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Radiation-agent combinations for glioblastoma: challenges in drug development and future considerations

Charles A. Kunos¹, Evanthia Galanis², Jeffrey Buchsbaum³, Qian Shi², Lewis C Strauss⁴, C Norman Coleman³, and Mansoor M. Ahmed³

¹Investigational Drug Branch, National Cancer Institute, Bethesda, MD, USA

²Mayo Clinic Cancer Center, Mayo Clinic, Rochester, MN, USA

³Radiation Research Program, National Cancer Institute, Bethesda, MD, USA

⁴Bristol-Myers Squibb, Lawrenceville, NJ, USA

Abstract

Glioblastoma is an aggressive disease characterized by moderate initial response rates to first-line radiation-chemotherapy intervention followed by low poor response rates to second-line intervention. This article discusses novel strategic platforms for the development of radiation-investigational agent combination clinical trials for primary and recurrent glioblastoma in a NCI-NCTN settings with simultaneous analysis of challenges in the drug development process.

Keywords

radiotherapy; experimental chemotherapy; precision medicine; clinical cancer research; cancer discovery; clinical trials

INTRODUCTION

The prognosis for patients diagnosed with glioblastoma remains dismal. The overarching intent of this article is to discuss new strategic platforms for clinical development of radiation-agent clinical trials for the management of patients diagnosed with glioblastoma.

The Cancer Moonshot's Blue Ribbon Panel report [1] emphasizes recommendations for accelerating cancer science in a single effort to bring together promising biological research and clinical development for cancer patients in the near-term. The National Cancer Institute (NCI) and its Cancer Therapy Evaluation Program (CTEP) have aligned some research efforts around a personalized medicine approach in order to predict a precise and individual response to treatment. Application of personalized medicine in glioblastoma is of primary

CORRESPONDING AUTHOR: Mansoor M. Ahmed, PhD, Radiotherapy Development Branch, Molecular Radiation Therapeutics, Radiation Research Program, National Cancer Institute, National Institutes of Health, 9609 Medical Center Drive, Rockville, MD 20892-9760, (P): 240 276 5700, mansoor.ahmed@nih.gov.

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importance to craft novel targeted therapies specifically tailored to molecular alterations that arise in a patient's tumor at any clinical stage. Given such a directive, glioblastoma biology, biomarkers relevant to radiation-agent clinical development, and radiomics are discussed as each relates to near-term and long-term strategies for glioblastoma patient care.

BIOLOGY

Similar to most other cancers that arise from stem cells within a particular tissue or organ, glioblastoma is driven by evolving molecular alterations in glioma cells [2,3]. Clinically, glioblastoma may arise in eloquent brain, creating challenges for effective neurosurgical management because of quality of life decisions that impact surgical approach [4]. Glioblastoma infiltrates normal brain diffusely, a factor recognized in treatment failure, relapse, and eventual patient death [5]. Further, glioblastoma resides 'behind' a relatively impermeable blood-brain barrier that can impede targeted delivery of a biological product or a chemotherapeutic drug [6]. Absolute immune privilege does not necessarily apply to all brain areas, but effective afferent or efferent immune responses mounted alone against glioblastoma are rare [7].

BIOMARKERS

The comprehensive genomic fingerprint of glioblastoma has led to putative identification of biomarkers [3], such as the methylation status of O⁶-methylguanine-DNA methyltransferase (*MGMT*), a gene encoding an enzyme responsible for removing alkylating lesions in host cell DNA [8]. Genomic fingerprints are currently utilized as integral biomarkers to stratify glioblastoma patients in clinical trials and to assign care.

Contemporary large-scale genome profiling studies indicate that glioblastomas segregate into different biomarker subgroups which can be classified by mutations or by DNA methylation profiles [3]. Two predominantly pediatric glioblastomas subgroups are listed by a missense mutation in the *H3F3A* gene encoding histone H3.3—labelled either *H3F3A* K27 (particularly in pontine or thalamic brain tumors) or *H3F3A* G34 (in hemispheric brain tumors) [9]. A third young adult glioblastoma subgroup is categorized by a missense mutation in the *IDH* gene. This young adult subgroup often has an improved clinical outcome [9]. Glioblastomas that are *IDH* mutant often lack a molecular profile of monosomy 10, trisomy 7, and epidermal growth factor receptor (*EGFR*) amplification that is frequent in *IDH* wild-type glioblastomas. Moreover, *H3F3A* and *IDH* mutant glioblastomas often carry *p53* gene alterations [9]. Platelet-derived growth factor receptor- α (*PDGFRA*) gene amplification, most prevalent in adolescent and young adult patients, identifies another subgroup [9]. Other subgroups include the 'classic' (or 'RTK II') and the 'mesenchymal' subgroups—characterized by distinct mRNA expression profiles and by RTK II tumors exhibiting *EGFR* amplification [9]. Whether therapies attuned to these biomarkers will elicit personalized responses remains to be determined.

