GEM ExTra™ Report

ASHION
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Report Date: 10/10/2019

Patient:	John Jones	Ordering Client:	Indiana Clinical
Gender:	Male	Specimen Type:	FFPE Block
DOB:	10/01/1980	Specimen Site:	Colon
Medical Record #:	MRN1234	Tumor Collection Date:	11/01/1999
Client Accession #:	CA1234	Normal Collection Date:	11/02/1999
Ordering Physician:	Dr. Jones	Received Date:	12/01/1999

Genomic Snapshot
- Analytes sequenced: DNA+RNA
- Actionable Targets: 13
- TMB: Low
- MSI: Stable
- Clinical Trials: Yes

Diagnosis: **Glioblastoma**

TUMOR GENOMIC ALTERATIONS[1]

CDKN2A CDKN2B EGFR MDM4 PIK3C2B PTEN RET ZNF33B/RET

GENOMIC TARGETS	FDA-APPROVED DRUGS -for patient's cancer	FDA-APPROVED DRUGS -for another cancer	DRUGS PREDICTED NON-BENEFICIAL	POTENTIAL CLINICAL TRIALS
13	0	19	0	Yes
CDKN2A (Deletion)		abemaciclib, palbociclib, ribociclib		Yes
CDKN2B (Deletion)				Yes
EGFR (A289V)		afatinib, erlotinib, gefitinib, lapatinib, neratinib		Yes
EGFR (Amplification)		afatinib, erlotinib, gefitinib, lapatinib, neratinib		Yes
EGFR (EGFRvIII)		afatinib, erlotinib, gefitinib		Yes
EGFR (EGFRvIVb)		afatinib, cetuximab, erlotinib, osimertinib		Yes

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TUMOR GENOMIC ALTERATIONS[1]

CDKN2A CDKN2B EGFR MDM4 PIK3C2B PTEN RET ZNF33B/RET

GENOMIC TARGETS	FDA-APPROVED DRUGS -for patient's cancer	FDA-APPROVED DRUGS -for another cancer	DRUGS PREDICTED NON-BENEFICIAL	POTENTIAL CLINICAL TRIALS
13	0	19	0	Yes
EGFR (R108K)		afatinib, gefitinib, lapatinib, neratinib		Yes
MDM4 (Amplification)				Yes
PIK3C2B (Amplification)				Yes
PTEN (Deletion)		copanlisib, everolimus, temsirolimus		Yes
RET (Amplification)		cabozantinib, lenvatinib, regorafenib, sorafenib, sunitinib, vandetanib		Yes
ZNF33B/RET (Fusion)		cabozantinib, lenvatinib, regorafenib, sorafenib, sunitinib, vandetanib		Yes

TUMOR MUTATION BURDEN (TMB)

LOW (1 mut/Mb)		No

[1]Alterations with predictive value according to Ashion's database and/or clinical trials identified by Ashion. For a complete list of alterations, please see the VUS section near the end of the report.

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MICROSATELLITE STATUS (MSI)		
STABLE		No

ADDITIONAL SIGNIFICANT ALTERATIONS		
TERT (c.-124C>T)		No

*NOTE: Certain drugs associated with variants detected in this case may not cross the blood-brain barrier; treating physician discretion is necessary.

**NOTE: The tumor sample harbors a focal gain at 1q32.1 and LOH at chr 9p, 10, and partial LOH at chr 13 and 22. Amplification at 1q32.1 (region contains MDM4, SOX13, ETNK2, KISS1, GOLT1A, PLEKHA6, REN and PIK3C2B genes, all of which are amplified in this tumor sample) is a frequent event in GBM, and reported at a frequency of 4% in one study (González-Tablas M et al., 2018; PMID: 29963263, Crespo I et al., 2012; PMID: 23029397). LOH of both 9p and 10 is a frequent occurrence in GBM that is associated with shorter survival and considered an independent prognostic factor in high-grade gliomas (Balesaria S et al., 1999; PMID: 10604735, Phillips HS et al., 2006; PMID: 16530701). Genomic deletions of 9p and 10q are typical in a subset of GBM, and studies suggest that LOH on 9p and 10q are associated with shorter survival in patients without adjuvant therapy (Tada K et al., 2001; PMID: 11596960, Terada K et al., 2002; PMID: 12164681, Wemmert S et al., 2005; PMID: 16242071). However, the studies also indicated that tumors with these alterations had significantly better response to temozolomide (TMZ) treatment (Wemmert S et al., 2005; PMID: 16242071). LOH of chr 22 at one or more microsatellite increased with increasing grade of the tumor ($P < 0.01$). LOH 22q was more frequent in astrocytic tumors versus mixed or oligodendroglial tumors, and correlated to 10q loss suggesting that LOH 22q is an alteration associated with malignant progression of gliomas (Laigle-Donadey F et al., 2006; PMID: 16283437).

***NOTE: The tumor sample harbors two truncating mutations in the PCLO gene (E3258* and V5064fs) (please see VUS section). In a recent report of a study on subtype-specific signaling pathways and mutations associated with prognosis of glioblastoma, in the mesenchymal subtype, patients with PCLO mutations showed poor prognosis (Park AK et al., 2019; PMID: 30053126).

****NOTE: The tumor sample harbors a novel EGFR fusion (EGFR/BMS1) (please see VUS section). In this fusion EGFR exon 16 is fused to exon 14 of BMS1 gene, with EGFR at the N-terminal end. Breakpoint at exon 16 is predicted to be upstream of the Kinase domain and hence it is not known if this fusion would retain the kinase domain. As this fusion has not been characterized, its impact on tumor behavior and drug response is not known. EGFR rearrangements have been identified in multiple tumor types, including NSCLC and GBM. The common fusion partners of EGFR involve RAD51, SEPT14 and PURB (Konduri K et al., 2016; PMID: 27102076). These fusions were oncogenic in pre-clinical studies and could be inhibited by treatment with EGFR TKI therapy. Certain common EGFR fusions have been reported in 4% of GBM samples (10/248) (Frattini V et al., 2013; PMID: 23917401). Treatment of tumor cells harboring the more common EGFR/SEPT14 fusion (which retained the kinase domain) demonstrated that lapatinib and erlotinib could significantly delay tumor growth (Frattini V et al 2013; PMID: 23917401).

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Genomic Alterations Detail

Genomic Alteration		Therapeutic Implication	
Alteration:	CDKN2A (Deletion)	**Drug**	**Status**
Coordinate:	chr9:21974865	abemaciclib (Verzenio)	PREDICTED BENEFICIAL
Origin:	DNA	palbociclib (Ibrance)	PREDICTED BENEFICIAL
		ribociclib (Kisqali)	PREDICTED BENEFICIAL

Biomarker Summary

CDKN2A gene codes for the tumor suppressor proteins p16INK4a and p14ARF (Stone et al., 1995; PMID: 7606716, Zhang et al., 1998; PMID: 9529249, Zhang et al., 2010; PMID: 20565749). CDKN2A is a frequently lost gene in glioblastomas (GBM) (Parsons DW., et al., 2008; PMID: 18772396). Because p16INK4a is known to inhibit Cdk4, tumors with CDKN2A loss may be sensitive to Cdk4/6 inhibitors (Stone et al., 1995; PMID: 7606716, Zhang et al., 1998; PMID: 9529249). In 47 melanoma cell lines with homozygous loss, methylation, or mutation of CDKN2A gene or loss of protein (p16INK4a), such alterations predicted sensitivity to the CDK4/6 inhibitor PD0332991, while RB1 loss predicted resistance (Young RJ et al., 2014; PMID: 24495407). The Cdk4/6 inhibitors palbociclib (PD-0332991) and ribociclib have been approved by the FDA for the treatment of certain breast cancer patients (Finn et al., 2015; PMID: 25524798). Recently, abemaciclib was approved for the treatment of certain breast cancer patients (Corona SP and Generali D, 2018; PMID: 29497278). These and other Cdk inhibitors are being studied in clinical trials. The p14ARF protein has been reported to function as a tumor suppressor through stabilization and activation of p53, via a mechanism of Mdm2 inhibition (Sherr et al., 2005; PMID: 16869746, Ozenne et al., 2010; PMID: 20549699, Zhang et al., 1998; PMID: 9529249). Therefore, some CDKN2A alterations may indicate sensitivity to Mdm2 inhibitors, and clinical trials of these agents are ongoing. Somatic loss of CDKN2A has been reported in about 53%-60% of astrocytoma grade IV samples (COSMIC, cBioPortal).

Molecular Function

CDKN2A deletion has been significantly correlated with loss of p16INK4a expression in glioblastoma (GBM) (Purkait S et al, 2013: PMID: 23311918). CDKN2A loss results in loss of both tumor suppressor proteins p16INK4a and p14ARF, leading to improper bypass of G1/S checkpoint, enhanced cell proliferation and decreased apoptosis (Zhang Y et al., 1998; PMID: 9529249, Gazzeri S et al., 1998; PMID: 9484839). Subsequently, this can result in dysregulated p16INK4a-CDK4/Cyclin/Rb, and Mdm2/p53 pathways, all of which lead to aberrant cell cycle progression (Gazzeri et al., 1998; PMID: 9484839, Zhang et al., 1998; PMID: 9529249). Loss of CDKN2A, which frequently co-occurs with loss of CDKN2B, as seen in this case, also contributes to tumor formation and progression (Schuster K et al, 2014: PMID: 24618618; Ortiz B et al, 2014: PMID: 25138050; Ortiz B et al, 2014: PMID: 24843164; Altomare D et al, 2011: PMID: 21526190; Camacho C et al, 2010: PMID: 20663777). Loss of CDKN2A may predict sensitivity to CDK4/6 inhibitors like palbociclib, ribociclib, and abemaciclib, which are approved in other tumor types.

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Genomic Alteration		Therapeutic Implication	
Alteration:	CDKN2B (Deletion)	**Drug**	**Status**
Coordinate:	chr9:22009362	See Clinical Trials Section	
Origin:	DNA		

Biomarker Summary

CDKN2B (cyclin dependent kinase inhibitor 2B) gene resides on 9p21.3, adjacent to the tumor suppressor gene CDKN2A, in a region that is frequently mutated or deleted in diverse tumor types, including GBM. CDKN2B codes for p15INK4B, which inhibits the binding of CDK4 and CDK6 to cyclin D, preventing the activation of the CDK kinases, thereby regulating cell growth and cell cycle progression through G1 phase (Canepa ET et al., 2007; PMID: 17654117). In greater than 90% of cancer tissues harboring CDKN2A deletion, the adjacent CDKN2B gene is also co-deleted (as seen in this tumor). CDKN2B loss is reported to promote progression of benign nevus melanocytes to melanoma (McNeal AS et al., 2015; PMID: 26183406). Somatic loss of CDKN2A/CDKN2B via deletions or promoter methylation are frequent in GBM. Complete loss of 9p21.3 locus has been significantly associated with worse prognosis for both tumor progres-sion/recurrence and overall survival in GBM patients (Geng J et al., 2012; PMID: 21713760). Tumors with CDKN2B loss may be sensitive to CDK4/6 inhibitors, and clinical trials are ongoing with CDK4/6 inhibitors in solid tumors. CDKN2B loss is a frequent event in GBM and reported in 54%-58% of GBM samples (COSMIC, TCGA).

Molecular Function

CDKN2B loss by homozygous deletion is a frequent event in GBM, and leads to loss of p15INK4b protein. Conse-quently, it leads to loss of inhibition of CDK4/6-cyclin D binding, causing uncontrolled cell-cycle progression at G1 phase. CDKN2B plays a critical role against tumorigenesis and its loss is associated with poor prognosis in GBM patients (Feng J et al., 2012; PMID: 21713760).

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Genomic Alteration		Therapeutic Implication	
Alteration:	EGFR (A289V)	**Drug**	**Status**
Alteration Type:	Missense	afatinib (Gilotrif)	PREDICTED BENEFICIAL
Coordinate:	chr7:55221822	erlotinib (Tarceva)	PREDICTED BENEFICIAL
Allele Frequency:	2%	gefitinib (Iressa)	PREDICTED BENEFICIAL
Transcript ID:	ENST00000275493	lapatinib (Tykerb)	PREDICTED BENEFICIAL
Origin:	DNA	neratinib (Nerlynx)	PREDICTED BENEFICIAL

Biomarker Summary

EGFR gene encodes for a transmembrane tyrosine kinase receptor, which upon activation, elicits activation of diverse signaling cascades, leading to cell growth, proliferation, cell survival, cell differentiation and metastasis (Oda K et al., 2005; PMID: 16729045). Several EGFR mutations in the kinase domain, as well as in the extracellular domain, are oncogenic in nature and activate the EGFR pathway. EGFR mutations are present in about 15% of GBM patients, are clustered in the extracellular domain, and are associated with increased gene amplification. EGFR ectodomain mutants are sensitive to EGFR kinase inhibitors in transformed cell lines, as well as in clinical samples (Zhou K et al 2017; PMID: 28611289; Vivanco I et al., 2012; PMID: 22588883). Clinical studies have shown that a GBM patient with EGFR ectodomain mutation R108K responded to erlotinib when PTEN was intact. Another GBM patient failed erlotinib therapy in presence of R108K EGFR mutation and loss of PTEN, which is known to cause resistance to EGFR kinase inhibitors in GBM patients (Lee JC et al., 2006; PMID:17177598, Mellinghoff IK et al 2005; PMID: 16282176). Loss of PTEN promotes cellular resistance to EGFR kinase inhibitor therapy by dissociating EGFR inhibition from downstream PI3K pathway inhibition; thus, downstream inhibition of PI3K pathway could be combined with EGFR kinase inhibitors in patients with PTEN-deficient tumors to promote responsiveness (Goudar RK et al., 2005; PMID:15657358, Mellinghoff IK et al 2005; PMID: 16282176). EGFR mutations have been reported in 11.3% of astrocytoma grade IV samples from brain (COSMIC) and 5.03% of all GBM samples in cBioPortal.

Molecular Function

EGFR (A289V) mutation is located in the cysteine-rich extracellular domain (ECD) of EGFR protein in an evolutionarily-conserved residue. Mutations at codon 289 are associated with EGFR gene amplification and are oncogenic in nature. Cell lines expressing A289V showed a marked increase in receptor autophosphorylation in the absence of ligand or serum and produced large tumors at the inoculation site in nude mice within 3-4 weeks (Lee JC et al., 2006; PMID:17177598). EGFR tyrosine kinase inhibitor erlotinib was able to induce cell death in cell lines expressing EGFR ECD mutations, including A289V (Lee JC et al., 2006; PMID:17177598), and lapatinib induced death in GBM cells expressing A289V (Vivanco I et al., 2012; PMID: 22588883). BAY846 is an irreversible inhibitor of EGFR and ERBB2, which is also effective against cells expressing A289V in experimental models in the presence of an intact PTEN (Longo SL et al., 2012; PMID: 22203214). The A289V is a highly recurrent hotspot mutation in GBM (COSMIC, cBioPortal).

