The role of advanced MRI techniques to differentiate glioblastoma pseudo-progression and tumor-progression during immunotherapy.

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Learning objectives

The purpose of this review is to outline the current research into MRI assessment for patients undergoing immunotherapy.
Background

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults [1], and carries a grim prognosis. Infiltrative nature of diffuse gliomas makes it difficult to eliminate microscopic disease despite macroscopic gross-total resection. Recurrence of GBM is inevitable and the median overall survival (OS) time of GBM patients receiving the standard treatment, which consists of maximal safe resection followed by radiation ad adjuvant temozolomide, is about 14-16 months [2,3]. At recurrence no standard approach has been established (further surgery, re-irradiation, chemotherapy, antiangiogenic therapy) and despite advances in treatment for GBM, the survival of patients has not significantly improved over the past two decades.

The central nervous system (CNS) has been traditionally considered an immune privileged system; however it has been proved that immune cells can cross the blood brain barrier (BBB) to gain access to the brain parenchyma and can leave the CNS to reach the cervical lymph nodes. Considering that the immune system has access to the brain and that GBM expresses multiple tumor antigens that can be targeted by immunotherapeutic approaches, the development of immunotherapy has gained considerable interest over the last decade [4].

Novel immunotherapeutic strategies being investigated to treat glioblastoma can be broadly divided into four major classes: active immunotherapy, passive immunotherapies immune-modulatory strategies and adoptive strategies [5] and include: vaccination therapy targeted against specific tumor antigens or whole tumor lysate, adoptive cellular therapy with cytotoxic T lymphocytes, chimeric antigen receptors and bi-specific T-cell engaging antibodies to bypass major histocompatibility complex restriction, aptamer therapy allowing a more efficient target delivery, and checkpoint blockade to release the tumor-mediated inhibition of the immune system.

Initial data show OS 23 to 38 months in GBM patients treated by vaccines [6]. Upcoming clinical trial results will clarify the efficacy of different cancer immunotherapy approaches in gliomas and other cancers with poor prognosis. Due to the heterogeneity of glioblastoma and its ability to mutate throughout the disease course, multiple treatment strategies of immunotherapy, in addiction with conventional therapy, will be most likely to succeed moving forward.

Efficacy of therapy is assessed by clinical examination and magnetic resonance imaging (MRI) features.

Pseudo-progression, i.e. imaging worsening not closely dependent on tumoral modifications, occurs in about 20% of patients within three months after radio-chemotherapy [7-9] but pseudoprogression after immunotherapy seems to occur more often and the timeframe for immunotherapy associated pseudo-progression remains to be defined, potentially differing by the class of immunotherapy given. The main issue is that
transient treatment-related changes mimicking progressive disease are being recognized on follow-up imaging [10]. Considering pseudo-progression as true tumor progression (and conversely) could lead to an inappropriate change in therapy and errors in assessing the efficacy of novel treatments.

Effective immune response might need time to evolve and early imaging might reflect true progressive disease; on the other hand, inflammatory response might mimic radiological features of tumor progression with increased enhancement and edema

To address these issues in 2015 the iRANO committee redefined the response assessment criteria for patient with neuro-oncological malignancies, undergoing immunotherapy: the "limbo" window when radiologic worsening does not allow the suspension of immunotherapy, has been widened to six months, then progression or pseudoprogression could be backdated [11].
Findings and procedure details

**Conventional MRI (cMRI)**

Several criteria have been proposed in literature and used in clinical trials along the last two decades to assess response to therapy in gliomas.

The standard method for the radiological evaluation of GMB is based on contrast-enhancing T1-weighted imaging and T2/FLAIR sequences.

Enhancement on T1 weighted imaging reflects non-specific impairment of the blood brain barrier; a reduction or lack of enhancement can be observed in real tumor response but also under antiangiogenic therapy, reflecting pseudoresponse.

T2 and FLAIR hyper-intensity can be associated to tumor infiltration, edema, ischemia, gliosis, demyelination or post-actinic alteration.

