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Targeting Glioma Stem Cells

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Introduction

Glioblastoma (GBM) remains to be the deadliest brain cancer in adults with almost all patients succumbing to the disease. The current standard of care, surgery followed by radiotherapy and temozolomide, prolongs the median survival from 2–3 months to 12–14 months¹. Reasons for treatment failure in GBM are multiple and include the dispersion of cancer cells into the normal brain parenchyma far beyond the bulk tumor detected by clinical imaging modalities, the almost always incomplete surgical resection of the tumor, the lack of blood-brain-barrier penetration for many systemic therapies, and the normal tissue radiation tolerance of the brain. Mounting evidence suggests that GBMs contain a small number of glioma-initiating cells (GICs)^{2–4} (often called glioma stem cells). The relative resistance of these GICs to chemo- and radiotherapy further contributes to the treatment resistance of GBM, making GICs an attractive target for novel treatment approaches against this disease^{5,6}.

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The History of the Cancer Stem Cell Hypothesis

Tumor cell heterogeneity in cancer has been recognized since 1875 when Julius Conheim published a case report on a sarcoma of the kidney⁷ and laid ground for the *cancer stem cell (CSC) hypothesis*. It argues that tumors are organized hierarchically with a small number of CSCs at the apex of this hierarchy, able to self-renew, repopulate a tumor after sublethal treatment, and give rise to the differentiated progeny, which lack these defining features of CSCs⁸. Given the resistance of CSCs to radiation^{5,9} and chemotherapy^{6,10} and their low frequency in many solid tumors including GBM¹¹, bulk tumor responses in these cancers do not necessarily reflect responses of CSCs to treatment. While this is, and always has been a well-understood phenomenon in radiation therapy, the efficacy of chemotherapeutic agents is often evaluated based on bulk tumor responses, and current classical radiosensitizers offer only marginal improvements in local control while adding significant toxicity¹². With the lack of marker systems to identify CSCs, the CSC hypothesis was mainly a theoretical concept until in 2003 Michael Clarke, Peter Dirks, and Harley Kornblum independently reported surface marker combinations that could prospectively identify tumor cell populations, highly enriched for tumor-initiating cells in breast¹³ and brain cancers^{2,4}.

In 1976, Peter Nowell introduced the *clonal evolution model* of tumor organization¹⁴. Similar to the CSC hypothesis, the model assumes a clonal origin of cancers, without proposing a hierarchical organization. The clonal evolution model postulates that the genetic instability of cancer cells leads to different clones of cells that contribute to the cellular heterogeneity of cancers; in turn, subsequent acquisition of additional mutations that favor cellular proliferation generate cells that outcompete other cell populations and become the driving cell population in a tumor. Considering the stochastic nature of acquiring additional genetic mutations, this model predicts that every cell in the tumor can acquire cancer stem cell traits through genetic changes, rather than epigenetic modifications. There is indisputable evidence supporting the genetically unstable nature of solid cancers and its role on the genetic heterogeneity of solid tumors, even if they originate from specific cell clones. What is less clear is whether or not CSC traits are shifting from one clone to another in a stochastic manner. There is evidence that the clonal evolution model may hold true for some cancers¹⁵, however the majority of solid tumors seem to follow a hierarchical model¹¹.

Finally, a lesser known interconversion model assumes multiple cellular states with differing tumorigenicities and growth rates depending on the context in which the process of differentiation is bidirectional. Evidence supporting interconversion of differentiated cells into leukemogenic cells has been reported for AML¹⁶. More recently, it has been increasingly recognized that these three models are not necessarily exclusive and that stemness is less of a binary state but rather a continuous variable contributing to the heterogeneity of tumors¹⁷.

