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The role of amino acid PET in radiotherapy target volume delineation for adult-type diffuse gliomas: A review of the literature

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ABSTRACT

Purpose: To summarise existing literature examining amino acid positron emission tomography (AA-PET) for radiotherapy target volume delineation in patients with gliomas.
Methods: Systematic search of MEDLINE and EMBASE databases.
Results: Twenty studies met inclusion criteria. Studies comparing MRI- and AA-PET- derived target volumes consistently found these to be complementary. Across studies, AA-PET was a strong predictor of the site of subsequent relapse. In studies examining AA-PET-guided radiotherapy at standard doses, including one study using reduced margins, survival outcomes were similar to historical cohorts whose volumes were generated using MRI alone. Four prospective single-arm trials examining AA-PET-guided dose-escalated radiotherapy reported mixed results. The two trials that used both a higher biologically-effective dose and boost-volumes defined using both MRI and AA-PET reported promising outcomes.
Conclusion: AA-PET is a promising complementary tool to MRI for radiotherapy target volume delineation, with potential benefits requiring further validation including margin reduction and facilitation of dose-escalation.

1. Introduction

Adult-type diffuse gliomas as defined in the WHO 2021 classification include glioblastoma isocitrate dehydrogenase (IDH)-wild-type (grade 4), astrocytoma IDH-mutant (grades 2–4) and oligodendroglioma IDH-mutant 1p/19q co-deleted (grades 2–3) (Louis et al., 2021). Most patients across this spectrum of disease receive radiotherapy as a component of their initial or subsequent treatment. In glioblastoma, despite aggressive multi-modal treatment, recurrence is almost universal (Stupp et al., 2005). Rates of central in-field failure following adjuvant chemoradiotherapy are in the order of 80 % (Chang et al., 2007), including in patients treated with novel systemic agents (Seaberg et al., 2023). Conversely, for patients with grade 2–3 IDH-mutant tumours, survival may exceed a decade (van den Bent et al., 2021; Buckner et al., 2016) and therefore minimising toxicity from treatments including radiotherapy is an important goal. More accurate delineation of the gross tumour volume (GTV) for radiotherapy planning may help achieve better tumour control by reducing the risk of geographic miss or possibly by facilitating focal dose-escalation. More reliable GTV delineation may also potentially allow margin reduction without loss of treatment efficacy and thereby reduce toxicity.

Magnetic resonance imaging (MRI) is the primary modality used to define radiotherapy target volumes. In the case of high-grade glioma, consensus guidelines (Niyazi et al., 2023; Kruser et al., 2019) recommend a margin of 15–20 mm around the gadolinium-enhancing disease on T1-weighted MRI sequences and surgical cavity to define the high-dose clinical target volume (CTV). European protocols (ESTRO--EANO) (Niyazi et al., 2023) recommend including the T2-FLAIR abnormality only if the clinician feels this represents non-enhancing tumour but acknowledge the challenges in making this distinction,

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whereas North American (NRG) (Kruser et al., 2019) guidelines use the T2-FLAIR abnormality with a 20 mm margin to define a second clinical target volume that is prescribed an intermediate dose. Gadolinium-enhancement in brain tumours relies on blood-brain barrier disruption (Essig et al., 2006). The area of gadolinium-enhancement may not tell the full story with respect to the location of high-density residual tumour as there may be regions of viable tumour that lack blood-brain barrier breakdown. Furthermore, the underlying pathology in the region of T2-FLAIR hyperintensity may be difficult to define and may include tumour cell infiltration, oedema, a combination of the two or unrelated pathology (Niyazi et al., 2023). The uncertainty in delineation of viable tumour using MRI images drives the large isotropic CTV margins used in current guidelines.

Amino acid positron-emission tomography (PET) tracers include ^{11}C-methionine (MET), ^{18}F-fluoro-L- dihydroxy-phenylalanine (FDOPA), ^{18}F-fluoroethyl-L-tyrosine (FET) and ^{11}C-alpha-methyl-L-tryptophan (AMT). These tracers have a shared pathway for uptake into cells, namely by amino acid transporters, primarily of the sodium-independent L-system (especially LAT1). Glioma cells commonly exhibit increased transmembrane transport of amino acids to facilitate higher rates of protein synthesis compared to normal cells (Laverman et al., 2002). Unlike gadolinium enhancement, blood brain barrier breakdown is not a pre-requisite for amino acid tracer accumulation (Langen et al., 2006).

MET was the first amino acid tracer to be extensively studied for imaging of brain tumours (Bergstrom et al., 1987; O'Tuama et al., 1988). Whereas the use of ^{18}F-fluoro-deoxyglucose (FDG) PET in this setting was limited by high glucose utilisation by normal brain tissue, MET (and subsequently the other amino acid tracers) demonstrated comparatively lower background brain uptake (Juhasz et al., 2014). MET also offered a convenient radiochemical production pathway with rapid synthesis and high yield (Jager et al., 2001). However, a major limitation is the short physical half-life of ^{11}C (20 minutes) which necessitates on-site cyclotron production that is not feasible for many centres.

This short half-life of MET drove the development in the 1990's of amino acid tracers utilising the radioisotope ^{18}F which has a much longer physical half-life (109 minutes), suitable for use at centres which do not have on-site production (Laverman et al., 2002; Wester et al., 1999). FET, a tyrosine analogue, was the dominant tracer that came out of this process and has been since demonstrated in comparative studies to offer similar performance to MET for brain tumour imaging with possibly improved specificity for tumour versus non-tumour pathologies (Juhasz et al., 2014; Grosu et al., 2011; Pauleit et al., 2005; Weber et al., 2000). While MET is incorporated into protein synthesis and also undergoes significant non-protein metabolism, FET is not incorporated into protein synthesis and does not result in significant metabolites (Langen et al., 2006; Heiss et al., 1999). Kinetic modelling is therefore simpler for FET than MET.

Initial interest in and development of FDOPA, which is a dopamine precursor, primarily focused on imaging of brain dopaminergic pathways (Bauer et al., 2000). However, it was subsequently recognised to be a useful tracer for brain tumour imaging, once again with similar performance when tested against MET in comparative studies (Becherer et al., 2003; Beuthien-Baumann et al., 2003). FDOPA also uses the ^{18}F radioisotope and is suitable for off-site production. Unlike MET and FET, there is increased physiologic uptake of FDOPA in the basal ganglia, particularly at later scan time-points (Becherer et al., 2003).

AMT is a tryptophan analogue that was developed primarily to image serotonin synthesis and studied in patients with epilepsy (Chugani et al., 1998; Diksic et al., 1990; Tohyama and Takada, 2000; Juhasz et al., 2003; Muzik et al., 1997). Intracellular pathways metabolise AMT to both serotonin and kynurenine (Tohyama and Takada, 2000). Application of AMT to brain tumour imaging has been through a single centre only. The body of evidence supporting the use of AMT PET for brain tumour imaging is therefore less extensive but nevertheless promising (Bosnyak et al., 2016; Christensen et al., 2014; John et al., 2019; Kamson et al., 2013, 2014). Like MET, AMT uses a ^{11}C radioisotope so is limited to centres with on-site cyclotron production capability.

For each of MET, FET, FDOPA and AMT stereotactic biopsy and/or resection studies have demonstrated tumour to be reliably present at sites of tracer uptake, including at sites where no contrast enhancement is present on MRI (Pauleit et al., 2005; Kamson et al., 2013; Harat et al., 2024, 2023; Pafundi et al., 2013; Song et al., 2020; Mosskin et al., 1989). Parameters such as standardised uptake value (SUV) and tumour-background ratio (TBR), as well as parameters derived from dynamic acquisitions have also been demonstrated to correlate with tumour grade and molecular features (Albert et al., 2016a; Song et al., 2021). As outlined by the Response Assessment in Neuro-oncology (RANO) and European Association of Neuro-oncology (EANO) groups (Albert et al., 2016b), amino acid PET has an emerging role in the pre-operative setting in differentiation of glioma from non-neoplastic lesions, guiding stereotactic biopsies and delineating glioma extent to guide surgical resection (Ort et al., 2021). Similarly, in the post-adjuvant therapy setting amino acid PET is establishing a role in distinguishing recurrence from treatment-related changes (Bashir et al., 2019) and assessment of treatment response (Prather et al., 2022). This review aims to summarise the published literature to date examining the role of post-operative amino acid PET in volume definition for radiotherapy planning in the adjuvant setting for adult-type diffuse gliomas.

2. Methods

2.1. Search strategy

The literature search was performed using both Medline and EMBASE databases on 5 January 2023 and subsequently updated on 6 September 2024. Search terms used related to PET, amino acid tracers, radiotherapy and gliomas. The full search strategies for Medline and EMBASE are included in supplementary appendices A and B respectively.

