BRAIN
REVIEW ARTICLE

Assessment and prediction of glioblastoma therapy response: challenges and opportunities

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Glioblastoma is the most aggressive type of primary adult brain tumour. The median survival of patients with glioblastoma remains approximately 15 months, and the 5-year survival rate is <10%. Current treatment options are limited, and the standard of care has remained relatively constant since 2011. Over the last decade, a range of different treatment regimens have been investigated with very limited success. Tumour recurrence is almost inevitable with the current treatment strategies, as glioblastoma tumours are highly heterogeneous and invasive. Additionally, another challenging issue facing patients with glioblastoma is how to distinguish between tumour progression and treatment effects, especially when relying on routine diagnostic imaging techniques in the clinic. The specificity of routine imaging for identifying tumour progression early or in a timely manner is poor due to the appearance similarity of post-treatment effects. Here, we concisely describe the current status and challenges in the assessment and early prediction of therapy response and the early detection of tumour progression or recurrence. We also summarize and discuss studies of advanced approaches such as quantitative imaging, liquid biomarker discovery and machine intelligence that hold exceptional potential to aid in the therapy monitoring of this malignancy and early prediction of therapy response, which may decisively transform the conventional detection methods in the era of precision medicine.

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Introduction

Glioblastoma is a biologically aggressive adult brain tumour and presents unique treatment challenges. Progress in understanding genomic characteristics and pathophysiological characteristics has generated notable insights into the next generation of disease management, including diagnosis, therapeutics, treatment response assessment and early prediction. Genomic analysis studies have revealed a series of the most frequently altered genes in glioblastomas, including *TP53*, *EGFR*, *CDKN2A*, *PTEN*, *PDGFRA*, *NF1*, *PIK3R1*, *PIK3CA*, *RB1*, *IDH1/2* and *TERT*.[1–4] Among them, *EGFR* amplification was found in >40% of all glioblastoma cases;[3,5,6] *IDH* mutations[7,8] have been used in the 2016 World Health Organization (WHO) classification, categorizing glioblastomas into IDH-wild-type (IDHwt), IDH-mutant (IDHmut) and not otherwise specified entities.[9] In the recently updated 2021 WHO classification, glioblastoma is defined as grade IV glioma with IDHwt ('glioblastoma' is no longer used in paediatric-type neoplasms). Three genetic alterations, including *TERT* promoter mutations, *EGFR* gene amplification and chromosome 7/10 status, have been incorporated into the criteria for glioblastoma diagnosis (Fig. 1).[10,11]

Glioblastoma is the most common primary brain malignancy in adults, accounting for approximately 48.6% of malignant brain and other CNS tumours.[12–17] The overall incidence of glioblastoma is approximately 3–5 cases per 100 000 adults per year in the USA.[18–21] The incidence of glioblastoma in males is slightly higher than that in females, overall trending up from the age of 45, and is much higher in the age group of 75–79 years than in other age groups.[19,20] The diagnosis of glioblastoma uses imaging techniques, including MRI, CT and PET, and histological confirmation and molecular characterization using tumour biopsy. The current standard of care treatment for newly diagnosed glioblastoma is multimodal, including surgical resection, radiation and concomitant or adjuvant chemotherapy (usually temozolomide, an anticancer alkylating agent).[22–27] With these first-line therapies, almost all patients suffer from post-treatment tumour progression or recurrence. Treatment options for progressive or recurrent glioblastoma may include reoperation, reirradiation, chemotherapy, tumour treating fields (small non-invasive portable devices damaging DNA in rapidly dividing cells)[28] and antiangiogenic therapy (e.g. VEGF inhibitors). With current therapy options, the median survival of glioblastoma patients is approximately 14.6 months,[14,29–35] with fewer than 10% of patients surviving 5 years and only 2–3% of patients surviving 10 years beyond diagnosis, representing a very dismal treatment outcome.[20,35,36]

Glioblastoma exhibits distinct pathophysiological characteristics. Its tumour grows faster than other types of cancer, e.g. the doubling time of glioblastoma cells is about four times faster than that of breast cancer cells.[37] Therefore, without treatment, the survival time of glioblastoma patients may only be a few months. According to transcriptional profiling, Wang *et al.*[38] divided glioblastomas with IDHwt into three subtypes, i.e. classic, proneuronal and mesenchymal, with the proneuronal subtype showing better survival than other subtypes and the mesenchymal subtype showing the worst survival. Hypermethylation of the *MGMT* (O^6-methylguanine-DNA methyltransferase) gene promoter region, which inactivates MGMT protein, has been associated with better prognosis, especially for temozolomide-treated patients.[39,40] Glioblastoma tumour cells of origin may control their generation and progression. An insightful review by Laug *et al.*[41] discussed gliomagenesis through the lens of development and proposed that the possible cell of origin for gliomas may be cells that retain proliferative and migratory capabilities, such as intermediate astrocyte precursors, oligodendrocyte progenitor cells, and glioma stem cells with glial precursor-like and neural stem cell-like properties. Current studies on astrocyte precursors and neural stem cells with mutations show their possibility as the origin of glioblastoma.[41–43] However, the complexity of cell subpopulations remains to be further investigated to fully understand how different lineages arrive at a stage of malignancy (Fig. 1).

From the research on glioblastoma by us and others, the challenges for glioblastoma therapy are summarized as follows: intratumoural heterogeneity, genetic defects and genotype–phenotype networks, malignant reprogramming evolution of glioblastoma stem cells, low or partial response to immunotherapy, crosstalk between oncogenic and immune response regulatory signalling pathways, the diffusely infiltrative nature of glioblastoma, the blood–brain barriers (BBBs) for delivering therapies (poor delivery of available drugs to the tumours in the brain), the inefficiency of current treatment options and inevitable tumour recurrence.[10,20,44–51] As mentioned before, after initial first-line treatment, glioblastoma patients often succumb to post-treatment progression or recurrence. The invasive nature of glioblastoma tumours means they easily infiltrate normal brain regions, which impedes surgical recession of the entire tumour and results in a high rate of post-treatment progression and recurrence for this cancer. More effective therapeutics are desirable to achieve better patient outcomes. In addition to the difficulties in therapy, there are also challenges regarding the monitoring and early prediction of treatment response for glioblastoma. The main method used in clinical settings to assess or predict treatment response is imaging, which largely helps clinicians monitor patient responses to therapy. However, there are also cases in which it is difficult to tell whether a radiographically suspicious lesion is a non-tumoural treatment effect (e.g. radiation injury) or a tumour lesion from conventional imaging scans, which may affect clinical decision making.[52] Therefore, more sensitive and reliable approaches for the early prediction of treatment response and the early detection of tumour progression are also dire, which will help guide treatment decisions at an earlier time point. Tools that are capable of forecasting response after treatment initiation hold the premise of tailored treatment enabling changes in therapy to prevent treatment ineffectiveness or adverse events.[53]

Current status and challenges of treatment response assessment

Currently, the treatment response of glioblastoma is assessed based on criteria including the Macdonald criteria[54] and Response Assessment in Neuro-Oncology (RANO), mainly depending on imaging techniques (e.g. MRI, CT, PET).[55] The Macdonald criteria combine contrast-enhancing tumour sizes with other clinical metrics to determine treatment response and tumour progression and categorize treatment response status into complete response, partial response, stable disease and progressive disease.[54] However, the fundamental basis of the Macdonald criteria is enhancing lesions, which sometimes fail to discern post-treatment effects (e.g. pseudoprogression, pseudoresponse, radiation necrosis) and true progressive diseases (Fig. 2).

