Review

Advances in the management of glioblastoma

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ABSTRACT

Glioblastoma (GB) is the most common and most malignant primary brain tumour in adults. Despite much effort, gold standard therapy has not changed since the introduction of adjuvant temozolomide in 2005 and prognosis remains poor. Despite this, there has been significant improvement in the surgical technology and technique, that has allowed for increased rates of safe maximal resection of the tumour. In addition, our increased knowledge of the biology of GB has revealed more potential targets, especially in the field of immunotherapy, which has been successful in revolutionising treatment of other cancers. We review the current best practice for the treatment of GB and explore some of the more recent advances in GB management from both a surgical and molecular therapeutic perspective.

INTRODUCTION

Glioblastoma (GB) is the most common and most malignant primary brain tumour in adults. Each year in the UK more than 4000 new cases of central nervous system (CNS) cancers are diagnosed, which equates to around 7 per 100 000 population. Although brain tumours account for less than 2% of all primary tumours they are responsible for 7% of the years of life lost from cancer before age 70 (Office for National Statistics 2006 Series MB1 No. 34). Unfortunately, despite significant research into these tumours, the latest survival trends for patients with CNS malignancies have remained largely static[1] reflecting the lack of new therapeutic options for patients. We review the current best practice for the treatment of GB and explore some of the more recent advances in GB management from a radiological, surgical and molecular therapeutic perspective (figure 1).

Current treatment paradigm

The current gold standard of treatment in the UK involves discussion at a dedicated neuro-oncology multidisciplinary team (MDT) meeting. Where deemed appropriate, patients undergo surgery for gross total resection (GTR, as defined by complete resection of contrast enhancing tumour on a post-operative scan performed within 72 hours of surgery) of the tumour where possible. This is then followed by concomitant chemoradiotherapy (150–200 mg/m^2/day temozolomide for 5 days every 28-day cycle plus fractionated radiotherapy of 60 gray (Gy) in 30 fractions over 6 weeks) and subsequent maintenance temozolomide chemotherapy.[2] Despite optimal treatment the median survival for such patients is still only 14–24 months and a 5-year survival of approximately 10%.[2 3]

Imaging for glioblastoma

In the UK, preoperative diagnosis of GB is based primarily on gadolinium-enhanced MRI. Classical features of GB on MRI include irregular peripheral enhancement around areas of necrosis with vasogenic oedema in the surrounding white matter. Fluid attenuation and inversion recovery sequences can also be used to highlight areas of peri-tumorous oedema, which has been shown to infiltrate with microscopic disease from which most tumour recurrences occur.[S1] Diffusion-weighted imaging can be useful for differentiation from abscess. MR spectroscopy (MRS) can also be used to demonstrate a markedly raised choline:N-acetylaspartate ratio in areas of solid enhancement. This can be useful in cases where there is equipoise regarding the grading of the diffuse glioma. More recently, MRS has also been used to identify other novel metabolites, including 2-hydroxyglutarate (2-HG).[4 S2,3] 2-HG is an oncometabolite, arising from the enzymatic activity of the mutated isocitrate dehydrogenase (IDH), found in certain gliomas. This is highly useful, as it is well established that IDH mutated GB represent a completely different tumour phenotype to IDH wild-type GB, conferring a much-improved prognosis.[5] The ability to predict molecular biomarkers preoperatively will enable clinicians to counsel patients much better pretreatment. To this end, other MRS markers, such as branched-chain amino acid transaminase-1 as well as other imaging modalities, such as positron emission tomography (PET),[S4] have also been trialled to predict molecular biomarkers of GB have also been used with some success, but all represent, at most, level III evidence for now.[6]

Surgery for glioblastoma

Surgery remains a mainstay of treatment and is essential in establishing a diagnosis. In addition, as surgical techniques have improved, the importance of obtaining a GTR is increasingly recognised[S5,6] and has been incorporated into European guidelines for the management of patients with GB.[7 8] With the caveat that patient safety is paramount, the primary aim of surgery is twofold: first, it allows for a histological, molecular genetic diagnosis to be made and second, by reducing tumour bulk, it provides a platform for further adjuvant therapy to act most effectively. However, GTR of GB can be technically challenging and is not possible in every case. As it is an intrinsic, infiltrating tumour it can sometimes be difficult to visualise a distinct tumour/brain margin and there is always functional-oncological

