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The Molecular Basis for Novel Therapies

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Abstract

The era of targeted therapy for glioblastoma has arrived, but results have been modest thus far. This review highlights the challenges inherent to treating glioblastoma with targeted therapy, and delves into the complex signaling networks that form the molecular basis of novel therapies. Past failures, current challenges, and future possibilities are discussed in the context of the classic ‘oncogenic’ signaling network, as well as the ‘non-oncogenic’ stress response network.

Keywords

glioblastoma; signaling pathways; targeted therapy; kinase inhibitors; personalized medicine

INTRODUCTION

The first wave of targeted molecular therapies for glioblastoma has come and gone, and at the risk of sounding glib, it is fair to say that the party did not quite live up to the hype. The rousing success of the small molecule multikinase inhibitor imatinib for CML certainly set the bar very high, and while there were reasons to suspect that effective targeted therapies for a diffuse, heterogeneous solid tumor such as glioblastoma would not be so easy to come by, the failure of targeted therapies to demonstrate efficacy in clinical trials has been disappointing nonetheless. There have been a few successes -- notably antiangiogenic therapy with bevacizumab -- but even the successes come with qualifications. To date, no patients have been cured, and durable responses are still rare. Thus far, small molecular inhibitors targeting EGFR, mTOR, VEGFR-1, VEGFR-2, and VEGFR-3, PKC β , PDGFR, SRC, c-MET, Raf, and Notch either individually or in combination have failed to significantly improve outcome (Table 1). Clinical trials of these agents have generally demonstrated radiographic response rates of less than 10–15%, with no significant improvement in time-to-progression or overall survival.¹

Why have these drugs and drug combinations failed? For many trials, the reasons are still unknown, though they are likely varied and multiple. Was drug delivery insufficient? It has

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been estimated that only 2% of small molecular weight drugs (and no large molecular weight drugs) are able to penetrate an intact blood-brain barrier.² Was the drug target sufficiently inhibited? If so, are tumors intrinsically resistant to inhibition of that target? Or does the tumor respond to effective drug target inhibition by utilizing or acquiring other means of sustaining growth? This form of resistance to therapy has been termed 'adaptive' or 'evasive' resistance.

We are still learning just how dense, interconnected, and robust the signaling networks within glioblastoma can be (Figure 1). Simple cartoon diagrams of signal transduction pathways with free-floating proteins connected by arrows have unfortunately failed to provide a portrayal of the disease sophisticated enough to explain the variety of mechanisms of resistance to treatment. To understand our past failures, it is helpful to first appreciate the complexity of the signaling networks the treatments were meant to target.

Glioblastoma - a dramatization

Here is the view of targeted therapy as understood by the average clinician: Glioblastoma cells have surface receptors like EGFR that can become overactivated. Overactivation of EGFR 'turns on' PI3-kinase, which 'turns on' AKT, which 'turns on' mTOR. Activation of mTOR results in a variety of changes within a cell that lead to growth and proliferation. Using this conceptual framework, it is difficult to understand why drugs like erlotinib and gefitinib (both small molecule EGFR inhibitors) and temsirolimus (an mTOR inhibitor) failed. The truth, of course, is that what really goes on in glioblastoma is far more complicated.

Every cell within a glioblastoma has thousands of cell surface receptors. Of the 90 receptor tyrosine kinase genes encoded in the human genome, those with particular relevance to glioblastoma include the EGFR family of receptors, PDGFR, c-MET, and the VEGF receptors. These receptors span the cell membrane and, in their normal wild-type form, are activated when ligand binds to the extracellular receptor domain. This binding triggers a conformational change that leads to dimerization and activation of the intracellular kinase domain. The kinase domain phosphorylates tyrosine residues along the intracellular portion of the receptor, creating binding sites for proteins that activate downstream signal transduction pathways. Each receptor has numerous autophosphorylation sites; PDGFR, for example, has at least ten. Each of these sites is phosphorylated and dephosphorylated more or less independently, meaning that the total number of phosphorylation states per PDGF receptor is 2^{10} . The exact state of phosphorylation helps determine which cytosolic effector proteins bind. More than one hundred effector proteins can bind a given receptor at any given time. Taken together, this combinatorial explosion of possibilities means that the idea of an 'activated' PDGF receptor is actually quite nebulous. Rather than function like on-off switches with easily determined outputs, these receptors are more accurately conceptualized as a probability cloud of possible receptor states. The consequences of inhibiting PDGFR, therefore, are similarly nebulous.

