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REVIEW ARTICLE

Glioblastoma multiforme: emerging treatments and stratification markers beyond new drugs

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ABSTRACT

Glioblastoma multiforme (GBM) is the most common primary brain tumour in adults. The standard therapy for GBM is maximal surgical resection followed by radiotherapy with concurrent and adjuvant temozolomide (TMZ). In spite of the extensive treatment, the disease is associated with poor clinical outcome. Further intensification of the standard treatment is limited by the infiltrating growth of the GBM in normal brain areas, the expected neurological toxicities with radiation doses >60 Gy and the dose-limiting toxicities induced by systemic therapy. To improve the outcome of patients with GBM, alternative treatment modalities which add low or no additional toxicities to the standard treatment are needed. Many Phase II trials on new chemotherapeutics or targeted drugs have indicated potential efficacy but failed to improve the overall or progression-free survival in Phase III clinical trials. In this review, we will discuss contemporary issues related to recent technical developments and new metabolic strategies for patients with GBM including MR (spectroscopy) imaging, (amino acid) positron emission tomography (PET), amino acid PET, surgery, radiogenomics, particle therapy, radioimmunotherapy and diets.

INTRODUCTION

Glioblastoma multiforme (GBM; including giant-cell glioblastoma and gliosarcoma) is the most common astrocytoma classified as World Health Organization (WHO) grade IV. The grade is assigned to the cytologically most malignant, mitotically active, necrosis-prone neoplasms typically associated with widespread infiltration into the surrounding tissue, microvascular proliferation, rapid pre- and post-operative disease evolution and fatal outcome.¹ Tumour grading is typically determined based on tissue characteristics observed with haematoxylin/eosin stainings.² Although histological discrimination of e.g. oligoastrocytomas and GBM is sometimes difficult,³ tumour grade is a key factor influencing the choice of therapy.¹

ORIGIN OF GLIOBLASTOMA MULTIFORME AND RECURRENT TUMOUR

Traditionally, it is presumed that GBM originates from malignant transformation of differentiated glia cells of the

brain. However, there is evidence that neural stem cells and lineage restricted progenitor cells might function as the origin of GBM or as a source of glioma-initiating cells.⁴ Tumour localizations involving the subventricular zone as well as the hippocampal subgranular zone have shown prognostic relevance.^{5,6} However, GBM have an infiltrating growth into the normal brain limiting the effectiveness of surgical resection of the primary tumour, which is aimed to be as radical as possible. Depending on size, localization and relation to eloquent areas, gross total resection of the neoplasm is often not possible without leading to further neurological and functional impairments such as motoric disorders with an adverse impact on quality of life. Studies examining patterns of recurrences following surgery, radiotherapy and chemotherapy have consistently found that 80–90% of recurrences are within the original treatment field.^{7–12} With the help of gene profiling, GBM was subgrouped in proneural, neural, classical, proliferative and mesenchymal types.^{13,14} However, it was found that the recurrences present different genetic properties compared

with the primary tumour,¹³ mediating possible treatment resistance. As a consequence, the main cause of treatment failure is the inefficacy to control the tumour at the original site and not distant invasion.

STANDARD TREATMENT

Prior to the last decade, there has been little improvement in outcome of patients with GBM although major technological progress was made in radiotherapy, surgery, as well as chemotherapy development.¹⁵ In spite of intense treatment, GBM is characterized by resistance to multimodal therapies, and survival is still reported in months. The current standard of care is a debulking surgery followed by fractionated radiotherapy (60 Gy, 6 weeks) with concomitant and adjuvant treatment of the cytostatic agent temozolomide (TMZ) (Figure 1). The regime is based on the landmark European Organization for Research and Treatment of Cancer (Brain Tumour and Radiotherapy Groups/National Cancer Institute of Canada Clinical Trials Group (EORTC/NCIC) study including 573 patients in 85 centres worldwide. In this study, the median and 2-year survival were significantly different after combined radiotherapy with TMZ (14.6 months and 26.5%) relative to radiotherapy alone (12.1 months and 10.4%). Moreover, the overall survival at 5 years was 9.8% after post-operative radiotherapy combined with TMZ treatment and 1.9% with radiotherapy alone. The strongest predictor for outcome and benefit from combined radiochemotherapy was methylation of the O-6-methylguanine-DNA methyltransferase (MGMT) promoter, which resulted in a 5-year survival of

13.8%.^{16,17} However, even after radiotherapy alone, survival was significantly better in patients with MGMT hypermethylation compared with wild-type tumours.¹⁷ This maximum but still palliative treatment of GBM is well tolerated by most patients with fair general conditions. Although most studies exclude elderly patients and patients with reduced general conditions, rethinking proceeded in the past years in the course of treatment individualization. Especially for elderly patients, there is growing evidence for isoefficacy of shorter fractionation concepts as well as sequential chemotherapy or discussion of chemotherapy instead of radiotherapy according to MGMT status in the latter situations.^{18,19} Although the EORTC/NCIC study found the greatest benefit in patients with MGMT promoter methylation, all patients with and without promoter methylation will still be treated using the simultaneous radiotherapy and TMZ approach established by Stupp *et al*¹⁶ owing to a lack of evidence-based clinical alternatives. Studies published in the last decade still have a significant number of patients included who were not treated with TMZ but with other alkylating agents simply due to the long recruitment times of clinical trials.

