Multiparametric MR approaches for non-invasive Glioblastoma therapy response follow-up (MAGRes)

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ABSTRACT

Glioblastoma is an aggressive brain tumour with no cure. Improved non-invasive imaging biomarkers are needed for early evidence-based therapy-related decisions. Magnetic Resonance-derived data (imaging, MRI and spectroscopic imaging, MRSI) can be translated into imaging biomarkers of successful preclinical glioblastoma therapy. MAGRes pursued a breakthrough combination of MRI+MRSI data, a step beyond present strategies based only in tumour volume. MAGRes also aimed to develop a software tool for MR post-processing, MRSI artefact removal and machine learning analysis, allowing combined visualisation. This open-source software will pave the way to a scalable system, which could handle clinical data in the future.

Keywords: Glioblastoma therapy; Magnetic resonance spectroscopic imaging; machine learning

1. INTRODUCTION

Glioblastoma (GB) is the most frequent aggressive primary brain tumor in adults. GB prognostic is invariably bad: average survival rates are 16-18 months after diagnosis, highlighting the need of improving therapy response assessment, pursuing early and confident information useful for personalizing therapy schedules. Therapy response follow-up is performed following strict guidelines, centered in aspects such as tumor volume and contrast uptake using defined categorizations. Magnetic resonance imaging (MRI) is often used for these categorizations, through criteria such as response assessment in neuro-oncology (RANO) [1] and Response Evaluation Criteria in Solid Tumors (RECIST) [2], which are not exempt of misinterpretation. Moreover, the participation of the immune system in therapy response is widely acknowledged, although there is a lack of noninvasive biomarkers to assess whether such participation is taking place, which is a determinant factor in response to therapy. There is still much room for improvement in therapy response follow-up in GB, which can be addressed considering multiparametric MR analysis.

MAGRes breakthrough approach uses changes in the tumour metabolome upon successful therapy, related to efficient host immune system action against GB [4]. This is initially sampled by magnetic resonance spectroscopic imaging data (MRSI) and later refined into an MRI-based biomarker for efficient immune system action with clear translational potential.

Results with treated (responding) and untreated GB-bearing immunocompetent mice showed that MRI classification analysis with a radiomics approach achieved 75% hold-out accuracy with only two features, rising up to 90% when using 10 radiomic features. This was based in T2w MRI acquisitions, suggesting that basic MR sequences contain the essential information for biomarker performance, when properly guided (MRSI-training) and analysed. Moreover, direct MRSI-based analysis yielded over 90% accuracy (14 -17 sources) and the paradigmatic information extracted showed metabolomic changes compatible with response in previous work [3,5], such as increases in Polyunsaturated fatty acids (PUFA) and lactate (Lac). This type of metabolomics changes in responding GB are related with immune system presence/action (e.g. macrophages/microglia). The next step will entail using

machine learning (ML) advanced multi-view learning methods to properly combine/synergize information from both MRI and MRSI. A software prototype has been launched for proper 3DMRSI/MRI visualisation, still pending the incorporation of part of the ML-based approaches.

2. STATE OF THE ART

MR-based techniques are the most common non-invasive approach for GB diagnosis and therapy response follow-up. Current procedures to assess whether a patient receiving therapy for GB is properly responding, are performed through MRI explorations, i.e. checking for changes in tumour size and contrast enhancement. However, this is not free of limitations such as the progression/ pseudoprogression dilemma [6]: one month after finishing the standard treatment, contrast-enhancing foci may appear on MR images reflecting either inflammation or relapse. *The only way to confirm patient outcome is to wait for the next MR exploration*, performed two months later. This means that *this temporal window is usually lost*, with no change in treatment: if treatment is failing, precious time and resources have been wasted. This may become even more challenging for the novel immunotherapy approaches for which good non-invasive biomarkers of early response are still lacking [7]. PET-based approaches for imaging immune system have been proposed [8] but it is not feasible to submit patients to repeated PET explorations, as opposed to MR. Efficient therapeutic strategies may recruit/enhance host immune system to fight against tumour, but there is no current way to assess it with the standard MR follow-up protocol. In other words, we are still missing MR image feature(s) that can inform the radiologist about anti-tumour immunity. Information originating from MRSI could help to approach this goal [4,5] but its use is not currently integrated in the clinical pipeline. The complex steps for processing, postprocessing and interpreting, in addition to the lack of standardized file formats (as opposed to the standard MRI formats) hamper this integration. MAGRes aims to tackle these issues and help to unlock the outstanding predictive power of MRSI information in combination with, MRI leading to its integration into clinical practice, as early biomarker of therapy response in GB.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