2.2. Study selection

Duplicates of articles retrieved through the above search were removed. Remaining articles were screened for eligibility based on the following criteria: (1) Original article (exclusions: review articles, case reports); (2) Population studied: Adult-type diffuse glioma, including glioblastoma IDH-wildtype WHO grade 4, astrocytoma IDH-mutant grades 2–4, oligodendroglioma IDH mutant 1p/19q co-deleted grades 2–3 as per the WHO 2021 tumour classification and previous iterations of these entities in earlier WHO classification schemes; (3) Clinical studies in adult patients (exclusions: paediatric population, animal studies, in vitro studies); (4) Minimum of 20 patients in study; (5) Evaluated role of amino acid PET (including MET, FET, FDOPA and AMT tracers) in radiotherapy planning; (6) PET performed in early post-operative setting (prior to adjuvant therapy) (exclusions: PET performed in recurrence setting, including prior to re-irradiation); (7) Outcomes reported: Volumetric comparison of PET- and MRI-derived volumes, correlation of PET-defined biologic tumour volume (BTV) to location of failure or progression and/or clinical outcomes of PET-guided radiotherapy (exclusions: reported only prognostic value of PET parameters without impact on target volumes, patterns of failure or clinical outcomes of PET-guided radiotherapy). Initial screening was performed based on title and abstract, followed by final selection of included studies based on full text review by a single reviewer using the Covidence systematic review software (Veritas Health Innovation, Melbourne Australia).

2.3. Quality assessment

Quality assessment was performed using the QUADAS 2 (Whiting et al., 2011) tool for studies comparing MRI- to PET-derived target

volumes, the QUAPAS tool (Lee et al., 2022) for studies that correlated the PET-defined BTV with location of failure and/or clinical outcomes and the Downs and Black checklist (Downs and Black, 1998) for studies reporting clinical outcomes of PET-guided radiotherapy.

2.4. Integrated WHO diagnosis and grading

Due to differences in WHO grading systems over the time period of included studies, there may have been discrepancies in tumour classification between studies. Tumour classification was taken as that given in the study and no attempt was made to reconcile tumour classification to the WHO 2021 version.

2.5. Standardisation of nomenclature

A broad range of nomenclature was used to describe similar radiotherapy target volumes across the included studies. For ease of reading for the purposes of this review, nomenclature used in individual studies has been changed to a standardised nomenclature. Gross tumour volumes defined using MRI are designated "GTV". The PET-avid volume is assigned "BTV". Clinical target volumes and planning target volumes whether defined using MRI, PET or a combination are designated "CTV" and "PTV" respectively. Additional letters and subscripts are used when relevant to distinguish between multiple GTVs, BTVs, CTVs and PTVs used in the same protocol. In particular subscripts "MRI", "PET" and "MRI-PET" are used to describe volumes derived based on MRI, PET and the combination of PET and MRI respectively. When describing how these volumes are derived, we use "T1g" to denote the gadolinium-enhancing disease on T1-weighted MRI sequences, "T2F" to denote the hyperintense volume on T2-FLAIR MRI sequences and "cavity" to denote the surgical cavity. Clinical target volumes used in the described studies defined by expansions of other volumes were clipped to anatomical boundaries unless otherwise stated. All volumes referred to in this review are based on post-operative imaging unless explicitly stated.

A range of terms were used in the literature to describe the ratio of SUV of a given voxel to that of background brain. Terms used included tumour-normal ratio (commonly abbreviated as TNR or T/N ratio) and tumour-brain ratio (TBR). For ease of reading, TBR has been used throughout this review irrespective of the term used in the original article. Many studies did not explicitly define the method used to define the region or volume of interest for background brain. For these studies the subscript 'X' has been applied to TBR (i.e. TBR_X). Three studies used a crescent-shaped volume of interest made up of 6 merged crescent-shaped regions of interest in the contralateral hemisphere including white and gray matter as described by Unterrainer et al (Unterrainer et al., 2017). For these studies, the subscript CresVOI has been applied. The remaining definitions were used in individual studies only and have been assigned subscripts as follows:

- Crescent shaped region of interest in healthy-appearing contralateral cortex encompassing gray and white matter: CresROI
- Cerebellum: Cerebellum
- Three to five round regions of interest of diameter 1 cm in gray matter of contralateral frontal or temporal lobe: Round
- Similarly sized VOI to tumour in contralateral hemisphere in symmetrical location to tumour: Symmetrical
- Contralateral normal brain tissue at the level of the centrum semiovale, the normal striatum and the normal white matter: Striatum

For all studies that describe the region or volume of interest of normal brain used to define the TBR, the mean SUV of that volume was taken as the denominator.

Several studies defined patterns of failure using the terms 'central', 'in-field', 'marginal' and 'ex-field'. For the purposes of this review, these terms will be defined as:

- Central: Recurrence volume >95 % covered by reference volume
- In-field: Recurrence volume 80–95 % covered by reference volume
- Marginal: Recurrence volume 20–80 % covered by reference volume
- Distant: Recurrence volume <20 % covered by reference volume and/or new lesion outside of reference volume.

The reference volume could either be the PTV (referred to as 'volumetric patterns of failure' for the purposes of this review) or the volume encompassed by the 95 % isodose line (referred to as 'dosimetric patterns of failure' for the purposes of this review). For each study that deviated from the above definitions of these terms, this review will omit these terms when describing that study and explicitly describe the patterns of failure methodology used.

3. Results

The search yielded 645 studies, of which 111 were duplicates, 534 underwent abstract and title screening, 71 were included for full text review and 20 met eligibility criteria for inclusion (Fig. 1).

3.1. Volumetric comparison of MRI-, PET- and combined MRI-PET-defined target volumes

Two prospective (Dissaux et al., 2020; Grosu et al., 2005) and six retrospective studies (Hayes et al., 2018; Hirata et al., 2019; Matsuo et al., 2012; Munck Af Rosenschold et al., 2015; Navarria et al., 2014; Sweeney et al., 2014) were identified that compared radiotherapy planning volumes generated based on MRI alone with those generated using PET and/or a combination of PET and MRI. Their characteristics and selected outcomes are presented in Table 1. One study included only patients with glioblastoma (Matsuo et al., 2012), five studies (Dissaux et al., 2020; Grosu et al., 2005; Hayes et al., 2018; Hirata et al., 2019; Munck Af Rosenschold et al., 2015; Navarria et al., 2014) included a mixture of grade 3 and 4 gliomas and one study (Sweeney et al., 2014) included 23 high grade gliomas as well as five grade 2 gliomas. The tracer used was FET in four studies (Dissaux et al., 2020; Hayes et al., 2018; Munck Af Rosenschold et al., 2015; Sweeney et al., 2014), MET in three studies (Grosu et al., 2005; Matsuo et al., 2012; Navarria et al., 2014) and a combination of MET and FDG in one study (Hirata et al., 2019). Four studies used a TBR-based threshold to define the BTV with cut offs of 1.7 in one study (Grosu et al., 2005), 1.6 in two studies (Dissaux et al., 2020; Munck Af Rosenschold et al., 2015) and 1.3 in one study (Matsuo et al., 2012). One study (Navarria et al., 2014) used a visual assessment and/or TBR cut-off of 1.5 and one study (Hayes et al., 2018) used a clinician-defined BTV with no threshold cut-off. One study (Sweeney et al., 2014) used two different thresholds – $SUV_{max} \geq 2.2$ and $SUV > 0.4 \times SUV_{max}$ respectively. One study (Hirata et al., 2019) defined the BTV using a decoupling score threshold (≥3) based on the magnitude of deviation of MET/FDG uptake in a region of interest voxel from a linear regression line of MET uptake plotted against FDG uptake in normal brain expressed as a Z-score.

The details of each study and selected outcomes are presented in Table 1. In summary, the major findings were:

1. The median BTV was consistently larger than T1g but smaller than T2F (Dissaux et al., 2020; Grosu et al., 2005; Hirata et al., 2019)
2. A substantial component of the BTV was commonly found outside of both T1g and T2F and vice versa (Dissaux et al., 2020; Grosu et al., 2005; Hayes et al., 2018; Hirata et al., 2019; Matsuo et al., 2012; Munck Af Rosenschold et al., 2015; Sweeney et al., 2014)
3. The proportion of cases in which the BTV was covered by T1g+20 mm ranged from 24 % to 90 % (Grosu et al., 2005; Hirata et al., 2019; Matsuo et al., 2012)
4. The margin required on T2F to cover the BTV in most or all cases (96.4–100 %) was 10 mm in one study (Navarria et al., 2014) that

