For the Next Trick: New Discoveries in Radiobiology Applied to Glioblastoma

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OVERVIEW

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults. Since the 1970s, radiotherapy together with surgery has constituted the cornerstone in multimodal management of patients with GBM.1 In 2005, the addition of alkylating agent temozolomide chemotherapy to radiotherapy was shown to prolong the median overall survival (OS) by 2.5 months from 12.1 to 14.6 months.2 Therefore, this regimen is considered current standard in management of this devastating disease. A recent phase III trial investigated the effect of dose escalation of maintenance temozolomide chemotherapy to dose-dense temozolomide (administered at 75–100 mg/m² for 21 consecutive days of a 28-day cycle) to dose-dense temozolomide (150–200 mg/m² days 1 to 5 of a 28-day cycle) to dose-dense temozolomide (administered at 75–100 mg/m² for 21 consecutive days of a 28-day cycle). Interestingly, the median OS was 16.6 months in standard-versus 14.9 months in the dose-dense temozolomide arm, respectively, and no statistically significant difference was observed between arms (hazard ratio [HR] 1.03; p = 0.63). Likewise, previous radiotherapy dose escalation studies did not improve OS in GBM.3 Toward improvement of GBM therapy, a better comprehension of distinct biology and mechanisms governing therapy resistance is needed. Histopathologic features distinguishing GBM from lower-grade astrocytomas include: (a) central necrotic foci with surrounding highly hypoxic cellular pseudopalisades that contribute via expression of angiogenic and extracellular matrix (ECM)-remodeling factors to the (b) microvascular hyperplasia, and (c) infiltrative growth in the tumor periphery.1

LOCAL RECURRENCE AS THE PREDOMINANT PATTERN OF THERAPY FAILURE

In contrast to most other solid tumors where systemic tumor progression often limits the efficacy of local treatments such as surgery and radiotherapy, in GBM, the predominant pattern of therapy failure is the development and rapid progression of local recurrences. Analysis of the GBM recurrence pattern post surgery and radiotherapy indicated that the vast majority of all recurrent tumors are localized in the high-dose radiation field. Based on the volume of the recurrent tumor localizing within the initial high-dose region of radiotherapy the term “central” recurrence (>95%), “in-field” recurrence (>80%), “marginal” recurrence (between 20% and 80%) and “distant” recurrence were used. Although more than 91% of recurrences were central (78%) or in-field (13%), respectively,
only 9% were marginal, and no distant recurrences were found.\(^4\) Later studies of recurrence pattern after radiochemotherapy confirm that more than 90% of recurrences are central.\(^5\)

The introduction of amino-acid positron emission tomography (PET) has fueled hopes that biologic imaging may guide radiation oncologists to better delineate the metabolically active tumor region. Consequently, dose escalation in these regions was postulated to increase local control and improve patients’ survival. Indeed, encouraging improvements of OS were reported, for example, in radiotherapy of recurrent gliomas by integrating biologic imaging in treatment planning.\(^6\) However, recent studies found only a detrimental effect of integrating the biologic target volumes on patients’ survival.\(^5,7\) Larger prospective trials are needed to confirm the effects of metabolic imaging in radiotherapy planning of GBM.

**A CROSS-TALK BETWEEN THE MOLECULAR DRIVERS OF GBM INVASION AND THERAPY RESISTANCE**

Post resection of tumor bulk, only a few tumor cells infiltrating surrounding normal tissue remain that survive irradiation doses up to 90 Gy. This quickly raises the question whether the two processes of tumor invasion and therapy resistance are not interconnected.\(^8\) In fact, invasive GBM cells were shown to gain tumor stem cell characteristics, activation of Akt signaling, and relative resistance to mTOR inhibition by rapamycin as compared with parental cells.\(^9\) In line with these observations, experimental evidences are provided that mechanisms governing the invasive program may also render GBM cells resistance to radiotherapy.\(^10\) The existence of such crosstalk between different dynamic pathways points toward the intratumoral heterogeneity and the lack of optimal experimental models to investigate the therapy response of this distinct invasive population rather than the tumor bulk in vivo. In this context, at least two pathways have been forwarded into the clinical evaluation phase and are briefly mentioned here. Inhibition of CD95L/CD95 signaling was shown to reduce glioma cell invasion and tumor stem cell characteristic\(^11\) and is currently investigated in combination with radiotherapy in GBM (phase II trial: NCT01071837). Integrin antagonists were shown to efficiently inhibit tumor invasion and enhance the effect of radiotherapy in preclinical glioma models.\(^12\) In contrast to promising phase II data, the first generation integrin antagonist cilengitide consisting of a cyclic pentapeptide harboring the integrin binding motif (RGD amino acid sequence) did not alter the infiltrative tumor growth pattern and failed to improve OS in combination with radiotherapy and temozolomide in newly diagnosed GBM (Centric NCT00689221 phase III trial\(^13\)).

