Cytoplasmic p53 couples oncogene-driven glucose metabolism to apoptosis and is a therapeutic target in glioblastoma

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Cross-talk among oncogenic signaling and metabolic pathways may create opportunities for new therapeutic strategies in cancer. Here we show that although acute inhibition of EGFR-driven glucose metabolism induces only minimal cell death, it lowers the apoptotic threshold in a subset of patient-derived glioblastoma (GBM) cells. Mechanistic studies revealed that after attenuated glucose consumption, Bcl-xL blocks cytoplasmic p53 from triggering intrinsic apoptosis. Consequently, targeting of EGFR-driven glucose metabolism in combination with pharmacological stabilization of p53 with the brain-penetrant small molecule idasanutlin resulted in synthetic lethality in orthotopic glioblastoma xenograft models. Notably, neither the degree of EGFR-signaling inhibition nor genetic analysis of EGFR was sufficient to predict sensitivity to this therapeutic combination. However, detection of rapid inhibitory effects on [18F]fluorodeoxyglucose uptake, assessed through noninvasive positron emission tomography, was an effective predictive biomarker of response in vivo. Together, these studies identify a crucial link among oncogene signaling, glucose metabolism, and cytoplasmic p53, which may potentially be exploited for combination therapy in GBM and possibly other malignancies.

Molecularly targeted therapies have revolutionized cancer treatment and paved a path for modern precision medicine. However, despite well-defined actionable genetic alterations, targeted drugs have failed in patients with GBM, largely because of insufficient brain penetration of most targeted agents to levels necessary for tumor killing. This insufficient abundance in the target tissue may induce the development of adaptive mechanisms that drive drug resistance. Whereas therapeutic combinations that target both the primary genetic lesion and the compensatory signaling pathways that promote resistance are appealing, these combination-therapy strategies have been hampered by toxicity, thereby requiring subthreshold dosing of each drug. Owing to the dismal prognosis for patients with GBM and the poor efficacy of conventional approaches, new therapeutic strategies are critically needed.

An alternative therapeutic approach—synthetic lethality—targets an oncogenic driver and consequently modifies an important functional property for tumorigenesis, thus rendering cells vulnerable to an orthogonal ‘second hit’. This strategy may be particularly attractive when oncogene-regulated functional networks modulate pathways of tumor cell death. In a notable example, oncogenic signaling drives glucose metabolism, thereby suppressing the intrinsic (or mitochondria-dependent) apoptotic pathway and preventing cell death. Consequently, inhibition of oncogenic drivers with targeted therapies may trigger the intrinsic apoptotic machinery as a direct consequence of attenuated glucose consumption. The intertwined nature of these tumorigenic pathways could present therapeutic opportunities for rationally designed combination treatments, but this possibility has yet to be investigated.

Previous work has demonstrated that the epidermal growth factor receptor (EGFR) is mutated and/or amplified in ~60% of patients with GBM and regulates glucose metabolism. Whether targeting EGFR-driven glucose utilization alters the dynamics of the intrinsic apoptotic machinery in cancer is unknown. Here we hypothesized that a deeper understanding of this relationship might reveal a pharmacological vulnerability allowing for enhanced tumor killing in GBM.
RESULTS
EGFR-inhibitor metabolic responders and nonresponders
We first characterized the changes in glucose uptake induced by acute EGFR inhibition across 19 patient-derived GBM cell lines. The cells were cultured as gliomaspheres in supplemented serum-free medium, conditions that, in contrast to serum-based culture conditions, preserve many of the molecular features of patient tumors11,12. Treatment with the EGFR tyrosine kinase inhibitor erlotinib revealed a subset of GBMs, hereafter termed ‘metabolic responders’, in which uptake of radiolabeled glucose ([18F]fluorodeoxyglucose, denoted [18F]FDG) was significantly attenuated (Fig. 1a and Supplementary Fig. 1a). Silencing of EGFR with siRNA confirmed that the decrease in glucose uptake was not due to off-target effects of erlotinib (Supplementary Fig. 1b,c). As determined in a randomly selected cohort of metabolic responders, lower [18F]FDG uptake was associated with lower lactate secretion, glucose consumption, and extracellular acidification rate (ECAR), yet glutamine levels remained unchanged (Fig. 1b and Supplementary Fig. 1d–g).

Suppressed glucose utilization also correlated with a decrease in signaling of the RAS-MAPK and PI3K-AKT-mTOR pathways, each of which regulates glucose metabolism in GBM and other cancers10,13,14 (Supplementary Fig. 2a).

In contrast, no nonresponder GBMs (Fig. 1a and Supplementary Fig. 1b,c) showed decreased glucose consumption, lactate secretion, or ECAR, despite robust inhibition of EGFR activity (Fig. 1b and Supplementary Figs. 1d–g and Supplementary Fig. 2b). Moreover, RAS-MAPK and PI3K-AKT-mTOR signaling was unchanged in nearly all metabolic nonresponders (Supplementary Fig. 2b). Notably, whereas all metabolic responders had alterations in EGFR (mutation and/or amplification, or polysomy), six GBM lines without metabolic responses also contained EGFR mutations and/or copy-number gains (Supplementary Fig. 3a,b). Together, these data illustrate two key points. First, acute inhibition of EGFR rapidly attenuates glucose utilization in a subset of primary GBM cells, and second, genetic alterations in EGFR alone cannot predict which GBMs have a metabolic response to EGFR inhibition.

Figure 1 Inhibition of EGFR-driven glucose metabolism induces minimal cell death but primes GBM cells for apoptosis. (a) Percentage change in [18F]FDG uptake after 4 h of 1 µM erlotinib treatment relative to vehicle in 19 patient-derived GBM gliomaspheres. The concentration of erlotinib was selected to achieve robust inhibition of EGFR activity across the panel of primary GBM cells (additional data in Supplementary Fig. 2). Metabolic responders (blue) are samples that showed a significant decrease in [18F]FDG uptake relative to vehicle; nonresponders (red) showed no significant decrease (mean ± s.d.). Number of gliomasphere samples (n) = 3 for GBM39, HK248, HK301, HK385, HK423, HK206, HK217, HK250, HK254, HK350, HK393, GS017, and GS025; n = 4 for HK229; n = 5 for HK390; n = 6 gliomasphere samples for HK157, HK296, HK336, GS024). (b) Percentage change in glucose consumption and lactate secretion after 12 h of 1 µM erlotinib treatment relative to vehicle. Measurements were made with a Nova Biomedical BioProfile Analyzer (mean ± s.d.). For glucose consumption: n = 5 for HK390, HK217, and HK393; n = 4 for GBM39, HK301, and HK254. For lactate secretion: n = 5 for HK390, GBM39, and HK301; n = 3 for HK217, HK393, and HK254. (c) Annexin V staining of metabolic responders (blue, n = 10 unique gliomaspheres) or nonresponders (red, n = 9 unique gliomaspheres) after treatment with 1 µM erlotinib for 72 h. Each point represents the mean apoptosis of two independent experiments conducted for each gliomasphere sample. (d) The percentage change, relative to vehicle control, in priming, as determined by cytochrome c release after exposure to each BH3 peptide (BIM, BID, or PUMA) in metabolic responders or nonresponders treated with 1 µM erlotinib for 24 h (mean ± s.d., n = 2). Statistical analysis was performed on the grouped metabolic responders versus nonresponders. Results are representative of two independent experiments. (e) Left, immunoblot of whole cell lysates of HK301 cells overexpressing GFP control or GLUT1 and GLUT3 (GLUT1/3). Right, changes in glucose consumption or lactate secretion of HK301-GFP or GLUT1/3. β-actin, loading control. Uncropped images of gels are shown in Supplementary Figure 13. For glucose consumption: n = 4 for HK301-GFP; n = 5 for HK301-GLUT1/3. For lactate secretion: n = 7 for HK301-GFP; n = 5 for HK301-GLUT1/3. For glucose consumption: n = 4 for HK301-GFP; n = 5 for HK301-GLUT1/3. For lactate secretion: n = 7 for HK301-GFP; n = 5 for HK301-GLUT1/3. β-actin, loading control. Uncropped images of gels are shown in Supplementary Figure 13. For glucose consumption: n = 4 for HK301-GFP; n = 5 for HK301-GLUT1/3. For lactate secretion: n = 7 for HK301-GFP; n = 5 for HK301-GLUT1/3. β-actin, loading control. Uncropped images of gels are shown in Supplementary Figure 13. For glucose consumption: n = 4 for HK301-GFP; n = 5 for HK301-GLUT1/3. For lactate secretion: n = 7 for HK301-GFP; n = 5 for HK301-GLUT1/3. β-actin, loading control. Uncropped images of gels are shown in Supplementary Figure 13.
Metabolic responders are primed for apoptosis