MAGRes proposes a change of paradigm in the follow-up of therapy response in GB patients, mostly centred in MRI features, acquired months apart. Yet it is true that Radiomics approaches are being currently evolved, these are focused only on MRI features. Our proposal goes one step beyond, integrating the MRSI prior knowledge with advanced ML analysis into the biomarker development protocols. The metabolomic and micro-environment of GB changes under successful therapy, preceding gross anatomical changes, thus relevant hints can be obtained incorporating MRSI data. This is not being currently used in the standard pipeline for therapy follow-up, mostly due to complex postprocessing and interpretation steps, in addition to the lack of standardization in file formats. Moreover, radiologists are used to "imaging-like" outputs and the use of crude spectroscopic information requires previous knowledge about the chemical environment of the observed compounds. The current vendor software packages can offer imaging transformations based on a single metabolite (or two metabolite ratio), disregarding the rich information coming from the whole spectral pattern. We propose to provide radiologists with an innovative output, built over a large dataset of MRI+MRSI data, where analyses use both types of information, and where output may be refined in comparison with studies carried out with MRI alone.

Our approach can provide clinicians with the possibility to 'image' the local immune system action through MRSI-guided information, which is a reliable hint about therapy effectiveness, not possible with current follow-up methods. This analysis will produce an output that confidently informs clinicians whether a therapeutic approach is producing suitable anti-tumour immune response or not. It will provide a robust basis for clinical decisions in therapy management, as early as 1-2 weeks after a new therapy start, as opposed to 2 months with the standard procedures (see Table 1). Our working hypothesis is that preclinical results obtained reflect local tissue changes due to immune system action that are reproducible regardless of the therapeutic approach used or the species being examined. In a second step, ML approaches will be integrated into an initial software version that, once proper MRI input is provided, must be able to process, postprocess, and evaluate MR-files. This will provide doctors with an estimation on how effective the current therapy is recruiting efficient immune system attack onto tumour, even before changes in tumour volume can be detected. This will save time and financial resources, relevant both for patients and health systems.

Tab. 1. Comparison of the estimated time for confident therapy response assessment

Method	Estimated time for confident therapy response output
Standard follow-up procedures	8-12 weeks after new therapy start
Follow-up proposed in MAGRes	2 weeks after new therapy start

4. PROJECT RESULTS

Our analysis within MAGRes was essentially centred in retrospective cases with available T2w MRI and single- or multi-slice MRSI of control (untreated) and Temozolomide (TMZ)-treated GB-bearing mice. This cohort secured that consistent results were achieved which could serve for future validation and translational studies.

MRI data analysis

Two feature selection procedures were used. The results for the feature subsets corresponding to the two used approaches, varying from 1 to 30 radiomic features, are presented in Fig. 1. For the basic statistical *t*-test, the features are directly ranked by relevance. In the more involved wrapper approach, instead, each subset corresponds to the best results for that number of features. Radiomic features discriminated fairly well between treated and control cases in our preclinical study.

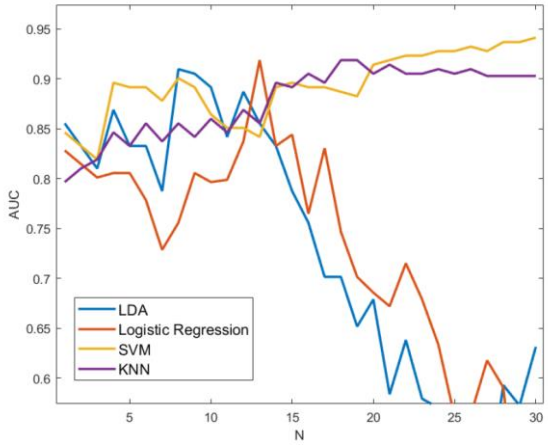

Fig. 1. Representation of performance of different classifiers approached over the number of Radiomics features selected (N most relevant features with N = 1…30) for MRI data. LDA: Linear Discriminant Analysis, SVM: Support Vector Machines, KNN k-Nearest Neighbour.

