status	Patients	Target drugs	Dose	Schedule and resection	Sample types	Drug level assessment	Biological relevance	Clinical finding and relevance	Registration number	References
Lee et al., 2025	Finished	nGBM ($n=57$)	adavosertib	200–425 mg (MTD) adavosertib daily	200 mg daily with or without RT/TMZ for 6 weeks and 425 mg MTD with adjuvant TMZ in three cohorts	Blood, tumor	PK/PD analysis, molecular analysis	Adavosertib (AZD1775) is a selective, ATP-competitive, small-molecule inhibitor of Wee1 kinase. The efficacy is demonstrated in preclinical models.	Investigate the MTD	NCT01849146	[76]
Rendo et al., 2025	Finished	rGBM ($n=21$)	navtemadlin	120 ($n=11$)/240 mg ($n=10$) navtemadlin daily	120/240 mg daily for 2 days before surgery and after surgery till tumor progression	Blood, tumor	PK/PD analysis, molecular analysis, IHC, IF and multi-omics sequencing	Navtemadlin is an oral small-molecule inhibitor for MDM2 that reactives TP53	OS and PFS	NCT03107780	[77]

Some phase 0/I trials are summarized from a previous RANO review (Ref [42]), we also updated the results with some new studies

Abbreviation: rGBM, recurrent glioblastoma; AGT, O6-alkylguanine-DNA alkyltransferase; MGMT, O6-methylguanine-DNA methyltransferase; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; mTOR, mammalian target of rapamycin; p4E-BP1, phosphorylated 4E-BP1; LRP-1, lipoprotein receptor–related protein-1; DRD2, dopamine receptor D2; qRT-PCR, quantitative reverse transcription polymerase chain reaction; IHC, immunohistochemistry; WB, western blot; FACS, fluorescence-activated cell sorting; BBB, blood brain barrier; CSF, cerebrospinal fluid; OS, overall survival; PFS, progression-free survival; AEs, adverse events; PK, pharmacokinetics; PD, pharmacodynamics; FDA, food and drug administration; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; IDH1, isocitrate dehydrogenase 1; 2-HG, 2-hydroxyglutarate; ATP, adenosine triphosphate; MTD, maximum tolerated dose; MDM2, murine double minute homolog 2; RT, radiotherapy; TMZ, temozolomide; IF, immunofluorescence; NA, not available

assays, including apoptosis, BH3 profiling and scRNA-seq. Both doses achieved PD effects with median PFS of 3.1 months and OS of 10.0 months. Intratumoral drug concentrations exceeded 25 nM in ≥ 80% of patients, with p53 pathway activation (e.g., CDKN1A upregulation) and reduced proliferation (Ki67 decrease). In functional assay, tumor cell death occurred in monotherapy process, combined therapy with TMZ enhanced apoptosis in TP53 wild-type, O[6]-methylguanine-DNA methyltransferase (MGMT)-methylated models while sparing bone marrow cells. Navtemadlin upregulated oligodendrocyte differentiation genes (e.g., OLIG2 enrichment at relapse). No TP53-inactivating mutations were acquired in three recurrent tumors. Navtemadlin penetrates GBM tissues and elicits PD responses at clinically achievable doses, inducing tumor cell death and adaptive oligodendrocyte-like states. Combined treatment with TMZ may enhance apoptosis and promote more durable survival benefits, warranting further clinical trials. Most incorporated studies primarily investigated the efficacy of novel drugs on recurrent tumors, justified by the feasibility of obtaining pre- and post-resection samples. Over time, analyses expanded beyond tumor tissues to systematically include blood and CSF samples, extending PK and PD evaluations to these fluids to demonstrate comparability with prior results on investigational agents. This comprehensive approach assessed drug-specific target effects across multiple dimensions.

In addition to the studies outlined in Table 1, ongoing clinical trials such as NCT01849146 (phase 1), NCT02101905 (pilot study), NCT02133183 (phase 1), NCT02933736 (early phase 1), NCT03893903 (early phase 1), NCT03122197 (phase 0/1), and others are currently underway, exploring the concept of the window-of-opportunity. In conclusion, the phase 0 window-of-opportunity study design remains an underutilized strategy for the development of systemically administered therapeutics in neuro-oncology. Despite variations in PK and PD analysis approaches and challenges in obtaining samples before neoadjuvant and surgical treatments, the encouragement of tissue-based assessments for the biological effects of treatment is crucial in the early clinical

development of novel therapeutics. This approach facilitates the advancement of highly effective drugs to higher levels of clinical trials [82].

