Pathway inhibition: emerging molecular targets for treating glioblastoma

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Abstract

Insights into the molecular pathogenesis of glioblastoma have not yet resulted in relevant clinical improvement. With standard therapy, consisting of surgical resection with concomitant temozolomide in addition to radiotherapy followed by adjuvant temozolomide, median survival is 12–14 months. Therefore, the identification of novel molecular targets and inhibitory agents has become a focus of research for glioblastoma treatment. Recent results with bevacizumab may represent a proof of principle that treatment with targeted agents can result in clinical benefits for patients with glioblastoma. This review discusses limitations in the existing therapy for glioblastoma and provides an overview of current efforts to identify molecular targets using large-scale screening of glioblastoma cell lines and tumor samples. Preclinical and clinical data for several novel molecular targets including growth factor receptors, phosphatidylinositol-3 kinase, SRC-family kinases, integrins, and CD95 ligand and agents that inhibit these targets, including erlotinib, enzastaurin, dasatinib, sorafenib, cilengitide, AMG102, and APG101 are discussed. By combining advances in tumor screening with novel targeted therapies, it is hoped that new treatment options will emerge for this challenging tumor type.

Keywords

VEGF signaling, receptor tyrosine kinase, PI3 kinase, SRC-family kinases, integrins

Introduction

Glioblastoma is the most common primary central nervous system tumor, accounting for approximately 60% of 17,000 primary brain tumors diagnosed annually in the US.[1] Patients diagnosed with glioblastoma have a dismal prognosis, typically dying within 3 months if untreated. Standard treatment increases median survival to 12 months, although disease tends to progress within 6–9 months and the 2-year survival rate is less than 25%.[2] In this review, we discuss the limitations of existing therapies for glioblastoma, before summarizing ongoing efforts to identify novel molecular targets and develop novel targeted agents for this disease.

Search Strategy and Selection Criteria

Relevant publications in the PubMed database published between January 1995 and February 2010 were identified using the search terms “glioblastoma”, “glioma”, “VEGF”, “EGFR”, “PI3K”, “SRC”, “PDGFR”, “integrin”, “CD95”, “TRAIL”, and “c-MET”. Only papers published in English were reviewed. Relevant clinical trials were identified by searching http://www.clinicaltrials.gov/ using the search terms “glioblastoma” and “glioma”.

Current Glioblastoma Therapy

The current standard of care for newly diagnosed glioblastoma is surgical resection with concomitant daily temozolomide (TMZ; 75 mg/m^2) and radiotherapy, followed by six cycles of adjuvant TMZ (150–200 mg/m^2) for 5 days during each 28-day cycle.[3] However, almost all patients with glioblastoma experience disease recurrence. Because no standard treatment option exists following recurrence, rechallenging with TMZ or switching to an alternative TMZ dosing regimen has become common practice. In a retrospective analysis (*N* = 80), 6-month progression-free survival (PFS) was similar in patients with recurrent glioblastoma or anaplastic astrocytoma following TMZ regimen change (26%) or rechallenge (29%).[4] The Canadian RESCUE study showed similar results using a low-dose metronomic TMZ schedule, reporting a smaller benefit from rechallenge if prior TMZ exposure exceeded 6

months.[5] Ongoing studies are investigating alternative TMZ regimens in first-line and second-line settings, including the Neurooncology Working Group (NOA)-08, Radiation Therapy Oncology Group (RTOG) 0525, and DIRECTOR trials.

Targeted Therapy for Glioblastoma

The introduction of molecularly targeted agents is one of the most significant advances in cancer therapy in recent years. Targeted therapies block activation of oncogenic pathways, either at the ligand–receptor interaction level or by inhibiting downstream signal transduction pathways, thereby inhibiting growth and progression of cancer. Because of their specificity, targeted therapies should theoretically have better efficacy and safety profiles than systemic cytotoxic chemotherapy or radiotherapy.

Because of the substantial neovascularization seen in glioblastoma, targeted antiangiogenic therapies have received considerable attention.[6] The main rationale for using antiangiogenic therapies in glioblastoma is to normalize the vasculature, restoring the selective permeability of the blood-brain barrier (BBB), rather than starving tumors of oxygen and growth factors as originally proposed.[7] However, animal models of glioblastoma have shown that antiangiogenic therapies may reduce the effectiveness of TMZ.[8] The sequence of combination regimens and effects of specific antiangiogenic therapies on the BBB should be fully characterized to optimize therapy.

Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), has shown unusually high response rates in recurrent grade 3 and 4 gliomas (6-month PFS = 46%, 6-month overall survival (OS) = 77%),[9] which led to US approval for glioblastoma. Whether these response rates are a valid surrogate for PFS and OS remains a matter of debate.[10] Recently, the worry of more distant recurrences with bevacizumab treatment was not substantiated in a matched-pair analysis.[11]

Identifying Novel Therapeutic Targets in Glioblastoma

Identifying biological mechanisms contributing to glioblastoma oncogenesis will help researchers and physicians to develop and select appropriate targeted therapies to improve patient outcomes. In a large-scale multidimensional analysis carried out by the Cancer Genome Atlas in 206 glioblastoma samples, 91 of which were also analyzed to identify nucleotide sequence aberrations, the most frequent gene amplifications were: *epidermal growth factor receptor* (*EGFR)* and *platelet-derived growth factor receptor* (*PDGFR)α*, two transmembrane receptors with tyrosine kinase activity; *cyclin-dependent kinase 4* (*CDK4*), a promoter of cell cycle progression; and *murine double minute (MDM)2* and *MDM4*, suppressors of P53 activity.[12] The most frequent homozygous gene deletions were: *CDKN2A*, *CDKN2B,* and *CDKN2C*, which encode tumor suppressor proteins that suppress activation of CDK4 and CDK6; *phosphatase and tensin homolog* (*PTEN*), a tumor suppressor that inhibits phosphatidylinositol-3 kinase (PI3K) signaling; *retinoblastoma* (*RB1*), a cell cycle inhibitor; *PARK2*, a regulator of dopaminergic cell death; and *neurofibromin* (*NF)1*, a negative regulator of the RAS signal transduction pathway. The most frequently mutated genes were: *P53*; *PTEN*; *NF1*; *EGFR*; *human epidermal growth factor receptor 2* (*HER2*); *RB1*; and *PIK3R1* and *PIK3CA,* two components/regulators of the PI3K signaling pathway. This study shows that signaling pathways involving receptor tyrosine kinases/PI3K, regulators of the cell cycle such as P53 and the cyclin/RB1 pathway are considerably altered in glioblastoma (Fig. 1).