Definitions/Background

Normal neural stem/progenitor cells in the CNS have been rigorously defined, and stem cell state and differentiation steps into neurons can be followed by well-defined marker combinations that have been carefully validated in functional assays¹⁸. The definition of

GICs is less uniform in the literature and ranges from correlating rigorous functional testing of patient-derived cells with marker profiles⁴ to less stringent studies that establish gliomaspheres from cell lines that have been cultures as monolayers for decades and labeling them glioma stem cell lines. The functional identification of tumor-initiating cells, including GICs, is traditionally performed using *in vivo* limiting dilution assays in which the frequency of GICs can be calculated retrospectively¹⁹. Time-intensive and resource-consuming, this assay is now often replaced or complemented with *in vitro* limiting dilution assays, where cells form clonal gliomaspheres from single cells under conditions that favor cells able to grow serum-free and where cells prone to die from anoikis are eliminated²⁰. Gliomaspheres in this assay do not necessarily derive exclusively from GICs but can give an approximation on the self-renewal capacity of GBM cell subpopulations.

In many studies, the prospective identification of GIC relies on the surface protein CD133 (AC133 or prominin-1), a pentaspan transmembrane glycoprotein that was first described on cellular protrusions of hematopoietic stem cells²¹. Its validity to identify GICs in GBM is discussed controversially^{22,23} with some studies viewing it as a measure of a bioenergetic stress, unrelated to stemness²⁴. Despite the controversy surrounding CD133, it is widely applied to enrich for tumorigenic GBM cells. Additional surface markers for GIC enrichment include stage-specific embryonic antigen-1 (SSEA-1)²³, a surface antigen expressed by glia and neural progenitors called A2B5²⁵, stem cell markers like nestin²⁶ and CD15²⁷, and integrin- α 6, often co-expressed with other markers and thought to contribute to both tumorsphere generation *in vitro* and tumor growth *in vivo*²⁸. Functional markers include high ALDH1 activity and lack of proteasome activity²⁹. An excellent review of surface and functional markers of GICs can be found in³⁰.

Discussion

Clinical relevance of Glioma-initiating cells

GICs constitute a small fraction of the tumor bulk³¹ but quietly play multiple critical roles in promoting relentless tumor progression. They resemble non-cancerous progenitor cells normally found in brain tissue and can produce neurons and other cell types, both *in vitro* and *in vivo*. By definition, they promote development of the initial lesion by producing differentiated cancer cells that possess the ability to rapidly divide. Additionally, when injected into immunologically deficient mice via secondary transplantation, GICs cause development of tumors phenotypically similar to the donor tissue³¹. Recent studies have demonstrated the role of GICs in promoting invasiveness, as well, especially subpopulations expressing A2B5 with or without CD133 expression^{25,32}.

In addition to their direct actions, GICs also manipulate and benefit from their microenvironment for optimal tumorigenesis. Residing in the perivascular space allows GICs to retain their stem cell properties via a steady supply of nutrients and vascular-derived signaling factors that promote self-renewal³³, and GICs further secrete extracellular matrix proteins to develop a specific microenvironment conducive to their proliferation and differentiation³⁴. Cross-signaling between GICs and other cell types also propagates tumor development³⁵. As another example, GICs effectively evade the immune system by inhibiting the proliferation of T-cells, by expressing defective antigen-processing machinery,

and by only weakly expressing MHC-I, MHC-II, and NKG2D ligand, important players in antigen recognition by T-cells and natural killer cells³⁶.

Finally, GICs remain infamous for their ability to survive both chemotherapy and radiation. Traditional chemotherapies target rapidly-dividing cells by taking advantage of unstable DNA repair mechanisms, but GICs remain rather quiescent relative to these typical tumor cells. GICs also overexpress important players in the DNA repair pathway like O-6 methylguanine-DNA-methyltransferase (MGMT)³⁷. This allows them to more efficiently correct DNA damage caused by temozolomide, which functions by methylating guanine moieties at the O-6 position, normally resulting in serious DNA damage. In fact, protein expression of MGMT in GIC cell lines predicts resistance to chemotherapy, though MGMT methylation status curiously does not strongly correlate with resistance³⁸. GICs may also express drug transporters - like multi-drug resistance 1 (MDR1) of the ABC transporter family - at higher levels to pump out therapeutic molecules³⁹. Interestingly, radiation seems to actively enrich the percentage of CD133+ GICs remaining after conventional fractionation⁵, and the GIC population that remains serves to heighten DNA repair efforts, via more efficient homologous recombination and aberrancies in the checkpoints that govern cell growth⁴⁰. As such, slowly dividing GICs allow for the universal recurrence that ultimately dampens patient survival.