GENETIC ALTERATIONS AND TREATMENT IMPLICATIONS

Older patients (mean age 55 years) are at a higher risk for developing GBM without clinical or histological evidence of evolution out of a low-grade tumour. These primary GBMs are generally characterized by epidermal growth factor receptor (EGFR) amplification and/or EGFR overexpression, phosphatase and tensin homologue mutations, *p16* deletion, mouse double minute 2 homologue (MDM2) amplification and/or MDM2 overexpression and loss of heterozygosity (LOH) of chromosome 10 (Figure 2).^{3,20–22} By contrast, the secondary GBM manifest in younger patients (mean age 40 years) and develop through malignant disease progression from low-grade astrocytomas. In the majority of the cases, a *TP53* mutation is present as well as loss of chromosomes 19q and 10q³ (Figure 2). In anaplastic oligodendrogliomas, co-deletion of 1p/19q is a predictive factor. The median survival of patients with co-deleted tumours increased from 7.3 to 14.7 years when radiotherapy was combined with chemotherapy (procarbazine, lomustine and vincristine).²³ By contrast, in 491 histologically confirmed GBM, co-deletion of 1p/19q evaluated by fluorescence *in situ* hybridization or polymerase chain reaction-based LOH had no impact on overall survival.²⁴ Isocitrate dehydrogenases (*IDH*) 1/2 is mutated more often in the secondary GBM than in the primary GBM.^{25,26} Given the better prognosis for the secondary GBM, *IDH* mutations could therefore be assumed as a predictive biomarker.²⁷ However, the most important prognostic biomarker in clinical use for both primary and secondary GBM is MGMT promoter methylation.^{16,17} In selected patient cohorts, MGMT promoter methylation might have even predictive value, *e.g.* in the management of elderly patients with GBM.¹⁹ Histological tumour grading criteria are subjected to changes and might soon include additional molecular criteria. The need to include specific molecular subtyping in clinical trials is obvious and might lead to the development of dynamic classification schemes for GBM in the nearer future.

Figure 1. Current standard of care and emerging treatment options for glioblastoma multiforme (GBM). Post debulking surgery, GBM is treated with radiotherapy (30 fractions, 2 Gy/fraction, 6 weeks) with concomitant temozolomide (TMZ) (75 mg m⁻²) followed by six cycle of adjuvant TMZ (150 mg m⁻²). Emerging therapies are multidisciplinary and cover areas such as omics, targeting, imaging and metabolic strategies.

Figure 2. Genetic alterations in the evolution of glioblastoma multiforme (GBM).^{2,3,20–22} ATRX, alpha thalassaemia/mental retardation syndrome X-linked; EGFR, epidermal growth factor receptor; IDH, isocitrate dehydrogenase; LOH, loss of heterozygosity; MDM2, mouse double minute 2 homologue; PTEN, phosphatase and tensin homologue; TP53, tumour protein p53; WHO, World Health Organization.

CHALLENGES OF CLINICAL TRIALS

Efforts to develop new treatment strategies have focused on new drugs or treatment targets as well as subgrouping of patients according to age and molecular cancer markers (Figure 1). The common goal of experimental protocols is to control disease, prolong progression-free and overall survival while lowering treatment associated toxicities, to improve quality of life or to preserve it for as long as possible. Currently, few molecular markers are guiding clinical decisions in modern neuro-oncology even though complex characterizations on genomic, epigenetic, transcriptional and proteomic levels are performed. Although the brain was for a long time thought to be immune privileged, the field of immunotherapy is incrementally investigated in GBM. Particular progress has been made with dendritic cell-based vaccines²⁸ and the peptide vaccine rindopepimut (CDX-110), targeting the most common EGFR mutation, variant III deletion mutation (EGFRvIII).²⁹ Following the standard treatment, rindopepimut injections staggered with TMZ administration showed in patients with EGFRvIII-expressing GBM a median progression-free survival of 9.2 months [95% confidence interval (CI) 7.4–11.3] and a median overall survival of 21.8 months (95% CI 17.9–26.5) from study entry (approximately 3 month after diagnosis).³⁰ The treatment was well tolerated and had no fatal adverse side effects, no cumulative toxicity over time and only moderate injection side reactions. A 2-arm, double-blinded, randomized Phase III trial will test the efficacy and safety of the addition of rindopepimut to the current standard of care in patients with newly diagnosed GBM (ACT IV, NCT01480479) while evaluation of rindopepimut for recurrent GBM (ReACT, NCT01498328) and paediatric pontine glioma (NCT01058850) is ongoing. Thus, advances in the understanding of molecular

mechanisms are rapidly translated into novel therapeutic approaches. It is beyond the scope of this review to provide a comprehensive overview of all the substance classes under investigation. We therefore refer the reader to recent reviews.^{31,32} However, initially promising results of pre-clinical and early-phase clinical trials often fail in further investigations. The majority of the investigated substances had minimal to no effect on patient survival in randomized Phase III trials.^{18,33–43} Unexpected negative results might be caused by redundancy or co-activation of targeted molecular pathways; as such, focusing on common pathways or critical nodes in signalling networks is necessary. Moreover, limited resources require well-designed clinical trials, including enrichment of the target population to increase the likelihood of success, e.g. genetic enrichment by sequencing.⁴⁴ These aspects refer to the pivotal role of patient selection not only in targeted therapies but also in translational research in general, which will help to further broaden our understanding of the therapeutic value of the most promising candidates. So far, none of the numerous published genetic and epigenetic alterations dividing GBM patients into sub-groups has implications on treatment decisions which underline the desperate need for more effective and less toxic therapies.

NEW THERAPEUTIC STRATEGIES

Advanced analysis of conventional imaging data for improved treatment

In GBM, important pre-treatment categorization is derived by structural and spatial information from imaging procedures. MRI is the primarily used modality providing excellent brain tissue delineation with the exception of contraindications, e.g. patients with cardiac pacemakers, where only CT is feasible. However, constantly developed and improved imaging

approaches are becoming available for GBM research, diagnosis and choice of therapy, including positron emission tomography (PET) or multivoxel spectroscopy.⁴⁵ The imaging targets of conventional MRI techniques are gadolinium (Gd) enhancement due to blood brain barrier deteriorations, the non-enhancing low-grade tumour extent or perifocal oedema presenting as T2 hyperintensities and their dynamic change with respect to ascertainable responses to new therapies.⁴⁶ Particularly, novel MRI sequences are developed and investigated that allow quantifying specific tissue characteristics and aimed to identify differences in tumour types and grades and post-treatment changes.