Post-treatment radiation effects, such as pseudoprogression, radiation necrosis, oedema and pseudoresponse, can develop in up to half of cases following first-line therapy, underscoring the clinical impact of this common diagnostic dilemma.[56,57] Common forms of post-treatment effects have been described in detail in the literature, such as the RANO criteria and some review articles.[55,58,59] Pseudoprogression, which usually occurs within 3–6

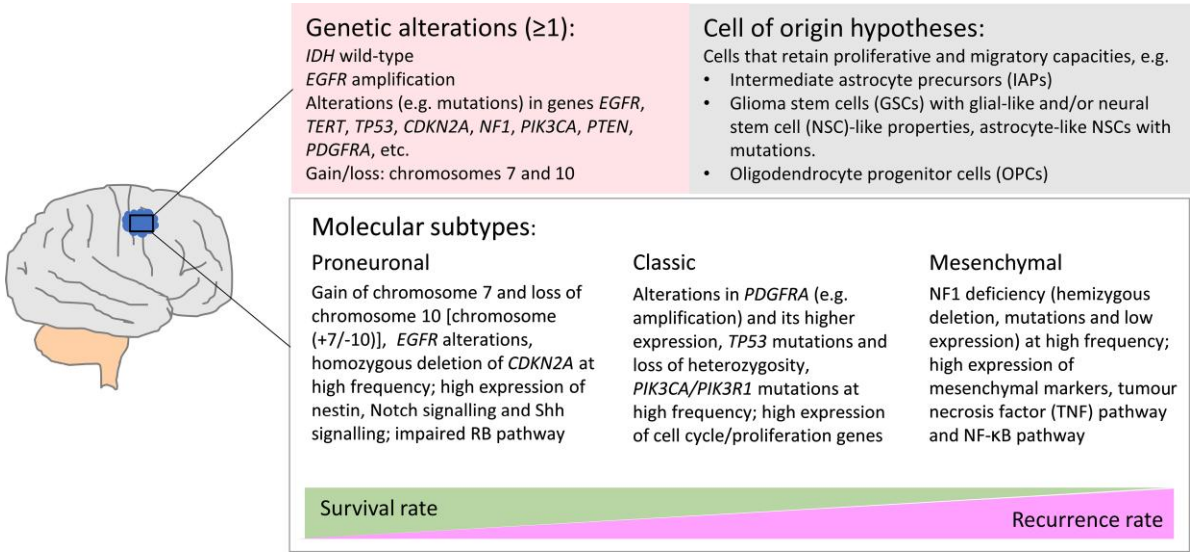

Figure 1 Molecular characteristics of glioblastoma. The 2021 WHO Classification for CNS Tumour has defined glioblastoma, IDHwt, in the setting of an IDHwt diffuse and astrocytic glioma in adults if there is microvascular proliferation, necrosis, *TERT* promoter mutation or *EGFR* gene amplification or +7/−10 chromosome copy number changes. Gene mutations are commonly seen in genes such as *TP53*, *EGFR*, *CDNK2A*, *PIK3CA* and *PDGFRA*. The possible cell of origin for gliomas is discussed by Laug *et al.*[41] from the developmental aspect: intermediate astrocyte precursors, glioma stem cells with glial-like properties and/or neural stem cell-like properties and oligodendrocyte progenitor cells in the context of tumorigenesis. On the basis of transcriptomic profiling, the molecular subtypes of glioblastomas are defined in to three subtypes: classic, proneuronal and mesenchymal. This is an updated classification from the previous four subtypes, in which the neural subtype is found to be non-tumour specific with low gene abnormalities and omitted in the updated subtypes. The common molecular features of each subtype are listed in the figure.

months of chemoradiation,[60] is incompletely understood but characterized by a mix of 'quiescent' tumour and non-hyalinized necrosis with proinflammatory mediators and cytokines yielding increased vascular permeability.[61,62] Radiation necrosis may occur months to years after treatment, with damaged tumour cells and thickened, hyalinized vessel walls, and can progress with no clinical outcome benefit. Oedema and contrast enhancement mimic tumour progression[63] and stabilize or resolve without further intervention with potential improvement in outcome.[64,65] Tumour progressive diseases signify treatment failure, while post-treatment effects, radiation necrosis and pseudoprogression indicate a positive response to treatment.[62,66–71] Post-treatment effects, such as pseudoprogression and radiation necrosis, pose significant challenges for clinical diagnosis and response assessment, as it could be difficult to distinguish lesions of post-treatment effects from lesions of progressed tumours by conventional imaging modalities such as contrast-enhanced MRI.[61,72]

RANO criteria included the evaluation of non-enhancing lesions from fluid-attenuated inversion recovery or T_2-weighted images, new lesions and issues associated with post-treatment effects and provided detailed recommendations on therapy response assessment for high-grade gliomas.[55] The recent modified RANO criteria further included modifications such as suggestions for volumetric response evaluation, use of the postradiation time point as a response assessment baseline for newly diagnosed glioblastoma, and 'treatment-agnostic' response assessment rubrics for identifying post-treatment effects.[54] Haider *et al.*,[73] in a recent review, proposed their idea from the clinical aspect of how to overcome the challenge in differentiating tumour progression from post-treatment effects, which in many cases confuses clinical status assessments and decisions for next-step treatments. The recommended methods include: (i) the generation of a rigorous definition of recurrent versus residual tumour in diagnostic pathology; (ii) the investigation of pseudoprogression in prospective cohorts with standardized pathological and radiographic correlates; (iii) the molecular analysis of recurrent tumour specimens; and (iv) the inclusion of assessment of tissue with post-treatment changes in clinical trials.

The criteria and basis of the guidelines for therapy response assessment are continuously defined in more detail for not only the standard of care but also the optimization of clinical trials, including trials for immunotherapies.[74] Since, at the moment, one single imaging modality has insufficient specificity to conclusively differentiate tumour progression from post-treatment effects, advanced quantitative imaging modalities for the assessment of cellularity, blood flow or volume and biochemistry (e.g. metabolites) have also been studied and applied in situations such as the differentiation of tumour progression and post-treatment changes.[75] Despite the wide application of these robust and standardized treatment assessment methods and guidelines, the insufficient part of current criteria based on imaging biomarkers exists, such as neurological assessment, response assessment for novel therapies, intratumour heterogeneity (i.e. inhomogeneous response), low tumour specificity, reduction of imaging variability and mostly the inability to predict or detect early therapy responses and tumour progression.

Potential solutions

On the basis of recent notable basic, translational and clinical studies, we compendiously review and propose potential and possible minimally invasive solutions from the facets of imaging modalities, liquid biomarkers and artificial intelligence for the early prediction of tumour progression or early assessment of therapy response: namely, multidisciplinary approaches to empower the establishment of next-generation therapy response assessment tools.

