Molecular Imaging and Advanced MRI findings following Immunotherapy in Patients with Brain Tumors

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ABSTRACT

Introduction: Currently, immunotherapy using vaccination strategies or oncolytic virus approaches, cell-based immunotherapy, and the blockade of immune checkpoints are under evaluation in patients with brain cancer. We here summarize clinically significant imaging findings such as treatment-related changes detected by advanced neuroimaging techniques following the most suitable immunotherapy options currently used in neuro-oncology. We, furthermore, provide an overview of how these advanced imaging techniques may help to overcome shortcomings of standard MRI in the assessment and follow-up of patients with brain cancer.

Areas covered: The current literature on neuroimaging for immunotherapy in the field of brain tumors, with a focus on gliomas and brain metastases is summarized.

Expert Commentary: Data suggest that imaging parameters primarily derived from amino acid PET, diffusion- and perfusion-weighted MRI, or MR spectroscopy are particularly helpful for the evaluation of treatment response and provide valuable information for the differentiation of treatment-induced changes from actual brain tumor progression following various immunotherapy approaches.

KEYWORDS

FET PET; ADC; vaccine; dendritic cells; checkpoint inhibitors; CAR-T cells; oncolytic virus immunotherapy

1. INTRODUCTION

Following various immunotherapy options for the treatment of brain cancer, imaging findings on conventional MRI after injection of a contrast agent can be highly variable, and the interpretation concerning the differentiation of treatment response from tumor progression is often difficult [1]. This uncertainty may negatively affect the assessment of response to treatment [2]. Specifically, inflammation triggered by immunogenic reactions and intratumoral infiltrates, including cytotoxic T cells, may lead to MR imaging findings that suggest tumor progression. Histopathology typically shows inflammatory cells [3], but not mitotically active tumor cells. On the other hand, following immunotherapy, first progressive imaging changes on anatomical MRI might represent an actual tumor progression that ultimately becomes controlled by a delayed immune response [1]. Although the immunotherapy Response Assessment in Neuro-Oncology (iRANO) Working Group recently recommended both clinical and standard MRI criteria to overcome the clinical problem of immunotherapy-related pseudoprogression [1], available immunotherapy options seem to impose demands on brain imaging beyond those offered by routine MRI techniques.

We here summarize clinically relevant imaging findings obtained from advanced neuroimaging techniques following the most relevant immunotherapy options currently used in neuro-oncology. Besides, we provide an overview of how these advanced imaging techniques overcome the shortcomings of standard MRI.

2. MOST IMPORTANT NEUROIMAGING TECHNIQUES

Contrast-enhanced anatomical MRI is exceptional in providing detailed structural information of the brain anatomy and intracranial neoplasms, although its specificity is comparatively poor [4-8]. Advanced MR techniques, including apparent diffusion

coefficients (ADC) obtained by diffusion-weighted MR imaging (DWI), perfusion-weighted MR imaging (PWI) techniques such as dynamic contrast-enhanced (DCE) or dynamic susceptibility contrast (DSC) PWI as well as arterial spin labeling (ASL), and proton MR spectroscopy (^{1}H-MRS) [2,9,10], yield additional information regarding tumor biology, especially at the molecular, physiological, and functional level.

The most relevant PET tracers in neuro-oncology are radiolabeled amino acids, especially O-(2-[^{18}F]-fluoroethyl)-L-tyrosine (FET), [^{11}C]-methyl-L-methionine (MET), and 3,4-dihydroxy-6-[^{18}F]-fluoro-L-phenylalanine (FDOPA), because of their well documented high clinical value for the differentiation of treatment-related changes from actual tumor progression, and for the evaluation of treatment effects [5,11,12]. In brain tumor patients with a preexisting disruption of the blood-brain barrier (e.g., patients with brain metastases), the PET tracer 3´-deoxy-3´-[^{18}F]-fluorothymidine (FLT, an analogue to the nucleoside thymidine), developed to assess cellular proliferation by tracking the thymidine salvage pathway, is also of great interest [13].