There is a growing body of evidence concerning the diagnostic, prognostic and/or predictive value of diffusion as well as perfusion parameters in glioma.⁴⁷ Diffusion imaging, probing the capability of water molecule movement within soft tissue, is used to investigate microscopic tumour tissue changes-related therapeutic responses. It is vastly available at clinical imaging sites and allows to retrieve an estimate of the hindrance of water molecule diffusion called apparent diffusion coefficient (ADC). As an example, low tumour tissue ADC values are known to be negative prognostic factors.⁴⁸

Diffusion tensor imaging, an advanced diffusion technique based on water movement properties within their respective surrounding, has also extensively been used to observe intrinsic tumour tissue structure with its inferred indices used to differentiate between low- and high-grade gliomas.^{49,50} Various perfusion MRI techniques aim to measure tumour vascular leakiness and underlying tumour-related vasculature. One of the most promising perfusion imaging approaches is dynamic susceptibility contrast-enhanced perfusion MRI⁵¹ that reveals microvascular changes in tumour tissue that are reported to be related to tumour grade and clinical outcome.^{52,53} However, multimodal imaging approaches propose to overcome conventional MRI limits to depict structural changes.⁵⁴ With respect to monitoring tumour response to therapy, one of the major limits of current conventional MRI is the lack of differentiating tumour progression from treatment-related effects, *e.g.* pseudoprogression, pseudoreponse and radiation necrosis.^{55–57}

The concept of radiogenomics takes quantitative imaging features and their impact on the clinical outcome into consideration while underlying aetiological and tumour-biological mechanisms are considered in the second step. With an increasing number of evolving imaging modalities from research towards clinical practice, there is growing need to carefully assess their practical use and to compare the additional benefit relative to the standard imaging, in an effort to complement and not to replace. Innovative imaging protocols will be multimodal either as hybrid or sequential scanning, the latter requiring co-registration algorithms.⁵⁸ In this respect, multimodal navigation systems have already proven to be more effective than conventional navigation systems in terms of decreasing tumour remnants and a significant prolonged survival.⁵⁹ The following three paragraphs will highlight ambitious imaging modalities in the context of the respective clinical situations.

Imaging implications in neurosurgery

Pre-surgical imaging should guide biopsy targeting to define cortical eloquent areas and white matter tracts to perform most accurate resection. Since resection status is an independent prognostic factor in GBM,⁶⁰ conventional neuronavigation is constantly improved. The current standard is comprised pre-operative MRI scans and intraoperative fluorescence microscopy utilizing 5-aminolevulinic acid (ALA). There is Level II evidence that advanced intraoperative MRI guidance is more effective than conventional neuronavigation concerning the extent of resection, enhancement of quality of life or prolongation of survival.⁶¹ On the other hand, there is sparse evidence that intraoperative MRI is superior to fluorescence with regard to residual tumour volumes, completeness of resection and neurological outcomes.⁶² In fact, ALA fluorescence technology revealed that GBM is more widespread infiltrating than the contrast-enhanced MRI had demonstrated, enabling intraoperative margin adaptation.⁶³ The intraoperative fluorescence signal corresponds well with the histopathological tumour infiltration in ALA-positive resection planes. Nevertheless, residual faint ALA uptake is often documented in the cortical eloquent areas towards the end of resections. In a small clinical study, ALA-positive areas exceeded post-operative [¹⁸F]fluoroethyl-L-tyrosine ([¹⁸F]-FET) uptake regions in PET scans, requiring more clinical data.⁶⁴ With the metabolic amino acid tracers such as [¹¹C] methionine (MET)-PET, additional information regarding both solid and infiltrative areas could be acquired at high sensitivity and specificity.⁶⁵ In a PET and MR fusion image study, the MET-positive area was larger than the Gd-enhanced area with the discrepancy increasing with tumour diameter.⁶⁶ The additional PET information in contrast to operative MRI alone is particularly valuable as recurrences originated in the MET-positive areas after complete resection.⁶⁶

Combining molecular analyses and imaging data

The term radiogenomics refers either to studies on genetic variation associated with radiation response (radiation genomics) or to correlations between cancer imaging features and gene expression (imaging genomics). The systems biology approach of combining radiogenomics with transcriptomics or proteomics is a very promising approach to find innovative prognostic or predictive biomarkers. Especially for MRI, imaging features such as contrast-to-necrosis ratio, putative stem cell zone involvement or infiltrative *vs* oedematous traits can be evaluated independently and correlated with matched molecular profiles.⁶⁷ Currently, most studies are of retrospective nature, generating hypotheses only. Nonetheless, with decreasing cost and time requirements for molecular testing and increasing numbers of imaging methods and availabilities, promising biomarker candidates could be validated in external cohorts and prospective clinical trials.

Imaging implications in radio-oncological treatment

Radiotherapy relies on post-surgical imaging to define residual tumour mass and areas of the highest risk for recurrence in order to guide target volume definition. Radio-oncologists as well as neuro-oncologists are faced with putative post-therapeutic changes such as pseudoprogression or radiation-induced necrosis during follow-up. Both are often difficult to

distinguish from disease progression, but a wait-and-see strategy may prohibit further local therapy attempts. However, there are well-recognized limitations of conventional MRI that are based solely on oedematous brain characteristics excludes tumour in normal appearing brain tissue in clinical target volumes (CTVs). Hence, besides an important gain in diagnostic accuracy, newer quantitative MRI techniques may play an important role in future radiation treatment planning.