Given their role in promoting recurrence, studies have shown prognostic value in the characterization of tumor GIC populations^{41,42}. A recent massive meta-analysis proved that higher levels of CD133 expression correlate with worse progression-free survival and worse overall survival, particularly among patients with grade IV - but not grade II or III - glioma, while higher levels of Nestin expression correlate with worse overall survival among patients with only grade II or III glioma⁴³. In addition, GICs can serve as a model for high-throughput experiments to identify molecules that target this quiescent population. One such study successfully cultured GICs as neurospheres with heterogeneity matching that of the original tumor and developed a high-throughput proliferation assay to identify multiple compounds with anti-GIC activity from a pilot experiment. Not all results of *in vitro* assays translate well *in vivo*, however, partly because multiple GIC lines have been identified and developed over time⁴⁴.

In reality, the phenotype of these powerful stem cells varies among patients, and a single tumor can harbor a handful of different phenotypes, as well. For example, GIC subpopulations even without CD133 expression also demonstrate similar properties. The A2B5+/CD133- subpopulation retains motility, invasiveness, and tumorigenic properties^{22,25,32}, as alluded above. The SSEA-1/CD15+ subpopulation of GICs reportedly can also form tumor spheres, albeit typically smaller than CD133+ tumor spheres and yet positive for Ki-67, rendering these smaller formations likely more proliferative than those positive for CD133⁴⁵. Such a diversity offers a mere glimpse into the complexity of various GIC subpopulations and harkens back to the heterogeneous nature of GBM, further encouraging the development of personalized combinatorial therapies for combating inevitable recurrence. The challenge of targeting GICs may very well present the most impactful existing opportunity to improve the standard-of-care for GBM, elucidated in greater depth below.

Glioma-initiating cells and the standard-of-care

The standard-of-care, surgery followed by radiotherapy and temozolomide, fails to provide cure for GBM patients and only prolongs median survival by about 12 months⁴⁶.

The benefit of surgery in patients with GBM is well-established and gross-total resection undeniably prolongs survival⁴⁷. Like all surgeries, every brain tumor resection creates a wound and sets a repair program into motion that is triggered by cytokines and hypoxia. However, hypoxia in particular is a key feature of GBM, associated with the niche requirements of GICs^{48,49} and known for supporting phenotypic plasticity of GBM cells⁵⁰. It remains to be seen if pathways engaged in GBM cell in those hypoxic resection margins can be employed against GICs.

One of the many reasons for treatment failure in GBM is that the standard-of-care differentially affects GICs and their progeny. First reported by Jeremy Rich's laboratory, GICs exhibit relative radioresistance as a result of a more efficient repair of DNA double strand breaks⁵. It is important to point out that this resistance is relative to that of more differentiated GBM cells, that GICs still respond to radiation and that radiotherapy prolongs median survival in a dose-dependent manner⁵¹ by about 6 to 9 months, thus making it the most effective agent against GBM so far. The dose-dependency of the response of GBM to radiation would imply that further dose escalation or alternative fractionation schemes could improve treatment outcome. However, standard fractionation schemes applying 2 Gy fractions, five times per week were empirically established and do not necessarily present an optimum. In fact, experimental evidence suggests that taking the heterogeneity of GBM into consideration, more unconventional fractionation schemes could improve the efficacy of radiotherapy against GBM⁵². Yet, clinical attempts at improving the impact of radiotherapy have so far largely failed⁵³ and dose escalation up to a total dose of 90 Gy did not improve survival^{54,55}.