The iRANO committee, integrating guidance for progressive imaging findings from the irRC [12] with RANO criteria [13], redefined the response assessment criteria for patients with brain tumors undergoing immunotherapy providing novel iRANO criteria [11]: in patients with early progressive imaging findings (i.e. # 25% increase in the sum of biperpendicular diameters of enhancing tissue, including the development of new lesions, or substantial worsened T2/FLAIR) within the first 6 months of immunotherapy regimen without substantial neurological decline, therapy should be continued and confirmation of radiographic progression by follow-up imaging should be sought 3 months after the initial radiographic evidence of progressive disease (Fig. 1)

The iRANO criteria for GBM are summarized in Table 1 (Fig. 2)

Conventional MRI alone can be misleading in assessing therapy response, providing limited information on tumor physiopathology and on the status of perilesional tissue and additional research into advanced imaging modalities is necessary to identify pseudo-progression or true progression during immunotherapy.

**Delayed-contrast MRI: TRAMs (Treatment Response Assessment Maps)**

In recent years, Zach and Mardor proposed a new method to distinguish active tumor and treatment induced effects [14]. This method consists on the acquisition of two high-resolution 3D T1- weighted sequences in the same MR session, 3 and 75 minutes after the injection of the contrast medium and in the following subtraction of the first one from the second. The map obtained is then colour-coded to differently represent areas in which contrast accumulates during time (red-coded) and regions in which contrast is rapidly cleared from the tissue (blue-coded). The maps obtained are defined as Treatment Response Assessment Maps (TRAMs).
Differently from the other methodologies, TRAMs are not user-dependent, less acquisition dependent, i.e. they need only a good quality 3D T1 sequence, and relatively simple to be acquired. The only inconvenience is that patient has to wait longer outside the scanner.

The rationale for applying TRAMs analysis to immunotherapy is the differentiation between tumor and "other tissue" and to discriminate the co-existing components within a mixed lesion and to monitor the volumetric modifications of each component along the treatment course.

Preliminary data show different components in enhancing lesions during immunotherapy with dendritic cells. Besides that, longer follow up in responder vs non-responder patients is needed to understand if this approach can define immuno-mediated pseudoprogression as it does in post-radiotherapy follow-up

**Diffusion weighted imaging (DWI)**

Apparent diffusion coefficient (ADC) values are inversely correlated with cellularity so that low ADC is expected in the hyper cellular enhancing parts of GBM as well in the non-enhancing but densely cellulated glioma volumes.

Pseudo-progressive tissue is supposed to have less cells than GBM tissue and to be associated with higher ADC levels. Anyway during immunotherapy an inflammatory reaction can be associated with both edema (reduced cell density - high ADC value) and immune cell accumulation (increased cell density - low ADC value) as reported in other brain inflammatory diseases such as encephalitis or lymphomas.

As a result, non-homogeneous [15-16] ADC patterns are most often observed in this kind of treatment and the rule of low ADC univocally linked to HGG may be biased.

In a pilot study on eight patients treated with dendritic cells immunotherapy ADC values were used as a potential marker to differentiate between immunotherapy-induced inflammatory response and recurrent GBM growth [17]. In the study ADC values were lower in enhancing lesions at progression compared to stable diseases. Furthermore, ADC values within non enhancing FLAIR hyper intense regions were lower in pre-progressive lesions than in stable ones, even if a statistical significance was not found.

In another study [18], ADC evaluation in 21 children with pontine glioma treated by peptide-based vaccination protocol following radiation therapy, showed a decreased ADC value in patients experiencing pseudo-progression compared with patients without pseudo-progression.

**Perfusion MRI**
**Dynamic Susceptibility Contrast (DSC)-MRI** has been performed in course of immunotherapy and elevation of cerebral blood flow (CBV) in a region with contrast enhancement supports the diagnosis of malignant tumor [17,19].

In a pilot study performed on eight patients treated with dendritic cell immunotherapy [17], maximum CBV has been proposed as a potential radiological marker to differentiate between therapy-induced inflammatory response (pseudo-progression) and recurrent GMB (true-progression): maximum normalized lesional CBV resulted highest in progressing tumors, intermediate in pre-progressing lesions and lowest in stable cases. Anyway, due to the small number of cases, a clear correlation between CBV and pseudo-progression was not achieved.