The intracellular proteins involved in these signaling networks do not float freely in space, but instead are restricted to distinct subcellular pools by various shifting molecular roadblocks and highways. The protein-protein interactions that drive signaling are typically of modest affinity with half-lives on the order of seconds or less. Posttranslational modifications such as phosphorylation are similarly transient, as kinases and phosphatases constantly undo the actions of the other. Because binding affinity is modest, ultimately protein-protein interactions are determined in large part not by affinity but by steric constraints and local availability. Within a given subcellular locale, some signaling proteins may number in the thousands, and others may be very rare. (This suggests that interactome maps are limited in their ability to represent functional signaling networks, as they reflect

measurements of binding affinity alone. Indeed, low-affinity interactions that fly under the radar of interaction screens may be of much more importance.)

The overall picture that emerges from this amalgum of probability clouds and stochastic events is an evershifting blend of signaling complexes, constantly forming and dissociating, flickering from one state to the next. With this view in mind, it is easier to understand how inhibiting one component of one pathway can fail to stop tumor growth. It's like dipping your finger in a cup of water -- all of the water molecules that are displaced simply find somewhere else to go.

Of course, intracellular signaling networks represent only one layer of complexity and heterogeneity within glioblastoma. Most receptors are able to bind several different ligands. Receptors may dimerize with themselves (homodimerization), or may dimerize with other receptors (heterodimerization). All of these possible interactions result in different outcomes. Dysregulation of transcription and translation result in the accumulation of misfolded proteins in abnormal stoichiometric ratios. Autocrine (*eg*, tumor cell to itself) and paracrine (*eg*, endothelial cell to tumor cell) loops are created when, for example, PDGF binds to PDGFR, which releases more PDGF. Glioblastoma cells are constantly interacting with their local environment, and gene expression (and ultimately cell behavior) is largely predicated on factors such as whether the cell borders a hypoxic necrotic environment, or is located amidst a swamp of distorted abnormal blood vessels, or is invading as single cells into normal brain, etc. The ability of glioblastoma cells to take on different identities in response to different environmental stresses is truly astounding. It was recently shown that stem-like cells within tumor are able to differentiate into tumor endothelium. In other words, even the concept of a glioblastoma cell as being distinct from the surrounding stroma is not as concrete as it might seem on the surface. Given all of these layers of complexity, the occasional modest success of targeted therapy for glioblastoma is perhaps more surprising than the failures.

GLIOBLASTOMA SIGNALING NETWORKS

With all of that as a preface, the rest of this review will focus on drug targets and signaling pathways in a much more linear and simplified manner. In general, the processes that propagate cancer can be broken down into those that drive growth and proliferation (the classic 'oncogenic' network) versus those that allow the cancer to mitigate and tolerate cellular stress (the 'non-oncogenic' network). In reality, there is significant overlap between these two networks, but as a heuristic device, it is helpful to discuss them separately.

The 'oncogenic' growth and proliferation signaling network

The signaling networks that drive growth are commonly triggered by receptor overactivation, with or without the presence of ligand. The downstream effects of receptor activation are usually pleiotropic, and the individual links between receptor activation to the ultimate end effectors are not always clear, but some of the major players are discussed below. (Angiogenesis is discussed elsewhere in this journal.)

EGFR

If ever there was a promising molecular target for glioblastoma, it is EGFR. The *EGFR* gene is amplified in 40–50% of primary glioblastomas, and the degree of amplification seen in glioblastoma is often far greater than seen with other cancers that are sensitive to EGFR inhibitors, such as non-small cell lung adenocarcinoma and colorectal cancer.³ Approximately 40% of tumors with EGFR amplification also possess a gene rearrangement that results in a ligand-independent variant of the receptor known as EGFRvIII. Yet, clinical trials of the small molecule EGFR inhibitors gefinitib and erlotinib, as well as the EGFR/

HER2 inhibitor lapatanib and the anti-EGFR monoclonal antibody cetuximab, have all failed to show a significant survival benefit.⁴ A phase II trial of gefitinib demonstrated no correlation between EGFR status and survival, though interestingly, patients with adverse effects attributable to anti-EGFR therapy (diarrhea and rash) survived longer.⁵