CLINICAL DEVELOPMENT OF AGENTS TARGETING GLIOBLASTOMA MULTIFORME

There are a number of new strategic directions for radiation plus experimental therapeutic agents that appear particularly promising for clinical development in glioblastoma over the next several years. Examples of four settings for radiation-agent or drug-agent discovery are discussed below.

DNA methylation

Modification of gene expression, rather than the genome itself, can impact glioblastoma biology. Initial findings from phase III clinical trials suggested that the addition of an alkylating chemotherapeutic agent (such as nitrosourea) given during or after radiotherapy led to small but clinically significant gains in patient survival [10,11]. The newer chemotherapeutic agent temozolomide acts as a cytotoxic alkylating agent, which is converted at physiologic pH to a short-lived active compound, monomethyl-triazenoimidazole-carboxamide (MTIC). Temozolomide cytotoxicity results from DNA methylation at the O⁶ or N⁷ positions of guanine, effectively blocking DNA replication [12]. A 573-patient randomized phase III clinical trial of radiotherapy alone or radiotherapy-temozolomide showed a significant 2.5-month prolongation in median survival after radiotherapy-temozolomide [13]. In a later performed subgroup analysis, patients with a methylated *MGMT* promoter had a median 22-month survival from radiotherapy-temozolomide, compared to a median 15-month survival after radiotherapy alone [14]. For patients with an unmethylated *MGMT* promoter, a smaller gain in survival was observed with the addition of temozolomide [14]. The prognostic importance of the *MGMT* promoter methylation status was confirmed in multiple subsequent phase III trials in newly diagnosed glioblastoma [15,16]. Emerging from such studies is the concept that the *MGMT* promoter methylation status might serve as an integral biomarker in an umbrella trial protocol—the rationale being, patients harboring methylated glioblastomas are most likely to benefit from temozolomide, and thus, a temozolomide-new agent combination might be warranted and studied, whereas patients having unmethylated glioblastomas appear to benefit little from temozolomide and a novel radiation-agent trial arm without temozolomide could be justified. An intriguing future direction in the methylated *MGMT* promoter patient populations involves methoxyamine. Methoxyamine is an oral small-molecule inhibitor that covalently binds to apurinic/apyrimidinic (AP) DNA damage sites and inhibits DNA base excision repair (BER), expanding the number of DNA strand breaks and inducing apoptosis [17]. Methoxyamine may increase the anti-tumor activity of alkylating agents like temozolomide [18], and perhaps, would be of clinical trial interest if preclinical data confirmed blood-brain barrier penetration.

Cell cycle

It is commonly held that the pathognomonic radiographic appearance of glioblastoma results from failure of blood supply to keep pace with rapid cell turnover. Glioblastoma's propensity for rapid cell turnover has been linked to deregulation of the phosphoinositide 3-kinase (*PI3K*), *p53*, and the cell cycle retinoblastoma (*Rb*) protein pathways [3] (Figure 1).

Attempts, to date, at targeting nodes of convergence within the *PI3K* pathway by small-molecule inhibitors have been unsuccessful [19–23]; however, novel approaches with improved drugs are in progress. As an example, temsirolimus acts as a rapamycin analog to pharmacologically block the *PI3K* pathway's mammalian target of rapamycin (*mTOR*), resulting in decreased expression of downstream effectors necessary for G1 phase cell cycle progression. Preclinical data demonstrated a radiation sensitization effect of rapalogs in glioblastoma [24] and modest brain penetration by drug was observed [19]. But, a clinical trial in glioblastoma patients reported poor clinical activity using radiation plus temsirolimus [20]. Future study for the glioblastoma *PI3K* pathway might involve sapanisertib (TAK-228), an orally bioavailable equipotent inhibitor of the raptor-*mTOR* complex (*TOR* complex 1 or *TORC1*) and the rictor-*mTOR* complex (*TOR* complex 2 or *TORC2*), that could possibly halt tumor cell proliferation and might better activate tumor cell apoptosis [25].