A similar study has identified characteristic mutations in the active site of *isocitrate dehydrogenase 1* (IDH1) in 12% of glioblastoma patients. *IDH1* mutations occurred in a high proportion of young patients and in the majority of secondary glioblastoma cases and were associated with increased OS (3.8 years) compared with wild-type *IDH1* (1.1 years).[13] This may be due to increased tumor sensitivity to chemotherapy,[14] although a large controlled series of the German Glioma Network did not find any association between prolonged survival of patients with tumors with *IDH1* mutations and administration of a specific therapy.[15] Mutation of the IDH1 active site prevents conversion of isocitrate to α-ketoglutarate but allows the mutated enzyme to catalyze the *nicotinamide dinucleotide*

phosphate-dependent reduction of α-ketoglutarate to R(-)-2-hydroxyglutarate (2HG). Accumulated 2HG appears to act as an oncometabolite that contributes to glioma formation and malignant progression. This observation is supported by data from patients with inherited 2-hydroxyglutaric aciduria in whom deficient 2HG dehydrogenase causes an accumulation of brain 2HG. These patients have an increased risk of developing brain tumors, possibly because of increased production of reactive oxygen species.[16]

Increased tyrosine kinase activity has also been associated with glioblastoma oncogenesis. In a tyrosine kinase activation catalog covering 130 human cancer cell lines, the most frequently activated tyrosine kinases were: EGFR; fibroblast growth factor receptor 3 (FGFR3); protein tyrosine kinase 2 (PTK2, also known as focal adhesion kinase, or FAK); and SRC, LCK, and LYN, three members of the SRC-family kinases (SFK).[17] SRC and SFKs mediate downstream signaling from several growth factor receptors and SRC is a key binding partner of FAK.[18] Screening of 31 primary glioblastoma samples showed similar patterns of tyrosine kinase activation, including SRC activation in 61% of the samples.[17] Overexpression of SFKs has been reported in previous studies,[19] although the Cancer Genome Atlas study did not identify any focal amplification or somatic missense mutations of *SRC* or SFKs.[12]

Studies have already been performed using novel agents that inhibit targets identified by screening methods discussed above, or based on preclinical studies and experience in other tumors (Table 1). However, further analyses of clinical and molecular data derived from these trials (Table 2)[20-44] are necessary to verify the relevance of these targets to glioblastoma.

Therapeutic Inhibition of Novel Molecular Targets in Glioblastoma

VEGF Signaling

Approval of the anti-VEGF antibody bevacizumab for glioblastoma has highlighted the potential for other antiangiogenic agents in glioblastoma therapy (Fig. 2). Cediranib is a potent, orally available, small-molecule inhibitor of VEGF-receptor (VEGFR) tyrosine kinase

activity that rapidly normalizes tumor blood vessels in patients with glioblastoma, leading to a clinical improvement in cerebral edema.[47] In mouse models, improvement in edema was associated with increased survival despite continued tumor growth.[48] The first clinical data of the REGAL trial of cediranib plus lomustine (CCNU) to investigate whether preclinical findings will translate into improvements for patients with recurrent glioma have been negative.[49] Six other clinical trials are underway to assess cediranib as either a monotherapy or in combination with other agents (Table 3).

Epidermal Growth Factor Receptor Family

Approximately 50% of glioblastomas overexpress EGFR and 25% express a constitutively active mutated form of EGFR.[50] EGFR overexpression and immunoreactivity are more common in primary tumors than in secondary glioblastomas.[51] These observations, in addition to the large body of preclinical data in glioblastoma[52] and successful targeting of EGFR in other tumors, make EGFR an attractive target for glioblastoma therapy. However, caution is needed with EGFR inhibitors because hypoxia and low glucose levels might convert the cytotoxic effects of EGFR inhibition into a cytoprotective effect.[53]

One agent that has been the subject of many clinical trials is erlotinib, an orally active inhibitor of the EGFR tyrosine kinase approved for treating some forms of nonsmall cell lung cancer (NSCLC) and pancreatic cancer. In a phase I study, patients with gliomas expressing high levels of EGFR and low levels of activated AKT had better responses to erlotinib (50% decrease in tumor cross-sectional area, than those with low EGFR expression and high levels of activated AKT.[54] However, phase II trials have so far shown limited clinical benefit of erlotinib in patients with either recurrent or newly diagnosed glioblastoma (Table 2), either in combination regimens[22, 23, 33, 34] or as monotherapy.[31] Studies to identify markers predicting response to EGFR inhibitors in patients with recurrent glioblastoma have shown significant correlation of response to therapy with coexpression of the PTEN tumor suppressor and the EGFR deletion mutant variant III (EGFRvIII) ($P < .001$; odds ratio, 51; 95% confidence

interval (CI), 4–669).[55] However, this has been suggested to be a prognostic phenomenon.[31] Ongoing clinical trials of erlotinib and other EGFR-directed drugs are summarized in Table 3.

Phosphatidylinositol-3 Kinase and Related Pathways

PI3K plays a role in intracellular signaling pathways regulating cell survival, growth, and proliferation. Activated PI3K is recruited to the cell membrane where it mediates signaling following receptor activation. Downstream signaling proteins include: AKT, a promoter of growth, proliferation, and survival; glycogen synthase kinase-3 (GSK-3), a regulator of c-MYC and cyclin degradation; and mammalian target of rapamycin (mTOR), a regulator of protein synthesis and negative regulator of PI3K.[56]

Regulators of PI3K signaling are frequently mutated in glioblastomas and preclinical studies suggest that inhibiting the PI3K pathway may have therapeutic potential.[12] NVP-BEZ235, an orally available kinase inhibitor of PDK1, mTOR, and PI3K, induced G1 arrest of a glioblastoma cell line in vitro, and enhanced TMZ efficacy in vivo.[57] Glioblastoma cells treated with LY294002, a specific PI3K inhibitor, became sensitized to chemotherapy-induced apoptosis.[58] These preclinical studies suggest that PI3K inhibitors have the potential to overcome TMZ resistance in recurrent glioblastoma. NVP-BEZ235 treatment is currently in phase I trials in patients with solid tumors (Table 3).

Enzastaurin, a PKC/PI3K/AKT inhibitor, suppressed proliferation and induced apoptosis via a caspase-dependent mechanism in glioblastoma cells in vitro[59] and inhibited growth of human glioblastoma xenografts, accompanied by decreased phosphorylation of downstream signaling molecules, including GSK-3β.[60] *In vivo* models showed that enzastaurin combined with radiotherapy synergistically reduced tumor volume, radiation-induced satellite tumor formation, upregulation of VEGF expression, neovascularization, and GSK-3β phosphorylation.[61] In a phase II study of enzastaurin in patients with recurrent heavily pretreated glioblastoma, an interim analysis showed that objective radiographic responses occurred in approximately 20% of patients.[62] The subsequent phase III trial comparing lomustine and enzastaurin at first or second recurrence was the first phase III trial

to evaluate a targeted therapy for recurrent glioblastoma. However, a planned interim analysis found that enzastaurin treatment did not significantly increase PFS, leading to enrolment being halted. The final analysis confirmed the absence of any significant difference across all efficacy endpoints (Table 2).[29]

Whilst ineffective in glioblastoma, enzastaurin monotherapy appears to have poor tolerability (thrombocytopenia and prolonged QTc as dose-limiting toxicities) and limited efficacy in patients with malignant glioma as shown by over 150 weeks of PFS in 2 patients.[63] In a phase I/II trial, enzastaurin had limited efficacy in patients with anaplastic glioma (6-month PFS = 16%) and negligible efficacy in patients with glioblastoma (6-month PFS = 7%).[28]

