

Implementing targeted therapies in the treatment of glioblastoma: Previous shortcomings, future promises, and a multimodal strategy recommendation

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Abstract

The introduction of targeted therapies to the field of oncology has prolonged the survival of several tumor types. Despite extensive research and numerous trials, similar outcomes have unfortunately not been realized for glioblastoma. For more than 15 years, the standard treatment of glioblastoma has been unchanged. This review walks through the elements that have challenged the success of previous trials and highlight some future promises. Concurrently, this review describes how institutions, through a multimodal and comprehensive strategy with 4 essential components, may increase the probability of finding a meaningful role for targeted therapies in the treatment of glioblastoma. These components are (1) prudent trial designs, (2) considered drug and target selection, (3) harnessed real-world clinical and molecular evidence, and (4) incorporation of translational research.

Keywords

glioblastoma | real-world evidence | targeted treatment | translational research | trial design.

Glioblastoma is the most common and most aggressive adult brain tumor.¹ The standard treatment for patients with glioblastoma in good performance status is surgery, concomitant temozolomide and radiation therapy, and adjuvant temozolomide for 6 months.² Temozolomide is a small lipophilic chemotherapeutic drug that can penetrate the blood-brain barrier and cause DNA damage and cytotoxicity by alkylating DNA.³ Despite years of research and numerous clinical trials with experimental agents, patients with glioblastoma have a poor prognosis with a median overall survival of 15 months.^{2,4} Nearly every single patient will have relapse of their cancer; the 5-year overall survival is less than 10%.⁵

With the advances in sequencing technology, frequent genomic alterations causing cancer have been identified, and treatments targeting these alterations have emerged across several tumor types. In tumor types such as breast cancer, colorectal cancer, and nonsmall cell lung cancer, targeted treatment based on the identification of genomic alterations is now standard.^{6–8} In contrast, no broadly applicable targeted treatments have been able to prolong overall survival in

glioblastoma.⁹ The vascular endothelial growth factor (VEGF) antibody, bevacizumab, prolonged the progression-free survival in the first and second line setting, but overall survival was unaffected.^{10,11} Attempts to target frequently modified cellular pathways, such as phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR), hepatocyte growth factor (HGF)/MET and retinoblastoma (RB) pathways likewise, failed to alter the course of the disease.^{4,9} Currently, targeting the commonly affected epidermal growth factor receptor (EGFR) has also fallen short.¹² Because multiple aspects could explain these failed attempts, the community should not yet renounce the idea that targeted treatments may have a role in the treatment of glioblastoma. In this review, we will steadily walk through elements that have challenged the success of previous trials and highlight future promises. Concurrently, we will describe how institutions, through a multimodal and comprehensive strategy with 4 essential components, may increase the probability of finding a meaningful role for targeted therapies in the treatment of glioblastoma. These components are (1)

prudent trial designs, (2) considered drug and target selection, (3) harnessed real-world clinical and molecular evidence, and (4) incorporation of translational research. The components are summarized and unfolded as short recommendations with selected advantages and challenges for each recommendation in [Table 1](#).

Prudent Trial Designs

A thorough consideration of the aspects of the trial design is essential to assess the efficacy of a drug in a timely and cost-effective manner. Likewise, in an adequately designed trial, the time and effort contributed by patients are well preserved. A few important design aspects for glioblastoma trials are discussed in the following sections.

Predictive Biomarkers

A predictive biomarker gives information on the effect of an intervention in a patient.¹³ Such a biomarker aims to grant the clinician the ability to predict, and thus stratify, patients having effects from a given treatment from the ones that do not. A recent large study assessing the use of these biomarkers, both exploratory and validated, as inclusion criteria in oncology clinical trials found that the biomarker-enriched trials had a clear increase in the likelihood of success.¹⁴ Glioblastoma trials were unfortunately not included in this study. This might be because biomarker-selected trials have been a rare feat in neuro-oncology.⁴ Most trials of targeted therapies have been conducted without using predictive biomarkers as inclusion or exclusion criteria. Because these trials possibly limit the likelihood of response and success, they should truly be avoided. Yet, there is also a legitimate explanation for their continued existence since, other than the O6-methylguanine-DNA methyltransferase (MGMT)-promoter methylation status, no independent association between molecular alterations and drug response or survival endpoints have been found.¹ As a result, every predictive biomarker applied in glioblastoma trials of targeted treatments has been exploratory. This factor might have prevented investigators from initiating such trials as they feared inappropriately selecting patients. The delayed validation of predictive biomarkers for glioblastoma is understandable. It requires randomization within a biomarker-positive population and thus large sample sizes, which is complicated and time-consuming in such a rare disease. Nevertheless, it is achievable, as shown in the innovative Bayesian adaptive platform trials INSIGHT¹⁵ and GBM-AGILE¹⁶ (further described below). These are 2 examples of biomarker-enriched trials where predictive biomarker evidence is generated efficiently. Generating this evidence is highly relevant as awareness of how the individual predictive biomarkers influence survival matters for interpreting the trial's final efficacy results, distinctively in non-randomized trials.¹⁷ However, significant progress has been made on biomarker frequencies and co-occurrence patterns. Large databases of glioblastoma genomic alterations can be queried through public portals such as cbiportal (<https://cbiportal.org/>) and the ICGC portal