Interestingly, the study described a mismatch in a few cases where enhancing volumes were larger than elevated CBV; histopathological evaluation of this mismatching patients showed a malignant tissue with aberrant vessels with thrombosis or wall damage [19]. These vessels may thus present an increased permeability with contrast enhancement not necessarily linked to increased microvascular volume. As a matter of fact all lesions grew in size over time.

Hypothetically, areas of non-hyperperfused enhancing tissue could be a sign of immune-mediated BBB impairment; this hypothesis is supported by histopathological studies on immune-treated brain metastatic melanoma, which showed reactive astrocytosis, scattered inflammatory cells and microglial cells surrounding isolated clusters of tumor cells [20].

**Dynamic Contrast Enhanced (DCE)-MRI** has been used in GBM immunotherapy preclinical studies in rat models at 7T field strength [21]; an increased Ve (extracellular-extravascular volume fraction) was found in tumors responding to treatment as a consequence to increased tumor cell death as indicated by the diminished growth index on histological measures. On the contrary a decreased Ve was found in progressive lesions, with highest growth index on histological measures.

The same study [21] focused on the evaluation of Ktrans (transvascular transport, an index related to permeability) which seems to be reduced in presence of progressive lesions; however vessel permeability can be affected by inflammation, as endothelial junctions become less tight, thus Ktrans might not reflect a tumor vascular feature but rather an immune-mediated alteration.

**Arterial Spin Labeling (ASL)** has not yet been used in immunotherapy follow-up. It may be an alternative to perfusional techniques in patients with renal failure and severe allergy to contrast agents or to limit the potential risk of chronic contrast accumulation [22].

**Susceptibility Weighted Imaging (SWI)**
SWI has been used for tumor grading [23-26], analyzing the presence of the so called "intra-tumoral susceptibility signals" (ITSS), defined as "low signal intensity and fine linear or dot-like structures, with or without conglomeration, seen within the tumor" [27]. Hypointense signal on SWI images reflect both vascularity and vascular integrity and can be a predictive marker in assessing treatment response as reported in antiangiogenic treatments or RT [28-30]. Additionally, significant higher concentrations of gadolinium are present at the border of the tumor on SWI sequences.

SWI might be helpful to differentiate enhancing hyper-cellulated GBM from immunecellulated volumes, given that ITSS can be found in high grade gliomas while are absent in lymphomas (i.e. hypercellulated lymphocytic tumors) [25]. Moreover, edema does not significantly interfere with SWI images.

Anyway SWI data in immunotherapy have not been published yet.

**Diffusion tensor imaging (DTI)**

DTI has increasingly been performed in the study of high-grade gliomas but only in few studies it was used to discriminate progression from pseudo-progression [31-33]. DTI after RT-CHT shows elevate levels of FA in progressing lesions compared to pseudo-progressing enhancing lesions; a longitudinal analysis approach was also proposed [33].

The main limit of DTI is the presence of edema due to inflammation. Also, DTI acquisition is time consuming and a huge post-processing is needed.

**Magnetic resonance spectroscopy (MRS)**

MRS has been used for glioma diagnosis and grading and for response monitoring [34-35].

The most common metabolites investigated by MRS are N-acetyl aspartate (NAA), a neuronal marker that decreases with any disease that adversely affects neuronal integrity, Choline (Cho) a marker of increased cellular turnover that can be elevated in tumors and inflammatory processes and Creatine (Cr) that gives a measure of energy stores.

Normalized Choline/creatinine (Cho/Cr) ratio seems the most accurate parameter to distinguish between progressive and stable GBMs with sensitivity 70% and specificity 78.6%, gained by a cut-off of 1.9 [36].

MRS can detect the presence of high Cho levels (and Cho/Cr or Cho/NAA ratios) within enhancing tissue thus the presence of glioma within post radio-therapeutic alterations, even though elevated Cho can be also be found in radionecrosis [37].
In a report of two GMB patients with pseudo-progression after immunotherapy MRS did not show an extensive increased Cho concentration within the enhancing areas [38].