Perturbations in glucose metabolism can induce the expression of proapoptotic factors and promote intrinsic apoptosis\(^{15}\), leading us to posit that decreased glucose uptake in response to EGFR inhibition would stimulate the intrinsic apoptotic pathway. Indeed, acute erlotinib treatment enhanced the expression of the proapoptotic BH3-only proteins BIM and PUMA, only in the metabolic-responder cultures (Supplementary Fig. 4a). However, annexin V staining revealed that metabolic responders had only modest (~17% of cells annexin V positive), albeit significantly higher, apoptosis than did nonresponders (~3% of cells annexin V positive) after 72 h of erlotinib exposure (Fig. 1c).

The relatively low level of apoptosis in metabolic-responder GBMs, despite pronounced induction of proapoptotic factors, led us to ask whether perturbing glucose uptake with erlotinib simply ‘primes’ GBM cells for apoptosis, thus increasing the propensity for apoptosis without inducing considerable cell death\(^{16}\). The induction of whether perturbing glucose uptake with erlotinib simply ‘primes’ GBM cells for apoptosis, thus increasing the propensity for apoptosis without inducing considerable cell death 16. This priming, we abrogated p53 expression in two

Cytoplasmic p53 is required for apoptotic priming

Next, we investigated the mechanism by which GBMs become primed for apoptosis after treatment with erlotinib. In primed cells, the antiapoptotic Bcl-2 family proteins (for example, Bcl-2, Bcl-xl, and Mcl-1) are largely loaded with proapoptotic BH3 proteins (for example, BIM, BID, PUMA, BAD, NOXA, and HRK); consequently, cells are dependent on these interactions for survival\(^{16}\). The tumor-suppressor protein p53 increases expression of proapoptotic proteins that subsequently must be sequestered by antiapoptotic Bcl-2 proteins to prevent cell death\(^{18}\). To examine whether p53 is required for erlotinib-induced priming, we abrogated p53 expression in two metabolic responders (HK301 and HK336) using CRISPR–Cas9 targeting TP53; the resulting cells are hereafter referred to as p53KO (Fig. 2a). Although the change in glucose uptake with erlotinib was unaffected in p53KO cells (Supplementary Fig. 6a), BH3 profiling revealed that p53KO exhibited nearly abolished erlotinib-induced apoptotic priming (Fig. 2b).

Figure 2 Cytoplasmic p53 links EGFR to intrinsic apoptosis.

(a) Immunoblots of the indicated proteins in two responders (HK301 and HK336) expressing CRISPR–Cas9 protein with control guide RNA (sgCtrl) or p53 guide RNA (p53KO). β-actin, loading control. (b) The percentage change, relative to vehicle control, in apoptotic priming, as determined by cytochrome c release after dynamic BH3 profiling with BIM peptides in sgCtrl and p53KO cells treated with 1 μM erlotinib for 24 h (mean ± s.d., number of gliomasphere samples (n) = 2). BIM was selected on the basis of exhibiting the greatest dynamic range from tested synthetic BH3 peptides (Supplementary Fig. 4). Results are representative of two independent experiments. (c) Immunoblot of the indicated proteins in HK301 sgCtrl, p53KO, p53KO + p53cyto, and p53KO + p53WT. Uncropped images of gels are shown in Supplementary Figure 12. (d) Immunofluorescence of p53 protein combined with DAPI nuclear staining to reveal protein localization in HK301 sgCtrl, p53KO + p53cyto, and p53KO + p53WT (scale bars, 20 μm). (e) Changes in levels of the indicated mRNAs after 100 nM doxorubicin treatment for 24 h in HK301 sgCtrl, p53KO, p53KO + p53WT, and p53KO + p53WT. Levels were normalized to respective dimethyl sulfoxide (DMSO)-treated cells (mean ± s.d., n = 3). (f) As in a, but in HK301 sgCtrl, p53KO, p53KO + p53wt, and p53KO + p53WT (mean ± s.d., n = 2). Results are representative of two independent experiments. (g) As in e, but in HK301 sgCtrl, p53KO, p53KO + p53R175H, p53KO + p53R273H, and p53KO + p53NES (mean ± s.d., n = 3). (h) As in b and f, but in HK301 sgCtrl, p53KO, p53KO + p53R175H, p53KO + p53R273H, and p53KO + p53NES (mean ± s.d., n = 2). Results are representative of two independent experiments. Comparisons were made with two-tailed unpaired Student’s t tests. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; NS, not significant.
Because transcription of p53-target genes has been shown to be enhanced under glucose limitation, we tested whether p53-mediated transcription was induced by EGFR inhibition. Erlotinib neither increased the expression of p53-regulated genes (for example, CDKN1A, also known as P21, MDM2, PIG3, and TIGAR) nor induced p53-luciferase reporter activity in HK301 metabolic-responder cells. In contrast, reconstitution of wild-type p53 (p53WT) in HK301 and HK336 p53KO gliomaspheres resulted in localization of p53 similar to that observed in the parental cells, and p53WT reconstitution rescued transcriptional activity (Figures 2c and Supplementary Fig. 6d) in a manner restricted to the cytoplasm (Fig. 2d and Supplementary Fig. 6e) and had no transcriptional activity (Fig. 2e and Supplementary Fig. 2f). In contrast, reconstitution of wild-type p53 (p53WT) in HK301 and HK336 p53KO cells displayed a localization of p53 similar to that observed in the parental cells, and p53WT reconstitution rescued transcription of p53-regulated genes (Fig. 2c–e and Supplementary Fig. 6d–f).

Stable introduction of p53WT significantly restored priming with erlotinib in both HK301 and HK336 p53KO cells to levels comparable to those of p53WT (Fig. 2f and Supplementary Fig. 6g), indicating that the cytoplasmic function of p53 is required for erlotinib-mediated priming. In support of this conclusion, introduction of a transcriptionally active (Fig. 2g) yet nuclear-confined p53 mutant (p53NES) into HK301 p53KO cells did not induce erlotinib-mediated apoptotic priming (Fig. 2g,h and Supplementary Fig. 6h). Finally, pharmacological inhibition of cytoplasmic-p53 activity with pifithrin-μ (ref. 24) markedly decreased priming with erlotinib (Supplementary Fig. 6i).

Collectively, these results show that cytoplasmic p53 engages the intrinsic apoptotic machinery after treatment with erlotinib in GBM metabolic-responder samples.