Along the same general approach, 85% of glioblastomas possess dysregulated *p53* pathway effectors [3], which are appealing anticancer drug targets (Figure 1). Clinical development is in progress of the murine double minute 2 (*MDM2*) inhibitor AMG 232, which is an oral piperidinone binder to the *MDM2* protein and disrupts its binding to the transcriptional activation domain of the tumor suppressor protein *p53*. By blocking a *MDM2-p53* protein-protein interaction, transcriptional activity of *p53* becomes restored in a manner that induces tumor cell apoptosis when DNA damage is detected [26,27]. Growing evidence suggests *MDM2* amplification contributes to glioblastoma pathogenesis [3], but it is not understood whether *MDM2* amplification drives an oncogenic phenotype or is a bystander of (epi-)genetic silencing [28]. Clinical development of AMG 232 in glioblastoma patients is underway.

As a third alternative, clinical investigation has begun into inhibitors of cyclin-dependent kinases to target the deregulation of the *Rb* pathway in glioblastoma (Figure 1). An agent like palbociclib, an oral inhibitor of cyclin-dependent kinase 4 (*CDK4*) and 6 (*CDK6*) [29], may hold promise in a radiation-agent management strategy for glioblastoma due to the deletion of *CDKN2A* and amplification of *CDK4* observed in glioblastomas [30].

Angiogenesis

Developing new or pruning old cerebral blood vessels generate tell-tale signs of glioblastoma tumorigenesis [31,32]. Bevacizumab, a recombinant humanized monoclonal antibody directed against the vascular endothelial growth factor (*VEGF*), a pro-angiogenic cytokine, binds to *VEGF* and prevents *VEGF* coupling with its cell surface receptor, thereby slowing the growth and the maintenance of tumor blood vessels. Bevacizumab has short-lived survival benefits in second-line therapy for patients with recurrent glioblastoma [33] and no overall survival benefit for patients in first-line disease management [15,16]. Attempts at targeting nodes of convergence within the *VEGF* receptor pathway by small-molecule inhibitors have been ineffective [34–37]. Modifying a stem cell-like transition following *VEGF* receptor blockade, such as through disruption of the *Hedgehog* (*Hh*) signaling pathway, may be more enticing in the near future. *Hh* is vital to the pattern and embryonic development of many organs, and both *Hh* ligand-dependent and ligand-

independent activation promote tumorigenesis and *VEGF*-mediated angiogenesis [38]. Study of one such inhibitor, vismodegib, an orally bioavailable small-molecule inhibitor of the *Hh* signaling pathway, has found that pharmacologically blocked *Hh*-ligand cell surface receptor signaling disrupts neuronal stem cell-like properties [39]. Whether Vismodegib has clinical activity in prospective glioblastoma clinical trials remains to be tested. Retrospective data indicates that *Hedgehog* signaling is an active and important pathway in glioblastoma [40].

Immunity

Tumor vascular and blood-brain integrity impact efferent and afferent immune responses against glioblastoma [7]. Expression and signaling activity of immune checkpoint effectors that inhibit CD8+ T cells—in particular, the cell surface programmed cell death protein 1 (PD-1)—have emerged as actionable targets in glioblastoma. Usually, activated PD-1 negatively regulates CD8+ T cell activation and serves a key role in glioblastoma's evasion of the immune system. Pembrolizumab is a humanized monoclonal antibody directed against PD-1 with immune checkpoint blocking and anticancer activity. Given a prominent role of PD-1 signaling in glioblastoma [41], there may be a future role for immune checkpoint inhibitors like pembrolizumab for glioblastoma, both as monotherapy and in combination with other anticancer agents (clinicaltrials.gov, NCT02337491). Much is to be learned in the immunotherapy oncology research space. Clinical trial results are eagerly anticipated, and particularly of prime interest, is when ionizing radiation is used in combination because it can cause robust immune-modulation and modification of the blood-brain barrier.

NEW COMPLIMENTARY TOOLS AND CLINICAL METHODS IN MANAGEMENT OF GBM

Radiomics

Beyond therapeutic developments stemming from novel combinatorial strategies of different therapeutic agents with radiation therapy, radiomics research can be equally important to lead incremental gains. Radiomics enables high-output extraction of medical image quantitative features, providing a comprehensive non-invasive assessment of tumor phenotype [42]. Radiomics has spurred interest in near-term research initiatives that include photon and particle radiotherapy delivery platforms driven by treatment planning software utilizing (a) biologic rather than physical dose [43], (b) grid therapy [44], and (c) avoidance algorithms for eloquent brain [45]. How best to integrate radiomics into clinical trials remains under study.

