

# Targeted Therapy for Malignant Glioma Patients: Lessons Learned and the Road Ahead

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**Summary:** Molecularly targeted therapies are transforming the care of patients with malignant gliomas, including glioblastoma, the most common malignant primary brain tumor of adults. With an arsenal of small molecule inhibitors and antibodies that target key components of the signal transduction machinery that are commonly activated in gliomas, neuro-oncologists and neurosurgeons are poised to transform the care of these patients. Nonetheless, successful application of targeted therapies remains a challenge. Strategies are lacking for directing kinase inhibitor or other pathway-specific therapies to individual patients most likely to benefit. In addition, response to targeted agents is determined not only by the presence of the key mutant kinases, but also by other critical changes in the molecular circuitry of cancer cells, such as loss of key tumor suppressor proteins, the selection for kinase-resistant mutants, and the deregulation of feedback loops. Understanding these

signaling networks, and studying them in patients, will be critical for developing rational combination therapies to suppress resistance for malignant glioma patients. Here we review the current status of molecular targeted therapies for malignant gliomas. We focus initially on identifying some of the insights gained to date from targeting the EGFR/PI3K/Akt/mTOR signaling pathway in patients and on how this has led toward a reconceptualization of some of the challenges and directions for targeted treatment. We describe how advances from the world of genomics have the potential to transform our approaches toward targeted therapy, and describe how a deeper understanding of the complex nature of cancer, and its adeptness at rewiring molecular circuitry to evade targeted agents, has raised new challenges and identified new leads. **Key Words:** Glioma, growth factors, molecular targeted therapy, microenvironment, coactivation.

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## INTRODUCTION

Molecularly targeted therapies are transforming the treatment of cancer. Instead of therapies based on relatively broad pathologic diagnoses, population-based risk assessments, and relatively nonspecific one-size-fits-all strategies, we are moving to an era of predictive care based on molecular classification and targeted kinase inhibitor therapies. The enthusiasm for molecularly targeted approaches to cancer treatment seems justified by the remarkable success of selected kinase inhibitors in the clinic for cancers such as chronic myeloid leukemia (CML) and gastrointestinal stromal tumor (GIST), and by an increasing ability to interrogate the cancer genome, transcriptome, and proteome in clinical tumor samples.

There is compelling evidence that activating mutations in signaling pathways can result in tumor cell addiction to these pathways and so allow the prediction of clinical responses to pathway inhibition.

The remarkable success of signal transduction inhibitors for the treatment of patients with CML and GIST has yet to translate to the treatment of patients with malignant gliomas. One potential explanation for the relative success lies in the fact that the relevant molecular targets (BCR-ABL and c-KIT mutations, respectively) are present in the overwhelming majority of CML and GIST patients. In contrast, in malignant gliomas many different molecular alterations are present in varying combinations, which cannot be readily detected by routine pathological examination. In fact, strategies are lacking for directing kinase inhibitor or other pathway-specific therapies to the individual patients most likely to benefit. In addition, response to kinase inhibitors is determined not only by the presence of the key mutant kinases, but also by other critical changes in the molecular circuitry of cancer cells,

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such as loss of key tumor suppressor proteins, the selection for kinase-resistant mutants, and the deregulation of feedback loops. Understanding these signaling networks, and studying them in patients, will be critical for developing rational combination therapies to suppress resistance for malignant glioma patients.

An arsenal of small molecule inhibitors and antibodies has been developed, and is continuing to be developed, to target key components of the signal transduction machinery, with a particular focus on growth factors and their corresponding receptors and canonical downstream signal transduction intermediates. Many of these inhibitors have potential for the treatment of glioblastoma. Testing some of these inhibitors, as well as using genetic approaches to untangling these signaling networks in well-designed model systems (including both highly representative xenograft models and mouse genetic models), is beginning to provide an emerging picture of the targetable molecular phenotype of glioblastoma. Complementary to these studies, application of powerful new technologies to interrogate molecular networks in clinical samples, particularly as applied to innovative clinical trials, has begun to yield important insights as to why (with the exception of the anti-VEGF antibody bevacizumab) targeted monotherapies have yet to significantly improve outcome for most patients with malignant gliomas. These insights are beginning to point the way toward alternative combination approaches that are more likely to yield effective treatments that can anticipate and suppress clinical resistance.

In this review, we set forth the rationale for targeting growth factors and their receptors and describe the challenges of translating an increasing knowledge of the underlying biology of gliomas into more successful treatments. We take as an example the lessons learned to date from targeting a paradigmatic example, the EGFR/PI3K/Akt/mTOR signaling network, in malignant glioma patients. We then discuss how a deeper understanding of canonical growth factor signaling networks commonly activated in malignant gliomas has opened up a range of other possibilities for targeted therapies. Finally, we conclude by setting forth a series of challenges, and suggest some potential approaches to developing more effective and less toxic treatments for patients with malignant gliomas.