Prior work has demonstrated that TP53 mutations detected in human tumors—specifically those in the DNA-binding domain—result in diminished cytoplasmic functions in addition to transcriptional deficiencies. We asked whether stable expression of either of two of these ‘hotspot’ p53 mutants, R175H and R273H, in HK301 p53KO cells might decrease EGFR-inhibitor-mediated apoptotic priming (Supplementary Fig. 6h). As expected, both mutants lacked transcriptional capabilities (Fig. 2g) and, in agreement with their low cytoplasmic activity, were incapable of priming with erlotinib (Fig. 2h). Therefore, in line with previous findings, oncogenic mutations in the DNA-binding domain of p53 result in ‘dual hits’, whereby both transactivation and cytoplasmic functions are abrogated, the latter having implications for apoptotic priming with EGFR inhibition. In agreement with the notion that erlotinib-mediated apoptotic priming of metabolic responders requires a functional p53, all metabolic responders lacked mutations in p53 (Supplementary Fig. 3a).
**Figure 4** Synthetic lethality with combined targeting of EGFR and p53. (a) Summary of alterations in EGFR and genes involved in p53 regulation across 273 GBM samples. Mut., mutation. (b) Table indicating the significant associations between alterations in EGFR and genes involved in the p53 pathway. Mut. excl., mutually exclusive. Significance was determined using two-tailed Fisher’s exact test. (c) Annexin V staining of a metabolic responder (left, HK301) and nonresponder (right, HK393) treated with varying concentrations of erlotinib, nutlin, and both in combination, represented as a 6 x 6 dose–titration matrix. (d) The dose titration of erlotinib and nutlin, as described in c, was conducted across ten metabolic responders and five nonresponders (all wild-type p53), and the synergy score was calculated (Online Methods) (mean ± s.d., number of gliomasphere samples (n) = 2). The dashed line indicates a synergy score of 1. Results are representative of two independent experiments. (e) Annexin V staining of HK301-GFP and HK301-GLUT1/3 after 72 h of treatment with 1 µM erlotinib, 2.5 µM nutlin, or both (mean ± s.d., n = 3). Results are representative of two independent experiments. (f) As in e, but in HK301 sgCtrl and HK301 p53KO (mean ± s.d., n = 3). Results are representative of two independent experiments. (g) HK301 was treated for 24 h with 1 µM erlotinib, 2.5 µM nutlin, or both in combination. Immunoprecipitation was performed with immunoglobulin G control antibody or anti-p53 antibody, and the immunoprecipitate was probed with the indicated antibodies. Below are respective preimmunoprecipitation lysates (input). β-actin, loading control. Comparisons were made with two-tailed unpaired Student’s t tests. **P < 0.01; ***P < 0.001; ****P < 0.0001. Uncropped images of gels are shown in Supplementary Figure 12.

**Inhibition of glucose uptake primes tumor cells for apoptosis**

Bcl-xL can sequester cytoplasmic p53 and prevent p53-mediated apoptosis, thus creating a primed apoptotic state and a dependency on Bcl-xL for survival\(^1^\). Indeed, BH3 profiling revealed a reliance on Bcl-xL for survival\(^2^\). Indeed, BH3 profiling revealed a reliance on Bcl-xL for survival\(^27\). This suggests that glucose metabolism by sequestering cytoplasmic p53 by Bcl-xL, leading to the sequestration of cytoplasmic p53 by Bcl-xL. To investigate this possibility, we performed coimmunoprecipitation to examine the dynamics of p53–Bcl-xL interactions in response to erlotinib in both responders (n = 2) and nonresponders (n = 2). Importantly, we observed increased formation of Bcl-xL-p53 complexes with erlotinib treatment in metabolic responders (Fig. 3a) but not in nonresponders (Fig. 3b). This result suggests that inhibition of EGFR-dependent glucose consumption results in sequestration of p53 by Bcl-xL. In agreement with this interpretation, ectopic expression of GLUT1/3, which rescued the erlotinib-mediated decrease in glucose uptake and apoptotic priming, prevented the association of p53 with Bcl-xL in two metabolic responders (HK301 and GBM39) (Fig. 3c and Supplementary Fig. 7b). These findings strongly indicate that erlotinib-mediated inhibition of glucose uptake primes GBM cells for apoptosis by promoting interaction between cytoplasmic p53 and Bcl-xL.

Disruption of the p53–Bcl-xL complex can ‘free’ cytoplasmic p53, thus allowing it to stimulate intrinsic apoptosis\(^27\). In light of the result that binding between Bcl-xL and p53 increases in metabolic responders in erlotinib, we asked whether the liberation of p53 from Bcl-xL might elicit apoptosis. To test this possibility, we treated a metabolic responder (HK301) with erlotinib and the specific Bcl-xL inhibitor WEHI-539 (ref. 28). The addition of WEHI-539 released p53 from Bcl-xL under erlotinib treatment (Fig. 3d), thereby leading to synthetic lethality in three metabolic responders (HK301, GBM39, and HK336) (Fig. 3e and Supplementary Fig. 7c). Notably, cytoplasmic p53 was sufficient for caspase-dependent apoptosis elicited by the drug combination (Supplementary Fig. 7c,e). However, WEHI-539 did not enhance apoptosis in a nonresponder (HK393) treated with erlotinib, thus suggesting that attenuation of glucose uptake with EGFR inhibition, and subsequent association between p53 and Bcl-xL, is necessary to lower the apoptotic threshold and generate a dependence on Bcl-xL for survival (Fig. 3e). In support of this possibility, enforced expression of GLUT1/3 significantly mitigated cell death with the drug-combination treatment in both HK301 and GBM39 cells (Fig. 3f and Supplementary Fig. 7d). Together, these observations indicate that Bcl-xL blocks GBM cell death in response to erlotinib-mediated inhibition of glucose metabolism by sequestering cytoplasmic p53.

**Combination-treatment efficacy in metabolic responders**

Our mechanistic studies revealed a potential therapeutic opportunity in EGFR-driven GBMs that is dependent on functional p53. Although the p53 signaling axis is one of the three core pathways altered in GBM\(^29\), analysis of the GBM data set from TCGA demonstrated that TP53 mutations were mutually exclusive with alterations in EGFR (Fig. 4a,b). In contrast, in most patients with EGFR...
mutations or gains, co-occurring alterations can lead to suppressed p53 activity, including amplification of MDM2 and/or deletions in p14 ARF, a negative regulator of MDM2, at the CDKN2A locus\(^3\),\(^\text{30,31}\) (Fig. 4a,b). Given these relationships, and the requirement of p53 for priming under erlotinib-attenuated glucose uptake, we hypothesized that stabilization of p53 via MDM2 inhibition might have similar therapeutic effects to Bcl-xL antagonism. Using nutlin, an extensively characterized inhibitor of MDM2 (ref. 32), we observed synthetic lethality in combination with erlotinib treatment in a metabolic-responder gliosarcoma. More than 90% of HK301 cells underwent apoptosis after combined erlotinib and nutlin treatment (Fig. 4c). In contrast, we observed no synergy between these drugs in a metabolic nonresponder (HK393; Fig. 4c). We then tested this combination across our panel of primary GBM cells (all wild-type p53) and found synthetic lethality only in GBMs with a metabolic response to erlotinib, albeit to a lesser extent in HK423 and HK296 metabolic responders (Fig. 4d and Supplementary Fig. 8a; methodology in ref. 33). Silencing of EGFR in combination with nutlin also showed selective synergy for metabolic-responder cells, thus suggesting that the effects of the drug combination were not due to off-target effects of erlotinib (Supplementary Fig. 8b). Importantly, enforced expression of GLUT1/3 in HK301 metabolic responder cells significantly decreased molecular markers of intrinsic apoptosis—including BAX oligomerization and cytochrome c release—as well as cell death after combined erlotinib and nutlin treatment (Fig. 4e and Supplementary Fig. 8c), thus supporting the concept that attenuated glucose metabolism with EGFR inhibition is required for the synthetic lethality of the drug combination.