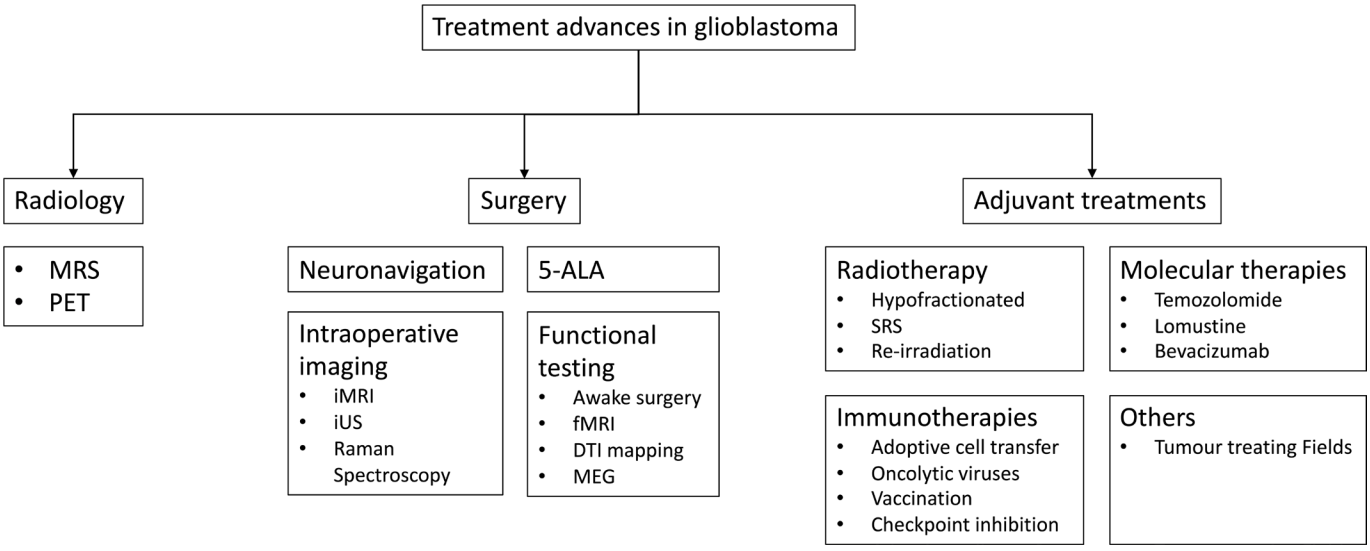

Figure 1 Schematic showing recent advances in the management of glioblastoma from imaging, surgery to adjuvant treatments. DTI, diffusion tensor imaging; fMRI, functional MRI; iMRI, intraoperative MRI; iUS, intraoperative ultrasound; MEG, magnetoencephalography; MRS, MR spectroscopy; PET, positron emission tomography; SRS, stereotactic radiosurgery; 5-ALA, 5-aminolevulinic acid.

balance and a potential trade-off between radical resection and causing permanent neurological deficit. Within the UK standard surgical practice consists of MRI-guided surgery with the addition of 5-aminolevulinic acid (5-ALA) to help visualise resection margins. There are many other more recent advances that are currently being trialled to increase the rates of safe surgical resection.

Neuronavigation

Structural and functional image guidance is now an essential part of glioma surgery. Neuronavigation is widely used in brain tumour surgery and can now be integrated with diffusion tensor imaging (DTI) tractography, magnetoencephalography (MEG) or functional MRI (fMRI) to produce both an anatomical and functional map of the brain. It is now possible to generate an individualised impression of functional areas and their relationship to tumours within the brain. Variability of individual neuroanatomy, distortion due to mass lesions and functional reorganisation caused by plasticity make classic anatomical identification of functional areas insufficient.[9] As we move increasingly towards a 'connectome' model of brain function, subcortical white fibre anatomy has become increasingly important.[10] DTI is being increasingly used to identify these important white fibre tracts both preoperatively and intraoperatively to allow for increased successful safe resection of tumours.[11] A multicentre randomised control trial in the UK, FUTURE-GB, investigating the additional benefit of intraoperative DTI and ultrasound is currently underway (ISRCTN38834571; https://future-gb.octru.ox.ac.uk/future-gb).

Compared with direct cortical and subcortical stimulation mapping, fMRI has a sensitivity and specificity of 91% and 64% for identification of Broca's area, 93% and 18% for identification of Wernicke's area, and 100% and 98% in motor areas.[12] By having an individualised map, a surgeon may not only use this preoperatively to plan their approach to a tumour, but also intraoperatively to ensure that maximal surgical resection of tumour is achieved while preserving important subcortical fibre tracts that are not normally identifiable under the microscope. In addition, further information on the localisation of metabolic function (single photon emission CT, PET, MRS) is also possible.[S7]

5-ALA

5-ALA is a haeme precursor which, if given exogenously, leads to increased accumulation of the fluorophore protoporphyrin IX (PpIX) within GB.[13] This fluorescence can be visualised intraoperatively through the operative microscope under blue light to help identify tumour, especially at the resection margins to increase resection rates (figure 2). Through the use of 5-ALA

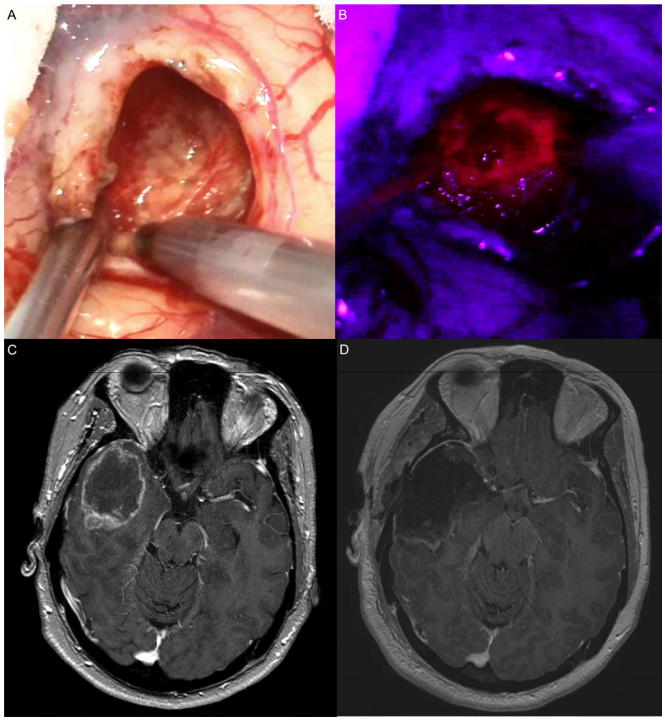

Figure 2 Example of 5-aminolevulinic acid induced fluorescence-guided surgery. (A+B) Photos of the same operative field as seen under (A) white light (B) blue light. Tumour can clearly be seen to fluoresce pink under blue light. (C+D) MRI scans showing the glioblastoma (C) before resection and (D) immediately after resection, showing gross total resection of the enhancing tumour.