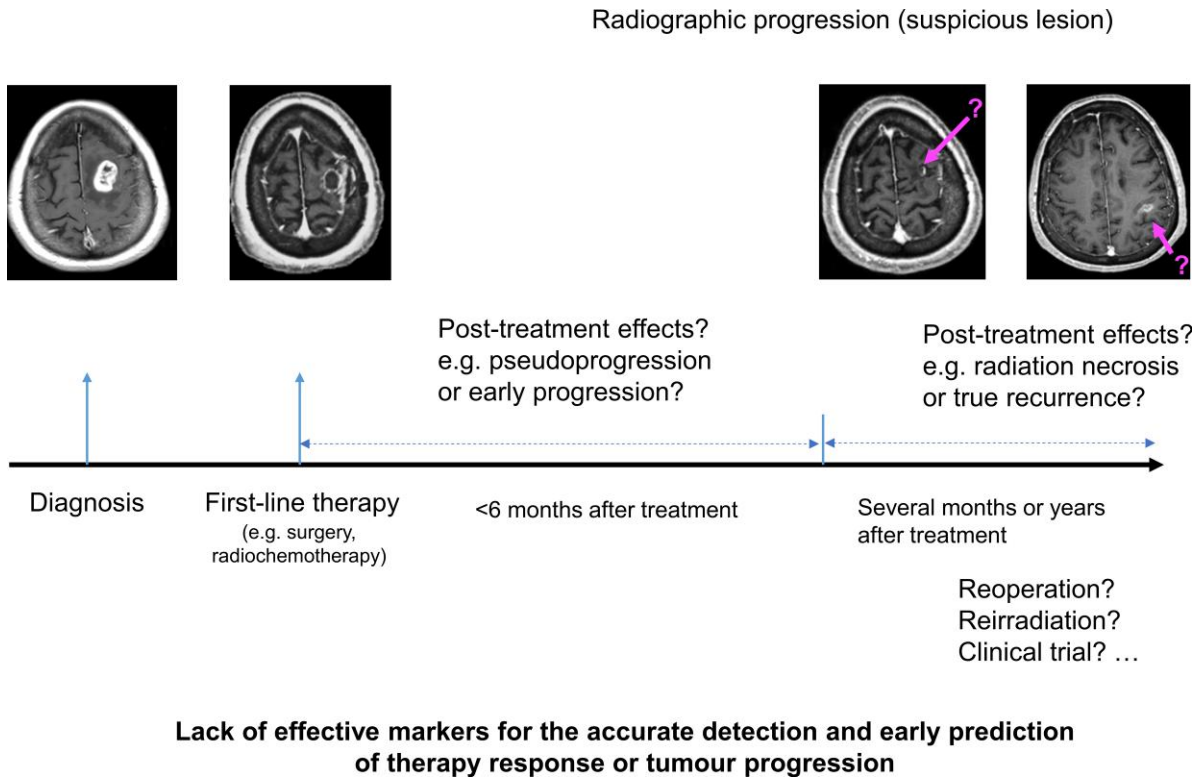

Figure 2 Challenges of image monitoring in the clinical management of glioblastoma. Clinicians face difficulties for patients showing radiographic progression, a suspicious gadolinium-enhancing lesion found on contrast-enhanced imaging, and not knowing exactly whether it is a progressed tumour lesion or treatment-related changes during MRI surveillance after standard first-line therapy. For some patients who undergo clinical trials, such as immune checkpoint inhibitor therapy, treatment-related inflammation effects may appear during MRI surveillance, which look similar to tumour lesions. In these cases, various imaging methods and/or further invasive brain biopsies may be needed to achieve an accurate diagnosis of the suspicious lesion; however, prolonged diagnostic time may delay clinical decision making.

Imaging modalities

While contrast-enhanced MRI represents the clinical standard for response assessment and detection of tumour lesions, it fails to distinguish progressive tumours from treatment-related changes such as the post-treatment radiation effects as described before. Tumour lesions and post-treatment effects similarly present as contrast-enhancing masses but represent distinct histoprognostic entities. Unlike contrast-enhanced MRI, physiological and metabolic imaging methods interrogate specific biological attributes of cancer and are more sensitive to glioblastoma progression and treatment response. At the time a suspicious enhancing lesion appears, perfusion imaging with arterial spin labelling or dynamic susceptibility contrast MRI (DSC-MRI),[76] diffusion-weighted imaging (DWI), magnetic resonance spectroscopy (MRS), chemical exchange saturation transfer (CEST) MRI and amino acid PET scans are the most widely available tools for lesion determination that have been validated with imaging-guided tissue histopathology. Given that imaging is the mainstay in clinics used in brain lesion diagnosis, a large body of literature has discussed and reported studies regarding suspicious enhancing lesion determination. For example, from a meta-analysis, van Dijken *et al.*[77] found that MRS and perfusion MRI showed better accuracy than routine MRI. Huang *et al.*[52] reviewed pitfalls of neuroimaging in glioblastoma management and discussed the pros and cons of each commonly used imaging modality in distinguishing non-tumoural enhancing lesions, especially treatment-related changes and true tumour lesions. Here, we highlight studies on advanced or multiparametric imaging and parameters derived from such imaging modalities in the aid of lesion differentiation and treatment response assessment or prediction; the studies that also combined advanced imaging with machine learning algorithms are discussed in a later section.

DSC-MRI is a method that relates dynamic T_2-weighted changes to gadolinium-based contrast agent concentration and pharmacokinetics.[78] DSC-MRI measures of relative cerebral blood volume (rCBV) enable the differentiation of glioma grades, tumour types and identification of tumour components in non-enhancing glioma,[79–83] the differentiation of post-treatment effects and tumour progression at the time of radiographic progression (e.g. high rCBV for tumours and low CBV for radiation necrosis, relative to normal appearing white matter)[56,69,84–89] and the prediction of tumoural response and patient survival after targeted therapy.[90–94] DSC-MRI measures of peak height and percentage of signal intensity recovery have also been studied for their diagnostic value in differentiating tumours and other treatment-related changes. A retrospective study analysed the peak height, CBV and percentage of signal intensity recovery of 57 patients with glioblastoma and found that relative peak height and rCBV values were higher in progressive or recurrent tumour lesions, whereas percentage of signal intensity recovery values were lower in tumour lesions than in radiation necrosis.[85] Another retrospective study analysed 135 patients with newly diagnosed glioblastoma to predict early treatment response at the first and second follow-up imaging after initial treatment and showed that the skewness (distribution) and kurtosis (outliers) changes in normalized CBV may have predictive value to distinguish early tumour progression from

pseudoprogression.[95] A prospective study by Hu *et al.*[56] analysed 13 subjects with high-grade glioma aiming to define a threshold for rCBV values to robustly distinguish post-treatment radiation effects and tumours. An rCBV of 0.71 was proposed as an optimal threshold with tumours having greater values, achieving an accuracy of ~95.9%.[56] In their later study that analysed 25 patients with recurrent glioblastoma, an rCBV of 1.0 was found to be an optimal threshold to differentiate true tumours from radiation necrosis, and the percentage of fractional tumour burden was shown to be correlated with patient overall survival.[96] Due to the regional variations in normalized rCBV values (normalized to a reference brain region defined by the user), the Hu group[88] recently studied the performance of standardized rCBV (without a need to define a reference region) in differentiating post-treatment effects and true tumour lesions by analysis of imaging metrics together with image-localized stereotactic biopsies in a high-grade glioma cohort of 38 patients. The results showed a similar performance of standardization (may be slightly superior) and normalization of rCBV and therefore may help optimize workflow and reduce variations.[88]