We next investigated the role of p53 in eliciting cell death in response to combined erlotinib and nutlin treatment. As expected, p53KO in two erlotinib metabolic responders (HK301 and HK336) abolished sensitivity to the drug combination (Fig. 4f and Supplementary Fig. 8g). Likewise, ectopic expression of Bcl-xL markedly suppressed cell death after combined treatment, in agreement with a critical function for Bcl-xL in antagonizing p53-mediated apoptosis (Supplementary Fig. 8d). Moreover, similar to our results with Bcl-xL inhibition (for example, WEHI-539), the addition of nutlin liberated p53 from Bcl-xL under erlotinib treatment (Fig. 4g). These data are consistent with prior observations that p53 stabilization stimulates cytoplasmic-p53-mediated apoptosis\(^27,28\). In support of the suggestion that cytoplasmic-p53 activity is required for the synergy of erlotinib and nutlin in metabolic responders, blocking cytoplasmic-p53 activity with pifithrin-µ significantly mitigated apoptosis elicited by combination treatment (Supplementary Fig. 8e), whereas HK301 cells containing the nuclear-confined p53 mutant p53\(^{\text{NLS}}\) did not show enhanced cell death when treated with the drug combination (Supplementary Fig. 8f). Finally, cells expressing the cancer-hotspot p53 mutants R175H and R273H, which have both transactivation and cytoplasmic deficiencies, were completely insensitive to the erlotinib and nutlin combination (Supplementary Fig. 8f).

Whereas cytoplasmic p53 was absolutely required to promote cell death with combined erlotinib and nutlin treatment, we observed in some instances that both the transcription-dependent (i.e., nuclear) and transcription-independent (i.e., cytoplasmic) functions of p53 were needed for optimal execution of synergistic apoptosis with nutlin (Supplementary Fig. 8g). These results are consistent with reports that the cytoplasmic functions of p53 can alone execute intrinsic apoptosis\(^34,35\), whereas, in other contexts, p53’s nuclear functions may be required to facilitate cytoplasmic-p53-mediated cell killing\(^27\). Collectively, our results show that combined targeting of EGFR-driven glucose metabolism and p53 induces marked synthetic lethality in primary GBM, in a manner dependent on the cytoplasmic functions of p53.

**Priming metabolic nonresponders for apoptosis**

Our data led us to propose a model wherein inhibition of EGFR-driven glucose metabolism primes the apoptotic machinery, thus resulting in synergy with proapoptotic stimuli such as p53 activation. A logical prediction of this model is that direct targeting of glucose metabolism should mimic the effects of EGFR inhibition. In agreement with this
prediction, addition of the glucose–metabolism inhibitor 2-deoxyglucose (2DG) stimulated apoptotic priming, binding of p53 to Bcl-xL, and synthetic lethality with nutlin in HK301 metabolic-responder cells. (Supplementary Fig. 9a,b,d). In contrast, inhibition of oxidative phosphorylation with oligomycin (complex V/ATP synthase) or rotenone (complex I) did not synergize with nutlin treatment in HK301 gliomaspheres (Supplementary Fig. 9c,d). Thus, decreased glucose metabolic flux alone, but not oxidative metabolism, appears to be sufficient for synergistic sensitivity to p53 activation.

This result prompted us to consider whether modulating glucose consumption in nonresponders might result in a similar p53-dependent vulnerability. To investigate this possibility, we tested whether direct inhibition of glucose uptake through 2DG treatment, or targeting the kinase PI3K, a well-characterized driver of glucose metabolism56, would elicit apoptotic priming in two erlotinib metabolic nonresponders. In contrast to erlotinib treatment, acute inhibition of PI3K with pictilisib abrogated PI3K-AKT-mTOR signaling (Supplementary Fig. 9e) and significantly decreased [18F]FDG uptake in HK393 and HK254 cells (Fig. 5a). The decrease in glucose consumption with pictilisib was associated with significantly higher apoptotic priming (Fig. 5b); 2DG treatment induced similar effects (Fig. 5a,b). Therefore, erlotinib metabolic nonresponders can be primed for apoptosis after inhibition of glucose uptake. Notably, CRISPR–Cas9 targeting of TP53 in HK393 cells significantly suppressed priming mediated by 2DG or pictilisib (Fig. 5c). Moreover, p53-dependent priming was associated with heightened Bcl-xL and p53 binding indicative of sequestration of p53 by Bcl-xL and blocking of apoptosis (Fig. 5d and Supplementary Fig. 9f). In agreement with this interpretation, combining 2DG or pictilisib with nutlin treatment caused significant p53-dependent synthetic lethality in erlotinib nonresponder cells (Fig. 5e,f). Together, these data demonstrate that acute inhibition of glucose metabolism, either directly or with targeted therapy, promotes p53-dependent apoptotic priming in GBM, thereby creating a targetable vulnerability.

A noninvasive biomarker for combination treatment in vivo

Our results in cell culture show that combined targeting of oncogene-driven glucose metabolism and p53 has synergistic activity in primary GBM. This result led us to investigate whether this approach might be effective in orthotopic GBM xenograft models. For these studies, we used the MDM2 inhibitor idasanutlin, which is currently in clinical trials for many malignancies87. Given the uncertainty of penetration of idasanutlin into the central nervous system, we first demonstrated that idasanutlin accumulated in the brains of mice with a completely intact blood–brain barrier (~35% relative to plasma levels) and stabilized p53 in orthotopic-tumor-bearing mice (Supplementary Fig. 10a,b).

Next, because perturbations in glucose metabolism with oncogene inhibition are required for synergistic sensitivity to p53 activation, we hypothesized that rapid attenuation of glucose uptake in vivo after erlotinib administration, as measured by [18F]FDG positron emission tomography (PET), might serve as a noninvasive predictive biomarker for the therapeutic efficacy of combined erlotinib and idasanutlin treatment (Fig. 6a). We observed, in orthotopic xenografts of a metabolic-responder gliomasphere (GBM39), that acute erlotinib treatment (75 mg/kg) rapidly decreased [18F]FDG uptake (15 h after erlotinib administration; Online Methods) (Fig. 6b and Supplementary Fig. 10c). In separate groups of mice, we tested the individual drugs and the combination of daily erlotinib (75 mg/kg) and idasanutlin (50 mg/kg) treatment for up to 25 d.
The drug combination was tolerable over the treatment period; we observed a ~10% decrease in body weight, which was comparable to that after erlotinib treatment alone (Supplementary Fig. 10d). Relative to single-agent controls, combined treatment with erlotinib and idasanutlin demonstrated synergistic growth inhibition—as determined by secreted Gaussia luciferase38—in GBM39 intracranial-tumor-bearing mice (Fig. 6e). In contrast, orthotopic xenografts of a nonmetabolic responder (HK393) showed neither changes in 18F]FDG uptake with acute erlotinib (Fig. 6d and Supplementary Fig. 10c) nor synergistic activity with erlotinib and idasanutlin combination treatment (Fig. 6e). Thus, noninvasive 18F]FDG PET, used to measure rapid changes in glucose uptake with EGFR inhibition, was effective in predicting subsequent synergistic sensitivity to combined erlotinib and idasanutlin treatment.

Finally, we evaluated the effects of the drug combination on overall survival in orthotopic xenografts of either two erlotinib metabolic responders (GBM39 and HK336) or two nonresponders (HK393 and GS025). All tumors were wild-type p53 (Supplementary Fig. 3a). On the basis of evidence of tumor growth (as determined by Gaussia luciferase), mice were treated with vehicle, erlotinib, idasanutlin, or both for up to 25 d, and then treatment was halted, owing to limited availability of idasanutlin for these studies. Although all tumors had genetic alterations in EGFR (for example, mutation and/or amplification, or polyploidy), the drug combination led to a pronounced increase in survival only in animals bearing erlotinib metabolic-responder GBM tumors (Fig. 6f-i). Together, these data show that combined targeting of EGFR and p53 synergistically inhibits growth and prolongs survival in a subset of wild-type p53 GBM orthotopic xenografts, and that 18F]FDG PET is a noninvasive predictive biomarker of sensitivity to this therapeutic strategy.

