Molecular imaging in the development of a novel treatment paradigm for glioblastoma (GBM): an integrated multidisciplinary commentary.

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Molecular Imaging in the Development of a Novel Treatment Paradigm for Glioblastoma: An Integrated Multi-Disciplinary Commentary

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Abstract

Current therapeutic strategies against glioblastoma (GBM) have failed to effectively prevent disease progression and recurrence. The role played by molecular imaging (MI) in the development of novel therapies has gained increasing traction in recent years. For the first time, leveraging expertise from an integrated multi-disciplinary authorship, we present herein a comprehensive evaluation of the state-of-the-art in GBM imaging and explore how advances facilitate emergence of new treatment options. We propose a novel next generation treatment paradigm based on the targeting of multiple ‘hallmarks of cancer’ evolution of which will heavily rely on MI.

Key words: glioblastoma (GBM), molecular imaging (MI), anti-GBM therapeutics

Teaser: This review highlights the role played by molecular imaging in current and future GBM treatment approaches.
**Introduction**

Based on estimations, 23,000 men and women (55% men) will be diagnosed with a primary brain or other nervous system malignancy this year in the United States with 13,700 men and women to succumb to these diseases [1]. Glioma is the most common primary central nervous system (CNS) tumour. Widespread infiltration of surrounding tissue, the presence of necrotic tissue and/or angiogenic activity characterize the most malignant form termed glioblastoma (GBM, grade IV) [2]. The standard of care for treatment of GBM consists of neurosurgical tumour resection and concomitant chemotherapy and radiotherapy (RT) followed by adjuvant chemotherapy. The most widely implemented treatment regimen has been developed by the European Organisation for Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada (NCIC) Clinical Trials Group, and is often referred to as “the Stupp protocol”. In a randomised trial including almost 600 patients they clearly demonstrated improved overall survival in patients who received a combined modality treatment of temozolomide (TMZ) chemotherapy in conjunction with concomitant radiotherapy followed by adjuvant or maintenance TMZ for 6 cycles (TMZ/ RT → TMZ), compared to patients who received only RT as initial treatment but may have received TMZ chemotherapy at recurrence [3].

Nevertheless despite the emergence of novel treatment strategies in recent years (discussed in detail below) survival rates for GBM patients continue to be low. According to the National Cancer Institute median survival for patients with GBM is 15 months from time of diagnosis [1], although it may be longer for selected patients receiving modern therapy.
Development of novel therapeutics having anti-GBM activity remains a critical and challenging objective. Moreover, targeting of a single ‘cancer hallmark’ [4] is unlikely to elicit significant benefit in patients presenting with advanced malignancies. Thus, developing molecular targeted drugs that concomitantly impact several GBM survival pathways/ ‘cancer hallmarks’ is likely to represent a new horizon within the field.

Nevertheless, notwithstanding the validity of this objective the average cost of developing a drug within the healthcare space has over several years increased to an average of USD 1.3 billion with sustained investment required over a period of no less than ten years. This is none more evident than in the oncology space, where molecular targeted therapies (in the context of the evolution of a personalised treatment paradigm) comprise a significant sector of the market.

Reducing the drug development time-line through implementation of molecular imaging (MI) strategies during pre-clinical and clinical development phases would significantly reduce the burden of cost. MI can improve the efficiency of drug screening and may be used to interrogate drug pharmacokinetics and bio-distribution, thus markedly reducing time and costs required for the development of a new drug. Moreover, identification of MI biomarkers yielding spatial and temporal information may also provide surrogate clinical endpoints. Critically, imaging of key biological processes which underpin the classical ‘cancer hallmarks’ [4] e.g. angiogenesis, invasion, proliferation may provide a more robust mechanistic assessment of newly emergent molecular targeted therapeutics which represents a cornerstone of personalized medicine. This is of note when one considers that chronic treatment with targeted therapies results in disease stabilization rather than the tumour shrinkage more evident in response to cytotoxic therapies. As such, classical gross anatomic imaging of tumour lesions may no longer provide for optimal therapeutic
follow up, but rather what is currently required, are imaging modalities that allow for sensitive assessment of drug effects at the level of specific targeted molecular features/ biological pathways.

MI optimises streamlines and refines the drug development process. The advantages of implementing a MI strategy both during the pre-clinical and clinical development stages are illustrated with translational research in primary brain tumours. The disease represents particular challenges towards development of novel therapeutics, as drugs must be amenable to crossing the blood brain barrier (BBB) (notwithstanding BBB breakdown during the disease process) and should efficiently compromise the key processes of tumour angiogenesis, invasion and cell proliferation. The ability to monitor drug effects on these key biological processes in real time will play a key role in elucidating the next generation of molecular targeted anti-GBM therapeutics.

Within the context of the current review, we have, for the first time gathered a multi-disciplinary team leveraging expertise in cell biology, pre-clinical modelling, neurosurgery, MI and neuro-oncology to critically assess the role played by MI in the development of a state-of-the-art ‘next-generation’ anti-GBM therapeutic paradigm which will seek to target multiple cancer hallmarks.
Pre-clinical and clinical molecular imaging strategies applied in GBM

Longitudinal monitoring of tumour progression and drug efficacy are possible thanks to real-time non-invasive imaging technologies. These technologies facilitate quantitative and qualitative imaging of the primary tumour and record the extent of infiltration into the brain parenchyma. State-of-the art MI modalities used to assess GBM in both pre-clinical and/ or clinical settings include tomographic imaging, nuclear radioisotope-based imaging, optical imaging, and intra-operative photodynamic navigation in brain tumour surgeries (See Figure 1 and Table 1).

Magnetic resonance imaging

A high degree of spatial resolution is facilitated using tomographic approaches. Computed tomography (CT) may be initially used in the diagnostic process. However, magnetic resonance imaging (MRI) is the gold standard for brain tumour imaging [5]. MRI continues to be refined in pre-clinical models and now provides both high-resolution anatomical information and functional measurements of tumour physiology [6].