DWI is sensitive to the rate and direction of water movement in tissue. The rate and direction of diffusion in cellular tissues can be represented by the apparent diffusion coefficient (ADC). DWI methods have been developed to map the ADC, a parameter that has been shown to correlate inversely with tissue cellularity over a range of tumour types[97,98] and detect treatment-related changes in cellularity before changes in tumour volume are detectable.[99,100] ADC may also predict radiographic response and long-term patient survival within several weeks after first-line therapy, distinguish post-treatment effects from tumour progression[101] and identify tumour components in non-enhancing lesions.[102,103] An increase in ADC after radiochemotherapy may predict a favourable response, whereas a decrease in ADC may indicate a progressive risk. Recently, a retrospective study by Song *et al.*[104] reported a multiparametric MRI approach to identify the early response of recurrent glioblastoma treated with immune checkpoint inhibitors in 19 patients. They calculated the mean values of relative ADC and rCBV from a volume of interest of the enhancing tumour and determined stable/improved versus progressive disease at the 6-month follow-up based on the modified RANO criteria. Their results indicated that interval changes in relative ADC may have an indicative value in assessing treatment response versus tumour progression following immune checkpoint inhibitor treatment in this small series.[104]

CEST imaging is a pH-weighted imaging technique that relies on the exchange between mobile protons in amide, amine and hydroxyl groups and bulk water.[105,106] CEST makes MRI sensitive to the concentrations of endogenous metabolites and their environments.[107] Amide CEST imaging, also termed amide proton transfer (APT) imaging, has shown robust performance in the assessment of ischaemia, brain tumours and breast and prostate cancers.[106] Recently, APT-MRI metrics such as the magnetization transfer ratio corresponding to the nuclear Overhauser effect (NOE) and amide protons were studied for their predictive value of therapy response in glioblastoma patients. Mehrabian *et al.*[108] reported that changes in MTR_{NOE} and MTR_{amide} 2 weeks after therapy showed significant separation performance of tumour progressors and non-progressors and may also have indicative value even before treatment after analysing 19 patients with newly diagnosed glioblastoma at various time points. Another study by Regnery *et al.*[109] analysed 20 previously untreated glioblastoma patients based on CEST imaging data before standard treatment predicting early progression, with the Lorentzian difference (LD) of NOE (NOE-LD) and downfield-NOE-suppressed (dns) APT (dns-APT) showing significant predictive value. Park *et al.*[110] performed a retrospective study on 54 patients with recurrent glioblastoma who received bevacizumab (BEV) therapy and showed that early reduction of mean APT-MRI signal intensity at 4–6 weeks may indicate a better response for 12 months or longer progression free survival.

In addition to the aforementioned MRI methods, MRS, which measures tissue metabolite concentrations, also has diagnostic value in glioblastoma management.[111] Lower lipid signals in MRS have been studied in the characterization of tumour progression.[112,113] Ratios of intralesional metabolites, such as choline/creatine (Cho/Cr) and choline/N-acetyl-aspartate (Cho/NAA), in MRS have been studied in patients to differentiate radiation changes and tumour progression, with high ratios being indicative of tumour cells.[112] The combination of DWI, DSC-MRI and MRS shows much higher accuracy in distinguishing true tumour progression in glioma.[112] Higher total Cho/total NAA ratios together with low ADCmean values were shown to correlate with tumour progression/recurrence from MRS and DWI data of glioblastoma patients.[114]

Tumours are often proliferative and have higher metabolic activity, such as high glucose uptake and fast amino acid transport. By using tracer agents such as radiolabelled glucose analogues or amino acids, PET scans are also used to detect tumours. For instance, PET scans (e.g. ^{11}C- and ^{18}F- PETs) detecting the tumour-to-normal (T/N) ratio of suspicious lesions combined with DSC-MRI are reported to give higher accuracy to assess enhancing lesions than a single imaging modality.[58] Amino acid PET, using agents such as O-(2-[^{18}F] fluoroethyl)-L-tyrosine (FET) PET (FET-PET), can detect the increase in tumour metabolism and shows higher specificity in the assessment of treatment response than conventional MRI. A prospective study on 21 patients with glioblastoma who received an antiangiogenic treatment regimen with BEV and lomustine at first progression to determine the value of FET-PET obtained 8–10 weeks post-treatment in the prediction of treatment response using overall survival > 9 months as a reference showed that FET metabolic tumour volumes below 5 ml survived significantly longer, while RANO criteria did not provide indicative values.[115] Bolcaen *et al.*[116] studied ^{18}F fluoromethylcholine (^{18}F-FCho) PET and contrast-enhanced MRI in 11 glioblastoma patients following chemoradiation therapy to determine which modality was able to predict responders and non-responders early at the 6-month follow-up. They found that ^{18}F-FCho PET could predict response using metabolic tumour volume (MTV) × standardized uptake mean value 4 weeks after treatment, and a decrease in enhancing tumour volume on T_1-weighted MRI (gadolinium TV) between Weeks 2 and 6 of at least 31% could predict response with 100% sensitivity and specificity in this cohort.[116]

The studies described here are summarized in Table 1. In summary, these studies highlight that biologically sensitive image parameters, individually or in combination, provide a more robust way to predict and evaluate tumour progression. Additionally, evaluating these image-based biomarkers dynamically and spatially can lead to further enhancements in accuracy. With a growing number of immunotherapy-based clinical trials, there have also been efforts to establish image-based biomarkers and analysis methods that can reliably detect treatment response and differentiate pseudoprogression (e.g. therapy-induced inflammation).[52,74,117] To facilitate the clinical application of physiological and metabolic imaging methods, federal sponsored research activities, such as the US National Cancer Institute's Quantitative Imaging Network and academic–industrial partnerships, are specifically focused on funding and accelerating the validation and benchmarking of acquisition and analysis solutions for cancer imaging biomarkers. Simultaneously, the exploration and development of alternative detection approaches are also desirable to

improve early prediction or detection of treatment response for brain tumours, especially glioblastoma.