(<https://dcc.icgc.org>), and such information should be considered when estimating accrual or when constructing prioritization algorithms for treatment-arm allocations. Finally, following the selection of biomarker(s) for trial enrichment, considering having a central biomarker assessment is appropriate. It is highly relevant for multicenter trials and nonstandardized analysis procedures, as it increases the validity of the generated biomarker evidence. To avoid potential delay of patient inclusion by awaiting central assessment, it can also serve as a validation of a local assessment, as it is done in INSIGHT.¹⁵

Moving Targeted Treatment to the First Line

The classical paradigm of initiating clinical trials of new drugs with assessments for single-agent efficacy, in the relapse setting, has yet to bear fruit in glioblastoma.⁴ Apart from the experimental drugs' lack of intrinsic efficacy, a possible explanation is that the patient's inherent poor prognosis may have concealed the drug's true effect. This situation could be the case if it appears the drug must be administered a few cycles to show the true effect. The worsening heterogeneity at relapse is another, more broadly accepted explanation.¹⁸ These reasons justify a new initial drug assessment strategy and further explorations of drugs with missing single agent efficacy.¹⁹ As a result, in recent years, there has been a slight trend towards moving trials to the first line, where the abovementioned aspects are believed to be less pronounced. Thus, the probability of response is expected to be higher.²⁰ Different ways of combining experimental agents with standard therapy have been observed.⁴ An interesting approach is the specific inclusion of patients with MGMT-promoter unmethylated glioblastoma, where the experimental agent is given alone in the adjuvant setting of the initial standard treatment.^{15,21} As temozolomides effect in this patient group is marginal, this design permits the administration of the potential promising experimental agent with minimal risk of compromising the current standard of care. Moreover, it dismisses the need for a separate phase I dosing escalation study with temozolomide. In cases where the experimental agent is added to standard therapy, the risk is greater, as it may lead to potential adverse drug interactions or treatment discontinuation due to toxicity. The last argument for advancing trials to newly diagnosed patients is the larger pool of patients available for enrollment.

Control Arm

Randomized trials are the gold standard for efficacy trials.^{20,22} Unfortunately, for glioblastoma, randomization in Phase II trials have been scarce. Consequently, the widespread use of historic controls is a commonly mentioned explanation for the failed transitions between phase II and phase III trials.²³ All experimental agents that completed efficacy assessments in Phase II and thus transitioned onto a phase III trial have universally failed since 2005.^{1,9} The drawback of single-arm studies with historic controls is that skipping contemporary controls might overestimate the effect of the experimental drug, especially if the

Table 1. Recommendations to Increase the Likelihood of Success for Targeted Treatments

Strategy components	Recommendations	Selected advantages and challenges for each recommendation	
		Advantages	Challenges
Prudent trial designs	Apply and validate predictive biomarkers	Increases the likelihood of trial success	Requires larger sample sizes or innovative trial designs
	Move trials to the first line	May increase the probability of response and larger pool of patients	Risk of not providing current standard of care
	Randomize or apply leveraged control arms	Mitigates false positive survival results	Requires either larger samples sizes or the means and data to leverage a control arm
	Utilize prognostic models for patient selection	Improves patient stratification and selection	May increase time for trial inclusion
	Apply overall survival as the primary endpoint	Mitigates false surrogate survival results	Requires larger samples sizes
	Consider designing a platform trial	Efficient and flexible large-scale trial design	Logistically demanding to set up and requires multi-institution collaborations
Considered drug and target selections	Assess the drug pharmacokinetics	Prevents potential inevitable efficacy trial failure and explains the reason for failure	Determining adequate intra-tumoral concentrations
	Assess the drug pharmacodynamics	Prevents potential inevitable efficacy trial failure and explains the reason for failure	Resource demanding and laborious process
	Weigh potential new target strategies	Opportunity for new meaningful findings	Risk of failure, although not larger than for previous targeting strategies
Harnessed real-world and molecular evidence	Establish clinical and molecular databases	Improves the bridging between basic and clinical science and capitalizes on all available data	Logistically demanding to create and maintain a quality database
	Establish multi-institutional collaborations	Speeds data collection	Standardizing data across institutions
Incorporation of translational research	Combine clinical trials and translational research	Improves the bridging between basic and clinical science	Requires collaborations with basic researchers and is resource demanding