**DISCUSSION**

Here we found that acute EGFR inhibition rapidly decreases glucose utilization in a subset of patient-derived GBMs. As a consequence of this altered metabolic state, cells unexpectedly become primed for apoptosis via the cytoplasmic functions of p53. Accordingly, pharmacological p53 stabilization with a novel brain-penetrant small molecule was synthetically lethal with inhibition of EGFR-driven glucose uptake in primary orthotopic GBM models. Although these preclinical systems do not fully recapitulate the features of human GBM—consisting of an active immune system, pseudopalisading necrosis, and microvasculature proliferation—our results provide a proof of concept that deployment of targeted agents to perturb and exploit altered tumor metabolism may be an effective therapeutic strategy in GBM.

Most previous studies have suggested that the apoptotic functions of p53 are primarily exerted through its transcriptional activity. However, recent work supports the suggestion that the nontranscriptional functions of p53 have a critical role in triggering intrinsic apoptosis26. Our results demonstrate that cytoplasmic p53 couples oncogenic signaling to intrinsic apoptosis, which in this case is dependent on alterations in glucose utilization. However, the metabolic pathways downstream of glucose uptake that are responsible for this effect remain unknown. The observation that direct inhibition of oxidative phosphorylation does not synergize with p53 activation suggests that oxidation of glucose or other metabolites (for example, glutamine) is not required. Glucose can feed into many metabolic pathways including those for anabolic processes (for example, lipids, nucleotides, and amino acids), energetics, and enzyme function (for example, glycosylation and acetylation). Thus, attenuated glucose consumption may affect multiple pathways and induce metabolic stress39 and/or decrease donor metabolic substrates40,41 to levels sufficient to stimulate the cytoplasmic functions of p53. Future studies are required to specifically define these metabolic nodes that render GBM cells exquisitely susceptible to cytoplasmic-p53-mediated apoptosis. The results may reveal analogous therapeutic vulnerabilities to exploit GBM tumors for p53-dependent cell death.

More work is also needed to understand precisely how cytoplasmic p53 triggers intrinsic apoptosis in GBM cells. Considerable evidence indicates that cytoplasmic p53 possesses similar functionality to that of proapoptotic BH3 proteins; for example, it can activate the proapoptotic effectors BAK22,24 or BAX both directly21 and indirectly via neutralizing antiapoptotic Bcl2 proteins22. Our results support this role for cytoplasmic p53, whereby, after glucose metabolism is attenuated, p53 engages the intrinsic apoptotic machinery via binding to the antiapoptotic protein Bcl-XL. Despite minimal cell death, the increased occupancy of Bcl-XL with p53 lowers the apoptotic threshold and creates a dependency on Bcl-XL to block p53-mediated cell death. Targeting this interaction (for example, BCL-XL inhibition or MDM2 antagonism) liberated p53 from Bcl-XL, a result that coincided with BAX activation and cytoplasmic-p53-dependent intrinsic apoptosis. Thus, ‘free’ cytoplasmic p53 may directly activate BAX and promote apoptosis in response to this therapeutic combination. Finally, it is important to note that although cytoplasmic p53 was necessary for the execution of synergistic apoptosis with either Bcl-XL or MDM2 inhibition, it was universally sufficient only in the context of Bcl-XL inhibition. This apparent discrepancy may be explained through observations that, in some instances, the displacement of cytoplasmic p53 from Bcl-XL requires the binding of the p53-transcriptional-target gene PUMA (official symbol BBC3)27,43. Because MDM2 antagonists stimulate nuclear p53 transcriptional activity, including expression of PUMA, it is possible that in some contexts the transcription-dependent functions of p53 are required to facilitate cytoplasmic-p53-mediated apoptosis in GBM.

Notably, neither genetic alterations in EGFR nor the degree of EGFR-activity inhibition was sufficient to predict a metabolic response to EGFR tyrosine kinase inhibition in our GBM samples. Several molecular mechanisms have been described that enable dynamic compensatory responses to EGFR-directed therapy in GBM44. Thus, it is likely that, despite robust inhibition of EGFR, some tumors quickly reconfigure their molecular circuitry to preserve downstream signaling flux and drive glucose consumption45. Given the breadth of potential adaptive mechanisms, coupled with the molecular heterogeneity of GBM, genetic biomarkers alone may be insufficient to predict responses to this approach. Our results emphasize the value of a functional biomarker, in this case changes in glucose uptake46, as a means to rapidly stratify metabolic responders and nonresponders.

Together, our findings provide a rationale for the clinical evaluation of combined targeting of oncogene-driven glucose metabolism (for example, inhibition of EGFR or PI3K) and p53 in patients with GBM. Furthermore, we propose a new clinical application of 18F]FDG PET to assess whether targeted drugs induce a metabolic vulnerability that can be exploited. On the basis of our data showing that changes in 18F]FDG accumulation can be observed within hours after EGFR-inhibitor treatment, 18F]FDG PET may serve as a rapid, noninvasive functional biomarker to predict synergistic sensitivity to p53 activation. This noninvasive analysis may be particularly valuable for malignant brain tumors, in which pharmacokinetic and pharmacodynamic assessment is extremely difficult and impractical. Although there are concerns that 18F]FDG PET cannot properly
delineate glucose uptake in tumor tissue versus healthy brain tissue, delayed imaging protocols\(^{47}\) (used here for the mouse studies) and parametric-response maps with magnetic resonance imaging fusion can be useful for quantifying the changes in tumor \(^{[18]}\)FDG consumption. Finally, targeting oncopgenes that drive glucose uptake in other cancers may evoke similar p53-dependent vulnerabilities. Future work is required to assess the applicability of this concept to other oncogenic drivers and cancers.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

**ACKNOWLEDGMENTS**

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**AUTHOR CONTRIBUTIONS**

W.X.M., T.F.C., and D.A.N. conceived the study. W.X.M., L.G., V.W.D., I.T., J.E.T., B.H., W.B.G., N.A.B., M.D.H., J.T.L., W.H.Y., P.N.K., A.L., and D.A.N. designed and/or conducted the experiments and analyzed data. B.H., H.I.K., S.J.B., P.S.M., P.M.C. and A.L. provided reagents, cell lines, and critical input. W.X.M. and D.A.N. wrote the original manuscript. All authors read and edited the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare competing financial interests: details are available in the online version of the paper.
42. Leu, J.I.J., Dumont, P., Hafey, M., Murphy, M.E. & George, D.L. Mitochondrial p53 activates Bak and causes disruption of a Bak–Mcl1 complex. Nat. Cell Biol. 6, 443–450 (2004).

43. Follis, A.V. et al. PUMA binding induces partial unfolding within BCL-xL to disrupt p53 binding and promote apoptosis. Nat. Chem. Biol. 9, 163–168 (2013).

44. Reardon, D.A., Wen, P.Y. & Mellinghoff, I.K. Targeted molecular therapies against epidermal growth factor receptor: past experiences and challenges. Neuro. Oncol. 16 (Suppl. 8), vii7–viii13 (2014).

45. Wei, W. et al. Single-cell phosphoproteomics resolves adaptive signaling dynamics and informs targeted combination therapy in glioblastoma. Cancer Cell 29, 563–573 (2016).

46. Clark, P.M., Ebiana, V.A., Gosa, L., Cloughesy, T.F. & Nathanson, D.A. Harnessing preclinical molecular imaging to inform advances in personalized cancer medicine. J. Nucl. Med. 58, 689–696 (2017).