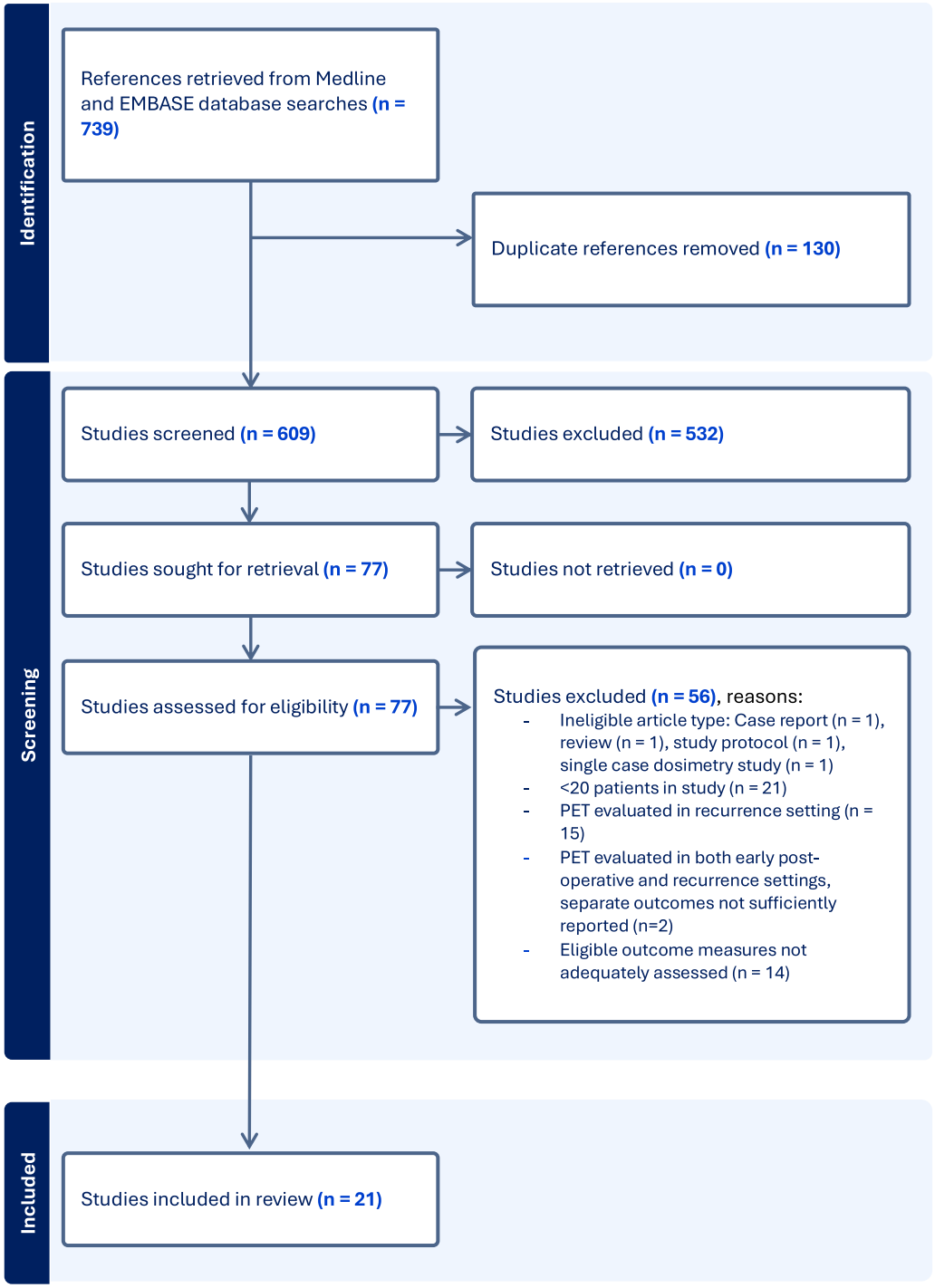

Fig. 1. PRISMA flowchart.

used the pre-operative T2F and 20 mm in another that used the post-operative T2F (Matsuo et al., 2012)

5. The method used to define the BTV was not consistent across studies

In aggregate, these studies suggest that PET and MRI may have complementary roles in defining tumour volumes for adjuvant radiotherapy planning. They suggest that if PET data are not taken into account, a 20 mm margin on residual enhancing disease may be inadequate to cover residual tumour in anywhere from a substantial minority to a majority of patients. An isotropic 10–20 mm margin on the T2F volume may cover the BTV in nearly all cases albeit at the cost of large target volumes. Incorporation of PET into the radiotherapy planning process may therefore reduce the volume of brain that is necessary to irradiate in order to achieve similar tumour coverage to that achieved using planning based on MRI alone. Quality assessment of studies from this section using the QUADAS-2 tool (Whiting et al., 2011) is presented in supplementary appendix C.

3.2. Patterns of failure following radiotherapy, PET not used to guide volume delineation

Three prospective (Lee et al., 2009; Harat et al., 2016; Allard et al., 2022) and five retrospective studies (Hirata et al., 2019; Navarria et al., 2014; Fleischmann et al., 2020; Harat et al., 2018; Iuchi et al., 2015)

Table 1
Characteristics and selected outcomes of studies comparing PET-derived volumes with MRI-derived volumes.

Study	Population	N	PET tracer	Volumes	Selected outcomes
Grosu et al. (2005) Prospective single institution trial	HGG - GB NOS 27 - AA 9 - AO 3	39	MET	GTV_{T1g}: T1g GTV_{T2F}: T2F+cavity BTV: $TBR_X \geq 1.7$	Comparison with GTV_{T1g} (N=39): - Mean volume BTV vs. GTV_{T1g} 19 vs. 11 cc - GTV_{T1g} extended outside BTV in 27/39 (69 %) - Mean GTV_{T1g} outside BTV: 6cc (range 0–60cc) - BTV extended outside GTV_{T1g} in 29/39 (74 %) - Mean BTV outside GTV_{T1g}: 13cc (range 0–101cc) - BTV extended >25 mm from GTV_{T1g} in 11/39 (28 %) Comparison with GTV_{T2F} (N=18): - Mean volume BTV vs. GTV_{T2F} 23cc vs. 42cc - GTV_{T2F} extended outside BTV in 18/18 (100 %) - Mean GTV_{T2F} outside BTV: 29cc (range 1–138cc) - BTV extended outside GTV_{T2F} in 9/18 (50 %) - Mean BTV outside GTV_{T2F}: 10cc (range 0–78cc)
Matsuo et al. (2012) Retrospective, observational	GB NOS	32	MET	GTV_{T1g}: T1g GTV_{T2F}: T2F BTV: $TBR_X \geq 1.3$	Mean percentage of BTV covered by GTV with 0 | 2 | 5 | 10 | 20 mm margin respectively: - GTV_{T1g}: 28.6 % | 44.3 % | 55.6 % | 72.0 % | 86.4 % - GTV_{T2F}: 61.1 % | 72.4 % | 81.9 % | 89.4 % | 96.4 %
Sweeney et al. (2014) Retrospective, observational	Glioma - Grade 4: 11 - Grade 3: 12 - Grade 2: 5	28	FET	GTV_{MRI}: T1g+T2F BTV1: SUV>(0.4×SUVmax) BTV2: SUV≥2.2	Median (range) tumour volumes: - GTV_{MRI} 88.6cc (2.6–467.4cc) - BTV1 26.2cc (1.6–261cc) - BTV2 3.7cc (range 0–135cc) Median volume of non-overlap between GTV and BTV: - GTV_{MRI} and BTV1: 4.6cc (0.6–83cc) - GTV_{MRI} and BTV2: 0.1cc (0–13cc)
Navarria et al. (2014) Retrospective observational	HGG - GB NOS 52 - AA 14 - AO 3	69	MET	CTV1: [T2F(preop)+10 mm] +T1g+cavity CTV2: T1g(preop)+20 mm BTV: Visual assessment and/or $TBR_X \geq 1.5$	Median (range) volumes: CTV1: 230cc (134–533cc) CTV2: 407 cc (143–594cc) BTV: 4.3cc (0.4–17.5cc) BTV was entirely included in CTV1 in all cases BTV was at least partly outside CTV2 in 35/69 (50 %) cases
Munck Af Rosenschold et al. (2015) Retrospective, observational	HGG - GB NOS 34 - GS 1 - AA 5 - AOA 3 - AO 10 - Gliomatosis 1	54	FET	GTV_{MRI}: T1g+cavity BTV: $TBR_{CresROI} \geq 1.6$	20 mm margin required on GTV_{MRI} to cover BTV in ~90 % of cases
Hayes et al. (2018) Retrospective, observational	HGG - GB IDHwt 18 - GB IDHmut 1 - AA IDHwt 5	24	FET	$CTV1_{MRI}$: (T1g+cavity)+20 mm $CTV2_{MRI}$: T2F $CTV1_{MRI\text{-}PET}$: $CTV1_{MRI}$+adjacent BTV* $CTV2_{MRI\text{-}PT}$: $CTV2_{MRI}$+rest of BTV* *Clinician-defined, $TBR_X \geq 1.6$ considered pathological	Comparison $CTV1_{MRI\text{-}PET}$ vs. $CTV1_{MRI}$ (T1g-defined volumes): Median volume: 94.7cc vs. 83.6cc (median difference 11cc) ≥10 % volumetric increase in 6/24 (25 %) Comparison $CTV2_{MRI\text{-}PET}$ vs. $CTV2_{MRI}$ (T2F-defined volumes): Median volume: 62.8cc vs. 57.1cc (median difference 5.7cc) ≥10 % volumetric increase in 9/24 (38 %)
Hirata et al. (2019) Retrospective observational	HGG - GB IDHwt 11 - GB IDHmut 3 - GS IDHwt 1 - AA IDHwt 6 - AA IDHmut 2 - AO IDHwt 1 - AOA IDHmut 1	25	MET and FDG	GTV_{T1g}: T1g GTV_{T2F}: T2 BTV: FDG-MET decoupling score ≥3	Median volumes: GTV_{T1g} - 9.9cc GTV_{T2F} - 91.1cc BTV - 60.4cc T1g+20 mm covered entirety of BTV in only 24 % GTV_{T1g} extended beyond BTV in 60 %
Dissaux et al. (2020) Prospective observational	HGG - Grade 4 25 - Grade 3 5	30	FET	GTV_{T1g}: T1g GTV_{T2F}: T2F BTV: $TBR_{CresVOI} \geq 1.6$ within 30 mm of T1g CTV: T1g+20 mm	Median volumes: - BTV: 43.8cc - GTV_{T1g}: 23.8cc - GTV_{T2F}: 78.7cc >5cc BTV outside volume: - GTV_{T1g}: 25/30 (83.3 %) - GTV_{T2F}: 23/28 (82.1 %) - CTV: 5/30 (16.7 %)