Several investigators have attempted to understand resistance to EGFR-directed therapy in the context of the broader downstream signaling network. EGFR activates two major signaling cascades – the RAS/RAF/MEK/ERK pathway and the PI3K/AKT/mTOR pathway. Researchers have hypothesized that if the signal transduction cascade triggered by EGFR is also aberrantly overactivated by another mechanism, then inhibiting EGFR may not sufficiently inhibit the downstream pathway enough to terminate tumor growth and proliferation. This appears to explain why colorectal cancer typically only responds to EGFR inhibition if normal wild-type KRAS is present. Similarly, in glioblastoma, sensitivity to EGFR inhibition may occur only if the PI3K pathway is not otherwise overactivated. The phosphatase PTEN catalyzes the reverse reaction of PI3K – it forms PIP₂ by dephosphorylating PIP₃ – and in doing so acts as a negative regulator of the pathway. Loss of PTEN expression is common in glioblastoma. In a sense, this removes the ‘brake’ on the PI3K pathway. Mellinghoff *et al* reported that glioblastoma patients with retained PTEN expression and aberrant EGFR pathway activation (due to the presence of EGFRvIII) were more sensitive to erlotinib than patients with tumors characterized by the loss of PTEN expression.⁶ However, subsequent studies have failed to corroborate this finding.⁷ Others have reported that tumors with low levels of AKT phosphorylation may be more likely to respond to EGFR inhibition.⁸

In addition to its role as a cell surface receptor, EGFR (and EGFRvIII) can also be found on the inner nuclear membrane and in the nucleoplasm. Nuclear EGFR has been shown to be a negative prognostic factor for breast carcinoma, oropharyngeal squamous cell carcinoma, and ovarian cancer.⁹ Its presence has been demonstrated in glioblastoma, but significance remains unknown. Nuclear EGFR interacts with DNA-binding transcription factors to induce expression of genes that promote proliferation. Cell surface EGFR is also able to modulate gene expression by phosphorylating STAT3, which subsequently dimerizes and translocates to the nucleus to act as a transcription factor. STAT3 has been shown to play an oncogenic role in a variety of malignancies, and is highly activated in glioblastoma.

In response to treatment with EGFR inhibitors, other members of the EGFR family of receptors (HER2/NEU, HER3, and HER4) available in the local pool may step in and serve many of the same functions as EGFR. HER2/NEU, in particular, may represent a promising treatment target, as a genomic survey demonstrated that *ERBB2* (the gene that codes for HER2/NEU) is mutated in 8% of glioblastomas. The dense cross-talk between signaling pathways also suggests that other receptor tyrosine kinases, such as PDGFR, c-MET, and IGF-1R, are likely able to activate the same pro-growth programs as EGFR. IGF-1R expression has been shown to be upregulated following treatment with an EGFR inhibitor.¹⁰ Trials of irreversible EGFR inhibitors and multikinase inhibitors are currently underway. Combination therapies are also being evaluated, as discussed below. And of course, the search for additional synergistic targets continues. A recent study using a siRNA library to screen the EGFR signaling network identified 61 genes that may be associated with resistance to anti-EGFR therapy.¹¹ Interestingly, most of the candidate genes identified in this screen were not found to have a high level of gene expression or mutation frequency, and thus would not have been detected using conventional high-throughput screening methods.

mTOR

A major downstream mediator of PI3K/AKT activation is the kinase mTOR. (AKT inhibits an inhibitor of mTOR.) mTOR integrates inputs from a number of upstream pathways, and in normal cells responds to signals such as nutrient levels and energy status. mTOR actually exists as a key component of two multiprotein complexes, mTORC1 and mTORC2. Activation of mTORC1 facilitates cell growth and proliferation by inducing protein and lipid synthesis, as well as the formation of ribosomes. The first generation of mTOR inhibitors (sirolimus, temsirolimus, everolimus, and ridaforolimus – known collectively as ‘rapalogs’) form a complex with the cytosolic receptor FKBP12, which interferes with mTORC1 activity. mTORC2 was initially thought not to be inhibited by the rapalogs, but subsequent studies have shown that continuous exposure to sirolimus is able to inhibit mTORC2.

Because mTOR plays such a central role in determining cellular activity, rapalog treatment for glioblastoma was initially thought to be very promising. However, monotherapy trials have demonstrated modest activity at best.¹²¹³ It is now recognized that the downstream consequences of inhibiting mTORC1 may be somewhat counterproductive. One of the main targets of mTORC1 signaling – the ribosomal protein S6K1 – exerts negative feedback by downregulating IGF signaling, which dampens the activity of PI3K and AKT. Loss of this negative feedback can therefore result in activation of the PI3K/AKT pathway. Furthermore, inhibition of mTORC1 pushes the equilibrium in favor of mTORC2 signaling. One of the main phosphorylation targets of mTORC2 is Ser473 on AKT. In fact, this ‘second’ phosphorylation of AKT by mTORC2 is necessary for AKT to become maximally active. Therefore, rapalog treatment may activate the very pathway it was meant to inhibit. This premise is supported by the results of a phase I trial of everolimus, in which it was shown that an increasing dose of drug was associated with a corresponding increase in AKT.¹⁴ A small study of 15 glioblastoma patients treated with everolimus demonstrated that patients with tumors with phosphorylated AKT had shorter time-to-progression.¹⁵