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Genomic Alteration		Therapeutic Implication	
Alteration:	EGFR (Amplification)	**Drug**	**Status**
Coordinate:	chr7:55279321	afatinib (Gilotrif)	PREDICTED BENEFICIAL
Origin:	DNA	erlotinib (Tarceva)	PREDICTED BENEFICIAL
		gefitinib (Iressa)	PREDICTED BENEFICIAL
		lapatinib (Tykerb)	PREDICTED BENEFICIAL
		neratinib (Nerlynx)	PREDICTED BENEFICIAL

Biomarker Summary

EGFR gene codes for a transmembrane tyrosine kinase receptor (Epidermal Growth Factor Receptor). EGFR amplification is one of the most common alterations in GBM, leading to protein over-expression as measured by IHC, although this correlation is not consistent for low-level gene amplification (Hemmings et al., 2009; PMID: 19404848, Liang et al., 2010; PMID: 20637128, Yang et al., 2012; PMID: 22490401, Bhargava et al., 2005; PMID: 15920544). Massive amplification of EGFR has been reported in GBM and somatic copy number alterations are present in 43% of the patients (Libermann TA et al., 1995; PMID: 2981413, Maire CL and Ligon KL, 2014; PMID: 25342599). EGFR activation may lead to activation of the PI3K and MAPK pathway and confer sensitivity to PI3K and MAPK pathway inhibitors (Bancroft CC et al., 2002; PMID:11992543). EGFR activating mutations or amplification may also predict sensitivity to targeted therapies, including inhibitors of multiple members of the ERBB family, of which several have received FDA approval in other tumor types (Mok TS et al., 2009; PMID: 19692680, Rosell R et al., 2009; PMID: 19692684, Tsao et al., 2005; PMID:16014883). Dacomitinib, a selective, irreversible inhibitor of EGFR, had limited single-agent activity in recurrent GBM with EGFR amplification but not with EGFRvIII patients (Sepúlveda-Sánchez JM et al., 2017; PMID: 28575464). Depatuxizumab mafodotin (depatux-m) (formerly ABT414) is an antibody-drug conjugate that binds to cells with EGFR amplification and releases an anti-microtubule agent (van den Bent M et al., 2017; PMID: 29075855). A phase II study on Depatux-M alone, Depatux-M plus temozolomide (TMZ), and either TMZ or lomustine (LOM) single agent in recurrent EGFR-amplified glioblastoma (NCT02343406) confirmed an improvement in OS in EGFR-amplified recurrent GBM (Van Den Bent MJ et al., J Clin Oncol 36, 2018 (suppl; abstr 2023). Among anti-EGFR monoclonal antibodies, cetuximab failed to demonstrate efficacy as a single agent or in combination with chemo- or radiotherapy, which is attributed to delivery issues (Westphal M et al., 2017; PMID: 28791656). Nimotuzumab is another antibody that binds to EGFR over-expressing cells and has shown some efficacy in phase II/III trials for glioma. In newly diagnosed diffuse intrinsic pontine glioma (DIPG), radiation plus nimotuzumab plus chemo-agent resulted in a median survival of 15 months (Massimino M et al., 2014; PMID: 24696052). In a Phase III study of nimotuzumab with radiotherapy, the GBM patients with the greatest median OS had EGFR amplification and unmethylated MGMT, although there was no statistically significant difference in OS of patients (Bode U et al., 2012; PMID: 23043252). Currently, FDA-approved EGFR TKIs erlotinib, gefitinib, afatinib, and lapatinib have not shown efficacy, either alone or in combination in phase I/II trials in GBM (Westphal M et al., 2017; PMID: 28791656). In addition to efficacy, issues of brain penetrance, which are difficult to measure except in animal models, have been attributed to some drugs like erlotinib, which had 5-7% accumulation; however, gefitinib showed 2- to 3- fold concentration and, hence, lack of concentration could not be the cause of poor efficacy (Lassman AB et al., 2005; PMID: 16278407). EGFR amplifications have been reported in 46.4% of astrocytoma grade IV samples from brain (COSMIC).

Molecular Function

EGFR amplification may lead to increased EGFR signaling, which may trigger diverse signaling cascades leading to cell growth, proliferation, cell survival, cell differentiation and, metastasis. EGFR undergoes dimerization and activation upon binding to its ligand at the extracellular domain of the protein (Oda K et al., 2005; PMID: 16729045).

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Genomic Alteration		Therapeutic Implication	
Alteration:	EGFR (EGFRvIII)	**Drug**	**Status**
Alteration Type:	Alternative Transcript	afatinib (Gilotrif)	PREDICTED BENEFICIAL
Coordinate:	chr7:55087058; chr7:55223523	erlotinib (Tarceva)	PREDICTED BENEFICIAL
Transcript ID:	ENST00000275493; ENST00000275493	gefitinib (Iressa)	PREDICTED BENEFICIAL
Origin:	RNA		

Biomarker Summary

EGFR codes for a transmembrane tyrosine kinase receptor with a critical role in cellular functions. The wild-type EGFR contains an extracellular ligand binding domain (LBD) to which the ligands bind and mediate dimerization and activation. Activated EGFR then triggers diverse signaling cascades, leading to cell growth, proliferation, survival, cell differentiation, and in tumor cells, metastasis (Oda K et al., 2005; PMID: 16729045). However, the EGFRvIII mutation, generated by an in-frame deletion of exons 2–7, codes for a unique tumor-specific protein that lacks a ligand-binding domain. Consequently, the EGFRvIII encoded protein is constitutively active in the absence of ligand binding (Guo G et al., 2015; PMID: 26282175). EGFRvIII has been associated with longer OS (P = .0023) and favorable prognosis in association with Ki67 of 20% or less, normal PTEN, and methylated MGMT (Montano N et al., 2011; PMID: 22241957). Other studies have reported that concomitant EGFRvIII over-expression and amplification confers poor prognosis in GBM (Shinojima N et al., 2003; PMID: 14583498). Currently, there are no approved drugs with proven efficacy against EGFRvIII. EGFRvIII has been associated with resistance to the EGFR TKI erlotinib by some investigators (Gallego et al., 2014; PMID: 24352766, Schulte A et al., 2013; PMID: 23877316). According to another study, response to EGFR TKIs (erlotinib, gefitinib) in EGFRvIII GBM required an intact PTEN (Mellinghoff IK et al., 2005; PMID: 16282176), while other studies failed to show any efficacy at all (Reardon DA et al., 2010; PMID: 19562254; van den Bent MJ et al., 2009; PMID: 19204207; Brown PD et al., 2008; PMID: 18955445). Response to afatinib in EGFRvIII-mutant GBM have been modest (Alshami J et al., 2015; PMID: 26423602, Reardon DA et al., 2015, PMID: 25140039), while osimertinib activity was lower than that of afatinib (Cross DA et al., 2014; PMID: 24893891). Lapatinib, an irreversible inhibitor of EGFR, suppressed GBM cell lines with EGFR ECD mutations, including EGFRvIII, but not EGFR wild-type (Vivanco I et al., 2012; PMID: 22588883, Frattini V et al., 2013; PMID: 23917401), while another study reported lack of efficacy (Thiessen B et al., 2010, PMID: 19499221). In regard to anti-EGFR MAbs, combination of panitumumab and VEGFR inhibitors demonstrated anti-tumor efficacy in pre-clinical models of EGFRvIII GBM (Greenall SA et al., 2015; PMID: 25659577), but cetuximab had no efficacy (Lv S et al., 2012; PMID: 22752145, Sok JC et al., 2006; PMID: 16951222, Wheler DL et al., 2008, PMID: 18297114). Pre-clinical data on an anti-EGFR MAb, ABT-806, reported response in EGFRvIII-mutant murine lung cancer (Li D et al., 2007; PMID: 17256054). EGFRvIII, being a tumor specific antigen, is an ideal target for immunotherapy, including CAR T-cell, vaccines, and Bi-specific T Cell Engager (Yang J et al., 2017; PMID: 28649003), many of which are in clinical trials. Peptide vaccines in combination with chemotherapy have shown some benefit in EGFRvIII GBM tumors (Heimberger AB et al., 2008; PMID: 18079360), and are being evaluated in clinical trials. Reports of a first in-human study of single dose CAR T-cells against EGFRvIII mutation in 10 recurrent GBM patients showed one patient with residual SD for >18 months, and antigen decrease in 5/7 patients (O'Rourke DM et al., 2017; PMID: 28724573, Brown CE et al., 2016; PMID: 28029927). Osimertinib has also shown activity in mice models implanted with EGFRvIII glioblastoma stem cells (Kwatra M et al., Neuro-Oncology, Volume 19, abstract, November 2017).

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Molecular Function

EGFRvIII mutation results from a deletion in the extracellular domain from exons 2 to 7, which leads to loss of amino acids 6-273, rendering the mutant receptor incapable of binding to its ligand. EGFRvIII confers constitutively active signaling in the absence of ligand binding, which results in increased cell proliferation, enhanced ability to form tumor xenografts, reduced apoptosis, increased angiogenesis, and enhanced invasiveness in GBM cell lines (Keller S and Schmidt MHH., 2017; PMID: 28629170). In addition, impaired internalization and degradation enhances the tumorigenic potential of the mutant protein (Wikstrand CJ et al., 1998; PMID: 9584952). EGFRvIII mutant has also been reported to increase activation of the PI3K-AKT-mTOR pathway (Vivanco I et al., 2010; PMID: 20308550).

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Genomic Alteration		Therapeutic Implication	
Alteration:	EGFR (EGFRvIVb)	**Drug**	**Status**
Alteration Type:	Alternative Transcript	afatinib (Gilotrif)	PREDICTED BENEFICIAL
Coordinate:	chr7:55268106; chr7:55270210	cetuximab (Erbitux)	PREDICTED BENEFICIAL
Transcript ID:	ENST00000275493; ENST00000275493	erlotinib (Tarceva)	PREDICTED BENEFICIAL
Origin:	RNA	osimertinib (Tagrisso)	PREDICTED BENEFICIAL

Biomarker Summary

Epidermal growth factor receptor (EGFR), a member of the ErbB family of receptor tyrosine kinases, is a common genetically altered gene primarily in GBM. Different classes of EGFR somatic mutations have been identified, which include various exon deletion mutations, including exon 27, exon 25–27 and exon 25–28 deletion mutations, which result in the truncation of the C-terminal domain of EGFR (Brennan CW et al., 2013; PMID: 24120142; Cho J et al., 2011; PMID: 22001862). The carboxyl terminal deletion mutants, collectively termed EGFRvIV, lack either three exons (numbered 25–27; also known as EGFRvIVa) or two exons (25 and 26; also known as EGFRvIVb), with the deletion initiating immediately downstream to the kinase domain. EGFR C-terminal deletion mutants are able to induce cellular transformation in vitro and in vivo in the absence of ligand and receptor autophosphorylation. Treatment with the EGFR-targeted monoclonal antibody cetuximab, or the small molecule EGFR inhibitor erlotinib, effectively impaired tumorigenicity of oncogenic EGFR CTD deletion mutants. Certain EGFR fusions, such as EGFR/RAD51 and EGFR/PURB, which are oncogenic and able to mediate downstream signaling through the MAPK and PI3K/AKT pathways, also lack the C-terminal tail (spanning exons 25-28) known to be important for EGFR signal transduction. Clinical data reported durable partial responses in all 4 NSCLC patients with EGFR/RAD51 and EGFR/PURB fusions, which resulted in C-terminal deletion of EGFR when treated with erlotinib (N=3 EGFR/RAD51, N=1 EGFR/PURB). Further, pre-clinical data showed that erlotinib, afatinib and osimertinib could inhibit growth and signaling in EGFR/RAD51 expression Ba/F3 cells (Konduri K et al., 2016; PMID: 27102076). 4 out of 5 TCGA samples with mutant EGFR genes harboring CTD deletion exhibit high amplification at EGFR and do not harbor any other EGFR mutation. In cell lines and animal xenograft models of EGFR C-terminal domain mutants, including those harboring deletion of Exons 25-28, both erlotinib and cetuximab were able to effectively suppress the growth by decreasing the constitutive phosphorylation of Stat5 in these cells (Cho J et al., 2011; PMID: 22001862). Anti-EGFR therapies may be a promising therapeutic strategy in patients harboring EGFR CTD deletion mutants.

Molecular Function

EGFRvIVb includes deletion of exon 25 and 26 in the C-terminal domain immediately downstream to the kinase domain. The internal deletions of EGFRvIV enhance basal kinase activity and confer oncogenic phenotypes. The C-tail distal to the kinase domain of EGFR possesses an inhibitory role, which interferes with catalytic activation in the absence of ligand-induced dimerization. Upon deletion in brain tumors, mutant EGFRvIV acquires basal tyrosine phosphorylation that instigates unique sets of signaling proteins and culminates in malignant transformation (Pines G et al., 2010; PMID: 20676128). The vIVb mutant protein conferred anchorage-independent growth of cell colonies in vitro, similar to EGFRvIII, and formed tumors in vivo. Further, the vIVb, which is a shorter deletion than vIVa, resulted in stronger transforming activity of the two, further suggesting the growth inhibitory roles of the C-terminal domain (Pines G et al., 2010; PMID: 20676128). The vIV mutations, which increase EGFR stability, are specific to GBM, and pre-clinical studies have shown to be responsive to erlotinib, afatinib, dacomitinib and cetuximab (Cho J et al., 2011; PMID: 22001862).

