**Multiparametric Magnetic Resonance Imaging in the Assessment of**

**Anti-EGFRvIII Chimeric Antigen Receptor T cell**

**Therapy in Patients with Recurrent Glioblastoma**

ABSTRACT:

BACKGROUND: EGFRvIII targeted chimeric antigen receptor T (CAR-T) cell therapy has recently been reported for treating glioblastomas (GBMs), however, physiology-based MRI parameters have not been evaluated in this setting.

METHODS: Ten patients underwent multiparametric MRI at baseline, 1, 2 and 3-months after CAR-T therapy. Logistic regression model derived progression probabilities (PP) using imaging parameters were used to assess treatment response.

RESULTS: Four lesions from “early surgery” group demonstrated high PP at baseline suggestive of progression which was confirmed histologically. Out of eight lesions from remaining 6 patients, three lesions with low PP at baseline remained stable. Two lesions with high PP at baseline were associated with large decreases in PP reflecting treatment response, whereas other two lesions with high PP at baseline continued to demonstrate progression.

CONCLUSION: Our findings indicate that multiparametric MRI may be helpful in monitoring CAR-T related early therapeutic changes in GBM patients.

**Introduction**

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults with poor prognosis. Recurrence is almost inevitable and the median survival for these recurrent patients is only 6.6-9.6 months 1. Epidermal growth factor receptor variant III (EGFRvIII) is expressed in about one third of GBM patients, promotes oncogenesis and is associated with poor prognosis 2. A recent study demonstrated successful synthesis, delivery, and acceptable safety profile of chimeric antigen receptor T (CAR-T) cell therapy targeting against EGFRvIII epitope in patients with recurrent GBM 3. Since immunotherapy including CAR-T therapy, triggers patient’s immune system to fight cancer cells, a pronounced inflammatory response occurs within the tumor bed 4, complicating the appearance on conventional MRI for evaluation of therapeutic response. Multiparametric analysis using diffusion tensor imaging (DTI) 5, dynamic susceptibility contrast (DSC) perfusion imaging 6 and proton MR spectroscopy 7 have been reported to distinguish true progression (TP) from pseudoprogression (PsP) with high accuracy 5,8. This rationale formed the basis of this study in which treatment response of CART-EGFRvIII immunotherapy in recurrent GBMs was evaluated using DTI, DSC and spectroscopic imaging.

Methods

The study was approved by the Institutional Review Board. Informed consent was obtained from all patients. Ten recurrent GBM patients (5M/5F, mean age: 60.56±10.31 years) were included; inclusion criteria (Supplementary Material). Clinical/demographic information, EGFRvIII expression levels, and overall survival (OS) described in Table S1. Baseline images were acquired within one week before CAR-T infusion. Tumor progression was determined based on a combination of clinical status and RANO criteria.

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Seven of 10 patients underwent resection after demonstrating progression following CAR-T cell infusion. Patients were divided into 3 groups according to the time of repeat surgery after CAR-T cell infusion: 1) no surgery (***no surgery group***, n=3); 2) surgery within a month (***early surgery group***, n=4); 3) surgery > a month (***late surgery group***, n=3) (Table S1).

Data acquisition/analysis of DTI, DSC and 3-D echo planar spectroscopic imaging (3D-EPSI) sequences were performed as previously described 5,7,9. All contrast enhancing lesions (n=12) ≥ 1cm3 were selected for quantitative analysis. A semi-automatic segmentation approach was used to generate a mask from the enhancing region of the neoplasm (Supplementary Material). The enhancing and central non-enhancing regions were used to compute tumor volume. Mean diffusivity (MD), fractional anisotropy (FA), linear anisotropy (CL), planar anisotropy (CP), spherical anisotropy (CS), relative cerebral blood volume (rCBV) and choline/creatine (Cho/Cr) ratio from enhancing lesions were estimated at each time point. Percent changes for each parameter between baseline and subsequent scans (N) were calculated as (N – baseline)/baseline × 100 for the non-surgery and late surgery groups. Baseline 3D-EPSI data were available from two lesions in one patient. Hence, percentage changes in Cho/Cr from these two lesions were also evaluated.

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In our previous study5, for patients who underwent surgery and chemoradiation therapy (CRT) and exhibited new contrast enhancing lesions on follow up imaging within six months , a combination of FA, CL and rCBVmax was reported to be the best model in differentiating PsP from TP with high accuracy (AUC 0.91). This model was determined based on the histological analysis of surgical samples. Therefore, these three parameters were used in this study to compute the progression probabilities (PP) of each lesion at each time point using the following regression equation 5:

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where β0 = -16.17, β1 = 194.01, β2 = -285.65, and β3 = 1.21. Lesions were considered TP (predominant viable tumor) if the predictive PP was ≥ 50% and PsP (predominant treatment effects) if predictive PP was ≤ 50%.