survival of the control population has risen over time.²³ However, these trials tend to be favored by clinicians as they require fewer recruited patients.²⁴ Generally, randomization can dismiss this risk of false positive results and mitigate confounders, although as mentioned they often require large sample sizes and are costly. Glioblastoma is a rare disease, so the considerable patient enrollment needed for randomized trials is challenging. However, a new alternative design has been identified for investigators and institutions where the necessary enrollment of patients for a randomized trial is not feasible. The helpful design is to leverage externally controlled data in single-arm trials and thus significantly decrease the probability of a false positive trial.²² This feature, where you methodically exploit both real-world data and data from prior clinical trials, diminishes the required sample size and is a valid option in institutions with prospectively collected databases with relevant prognostic data. Alternatively, the umbrella design is also a new way of easing the numbers needed for enrollment while still maintaining quality. The umbrella design, a member of the master protocol family further described below, allows a common control arm for several experimental arms stratified by molecular alterations, all within a single histology.²⁵ There is no randomization, but the common control arm can be leveraged to increase the quality.

Patient Selection

Patient selection is a design consideration that gains importance if a randomized trial is considered too challenging to initiate. Since, without randomization, the balancing of treatment arms does not automatically occur. Choosing the optimal patient selection criteria for single-arm trials is, therefore, critical, as a skewed selection can bias the results. Presently, most clinical trials use performance scores to assess eligibility.²⁰ The postoperative performance score is a statistically reliable predictor of survival with low intra- and interobserver variability.²⁶ Yet, it does not consider fixed neurological deficits,²⁷

and we still experience a considerable variance in survival among patients deemed fit for treatment.²⁸ For non-randomized trials, an improved tool that more efficiently predicts survival would minimize the risk of a skewed selection. Furthermore, accurately selecting patients with a favorable prognosis would reduce the number of patients progressing or passing away before an experimental treatment has been administered an appropriate number of times. This would consequently minimize the risk of concealing the efficacy of an experimental drug. There have been attempts to develop such tools by combining prognostic factors into an applicable prognostic model (Table 2). Attempts by Gittleman et al. and Gorlia et al., have solely been based on patients already selected for clinical trials.^{29,30} They include factors like performance score, age, MGMT-promoter methylation status, the extent of resection as well as gender for Gittleman et al. and Mini-Mental State Examination for Gorlia et al. Recently, Abedi et al. established a new prognostic model by analyzing a prospectively collected and non-selected large cohort of patients treated with standard therapy. The model is based upon 6 factors: performance status, age, MGMT status, resection or biopsy, multifocality of disease at diagnosis, and corticosteroid use.²⁸ Still, this newer model only has a 65% probability of concordance between estimated and actual survival, implying that there are still undiscovered factors significantly influencing survival.

Endpoints

Another reason for the unprofitable Phase II to III transition is the extensive use of surrogate endpoints, such as overall response rate and progression-free survival at 6 months, in Phase II trials.⁹ Surrogate endpoints reduce the time and cost of conducting trials. However, in glioblastoma, apart from the correlation of progression-free survival and overall survival from temozolomides effect, no surrogate endpoint is proven to strongly correlate with overall survival.³¹ As Phase III trials commonly use overall survival as the primary endpoint, encouraging

Table 2. Selected Prognostic Models for Patients Treated With Standard Therapy

Ref.	Patients from:	CI	Independent prognostic factors:							
			PS	Age	MGMT-status	Gender	Resection [†]	MMSE	Multif.	Cortico.
Gorlia et al.	³⁰	Clinical trials (pop. 3*)	0,66	X	X	X		Extent of resection	X	
Gittleman et al.	²⁹	Clinical trials	0,66	X	X	X	X	Extent of resection		
Abedi et al.	²⁸	Prospective non-selected cohort	0,65	X	X	X		Resection or biopsy	X	X

Index: Ref: Reference number; CI = Concordance index; PS = Performance status; Multif. = Multifocality at diagnosis; Cortico = Corticosteroid use at diagnosis; MGMT-status = O6-methylguanine-DNA methyltransferase promoter methylation status.

*We have only included the normogram for population 3 where the patients underwent partial or complete resection, were treated with temozolomide and radiotherapy and where the MGMT status was known.