Utility of window-of-opportunity design in phase 1–2 trials

Given the evidence delineated in this review, numerous methodologies have been proposed for tailoring phase 1–2 early-stage trials to incorporate biological endpoints and achieve the objectives of window-of-opportunity trials for brain tumors. Phase 1 clinical investigations are traditionally perceived as the inaugural phase in the clinical advancement of novel drugs. The principal objective of a phase 1 trial is to evaluate the safety profile of the investigational drug, with a secondary focus on assessing its efficacy. Notably, the phase 1 study aims to determine the MTD by progressively escalating dose levels until reaching the pre-defined dose-limiting toxicity (DLT) rate [83]. 3 + 3 design is always adopted by the classic phase I trial due to its simplicity and accessibility. While an unexpectedly prolonged trial duration could emerge, and predicting the final sample size beforehand for optimal randomization poses a formidable challenge [84, 85].

Designs akin to those proposed for phase 1 trials can likewise be applied to phase 2 trials, acknowledging that these phase 2 trials maintain the objective of determining clinical efficacy while integrating biological endpoints. Ideally, patients would undergo a biopsy before investigational treatment and after which they are treated with therapeutic agent at defined dose in phase 1. Post-treatment specimens are feasible after surgical resection and can be compared with pre-treatment specimens. Molecular assays can be conducted to ascertain whether the agent continues to target its intended site and modifies the TME established in the Phase I setting. Once the agent consistently targets its intended site and elicits the desired molecular effects, it is considered effective [86, 87]. Efficacy analysis can be extended further by administering the experimental agent in the adjuvant setting after surgery to determine PFS and/or OS and recurrence [47]. The window-of-opportunity design in phase 2 trials provides significant biological insights in addition to standard clinical outcome data and informs decision-making regarding whether the agent merits further study. If the agent proves effective, it should progress to phase 3 trials; conversely, if the agent fails to target its intended site or elicit a clinical response, it may be abandoned. However, the window-of-opportunity approach may offer some insights at the molecular and cellular levels into the cause of failure. It is possible that the agent is only partially effective or that the defined dose is not as potent as anticipated in inducing a durable response. Therefore, careful evaluation and discussion of these molecular/cellular results are necessary before completely abandoning the agent. Subsequently, trial design improvements should be made, the agent should be further developed, and novel targets should be pursued. If there is a phenomenon where the agent demonstrates efficacy in preclinical studies but fails to show robust clinical benefits, one hypothesis is that the target is not an independent driver of tumor growth. In this circumstance, combination therapy with other agents may prove effective, prompting the rational development of the agents in trials with combination arms. Overall, given the fact that new drug discovery efficiency is low for GBM and the median duration from phase 2 to phase 3 trials is quite long of which many fail to promote the new drug into real clinical approval, the window-of-opportunity trial design can be considered as a good paradigm to improve new drug development efficiency, success rate and reduce unexpected cost.

Lessons from phase 0/window-of-opportunity trials on future glioma clinical studies

Although phase 0 and window-of-opportunity studies accelerate the development for novel drug agents against glioma, challenges keep persisting. To optimize glioma drug development, interdisciplinary collaboration and novel technologies must be leveraged with and beyond these novel trial design. First of all, artificial intelligence (AI)-driven predictive modeling can optimize patient selection and dose-escalation strategies reducing trial duration and enhancing success rate [88, 89]. With the state-of-the art machine learning screening algorithms, investigators could identify novel glioma treatment candidates more effectively. Then aggregated big data, imaging data and/or real-world data from multi-centers on glioma patients' clinical response information will facilitate more tailored therapeutic approaches [90, 91]. Next, addressing the collaboration among neurosurgeons, oncologists and pharmacologists help to refine clinical trial methodologies and ensure translational success. Encouraging global partnerships in glioma study benefits to generate more diverse and representative patient cohorts. The diversity and heterogeneity limits its representation of glioma patients in current trials, expanding recruitment internationally could enhance the generalizability of findings. Policymakers should also consider developing streamlined regulatory pathways to fast approve early-stage trials. In the future, Early-stage trials should focus on evaluating novel therapies' efficacy in glioma patients, particularly in combination with BBB-penetrating agents. Using gene editing tools such as CRISPR/Cas9 opens up new potentials for precise therapeutic interventions in gliomas [92–94]. Future trials could explore how gene-editing approaches can be incorporated into Phase 0 trials to test target modulation strategies. Innovatively, wearable devices capable of tracking patient biometrics, neurological function, and treatment

responses in real time could play a crucial role in future glioma clinical trials. Those kinds of technologies could provide a non-invasive means of assessing therapy efficacy over extended periods [95, 96].