Abbreviations: PET: Positron-emission tomography; HGG: High grade glioma; GB: Glioblastoma; AA: Anaplastic astrocytoma; AO: Anaplastic oligodendroglioma; NOS: Not otherwise specified; GS: Gliosarcoma; AOA: Anaplastic oligoastrocytoma; IDH: Isocitrate dehydrogenase; IDHwt: IDH-wildtype; IDHmut: IDH-mutant; MET: ^{11}C-Methionine; FET: ^{18}F-Fluoroethyltyrosine; FDG: ^{18}F-Fluorodeoxyglucose; GTV: Gross tumour volume; T1g: Gadolinium-enhancing tumour on post-operative MRI; T2F: T2-hyperintense tumour on MRI T2-FLAIR sequence; Cavity: Surgical cavity; BTV: Biologic tumour volume; TBR: Tumour-brain ratio; SUV: Standardised uptake value; CTV: Clinical target volume

were identified that examined patterns of failure in patients where PET data was not used to guide radiotherapy. An overview of these studies is provided in Table 2. Two studies (Harat et al., 2016, 2018) from the same group had an overlapping cohort of 29 patients. Five (Lee et al., 2009; Harat et al., 2016; Fleischmann et al., 2020; Harat et al., 2018; Iuchi et al., 2015) of the seven studies included only patients with glioblastoma. One study (Navarria et al., 2014) included 69 patients of whom 52 had glioblastoma, 14 had anaplastic astrocytoma and 3 had anaplastic oligodendroglioma. One further study (Hirata et al., 2019) included 25 patients, 14 with glioblastoma (3 of which were IDH mutant), one with gliosarcoma, and 8 with anaplastic astrocytoma (2 of which were IDH-mutant), one with anaplastic oligodendroglioma (IDH wild-type) and one with anaplastic oligoastrocytoma (IDH-mutant). A final study (Allard et al., 2022) included 23 patients, 20 with glioblastoma and 3 classified grade 3 astrocytoma although notably all cases in that study were IDH-wildtype. In 4 (Navarria et al., 2014; Lee et al., 2009; Harat et al., 2016, 2018) of the 8 studies IDH status was not reported. Fleischmann et al (Fleischmann et al., 2020). reported IDH status and included 28 IDH1 wild-type, 5 IDH1-mutant and 3 IDH1-unknown glioblastoma cases respectively. Iuchi et al (Iuchi et al., 2015). included 20 patients with IDH wild-type and 2 patients with IDH-mutant glioblastoma. The tracers used were FET in 4 studies (Harat et al., 2016; Allard et al., 2022; Fleischmann et al., 2020; Harat et al., 2018), MET in 3 studies (Navarria et al., 2014; Lee et al., 2009; Iuchi et al., 2015) and both MET and FDG in one study (Hirata et al., 2019). Thresholds used to define the BTV were TBR >1.6 in 3 of the FET studies (Harat et al., 2016; Fleischmann et al., 2020; Harat et al., 2018) with this parameter as well as a range SUV threshholds tested in the fourth (Allard et al., 2022). In the MET studies, TBR>2 and/or region of highest MET avidity prior to radiotherapy (Iuchi et al., 2015), 1.5 times mean cerebellar uptake (Lee et al., 2009) and either the clinician-defined area of MET uptake or TBR>1.5 (Navarria et al., 2014) were used respectively. As described in Section 3.1, Hirata et al (Hirata et al., 2019). defined the BTV using a MET-FDG decoupling score threshold of ≥3. All studies compared the location of disease on pre-radiotherapy amino acid PET to sites of subsequent failure defined by MRI and/or PET imaging. In one study (Lee et al., 2009), patients were treated with an MRI-guided dose-escalated boost to the GTV (66–81 Gy in 30 fractions) and one (Iuchi et al., 2015) used a range of doses including hypofractionated radiotherapy to a high BED in a subgroup. The six remaining studies (Hirata et al., 2019; Navarria et al., 2014; Harat et al., 2016; Allard et al., 2022; Fleischmann et al., 2020; Harat et al., 2018) used standard radiotherapy doses (60 Gy in 30 fractions). Eligible patients in all 8 studies were treated with concurrent temozolomide.

The details and selected outcomes of individual studies in this group are presented in Table 2. The main concordant finding across studies was that the location of uptake on amino acid PET prior to radiotherapy is consistently a strong predictor of the site of subsequent recurrence. Individual studies also provided several further relevant insights:

1. Coverage of the BTV by the 60 Gy volume was found to be associated with prolonged progression-free survival (assessed using RANO criteria in each case) in studies from both Harat et al. (2018). (using FET PET) and Hirata et al. (2019). (using MET and FDG PET)
2. Fleischmann et al. (2020), using FET PET, found that the median minimal margin to encompass all recurrent tumour was smaller for T1g+BTV (12.5 mm) compared to T1g alone (16.5 mm) suggesting that a smaller CTV margin may be feasible if PET is incorporated into volume definition.
3. Despite the BTV falling within the 60 Gy CTV in all cases, all recurrences corresponded to the location of the BTV in the Navarria study (Navarria et al., 2014) (which used MET-PET), suggesting that 60 Gy may remain inadequate for ultimate tumour control even when the BTV is covered.
4. In the two studies that used dose-escalated radiotherapy, there was a signal that coverage of the BTV (defined using MET PET by both studies) by the higher dose may improve tumour control. Lee et al. (2009) found that all 5 cases where the BTV was not covered by 95 % the dose-escalated prescription (66–81 Gy in 30 fractions) non-central failure occurred (compared to 2 of 11 cases where the BTV was covered). Iuchi et al. (2015) also found that in patients treated with up to 68 Gy in 8 fractions to the highest-dose volume, the ratio of MET uptake to the biologically-effective dose delivered to a region of interest was significantly associated with whether that region was ultimately controlled or not.
5. Allard et al. (2022) explored multiple methods to define the BTV using FET PET and found that BTV defined using $TBR_{CresVOI} \geq 1.6$ provided the best combination of spatial similarity scores assessed using the dice similarity coefficient (DICE), Jaccard similarity coefficient (JSC) and overlap fraction (OV) metrics with the recurrence volume for both patients who had biopsy or partial surgery (DICE=0.488, JSC=0.339, OV=0.757) and those who had total or subtotal surgery (DICE=0.233, JSC=0.144, OV=0.434). This was suggested by the authors as the best of the examined options to define the standard-dose radiotherapy target volume.

Important caveats are that all studies were small (22–69 patients), only three were prospective and none were randomised. Caution should be applied extrapolating this data beyond glioblastoma, as this was the diagnosis in the vast majority of cases across all seven studies. Formal quality assessment of these studies using the QUAPAS tool (Lee et al., 2022) is included in supplementary appendix D.

3.3. Clinical outcomes and patterns of failure following PET-guided standard-dose radiotherapy

One prospective (Seidlitz et al., 2021) and two retrospective studies (Lundemann et al., 2017; Munck Af Rosenschold et al., 2019), met the eligibility criteria including patients treated with PET-guided radiotherapy using standard doses (Table 3). One further previously-described study (Sweeney et al., 2014) included patients treated with PET-guided radiotherapy at standard doses but was excluded from this section of the review because the outcomes reported did not meet the inclusion criteria. All three included studies reported on only patients with glioblastoma. None of the three studies reported IDH mutation status. The tracer used was MET in one study (Seidlitz et al., 2021) and FET in two studies (Lundemann et al., 2017; Munck Af Rosenschold et al., 2019). The BTV was defined by TBR > 1.6 in the two FET studies (Lundemann et al., 2017; Munck Af Rosenschold et al., 2019) and was clinician-defined with no threshold for the study that used a MET tracer (Seidlitz et al., 2021). Across all studies, radiotherapy was delivered in 30 fractions to a total dose of 60 Gy, with one study (Seidlitz et al., 2021) also utilising an intermediate dose region that was prescribed 50 Gy. All three studies used concurrent temozolomide for eligible patients.

