Title

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Journal

Clinical Cancer Research, 26(11)

ISSN

1078-0432

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Publication Date

2020-06-01

DOI

10.1158/1078-0432.ccr-19-3924

Peer reviewed

Published in final edited form as:

Clin Cancer Res. 2020 June 01; 26(11): 2457–2460. doi:10.1158/1078-0432.CCR-19-3924.

Translating basic science discoveries into improved outcomes for glioblastoma

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Introduction.

Glioblastoma is the most common primary malignant brain tumor in adults and also occurs commonly in children. It remains among the most intractable of cancers, with short survival times and significant neurological morbidity. A hallmark feature of glioblastoma is infiltration into normal brain, often involving critical deep brain structures, which makes it incurable by surgery. Over the past 10+ years, hundreds of clinical trials in both adults and in children, including large phase III international trials in adults have ended without any clinical benefit or any change in clinical care. This failure is despite enormous strides in understanding the genetic, epigenetic, metabolic, and biologic basis for the disease over the same time frame.

The Banbury Center meeting, which was held from April 7–10, 2019, hosted 33 scientists and clinicians who discussed the topic “Glioblastoma: Why is impactful science so hard to translate?” The participants sought to identify roadblocks to the translational application of fundamental basic science discoveries. The consensus of the group was that multiple factors spanning the full range from basic science through clinical trial design and implementation contribute to the challenge in achieving improved survival and ultimate cure.

The molecular hierarchy in glioblastoma was a major focus of the meeting, recognizing that the identification of true “driver” mutations, the potentially key targets, is complex. Are there truncal mutations that remain essential targets or act only in the earliest stages of disease evolution, thereby reducing their therapeutic importance? An example is the *IDH* mutation, one of the earliest to occur in low grade gliomas, which may have no relevance after transformation to glioblastoma. Similarly, targeting mutations or pathways present at

glioblastoma diagnosis may not be relevant in the setting of recurrent disease. There may be redundancy in the pathways of a potentially key mutation. Moreover, the patient may present with primary refractory disease. Discussion throughout the meeting also focused on the importance of preclinical testing and the choice of a wide range of model systems for preclinical validation of targets, choosing the most informative validation system for a particular target, including clear documentation of blood-brain barrier penetrance and target inhibition. These questions and issues significantly impact design of clinical trials and interpretation of outcomes. Additionally, generating international standards for collection and analyses of tumors was a common theme, in terms of improving clinical trial design and patient stratification, as well as maximizing the potential impact of biomarkers of response or lack of response on understanding outcomes. The need for "look back" on failed clinical trials to identify where in the development of a therapy did the failure occur, was deemed highly valuable going forward.

Discussion

Natural history of disease.

Glioblastoma arises in the confines of a fixed boney structure (skull), and adjacent to eloquent functional areas, complicating the ability to gain local control or to obtain serial biopsies. The infiltrative nature of these tumors likely reflects the normal biology of glial cells that mobilize in the developing brain, or of cells that have engaged a mobile developmental state. Recent studies have clarified aspects of tumor evolution by obtaining and sequencing multiple samples from within a tumor at one point in time. However, there is minimal data from tumors sampled at multiple time points during tumor progression and almost no data on sampling during therapy, whether responding or not. As a result, the understanding of the natural history of disease and response to specific therapies (single agents or combination treatments), is very limited. Yet decisions regarding the further development and use of specific treatments, and decisions on therapies to use in the relapsed setting are being made with little more than radiographic and/or clinical features. A strong endorsement of serial biopsy, both within the tumor and at the tumor margin, was made by the group, recognizing a major limitation in the information content of the natural history or tumor response/lack of response, by rarely having tumor beyond the initial surgical specimen to study. Moreover, the vastly different situation in non-CNS tumors was highlighted along with the highly valuable data in these cancers from iterative tumor sampling. It was agreed that further discussions of implementing safe rebiopsy be a point of focus going forward in the field.