There is a need to complement structural information derived from the standard imaging with assessment of metabolic activity, *e.g. via* PET. There are a variety of tracers investigating processes such as DNA synthesis, membrane biosynthesis and resistance mechanism to hypoxia, oxygen metabolism and blood flow in brain tumours.⁶⁸ Imaging with radiolabelled glucose has a minor impact in brain tumours since the high fluorine-18 flu-deoxyglucose uptake in the grey matter limits its use. Therefore, tracers based on amino acid analogues, *e.g.* [¹⁸F]-FET or L-methyl-[^11C]-MET-PET, with a lower uptake in normal brain tissue are used to enable better contrast. Nevertheless, its application is subject to feasibility issues such as device parameters, threshold assessment or pharmacodynamics (*e.g.* the carbon-11 label of MET with the short half-life time of 20 min requires a cyclotron near the imaging facility). In addition, [¹¹C]-MET-PET imaging increases the sensitivity of CT and/or MRI scans up

to 97% in the post-surgical evaluation and even in the assessment during adjuvant therapy.⁶⁹ These aspects lend important information regarding the definition of target volume and margins. CTV margins of Gd-enhancing areas and suspect areas in T2 sequences differ considerably from those by amino acid PET. As in most cases, the latter combines areas of both MRI features, the brain volume remains feasible to treat based on this biological information.^{70,71} It was shown that the MET-PET-positive volumes are correlated with outcome in photon and in carbon ion radiotherapy.^{72,73} In both studies, pre-treatment MET-PET was performed but not used for target volume delineation. As MET uptake revealed high-risk areas associated with the site of subsequent failure, it is reasonable to test an intervention based on these observations.⁷² In our ongoing prospective study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT01873469) Identifier: NCT01873469), we aim to implement MET-PET imaging into radiation treatment planning of approximately 100 patients. If our hypothesis holds true that recurrences appear earlier and show a special correlation to former MET-PET-positive surgical residues, then an interventional trial will follow (Figure 3).

MR spectroscopy imaging (MRSI) also holds a tremendous potential to provide specific metabolite-defined treatment information. Targeting active tumour tissue by means of the choline (Cho) and the *N*-acetylaspartate (NAA) distribution and

Figure 3. Positron emission tomography (PET)-MRI imaging of a patient with glioblastoma multiforme (GBM) recruited in PETra trial. Imaging was performed for radiotherapy planning. [¹¹C]-methionine (MET)-PET uptake indicates metabolic active tumour, whereas corresponding MRI sequences show no evidence of glioblastoma multiforme residue after primary resection. (a) Fusion [¹¹C]-MET/T1, (b) [¹¹C]-MET-PET, (c) Fusion [¹¹C]-MET/T2, (d) MRI (gadolinium-T1), (e) MRI (native T₁), (f) MRI (T₂).

their Cho-to-NAA index of greater than two (CNI2) defined as abnormal, this technique may identify tumour tissue beyond conventional MRI and may also detect regions of high risk of recurrence based on metabolic activity.

A pre-treatment comparison of the MRSI-based CNI2 volume with conventional MRI in GBM, a pre-treatment, revealed just 25% overlap with contrast-enhancing T_1 weighted (T1W) and only 10% of T_2 weighted hyperintense non-enhancing tumour tissue. However, within the latter overlapping volume 80% of the new contrast-enhancing T1W, tumour tissue was found pointing towards a predictive value for the site of relapse.⁷⁴ Using the CNI2 approach to assess metabolic abnormalities, Pirzkall *et al*⁷⁵ found substantial variations in the spatial relationships of metabolic tissue changes compared with conventional MRI alone. The same group showed that this additional metabolic information would not only change the size but also would result in a substantial shift of the volumes if considered for radiation target delineation.⁷⁶

By this means, Parra *et al*⁷⁷ compared metabolic tumour volumes with standard RT volumes using an advanced volumetric three-dimensional (3D) MRSI approach and found in an observational study of $n = 19$ patients with GBM that on average, one-third Cho-defined volume was not covered by the CTV receiving 60 Gy. Park *et al*⁷⁸ studied the CNI2 volume at therapy initiation in another observational study of $n = 23$ patients with GBM and showed that one-third of recurrent-enhancing GBM volume within this metabolically active tumour region was not initially covered by the 60-Gy RT target. Moreover, the early change of the Cho-to-NAA index during RT has been shown to predict early progression within an observational study of GBM where the voxel-based analysis of 3D MRSI data retrospectively revealed a significant increase in median Cho/NAA in the group of patients with early recurrent tumour.⁷⁹

Alongside with the metabolites mentioned above, there is another promising MRSI approach examining lactate (Lac) accumulation as a surrogate for hypoxic and subsequently radioresistant areas that has also shown the potential to predict local recurrence. Therefore, lactate imaging might also serve as a biological targeting or dose-painting technique.⁸⁰

Although including only small patient numbers so far, longitudinal trials considering the correlation of patterns of recurrence with respect to MRSI and in comparison to the conventional MRI pattern before radiotherapy are indispensable before treatment-planning approaches may be adapted. On this account, the results of trials investigating the implications of radiotherapy planning using MRSI are ambitiously awaited. A projected whole-brain volumetric 3D MRSI guided selective dose-escalation RT Phase II trial will compare the efficacy and also toxicity of such specifically altered treatment approach with conventional MRI guidance based on the progression-free patient survival (NCT02394665). Furthermore, a currently recruiting multicentre Phase III trial intends to comprehend if MRSI guided intensity-modulated RT that applies simultaneous integrated boosts in comparison with 3D conformational RT

may provide advantage in progression or survival (SPECTRO GLIO; NCT01507506).