Given the variety of mechanisms by which tumors are able to surmount blockade of a single target, one appealing therapeutic strategy is to treat with drug combinations that inhibit signaling pathways at multiple points. A common approach is to inhibit an ‘upstream’ receptor in addition to the ‘downstream’ mediator, mTORC1. To this end, several clinical trials combining rapalogs with other targeted therapies have been performed. Results of trials combining EGFR inhibitors with rapalogs have on the whole been modest.¹⁶¹⁷ Studies using a rhabdomyosarcoma cell line demonstrate that the upregulation of AKT following treatment with sirolimus can be suppressed with concomitant administration of an IGF-1R inhibitor.¹⁸ Other studies have demonstrated increased MAPK pathway activity in the face of mTORC1 inhibition. This provides a rationale for combining mTOR inhibitors with MAPK pathway inhibitors. Given the numerous interactions between the two pathways, however, actual outcomes with these combinations are hard to predict. In fact, one systems analysis predicts that using a rapalog along with a MEK inhibitor will result in a reduction in cytotoxicity.¹⁹ Another growing concern with combination therapies is increased toxicity. A phase I/II trial of erlotinib with temsirolimus for patients with recurrent malignant glioma was complicated by higher than expected rates of rash and mucositis. The maximum tolerated dose of temsirolimus in this trial was only 15 mg weekly, as opposed to the single-agent dose of 170 mg weekly.²⁰

The second generation of mTOR inhibitors bind the ATP site of the mTOR kinase domain, and are able to inhibit both mTORC1 and mTORC2. In fact, because the catalytic domain of mTOR and the p110 α subunit of PI3K are structurally similar, some second generation inhibitors are able to inhibit both mTOR and PI3K. Laboratory studies demonstrate

suppression of both S6K1 and AKT using these compounds.²¹ Clinical trials of these inhibitors are currently underway.

Other kinases, proteins and targeted therapies

PDGFR gene amplification is present in thirteen percent of glioblastoma.²² The PDGF receptors (PDGF-R α and PDGF-R β) play important roles in multiple signaling pathways that promote tumor growth and progression. PDGF signaling is complex – the four subunits of PDGF combine to form five ligands. Small molecule inhibitors of PDGFR such as imatinib have been evaluated in glioblastoma trials, but results have been modest. Newer small molecule inhibitors (*eg*, nilotinib) are currently being evaluated, and monoclonal antibodies against PDGF are also being developed.

The activation of RAS leads to the phosphorylation of RAF, which then phosphorylates MEK, which then phosphorylates MAPK, ultimately resulting in a host of changes that promote mitosis and proliferation. Effective RAS inhibitors have thus far been difficult to design. Because RAS activity is dependent on the addition of a farnesyl moiety to the protein, drugs that inhibit the transfer of farnesyl groups – farnesyltransferase inhibitors such as tipifarnib and lonafarnib – have been evaluated in clinical trials for brain tumor patients. Results have been modest. The small molecule drug sorafenib inhibits RAF, VEGFR, and PDGFR, but use for CNS tumors has mostly been limited to small clinical trials.

The tyrosine kinase receptor c-MET plays an important role in embryonal development, and has been implicated in invasion and cancer stem cell biology. Rilotumumab, a monoclonal antibody against HGF/SF (the ligand for c-MET), was recently evaluated in a phase II clinical trial for patients with recurrent glioblastoma. Unfortunately, little activity was seen.

The non-receptor tyrosine kinase SRC activates multiple signaling pathways, many of which result in changes in the way cells interact with their extracellular environment. SRC overactivation triggers integrin-dependent changes that lead to cell movement and proliferation, resulting in increased tumor invasiveness.²³ The multikinase inhibitor dasatinib (which inhibits SRC, BCR-ABL, c-KIT, EPHA2, and PDGFR β) was evaluated in a small study of heavily pretreated patients who had failed bevacizumab, based on the idea that antiangiogenic therapy may cause tumors to adapt a SRC-driven invasive phenotype. Unfortunately, little activity was seen. Results of multicenter clinical trials of dasatinib are currently pending.²⁴