[†]Gorlia et al. dichotomized the extent of resection between "complete" or "partial" while Gittleman et al. trichotomized between "total/gross," "sub-total," and "other." Finally, Abedi et al. dichotomized between patients undergoing resection or a biopsy.

phase II data with surrogate endpoints have the potential for false signals. Further opposing surrogate endpoints is that, for glioblastoma, precisely determining true response and progression is challenging. Even though the Response Assessment in Neuro-Oncology (RANO) group has drafted consensual criteria improving reliability and using central assessment may reduce variability, concepts such as pseudo-progression and non-enhancing tumor progression truly complicate evaluations of response and progression.³² Illustrating examples of this endpoint mismatch are the large Phase III bevacizumab trials and the Phase III cilengitide trial, where a significant difference of several months in progression-free survival was lost in the final overall survival analysis.^{10,33,34} Thus, although time-consuming and costly, until a validated surrogate endpoint for a given targeted therapy is discovered, the optimal endpoint for Phase II trials, in both primary and recurrent settings, is overall survival.

Platform Trials

Master protocols, such as basket and platform trials have emerged in the glioblastoma trial landscape. These are trial designs optimized for precision oncology.²⁵ Basket trials, where an agent is evaluated on multiple histologies sharing a common molecular alteration, are commonly used for solid tumors, but unfortunately, trialed agents are rarely designed to cross the blood-brain barrier.³⁵ Mostly, they also include patients in the relapse setting and primarily consider the overall response rate, which, as previously discussed, is challenging for glioblastoma.^{32,36,37} In conclusion, although encouraging results with neurotrophic tyrosine receptor kinase (NTRK) gene fusions and BRAF V600E mutations have come from basket trials, they lack the necessary design capabilities to assess targeted therapies for glioblastoma efficiently.^{36,38} In contrast, platform trials allow the previously discussed features such as biomarker enrichment, common control arms, and testing experimental agents in the first line and have gained popularity in the glioblastoma trial landscape.^{15,16,21} INSIGHt and GBM-AGILE are two examples of adaptive platform trials with a Bayesian randomization.^{15,16} The distribution of patients across treatment arms is initially equal, but the allocation algorithm will adapt with time based on accumulating results and as biomarker evidence is generated. Thus, prioritizing arms with a treatment benefit and reducing the number of patients exposed to inefficient drugs. The trial algorithms' treatment effect estimation also allows dropping ineffective arms and adding new treatment arms as the trial progresses, identifying it as a platform trial. A second version of the platform trial design is the NCT Neuro Master Match (N2M2).²¹ This trial is nonrandomized and uses a combination of biomarker-selected arms and biomarker agnostic arms, allowing every screened patient to be eligible. In case a patient harbors several relevant biomarkers, prioritization is based on a predefined likelihood of treatment effect. And equal to INSIGHt, N2M2 only recruits patients with MGMT-promoter

unmethylated glioblastoma. Finally, as biomarkers often are present in less than 5% of cases, establishing a platform trial in a rare disease such as glioblastoma necessitates a large patient pool. Only multicenter or international collaborations can provide such numbers.³⁹ Such designs also improve generalization of the findings and the aforementioned trials are all examples of such collaborations.

Considered Drug and Target Selection

Pharmacokinetics and Pharmacodynamics

Different trial designs are not the sole culprits for the numerous failed trials for this disease. The trial drugs selected by investigators play a role as well. A thorough assessment of experimental agent(s) is required before a decision is made to expose patients in a clinical trial. When considering drugs for a potential Phase II efficacy trial, the most important necessary evaluation, besides toxicity, is if they can cross the blood-brain barrier. Clinical evidence that a specific drug reaches the tumor in therapeutic doses should be present before commencing such a trial, as this tight endothelial barrier restricts 98% of all small molecules from entering the brain.^{35,40} The proportion of failed trials as a direct consequence of the drug never reaching its target, is probably significant.⁴⁰ Only small, uncharged, lipid-soluble molecules can diffuse across, and regrettably, unlike temozolomide, most targeted therapies do not possess these features.^{3,35} Moreover, transporters on the luminal side of the barrier pump drugs and other unwanted chemicals back into the vasculature, further limiting access.^{1,35} Data from exclusively preclinical assessments of blood-brain barrier penetrance may be misleading, and should therefore not suffice as a scientific argument for trial initiation.⁴⁰ In addition, as the blood-brain barrier may be partially disrupted with varying drug concentrations in different parts of the tumor, pharmacokinetic assessments of both contrast-enhancing and noncontrast-enhancing areas should be performed.⁴¹ In a company with a satisfactory pharmacokinetic analysis, where adequate intra-tumoral concentrations follow the administration of a drug in a clinical dose, a demonstration of the drug's pharmacodynamic effects adds considerable value. For example, displaying proof of modified signaling in targeted pathways or showing a reduction in the number of clones harboring the target molecular alterations are additional ways to provide strong arguments in favor of a drug's further clinical evaluation.^{41,42} These valuable pharmacokinetic and pharmacodynamic evaluations can be obtained by a preceding Phase 0 trial. For instance, this was done for ribociclib, where the investigators also added a pharmacokinetic and pharmacodynamic-guided expansion cohort to ease accrual.⁴³ That is, if the drug crossed the blood-brain barrier and showed a pharmacodynamic effect, the patient would continue on ribociclib. Alternatively, if a standalone preceding phase 0 trial is deemed too resource demanding, another way of obtaining this information is to incorporate a "window of opportunity" analysis in a Phase II efficacy trial. Here, regardless of treatment failure, you administer the experimental agent to patients planning to undergo relapse surgery, thus enabling sampling and analysis of exposed tissue.⁴¹ If done rigorously, by applying the abovementioned