Conventional MRI produces high-resolution brain images that facilitates analysis of tumour size, location and extent of associated oedema and lesion necrosis, but does provide only little physiological information [5]. Most high-grade tumours, such as GBM, lead to a disruption of the BBB with leakage of contrast medium into the interstitial space, while low-grade glioma has typically an intact BBB. The increased blood flow due to the high density of neo-vasculature, however, does not facilitate quantification of contrast uptake as a means of monitoring disease [7].
Advanced MR techniques have now been validated to monitor tumour vasculature and study biochemistry of drug responses. MR modalities showing promise in early detection of response to treatment and disease progression include proton magnetic resonance spectroscopy (1H-MRS), perfusion/permeability MRI, Diffusion-Weighted MRI (DW-MRI) and functional MRI (fMRI). MRS techniques allow for Nuclear MR Spectroscopy (NMRS) in a living organism. 1H-MRS is available for clinical MR scanners and provides a non-invasive assessment of tumour biochemical features. 1H-MRS can be performed as part of a standard clinical MRI protocol [8]. Furthermore, it has been shown that 1H-MRS using short and intermediate echo time (TE) sequences facilitates the differentiation of high-grade cerebral gliomas from single metastatic brain tumours [9]. Perfusion MRI may be divided into three categories: Dynamic Susceptibility Contrast MRI (DSC-MRI), Dynamic Contrast Enhanced MRI (DCE-MRI) and Arterial Spin Labeling (ASL) [5]. DSC perfusion MRI allows measurement of cerebral blood volume (CBV), peak height (PH) and percentage of signal intensity recovery (PSR) within the brain [10]. DCE-MRI assesses tumour perfusion and angiogenesis by monitoring the pharmacokinetic uptake and rinsing of an MR contrast agent within the extracellular space of tumour lesions. Changes in volume transfer constant (Ktrans) or the initial area under the Gadolinium (Gd) concentration time curve in tumour, blood adjusted (IAUCBA) in one or more malignant target lesions following drug treatment have been used as primary measurements of drug activity [11]. The ASL technique is capable of measuring Cerebral Blood Flow (CBF). ASL facilitates cerebral perfusion mapping, without administration of a contrast agent or the use of ionizing radiation [12]. An important tool in tumour diagnosis is DW-MRI. This technique allows quantification of two values: apparent diffusion coefficient (ADC) and fractional anisotropy (FA) (the
diffusion of water in one plane). ADC is often correlated with tumour size (an increase of ADC values suggests response to therapy, a decrease of ADC values is associated with no response to therapy) [13]. Based on the principles of DW-MRI, Diffusion Tensor Imaging (DTI) tractography indicates white matter fiber tracts inside a tumour and in surrounding tissues [14]. 3D-volumetric sequences may be fused with DTI tractography on an intra-operative navigation computer [5]. Blood Oxygen Level Dependent (BOLD) contrast functional MRI (BOLD-fMRI) is well-established method for non-invasive localisation of eloquent brain regions in preoperative planning for tumour removal. A change in BOLD response (the change in the ratio of oxyhemoglobin to deoxyhemoglobin) occurs due to a change in blood flow to the active neurological area [15]. The neurosurgeon may integrate anatomic, physiologic and metabolic MR images preoperatively in an effort to delineate tumour margins and maximise resection (See also Table 1).

Radioisotope imaging

Compared to MR protocols, radioisotope imaging is less well established for GBM patients in the clinical setting. However, benefits from radioisotope imaging may be obtained at primary diagnosis, for planning of biopsy/ surgery targeting at the highest grade area, and in post-treatment radiological follow-up. Positron emission tomography (PET) based on the detection of pairs of gamma rays, which are produced when a positron emitted by a PET tracer encounters an electron. Computed image reconstruction is used to generate a three dimensional (3-D) image of tracer concentration at a given location [16].
The most common PET radiotracer used to visualise primary tumours and tumour metastases is the glucose analogue 18F-Fluorine-2-Deoxy-D-Glucose (FDG). Tumour cells exhibit high level of glucose metabolism and up-regulation of glucose transporters (GLUTs) when compared to non-tumour cells. However, due to high-glucose metabolism in normal brain tissue resulting in a significant physiological FDG uptake, 18F-FDG-PET application for detecting glioma or for interrogating anti-glioma therapeutics is limited [17].

As such, several alternative radiotracers having a lower background uptake in the brain have been developed to date, such as amino acid tracers (11C-methionine (MET), 18F- O-(2-18F-fluoroethyl)-L-tyrosine (FET) and α-11C-methyl-L-tryptophan (AMT)), the thymidine analog (18F- fluorothymidine (FLT)), 11C-choline (CHO), 18F-fluoromisonidazole (FMISO), 18F-Fluciclatide and 18F-Galacto-Arginine-glycine-aspartate (RGD). The most widely used alternatives for 18F-FDG are the radio-labelled amino acid tracers. It has been reported that amino acid PET tracers may have utility in microsurgical and radiotherapy [18]. The 11C-MET radiotracer visualises elevated amino acid transport in endothelial and tumour cells being valuable for detection of tumour size and glioma cell invasion [19]. The amino acid 18F-FET further represents a promising radiotracer for targeting biopsy sites, delineating tumour extent, diagnosing recurrence and determining tumour volume [20]. Further, 11C-AMT was shown to identify tumour-infiltrated brain tissue not detectable by conventional MRI in newly diagnosed GBMs. Moreover, elevated 11C-AMT uptake was not observed in areas of vasogenic oedema [18]. The thymidine analog, 18F- FLT has been developed as a PET tracer to image tumour cell proliferation in vivo. Uptake of 18F-FLT by proliferating tissues occurs through activation of thymidine kinase [21]. Evaluation of brain tumour malignancy and glioma
differentiation may also be assessed using 11C-CHO PET tracer. CHO is a natural blood constituent, which penetrates cell membranes. The uptake rate of 11C-CHO within tumour tissue has shown to be proportional to the rate of tumour cell duplication [17]. 18F- FMISO represents yet another PET radiotracer of interest with regards to brain tumours. FMISO is a pro-drug which becomes activated in hypoxic tissue and is subsequently inhibited in the presence of oxygen. [22]. PET radiotracers, such as 18F-Fluciclatide and 18F-RGD, have also been evaluated in GBMs. Fluciclatide binds with high affinity to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, whereas the tripeptide sequence, RGD, binds to $\alpha_v\beta_3$ only [23].

Single photon emission computed tomography (SPECT) represents a further imaging modality based on selective radioisotope uptake. A gamma camera is rotated around a subject to obtain multiple two dimensional (2-D) images of SPECT tracer distribution. A 3-D image is generated by computer processed tomographic reconstruction of the 2-D image dataset. SPECT tracers directly emit gamma radiation [24].

With respect to SPECT, Thallium-201 (201TI), Technetium-99-m methoxyisobutylisonitrile (99mTc-MIBI) and 3-123I-iodo-$\alpha$-methyl-L-tyrosine (123IMT) radiotracers have been implemented in evaluation of brain tumour progression. 201TI is preferentially taken up by neoplastic glial cells. Use of this tracer is limited as 201TI SPECT exhibits low sensitivity in the detection of low-grade lesions with an intact BBB [25]. More sensitive SPECT tracers, known are 99mTc-MIBI and 123IMT. 99mTc-MIBI is stored in mitochondria and cytoplasm of metabolically active cancer cells due to an active transport mechanism and has been shown to have utility in the determination of histological grade and to determine GBM follow up [26], whereas the amino acid derivate 123IMT has been evaluated to determine metabolic activity of
brain tumours and further to distinguish tumour recurrence from radiation necrosis and peri-tumoural oedema. Moreover, as the uptake of 123I
t is less dependent on BBB damage, it may be more suitable in the detection of low grade tumours than 201TI SPECT [27] (See also Table 1).
Optical imaging

An essential pre-clinical tool for monitoring disease progression and therapeutic response at the molecular level is non- (or minimally) invasive optical imaging.