Another strategy to decrease invasiveness is to inhibit integrin signaling directly. The cyclic pentapeptide cilengitide is an inhibitor of the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$. These integrins are expressed both on endothelial cells and glioma cells, suggesting that cilengitide may target both angiogenesis and invasion. Having demonstrated activity and safety in several phase I and phase II trials, cilengitide is currently being evaluated in phase III trials for patients with glioblastoma. Other targets in the ‘invasion’ pathway include tenascin C (an extracellular matrix glycoprotein expressed ubiquitously by glioma cells), matrix metalloproteinases (which help degrade and remodel the extracellular matrix), and FAK (which is needed for cell migration). A radiolabeled monoclonal antibody against tenascin-C has been developed, but the intracavitary injection used to deliver this treatment may fail to reach the leading invasive edge it was designed to target. A randomized placebo-controlled phase II trial of the matrix metalloproteinase inhibitor marimastat was disappointingly negative, perhaps due to compensatory activity by other enzymes.²⁵ Laboratory studies demonstrate that inhibiting FAK decreases invasiveness.²⁶

Protein kinase C (PKC)- β plays a role in both the VEGF and PI3K signaling pathways, and therefore represents an attractive therapeutic target. However, a phase III trial of the PKC- β

inhibitor enzastaurin for patients with recurrent glioblastoma was disappointingly negative.²⁷ The partial estrogen agonist tamoxifen is also a PKC inhibitor, but results of tamoxifen use in brain tumor patients have been modest.

The 'non-oncogenic' stress response network

The oncogenic signals that drive cancer growth and proliferation also create an assortment of cellular stressors, including stress from DNA damage, cell cycle stress, proteotoxic stress, and metabolic stress. These 'stress phenotypes' are as much a hallmark of malignancy as classic oncogenes, and targeting a cancer cell's ability to tolerate stress represents another promising area of therapy. As previously stated, in reality the 'oncogenic' and 'non-oncogenic' networks are closely related, and many adaptations to cellular stress stem directly from oncogenic signaling.

The DNA damage response

The elevated DNA replication rates seen in cancer result in elevated rates of DNA damage. In response to this damage, glioblastoma cells activate the PI3K-related kinases ATM, ATR and DNA-PK. These kinases phosphorylate multiple targets including the effector kinases CHK1 and CHK2, resulting in cell cycle checkpoint initiation or apoptosis. Loss of genes encoding components of these pathways is common in glioblastoma, with heterozygous loss of *CHEK2* occurring approximately 20% of the time.²⁸ In laboratory studies, ATM inhibitors have been shown to radiosensitize glioma cells, abrogate AKT and ERK signaling, inhibit migration and invasion, and lead to the accumulation of double-stranded DNA breaks in EGFRvIII overexpressing glioma cells.²⁹

DNA damage caused by radiation and adduct-forming agents such as temozolomide can be repaired by the enzyme MGMT. It is well-documented that patients with tumors in which the *MGMT* gene promoter is silenced by methylation have better outcomes than patients who don't, though it is still unclear whether this is due specifically to loss of MGMT activity.^{30,31} Clinical trials of O⁶-BG, a low molecular weight substrate of MGMT that depletes the enzyme, have been performed. Unfortunately, significant hematologic toxicity was noted when O⁶-BG was given systemically.³² It is also worth noting that the alkylation of DNA at the O⁶ position of guanine repaired by MGMT makes up only 7% of the alkylating events caused by temozolomide. It is far more common for temozolomide to alkylate DNA at positions that are mended by base excision repair pathways. The enzyme PARP plays a critical role in base excision repair, and PARP inhibitors are currently being evaluated in several clinical trials. While PARP inhibition by itself may not be lethal to the cancer cell, administering drug in conjunction with DNA-damaging chemotherapy may overwhelm the ability of the cancer cell to repair critical DNA strand breaks, resulting in lethal 'stress overload'. Inhibitors against CHK1 or both CHK1 and CHK2 are also in development.³³

Growth factor signaling also regulates the DNA damage response at many levels. EGFR in particular appears to play an integral role, and has been shown to translocate to the nucleus in response to radiation and chemotherapy, where it activates DNA-PK, a nuclear kinase that helps repair double-strand DNA breaks.