### **GROWTH FACTORS, THEIR RECEPTORS, AND DOWNSTREAM SIGNALING PATHWAYS AS TARGETS**

Self-sufficiency of growth signals is one of the classic hallmarks of cancer.<sup>1</sup> Tightly linked to this phenotype are resistance to antigrowth signals, evasion of apoptosis, limitless replicative capacity, tumor invasion, and enhanced angiogenesis. All these classic hallmarks of can-

cer are directly linked to deregulated growth factor receptor signaling. This should be no surprise: growth factor signaling in health is tightly regulated to orchestrate cell growth, cell division, and cell death. Genetic copy number alterations and mutations that activate growth factor signaling (e.g., common oncogene gains) or prevent negative regulation of their downstream effectors (e.g., common tumor suppressor losses) often result in persistently activated growth factor signaling networks that promote growth factor-independent proliferation, survival, invasion, and angiogenesis.

Activating mutations of growth factor receptors are, in fact, among the most common oncogenes; they were among the first to be identified,<sup>2</sup> and studies of nonviral cancers clearly demonstrate that DNA amplification and mutation mediated activation of growth factor receptors are common events across many types of cancer, including malignant gliomas. In addition, growth factor receptor ligands such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF) are known to be upregulated in malignant gliomas.<sup>3-9</sup>

Growth factor receptors that are amplified and/or mutated make appealing drug targets. Their cell surface location, and abundant expression in many cancer cells, have facilitated the development of small molecule inhibitors that target these receptors, frequently as ATP-competitive inhibitors (Table 1). In addition, the cell surface location makes them potential sites for antibody-mediated therapies. For example, antibodies targeting a specific mutant form of EGFR, EGFRvIII (described in detail below) are already in clinical trials. Although the blood-brain barrier presents delivery issues for antibodies, numerous studies have suggested that the leaky barrier found in most gliomas does allow some access.<sup>10-13</sup>

Mutated growth factor receptors use a series of canonical signal transduction cascades to promote tumor growth, survival and invasion. The PI3K/Akt signaling pathway and the Ras/MAPK pathways, among others, have emerged as central players in glioma pathogenesis by promoting proliferation, inhibiting apoptosis, stimulating invasion, and leading to angiogenesis.<sup>14-16</sup> As described below, mouse genetic models provide compelling functional evidence for importance of these signaling networks in glioma pathogenesis, and correlative human studies attest to their relevance.

The real challenge is to do the clinical experiment. Does inhibition of these crucial signaling networks in malignant glioma patients result in tumor shrinkage, longer time to progression, and improved survival? Unfortunately, the initial series of clinical trials with small molecule inhibitors of EGFR and mTOR (as will be described in more detail below) failed to show efficacy, in part because there were no strategies in place to direct these therapies to the patients whose tumor molecular composi-

**Table 1.** *Drugs Targeting Growth Factors and Growth Factor Receptors*

Therapeutic Agent	Target(s)	Company	Comments and Status*	GBM Trial
<b>EGFR/ERBB family kinase inhibitors</b>				
Gefetinib (Iressa) <sup>69</sup>	EGFR	AstraZeneca	Selective EGFR inhibitor; phase II trials for GBM demonstrated modest if any clinical benefit; FDA approved for NSCLC	Yes
Erlotinib (Tarceva) <sup>70,71</sup>	EGFR	Genentech	Selective EGFR inhibitor; trials indicate minimal efficacy as monotherapy for GBM patients; ongoing trials in combination with other drugs for treatment of GBM; FDA approved for NSCLC	Yes
Lapatinib (Tykerb)	EGFR, ERBB2	GlaxoSmithKline	Dual inhibitor; phase II trials underway for GBM; FDA approved for breast cancer	Yes
BMS-599626 <sup>72</sup>	EGFR, ERBB2	Bristol-Myers Squibb	Dual inhibitor; phase I trials underway for advanced solid tumors	No
<b>Anti-EGFR antibodies</b>				
Cetuximab (Erbix) <sup>73</sup>	EGFR	Eli Lilly/ImClone	Chimeric extracellular-binding antibody; small subgroup of patients responded in phase II trial for GBM; trials underway as a combination therapy; FDA approved for colorectal cancer	Yes
Panitumumab (Vectibix) <sup>74</sup>	EGFR	Amgen	Human extracellular-binding antibody; ongoing phase II trials for various solid tumors; FDA approved for colorectal cancer	No
Nimotuzumab <sup>75,76</sup>	EGFR	YM BioSciences	Humanized extracellular-binding antibody; promising results from early trials; phase II/III trials underway for GBM	Yes
Matuzumab <sup>77</sup>	EGFR	Merck	Humanized extracellular-binding antibody; development in question due to poor clinical trial results; may prove effective as a combination therapy	No
Zalutumumab <sup>78</sup>	EGFR	Genmab	Human extracellular-binding antibody; phase II trials for SCCHN in progress	No
IMC-11F8 <sup>79,80</sup>	EGFR	Eli Lilly/ImClone	Fully human extracellular-binding antibody; phase II trial for colorectal cancer in progress	No
MAb 806 (Ch806) <sup>81</sup>	EGFR		Chimeric extracellular-binding antibody that preferentially targets EGFRvIII and active EGFR; phase I trial complete	Yes
<b>Anti-ERBB2 antibodies</b>				
Trastuzumab (Herceptin) <sup>82</sup>	ERBB2	Genentech	Humanized extracellular-binding antibody; induces glioma cell death in preclinical study; FDA approved for breast cancer	No
Pertuzumab (Omnitarg)	ERBB2	Genentech	Humanized extracellular-binding antibody; interferes with receptor dimerization; ongoing phase II/III trials for breast cancer	No
<b>Anti-IGF-1R antibodies</b>				
AMG-479 <sup>83</sup>	IGF-1R	Amgen	Human extracellular-binding antibody; combined therapy phase I/II trials are starting for solid tumors	No
IMC-A12 <sup>84,85</sup>	IGF-1R	Eli Lilly/ImClone	Human extracellular-binding antibody; several phase II trials underway for solid tumors	No