Bioluminescence imaging (BLI) is based on the production of light following a multistep, enzymatic reaction of luciferase with its substrate (luciferin or coelenterazine), which can be visualised with an external detector. Firefly luciferase, in the presence of adenosine triphosphate (ATP) and molecular oxygen, catalyzes the oxygenation of luciferin to form a highly unstable, electronically excited oxy-luciferin. Photons of light are released upon relaxation of oxy-luciferin to its ground state [28]. BLI has been implemented in GBM tumour model systems and is established as a reliable tool in the assessment of tumour progression over time and in the pre-clinical evaluation of drug efficacy. The main advantages of BLI include the ability to non-invasively monitor tumour progression and treatment efficacy, ease of use, relatively inexpensive equipment, and the possibility to image more than one animal at the same time. The major disadvantage of BLI is that genetic expression of luciferase is a fundamental requirement. Therefore, its application is limited to the pre-clinical setting [29].

Fluorescence imaging (FLI) is based on the excitation of a fluorescent molecule with visible light, resulting in emitted light, which can be utilised for imaging purposes. Although extensively used in vitro, utilisation of FLI in vivo is limited by tissue light penetration [30]. Nevertheless, over the past several years significant advances have been made to implement this technology in vivo using wavelength tissue penetrating light.
In particular, near-infrared fluorescence (NIRF) imaging has been successfully applied as a powerful tool for imaging GBM at the molecular level in pre-clinical small animal models. NIRF imaging uses wavelengths in the 700- to 900-nm range where light absorbance and scattering are significantly lower and auto-fluorescence of normal tissues is minimally detected. As an example, the NIRF dye Cy5.5 associated with a targeted probe has proven to be a promising contrast agent for \textit{in vivo} tumour visualisation. \textit{In vivo} NIRF imaging of the integrin $\alpha_v\beta_3$ using an RGD-Cy5.5 conjugate has been further shown to represent a highly sensitive technique for tumour detection in GBM xenograft models [31].

Translation of optical imaging in the clinical GBM setting is limited to intra-operative applications. Implementation of intra-operative photodynamic navigation based on optically active imaging agents has been shown to provide benefits during GBM surgeries. 5-aminolevulinic (5-ALA) is a non-fluorescent naturally occurring precursor of haemoglobin, which can be administered orally and which is metabolized by tumour cells of epithelial and mesenchymal origin leading to intracellular accumulation of the fluorescent dye Protoporphyrin IX (PpIX). Once exposed to violet blue light, cells with high concentrations of PpIX illuminate with red light. When the extent of resection guided by 5-ALA fluorescence was compared to that achieved by resection under normal white light, higher rates of complete resection were observed in a group of patients assigned to the 5-ALA group [32]. Thus, fluorescent-guided resection provides the neurosurgeon with real time visual recognition of tumour material (See also Table 1).
Multimodality imaging strategies

Multimodal imaging approaches, which have reached the clinical setting, combine PET and/or SPECT with CT or MRI [18,19,33]. As discussed, PET enables non-invasive visualisation of tumour metabolism, neuronal function, and therapeutic gene expression (see Figure 1 for advantages and disadvantages of PET). CT or MRI completes this information with anatomical data (see Figure 1 for advantages and disadvantages). The utility of applying such a multi-modality approach in monitoring cancer progression or therapeutic efficacy is well established and will be discussed further in the next section [34].

The development pipeline for novel anti-GBM therapeutics often combines methods of imaging to deliver more detailed information on tumour growth and metabolism and to mechanistically interrogate response to treatment at the molecular level in a spatially sensitive (e.g. anatomic) setting. In pre-clinical studies it is now possible to implement BLI protocols in parallel with conventional MRI (see Figure 1 for advantages and disadvantages). This multimodal imaging approach provides valuable information with respect to early identification of tumours (BLI), tumour localisation (MRI), volumetric measurements (MRI), efficient measurement of response to therapeutics (MRI and BLI) and further facilitates high-throughput analyses (BLI). Generally, BLI signal has been found to correlate well with MR measurements of tumour volume [35]. However, some limitations relating to the use of BLI alone in the context of translational GBM research have been noted. As shown by Jost et al., at early time points following tumour implantation, false bioluminescence signal can be generated by the presence of intra-parenchymal haemorrhages and extensive hydrocephalus at the site of injection. Lack of correlation with MRI-determined tumour volumes has also been shown at late stages
of tumour growth, when bioluminescence signal is lost due to the presence of hemorrhagic and necrotic tissue, whereas tumour volumes continue to increase which is evident on MRI [36]. Therefore a BLI/ MRI multimodal strategy is of significant benefit within the pre-clinical setting.

PET and optical imaging have been recently combined. Radioactive materials are able to produce low energy visible photons (1.2 to 3.1 eV, 400 to 1000 nm) associated with Cerencov or Bremsstrahlung radiation. Thus, radioactive molecular probes may be non-invasively imaged using commercially available optical imaging instruments. The main advantages of imaging radioactive probes using optical imaging include lower capital costs, high-throughput imaging and wider accessibility of optical imaging instruments when compared with PET and SPECT scanners [37].
Application of MI strategies in the pre-clinical development and clinical evaluation of a novel GBM therapeutic paradigm

The increasing use of MI in translational GBM research as well as evolution of the GBM imaging field has facilitated pre-clinical and clinical interrogation of both gold-standard and ‘next generation’ therapeutics. The expanding complexity of the MI space, to include novel MR techniques and PET based approaches, support the emergence of a novel GBM treatment paradigm based on the targeting of multiple cancer hallmarks and is likely to result in the identification of new imaging as well as therapeutic targets. Examples of how MI contributes to the evolving GBM therapeutic paradigm are discussed below (See also Table 2).

Angiogenesis inhibitors

As with all neoplastic lesions, growth and survival of GBM is dependent on a blood supply. Complex interaction of angiogenic factors, such as vascular endothelial growth factor (VEGF), results in the formation of new blood vessels. Thus, angiogenic factors and pathways participating in the angiogenesis process are targets for GBM treatment.

Currently the sole angiogenesis inhibitor licensed by the Food and Drug Administration (FDA) for use in GBM is bevacizumab following two successful phase II trials, although its value in brain tumours remains a matter of debate [38,39]. Cediranib (AZD2171, Recentin™, a small molecule, orally bioavailable tyrosine kinase inhibitor (TKI) of VEGFR2, PDGF, receptor-α and –β, and stem growth factor –receptor (c-Kit) [40]) and cilingitide (EMD121974, a cyclic RGD-mimetic peptide
selective inhibitor of both αvβ3 and αvβ5 integrins) has been investigated in the treatment of recurrent GBM, but have failed to prolong survival in a phase III randomised trial. Other more conventional, multi-targeted tyrosine kinase inhibitors (TKIs), such as sunitinib and sorafenib, (which inhibit activation of downstream VEGF receptor (VEGFR-2), signalling pathways as well as targeting other pathways involved in angiogenesis including PDGFR, fms-related tyrosine kinase 3 (FLT3), c-KIT [41] etc) showed pre-clinical promise for application in the GBM setting [42]. However, to date there is lack of available clinical data demonstrating significant benefit of sunitinib and sorafenib in GBMs [43,44]. Resistance to angiogenesis inhibition is likely a complex and multi-factorial event which may at least partially be explained by activation of compensatory ‘cancer hallmark’ pathways [4].