A large number of groups have demonstrated resistance of GICs to many commonly used chemotherapeutic agents,^{6,56} including temozolomide^{38,57}. Several different mechanisms contribute to this resistance including the overexpression of ABC transporter proteins, which can be exploited to enrich GICs in side-population assays⁵⁸ and are responsible for an active efflux of chemotherapeutic agents, the preference to reside not only in a perivascular niche but also inside the hypoxic microenvironment of the tumor core^{59,60}, in which the efficacy of chemotherapy is drastically reduced^{61,62} and overexpression of free radical scavenging systems⁶³ able to detoxify drugs. Furthermore, a large number of targeted therapies against bulk tumor cell populations in GBM have largely failed to improve outcome⁶⁴.

Taken together, the standard-of-care against GBM and many of its iterations have hit a critical barrier and their inefficacy to eliminate GICs suggests that specific targeting of GICs could further improve treatment outcome for GBM patients.

Targeting Glioma-initiating cells

From the identification of tumor-initiating cells came considerable enthusiasm for novel therapies aimed at this rare population. With this in mind, the discussion of how gliomas are organized becomes of less academic and more of practical importance as it dictates possible

intervention points to target GICs. A hierarchical model of GBM implies a finite number of GICs, responsible for the repopulation of the tumor, and suggests that their successful elimination will improve treatment outcome. The understanding that those tumor-initiating cells potentially derive from normal stem cells implied to target developmental signaling pathways including the Wnt, BMP, and c-Met pathways in GICs⁶⁵, known to govern stem cell traits in normal neural/progenitor stem cells. However, if GICs are driven by the same pathways that maintain neural stem cells or progenitor cells, every attempt to target these pathways for therapeutic gain will then rely on the existence of a therapeutic window⁶⁶ that limits current established anti-tumor therapies. E.g., while CD133+ GBM cells can be targeted by inhibition of the Notch pathway by γ -secretase inhibitors in patients⁶⁷, normal stem cell compartments like that of the gastrointestinal tract rely on the same pathway and exhibit significant toxicity. In fact, clinical results fall short of the encouraging experimental findings that motivated the clinical trials⁶⁸.

If in fact the clonal evolution model more accurately describes the organizational structure of GBM, targeting GICs becomes more complicated. Different driver mutations in individual clones will emerge over time⁶⁹, leading to dysregulation of multiple pathways that potentially can maintain stemness. And even though the number of these mutations is most likely finite, surgical specimens will only provide snapshots of the evolutionary landscape of these mutations and will not be informative enough to select treatments against individual clones over time unless an overarching feature of GICs can be identified and targeted.

The problem of targeting GICs is further complicated when one factors the effects of cancer therapies⁷⁰ and the response of surviving tumor cells into the equation. Microenvironmental stress⁷¹, chemotherapy, and radiation⁷² induce interconversion of non-GICs into induced GICs, thus replenishing the pool of treatment-resistant GICs and fueling recurrences. In the case of ionizing radiation, this process involves re-expression of developmental transcription factors and global epigenetic changes⁷³. In a recent unbiased high-throughput screen, we were able to identify compounds that can interfere with the process of radiation-induced phenotype conversion⁷⁴. One group of compounds identified in this screen was that of dopamine receptor antagonists that easily cross the blood-brain barrier and not only prevent the induction of GICs but also target intrinsic GICs and non-GICs⁷³, thereby significantly prolonging survival in a mouse model of GBM. With novel dopamine receptor antagonists harboring more favorable side effect profiles in clinical development⁷⁵, this strategy could offer a novel approach to improve the efficacy of the standard-of-care in GBM.

Summary

GICs accomplish several tasks critical to tumor growth and recurrence by promoting invasiveness, immune system evasion, and resistance to existing therapeutic options via conversion of non-GICs into new GICs following radiation. While GICs can be identified by a handful of different markers (CD133, SSEA-1, A2B5, nestin, integrin- α 6), the scientific community still lacks a complete understanding of different such subtypes. Furthermore, translating the excitement surrounding experimental therapies into successful clinical outcomes has proved difficult. Hampering the effect of GICs may be possible, however, by

inhibiting the treatment-induced phenotype conversion that replenishes residual tumor of its GIC population and helps promote recurrence.