47. Spence, A.M. et al. 18F-FDG PET of gliomas at delayed intervals: improved distinction between tumor and normal gray matter. J. Nucl. Med. 45, 1653–1659 (2004).
ONLINE METHODS

Mice. Female NOD scid gamma (NSG) mice, 6–8 weeks of age, were purchased from the University of California Los Angeles (UCLA) Medical Center animal-breeding facility. Male CD-1 mice, 6–8 weeks of age, were purchased from Charles River. All mice were kept under defined pathogen-free conditions at the AAALAC-approved animal facility of the Division of Laboratory Animals (DLAM) at UCLA. All animal experiments were performed with the approval of the UCLA Office of Animal Resource Oversight (OARO).

Patient-derived GBM cells. After explicit informed consent was obtained from patients, all patient tissue used to derive GBM cell cultures was obtained through the UCLA Institutional Review Board (IRB) protocol 10-000655. As previously described,12 primary GBM cells were established and maintained in gliomasphere conditions, under the appropriate drug treatments. 12 h after drug treatment, 1 mL of medium was removed from each sample and analyzed in the Nova BioProfile analyzer. Measurements were normalized to cell number.

Annexin V apoptosis assay. Cells were collected and analyzed for annexin V and PI staining according to the manufacturer's protocol (BD Biosciences). Briefly, cells were plated at 5 × 10^6 cells/mL and treated with the appropriate drugs. At the indicated time points, cells were collected, trypsinized, washed with PBS, and stained with annexin V and PI for 15 min. Samples were then analyzed with a BD LSRII flow cytometer.

Immunoblotting. Cells were collected and lysed in RIPA buffer (Boston BioProducts) containing Halt Protease and Phosphatase Inhibitor (Thermo Fisher Scientific). Lysates were centrifuged at 14,000g for 15 min at 4 °C. Protein samples were then boiled in NuPAGE LDS Sample Buffer (Invtro) and NuPAGE Sample Reducing Agent (Invtro), separated with SDS–PAGE on 12% Bis–Tris gels (Invitrogen) and transferred to nitrocellulose membranes (GE Healthcare). Immunoblotting was performed per the antibody manufacturers' specifications, as mentioned previously. Membranes were developed with the SuperSignal system (Thermo Fisher Scientific).

Immunoprecipitation. Cells were collected, washed once with PBS, and incubated in IP lysis buffer (25 mM Tris–HCL, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, and 5% glycerol) at 4 °C for 15 min. 300–500 µg of each sample was then precleared in Protein A/G Plus Agarose Beads (Thermo Fisher Scientific) for 1 h. After preclearing, samples were then incubated with antibody–bead conjugates overnight according to the manufacturer's specifications and as mentioned previously. The samples were then centrifuged at 1,000g for 1 min, and the beads were washed with 500 µL of IP lysis buffer five times. Proteins were eluted from the beads by boiling in 2× LDS Sample Buffer (Invtro) at 95 °C for 5 min. Samples were analyzed by immunoblotting as previously described. Immunoprecipitation antibodies were diluted according to the manufacturer's instructions (1:200 for p53).

Dynamic BH3 profiling. GBM gliomaspheres were first dissociated into single-cell suspensions with TrypLE (Gibco) and resuspended in MEB buffer (150 mM mannitol, 10 mM HEPES-KOH, 50 mM KCl, 0.02 mM EDTA, 0.02 mM EGTA, 0.1% BSA, and 5 mM succinate). 50 µL of cell suspension (3 × 10^6 cells/well) was plated in wells holding 50 µL MEB buffer containing 0.002% digitonin and the indicated peptides in 96-well plates. Plates were then incubated at 25 °C for 50 min. Cells were then fixed with 4% paraformaldehyde for 10 min and neutralized with N2 buffer (1.7 M Tris and 1.25 M glycine, pH 9.1) for 5 min. Samples were stained overnight with 20 µL of staining solution (10% BSA and 2% Tween 20 in PBS) containing DAPI and anti-cytochrome c (BioLegend clone 6H2.B4, cat. no. 612310). The following day, cytochrome c release was quantified with a BD LSRII flow cytometer. Measurements were normalized to appropriate controls that do not promote cytochrome c release (DMSO and inactive PUMA2A peptide). Change in priming refers to the difference in the amount of cytochrome c release between vehicle-treated cells and drug-treated cells.

Plasma-membrane protein extraction. 1 × 10^7 cells were treated with the indicated drugs. After 4 h of treatment, cells were collected, washed once with ice-cold PBS, and lysed with a Dounce homogenizer. Plasma-membrane protein extraction was performed according to the manufacturer's protocol (BioVision), and isolated proteins were then subjected to immunoblotting.

BAX oligomerization. 7.5 × 10^6 cells were treated with the indicated drugs. After 24 h of treatment, cells were collected, washed once with ice-cold PBS, and resuspended in 1 mM bismaleimidoethane (BMH) in PBS for 30 min. Cells were then pelleted and lysed for immunoblotting, as described above.

Cytochrome c detection. 5 million cells were plated at a concentration of 1 × 10^6 cells/mL and treated with the indicated drugs. After 24 h of treatment, cells were collected and washed once with ice-cold PBS. Subcellular fractionation was then performed with a mitochondrial isolation kit (Thermo Fisher Scientific, 89874). Both cytoplasmic and mitochondrial fractions were subjected...
to immunoblotting, and cytochrome c was detected with anti-cytochrome c antibody at a dilution of 1:1,000 (Cell Signaling, 4272).

**Mouse xenograft studies.** For intracranial experiments, GBM39, HK336, HK393, and G5025 cells were injected (4 × 10^6 cells per injection) into the right striatum of the brain in female NSG mice (6–8 weeks old). Injection coordinates were 2 mm lateral and 1 mm posterior to the bregma, at a depth of 2 mm. Tumor burden was monitored on the basis of secreted Gaussia luciferase, and after three consecutive growth measurements, mice were randomized into four treatment arms consisting of the appropriate vehicle, 75 mg/kg erlotinib, 50 mg/kg idasanutlin, or a combination of both drugs. Vehicle consisted of 0.5% methylcellulose in water, which was used to dissolve erlotinib, and a proprietary formulation obtained from Roche, which was used to dissolve idasanutlin. Tumor burden was assessed twice per week on the basis of secreted Gaussia luciferase.

When possible, mice were treated for 25 d and then taken off treatment and monitored for survival. Drugs were administered through oral gavage. Sample sizes were chosen on the basis of estimates from pilot experiments and results from previous literature12. Investigators were not blinded to group allocation. Sizes were chosen on the basis of estimates from pilot experiments and results from previous literature12. Investigators were not blinded to group allocation.

**Intracranial delayed PET/CT mouse imaging.** For baseline [18F]FDG scans, mice were treated with vehicle and 15 h later were prewarmed, anesthetized with 2% isoflurane, and intravenously injected with 70 µCi of [18F]FDG. After 1 h of unconscious uptake, the mice were taken off anesthesia but kept warm for another 5 h of uptake. 6 h after the initial administration of [18F]FDG, mice were imaged with a G8 PET/CT scanner (Sofie Biosciences). After imaging, all mice were then dosed with erlotinib (75 mg/kg) and 15 h later were subjected to the same imaging procedure. As described above, quantification was performed by drawing 3D regions of interest (ROI) in AMIDE software, as previously described48. The 15-h treatment time point was the earliest time point that fit within logistical constraints including the amount of time required for adequate probe decay for subsequent imaging, the [18F]FDG production schedule and the hours of operation of the imaging center.