Angiogenesis inhibitors nevertheless still represent a potentially promising approach in targeted GBM therapy though it is likely that the future of this class of agents will be based on application within combination treatment regimens. Defining AI treatment response using imaging is challenging as AIs may be cytostatic rather than cytotoxic. Obvious reductions in tumour size (according to Response Evaluation Criteria in Solid Tumours (RECIST) criteria) as measured by conventional imaging techniques may not be evident despite an apparent clinical benefit. Thus, it has proven difficult to obtain accurate response rates when using standard T1- and T2-weighted MR images to analyse benefits of angiogenesis therapy in GBM patients [45]. Despite limited availability, novel GBM imaging methods have proven effective in measuring treatment response. These methods include DSC-MRI, DCE-MRI, DW-MRI and PET as discussed above.

DSC-MRI can further provide information on vascular re-modelling and may be used as an early indicator of response to AI therapy as shown in patients treated with
enzastaurin in combination with TMZ [46]. Sorenson et al. have used DCE-MRI to measure $K^{\text{trans}}$ in patients treated with the TKI cediranib. By combining $K^{\text{trans}}$ values with biomarkers (micro vessel volume and circulating collagen IV) the authors calculated a ‘vascular normalization index’ which was predictive of overall survival (OS) and progression-free survival (PFS) following a single dose of cediranib [47].

DW-MRI can provide information on tumour cellularity and response to treatment by estimating ADC [48,49]. DW-MRI has been used to examine ADC voxel changes in the same patient over time, creating functional diffusion maps (fDMs). In GBM patients treated with bevacizumab, nonlinear registration of pre-treatment ADC maps to post-treatment ADC maps demonstrated improvement in clinical predictability, sensitivity and specificity of fDMs for both PFS and OS [50]. Moreover, Gerstner et al. have further elucidated the benefits of ADC maps in assessing cell infiltration after treatment with anti-VEGF agent, cediranib. Regions of tumour growth that are not visible on contrast-enhanced MRI may be visualized using DW-MRI technique [13].

PET further represents an evolving technique for assessing GBM therapeutic response to anti-angiogenic therapies [51]. In comparison to anatomic imaging, tumour metabolic response to anti-angiogenic treatment has been suggested as a more powerful method for predicting OS. A pilot clinical study demonstrated a correlation between reduced FLT uptake and improved OS in GBM patients treated with a combination of irinotecan and bevacizumab. Thus, FLT-PET has been suggested as an imaging biomarker for predicting survival benefit in response to anti-VEGF therapy in patients with recurrent GBMs [52]. Moreover, a pre-clinical study using the novel tracer 18F-Fluciclatide in a U87MG-based xenograft model provided evidence of a method to measure tumour response to anti-angiogenic therapy with sunitinib. This study demonstrated that assessment of the response to anti-
angiogenic therapy using 18F-Flucilatide-PET to measure $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin expression on GBM cells and the vasculature was observed before any significant changes in tumour size were evident using the current standard tumour calliper measurements. Moreover, 18F-Flucilatide tumour uptake was useful in assessment of tumour necrosis and integrity [23].

Thus, the multitude of tracers currently under investigation suggests that PET may play an important role in for early evaluation of the efficacy of AIs and other targeted agents [53]. Moreover, with the emergence of AIs in the treatment of GBM it is imperative that the most appropriate imaging modalities are used to determine tumour response to treatment. Importantly, in light of the resistance to AI therapy evident in the clinic [54] and given the most recent data which has emerged to suggest a paradoxical up-regulation of invasive pathways in response to AI treatment, a future therapeutic strategy may include targeted therapies to compromise both angiogenic and invasive pathways [55]. Thus, the ability to sensitively monitor GBM tumour cell invasion in vivo is likely to become increasingly important.

**EGFR inhibitors**

The Epithelial Growth Factor Receptor (EGFR) family comprises an important group of receptor tyrosine kinase (RTK) molecules. These molecules include the closely related trans-membrane proteins: ErbB-1 (EGFR, HER1), ErbB-2 (neu, HER2), ErbB-3 (HER3) and ErbB-4 (HER4). Major downstream signalling pathways activated by EGFR include Ras/Rapidly Accelerated Fibrosarcoma (Raf)/MAPK Kinase (MEK)/Extracellular signal Related Kinase (ERK) 1/2/MAPK and PI3K/AKT.
Physiologically, EGFR is activated by epithelial growth factor (EGF) and related ligands thus mediating cellular growth and differentiation in both embryo and adult [56]. Deregulation of this pathway occurs in approximately 40 % of patients with GBM and results in increased proliferation, invasion, angiogenesis and tumour survival. Recently EGFR expression has been associated with resistance to standard chemo- and radiotherapy.

Strategies exist to target EGFR, most notably monoclonal antibodies (mAbs) targeting the extracellular epitope (e.g. cetuximab) or small molecule receptor tyrosine kinase inhibitors that target the intracellular kinase domain (gefitinib and erlotinib). Gefitinib and erlotinib are small molecule TKIs, which have undergone numerous phase I/II trials either in single agents or combination protocols. Both are orally bio-available, well tolerated and have demonstrated significant anti-GBM activity in pre-clinical trials. However, these findings did not translate consistently in clinical trials [57,58].

Using MI, Mukherjee et al. investigated the molecular mechanism by which the EGFRvIII mutation confers resistance to radiotherapy in vivo. An orthotopic intracranial luciferase expressing U87-EGFRvIII mouse model was implemented to assess tumour growth dynamics following whole brain radiotherapy. The authors provided evidence that EGFRvIII expression activates a DNA double-strand break repair enzyme by up-regulation of the PI3K-AKT-1 pathway. Activation of this enzyme reverses the DNA damage induced by ionizing radiation. The authors were able to demonstrate that combining EGFR inhibition with a PI3K inhibitor attenuated the rate of DSB repair providing a rationale for combining these drugs [59].
A chimeric antibody (Ch806) binds to the EGFR deletion variant de2-7 EGFR (EGFRvIII) and has shown efficacy in pre-clinical GBM models [60]. Ch806 labelled with a positron emitter characterised by a relatively long half-life, such as 124I or 89Zr facilitates non-invasive quantification of de2-7 EGFR expression in vivo via immuno-PET, which represents an attractive and novel approach to improve tumour characterisation. As mAbs target specific tumour markers or predominant antigens, immuno-PET provides not only higher-resolution images but also facilitates tumour targeting and mAb quantification in targeted and non-targeted tissues. Implementation of immuno-PET in experimental studies for newly developed antibody-based anti-GBM therapies may facilitate the selection of patients which can benefit from expensive mAb-based therapy [61,62]. Immuno-PET may be developed to non-invasively characterise the growth factor receptor status (EGFR, PDGFR and VEGFR) of patients with GBM before surgery, which would allow us to predict response and tailor therapy accordingly. Along a similar line, but combining MI with a therapeutic strategy, Hadjipanayis et al. orthotopically inoculated U87ΔEGFRvIII GBM cells into nude mice. EGFRvIII antibody was conjugated to iron oxide nanoparticles and on day 7 post-inoculation particles were delivered by intracerebral convection enhanced delivery. The authors were able to demonstrate not only a survival benefit but also specific uptake of the nanoparticles by GBM cells as noted by a signal drop on T2-weighted images. This approach combines treatment with a sensitive imaging modality as the conjugated iron oxide particles act as a specific MRI contrast agent for glioma cells [63].