PARTICLE THERAPY

The main rationale to invest in particle therapy facilities has been poor local disease control with conventional radiotherapy and the close proximity of tumour and dose-limiting critical structures. The depth-dose profile of particle radiation offers the theoretical benefit of more localized dose delivery and improved dose distribution. The tumour volume can be exposed to higher gray equivalent (Gy-E) doses, which is the physical dose multiplied by the relative biological effectiveness (RBE). The RBE for carbon ions is in the range⁸¹ of 2–5 while the RBE for protons is assumed to be 1.1.⁸² Recent results from pre-clinical models suggest a higher proton RBE in the distal end of the Bragg peak.^{83–85} It has been concluded (1) that if cost-effectiveness and reimbursement would be no issues, proton therapy can be performed instead of photon therapy without any further clinical trial when the same dose and fractionation schedule is applied and (2) that the clinical trials determining the advantage of protons should deal with finding the optimal radiation dose by, *e.g.* dose escalation and the optimal fractionation schedule.⁸⁶ However, the infiltrating growth pattern of GBM requires significant margins to cover possible microscopic extensions, *i.e.* potential dose-escalation strategies need to deal with shrinking-field techniques. It should be noted that dose-escalation attempts with photons beyond 60 Gy have resulted in increased toxicity but no additional survival benefit.⁸⁷ In a Phase II trial, the conventional 55–65 Gy photons dose was escalated to 90 Gy-E with a combination of protons and photons in accelerated fractionation.⁸⁸ The treatment led to a central tumour control in most cases, but tumours recurred in the periphery, and median survival time was 20 months. Patients with radiation necrosis only seem to live longer than those with recurrent tumour and radiation necrosis with median survival times of 29 and 16 months, respectively. The improvement in median survival time was suggested to be too modest relative to the high toxicity level.⁸⁸

Clinical evidence for particle therapy in glioblastoma multiforme

A limited number of studies on the use of particle therapies in GBM have been published so far, most likely due to the low number of proton and carbon ion treatment facilities worldwide. Although randomized trials are missing, proton therapy is widely accepted for treating paediatric malignancies including GBM since the reduced normal-tissue dose has the potential to decrease late toxicity in the developing brain.⁸⁹ This aim is in conjunction with the objective to decrease the risk for secondary malignancies. Currently, there are no randomized trials published that prove the superiority of particles over photons based on either overall or progression-free survival or on late toxicity in the central nervous system. Thus, the value of proton or heavy ion therapy *vs* photon therapy in adults is still controversially discussed.^{90,91} The lack of available evidence does not imply that particle radiation is not useful for selected tumours and clinical situations. More prospective and specifically comparative, *i.e.* randomized or at least matched pair studies are needed to prove potentially higher effectiveness and/or lower toxicity than

Table 1. Clinical studies with radioimmunotherapy in patients with glioblastoma multiforme (GBM)

Radiotherapeutic	Previous treatment	Treatment	Phase	Karnofsky performance status	Patient numbers	Status	Application	Activity ^a	Toxicity	Survival [95% confidence interval] 1 month = 4.4 weeks ^b	Study
¹³¹ I 81C6 murine mAb (anti-tenascin)	RT ± chemotherapy	RIT	I	≥70	26	Recu	i.c.	20–120 mCi (0.74–4.44 GBq)	Grade 3–4, hema, no significant neuro <120 m Ci	^c 56.0 weeks [35.3–∞] (12.7 months) ^d 0.57% [0.43–0.77]	Bigner et al ⁹⁸
²¹¹ At 81C6 chimeric mAb (anti-tenascin)	RT ± chemotherapy	RIT + salvage chemotherapy	I	≥70	14	Recu	i.c.	0.071–0.347 GBq	Grade 2 neuro No grade 3 or 4	^c 52.0 weeks [33–76] (11.8 months) ^d 0.50% [0.30–0.84]	Zalutsky et al ⁹⁹
¹³¹ I 81C6 murine mAb (anti-tenascin)	RT + chemotherapy	RIT + salvage chemotherapy	II	≥70	33	Recu	i.c.	100 mCi (3.7 GBq)	Acute grade 3–4 hema, acute grade 3 neuro	^c 63.9 weeks [38.8–90.0] (14.5 months) ^d 0.60% [0.46–0.76]	Reardon et al ⁹³
⁹⁰ Y BC-4 mAb (anti-tenascin)	RT ± chemotherapy	RIT ± TMZ	I/II	≥70	73	Recu	i.c.	0.370–0.925 GBq ≥2 cycles	Grade 2–3 hema	^c RIT: 17.5 months [17–20] (77 weeks) ^c RIT + TMZ: 25 months [23–30] (110 weeks)	Barborea et al ⁹⁷
⁹⁰ Y BC-4 mAb (anti-tenascin)	chemotherapy + RT	Systemic chemotherapy + RIT ± loco regional chemotherapy		≥60	26	Recu	i.c.	5–25 mCi, >2 cycles (0.185–0.925 GBq)	Grade 3–4 hema Grade 3 neuro	^c RIT: 20 months (88 weeks) ^c RIT + chemotherapy: 22 months (96.8 weeks)	Boiardi et al ¹⁰¹
¹³¹ I 81C6 chimeric mAb (anti-tenascin)	—	RIT ± RT + chemotherapy RIT + chemotherapy	I	≥60	30 8	New Recu	i.c.	80–120 mCi (2.96–4.44 GBq)	Dose-limiting neuro; hema	^c New: 86.1 weeks [70.1–99.1] (19.6 months) ^c Recu: 48.9 weeks [30.4–83.3] (11.1 months)	Reardon et al ¹⁰²
¹³¹ I 81C6 murine mAb (anti-tenascin)	—	RIT + RT ± chemotherapy	I	≥60	32	New	i.c.	20–180 mCi (0.74–6.66 GBq)	Grade 3–4 hema, neuro	^c 69 weeks [52–106] (15.7 months)	Cokgor et al ¹⁰³
¹³¹ I 81C6 murine mAb (anti-tenascin)	—	RIT + RT + chemotherapy	Pilot	≥70	15	New	i.c.	44 Gy-E boost	Reversible grade 3 hema No grade 4	^c 90.6 weeks [73.3–97.1] (20.6 months) ^d 0.86% [0.72–1.00]	Reardon et al ¹⁰⁴