*(Table continues)*

Table 1. Continued

Therapeutic Agent	Target	Company	Comments and Status*	GBM Trial
CP-751,871 <sup>86,87</sup>	IGF-1R	Pfizer	Humanized extracellular-binding antibody; phase I/II trials ongoing for a variety of solid tumors; encouraging results in combination with chemotherapy for NSCLC	No
<b>HGF/MET antagonists</b>				
NK4 <sup>88</sup>	MET	Kringle Pharma	Competitively inhibits HGF binding to MET; proven efficacy in GBM preclinical studies	No
Uncleavable HGF <sup>89</sup>	MET, HGF		Immune to protease activation yet binds MET; antitumor activity in preclinical development	No
Recombinant Sema domain <sup>90</sup>	MET, HGF	Genentech	Extracellular domain of MET binds HGF and interferes with MET dimerization; antitumor activity in preclinical development	No
Decoy MET <sup>91</sup>	MET, HGF		Soluble extracellular portion of MET receptor; antitumor activity in preclinical development	No
Cgen-241 <sup>92</sup>	MET, HGF	Compugen	MET ectodomain/IgG fusion protein; preclinical study demonstrated antiproliferative effect on GBM cells	No
<b>Anti-HGF/SF antibodies</b>				
L2G7 <sup>93</sup>	HGF	Galaxy Biotech	HGF neutralizing antibody; effective in a preclinical GBM model	No
AMG 102 <sup>94</sup>	HGF	Amgen	Fully human HGF neutralizing antibody; phase II trial for GBM is ongoing and preliminary data indicate some cases of clinical benefit	Yes
<b>Anti-MET antibodies</b>				
OA-5D5 (MetMab) <sup>95</sup>	MET	Genentech	Humanized extracellular-binding antibody; inhibits GBM tumor growth in preclinical study; planning phase II trial for NSCLC	No
CE-355621 <sup>96</sup>	MET	Pfizer	Extracellular-binding antibody; effective in preclinical GBM study	No
DN-30 <sup>97</sup>	MET	Methersis	Extracellular-binding antibody; impairs GBM tumor growth in a preclinical study	No
<b>MET kinase inhibitors</b>				
AM7 <sup>98</sup>	MET, LCK, BTK	Amgen	Effective against several mutant forms of MET; efficacy demonstrated in GBM preclinical model	No
ARQ 197 <sup>99,100</sup>	MET	ArQule	Selective MET inhibitor; phase I/II trials in progress for advanced solid tumors	No
MK-2461 <sup>101</sup>	MET	Merck	Selective MET inhibitor; ongoing phase I/II trials for advanced solid tumors	No
MP470 <sup>102</sup>	MET, PDGFR, KIT	SuperGen	Multikinase inhibitor that also stifles DNA repair by suppressing Rad51; preclinical studies in GBM; ongoing phase I trial for solid tumors	No
JNJ-38877605	MET	Johnson & Johnson	Selective MET inhibitor; phase I trial underway for advanced solid tumors	No
PF-2341066 <sup>103</sup>	MET, ALK	Pfizer	Potent MET and ALK inhibitor; ongoing phase I/II trials for stomach tumors and lymphomas	No
PHA665752 <sup>104</sup>	MET	Pfizer	Selective MET inhibitor; abrogates signaling in preclinical setting	No

(Table continues)