Clinical trial design for glioblastoma

As the proportion of unselected patients truly benefiting from use of any specific targeted agent is expected to be relatively low, a trial design with a properly selected limited sample size could quickly generate clinical activity signal or screen out an inactive agent. An appealing trial design could be the enrichment or patient-matched trial design. In the enrichment trial design, only patients with a biomarker-positive status are enrolled and are treated with the targeted biologic agent regimen. Simon [46] and Maitouram [47] showed

that the enrichment trial design has a higher relative efficiency in terms of required sample size and number of patients to be screened, when compared to a traditional randomized clinical trial design without incorporating biomarkers. However, study successes depend on proper biomarker enrichment strategies to correctly identify the patient-matched population. When multiple potentially therapeutic agents honing-in on similar pathways are under clinical development, it is critical to optimally utilize limited patient and clinical trial infrastructure resources. Typically, a phase II selection design can facilitate the prioritization among competing targeted biologic agents. One phase II selection design example is the “pick-the-winner” design [48]. In this particular design, patients are randomized to two or more treatment groups. Within each treatment group, the outcome is compared to a historical control group rather than each other. This affords common single-arm trial designs (e.g., Simon’s two-stage design) to be used for each group rather than a more complex format. If more than one competing targeted biologic agent shows efficacy, a winner will be declared for further investigation based on the best outcome estimate. This design always selects a winner, even with nominal difference, and is efficient to narrow choice of targeted biologic agent for phase III studies.

One caveat of a pick-the-winner design is that the statistical significance comparison is not the purpose of the study, and thus, it is not properly powered for this type of endpoint. When the treatment mechanism of targeted biologic agents is cytostatic, response endpoints may not be suitable. Progression-free survival (PFS) in these cases may capture the treatment effects better than response endpoints. However, historical PFS data are not always readily available and are commonly subject to patient selection bias and sensitive to miscellaneous prognostic factors. In this latter case, a randomized design with a concurrent control arm is needed to protect against such biases. A randomized phase II screening design [49], which applies less stringent type I error rate and power allows preliminary comparison between new targeted biologic agent versus control regimen, provides insight into whether a targeted biologic agent warrants further definitive evaluation in phase III studies. For the designs mentioned above, efficacy evaluations are fixed at (a) the number of treatment groups, (b) the number of patients within treatment group, and (c) pre-specified time points.

An adaptive design clinical trial for human drugs and biological products has been defined as a prospectively planned opportunity for modification of one or more aspects of trial design based upon interim in-trial subject data [50]. For this definition, the term ‘prospectively’ implies that the modification was preplanned and specified in detail before unblinded analysis.

The traditional clinical development plan for oncology agents typically involves ‘isolated’ trial phases—from dose finding (pilot or phase I) to efficacy screening (single-arm or randomized phase II) to confirmatory (phase III) trials. Lead time between phases, duplicated efforts for trial protocol development, and unawareness of data collected on earlier phase trials are the major inefficiencies of such a clinical development program. Nowadays, seamless trial designs which integrate both early and late phase trial hypotheses under an umbrella trial protocol are a new trend in oncology. Under umbrella trial protocols, data collected in the ‘learning’ early phases (i.e., phase I or II studies in traditional paradigm) informs final analyses. During the transition from the early phase to the late phase

of an umbrella trial protocol, the seamless design allows adaptability within a single protocol for (a) testing different hypotheses, (b) selecting different endpoints, (c) integrating biomarker validations as results mature, and (d) adjusting agent dose and intensity as safety data matures. A biomarker-enriched glioblastoma clinical trial (clinicaltrials.gov, NCT02152982) trial is employing a seamless phase II/III design to expeditiously and to efficiently answer the question if blocking DNA damage repair by using the poly(ADP-ribose) polymerase (PARP) inhibitor veliparib can improve survival outcome in *MGMT* methylated newly diagnosed glioblastoma patients.