criteria of (1) relevant clinical drug doses to ensue by, (2) pharmacokinetic analysis in both contrast-enhancing and non-enhancing tumor regions as well as by (3) pharmacodynamic analysis, you enable the discovery of potential pharmacological reasons for a drug failure. Unfortunately, these fundamental drug evaluations are rarely performed. A possible explanation may be that, even when rigorously designed, these trials still carry some limitations. Their small sample sizes, arbitrarily chosen adequate intra-tumoral concentrations and patient selection bias, all challenge their general validity.^{43,44} In 2020, Vogelbaum et al. published a systematic review displaying the scarcity of meticulous published and ongoing Phase 0 or “window of opportunity” trials for glioblastoma.⁴¹ Twenty-two published trials matched their search criteria, and only three trials fulfilled all previously mentioned criteria enabling a thorough pharmacological assessment of a compound.^{43,45,46}

Table 3 recapitulates recommendations for the data necessary for an informed go/no-go decision from phase II to phase III to improve the estimated probability of success of a phase III trial. To date, no consensus paper on the matter has been published.

Choosing the Right Targets

In the last 20 years, 257 phase I/II to phase III trials of targeted therapies for glioblastoma have been initiated.⁴ In one-third of trials ($n = 85$), a tumor cell transmembrane receptor tyrosine kinase acts as a drug target. And most common among those, not surprisingly, is EGFR, serving in 40 trials. Another frequent target, influencing angiogenesis, is vascular endothelial growth factor/-receptor (VEGF/-R), appearing in 75 trials.⁴ (Figure 1 summarizes the most common targets used in glioblastoma trials). These two treatment strategies have been extensively trialed, and numerous reasons for their failures and enduring promises.^{4,9} However, as new molecular pathways have been identified, it may be time to shift focus towards other potential targets.^{47,48} For that reason, we will not discuss EGFR, VEGFR, or other frequent targets such as the PI3K/AKT-pathway, as they have recently been reviewed elsewhere, but we will swiftly walk through selected alternative treatment strategies where drugs are currently in trial.^{12,49,50} The following list of strategies is far from absolute. However, it covers a few biologically pertinent strategies yet not extensively trialed and, therefore, may be interesting options for future trials.

Targeting the Glioblastoma-Initiating Cells

The discovery of tumor-initiating cells with self-renewing capacity, also known as glioblastoma stem cells (GSCs), has

transformed our understanding of glioblastoma initiation and recurrence.⁵¹ The cancer stem cell model is not incompatible with the previous dogma of tumor growth by clonal evolution but poses that a rare population of cancer cells retain the capacity to self-renew and replenish the tumor heterogeneity and therefore represent prime suspects in treatment resistance and relapse.⁵² Having targeted therapy in mind, such a cell type would arguably be the ultimate target. Even though identifying a consistent target for GSCs has proven challenging, several drugs acting upon pathways influencing cells’ stem-like properties have been developed and undergone clinical trials.^{4,53} These are drugs inhibiting targets such as Notch, Wnt, and Smoothened.⁴ The latter is the only one currently in trial—NCT03466450 and NCT03734913; and is a component of the Sonic hedgehog pathway. This pathway plays a role in the maintenance of GSCs by, amongst other mechanisms, controlling cell interactions.⁵⁴

Targeting the DNA Damage Repair

Targeting the DNA damage repair system has been a success in several cancer types with poly-ADP-ribose polymerase (PARP)-inhibitors.⁵⁵ These drugs perturb the repair of single-strand breaks, and in cells with impaired homologous recombination repair, such as BRCA-deficient cells, a synthetic lethality is created.⁵⁵ This concept may be propitious for glioblastoma as well. The fact that present treatments such as radiation therapy and temozolomide induce DNA damage and that the expression of the DNA repair enzyme MGMT clearly influences survival indicates that DNA repair has a major role in glioblastoma.^{3,56,57} It also encourages the hypothesis that administering DNA damage repair inhibitors alongside standard therapy may induce attractive synthetic lethality and, thus, enhance the effect of the latter. There are several ongoing trials where radiation and/or temozolomide are given alongside inhibitors of vital DNA repair effector proteins. To the best of our knowledge, the inhibitors currently in trial are PARP, DNA-dependent protein kinase—NCT04555577 and ataxia-telangiectasia-mutated—NCT03423628.^{58,59} PARP-inhibitors for glioblastoma are already well studied and comprehensively reviewed elsewhere.⁵⁹