(Continued)

Table 1. (Continued)

Radiotherapeutic	Previous treatment	Treatment	Phase	Karnofsky performance status	Patient numbers	Status	Application	Activity ^a	Toxicity	Survival [95% confidence interval] 1 month = 44 weeks ^b	Study
¹³¹ I 81C6 murine mAb (anti-tenascin)	—	RIT + RT + chemotherapy	II	≥70	27	New	i.c.	120 mCi	Grade 3–4 hema	^c 79.4 weeks [61.4–∞] (18.0 months)	Reardon et al ¹⁰⁵
								(4.44 GBq)	Grade ≥ 3 neuro		
¹²⁵ I 425 murine mAb (anti-EGFR)	—	RT + RIT ± TMZ	II	≥60	192	New	i.v.	120–150 mCi	No grade 3 or 4	^c RIT: 14.5 months [12.1–16.7] (63.8 weeks)	Li et al ¹⁵
								(4.44–5.55 GBq)		^c TMZ + RIT: 20.4 months [14.9–25.8] (89.8 weeks)	
										^d RIT: 0.66%	
¹⁸⁸ Re Nimotuzumab (anti-EGFR)	RT	RIT	I	≥70	8	Recu	i.c.	10–15 mCi	Transient—very severe acute and late neuro	^c 6.07–18.7 months (26.7–82.3 weeks)	Casaco et al ¹⁰⁶
								(0.37–0.555 GBq)			

hema, haematological; i.c., intracranially; i.v., intravenously; m, month; mAb, monoclonal antibody; neuro, neurological; New, newly diagnosed GBM; Recu, recurrent disease; RIT, radioimmunotherapy; RT, radiotherapy; TMZ, temozolomide; wk, week.

^amCi was converted in SI-unit GBq; converted values are given in parenthesis.

^bSurvival was converted in month or weeks; converted values are given in parenthesis.

^cMedian overall survival.

^d1-year overall survival.

for other radiotherapy techniques. In order to avoid severe toxicities, such studies need to include the best available imaging techniques to define high-risk regions where boost doses can be applied to a limited volume. Whether these high-risk regions can indeed be defined by, *e.g.* PET, also awaits results from clinical trials.

Clinical experience with particle therapy in glioblastoma multiforme

A recent explorative hypothesis-generating retrospective analysis of patients from multiple trials who received photon radiation alone (60 Gy), photon radiation (60 Gy) with TMZ or photon radiation (50 Gy) followed by a carbon boost (16.8–24.8 Gy-E) in combination with nimustine hydrochloride (ACNU) found that neither the median overall survival nor the median progression-free survival of patients with GBM are statistically different between the radiochemotherapy with TMZ and the carbon boost cohort.⁹² Nonetheless, the authors simulated a patient cohort with TMZ treatment and carbon boost irradiation concluding that there might be an additional benefit for the outcome.⁹² The CLEOPATRA Phase II trial is currently evaluating the combined standard radiochemotherapy with TMZ up to 48–52 Gy of photons followed by 5 fractions of 2 Gy-E protons boost (standard arm) or 6 fractions of 3 Gy-E carbon ions boost (experimental arm).⁸¹ In a Phase I/II clinical trial, patients were treated with 50 Gy photons in 25 fractions followed by 8 fractions of carbon ions with increasing carbon ion doses (16.8–24.8 Gy-E) and concomitant chemotherapy with ACNU. There was no Grade 3 or higher acute or late brain toxicities. Comparison between the lowest and the highest carbon dose group revealed an increase of the median progression-free survival from 4 to 14 months and the median survival time from 7 to 26 months.⁹³ In a Japanese trial, 20 patients with GBM were treated with a hyperfractionated regime of photon irradiation (50.4 Gy, 28 fractions) followed by a proton boost (46.2 Gy-E, 28 fractions), *i.e.* total radiation doses of 96.6 Gy-E, in combination with ACNU administration in week 1 and 4.⁹⁴ Overall, toxicities were acceptable with one case having radiation necrosis and six patients showing a mixture of radiation necrosis, oedema and degraded GBM cells. The median survival period was 21.6 months while the 1- and 2-year progression-free survival rates were 45.0% and 15.5%, respectively.⁹⁴

Another important issue is the reirradiation in case of GBM recurrence. Here, particle radiotherapy competes with stereotactic photon radiotherapy. In both cases, the treated volume is the visible tumour with a small margin of normal brain tissue. Clinical trials are ongoing that evaluate dose escalation with protons or carbon.^{95,96}