in a randomised trial, GTR rates have been shown to significantly increase from 36% to 65% with a concomitant increase in 6-month progression-free survival (PFS) from 21.1% to 41%[13] and overall survival (OS) from 11.8 months to 16.7 months.[14] This increase in resection is associated with an increase in early postoperative neurological deficit at 48 hours (26.2% vs 14.5%) but not at 3 months (19.6% vs 18.6%).[15] Use of 5-ALA is now approved by the National Institute for Health and Care Excellence (NICE) for routine use in all patients undergoing surgical resection of GB in the UK.

In addition to the ability of PpIX to fluoresce under blue light, it also acts as a photosensitiser. Activated in the presence of bright light, it reacts produce reactive oxygen species resulting in local cytotoxicity to the cancer cells. It has been used for local photodynamic therapy (PDT) in other cancers, such as bladder and skin cancers. It has recently been the subject of trials in GB where patients are subjected to PDT following resection of their tumour. Initial evidence support the feasibility and safety of this approach[16 17] further studies will help determine the efficacy of this.

Intraoperative imaging (MRI and ultrasound)

The big issue with intraoperative image-guided neuronavigation is the problem of brain shift due to resection of tumour, loss of cerebral spinal fluid and cerebral oedema. This can now be partially overcome with intraoperative MRI (iMRI) as well as intraoperative ultrasound (iUS) scanning after the surgeon feels that they have macroscopically achieved GTR. This allows the surgeon to carry on with surgery if any residual disease is identified. A recent systematic review, although hindered by various sources of bias and other limitations, provides level 2 evidence to support the increased extent of resection (EoR) and survival with iMRI.[18] The use of iMRI is limited in the UK by its high set-up costs and, while it is available in some neurosurgical units, its use, unlike 5-ALA, is not standard of care. iUS has the advantage of being considerably cheaper and easier to use intraoperatively but is much more user dependent and has a longer learning curve. This is reflected in the relatively low sensitivity (49.6%) as determined in a recent meta-analysis.[19] However, it is hoped that as its use becomes more widespread, this will improve with time. Studies such as FUTURE-GB will hopefully accelerate the learning curve and uptake as well as shine a light on its usefulness as a surgical adjunct.

Awake surgery

Surgery in eloquent areas of the brain is often fraught with difficulty between achieving maximal resection and causing permanent neurological damage. Awake surgery helps in this regard as it allows for mapping of eloquent brain areas intraoperatively, allowing for maximal resection while preserving eloquent brain.[20] Due to the infiltrative nature of these tumours, often in eloquent locations, awake surgery has predominantly been performed for low-grade gliomas. As they are more aggressive, GB often have a more distinct margin between normal brain with little functional tissue within the tumour. However, it is still a diffuse infiltrative disease, which can be located close to eloquent brain. Awake surgery can be used, in these situations, to both increase the extent of resection and reduce postoperative morbidity. A recent meta-analysis of 14 studies involving 278 patients with GB who underwent awake surgery showed a GTR of 74.7% with an early postoperative deficit rate of 34.5%, which falls to only 1.9% beyond 3 months.[21] The SAFE-Trial (NCT03861299), a randomised control trial comparing awake craniotomy to surgery under general anaesthesia, is ongoing and will shed more light on this.

Raman spectroscopy

Raman spectroscopy is a biophotonic tool that has been used to differentiate between different tissue types. It has been investigated for the detection of GB and results have shown that it is capable of distinguishing both between tumour tissue and normal brain, with greater sensitivity than 5-ALA[S8] as well as being able to separate IDH wild-type and mutated tumours.[S9] It has been used alongside machine learning to produce real-time intraoperative diagnosis of brain tumour that is non-inferior to traditional pathology (S10), although the utility of this at tumour margins was not tested. Finally, a hand-held probe has also been developed to be used in real-time intraoperatively within the tumour cavity with a sensitivity of 93% and specificity of 91%.[S11] If shown to be true in follow-up studies, this could provide another surgical adjunct available to surgeons to maximise resection. The disadvantage of Raman spectroscopy is that it is not 'diagnostic' in the classical sense. It will produce a spectra for each tissue which is then compared with known standards. These spectra will differ between different probes and conditions and requires a significant amount of learning and validation for each probe in each location before it can be used clinically, which may limit its widespread use.