Despite immune-PET, which showed to be promising in non-invasive quantification of receptor expression, an association between MR perfusion and the identification of EGFRvIII-positive GBMs has been demonstrated in large group of human GBMs and
showed to have clinical significance in personalised treatment (e.g. patient selection, investigation of therapeutic sensitivity). It has been reported that the EGFRvIII-expressing GBM patients would demonstrate increased relative tumour blood volume (rTBV). One of the factor which contribute to the increased rTBV is EGFRvIII-mediated upregulation of VEGF and enhancement of angiogenesis. Moreover the association between rTBV and EGFRvIII was demonstrated regardless system that has been used and showed weaker correlation between rTBV and VEGF. This imaging-based analysis may be of particular use in postoperative diagnosis of EGFRvIII expression in residual tumour and EGFRvIII-directed therapy choice. The use of MR perfusion as a noninvasive imaging of GBM EGFRvIII status encourages for further exploration (figure 2).

Measuring the downstream effects of EGFR inhibitors on tumour burden may be successfully achieved using optical methods in the pre-clinical setting or by MR protocols in both pre-clinical and clinical settings. Using these MI strategies as described it is becoming clear as to why EGFR inhibitors when delivered as a monotherapy have not translated well in the clinical setting and helps establish a rationale for using combinations treatment approaches. Importantly MI has further helped establish a mechanism for the role played by EGFR amplification in the observed resistance to standard therapy. These findings highlight the prognostic significance of quantifying EGFR status in the tumour. Non-invasive quantification of receptor expression using an antibody tagged with a PET tracer and/or MR perfusion may be of translational relevance.

*mTOR inhibitors*
mTOR is a serine/threonine kinase which belongs to the PI3K-related family. It is a central integrator of intracellular and extracellular signals involving growth, proliferation, nutrient and energy status [64]. mTOR acts both as an upstream regulator and downstream effector of PI3K [65] and is found in two distinct protein complexes (mTORC1 and mTORC2). The PI3K/ mTOR signalling pathway is deregulated in approximately 50% of patients with GBMs. First generation inhibitors, which are analogues of rapamycin, are termed rapalogs and inhibit mTORC1. The two main categories of second generation mTOR inhibitors are mTOR/ PI3K dual inhibitors and mTORC1/ mTORC2 dual inhibitors, which are at various stages of phase I and phase II clinical development for solid and haematological malignancies [66].

Cellular metabolism and energy status is mediated by the mTORC1 complex. Glucose uptake and therefore intracellular AMP/ ATP ratio modulate mTORC1 activity via an AMPK dependent pathway, whereas growth and proliferation are mediated by both mTORC1 and mTORC2. Using small animal PET, Wei et al. have utilized a subcutaneous GBM mouse model to investigate the effect of rapamycin on tumour metabolism and growth. Over a 72 hour period rapamycin decreased 18F-FDG and 18F-FLT uptake. Thus, it has been proposed that 18F-FDG may be used to monitor tumour response in clinical trials involving mTOR inhibitors [67]. Within the context of the ‘Warburg effect’, GBM cells convert most glucose to lactate at the expense of ATP production. This occurs independently of O₂ availability by the enzyme hexokinase 2. The resulting glucose metabolites are utilized for anabolic processes. As well as sensing AMP/ ATP ratios via AMPK, the PI3K/ mTOR pathway also regulates other aspects of glycolysis such as glucose transporters and glycolytic enzymes. To interrogate this feature of GBM tumour cell metabolism, Chaumeil et al.
studied the effect of everolimus, a rapamycin analogue, on the GBM cell line GS-2 in an orthotopic rat model. Through implementation of hyperpolarized 13C-MRS imaging, the authors noticed a significant reduction in lactate-to-pyruvate production by day 7 of treatment where conventional MRI was only able to detect a change in tumour volume at day 14. Thus, the use of hyperpolarized 13C-MRS imaging in clinical MR application was recommended to monitor early response to molecularly targeted treatments (figure 3) [68]. These findings were in line with other reports proposing the use of hyperpolarized 13C-MRS imaging to detect tumour and response to treatment [69]. Over-expression of platelet derived growth factor receptor (PDGFR), a common genetic mutation in GBM, leads to deregulation of the PI3K/ Akt/ mTOR pathway. Pitter et al. investigated the combination of CCI-779 (a lipid soluble analogue of rapamycin) with perifosine (an alkylphospholipid that interferes with Akt recruitment) in genetically engineered PTEN-intact and PTEN-deficient PDGF-driven mouse models of GBM. Activated Akt modulates mTOR activity. Using standard MR sequences, T1 with gadolinium and diffusion weighted sequence analysis, the authors observed reduced tumour enhancement and a corresponding decline in ADC values. Their findings supported the rationale for testing this novel therapeutic combination in the human PDGF-subgroup of GBM and highlighted the importance of inhibiting Akt and mTOR simultaneously [70].

As single-agent mTOR inhibitors continue to show no significant benefit, design of novel molecular targeting combinations is warranted. At present, mTOR inhibitors are undergoing assessment in clinical trials in combination with TMZ with or without radiation in patients with GBM. Undoubtedly, use of metabolic MI strategies (such as hyperpolarized 13C magnetic resonance spectroscopic imaging and PET) in the...
interrogation of mTOR pathway inhibition has highlighted its central role in GBM metabolism.

*Gene therapy*

Use of nucleic acids to deliver therapeutic genes to GBM cells provides an alternative anti-GBM therapeutic approach [71]. To date, the use of recombinant adenoviruses [72] and herpes simplex virus type 1 (HSV-1) vectors [73,74] has shown some promise, although they failed to improve outcome in randomised clinical trials. Gene delivery vectors locally transduce brain tumour cells with therapeutic genes, which may then influence biological properties of cancer cells by inhibiting angiogenesis [75], stimulating the immune response [76] or triggering apoptosis thus targeting several ‘cancer hallmark’ pathways [77]. Nevertheless, efficacy of gene therapy in GBM patients may be compromised due to tumour tissue heterogeneity causing restricted transduction efficiency. Several strategies for non-invasive and quantitative imaging of gene expression *in vivo* have been developed over the past years. Implementation of MI in gene therapy protocols provide better clinical outcome of novel gene therapy approaches through non-invasive assessment of the dynamics of gene regulation, signal transduction and monitoring the induction and regulation of therapeutic genes [71].