Liquid biomarkers

Liquid biopsies have become promising media for the development of minimally invasive diagnostic methods.[118] Human blood, in particular, is an attractive material for the identification of disease biomarkers because of its critical role in circulation, immune response, metabolism, communication with cells and formation of extracellular matrices in various tissues and organs in the human body, as well as the simplicity and less invasive nature of sample collection.[119–128] Blood-based biomarkers are considered reflections of systemic collective cellular behaviours that underlie cancer processes such as cancer initiation, progression, metastasis and/or response to treatment. One unique feature of the human brain is its special protective structure, the BBB. The BBB is composed of tightly packed cells, and substances passing through the barrier are highly selective. However, the BBBs of brain tumour patients are disrupted due to the loss of tight junction proteins, e.g. a deficiency or mutation in claudin-1,[129–132] among other reasons. Therefore, human blood may act as a 'sentinel' that effectively reflects physiological and pathological changes in the brain.[133]

Tumour-specific materials falling into the bloodstream across the BBB in glioblastoma patients are supported by the following evidence. First, glioblastoma can metastasize to distant organs, i.e. rare extracranial metastasis[134–137] and lung metastasis.[138,139] Second, studies exploring the detection of glioblastoma circulating tumour cells (CTCs) showed that glial fibrillary acidic protein positive CTCs were detected in 29 of 141 (approximately 20.6%) glioblastoma patient blood samples,[140] and STEAM (SOX2, Tubulin beta-3, EGFR, A2B5 and c-MET)-positive CTCs were detected in 13 of 33 (approximately 39.4%) glioblastoma blood specimens with approximately 80% accuracy via a microfluidic device (CTC-iChip).[138,141] These studies supported the concept that secreted tumourous stimuli may trigger a detectable response in circulating cells,[142] making it possible to detect glioblastoma-associated circulating molecules in peripheral blood. By analysing EGFRvIII in patients' tumour and plasma specimens, Salkeni *et al.*[143] showed that EGFRvIII deletion was detected in both the tumour sample and presurgery plasma but not in postsurgery plasma for glioblastoma patients who received complete tumour resection. Wang *et al.*[144] identified two fusion transcripts (FGFR3-TACC3 and VTI1A-TCF7L2) in both tumour tissue and matched plasma samples from glioblastoma patients. Moreover, recent reports have shown that blood is affected by the existence of tumours and is sensitive to tumour burden (e.g. metabolites, cell-free nucleic acids, mRNAs in tumour-educated platelets and extracellular vesicles).[145–148] For instance, the blood of tumour patients may have elevated levels of certain types of T cells, tumour cells and microvesicles shed by the primary tumour mass, including glioblastoma.[149–151] These studies provide the opportunity to develop blood-based biomarkers to aid in the diagnosis and management of glioblastoma.

Due to its anatomical structure, CSF has also become a promising material to develop biomarkers for patients with CNS disorders.[152] For example, a recent study found that p-tau181 in blood exhibits attractive diagnostic value for Alzheimer's disease, with accuracy similar to that of Tau PET scans and p-tau181 detection in CSF, demonstrating the usefulness of blood tests and CSF tests in brain disease diagnosis.[153,154] In the case of brain tumours, an early study reported that the detection of seven microRNAs, i.e. miR-10b, miR-21, miR-125b, miR-141, miR-200a, miR-200b and miR-200c, in CSF showed over 90% accuracy in differentiating glioblastoma from metastatic brain tumours.[155] A meta-analysis revealed that miR-21 was detectable in both the blood serum and CSF of several glioblastoma patients.[156] Promoter hypermethylation in *MGMT*, *CDKN2A*, *TIMP3* and *THBS1* genes was detected in CSF, serum and tumour tissue specimens in glioblastoma patients but not in healthy donors.[145,157] Compared with healthy donors, higher levels of osteopontin or its cleaved fragments were found in CSF samples of glioblastoma patients, which may be linked to the aberration of VEGF and TNFα signalling pathways in glioblastoma.,[145,158,159] Another recent study showed that circulating tumour DNA reflected patient tumour-specific mutations in CSF samples, including mutations in the genes *NTRK1*, *JAK2*, *EGFR*, *PIK3R1*, *ATRX*, *SMARCA4*, *IDH1*, *TP53* and *H3F3A*.[160] In addition, a study reported that CSF-derived tumour DNAs were present in 74% of 35 patients, and CSF-derived tumour DNAs may be useful for tracking recurrence after initial treatment.[161]

One important benefit of developing liquid biomarkers for the early prediction of therapy response and tumour progression is to mechanistically enable a better understanding of glioblastoma tumour biology and its evolution, as well as the pathogenesis of post-treatment effects. Many studies comparing recurrent tumours and primary tumours have been reported; however, research focusing on identifying the molecular differences in post-treatment effects and progressed or recurrent tumours remains limited. Nevertheless, a few studies have touched on this area. By detecting the above-mentioned STEAM+ CTCs, Sullivan *et al.*[138] reported that patients with progressive glioblastoma had approximately 11.8 cells/ml and a patient with metastatic tumours had 4.2 cells/ml, while patients with stable disease had approximately 2.1 cells/ml.[162] Patients with methylated MGMT were shown to have higher rates of pseudoprogression (91%) than patients with unmethylated MGMT.[163] Overexpression of p53, interferon regulation factor 9 and X-ray repair cross-complementing 1 was correlated with pseudoprogression.[164–166] EGFR+ microvesicle counts were higher in patients with recurrent glioblastoma than in those with pseudoprogression.[167,168] A study reported that the ratio of HLA-DR (human leukocyte antigen – DR isotype) and VNN2 (vascular non-inflammatory molecule 2) expression on CD14-positive myeloid-derived suppressor cells isolated from patient peripheral blood monocytes may distinguish true tumours from radiation necrosis.[169] A study assessed the expression levels of matrix metalloproteinase 2 and neutrophil gelatinase-associated lipocalin in the serum and urine from glioblastoma patients, and the results showed that their preoperative levels correlated with overall survival and progression free survival but did not differentiate tumour growth and post-treatment effects.[170] Sabedot *et al.*[171] performed DNA methylation profiling using serum cfDNA and tumour tissue DNA from glioma patients and developed a score metric to optimally distinguish patients with or without glioma. The score metric of a glioblastoma patient showed an increasing trend with disease progression.

These studies (summarized in Table 2) support the idea that tumour-related molecules in patient blood and/or CSF may be useful as indicators of treatment response or tumour progression and may be detected early before a suspicious lesion appears in imaging surveillance. Müller Bark *et al.*[164] and Raza *et al.*[167] also provided informatic reviews of circulating biomarker studies for glioblastoma and blood biomarker studies in therapy response assessment for glioma. More studies to extend our knowledge of post-treatment effects will be helpful for the development of liquid molecular biomarkers serving in this scenario. To this end, it is important to select proper patient cohorts and set up effective approaches given

Table 1 Notable studies of imaging biomarkers for the early prediction of therapy response or tumour progression for glioblastoma