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Genomic Alteration		Therapeutic Implication	
Alteration:	EGFR (R108K)	**Drug**	**Status**
Alteration Type:	Missense	afatinib (Gilotrif)	PREDICTED BENEFICIAL
Coordinate:	chr7:55211080	erlotinib (Tarceva)	PREDICTED BENEFICIAL
Allele Frequency:	31%	gefitinib (Iressa)	PREDICTED BENEFICIAL
Transcript ID:	ENST00000275493	lapatinib (Tykerb)	PREDICTED BENEFICIAL
Origin:	DNA	neratinib (Nerlynx)	PREDICTED BENEFICIAL

Biomarker Summary

EGFR codes for a transmembrane tyrosine kinase receptor, which, upon activation, triggers diverse signaling cascades leading to cell growth, proliferation, cell survival, cell differentiation, and, in tumor cells, metastasis (Oda K et al., 2005; PMID: 16729045). EGFR kinase domain (KD) mutations, as well as the extracellular domain (ECD) mutations, are oncogenic in nature and activate EGFR signaling. In GBM, EGFR mutations are present in about 15% of patients, and, importantly, clustered in the ECD and associated with EGFR amplification (Lee JC et al., 2006; PMID: 17177598). EGFR ECD mutants are sensitive to EGFR kinase inhibitors in transformed cell lines, as well as in clinical samples (Zhou K et al 2017; PMID: 28611289; Vivanco I et al., 2012; PMID: 22588883; Lee JC et al., 2006; PMID:17177598). In a pre-clinical model, afatinib was found to be more effective than erlotinib against the extracellular domain point mutations A289V and R108K (Li D et al., 2008; PMID: 18408761). EGFR activating mutations or amplification may predict sensitivity to targeted therapies, including inhibitors of multiple members of the ERBB family, of which several have received FDA approval in other tumor types (Mok TS et al., 2009; PMID: 19692680, Rosell R et al., 2009; PMID: 19692684, Tsao et al., 2005; PMID:16014883). EGFR activation may also lead to activation of the PI3K and MAPK pathway and confer sensitivity to PI3K and MAPK pathway inhibitors as well (Bancroft CC et al., 2002; PMID:11992543).

Molecular Function

EGFR (R108K) is an ectodomain mutation in a highly conserved residue and is oncogenic. It is a recurrent hotspot mutation in CNS tumors (COSMIC, TCGA). In GBM, R108K substitution has been observed to confer anchorage-independence in experimental systems. In addition, this mutation produced large tumors at the inoculation site in nude mice within 3-4 weeks (Lee JC et al., 2006; PMID: 17177598). The R108K, like other ectodomain mutations, showed an increased auto-phosphorylation in the absence of ligand or serum-deprivation. This mutation, like other ectodomain mutations, is associated with EGFR amplifications, and is sensitive to erlotinib in clinical studies. However, the sensitivity was limited to tumors with an intact PTEN, while tumors deficient for PTEN were not responsive (Lee JC et al., 2006; PMID:17177598, Mellinghoff IK et al 2005; PMID: 16282176).

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Genomic Alteration		Therapeutic Implication	
Alteration:	MDM4 (Amplification)	**Drug**	**Status**
Coordinate:	chr1:204527248	See Clinical Trials Section	
Origin:	DNA		

Biomarker Summary

MDM4 (MDMX) encodes a nuclear protein that contains a p53 binding domain at the N-terminus and a RING finger domain at the C-terminus, and is structurally similar to p53-binding protein MDM2. Both MDM2 and MDM4 proteins bind to p53 tumor suppressor protein and inhibit its activity, and are found to be overexpressed in a variety of human cancers. However, unlike MDM2, which degrades p53, MDM4 inhibits p53 by binding its transcriptional activation domain (TAD). MDM4 also interacts with MDM2 protein via the RING finger domain, and inhibits the latter's degradation (RefSeq). Like MDM2, MDM4 acts as oncogenic inhibitor/negative regulator of p53's tumor suppressive activity. The tumor suppressor activity of p53 is highly diminished in GBM by various mechanisms, one of which is amplifications of MDM2/MDM4 (Zhang Y et al., 2015; PMID: 30200436). Currently, there are no approved therapies targeting MDM4, although a few investigational agents are being studied at pre-clinically. A dual Mdm2/Mdm4 antagonist, RO-5963, was identified that blocked p53 interaction with both Mdm2 and Mdm4. In the p53 wild-type but not mutant tumor cells, the dual antagonist induced apoptosis, even in the presence of high levels of Mdm4, while Nutlin-3a (MDM2 inhibitor) induced only a weak apoptotic response (Graves B et al., 2012; PMID: 22745160, L1 Q and Lozano G, 2013; PMID: 23262034). Further, wild-type p53-carrying tumor cells with high levels of Mdm4 showed minimal response to Nutlin-3 treatment (Bernal F et al., 2012; PMID: 21075307).

Molecular Function

MDM4 amplification can inactivate p53, leading to loss of various tumor suppressor functions, including growth arrest, apoptosis, DNA repair, and senescence. MDM4 (MDMX) gene, which is located on 1q32, is a target for amplification in a subset of malignant gliomas without TP53 mutation or MDM2 amplification (Riemenschneider MJ et al., 1999; PMID: 10626796). MDM4 amplifications have been reported in 7% of GBMs (England B et al., 2013; PMID: 23737287, Crespo I et al., 2015; PMID: 25976245).

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Genomic Alteration		Therapeutic Implication	
Alteration:	PIK3C2B (Amplification)	**Drug**	**Status**
Coordinate:	chr1:204459552	See Clinical Trials Section	
Origin:	DNA		

Biomarker Summary

PIK3C2B codes a protein that is a member of the phosphoinositide 3-kinase (PI3K) family. PI3-kinases play a major role in signaling pathways involved in cell proliferation, oncogenic transformation, cell survival, cell migration, and intracellular protein trafficking. It is a class II PI3-kinase (RefSeq). As PIK3C2B is a part of PTEN/PI3K/AKT/mTOR signaling pathway, mTOR inhibitors may have clinical benefit in tumors with PIK3C2B dysregulation (de Melo AC et al., 2017; PMID: 28286604). Currently, mTOR inhibitors are being evaluated in PIK3C2B amplified GBM (NCT03834740).

Molecular Function

PIK3C2B (amplification) is located in chr 1q32.1, a region that is frequently amplified in GBM (Knobbe CB and Reifenberger G, 2003; PMID: 14655756). Expression analysis of selected genes pertaining to the primary and secondary pathways of GBM pathogenesis identified PIK3C2B as a gene whose expression significantly correlated with cellular resistance towards erlotinib, suggesting that resistance towards this EGFR TKI may be acquired during the natural evolution of GBM (Löw S et al., 2008; PMID: 19189657).

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Genomic Alteration		Therapeutic Implication	
Alteration:	PTEN (Deletion)	**Drug**	**Status**
Coordinate:	chr10:89731687	copanlisib (Aliqopa)	PREDICTED BENEFICIAL
Origin:	DNA	everolimus (Afinitor)	PREDICTED BENEFICIAL
		temsirolimus (Torisel)	PREDICTED BENEFICIAL

Biomarker Summary

PTEN codes for a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase enzyme that negatively regulates cellular levels of phosphatidylinositol-3,4,5-trisphosphate and, thereby, the AKT/mTOR signaling pathway. PTEN thus functions as a tumor suppressor, and loss or inactivating mutations can lead to increased activation of the PI3K/AKT/mTOR pathway. In glioblastomas (GBM), PTEN mutations are late events in the progression of the disease, and significantly associated with patients' short-term survival (Yang Y et al., 2012; PMID: 20393024, Smith JS et al., 2001; PMID: 11504770). PTEN, via PI3K activation, contributes to cell-cycle progression, reduced apoptosis, and increased metastasis (Mills GB et al., 2001; PMID: 11706404). Therefore, inhibitors of PI3K pathway may be relevant in tumors with PTEN loss or mutation (Courtney et al., 2010; PMID: 20085938, Wu et al., 2011; PMID: 21903772, Simpson and Parsons, 2001; PMID: 11237521). The mTOR inhibitor (mTORi) rapamycin has been reported to have anti-cancer activity in PTEN-deficient GBM (Cloughesy TF et al., 2008; PMID: 18215105). The mTOR inhibitors temsirolimus and everolimus, and the PI3K inhibitor copanlisib, have been approved by the FDA for use in other solid tumors. These and other mTOR inhibitors, as well as inhibitors of PI3K and AKT pathways, are currently in clinical trials, alone or in combination with other therapies. However, it is also to be noted that both pre-clinical and clinical studies have reported that PTEN loss maybe insufficient to predict response to everolimus in GBM (Yang L et al., 2008; PMID: 18559622, Ma DJ et al., 2015; PMID: 25526733). Besides mTOR and AKT inhibitors, PARP inhibitors are also being considered for PTEN-deficient tumors. Pre-clinical studies have shown that GBM cell-lines harboring homozygous loss of PTEN are sensitive to PARP inhibitors (Mendes-Pereira AM et al., 2009; PMID: 20049735). It is to be noted that in GBM patients, loss of PTEN promotes cellular resistance to EGFR TKI therapy by dissociating EGFR inhibition from downstream PI3K pathway. Therefore, downstream inhibition of PI3K pathway could be combined with EGFR kinase inhibitors in patients with PTEN deficient tumors to promote responsiveness (Goudar RK et al., 2005; PMID:15657358, Mellinghoff IK et al 2005; PMID: 16282176).

Molecular Function

PTEN loss via homozygous deletion leads to activation of PI3K/AKT pathway and potential resistance to anti-EGFR monotherapy (Mellinghoff IK et al 2005; PMID: 16282176). Homozygous loss of PTEN has been reported to be common in GBM patients > 45-years of age and associated with shorter survival (Srividya MR et al., 2011; PMID: 21134002).

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Genomic Alteration		Therapeutic Implication	
Alteration:	RET (Amplification)	**Drug**	**Status**
Coordinate:	chr10:43625799	cabozantinib (Cometriq)	PREDICTED BENEFICIAL
Origin:	DNA	lenvatinib (Lenvima)	PREDICTED BENEFICIAL
		regorafenib (Stivarga)	PREDICTED BENEFICIAL
		sorafenib (Nexavar)	PREDICTED BENEFICIAL
		sunitinib (Sutent)	PREDICTED BENEFICIAL
		vandetanib (Caprelsa)	PREDICTED BENEFICIAL

Biomarker Summary

RET (Rearranged during Transfection) encodes Ret, a receptor tyrosine kinase and proto-oncogene that results in transformation of cells upon recombination with a partner gene (Takahashi et al., 1985; PMID:2992805). RET is activated by receptor dimerization, which is facilitated by RET binding to a complex formed through the binding of a ligand of the glial cell line-derived neurotrophic factor (GDNF) family to a GDNF family receptor. RET-mediated signaling regulates cell survival, cell growth, and cell differentiation and migration (Castellone MD and Santoro M, 2008; PMID: 18502331). RET amplification, activating mutations, or fusions may lead to activation of the Ret kinase. Therefore, kinase inhibitors targeting Ret may be relevant in a tumor with one of these RET alterations (Subbiah et al., 2014; PMID:25085632, Mei et al., 2014; PMID:24375110, Mologni et al., 2013; PMID:23811235, Sim and Cohen, 2014; PMID:24299515, Bentzien et al., 2013; PMID:23705946, Wilhelm et al., 2011; PMID:21170960, Tohyama et al., 2014; PMID:25295214, Okamoto et al., 2013; PMID:23856031). Several multi-tyrosine kinase inhibitors (TKIs), including regorafenib, sunitinib, vandetanib, cabozantinib, sorafenib, ponatinib, and lenvatinib, which target Ret, as well as other RET inhibitors, have received FDA approval in cancer therapy. In regards to ponatinib, despite its limited drug distribution or potential inability to cross the blood–brain barrier (demonstrated in clinical studies), it is being evaluated in GBM patients (Tan FH et al., 2019; PMID: 30705592).

Molecular Function

RET amplification can result in gain of function that results in ligand-independent kinase activation (Kato S et al., 2017; PMID: 27683183). RET amplification has been reported in certain tumor types and has been associated with RET mRNA overexpression (Nakashima et al., 2007; PMID:17270245, Platt et al., 2013; ASCO 2013, Abstract 8045, Huang et al., 2003; PMID:12519890, Ciampi et al., 2012; PMID:21867742). RET alterations including amplifications have been rarely reported in GBM (Kato S et al., 2017; PMID: 27683183).

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Genomic Alteration		Therapeutic Implication	
Alteration:	TERT (c.-124C>T)	**Drug**	**Status**
Alteration Type:	Upstream Gene Variant	See Clinical Trials Section	
Coordinate:	chr5:1295228		
Allele Frequency:	35%		
Transcript ID:	ENST00000310581		
Origin:	DNA		

Biomarker Summary

TERT (telomerase reverse transcriptase) encodes a ribonucleoprotein polymerase, an enzyme that maintains telomere ends by addition of the telomere repeat (TTAGGG). The enzyme consists of a protein and an RNA component (RefSeq). The protein component possesses reverse transcriptase activity, while the RNA component serves as a template for the telomere repeat. TERT plays a critical role in cellular aging and is repressed in somatic cells, resulting in progressive shortening of telomeres (Patel PL et al., 2016; PMID: 27503890). TERT may also play a role in chromosomal repair as a part of de novo synthesis of telomere repeats. Deregulation of TERT function, as a result of TERT promoter mutations, is associated with oncogenic events in certain tumor types (Pezzuto F et al., 2016; PMID: 27276713). In gliomas, the TERT promoter mutation (TPM) and resultant overexpression of TERT are observed mainly in the most aggressive (primary glioblastoma/grade IV astrocytoma) and the least aggressive (grade II oligodendroglioma) cases (Killela PJ et al., 2013; PMID: 23530248). TERT promoter mutations in adult GBM have been identified as a crucial step in glioma pathogenesis. Approximately 83% of primary GBMs exhibit a mutation of C to T in the promoter of TERT at either -124 or -146 bp upstream from the transcription start site (Bollam SR et al., 2018; PMID: 29525892). Investigational agents, like imetelstat (GRN163L), telomestatin, and telomelysin, have been found to be effective in GBM cells in vitro and in clinical studies (Bollam SR et al., 2018; PMID: 29525892). Glioblastoma patients with C228T TERT mutation and MGMT promoter methylation appeared to derive more benefit from temozolomide chemoradiotherapy (median overall survival 26.5 months, 95% CI 20.3-32.7) than patients with the C250T mutation (median overall survival 16.2 months, 95% CI 8.5-23.8) or patients without TERT mutation (median overall survival 23.7 months, 95% CI 18.7-28.8) (Weller M et al., J Clin Oncol 36, 2018 (suppl; abstr 2013). Further, GBM patients with TERT promoter mutation have a significantly decreased overall survival compared to those with wild type TERT, indicating an association with clinical aggressiveness (Jeong DE et al., 2017; PMID: 29344264).

Molecular Function

TERT c.-124C>T (also known as C124T) is a mutation in the GC-rich promoter sequence of the TERT gene. The C124T mutation leads to a structural misfolding of the promoter sequence, which functions as a transcription silencer, leading to structural and functional loss of TERT, and, consequently, TERT overexpression (Balasubramanian S et al., 2011; PMID: 21455236). The C124T is a recurrent hotspot mutation crucial for glioma pathogenesis (COSMIC, cBioPortal). Recently, a new molecular classification of gliomas using the TERT promoter mutation status has been reported to be highly predictive for survival (Schwaederle M et al., 2018;PMID: 29211306).