The embedded method found excellent discriminant values with 7 to 9 radiomics features (Area Under the ROC Curve: AUC, used for performance evaluation, 0.95-0.97). With the wrapper approach, an AUC of 0.96 was achieved with only 3 features, namely *Gray Level Run Length Matrix* type GLRLM-SRHGE and GLRLM-RLV, and *Gray Level Size Zone* type GLSZM-LZHGE. These results agree with the available literature; for example [9] describes features such as GLRLM correlating with histopathological features such as Ki67 in high-grade glioma. Recent work [10] also described the value of GLRLM and GLSZM for evaluating response to therapy in GB, being able to distinguish pseudoprogression from true progression. The ten most relevant radiomics features found in our approach are listed in Table 1, while Fig. 2 shows representative T2w MRI from different mice with evident changes observed in tumour zones, derived from treatment. More details can be found in [11].

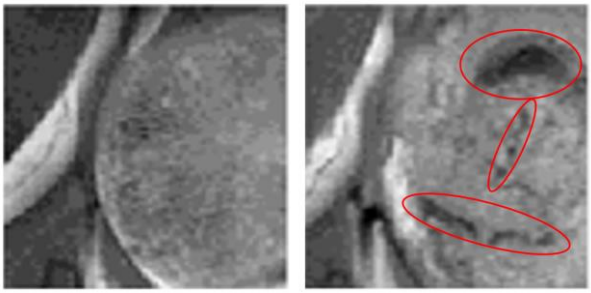

Fig. 2. Examples of control (left, mouse C583) and treated, transiently responding to TMZ according to histopathological parameters (right, mouse C574) murine GL261 GB tumours. Note the appearance of hypointense zones (red ovals) in T2w MRI from the treated mouse, noticeably different from the more homogeneous appearance observed in the control case.

Tab. 1. Feature ranking by t-test and wrapper feature selection methods (first 10 features).

Ranking Position	t-test method	Wrapper method
1	Perimeter9	GLCMEntropy
2	Perimeter8	Perimeter9
3	GLRLMRLV	GTDMComplexity
4	Perimeter7	GLSZMSZLGE
5	Euler7	Area16
6	Euler6	Area13
7	GLRLMGLN	GLRLMRLV
8	Perimeter6	GLRLMRLN
9	GLCMVariance	Euler1
10	GLSZMGLN	GLRLMSRE

MRSI data analysis

The results obtained with MRSI through convex-NFM analysis of sources (paradigmatic spectra extracted from tumour regions from all studied mice, control and treated) were overall better than those achieved by the best model applied to the radiomic features, being also more stable in their evolution over the selection of features (sources in this case). A 20-fold validation method was used for analysis. The best results were obtained with SVM using embedded+wrapper methods for feature selection with AUC higher than 0.997 (14 to 17 sources used). The aforementioned sources showed the expected changes in metabolites previously described by us in [3] as relevant for distinguishing among control and responding GL261 murine GB, such as PUFA, Lac, glutamate-glutamine (Glx) and alanine (Ala). It is worth noting that the appearance of PUFAs after preclinical brain tumour treatment has been described by others [12], probably reflecting local apoptosis as a

consequence of therapeutic protocols. One of the benefits of the MRSI per-voxel analysis is the possibility to ascertain how each part of the tumour is responding, allowing us to unravel its heterogeneity. Even if considering a mouse as responding to a specific therapy as a whole, we still cannot be sure whether the tumour is consistently and homogeneously responding to therapy or not. The key advantage of our analytical pipeline is that the classification results come with a quantification of the certainty of the classification prediction that is anatomically bounded. This means that we can graphically represent, using nosological images, such level of certainty over the anatomy of the tumour, as exemplified by the images in Figure 3.