Surgery for recurrent tumours

Surgery for recurrent GB has historically been viewed with an air of futility. Unlike for primary GB, there is much less data in the literature reporting outcomes of repeat surgery for recurrent GB. However, there is now increasing evidence that repeat operations for recurrent GB provides increasing survival advantages[S12,13] and that much like for newly diagnosed GB, maximal resection will also increase survival benefit (table 1). The survival benefit seen is similar, if not greater in recurrent GB with similar EoR required for maximal benefit. This increased survival benefit may be surprising given the worse prognosis

Table 1 Summary of literature of extent of resection in recurrent glioblastoma

Study	No. of patients	Maximum survival advantage	Volumetric study	Minimum resection required
Bloch *et al* (S14)	107	3.4 months	Yes	95%
McGirt *et al* (S15)	294	2 months (GTR vs NTR) 6 months (GTR vs STR)	No	GTR
Oppenlander *et al* (S16)	170	10.8 months	Yes	80%
Quick *et al* (S13)	40	6.7 months	Yes	100%
Ringel *et al* (S17)	503	4.4 months	No	GTR
Suchorska *et al* (S18)	71	6.4 months	Yes	100%
Yong *et al* (S19)	97	7.5 months	Yes	<3cm3 RTV

GTR, gross total resection; NTR, near total resection; RTV, residual tumour volume; STR, subtotal resection.

Table 2 Summary of important molecular biomarkers in glioblastoma multiforme, their significance, prevalence and functional relevance

Biomarker	Significance	Prevalence	Functional relevance
IDH mutations	Prognostic marker	~12%	Production of 2-HG leading to change in metabolism and hypermethylation and better prognosis. Key driver mutation.
MGMT	Prognostic/predictive marker	50%–60%	MGMT methylation leads to increased response to alkylating chemotherapy.
EGFR	Prognostic marker*/therapeutic target	40%–50%	EGFR amplification activates downstream receptor tyrosine kinase pathways and change in methylation. Target for multiple therapeutics with limited success.
TP53	Prognostic marker*	25%–30%	Associated with pro-neural subtype. Deregulation of TP53 linked to tumour progression.
TERT	Prognostic marker*	60%–70%	Activating mutation of TERT leads to tumour immortality and oncogenesis.
G-CIMP	Prognostic marker	5%–10%	Associated with IDH mutations and proneural subtype. Confers a better prognosis.
Chromosome 10 loss	Prognostic marker*	70%–80%	Loss of tumour suppressor genes on chromosome 10, eg, PTEN, TP53 and NF1 leads to oncogenesis.
IL13Rα2	Therapeutic target	40%–50%	High-affinity IL13 receptor variant. Reduces STAT6 signalling leading to apoptosis inhibition. Target for immunotherapies.

*Role as prognostic marker is still debated.
EGFR, epidermal growth factor receptor; G-CIMP, Glioma CpG Island Methylator Phenotype; 2-HG, 2-hydroxyglutarate; IDH, isocitrate dehydrogenase; IL13Rα2, interleukin receptor 13Rα2; MGMT, O(6)-methylguanine-DNA methyltransferase; NF1, Neurofibromin 1; PTEN, Phosphatase and TENsin homolog deleted on chromosome 10; STAT6, Signal transducer and activator of transcription 6; TERT, Telomerase Reverse Transcriptase; TP53, Tumour protein 53

following recurrence. In the UK, there is a wide variation in clinical practice for patients with recurrent GB,[S20] reflecting the lack of consensus guidelines. Large, randomised trials, such as RESURGE (NCT02394626), investigating the use of surgery for recurrence will no doubt help with clinical decision-making and the formation of guidelines.

Molecular biomarkers for GB

With the increasing availability and use of genomic sequencing of tumours, coupled with the formation of large repositories of data, knowledge on the molecular biology of GB has steadily increased and now forms an important part of the new WHO classification of brain tumours.[5] Their importance is reflected in their increasing use in precision medicine to find targeted therapies (table 2). The three most commonly used biomarkers are described below.

IDH mutations

In 2008, a genome-wide sequencing study of glioblastoma multiforme (GBM) identified mutations in the gene encoding isocitrate dehydrogenase 1 (IDH1) in 18/149 tumours.[S21] Interestingly, these mutations occurred predominantly in younger patients with GB, which had progressed from lower-grade gliomas and were associated with increased survival.[22] This finding has sparked a raft of studies investigating the role of IDH in GB and has developed into a whole field of study in itself. The most common mutation is a single base transition substitution of arginine for histidine, which accounts for about 90% of all mutations, the so-called R132H mutation.[S22] This affects the binding site of isocitrate and changes the metabolic activity of the enzyme. Instead of converting isocitrate into α-ketoglutarate, it concerts α-ketoglutarate into 2-hydroxyglutarate. This leads to a change in both the metabolic state and the epigenetic state of the tumour cells.[S23] Reflecting its importance, IDH mutational status is a key molecular marker in the new WHO classification of brain tumours[5] and is an important prognostic marker. IDH-mutant GB have a significantly better prognosis[22] with survival rates similar to that of low-grade gliomas. Given its prognostic advantage and relative ease of testing, IDH status is now an essential molecular marker to factor in for all ongoing therapeutic trials. Additionally, there are many trials looking to specifically treat IDH-mutant gliomas, targeting the enzyme directly or using target treatment for IDH-mutant tumours, such as epigenetically modulating drugs or targeting DNA repair enzymes.[S23] Most recently, there has been a report of a novel vaccine targeting a neoantigen arising from the IDH-1 mutation.[S24]