**Immunohistochemistry.** Immunohistochemistry was performed on 4-µm sections cut from FFPE (formalin-fixed, paraffin-embedded) blocks. Sections were then deparaffinized with xylene and rehydrated through graded ethanol. Antigen retrieval was achieved with a pH 9.5 Nuclear Decloaker (Biocare Medical) in a decloaking pressure cooker at 95 °C for 40 min. Tissue sections were then treated with 3% hydrogen peroxide (lot 161509; Fisher Chemical) and with Background Sniper (Biocare Medical) to decrease nonspecific background staining. Primary anti-p53 (Cell Signaling, 2527) was applied in a 1:50 dilution for 80 min, and detection was then performed with a MACH3 Rabbit HRP-Polymer Detection kit (Biocare Medical). Visualization was achieved with VECTOR NovaRED (SK-4800; Vector Laboratories) as a chromogen. Finally, sections were counterstained with Tach’s Automated Hematoxylin (Biocare Medical).

**Quantitative RT–PCR.** RNA was extracted from all cells with a PureLink RNA Kit (Invitrogen). cDNA was synthesized with an iScript cDNA Synthesis Kit (Bio-Rad), per the manufacturer’s instructions. Quantitative PCR (qPCR) was conducted on a Roche LightCycler 480 with SYBRGreen Master Mix (Kapa Biosciences). Relative expression values are normalized to the control gene GAPDH. Primer sequences were as follows (5′ to 3′): CDKN1A (forward GACCTTGTACCCGAGACACC, reverse GACAGGTCACATGTTCTTC), PUMA (forward ACGACGCTCAACGCACATCGAC, reverse GTAAGGCGCAGATGCCATGATG), GAPDH (forward TGCCATGATACCCCTTGAAG, reverse ATGGTGATCATGACAAGTGCGG), MDM2 (forward CGCTGTCTGCAATGAGATGATG, reverse GAGGTTGCTGTGGCAGGAG), and PIG3 (forward GCAGCTGCTGACGGTGTCG, reverse TCTGGAGTTGATCGCGCTTAT).

**p53 reporter activity.** Cells were first infected with lentivirus synthesized from a p53 reporter plasmid encoding luciferase under the control of a p53-responsive element: TACAGAAATGCTAAGCATGTGCGTGGCTTGCAGAGCTTGCGCCCTTGAGG. Infected cells were then plated into a 96-well plate at 5,000 cells/well, treated with the indicated drugs for 24 h and then incubated with 1 nM D-luciferin for 2 h. Bioluminescence was measured with an IVIS Lumina II system (Perkin Elmer).

**Genetic manipulation.** In general, lentivirus particles used for genetic manipulation were produced by transfection of 293-FT cells (Thermo) with Lipofectamine 2000 (Invitrogen). Virus particles were collected 48 h after transfection. The lentiviral sg53 vector and sgControl vector contained the following guide RNAs, respectively: CCGGTTCATCCGCCGCCCCATGC and GATACTCAGATCCTTATGAG. LentriCRISPR-v2 was used as the backbone. Glut1 and Glut3 cDNAs were cloned from commercially available vectors and incorporated into the pLenti-GLuc-IRE5-EGFP lentiviral backbone containing a CMV promoter (Glut1 was a gift from W. Frommer (Addgene no. 18085 (ref. 49)), Glut3 was obtained from OriGene (no. SC115791), and the lentiviral backbone was obtained from Targeting Systems (no. GL-GFP). pMIG Bcl-Xl was a gift from S. Korsmeyer (Addgene no. 8790 (ref. 50)) and cloned into the lentiviral backbone described above (Targeting Systems). Cytoplasmic (K365A and R306A) and wild-type p53 constructs were a kind gift from R. Agami and G. Lahav. The genes of interest were cloned into a lentiviral vector containing a PGK promoter. Constructs for p53 DNA-binding-domain mutants (R175H) and (R273H) as well as the nuclear mutant (L348A and L350A) were generated with site-directed mutagenesis (New England Biolabs no. E05S45) on the wild-type p53 construct.

For EGFK-knockdown experiments, siRNA against EGFR (Thermo Fisher, s563) was transfected into cells with DharmaFECT 4 (Dharmacon). After 48 h, cells were harvested and used for the indicated experiments.

**Immunofluorescence.** For immunofluorescence, gliomaspheres were first dissociated into single cells and adhered to the 96-well plates with Cell-Tak (Corning) according to the manufacturer instructions. Adhered cells were subsequently fixed with ice-cold methanol for 10 min, then washed with PBS. Cells were then incubated with blocking solution containing 10% FBS and 3% BSA in PBS for 1 h and subsequently incubated with anti-p53 (Santa Cruz, SC-126, 1:50 dilution) overnight at 4 °C. The following day, cells were incubated with secondary antibody (Alexa Fluor 647 pAb, Thermofisher) for 2 h at 37 °C. Cells were then incubated with blocking solution containing 10% FBS and 3% BSA in PBS for 1 h and subsequently incubated with anti-p53 (Santa Cruz, SC-126, 1:50 dilution) overnight at 4 °C. The following day, cells were incubated with secondary antibody (Alexa Fluor 647 pAb, Thermofisher, cat. no. A-21244, dilution 1:2,000) for 1 h and DAPI stained for 10 min, then imaged with a Nikon Ti Eclipse microscope equipped with a Cascade II fluorescence camera (Roper Scientific). Cells were then imaged with an microscope and DAPI stained at 37 °C for 30 min before OCT and ECAR measurements. Basal ECAR measurements were performed on a 100× 2.1-mm Phenomenex Kinetex C18 column (Kinetex).

**Mass spectroscopy sample preparation.** Male CD-1 mice (6–8 weeks old) were treated with 50 mg/kg idasanutlin in duplicate through oral gavage. At 0.5, 1, 2, 4, 6, 8, 12, and 24 h after administration, mice were sacrificed, blood was harvested by retro-orbital bleeding, and brain tissue was collected. Whole blood from mice was centrifuged to isolate plasma. Idasanutlin was isolated by liquid-liquid extraction from plasma: 50 µL plasma was added to 2 mL internal standard and 100 µL acetonitrile. Mouse brain tissue was washed with 2 mL cold PBS and homogenized with a tissue homogenizer with another 2 mL of cold PBS. Idasanutlin was then isolated and reconstituted in a similar manner by liquid-liquid extraction: 100 µL brain homogenate was added to 2 mL internal standard and 200 µL acetonitrile. After vortex mixing, the samples were centrifuged. The supernatants were removed and evaporated with a rotary evaporator and reconstituted in 100 µL 50:50 water/acetonitrile.

**Idasanutlin detection by mass spectrometry.** Chromatographic separations were performed on a 100× 2.1-mm Phenomenex Kinetex C18 column (Kinetex).
with a 1290 Infinity LC system (Agilent). The mobile phase was composed of solvent A consisting of 0.1% formic acid in Milli-Q water and solvent B consisting of 0.1% formic acid in acetonitrile. Analytes were eluted with a gradient of 5% B (0–4 min), 5–99% B (4–32 min), and 99% B (32–36 min), and then returned to 5% B for 12 min to reequilibrate between injections. Injections of 20 µL into the chromatographic system were used with a solvent flow rate of 0.10 mL/min. Mass spectrometry was performed on a 6460 triple-quadrupole LC/MS system (Agilent). Ionization was achieved by using electrospray in the positive mode, and data acquisition was performed in multiple reaction monitoring (MRM) mode. The MRM transition used for idasanutlin detection was m/z 616.2 → 421.2 with a fragmentor voltage of 114 V and a collision energy of 20 eV. The analyte signal was normalized to the internal standard, and concentrations were determined by comparison to the calibration curve (0.5, 5, 50, 250, 500, and 2,000 nM). Idasanutlin brain concentrations were adjusted by 1.4% of the mouse brain weight for the residual blood in the brain vasculature, as described by Dai et al.51.

**Synergy-score calculations.** Cells were infected with a lentiviral vector containing a secreted Gaussia luciferase (sGluc)-encoding reporter gene (Targeting Systems no. GL-GFP) and intracranially implanted into the right striatum in mice (4 × 106 cells/mouse). To measure the levels of secreted Gaussia luciferase (sGluc), 6 µL of blood was collected from the tail vein and immediately mixed with 50 mM EDTA to prevent coagulation. Gluc activity was obtained by measuring chemiluminescence after injection of 100 µL of 100 mM coelenterazine (Nanolight) in a 96-well plate, as described before48.