SRC and SRC-family Kinases

SRC and SFKs are frequently activated in glioblastoma cell lines and patient samples,[17] and SFK overexpression has also been reported,[19] although not in the Cancer Genome Atlas study.[12] SRC and SFKs are promiscuous regulators of multiple signaling pathways regulating cell growth, proliferation, adhesion, migration, and invasion, which are important processes in tumor invasion and metastasis. In particular, SFKs mediate signaling from growth factor receptors commonly overexpressed in glioblastomas, providing a potential mechanism for SFK activation. Recently, SRC and FYN (a SFK) were shown to mediate oncogenic EGFR and EGFRvIII signaling in a rodent glioblastoma model.[19] SRC inhibition also reduced glioblastoma cell viability and migration in vitro and decreased growth in vivo.[17] Transgenic mice expressing v-SRC, a viral oncogenic homolog of cellular SRC, develop brain tumors that rapidly progress to mimic the morphological and molecular characteristics of human glioblastoma, providing additional strong evidence that SFKs may be a promising target for glioblastoma therapy.[64]

Dasatinib is a potent inhibitor of SRC and SFK tyrosine kinase activity and is approved for treatment of certain types of leukemia based on activity against BCR-ABL.[65] Dasatinib also has inhibitory activity against c-KIT and PDGFR.[66] In glioblastoma cells,

dasatinib inhibited migration and induced autophagic cell death, and autophagy was increased by combining dasatinib with TMZ.[19, 67] In vivo, dasatinib inhibited invasion, promoted tumor regression, and induced apoptosis in EGFRvIII-expressing glioblastomas, and enhanced the activity of anti-EGFR antibodies.[19]

Trials of dasatinib are ongoing in several solid tumors, including glioblastoma (Table 3). A phase I/II trial in patients with newly diagnosed glioblastoma is assessing dasatinib combined with radiotherapy and concomitant TMZ followed by adjuvant dasatinib plus TMZ. Trials of dasatinib in recurrent glioblastoma include a phase II trial of dasatinib monotherapy, a phase I trial in combination with erlotinib, and a randomized phase I/II trial in combination with CCNU that has started its phase I part in patients with recurrent glioblastoma as part of an EORTC initiative (Table 3).

Platelet-derived Growth Factor Receptor

PDGFR is a receptor tyrosine kinase with α and β isoforms. Overexpression of *PDGFR-*α has been demonstrated in all grades of astrocytoma, including in one in six glioblastomas,[49] indicating a potential role in tumor development.[68] Several PDGFR-targeting agents have been developed that may have therapeutic potential against tumors with elevated PDGFR expression.

Sorafenib is an orally available antiangiogenic agent that inhibits tumor cell growth and proliferation by blocking the action of intracellular and receptor kinases including PDGFR, RAF kinase, VEGFR2, and c-KIT.[69] In human glioblastoma cell lines, sorafenib inhibited proliferation synergistically in combination with bortezomib, a proteosome inhibitor,[70] and rottlerin, an experimental inhibitor of protein kinase C.[71] A phase II trial found that first-line TMZ and radiotherapy followed by TMZ plus sorafenib was tolerated by patients with glioblastoma, although preliminary efficacy data for this regimen (median PFS = 6 months, 12-month PFS = 16%) were similar to standard therapy (Table 2).[24] Clinical trials of sorafenib are summarized in Tables 2 and 3.

Preclinical trials of imatinib, a small-molecule inhibitor of PDGFR, ABL, and c-KIT, have shown growth inhibition in a subpopulation of CXCL12-expressing glioblastoma cells[72] and radiosensitizing activity.[73] However, in phase II trials in recurrent glioblastoma, imatinib alone or combined with hydroxyurea had limited antitumor activity (Table 2).[37-41] The combination of imatinib, hydroxyurea, and vatalanib, a VEGFR inhibitor, was well tolerated in a phase I trial and has been suggested as a possible multitargeted regimen for glioblastoma.[36] Ongoing trials include a trial of imatinib and TMZ in patients with either newly diagnosed or recurrent glioblastoma and six trials in recurrent glioblastoma of imatinib monotherapy, or combined with TMZ or hydroxyurea (Table 3).

Tandutinib is an orally active inhibitor of PDGFR, FLT3, and c-KIT tyrosine kinase activity. Although no preclinical data have been reported for tandutinib in glioblastoma, two early-phase trials are assessing tandutinib in recurrent or progressive glioblastoma as monotherapy or combined with bevacizumab (Table 3).

Although gene expression and preclinical data suggest that PDGFR may be a promising target for treating glioblastoma, the available clinical data suggest otherwise. Trial data are awaited from novel combination regimens involving PDGFR inhibitors.

Integrins

Integrins play key roles regulating cellular adhesion, migration, and invasion. In addition to a structural role, integrins also activate intracellular signaling proteins, including SRC. In various tumors, integrins have an established role in metastasis, and angiogenesis.[74] Therefore, targeting integrin function may have potential for treating glioblastoma.

Cilengitide is a specific α_V integrin inhibitor in clinical development. In vitro, cilengitide blocked glioma cell adhesion without effecting tumor radiosensitivity, despite increasing radiation-induced vascular endothelial cell death. However, cilengitide combined with radiotherapy in vivo more than doubled survival time to over 110 days compared with radiotherapy alone (50 days survival).[75] A second study showed inconsistent effects of

cilengitide on cell migration or invasiveness across several glioma cell lines, although additive effects were observed for cilengitide combined with TMZ.[76]

Cilengitide has been assessed in clinical trials (Table 2). In a phase I/IIa trial, cilengitide combined with the current standard of therapy in patients with newly diagnosed glioblastoma was well tolerated with an encouraging 6-month PFS of 69%. Tumor *O6-methylguanine-DNA methyltransferase (MGMT)* promoter methylation predicted a higher likelihood of achieving 6-month PFS as shown by increases in PFS and OS to 13.4 months and 23.2 months, respectively, compared with 3.4 and 13.1 months in patients without MGMT promoter methylation.[21] Based on these findings, a similar regimen is being compared with radiotherapy/TMZ alone in the phase III CENTRIC trial in patients with newly diagnosed glioblastoma whose tumors have a hypermethylated *MGMT* promoter (Table 3). In a phase IIa study in recurrent glioblastoma, cilengitide monotherapy was well tolerated but was largely inactive (6-month PFS = 15%): long-term disease stabilization was seen in a small subset of patients, 10% were progression-free for greater than 12 months and 5% were progression-free for greater than 24 months.[27]

A recent preclinical study has suggested that integrin inhibitors may paradoxically stimulate tumor growth and angiogenesis when doses are missed.[77] However, because of the artificial dosing schedule and nonglioma models used for preclinical investigations, this may not represent an issue for ongoing trials in glioblastoma.[78]

c-MET Inhibitors

Aberrant signaling by the MET receptors and its ligand, hepatocyte growth factor (HGF), has been observed in various tumors, including glioblastoma, and potential involvement in tumorigenesis and metastasis has been reported.[79] In a recent study, c-MET overexpression was detected in 18/62 glioblastoma samples (29%) and patients with c-MET overexpression had shorter median survival than those with little or no c-MET expression (median survival 11.7 months vs 14.3 months).[80]

Inhibitors of HGF or c-MET have shown preclinical activity against glioblastoma cell lines.[79] The anti-HGF antibody AMG102 enhanced TMZ-induced inhibition of glioblastoma cell line growth in vitro and in xenografts[81] and in an ongoing phase II trial in patients with recurrent glioblastoma AMG102 was well tolerated with initial evidence of response seen in a small proportion of patients (Table 2).[26] PF02341066, an orally available ATP-competitive small-molecule inhibitor of c-MET that inhibited glioblastoma growth and cMET phosphorylation in preclinical studies,[82] is under clinical investigation in patients with advanced cancers.