Table 1. Continued

Therapeutic Agent	Target	Company	Comments and Status*	GBM Trial
SU11274 <sup>58,105,106</sup>	MET	Pfizer	Selective MET inhibitor effective against several mutant variants of MET; potent antagonist in GBM cell lines	No
XL880/GSK1363089 <sup>107</sup>	MET, VEGFR2	Exelixis	Multikinase inhibitor that primarily targets MET and VEGFR2; phase I trials for solid tumors; phase II trials for PRC, SCCHN and gastric cancer	No
XL184 <sup>108</sup>	MET, VEGFR2, RET	Exelixis	Multikinase inhibitor; phase II trial underway for GBM	Yes
<b>PDGFR kinase inhibitors</b>				
Dasatinib (Sprycel) <sup>109</sup>	PDGFR, SFKs, BCR-ABL	Bristol-Myers Squibb	Potent inhibitor of many kinases; promising preclinical data; phase I/II trials underway for GBM; FDA approved for CML	Yes
Imatinib (Gleevec) <sup>110-113</sup>	PDGFR, KIT, BCR-ABL	Novartis	Multikinase inhibitor; limited anti glioma activity as monotherapy; effect in some GBM patients when combined with hydroxyurea; phase I/II trials in combination with other drugs are ongoing; FDA approved for CML and GIST	Yes
Tandutinib	PDGFR, FLT3, KIT	Millennium Pharmaceuticals	Multikinase inhibitor; phase II trial in combination with Avastin underway	Yes
<b>VEGF antibodies</b>				
Bevacizumab (Avastin) <sup>114,115</sup>	VEGF	Genentech	Humanized VEGF neutralizing antibody; several phase II trials have reported significant anti glioma activity; many ongoing trials are combining Avastin with other therapeutic agents; FDA approved for previously treated GBM	Yes
<b>VEGF antagonists</b>				
Aflibercept/VEGF Trap <sup>116,117</sup>	VEGF	Regeneron	VEGFR ectodomain/IgG fusion protein; phase II trial found single-agent efficacy in GBM; combined therapy trials imminent	Yes
<b>VEGFR kinase inhibitors</b>				
Cediranib/AZD2171 (Recentin) <sup>118,119</sup>	VEGFR, PDGFR, KIT, FGFR1	AstraZeneca	Most potent against VEGFR; phase II trial showed promising GBM tumor responses; phase II/III trials are underway in combination with cytotoxic therapy	Yes
Pazopanib <sup>120</sup>	VEGFR, PDGFR, KIT	GlaxoSmithKline	Multikinase inhibitor; promising phase II/III results for RCC; phase II trials in progress for GBM and other solid tumors	Yes
Sorafenib (Nexavar) <sup>121</sup>	VEGFR, c-Raf, B-Raf, PDGFR	Bayer	Multikinase inhibitor; combined with other targeted therapies in ongoing phase II trials for GBM; FDA approved for RCC	Yes
Sunitinib (Sutent)	VEGFR, PDGFR, KIT, RET, FLT3	Pfizer	Multikinase inhibitor; several phase II trials underway for GBM; FDA approved for RCC and GIST	Yes
Vandetanib/ZD6474 <sup>122-124</sup>	VEGFR, EGFR	AstraZeneca	Dual inhibitor; phase I/II trials in progress for GBM; mixed results from other solid tumor trials; candidate for combination therapy	Yes

(Table continues)

**Table 1. Continued**

Therapeutic Agent	Target	Company	Comments and Status*	GBM Trial
Vatalanib/PTK787 <sup>113,125</sup>	VEGFR, PDGFR, KIT	Novartis	Most potent against VEGFR; phase I/II trials ongoing for GBM; encouraging results as combination therapy	Yes

ALK = anaplastic lymphoma kinase; BCR-ABL = a gene fusion protein product; B-Raf = a proto-oncogenic kinase; BTK = Bruton's tyrosine kinase; CML = chronic myeloid leukemia; c-Raf = a protein kinase involved in MAPK pathway; EGFR = epidermal growth factor receptor; ERBB2 = a member of the epidermal growth factor (EGF) receptor family; FDA = U.S. Food and Drug Administration; FGFR1 = fibroblast growth factor receptor 1; FLT3 = fms-related tyrosine kinase 3; GBM = glioblastoma multiforme; GIST = gastrointestinal stromal tumor; HGF/SF = hepatocyte growth factor/scatter factor; IGF-1R = insulin-like growth factor receptor type 1; IgG = immunoglobulin G; KIT = a protein-tyrosine kinase receptor specific for stem cell factor; LCK = lymphocyte specific protein tyrosine kinase p56(lck); MET = cell surface protein-tyrosine kinase receptor for hepatocyte growth factor; NSCLC = non-small cell lung cancer; PDGFR = platelet-derived growth factor receptor; PRC = papillary renal-cell carcinoma; Rad51 = repairs DNA double strand breaks; RCC = renal cell carcinoma; RET = a receptor protein-tyrosine kinase involved in signaling of glial cell-line derived neurotrophic factor ligands; SCCHN = squamous cell cancer of the head and neck; SFK = Src family kinases; VEGF(R) = vascular endothelial growth factor (receptor). \*Status reflects progress in the treatment of gliomas when applicable. Agents may be further along for other malignancies. Refer to <http://www.clinicaltrials.gov> for more detailed information.

tion was most likely to yield a favorable response. Furthermore, even in patients fortunate enough to have a clinical response, for most of them the duration of response was limited. This should not be considered evidence of the lack of feasibility of effective targeted therapy. Rather, it must be considered a humbling lesson in our relative lack of understanding of the remarkable capacity of cancer cells to adapt and rewire to overcome targeted monotherapy.