Besides other techniques, the latter mentioned advanced MR techniques and PET tracers have predominantly been used to monitor treatment effects related to various immunotherapy strategies in patients with brain tumors. Table 1 provides an overview.

3. IMMUNOTHERAPY STRATEGIES AND NEUROIMAGING FINDINGS

3.1 Neuroimaging Findings following Vaccine Immunotherapy

Vaccine immunotherapy strategies aim to load antigen-presenting cells (APC) with tumor antigens for major histocompatibility complex (MHC) presentation to T-cells. After encountering T-cells with tumor antigen-primed APCs, these T-cells clonally expand and mediate an immune response against the antigen-bearing tumor cells,

thereby destroying tumor cells, either directly through cytolytic mechanisms or indirectly through cytokines [14]. Vaccine immunotherapies employed in neuro-oncology basically include glioma-specific peptide vaccines (e.g., rindopepimut) and vaccination strategies with dendritic cells (a heterogeneous group of functionally specialized APCs that initiate immune responses). In clinical trials, single glioma-specific mutant proteins such as the isocitrate dehydrogenase 1 (IDH1), the epidermal growth factor receptor variant III (EGFRvIII) [15,16], or a panel of tumor-associated antigens have been targeted [17].

A prospective study assessed whether serial ADC metrics obtained from diffusion-weighted MRI following peptide-based vaccination targeting three glioma-associated antigens (IL13Rα2, EphA2, and survivin) might help to distinguish pseudoprogression from actual tumor progression in pediatric patients with diffuse intrinsic pontine gliomas [18]. The rate of pseudo-progressive patients in that study was 19% (4 of 21 children). ADC metrics such as fractional decreased ADCs were significantly different in patients with pseudoprogression [18]. Furthermore, a prolonged median survival was observed in pseudo-progressive patients compared to those without pseudoprogression (19.1 vs. 12.5 months).

Further studies in glioblastoma patients evaluated imaging parameters derived from perfusion-weighted MRI to differentiate immunotherapy-induced inflammatory MR imaging changes from progressive glioblastoma tumor growth following dendritic cell vaccination [19] or autologous tumor cell vaccination [20]. Relative cerebral blood volumes obtained from perfusion-weighted MRI seem to have added relevant diagnostic information, but the number of examined patients in these studies was relatively low (< 10 patients).

Regarding MR spectroscopy, a ^{1}H-MRS study in two glioblastoma patients, multimodally treated with radiotherapy, temozolomide chemotherapy, and intralesional interleukin-4 toxin conjugate immunotherapy after resection, observed in contrast to the progressive MRI at follow-up a low choline concentration, suggesting that the enhancement on MRI did not reflect vital tumor tissue [21]. Clinical and radiological follow-up confirmed these imaging findings.

Amino acid PET has also been used for the evaluation of vaccine immunotherapy effects. Using MET PET, a prospective study with 14 recurrent glioblastoma patients treated with the WT1 peptide-based vaccine suggested that metabolic responders (threshold, 5% decrease at follow-up on voxel-wise parametric response maps) showed a significant correlation with the overall survival [22]. In contrast, anatomical MRI was not helpful for the evaluation of treatment effects. Furthermore, FET PET has recently been used in glioblastoma patients to follow immunotherapy with dendritic cell vaccination [23]. The results of that study suggest that FET PET has a valuable impact on the diagnosis of post-therapeutic MRI changes related to inflammation triggered by immunogenic reactions (Fig. 1).

Furthermore, PET probes that specifically aim at distinguishing immune cells from cancer cells have prompted considerable clinical interest when evaluating therapeutic immune responses. In particular, the efficacy of PET probes for deoxycytidine kinase, such as [^{18}F]-clofarabine (CFA PET), has been assessed for differentiating immune-inflammatory responses from other sources of contrast-enhancement on MRI, i.e., actual tumor progression [24]. In a series of three glioblastoma patients treated with dendritic cell vaccination in combination with the immune checkpoint inhibitor

pembrolizumab, CFA PET noninvasively localized and quantified the immune responses induced by immunotherapy [24].