MGMT promoter methylation

O(6)-methylguanine-DNA methyltransferase (MGMT) is a suicide DNA repair protein which irreversibly transfers a methyl group from O6 of guanine to a cysteine residue on itself at position 145. This one-way process produces alkylated MGMT, which is targeted ultimately for destruction.[S25] This is important in GB as the mainstay of chemotherapy is through the use of alkylating agents such as temozolomide/lomustine. MGMT counteracts the action of such drugs, conferring drug resistance to such tumour cells. This is supported by clinical data showing that GB with MGMT silenced through hypermethylation of the promoter respond better to temozolomide.[S26,27] However, patients with unmethylated MGMT still responded to therapy, although less well. Given the lack of alternative treatment options, MGMT methylation is currently not important in stratifying patients to adjuvant therapy. However, it is an important biomarker to take into account for trials of new therapeutics. In addition, a subset of GB become hypermutated following temozolomide treatment. This has been found to be due to mutations in the DNA mismatch repair gene MSH6 and is thought to be

temozolomide-induced mutagenesis, associated with MGMT promoter methylation.[23 S28]

EGFR amplification

The most commonly amplified and altered proto-oncogene seen in GB is the epidermal growth factor receptor (EGFR) seen in roughly 40% of all GB.[23] The most common mutation seen is the variant III (EFGRvIII), which is a constitutively active form of the EGFR. The role of both EGFR amplification as well as the specific EGFRvIII mutation as a prognostic marker remains controversial with no clear evidence to suggest it is associated with a significant change in overall survival.[S29] Given the prevalence of this alteration, there have been numerous studies investigating EGFR targeting drugs. Additionally, it is also the subject of a wide range of immunotherapy trials as discussed below. Unfortunately, to date, there has been little efficacy seen in drugs targeting this pathway.[S30] There are a multitude of potential reasons for this. GB is a highly heterogenous tumour and as such, EGFR inhibition will be insufficient to target the whole GB,[24] leading to tumour escape. Second, EGFR are receptor tyrosine kinases with multiple upstream and downstream pathways. Therefore, targeting a specific pathway may be insufficient to inhibit the downstream signalling cascade due to other upstream inputs, such as insulin-like growth factor-1 (IGF-1), or downstream activating mutations such as Phosphatase and TENsin homolog deleted on chromosome 10/phosphoinositide-3-kinase (PTEN/PI3K). No EGFR-targeted drugs are currently in clinical use for patients with GB other than in the trial setting.

Adjuvant therapeutic advances

Discovery of efficacious adjuvant therapy for GB has proven to be as difficult if not more so than successful surgical resection of this diffuse infiltrative tumour. Gold standard therapy of adjuvant chemo-radiotherapy followed by temozolomide has not changed since the introduction of the Stupp regimen in 2005.[2] Since then, there have been many agents trialled with varying preliminary evidence of activity in both standard chemotherapies as well as, more recently, immunotherapies. Of note, although in its relative infancy, tumour treating fields (TTF) offer a novel approach to adjuvant treatment of GB.

Radiotherapy

Fractionated radiotherapy remains one of the most effective adjuvant treatments for GB. In the UK fractionated radiotherapy with concomitant temozolomide is started within 6 weeks of surgery. More recently, various fractionations and stereotactic radiosurgery (SRS) have been trialled for the treatment of GB. However, most studies have been heterogenous, small, non-randomised trials making interpretation difficult. A recent meta-analysis of the available data suggests that hypofractionation is comparable to standard treatment with the benefit of shortened duration, worthwhile for elderly glioblastoma.[25] A large, randomised Radiation Therapy Oncology Group study (RTOG 93–05) investigating the use of SRS in addition to standard radiotherapy showed no survival benefit (13.5 vs 13.6 months) compared with conventional therapy. In addition, there was also no benefit to quality of life or cognitive function. There are also increasing numbers of studies investigating re-irradiation following tumour recurrence. Again, analysis of these studies is hampered by low numbers, differing treatment regimens and heterogenous patient cohorts. However, it does seem that there may potentially be a role to play for re-irradiation on recurrence, especially for patients treated with a short hypofractionated regime or brachytherapy. There did not seem to be a dose dependent response in patients treated with standard radiotherapy.[26] Given the lack of high-quality data, neither SRS nor hypofractionated radiotherapy is given to patients in the UK and re-irradiation is considered on a case-by-case basis by the MDT.

Chemotherapeutics

Temozolomide

Temozolomide is the first-line chemotherapy treatment of choice for primary GB. Discovered in the 1970s, it is an alkylating agent, causing DNA damage and leading to cell death. Stupp *et al* showed in their landmark paper that addition of temozolomide to adjuvant radiotherapy followed by six further cycles of temozolomide increase OS by over 2.5 months (14.6 months vs 12.1 months) with a 2-year survival advantage of 16.1% (26.5% vs 10.4%).[2] This was associated with a toxicity (grade 3/4 haematological adverse event) of 7%. Introduction of this protocol to standard of care in 2005 remains the last significant change in the management of primary GB. The effects of temozolomide are accentuated in patients who have MGMT silenced through methylation of its promoter.[27] However, given that it is still efficacious in patients with active MGMT and a lack of other treatment options, in the UK, temozolomide is given to all patients under 70 regardless of MGMT status.