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Genomic Alteration		Therapeutic Implication	
Alteration:	ZNF33B/RET (Fusion)	**Drug**	**Status**
Alteration Type:	Fused Genes	cabozantinib (Cometriq)	PREDICTED BENEFICIAL
Coordinate:	chr10:43127377; chr10:43617394	lenvatinib (Lenvima)	PREDICTED BENEFICIAL
Transcript ID:	ENST00000359467.3; ENST00000355710.3	regorafenib (Stivarga)	PREDICTED BENEFICIAL
Origin:	RNA	sorafenib (Nexavar)	PREDICTED BENEFICIAL
		sunitinib (Sutent)	PREDICTED BENEFICIAL
		vandetanib (Caprelsa)	PREDICTED BENEFICIAL

Biomarker Summary

RET (Rearranged during Transfection) encodes Ret, a receptor tyrosine kinase and proto-oncogene that results in transformation of cells upon recombination with a partner gene (Takahashi et al., 1985; PMID 2992805). It functions as the receptor of growth factors of the glial cell line–derived neurotropic factor family. Binding of ligand facilitates RET kinase activation, which leads to activation of multiple downstream effectors, including MAPK and PI3K pathways. RET aberrations can result in gain-of-function via amplification, activating mutations or gene fusions. Therefore, kinase inhibitors targeting Ret may be relevant in a tumor with one of these RET alterations (Subbiah et al., 2014; PMID 25085632, Mei et al., 2014; PMID 24375110, Mologni et al., 2013; PMID 23811235, Sim and Cohen, 2014; PMID 24299515, Bentzien et al., 2013; PMID 23705946, Wilhelm et al., 2011; PMID 21170960, Tohyama et al., 2014; PMID 25295214, Okamoto et al., 2013; PMID 23856031). Several multi-tyrosine kinase inhibitors (TKIs), including regorafenib, sunitinib, vandetanib, cabozantinib, sorafenib, ponatinib, and lenvatinib, which target Ret, as well as other RET inhibitors, have received FDA approval in cancer therapy. In a neuroblastoma pre-clinical study, regorafenib showed efficacy by inhibiting tumor growth (Chen Z et al., 2017; PMID: 29262623). Sorafenib has conflicting efficacy of no or limited response as demonstrated by clinical studies of pediatric patients with refractory tumors (Okada K et al., 2016; PMID: 27264843; Kim A et al., 2015; PMID: 26207356).

Molecular Function

RET fusions have been commonly observed in several tumor types (Cerrato A et al., 2018; PMID: 29455670; Le Rolle AF et al., 2015; PMID:26078337; Santoro M et al., 2013; PMID: 24296167).The ZNF33B/RET is a novel fusion with a breakpoint in RET exon 16, which is expected to retain most of the kinase domain in the fusion protein. In the known RET fusions, the N-terminal partner contributes the promoter and N-terminal portion necessary for constitutive activation of RET kinase domain (Cerrato A et al., 2018; PMID: 29044514). Most fusions occur at a breakpoint in Intron 11 of RET (Mizukami T et al., 2014; PMID: 24722152). RET fusions are known to form homodimers that activate the MAPK/ERK pathway and reduce DNA repair efficacy in TK-activated cancer cells (Cerrato A et al., 2018; PMID: 29044514). In addition, an increase in EGFR activity can induce RET TKI resistance. Therefore, reversible EGFR-TKI compounds, like gefitinib, and combination therapy of RET and EGFR TKIs may be beneficial to overcome RET TKI resistance (Chang H et al., 2017; PMID: 27873490).

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Drug Evidence Detail

Literature Supporting Therapeutic Implication

Drug	Gene	Therapeutic Implication
abemaciclib (Verzenio)	CDKN2A (Deletion)	PREDICTED BENEFICIAL

A phase I dose escalation study of abemaciclib in 225 patients with solid tumors was followed by evaluation in tumor-specific cohorts in 192 patients with solid tumors including breast cancer (n=47), NSCLC (n = 68), GBM (n = 17), melanoma (n = 26) and CRC (n = 15). Consistent with detection of abemaciclib in cerebrospinal-fluid from patients at concentrations associated with target inhibition, 3 patients with GBM achieved SD, and 2 of these patients continued to receive treatment without progression for 19 and 23 cycles, respectively. Both GBM cases with durable disease control on single-agent abemaciclib had TP53 mutations, and one also had a frameshift mutation in EGFR. These results are compatible with distribution of abemaciclib to the central nervous system.

https://www.ncbi.nlm.nih.gov/pubmed/27217383 *(Patnaik A et al., Cancer Discov. 2016 Jul;6(7):740-53)*

Drug	Gene	Therapeutic Implication
palbociclib (Ibrance)	CDKN2A (Deletion)	PREDICTED BENEFICIAL

In a phase 2 trial of palbociclib in adult patients with RB expressing recurrent, heavily pre-treated GBM, 22 patients were administered oral palbociclib. The median progression free survival for all patients was 5.14 weeks (range 5 days – 142 weeks) and median overall survival was 15.4 weeks (range 2 – 274 weeks). Two patients (10%) had treatment related AEs that were grade ≥3.

https://doi.org/10.1093/neuonc/nox036.314 *(Taylor JW et al., Neuro-Oncology, Volume 19, Issue suppl_3, 1 May 2017)*

Drug	Gene	Therapeutic Implication
ribociclib (Kisqali)	CDKN2A (Deletion)	PREDICTED BENEFICIAL

In a phase 0/2 study of ribociclib in patients with recurrent GBM, 12 eligible patients with intact RB expression and CDKN2A deletion or CDK4/6 amplification were enrolled into the Phase 0 component to receive ribociclib (900mg daily) for 5 days prior to tumor resection. Ribociclib penetrated both enhancing and non-enhancing regions of the tumor. In non-enhancing tissue, median unbound brain-to-plasma ratio was 1.78 and median ribociclib concentration was 0.54 nmol/mL, exceeding the in vitro IC50 for CDK4/6 (0.04 nmol/mL). Suppression of G1-to-S phase was inferred by a decrease in RB phosphorylation (p<0.01) and cell proliferation (p<0.05). Six patients (50%) were graduated to the Phase 2 component and demonstrated a median progression-free survival of 9.7 weeks. It was concluded that Ribociclib penetrates the tumor-brain barrier, achieving pharmacologically-active concentrations in human GBM and suppressing tumor proliferation. However, ribociclib would be ineffective as a monotherapy due to secondary resistance induced by mTOR pathway activation, and analysis of ribociclib-resistant tumors identified the addition of an mTOR inhibitor as a dual-drug strategy for recurrent glioblastoma.

https://academic.oup.com/neuro-oncology/article-abstract/20/suppl_6/vi21/5154238 *(Tien A-C et al., Neuro-Oncology, Volume 20, Issue suppl_6, 5 November 2018, Pages vi21)*

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Drug	Gene	Therapeutic Implication
afatinib (Gilotrif)	EGFR (A289V)	PREDICTED BENEFICIAL

A 58-year-old female patient diagnosed with multifocal grade IV GBM which harbored a number of EGFR aberrations including EGFR amplification, EGFRvIII mutation and EGFR P596L and G598V subclonal somatic mutations. An additional VUS mutation D247Y was seen in the extracellular domain of EGFR along with a null mutation in PTEN (R130). After 1 cycle of 2nd-line treatment with afatinib, MRI revealed minimal decrease in lesion size of patient. After 5 cycles, significant disease regression was observed and maintained in subsequent assessments to 54 months. A full spine MRI at this time showed no evidence of metastases in the cervical, thoracic or lumbar regions, and the most current MRI showed SD. At the time of publication (April 2015), the patient had completed 63 cycles and treatment was ongoing.*

https://www.ncbi.nlm.nih.gov/pubmed/26423602 *(Alshami J et al., Oncotarget. 2015 Oct 20;6(32):34030-7)*

A Phase 2 trial of afatinib, in solid tumor patients harboring EGFR/HER2 gene amplification (n=20) reported objective response. Of 385 prescreened patients, 38 had FISH-positive tumors (10 with EGFR amplification and 29 with HER2 amplification or high polysomy [1 tumor had EGFR/HER2 high polysomy]; none had EGFR-activating mutations), and 20 patients received treatment with afatinib 50 mg daily. The objective response rate was 5% (1 of 20 patients), and the best objective response included 1 complete response. Eight patients experienced stable disease.

http://www.ncbi.nlm.nih.gov/pubmed/23775486 *(Kwak EL et al., Cancer, 2013; 119(16): 3043-51)*

A Phase I/randomized phase II study of afatinib, an irreversible ERBB family blocker, with or without protracted temozolomide in adults with recurrent glioblastoma showed limited single agent activity in unselected GBM patients. Among participants with EGFR amplification demonstrated by FISH analysis (there was no correlation with EGFRvIII status), a median PFS of 2.73 months was noted for afatinib plus temozolomide and 1.02 months for temozolomide monotherapy.

https://www.ncbi.nlm.nih.gov/pubmed/25140039 *(Reardon DA et al., Neuro Oncol. 2015 Mar;17(3):430-9)*

Drug	Gene	Therapeutic Implication
erlotinib (Tarceva)	EGFR (A289V)	PREDICTED BENEFICIAL

A case report of a 31-year-old male patient presenting with GBM showed brief response to erlotinib therapy. After PD with conventional treatment, NGS analysis revealed EGFR A289V and P773delinsPP mutations along with 14-fold EGFR amplification. Erlotinib was started based on EGFR alterations, and in the first 3 months there was improvement in response with less edema and reduced compression of the lateral ventricles. However, after 4 months patient developed progressive neurological deterioration and died due to secondary disease progression.

https://www.ncbi.nlm.nih.gov/pubmed/28611289 *(Zhou K et al., Oncotarget. 2017 Jul 25;8(30):50305-50313)*

Following a GBM clinical trial with EGFR kinase inhibitors, genomic analysis was performed to identify mutations linked to clinical response. Analysis of available tumor DNA samples from the trial identified an EGFR ectodomain mutation in one sample derived from a responder to erlotinib, and another who failed. The latter sample also carried loss of PTEN. It was concluded that failure to respond to erlotinib in the second case might be attributed to PTEN loss, and that response to EGFR TKI may be dependent on the presence of an intact PTEN.

https://www.ncbi.nlm.nih.gov/pubmed/17177598 *(Lee JC et al., PLoS Med. 2006 Dec;3(12):e485)*

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Drug	Gene	Therapeutic Implication
gefitinib (Iressa)	EGFR (A289V)	PREDICTED BENEFICIAL

A Phase 2 trial of gefitinib in recurrent glioblastoma patients reported 6 month event-free survival in 13% (7/53) of patients, median OS of 39.4 weeks and median event-free survival of 8.1 weeks; EGFR expression did not correlate with either EFS or OS.
https://www.ncbi.nlm.nih.gov/pubmed/14638850 *(Rich JN et al., J Clin Oncol, 2004; 22(1): 133-42)*

In a cohort of GBM patients treated with gefitinib, EGFR mutations with wildtype PTEN was associated with significantly better PFS and OS. Patients positive for both EGFR/PTEN mutations had lower DFS and OS of 6 & 9 months as compared to 6 and 14 months for those negative for both EGFR/PTEN mutations.
https://www.ncbi.nlm.nih.gov/pubmed/29444555 *(Arif SH et al., 2018; PMID: 29444555)*

Drug	Gene	Therapeutic Implication
lapatinib (Tykerb)	EGFR (A289V)	PREDICTED BENEFICIAL

In a pilot study of lapatinib dosing in 10 patients with pre-treated GBM, high dose lapatinib was well tolerated based on which expanded phase II trial was suggested for evaluation.
http://ascopubs.org/doi/abs/10.1200/jco.2014.32.15_suppl.2045 *(Nghiemphu PL et al., JCO Precision Oncology, 2014 May; 2045)*

A phase I study of temozolomide and lapatinib combination in patients with recurrent high-grade gliomas enrolled 16 patients. Median progression-free survival (PFS) and survival were 2.4 and 5.9 months, respectively. EGFR amplification and EGFRvIII expression were not related to PFS. Combination of TMZ and LP is feasible with manageable toxicity.
https://www.ncbi.nlm.nih.gov/pubmed/23292205 *(Karavasilis V et al., J Neurol. 2013 Jun;260(6):1469-80)*

Drug	Gene	Therapeutic Implication
neratinib (Nerlynx)	EGFR (A289V)	PREDICTED BENEFICIAL

In a phase I study with neratinib in patients with advanced solid tumors, PR was observed for 8 (32%) of the 25 evaluable patients with breast cancer. Stable disease >or=24 weeks was observed in one breast cancer patient, and 6 (43%) of the 14 evaluable NSCLC patients.
http://www.ncbi.nlm.nih.gov/pubmed/19318484 *(Wong KK et a., Clin Cancer Res, 2009; 15(7): 2552-2558)*

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Drug	Gene	Therapeutic Implication
afatinib (Gilotrif)	EGFR (Amplification)	PREDICTED BENEFICIAL

A 58 year old female patient diagnosed with multifocal glioma, GBM grade IV which harbored a number of EGFR aberrations. Tumor was positive for EGFR amplification, EGFRvIII mutation and EGFR P596L and G598V subclonal somatic mutations. An additional VUS mutation D247Y was seen in the extracellular domain of EGFR along with a null mutation in PTEN (R130). After 1 cycle of second-line treatment with afatinib, MRI revealed minimal decrease in lesion size of patient. After 5 cycles, significant disease regression was observed and maintained in subsequent assessments to 54 months. A full spine MRI performed in November 2014 showed no evidence of metastases in the cervical, thoracic or lumbar regions and the most recent MRI (January 2015) showed stable disease. At the time of publication (April 2015), the patient had completed 63 cycles and treatment was ongoing.*
https://www.ncbi.nlm.nih.gov/pubmed/26423602 *(Alshami J et al., Oncotarget. 2015 Oct 20;6(32):34030-7)*

A Phase 2 trial of afatinib, in solid tumor patients harboring EGFR/HER2 gene amplification (n=20) reported objective response. Of 385 prescreened patients, 38 had FISH-positive tumors (10 with EGFR amplification and 29 with HER2 amplification or high polysomy [1 tumor had EGFR/HER2 high polysomy]; none had EGFR-activating mutations), and 20 patients received treatment with afatinib 50 mg daily. The objective response rate was 5% (1 of 20 patients), and the best objective response included 1 complete response. Eight patients experienced stable disease.
http://www.ncbi.nlm.nih.gov/pubmed/23775486 *(Kwak EL et al., Cancer, 2013; 119(16): 3043-51)*