Tumor heterogeneity was also a topic of discussion, especially in the light of increasing data available from single-cell transcriptome analysis of patient samples (1). These studies reveal a remarkable degree of genetic and cellular heterogeneity within patient tumors. How this diverse cellular context (including myeloid and lymphoid cell types that are now well-recognized microenvironmental components of glioblastoma) changes over time and with therapy will need to be understood. Issues related to heterogeneity raise critical questions. Are the numerous parenchymal cell states identified in glioblastoma stable or changeable? Are precursor and more differentiated cell identities reversible or hierarchically related?

There was an extensive discussion about the course of evolution of glioblastoma. Is there an extended period of early disease that then undergoes an explosive secondary change at the time of presentation (2) ? Issues of latency and evolution have implications for understanding initiating genetic or molecular lesions, and events that might drive eruptive secondary behavior. Important areas discussed included whether glioblastoma after treatment (in therapy-responsive patients) moves into a quiescent or dormant state, perhaps to a state in symbiosis with normal brain. Clearly, there is a point of disease progression/recurrence when cells enter back into the cell cycle, resuming a highly proliferative state, most often resistant to treatment. This “switch” from dormancy to proliferation is similar in low-grade gliomas where 1–3% of cells are proliferating for an extended period of time (often years), that is marked by a clear point of transition (radiographically and often clinically) to the rapidly proliferative state. The “switch” is currently a black box both in timing and mechanism, although low- to high- grade glioma is characterized by acquisition of additonal genetic mutations in key proliferative pathways.

It is likely that many patients with glioblastoma never achieve quiescence or dormancy, instead progress inexorably through therapy. It is therefore essential that genetic markers are identified in the primary tumor to enable physicians to stratify patients with primary resistance upfront, enabling these patients to be studied in greater depth and to be treated with more aggressive frontline therapy. The retrospective history of molecular subgroups with poor outcomes suggests that the majority of these patients die before being enrolled on any trial.

Based on TCGA data, approximately 10% of IDH wild-type glioblastoma survive are long term survivors, living byond three year from diagnosis (3, 4) A reference landscape of this patient population generated by exome sequence and copy number (3) identifies approximately 40% of the IDH wild-type glioblastomas as having a molecular structure predicting a very low likelihood of re-resection. (4) This group has a shorter median survival than the group as a whole and does not contain any of long term survivors noted above. Further, a refined subset of this group containing approximately a third of the total IDH wild-type glioblastoma has a median survival of less than a year, statistically shorter than the remainder of the IDH wild-type glioblastoma ($p < .0005$). (5)

Therefore, current clinical trials in glioblastoma do not sample this population of the worst prognosis patients. Even autopsy data, for patients with primary therapy resistance, would allow us to study evolution at two points in time, potentially providing insights into evolution that could impact therapy. At the opposite end of this spectrum, the group agreed that there was insufficient focus on identifying markers of long-term survivors. Insights from both groups could lead to a better understanding of differences that could be exploited therapeutically.

The discussion of needing to focus on improved understanding of these dormant vs. proliferative states and the switch between the two again highlights the importance of considering rebiopsy for tumor resampling. The community could establish international standards for sample collection and analysis, collection of viable tissue, routine generation of primary serum-free cultures and patient-derived orthotopic models, a national or

international registry, and input/support from patient advocates. There were discussions concerning imaging opportunities, better or even routine use of autopsy tissue, and liquid biopsies (CSF versus plasma (6)), although the amount of DNA in plasma appears too low currently for reproducible analysis,