New collaborations for glioblastoma science and clinical development

As new settings for radiation-agent discovery evolve, there is an increasing impetus for collaborations among research stakeholders in academia, industry, and government. For example, NCI CTEP and the NCI Molecular Radiation Therapeutics (MRT) of the Radiation Research Program facilitates a working group of peers with expertise in primary brain tumors (and brain metastasis) whose tasks include scientific comment early-on in radiation-agent clinical development plans, harmonization of pre-clinical data and radiobiological biomarkers in trials strongly supported by providing inputs for the evaluation of radiation-agent preclinical performance. Such input impacts the enthusiasm for the overall radiation-agent clinical development process at the intramural agent project team, extramural agent project team, and NCI CTEP consensus reviews. In addition, RRP-MRT play an important role in bridging companies that are funded with SBIR projects from NCI with the members of working groups within MRT with a focus to promote cancer drug development with radiation. In this mission, MRT also interacts with FDA to discuss and educate particularly guidelines for non-clinical and clinical considerations. A recent publication from FDA highlights the existing regulatory framework in which cancer drugs may be developed for use in combination with radiation therapy and describe the mechanisms in place for sponsors to obtain feedback from the Agency during the process of drug development [51]. Moving forward, NCI CTEP MRT working group participation is encouraged among the research community to help develop optimal smart clinical trial designs that can potentially lead to the development of new standard-of-care glioblastoma management.

CONCLUSION

In summary, these are exciting times for personalized medicine initiatives in glioblastoma cancer care. Novel early-phase and late-phase clinical trial designs investigating experimental therapeutics paired with radiotherapy hold promise for effective therapy against glioblastoma over the next five years.

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References

1. Jacks, T., Jaffee, E., Singer, D. National Cancer Advisory Board; 2016. Cancer Moonshot Blue Ribbon Panel Report 2016. cancer.gov/brp [Accessed 04 January 2017]
2. Ostrom QT, Gittleman H, Fulop J, Liu M, Blanda R, Kromer C, et al. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008–2012. *Neuro Oncol.* 2015; 17(Suppl 4):iv1–iv62. DOI: 10.1093/neuonc/nov189 [PubMed: 26511214]
3. Brennan CW, Verhaak RG, McKenna A, Campos B, Nounshmehr H, Salama SR, et al. The somatic genomic landscape of glioblastoma. *Cell.* 2013; 155(2):462–477. DOI: 10.1016/j.cell.2013.09.034 [PubMed: 24120142]
4. Osorio JA, Aghi MK. Optimizing glioblastoma resection: intraoperative mapping and beyond. *CNS Oncol.* 2014; 3(5):359–366. DOI: 10.2217/cns.14.36 [PubMed: 25363008]
5. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol.* 2006; 7(5):392–401. DOI: 10.1016/S1470-2045(06)70665-9 [PubMed: 16648043]
6. Jue TR, McDonald KL. The challenges associated with molecular targeted therapies for glioblastoma. *J Neurooncol.* 2016; 127(3):427–434. DOI: 10.1007/s11060-016-2080-6 [PubMed: 26900075]
7. Platten M, Bunse L, Wick W, Bunse T. Concepts in glioma immunotherapy. *Cancer Immunol Immunother.* 2016; 65(10):1269–1275. DOI: 10.1007/s00262-016-1874-x [PubMed: 27460064]
8. Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. *Nat Rev Cancer.* 2004; 4(4):296–307. DOI: 10.1038/nrc1319 [PubMed: 15057289]
9. Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell.* 2012; 22(4):425–437. DOI: 10.1016/j.ccr.2012.08.024 [PubMed: 23079654]
10. Levin VA, Wilson CB, Davis R, Wara WM, Pischer TL, Irwin L. A phase III comparison of BCNU, hydroxyurea, and radiation therapy to BCNU and radiation therapy for treatment of primary malignant gliomas. *J Neurosurg.* 1979; 51(4):526–532. DOI: 10.3171/jns.1979.51.4.0526 [PubMed: 225456]
11. Gutin PH, Wilson CB, Kumar AR, Boldrey EB, Levin V, Powell M, Enot KJ. Phase II study of procarbazine, CCNU, and vincristine combination chemotherapy in the treatment of malignant brain tumors. *Cancer.* 1975; 35(5):1398–1404. [PubMed: 1122488]
12. Tang JB, Svilar D, Trivedi RN, Wang XH, Goellner EM, Moore B, et al. N-methylpurine DNA glycosylase and DNA polymerase beta modulate BER inhibitor potentiation of glioma cells to temozolomide. *Neuro Oncol.* 2011; 13(5):471–486. DOI: 10.1093/neuonc/nor011 [PubMed: 21377995]
13. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005; 352(10):987–996. DOI: 10.1056/NEJMoa043330 [PubMed: 15758009]
14. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005; 352(10):997–1003. DOI: 10.1056/NEJMoa043331 [PubMed: 15758010]
15. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med.* 2014; 370(8):699–708. DOI: 10.1056/NEJMoa1308573 [PubMed: 24552317]
16. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N Engl J Med.* 2014; 370(8):709–722. DOI: 10.1056/NEJMoa1308345 [PubMed: 24552318]
17. Liu L, Taverna P, Whitacre CM, Chatterjee S, Gerson SL. Pharmacologic disruption of base excision repair sensitizes mismatch repair-deficient and -proficient colon cancer cells to methylating agents. *Clin Cancer Res.* 1999; 5(10):2908–2917. [PubMed: 10537360]