References

1. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987–996. [PubMed: 15758009]
2. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res*. 2003;63(18):5821–5828. [PubMed: 14522905]
3. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature*. 2004;432(7015):396–401. [PubMed: 15549107]
4. Hemmati HD, Nakano I, Lazareff JA, et al. Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci U S A*. 2003;100(25):15178–15183. [PubMed: 14645703]
5. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756–760. [PubMed: 17051156]
6. Eramo A, Ricci-Vitiani L, Zeuner A, et al. Chemotherapy resistance of glioblastoma stem cells. *Cell Death Differ*. 2006;13(7):1238–1241. [PubMed: 16456578]
7. Congenitales Cohnheim J., quergestreiftes Muskelsarkom der Nieren. *Virchows Archiv für Pathologische Anatomie und Physiologie und für Klinische Medizin*. 1875;65(1):64–69.
8. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature*. 2001;414(6859):105–111. [PubMed: 11689955]
9. Phillips TM, McBride WH, Pajonk F. The response of CD24(–/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst*. 2006;98(24):1777–1785. [PubMed: 17179479]
10. Li X, Lewis MT, Huang J, et al. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *Journal of the National Cancer Institute*. 2008;100(9):672–679. [PubMed: 18445819]
11. Ishizawa K, Rasheed ZA, Karisch R, et al. Tumor-initiating cells are rare in many human tumors. *Cell Stem Cell*. 2010;7(3):279–282. [PubMed: 20804964]
12. Overgaard J Chemoradiotherapy of head and neck cancer--can the bumble bee fly? *Radiother Oncol*. 2009;92(1):1–3. [PubMed: 19539125]
13. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA*. 2003;100:3983–3988. [PubMed: 12629218]
14. Nowell PC. The clonal evolution of tumor cell populations. *Science*. 1976;194(4260):23–28. [PubMed: 959840]
15. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. *Nature*. 2008;456(7222):593–598. [PubMed: 19052619]
16. McKenzie MD, Ghisi M, Oxley EP, et al. Interconversion between Tumorigenic and Differentiated States in Acute Myeloid Leukemia. *Cell Stem Cell*. 2019;25(2):258–272 e259. [PubMed: 31374198]
17. MacArthur BD, Lemischka IR. Statistical mechanics of pluripotency. *Cell*. 2013;154(3):484–489. [PubMed: 23911316]
18. Bazan E, Alonso FJ, Redondo C, et al. In vitro and in vivo characterization of neural stem cells. *Histol Histopathol*. 2004;19(4):1261–1275. [PubMed: 15375770]
19. Hu Y, Smyth GK. ELDA: extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. *J Immunol Methods*. 2009;347(1–2):70–78. [PubMed: 19567251]
20. Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol*. 1994;124(4):619–626. [PubMed: 8106557]
21. Yin AH, Miraglia S, Zanjani ED, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood*. 1997;90(12):5002–5012. [PubMed: 9389720]