The failure of surgery to cure glioblastoma, even when an extensive gross total resection is achieved, suggests a high degree of infiltration at diagnosis. However, the majority of data demonstrating extensive infiltration is from autopsy at end-stage and, in fact, approximately 80% of patients recur at the tumor margin. Participants debated as to whether the biology of primary nodular disease might differ from that of distantly infiltrating disease, and whether there would be value in obtaining biopsies from the subventricular zone, a neurogenic region from which glioblastoma may arise. At the time of first resection, multiple biopsies from regions including the subventricular zone, and of normal brain from the surgical tract (on the way to a deeper contrast enhancing tumor), or lobectomy specimens, could clarify the extent of infiltration at presentation, as compared to that at autopsy. There was a discussion as to whether this information would impact therapeutic response. While it is important to understand how tumor cells respond to their environment, it is unclear whether distinct genetic mutations that differ between primary tumor and infiltrating tumor in the subventricular zone or other brain regions, are likely to represent robust targets for therapy. An analysis of normal brain regions in glioblastoma patients could be undertaken to investigate these issues. However, unless it becomes clear that this would change the management of patients, the costs of these procedures would need to be covered by the research community.

There was a general discussion of rigor and standards being utilized strategically to achieve publication. Perhaps, as a group, we could better serve the community by increasing rigor, and drawing realistic rather than over-reaching conclusions about translational applications in preclinical studies in glioblastoma. Again, there was emphasis on the need for standards of performance of the biopsy, analysis and standard operating procedures for tissue processing, to be sure that results obtained from multiple centers are comparable.

Cells of origin

Glial cells exist throughout the nervous system, with recent studies demonstrating both regional and functional specificity for astrocytes in distinct brain regions (7). Might this diversity contribute to restrictions in those astrocyte populations capable of generating glial tumors? And what is the role of more primitive cell types such as neural stem cells? Recent studies layered single-cell RNAseq data from human pediatric embryonal brain tumors onto a developmental atlas of single cell RNA-seq during brain development in the mouse (8,9). These studies identified candidate developmental cell lineages of origin for pediatric brain tumors, including cells that exist only transiently during embryonic development. Similar studies, extended to glioblastoma, could identify candidate cells of origin relevant to both pediatric and adult glioma. By revealing the lineage of brain tumors, researchers can conceivably nominate pathways that represent new drug targets for these malignancies, and start to more robustly probe mechanisms of tumor initiation that might have a bearing on

earlier diagnosis or treatment. This, coupled with improved model systems, could be used for preclinical testing of treatments that could impact very early stage disease.

Improved patient stratification

Discussants agreed on the need to test and identify prospective markers for outcome and for therapy. There was discussion on use of copy number aberrations to stratify patients in clinical trials, and discussion of trunk versus branch abnormalities as therapeutic targets. The *IDH* mutation appears to be a main trunk target in a subset of gliomas because it is present in all glioblastoma cells sampled at different regions in time and space (10,11), although this may or may not be required for maintenance of these tumors once they have transformed to glioblastoma. Using that criteria, copy number aberrations in IDH wildtype gliomas also appear to be truncal events (12). An improving understanding of tumor evolution, including sampling tumors at multiple points in both time and space, may clarify this issue; however, once a tumor is clinically observable, it may be late by definition. If such results establish copy number aberrations as truncal events, then these aberrations could/should be targeted, perhaps by synthetic lethality approaches. However, there are concerns that the synthetic lethality approach may have more success in less heterogeneous tumors where the synthetic lethality partners may not be uniform, given the many tumor subclones in glioblastoma.

As noted above, there was discussion of stratifying patients based on copy number aberrations, and other genetic events, into groups likely to represent long-term survivors, groups likely to have a short but measurable clinical response, achieving transient dormancy in response to therapy, and those likely to progress and never be resampled. Finding “truncal” targets may be too ambitious as a first step. Could genomic analysis and phylogeny at diagnosis be used as a predictor of outcome and future mutational patterns? These analyses would provide some insights into the early clonal diversity of an individual cancer, thereby enabling the analysis of subsequent recurrent tumor samples to determine if treatment of the primary cancer can alter the evolution of the cancer or anticipate the development of resistance mechanisms. A similar strategy determined that the addition of a MEK inhibitor to a BRAF inhibitor improved outcomes in patients with $BRAF^{V600E}$ mutated melanoma. Furthermore, it is clear that glioblastoma has a close relationship to neural precursor biology despite the diversity of mutations identified. Efforts to understand the common precursor characteristics at the level of transcription factors or epigenetic states is deserving of intense effort, although targeting these processes will be challenging.