An analogy with the development of combination antiretroviral therapy for HIV is apt. The first attempts in treatment of HIV with antiretroviral therapy yielded excitement, but then disappointment, as patients rapidly failed monotherapy. However, careful analysis of the mechanisms of resistance was critical for developing rational combination therapies that anticipate and suppress resistance, an achievement that has transformed a deadly disease into a chronic one that can be managed. The future of targeted therapies hinges on understanding how the molecular circuitry rewires to promote resistance, and then developing combination therapies to more effectively suppress it.

#### DEFINING CORE PATHWAYS IN GBM: HIGH-THROUGHPUT TECHNOLOGIES TO INTERROGATE THE MUTATIONAL LANDSCAPE OF PATIENT SAMPLES MEET FUNCTIONAL BIOLOGY

The application of powerful technologies to globally survey the genome, transcriptome, and epigenome of human cancer samples has greatly increased our knowledge of the molecular underpinnings of malignant glioma and has provided a striking concordance with lessons learned from mouse genetic models. Two recent large-scale multidimensional analyses of glioblastoma shed light on the mutational landscape of glioblastoma. The studies by the Cancer Genome Atlas (TCGA) group<sup>17</sup> and by Parsons et al.<sup>18</sup> through integrated anal-

yses of multidimensional genomic data from complementary technology platforms identified three core genetically altered pathways: 1) RTK/RAS/PI3K, 2) p53, and 3) RB, which are present in nearly all glioblastomas. These studies offer the promise that application of global methods can identify previously unrecognized molecular targets, and the convergence of findings upon these three signaling networks further highlights their importance. Mouse genetic models also have strongly implicated these pathways.<sup>19-27</sup> Furthermore, activation of these pathways has also been detected and maintained in elegant, serially passaged human glioblastoma intracranial xenograft models.<sup>28</sup>

RTK/RAS/PI3K pathway activation appears to be an obligatory event in most, and perhaps all, glioblastomas.<sup>17,18</sup> This observation is entirely consistent with previous studies that have used less global approaches to analyzing this signaling network in clinical samples from glioblastoma patients.<sup>29-31</sup>

#### THE EGFR/PI3K/AKT/mTOR SIGNALING NETWORK: A PARADIGMATIC EXAMPLE

In malignant gliomas, EGFR amplification and mutation is by far the most common receptor tyrosine kinase (RTK) alteration.<sup>17,18</sup> EGFR amplification and/or mutation, in combination with loss of the PTEN tumor suppressor protein, results in constitutive PI3K pathway activation, which highlights the role of this signaling network as a molecular target. Normally, EGFR dimerizes and autophosphorylates to activate signal transduction through PI3K, MAPK, and other pathways upon binding to one of its two main ligands, EGF and transforming growth factor- $\alpha$  (TGF- $\alpha$ ). These ligands have been shown to be upregulated in glioblastoma, suggesting one possible mechanism for their activation.<sup>32-37</sup> However, EGFR is most frequently activated in malignant gliomas through DNA amplification, which occurs in up to

45% of cases.<sup>17,18</sup> In addition, activating mutations in the extracellular domain are common, including the most frequent form of activation, the EGFRvIII variant.<sup>32,34,36</sup> This variant results from genomic deletion of exons 2-7; it is detected in between 20% and 30% of glioblastomas.<sup>17,18,38</sup> It lacks the ligand-binding domain, yet also fails to be internalized regularly, resulting in a constitutive signal. A number of studies have suggested that EGFRvIII has a particularly strong effect on PI3K signaling,<sup>29</sup> and recent proteomic studies suggest that EGFRvIII favors different canonical pathways relative to wild-type EGFR, including a greatly enhanced effect on PI3K pathway activation.<sup>39</sup> In addition to EGFRvIII, genomic studies have identified a number of other genomic deletion variants,<sup>40,41</sup> and recent work identifies a series of novel extracellular domain missense mutations, occurring in up to 14% of glioblastoma patients, that appear to be associated with gain of function.<sup>42</sup>

Thus, in glioblastoma, EGFR, potentially through its activation of the PI3K and MAPK pathways, and potentially through other less well understood signaling cascades, regulates tumor proliferation and growth, leads to growth factor independence,<sup>43</sup> inhibits apoptosis,<sup>44,45</sup> increases invasion,<sup>46</sup> and promotes angiogenesis.<sup>44</sup> The relevance of constitutive EGFR signaling for all of these hallmarks of cancer, coupled with its relatively high frequency in malignant gliomas, has suggested that EGFR could be for glioblastoma what BCR-ABL has become for chronic myeloid leukemia.

### TARGETING EGFR IN THE CLINIC: IT'S NOT SO SIMPLE

The ability to target EGFR with ATP-competitive small molecule inhibitors such as erlotinib and gefitinib generated great excitement in the neuro-oncology community. Regrettably, the experience to date has not lived up to the promise. Clinical responses have been infrequent, and short-lived. This does not mean, however, that EGFR is not a compelling target, or even that glioblastoma cells are not addicted to it.