**RECURRENT-GLIOMA-INITIATING CELLS (R-GIC)**

More than a decade has passed since the introduction of the modern cancer stem cell theory in solid tumors, and still controversies exist on definition, proper cellular, and molecular characterization of this subpopulation in different tumor entities. The original model suggested a static intratumoral hierarchy, with a cancer stem cell population in the apex exhibiting high self-renewal and DNA double-strand breakage (DSB) repair capacities that consequently leads to their inherent resistance to conventional cancer therapies, such as radiotherapy.\(^14\) The key implication for therapy was that eradication of only these cells, and not the entire tumor bulk, was required to prevent tumor recurrence or regress tumors. Interestingly, others have shown that glioma stem cells do not necessarily present a higher radioresistance phenotype as compared with, for example, classical human GBM cell lines.\(^15\)

It is increasingly apparent that intratumoral hierarchies are more dynamic, and acquisition of tumor stem cell traits could be influenced by a number of niche factors, such as signalings induced by intratumoral hypoxia, or as mentioned earlier, as a result of transition of tumor cells into the invasive state, or via intercellular communication in the tumor-stroma interface for example, glioma-microglia, glioma-BMDC, or glioma-microvascular endothelium.\(^16\) Based on the premise that recurrent GBM must be generated from few residual tumor cells post surgery, the term recurrent glioma-initiating cells (R-GIC) seems to better describe this population. Novel tools are needed to longitudinally trace the fate of the R-GIC population in vivo and to better understand the temporal dynamic and molecular mechanisms under-
lying their relative resistance to radiochemotherapy. Invari-
ably, the low-cycling R-GICs population residing in sur-
rounding tissue or coopting preexistent vessels will ulti-
mately escape from their dormant state and switch to the an-
гиогенic phenotype to grow exponentially and develop recurrent macroscopic tumors. Although the transcriptional con-
sequence of this switch is well studied, and recruitment of 
BMDCs seems to play an important role in this process, the 
molecular determinants triggering the switch are still elu-
sive. In addition to BMDC, a growing body of data indi-
cate a pivotal role for glioma—microglia communication in 
GBM development and therapy refractoriness. In analogy to 
the role of tumor-associated macrophages (TAM, M1/2) and 
myofibroblasts in modulating the niche of other solid tu-
mors, microglia are involved in numerous processes reach-
ing from immune modulation to ECM remodeling. The 
intratumoral preponderance and multifaceted functions of 
microglia make them an attractive yet less-explored target for 
treatment of GBM. Together, gliomas may exhibit different 
sensitivities to conventional or targeted tumor/angiogio-
genic therapies at different stages of their development start-
ing from the residual invasive cells, to low-cycling dormant 
R-TIC state, to fast growing macroscopic recurrent tumor 
mass. To identify the best sequence of treatment, these tem-
poral specificities of tumor-niche communication need to be 
better understood and considered, in particular, when mul-
timodal trials are designed.

ANTIANGIOGENIC THERAPY
Angiogenesis is a prerequisite for all solid tumors to grow 
above ~1 mm³, therefore, the tumor microvessel endothe-
lum has emerged as a critical target for cancer therapy.1 
Intriguingly, radiotherapy was shown to exert direct anti-
angiogenic effects. However, paracrine release of angiogenic 
growth factor by tumor/stroma and the corresponding up-
regulation of angiogenic receptors in the endothelium may re-
present a coordinated mechanism by which radiation-
induced endothelial cell damage and apoptosis are effectively 
evaded. Consequently, it was shown that resensitizing tumor 
endothelium via blockage of these evasive proangiogenic 
mechanisms by, for example, inhibiting VEGF, hFGF, EGF, 
mTOR, PDGF, or integrin signaling enhances the antiangiogio-
genic and antitumor effect of radiotherapy. Some of these 
concepts have already been successfully translated in ad-
vanced phase II/III clinical trials.

Antiangiogenic therapy is efficient in blocking exponential 
angiogenesis-dependent tumor growth, whereas dissemi-
nated invasive tumor cells co-opting pre-existing vessels 
could survive this type of therapy. Of note, the exponential 
growth of these microscopic tumor satellites that surround large vessels is still angiogenesis dependent. This provides a 
plausible explanation for the success of radiotherapy in reir-
radiation of relapsed radioresistant GBM derived from the 
R-GIC population. It was recently reported that augmented 
influx of CD11b+ BMDC to the irradiated tumor bed via ac-
tivation of the HIF1alpha-SDF-1/CXCR4 axis contribute to 
glioma relapse. In contrast to angiogenesis, that is, genera-
tion of new vessels from the local preexisting vessels, it was 
postulated that vasculogenesis mediated by differentiation 
of BMDC into new vessels govern tumor relapse after radio-
therapy.21 Observations in most other solid tumors contra-
dict the relevance of BMDC-derived endothelial progenitors 
in revascularization of relapsed tumors post radiotherapy.22,23 
Undoubtedly, BMDC are playing a critical role in tumor 
therapy refractoriness, the switch of dormant tumors to an-
гиогenic phenotype, and ultimately tumor relapse. Recent 
correlation of enhanced influx of myeloid cells in relapsed 
GBM specimens after antiangiogenesis and radiochemo-
therapy underscores the relevance of this stromal compart-
ment in therapy referactoriness.23