Improved therapeutic options and outcomes.

The group discussed the pharmacological armamentarium available to patients with glioblastoma. There is a general lack of glioblastoma-specific activating point mutations in kinases, and of drugs that can offer some index for a glioblastoma-associated kinase over a wild-type kinase. Nevertheless, there was some agreement that PDGFR and EGFR may remain viable as targets, that the failure of trials in patients whose tumors are driven by PDGFR or EGFR may reflect use of drugs with poor brain:plasma concentration ratios, and failure to effectively hit the target, rather than failures of an adequately blocked target to impact progression. These potential targets highlight the basic premise that there is inadequate information available when taking a drug into a clinical trial, as discussed

previously. At a minimum, there was a strong endorsement for using drugs with favorable brain:plasma ratios that inhibit these targets and are documented preclinically. There was also discussion about direct delivery of drugs to the CNS, via the cerebrospinal fluid (CSF) or convection-enhanced delivery. Intrathecal administration of some cytotoxic agents has made in-roads in some aggressive pediatric brain tumors such as atypical teratoid rhabdoid tumors, and alternative modes of therapeutic delivery should be considered.

Consensus statement

Conference attendees concurred that this meeting was valuable, and agreed on the importance of having meetings similar to the Banbury conference, bringing together basic and clinical investigators working on many different aspects of cancer and glioblastoma biology. The clinicians felt strongly that as a field, we need to step up our preclinical and clinical studies to improve outcomes.

Our preclinical recommendations are to:

1. Elucidate connections between brain development and tumorigenesis, to inform potential treatment avenues. Given the diversity of cell types in the nervous system, and species-specific differences in both development and in cell types between rodents and humans, there is need for a normal atlas of human brain development, on which to overlay human brain tumors, to elucidate candidate cells of origin.
2. Clarify how the brain tumor microenvironment changes with tumor progression and in response to therapy, information critical for development of new therapies, especially immunotherapy. While the vast majority of preclinical studies are done in treatment naïve tumors, most agents are introduced into the recurrent setting. There is need to develop accurate preclinical models for tumor recurrence, in order to improve confidence that efficacy in a preclinical setting justifies moving forward with clinical trials. New methods for cataloguing an immune profile for brain cancers, mass cytometry and multiplexed ion beam imaging could provide rapid insights here.
3. Perform preclinical and ex vivo testing to validate pharmacodynamic assays before incorporation into clinical studies, where patients are undergoing an invasive procedure.

Our recommendations for clinical trials are to:

4. Enhance the utilization of molecularly characterized preclinical models to better recapitulate subgroups of patients where inter-tumoral heterogeneity has contributed to a negative clinical trials that often have a small responding subpopulation.
5. Utilize these models along with state of the art assay development to insure that the bioassays for tumor effect (pharmacodynamics) and delivery (pharmacokinetics) are accurate and reproducible.

6. Using the refined assay systems, increase the use of Phase 0 “Proof of target engagement” approaches to study pharmacodynamics and pharmacokinetics in clinical studies prior to launching large, resource intensive clinical trials.

7. Document in patients that drugs cross the blood-brain barrier, hit their targets, and modify downstream effectors. These critical issues will require rebiopsy on treatment, and promise to:

   a. Clarify the importance of the blood-brain barrier, and

   b. Establish standard criteria for pharmacodynamic studies, assessing tumor tissue for molecular changes.