A number of lessons have been learned from this clinical experience. First, perhaps the most critical lesson from the first forays into targeted therapies for malignant glioma patients is that tumor cell responses are determined not only by the presence of the target, but rather also by the molecular circuitry in which these activating mutations occur. This concept of context-dependent oncogene addiction has important implications for the design of molecularly targeted combination therapies (as will be discussed below). Second, unlike cytotoxic agents, for which maximal tolerated dose can be used as a guide for determining effective drug dose, targeted agents require a different type of evaluation. Biomarkers of pathway inhibition need to be developed and incor-

porated into clinical trial design in order to determine whether the study dose effectively inhibits its target in patients. It would be premature and misleading to assume that a target is not valid if our inhibitors have not achieved necessary inhibition of it *in vivo*. Third, pathways are not simply linear vectors moving from one molecule to the next, but rather complex interactive networks characterized by cross-talk and homeostatic feedback loops that can greatly influence response to therapy. Fourth, cancer cells are characterized by genomic instability, which favors mutations. Cancer patients are treated with cytotoxic agents and radiotherapy, which accelerate the rate of mutagenesis, and they are then subject to immense selection pressures as we treat them with targeted and nontargeted agents. Thus, a broader view of the ecology of the cancer system is needed. The initial experience with EGFR/PI3K pathway targeted therapies has been informative with regard to each of these points.

### LESSON 1: CONTEXT-DEPENDENT ONCOGENE ADDICTION

Initial results with the EGFR tyrosine kinase inhibitors gefitinib and erlotinib suggested relatively low response rates, on the order of 10% to 15%.<sup>47,48</sup> This was difficult to reconcile with the perceived importance of the EGFR target, and two important studies have shed light on this problem. Work from our group demonstrated that expression of the constitutively active mutant EGFRvIII sensitized tumors to EGFR inhibitors *in vitro*, and also in patients on clinical trials, but only if the PTEN tumor suppressor protein was intact. In fact, loss of PTEN uncoupled the inhibition of EGFR from the inhibition of downstream PI3K signaling, demonstrating that PTEN loss was a critical factor in promoting up-front resistance to EGFR inhibitors.<sup>49</sup> Concurrently, Haas-Kogan et al.<sup>47</sup> demonstrated, *in vitro* and in glioma patients, that high levels of EGFR coupled with low levels of activated Akt (a critical effector of PI3K signaling) were associated with favorable response. These two studies demonstrated that intact regulation of PI3K signaling appears to be critical for effective response to EGFR, a finding that has also been shown in the human serially passaged xenograft system.<sup>28</sup> Furthermore, recent work by Fan et al.<sup>14</sup> clearly demonstrates that EGFR signaling through PI3K, particularly through mTOR, is critical for mediating the response to EGFR tyrosine kinase inhibitors.

These findings all teach a critical lesson, that there are many paths toward resistance, whereas a few critical mediators must be inhibited for sensitivity. Perhaps EGFRvIII and PTEN expression, can potentially be used as biomarkers to identify patients more likely to respond to EGFR inhibitors (a large prospective trial through the RTOG is ongoing to test this hypothesis). However, it is

likely that additional mechanisms of resistance will be present. It will be important to determine whether these resistance factors maintain constitutive PI3K signaling despite EGFR inhibition, to determine whether mTOR signaling will provide an accessible biomarker of response to EGFR kinase inhibitors.

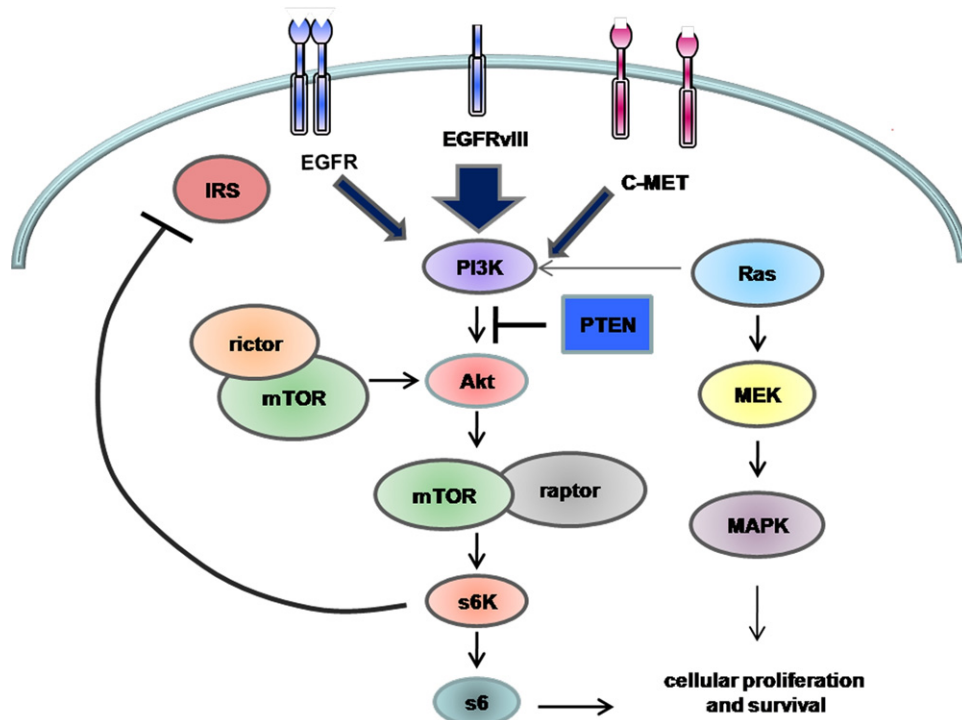
## LESSON 2: BIOMARKERS OF PATHWAY INHIBITION NEED TO BE DEVELOPED AND INCORPORATED INTO CLINICAL TRIAL DESIGN