Glutamate Receptor Inhibition

Alpha-amino-3-hydroxy-5 methyl-4-isoxazolepropionate (AMPA) glutamate receptor antagonists have been used to prevent neurotoxicity in several nontumor neurologic disorders. Because glioblastomas secrete glutamate and preclinical evidence suggests a role of the glutamate/AMPA system in proliferation and migration, talampanel, an orally available BBB-permeable AMPA inhibitor, has been assessed in clinical trials. Initial phase I/II data for first-line talampanel combined with standard of care have suggested improved efficacy compared with recent historic controls demonstrated a median OS of 18.3 months (95% CI, 14.6–22.5 months).[25] However, a phase II trial of talampanel monotherapy in patients with recurrent disease found no significant activity (6-month PFS was 4.6%, median PFS was 5.9 weeks, median OS was 13 weeks) (Table 2).[44]

HDAC inhibition

Histone deacetylases (HDAC) are involved in multiple processes shaping the malignant phenotype of glioma including maintanance of stemness, angiogenesis and resistance to DNA damage. Vorinostat (Zolinza™), an orally available inhibitor of class I and II HDAC approved for advance cutaneous T cell lymphoma. In a phase II study in recurrent glioblastoma vorinostat monotherapy was well tolerated and had modest clinical activity (6-

month PFS was 15.2%, median OS was 5.7 months.[45] Vorinostat is currently being evaluated in newly diagnosed and recurrent glioblastoma as a combination therapy.

Death-receptor targeting has been an experimental approach for malignant glioma for more than a decade.[83] Death-receptor ligand activation can also have nonapoptotic effects, as demonstrated using anti-***CD95*** antibody treatment of mouse glioblastoma models.[84] APG101 is an inhibitor of CD95 ligand consisting of the CD95 receptor extracellular domain fused to the Fc domain of IgG. A randomized phase II trial of APG101 plus radiotherapy vs radiotherapy has recently been initiated in patients with recurrent glioblastoma. Future research will determine whether inducing apoptosis or relying on the nonapoptotic properties of death ligands will be advantageous for glioblastoma treatment.

Poly [ADP-ribose] polymerase (***PARP***) is a DNA repair enzyme implicated in the resistance of tumors to DNA damaging anticancer agents and radiotherapy.[85] Iniparib (BSI-201), which has recently demonstrated clinical efficacy in triple-negative breast cancer[86], is currently being explored in a phase I/II study in patients with newly diagnosed glioblastoma.

Discussion

Targeted therapies have revolutionized oncology, causing a shift from systemic and/or slow-release implants of cytotoxic drugs towards highly specific agents that are more selective at targeting tumor cells. Clinical studies with EGFR and PDGFR inhibitors as monotherapy, however, have so far failed to show any efficacy in glioblastoma. New data indicate that subtypes of glioblastoma exist with distinct molecular characteristics, suggesting that to fully evaluate targeted agents, patient selection based on tumor subtype may be needed. Because of the progressive nature of glioblastoma and the accumulation of genomic and proteomic changes, it is also possible that a recurrent tumor may have different characteristics from the primary tumor, suggesting that tumors should be rebiopsied at recurrence to ensure that an appropriate therapy is selected. Translation of promising

preclinical data into a clinically useful therapy remains challenging, with data frequently generating new questions or hypotheses that need to be addressed in the laboratory.

Targeted agents are likely to have greatest potential when used in combination to increase the activity of standard chemotherapies, broadening the range of pathways inhibited by treatment, and/or counteracting mechanisms of resistance. In addition, as for classic cytotoxic agents, an intact blood-brain-barrier may represent an important impediment limiting the efficacy of targeted therapies. Numerous trials are ongoing to investigate combinations of targeted therapies with other agents, potentially accompanied by novel methods of patient monitoring or assessment based on the mechanism of action, allowing for more individualized patient therapy. Dialog between preclinical and clinical research will allow us to address the questions or hypotheses arising from the use of novel therapies, leading to fine-tuning of clinical trial regimens and a better understanding of which patients may benefit from a particular therapy. Coupled with advances in tumor screening and outcome assessment, this will hopefully result in new treatment options and meaningful patient benefits.

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Figure legends

Fig. 1. Genetic alterations in glioblastoma signal transduction pathways (adapted from Parsons et al.)[13] (A) Proliferation and survival signaling is altered in 88% of glioblastomas. (B) p53 signaling is altered in 87% of glioblastomas. (C) RB signaling is altered in 78% of glioblastomas.

CCND2, cyclin-D2; CDK, cyclin-dependent kinase; CDKN, cyclin-dependent kinase inhibitor; EGFR, epidermal growth factor receptor; FOXO, forkhead box-O; HER2, human epidermal growth factor receptor-2; NF1, neurofibromin; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; RB1, retinoblastoma protein-1; SRC* = activated (phosphorylated) SRC.

Fig. 1 is adapted from The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008;455(7216):1061–1068.

Fig. 2. Molecular targets of antiangiogenic therapies investigated in glioblastoma.

ANG, angiopoietin; CKII, casein kinase II; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinases; FAK, focal adhesion kinase; GSK3β, glycogen synthase kinase 3β; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; PDGF(R), platelet-derived growth factor (receptor); PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLCγ, phopholipase Cγ; VEGF(R), vascular endothelial growth factor (receptor).