A Phase I/randomized phase II study of afatinib, an irreversible ERBB family blocker, with or without protracted temozolomide in adults with recurrent glioblastoma showed limited single agent activity in unselected GBM patients. Among participants with EGFR amplification demonstrated by FISH analysis (there was no correlation with EGFRvIII status), a median PFS of 2.73 months was noted for afatinib plus temozolomide and 1.02 months for temozolomide monotherapy.
https://www.ncbi.nlm.nih.gov/pubmed/25140039 *(Reardon DA et al., Neuro Oncol. 2015 Mar;17(3):430-9)*

Drug	Gene	Therapeutic Implication
erlotinib (Tarceva)	EGFR (Amplification)	PREDICTED BENEFICIAL

A Phase 1/2 study of erlotinib in combination with temsirolimus in glioblastoma patients reported SD in 29% (12/41) of patients; patients with elevated phospho–extracellular signal-regulated kinase or reduced phosphatase and tensin homolog protein expression had decreased progression-free survival at 4 months.
http://www.ncbi.nlm.nih.gov/pubmed/?term=24470557 *(Wen PY et al., Neuro Oncol, 2014; 16(4): 567-78)*

A case report of a 31-year-old male patient presenting with GBM showed brief response to erlotinib therapy. After PD with conventional treatment, NGS analysis revealed EGFR A289V and P773delinsPP mutations along with 14-fold EGFR amplification. Erlotinib was started based on EGFR alterations, and in the first 3 months there was improvement in response with less edema and reduced compression of the lateral ventricles. However, after 4 months patient developed progressive neurological deterioration and died due to secondary disease progression.
https://www.ncbi.nlm.nih.gov/pubmed/28611289 *(Zhou K et al., Oncotarget. 2017 Jul 25;8(30):50305-50313)*

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Drug	Gene	Therapeutic Implication
gefitinib (Iressa)	EGFR (Amplification)	PREDICTED BENEFICIAL

A Phase 2 trial of gefitinib in recurrent glioblastoma patients reported 6 month event-free survival in 13% (7/53) of patients, median OS of 39.4 weeks and median event-free survival of 8.1 weeks; EGFR expression did not correlate with either EFS or OS.
http://www.ncbi.nlm.nih.gov/pubmed/14638850 *(Rich JN et al., J Clin Oncol, 2004; 22(1): 133-42)*

In a cohort of GBM patients treated with gefitinib, EGFR mutations with wildtype PTEN was associated with significantly better PFS and OS. Patients positive for both EGFR/PTEN mutations had lower DFS and OS of 6 & 9 months as compared to 6 and 14 months for those negative for both EGFR/PTEN mutations.
https://www.ncbi.nlm.nih.gov/pubmed/29444555 *(Arif SH et al., 2018; J Neurosurg Sci. 2018)*

Drug	Gene	Therapeutic Implication
lapatinib (Tykerb)	EGFR (Amplification)	PREDICTED BENEFICIAL

In a pilot study of lapatinib dosing in 10 patients with pre-treated GBM, high dose lapatinib was well tolerated based on which expanded phase II trial was suggested for evaluation.
http://ascopubs.org/doi/abs/10.1200/jco.2014.32.15_suppl.2045 *(Nghiemphu PL et al., JCO Precision Oncology, 2014 May; 2045)*

A phase I study of temozolomide and lapatinib combination in patients with recurrent high-grade gliomas enrolled 16 patients. Median progression-free survival (PFS) and survival were 2.4 and 5.9 months, respectively. EGFR amplification and EGFRvIII expression were not related to PFS. Combination of TMZ and LP is feasible with manageable toxicity.
https://www.ncbi.nlm.nih.gov/pubmed/23292205 *(Karavasilis V et al., J Neurol. 2013 Jun;260(6):1469-80)*

Drug	Gene	Therapeutic Implication
neratinib (Nerlynx)	EGFR (Amplification)	PREDICTED BENEFICIAL

In a phase I study with neratinib in patients with advanced solid tumors, PR was observed for 8 (32%) of the 25 evaluable patients with breast cancer. Stable disease >or=24 weeks was observed in one breast cancer patient, and 6 (43%) of the 14 evaluable NSCLC patients.
http://www.ncbi.nlm.nih.gov/pubmed/19318484 *(Wong KK et a., Clin Cancer Res, 2009; 15(7): 2552-2558)*

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Drug	Gene	Therapeutic Implication
afatinib (Gilotrif)	EGFR (EGFRvIII)	PREDICTED BENEFICIAL

A 58 year old female patient diagnosed with multifocal glioma, GBM grade IV which harbored a number of EGFR aberrations. Tumor was positive for EGFR amplification, EGFRvIII mutation and EGFR P596L and G598V subclonal somatic mutations. An additional VUS mutation D247Y was seen in the extracellular domain of EGFR along with a null mutation in PTEN (R130). After 1 cycle of second-line treatment with afatinib, MRI revealed minimal decrease in lesion size of patient. After 5 cycles, significant disease regression was observed and maintained in subsequent assessments to 54 months. A full spine MRI performed in November 2014 showed no evidence of metastases in the cervical, thoracic or lumbar regions and the most recent MRI (January 2015) showed stable disease. At the time of publication (April 2015), the patient had completed 63 cycles and treatment was ongoing.*

https://www.ncbi.nlm.nih.gov/pubmed/26423602 *(Alshami J et al., Oncotarget. 2015 Oct 20;6(32):34030-7)*

In a phase I/II randomized study of afatinib in adults with recurrent GBM, median PFS was longer in afatinib-treated participants with EFGRvIII-positive tumors versus EGFRvIII-negative tumors.

https://www.ncbi.nlm.nih.gov/pubmed/25140039 *(Reardon DA et al., Neuro Oncol. 2015 Mar;17(3):430-9)*

In a phase II study of afatinib (A) with or without temozolomide (T) in the treatment of patients with recurrent glioblastoma, 119 pts were randomized 1:1:1 to receive A, AT or T. PFS-6 by investigator assessment was 3%, 17% and 22% in the A, AT and T arms, respectively. Best overall response included partial response in one, five and six pts and SD in 22, 20 and 21 pts in A, AT and T, respectively.Preliminary biomarker data in 54 pts suggested durable disease control in EGFRvIII-positive pts treated with A/AT.

http://ascopubs.org/doi/abs/10.1200/jco.2011.29.15_suppl.2010 *(Eisenstat DD et al., Journal of Clinical Oncology 29, no. 15_suppl (May 2011) 2010-2010)*

Drug	Gene	Therapeutic Implication
erlotinib (Tarceva)	EGFR (EGFRvIII)	PREDICTED BENEFICIAL

A case report of a patient with recurrent GBM who showed coexpression of EGFRvIII and PTEN achieved partial response with improvement of neurologic symptoms when treated with erlotinib for 4 months.

https://www.ncbi.nlm.nih.gov/pubmed/20462843 *(Custodio A et al., Clin Transl Oncol. 2010 Apr;12(4):310-4)*

In a cohort of 49 patients with recurrent malignant glioma who were treated with EGFR kinase inhibitors, 9 had tumor shrinkage of at least 25 percent. Pretreatment tissue was available for molecular analysis from 26 patients, 7 of whom had had a response and 19 of whom had rapid progression during therapy. Coexpression of EGFRvIII and PTEN was significantly associated with a clinical response (P<0.001). These findings were validated in 33 patients who received similar treatment for glioblastoma at a different institution.

https://www.ncbi.nlm.nih.gov/pubmed/16282176 *(Mellinghoff IK et al., 2005; PMID: 16282176)*

Drug	Gene	Therapeutic Implication
gefitinib (Iressa)	EGFR (EGFRvIII)	PREDICTED BENEFICIAL

In a cohort of 49 patients with recurrent malignant glioma who were treated with EGFR kinase inhibitors, 9 had tumor shrinkage of at least 25 percent. Pretreatment tissue was available for molecular analysis from 26 patients, 7 of whom had had a response and 19 of whom had rapid progression during therapy. Coexpression of EGFRvIII and PTEN was significantly associated with a clinical response (P<0.001). These findings were validated in 33 patients who received similar treatment for glioblastoma at a different institution.

https://www.ncbi.nlm.nih.gov/pubmed/16282176 *(Mellinghoff IK et al., 2005; PMID: 16282176)*

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Drug	Gene	Therapeutic Implication
afatinib (Gilotrif)	EGFR (EGFRvIVb)	PREDICTED BENEFICIAL

A 58 year old female patient diagnosed with multifocal glioma, GBM grade IV which harbored a number of EGFR aberrations. Tumor was positive for EGFR amplification, EGFRvIII mutation and EGFR P596L and G598V subclonal somatic mutations. An additional VUS mutation D247Y was seen in the extracellular domain of EGFR along with a null mutation in PTEN (R130). After 1 cycle of second-line treatment with afatinib, MRI revealed minimal decrease in lesion size of patient. After 5 cycles, significant disease regression was observed and maintained in subsequent assessments to 54 months. A full spine MRI performed in November 2014 showed no evidence of metastases in the cervical, thoracic or lumbar regions and the most recent MRI (January 2015) showed stable disease. At the time of publication (April 2015), the patient had completed 63 cycles and treatment was ongoing.*

https://www.ncbi.nlm.nih.gov/pubmed/26423602 *(Alshami J et al., Oncotarget. 2015 Oct 20;6(32):34030-7)*

In a phase I/II randomized study of afatinib in adults with recurrent GBM, median PFS was longer in afatinib-treated participants with EFGRvIII-positive tumors versus EGFRvIII-negative tumors.

https://www.ncbi.nlm.nih.gov/pubmed/25140039 *(Reardon DA et al., Neuro Oncol. 2015 Mar;17(3):430-9)*

In a phase II study of afatinib (A) with or without temozolomide (T) in the treatment of patients with recurrent glioblastoma, 119 pts were randomized 1:1:1 to receive A, AT or T. PFS-6 by investigator assessment was 3%, 17% and 22% in the A, AT and T arms, respectively. Best overall response included partial response in one, five and six pts and SD in 22, 20 and 21 pts in A, AT and T, respectively.Preliminary biomarker data in 54 pts suggested durable disease control in EGFRvIII-positive pts treated with A/AT.

http://ascopubs.org/doi/abs/10.1200/jco.2011.29.15_suppl.2010 *(Eisenstat DD et al., Journal of Clinical Oncology 29, no. 15_suppl (May 2011) 2010-2010)*

Drug	Gene	Therapeutic Implication
cetuximab (Erbitux)	EGFR (EGFRvIVb)	PREDICTED BENEFICIAL

A phase 2 clinical trial of cetuximab in patients with recurrent glioblastoma reported superior progression free survival (PFS) [median PFS 3.03 vs. 1.63 months (p=0.006)] and better overall survival [median OS 5.57 vs. 3.97 months (p=0.12] in patients with an EGFR amplification lacking EGFRvIII expression.

http://www.ncbi.nlm.nih.gov/pubmed/22752145 *(Lv S, Int J Oncol, 2012;41(3):1029-35)*

Fifty-five eligible high grade glioma patients (28 with and 27 without EGFR amplification) tolerated cetuximab well. Three patients (5.5%) had a PR and 16 patients (29.6%) had SD. The median TTP was 1.9 months. Whereas PFS was <6 months in the majority (n = 50/55) of patients, five patients (9.2%) had a PFS on cetuximab of >9 months. Median OS was 5.0 months (95% CI 4.2–5.9 months). No significant correlation was found between response, survival and EGFR amplification.

https://www.ncbi.nlm.nih.gov/pubmed/19491283 *(Neyns B et al., Ann Oncol. 2009 Sep;20(9):1596-603)*

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Drug	Gene	Therapeutic Implication
erlotinib (Tarceva)	EGFR (EGFRvIVb)	PREDICTED BENEFICIAL

A Phase 1/2 study of erlotinib in combination with temsirolimus in glioblastoma patients reported SD in 29% (12/41) of patients; patients with elevated phospho–extracellular signal-regulated kinase or reduced phosphatase and tensin homolog protein expression had decreased progression-free survival at 4 months.
http://www.ncbi.nlm.nih.gov/pubmed/?term=24470557 *(Wen PY et al., Neuro Oncol, 2014; 16(4): 567-78)*

A case report of a 31-year-old male patient presenting with GBM showed brief response to erlotinib therapy. After PD with conventional treatment, NGS analysis revealed EGFR A289V and P773delinsPP mutations along with 14-fold EGFR amplification. Erlotinib was started based on EGFR alterations, and in the first three months there was improvement in response with less edema and reduced compression of the lateral ventricles. However, at the fourth month, patient had progressive neurological deterioration and died due to secondary disease progression.
https://www.ncbi.nlm.nih.gov/pubmed/28611289 *(Zhou K et al., Oncotarget. 2017 Jul 25;8(30):50305-50313)*

In a study of 49 recurrent malignant glioma patients treated with erlotinib or gefitinib, 9 patients had tumor shrinkage of at least 25%. Out of 7 patients that responded to EGFR inhibitors, 3 patients had EGFR amplification and no PTEN loss. Out of patients with EGFR amplification that did not respond to EGFR kinase inhibitors, majority had PTEN loss which may have contributed to treatment failure. However, due to low sample size the authors did not find an association between EGFR amplification and response to erlotinib.
https://www.ncbi.nlm.nih.gov/pubmed/16282176 *(Mellinghoff IK et al., N Engl J Med. 2005 Nov 10;353(19):2012-24)*

Drug	Gene	Therapeutic Implication
osimertinib (Tagrisso)	EGFR (EGFRvIVb)	PREDICTED BENEFICIAL

The randomized Phase 3 FLAURA trial of osimertinib versus gefitinib or erlotinib in TKI-naïve advanced NSCLC harboring EGFR exon 19 deletion or L858R mutation, has reported progression-free survival (PFS) of 18.9 months, an overall response rate of 80% (223/279), and a median duration of response (DoR) of 17.2 months in the osimertinib arm, while the other treatment arm reported PFS of 10.2 months, an overall response rate of 76% (211/277), and median DoR of 8.5 months.
https://www.ncbi.nlm.nih.gov/pubmed/29151359 *(Soria JC et al., 2018; N Engl J Med. 2018 Jan 11;378(2):113-125)*

Drug	Gene	Therapeutic Implication
afatinib (Gilotrif)	EGFR (R108K)	PREDICTED BENEFICIAL