18. Taverna P, Liu L, Hwang HS, Hanson AJ, Kinsella TJ, Gerson SL. Methoxyamine potentiates DNA single strand breaks and double strand breaks induced by temozolomide in colon cancer cells. *Mutat Res.* 2001; 485(4):269–281. [PubMed: 11585361]
19. Kuhn JG, Chang SM, Wen PY, Cloughesy TF, Greenberg H, Schiff D, et al. Pharmacokinetic and tumor distribution characteristics of temsirolimus in patients with recurrent malignant glioma. *Clin Cancer Res.* 2007; 13(24):7401–7406. DOI: 10.1158/1078-0432.ccr-07-0781 [PubMed: 18094423]
20. Wick W, Gorlia T, Bady P, Platten M, van den Bent MJ, Taphoorn MJ, et al. Phase II Study of Radiotherapy and Temsirolimus versus Radiochemotherapy with Temozolomide in Patients with Newly Diagnosed Glioblastoma without MGMT Promoter Hypermethylation (EORTC 26082). *Clin Cancer Res.* 2016; [accessed 03 January 2017] published online 03 May 2016. doi: 10.1158/1078-0432.ccr-15-3153
21. Brown PD, Krishnan S, Sarkaria JN, Wu W, Jaeckle KA, Uhm JH, et al. Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. *J Clin Oncol.* 2008; 26(34):5603–5609. DOI: 10.1200/jco.2008.18.0612 [PubMed: 18955445]
22. Ma DJ, Galanis E, Anderson SK, Schiff D, Kaufmann TJ, Peller PJ, et al. A phase II trial of everolimus, temozolomide, and radiotherapy in patients with newly diagnosed glioblastoma: NCCCTG N057K. *Neuro Oncol.* 2015; 17(9):1261–1269. DOI: 10.1093/neuonc/nou328 [PubMed: 25526733]
23. Pitz MW, Eisenhauer EA, MacNeil MV, Thiessen B, Easaw JC, Macdonald DR, et al. Phase II study of PX-866 in recurrent glioblastoma. *Neuro Oncol.* 2015; 17(9):1270–1274. DOI: 10.1093/neuonc/nou365 [PubMed: 25605819]
24. Eshleman JS, Carlson BL, Mladek AC, Kastner BD, Shide KL, Sarkaria JN. Inhibition of the mammalian target of rapamycin sensitizes U87 xenografts to fractionated radiation therapy. *Cancer Res.* 2002; 62(24):7291–7297. [PubMed: 12499272]
25. Guo Y, Kwiatkowski DJ. Equivalent benefit of rapamycin and a potent mTOR ATP-competitive inhibitor, MLN0128 (INK128), in a mouse model of tuberous sclerosis. *Mol Cancer Res.* 2013; 11(5):467–473. DOI: 10.1158/1541-7786.mcr-12-0605 [PubMed: 23386687]
26. Canon J, Osgood T, Olson SH, Saiki AY, Robertson R, Yu D, et al. The MDM2 Inhibitor AMG 232 Demonstrates Robust Antitumor Efficacy and Potentiates the Activity of p53-Inducing Cytotoxic Agents. *Mol Cancer Ther.* 2015; 14(3):649–658. DOI: 10.1158/1535-7163.mct-14-0710 [PubMed: 25567130]
27. Werner LR, Huang S, Francis DM, Armstrong EA, Ma F, Li C, et al. Small Molecule Inhibition of MDM2-p53 Interaction Augments Radiation Response in Human Tumors. *Mol Cancer Ther.* 2015; 14(9):1994–2003. DOI: 10.1158/1535-7163.mct-14-1056-t [PubMed: 26162687]
28. Lambiv WL, Vassallo I, Delorenzi M, Shay T, Diserens AC, Misra A, et al. The Wnt inhibitory factor 1 (WIF1) is targeted in glioblastoma and has a tumor suppressing function potentially by induction of senescence. *Neuro Oncol.* 2011; 13(7):736–747. DOI: 10.1093/neuonc/nor036 [PubMed: 21642372]
29. Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. *Mol Cancer Ther.* 2004; 3(11):1427–1438. [PubMed: 15542782]
30. Furgason JM, Koncar RF, Michelhaugh SK, Sarkar FH, Mittal S, Sloan AE, et al. Whole genome sequence analysis links chromothripsis to EGFR, MDM2, MDM4, and CDK4 amplification in glioblastoma. *Oncoscience.* 2015; 2(7):618–628. DOI: 10.18632/oncoscience.178 [PubMed: 26328271]
31. Veeravagu A, Hou LC, Hsu AR, Cai W, Greve JM, Chen X, Tse V. The temporal correlation of dynamic contrast-enhanced magnetic resonance imaging with tumor angiogenesis in a murine glioblastoma model. *Neurol Res.* 2008; 30(9):952–959. DOI: 10.1179/174313208x322761 [PubMed: 18662497]
32. de Groot JF, Fuller G, Kumar AJ, Piao Y, Eterovic K, Ji Y, Conrad CA. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. *Neuro Oncol.* 2010; 12(3):233–242. DOI: 10.1093/neuonc/nop027 [PubMed: 20167811]