3.2 Neuroimaging Findings following Cell-based Immunotherapy

Cell-based immunotherapy aims at triggering the patient's immune system against cancer by expanding and enhancing the functions of effector T-cells. One approach is to isolate T-cells from the patient or an HLA-matched donor and to expand and activate *in vitro* these cells via mitogenic stimulation. Expanded cells are re-infused to the patient to promote anticancer activity, potentially leading to tumor shrinkage.

Another cell-based immunotherapy approach employs effector immune cells that are genetically modified. Typically, T-cells were genetically modified to express a chimeric antigen receptor (CAR) that recognizes a prespecified tumor antigen [25]. Before modification, precursor cells from the immune system are collected from healthy donors or patients. These *ex vivo* genetically modified cells are then infused into the patient as effector immune cells that recognize and subsequently destroy specific types of antigen-presenting tumor cells, independent from a tumor antigen-presentation by the MHC. Recently, a case report suggests improvement of conventional MRI findings following CAR T-cell therapy targeting the tumor-associated antigen interleukin-13 receptor alpha 2 in a glioblastoma patient [26].

Regarding patients undergoing cell-based immunotherapy monitored by advanced neuroimaging techniques, the number of available studies is currently low. A recent study used multiparametric MRI parameters obtained from diffusion tensor imaging, DSC-PWI, and ^{1}H-MRS for treatment response assessment of anti-EGFRvIII CAR-T cell therapy [27] in 10 glioblastoma patients. Based on a subset of the obtained

parameters, the authors computed progression probabilities using logistic regression models and observed that this approach might help to evaluate CAR-T related therapeutic effects early after treatment initiation (i.e., after 1, 2, and 3 months) [27]. Another study with 7 recurrent high-grade glioma patients suggested that PET using 9-[4-[^{18}F]fluoro-3-(hydroxymethyl)butyl]guanine (FHBG) can detect reporter gene expression in CAR-engineered cytotoxic T-lymphocytes [28]. Thus, this approach seems to be helpful to locate and to quantify therapeutic CAR-T cells within the recurrent tumor, which is difficult by conventional MRI alone.

3.3 Neuroimaging Findings following Checkpoint-Inhibitor Immunotherapy

Checkpoint inhibitor immunotherapy has been designed to overcome immunosuppressive cell-cell signaling mechanisms between immune effector cells and tumor cells. This form of immunotherapy uses specific monoclonal antibodies that bind to immune checkpoints such as the programmed cell death protein 1 (PD-1) or the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on immune cells, as well as antibodies that target the programmed cell death ligand 1 (PD-L1) on tumor cells to prevent the inhibition of cytotoxic T-lymphocyte activity by tumor cells [29].

Qin and colleagues evaluated ADC volumes obtained from diffusion-weighted MRI for the prediction of the benefit of a treatment using checkpoint inhibitors in 10 patients with recurrent glioblastoma enrolled in clinical trials [30]. The average time on trial for the benefit group was longer than 6 months (194 days), whereas patients without therapeutic benefit were on trial less than three months (81 days). In contrast to anatomical MRI, the authors found that stabilization or decrease of ADC volumes at follow-up appear to better predict therapeutic benefit from checkpoint inhibitors.

A small prospective PET imaging study suggested in a subset of patients with melanoma brain metastases treated with checkpoint inhibitors or targeted therapy that metabolic responders may have improved survival of more than 12 months after therapy initiation. Importantly, FLT PET responders showed a reduction of the proliferative tumor activity despite unchanged findings on standard MRI [31].

A further study suggested that amino acid PET using FET has the potential to identify pseudoprogression in patients with melanoma brain metastases [32] treated with the checkpoint inhibitor ipilimumab. In that small pilot study (n=5 patients), imaging findings were correlated with the clinical course after the initiation of the checkpoint inhibitor therapy. In the case of pseudoprogression, FET PET showed, in contrast to progressive MRI findings, only low tracer uptake and a favorable outcome with a survival longer than 6 months. In a larger series of patients (n=31) with brain metastases (n=74) secondary to melanoma or non-small cell lung cancer treated with checkpoint inhibitors or targeted therapy in combination with radiotherapy, FET PET provided important diagnostic information in terms of both response assessment and diagnosis of pseudoprogression related to inflammation triggered by immune responses [2,33].