8. Use the results from the preclinical and Phase 0 studies to either molecularly stratify or enrich patient enrollment in Phase I-II clinical trials. These trials need to incorporate tumor sampling, biomarker studies, and outcome analyses in both responders and non-responders, setting the stage for the next generation of clinical trials.

Acknowledgements:

We thank Rebecca Leshan and David Tuveson for help and support in organizing this meeting, and David Tuveson for critical review of this manuscript.

Members of the scientific and clinical neuro-oncology community met in April 2019 to discuss the current challenges and opportunities associated with translating basic science discoveries in glioblastoma to improved survival for patients. A summary of key points of these discussions is presented in this report.

Abbreviations:

CNS	Central Nervous System
CSF	Cerebrospinal Fluid
EGFR	Epidermal Growth Factor
IDH	Isocitrate Dehydrogenase
MEK	Mitogen-Activated Protein Kinase
PDGFR	Platelet Derived Growth Factor
RAF	Rapidly Accelerated Fibrosarcoma

References

1. Suvà ML, Tirosh I. Single-Cell RNA Sequencing in Cancer: Lessons Learned and Emerging Challenges. Mol Cell. 2019; 75:7–12. [PubMed: 31299208]
2. Körber V, Yang J, Barah P, Wu Y, Stichel D, Gu Z, et al. Evolutionary Trajectories of IDHWT Glioblastomas Reveal a Common Path of Early Tumorigenesis Instigated Years ahead of Initial Diagnosis. Cancer Cell. 2019; 35:692–704 PMID: 30905762 [PubMed: 30905762]
3. Bolouri H, Zhao LP, Holland EC. Big data visualization identifies the multidimensional molecular landscape of human gliomas. Proc Natl Acad Sci U S A. 2016; 113:5394–9. [PubMed: 27118839]

4. McFerrin LG, Zager M, Zhang J, Krenn G, McDermott R, Horse-Grant D, et al. Analysis and visualization of linked molecular and clinical cancer data by using Oncoscape. Nat Genet. 2018;50:1203–4. [PubMed: 30158685]

5. Cimino PJ, Zager M, McFerrin L, Wirsching H- G, Bolouri H, Hentschel B, et al. Multidimensional scaling of diffuse gliomas: application to the 2016 World Health Organization classification system with prognostically relevant molecular subtype discovery. Acta Neuropathol Commun. 2017; 5:39. [PubMed: 28532485]

6. Miller AM, Shah RH, Pentsova EI, Pourmaleki M, Briggs S, Distefano N, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. Nature. 2019;565(7741):654–58. [PubMed: 30675060]

7. Ben Haim L, Rowitch DH. Functional diversity of astrocytes in neural circuit regulation. Nat Rev Neurosci. 2017;18:31–41. [PubMed: 27904142]

8. Vladoiu MC, El-Hamamy I, Donovan LK, Farooq H, Holgado BL, Sundaravadanam Y, et al. Childhood cerebellar tumours mirror conserved fetal transcriptional programs. Nature. 2019 572:67–73. [PubMed: 31043743]

9. Hovestadt V, Smith KS, Bihannic L, Filbin MG, Shaw ML, Baumgartner A, et al. Resolving medulloblastoma cellular architecture by single-cell genomics. Nature. 2019 572:74–79. [PubMed: 31341285]

10. Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, McLean CY. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science. 2014 343:189–93. [PubMed: 24336570]

11. Mazor T, Pankov A, Johnson BE, Hong C, Hamilton EG, Bell RJA. DNA Methylation and Somatic Mutations Converge on the Cell Cycle and Define Similar Evolutionary Histories in Brain Tumors. Cancer Cell. 2015 28:307–17. [PubMed: 26373278]

12. Johannessen T- CA, Mukherjee J, Viswanath P, Ohba S, Ronen SM, Bjerkvig R, et al. Rapid Conversion of Mutant IDH1 from Driver to Passenger in a Model of Human Gliomagenesis. Mol Cancer Res MCR. 2016;14:976–83. [PubMed: 27430238]