Table 1. Targeted therapies in clinical trials for glioblastoma

Agent	Target molecules	Approved cancer indications	Combination treatments under investigation
APG101	CD95	None	RT
AMG102	c-MET	None	Monotherapy
Cetuximab	EGFR	CRC, HNSCC	TMZ+RT
Erlotinib	EGFR	NSCLC, pancreatic	TMZ+RT CCNU Carboplatin Sorafenib Sirolimus Temsirolimus Bevacizumab
Gefitinib	EGFR	NSCLC	Monotherapy
BIBW2992	EGFR, HER2	None	Monotherapy TMZ TMZ+RT
Lapatinib	EGFR, HER2	MBC	Monotherapy Pazopanib
Cilengitide	α_V integrins	None	TMZ+RT
Imatinib	PDGFR	CML, Ph+ ALL, KIT+GIST	Vatalanib+hydroxyurea TMZ Hydroxyurea
Tandutinib	PDGFR	None	Monotherapy Bevacizumab

NVP-BKM120	PI3K	None	Monotherapy
Enzastaurin	PKC/PI3K/AKT	None	Monotherapy RT TMZ+RT
Dasatinib	SRC	CML, Ph+ ALL	Monotherapy TMZ TMZ+RT CCNU Erlotinib
Bevacizumab	VEGF	CRC, MBC, GBM, RCC, NSCLC	Cetuximab+irinotecan
Cediranib	VEGFR	None	Monotherapy TMZ CCNU Bevacizumab Cilengitide
Vandetanib	VEGFR, EGFR	None	Monotherapy TMZ Carboplatin Imatinib Sirolimus Etoposide
Vorisnostat	HDAC I/II	T cell lymphoma	Monotherapy TMZ Bevacizumab Irinotecan

			Bortezomib
Sorafenib	VEGFR, PDGFR, MAPK	RCC, HCC	Monotherapy TMZ+RT TMZ Temsirolimus Erlotinib Bevacizumab

CCNU, lomustine; CML, chronic myeloid leukemia; CRC, colorectal carcinoma; EGFR, epidermal growth factor receptor; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; HNSCC, head and neck squamous cell carcinoma; MAPK, mitogen-activated protein kinase signaling; MBC, metastatic breast cancer; NSCLC, nonsmall cell lung carcinoma; PDGFR, platelet-derived growth factor receptor; Ph+ ALL, Philadelphia chromosome-positive acute lymphoblastic leukemia; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; RCC, renal cell carcinoma; RT, radiotherapy; TMZ, temozolomide; VEGFR, vascular endothelial growth factor (receptor).

Table 2. Published clinical data for targeted therapies in glioblastoma

Agents	Phase	Primary/recurrent disease	Patients (*n*)	Primary outcomes	Positive prognostic indicator(s)	Reference
Cetuximab+TMZ+RT	I/II	Primary	39	12-month OS = 89%, 24-month OS = 42%, 6-month PFS = 76%, 12-month PFS = 45%	EGFR and PTEN coexpression significantly correlated with PFS (*P* = .005)	[20]
Cilengitide+TMZ+RT	I/IIa	Primary	52	6-month PFS = 69%	MGMT promoter methylation	[21]
Erlotinib+TMZ+RT	I/II	Primary	97	OS = 15.3 months median PFS = 7.2 months	None	[22]
Erlotinib+TMZ+RT	II	Primary	65	Median OS = 19.3 months median PFS = 8.2 months	MGMT promoter methylation and PTEN+ (*P* = .04)	[23]

RT+TMZ→ TMZ+sorafenib	II	Primary	47	Median PFS = 6 months (95% CI 3.7 to 7.0 months) 12-month PFS = 16%	NA	[24]
Talampanel	II	Primary	72	OS = 18.3 months (median)	NR	[25]
AMG102	II	Recurrent	20	Response rate by Macdonald criteria: one cPR, two SD, 14 PD, one minor response	NA	[26]
Cediranib +/- CCNU	III	Recurrent	300	PFS Cediranib 30 mg = 3 months Cediranib 20 mg + CCNU = 4 months CCNU = 2.7 months	NA	[47]
Cilengitide	IIa	Recurrent	81	6-month PFS = 16%	NA	[27]
Enzastaurin	II	Recurrent	85	Objective radiographic responses in 14 patients (10 GBM), including one CR 6-month PFS = 7%	NA	[28]

Enzastaurin	III	Recurrent	266	No significant effect on PFS (1.5 vs 1.6 months), OS (6.6 vs 7.1 months), 6-month PFS (*P* = .13), SD (38.5 vs 35.9%), or OR (2.9 vs 4.3%) respectively for enzastaurin vs lomustine	NA	[29]
Erlotinib+ temsirolimus	I/II	Recurrent	22 (phI) 56 (phII)	6-month PFS = 12.5%	NR	[30]
Erlotinib+ TMZ/CCNU	II	Recurrent	110	6-month PFS = 11.4%	Low phospho-AKT (*P* = .068)	[31]
Erlotinib+ bevacizumab	II	Recurrent	24 (MG) 32 (AG)	6-month PFS = 25%	NR	[32]
Erlotinib+ carboplatin	II	Recurrent	43	Median PFS = 9 weeks, 6-month PFS = 14%	None	[33]
Erlotinib+sirolimus	II	Recurrent	32	6-month PFS = 3.1%	Increased phospho-AKT (*P* = .045)	[34]
Gefitinib	II	Recurrent	28	6-month PFS = 14.3%	None	[35]

Imatinib+vatalanib+ hydroxyurea	I	Recurrent	37	Vatalanib MTD = 1000 mg BID DLTs = hematologic, GI, renal, and hepatic 6-month PFS = 25%	NA	[36]
Imatinib	II	Recurrent	39	6-month PFS = 24%	NA	[37]
Imatinib	II	Recurrent	50/55	MTD = 800 mg/day two PR, six SD (GBM) zero PR, five SD (AG) 6-month PFS = 3% (GBM), 10% (AG)	NA	[38]
Imatinib	II	Recurrent	112	PR = five (three GBM) 6-month PFS rate = 16% (95% CI, 8.0% to 34.0%) in GBM	NA	[39]
Imatinib	II	Recurrent	231	Radiographic response rate = 3.4% 6-month PFS = 10.6%	NA	[40]
Hydroxyurea +/- Imatinib	III	Recurrent	240	Median PFS = 6 weeks (both arms) 6-month PFS = 7% (combination)	NA	[41]

Lapatinib	I/II	Recurrent	7 (phI) 17 (phII)	DLT = none, efficacy (SD = four, PD = 13)	None	[42]
Sorafenib+erlotinib	I/II	Recurrent	17 (phI) 19 (phII)	6-month PFS = 16%	NR	[43]
Talampanel	II	Recurrent	30 (22 GBM)	6-month PFS = 4.6%	NA	[44]
Vorinostat	II	Recurrent	66	6-month PFS = 15.2% OS 5.7 months	NA	[45]
Temsirolimus	II	Recurrent	65	6-month PFS = 7.8% OS 5.7 months	p70S6 kinase	[46]

AG, anaplastic glioma; BID, twice daily; CCNU, lomustine; CI, confidence interval; cPR, confirmed partial response; CR,= complete response; GI, gastrointestinal; DLT, dose-limiting toxicities; EGFR, epidermal growth factor receptor; GBM, glioblastoma multiforme; MG, malignant glioma; MGMT, methyl guanine methyltransferase; MTD, maximum tolerated dose; NA, not available; NR, not recorded; OR, objective response; OS, overall survival; ph, phase; PD, progressive disease; PFS, progression-free survival; PR, partial response; PTEN, phosphatase and tensin homolog; RT, radiotherapy; SD, stable disease; TMZ, temozolomide.