22. Ogden AT, Waziri AE, Lochhead RA, et al. Identification of A2B5+CD133– tumor-initiating cells in adult human gliomas. *Neurosurgery*. 2008;62(2):505–514; discussion 514–505. [PubMed: 18382330]
23. Son MJ, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell stem cell*. 2009;4(5):440–452. [PubMed: 19427293]
24. Griguer CE, Oliva CR, Gobin E, et al. CD133 is a marker of bioenergetic stress in human glioma. *PLoS ONE*. 2008;3(11):e3655. [PubMed: 18985161]
25. Tchoghandjian A, Baeza N, Colin C, et al. A2B5 cells from human glioblastoma have cancer stem cell properties. *Brain pathology (Zurich, Switzerland)*. 2010;20(1):211–221.
26. Jin X, Jin X, Jung JE, Beck S, Kim H. Cell surface Nestin is a biomarker for glioma stem cells. *Biochemical and biophysical research communications*. 2013;433(4):496–501. [PubMed: 23524267]
27. Mao XG, Zhang X, Xue XY, et al. Brain Tumor Stem-Like Cells Identified by Neural Stem Cell Marker CD15. *Translational oncology*. 2009;2(4):247–257. [PubMed: 19956386]
28. Lathia JD, Gallagher J, Heddleston JM, et al. Integrin alpha 6 regulates glioblastoma stem cells. *Cell stem cell*. 2010;6(5):421–432. [PubMed: 20452317]
29. Vlashi E, Kim K, Lagadec C, et al. In vivo imaging, tracking, and targeting of cancer stem cells. *Journal of the National Cancer Institute*. 2009;101(5):350–359. [PubMed: 19244169]
30. Ludwig K, Kornblum HI. Molecular markers in glioma. *J Neurooncol*. 2017;134(3):505–512. [PubMed: 28233083]
31. Lathia JD, Gallagher J, Myers JT, et al. Direct in vivo evidence for tumor propagation by glioblastoma cancer stem cells. *PloS one*. 2011;6(9):e24807. [PubMed: 21961046]
32. Sun T, Chen G, Li Y, Xie X, Zhou Y, Du Z. Aggressive invasion is observed in CD133(–)/A2B5(+) glioma-initiating cells. *Oncology letters*. 2015;10(6):3399–3406. [PubMed: 26788141]
33. Calabrese C, Poppleton H, Kocak M, et al. A perivascular niche for brain tumor stem cells. *Cancer cell*. 2007;11(1):69–82. [PubMed: 17222791]
34. Niibori-Nambu A, Midorikawa U, Mizuguchi S, et al. Glioma initiating cells form a differentiation niche via the induction of extracellular matrices and integrin α V. *PloS one*. 2013;8(5):e59558. [PubMed: 23704872]
35. Jeon HM, Kim SH, Jin X, et al. Crosstalk between glioma-initiating cells and endothelial cells drives tumor progression. *Cancer research*. 2014;74(16):4482–4492 [PubMed: 24962027]
36. Di Tomaso T, Mazzoleni S, Wang E, et al. Immunobiological characterization of cancer stem cells isolated from glioblastoma patients. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2010;16(3):800–813. [PubMed: 20103663]
37. Kitange GJ, Carlson BL, Schroeder MA, et al. Induction of MGMT expression is associated with temozolomide resistance in glioblastoma xenografts. *Neuro-oncology*. 2009;11(3):281–291. [PubMed: 18952979]
38. Blough MD, Westgate MR, Beauchamp D, et al. Sensitivity to temozolomide in brain tumor initiating cells. *Neuro-oncology*. 2010;12(7):756–760. [PubMed: 20388697]
39. Nakai E, Park K, Yawata T, et al. Enhanced MDR1 expression and chemoresistance of cancer stem cells derived from glioblastoma. *Cancer investigation*. 2009;27(9):901–908. [PubMed: 19832037]
40. Lim YC, Roberts TL, Day BW, et al. A role for homologous recombination and abnormal cell-cycle progression in radioresistance of glioma-initiating cells. *Molecular cancer therapeutics*. 2012;11(9):1863–1872. [PubMed: 22772423]
41. Zeppernick F, Ahmadi R, Campos B, et al. Stem cell marker CD133 affects clinical outcome in glioma patients. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2008;14(1):123–129. [PubMed: 18172261]
42. Zhang M, Song T, Yang L, et al. Nestin and CD133: valuable stem cell-specific markers for determining clinical outcome of glioma patients. *Journal of experimental & clinical cancer research : CR*. 2008;27(1):85. [PubMed: 19108713]
43. Wu B, Sun C, Feng F, Ge M, Xia L. Do relevant markers of cancer stem cells CD133 and Nestin indicate a poor prognosis in glioma patients? A systematic review and meta-analysis. *Journal of experimental & clinical cancer research : CR*. 2015;34(1):44. [PubMed: 25967234]