The cell cycle stress response

In normal cells, mitosis is a tightly regulated and carefully orchestrated process. Not so in cancer cells, which is why aneuploidy is common. The kinases Aurora A and Aurora B normally help regulate mitosis at the spindle checkpoint, but their dysfunction can contribute to tumor progression. One review of 25 glioblastoma patients demonstrated a correlation between increased Aurora B expression and poor survival.³⁴ Several Aurora kinase

inhibitors are currently being evaluated in clinical trials. Adverse effects of these inhibitors include neutropenia, and the theoretical induction of polyploidy.³⁵

The kinases that regulate the cell cycle are another potential therapeutic target. The genes encoding CDK4 and CDK6 are frequently amplified in glioblastoma. Pan-CDK inhibitors have been evaluated in several clinical trials, but activity has been modest. PD-0332991, a new generation CDK4/CDK6-specific inhibitor, arrests glioblastoma growth in an intracranial xenograft model.³⁶ A clinical trial of this drug for patients with Rb-positive recurrent glioblastoma is currently underway.

The proteotoxic stress response

The proper folding, assembly, and transport of proteins is critical to their function. These processes are guided by a host of highly conserved molecular chaperones, of which the heat shock proteins are the best characterized. HSPs constitute 10% of proteins found in a normal cell, but in response to the stress phenotypes seen with cancer, expression levels can increase several-fold. In glioblastoma, HSP90 binds and stabilizes oncogenic proteins such as EGFRvIII, PDGFR, MAPK, PI3K, AKT, RAF, and SRC. It also stabilizes HIF1 α , the major intracellular sensor of hypoxia and driver of angiogenesis. Preclinical data indicates that HSP90 inhibitors have antiglioma activity, and clinical trials for other malignancies are currently being performed.³⁷ HSPs also have immunogenic properties. This has spurred the development of HSP-peptide vaccines for several malignancies, including glioblastoma.

Orderly degradation of proteins is also an important part of normal cellular function. This process is carried out in large part at the proteasome via the process of ubiquitination, but is often dysregulated in malignancy. In fact, the ‘constitutive activity’ of the EGFRvIII receptor stems in part from its inability to be ubiquitinated and degraded as efficiently as wild-type EGFR.³⁸ Downregulation of the tumor suppressor p53 can also result from abnormal ubiquitination. The gene *MDM2*, which is amplified in 8–10% of glioblastomas, encodes for a protein that ubiquitinates p53, leading to its degradation.³⁹ MDM2 inhibitors may therefore be a promising mode of therapy. Inhibiting proteasome function has proven to be an effective treatment for hematologic malignancies such as multiple myeloma. Preclinical studies suggested that glioblastoma is sensitive to the proteasome inhibitor bortezomib. However, a phase II clinical trial of bortezomib and bevacizumab for patients with recurrent glioblastoma did not demonstrate efficacy superior to single-agent bevacizumab.⁴⁰ Results from a phase II trial of bortezomib plus the histone deacetylase inhibitor vorinostat have not yet been published.⁴¹

The metabolic stress response

Tumor growth requires the generation of lipids and amino acids needed to make new cells. This is an energy-consuming process, and there has been great interest recently in elucidating whether the unique nature of cancer cell metabolism can be exploited for therapeutic gain. It has long been recognized that cancer cells generally favor glycolytic pathways as their source of energy. This, of course, is the basis for using radiolabeled glucose to image cancer ‘hot spots’ by PET scan. Glucose transport into glioblastoma cells is mediated in part by one of the kinase-independent functions of EGFR, which is to stabilize sodium-glucose cotransporter 1. The elevated rate of glycolysis in glioblastoma cells results in increased lactate production, which is rapidly shuttled out to the microenvironment using monocarboxylate transporters. Inhibiting the function of these transporters reduced invasiveness and increased necrosis in a glioblastoma xenograft model.⁴² Other investigators have used dichloroacetate to enhance mitochondrial oxidative phosphorylation and decrease glycolysis. Disease stabilization was seen in four of the five patients who received treatment.⁴³ Further study is warranted. Interestingly, the preference

for glycolysis within a tumor may not be universal. A recent study demonstrated that glioma stem-like cells utilize oxidative phosphorylation more than their non-stem cell counterparts.⁴⁴ Recent findings have shown that the majority of grade II and III gliomas and secondary glioblastomas bear mutations in one of two NADP⁺-dependent isocitrate dehydrogenase enzymes (IDH1 and IDH2)^{45,46}. Although the role of IDH1 mutation in tumorigenesis has not been determined, changes in enzymatic functions driven by IDH1 mutation are thought to contribute to tumor formation⁴⁷. Interestingly, glioma cells with mutant IDH1 are particularly sensitive to inhibition of glutamine metabolism⁴⁸. There is an active search for drugs modulating this pathway.