What the neurosurgical oncologists do on phase 0 trials

Neuro-oncology phase 0 trials are experiencing a surge in the realm of clinical trials within neurosurgery, necessitating collaborative efforts from a substantial number of investigators [97, 98]. Notably, the neurological oncologist plays a pivotal role in the study design, patient accrual, and surgical phases [98]. A comprehensive understanding of the clinical and translational science trial elements is imperative for all team members and constitutes an essential aspect for the neurosurgeon. Initially, the neurosurgical oncologist assumes a critical role in patient selection and study consent [98]. In the context of phase 0 studies, it is crucial to conduct an assessment of tumor operability during the patient selection process. This determination must account for the timing of the planned surgery. Unlike other conventional clinical trials, brain tumor phase 0 studies necessitate a significant lead-in time prior to tumor resection. Molecular entry criteria are standard in phase 0 studies and typically involve 1 to 2 weeks of testing a patient's archived tumor tissues. Overall, eligible patients should be clinically stable, and the neurosurgical oncologist must evaluate the safety of timing an indicated operation to allow for trial pretesting and pretreatment. Timely PK analysis is imperative for the prompt acquisition of blood, CSF, and tumor tissue in phase 0 trials. Therefore, the feasibility of the sample achievement parameter heavily relies on operating room logistics and surgical timing. The key to fulfilling this requirement is to establish deliberate coordination with anesthesiologists, nurses, and surgical technologists, among other operating room personnel, to mitigate potential delays [98]. Furthermore, the neurosurgical oncologists should envisage the impact of data biases and tumor heterogeneity.

Conclusion

The phase 0 and window-of-opportunity clinical trial design, originally derived from the FDA and proposed for general drug development, encounters a slow pace in new drug development for brain tumors. This slowness is attributed not only to the aggressive nature of gliomas but also to challenges such as the absence of predictive animal models, risks associated with tumor acquisition, limitations in microdosing applicability, the presence of the BBB, substantial inter- and intratumoral heterogeneity, an incomplete understanding of the TME, and limited coordination among neurosurgeons, oncologists, and biostatisticians. Implementing these trial paradigms in brain tumors proves to be an effective strategy for acquiring direct evidence of drug delivery and target modulation. In the future, more steps like utilizing novel AI technologies, incorporating multi-disciplinary cooperation and partnership and linking currently available data to large public, real-world databases should be thoroughly considered to expand the impact from phase 0 and window-of-opportunity trial designs on glioma research. Overall, phase 0 window-of-opportunity trials demonstrate unique advantages for discovering new drugs for gliomas. These innovative trial designs and PK/PD analyses promise more efficient and effective clinical trials, aiming to avoid ineffective therapies and accelerate the discovery of effective treatments for gliomas.

Abbreviations

CNS	Central nervous system
GBM	Glioblastoma
OS	Overall survival
FDA	Food and drug administration
TMZ	Temozolomide
TTF	Tumor-treating field
ICIs	Immune checkpoint inhibitors
NSCLC	Non-small cell lung cancer
TME	Tumor microenvironment
PK	Pharmacokinetic
BBB	Blood-brain barrier
PD	Pharmacodynamics
PFS	Progression-free survival
RECIST	Response evaluation criteria in solid tumors
18F-FDG-PET	[fluorine-18]-fluoro-2-deoxy-D-glucose positron emission tomography
SNBs	Stereotactic needle biopsies
CSF	Cerebrospinal fluid
IHC	Immunohistochemistry
WB	Western blot
CyTOF	Cytometry by time-of-flight
scRNA-seq	Single-cell RNA sequencing
RANO	Response assessment in neuro-oncology
IDH-mt	Isocitrate dehydrogenase-mutant
2HG	2-hydroxyglutarate
ORR	Objective response rate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
snRNA-seq	Single nucleus RNA sequencing
FLAIR	Fluid-attenuated inversion recovery
MRI	Magnetic resonance imaging
SG	Sacituzumab govitecan
IDD	Intratumoral drug distribution
MDM2	Murine double minute homolog 2
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
t-CyCIF	Issue-based cyclic immunofluorescence
MGMT	O6-methylguanine-DNA methyltransferase
MTD	Maximum tolerated dose
DLT	Pre-defined dose-limiting toxicity
AI	Artificial intelligence

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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