44. Mock A, Chiblak S, Herold-Mende C. Lessons we learned from high-throughput and top-down systems biology analyses about glioma stem cells. *Current pharmaceutical design*. 2014;20(1):66–72. [PubMed: 23530497]
45. Ahmed AU, Auffinger B, Lesniak MS. Understanding glioma stem cells: rationale, clinical relevance and therapeutic strategies. *Expert review of neurotherapeutics*. 2013;13(5):545–555. [PubMed: 23621311]
46. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009;10(5):459–466. [PubMed: 19269895]
47. Sanai N, Polley MY, McDermott MW, Parsa AT, Berger MS. An extent of resection threshold for newly diagnosed glioblastomas. *J Neurosurg*. 2011;115(1):3–8. [PubMed: 21417701]
48. Li Z, Bao S, Wu Q, et al. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell*. 2009;15(6):501–513. [PubMed: 19477429]
49. Li Z, Rich JN. Hypoxia and hypoxia inducible factors in cancer stem cell maintenance. *Curr Top Microbiol Immunol*. 2010;345:21–30. [PubMed: 20582533]
50. Heddleston JM, Li Z, McLendon RE, Hjelmeland AB, Rich JN. The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. *Cell Cycle*. 2009;8(20):3274–3284. [PubMed: 19770585]
51. Walker MD, Strike TA, Sheline GE. An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int J Radiat Oncol Biol Phys*. 1979;5(10):1725–1731. [PubMed: 231022]
52. Leder K, Pitter K, LaPlant Q, et al. Mathematical modeling of PDGF-driven glioblastoma reveals optimized radiation dosing schedules. *Cell*. 2014;156(3):603–616. [PubMed: 24485463]
53. Laperriere N, Zuraw L, Cairncross G, Cancer Care Ontario Practice Guidelines Initiative Neuro-Oncology Disease Site G. Radiotherapy for newly diagnosed malignant glioma in adults: a systematic review. *Radiother Oncol*. 2002;64(3):259–273. [PubMed: 12242114]
54. Badiyan SN, Markovina S, Simpson JR, et al. Radiation therapy dose escalation for glioblastoma multiforme in the era of temozolomide. *Int J Radiat Oncol Biol Phys*. 2014;90(4):877–885. [PubMed: 25257812]
55. Wegner RE, Abel S, Horne ZD, et al. National trends in radiation dose escalation for glioblastoma. *Radiat Oncol J*. 2019;37(1):13–21. [PubMed: 30947476]
56. Kenig S, Faoro V, Bourkoula E, et al. Topoisomerase II β mediates the resistance of glioblastoma stem cells to replication stress-inducing drugs. *Cancer cell international*. 2016;16:58. [PubMed: 27462186]
57. Liu G, Yuan X, Zeng Z, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer*. 2006;5:67. [PubMed: 17140455]
58. Hirschmann-Jax C, Foster AE, Wulf GG, et al. A distinct “side population” of cells with high drug efflux capacity in human tumor cells. *Proc Natl Acad Sci U S A*. 2004;101(39):14228–14233. [PubMed: 15381773]
59. Smith SJ, Diksin M, Chhaya S, Sairam S, Estevez-Cabrero MA, Rahman R. The Invasive Region of Glioblastoma Defined by 5ALA Guided Surgery Has an Altered Cancer Stem Cell Marker Profile Compared to Central Tumour. *Int J Mol Sci*. 2017;18(11).
60. Sattiraju A, Sai KKS, Mintz A. Glioblastoma Stem Cells and Their Microenvironment. *Adv Exp Med Biol*. 2017;1041:119–140. [PubMed: 29204831]
61. Teicher BA. Hypoxia and drug resistance. *Cancer Metastasis Rev*. 1994;13(2):139–168. [PubMed: 7923547]
62. Musah-Eroje A, Watson S. A novel 3D in vitro model of glioblastoma reveals resistance to temozolomide which was potentiated by hypoxia. *J Neurooncol*. 2019;142(2):231–240. [PubMed: 30694423]
63. Dokic I, Hartmann C, Herold-Mende C, Regnier-Vigouroux A. Glutathione peroxidase 1 activity dictates the sensitivity of glioblastoma cells to oxidative stress. *Glia*. 2012;60(11):1785–1800. [PubMed: 22951908]
64. Bai RY, Staedtke V, Riggins GJ. Molecular targeting of glioblastoma: Drug discovery and therapies. *Trends Mol Med*. 2011;17(6):301–312. [PubMed: 21411370]