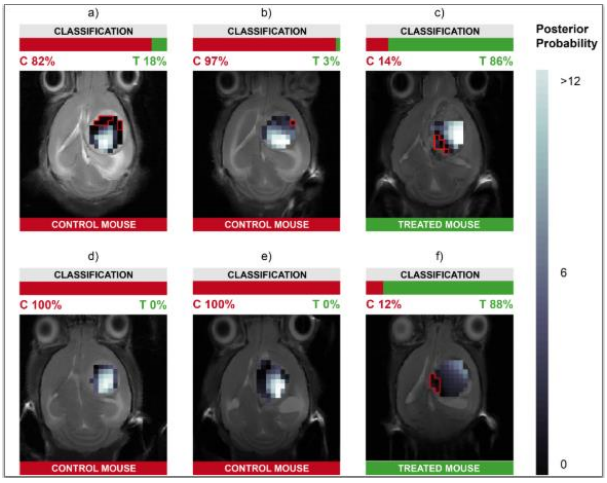

Fig. 3. Examples of nosological visual representation of the classification results for MRSI data from extracted sources. Horizontal T2w MRI images of GL261 GB afflicted mice, superimposed with representative nosological maps of the classification reliability in different tumour regions for the SVM classifier with 10 features (sources). The color-coding (scale on the right) shows how reliable the model classification output is, representing a posterior probability. The lighter the colour, the more reliable and vice versa. The red contour over some voxels represents those misclassified by that model. The colour bars at the bottom represent the true class of the case, whereas the colour bars at the top represent the percentage of voxels classified as treated or control for each case.

Preliminary software version launched

A preliminary software version for visualization of 3D MRSI-based nosological images of therapy response acquired as in [5] superimposed to MRI was launched by month 6 of ATTRACT (see a screenshot in Fig. 4).

This software called IMAGINEs (Imaging Immune System) is based on open source packages like 3D slicer, so that it can be made available to interested researchers, being the initial step towards having a fully functional programme including the ML approaches developed. Unfortunately, it was not possible to integrate all functions due to several challenges faced during 2020 and some processing steps should still be performed with other software modules. A possible ATTRACT Phase 2 may allow for proper software development, testing and validation, first with preclinical data and later on, with clinical data, as described in section 5.3.

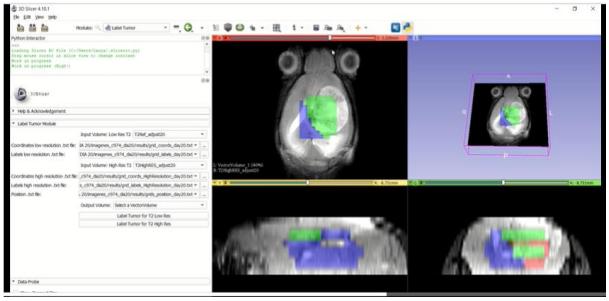

Fig. 4. Screenshot of the preliminary version for 3D-MRSI visualisation launched. Left, menu for uploading files. Right, different views of the acquired MRI (horizontal, sagittal, coronal) with the MRSI-based nosological image of therapy response [3,4,5] superimposed in colour.

5. FUTURE PROJECT VISION

5.1. Technology Scaling

The MAGRes technology is presently at TRL level 3-4, i.e. preclinical proof-of-concept performed and under molecular/cellular validation. Scaling up to TLR 5-7 requires coordination with scientific, commercial and clinical actors and application of cutting-edge ML approaches such as transfer learning (ensures applicability of preclinical findings to clinical pipelines). It is worth mentioning that MAGRes members are involved in a recently granted network (XARTEC Salut XARDI-00016, *Generalitat Catalunya*, 2020-2022, 47 partners, 1.3 M€) which contribute to increase TRL for a sustained level 4 and prepare MAGRes for Phase 2.

5.2. Project Synergies and Outreach

Project Synergies

Processing/postprocessing MR pipelines evolution will benefit from participation of long-term collaborators at LJMU. Major clinical scanner vendors (Philips, GE HealthCare, Siemens) are already familiarized with the MAGRes concept through joint participation in EU ITN requests with CIBER and UPM. They, jointly with Bruker, the main preclinical scanner manufacturer, will be approached for MAGRes Phase 2 participation/advice. Prototype validation requires participation of academic hospitals such as UMCU, UKER (also involved in joint ITN requests), as well as Hospital Sant Joan de Déu for paediatrics. Partnership with a nationwide infrastructure network, NANBIOSIS is also envisioned.

Regarding other ATTRACT Phase 1 projects, synergies are foreseen with TOPiomics pursuing early variations in

tumour phenotype influencing outcome. They also aim to model multi-view spaces with Small Sample Size data which is relevant for both projects.

Dissemination/Outreach
The following actions will be taken in a potential ATTRACT Phase 2 (some already implemented in Phase 1):
- Open access reporting to high impact factor journal/conferences.
- Participation in national, European, worldwide scientific conferences to communicate results and demonstrate prototype performance.
- Software beta versions made available from public repositories

Public engagement strategy
General scientific community: scientific conferences and open access publication. **Clinical community:** presentations targeting clinical audience focusing in the impact of our results in patient's quality of life and survival. **Scanner Vendors**: Non-participating Phase 2 companies invited to dissemination sessions. **Brain cancer patients:** "Helping Cancer" will be contacted to provide information to cancer patients with appropriate language. **General public**: make MAGRes topics understandable to EU citizens. Open day meetings, talks in science pubs and use of social media.