The steadily increasing use of checkpoint inhibitors has also prompted the development of PET probes to quantify non-invasively the expression of immune targets such as PD-1 or PD-L1 [9]. Initial animal [34,35] and first-in-human studies [36] suggest that these PET probes may be of value for response assessment. In the latter study, all extracranial tumors of 13 patients with non-small lung cancer exhibited increased uptake using the PD-1 and PD-L1 PET tracer in whole-body PET/CT scans. Furthermore, tracer accumulation in selected, but not in all brain metastases was

observed (2 of these 13 patients had brain metastases). This finding was most probably related to the lesion size, low CNS tracer penetration, and/or variation in PD-1 and PD-L1 expression [36].

3.4 Neuroimaging Findings following Oncolytic Virus Immunotherapy

Oncolytic virus immunotherapy uses attenuated and immunogenic viruses to infect tumor cells and generate a *de novo* or boost a pre-existing immune response (e.g., responses mediated by natural killer cells or macrophages) [37]. Oncolytic viruses are genetically modified to reduce virulence for non-neoplastic cells and enhance tumor tropism. These viruses selectively replicate in tumor cells and can directly lyse the tumor cells, thereby releasing additional tumor antigens that can trigger further immune responses. This antigen release promotes a proinflammatory environment and a subsequent immune activation against remaining malignant cells. Currently, in neuro-oncological patients, the evaluation of several viral immunotherapy strategies is on the way [38,39].

Besides imaging of reporter gene expression for the location and quantification of infected tumor cells following oncolytic virus immunotherapy [40], data on the value of advanced neuroimaging techniques for the differentiation of treatment-related changes and actual tumor progression as well as for treatment response assessment remain currently scarce and controversial [41].

4. CONCLUSIONS

The present literature provides evidence that amino acid PET and newer PET probes, metrics obtained from diffusion- and perfusion-weighted MRI, or from MR spectroscopy are able to monitor effects induced by various forms of immunotherapy and have the

potential to provide valuable additional diagnostic information for the diagnosis of treatment-related changes following immunotherapy.

5. EXPERT OPINION

When evaluating the imaging findings related to various immunotherapy approaches, one should keep in mind that at present the results of most advanced imaging studies are based on a low number of patients and, additionally, have frequently an explorative character. Furthermore, advanced imaging protocols are not yet sufficiently standardized for routine clinical use, thereby also hampering comparability of study results. Notwithstanding, current efforts aim at standardizing neuroimaging protocols. Major medical societies for nuclear medicine and neuro-oncology, i.e., the EANM (European Association of Nuclear Medicine), the SNMMI (Society of Nuclear Medicine and Molecular Imaging), the Response Assessment in Neuro-Oncology group, and the EANO (European Association of Neuro-Oncology), have published in 2019 joint practice guidelines for PET imaging in patients with brain tumors [42]. These guidelines are essential for the comparability of study results and to reach consensus across studies and institutions regarding acquisition parameters. Furthermore, besides practice guidelines for standard MRI [43], additional recommendations have also been reported for clinical applications using advanced MRI techniques [44,45].

Overall, little data is available concerning the evaluation of imaging findings following immunotherapeutic approaches for brain tumors using advanced imaging techniques. To confirm and to further evaluate the reported encouraging imaging findings, which suggest a relevant clinical impact of advanced imaging for assessing treatment response, further (multi-centric) studies with higher number of patients are warranted

in which standardized imaging protocols as well as post-processing procedures are utilized.