ANTI-VEGF THERAPIES IN GBM
Among the most advanced concepts translated into the clinic 
is the inhibition of angiogenesis by the VEGF-neutralizing 
antibody bevacizumab. Two recent phase III trials revealed a 
signal for improved progression-free survival (PFS) after be-
vacizumab and radiochemotherapy in newly diagnosed pa-
ients with GBM.24,25 However, the beneficial effects of 
adding bevacizumabs on PFS were not translated into a pro-
longed OS. Moreover, the studies produced diametral opposing 
results with regard to the endpoints neurotoxicity and 
general deterioration of patients’ health status. Whereas 
RTOG-0825 reported more frequent decline in neurocogni-
tive function and a worse quality of life in bevacizumab ver-
sus placebo arm, the AVAglio study group reported a longer 
conservation of the health-related quality of life and perform-
ance status (“deterioration-free-survival”), and lower glu-
corticoid requirement in the bevacizumab arm. Therefore, 
further studies are required to clarify the effect of bevaci-
uzumab in concurrent and adjuvant settings in GBM. But, 
what could we still learn from these large randomized trials, 
and how could we design better rationally designed trials in 
the future? Does maintenance bevacizumab therapy lead to 
partial blockage of VEGF-dependent angiogenesis and con-
sequently select for tumor cells that demonstrate a high 
degree of fitness to survive the hypoxic conditions resulting 
from the impaired tumor perfusion? Does bevacizumab ther-
agy select for tumor cells with an augmented capacity to in-
vade the surrounding tissue and co-opt preexisting vessels 
that are less prone to anti-VEGF treatments? The exponential 
growth of these microscopic tumor satellites that surround 
large vessels is most likely still angiogenesis dependent. 
Therefore, why does anti-VEGF therapy not stop or at least 
delay the growth of macroscopic tumors out of these tumor 
satellites? The improved PFS observed in these trials is in line 
with current understanding of the mechanism of action of 
VEGF-targeting antiangiogenic therapy. We need to identify 
the evolutionary landscape underlying tumor referactoriness 
to anti-VEGF therapy and precisely target those mechanisms 
to produce more enduring responses. For example, glioma-
evasive mechanisms might be circumvented by multimodal 
therapies more efficiently targeting hypoxic tumor cells, such
as carbon radiotherapy (see later in this article), preventing tumor invasion, and compensatory pro-angiogenic mechanisms to single-agent anti-VEGF therapy.\(^1\)

However, if proper salvage therapies are not yet available, may the generation of hypoxic and invasive tumors by anti-VEGF therapy not impair the efficacy of reirradiation of relapsed tumors? Preliminary data suggest promising outcomes when bevacizumab is added to reirradiation in the recurrent setting in antiangiogenesis-naïve GBM.\(^{26,27}\) RTOG-1205 will provide more definitive evidence for this alternative strategy.

Another valuable result from the RTOG-0825 study is that no correlation was found between the 9-gene expression signature and the clinical outcome. The gene-expression signature was a composite of most robustly detected genes in FFPE material identified after integration of four independent microarray datasets.\(^{28}\) These data indicate that the gene expression-based diagnostic is still not ready for the prime time in GBM. Perhaps microRNAs or DNA-methylation patterns, which are more robust against processing bias introduced by FFPE conservation, may provide a better alternative for identification of a biomarker. Another source for bias might be the relatively heterogeneous treatments of patients studied in large cohorts such as The Cancer Genome Atlas (TCGA) as compared with the RTOG-0825. Therefore, caution is warranted in interpretation and classification of GBM into, for example, proneural, classical, and mesenchymal subtypes solely based on matched clustering of gene expression. Additional prospective trials are needed to validate the value of these classifications. Likewise, the predictive value of \(O^6\)-methylguanine-DNA methyltransferase (MGMT) promoter methylation status to stratify patients most benefiting from radiochemotherapy is, at least within the radiotherapy community, still controversial debated.\(^{29}\) Whereas the biologic basis for impaired DNA repair is given for alkylating agents like temozolomide, it is conceivable that epigenetic silencing of MGMT does not chiefly affect the nonhomologous end joining (NHEJ) relevant to radiation—induced DSB. Interestingly, dose-dense temozolomide treatment added no benefit to PFS and OS as compared with the standard-treatment arm, regardless of the MGMT methylation status.\(^3\)

However, the MGMT status correlated well with all endpoints irrespective of temozolomide dose used, suggesting a dose threshold for combined effect of the alkylating agent with radiotherapy. In conclusion, large prospective trials provide an excellent platform to validate diagnostic markers. In contrast to temozolomide, specific and robust predictors of radiotherapy response are urgently needed. In addition to molecular markers, integration of longitudinal measures of, for example, tumor perfusion, vascularity, and permeability, and cellularity via modern diffusion weighted (DWI) and dynamic contrast-enhanced (DCE) MRI imaging may facilitate the identification of those patients that most probably benefit from combined antiangiogenics and radiochemotherapy.\(^{30}\)

**Disclosures of Potential Conflicts of Interest**

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**References**