Lundemann et al. (2017) retrospectively reviewed outcomes for 50 patients (21 with MGMT methylated and 29 with un-methylated tumours) treated with radiotherapy guided by both FET-PET and MRI. The CTV was defined by the union of the MRI-defined GTV (T1g + surgical cavity) and the PET-defined BTV (TBR_X>1.6) with a further 20 mm margin. The predominant pattern of recurrence was central (82 %), similar to historical data (Chang et al., 2007). The composite MRI- and PET-generated GTV/BTV volume demonstrated greater overlap with the site of recurrence (39 %) compared to either the BTV (31 %) or MRI-GTV (26 %) alone. The authors also found that a 12 mm margin from the combined GTV/BTV to CTV would have maintained the same rate of central recurrence as the 20 mm margin used to treat patients in this study. Survival data were not reported.

In the largest study of amino-acid PET-guided radiotherapy, Munck Af Rosenschold et al. (2019) conducted a retrospective analysis comparing outcomes for 190 patients treated with radiotherapy guided by both FET-PET and MRI to those of 521 patients treated with

Table 2
Characteristics and selected outcomes of studies comparing location of PET uptake prior to radiotherapy with subsequent site of failure where PET not used to guide radiotherapy.

Study	*N*	Population	PET tracer	Treated volumes	Comparison volumes (not used for treatment)	Dose levels	Selected outcomes
Lee et al. (2009) Prospective single-arm trial	26	GB NOS	MET	GTV: T1g+cavity CTV: GTV+15 mm PTV1: CTV+5 mm PTV2: GTV+5 mm	BTV: $TBR_{Cerebellum}$>1.5	PTV1: 60 Gy/30# PTV2: 66–81 Gy/30#	Dosimetric patterns of failure BTV >95 % (N=11) vs. <95 % (N=5) covered by 95 % isodose line for PTV2 - Central*: 9 vs. 0 - Non-central: 2 vs. 5 Note: 7 patients with no appreciable BTV and 3 patients who had not failed not included
Navarria et al. (2014) Retrospective observational	69	HGG - GB 52 - AA 14 - AO 3	MET	CTV1: [T2F (preop)+10 mm] +T1g+cavity PTV: CTV1+3 mm	BTV: Visual assessment and/or $TBR_X \geq 1.5$ CTV2: T1g(preop)+20 mm	60 Gy/30#	Proportion of recurrence volume located within target volume: CTV1: 0.97 (range 0.91–1.0) CTV2: 0.83 (range 0.53–1.0) BTV: 0.97 (range 0.91–1.0)
Iuchi et al. (2015) Retrospective observational	22	GB - IHDwt 20 - IDHmut 2	MET	IMRT (N=16): PTV1: T1g+5 mm PTV2: PTV1+15 mm PTV3: T2F+0 mm 3DCRT (N=6): PTV1: T1g+5 mm PTV2: T2F+5 mm	Cases where recurrence occurred: - Uncontrolled region of interest (ROI): Region of recurrence - Controlled ROI: TBR_{Round}>2 prior to RT but no recurrence in ROI for ≥12 months after RT Cases without recurrence: - Controlled ROI: Highest MET uptake prior to RT	IMRT (SIB): PTV1: 48–68 Gy/8# PTV2: 40 Gy/8# PTV3: 32 Gy/8# 3DCRT (Sequential boost): PTV1: 60 Gy/30# PTV2: 40 Gy/20#	18 controlled and 12 uncontrolled ROIs in 22 patients Median (range) TBRmax (p=0.021): Controlled ROIs: 2.12 (1.39–3.63) Uncontrolled ROIs: 3.48 (1.13–6.16) MET uptake to BED ratio (p=0.003): Controlled ROIs: 2.39×10^{-2} Uncontrolled ROIs: 3.99×10^{-2}
Harat et al. (2016) Prospective single centre observational	34	GB NOS	FET	GTV: T1g+cavity CTV: GTV+20 mm PTV: CTV+3 mm	BTV: $TBR_{Symmetrical}$>1.6	60 Gy/30#	Patterns of failure based on MRI- vs. PET-defined volumes: - In contact with GTV vs. BTV: 57 % vs. 70 % - Inside CTV, outside GTV vs. BTV: 13 % vs. 0 % - Outside CTV: 21 % vs. 21 % - Multifocal: 9 % vs. 9 %
Harat et al. (2018) Retrospective observational	29	GB NOS	FET	GTV: T1g+cavity CTV: GTV+20 mm PTV: CTV+3 mm	BTV: $TBR_{Symmetrical}$>1.6	60 Gy/30#	V100 % for BTV was correlated with PFS* (Spearman's rho = 0.417, p=0.038) *Assessed using RANO criteria
Hirata et al. (2019) Retrospective observational	25	HGG - 14 GB - 1 GS - 8 AA - 1 AO - 1 AOA	MET and FDG	GTV: T1g CTV60: GTV+20 mm CTV40: T2F+20 mm	BTV: MET-FDG decoupling score≥3	Sequential boost PTV60:60 Gy/30# PTV40: 40 Gy/20#	Poor BTV coverage by T1g+20 mm was associated with: Disease recurrence outside T1g (HR 11.7, p=0.012) Trend to reduced PFS* (p=0.05) *Assessed using RANO criteria
Fleischmann et al. (2020) Retrospective observational	36	GB - 26 IDHwt - 5 IDHmut - 5 NOS	FET	GTV: T1g CTV: GTV+20 mm PTV: CTV+3 mm	BTV: $TBR_{CresVOI}$>1.6 $GTV_{PET\text{-}MRI}$: GTV+BTV $CTV_{PET\text{-}MRI}$: $CTV_{PET\text{-}MRI}$+15 mm $PTV_{PET\text{-}MRI}$: $CTV_{PET\text{-}MRI}$+3 mm	60 Gy/30#	Median minimal margin to encompass all recurrent tumour (p<0.001): - GTV: 16.5 mm - $GTV_{PET\text{-}MRI}$: 12.5 mm Volumetric patterns of failure MRI-(CTV) vs. MRI+PET-($CTV_{PET\text{-}MRI}$) defined volumes: - Central: 32 (89 %) vs. 30 (83 %) - In-field: 2 (5.6 %) vs. 4 (11 %) - Marginal: 0 vs. 0 - Ex-field: 2 (5.6 %) vs. 2 (5.6 %)
Allard et al. (2023) Prospective observational	23	HGG IDHwt - 20 grade 4 - 3 grade 3	FET	Not described	Compared BTVs defined as: SUV >30 % of SUVmax SUV >40 % of SUVmax SUV >50 % of SUVmax SUV >60 % of SUVmax SUV >70 % of SUVmax SUV >80 % of SUVmax SUV >90 % of SUVmax $TBR_{CresVOI} \geq 1.6$	60 Gy/30#	Each definition of BTV compared to enhancing volume at relapse BTV = $TBR_{CresVOI} \geq 1.6$ provided best DICE/JSC/OV similarity with recurrence (suggested for standard-dose volume)

Abbreviations: PET: Positron-emission tomography; HGG: High grade glioma; GB: Glioblastoma; AA: Anaplastic astrocytoma; AO: Anaplastic oligodendroglioma; NOS: Not otherwise specified; GS: Gliosarcoma; AOA: Anaplastic oligoastrocytoma; IDH: Isocitrate dehydrogenase; IDHwt: IDH-wildtype; IDHmut: IDH-mutant; MET: ^{11}C-Methionine; FET: ^{18}F-Fluoroethyltyrosine; FDG: ^{18}F-Fluorodeoxyglucose; GTV: Gross tumour volume; CTV: Clinical target volume; PTV: Planning target volume; T1g: Gadolinium-enhancing tumour on post-operative MRI; Cavity: Surgical cavity; T2F: T2-hyperintense tumour on post-operative MRI T2-FLAIR sequence; T2F(preop): T2-hyperintense tumour on pre-operative MRI T2-FLAIR sequence; IMRT: Intensity-modulated radiotherapy; 3DCRT: 3-dimensional conformal radiotherapy; BTV: Biologic tumour volume; TBR: Tumour-brain ratio; SUV: Standardised uptake value; ROI: Region of interest; BED: Biologically-effective dose; MRI: Magnetic resonance imaging SIB: Simultaneous integrated boost, DICE: Dice similarity coefficient; JSC: Jaccard similarity coefficient; OV: overlap fraction

Table 3
Characteristics and selected outcomes of studies examining PET-guided radiotherapy without dose-escalation.