To validate imaging findings following immunotherapy obtained by these advanced neuroimaging techniques, neuropathological confirmation of imaging findings, preferentially by the extraction of tissue samples using stereotactic biopsy, is necessary and should be performed more frequently within the next years. In order to overcome contraindications or possible ethical issues related to stereotactic biopsy, the investigation of liquid biopsies as a surrogate for tumor tissue seems to be a promising alternative diagnostic method for the detection of tumor-associated markers (e.g. circulating tumor cells, cell-free tumor DNA) in body fluids such as cerebrospinal fluid or blood plasma [46].

ARTICLE HIGHLIGHTS

- Vaccine immunotherapy, cell-based immunotherapy including CAR-T cells, checkpoint inhibitor immunotherapy, and virus immunotherapy are the most relevant immunotherapy approaches
- Amino acid PET, diffusion- and perfusion-weighted MRI, or MR spectroscopy are particularly helpful for the evaluation of treatment response following immunotherapy
- Advanced neuroimaging techniques may provide valuable information for the differentiation of treatment-induced changes from actual brain tumor progression related to immunotherapy

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Declaration of Interest

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Table 1: Overview of main results obtained from advanced MR and PET imaging in patients undergoing immunotherapy

	ADVANCED MRI				**PET IMAGING**		
	DWI	***PWI***	***MRS***	***Other techniques***	***Amino acid PET***	***FLT PET***	***Other PET probes***
Vaccine Immunotherapy	ADC metrics of value for TRC diagnosis [18]	rCBV ratios obtained from DSC-PWI of value for TRC diagnosis [19,20]	Choline concentrations obtained from ^{1}H-MRS of value for TRC diagnosis [21]	n.a.	MET of value for response assessment [22], FET of value for TRC diagnosis [23]	n.a.	CFA PET targets immune cells, potentially of value for TRC diagnosis [24]
Cell-based Immunotherapy	n.a.	n.a.	n.a.	Multiparametric MRI of value for response assessment [27]	n.a.	n.a.	FHBG PET helpful to locate and quantify therapeutic CAR-T cells [28]
Checkpoint Inhibitor Immunotherapy	ADC metrics of value for response assessment [30]	n.a.	n.a.	n.a.	FET of value for response assessment and TRC diagnosis [2,12,32,33]	FLT of value for response assessment [31]	PD-1 or PD-L1 PET of value for response assessment, but uptake only in selected tumors [36]
Oncolytic Virus Immunotherapy	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	FHBG and FIAU PET potentially helpful for imaging of reporter gene expression, but controversial results [40,41]

ADC = apparent diffusion coefficient; **CFA** = [^{18}F]-clofarabine; **DSC** = dynamic susceptibility contrast PWI; **FDOPA** = 3,4-dihydroxy-6-[^{18}F]-fluoro-L-phenylalanine; **FET** = O-(2-[^{18}F]-fluoroethyl)-L-tyrosine; **DWI** = diffusion-weighted imaging; **FIAU** = 2'-fluoro-2'-deoxy-1b-D-arabino-furanosyl-5-iodo-uracil labeled with I-124; **FHBG** = 9-[4-[^{18}F]fluoro-3-(hydroxymethyl)butyl]guanine; **FLT** = 3´-deoxy-3´-[^{18}F]-fluorothymidine; **MET** = [^{11}C]-methyl-L-methionine; **^{1}H-MRS** = proton MR spectroscopy; **n.a.** = not available; **PWI** = perfusion-weighted imaging; **rCBV** = relative cerebral blood volume; **TRC** = treatment-related changes associated with inflammatory immune reactions

FIGURE LEGEND

Figure 1: Pseudoprogressive findings in a glioblastoma patient following immunotherapy using dendritic cell vaccination in combination with chemoradiation with temozolomide (image modified from Schmitz et al. [23]). In comparison to baseline imaging (top row), the contrast-enhanced and T2-weighted MRI at follow-up (bottom row) suggested tumor progression. In contrast, serial FET PET imaging revealed a decline of metabolic activity (27%) as assessed by tumor-to-brain ratios indicating treatment-related changes. Neuropathological examination of extracted tissue samples revealed reactive and necrotic tissue as a result of the therapy and no signs of tumor progression.

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