65. Mehta S, Lo Cascio C. Developmentally regulated signaling pathways in glioma invasion. *Cell Mol Life Sci.* 2018;75(3):385–402. [PubMed: 28821904]
66. Holthausen H Erfahrungen über die Verträglichkeitsgrenze für Röntgenstrahlen und deren Nutzenanwendung zur Verhütung von Schäden. *Strahlentherapie.* 1936(57):254–269.
67. Xu R, Shimizu F, Hovinga K, et al. Molecular and Clinical Effects of Notch Inhibition in Glioma Patients: A Phase 0/I Trial. *Clin Cancer Res.* 2016;22(19):4786–4796. [PubMed: 27154916]
68. McCaw TR, Inga E, Chen H, et al. Gamma secretase inhibitors in cancer: a current perspective on clinical performance. *Oncologist.* 2020.
69. Abou-El-Ardat K, Seifert M, Becker K, et al. Comprehensive molecular characterization of multifocal glioblastoma proves its monoclonal origin and reveals novel insights into clonal evolution and heterogeneity of glioblastomas. *Neuro Oncol.* 2017;19(4):546–557. [PubMed: 28201779]
70. Wang J, Cazzato E, Ladewig E, et al. Clonal evolution of glioblastoma under therapy. *Nat Genet.* 2016;48(7):768–776. [PubMed: 27270107]
71. Lee G, Auffinger B, Guo D, et al. Dedifferentiation of Glioma Cells to Glioma Stem-like Cells By Therapeutic Stress-induced HIF Signaling in the Recurrent GBM Model. *Mol Cancer Ther.* 2016;15(12):3064–3076. [PubMed: 27765847]
72. Safa AR, Saadatzadeh MR, Cohen-Gadol AA, Pollok KE, Bijangi-Vishehsaraei K. Glioblastoma stem cells (GSCs) epigenetic plasticity and interconversion between differentiated non-GSCs and GSCs. *Genes Dis.* 2015;2(2):152–163. [PubMed: 26137500]
73. Bhat K, Saki M, Vlashi E, et al. The dopamine receptor antagonist trifluoperazine prevents phenotype conversion and improves survival in mouse models of glioblastoma. *Proc Natl Acad Sci U S A.* 2020;117(20):11085–11096. [PubMed: 32358191]
74. Zhang L, Bochkur Dratver M, Yazal T, et al. Mebendazole Potentiates Radiation Therapy in Triple-Negative Breast Cancer. *Int J Radiat Oncol Biol Phys.* 2019;103(1):195–207. [PubMed: 30196056]
75. Prabhu VV, Morrow S, Rahman Kawakibi A, et al. ONC201 and imipridones: Anti-cancer compounds with clinical efficacy. *Neoplasia.* 2020;22(12):725–744. [PubMed: 33142238]

KEY POINTS

- The concept of tumor-initiating cells is an old concept in oncology that was recently revived with the discovery of markers, able to enrich for tumor-initiating cells
- Intratumoral heterogeneity and plasticity during undisturbed growth and in response to anti-cancer affect the efficacy of the standard-of-care in glioblastoma
- Targeting cellular plasticity in glioblastoma emerges as a means to improve treatment outcome in glioblastoma

SYNOPSIS

Only a small fraction of the tumor cell population, glioma-initiating cells (GICs) help glioblastoma propagate, invade, evade immune recognition, repair DNA in response to radiation, remodel the microenvironment for optimal growth, and actively pump out chemotherapies. Recent data hints that efforts toward GIC characterization and quantification can help predict patient outcomes, and yet the different sub-populations of GICs remain incompletely understood. A better understanding of GIC sub-types and functions proves critical for engineering targeted therapies. Challenges for doing so are discussed, and dopamine receptor antagonists are introduced as new means to enhance the efficacy of the current standard-of-care against GICs.

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CLINICS CARE POINTS

- GICs drive recurrences and are a potential target against GBM
- Prospective identification of GICs is hampered by the fact that marker systems only enrich for GICs
- Intratumoral heterogeneity and plasticity of GBM allow tumors to escape therapies aimed at GICs

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