RADIOIMMUNOTHERAPY

Antibodies and radionuclides

Radioimmunotherapy (RIT) with monoclonal antibodies (mAb) labelled with therapeutic radionuclides are promising candidates to be applied alone or in combination with radio(chemo)therapy to improve treatment outcomes. The application of mAb is restricted by factors such as the high interstitial pressure inside the tumour, limited blood supply, inhomogeneous or inconstant expression of antigens, necrosis or fibrosis as physiological

barriers, formation of immune complexes, catabolism of immunoglobulins, the blood–brain barrier and possible unfavourable biodistribution of the mAb leading to radiation burden in normal tissue.⁹⁷ Owing to the infiltrating growth pattern of GBM,¹ complete removal of the tumour is impossible because of microscopic tumour cell clusters in the peritumoral brain tissue, which very often become the origin for early tumour recurrences.¹³ As shown in early clinical trials (Table 1),^{15,97–106} the injection of radiolabelled mAb in the post-surgical resection cavity allows for high local radiation doses in the tumour and low-to-no dose in the surrounding normal brain and adjacent organs at risk, *e.g.* optical nerve, chiasm or brain stem. Tumour dosimetry and biodistribution studies indicate that the maximum diffusion efficacy of radioimmunoconjugate is 1–2 cm from the intracranial injection site.^{107,108} A variety of radionuclides are of therapeutic interest such as ⁹⁰Y, ¹²⁵I, ¹³¹I, ¹⁷⁷Lu, ¹⁸⁶Re, ¹⁸⁸Re, ²¹¹At, ²¹²Bi and ²¹³Bi.¹⁰⁹ The radionuclides differ in emission characteristics as well as half-life times and thereby in mean radiation range and tumour cell killing potential. In particular, the high-energy β emitters ⁹⁰Y and ¹⁸⁸Re, with a mean range of 5 mm, are suggested to have the potential of killing cell clusters with $>10^8$ tumour cells. The α emitters ²¹¹At and ²¹²Bi (range 50–100 μ m) might be applied to inactivate single cells or small cell clusters of 10^4 tumour cells, but their short half-life time (7.2 h and 1.0 h, respectively), high costs, as well as poor availability, question their usefulness.¹¹⁰ When ¹²⁵I or ¹³¹I are used as radionuclide, thyroid blockage with potassium iodine solution is needed prior to and after application to prevent the unlimited uptake of radioactive iodine in the thyroid.^{15,100,105} The iodine isotopes ¹²⁵I or ¹³¹I differ in their therapeutic potential. ¹²⁵I emits low-energy Auger electrons with a range <1 μ m. The ¹²⁵I-mAb should therefore target structures associated with the tumour cell DNA to be effective. By contrast, ¹³¹I is a medium-energy β (range 0.9 mm) and γ radiation emitter that significantly contributes to the whole-brain radiation dose.¹¹⁰

Clinical experience with radioimmunotherapy

The majority of RIT studies in gliomas are performed with anti-tenascin-C or antiEGFR mAb. The expression of tenascin and of EGFR increases with advancing brain tumour grade with $>90\%$ of GBM biopsies exhibiting tenascin overexpression,¹¹¹ while EGFR overexpression varies between 31% and 95% in patients with GBM.^{112,113} In clinical Phase I and II studies, the radiolabelled mAb 81C6 and BC-4 targeting tenascin as well as 425 anti-EGFR are extensively studied. It is beyond the scope of this review to discuss all published studies. For a detailed review on RIT, see Zalutsky,¹¹¹ Carlsson et al,¹¹⁰ Seidl¹¹⁴ and Dietrich¹¹⁵. Table 1 summarizes the studies reviewed here. The mean cumulative activity used in the studies is in the range of 5 mCi (0.185 GBq) ⁹⁰Y-labelled anti-tenascin mAb¹⁰¹ up to 180 mCi (6.66 GBq) ¹³¹I 81C6 murine mAb.¹⁰³ No correlation between tenascin expression or EGFR expression and treatment outcome was made with the exception of Casaco et al.¹⁰⁶ Although no correlation between EGFR expression and treatment response was found (¹⁸⁸Re-nimotuzumab), a median survival of 25.1 months was reported suggesting mAb independent treatment efficacy of the radionuclide ¹⁸⁸Re.¹⁰⁶ In all Phase I/II clinical trials, the maximal tolerable dose of RIT was explored.

Dose-limiting toxicities were of neurological and haematological nature. Promising results in patients with newly diagnosed GBM were archived with a median (overall) survival of 18.0–20.6 months.^{15,102,104,105} Combination with radiotherapy and/or chemotherapy was feasible and tolerated. For recurrent GBM, single administration of RIT (without combination with any standard treatment) seems to be less effective than combined treatment with chemotherapy.^{97,101} By contrast to these encouraging results, equivalent or lower median survival were found^{98,99,103} in comparison to the standard therapy outcome in historic controls of 14.6 months.^{16,17}

Caution must be taken with interpretation of the study results. None of the listed trials were randomized, and all were performed at single institutes; thus, treatment efficacy is not conclusive. Moreover, the recruited patient numbers are generally low, except for Li *et al*¹⁵ with 192 patients, and all patients had a relatively favourable Karnofsky performance status of $\geq 60\%$. The intracranial administration and dosimetry of the radiolabelled mAb is influenced by the volume of the surgical cavity leading to dose variations. RIT was tested for combined administration with radio(chemo)therapy in patients with liver metastases from colorectal cancer,¹¹⁶ recurrent lymphomas,¹¹⁷ as well as in pre-clinical models of head and neck cancer¹¹⁸ and colon cancer.^{119,120} Although RIT alone is not a curative treatment, combined chemo-RIT in patients with recurrent GBM and chemoradiation-RIT in patients with newly diagnosed GBM seems to be a promising treatment strategy.