Our co-author (A.H. Jacobs), has successfully employed PET to non-invasively identify viable target tissue. Radiotracers implemented (18F-FDG, 11C-MET and 18F-FLT) were well correlated with histological signs of viable and necrotic tissues. Multimodal, image-guided vector application was used to deliver vector particles into viable tumour tissue. Tumour localization was based on MRI whereas viable target
tissue was successfully identified using 18F-FDG-PET. PET was also used to co-register and determine total tissue dose of vector-mediated gene expression (18F-FHBG), and to assess tumour proliferation prior to and following therapy (18F-FLT) [78]. Another radiotracer 18F-Fluoro-3-[hydroxymethyl]-butyl)-guanine (FHBG) employed in this study sensitively and specifically detects PET reporter genes. 18F-FHBG normally cannot cross the BBB, however it may accumulate within GBM tumours where the BBB is compromised [79]. The study revealed that multi-tracer PET scanning is of use in the development of safe and efficient gene therapy protocols. Multimodal imaging approaches have also been successfully employed by Winkeler et al. in experimental GBMs. Tumours were localized using MRI and PET (18F-FDG/-FLT) protocols were implemented to assess viable target tissue for vector application. Doxycycline-dependent gene expression over time was further assessed by 18F-FHBG-PET and BLI [80]. In the clinical setting, gene therapy efficacy may be assessed using MRI. As an example, a group of patients with recurrent GBM treated with the combined suicide (thymidine kinase of HSV-1, HSV-TK) and immuno-modulating (human interleukin-2, IL-2), suicide/ cytokine gene therapy was monitored using MRI. Using serial MRI scans monitoring tumour regression, presence of necrosis and/or inflammation around the injection site of the vector and tumour recurrence was assessed [81]. Nevertheless, in order to provide more detailed information (e.g. to identify targeted tissue and/or assess vector-distribution volume) multi-modality imaging ideally should be implemented in clinical protocols. Voges et al. employed MRI and PET to monitor response of patients with recurrent GBM to stereotactically guided intra-tumoural convection-enhanced delivery of HSV-TK gene–bearing liposomal vector and systemic ganciclovir. MRI was shown to be essential for visualizing therapeutic effects on tumour anatomy, metabolism and
gene expression. Focal therapeutic effects on tumour metabolism, as assessed by 11C-MET-PET, revealed reduced MET activity. However, results obtained from T1-weighted MRI scans indicated increased tissue volume based on Gd uptake indicating increased therapy-induced BBB permeability. The complementary information provided by PET and MRI indicates that multi-modal imaging for the identification of target tissue and response to gene therapy represents an essential technology in this evolving field (Figure 4) [82].

As highlighted above, implementing MI (mainly novel PET approaches) into gene therapy protocols at both pre-clinical and clinical stages is of significant utility in identifying target tissue and enabling application of targeted vectors.

**Therapeutic stem cell approaches**

It is now widely believed that GBM tumours recur due to the presence of a subpopulation of cancer cells having stem cell-like properties (CSC) [83]. The CSC hypothesis assumes that CSCs remain unresponsive due to high resistance to radio- and/or chemotherapy. CSCs are thought to be responsible for tumour re-growth post multimodal therapy [84]. The CSC hypothesis suggests that tumour bulk therapy (targeting only differentiated tumour cells) may fail due to GBM heterogeneity and invasive phenotype, thus allowing tumour progression and relapse. As such, molecular targeting of GBM CSCs subpopulations may lead to long-term treatment response and halt tumour progression [85]. As GBM CSCs represent only a small subpopulation of the entire tumour, their detection in vivo is likely to be challenging. Therefore, experimental protocols which interrogate the potential efficacy of GBM
CSC targeted therapeutics should ideally comprise highly sensitive MI approaches [86].

GBM CSCs may be targeted through inhibition of the nitric oxide (NO) pathway. The critical enzyme in this pathway is nitric oxide synthetase-2 (NOS2), which catalyzes the production of NO from L-arginine. NOS2 is expressed in glioma cells exhibiting high levels in GBM CSCs stimulating tumor growth and reducing tumor response to chemotherapy. The anti-GBM efficacy of BYK191023, a lipophilic NOS2-selective inhibitor has been effectively tracked using BLI in two different patient-derived, luciferase-expressing GBM xenografts. Although BYK191023 did not reduce tumor growth completely, significant tumor growth delay was successfully demonstrated by non-invasive imaging (Figure 5) [87]. Another potential therapeutic target may be inhibition of the NOTCH signalling pathway. NOTCH signalling activates several genes essential for maintenance and renewal of neural stem cells. Increased NOTCH activity has been linked with tumor growth promotion, whereas blockade of the NOTCH pathway inhibits proliferation and/or survival. *In vivo* studies have shown that blockade of NOTCH signalling pathway through inhibition of γ-secretases can restrict GBM CSCs activity *in vitro* and tumor formation *in vivo*. Blockade of the NOTCH signalling pathway has been targeted using the γ-secretase inhibitor (GSI-18) and tumor response monitored using MRI. Multiple T2-weighted scan slices confirmed that GSI-18 treatment blocked formation of tumors and prolonged survival [88]. In the past decade, interest has focused not only on identifying therapies, which target GBM CSCs, but also towards utilizing stem cells as a potential tool for cell-based treatment of GBM. The use of stem cells as vehicles for therapeutic agents is based on their ability to migrate to brain tumors irrespective of the BBB. Thus far, three types of stem cells have been tested as vehicles for various therapeutic agents:
embryonic, neural and mesenchymal. Several MI strategies have been implemented to examine and track stem cells used as delivery vehicles for anti-GBM therapeutics [89]. As proposed by Chien et al., a cellular MR technique can be successfully used to track mesenchymal stem cells in vivo once the cells are labelled with suitable MRI-visible particles prior to transplantation, such as superparamagnetic iron oxide (SPIO) nanoparticles. SPIO nanoparticles used with clinical 1.5-T MRI system revealed that mesenchymal stem cells used as delivery vehicles for therapeutic agents are able to migrate toward GBM in vivo being a promising strategy in anti-GBM treatment [90]. In order to visualise stem cell-based therapeutic interventions in a mouse model of GBM, optical imaging has also been used [91,92]. As demonstrated by Doucette et al., BLI successfully tracked mesenchymal stem cells and detected them in the right frontal lobe where tumour was identified. Moreover, through optical imaging the viability of mesenchymal stem cells may be confirmed [91]. Efficacy of therapeutic stem cells encapsulated in biodegradable, synthetic extracellular matrix (sECM) has been non-invasively monitored using BLI employing a clinically relevant GBM mouse model following tumour resection. BLI was efficiently used to follow both un-resected and resected intracranial tumours and to track stem cells in vivo. Serial monitoring revealed that stem cells encapsulated in sECM survived longer in the GBM resection cavity as compared to non-sECM-encapsulated cells. Furthermore, bioluminescence measurements revealed that TRAIL-secreting sECM-encapsulated stem cells transplanted in the resection cavity significantly delayed tumour re-growth in well established U87 and primary invasive GBM8 mice bearing GBM models. Stem cells encapsulated in sECM effectively extended the drug exposure time to tumour cells resulting in prolonged survival of mice treated with S-TRAIL [92].
Therefore, as indicated, optical imaging has provided evidence that stem cell-mediated GBM therapy offers a continuous, selective and concentrated local delivery of S-TRAIL, showing higher treatment efficiency. Sensitive MI approaches, which provide high resolution images, are important in stem cell research most critically due to stem cell infiltrative phenotype and low number of CSCs present.
Conclusions and future directions