The observation that successful inhibition of EGFR signaling is associated with suppression of mTOR activity suggested the possibility that dual EGFR/mTOR inhibition could be used to target EGFR-activated, PTEN-deficient tumors. A number of studies have demonstrated the efficacy of this approach in model systems.<sup>50,51</sup> However, early clinical trials with the mTOR complex inhibitor rapamycin and its derivatives did not show impressive clinical responses.<sup>48,52</sup> Our group completed and published a clinical trial of the mTOR inhibitor rapamycin in patients with relapsed, PTEN-deficient glioblastomas and identified key determinants of sensitivity and resistance.<sup>53</sup> We showed that: 1) the mTOR inhibitor rapamycin is present in potentially therapeutic levels in tumor tissue *in vivo*; that 2) rapamycin significantly inhibits mTOR signaling in all patients although the extent of inhibition is variable (from 10% to 80% pathway

inhibition) and that 3) the extent of pathway inhibition is critical. mTOR pathway inhibition of greater than 50% resulted in significantly inhibited proliferation; lower levels of mTOR inhibition did not translate into biological or clinical response. We further showed that this was not due to cell intrinsic resistance, but more likely was associated with failure of the drug to fully access its target *in vivo*.<sup>53</sup> These results, leveraging a novel biopsy-treat-biopsy paradigm, facilitated analysis of the effects of the inhibitor in patients leading to a critical lesson: target signaling may be insufficiently suppressed *in vivo*.

## LESSON 3: CROSS-TALK AND FEEDBACK LOOPS COMPLICATE TARGET SELECTION

mTOR is an unusually important integrator of multiple signaling cascades. It mediates signaling through the canonical PI3K pathway via two distinct complexes, mTORC1 (in which mTOR is paired with raptor within the protein complex) and mTORC2 (in which mTOR is paired with rictor within the protein complex) to mediate cell growth and proliferation. mTORC1 uniquely integrates growth factor signaling through S6K1 with cellular metabolism, underscoring its value as a cancer target.<sup>54</sup> mTORC1 signaling through S6K1 has recently been identified as a critical step in glial transformation.<sup>55</sup> mTORC2 regulates Akt signaling in a rapamycin-insensitive fashion, placing mTOR as both a downstream target and an upstream activator of Akt (FIG. 1).<sup>56</sup> Thus,



**FIG. 1.** The EGFR/PI3K/Akt/mTOR signaling pathway. The complexity of the signaling cascade is demonstrated by mTOR. Depending on its binding partner, raptor or rictor, the complex can be both a downstream target and an upstream activator of Akt.

mTOR is part of a complex, nonlinear canonical signaling network. Furthermore, S6K1, the key downstream effector of mTORC1, is known to regulate a homeostatic negative regulatory loop inhibiting PI3K signaling via IRS when mTORC1 activity is high.

Our group demonstrated that rapamycin treatment in glioblastoma patients results in S6K1 inhibition, which releases the negative feedback, leading to PI3K activation at the level of Akt and resulting in more rapid clinical progression.<sup>53</sup> Furthermore, recent studies from Pandolfi's group have shown that inhibition of mTOR signaling can activate the MAPK pathway,<sup>57</sup> highlighting the plasticity of the cancer circuitry in evading response to targeted monotherapy. It will be important to determine whether similar effects are seen in malignant glioma patients, and, if so, to develop combination approaches to suppress it.

#### LESSON 4: CANCER IS A MICROENVIRONMENT OF ADAPTATION, MUTATION, AND SELECTION

Some PTEN-intact malignant glioma patients relapse after enjoying a relatively short window of clinical response to EGFR kinase inhibitors. Recent work by DePinho's group sheds light on an alternative mechanism of acquired resistance. Notably, Stommel et al.<sup>58</sup> demonstrated that other receptor tyrosine kinases such as c-MET and PDGFR are commonly coactivated with EGFR and that when treated with EGFR inhibitors, c-MET and/or PDGFR engage PI3K to maintain downstream pathway activation despite EGFR inhibition. This suggests an noteworthy model in which nongenetic adaptations in the tumor result in resistance to treatment. Studies to assess this phenomenon in clinical samples from patients treated with EGFR inhibitors will be important for extending this model; however, these findings raise the concept that multiple inhibitors will be needed to suppress resistance. This is consistent with the lessons from pathway cross-talk and feedback described above. Thus, multiple lines of evidence are converging on the idea that successful targeted therapy will require carefully designed combinations of inhibitors with the objective of minimizing toxicity while maximizing benefit, based on an informed anticipation of mechanisms of resistance.