Study	N	Population	PET tracer	Volumes	Dose levels	Selected outcomes
Lundemann et al. (2017) Retrospective observational	50	GB - 21 MGMT methylated - 29 MGMT un-methylated	FET	GTV: T1g+cavity with reference to T2F BTV: TBR_X>1.6 CTV: GTV+BTV+20 mm PTV: CTV+2 mm	60 Gy/30#	Dosimetric patterns of failure: - Central (>95 %): 82 % - In-field (80–95 %): 10 % - Marginal (20–80 %): 2 % - Distant (<20 %): 6 % Volumetric overlap with recurrence volume: - MRI volume (GTV): 26 % - PET volume (BTV): 31 % - Composite MRI-PET volume: 39 %
Munck Af Rosenschold et al. (2019) Retrospective comparative cohort study	711 - 159 CRT cohort - 172 IG-VMAT cohort - 190 PET-IG-VMAT cohort	GB CRT cohort - 52 MGMT methylated - 42 MGMT un-methylated IG-VMAT cohort - 56 MGMT methylated - 93 MGMT un-methylated PET-IG-VMAT cohort - 58 MGMT methylated - 111 MGMT un-methylated	FET	GTV: T1g+cavity BTV: TBR_X≥1.6 MRI only (N=521) CTV: GTV+20 mm PTV: CTV+2–5 mm PET+MR (N=190) $GTV_{MRI-PET}$: GTV+BTV $CTV_{MRI-PET}$: $GTV_{MRI-PET}$+20 mm $PTV_{MRI-PET}$: $CTV_{MRI-PET}$+2–5 mm	60 Gy/30#	Median OS 15 months Use of FET-PET for radiotherapy planning was not associated with a difference in OS (HR 0.905, p=0.638)
Seidlitz et al. (2021) Prospective, single arm single-centre	89	GB - 30 MGMT methylated - 59 MGMT un-methylated	MET	BTV: clinician-defined $GTV_{MRI-PET}$: cavity+T1g+BTV CTV50Gy: ($GTV_{MRI-PET}$+20 mm)+T2F CTV60Gy: $GTV_{MRI-PET}$+5 mm PTV margins not specified	PTV60: 60 Gy PTV50: 50 Gy	Median OS 17.2 months Component of local failure in 80.3 %

Abbreviations: PET: Positron-emission tomography; GB: Glioblastoma; MGMT: MGMT: O^6-methylguanine-DNA methyltransferase; CRT: 3-D conformal radiotherapy; IG-VMAT image-guided volumetric modulated arc-therapy; PET-IG-VMAT: PET-guided IG-VMAT; MET: ^{11}C-Methionine; FET: ^{18}Fluoroethyltyrosine; GTV: Gross tumour volume; CTV: Clinical target volume; PTV: Planning target volume; BTV: Biologic tumour volume; T1g: Gadolinium-enhancing tumour on post-operative MRI; Cavity: Surgical cavity; T2F: T2-hyperintense tumour on post-operative MRI T2-FLAIR sequence; TBR: Tumour-brain ratio; MRI: Magnetic resonance imaging; PFS: Progression-free survival; OS: Overall survival; HR: Hazard ratio

radiotherapy guided by MRI alone. A 20 mm GTV (+/- BTV) to CTV margin was used for both MR/PET and MR-guided radiotherapy volumes. The median overall survival for the whole group was 15 months, and no difference was observed in overall survival with the utilisation of FET-PET for radiotherapy planning (HR 0.905, p=0.638 on univariate cox model). Notably however, there was an imbalance in MGMT promoter methylation status between the three arms (see Table 3) that the authors hypothesised could have contributed to this finding.

Seidlitz et al. (2021) conducted a single-arm, single-centre prospective trial (*N*=89, 30 MGMT methylated and 59 MGMT un-methylated glioblastoma cases) that incorporated both MET-PET and MRI into GTV delineation with a reduced (compared to EORTC or RTOG) 5 mm margin from T1g + cavity to the CTV60Gy, but a larger 20 mm expansion to the CTV50Gy that also included the non-enhancing lesions or edema identified on T2-FLAIR sequences. Median overall survival was 17.2 months, comparable to contemporary trials (Chinot et al., 2014) that used a larger margin from GTV to the 60 Gy CTV. The predominant pattern of failure remained local, with 80.3 % of cases having a component of local failure (defined as recurrence within the region of the former tumour or resection cavity).

These three studies are somewhat complementary. The Seidlitz et al. study (Seidlitz et al., 2021) suggests that incorporating PET into radiotherapy planning may facilitate reduction in GTV to CTV margins to 5 mm without detriment to survival which may ultimately translate into reduced toxicity. Disappointingly, the large but non-randomised comparative study from Munck Af Rosenschold et al. (2019) suggests that at a population level, when using generous 20 mm margins on the GTV, incorporation of the BTV is unlikely to improve survival outcomes. Hypotheses for why this might be the case could include that for the majority of patients the BTV is already included within the 20 mm expansion or alternatively that improved local control is ultimately of little consequence because the same patients then fail distantly with no ultimate impact on survival. Alternatively this finding may relate to the retrospective non-randomised study design and in particular the imbalance of confounders such as MGMT promoter methylation between cohorts examined that may have obscured a benefit from PET-guided treatment. Finally, both the Seidlitz et al. (2021) and Lundemann et al. (2017) studies, demonstrate (in concordance with the previously-discussed Navarria et al. study (Navarria et al., 2014)) that despite including the BTV in the 60 Gy volume, the predominant pattern of recurrence remains central (i.e. distant failure does not appear to be driving the lack of improvement observed in survival despite better coverage of the BTV by 60 Gy when PET is incorporated into planning). The logical question that arises is whether dose-escalation to combined MRI-PET target volumes can improve outcomes and this is explored in the next set of studies discussed in Section 3.4.

Caveats once again include the relatively small numbers in 2 of the 3 studies and that none of the studies were randomised. All conclusions are hypothesis-generating only. Furthermore, all patients included had glioblastoma, different principles are likely to apply for IDH-mutant tumours. Formal assessment of the quality of these studies using the Downs and Black checklist[47]is included in supplementary appendix E.

3.4. Clinical outcomes and patterns of failure following PET-guided dose-escalated radiotherapy

Four studies (Laack et al., 2021; Miwa et al., 2014; Navarria et al., 2017; Piroth et al., 2012), all prospective and single-arm, met the eligibility criteria and examined patients treated with FET-guided dose-escalated radiotherapy (Table 4). All patients across the four studies had glioblastoma. No study reported IDH mutation status. Two studies used a MET tracer (Miwa et al., 2014; Navarria et al., 2017), one used FET (Piroth et al., 2012) and one used F-DOPA (Laack et al., 2021). All studies used TBR-based thresholds for BTV delineation, no two studies used the same threshold. In all studies there was an escalation of biologically effective dose above the standard equivalent dose in 2 Gy fractions (EQD2) of 60 Gy. For the purposes of EQD2 calculations we have assumed an alpha/beta ratio of 10 for glioblastoma ($EQD2_{10}$). There was no consistency in dose and fractionation schedule across studies. All four studies used temozolomide concurrent with radiotherapy for eligible patients.

Piroth et al (Piroth et al., 2012). undertook a small prospective phase 2 study including 22 patients (5 MGMT promoter methylated, 13 un-methylated and 4 methylation status unknown). 60 Gy was prescribed to PTV1, which was a 5 mm expansion on the MRI-defined CTV (T1g+5 mm+edema). A modest simultaneous integrated boost of 72 Gy ($EQD2_{10}$ = 74 Gy) was applied to the BTV (defined on FET-PET using a threshold of $TBR_X \geq 1.6$) with no margin. Notably the gadolinium-enhancing disease on MRI was not considered in designing the boost volume. No radionecrosis or grade 3+ toxicity was observed. However there was no apparent improvement in efficacy, with overall survival (median 14.8 months) similar to the EORTC-NCIC (Stupp) trial (Stupp et al., 2009).

Miwa et al (Miwa et al., 2014). conducted a prospective single-arm study that included 45 patients treated for glioblastoma (MGMT promoter methylation status not reported) with a hypofractionated dose-escalated regimen: 68 Gy to the GTV, 56 Gy to the CTV and 40 Gy to the PTV in 8 fractions via a simultaneous integrated boost. These doses correspond to an $EQD2_{10}$ of 105, 79 and 50 Gy. This was by far the highest biologically effective dose used in any of the four studies. The GTV included both the enhancing disease on MRI and the high-avidity BTV on MET-PET ($TBR_X > 1.7$). The CTV was a modest 5 mm expansion on the GTV, but the PTV included a generous 15 mm expansion on the CTV as well as areas of 'moderate' PET avidity ($TBR_X > 1.3$). The median overall survival of 20 months compares favourably with contemporary trials (Chinot et al., 2014). Particularly intriguing was the very low 25 % rate of local failure in this trial with 14 % of patients exhibiting distant in-brain failure and 61 % failing with cerebro-spinal fluid (CSF) dissemination. Notably these terms were relatively loosely defined as: Local: Enlargement of a local lesion on MRI, treated with corticosteroids then confirmed on repeat MRI (excluding cases in which repeat surgery confirmed radionecrosis); Distant: New intra-parenchymal lesion distant from the original tumour site; CSF dissemination: New lesion distant from the original tumour site and exposed to the CSF space. Rates of grade 3 and 4 radionecrosis were 11.1 % and 4.4 % respectively.

Navarria et al. (2017) completed a prospective single-arm trial that included 97 patients with glioblastoma (61 MGMT methylated, 36 un-methylated) treated with a dose-escalated, hypofractionated, accelerated schedule in 15 fractions over 15 consecutive days. 60 Gy ($EQD2_{10}$ = 70 Gy) was delivered to the surgical cavity, residual contrast-enhancing tumour on MRI and BTV (clinician-defined using MET-PET) with no CTV margin and a 5 mm margin to PTV. 42 Gy was delivered to a volume that included the T2-FLAIR abnormality with a 5 mm margin. The median overall survival was 15.9 months, similar to contemporaneous trials (Chinot et al., 2014). Radionecrosis rates were relatively modest (grade 1–2 in 23 %, no grade 3+ radionecrosis).