MI is increasingly emerging as a critical tool existing at the interface between pre-clinical and clinical translational research towards identification of a next-generation GBM therapeutic paradigm.

Availability of reliable pre-clinical animal models, which recapitulate human pathology, when combined with suitable, state-of-the art translational, imaging modalities, has to-date facilitated significant advances in the field. Implementation of imaging protocols during pre-clinical development of novel anti-GBM therapeutics is now well recognized as a key strategy for non-invasive and longitudinal assessment of therapeutic effects, reduction of drug development costs, time and animal requirements. Pre-clinical assessment of therapeutic response with molecular imaging also may offer greater potential to facilitate translational development research of novel anti-GBM therapies.

The ubiquity of MI approaches in clinical practice coupled with its versatility (providing information on anatomic, physiologic and metabolic processes) underscores the large utility of this modality. Combination of anatomical (MRI or CT) with metabolic (PET or SPECT) imaging techniques may provide even more detailed information on response to drug treatment compared to single PET/ SPECT or MRI/ CT imaging alone. Implementation of and early imaging biomarker studies may be useful in predicting failure of therapy, planning the development of a drug and allow personalised cancer therapy [93].

As described above, the challenges exist in interpretation of conventional MRI as the primary imaging modality for initial diagnosis and assessment of treatment response
in patients with GBM. Therefore, new types of imaging are currently investigated aiming to improve accuracy of tumour assessment following treatment.

The standard, 2-dimensional measurement of contrast enhancement (the Macdonald Criteria) [94] to evaluate treatment response in GBMs has been used in neuro-oncology for many years. However, these criteria do not reflect functional, morphologic, or metabolic changes that may occur with conventional chemotherapy or targeted chemotherapy. The use of MI modalities may improve the study of complexity and heterogeneity of GBMs not only at the time of initial diagnosis but also during the treatment. Therefore, a rational selection of the imaging modality is important for the assessment of tumour response to treatment and should take into account the mechanism of action of the drug.

New criteria for tumour response assessment (the Response Assessment in Neuro-Oncology (RANO) Criteria), that have recently been published incorporate non-enhancing tumour progression in evaluation of treatment response in GBMs [95]. Nevertheless, detection of non-enhancing tumour and pseudoprogression are still the two major challenges in neuro-oncologic imaging.

Non-invasive MI may improve personalised medicine by providing detailed molecular information relating to drug mechanism of action. Current efforts to define a new treatment paradigm for GBM are primarily focused on targeted therapies. Nevertheless, it is becoming increasingly apparent that targeting a single ‘cancer hallmark’ in GBM may be insufficient to affect a sustained response. Thus, a combined ‘anti-cancer hallmark’ approach is warranted, for example concomitantly targeting angiogenesis and invasion pathways or angiogenesis and metabolic pathways. Moreover, the contribution of GBM CSCs to drug resistance and thus

32
evolution of GBM CSC targeted therapies will no doubt contribute to the emerging next generation GBM therapeutic paradigm. Evolution of increasingly sensitive MI approaches, in particular to provide information on extent of tumour and CSC invasion into normal tissue, will likely play a key role in this effort. In short, considering GBM heterogeneity and the importance of personalized therapy within the context of patient care, state-of-the-art MI approaches which inherently facilitate GBM targeted therapeutic follow up should ideally be implemented in clinical development.

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34


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Figure 1 Imaging modalities employed in the pre-clinical and clinical development of novel GBM treatment strategies
Images used show examples of: photodynamic navigation – OPMI PENTERO 900 Microscope and fluorescent screenshot reproduced with permission from Carl Zeiss Ltd, mouse computed tomography (CT) [96], mouse single-photon emission computed tomography (SPECT) [97], human single-photon emission computed tomography (SPECT) [98], human positron emission tomography (PET) [99], mouse positron emission tomography (PET) [100]. Advantages and limitations of each imaging modality are highlighted.

Figure 2 DCE-MRI used to assess the potential efficacy of various GBM therapies in individual patients

The image shows a distinct decrease in volume transfer constant ($K^{\text{trans}}$) and increase in microvessel cerebral blood volume (CBV). It was associated with high survival rates (A), compared to low survival rates in the patient with little change in
$K_{\text{trans}}$ and microvessel CBV (B). “Vascular normalization index” values obtained from DCE-MRI provide indication of progression-free survival (PFS) (C) and overall survival (OS) (D) in patients treated once with the angiogenesis inhibitor cediranib. Image adapted from Sorensen et al., 2009 [47]

Figure 3 PET used to assess the potential efficacy of mTOR inhibitors

The figure shows 18F-fluorodeoxyglucose- (18F-FDG) and 3′-18F-fluoro-3′-deoxythymidine- (18F-FLT) positron emission tomography (PET)/ computed tomography (CT) scans of U87 and LN-229 xenografts before and after one dosage of rapamycin (3 mg/kg). Axial 18F-FDG PET/ CT scans of representative tumour are shown in (A) and (B). Changes in tumour 18F-FDG uptake are summarized in (C) and (D). 18F-FLT PET scans of representative tumour are shown in (E) and (F).
Changes in tumour 18F-FLT uptake are summarized in (G) and (H). Figure adopted from Wei et al., 2008 [67]

Figure 4 Discrepancy between post-therapeutic image changes on MRI and 11C-MET PET scans

Images in (A) and (B) represent the treatment planning at two distinct levels of the tumour. T1-weighted magnetic resonance imaging (MRI) and 11C-methionine positron emission tomography (11C-MET PET) scans were fused with intraoperative stereotactic computed tomography images. The same procedure was performed with follow-up data taken 8 weeks after intra-tumoural infusion of LIPO-HSV-1-tk (C, D). The position of the two infusion catheters is indicated (red cross) and baseline extension of the tumour is outlined (blue dotted line: summarized information from T1- and T2-weighted series; yellow dotted line: 11C-MET PET scans). Eight weeks after intra-tumoural infusion of LIPO-HSV-1-tk, 11C-MET PET scans displayed a 50% reduction of the tumour volume if compared with baseline (Patient 3). In contrast,
the volume of gadolinium with diethylenetriaminepentaacetic acid (Gd-DTPA) uptake was increased by 300 % most likely due to gene therapy-induced alteration of blood–brain barrier (BBB). Figure adopted from Voges et al, 2003 [82]