Imaging type	Measure	Patient cohort	Time of measure	Predictive/assessible power	Reference
DSC-MRI	rPH, normalized rCBV, PSR	57 patients with glioblastoma	At the time of suspected recurrence	Distinguish tumour from radiation necrosis	Barajas *et al.*[85]
DSC-MRI	Normalized rCBV	13 patients with high-grade glioma	Prior to surgical reresection	Distinguish tumour from post-treatment effects	Hu *et al.*[56]
DSC-MRI	Normalized rCBV	25 patients with recurrent glioblastoma	Prior to surgical reresection	Distinguish tumour from post-treatment effects	Hu *et al.*[96]
DSC-MRI	Standardized rCBV	38 patients with high-grade glioma	Prior to surgical reresection	Distinguish tumour from post-treatment effects	Hoxworth *et al.*[88]
DSC-MRI	Normalized CBV histogram	135 patients with newly diagnosed glioblastoma	First and second follow-up[a]	Predict early tumour progression and distinguish it from pseudoprogression	Baek *et al.*[95]
DWI	Interval changes of relative ADC	19 patients with recurrent glioblastoma received immunotherapy	6 months	Predict treatment response	Song *et al.*[104]
CEST-MRI	MTR_{NOE}, MTR_{amide}	19 patients with newly diagnosed glioblastoma	2 weeks	Predict tumour progressors early after treatment	Mehrabian *et al.*[108]
CEST-MRI	NOE-LD, dns-APT	20 patients with newly diagnosed glioblastoma	Pretreatment	Predict early tumour progressors	Regnery *et al.*[109]
APT-MRI	Mean APT- weighted signal intensity	54 recurrent glioblastoma patients received BEV therapy	4–6 weeks	12 months or longer progression free survival prediction	Park *et al.*[110]
MRS, DWI	Total Cho/total NAA, ADCmean	39 patients with glioblastoma	At the time of suspected progression	Distinguish tumour from pseudoprogression	Kazda *et al.*[114]
FET-PET	MTV	21 glioblastoma patients who are IDHwt and received antiangiogenic treatment at first progression	8–10 weeks	>9 months overall survival prediction	Galldiks *et al.*[115]
T_1-weighted MRI, ^{18}F-FCho PET	Enhancing tumour volume, MTV × SUVmean	11 glioblastoma patients	Week 2–6 (T_1), and 1 month (PET)[a]	Predict response at 6 months post-treatment	Bolcaen *et al.*[116]

PH = peak height; PSR = percentage of signal intensity recovery; SUVmean = mean standardized uptake value.
[a]Post-treatment.

Table 2 Summary of several studies of molecular biomarkers, especially liquid biomarkers, for glioblastoma or post-treatment effects

Detection type	Molecule	Rate or result	Clinical applicability	Reference
CTC in blood	GFAP+	~20.6% (29 of 141)[a]	Glioblastoma diagnosis	Muller *et al.*[140]
CTC in blood	STEAM-positive (SOX2, Tubulin beta-3, EGFR, A2B5 and c-MET)	~39.4% (13 of 33)[a]	Glioblastoma diagnosis and progression prediction	Sullivan *et al.*,[138] Ozkumur *et al.*[141]
Plasma and tumour tissue	FGFR3-TACC3 and VTI1A-TCF7L2 fusion transcripts	~22.2% (2 of 9)[a]	Glioblastoma detection	Wang *et al.*[144]
CSF, plasma, tumour tissue	miR-21 expression	n/a	Glioblastoma detection (meta-analysis)	Qu *et al.*[156]
Tumour tissue	Methylated MGMT	91% (21 of 23 patients with methylated MGMT)[a]	Pseudoprogression prediction	Brandes *et al.*[163]
Tumour tissue	Overexpression of p53	87.5% (7 of 8 pseudoprogression)[a]	Distinguish early progression and pseudoprogression	Muller Bark *et al.*,[164] Kang *et al.*[165]
Tumour tissue	Overexpression of interferon regulation factor 9 and X-ray repair cross-complementing 1	n/a	Distinguish tumour progression and pseudoprogression	Muller Bark *et al.*,[164] Qian *et al.*[166]
Microvesicle count in blood		Microvesicle counts are high for true progression	Distinguish true progression from stable disease or pseudoprogression	Raza *et al.*,[167] Koch *et al.*[168]
$CD14^+$ myeloid-derived suppressor cells in blood	The ratio of HLA-DR and VNN2 expression, termed HLA-DR-VNN2 Index (DVI)	DVI values are high for true tumours	Distinction of glioblastoma and radiation necrosis	Soler *et al.*[169]

n/a = not available.
[a]Percentage of positive numbers in total studied subject numbers.

Table 3 Notable studies of ML-applied studies for the early prediction of therapy response or tumour progression for glioblastoma

Data source	Patient cohort	Features	Clinical applicability	Reference
Routine blood test data	15 176 neurological patients	Blood parameters, e.g. glucose, neutrophils (%), etc.	Predict brain tumours	Podnar *et al.*[185]
Pretreatment (<2 weeks) and post-treatment (<3 months) MRI	106 glioblastoma patients treated with BEV	Clinical, volumetric, shape, texture, parametric and histogram features	Predict overall survival	Chang *et al.*[197]
rCBV and patient histological data	82 biopsy samples from 18 glioblastoma patients	MRI features, e.g. rCBV, mean diffusivity and FA, etc.	Quantify regional cell density in glioblastoma tumour tissue and detect interpatient tumour variabilities	Hu *et al.*[193]
MRI data and clinical features	78 glioblastoma patients	Age, gender, MGMT methylation status, IDH mutation status, the dose and number of fractions of radiotherapy, interval between end of radiation and appearance of enhancing lesion	Distinguish pseudoprogression from tumour progression	Jang *et al.*[200]
Clinical, radiomic and molecular information	76 glioblastoma patients with early enhancing disease	Age, MGMT methylation status, two shape-based features from enhancing mask, three radiomic features from enhancing mask on ADC, and one radiomic feature from perilesional oedema mask on T_2-weighted images	Distinguish true progression and pseudoprogression	Patel *et al.*[201]
Tissue characteristics, and imaging data from TCIA and local hospital	152 glioblastoma patients' data from TCIA and local hospital	Three texture, one shape, four intensity features	Predict overall survival of individual glioblastoma patients	Pan *et al.*[198]
Quantitative MRI data	51 newly diagnosed glioblastoma patients	Magnetization transfer parameters and CEST parameters	Distinguish early from late-progression glioblastoma cohorts	Chan *et al.*[202]
Clinical and radiomics information from TCGA, TCIA, ImageNet and local hospital	High-grade glioma patients 55 from local hospital and 128 from TCGA	Deep features and radiomics features (e.g. tumour volume, solidity, histogram data, energy, etc.)	Classify long- and short- term survivor groups	Han *et al.*[199]
Quantitative MRI data	63 glioblastoma patients	Imaging features reflecting angiogenesis, cellularity and water concentration	Distinguish true progression from pseudoprogression	Akbari *et al.*[203]
Clinical characteristics and imaging data	98 glioblastoma patients	Mainly K_{trans} and rCBV	Discriminate pseudoprogression and progressive diseases	Elshafeey *et al.*[204]
Imaging data (preoperative MRI scans)	83 glioblastoma patients	Four fractional anisotropy features and six CBV features	Predict 6-month local progression	Kim *et al.*[205]
Imaging data (preoperative MRI scans) and clinical data	83 glioblastoma patients	Fractional anisotropy and CBV features and clinical data (age, sex, KFS, extent of surgical resection)	Predict survival using Harrell's C-index	Kim *et al.*[205]
Imaging data	61 glioblastoma patients	Three from conventional (e.g. covered image intensity range, etc.), two from DWI (e.g. long-run low grey-level emphasis and correlation) and seven from perfusion MRI (sum of intensities, etc.)	Differentiate pseudoprogression from early tumour progression	Kim *et al.*[206]