Table 4
Characteristics and selected outcomes of studies examining PET-guided dose-escalated radiotherapy.

Study	N	Population	PET tracer	Volumes	Dose levels	Selected outcomes
Piroth et al. (2012) Prospective, single-arm	22	GB - 5 MGMT Methylated - 13 MGMT Un-methylated - 4 MGMT unknown	FET	BTV: $TBR_X \geq 1.6$ CTV: (T1g+1.5 cm)+edema PTV1: BTV+0 mm PTV2: CTV+0.5 mm	30# SIB: PTV1: 72 Gy PTV2: 60 Gy	Median OS 14.8 months No grade 3+ toxicity, no radionecrosis observed
Miwa et al. (2014) Prospective, single-arm	45	GB - MGMT methylation not reported	MET	$BTV_{Moderate}$: $TBR_X \geq 1.3$ BTV_{High}: $TBR_X \geq 1.7$ $GTV_{PET\text{-}MRI}$: T1g+BTV_{High} CTV: $GTV_{PET\text{-}MRI}$+5 mm PTV: CTV+15 mm+$BTV_{Moderate}$	8# SIB: $GTV_{PET\text{-}MR}$: 68 Gy CTV: 56GY PTV: 40 Gy	Median OS 20.0 months Patterns of failure: - Local 25 % - Distant 14.3 % - CSF dissemination 60.7 % Grade 3 radionecrosis 5 patients (11.1 %); Grade 4 radionecrosis 2 patients (4.4 %)
Navarria et al. (2017) Prospective, single-arm	97	GB - 61 MGMT methylated - 36 MGMT un-methylated	MET	BTV: Clinician-defined CTV1: Cavity+T1g+BTV CTV2: CTV1+T2F PTV1: CTV1+5 mm PTV2: CTV2+5 mm	15# SIB over 15 consecutive days: PTV1: 60 Gy PTV2: 42 Gy	Median OS 15.9 months Grade 1–2 radionecrosis 22.7 % No grade 3+ radionecrosis
Laack et al. (2021) Prospective, single arm	75	GB - 24 MGMT methylated - 39 MGMT un-methylated - 12 MGMT unknown	FDOPA	GTV51: T2F GTV60: T1g+cavity BTV51: $TBR_{Striatum} \geq 1.2$ BTV60: $TBR_{Striatum} > 2.0$ CTV51: (GTV51+BTV51)+10 mm CTV60: (GTV60+BTV60)+10 mm CTV76: GTV60+BTV60 PTV51: CTV51+3 mm PTV60: CTV60+3 mm PTV76: CTV76+3 mm	30# SIB: PTV51: 51 Gy PTV60: 60 Gy PTV76: 76 Gy	MGMT promoter un-methylated (N=39): - Median OS 16.0 months - Median PFS 8.7 months MGMT promoter methylated (N=24): - Median OS 35.5 months - Median PFS 10.7 months Toxicity: - Grade 3 radionecrosis in 13 %

Abbreviations: PET: Positron-emission tomography; GB: Glioblastoma; MGMT: O^6-methylguanine-DNA methyltransferase; MET: ^{11}C-Methionine; FET: ^{18}F-fluoroethyltyrosine; FDOPA: ^{18}F-fluoro-L-dihydroxy-phenylalanine; BTV: Biologic tumour volume; GTV: Gross tumour volume; CTV: Clinical target volume; PTV: Planning target volume; T1g: Gadolinium-enhancing tumour on post-operative MRI; Cavity: Surgical cavity; T2F: T2-hyperintense tumour on post-operative MRI T2-FLAIR sequence; TBR: Tumour-brain ratio; OS: Overall survival; PFS: Progression-free survival; DFS: Disease-free survival; MGMT: O6-Methylguanine-DNA methyltransferase

Laack et al. (2021) reported outcomes from a prospective single-arm phase 2 trial of dose-escalated radiotherapy that enrolled 75 patients with glioblastoma (24 MGMT methylated, 39 un-methylated and 12 MGMT methylation status unknown) who were compared to a historical control cohort of 139 patients treated with standard radiotherapy. For the dose-escalated cohort, radiotherapy was delivered in 30 fractions with dose levels of 76 Gy ($EQD2_{10}$ = 79 Gy), 60 Gy and 51 Gy to the PTV76, PTV60 and PTV51 respectively via simultaneous integrated boost. The 76 Gy volume included the surgical cavity, the gadolinium-enhancing tumour and the high-density BTV (defined on FDOPA-PET by $TBR_{Striatum}$>2.0) with a 3 mm PTV margin but no margin for subclinical disease. The 60 Gy and 51 Gy dose levels included 1 cm CTV margins and 3 mm PTV margins on volumes defined by the combination of MRI and PET as illustrated in Table 4. Progression was determined only when patients both met RANO criteria for progression and had progression as assessed by a clinician. For the 39 patients with MGMT promoter un-methylated tumours, median progression-free survival (PFS) was superior to un-methylated historical controls (median 8.7 vs. 6.6 months, p=0.017) and overall survival was numerically longer but the difference was not statistically significant (median 16.0 vs. 13.5 months, p=0.13). For the 24 MGMT promoter methylated tumours, PFS was no different from historical controls (median 10.7 vs. 9.0 months, p=0.26), however overall survival was significantly longer (median 35.5 vs. 23.3 months, p=0.049). Grade 3 CNS necrosis occurred in 13 % of patients.

Of these four prospective studies, two (Laack et al., 2021; Miwa et al., 2014) appear to indicate a benefit to overall and/or progression-free survival with dose-escalated PET-guided radiotherapy compared to historical studies or controls, whilst two (Navarria et al., 2017; Piroth et al., 2012) do not. A possible explanation lies in both the dose and target volumes used. The Piroth (Piroth et al., 2012) study escalated dose to only the PET-avid volume, whereas the other three trials also included the residual gadolinium-enhancing tumour on MRI in the dose-escalated target volume. The dose-escalated target volumes in both the Navarria et al. (2017) and Piroth et al. (2012) trials also received a lower $EQD2_{10}$ (70 Gy and 74 Gy respectively) compared to the Laack et al. (2021) and Miwa et al. (2014) trials (79 and 105 Gy respectively) which reported more promising survival outcomes. The exceptionally low rate of local recurrence (25 %) found in the Miwa trial, which used the highest $EQD2_{10}$ was particularly notable and suggests the tantalising hypothesis that optimised radiotherapy dose and target volumes may provide a pathway to local control in glioblastoma. Importantly none of the trials demonstrated an unacceptable rate of radionecrosis, although the 4.4 % rate of grade 4 radionecrosis in the Miwa trial is of some concern. Our interpretation is that amino acid PET-guided dose-escalation is a promising strategy appropriate for further prospective investigation. Based on the existing literature, future studies should consider inclusion of both the BTV and gadolinium-enhancing tumour in the dose-escalated volume and treating this to a high $EQD2_{10}$ (in the range of 79–105 Gy).

Limitations include that all studies were relatively small and none were randomised, conclusions are hypothesis-generating only. Caution is required in extrapolation to tumours other than glioblastoma, which was the diagnosis in all patients across the four studies. Quality assessment for these studies using the Downs and Black checklist (Downs and Black, 1998) is provided in supplementary appendix F.

4. Commentary and future perspectives

This review identified 21 studies that examined the role of amino acid PET in radiotherapy planning for gliomas. The vast majority of cases in the included studies were patients with glioblastoma. Four categories of study were identified. The fundamental insight from the studies that compared PET- to MRI-derived target volumes was that these are complementary with the implication that optimal target volume delineation would employ use of both imaging modalities. Studies that compared sites of PET uptake to location of subsequent failure consistently found the former to be a strong predictor of the latter, providing some validation of the rationale to include the BTV in target volume delineation. In the three studies (Seidlitz et al., 2021; Lundemann et al., 2017; Munck Af Rosenschold et al., 2019) that reported outcomes of PET-guided radiotherapy at standard doses there was no signal to suggest improved survival outcomes or alteration from the usual predominantly central recurrence pattern, perhaps implying that 60 Gy in 30 fractions remains an insufficient dose for tumour control even when all high-density tumour is reliably covered. However, the study conducted by Seidlitz et al. (2021) did provide early evidence to suggest that when amino acid PET is incorporated into radiotherapy planning, it may be feasible to reduce the margin for subclinical disease to 5 mm (compared to the 15–20 mm currently recommended by consensus guidelines (Niyazi et al., 2023; Kruser et al., 2019) without apparent detriment to oncologic outcomes. This may ultimately reduce the toxicity of treatment. Finally, the studies that reported outcomes of PET-guided dose-escalated radiotherapy warrant nuanced interpretation. Although there was no apparent signal for improved survival in two of the four studies (Navarria et al., 2017; Piroth et al., 2012), one reading is that this may have been the result of insufficient biologically-effective dose and, in one of the trials, not including the gadolinium-enhancing tumour in the dose-escalated volume. The two trials (Laack et al., 2021; Miwa et al., 2014) that used a higher biologically-effective dose and included both the BTV and gadolinium-enhancing tumour volume within the highest dose volume both provided promising signals for improved tumour control and possibly improved survival. Clearly this evidence is hypothesis-generating only. Larger randomised prospective trials are warranted to address this question directly.