Table 3. Ongoing clinical trials of targeted therapies

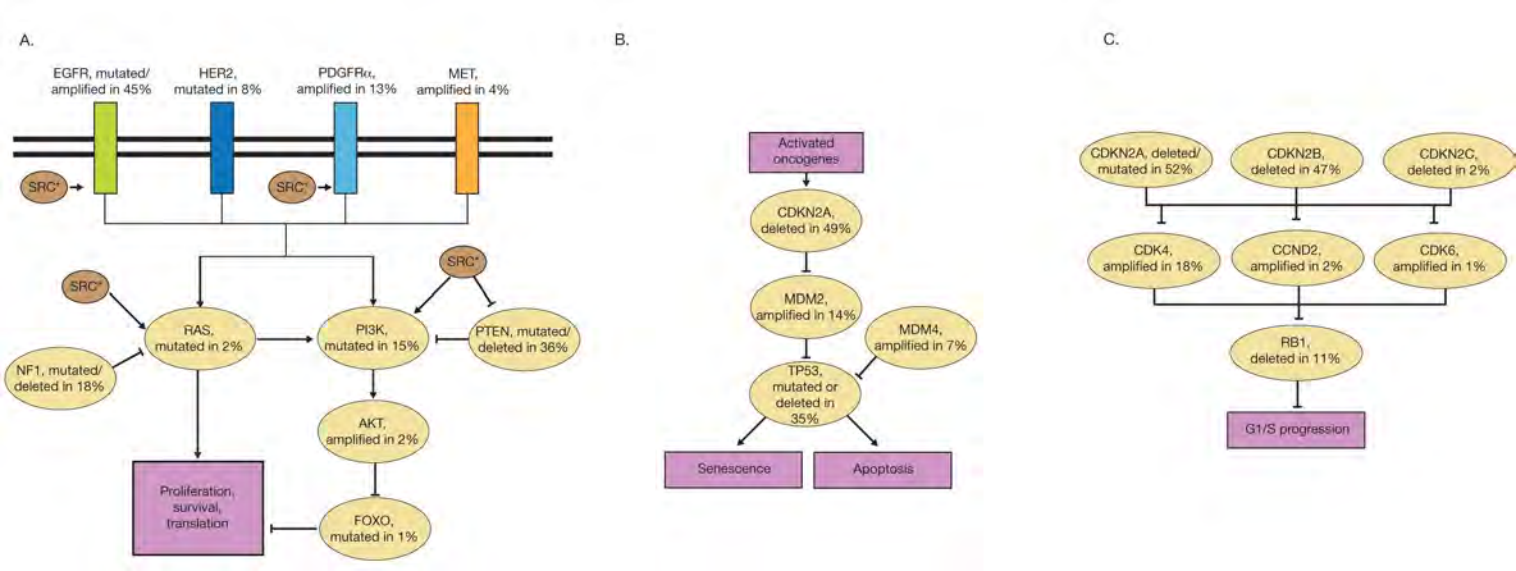
Treatment	Phase	Primary/recurrent disease	Patients (*n*)	Primary outcomes	Trial identifier
APG101+RT	II	Recurrent	83	6-month PFS	NCT01071837
BIBW2992+RT+TMZ	I	Primary	38	NA	NCT00977431
BIBW2992+TMZ	I/II	Recurrent	140	NA	NCT00727506
BIBW2992	II	Recurrent	60	NA	NCT00875433
BSI-201	I/II	Primary	100	Safety, OS	NCT00687765
Bevacizumab+Dasatinib	I/II	Recurrent	183	Safety, PFS, OS	NCT00892177
Bevacizumab+TMZ+RT vs TMZ+RT	III	Primary	942	PFS, OS	NCT00884741
Bevacizumab+TMZ+RT vs TMZ+RT	III	Primary	920	PFS, OS	NCT00943826
Cediranib+TMZ	I/II	Primary	80	Safety, PFS	NCT00662506
Cediranib	I	Any	55	MTD, DLT	NCT00326664
Cediranib+bevacizumab	I	Recurrent	51	MTD, PK, toxicity	NCT00458731
Cediranib+cilengitide	I	Recurrent	52	Safety	NCT00979862
Cetuximab+bevacizumab+irinotecan	II	Recurrent	32	NA	NCT00463073

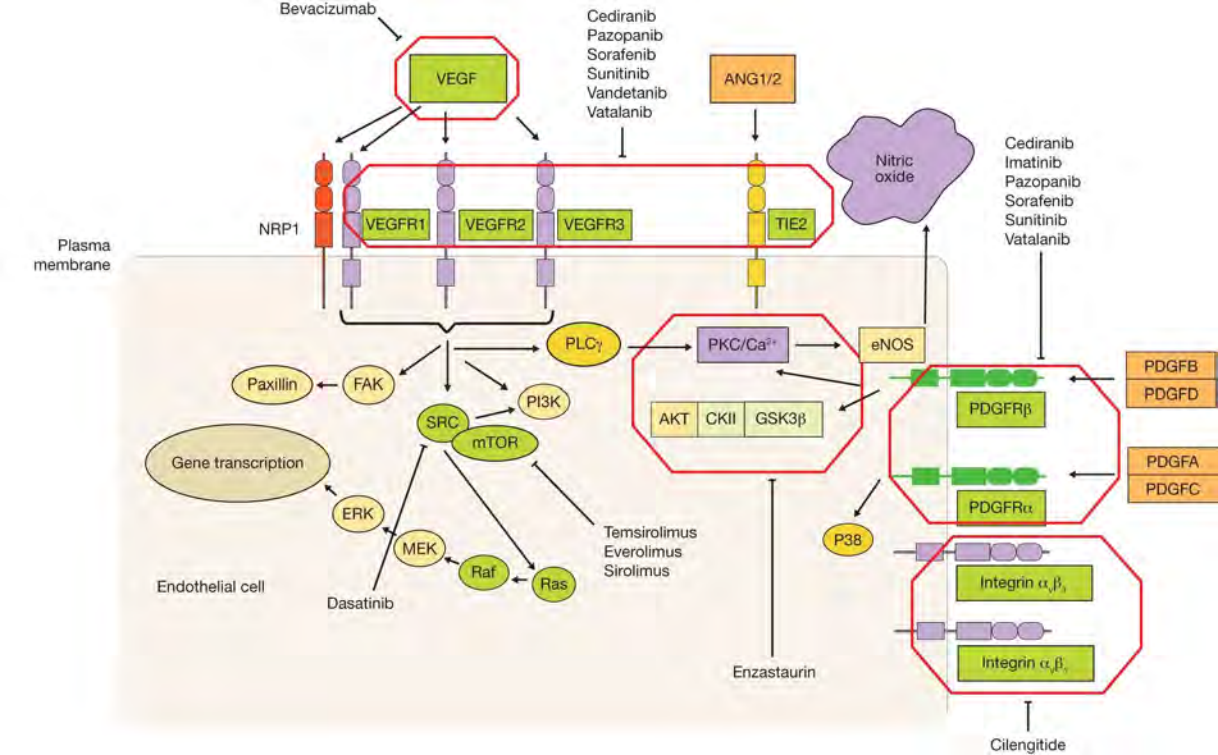
Cilengitide+TMZ+RT vs TMZ+RT	II	Primary	177	PFS	NCT01062425
Cilengitide+TMZ+RT vs TMZ+RT	III	Primary	504	OS	NCT00689221
Dasatinib+TMZ+RT vs TMZ+RT	I/II	Primary	217	Safety/OS	NCT00869401
Dasatinib+TMZ+RT	I/II	Primary	72	MTD/OS	NCT00895960
Dasatinib+erlotinib	I	Recurrent	48	MTD, DLT	NCT00609999
Dasatinib+CCNU	I/II	Recurrent	108	Safety/PFS	NCT00948389
Dasatinib	II	Recurrent	113	6-month PFS	NCT00423735
Erlotinib+TMZ+RT	II	Primary	30	6-month PFS	NCT00274833
Erlotinib+sirolimus	I/II	Recurrent	99	NA	NCT00509431
Erlotinib+sirolimus	II	Recurrent	20	6-month PFS	NCT00672243
Erlotinib+sorafenib	II	Recurrent	56	OS	NCT00445588
Everolimus+TMZ+RT	I/II	Primary	108	MTD/OS	NCT00553150
Imatinib+TMZ	I	Any	40	Safety, PK, antitumor activity	NCT00354068
Imatinib+TMZ	I	Recurrent	40	Safety, PK, antitumor activity	NCT00354068
Imatinib+hydroxyurea	I	Recurrent	48	MTD, DLT	NCT00613054