Figure 5 The tumour initiation and maintenance potential of GSCs is reduced by NOS2 inhibition

Luciferase-expressing GBM stem cells (GSC)-derived intracranial xenografts were treated with intraperitoneal vehicle or BYK191023 (n = 17 per group) after engraftment and tracked by bioluminescence imaging (BLI). Real-time images from median three animals on day 9 are shown (right). Mean tumour bioluminescence signal for each group over time is shown (left). *p < 0.05. Error bars represent the mean ± SEM of the indicated number of animals. Figure adopted from Eyler et al., 2011 [87]
Table legend

Table 1. Application of preclinical and clinical molecular imaging strategies in brain tumours

<table>
<thead>
<tr>
<th>MI</th>
<th>Main applications</th>
<th>Ref</th>
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<tbody>
<tr>
<td>MRI</td>
<td></td>
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<tr>
<td>T1-weighted</td>
<td>• Anatomical investigations (e.g. tumour location, volume)</td>
<td>[5]</td>
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<tr>
<td></td>
<td>• Pathological investigations (e.g. assessment of blood brain barrier function) when combined with contrast enhancement</td>
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<tr>
<td>T2-weighted</td>
<td>• Pathological investigations (e.g. visualisation of oedema, accumulation of cerebral spinal fluid)</td>
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<td>1H-MRS</td>
<td>• Detection of tumour cell infiltration</td>
<td>[9]</td>
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<tr>
<td>DSC</td>
<td>• Measures rCBV</td>
<td>[45]</td>
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<td></td>
<td>• Monitoring of tumour response to anti-angiogenic treatment</td>
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<tr>
<td>DCE</td>
<td>• Measures Ktrans</td>
<td>[46]</td>
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<td></td>
<td>• Monitoring of tumour response to anti-angiogenic treatment</td>
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<tr>
<td>ASL</td>
<td>• Measures CBF</td>
<td>[97]</td>
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<tr>
<td></td>
<td>• Monitoring of tumour response to anti-angiogenic treatment</td>
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<tr>
<td>DW</td>
<td>• Identification of tumour grade</td>
<td>[13]</td>
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<tr>
<td></td>
<td>• Assessment of tumour cell infiltration</td>
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<tr>
<td>DT</td>
<td>• Detection of tumour cell invasion</td>
<td>[98]</td>
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<td></td>
<td>• Pre-operative planning of tumour removal</td>
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<tr>
<td>BOLD-fMRI</td>
<td>• Localisation of eloquent brain regions</td>
<td>[15]</td>
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<td></td>
<td>• Pre-operative planning of tumour removal</td>
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<td>PET</td>
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<td>18F-FDG</td>
<td>• Glucose metabolism</td>
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<td></td>
<td>• Tumour visualization</td>
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<tr>
<td>18F-FLT</td>
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<td></td>
<td>• Assessment of therapy response</td>
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<tr>
<td>18F-MET</td>
<td>• Amino acid transport/ protein synthesis</td>
<td>[18]</td>
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<td></td>
<td>• Assessment of tumour size</td>
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<tr>
<td></td>
<td>• Detection of post-therapeutic changes (e.g. radiation)</td>
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</tbody>
</table>
| **18F-FET** | • Amino acid transport/ protein synthesis  
• Detection of recurrent tumour and biopsy site  
• Assessment of tumour location and extent | [19] |
| **11C-CHO** | • Tumour detection and grading | [17] |
| **18F-FMISO** | • Assessment of tumour hypoxia | [21] |
| **18F-Fluciclatide** | • Assessment of α,β₃ and α,β₅ integrins expression | [22] |
| **18F- Galacto-RGD** | • Assessment of α,β₅ integrin expression | [99] |
| **201Tl** | • Tumour detection | [24] |
| **99mTc-MIBI** | • Assessment of tumour progression and grading | [25] |
| **123I-IMT** | • Differentiation between tumour recurrence, radiation necrosis and oedema | [26] |
| **BLI** | • Tumour localisation  
• Tumour progression  
• Treatment response | [28] |
| **Fluorescence** | • Tumour localisation  
• Tumour progression  
• Treatment response | [30] |
| **Photodynamic navigation** | • Visual recognition of tumour tissue during surgical resection | [31] |

(Abbreivations: 1H-MRS - proton magnetic resonance spectroscopy, MRI - magnetic resonance imaging, DSC - dynamic susceptibility contrast, ASL - arterial spin labelling, DW - diffusion-weighted, DT - diffusion tensor, BOLD-fMRI - blood oxygenation level dependent functional magnetic resonance imaging, PET – positron emission tomography, 18F-FDG - 18F-fluorodeoxyglucose, 18F-FLT - 3′-18F-fluoro-3′deoxythymidine, 11C-MET - 11C-methionine, 18F-FET - O-(2-18F-fluoroethyl)-L-tyrosine, 11C-CHO - 11C-choline, 18F-FMISO - 18F-fluoromisonidazole, 18F-
Galacto-RGD – 18F-galacto-arginine-glycine-aspartic acid, SPECT - single photon emission computed tomography, 201Tl - thallium-201, 123IMT - iodine-123-alpha-methyl-tyrosine, BLI – bioluminescence imaging)

<table>
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<td>^18F-Fluciclatide-PET</td>
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<td>VEGF</td>
<td>DCE-MRI, DW-MRI</td>
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<td>[58]</td>
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<td>[60]</td>
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<td>mTOR, FRAP1</td>
<td>^18F FDG-PET, ^18F FLT-PET</td>
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<td></td>
<td>Everolimus</td>
<td>mTOR</td>
<td>^13C MRI</td>
<td>[67]</td>
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<td>NOS2</td>
<td>BLI</td>
<td>[86]</td>
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</table>
Table 2. Examples of molecular imaging approaches used in development of anti-GBM therapies

(Abbreviations: VEGF – vascular endothelial growth factor; R – receptor; PDGF – platelet derived growth factor; RTK – receptor tyrosine kinase; PKCβ – protein kinase C beta; PI3-K – phosphatidylinositol 3-kinase; EGF – epidermal growth factor; NOS2 – nitric oxide synthase 2; mTOR – mammalian target of rapamycin; FRAP-1 – FK506 binding protein 12-rapamycin associated protein 1; MRI – magnetic resonance imaging; DW – diffusion weighted; DCE – dynamic contrast enhanced; DSC – dynamic susceptibility contrast; PET – positron emission tomography; FLT – fluoro-3’-deoxy-L-thymidine; F – fluorine; FDG – fluorine-2-deoxy-D-glucose; BLI – bioluminescence imaging)