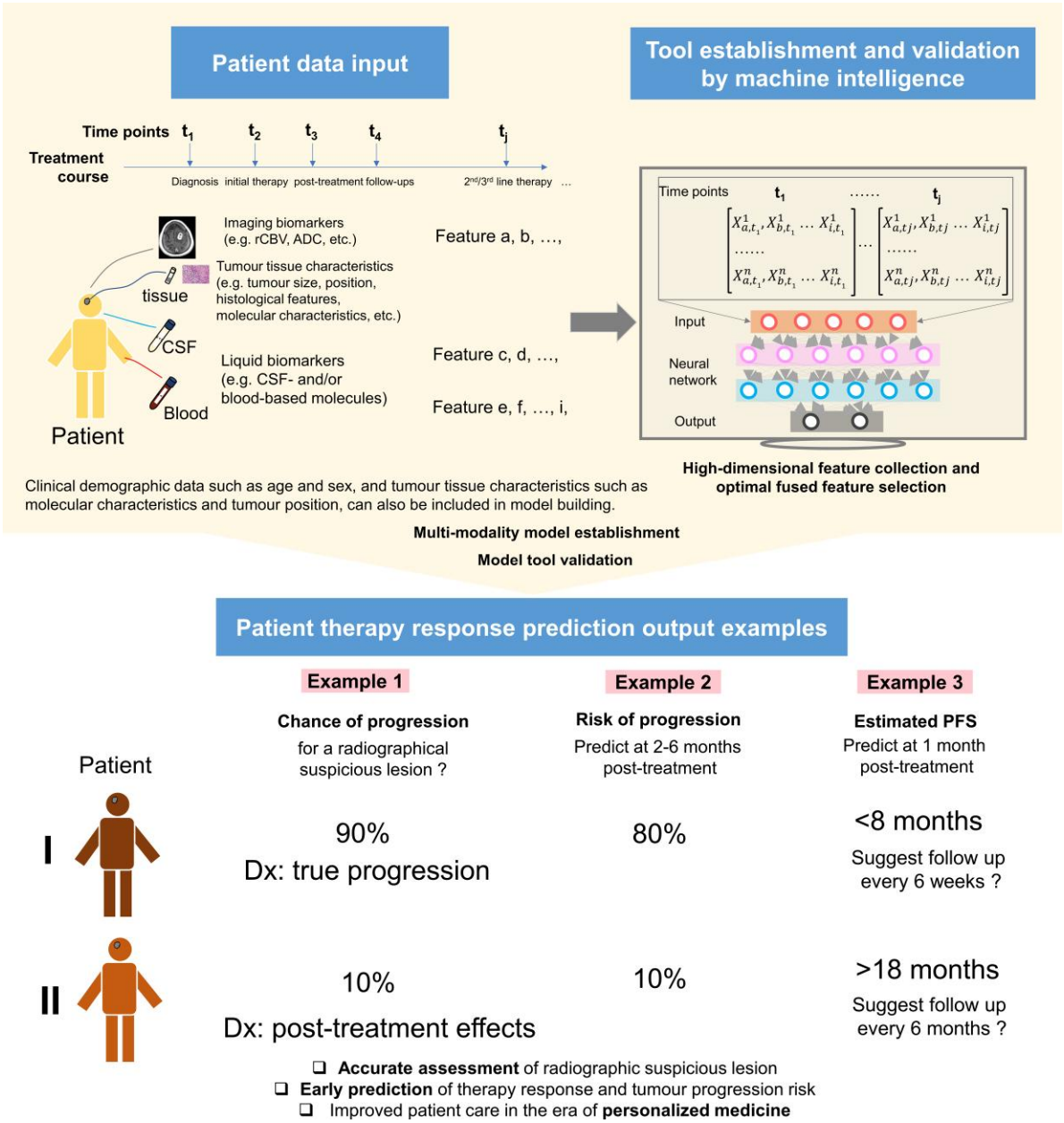

Figure 3 Potential approaches that may assist in the early prediction of therapy response and tumour progression. Patient data, including demographics, tumour tissue samples, liquid specimens and imaging data, can be collected and comprehensively analysed by using a machine learning method. Longitudinally collected patient data at various time points (t1, t2, …, tj) will allow the establishment of a dynamic machine learning model, such as by using a deep learning neural network approach. Patient (1, 2, …, *n*) represents samples included in the patient cohort, and patient data (input) can be summarized as features (a, b, …, i). High-dimensional features could then be analysed for optimal selection and model building. The resulting predictive model will be used for validation and adjustment. Such an approach may enable a more accurate assessment of radiographic suspicious lesions and achieve a reliable prediction of progression risk at an early time point (output). Empty circles represent neurons in layers of a neural network. I and II represent random patients with glioblastoma. Dx = diagnosis.

the heterogeneity of glioblastoma. Detection of differential gene expression[172] by transcriptome profiling, exploration of differential methylation profiles by methylome profiling, characterization of metabolite changes by metabolome profiling and identification of condition-specific extracellular vesicles in patient biofluids may aid in the diagnosis of tumours and their progression. It will also be helpful to take advantage of state-of-the-art single-cell technologies to analyse circulating blood or CSF from glioblastoma patients over the course of treatment. The molecular features of the treatment-related cell subsets captured from such approaches may complement imaging biomarkers.

Artificial intelligence technologies

Artificial intelligence simulates natural intelligence, including human intelligence, using computer algorithms to execute tasks.[173,174] In the era of artificial intelligence-based medicine, machine learning algorithms are making an immense impact on healthcare,[175]

especially on the development of novel computational tools for the stratification, grading, classification and prognostication of patients using large-scale patient datasets.[176–180] The fundamental principle of machine learning is to establish a mapping between significant features (e.g. image representation, gene expression changes, genetic alterations, histological characteristics) and a given outcome (e.g. disease type, tumour grade, risk group) from training datasets; the trained mapping (also known as a model) would then allow for predicting the outcome variables for new patients. One evident advantage of machine intelligence is its capability of handling big data and drawing insights that are difficult to obtain by human intelligence. Additionally, machine learning allows accurate classification and clustering using high-dimensional feature selection[181,182] and computer-based data analysis, making it a promising tool to address clinical challenges. The way in which artificial intelligence, particularly machine intelligence, is applied and transforming healthcare has been discussed in many publications, such as Esteva *et al.*[175] and Rajkomar *et al.*[177] Here, we will mainly focus on the applications of machine learning in glioblastoma studies.

Medical imaging and genomic datasets have high dimensionality and complex structures and thus have garnered considerable attention from the research community to develop machine learning algorithms, including deep learning (a subset of machine learning algorithm-based neural networks), to enable automatic data analysis to facilitate clinical decision making. For instance, a study developed a machine learning model for predicting the risk of developing breast cancer based on mammography images and improved the accuracy compared with the current model making it ready-to-use along with clinical imaging acquisition.[183] A study using swarm learning, a machine learning approach, analysed peripheral blood mononuclear cell transcriptomes from >12 000 individuals to predict leukaemia and achieved high accuracy.[184] Podnar *et al.*[185] curated routine blood test data from 15 176 neurological patients and built a machine learning predictive model for the detection of brain tumours with a sensitivity and specificity of 96% and 74%, respectively.