A 58-year-old female patient diagnosed with multifocal grade IV GBM which harbored a number of EGFR aberrations including EGFR amplification, EGFRvIII mutation and EGFR P596L and G598V subclonal somatic mutations. An additional VUS mutation D247Y was seen in the extracellular domain of EGFR along with a null mutation in PTEN (R130). After 1 cycle of 2nd-line treatment with afatinib, MRI revealed minimal decrease in lesion size of patient. After 5 cycles, significant disease regression was observed and maintained in subsequent assessments to 54 months. A full spine MRI at this time showed no evidence of metastases in the cervical, thoracic or lumbar regions, and the most current MRI showed SD. At the time of publication (April 2015), the patient had completed 63 cycles and treatment was ongoing.*
https://www.ncbi.nlm.nih.gov/pubmed/26423602 *(Alshami J et al., Oncotarget. 2015 Oct 20;6(32):34030-7)*

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Drug	Gene	Therapeutic Implication
erlotinib (Tarceva)	EGFR (R108K)	PREDICTED BENEFICIAL

A case report of a 31-year-old male patient presenting with GBM showed brief response to erlotinib therapy. After disease progression with conventional treatment, NGS was done and sample harbored EGFR A289V and P773delinsPP mutations along with 14-fold EGFR amplification. Erlotinib was started based on EGFR alterations and in the first three months GBM tumor was improved with less observed edema and reduced compression of the lateral ventricles. However, at the fourth month, patient had progressive neurological deterioration and died due to secondary disease progression.

https://www.ncbi.nlm.nih.gov/pubmed/28611289 *(Zhou K et al., Oncotarget. 2017 Jul 25;8(30):50305-50313)*

In a GBM clinical trial, ectodomain mutations in EGFR were noted in GBM patients. The ectodomain mutant R108K-EGFR was noted in 14% (1/7) gliomas that responded to erlotinib. This tumor, however, also expressed EGFRvIII, along with EGFR amplification and no PTEN loss, raising the possibility of independent clones arising from a common progenitor with EGFR amplification. The R108K EGFR mutation was also identified in 7% (1/5) gliomas that failed erlotinib, but loss of PTEN in the tumor sample provides a potential explanation for treatment failure.

https://www.ncbi.nlm.nih.gov/pubmed/17177598 *(Lee JC et al., PLoS Med. 2006 Dec;3(12): e485)*

Drug	Gene	Therapeutic Implication
gefitinib (Iressa)	EGFR (R108K)	PREDICTED BENEFICIAL

In a cohort of GBM patients treated with gefitinib, EGFR mutations with wildtype PTEN was associated with significantly better PFS and OS. Patients positive for both EGFR/PTEN mutations had lower DFS and OS of 6 & 9 months as compared to 6 and 14 months for those negative for both EGFR/PTEN mutations.

https://www.ncbi.nlm.nih.gov/pubmed/29444555 *(Arif SH et al., 2018; PMID: 29444555)*

Drug	Gene	Therapeutic Implication
lapatinib (Tykerb)	EGFR (R108K)	PREDICTED BENEFICIAL

A phase I study of temozolomide and lapatinib combination in patients with recurrent high-grade gliomas enrolled 16 patients. Median progression-free survival (PFS) and survival were 2.4 and 5.9 months, respectively. EGFR amplification and EGFRvIII expression were not related to PFS. Combination of TMZ and LP is feasible with manageable toxicity.

https://www.ncbi.nlm.nih.gov/pubmed/23292205 *(Karavasilis V et al., J Neurol. 2013 Jun;260(6):1469-80)*

In a pilot study of lapatinib dosing in 10 patients with pre-treated GBM, high dose lapatinib was well tolerated based on which expanded phase II trial was suggested for evaluation.

http://ascopubs.org/doi/abs/10.1200/jco.2014.32.15_suppl.2045 *(Nghiemphu PL et al., JCO Precision Oncology, 2014 May; 2045)*

Drug	Gene	Therapeutic Implication
neratinib (Nerlynx)	EGFR (R108K)	PREDICTED BENEFICIAL

In a phase I study with neratinib in patients with advanced solid tumors, partial response was observed for 8 (32%) of the 25 evaluable patients with breast cancer. Stable disease >or=24 weeks was observed in one evaluable breast cancer patient and 6 (43%) of the 14 evaluable non-small cell lung cancer patients. Antitumor activity was observed in patients with breast cancer who had previous treatment with trastuzumab, anthracyclines, and taxanes, and tumors with a baseline ErbB-2 immunohistochemical staining intensity of 2+ or 3+.

http://www.ncbi.nlm.nih.gov/pubmed/19318484 *(Wong KK et a., Clin Cancer Res, 2009; 15(7): 2552-2558)*

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Drug	Gene	Therapeutic Implication
copanlisib (Aliqopa)	PTEN (Deletion)	PREDICTED BENEFICIAL

In a phase I dose escalation study of copanlisib plus gemcitabine or with cisplatin plus gemcitabine in advanced cancer patients, two cholangiocarcinoma patients with objective tumor response had PTEN protein loss, one with a co-occuring KRAS mutation and the other with a BRAF mutation. Response rates were as follows: copanlisib plus gemcitabine, 6.3% (one partial response in a patient with peritoneal carcinoma); copanlisib plus CisGem, 12% (one complete response and three partial responses all in patients with biliary tract cancer (BTC) (response rate 17.4% in patients with BTC).

https://www.ncbi.nlm.nih.gov/pubmed/29348486 *(Kim RD et al., Br J Cancer. 2018 Feb 20;118(4):462-470)*

Drug	Gene	Therapeutic Implication
everolimus (Afinitor)	PTEN (Deletion)	PREDICTED BENEFICIAL

A phase I trial was designed to determine the recommended phase II dose(s) of everolimus (RAD001) with temozolomide (TMZ) in glioblastoma (GBM) patients. In the subset of 28 patients with measurable disease, 3 had partial responses (all NEIAEDs) and 16 had stable disease.

https://www.ncbi.nlm.nih.gov/pubmed/?term=22160854 *(Mason WP et al., Invest New Drugs. 2012 Dec;30(6):2344-51)*

A Phase 1 trial of everolimus and temozolomide in combination with radiation therapy in glioblastoma multiforme patients reported partial response/regression in 12% (2/18) of patients and stable disease in 83% (15/18) of patients.

http://www.ncbi.nlm.nih.gov/pubmed/20864273 *(Sarkaria JN et al., Int J Radiat Oncol Biol Phys, 2011; 81(2): 468-75)*

A Phase 2 trial of combination of radiation therapy, temozolomide, and bevacizumab followed by bevacizumab/everolimus as first-line treatment in 68 glioblastoma patients reported median PFS of 11.3 months and median OS of 13.9 months.

http://www.ncbi.nlm.nih.gov/pubmed/22706484 *(Hainsworth JD et al., Clin Adv Hematol Oncol, 2012; 10(4): 240-46)*

Drug	Gene	Therapeutic Implication
temsirolimus (Torisel)	PTEN (Deletion)	PREDICTED BENEFICIAL

A Phase 2 trial of temsirolimus in recurrent glioblastoma multiforme patients (n=65) reported PFS of 7.8% at 6 months and median OS of 4.4 months. Radiographic improvement was reported to be significantly correlated with high levels of phosphorylated p70s6 kinase in baseline tumor samples (P = .04).

http://www.ncbi.nlm.nih.gov/pubmed/?term=15998902 *(Galanis E et al., J Clin Oncol, 2005; 23(23): 5294-304)*

In a phase 1 trial of gefitinib plus sirolimus in 34 adults with malignant glioma who were pre-treated with radio- and chemotherapy, gefitinib exposure was not affected by sirolimus administration. Two patients (6%) achieved a partial radiographic response, and 13 patients (38%) achieved SD.

https://www.ncbi.nlm.nih.gov/pubmed/16467100 *(Reardon DA et al., Clin Cancer Res. 2006 Feb 1;12(3 Pt 1):860-8)*

Malignant gliomas frequently harbor amplification of the epidermal growth factor receptor (EGFR) and loss of PTEN tumor suppressor gene. Twenty-eight heavily pretreated patients with recurrent malignant gliomas were administered EGFR inhibitors (gefitinib or erlotinib) in combination with the mTOR (mammalian target of rapamycin) inhibitor sirolimus. The regimens were reasonably well tolerated. Nineteen percent of patients experienced a partial response and 50% had stable disease. Six-month progression-free survival for glioblastoma patients was 25%.

https://www.ncbi.nlm.nih.gov/pubmed/16832099 *(Doherty et al., Neurology. 2006 Jul 11;67(1):156-8)*

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Drug	Gene	Therapeutic Implication
cabozantinib (Cometriq)	RET (Amplification)	PREDICTED BENEFICIAL

A phase I trial of cabozantinib with temozolomide and radiotherapy or temozolomide after radiotherapy in 26 newly diagnosed patients with high grade glioma patients was well tolerated and demonstrated no pharmacokinetic interactions with concurrent temozolomide.
https://www.ncbi.nlm.nih.gov/pubmed/26588662 *(Schiff D et al., Cancer. 2016 Feb 15;122(4):582-7)*

In a phase II study of cabozantinib in patients with progressive GBM, a subset of 70 patients who had received prior anti-angiogenic therapy, the objective response rate was 4.3%, and the median duration of response was 4.2 months. The proportion of patients alive and progression free at 6 months was 8.5%. Median PFS was 2.3 months, and median OS was 4.6 months. Cabozantinib treatment appeared to have modest clinical activity with a 4.3% response rate in patients who had received prior antiangiogenic therapy for GBM.
https://www.ncbi.nlm.nih.gov/pubmed/29036345 *(Cloughesy TF et al., Neuro Oncol. 2018 Jan 22;20(2):259-267)*

In a phase II study of cabozantinib in patients with progressive GBM, a subset of 152 patients naive to prior antiangiogenic therapy, the objective response rate was 17.6% and 14.5% in the 140 mg/day and 100 mg/day groups, respectively, which did not meet the predefined statistical target for success. The proportions of patients alive and progression free at 6 months were 22.3% and 27.8%, respectively. Median progression-free survival was 3.7 months in both groups, and median overall survival was 7.7 months and 10.4 months, respectively. It was concluded that cabozantinib showed evidence of clinical activity in patients with recurrent GBM naive to antiangiogenic therapy, although the predefined statistical target for success was not met.
https://www.ncbi.nlm.nih.gov/pubmed/29016998 *(Wen PY et al., Neuro Oncol. 2018 Jan 22;20(2):249-258)*

Drug	Gene	Therapeutic Implication
lenvatinib (Lenvima)	RET (Amplification)	PREDICTED BENEFICIAL

A phase 1 study of lenvatinib in patients with advanced, refractory solid tumours, reported a clinical benefit (PR or SD) in 55% of patients treated with lenvatinib, where 7/82 (9%) had a PR and 38/82 patients (46%) had SD as best response.
http://www.ncbi.nlm.nih.gov/pubmed/22516948 *(Boss D et al., British Journal of Cancer, 2012; 10:1598-604)*

A phase 1 study of lenvatinib in patients with solid tumors reported 12/77 (15.6%) patients achieved confirmed PR (n=9) or unconfirmed PR (n=3), and 19 (24.7%) achieved SD ≥23 weeks. Total PR/uPR/SD ≥23 weeks was 40.3% (n=31). Responses (PR/uPR) by disease were: melanoma, 5/29 patients (includes 1 patient with NRAS mutation); thyroid, 3/6; pancreatic, 1/2; lung, 1/1; renal, 1/1; endometrial, 1/4; and ovarian, 1/5.
http://www.ncbi.nlm.nih.gov/pubmed/26169970 *(Hong D et al., Clin Cancer Res, 2015;21(21):4801-10)*

A phase 1 study of lenvatinib in patients with advanced solid tumors, including colon cancer (n=9), sarcoma (n=7) and NSCLC (n=5), reported SD as best overall response recorded in 21/27 (84%) of the evaluable patients.
http://www.ncbi.nlm.nih.gov/pubmed/21372218 *(Yamada K et al., Clin Cancer Res 2011; 17(8):2528-37)*

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Drug	Gene	Therapeutic Implication
regorafenib (Stivarga)	RET (Amplification)	PREDICTED BENEFICIAL

A phase I study of regorafenib and cetuximab in advanced cancer patients showed early signals of activity. Of 24 evaluable patients, 11 (46%) patients had clinical benefit (stable disease > 6 cycles or partial response [PR]) (CRC n = 8, one patient each with head and neck cancer, carcinoma of unknown primary, and glioblastoma).
https://www.ncbi.nlm.nih.gov/pubmed/28422758 *(Subbiah V et al., JCI Insight. 2017 Apr 20;2(8). pii: 90380)*

In a multicenter, open-label, randomized, controlled, phase 2 trial of Regorafenib compared with lomustine in patients with relapsed glioblastoma (REGOMA), 124 patients were screened and 119 eligible patients were randomly assigned to receive regorafenib (n=59) or lomustine (n=60). Median follow-up was 15·4 months (IQR 13·8–18·1). At the analysis cutoff date, 99 (83%) of 119 patients had died: 42 (71%) of 59 in the regorafenib group and 57 (95%) of 60 in the lomustine group. Overall survival was significantly improved in the regorafenib group compared with the lomustine group, with a median overall survival of 7·4 months (95% CI 5·8–12·0) in the regorafenib group and 5·6 months (4·7–7·3) in the lomustine group (hazard ratio 0·50, 95% CI 0·33–0·75; log-rank p=0·0009).
https://www.ncbi.nlm.nih.gov/pubmed/30522967 *(Lombardi G et al., Lancet Oncol. 2019 Jan;20(1):110-119)*

Drug	Gene	Therapeutic Implication
sorafenib (Nexavar)	RET (Amplification)	PREDICTED BENEFICIAL

A phase II study of Sorafenib plus daily low-dose temozolomide in 43 relapsed glioblastoma reported five patients (12%) achieved partial response, 18 (43%) stable disease, 20 (48%) showed progression. The median time-to-progression was 3.2 months, 6-month progression-free survival was 26%, and median overall survival was 7.4 months.
https://www.ncbi.nlm.nih.gov/pubmed/23898124 *(Zustovich F et al., Anticancer Res. 2013 Aug;33(8):3487-94)*

Drug	Gene	Therapeutic Implication
sunitinib (Sutent)	RET (Amplification)	PREDICTED BENEFICIAL

In this phase 1 study of ixabepilone (Ix) and sunitinib (S) in advanced solid tumors (CRC (n=16), pancreas (n=2), prostate (n=2)), reported 3/28 patients achieved PR (2 with CRC), 8/28 patients had SD (5 with CRC) and 10/28 patients had PD.
http://meetinglibrary.asco.org/content/78559-102 *(Kittaneh M et al., J Clin Oncol, 2011;29: Abstract 3081)*