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In order to evaluate CAR-T treatment efficacy, we included 10 recurrent GBM patients without CAR-T therapy (Table S2) and calculated PP values.

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OS was measured from the date of diagnosis and CAR-T infusion to the date of death for deceased patients, or the date of last clinical follow up for surviving patients.

# Results

Three out of 10 patients died within 6 months after CAR-T infusion. Six patients survived > 6 months before succumbing to the disease. One patient (209) was still alive at the time of the writing of this manuscript with a survival of 34.0 months (1033 days). Median OS from all 10 patients was 247 days (Table 1).

Serial anatomical images, parametric maps and histologic findings from patient 209 at baseline and follow-up periods are shown in Figure S1. Baseline imaging parameters and PP obtained using the classification model for the 3 groups are presented in Table 1. Percentage changes in tumor volume and imaging parameters at follow-up periods in comparison to baseline are shown in Figure 1A. Six out of 8 lesions demonstrated increased tumor volume at all follow-up periods relative to baseline.

The enhancing lesions from four patients in “early surgery” group demonstrated high PP (72-99%) and were classified as progressive disease. These four patients underwent repeat surgery within a month following CAR-T infusion and were excluded from longitudinal analysis. Histopathological findings confirmed the diagnosis of TP in these patients.

For remaining 6 patients (total of 8 lesions),three lesions (patients 204, 205 and 209 L2) with low PP at baseline remained stable at follow-up. Two lesions (patient 202, 209 L1) initially with high PP at baseline were associated with large decreases in PP and were classified as PsP at follow-up. The remaining 2 lesions (207 L1 and L2) with high PP at baseline continued to demonstrate aggressive imaging features at follow-up. No baseline data was available for patient 201. However, this patient showed features of TP at follow-up. PP values using multiparametric classification model at baseline and follow-up periods are shown in Table S3. The plots of these predictive PPs are shown in Figure 1B. Predicted PPs obtained from our classification model were confirmed on histopathology for all patients in the early and late surgery groups. Predicted PPs for recurrent GBMs without CAR-T therapy are shown in Table S4 and Figure 1C. 8 patients showed increased PP at follow-up time points.

# Discussion

We used a predictive model from multiparametric MRI to assess the behavior of neoplastic lesions following anti-EGFR CAR-T cell immunotherapy. All patients with available histopathology were correctly predicted as TP or PsP, indicating utility of multiparametric MRI in evaluating therapeutic response to CAR-T cell immunotherapy.

Harnessing of immune response involves inflammatory sequelae that complicates conventional imaging appearance limiting application of RANO criteria. As immunotherapies enter clinical trials for treating GBM, there is an urgent need to reliably assess the efficacy of these treatment modalities in detecting elusive disease and redefining response 4.

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Multiparametric MRI have been widely used to predict treatment response in GBM patients 5-7. When percent changes in individual imaging parameters were assessed from enhancing lesions at different follow-up periods relative to baseline, no definite trends were observed, indicating that imaging parameters, in isolation, may have a limited role in assessing heterogeneity of treatment response to EGFRvIII CAR-T cell therapy. However, when we used the PP model derived from multiparametric MRI, we were able to objectively characterize each lesion as either progression or response at each individual time point suggesting that a multiparametric approach may allow more accurate characterization of treatment response in GBM patients treated with immune/targeted therapies. These results need to be validated in a larger patient cohort and correlated with clinical endpoints of progression free survival and OS.

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**Figure legends**

**Figure 1.** Percent changes of MR parameters and tumor volume after CAR-T treatment for 8 lesions in 6 patients (A). Baseline and follow up MRSI data was only available for 2 lesions to compute the percent changes. Changes of progression probabilities (PP) using the predictive model for 8 lesions in 6 patients (B). Changes of PP in recurrent GBM patients without CAR-T therapy are shown in Figure 1C. The probability of true progression is 50-100% whereas the probability of pseudoprogression is 0-50%.

**Figure S1**. Representative MR and histology images of large lesion (L1) in Case 209. No appreciable changes of imaging parameters were observed (A). MRS spectra demonstrated decreased Cho/Cr ratios at 1, 2, 3-month follow up periods compared with the baseline (B). Histology (hematoxylin-eosin stain, 20 x magnification, a and b) and CD3 immunohistochemistry stain (c and d) (C) showed predominant treatment-related changes including extensive geographic necrosis and hyalinized vessels (b) and increased T cells (d) 104 days after CAR-T cell infusion.