DIETS

Ketogenic diet

With the current standard of care, GBM are not curable and dietary interventions have been proposed as supportive metabolic therapy.¹²¹ One of the most extensively studied diets, not only in GBM but also in other solid tumours, is the ketogenic diet (KD).¹²² KD is a high-fat and low-carbohydrate diet providing ketone bodies instead of glucose. In contrast to normal cells, malignant cells largely need glucose as their primary energy source and are unable to use ketones instead. In GBM management, application of steroid treatment (*e.g.* dexamethasone) to reduce brain oedemas has the potential to further raise the blood glucose level due to their glucocorticoid effect. However, it was suggested that elevated serum glucose levels might be associated with poor clinical outcome of patients with GBM.¹²³ The clinical safety of KD was assessed in six patients with GBM treated with the standard radiochemotherapy.¹²³ The mean blood glucose level decreased from 122 mg dl^{-1} (approximately 6.8 mmol l^{-1} , standard diet) to 84 mg dl^{-1} (approximately 4.7 mmol l^{-1} , KD). Overall, KD was well tolerated with no Grade III toxicity. The median follow-up was 14 months, but no correlation with survival was performed due to low patient number.¹²³ In two paediatric patients with astrocytoma and in a case report study of an elderly female patient with GBM, it was reported that the patients experienced no further tumour progression on KD.^{124–126} A study restricting the caloric intake of the KD to 600–1200 kcal per day was poorly tolerated, and patients had poor compliance due to constant hunger.¹²⁷ Considering the plausible rationale for the KD and the low number of established treatment options for recurrent GBM, a pilot study with 20 patients with recurrent GBM was initiated.¹²⁸

16 patients completed the unrestricted KD. The median progression-free survival and the median overall survival were 5 weeks (range 3–13 weeks) and 32 weeks (range 6–86+ weeks), respectively. 6 patients underwent a salvage therapy after disease progression combining KD with bevacizumab. The median progression-free survival was 20.1 weeks (range 12–124 weeks) for the combined treatment of KD and bevacizumab, which was not significantly different to bevacizumab administration alone (16.1 weeks; range 4–90+ weeks). Overall, KD is feasible and relatively well tolerated but had no significant clinical activity as a single agent in recurrent GBM.¹²⁸

Methionine-modified diets

The term amino acid auxotrophy refers to the dependence of cancer cells on exogenous essential amino acids such as MET. In the absence of MET, but in the presence of the MET-precursor homocysteine, normal cells can elevate the level of MET-synthase which methylates homocysteine to MET.¹²⁹ Therefore, MET-synthase is discussed to serve as a biomarker for MET auxotrophy. Pre-clinical studies with brain tumour bearing xenografts showed reduction of MET plasma levels as well as tumour growth retardation and inhibition under dietary MET depletion,^{130,131} suggesting MET restricted diets as an intervention to treat GBM. The clinical feasibility of a MET reduced diet ($2 \text{ mg kg}^{-1} \text{ d}^{-1}$) was assessed in eight patients with metastatic cancer. Within 8 weeks, MET plasma level decreased from 21.6 ± 6 to $9 \pm 2 \text{ } \mu\text{mol l}^{-1}$. The tumour response was not assessed, but the diet was well tolerated with minimal weight loss of 0.5% of body mass index per week.¹³² In a Phase I trial with 10 patients with recurrent glioma and metastatic melanoma, the optimal duration of a MET-free diet for maximum depletion of plasma level was determined to be 1 day, leading to a depletion level of 41%.¹³³ The patients tolerated four cycles of 2 weeks of a MET-free diet in combination with cystemustine treatment.¹³³ Based on these results, a clinical Phase II trial was initiated with 20 patients with metastatic melanoma and two recurrent glioma receiving four cycles of cystemustine every second week in combination with a MET-free diet.¹³⁴ The MET plasma level decreased to $53.1\% \pm 21.8\%$. The median time to progression and the median overall survival were 1.8 months (range 0.4–29.4 months) and 4.6 months (range 0.4–55 months), respectively, which is comparable to cystemustine administration alone. Compliance of the patients to the MET-free diet was good, allowing a high calorie intake of 2500 kcal per day for a patient with 70 kg body weight. Observed toxicities were mainly haematological (WHO grade 3–4) and most likely associated with the cystemustine treatment. Nonetheless, the authors concluded that the effect of the diet on the tumour might still be insufficient due to too high MET plasma levels.¹³⁴ In mice and patients, the MET serum levels can be reduced by intravenous injections of MET-degrading/cleaving enzymes,^{135,136} but more investigations are needed as risks for anaphylactic shocks and allergic reactions in primates after repeated injection have been reported.^{137,138}

The application of restricted diets needs careful evaluation as the dietary plan might negatively influence the patient's quality of life. It is questionable if all dietary treatment plans can be integrated in clinical routine. Evidence for an improved overall survival of patients with GBM owing to a change of diet is still missing. However, most animal experiments and clinical Phase I and II

trials showed good tolerance and no antagonistic effects when diets were combined with chemotherapy or radiotherapy. Nonetheless, further investigations are needed to test the most promising diets in combination with the standard treatment.

CONCLUSION

The last significant improvement in survival of patients with GBM was reached by the addition of TMZ to post-operative radiotherapy 10 years ago. Since then, numerous clinical trials on new combined drug treatments with radiotherapy have been performed, all so far with no significant effect on patient survival. This may partly be explained by a limited pre-clinical basis of combined mechanistic and functional studies that would allow effective patient stratification based on molecular or imaging biomarkers. Such biomarker-based approaches are now increasingly used in clinical trials with imaging giving implications for surgery and radio-oncological treatment. Integration of

functional imaging with metabolic tracers is also a promising approach for selective radiation dose escalation on well-defined small volumes in order to circumvent the problem of severe late brain necrosis after high radiation doses. Patients with GBM could further benefit from reirradiation or boost irradiation of a metabolically active tumour region with particle therapy. Further promising research avenues beyond combined drug treatment, which are currently in translational development, are immunotherapy, RIT or dietary treatments combined with the standard radio(chemo)therapy.

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