33. Kreisl TN, Kim L, Moore K, Duic P, Royce C, Stroud I, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. *J Clin Oncol.* 2009; 27(5):740–745. DOI: 10.1200/jco.2008.16.3055 [PubMed: 19114704]
34. Galanis E, Anderson SK, Lafky JM, Uhm JH, Giannini C, Kumar SK, et al. Phase II study of bevacizumab in combination with sorafenib in recurrent glioblastoma (N0776): a north central cancer treatment group trial. *Clin Cancer Res.* 2013; 19(17):4816–4823. DOI: 10.1158/1078-0432.ccr-13-0708 [PubMed: 23833308]
35. Hainsworth JD, Ervin T, Friedman E, Priego V, Murphy PB, Clark BL, Lamar RE. Concurrent radiotherapy and temozolomide followed by temozolomide and sorafenib in the first-line treatment of patients with glioblastoma multiforme. *Cancer.* 2010; 116(15):3663–3669. DOI: 10.1002/cncr.25275 [PubMed: 20564147]
36. Batchelor TT, Mulholland P, Neyns B, Nabors LB, Campone M, Wick A, et al. Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. *J Clin Oncol.* 2013; 31(26):3212–3218. DOI: 10.1200/jco.2012.47.2464 [PubMed: 23940216]
37. Hutterer M, Nowosielski M, Haybaeck J, Embacher S, Stockhammer F, Gotwald T, et al. A single-arm phase II Austrian/German multicenter trial on continuous daily sunitinib in primary glioblastoma at first recurrence (SURGE 01-07). *Neuro Oncol.* 2014; 16(1):92–102. DOI: 10.1093/neuonc/not161 [PubMed: 24311637]
38. Chen W, Tang T, Eastham-Anderson J, Dunlap D, Alicke B, Nannini M, et al. Canonical hedgehog signaling augments tumor angiogenesis by induction of VEGF-A in stromal perivascular cells. *Proc Natl Acad Sci USA.* 2011; 108(23):9589–9594. DOI: 10.1073/pnas.1017945108 [PubMed: 21597001]
39. Robinson GW, Orr BA, Wu G, Gururangan S, Lin T, Qaddoumi I, et al. Vismodegib Exerts Targeted Efficacy Against Recurrent Sonic Hedgehog-Subgroup Medulloblastoma: Results From Phase II Pediatric Brain Tumor Consortium Studies PBTC-025B and PBTC-032. *J Clin Oncol.* 2015; 33(24):2646–2654. DOI: 10.1200/jco.2014.60.1591 [PubMed: 26169613]
40. Chandra V, Das T, Gulati P, Biswas NK, Rote S, Chatterjee U, et al. Hedgehog signaling pathway is active in GBM with GLI1 mRNA expression showing a single continuous distribution rather than discrete high/low clusters. *PloS One.* 2015; 10(3):e0116390.doi: 10.1371/journal.pone.0116390 [PubMed: 25775002]
41. Berghoff AS, Kiesel B, Widhalm G, Rajky O, Ricken G, Wohrer A, et al. Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. *Neuro Oncol.* 2015; 17(8):1064–1075. DOI: 10.1093/neuonc/nou307 [PubMed: 25355681]
42. Zhang HH, Molitoris JK, Tan S, Giacomelli I, Scartoni D, Gzell C, et al. Radiomics for Survival Analysis and Prediction in Glioblastoma (GBM)-A Preliminary Study. *Int J Radiat Oncol Biol Phys.* 2016; 96(2S):E106–E107. DOI: 10.1016/j.ijrobp.2016.06.860
43. Qutub MA, Klein SB, Buchsbaum JC. Rapid RBE-Weighted Proton Radiation Dosimetry Risk Assessment. *Technol Cancer Res Treat.* 2015; 15(5):NP1–7. DOI: 10.1177/1533034615599313
44. Prasanna A, Ahmed MM, Mohiuddin M, Coleman CN. Exploiting sensitization windows of opportunity in hyper and hypo-fractionated radiation therapy. *J Thorac Dis.* 2014; 6(4):287–302. DOI: 10.3978/j.issn.2072-1439.2014.01.14 [PubMed: 24688774]
45. Kovacs A, Emri M, Opposits G, Pisak T, Vandulek C, Glavak C, et al. Changes in functional MRI signals after 3D based radiotherapy of glioblastoma multiforme. *J Neuro Oncol.* 2015; 125(1):157–166. DOI: 10.1007/s11060-015-1882-2
46. Simon R, Maitournam A. Evaluating the efficiency of targeted designs for randomized clinical trials. *Clin Cancer Res.* 2004; 10(20):6759–6763. DOI: 10.1158/1078-0432.ccr-04-0496 [PubMed: 15501951]
47. Maitournam A, Simon R. On the efficiency of targeted clinical trials. *Stat Med.* 2005; 24(3):329–339. DOI: 10.1002/sim.1975 [PubMed: 15551403]
48. Simon R, Wittes RE, Ellenberg SS. Randomized phase II clinical trials. *Cancer Treat Rep.* 1985; 69(12):1375–1381. [PubMed: 4075313]