Targeting the Tumor Microenvironment

Glioblastoma tumors consist of several non-neoplastic cells such as neurons, astrocytes, macrophages, microglia, and T-cells.⁶⁰ All these non-neoplastic cells within the tumor comprise the tumor microenvironment (TME) and have risen as an interesting therapeutic target.⁶¹ The

Table 3. Proposed Data Necessary for an Informed go/no-go Decision from Phase II to Phase III

1	Data from PK analysis of drug in clinical dose in both CE and non-CE tumor
2	Data from PD analysis of drug in clinical dose
3	Overall survival data compared to a randomized control arm and/or leveraged historical controls

Index: PK = pharmacokinetics; CE = contrast-enhancing; PD = pharmacodynamics.

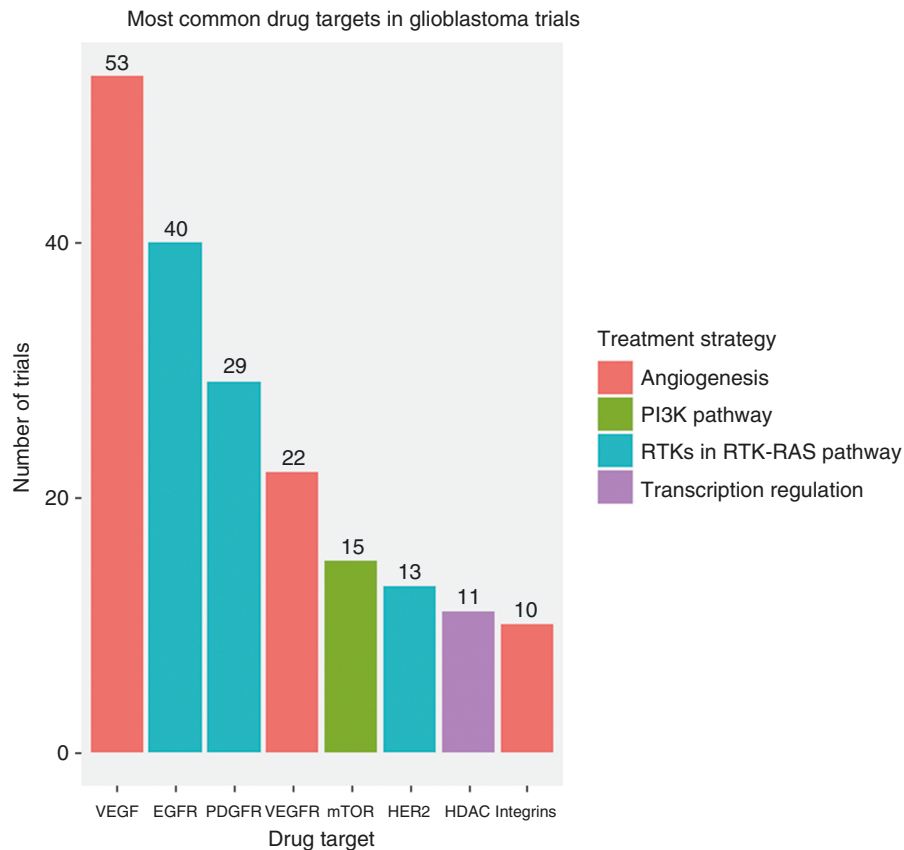


Figure 1. Most common targets in concluded and ongoing glioblastoma trials (up to 1st of April 2020) Adapted from data collected by Cruz Da Silva et al.⁴ Index: VEGF: Vascular endothelial growth factor; RTK: Receptor tyrosine kinase; EGFR: Epidermal growth factor receptor; PDGFR: Platelet-derived growth factor receptor; VEGFR: Vascular endothelial growth factor receptor; mTOR: Mammalian target of rapamycin; HER2: Human epidermal receptor 2; HDAC: Histone deacetylase; PI3K-pathway: Phosphoinositide 3-kinases pathway.

largest components of the TME are tumor-associated macrophages and microglia (TAMs). Glioblastoma TAMs are mostly pro-tumorigenic and immuno-suppressive, so-called M2 type, and are thought to engage in cross-talk with tumor cells and influence several aspects, including stemness, angiogenesis, migration, and immune suppression.⁶¹ TAMs can be recruited onto the tumor site and phenotypically transformed into the M2-type as a result of cytokines secreted by glioblastoma cells.⁶² And since macrophages and microglia have less genetic heterogeneity than tumor cells, they might be a more appropriate aim for targeted therapies. Plerixafor, an immunostimulant drug aiming to prevent the recruitment of TAMs to the tumor site, is currently in trial—NCT03746080.^{63,64} Exhaustive reviews of ongoing trials targeting TME have recently been published, showing activity in the field and the potential of this strategy.⁶¹