Borrowing again from lessons learned in treating CML patients with imatinib, the cancer community has recognized that rare secondary mutations in the kinase domain of BCR-ABL are selected for during imatinib treatment, leading to clinical resistance. A similar phenotype has been found in EGFR inhibitor-resistant lung cancer with detection of analogous mutations in the kinase domain of the receptor. It is not yet known whether such mutations are common in patients treated with

EGFR-inhibitors. If so, this strongly suggests the need to develop compounds that can inhibit such mutant kinases. Furthermore, it remains to be determined whether resistance is mediated by multiple mechanisms, including selection for kinase inhibitor-resistant mutations, and/or through adaptations such as pathway cross-talk or kinase switching. Understanding these escape routes will be essential for anticipating resistance and developing combination therapies to suppress it.

It is recognized that malignant gliomas are highly heterogeneous, and that selection for subpopulations of tumor cells, such as cancer stem cells,<sup>59-62</sup> may be critical for therapeutic resistance. PTEN loss in gliomas is heterogeneous. It will be important to determine whether acquired resistance to EGFR inhibitors can be mediated by selection against EGFR-activated, PTEN-intact tumor cells. New tools are needed to facilitate single-cell analysis of key mutations and pathways and that can be used to profile the dynamic changes within the tumor environment during treatment.

#### TARGETING MULTIPLE CORE PATHWAYS

This review has focused primarily on growth factor signaling pathways through the PI3K and MAPK signaling networks. Mutational landscape studies, however, as well as previous population-based targeted mutational analyses, highlight the co-occurrence of p53 pathway and Rb pathway mutations in EGFR/PI3K-activated tumors (highlighted, for example, by some of the mouse models).<sup>24-27</sup> Growth factor signaling has provided an attractive therapeutic target because it is easier to use small molecule inhibitors and antibodies to target a gain-of-function molecule. In contrast, p53 and RB1 loss are less amenable, although a variety of small molecules that modulate signaling through both of these pathways are under investigation.<sup>63-66</sup> Will it be necessary to concurrently target these pathways along with targeting the RTK/RAS/PI3K signaling network? Future studies will be needed. In addition, there may be efficacy in combining pathway-targeted therapies with other agents such as immunotherapies, radiotherapy, and chemotherapy, all of which are currently under investigation. Parsa et al.<sup>67</sup> showed that mTOR, the same signaling pathway that is critical for RTK/RAS/PI3K core pathway activation, may also be a key regulator of the immune response, suggesting a possible benefit from combining small molecule inhibitor and immunotherapeutic approaches.

#### SUMMARY: WHERE DO WE GO FROM HERE?

In this review, we have examined the current status of molecular targeted therapies for malignant gliomas, focusing primarily on lessons learned from targeting the

EGFR/PI3K/Akt/mTOR signaling pathway in patients. From studying patients in well-designed clinical trials, and integrating this knowledge with rigorous studies in model systems, including mouse models, we have learned: that 1) context-dependent oncogene addiction may determine clinical response; 2) we need to develop ways to monitor adequate target inhibition (particularly with noninvasive assays such as serum-based tests or molecular imaging), and inhibition of relevant downstream effectors, in order to understand the efficacy of targeted agents; 3) signal transduction networks are dynamic and interactive, and the cross-talk and feedback between different components of the signaling network may determine response to therapy; and 4) multiple mechanisms may contribute to acquired resistance, including adaptation, possibly additional mutation and selection.

These observations lay out a series of challenges. To improve the outcome of malignant glioma patients treated with targeted therapies, we will need to link targeted agents with molecular diagnostics that not only identify targets, but also anticipate the molecular circuitry of resistance to help guide more effective combination treatments. This will require a suite of new tools to facilitate multiparameter measurement of signaling networks in multiple cell types within the tumor microenvironment, such as between cancer cells and other cell types in the microenvironment (as, for example, vascular endothelial cells play a supportive role in maintaining the self-renewing phenotype of glioblastoma stem cells).<sup>68</sup> Based on our developing understanding of the communication networks within and between cancer cells, and between cancer cells and their partners in the tumor microenvironment, new combinations of targeted agents (or targeted agents in combination with immunotherapeutic or cytotoxic agents) can be designed to suppress resistance in small, well-designed clinical trials. The good news is that our experience has taught us how much can be learned from studying a small number of patients in great detail. The challenge for successfully treating gliomas with targeted agents is one of translating genomics into functional biology, and functional biology into better therapy for patients. The journey has begun.

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