A phase 1 study of sunitinib malate (S) and gemcitabine (G) in solid tumors (n=31), including pancreatic adenocarcinoma patients (n=9) reported that four patients had a confirmed PR: 2 with pancreatic adenocarcinoma (dose level 1), 1 with pancreatic neuroendocrine cancer (dose level 2) and 1 with thymic carcinoma (dose level 2) and 9 patients had SD (median 20 weeks, range 14-49 weeks).
http://meetinglibrary.asco.org/content/47624-74 *(Krishnamurthi SS et al., J Clin Oncol, 2010;28: Abstract 3046)*

In this phase 1B dose escalation study of bortezomib and sunitinib in patients with refractory solid tumors (n=25) reported 3/24 PR (1 Hurthle cell carcinoma [CA], 1 squamous cell CA of the nasopharynx, 1 papillary thryoid CA) and SD lasting > 6 months in 5 patients (1 papillary thyroid CA, 1 neuroendocrine CA of the pancreas, 1 Hurthle cell CA, 1 medullary thyroid CA, 1 pleomorphic sarcoma). The combination of bortezomib and sunitinib was well tolerated and demonstrated anticancer activity in various solid tumors.
http://meetinglibrary.asco.org/content/50451-74 *(Kauh JS et al., J Clin Oncol, 2010;28: Abstract 2538)*

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Drug	Gene	Therapeutic Implication
vandetanib (Caprelsa)	RET (Amplification)	PREDICTED BENEFICIAL

In this phase 1 study of vandetanib and oral etoposide (VP-16) for recurrent malignant gliomas (MG) reported clinical activity, with patients remaining stable on the study for multiple cycles.
http://meetinglibrary.asco.org/content/50819-74 *(Brickhouse A et al., J Clin Oncol, 2010;28 Abstract 2038)*

A phase I/II trial of vandetanib for patients with recurrent malignant glioma reported minimal activity in unselected patients. Six patients (4 GBM, 2 AG) had radiographic response. PFS6 was 6.5% in the GBM arm and 7.0% in the AG arm. Median overall survival was 6.3 months in the GBM arm and 7.6 months in the AG arm.
https://www.ncbi.nlm.nih.gov/pubmed/23099652 *(Kreisl TN et al., Neuro Oncol. 2012 Dec;14(12):1519-26)*

In this multicenter, phase 2, randomized, noncomparative clinical trial of radiation and temozolomide with (n=76) or without (n=38) vandetanib in newly diagnosed glioblastoma (GBM) reported that vandetanib is reasonably well tolerated when combined with standard chemoradiation.
http://meetinglibrary.asco.org/content/80319-102 *(Quant EC et al., J Clin Oncol, 2011;29: Abstract 2069)*

Drug	Gene	Therapeutic Implication
cabozantinib (Cometriq)	ZNF33B/RET (Fusion)	PREDICTED BENEFICIAL

In a small set of case studies of 14 patients with NSCLC, KIF5B-RET fusion was identified in 10 patients, and CCDC6-RET fusion in 4. Six of 10 patients with KIF5B-RET fusion, and 1 of 4 with CCDC6-RET fusion were treated with cabozantinib, with rest were treated with combinations of chemotherapy or immunotherapy. Overall, treatment with cabozantinib showed an ORR of 60%, and median PFS of 2.5 months. One patient with KIF5B-RET fusion had a CR for 7 months. The median survival for the 10 patients with KIF5B-RET fusion was 13.3 months and for patients with CCDC6-RET fusion it was 22.8 months.
https://www.ncbi.nlm.nih.gov/pubmed/?term=28082048 *(Sarfaty M et al., Clin Lung Cancer. 2017 Jul;18(4):e223-e232)*

In an open-label, Simon two-stage, phase II trial of 26 patients with lung adenocarcinoma and RET rearrangement were treated with cabozantinib. 16 patients (62%) had KIF5B-RET fusion. Partial response was observed in 7 patients with ORR of 28% which met primary end point. Three patients (20%) with KIF5B-RET showed response, while none of the patients with CCDC6-RET fusion showed response.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5143197/ *(Drilon A et al., Lancet Oncol. 2016 Dec; 17(12): 1653–1660.)*

Drug	Gene	Therapeutic Implication
lenvatinib (Lenvima)	ZNF33B/RET (Fusion)	PREDICTED BENEFICIAL

In this open label, Phase 2 study 25 patients with NSCLC and RET rearrangements received lenvatinib until disease progression or unacceptable toxicity. Tumor shrinkage occurred in majority of patients, with ORR at 16%, and DCR at 76%.
https://oncologypro.esmo.org/Meeting-Resources/ESMO-2016/Phase-2-study-of-lenvatinib-LN-in-patients-Pts-with-RET-fusion-positive-adenocarcinoma-of-the-lung *(Velcheti V et al., Ann Oncol 2016; 27:416-54.)*

In a phase 1/2 dose finding study 23 patients between 2 -18 years old were enrolled. The most common tumors were rhabdomyosarcoma (n = 5), Ewing sarcoma (n = 4), and neuroblastoma (n = 3). The recommended dose in children and adolescents was similar to the adult dose. Pharmacokinetics did not differ significantly from that in adults. The phase 1b dose-finding study in combination with chemotherapy in osteosarcoma (OS) and phase 2 monotherapy (RD 14 mg/m2) parts are ongoing.
http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.10544 *(Gaspar N et al., J Clin Onc. 2017 May;35(15)_suppl 10544-10544)*

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Drug	Gene	Therapeutic Implication
regorafenib (Stivarga)	ZNF33B/RET (Fusion)	PREDICTED BENEFICIAL

Case report of a 63-year-old woman diagnosed with stage IV sigmoid colon adenocarcinoma and widespread metastases most notable for an enlarged liver that was nearly replaced by tumor. Patient declined chemotherapy-based treatment and was treated with regorafenib instead. Clinically, patient had resolution of her early satiety and abdominal discomfort within 1 week of regorafenib initiation. She had a rapid CEA response from 471 to 158 and LDH response from 3310 to 1651 after 18 days of treatment. No further follow-up was available as the patient succumbed to urosepsis shortly thereafter. Her clinical and CEA tumor marker responses suggested regorafenib has single agent activity in RET fusion positive CRC.

http://www.ncbi.nlm.nih.gov/pubmed/26078337 *(Le Rolle AF et al., Oncotarget, 2015; 6(30):28929-37)*

In a phase I dose escalation study pediatric patients of 6 months to <18 years with histologically confirmed recurrent/refractory solid tumors received tablets or granulates QD for the first 21 days of each 28-day cycle. 41 patients, median 13 years (3–17), 20 with central nervous system tumors, 11 with sarcomas, received a median of 2 cycles (1–16). One partial response was seen in a patient with rhabdomyosarcoma, with stable disease of at least 15 weeks in 8 patients, including 31 and 56+ weeks in 2 anaplastic ependymomas. The toxicity of regorafenib in pediatric patients was tolerable and consistent with the known adult profile, although increased hematologic toxicity was observed in heavily pretreated patients.

http://ascopubs.org/doi/abs/10.1200/JCO.2016.34.15_suppl.10542 *(Geoerger B et al., Journal of Clinical Oncology 2016 May;34, no. 15_suppl 10542-10542)*

Drug	Gene	Therapeutic Implication
sorafenib (Nexavar)	ZNF33B/RET (Fusion)	PREDICTED BENEFICIAL

Three Japanese patients with NSCLC and RET rearrangement were enrolled in this study. No dramatic response was observed for either of the patients, however some tumor shrinkage was observed for all enrolled. One patient with a CCDC6-RET fusion showed slightly reduced tumor and reduced tumor related pain. Her response for sorafenib was maintained for 12 months.

https://www.ncbi.nlm.nih.gov/pubmed/26898613 *(Horrike A et al., Lung Cancer. 2016 Mar;93:43-6)*

Drug	Gene	Therapeutic Implication
sunitinib (Sutent)	ZNF33B/RET (Fusion)	PREDICTED BENEFICIAL

In this study of pediatric patients with refractory solid tumors, 23 patients were treated with sunitinib. The median age was 13.9 years (range:3.9-20.6). Patients had brain tumors (n=8), tissue sarcomas (n=4), Ewing sarcoma (n=2), Neuroblastoma (n=2), osteosarcoma (n=2), and other solid tumors (n=5). Patients received sunitinib once daily for 28 days followed by a 14-day break between each cycle. Dose levels of 15 and 20 mg/m2/day were evaluated, with dose escalation based on a 3+3 design. No objective responses were observed in 17 patients evaluable for response. Four patients with sarcoma and glioma had stable disease for 2 - 9 cycles as best response including patients with diffuse pontine glioma (2 cycles), osteosarcoma (4 cycles), epithelioid sarcoma (9 cycles), and ganglioglioma (9 cycles).

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3149978 *(DuBois SG et al., Clin Cancer Res. 2011 Aug 1; 17(15): 5113–5122.)*

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Drug	Gene	Therapeutic Implication
vandetanib (Caprelsa)	ZNF33B/RET (Fusion)	PREDICTED BENEFICIAL

In a single arm, phase 2 study of 18 patients with RET rearrangements and metastatic or recurrent NSCLC were recruited. Of the 17 evaluable patients, 3 exhibited partial response (18%), and eight (47%) had stable disease. These responses were durable for 6 months in eight (47%). The ORR was 18% and the DCR was 65%. Two patients with CCDC6-RET fusion showed primary tumor shrinkage although one of them showed an early progression of an extrathoracic lesion. In contrast, no objective response was observed in patients with the KIF5B-RET rearrangement.
https://www.ncbi.nlm.nih.gov/pubmed/27803005 *(Lee Sh et al., Ann Oncol. 2017 Feb 1;28(2):292-297)*

A multicenter, single arm, phase 2 LURET study, enrolled 19 RET rearranged NSCLC patients who had failed at least 1 prior line of chemotherapy. Among the 17 evaluable patients, 53% (95% CI, 31-74) achieved an overall response, including 9 partial responses. The disease control rate was 90%; median progression-free survival was 4.7 months (95% CI, 2.8-8.5). Subgroup analyses demonstrated that the overall response rates were 83%, 20%, and 67% for patients with KIF5B-RET, 6 CCDC6-RET, and unknown rearrangements, respectively; median progression-free survival was 8.3, 2.9, and 4.7 months, respectively.
https://doi.org/10.1093/annonc/mdw383.04 *(Horiike A et al., abstract ESMO 2016)*

In an open label, multicenter, phase 2 trial, patients with advanced RET rearranged NSCLC received vandetanib daily (n=19). Among 17 eligible patients included in primary analysis, nine (53%) achieved an objective response, which met the primary endpoint. In the intention-to-treat population of all 19 patients treated with vandetanib, nine (47%) achieved an objective response. At the data cutoff, median progression-free survival was 4·7 months.
https://www.ncbi.nlm.nih.gov/pubmed/?term=27825616 *(Yoh K et al., Lancet Respir Med. 2017 Jan;5(1):42-50)*

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Clinical Trials Report

Potential trials based on genomic targets indicated in the GEM ExTra Report

Genomic Alterations	Targeted Investigational Agents	Trial IDs
CDKN2A (Deletion)	CDK4/6 dual kinase inhibitor: (Palbociclib, Abemaciclib, Ribociclib, G1T38, SHR6390), CCDN1/CCND3 inhibitor: (Alvocidib [Flavopiridol, FLAVO, HMR 1275, L-868275])	NCT02693535 NCT01037790 NCT03220646 NCT02977780 NCT02703571 NCT02857270 NCT02791334
CDKN2B (Deletion)	CDK4/6 dual kinase inhibitor: (Palbociclib, Abemaciclib, Ribociclib, G1T38, SHR6390), CCDN1/CCND3 inhibitor: (Alvocidib [Flavopiridol, FLAVO, HMR 1275, L-868275])	NCT01037790 NCT03220646 NCT02703571 NCT02857270 NCT02791334
EGFR (A289V)	Mab/Immune therapy: (Cetuximab, Panitumumab, Necitumumab, Nimotuzumab, Depatuxizumab mafodotin [ABT414], HLX07, ABBV-321, MCLA-158, SYN004), EGFR TKI/Anti-EGFR: (Erlotinib, Gefitinib, Osimertinib, EGF816, Icotinib [Conmana, BPI-2009], Avitinib [AC0010MA], Olmutinib [HM61713, BI 1482694], Alflutinib [AST2818], Dacomitinib [PF-00299804, PF-00299804-03], Naquotinib [ASP8273], Epitinib [HMPL-813], BPI-7711, D-0316, AZD3759, BPI-15086, DBPR112, YH25448, CK-101), EGFR/ERBB2 dual kinase inhibitor: (Neratinib [Nerlynx, HKI-272], AP32788, Lapatinib), EGFR/ERBB2/ ERBB4 MK inhibitors: (Afatinib, Poziotinib [HM781-36B, NOV120101], Pirotinib [KBP5209, KBP-5209])	NCT02977780 NCT01953926 NCT02091141 NCT03065387 NCT02423525 NCT01920061
EGFR (Amplification)	EGFR TKI/Anti-EGFR: (Erlotinib [Tarceva, CP-358,774, CP358774, OSI774, OSI-774], Gefitinib [Iressa, ZD 1839], Osimertinib [Tagrisso, Mereletinib, AZD9291], EGF816, Icotinib [Conmana, BPI-2009], Avitinib^ [AC0010, AC0010MA], Olmutinib [HM61713, BI 1482694], Alflutinib [AST2818, AST 2818], Dacomitinib [PF-00299804, PF-00299804-03], Naquotinib [ASP8273], Epitinib [HMPL-813], BPI-7711, D-0316, AZD3759, BPI-15086, DBPR 112 [DBPR112], YH25448, CK-101), EGFR Mab/Immune therapy: (Cetuximab [Erbitux, C225, IMC-C225], Panitumumab [Vectibix, ABX-EGF], Necitumumab [Portrazza, IMC-11F8], Depatuxizumab mafodotin [ABT414, ABT-414, ABT 414], HLX07, ABBV-321, MCLA-158, SYN004), EGFR/ERBB2 dual kinase inhibitor: (Neratinib [Nerlynx, HKI-272], AP32788, Lapatinib [Tykerb, GSK572016, GW2016, GW-572016]), EGFR/ERBB2/ ERBB4 MK inhibitors: (Afatinib [Gilotrif, BIBW 2992 MA2], Poziotinib [HM781-36B, NOV120101], Pirotinib [KBP5209, KBP-5209]), EGFR/ERBB2/VEGFR/EPHB4 MK inhibitors: (Tesevatinib [KD019, XL647])	NCT02977780 NCT01953926 NCT02465060 NCT01552434 NCT01582191 NCT03065387 NCT02423525 NCT02101905 NCT01920061