For elderly patients, standard treatment for GB may not be possible due to patient comorbidities and the toxic nature of treatment. For such patients, there is evidence to show that treatment with temozolomide alone confers a greater survival advantage compared with standard fractionated radiotherapy (8.3 months vs 6 months, p=0.01) and was as effective as hypofractionated radiotherapy (8.4 months vs 7.4 months, p=0.12).[S31] Similar to younger patients, patients with MGMT promoter methylation responded better to temozolomide (9.7 months vs 6.8 months, p=0.02). More recent evidence also shows that addition of temozolomide to hypofractionated radiotherapy (40 Gy in 15 fraction over 3 weeks) in the elderly improved median survival from 7.7 months to 13.5 months (p<0.001).[S32]

Reflecting the paucity of effective treatment options, there are no established guidelines for the treatment of recurrent GB. The mainstay of chemotherapy is lomustine (see Lomustine/PCV). However, there is evidence to suggest that temozolomide may be useful in the treatment of recurrent GB. A large phase II study showed that continuous dose intense temozolomide was a viable treatment option for patients who developed disease progression within their first six cycles of temozolomide or those who had a disease-free interval and were rechallenged.[S33]

Lomustine/PCV

Lomustine, like temozolomide, is an alkylating agent and was commonly given with procarbazine and vincristine as part of the PCV regimen for GB. PCV was given as first-line treatment for GB prior to the discovery of greater efficacy of temozolomide. Of the PCV regimen, it is thought that lomustine is the agent with the greatest anti-GB activity. In the UK, PCV has been superseded by temozolomide for the adjuvant treatment of patients with primary GB and is reserved for treatment of patients with recurrent GB.[S34] However, a recent open-label phase-III trial showed that addition of lomustine to temozolomide chemo-radiotherapy, may increase survival for patients with primary MGMT methylated GB.[28] However, this trial was stopped early due to slow recruitment and consequently, the

results do not carry the necessary statistical power. A larger trial is being planned to address this issue.

Targeted molecular therapies

Bevacizumab

GB is characterised by significant microvascular proliferation and disruption of the blood brain barrier, hence the contrast-enhancement on CT and MRI. It has been found that there is an over-expression of vascular endothelial growth factor (VEGF), hence the logical extension that bevacizumab, a monoclonal antibody against VEGF-A, should help in the treatment of GB. There have been multiple large clinical trials to investigate this, including two large phase-III randomised controlled trial (RCT), which showed that although there can potentially be marked radiological response seen following treatment, with increased PFS (10.6–10.7 months vs 6.2–7.3 months) there was no benefit to OS (15.7–16.8 months vs 16.1–16.7 months) in patients with primary GB.[29 30] As such, the use of bevacizumab is not licensed in the UK but is for recurrent GB in the USA, Switzerland, Japan and Australia.[S35]

An interesting offshoot from these clinical trials is that bevacizumab also has steroid-sparing effects on surrounding oedema, allowing for reduced steroid use and the concomitant health benefits of this.[31] This is potentially highly important, given the increased interest in immunotherapy and the well-known immunosuppressive effects of high-dose steroids.

Immunotherapies

Interest and success in the use of immunotherapy for the treatment of cancers has rapidly increased in the last decade. There are multiple therapeutic strategies employed, including vaccination, adoptive cell transfer, chimeric antigen receptor T (CAR-T) cell and checkpoint inhibitor therapy. Within the UK, other than in the trials setting, immunotherapies are not part of the treatment algorithm for patients with GB.

Adoptive cell transfer

Adoptive cell transfer techniques involve the expansion of purportedly tumour specific T-cells (either endogenously expanded ex vivo, T cell receptor-transduced or CAR-T-cells) into patients, where they will traffic to the tumour and have anti-tumour effects. The focus of cellular based immunotherapies in GB have been CAR-T cell therapy. CAR-T cells are engineered to express single chain receptors that have an antibody-like antigen binding domain linked to an intracellular T-cell activating domain. They are therefore like antibodies in the way they recognise antigens, in an major histocompatibility complex (MHC)-independent manner, but they are also able to directly lead to T-cell activation rather than relying on antibody dependent cell-mediated cytotoxicity. There have been various CAR-T cells trialled, with the most common targets undergoing clinical trials including EGFRvIII (NCT01454596, NCT02664363, NCT02209376), interleukin receptor 13Rα2 (IL13Rα2) subtype (NCT02208362), human epidermal growth factor receptor 2 (HER2) (NCT02442297, NCT01109095) and ephrin type-A receptor 2 (NCT02575261).

While there has been some success, notably with IL13Rα2 CAR-T cells[32] providing tumour regression and maintenance for over 7 months with repeated infusion into the CSF, the efficacy of such treatments is limited in durability. This is because none of the common tumour associated antigens targeted are present in all GB tumours and even if present are only expressed in a proportion of the tumour cells. Even the most common mutation in GB, the EGFRvIII mutation is only seen in 30% of patients and of those 30% only up 86% of the tumour express the mutation[24] meaning tumour escape is therefore inevitable. More recent studies looking at bispecific CAR-T cells may help address this problem[S36,37] and it will be interesting to see how they perform in clinical trials.

Oncolytic viruses

Oncolytic virus therapies use native or genetically modified viruses that selectively infect and replicate within tumour cells. The ability of these viruses to preferentially target and replicate in cancer cells is thought to be a consequence of the cancer cells adaptation to escape the host immune system. Various types of oncolytic virus have been trialled for the treatment of GB including herpes simplex virus (HSV), adenovirus, poliovirus, parvovirus, reovirus, measles virus and more recently Zika virus. The success of such an approach, regardless of virus used, requires the successful replication of the virus balanced with an active immune response against infected cells. Any shift in this fine balance can lead to either uncontrolled viral infection, or more likely, eradication of the virus and lack of any therapeutic effect.