5.3. Technology application and demonstration cases

The MAGRes technology will have a positive impact in Health, Demographic Change and Wellbeing. Aspects such as personalised medicine, innovative health and systems and big data solutions will be approached.

The development of a software package must have in mind users' needs and desired outputs, in our case to produce a product that fulfils actual needs faced in clinics. For this, a user requirements list will be built to target clients. Still, although the idea is to benefit from MRSI to improve MRI algorithms, we may also consider providing users the possibility to directly deal with their own MRSI files. Having this in mind, we envision two validation levels.

A first round of validation at preclinical level:
1. Integration of the ML algorithms and output visualization.
2. Incorporation of MRI and MRSI processing steps, (preclinical first).
3. Validation with 'new test cases': A) longitudinal cases along standard GB treatment, B) longitudinal cases with different therapeutic strategies, C) chosen cases for molecular/cellular validation.

This will increase robustness and future applicability of our response biomarker and help to clarify methodological questions at preclinical level. However, MAGRes aims to help in GB patient management and a clinical demonstration must be planned with chosen clinical centres. A prospective study would be the ideal scenario, envisaged as follows:

1. Early MRI follow-up planned with GB patients under therapy. Predicted outcome registered both with MAGRes biomarker and MRI using standard criteria.

2. Patient 'standard' follow-up performed in the usual way and outcome registered as in point 1. Added value will be assessed when comparing how using the MAGRes biomarker would have changed clinical decision at early times, not possible with the standard approach.

A final evaluation from clinicians about software usability, user-friendly characteristics, and willingness to incorporate it into clinical-decision making will be asked for. The expected work packages in such project would be 1) Management, 2) Preclinical data acquisition and cellular validation, 3) ML approaches, 4) Software development 5) Clinical data acquisition and software testing.

The expected gross budget, considering different levels of technology validation, would be of 400.000€ for the preclinical setting and ca. 1.000.000€ for joint software development, clinical data acquisition and evaluation, and software evaluation procedures.

5.4. Technology commercialization

The biomarker and software development approach proposed in MAGRes, although firstly built over preclinical data, should be of great interest in a clinical environments. We foresee that the main MR clinical/preclinical scanner vendors will be interested in integrating it in their own machine/offline packages. Companies such as **Philips, Siemens and GE Healthcare** have demonstrated interest and participated in a parallel, extended approach presented as a Marie Sklodowska-Curie action (Innovative Training Networks, ITN) in which CIBER was also involved. Integrating our software approach may enable clinicians to use MRSI information in a robust platform without the need of large time-consuming learning curves.

5.5. Envisioned risks

Tab. 1. Risks to be considered in a potential ATTRACT Phase 2 project and the corresponding mitigation strategies proposed.

Risk	Mitigation Strategy
Poor predictive performance from biomarkers found in	Low to Moderate risk based on previous research. Increase the

preclinical models, so that biomarkers need to be found de novo in humans within a limited timeframe	number of human cases for the development of predictive pattern recognition models
Delay in the implementation of the software tools for image analysis and predictive modelling	Low risk. Existing in-house code will be made available together with training a set of publicly available libraries.
Absence of some commercial vendors in the project prevent us to develop proper strategies for processing/postprocessing strategies in the specific frame of this software	Moderate risk. Collaboration with researchers from previous jMRUI project, currently involved in INSPIRE-MED ITN, in which part of ATTRACT researchers also participate. Software package can be offered as an independent module, regardless of scanner vendors

5.6. Liaison with Student Teams and Socio-Economic Study

Student teams: it was not feasible to interact with M.Sc. level students along the Phase 1 project. However, researchers involved in MAGRes have ample expertise in supervising MSc and PhD students. Facing a possible Phase 2, students will benefit from transversal knowledge ranging from preclinical tumour management, MR processing/postprocessing, advanced ML + biomedical signal analysis, and software development/methodology integration.

Socio-economic study: MAGRes researchers will contribute to the socio-economic study in interviews or enquiries organized in Phase 2. We can provide up-to-date information on how GB therapy-response follow-up is evolving at collaborating clinics. An estimation on how health system resources will be saved with MAGRes approaches, in comparison with the standard currently applied, can be provided upon request.

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