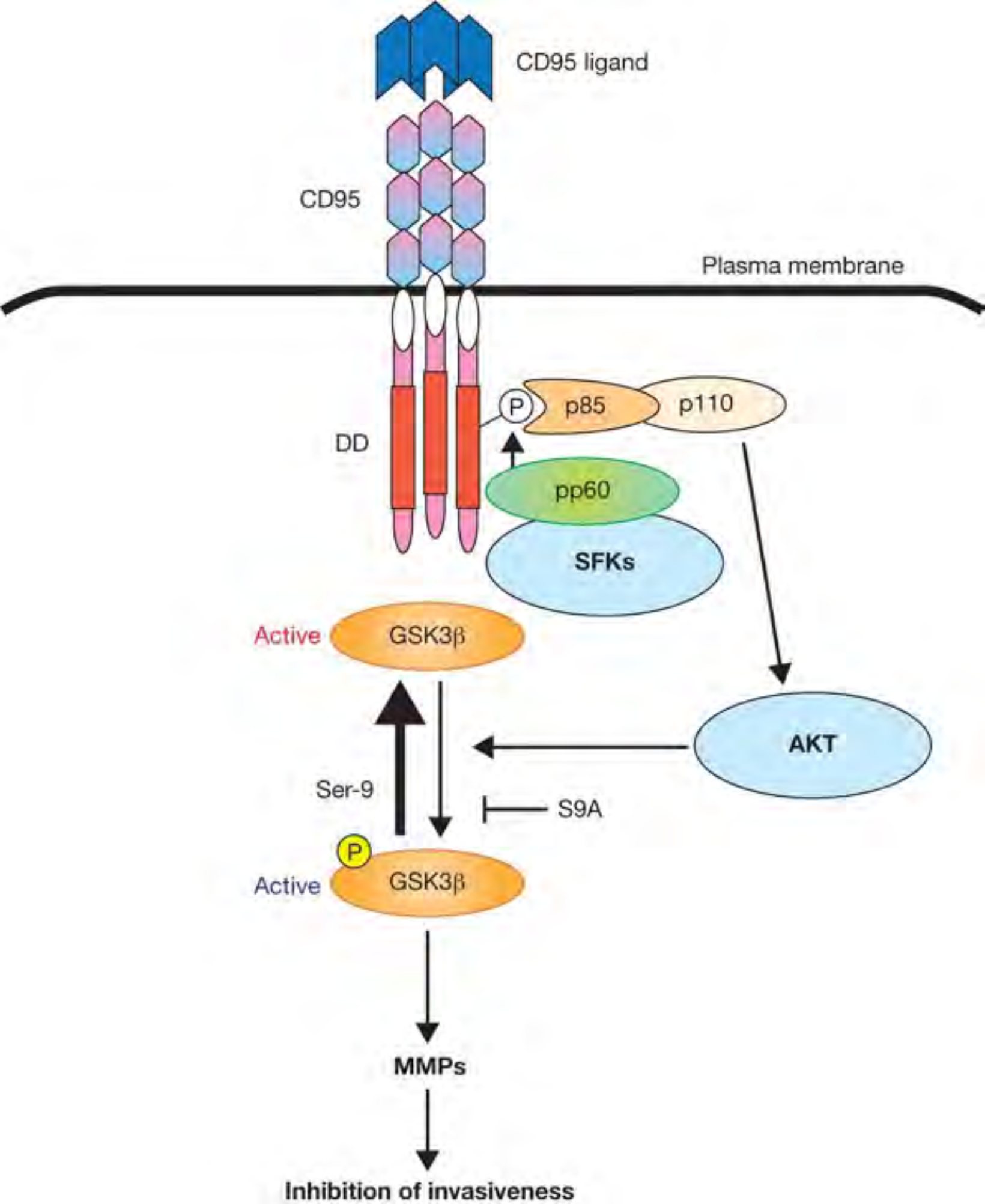
Imatinib+hydroxyurea	I	Recurrent	72	MTD, DLT	NCT00613132
Imatinib	II	Recurrent	77	6-month PFS	NCT00039364
Imatinib+hydroxyurea	II	Recurrent	21	6-month PFS	NCT00611234
Imatinib+hydroxyurea	II	Recurrent	64	12-month PFS	NCT00615927
Lapatinib+pazopanib	I/II	Recurrent	105	NA	NCT00350727
Lapatinib	II	Recurrent	44	NA	NCT00103129
Sorafenib	I	Primary	18	NA	NCT00884416
Sorafenib+RT+TMZ	I/II	Primary	51	NA	NCT00734526
TMZ→TMZ+RT+sorafenib	II	Primary	46	NA	NCT00544817
Sorafenib	I	Recurrent	36	NA	NCT00093613
Sorafenib+temsirolimus	I/II	Recurrent	141	NA	NCT00329719
Sorafenib	II	Recurrent	32	NA	NCT00597493
Sorafenib+bevacizumab	II	Recurrent	53	NA	NCT00621686
Tandutinib	I/II	Any	85	MTD, safety, response	NCT00379080
Tandutinib+bevacizumab	II	Any	80	6-month PFS	NCT00667394
Vandetanib+TMZ	I/II	Primary	114	NA	NCT00441142
Vandetanib+etoposide	I	Recurrent	48	NA	NCT00613223