In addition, machine learning algorithms have been developed to combine data from imaging modalities, histopathology and/or genomics to improve the predictive power for diseases. Research has been performed on the optimal fusion of features[186–188] and data fusion with modality-wise missing patterns.[189] Optimal fusion of multiple imaging modality data is considered to provide superior accuracy over the use of a single modality in the early detection of diseases, such as discerning subtypes of migraine[190] and dissolving genomic and molecular heterogeneity of glioblastoma.[191–194] Hu *et al.*[193] built a patient-specific transfer learning (building algorithms based on models established from other datasets) predictive model using rCBV data obtained from DSC-MRI and individual patient histological data. They demonstrated that this model provides an improved performance for quantifying regional cell density in glioblastoma tumour tissue and detecting interpatient tumour variabilities compared to other reported models. By using support vector machine and random forest classifiers (approaches used in machine learning), a study assessed the predictive power of the built model to predict *MGMT* methylation status in preoperative glioblastoma tumours using MRI texture features and achieved an area under the curve (AUC) of 0.85.[195] Another study using the support vector machine approach to classify brain tumour types by analysing MRS metabolite data and microarray gene expression data achieved an enhanced classification accuracy compared to each single-omics dataset.[196] Chang *et al.*[197] used the random forest method and generated a classifier using pre- and post-therapy MRI data to predict overall survival and showed high potential to assist in the clinical decision of recurrent glioblastoma.[197] Pan *et al.*[198] used glioblastoma patient data from The Cancer Imaging Archive (TCIA) and built a machine learning model that generated a multiparametric and multiregional radiomics signature with eight selected features (three textures, one shape and four intensity features). The model predicted overall survival with a concordance index of 0.7, which increased to 0.76 after the model was further combined with preoperative clinical risk factors.[198] Han *et al.*[199] studied data from 55 patients with high-grade glioma from a local hospital and 128 patients with glioblastoma from the The Cancer Genome Atlas (TCGA). They used a deep learning-based method to conduct feature selection from 384 handcrafted radiomics features and 8192 deep features from a pretrained convolutional neural network (a deep learning approach) and successfully classified long- and short-term survivor groups.

Specifically related to this paper, machine learning has been studied by researchers to distinguish post-treatment effects and tumour progression using clinical and imaging data (studies discussed in this review are listed in Table 3). Jang *et al.*[200] investigated the feasibility of machine learning algorithms to distinguish pseudoprogression from tumour progression in patients with glioblastoma by incorporating MRI data and clinical features with an AUC score of 0.83 and an F1-score of 0.74. Patel *et al.*[201] performed a retrospective study of building machine learning-based models combining clinical, radiomic and molecular data to distinguish true progression from pseudoprogression in 76 patients with early enhancing disease following the standard of care. They showed that the top selected features by bootstrapped cross validation were age, *MGMT* methylation status, two shape-based features from the enhancing mask, three radiomic features from the enhancing mask on ADC and one radiomic feature from the perilesional oedema mask on T_2-weighted images. The model built using these features resulted in an AUC of 0.8, a sensitivity of 78.2%, a specificity of 66.7% and an accuracy of 73.7% by 5-fold cross validation.[201] By using quantitative MRI, Chan *et al.*[202] built a multivariable least absolute shrinkage and selection operator model and identified a CEST/magnetization transfer approach showing potential in distinguishing early- from late-progression cohorts after standard chemoradiation therapy for glioblastoma patients. Akbari *et al.*[203] evaluated suspicious radiographic changes in 63 glioblastoma patients and extracted quantitative characteristics from multiparametric MRI. They reported that imaging features reflecting higher angiogenesis, higher cellularity and lower water concentration suggest true progression over pseudoprogression.[203] Elshafeey *et al.*[204] retrospectively studied imaging data of 98 glioblastoma patients and used a support vector machine to build a classifier based on radiomic features of both the volume transfer constant (K_{trans}) and rCBV maps. The model discriminated pseudoprogression and progressive diseases with an accuracy of 90.82%.[204] Considering that non-enhancing lesions are not frequently removed during surgery, Kim *et al.*[205] retrospectively studied preoperative MRI scans from 83 glioblastoma patients and reported that a diagnostic model combining four fractional anisotropy features and six CBV features presented a better predictive value for the prediction of 6-month local progression in patients with glioblastoma. By analysing imaging data from 61 glioblastoma patients obtained within 3 months post-treatment, Kim *et al.*[206] established a least absolute shrinkage and selection operator model that incorporated 12 significant radiomic features, including three from conventional MRI, two from DWI and seven from perfusion MRI, to robustly identify pseudoprogression with an AUC of 0.9.

As described before, a massive number of studies have applied machine learning in the diagnosis, prognosis and therapy response prediction of glioblastoma patients using their histological and imaging data, sometimes juxtaposed with their genomic and demographic data. These studies unequivocally support the idea that machine learning will be an indispensable tool in future personalized clinical care for glioblastoma patients. Currently, more effective treatment regimens need to be developed to treat glioblastoma, specifically killing tumour cells while protecting healthy brain tissue to maintain normal brain function for patients with glioblastoma. To this end, more advanced assessment and prediction strategies for glioblastoma therapy response must be exploited.

Perspective

Glioblastoma is a challenging deadly disease with a high recurrence rate attributed, in part, to the lack of sensitive diagnostic tools and effective treatment strategies. The inter- and intra-patient heterogeneity makes this cancer even harder to detect earlier and treat better. A more in-depth understanding of glioblastoma tumour heterogeneity (e.g. clonal development) and its microenvironment (e.g. immune infiltration) will certainly help in the development of new effective therapeutics. Sensitive and reliable tools for diagnosis, prognosis, therapy response assessment and early prediction of tumour progression are also in great need to improve the survival outcomes of patients with this malignancy. Many efforts have been made using machine learning-based methods to analyse non-specific imaging data and in combination with clinical data and histological data owing to advances in computer vision. More research will be needed to discover reliable liquid biomarkers and develop therapy assessment and early prediction tools based on features specific to a given disease condition, such as tumour progression or pseudoprogression.

Integration of patient medical history and demographic data, tumour molecular features (e.g. histopathological data), longitudinally collected imaging metrics and liquid biomarker signatures reflecting individual patient characteristics and tumour evolution patterns, which will generate a wealth of patient-specific data, may provide a more comprehensive and accurate assessment of treatment response at time points far earlier in the course of therapy (Fig. 3). Advanced analytic strategies using artificial intelligence, particularly machine learning, will enable automatic sifting through these data, discovery of image patterns and combinatory biomarker signatures, quantification of their interactive and dynamic evolutions and optimal combination of multiple modalities, providing an up-and-coming opportunity to improve the early prediction of therapy response and tumour progression for glioblastoma. Meanwhile, challenges still exist, such as missing data handling, data standardization across hospitals and institutions, optimized study design for algorithms and representation generalization. Moving forward, rigorous evaluation, prospective clinical validation of machine learning models and generalization of such models across sites will be of vital importance to ultimately establish standard operation protocols and policy frameworks in clinical settings and guide clinical decisions in both standard of care and clinical trials.

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Competing interests

The authors report no competing interests.

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