CONCLUSION

Just as it is impossible to solve a Rubik's cube by focusing on a single side, focusing on a single drug target is unlikely to yield a therapeutic solution. Instead, new drugs should be evaluated in combination trials as soon as their safety profiles have been established. Yet, combination trials are also not without disadvantages. It can be difficult to tease out the effect of a single drug within a combination, and as has already been seen, toxicities can be synergistic as well. Additionally, the number of potential drug combinations is far greater than the number of sustainable clinical trials – to test all the three-drug combinations that can be made from, for example, a panel of ten drugs, it would be necessary to test 120 drug combinations. And this ignores all the possible permutations in dosing schedules that are available.

What needs to happen to make trials of targeted therapies work? First, drug combinations need to be designed rationally with mechanisms of action in mind. It would be counterproductive, for example, to combine a drug that requires cells to be cycling with a drug that induces cell cycle arrest. Second, trials should select for patients with tumors that are likely to respond to inhibition of the targets of interest. For this oft-stated goal to become a reality, better biomarkers need to be developed and validated. This will not be an easy task, as prior experience has shown that traditional methods of genomic analysis, gene expression profiling, and assessment of target inhibition do not necessarily correlate with outcome. The goal of designing biomarker-enriched trials needs to be balanced against the risk of restricting patient access to trials on the basis of unproven biomarkers. Third, trials should be designed in ways that minimize the number of patients exposed to therapies that are unlikely to benefit them. Adaptive designs that utilize Bayesian methodology may be helpful in this regard. Lastly, we need to accelerate the learning curve by interrogating tumors as frequently as is feasible. Currently, for most patients, the only time tumor tissue is obtained is at diagnosis. (For all that we have learned from the TCGA glioblastoma screen, it only analyzed patients with newly diagnosed disease.) Recently, there has been a push to design trials in which surgery is performed after receiving drug in order to assess drug penetration and target inhibition. Any time surgery is performed at recurrence, tumor tissue should be analyzed rigorously. Other investigators are developing less invasive methods of assessing tumor status and response to treatment. Analyzing tumor exosomes in peripheral blood, for example, may provide useful information regarding response to treatment and mechanisms of resistance.

As our understanding of disease biology grows, we will gradually be able to shift our attention from individual drug targets to entire signaling pathways, and eventually to whole signaling networks. The complexity of these networks in glioblastoma is overwhelming, but we are making more sense of it every day. Einstein famously came up with theory of relativity after imagining himself riding on a beam of light. Sometimes all it takes is a new perspective for breakthroughs to be made.

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Figure 1. Major Signaling Pathways in Malignant Gliomas and the Corresponding Targeted Agents in Development for Glioblastoma

RTK inhibitors that target epidermal growth factor (EGF) receptor include gefitinib, erlotinib, lapatinib, BIBW2992, and vandetanib; those that target platelet-derived growth factor (PDGF) receptor include imatinib, dasatinib, and tandutinib; those that target vascular endothelial growth factor (VEGF) receptor include cediranib, pazopanib, sorafenib, sunitinib, vatalanib, vandetanib, and XL184. EGF receptor antibodies include cetuximab and panitumumab. Farnesyl transferase inhibitors (FTI) include lonafarnib and tipifarnib; HDAC inhibitors include depsipeptide, vorinostat, and LBH589; PI3K inhibitors include BEZ235 and XL765; mTOR inhibitors include sirolimus, temsirolimus, everolimus, and deforolimus; and VEGF receptor inhibitors include bevacizumab, aflibercept (VEGF-trap), and CT-322. Growth factor ligands include EGF, PDGF, IGF, TGF, HGF/SF, VEGF, and FGF. Stem-cell pathways include SHH, wingless family, and Notch. Akt denotes murine thymoma viral oncogene homologue (also known as protein kinase B), ERK extracellular signal-regulated kinase, FGF fibroblast growth factor, FTI farnesyl transferase inhibitors, GDP guanine diphosphate, Grb 2 growth factor receptor-bound protein 2, GTP guanine triphosphate, HDAC histone deacetylase, HGF/SF hepatocyte growth factor/scatter factor, IGF insulin-like growth factor, MEK mitogen-activated protein kinase kinase, mTOR mammalian target of rapamycin, NF1 neurofibromin 1, PIP2 phosphatidylinositol (4,5) biphosphate, PIP3 phosphatidylinositol 3,4,5-triphosphate, PI3K phosphatidylinositol 3-kinase, PKC protein kinase C, PLC phospholipase C, PTEN phosphatase and tensin homologue, RAF v-raf 1 murine leukemia viral oncogene homologue 1, RAS rat sarcoma viral oncogene homologue, RTK receptor tyrosine kinase inhibitor, SHH sonic hedgehog, SOS son of sevenless, Src sarcoma (Schmidt-Ruppin A-2) viral oncogene homologue, TGF transforming growth factor family, and TSC1 and 2 tuberous sclerosis gene 1 and 2. Red text denotes inhibitors.