49. Rubinstein LV, Korn EL, Freidlin B, Hunsberger S, Ivy SP, Smith MA. Design issues of randomized phase II trials and a proposal for phase II screening trials. *J Clin Oncol.* 2005; 23(28): 7199–7206. DOI: 10.1200/jco.2005.01.149 [PubMed: 16192604]
50. U.S. Department of Health and Human Services, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Guidance for Industry: Adaptive design clinical trials for drugs and biologics. Food and Drug Administration; Silver Spring, MD: 2010. www.fda.gov/downloads/drugs/guidances/ucm201790.pdf [Accessed 04 January 2017]
51. Walker AJ, Kim H, Saber H, Kim G, Kleutz P, Pazdur R. Clinical Development of Cancer Drugs in Combination with External Beam Radiation Therapy: FDA Perspective. *International Journal of Radiation Oncology, Biology and Physics.* 2017 (accepted).

Figure 1. Pathway alterations in glioblastoma multiforme. Canonical signaling pathway and tumor suppressor pathways are summarized for glioblastoma, highlighting potential nodes of convergence amenable to blockade by experimental therapeutics as monotherapy or in combination with radiation therapy. Percentages represent the proportion of glioblastoma cases harboring amplification (+) or mutation or homozygous deletion (–) of indicated pathway node. Figure adapted from reference [3]. Abbreviations: EGFR = epidermal growth factor receptor, PDGFT = platelet-derived growth factor receptor, c-MET = hepatocyte growth factor receptor, FGFR = fibroblast growth factor receptor, PTEN = phosphatase and tensin homolog, PI3K = phosphoinositide 3-kinase, NF1 = neurofibromatosis type 1, Akt = protein kinase B, mTOR = mammalian target of rapamycin, MAPK = mitogen-activated protein kinase, MDM2 = mouse double minute 2 homolog, CDK = cyclin-dependent kinase, RNR = ribonucleotide reductase, Rb = retinoblastoma protein.