Targeting Gene Fusions

When a genomic alteration causes a fusion of two coding and/or regulatory parts of a gene, it may cause an oncogenic new gene called a gene fusion.⁶⁵ Although infrequently occurring in all tumor types, when present, they are often

remarkably relevant for tumor growth.⁶⁶ In cancers like chronic myeloid leukemia and lung cancers, targeting rare oncogenic gene fusions are part of standard therapy.^{6,65} Regarding glioblastoma, recurring oncogenic gene fusions are similarly uncommon and mostly feature previously discussed receptor tyrosine kinases. Yet, interest in detecting and targeting these has surged due to clinically interesting results. NTRK gene fusions and fibroblast growth factor receptor 3 and acidic coiled-coil 3 (FGFR3-TACC3) fusions have shown to be druggable and larger biomarker-driven trials are now ongoing—NCT04142437, NCT02568267, and NCT05267106.^{37,38,67,68} Other oncogenic fusions, such as EGFR fusions, FIG-ROS fusions, and non-coding RNA, also hold the potential of becoming future targets.⁶⁵

Harnessed Real-World Clinical and Molecular Evidence

As the vast advances in molecular comprehension of glioblastoma have yet to unfold as clinical improvements, an inventive and efficient framework is necessary to facilitate the bridging of these two fields and thus precipitate the positioning of targeted therapies and precision medicine.

The current framework is the interventional clinical trial focusing on drug testing with standardized rules and regulations, often generating solid evidence but only providing answers to a single or a few questions. Although important, such trials have not kept pace with breakthroughs in the basic sciences and the development of new targeted therapies.⁶⁹ A feasible approach to supplement clinical evidence generated by clinical trials is establishing a prospectively collected clinical database with matched molecular information. A prospective, thorough, and systematically built database enables one to capitalize on all data created outside clinical trials, thus facilitating clinicians' hypothesis testing. Considering that only 8%–11% of newly diagnosed glioblastoma patients are enrolled in a clinical trial, incorporating all this added real-world data would truly increase the amount of collected information.⁷⁰ A sufficiently large database can support hypothesis-generating studies, captures the whole glioblastoma patient population as opposed to only the ones eligible for a trial and permits studies on smaller subsets of patients too demanding to recruit in a separate trial. Such a database would also greatly facilitate and improve leveraging externally controlled data for single-arm trials, as previously mentioned.²² The possibilities for hypothesis testing are only limited by the amount and quality of the data collected in the database. As a single institution, the estimated time necessary to reach a significantly large cohort of patients with matched relevant biomarker analysis is probably significant. Consequently, collaborations across multiple institutions are key to producing the sort of real-world evidence necessary for progress in this field.⁷¹ The concept has already been adopted and is under development for several cancers and institutions. For prostate cancer, the effort is led by the Prostate Cancer Precision Medicine Multi-Institutional Collaborative Effort (PROMISE) consortium, currently composed of 16 institutions.⁷² Clinical data with matching molecular data are methodically collected and, afterward, de-identified and standardized. In addition, it intends to make all data publicly available for future research. Another approach to prospectively collecting data is within a master observational trial framework. This new construct aims to combine a master interventional trial design with a prospective observational trial and a structured way of collecting molecular data.⁷¹ A master observational trial aims to provide large amounts of structured data that can answer a broad range of questions. The structure also mitigates common limitations of real-world data such as physician care bias, incomplete, and inconsistent medical record data, non-standardized molecular data, and biased reporting of adverse events.^{71–73} The Registry of Oncology Outcomes Associated with Testing and Treatment (ROOT) trial—NCT04028479T is pan-cancer and one of the first master observational trials.

Incorporation of Translational Research

A second approach to strengthen the link between basic and clinical research is to leverage clinical trials by incorporating translational research. This approach accelerates and facilitates the clarification of essential unanswered questions and has fortunately been a steadily

increasing trend in cancer clinical trials.⁷⁴ Following are examples of how this incorporation can be achieved in practice with a focus on how it can aid in understanding the mechanisms of treatment resistance.