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Genomic Alterations	Targeted Investigational Agents	Trial IDs
EGFR (EGFRvIII)	EGFRvIII specific therapy: (AMG 596, EGFRvIII CAR T cells), EGFR TKI/Anti-EGFR: (Erlotinib, Gefitinib, Osimertinib, EGF816, Icotinib [Conmana, BPI-2009], Avitinib [AC0010MA], Olmutinib [HM61713, BI 1482694], Alflutinib [AST2818], Dacomitinib [PF-00299804, PF-00299804-03], Naquotinib [ASP8273], Epitinib [HMPL-813], BPI-7711, D-0316, AZD3759, BPI-15086, DBPR112, YH25448, CK-101), EGFR/ERBB2 dual kinase inhibitor: (Neratinib [Nerlynx, HKI-272], AP32788, Lapatinib), EGFR/ERBB2/ ERBB4 MK inhibitors: (Afatinib, Poziotinib [HM781-36B, NOV120101], Pirotinib [KBP5209, KBP-5209])	NCT02423525 NCT01920061
EGFR (EGFRvIVb)	EGFR TKI/Anti-EGFR: (Erlotinib, Gefitinib, EGF816, Osimertinib, Icotinib [Conmana, BPI-2009], Avitinib [AC0010MA], Olmutinib [HM61713, BI 1482694], Alflutinib [AST2818], Dacomitinib [PF-00299804, PF-00299804-03], Naquotinib [ASP8273], Epitinib [HMPL-813], BPI-7711, D-0316, AZD3759, BPI-15086, DBPR112, YH25448, CK-101), EGFR/ERBB2 dual kinase inhibitor: (Neratinib [Nerlynx, HKI-272], AP32788, Lapatinib), EGFR/ERBB2/ ERBB4 MK inhibitors: (Afatinib, Poziotinib [HM781-36B, NOV120101], Pirotinib [KBP5209, KBP-5209]); EGFR Mab/Immune therapy: (Cetuximab)	NCT02423525 NCT01920061
EGFR (R108K)	EGFR TKI/Anti-EGFR: (Erlotinib, Gefitinib, EGF816, Icotinib [Conmana, BPI-2009], Avitinib [AC0010MA], Olmutinib [HM61713, BI 1482694], Alflutinib [AST2818], Dacomitinib [PF-00299804, PF-00299804-03], Naquotinib [ASP8273], Epitinib [HMPL-813], BPI-7711, D-0316, AZD3759, BPI-15086, DBPR112, YH25448, CK-101), EGFR/ERBB2 dual kinase inhibitor: (Neratinib [Nerlynx, HKI-272], AP32788, Lapatinib), EGFR/ERBB2/ ERBB4 MK inhibitors: (Afatinib, Poziotinib [HM781-36B, NOV120101], Pirotinib [KBP5209, KBP-5209])	NCT02977780 NCT01953926 NCT02091141 NCT03065387 NCT02423525 NCT01920061
MDM4 (Amplification)	MDM2/MDMX Inhibitor: (ALRN-6924)	NCT03725436 NCT02264613
PIK3C2B (Amplification)	mTOR inhibitor: (AZD8055, Everolimus, Temsirolimus, CC-223, GDC0349 [GDC-0349, RG7603], ME-344, Sapanisertib [MLN0128], Ridaforolimus, Vistusertib [AZD2014], OSI-027, PQR309), PI3K/mTOR inhibitor: (BEZ235, DS-7423, GDC-0980, LY3023414, P7170, PWT33597)	NCT03834740

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Genomic Alterations	Targeted Investigational Agents	Trial IDs
PTEN (Deletion)	AKT inhibitor: (Archexin [RX-0201], Afuresertib [GSK2110183], Miransertib [ARQ092], ARQ751, Capivasertib [AZD5363], Ipatasertib [GDC-0068, RG-7440, GDC-0068], MK-2206, MSC2363318A, Triciribine, Uprosertib [GSK2141795]), AKT/BRAF dual kinase inhibitor: (ASN003), mTOR inhibitor: (AZD8055, Everolimus, Temsirolimus, CC-223, GDC0349 [RG7603], ME-344, Sapanisertib [MLN0128], Ridaforolimus, Vistusertib [AZD2014], OSI-027, PQR309), PI3K/mTOR inhibitor: (BEZ235, DS-7423, GDC-0980, LY3023414, P7170, PWT33597), PI3K inhibitor: (Alpelisib [BYL719], AZD8186, Apitolisib [GDC-0980, RG7422], Buparlisib [BKM120], Copanlisib, CLR457, Taselisib [GDC-0032], GDC-0077 [RG6114, RG 6114, RG-61114, GDC0077, GDC 0077], Gedatolisib [PKI-587], GSK2636771, Idelalisib [CAL-101, GS-1101], INK1117, LY294002 [SF1101], PF-04691502, PF-05212384 [PKI-587], VS-5584 [SB2343], XL147, XL765), PI3K/HDAC dual inhibitor: (CUDC-907), PARP inhibitor: (Niraparib, Olaparib, Rucaparib, Talazoparib [BMN-673], Veliparib [ABT-888], Simmiparib [SMOCL-9112], Fluzoparib, BGB-290, SC10914)	NCT02465060 NCT03522298 NCT02921919 NCT02286687 NCT03207347 NCT02857270 NCT01552434 NCT01582191 NCT01920061
RET (Amplification)	Multi-kinase inhibitors: (Cabozantinib, Lenvatinib, Vandetanib, Regorafenib, Sorafenib, Alectinib, LOXO-292, MGCD516, Ponatinib, BLU-667, RXDX-105, Sunitinib)	NCT02693535 NCT02272998 NCT01582191
TERT (c.-124C>T)	TERT related therapies: Telomelysin [OBP-301], Telomestatin, Imetelstat [GRN163L], INO-1401, INO-1400	Not recruiting for tumor type
ZNF33B/RET (Fusion)	Multi-kinase inhibitors: (Cabozantinib, Lenvatinib, Vandetanib, Regorafenib, Sorafenib, Alectinib, LOXO-292, MGCD516, Ponatinib, BLU-667, RXDX-105, Sunitinib)	NCT02693535 NCT02272998 NCT01582191

Disclaimer:

These clinical trial results were procured by keyword search on www.ClinicalTrials.gov, last updated on . The information contained in this site changes frequently and may be out of date. Search terms were based on alterations identified in the GEM ExTra report, drugs indicated in the GEM ExTra Report, and the reported cancer type of the patient. The search strategy was not exhaustive and may not have retrieved every relevant trial for this patient. Healthcare professionals are encouraged to investigate other possibilities through additional searches at this s i t e .The identified trials may have specific inclusion or exclusion criteria that would make a trial inappropriate for the patient. Consideration of any listed option should be made in the context of the patient's complete medical history.

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Variants of Unknown Significance

Alteration	Alteration Type	Allele Freq
ABCG1 (T137M)	Missense	38
ADCY4 (L470H)	Missense	27
AGAP10	Deletion	.
AGAP4	Deletion	.
ATAD1	Deletion	.
ATP2B4	Amplification	.
BMS1	Amplification	.
CASR (V827I)	Missense	40
CCDC71L (G119D)	Missense	21
CCNB3 (A792V)	Missense	83
CCT8L2 (T258M)	Missense	41
CHRND (S283L)	Missense	39
CNTN2	Amplification	.
COBL (A6S)	Missense	28
CSGALNACT2	Amplification	.
CTC-273B12.7 (E32G)	Missense	39
CYTH2 (E48G)	Missense	39
DMRTA1	Deletion	.
DSTYK	Amplification	.
EGFR (D126H)	Missense	32
EGFR/BMS1	Fused Genes (RNA)	.
ELAVL2	Deletion	.
ERBB2 (P1158L)	Missense	27
ETNK2	Amplification	.
FAP (c.1969+1G>A)	Splice Donor Variant	43
FMOD	Amplification	.
FMOD/CNTN2	Fused Genes (RNA)	.
FREM2	Breakpoint: Inversion	.
GFRA1	Deletion	.
GGT7 (R448W)	Missense	41
GLG1 (V784M)	Missense	44
GLP1R (T35M)	Missense	39
GLT6D1 (c.72-1G>A)	Splice Acceptor Variant	45
GOLT1A	Amplification	.
GSTT1	Deletion	.
HRNR (R2207H)	Missense	10
IFNA10	Deletion	.
IFNA14	Deletion	.
IFNA16	Deletion	.
IFNA17	Deletion	.
IFNA2	Deletion	.
IFNA21	Deletion	.
IFNA4	Deletion	.
IFNA5	Deletion	.
IFNA6	Deletion	.
IFNA7	Deletion	.
IFNA8	Deletion	.
IFNB1	Deletion	.
IFNE	Deletion	.
IFNW1	Deletion	.

Alteration	Alteration Type	Allele Freq
KISS1	Amplification	.
KLHDC8A	Amplification	.
KLHL9	Deletion	.
KLLN	Deletion	.
LAX1	Amplification	.
LGSN (N18S)	Missense	34
LRRN2	Amplification	.
MAGEB1 (R155H)	Missense	87
MAN2B2 (R66H)	Missense	39
MARCH11 (K202N)	Missense	43
MDC1 (R1079H)	Missense	42
MINPP1	Deletion	.
MMRN1 (P278L)	Missense	35
MOGAT3 (L103M)	Missense	17
MTAP	Deletion	.
NFASC	Amplification	.
NRK (Q1489H)	Missense	81
NUAK2	Amplification	.
OPTC	Amplification	.
PAPSS2	Deletion	.
PCDHA13 (R538H)	Missense	42
PCLO (E3258*)	Stop Gain	20
PCLO (V5064fs)	Frameshift	14
PLEKHA6	Amplification	.
PPP1R15B	Amplification	.
PRELP	Amplification	.
PRKCD (R569H)	Missense	38
PTCHD3	Deletion	.
RAB18 (E130Q)	Missense	81
RASGEF1A	Amplification	.
RBBP5	Amplification	.
RBPJL (G151S)	Missense	35
REN	Amplification	.
RP11-145E5.5	Deletion	.
RXFP3 (T162M)	Missense	43
SEC61G	Amplification	.
SLC1A6 (R84H)	Missense	34
SLC1A6 (T204M)	Missense	36
SNRPE	Amplification	.
SOX13	Amplification	.
SPATA31A7	Deletion	.
SPTA1 (A1413E)	Missense	37
SSX4 (D26N)	Missense	38
SSX4B (D26N)	Missense	41
TMCC2	Amplification	.
TMEM81	Amplification	.
TTC6 (R1230Q)	Missense	42
UNC5D (G513A)	Missense	22
VSTM2A	Amplification	.
ZBED6	Amplification	.

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Alteration	Alteration Type	Allele Freq
ZC3H11A	Amplification	.
ZNF33B	Amplification	.

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General Information

Methodology:
GEM ExTra™, a Next Generation Sequencing tumor/normal exome and tumor RNA Seq assay, provides for the detection of SNVs, indels, copy number events, and fusions in tumor tissue. MET exon 14 skipping, AR-v7, and EGFRvIII variants are also detected in RNA. Genomic DNA is extracted from the patient's normal and tumor samples. The isolated DNA is then prepared using a custom xGen target capture (IDT). This library preparation includes shearing, purification, adaptor ligation and PCR amplification. Total RNA is extracted from the patient's tumor sample. The isolated RNA is then prepared using KAPA HyperPrep with Riboerase (Kapa Biosystems). Libraries are then clustered on a flow cell and sequenced using the Illumina HiSeq 2500 or NovaSeq 6000.
Sequence data are analyzed using various validated bioinformatics tools (GEM ExTra™ pipeline 3.0). The reference genome assembly used for alignment is NCBI GRCh37. Each tumor's cancer-specific mutations are then queried against a proprietary gene-drug database based on peer-reviewed literature to identify potential therapeutic associations. Additional analysis and annotation may be provided by N-of-One, Inc.
Copy number events (amplifications/deletions) reported are focal in nature (<25mb).
Allele frequency is dependent on tumor purity. Tumor purity is not taken into account when reporting allele frequencies.
Tumor Mutation Burden (TMB) is determined by measuring the number of somatic mutations occurring in sequenced genes, counting all mutations expected to change the amino acid sequence of the impacted protein. TMB results are rounded to the nearest integer and are classified as follows: TMB-High: >= 20 mutations per megabase (mut/Mb); TMB-Intermediate: 6-19 mut/Mb inclusive; TMB-Low: <= 5 mut/Mb. "Indeterminate" results may be due to poor sample quality or sequencing coverage.
Mean target coverage for tumor sample DNA averages 440x (unique reads). Tumor sample RNA averages 121 million reads.

Limitations:
Samples with a tumor content of less than 30% may have reduced sensitivity and lead to false negative results. It is also possible that the sample contains a mutation below our established limit of detection (5% allele frequency), or in a region not included in our assay.
Alterations present in repetitive or high GC content region or non-coding areas may not be detected. Indels larger than 40bp may not be detected. Copy number signal relative to background noise inherent in DNA from FFPE samples may affect sensitivity of reporting amplifications/deletions.
The lack of a variant call does not necessarily indicate the absence of a variant since technical limitations to acquire data in some genetic regions may limit assay detection.
Given the nature of RNA isolated from FFPE, sequencing failures may be seen with highly degraded samples, as they may produce sequence reads too short to align informatically.
Previously unspecified fusions cannot be called by the informatics pipeline if the partner genes occur between two closely adjacent genes on the same strand of the same chromosome.

Disclaimer:
This test was developed, and performance characteristics determined by Ashion Analytics. This test has not been approved by the U.S. FDA. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. Ashion Analytics is certified under the Clinical Laboratory Improvement Amendments (#03D2048606) as qualified to perform high complexity clinical laboratory testing.
This test is used for screening purposes and is not intended to be a stand-alone diagnostic tool. It should also be noted that the data interpretations are based on our current understanding of genes and variants and are current as of the report date. Alterations are listed alphabetically, and not in order of strength of evidence or appropriateness for the patient's disease. When the report does identify variants with therapeutic implications, this does not promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient, and the selection of any drug for patient treatment is done at the discretion of the treating physician.
General genomic alterations should be considered in the context of the patient's history, risk factors and any previous genomic testing. Consideration of germline evaluation testing in light of such information is at the discretion of the ordering physician.
Variants of Unknown Significance (VUS) may associate with potential therapies in the future. Ashion does not update reports or send notification regarding reclassification of these alterations.
Standard lab limitations caused by human error, such as sample contamination or sample mix-up, may occur but are unlikely.

Electronically Signed By

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