Table 1

Small molecule inhibitors with activity against signaling pathways relevant to malignant gliomas

Pathway	Name of agent	Signaling target(s)
<i>EGFR inhibitors</i>		
	Gefitinib (ZD1839; Iressa®)	EGFR
	Erlotinib (OSI-774; Tarceva®)	EGFR
	Lapatinib (GW-572016)	EGFR+ErbB-2
	AEE788	EGFR+VEGFR
	Vandetanib (ZD6474; Zactima®)	EGFR+VEGFR+RET
	Nimotuzumab	Anti-EGFR monoclonal Ab
	Cetuximab (C225; Erbitux®)	Anti-EGFR monoclonal Ab
<i>PDGFR inhibitors</i>		
	Imatinib mesylate (ST1571; Gleevec®)	PDGFR
	Vatalanib (PTK787; ZK222584)	PDGFR+VEGFR
	Dasatinib (Sprycel®)	PDGFR+Src+c-kit+Bcr-Abl
	Tandutinib (MLN-518)	PDGFR+c-Kit+FLT-3
	Sunitinib (Sutent®)	PDGFR+VEGFR+c-Kit+FLT-3
	CP-673, 451	PDGFR
	AMG706	PDGFR+VEGFR+c-kit+Raf
	Pazopanib (GW-786034)	PDGFR+VEGFR+c-kit
	SUO11248	PDGFR+VEGFR+c-kit
	Sorafenib (Bay43-9006; Nexavar®)	PDGFR+VEGFR+c-Kit+Raf
	OSI-930	PDGFR+VEGFR
	TKI258	PDGFR+VEGFR
<i>VEGF inhibitors</i>		
	Bevacizumab (Avastin®)	Anti-VEGF-A monoclonal Ab
	Aflibercept (VEGF-Trap)	Anti-VEGF-A/B Ab
	XL184	VEGFR+c-Met
	Pazopanib (GW786034)	VEGFR, PDGFR, c-Kit
	Cediranib (AZD2171; Recentin®)	VEGFR+PDGFR+c-Kit
	Sorafenib (Bay43-9006; Nexavar®)	VEGFR+PDGFR+c-Kit+Raf
	Vandetanib (ZD6474; Zactima®)	VEGFR+EGFR+Ret
	Vatalanib (PTK787; ZK222584)	VEGFR+PDGFR+c-kit
	Sunitinib (Sutent®)	VEGFR+PDGFR+c-Kit
	AEE788	VEGFR+EGFR
	SUO11248	VEGFR+PDGFR+c-kit
	SU6668	VEGFR+FGFR+PDGFR+c-kit
	OSI-930	VEGFR+PDGFR
	TKI258	VEGFR+PDGFR+FGFR
<i>Raf kinase inhibitors</i>		
	Sorafenib (Bay43-9006; Nexavar®)	Raf+VEGFR+PDGFR
	Bay549805	Raf
	AMG706	Raf+PDGFR+VEGFR+c-kit

Pathway	Name of agent	Signaling target(s)
<i>mTOR</i>	AAL881	Raf, VEGFR
	Temsirolimus (CCI-779)	mTOR
	Everolimus (RAD001; Certican®)	mTOR
	Sirolimus (Rapamycin; Rapimmune®)	mTOR
	AP23573	mTOR
<i>PKC/AKT inhibitors</i>	Enzastaurin	PKC- β 2 and Akt
	Perifosine	Akt
	Tamoxifen	
<i>Farnesyltransferase inhibitors</i>	Tipifarnib (R115777; Zarnestra®)	
	Lonafarnib (SCH66336; Sarasar®)	
<i>Notch</i>	MK-0752	Gamma-secretase inhibitor
	PF-03084014	Gamma-secretase inhibitor
<i>Sonic hedgehog</i>		
	GDC-0449	Smoothened inhibitor

Abbreviations: FGF, fibroblast growth factor; FLT-3, fms-tyrosine kinase; HGF, hepatocyte growth factor; PDGF-R, platelet-derived growth factor receptor; PKC-b, protein kinase C beta; PLGF, placenta growth factor; VEGF-R, vascular endothelial growth factor receptor