Several biopsy validation studies complement the findings of studies in this review. The study with the largest number of evaluable biopsy specimens (284 samples from 23 patients) using FET PET was performed by Harat et al. (2023). The authors demonstrated that regions of gadolinium enhancement and FET hotspot (positive predictive value (PPV) = 96 %), regions of gadolinium enhancement outside FET hotspot (PPV = 72.7 %) and regions of FET hotspot without gadolinium enhancement (PPV 91.8 %) were all highly likely to contain high-grade tumour on biopsy. This is consistent with prior studies using FET PET (Pauleit et al., 2005; Song et al., 2020) and similar studies with similar findings have also been reported for MET (Mosskin et al., 1989), FDOPA (Pafundi et al., 2013) and AMT (Kamson et al., 2013) tracers. These studies support the conclusion that optimal target volumes should cover both the gadolinium-enhancing and PET-avid tumour volumes.

Studies identified in this review used heterogenous methods to delineate the BTV. The majority of studies used TBR, however both the threshold TBR to define tumour and the method used to delineate the normal brain (the denominator in the tumour-brain-ratio) was not consistent. The most commonly used TBR thresholds reflect the outcomes of biopsy validation studies. For FET, most referenced is the seminal work by Pauleit et al. (2005), which found that a TBR≥1.6 threshold provided the optimal sensitivity (92 %) and specificity (81 %) for tumour. Similarly for FDOPA PET, the work by Pafundi et al. (2013). proposed TBR≥2.0 as the threshold for high density/cellularity tumour. For these TBR thresholds to remain valid, presumably the method to delineate normal brain should reflect that used in the original biopsy study. For FDOPA the Pafundi biopsy-validation study used the $TBR_{Striatum}$ method described in Section 2.5 of this review and this was implemented in the Laack et al. (2021) prospective dose-escalation trial. For FET, more recent studies (Dissaux et al., 2020; Allard et al., 2022; Fleischmann et al., 2020) adopted recommendations from Unterrainer et al. (2017) who compared several methods to define the normal brain background and found the $TBR_{CresVOI}$ approach (also described in Section 2.5 of this review) to be the most reproducible. This method has also been recommended by a recent guide (Holzgreve et al., 2024) for FET-PET based target volume delineation.

Two of the dose-escalation studies (Laack et al., 2021; Miwa et al., 2014) included in this review, as well as a smaller FDOPA study (Kazda et al., 2018) that did not meet eligibility criteria (due to sample size <20) also used a second TBR threshold to aid (in conjunction with the T2-FLAIR abnormality in one of the studies) in definition of a lower-dose target volume. The role of T2-FLAIR sequences in defining target volumes has been controversial, with divergent consensus guideline recommendations (Niyazi et al., 2023; Kruser et al., 2019), due primarily to the challenges in distinguishing tumour from edema and other pathology. For example Harat et al. (2023) found that, outside of areas of gadolinium enhancement and FET hotspot, the T2-FLAIR abnormality carries moderate specificity (29.6 %) and positive predictive value (47.2 %) for tumour. Biopsy studies (Harat et al., 2023; Pafundi et al., 2013) have found that individual tumours contain higher grade / higher density regions as well as lower grade / lower density regions that correlate to corresponding (albeit overlapping) TBR thresholds on amino acid PET. Moderate TBR thresholds therefore may have the potential to aid in delineating regions of lower grade/lower density tumour. For FDOPA PET, Pafundi et al. (2013) proposed thresholds of TBR >2.0 and TBR >1.2 for high and low grade/density disease respectively and these were implemented in the Laack et al. (2021) prospective trial (described in Section 3.4). For FET PET, Harat et al. (2024) recently found that the choroid plexus performed better as the denominator for the TBR than normal brain for predicting positive biopsies, and that a tumour-plexus threshold of 1.0 using early-acquisition (10 minute) images provided optimal performance for biopsies taken from the non-enhancing T2-FLAIR abnormality (AUC 0.75, sensitivity 0.90, specificity 0.61). Inclusion of a PET-defined (possibly in conjunction with the T2-FLAIR abnormality) lower dose volume to encompass lower density tumour is a potentially attractive concept that but one that requires further prospective validation.

All the included studies used static PET parameters (predominantly TBR) to define the BTV. However single time-point SUV values discard much of the information provided by the time-activity curve (TAC) that can be generated for a region of interest or each voxel in a dynamic acquisition (Verger et al., 2021). Dynamic PET parameters such as time-to-peak, post-peak TAC slope, 'curve type' as well as those from kinetic models such as Patlak analysis have been found to improve the accuracy of predicting glioma grade and molecular features (Albert et al., 2016b; Verger et al., 2018), distinguishing treatment-related changes from tumour recurrence in the post-adjuvant treatment setting (Galldiks et al., 2015) (Alkonyi et al., 2012) and assessing treatment response (Prather et al., 2022). Although often evaluated for a region of interest, dynamic parameters can also be evaluated in a voxelwise-manner (Vomacka et al., 2018) to produce maps similar to the SUV map that radiation oncologists are most familiar with. Conceivably, dynamic parameters may offer improved discrimination between normal tissue and tumour tissue for target delineation purposes, however this is yet to be validated.

Studies included in the systematic component of this review used either MET, FET or FDOPA tracers. AMT PET was not represented in the studies that met the inclusion criteria. A single centre has driven investigation of this tracer in the setting of gliomas including in the pre-operative (Juhasz et al., 2012; Jeong et al., 2015) and post-adjuvant therapy (Alkonyi et al., 2012) settings. This group has also compared AMT PET-defined radiotherapy target volumes with those defined using MRI (Christensen et al., 2014) and compared the AMT PET-defined BTV to locations of failure (Bosnyak et al., 2016). These studies were excluded from the systematic component of this review due to small sample size (<20 patients) but had findings concordant with those for other tracers that met the inclusion criteria. To our knowledge there are no published studies that report outcomes of AMT PET-guided radiotherapy.

It should also be noted that amino acid PET is not the only advanced imaging technique that may offer complementary information to standard MRI sequences for radiotherapy planning. Advanced MRI techniques such as diffusion-weighted imaging, perfusion-weighted imaging, diffusion-tensor imaging and magnetic resonance spectroscopy (Castellano et al., 2021; Brighi et al., 2023) as well as hypoxia imaging (e.g. FMISO-PET and oxygen-enhanced MRI) (Brighi et al., 2023; Leimgruber et al., 2020) are also active areas of research. The optimal combination of these imaging modalities to guide target volume delineation is yet to be defined.

The vast majority of patients in studies included in this review were patients with IDH-wildtype glioblastoma. IDH-mutant tumours are more difficult to study including due to their markedly lower incidence and longer time-frame to progression and death hence requiring substantially longer follow up for patterns of failure and clinical outcomes research. Nevertheless, optimisation of radiotherapy target volume delineation remains an important goal as due to long survival times minimisation of late treatment toxicity is imperative. Dedicated studies in this group of patients are called for to validate whether amino acid PET may facilitate margin reduction in this setting without introducing geographic miss.

Several relevant trials are ongoing. The Trans-Tasman Radiation Oncology Group (TROG) 18.06 FIG trial (Koh et al., 2023), is a multi-centre trial in which investigators are blinded to the FET PET result at the time of radiotherapy planning and will provide further information regarding the impact of FET PET on radiotherapy target volumes for glioblastoma (amongst other objectives). GlioMET (Lakomy et al., 2024) (NCT05608395), is a single-centre single-arm prospective trial recruiting patients with glioblastoma who experience rapid early progression within 6 weeks of surgery. Patients will undergo MET PET which will be used to define radiotherapy target volumes in conjunction with MRI using standard doses with the primary outcome being progression-free survival. The PRIDE trial (Bodensohn et al., 2024) (NCT05871021; NOA-28; ARO-2022–12) is a single-arm phase 2 multi-centre trial that combines dose-escalated radiotherapy up to 75 Gy in 30 fractions with concurrent bevacizumab. The study population is patients with glioblastoma, IDH wild-type, MGMT un-methylated. Target volume delineation will use both MRI and FET PET.

5. Conclusion

Amino acid PET is a promising tool to complement standard MRI sequences for the purpose of radiotherapy target volume delineation in gliomas. Existing data is hypothesis-generating and suggests a role in facilitating both margin reduction and dose-escalation. The evidence to date largely pertains to glioblastoma, focused research for IDH-mutant tumours is warranted.

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Patrick J Horsley: Conceptualisation, methodology, investigation, writing – original draft **Dale L. Bailey**: Conceptualisation, methodology, writing – review and editing, supervision **Geoffrey Schembri:** Conceptualisation, writing – review and editing **Edward Hsiao:** Conceptualisation, writing – review and editing **James Drummond:** Conceptualisation, writing – review and editing **Michael F Back:** Conceptualisation, methodology, writing – review and editing, supervision

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.critrevonc.2024.104552.

Data Availability

Research data are not available at this time

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