The first large study to show preclinical activity of an oncolytic virus approach was published in 1991 using a modified HSV that specifically targeted rapidly dividing GB cells in vitro and tumour regression in vivo.[33] This has been followed up with many phase-I trials in humans which proved safety. However, reports looking into their efficacy are still awaited. The added benefit of viral therapy is that it is relatively easy to modify viruses to also include extra transcripts to make infected cells produce immunoinflammatory cytokines and increase the local inflammatory response. The M032 is such an example of a HSV virus that has been additionally modified to include the human IL12 gene which is currently undergoing a phase-I trial (NCT02062827).

In addition to their role as oncolytic viruses directly, researchers have used adenoviruses as vehicles for gene-mediated cytotoxic immunotherapy. Adenoviral vectors encoding the thymidine kinase gene of the HSV have been used in conjunction with systemic injection of a prodrug such as ganciclovir. The prodrug is phosphorylated inside infected cells, converting the prodrug into its active form leading to termination of DNA replication and cell death. This has been tested in phase-I trials showing safety and potentially some clinical efficacy.[S38] However, while a larger phase-III trial did show some improvement in PFS, there was no effect on OS.[34]

More recently, following an outbreak of the virus in 2017, Zika virus has been explored for the treatment of GB given its propensity to infect neural precursor cells. So far only experimental models have been trialled but there is some evidence to support its efficacy in targeting GB stem cells for therapeutic benefit[S39] although much more work is required before it can be tested clinically.

Vaccination strategies

Vaccination strategies rely on artificially priming the host immune system against the tumour to induce, hopefully, a long-lasting immune response against the tumour. They can broadly fall into three major categories: (1) peptide; (2) genetic (RNA or DNA) or (3) cellular.

Among peptide vaccines, the EGFRvIII targeted vaccinations have received the most attention. The most advanced vaccination against this mutation, rindopepimut with human

Granulocyte-macrophage colony-stimulating factor (GM-CSF) adjuvant, has been the target of many in-human studies. Early-phase trials have shown it is safe and induces an effective immune response against tumours cells expressing the mutation, leading to loss of the mutation in the recurrent tumour.[S40] However, PFS after 5.5 months was only 66% in the ACT-III trial and a larger phase-III study was withdrawn early due to concerns over lack of effect on OS.[35] One of the pitfalls of a single peptide approach is the heterogeneity of expression of mutations leading to tumour escape. In addition, single peptide vaccinations are often restricted to a single HLA haplotype, further limiting the utility of such vaccinations to a smaller group of patients. Like bispecific CAR-T cells, this can be overcome by using multiple peptide vaccinations. Such examples include the IMA-950 peptide vaccination, which is an 11-peptide vaccination chosen based on enriched expression in GB compared with normal brain from mass spectrometry experiments. Early-phase trials using IMA-950 adjuvanted with poly-ICLC showed a CD8 T-cell response to one or more peptides in 63.2% or 36.8% of patients, respectively, and a median survival of 19 months for the 16 patients with GB in the trial.[36] Other approaches have been to provide personalised vaccinations targeted specifically at neoantigens[37] or a combination of both.[38] In the pure neoantigen vaccination trial, the eight patients enrolled had a median PFS of 7.6 months and OS of 16.8 months. Only three patients completed the vaccination protocol due to disease progression in the others. Neoantigens-reactive T-cells were only detected in the blood of patients who did not receive dexamethasone.[37] In a study where a standard TAA vaccination was first given followed by neoantigen vaccinations, 16 patients had a median PFS of 14.2 months and an OS of 29 months. Of the patients who received the vaccinations, 92% were able to generate an MHC class I-restricted T-cell reaction to the tumour associated antigens and 80% were able to generate a response to the neoantigens although many of these tumour-infiltrating lymphocytes (TILs) were still exhausted.[38] This would suggest there is a potential role for mixed tumour associated/neoantigen pooled peptide vaccinations in the treatment of GB.

In addition to peptide vaccines, there have been many dendritic cell (DC)-based vaccination strategies trialled for GB. At the simplest level, individual and multiple peptide-loaded DCs have been trialled in a small numbers of patients with some success. ICT-107 is a DC vaccine loaded with six cancer testis and GB associated antigens (absent in melanoma 2 [AIM-2], melanoma antigen 1 [MAGE-1], tyrosinase-related protein 2 [TRP-2], glycoprotein 100 [gp100], HER2, and IL13Rα2) and achieved a median PFS of 16.9 months and a median survival of 38.4 months. However, a randomised phase-II study showed only a slight 2.2 months PFS advantage and no significant improvement in survival.[S41] More commonly, tumour cells or cell lysates have been pulsed into DCs. Advantages of this system is that it allows for unbiased loading of the complete spectra of tumour peptides on to all of the relevant MHC class I and II alleles. The most advanced vaccine, DC Vax-L (autologous DCs pulsed with tumour lysate) has progressed to a randomised phase-III trial, in which initial reports suggest there may be an improvement in survival on an intention-to-treat analysis.[39] However, the reliability of this report is questionable given it is an interim report of the data with lack of unblinding and detailed analysis of all factors including PFS, which was their started primary end point.