In several cancer types, Darwinian selection of a preexisting resistant cell clone has long been viewed as one of the culprit mechanisms for recurrences and for mitigating the effect of targeted treatments.⁷⁵ Regarding gliomas, this mechanism of clonal selection seems to have a smaller role. Several studies with large cohorts of paired samples were sequenced longitudinally at diagnosis and recurrence(s), thus, assessing clonal evolution. These studies found that standard therapy rarely applied significant selective pressure and that most of the original truncal driver genes persisted.^{42,48,76} Furthermore, the mutational load in known cancer genes was fairly consistent between different time points. This finding could argue for a GSC model of initiating cells with high drug resistance, as discussed previously. This finding also indicates that standard therapy infrequently influences target expression over time. And thus, it may legitimize using newly diagnosed tissue for treatment selection in the relapse setting.⁷⁷ Yet, the most common mutational profiling method remains to be panel sequencing of specific cancer genes, but the expansion to whole exome sequencing and, more recently, whole genome sequencing reveals more driver mutations, including alterations outside coding regions.^{78,79} Also, recent longitudinal studies have focused on samples treated with standard therapy, leaving the molecular imprint on the tumor genome by targeted treatments undiscovered. With little effort, this last insight can be obtained by combining longitudinal sequencing and clinical trials. Accordingly, by integrating genome sequencing, at diagnosis and recurrence(s), you allow for the assessment of drug efficacy on the relevant clones and the possible discovery of genetic resistance mechanisms.^{42,46,48} Most importantly, this feat can also generate evidence that might justify an alternated treatment in the first line. For example, the discovery of recurring targetable clones, at relapse, may produce the biological rationale for combining targeted therapies upfront. A recent systematic review on combination therapies, actually showed that such an improved biological understanding often was absent ahead of new trials with combinations of targeted agents.⁸⁰

A drawback of longitudinal studies is the dependency on brain surgery to extract tumor tissue, which restricts the number of procurable samples. Moreover, molecular analysis of a tumor sample solely describes the analyzed region, and therefore cannot fully capture the intra-tumoral heterogeneity. The emerging field of liquid biopsies can solve both disadvantages of the classical tumor sample.⁸¹ Although new and still under development, research has yielded promising results, such as circulating tumor DNA in cerebrospinal fluid mirroring the gliomagenome in 50% of patients.⁸² Attempts to mimic these results from blood analytes are underway. If successful, systematically collecting liquid biopsies could offer a minimally invasive way of monitoring treatment response, diagnosing relapse, and following tumor heterogeneity throughout time.⁸²

In recent years, sequencing technologies have also introduced single-cell sequencing.⁸³ New knowledge on the glioblastoma transcriptome has been acquired, hinting

that the responsible resistance mechanisms in glioblastoma might not only be on the genetic level.⁸⁴ Resistance is rather a result of phenotypic plasticity where glioblastoma cells influenced by genomic, microenvironmental, and anatomical stress can shift between cellular states with varying susceptibility to the different treatments, and thus, induce treatment resistance.⁸⁵ This theory is gaining greater recognition, but the epigenetic regulators of these plastic cellular states are still unknown and possible treatment strategies have yet to be tested.⁸⁵

A way to further capitalize on the data produced by the abovementioned technologies is to bridge it back to the laboratory and improve current preclinical models. Present efficacy data of targeted therapies from cell cultures, organoids, and murine models have displayed a poor correlation with clinical outcomes.⁸⁶ By applying findings from the sequencing of human tumors, researchers can now develop genetically engineered mice that develop spontaneous gliomas. This approach allows one to recapitulate and discover molecular processes driving glioma formation and treatment resistance.⁸⁷ Thus, although more technically challenging, time-consuming, and costly than other murine models, genetically engineered mice may have an improved ability to predict clinical success.^{87,88}

In summary, continued research to close the vast knowledge gap concerning resistance mechanisms should still be pursued by integrating sequencing with clinical trials and requires interdisciplinary collaborations between basic researchers and clinicians. As the genomic reason for a drug's potential failure may emanate from this translational integration, we believe it should be a part of all trial protocols and financed by the trial sponsor.

Conclusion

The introduction of targeted therapies to treat glioblastoma has not been as successful as anticipated in the first place. The discovery of an effective treatment for this lethal disease almost seems to be more distant with every breakthrough in the basic and molecular sciences and for every negative clinical trial. To succeed, institutions must adopt an approach involving several well-thought components in an all-encompassing strategy. In this review, we have highlighted previous shortcomings and future promises. We discussed feasible improvements considering trial design, drug assessment and target selection, and the importance of real-world data and translational research. These are essential components of the demanded multimodal strategy. Our review is far from comprehensive, but offers valuable recommendations for how the neuro-oncology community can find a meaningful role for targeted therapies in the treatment of glioblastoma.

Funding

This research has not received any funding.

Conflicts of Interest: The authors do not have any conflicts of interest related to the information in this manuscript.

Author Contribution

Conception and design: VF, BH, TU, HSP. Manuscript writing: all authors. Final approval of the manuscript: all authors.

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