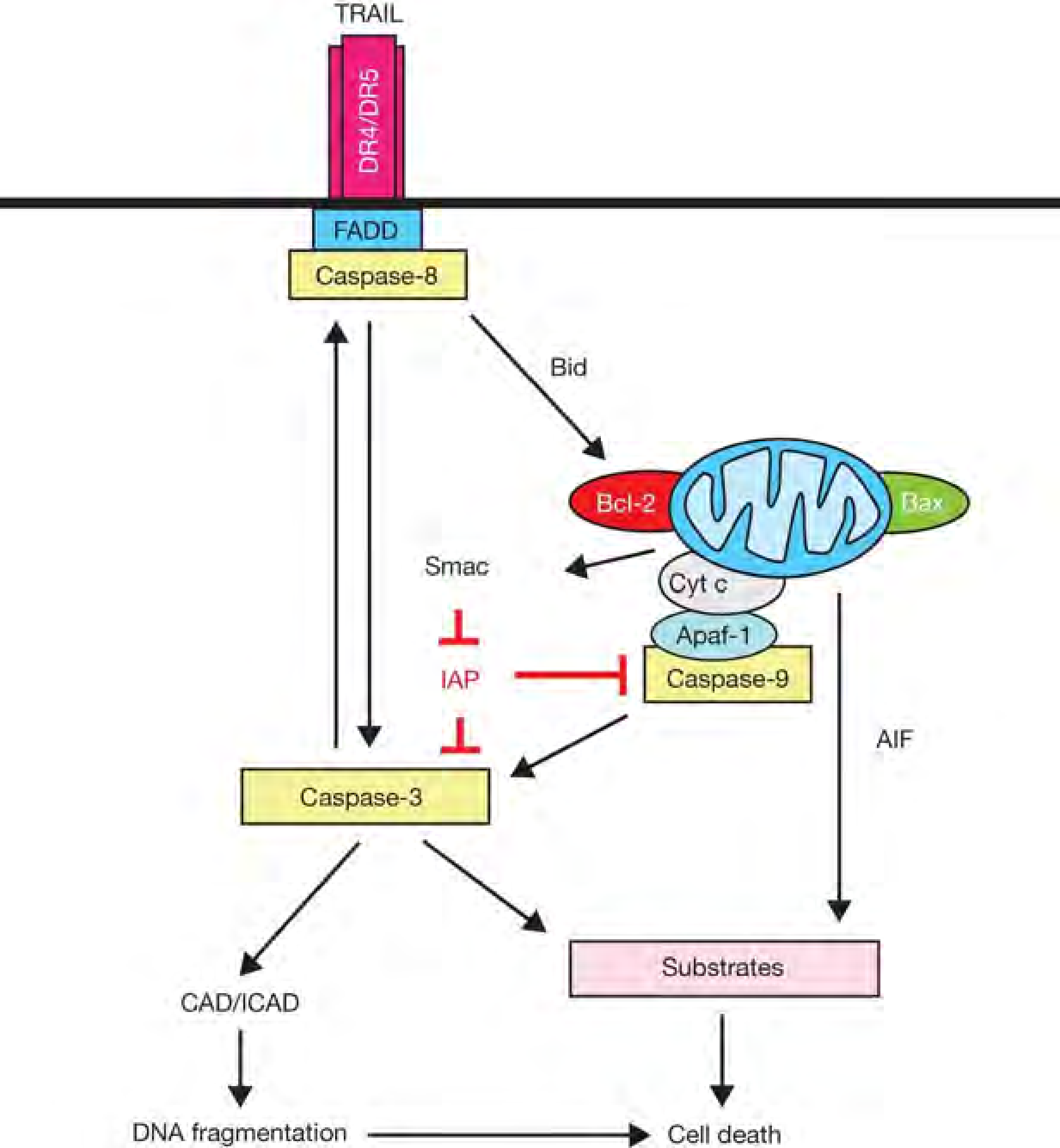
Vandetanib+imatinib+hydroxyurea	I	Recurrent	48	NA	NCT00613054
Vandetanib+sirolimus	I	Recurrent	33	NA	NCT00821080
Vandetanib	I/II	Recurrent	94	NA	NCT00293566
Vandetanib+carboplatin vs vandetanib→carboplatin	II	Recurrent	128	NA	NCT00995007
Vorinostat	II	Recurrent	94	PFS	NCT00238303
Vorinostat+RT+TMZ	I/II	Primary	132	Safety/OS	NCT00731731
Vorinostat+bevacizumab+irinotecan	I	Recurrent	21	NA	NCT00762255
Vandetanib+bevacizumab vs bevacizumab	I/II	Recurrent	108	Safety/PFS	NCT01266031
Vorinostat+TMZ	I/II	Recurrent	52	Safety/PFS	NCT00939991
Vorinostat+TMZ	I	Recurrent	77	NA	NCT00268385
Vorinostat+Bortezomib	II	Recurrent	68	PFS	NCT00641706
XL184+RT+TMZ	I	Primary	85	Safety	NCT00960492
XL765+TMZ	I	Maintainance	80	Safety	NCT00704080

CCNU, lomustine; DLT, dose-limiting toxicities; MTD, maximum tolerated dose; NA, not available; OS, overall survival; PFS, progression-free survival; PK, pharmacokinetics; RT, radiotherapy; TMZ, temozolomide.


A.
EGFR, mutated/ amplified in 45%
HER2, mutated in 8%
PDGFRα, amplified in 13%
MET, amplified in 4%
SRC*
SRC*
SRC*
SRC*
RAS, mutated in 2%
PI3K, mutated in 15%
PTEN, mutated/ deleted in 36%
NF1, mutated/ deleted in 18%
AKT, amplified in 2%
Proliferation, survival, translation
FOXO, mutated in 1%
B.
Activated oncogenes
CDKN2A, deleted in 49%
MDM2, amplified in 14%
MDM4, amplified in 7%
TP53, mutated or deleted in 35%
Senescence
Apoptosis
C.
CDKN2A, deleted/ mutated in 52%
CDKN2B, deleted in 47%
CDKN2C, deleted in 2%
CDK4, amplified in 18%
CCND2, amplified in 2%
CDK6, amplified in 1%
RB1, deleted in 11%
G1/S progression


Bevacizumab
VEGF
Cediranib
Pazopanib
Sorafenib
Sunitinib
Vandetanib
Vatalanib
ANG1/2
Nitric oxide
Cediranib
Imatinib
Pazopanib
Sorafenib
Sunitinib
Vatalanib
NRP1
VEGFR1
VEGFR2
VEGFR3
TIE2
Plasma membrane
PLCγ
PKC/Ca2+
eNOS
Paxillin
FAK
PI3K
SRC
mTOR
AKT
CKII
GSK3β
PDGFB
PDGFD
PDGFRβ
PDGFA
PDGFC
PDGFRα
Gene transcription
ERK
MEK
Raf
Ras
Temsirolimus
Everolimus
Sirolimus
P38
Integrin αvβ3
Integrin α5β1
Endothelial cell
Dasatinib
Enzastaurin
Cilengitide


CD95 ligand
CD95
Plasma membrane
DD
P
p85
p110
pp60
SFKs
Active
GSK3β
AKT
Ser-9
S9A
P
Active
GSK3β
MMPs
Inhibition of invasiveness


TRAIL
DR4/DR5
FADD
Caspase-8
Bid
Bcl-2
Bax
Smac
Cyt c
Apaf-1
IAP
Caspase-9
AIF
Caspase-3
Substrates
CAD/ICAD
DNA fragmentation
Cell death