Immune checkpoint inhibitors

Check point inhibitors, notably anti-programmed cell death protein 1 (PD1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4), have revolutionised the management of some previously untreatable cancers such as metastatic melanoma, non-small cell lung cancer and renal carcinoma. GB has been found to have very high levels of Programmed death-ligand 1 (PD-L1) expression on the tumours of up to 88%.[S42] Coupled with the knowledge that the majority of TIL in GB are PD1 positive and exhibit an exhausted phenotype,[S43] there was hope that check point inhibitors would prove to be an effective treatment. There are many clinical trials ongoing, but very few have published results. The largest study so far, the CheckMate 143 trial, showed that although some patients (3/40) showed partial response and 8/40 had stable disease with checkpoint inhibitor therapy, there was no difference in OS in any of the cohorts.[40] Furthermore, two large, randomised trials investigating the use of anti-PD1 (nivolumab) for the treatment of patients with primary GBM with (CheckMate 548) and without MGMT promoter hypermethylation (CheckMate 498) showed no survival advantage for patients treated with adjuvant nivolumab. Proponents of check point inhibitor therapy may argue that treatment of recurrent GB is very different to targeting primary GB as patients are often less well and have had immune-ablating therapies such as temozolomide and corticosteroids. The timing of check point inhibitor therapy has also come into question, with some more recent studies suggesting that neo-adjuvant therapy is more effective than adjuvant therapy as it gives pre-existing TILs the chance to become rejuvenated in the presence of cognate antigen, which is not present postoperatively as the tumour has been removed.[41 42] Within the UK, the Ipi-Glio trial is currently active and investigating the use of ipilimumab (anti-CTLA4) for patients diagnosed with primary GB.[S44]

TTF

TTF are a novel anti-mitotic therapy delivering low-intensity, intermediate-frequency (200 KHz) alternating electrical fields through transducers placed on the scalp. The alternating electrical field interrupts cellular mitosis at the metaphase–anaphase transition, leading to cell cycle arrest and apoptosis.[S45] Initial results of TTF for recurrent GB were controversial in that they did not show any survival advantage but still led to Food and Drug Administration (FDA) approval for this device.[S46] However, the EF-14 trial, a randomised controlled trial comparing TTF, given following completion radiotherapy, to standard of care for primary GB showed an increase in PFS from 4 months to 6.7 months and OS from 16 months to 20.9 months.[43] This increase in OS was not at the cost of increased morbidity.[S47] In fact, deterioration-free survival was increased in patients treated with TTF (4.8 vs 3.3 months), which likely reflects the increased PFS. A new trial (EF32, NCT04471844) is currently underway investigating the use of TTF concurrent to radiotherapy. This provided a more robust support for the use of TTF.

However, despite being licensed for use in the treatment of both primary and recurrent/refractory GB in the USA, uptake remains low. It is not licensed and hence its use is much more limited in the UK and Europe. This is due to the fact only safety and efficacy data are required for FDA approval in the USA. In order to gain NICE approval for

use in the UK, additional cost-effectiveness and clinical-effectiveness measures need to be reached, which is not yet the case. There are still significant differences of opinion regarding this among clinicians[S48] as well as patients.[S49] Reasons given are the lack of a sham device control in the RCT, paucity of corroborating evidence from other studies, difficulty in stratifying patient subgroups that benefit and patients being unwilling to wear the cap every day as it serves a visible reminder to themselves and others of their illness. Finally, this treatment is not cheap and in a publicly funded health service, such as the National Health Service, it does not fulfil the necessary criteria to be approved for routine use for patients. Even in a privately funded healthcare system, such as the USA, cost is still a significant barrier to uptake. It will be interesting to see if, over time, the initial scepticism shown for novel treatment modality can be overcome with increasing robust data to support the use of TTF.

Combination therapies

Given the inherent limitations of each therapy, researchers and clinicians are increasingly moving towards combinatorial therapies. Combinations involving checkpoint inhibitor therapies are especially attractive due to their efficacy in other tumours, such as melanoma. Various combinations have been trialled to try and increase their efficacy in GB including: (1) increasing mutations by radiation therapy[S50] or following temozolomide treatment (NCT02658279); (2) increasing local inflammatory response and antigen presentation through gene-mediated cytotoxic therapy;[S51] (3) increasing pool of tumour reactive T-cells by vaccination (NCT04201873) or adoptive cell transfer (NCT03726515) and (4) a combination of the above (NCT03018288). Such strategies are in their infancy and results from these are eagerly awaited. However, it must be also recognised that while combination therapies present opportunities for treatment of GB, they are also difficult to develop and analyse. Often, patients are receiving a combination of highly toxic drugs which may potentially have antagonistic side effects, such as temozolomide and immunotherapies, or in which the side effects become intolerable, such as erlotinib/rapamycin.[S52] Even when tolerated, it then becomes difficult to determine the contribution of each therapy to the efficacy of the combination.

Summary

Glioblastoma is the most common, and most aggressive primary brain tumour in adults. With a median survival of only 14–24 months, prognosis remains poor despite much effort into finding novel efficacious therapies. Mainstay of treatment remains gross total surgical resection followed by adjuvant temozolomide/radiotherapy. Surgical advances have improved the ability of surgeons to achieve maximal, and sometimes, supramaximal resection with reduced morbidity. Many small molecule inhibitors and immunotherapeutic strategies have failed to improve prognosis, although there is hope, especially with immunotherapy, that novel combination therapies can provide some much-needed improvement in survival.

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