A recent trend is focused on MRS quantification of lipids that are considered as substrate of NK T-cells [39]: the presence of a lipidic peak might be associated to a better immunotherapy response. In this view, MRS data need to be correlated to immunological findings and specifically NK activity [40].
### RANO Criteria for high-grade Glioma

| Complete Response | - Disappearance of all enhancing disease for ≥ 4 weeks AND  
|                  | - No new lesions AND  
|                  | - Stable/improved T2/FLAIR AND  
|                  | - No more than physiologic steroids AND  
|                  | - Stable/improved clinically |
| Partial Response  | - ≥ 50% ↓ sum of biperpendicular diameters of enhancing disease for ≥ 4 weeks AND  
|                  | - No new lesions AND  
|                  | - Stable/improved T2/FLAIR AND  
|                  | - Stable/improved steroids AND  
|                  | - Stable/improved clinically |
| Stable Disease    | - Does not qualify for CR, PR, PD AND  
|                  | - No new lesions AND  
|                  | - Stable/improved T2/FLAIR AND  
|                  | - Stable/improved steroids AND  
|                  | - Stable/improved clinically |
| Progressive Disease | - ≥ 25% ↑ sum of biperpendicular diameters of enhancing disease OR  
|                   | - New lesions OR  
|                   | - Significant worsened T2/FLAIR OR  
|                   | - Significant clinical decline |

### iRANO Criteria

<table>
<thead>
<tr>
<th>Is a repeat scan required to confirm radiographic PD for patients without significant clinical decline?</th>
<th>if ≤ 6 months after start of IT</th>
<th>if &gt; 6 months after start of IT</th>
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<td></td>
<td>Yes</td>
<td>No</td>
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<tr>
<th>Minimal time interval for confirmation of progression for patients without significant clinical decline?</th>
<th>≥3 months</th>
<th>Not applicable</th>
</tr>
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<tr>
<th>Is further immunotherapy treatment allowed after initial radiographic PD (if clinically stable) pending progression confirmation</th>
<th>Yes</th>
<th>Not applicable</th>
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| Does a new lesion define PD? | No | Yes |

**Fig. 1:** Table 1 iRANO criteria

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Fig. 2: Figure 2 FLAIR (a-e) and contrast enhanced-T1w.i. (f-j): post-surgical (a, f); increasing edema (b, c) and enhancement (g, h) and subsequent reduction of both (d, e, i) and remission of the enhancing lesion (j) in course of immunotherapy with dendritic cells vaccine.

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Fig. 3: Figure 3 Enhancing lesion (a) during immunotherapy with dendritic cells vaccine. Mismatch between T1w.i. enhancing volume and CBV (b), the last being just slightly elevated; permeability (Ktrans) is increased (c); ADC is low (d) suggesting hypercellularity.

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Conclusion

Conventional MRI alone can be misleading in assessing therapy response, providing limited information on tumor physiopathology and on the status of perilesional tissue.

Mixed scenarios with coexistence of glioma and treatment alterations are often the rule.

On MR-Perfusion main biomarkers are CBV-DSC, reflecting neoangiogenesis, and Ktrans-DCE, reflecting vessel permeability. Contrast-enhancing areas secondary to immunotherapy inflammation should be less perfused than progressive/recurrent tumor but vessel disruption and thrombosis due to high malignancy may inversely affect the perfusional pattern. Nevertheless, inflammation increases vessel permeability with effects on perfusional parameters.

Low ADC, inversely related to cellularity, can be associated to both tumoral and immune hypercellularity and specific analysis has to be performed to discriminate (Fig. 3).

MRS is useful to obtain metabolic information within the enhanced areas, by determining high choline concentration and therefore identifying glioma within treatment alterations.

A combination of different techniques is necessary to differentiate between pseudo-progression and tumor-progression.

Imaging approaches that take into account whole-lesion heterogeneity, and parametric modifications in course of treatment may better enlighten different patterns; evolving in-depth analyses of MRI data such as parametric maps, TRAMs and histogram analyses are promising to answer the challenge of immune-mediated pseudo-progression.

The adjunct of aMRI to cMRI needs clinical validation to become the standard for response assessment.
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References


34. Seeger A, Braun C, Skardellly M, Paulsen F, Schittenhelm J, Ernemann U, Bisdas S. Comparison of three different MR perfusion techniques and MR spectroscopy for