**Fluorescence in situ hybridization (FISH).** Fluorescence in situ hybridization (FISH) was performed with a commercially available fluorescently labeled dual-color EGFR (red)/CEP7 (green) probe (Abbott-Molecular). FISH hybridization and analyses were performed on cell lines, by following the manufacturer’s suggested protocols. The cells were counterstained with DAPI, and the fluorescent-probe signals were imaged under a Zeiss (Axiophot) Fluorescence Microscope equipped with dual- and triple-color filters.

**Statistical analysis.** Comparisons were made with two-tailed unpaired Student’s t tests, and P values <0.05 were considered statistically significant. All data from multiple independent experiments were assumed to be of normal variance. For each experiment, replicates are as noted in the figure legends. Data represent mean ± s.d. values unless otherwise indicated. All statistical analyses were calculated in Prism 7.0 (GraphPad). For all in vitro and in vivo experiments, no statistical method was used to predetermine sample size, and no samples were excluded. For in vivo tumor measurements, the last data sets were used for comparisons between groups. As described above, all mice were randomized before studies.

**Data availability.** Data presented in this manuscript are available from the corresponding author upon request. WES-analysis data have been deposited in the Sequence Read Archive under accession number SRP119012. A Life Sciences Reporting Summary for this paper is available.

48. Nathanson, D. et al. Co-targeting of convergent nucleotide biosynthetic pathways for leukemia eradication. J. Exp. Med. 211, 473–486 (2014).
49. Takanaga, H. & Frommer, W.B. Facilitative plasma membrane transporters function during ER transit. PASEB J. 24, 2849–2858 (2010).
50. Cheng, E.H. et al. BCL-2, BCL-XL) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol. Cell. 8, 705–711 (2001).
51. Dai, H., Marbach, P., Lemaire, M., Hayes, M. & Elmqquist, W.F. Distribution of STI-571 to the brain is limited by P-glycoprotein-mediated efflux. J. Pharmacol. Exp. Ther. 304, 1085–1092 (2003).
52. Magni, A. et al. EXCAVATOR: detecting copy number variants from whole-exome sequencing data. Genome Biol. 14, R120 (2013).
53. Gao, J. et al. Integrative analysis of complex cancer genomics and clinical profiles using the cbioPortal. Sci. Signal. 6, p1 (2013).
54. Cerami, E. et al. The cbio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2, 401–404 (2012).
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1. **Experimental design**

   1. **Sample size**
      
      Describe how sample size was determined. No formal statistical method was used to pre-determine sample size. Sample sizes were estimated based off pilot experiments and from previous literature.

   2. **Data exclusions**
      
      Describe any data exclusions. No data were excluded from these studies.

   3. **Replication**
      
      Describe whether the experimental findings were reliably reproduced. All attempts at replication were successful for all experiments.

   4. **Randomization**
      
      Describe how samples/organisms/participants were allocated into experimental groups. For mouse xenograft studies, mice were allocated into the various treatment groups so that each treatment arm had no statistically significant difference in mean tumor burden, as assessed by gaussia luciferase, measured immediately preceding group allocations.

   5. **Blinding**
      
      Describe whether the investigators were blinded to group allocation during data collection and/or analysis. Investigators were not blinded to group allocation.

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6. **Statistical parameters**

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   - n/a
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   - ☒ The exact sample size \( n \) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

   - ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

   - ☒ A statement indicating how many times each experiment was replicated

   - ☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)

   - ☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons

   - ☒ The test results (e.g. \( P \) values) given as exact values whenever possible and with confidence intervals noted

   - ☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

   - ☒ Clearly defined error bars

   See the web collection on statistics for biologists for further resources and guidance.
Software

Describe the software used to analyze the data in this study.

GraphPad Prism 7.0 was used to analyze data for this study.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All primary GBM cells generated for these studies are available upon request subject to an approved materials transfer agreement (MTA). All other reagents were either obtained from Roche via MTA (Idasanutlin) or purchased from the following commercial vendors: Cell Signaling Technologies, Sigma, Thermo Fisher, Selleck Chemicals, Chemietek, and Abcam.

All antibodies used purchased and validated from a commercial manufacturer. These include: β-actin (8H10D10) Mouse mAb (Cell signaling, 3700); tubulin (DM1A) Mouse mAb (Cell signaling, 3873); p-EGFR Y1086 (2533287) Rabbit pAb (Thermo Fischer Scientific, 36-9700); t-EGFR Rabbit pAb (Millipore, 06-847); t-AKT (11E7) Rabbit mAb (Cell Signaling, 4685); p-AKT T308 (D25E6) Rabbit mAb (Cell Signaling, 13038); p-AKT S473 (D9E) Rabbit mAb (Cell Signaling, 4060); t-ERK (137F5) Rabbit mAb (Cell Signaling, 4695); p-ERK T202/Y204 (D13.14.4E) Rabbit mAb (Cell Signaling, 4370); t-S6 (S235/236) (D57.2.2E) Rabbit mAb (Cell Signaling, 4858); t-4EBP1 (53H11) Rabbit mAb (Cell Signaling, 9644); p-4EBP1 S65 Rabbit pAb (Cell Signaling, 9451); Glut3 Rabbit pAb (Abcam, ab15311); Glut1 Rabbit pAb (Millipore, 07-1401); p53 (DO-1) Mouse mAb (Santa Cruz Biotechnology, SC-126); BAX (D2E11) Rabbit mAb (Cell Signaling, 2870); Bcl-xL (54H6) Rabbit mAb (Cell Signaling, 2764); Mcl-1 (D3S5) Rabbit mAb (Cell Signaling, 5453); Cytochrome c Rabbit pAb (Cell Signaling, 4272); and Cleaved Caspase-3 Rabbit pAb (Cell Signaling, 9661). Antibodies used for immunoprecipitation were obtained from the listed sources: p53 Rabbit pAb (Cell Signaling, 9282). Secondary antibodies were obtained from the listed sources: Anti-rabbit IgG HRP-linked (Cell Signaling, 7074) and Anti-mouse IgG HRP-linked (Cell Signaling, 7076).

Eukaryotic cell lines

All GBM cells used were of patient origin. 293FT cells for virus generation were purchased from Thermo Fisher

Authentication of cells was carried out by short-tandem repeat (STR) analysis

All cells were routinely tested and free of mycoplasma contamination.

No commonly misidentified cells were used.

Animals used for this study were female, nod-scid gamma null (NSG) mice aged 6-8 weeks.
12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.
Flow Cytometry Reporting Summary

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Data presentation

For all flow cytometry data, confirm that:

- 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- 3. All plots are contour plots with outliers or pseudocolor plots.
- 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.

Patient-derived GBM cells (gliomaspheres) that are first disassociated into single cell suspension with trypsin, and then washed once with FACS buffer (2% FBS in PBS). Samples are then processed with appropriate staining.

6. Identify the instrument used for data collection.

BD LSR II

7. Describe the software used to collect and analyze the flow cytometry data.

Data collected from BD LSR II was analyzed using FlowJo v10.

8. Describe the abundance of the relevant cell populations within post-sort fractions.

N/A - Cells were not sorted

9. Describe the gating strategy used.

Total cells were gated for based on forward scatter area (FSC-A) and side scatter area (SSC-A). This population was then gated for singlets using side scatter area (SSC-A) and side scatter width (SSC-W). Singlet population was then used to examine Annexin V+ and PI staining. For BH3 profiling, singlets were gated for DAPI staining to select out sub-G1 cells. Cytochrome c staining was then analyzed using SSC-A and APC.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☑